Vidya Bharati Shaikshanik Mandal, Amravati's

VIDYA BHARATI MAHAVIDYALAYA, AMRAVATI

Affiliated to Sant Gadge Baba Amravati University, Amravati

Re-accredited 'A' Grade by NAAC (CGPA : 3.26 Second Cycle)

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3.3.2 Number of research papers per teachers in the Journals notified on UGC website during the Academc Year 2016-17

SN	Title of paper	Name of the author/s	Department of the teacher	Name of journal	Year of publication	ISSN number	Link to the recognition in UGC enlistment of the Journal
124	Identification of nigella sativa secel & its adultrants using dna barcode marker	DR. LALIT K. VYAS	Cosmetic Technology	American journal of life sciences	2016-17	23285737	Not listed in present UGC approved list as well as in deleted approved UGC List
125	English Teaching and Learning with Computer	Prof. V. P.Shekokar	English	Research Bulletin An International Quarterly Refereed Research Journal	2016-17	22311025	Not listed in present UGC approved list as well as in deleted approved UGC List
126	Various Problems of Women and Their Empowerment in India	Dr. Arunsingh D. Chauhan	Sociology	EPRA International Journal of Environmental Economics, Commerce and Educational Management	2016-17	2348814X	Not listed in present UGC approved list as well as in deleted approved UGC List
127	Problems of Elderly in Rural Society	Dr. Arunsingh D. Chauhan	Sociology	EPRA International Journal of Socio-Economic and Environmental Outlook	2016-17	23484101	Not listed in present UGC approved list as well as in deleted approved UGC List
128	Effect of azadirachtin on some fertility aspects of male albino rat *Rattus norvegicus*	Dr. Y. D. Akhare	Zoology	VidyaBharati International Interdisciplinary Research Journal	2016-17	23194979	http://www.viirj.org/declaration.html
129	Study of stability constants of Cu (II), Co (II), Ni (II), Mn (II) complexes with substituted Δ^2-pyrazole in DMF solvent using pH-Meter.	P.S.Nandurkar,M. M. Rathore, P. R. Rajput	Chemistry	Res. J. Chem. Sci. Vol. 6(6), 2016, 44-46.	2016-17	2231606X	Not listed in present UGC approved list as well as in deleted approved UGC List
130	Synthesis, characterisation and screening of some new chlorosubstituted imidazolo-pyrazolines with special reference to their growth promoting and curative impact on *Oyster mushroom* crop.	N.G.Ghodile,P.R.Rajput and Padma Rajput	Chemistry	IJAPSA, 2(7) 2016, 66-71. ISSN: 2394-5532	2016-17	23945532	Not listed in present UGC approved list as well as in deleted approved UGC List
131	Synthesis of some chlorosubstituted thiazoles, imidazolo-thiazoles-as efficient antibacterial agents.	M.W.Bhade and P.R.Rajput	Chemistry	EJBPS, 3(8), 2016, 527-530.	2016-17	23498870	Not listed in present UGC approved list as well as in deleted approved UGC List

132	Design and synthesis of some imidazole derivatives containing 4-(3,5-dichloro-2-hydroxyphenyl) imidazole moiety as antibacterial agents.	M.W.Bhade and P.R.Rajput	Chemistry	IJAPSA, 2(11), 2016, 80-84.	2016-17	23945532	Not listed in present UGC approved list as well as in deleted approved UGC List
133	Synthesis and study of substituted 1,3-thiazoles and their nanoparticles on phytotic growth of some vegetable crops.	C.D.Badnakhe and P.R.Rajput	Chemistry	IJAPSA, 2(10), 2016, 139-153.	2016-17	23945532	Not listed in present UGC approved list as well as in deleted approved UGC List
134	Synthesis and study of substituted 1,3-thiazoles and their nano-particles on phytotic growth of some vegetable crops.	C.D.Badnakhe and P.R.Rajput	Chemistry	Int. J Basic and App. Chem. Sci., 6(3), 2016, 5-21.	2016-17	22772073	Not listed in present UGC approved list as well as in deleted approved UGC List
135	Phytochemical screening, antimicrobial and antioxidant activity of whole extract of *Cardiospermum halicacabum* Linn. (*Sapindaceae*).	M.O.Malpani,P.R.Rajput, P.S.Pande and M.M.Sapkal	Chemistry	Am. J. Pharm Tech Res., 6(5), 2016, 503-508.	2016-17	22493387	Not listed in present UGC approved list as well as in deleted approved UGC List
136	Quantitative structure–activity relationships (QSARs) and pharmacophore modeling for human African trypanosomiasis (HAT) activity of pyridyl benzamides and 3-(oxazolo[4,5-b]pyridin-2-yl)anilides	Vijay H. Masand, Devidas T. Mahajan, Atish K. Maldhure, Vesna Rastija	Chemistry	Med Chem Res, 2016, 25:2324–2334	2016-17	10542523	Not listed in present UGC approved list as well as in deleted approved UGC List
137	Computational Strategies to Explore Antimalarial Thiazine Alkaloid Lead Compounds Based on an Australian Marine Sponge Plakortis Lita	Lilly Aswathy, Radhakrishnan S. Jisha, Vijay H. Masand, Jayant M. Gajbhiye & Indira G.	Chemistry	Journal of Biomolecular Structure and Dynamics, 35 (11), 2017, 2407-2429	2016-17	7391102	Not listed in present UGC approved list as well as in deleted approved UGC List
138	QSAR modeling for anti-human African trypanosomiasis activity of substituted 2-Phenylimidazopyridines	Vijay H. Masand a, Nahed N.E. El-Sayed ,Devidas T. Mahajan , Andrew G. Mercader , Ahmed M. Alafeefy, I.G.	Chemistry	Journal of Molecular Structure	2016-17	222860	Not listed in present UGC approved list as well as in deleted approved UGC List
139	Synthesis, Antiphospholipase A2, Antiprotease, Antibacterial Evaluation and Molecular Docking Analysis of Certain Novel Hydrazones	Nahed N. E. El-Sayed, Ahmed M. Alafeefy, Mohammed A. Bakht, Vijay H. Masand, Ali Aldalbahi, Nan Chen,	Chemistry	Molecules 2016, 21, 1664-1681	2016-17	14203049	Not listed in present UGC approved list as well as in deleted approved UGC List

140	A STUDY OF IMPACT OF SAVINGS ON INVESTMENT PREFERENCES OF INVESTORS" A CASE STUDY OF BHMS DOCTORS OF AMRAVATI CITY, MAHARASHTRA, INDIA	S A Bothra and S S Kavitkar	Management Studies	ISRJ	2016-17	22307850	Not listed in present UGC approved list as well as in deleted approved UGC List
141	Preliminary phytochemical analysis of M.tomentosa Roxb. (J) Sinclair by using various organic solvent.	L.P.Khalid,P.V. Pulate and N.A. Wagay	Botany	European Journal of Biomedical and Pharmaceutical Sciences	2016-17	23498870	Not listed in present UGC approved list as well as in deleted approved UGC List
142	GOODS AND SERVICE TAX	Dr. S. B. Kadu & M.K.Gawande	Commerce	VIDYABHARTI INTERNATIONAL INTERDISCIPLINARY RESEARCH JOURNAL	2016-17	23194979	http://www.viirj.org/declaration.html
143	RURAL DEVELOPMENT AND DIGITAL VILLAGE	Dr. S. B. Kadu, Mr.S.K.Rodde	Commerce	VIDYABHARTI INTERNATIONAL INTERDISCIPLINARY RESEARCH JOURNAL	2016-17	23194979	http://www.viirj.org/declaration.html
144	Investigation of sodium hyaluronate skin serum by using nano technology	MS.BHAVIKA BHOKARE & DR. MADHURI D.	Cosmetic Technology	International journal of research in engineering & applied sciences (ijreas)	2016-17	22493905	Not listed in present UGC approved list as well as in deleted approved UGC List
145	LRS Bianchi Type I Magnetized Anisotropic dark energy models with variable equation of state	A. P. Wasnik, S. P. Kandalkar, P. P. Khade	Mathematics	Elixir Space Science	2016-17	2229712X	Not listed in present UGC approved list as well as in deleted approved UGC List
146	Challenges in rural development and digital village	Dr. D. S. Wankhade	Physical Education and Sports	Smart India Vision 2020 – innovation in computer application management and commerce	2016-17	23194979	http://www.viirj.org/declaration.html
147	Potentiometric titration of complexes with flavones and metal	T.S. Bante, M.M. Rathore , P.R. Rajput	Chemistry	Research Journal of Chemical Vol. 7(1), 1-4, January (2017)	2016-17	2231606X	Not listed in present UGC approved list as well as in deleted approved UGC List
148	QSAR analysis for 6-arylpyrazine-2-carboxamides as Trypanosoma brucei inhibitors	V. H. Masand, N. N. E. El-Sayed, D. T. Mahajan & V. Rastija	Chemistry		2016-17	1062936X	Not listed in present UGC approved list as well as in deleted approved UGC List
149	Eco-Friendly Synthesize and Biological Evaluatiion of 2-Amino -5- substituted-1,3,4-thiadiazoles	Shubhangi Athawale, V.H. Masand, and S. E. Bhandarkar	Chemistry	Research Journal of Chemical Science, Vol. 6,40-43, 2016	2016-17	2231606X	Not listed in present UGC approved list as well as in deleted approved UGC List

150	Synthesis of 3-Aroyl Flavanones by Using Microwave Irradiation and Study of Its Antibacterial Activity	Dr Pravin S. Bodkhe and J.N. Angaitkar	Chemistry	Journal of Chemical, Biological and Physical Sciences An International Peer Review E-3 Journal of Sciences Available online atwww.jcbsc.org Section A:	2016-17	22491929	Not listed in present UGC approved list as well as in deleted approved UGC List
151	Synthesis, Characterization and Antimicrobial Activity of Newly Substituted 3-Aroyl Flavanones	Dr Pravin S. Bodkhe and J.N. Angaitkar	Chemistry	J.of chemistry and chemical sciences	2016-17	2229760X	Not listed in present UGC approved list as well as in deleted approved UGC List
152	pH Metric Study of Synthesised Substituted Propane -1,3-Diones with CU(II), CO(II) and NI(II) Cataions at 0.1 M Ionic Strength	Dr Pravin S. Bodkhe ,J.N.Angaitkar and M.L.Narwade	Chemistry	Journal of Chemistry and Chemical Sciences, Vol.6(11), 1074-1079, November 2016 (An International Research Journal), www.chemistry-journal.org	2016-17	2229760X	Not listed in present UGC approved list as well as in deleted approved UGC List
153	Synthesis, Characterization and Antimicrobial Screening of Azo Compounds containing 4-Hydroxybenzaldehyde moiety	Dr Pravin S. Bodkhe,S.K.Pagariya and R.M.Pathade	Chemistry	Research Journal of Chemical sciences Vol. 6(9), 1-5, September (2016)	2016-17	2231606X	Not listed in present UGC approved list as well as in deleted approved UGC List
154	Spectrophotometric determination of pka of schiff base ligand	Lawankar TR, Mahajan DT	Chemistry	International Journal for Pharmaceutical Research Scholars (IJPRS)	2016-17	22777873	Not listed in present UGC approved list as well as in deleted approved UGC List
155	PH -METRIC ANALYSIS OF COMPLEX FORMATION OF CU(II),CO(III) AND FE(III) METAL IONS AND SUBSTITUTED HYDROXY SCHIFF'S BASES IN 70% MIXED SOLVENT MEDIA.	Lawankar TR, Mahajan DT	Chemistry	BIONANO FRONTIER	2016-17	9740678	Not listed in present UGC approved list as well as in deleted approved UGC List
156	STUDY OF METAL-LIGAND STABILITY CONSTANTS OF La (III), Sm(III) & Nd(III) METAL ION COMPLEXES WITH SUBSTITUTED SCHIFF'S BASES AT 0.1 M IONIC STRENGTH PH-METRICALLY	Truptanjali R. Lawankar*, Devidas T. Mahajan and Syed. Azhar. Quazi	Chemistry	european Journal of Biomedical AND Pharmaceutical sciences	2016-17	23498870	Not listed in present UGC approved list as well as in deleted approved UGC List

157	A STUDY OF IMPACT ON ECONOMIC EMPOWERMENT OF WOMEN THROUGH SELF HELP GROUPS WITH SPECIAL REFERENCE ON AMRAVATI DISTRICT	P G Dammani	Management Studies	ISRJ	2016-17	22307850	Not listed in present UGC approved list as well as in deleted approved UGC List
158	DIGITAL VILLAGE: BREAKING THE TRADITIONAL PERCEPTION TOWARDS RURAL DEVELOPMENT	P.G.Dammani and N.P.Agrawal	Management Studies	VIIRJ	2016-17	23194979	http://www.viirj.org/declaration.html
159	INDIA ON ITS URGE TOWARDS CASHLESS ECONOMY	S B Tripathi	Management Studies	VIIRJ	2016-17	23194979	http://www.viirj.org/declaration.html

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Identification of *Nigella sativa* Seed and Its Adulterants Using DNA Barcode Marker

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Abstract: Adulteration, misidentification, and substitution are the biggest challenges in maintaining safety and therapeutic efficacy of medicinal herbs. *Nigella sativa* seed, which is well known medicinal herb susceptible to adulteration or substitution due to its great therapeutic value. Adulteration and substitution by morphologically similar seeds are the primary concern in commercially available *Nigella sativa* seed. In this study, we have used DNA barcode marker to find out adulteration, misidentification, and substitution of *Nigella sativa* seed sold in various markets. We collected 10 samples, which were labelled as Black seed/*Nigella sativa* seed from open markets in India (1 No.), Pakistan (1 No.), Saudi Arabia(1 No.), Egypt (2 No.), Turkey (1 No.), Syria (1 No.), Tunisia(2 No.) and Oman (1 No.). All samples collected from different geographies were studied morphologically. Although few samples were quickly identified as *Nigella sativa* seeds, few were tough to detect and differentiate accurately. This is where DNA barcode marker proved to be useful. Plant DNA were obtained from seed coat cells of samples, was amplified by PCR with forward and reverse rbcl and matK primers as recommended by CBOL (The Consortium for the Barcode of Life). PCR amplification of plastid genome with matK was not very successful, while PCR amplification with rbcl primers was quite successful. We used rbcl sequences for alignment and further analysis. PCR products obtained were subjected to electrophoresis on 1.5% agarose plate. PCR products were sent to Macrogen (Seoul, South Korea) for DNA sequencing. DNA reads obtained with rbcl sequences were aligned and analyzed for nucleotide composition, conserved sites, variable sites, singleton sites and parsimony-informative sites, genetic distance and phylogenetic tree using MEGA 7. The phylogenetic tree was constructed using UPGMA method. NCBI Blast along with phylogenic tree and nucleotide characteristic were used to identify *Nigella sativa* seeds from different geographies and discriminate two adulterants as *Allium cepa* seed and *Clitoria guianensis* seed. Both of these adulterants are different regarding their active medicinal contents and therapeutic utility from *Nigella sativa* seed. This study proved the utility of DNA marker, especially rbcl loci in accurately identifying medicinal herb and its adulterants.

Keywords: *Nigella sativa*, Kalongi, DNA Barcoding, Molecular Markers, rbcl, Matk, Adulteration, Misidentification

1. Introduction

Worldwide trade of medicinal herb is about $ 60 billion dollar business annually. There are about 1000 companies from different countries involved in the trading of medicinal herbs, Business of medicinal herbs is growing at the rate of 15 to 20% per year [1]. This growth in the trade of herbal medicine is due to significant demand for natural, safe and reliable therapeutic agents. Patients want a more safe, secure and natural way of treatment of diseases.

This study is to report, the utility of rbcl and matK DNA barcode marker to identify substitution and adulteration in the *Nigella sativa* seed of various geographies.

2. Material and Methods

2.1. Samples

Samples of Nigella sativa seeds were collected from various geographies like India (1 No.), Pakistan (1 No.), Saudi Arabia (1 No.), Egypt (2 No.), Turkey (1 No.), Syria (1 No.), Tunisia (2 No.) and Oman (1 No.). Voucher specimens were deposited at Institute Herbarium.

2.2. DNA Extraction and PCR Amplification

Plant DNA was separated from Seed coat cells using plant/fungi DNA isolation kit from Norgen Biotek, Canada (DNA Isolation Kit Product # 26200) following manufacturer's protocol. Purified DNA was preserved at -20°C till further use. Further, extracted DNA was examined using 0.8% agarose gel electrophoresis stained with ethidium bromide.

Well 1 – DNA ladder (10, 8, 6, 5, 4, 3, 2.5, 2, 1.5, 1, 0.7, 0.5, 0.3 Kb)
Well 2- 11 = DNA of seed samples (S1-10)

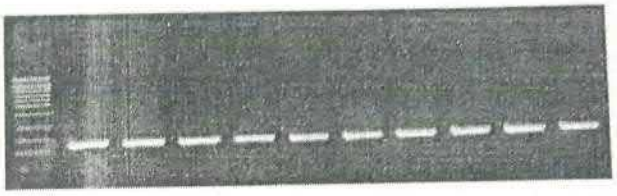
Figure 2. Quality Check for extracted DNA from Seed samples on 0.8% Agarose Gel.

2.3. DNA Amplification and Sequencing

The target DNA regions, namely rbcL and matK were amplified with respective universal DNA barcoding primers as prescribed by CBOL Plant working group, 2009 [16]. Universal primers for rbcl gene, rbcLa-F: ATGTCACCACAAACAGAGACTAAAGC and rbcLa-R: GTAAAATCAAGTCCACCRCG; for matK gene, matK-KIM1R: ACCCAGTCCATCTGGAAATCTTGGTTC and matK-KIM3F: CGTACAGTACTTTTGTGTTTACGAG were used. PCR was performed using a reaction mixture of a total volume of 50 µl for either of the genes: 25 µl of Taq PCR Master Mix (Norgen Biotek, Canada), 22 µl distilled water, 1 µl forward primer (10µM), 1 µl reverse primer (10 µM) and 1µl of the DNA template (50-80 ng/ µl). The PCR conditions maintained were as follows, one cycle (94°C for 3 min), 35 cycles (94°C for 1 min, 55°C for 1 min, 72°C for 1 min) and one cycle 72°C for 7 min. Amplified PCR products of rbcL and matK primers, each of 5 µl were checked on 1.5% agarose gel electrophoresis for the respective bands and sent to Macrogen (Seoul, South Korea) for DNA sequencing.

Well 1 – DNA ladder (10, 8, 6, 5, 4, 3, 2.5, 2, 1.5, 1, 0.7, 0.5, 0.3 Kb)
Well 2- 11 = mat K gene PCR product of samples (S1-10)
2, 3,4 6 wells shown light bands

Figure 3. PCR amplification with matK primer.



Well 1 – DNA ladder (10, 8, 6, 5, 4, 3, 2.5, 2, 1.5, 1, 0.7, 0.5, 0.3 Kb)
Well 2- 11 = rbcL gene PCR product of samples (S1-10)

Figure 4. PCR amplification with rbcl primer.

From above figures, it is clear that PCR amplification with rbcl primer was observed to be good in quality, while PCR amplification with matK primer not of high quality. Nucleotide bands of DNA with matK primer were not well separated. DNA sequencing was done by sending PCR products to specialized research laboratory Macrogen (Seoul, South Korea). As high-quality reads were obtained with the single direction, markers were sequenced in the single direction only.

3. Data Analysis

All data analysis were performed using MEGA 7 (Molecular Evolutionary Genetics Analysis Version: 7.0.18), NCBI (National Center for Biotechnology Information)website using 'blastn' application (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and Microsoft Excel 2010.

Obtained sequences were aligned by MUSCLE [17], which generates multiple alignments of amino acid and nucleotide sequences. MUSCLE program is much better regarding speed and accuracy when compared with T-Coffee, MAFFT, and CLUSTALW in all tests. Aligned sequences by MUSCLE were used to locate conserved, variable, singleton, parsimony informative site and compared with other obtained sequences of other *Nigella sativa* seed and its adulterant samples using MEGA 7 [18]. Primary sequence analysis of nucleotide composition, conserved sites, variable sites, singleton sites, parsimony informative sites and phylogenetic tree provided adequate information to discriminate *Nigella sativa* seeds from adulterants. Further all aligned sequences were submitted to NCBI (National Center for Biotechnology Information) website and identified using blastn application (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

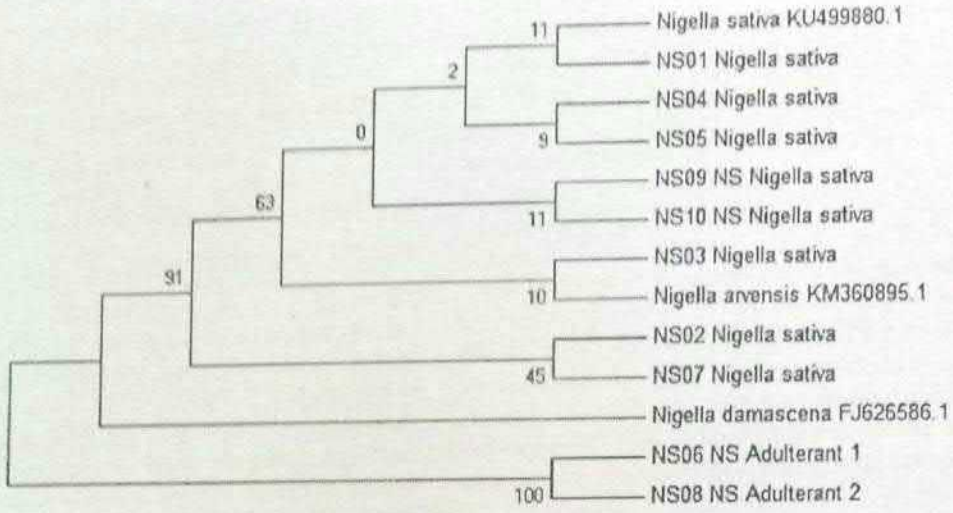


Figure 7. *Phylogenic tree of Nigella sativa (The bootstrap consensus tree).*

In figure 6 and 7, it can be clearly seen that sample NS06 and NS08 formed a separate clade since they are genetically different and evolved from different ancestor. This observation further confirms species discrimination power of rbcl sequences.

Further to above sequence analysis, aligned rbcl sequences of seed samples were blasted individually on NCBI website (http://blast.ncbi.nlm.nih.gov/Blast.cgi) for identification of samples. Following are the details of identification of rbcl sequences from NCBI blastn tool.

Table 7. *Matching and Identification of nucleotide sequences using NCBI blastn.*

Sample ID	Matches with Accession	Description	Max score	Total score	Query cover	E value	Ident
NS01	KU499880.1	*Nigella sativa* voucher A1 rbcL gene, partial cds; chloroplast	1027	1027	0.9	0	1
NS02	KU499880.1	*Nigella sativa* voucher A1 rbcL gene, partial cds; chloroplast	1027	1027	0.91	0	0.99
NS03	KU499880.1	*Nigella sativa* voucher A1 rbcL gene, partial cds; chloroplast	1025	1025	0.92	0	1
NS04	KU499880.1	*Nigella sativa* voucher A1 rbcL gene, partial cds; chloroplast	1029	1029	0.9	0	0.99
NS05	KU499880.1	*Nigella sativa* voucher A1 rbcL gene, partial cds; chloroplast	1033	1033	0.92	0	0.99
NS07	KU499880.1	*Nigella sativa* voucher A1 rbcL gene, partial cds; chloroplast	1021	1021	0.9	0	0.99
NS09	KU499880.1	*Nigella sativa* voucher A1 rbcL gene, partial cds; chloroplast	1027	1027	0.9	0	1
NS10	KU499880.1	*Nigella sativa* voucher A1 rbcL gene, partial cds; chloroplast	1042	1042	0.92	0	0.99
NS06_AD1	AB292286.1	Allium cepa chloroplast DNA, rbcL and ORF106, partial sequence	1037	1037	0.91	0	0.99
NS08_AD2	JQ591652.1	Clitoria guianensis voucher BioBot00900 rbcL gene, partial cds; chloroplast	1021	1021	0.9	0	0.99

Blastn tool identified rbcl sequences of two seed samples NS06 and NS08 as *Allium cepa* and *Clitoria guianensis*. These samples were further compared with standard morphological features of *Allium cepa* and *Clitoria guianensis*. Morphological features of samples found matching with physical samples used in study.

5. Discussion

Prophet Mohammed in Islamic literature has described black seed as the seed of blessings which has a property of curing any disease of humankind. *Nigella sativa* seed looking at its therapeutic utility can be considered as mentioned black seed.

Nigella sativa seed is one of the noble herbs which is extensively used as medicine and spices in the Middle East, South East Asia, and Europe It is one of the great spices used for culinary purposes. Recently many researchers proved the great therapeutic uses of *Nigella sativa* seeds and its extracts. Looking at huge benefits, its demand is growing and hence

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Site Number	2 5 8	2 7 6	3 0 9	3 2 4	3 3 0	3 3 9	3 4 9	3 5 1	3 5 8	3 6 6	3 6 9	3 8 1	3 9 6	4 2 0	4 4 1	4 4 4	4 7 4	4 8 3	4 8 9	4 9 2	4 9 5	4 9 8	5 1 6	5 2 5	5 2 7
Nigella arvensis KM360895.1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Nigella damascena FJ626586.1	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
NS06 NS Adulterant 1	.	.	.	T	.	A	T	.	.	T	C	C	.	A	.	A	.	.	C	A	A	.	C	A	.
NS08 NS Adulterant 2	C	.	T	.	G	.	.	G	T	.	.	.	T	.	C	C	T	G	.	.	.	T	.	.	.

(.)Identical site

When we studied singleton sites in 13 nucleotide sequences, it is observed that sample NS06 and NS08 showed 28 and 20 singleton sites respectively, which qualifies NS06 and NS08 samples as different plant genus or species from group consists of NS01, NS02, NS03, NS04, NS05, NS07, NS09, NS10 and Accession KU499880.1, KM360895.1.

4.2.3. Parsimony-Informative Site

A site is parsimony-informative if it contains at least two types of nucleotides (or amino acids), and at least two of them occur with a minimum frequency of two.

Table 5. Details of Parsimony-informative sites.

Sites Number	1 4	3 5	3 6	4 8	5 3	6 0	1 4	1 3 5	1 5 0	1 7 4	1 9 2	2 4 1	2 4 2	2 5 2	2 5 5	3 3 5	3 6 1	3 7 0	3 7 1	3 7 9	3 8 0	3 8 7	4 5 5	5 0 1	5 1 0
Nigella sativa KU499880.1	A	T	G	G	C	G	T	A	C	C	C	T	G	C	C	G	A	G	T	G	T	C	T	T	G
NS01 Nigella sativa	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
NS02 Nigella sativa	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.
NS03 Nigella sativa	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
NS04 Nigella sativa	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
NS05 Nigella sativa	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
NS07 Nigella sativa	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.
NS09 NS Nigella sativa	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
NS10 NS Nigella sativa	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Nigella arvensis KM360895.1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Nigella damascena FJ626586.1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
NS06 NS Adulterant 1	C	A	A	T	T	A	G	T	T	T	A	G	C	T	T	C	C	C	C	T	C	T	C	C	A
NS08 NS Adulterant 2	C	A	A	T	T	A	G	T	T	T	A	G	C	T	T	C	C	C	C	T	C	T	C	C	A

Parsimony-informative sites indicate NS01, NS02, NS03, NS04, NS05, NS07, NS09, NS10 and Accession KU499880.1, KM360895.1 are quite similar in genetic makeup while, NS06 and NS08 are quite different.

4.3. Estimation of Genetic Distance Between Sequences

Estimation of genetic distance between sequences is done basis number of base substitutions per site from between sequences. Analyses were conducted using the Kimura 2-parameter model [23].

Table 6. Intra and Inter-specific Genetic Distances and Species identification (Pairwise genetic distances).

	Nigella_sativa_KU499880.1	NS01_Nigella_sativa	NS02_Nigella_sativa	NS03_Nigella_sativa	NS04_Nigella_sativa	NS05_Nigella_sativa	NS07_Nigella_sativa	NS09_NS_Nigella_sativa	NS10_NS_Nigella_sativa	Nigella_arvensis_KM360895.1	Nigella_damascena_FJ626586.1	NS06_NS_Adulterant_1	NS08_NS_Adulterant_2
Nigella_sativa_KU499880.1													
NS01_Nigella_sativa	0.00000												
NS02_Nigella_sativa	0.00376	0.00376											
NS03_Nigella_sativa	0.00000	0.00000	0.00376										
NS04_Nigella_sativa	0.00000	0.00000	0.00376	0.00000									
NS05_Nigella_sativa	0.00000	0.00000	0.00376	0.00000	0.00000								
NS07_Nigella_sativa	0.00188	0.00188	0.00188	0.00188	0.00188	0.00188							
NS09_NS_Nigella_sativa	0.00000	0.00000	0.00376	0.00000	0.00000	0.00000	0.00188						

	Nigella_sativa_KU499880.1	NS01_Nigella_sativa	NS02_Nigella_sativa	NS03_Nigella_sativa	NS04_Nigella_sativa	NS05_Nigella_sativa	NS07_Nigella_sativa	NS09_NS_Nigella_sativa	NS10_NS_Nigella_sativa	Nigella_arvensis_KM360895.1	Nigella_damascena_FJ626586.1	NS06_NS_Adulterant_1	NS08_NS_Adulterant_2
NS10_NS_Nigella_sativa	0.00000	0.00000	0.00376	0.00000	0.00000	0.00000	0.00188	0.00000	0.00000				
Nigella_arvensis_KM360895.1	0.00000	0.00000	0.00376	0.00000	0.00000	0.00000	0.00188	0.00000	0.00000	0.00944			
Nigella_damascena_FJ626586.1	0.00944	0.00944	0.01325	0.00944	0.00944	0.00944	0.01134	0.00944	0.00944	0.10729	0.11617		
NS06_NS_Adulterant_1	0.10729	0.10729	0.10934	0.10729	0.10729	0.10729	0.10720	0.10729	0.10729	0.09021	0.09870	0.09041	
NS08_NS_Adulterant_2	0.09021	0.09021	0.09222	0.09021	0.09021	0.09021	0.09013	0.09021	0.09021	0.09021	0.09870	0.09041	

From the above chart, it is clear that intra-specific genetic distance is from 0.0000 to 0.01325, which is tiny while inter-specific genetic distance among all sequences were almost 0.1161, which is quite high. This genetic distance further helped in identification of adulterants as sequence from NS06 samples had shown genetic distances of 0.10720 to 0.11617, while NS08 sample showed genetic distances of 0.09013 to 0.09870, which are quite high as compared to inter –specific genetic distance of a maximum of 0.01323.

4.4. Phylogenic Tree with UPGMA Method

Typically the evolutionary history is inferred using the UPGMA method [20]. On this study, we wanted to understand whether adulterants show different cluster in the phylogenic tree. The bootstrap consensus tree inferred from 500 replicates is taken to represent the evolutionary history of the taxa analyzed Branches corresponding to partitions reproduced in less than 50% bootstrap replicates. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches. The evolutionary distances were computed using the Kimura 2-parameter method [19] and are in the units of the number of base substitutions per site.

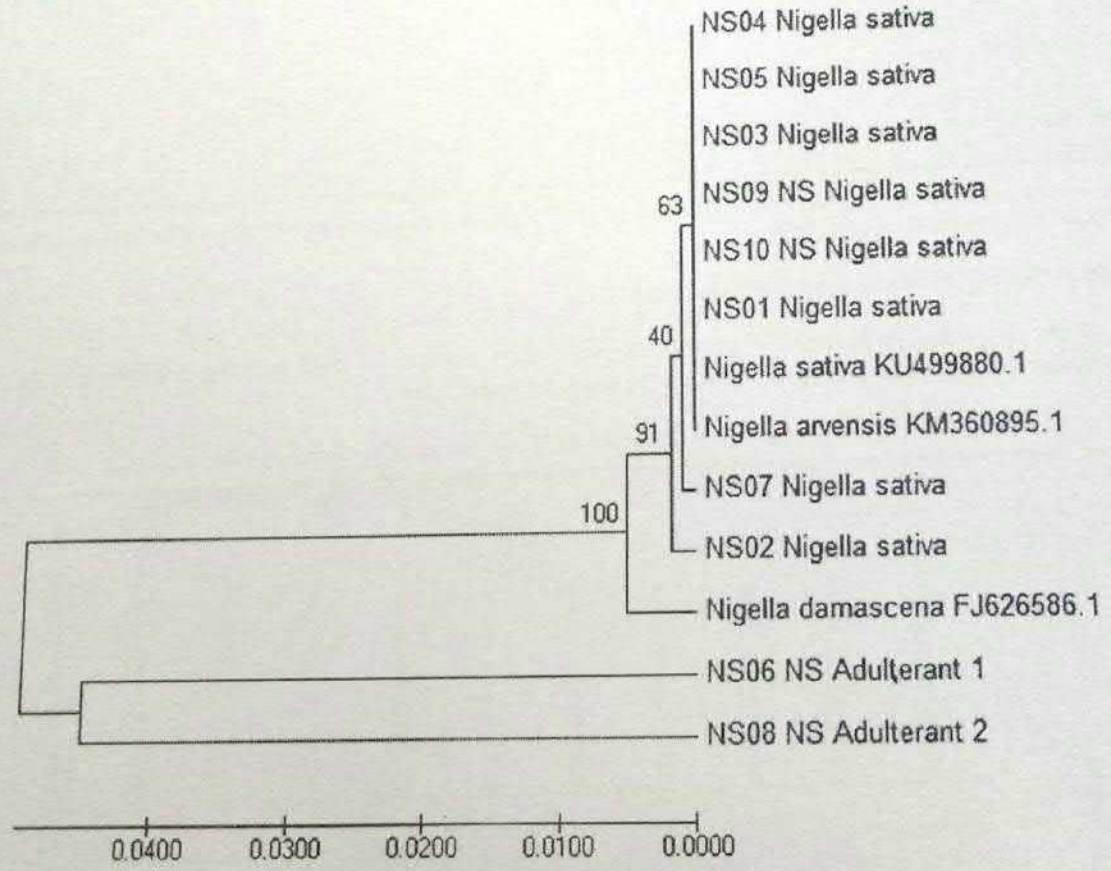

Figure 6. Phylogenic tree of Nigella sativa (Original Tree).

From Table 1 and Figure 5, it is quite clear that nucleotide composition of two samples (NS06 and NS08) are entirely different from all 11 samples majorly in terms of A and G nucleotide, which indicate that these two samples (NS06 and NS08) have different genetic makeup and hence could belong to different plant genus or species. Sample NS01, NS02, NS03, NS04, NS05, NS07, NS09, NS10 and Accession KU499880.1, KM360895.1, FJ626586.1 shows very similar nucleotide composition, which indicates all these samples are belong to single plant genus or species.

Table 2. Details of nucleotide pair frequencies (Directional) observed in entire 13 nucleotide sequences.

	ii	si	sv	R	TT	TC	TA	TG	CT	CC	CA	CG	AT	AC	AA	AG	GT	GC	GA	GG	Total	Domain Info
Avg	517	9	6	1.42	147	3	1	1	3	110	1	1	1	1	138	2	1	1	1	123	533	Data
1st	176	0	1	0.32	37	0	0	0	0	33	0	0	0	0	42	0	0	0	0	64	178	1st Pos Data
2nd	175	1	1	1.00	42	0	0	0	1	45	0	0	0	0	54	0	0	0	0	34	178	2nd Pos Data
3rd	166	7	4	2.00	68	3	0	1	2	31	0	1	0	1	43	2	1	0	1	25	177	3rd Pos Data

ii = Identical Pairs,si = Transitional Pairs,sv = TransversionalPairs,R = si/sv

In the entire group, the pair nucleotide frequencies provide the proper indication about diversity in the genetic makeup of various samples. In NS group, the nucleotide pair frequencies provide the precise evidence about highest % identical sites and lowest rate of Transversional Pairs in the group.

4.2. Analysis of Nucleotide Sequence

4.2.1. Conserved and Variable Sites

Out of 533 sites, 458 sites found to be conserved sites, while 75 sites found to be variable.

Table 3. Details of variable sites.

	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2
Site Number	0	1	1	2	2	3	3	4	5	5	6	7	9	0	1	1	2	3	3	4	4
	6	8	1	4	0	3	5	8	1	0	9	5	4	2	7	1	8	2	0	6	1	2
Nigella sativa KU499880.1	C	G	T	T	T	C	A	T	G	C	C	A	C	C	C	C	C	A	A	A	T	G
NS01 Nigella sativa	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
NS02 Nigella sativa	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
NS03 Nigella sativa	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
NS04 Nigella sativa	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
NS05 Nigella sativa	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
NS07 Nigella sativa	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
NS09 NS Nigella sativa	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
NS10 NS Nigella sativa	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Nigella arvensis KM360895.1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Nigella damascena FJ626586.1	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.
NS06 NS Adulterant 1	.	.	.	G	C	.	T	.	C	T	T	.	T	A	T	G	T	G	.	.	G	C
NS08 NS Adulterant 2	T	T	C	G	.	T	T	.	A	T	.	G	T	A	.	.	.	.	G	T	G	C

Contd..

	2	2	2	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Site Number	5	5	5	7	0	2	3	3	3	4	5	5	6	6	6	7	7	7	8	
	2	5	8	6	9	4	0	3	9	9	1	8	1	6	9	0	1	9	0	
Nigella sativa KU499880.1	C	C	T	A	G	G	A	G	C	C	A	C	A	C	T	G	T	G	T	
NS01 Nigella sativa	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
NS02 Nigella sativa	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
NS03 Nigella sativa	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
NS04 Nigella sativa	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
NS05 Nigella sativa	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
NS07 Nigella sativa	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
NS09 NS Nigella sativa	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
NS10 NS Nigella sativa	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
Nigella arvensis KM360895.1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
Nigella damascena FJ626586.1	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
NS06 NS Adulterant 1	T	T	.	.	.	T	.	C	A	T	.	.	C	T	C	C	C	T	C	
NS08 NS Adulterant 2	T	T	C	.	T	.	G	C	.	.	G	T	C	.	.	C	C	T	C	

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CONTENT

English Teaching and Learning with Computer

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Amravati

The tremendous progress in Information and Communication Technology has changed the way of teaching and learning English language. Computer-assisted teaching refers to the use of ICT tools, multimedia, and computer-based activities by the teachers as well as learners. The teaching techniques are radically changing. As technology has become an integral part of our daily lives, the field of Education could not possibly stay unaffected. The computers and the Internet offer a wide range of activities which are very effective in English language learning and teaching. These activities can be used either independently or in combination with traditional teaching techniques. This way students are offered a fully modified learning experience based on their individual requirements.

Computer Assisted Language Learning (CALL) is defined as "the search for and study of applications of the computer in language teaching and learning" (Levy,1997). The purpose of CALL is to find ways for using computer technology for the teaching and learning language. It included word processing, presentations, practice, multimedia, internet applications for language learning. There are several reasons to use CALL by English language teacher and learner. "Computers can do some of the work of the teacher and provide great assistance to the learner even without the presence of the teacher" (Pennington and Steven,1992). Today we have many useful designed CALL applications available for the teacher and student. "New technologies have seen computers become smaller, faster, and easier for the teacher to use (Evy,1997)". Technologies allow computers to do multimedia applications, incorporating video, sound, and text, and this capacity allows the learner to interact with both the program and other learners.(Felix,1998). The computer offers great flexibility for class scheduling and pacing of individual learning, choosing activities and content to suit individual learning styles. (Oxford and others,1998).

This interactive way of teaching appears to be more appealing to students, as today's children grow up in highly mechanized environments and find technology fascinating. Computer-based lessons give learners the chance to explore ideas in a fun and intriguing way. Therefore, they are less prone to get bored during classes and more likely to actively participate in the activities taking place in the classroom.

Children, however, are not the only type of students that can benefit from ICT-assisted teaching. Video instruction and other interactive activities have also been introduced in university classes around the world. These tools give students the opportunity to take a closer, more detailed look into complicated processes, such as medical procedures or scientific

experiments. It is important to say that computers cannot fully substitute the presence of a teacher who will guide students and answer all of their questions. After all, a computer activity is only as good as its programming; furthermore, a student's inability to adjust to the demands of a mechanized learning process would cause him to feel frustrated and left behind without his teacher's help. To conclude, computer-assisted teaching can offer great benefits to both teachers and students. Combined with traditional teaching methods, it can optimize the results of the learning process and make learning an interesting and fun experience.

Computer can be used as tutor to present learner the content of the lesson such as text, animation, slides, learning activities and practice. The computer serves as a means for delivering instructional material. Interactivity is the most important strength of the computer. The user can have control over learning so he/she becomes an active participant in learning and teaching process. It is also provide the instant feedback from the computer. It also helps to use students centered teaching method.With the rapid growth of science and technology, the use of multimedia technology in language teaching has created a favorable context for reforming and exploring English language teaching models in the new age. Multimedia is considered truly revolutionary for language teaching and learning. The use of multimedia in classroom cannot be denied in the present educational environment. In the present situation the technology plays an important role in the life teachers and students. Unlike the traditional classroom setting, the multimedia classroom setting has more facilities; all the equipments needed for teaching will be arranged inside the classroom. The print texts, video, audio, pictures and internet is being effectively used to enhance teaching and learning of the language. Using print, film and other internet as resources for studying provides students with opportunities to gather information through stimuli that will stimulate their imaginations, engage their interest and introduce them to the raw material for analysis and interpretation of both language and context. Thus we can greatly increase their overall knowledge base, as well as their English language and critical literacy skills, facilitating their performance in future courses. The growth of the internet has facilitated the growth of the English language. In this sense, computers are no longer the exclusive domain of a few individuals, but rather they are available to everyone.

As the computers become more readily available to all of us, it seems appropriate that the language teachers should integrate it into their lessons. The students are surrounded by technology and this technology can provide interesting and new approaches to language teaching and learning. The teachers of English can take full advantage of this technology to teach English as second language. The traditional teaching methods are unpopular in the English language classrooms. Computer technology with video, audio, graphs, power point presentation, animation effects motivates the students to learn English quickly. Technology also plays very supportive role in enhancing student's communication skills. Students can enhance both their written and oral communication skill using technology under the sound guidance of

their teachers. Computer technology has been a great help to integrate teaching and learning and provides the students greater incentives. The use of audiotape is essential in the oral skills class. For receptive skills development, the tape player or podcasts are the easiest way for students to listen to a variety of speakers on a variety of topics in a variety of genres such as dialogues, interviews, lectures, stories, songs and poems. Language Lab is invaluable for the promotion of listening and speaking skills. It does many things that benefit oral skills development better than the regular not-tech classroom. There are numerous apps to build oral communication skills. Using computer technology in the language classrooms improves teaching contents and makes the best of class time. It breaks the teacher centered traditional teaching method and improves the teaching methods. This technology goes beyond time and space so it creates more real life environment for English teaching. It stimulates students' initiatives and economizes class time, providing more information to the students.

Some Disadvantages of the Use

There are many disadvantages of using multimedia technology in English language teaching.The language learning programs start with expenses that are related to implementing new technologies in education. The expenses usually entail hardware, software, staffing and training for at least one networked computer laboratory where teachers and students can come and use it.

Though the use of multimedia technology in the language classrooms enhances the interest of the students through audio and video, it lacks interaction between teachers and students. The English language classroom becomes a show case and the students are considered only as viewers rather than the active participants in the classroom. Teachers may lack training and skills in using the CALL.

Summing up

Computer networks have allowed connecting to information around the world, and share millions of documents as texts, graphics, sounds and video. The internet provides a rich resource of good materials for language teaching and learning. Computer assisted teaching has important potential for English language teaching. It can motivate the learners, increase information access to the learner and teachers. It can also provide learners to control their own learning process and progress. It is also true that CALL will never replace the teacher. CALL is not a magic solution to language teaching. The effectiveness of CALL relies on how it is used to meet language learning goals for individualized learners in specific educational setting.

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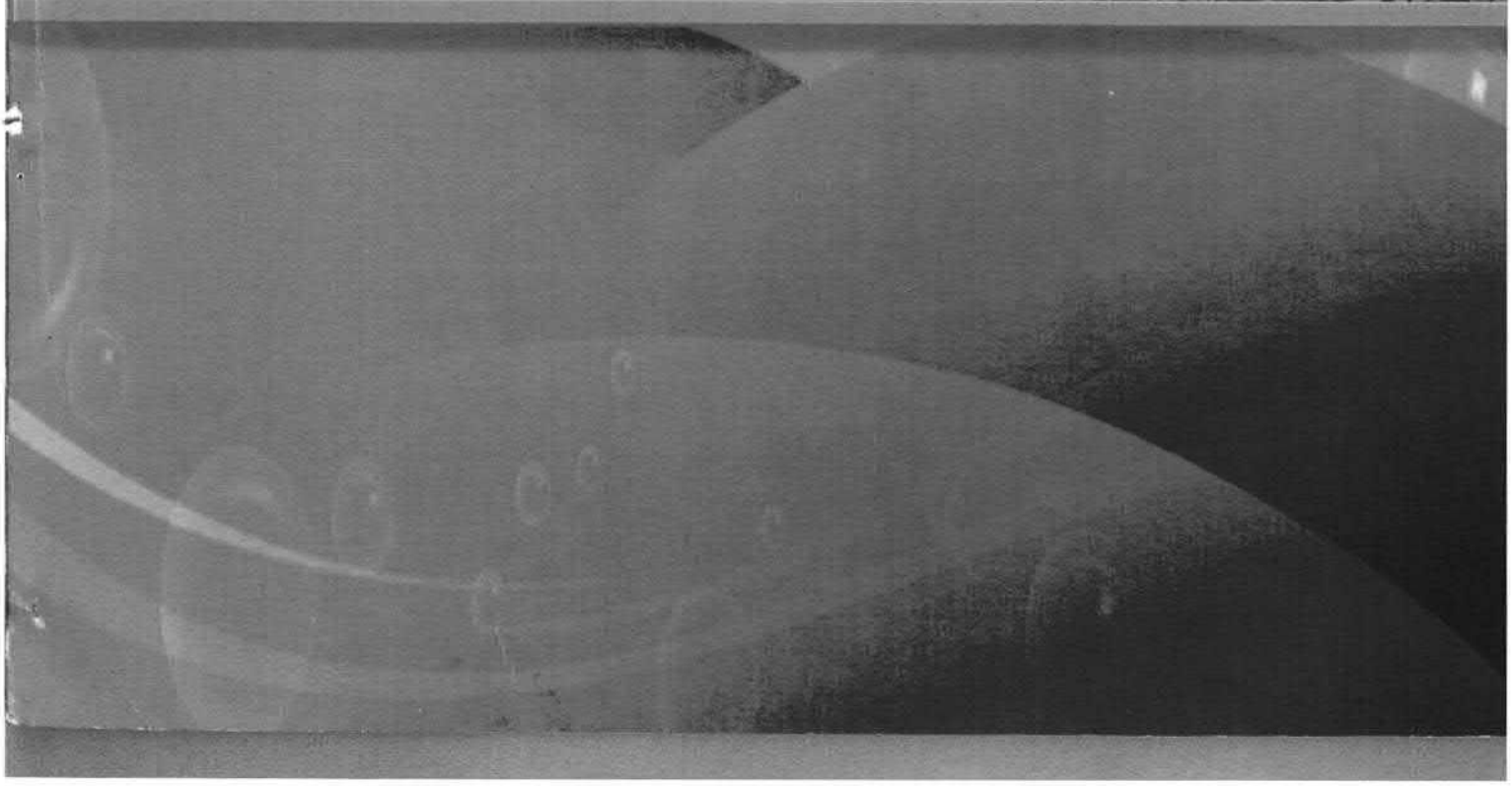

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THE VARIOUS PROBLEMS OF WOMEN AND THEIR EMPOWERMENT IN INDIA

Dr. A.D.Chauhan[1]

ABSTRACT

After independence in India, there are the special women related articles created by experts. In the Indian constitution the principle of gender equality is enshrined with preamble, fundamental rights, fundamental duties and directive principles. The Constitution not only grants equality to women, but also empowers the state to adopt measures of positive discrimination in favour of women. Empowerment is the one of the key factors in determining the success of development is the status and position of women in the society. For the healthy development of society there is a need to special focus on social, economical and political overall development of women. We need to augment our efforts for empowering women and enhance their progress. It is our moral, social and constitutional responsibility to ensure their progress by providing them with equal rights and opportunities. Today women with their smartness, grace and elegance have conquered the whole world.

KEYWORDS: *gender equality, women, women commission, female children,*

POSITION OF WOMEN IN CURRENT SCENARIO

In this contemporary world, women need to gain the same amount of power that men have. Now, it is time to forget that men are the only holders of power. In India, women are still facing different obstacles in male-dominated cultures. Now a day's women are coming the main stream of development. The government of India established a special commission for the women development i s. women commission in India.

Today, women are having the position in every place. Topmost organizations in India most of women are the CEOS in various field. Recently feminism concept added in the women empowerment which stress on the fundamental and human rights of women.

In India, women are facing various problems, heinous practice of female feticide and infanticide wherein nearly 10 million baby girls have been killed in the last twenty years alone. In fact, safety is an obsolete word in today's India. Even law enforcement is unable to control the situation in India.

Every person should think about women development. This is the need of the hour. Everyone must think of changing society. If we all abide by the rules, women in our cities will surely be safer. This situation has caused immense loss to their self-dignity as human beings and also their independent entities, associated with men, apart from other matter, in context with intellectual and professional capability.

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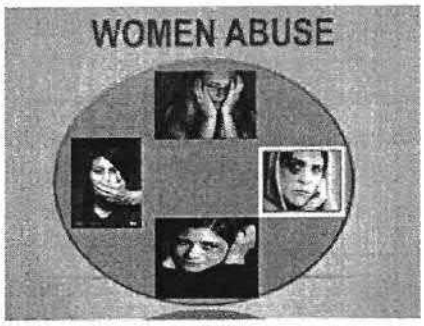
PROBLEMS OF WOMEN



1. Violence against Women:-

The life Indian women are full of sorrow and anxiety. There are various types of crime like rape, molestation, dowry harassment, wife-battering, kidnapping, female children to be sold into brothel homes, forcible embracement etc. Problems faced by Indian women.

2. Gender Discrimination:-

Gender discrimination refers to "the practice whereby one sex is given preferential treatment over the others. After overpopulation second number greatest problem in India is the female foeticide and discrimination. The practice of giving social importance to the biological differences between men and women is everywhere. In some societies, these differences are very much pronounced while in others, they are given less importance.

3. Neglegence and poor health:-

Indian women are the most exploited in the world. Socially, psychologically, politically and economically she is always on secondary place. Improper haemoglobin, different medical problems, malnutrition and high death rate are the feachers of Indian women.

4. Unequal sex ratio:-

Normally, in the population of any country, male- female ratio remains more or less the same. That is 50:50. in India as the census reports reveal female population has been steadily declining ever since 1901.This is serious indicator in society. Efforts should be takes place for identification and sort out this problem.

5. Un-attraction of Female Education:-

Since ancient time we have been seen that generally women ignored from the education.'Ladki to paraya dhan hoti hai' is common tendency observe among the Indians. Accordingly, much attention is paid to the education of women after independence. The female literacy level is also increasing steadily. It has increased from 18.7% in 1971 to 39.42% in 1991 and to 64% in 2001. In spite of this change in the trend towards literacy, some problem has cropped up.

6. Dowry a curse:-

At the time of marriage ceremony, the gift or amount given by the parents of girl is general trend in India. In later stage it became problem called dowry. Every year so many cases of dowry exposed in India. It is a very serious problem faced by Indian women and their parents.

7. Violence against women:-

Sexual exploitation, female foeticide, dowry, domestic violence etc are the common practices can see in Indian society. The rate of such problems is high in rural society. Main cause of it is that spoil mentality with old costomes and traditions.

8. Sexual Harassment:-

Now days so many cases are exposing related to sexual harassment of women. Delhi gang rape and so many incidents' taking place in India. Child abuse, sexual exploitation, human trafficking, child labour etc are the various problems are present in Indian society.

9. Organizational problems:-

In working place, women face a lot of problems regarding various matters. May be some times sexual harassment and other conflicts can creates at working place. Excessive bossing, unequal shifts, unwanted demands by high authority etc are the factors responsible for women exploitation in organization.

10. Familiar and Social Problems:-

Family and society quickly takes the cognizance about women issues. The intensity and proportion of works is always more than men. In religious and cultural activities women generally ignore and put secondary place. Whatever the situation is there the women assume a responsible for that act. Also there is a tendency that women are the factories of child creation.

THE ROLE OF WOMEN EMPOWERMENT IN INDIA

Empowering women usually involves giving them opportunity for better education. Focus on the overall development in India is the main work of women empowerment commission in India. Basically as per the human rights there should be the same place to men and women but society doesn't accept this situation and made the discrimination in society.

When we study the evolution of man , it is noticed that gradually women tilled towards secondary place and started the exploitation through various problems.

For stop and eradication of these problems, the women commission established by Govt. of India.

After independence the direction has been fixed and various acts, plans have been started for the women development in India. Definitely we can say that India has done the improvement in social, economical and political status of women. Again there is need to strictly implementation and development of scheme and plans started by Government of India.

FOLLOWING ARE SOME PLANS FOR THE WOMEN DEVELOPMENT AND EMPOWERMENT

1.Mahila cosh yojana:-

This is first plan started for especially rural women of India in which self employment, stress on msme and supplementary occupation are the most priority factors.

2. Training and employment programme for women (TEPW):-

To build up the confidence, economically strong and for enhancing the productivity are the main targets of this plan.

3.Rashtriya Mahila Kosh (RMK):-

For social and economical changes, financial improvements through various programmes are the main objects of this plan. Micro finance to poor women, agriculture women, shop keeping and handcrafts etc are important objects of this plan.

4.Rajiv Gandhi Scheme for Empowerment of Adolescent Girls (RGSEAG):-

This is especially well-known for the overall development of teen age girls for the issues like nutrition, education, medical facalities and eradication of the different problems

5.Central Social Welfare Board (CSWB):-

This scheme is especially famous for stimulation of the NGO which work for development of women.

6. Indira Gandhi Matritva Sahyog Yojana (IGMSY):-

For the improvement to the health and nutrition status of pregnant, lactating women and infants, child vaccination with sort out the various problems.

7. Swayam Siddha yojana:-

Creation of self help groups with financial support and availability the fund for poor women in society.

8. Short Stay Home for Women and Girls (SSH):-

Arrangement of temporary accommodation of deprived, mentally affected, very poor, widow, exploited and rejected by society and family. With the help of this plan various works knowledge given and try to become self to such type of women.

9. Swadhar:-

This plan is especially for the support of women those really want to do the advance type of work. Some financial support given by government to start the occupations.

SUGGESTIONS FOR WOMEN EMPOWERMENT

1. **Security of women-**
   As per the human rights every woman should safe and respectable in society as well as in organization. There are strict rules and regulations have been inserted and implemented by government time to time. Also there is a need to the strict implementation of this legislation for the success of women empowerment.
2. **Equality and no place to gender discrimination-**
   Women should be properly treated in everywhere. The contents like sexual harassment, rape, exploitation should be stopped by the efforts of all the contents from society. Women are equally entitled and qualified for jobs as men. We should not forget that some of the greatest characters in history are women such as Rani Laxmibai, Indira Gandhi, and Mother Teresa etc.
3. **Eradication of common and basic problems of women in society-**
   We see the various problems regarding social, economical and political aspects. The society should always try for maintain the equal status and position in society. Other women related issues immediately solve by government and implement the correct strategy for development of women.

4. **The role of sociologists, social workers, planners and NGOS-**
   For overall development of women, there is need to do the collectively efforts from all the levels of society. The intellectual class like sociologists, social workers, planners and NGOs have to give the best efforts for the success of women empowerment.
5. **Proper implementation and preparation of plans-**
   After independence, the speed of women empowerment has been raised and it shown the positive effects on society, but still again there is serious need of collectively efforts from government and properly implementation of various plans by administration.
6. **Improvement in politically and economically status-**
   In the suitable proportion of population government has to give the reservation to women. In that way women can pull into the main stream of development.

CONCLUSION

For the proper construction of society there is need to give special attention on women empowerment in India. Also traditional attitude has to change regarding women. Awareness programme, education and positive role of every indivisible will help to development in women empowerment in India.

Strictly implementation, creation and support of legislative, judiciary will be beneficial to sort out the women related problems in India. Stop the women exploitation, rape sexually harassment, acid throwing, domestic violence, child marriages and female foeticide with proper instruments and control on these problems.

Various issues like women health, education, sports, schemes, equal sex ratio, entertainment, basic facilities, freedom, protection, sanitation arrangement should provide to women. Then definitely we can develop the healthy India.

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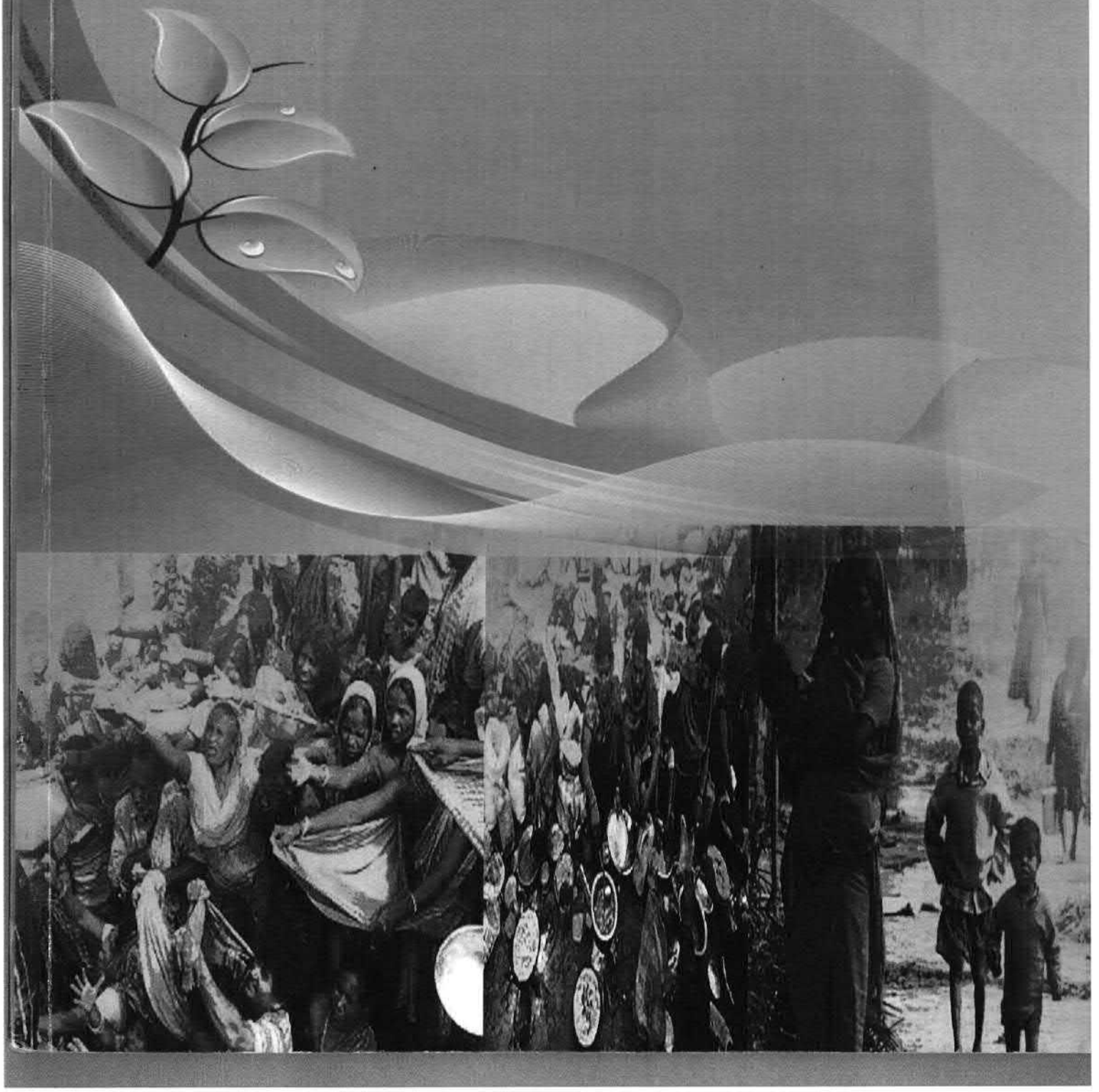

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PROBLEMS OF ELDERLY IN RURAL SOCIETY

✍ **Dr.A.D.Chauhan[1]**

ABSTRACT

Rural society is an important society in India. Around 74% population of India is scattered six lakhs villages. Total 65% proportion is present in an Indian economy. Elderberry population still exist and works in the farming sector. Rural elderly an important part rural of society. But in the study, it is notice that rural elderly sometimes ignored and exploited by rural system. As Population ageing is a recognized international reality, both in developed and developing countries. India is also having same situation about elderly in rural society. The number of elderly in the developing world is increasing per the demographic proportion.

KEYWORDS: *Farming Sector, Migration, Rural Society, Rural Culture, Agriculture Country.*

INTRODUCTION

Rural society is an important society in India. Around 74% population of India is scattered six lakhs villages. Total 65% proportion is present in an Indian economy. Elderberry population still exist and works in the farming sector. Rural elderly an important part rural of society. But in the study, it is notice that rural elderly sometimes ignored and exploited by rural system. As Population ageing is a recognized international reality, both in developed and developing countries. India is also having same situation about elderly in rural society. The number of elderly in the developing world is increasing per the demographic proportion.

The situation of elderly is common everywhere. Elderly having many social, economical and familiar problems with various diseases. In this stage they decline their psychological and physical problems. Earlier in rural society, joint family was executing but as per the span of time there is a frequently conversion and transfer it into the nuclear family.

While perusing the study on elderly, to be focus on their various problems and identify the overall situation. As per the dictionary of medical sources, the man who has crossed 60 years accommodate in the category of elderly. Elderly person also assume that he or herself weak and outdated. In today's age of globalization the status, structure and various problems are reflected to old persons.

OLD PERSON IN RURAL SOCIETY

India is an agriculture country. Around 75% Indian population lives in Six lakh villages. India is famous in the world due to Indian rural society and rural culture.

Indian rural culture always accepted and gives the place to "Atithi Deo Bhav". Giving the respect to

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senior and old person is a trend and tendency of Indians. Therefore respect,prestige and proper place to old person is tradition in rural society.

Rural society has the joint family system where all family members live together. Senior and old person monitor and control the familiar, social activities with sorting the problems. Guidance, respect, involment in cultural and religious activities of elderly is the part of rural life. Elderly have the basic agriculture knowledge which they hand over to young generation. Years to years this system has been carry forward in village society. Place in the Panchayat Raj system, Mukhiya, Judge etc role also used to play by elderly in rural society.

ROLE, STRUCTURE AND PROBLEM OF RURAL ELDERLY IN CURRENT SCENARIO –

Old is gold is the proverb available in rural society. Earlier old person was treated respectfully. But unfortunately in current scenario the status, position and role have been change d and so many problems are rising in front of rural elderly.

1) Transformation of joint family to nuclear family:-

Every day, people start towards cities. Therefore joint family is converting into nuclear family. Joint family preserves the things for elderly but nuclear family nuclear family doesn't support for elderly. Nuclear family is relative to time. Therefore most of the old person keeps in old age home by their sons.

2) Migration: –

For migration various causes are responsible. After independence, most of the people have been migrated towards cities. Bombay is well-known examples of migration. Obsiosaly problem of old person create in village and they absconded from society and family.

3) Rural poverty: –

In rural society, maximum part of the poverty centralized and affect on their life style. Problem of food, shelter and basic needs raised for elderly. Head of the family unable to focus on old person. Therefore elderly face the problems in rural family.

4) Medical problems: –

Physical and mental various disorders present in the elderly in villages. No experts doctors, medicine available in the villages. Therefore elderly death is high. Even 50 to 60 year old elderly died due to the very common diseases. The main reason is lack of proper diseases diagnosis and treatment.

5) Uncleanness and Hygienic related issues: –

Lack of toilet, bathroom facility, unhygienic problems etc are face by rural elderly. Rural elderly looks dirty and their cloths always unclean and unsafe.

6) Familiar Dispute and conflict:-

Sometimes due to various psychological problems, elderly are responsible for raise the conflict in family. Among family members on various issues are responsible for familiar tension, stress and spoiled the health of family as well as society.

7) Generation Gap: –

There is gap between old and present generation. They are not ready to listen them and old people sometimes addict to force their thoughts on others. This is the one of the most responsible causes to create the problems for elderly.

8) Industrialization and Globalization: –

Today is a global age. Every one gives the importance to time. Busy schedule, much assignment, lack of morals and various bad habits are the gifts of globalization and modernization.

9) Avoidance of ethics and morals: –

The effect of urban lifestyle failed on rural society. Villages also started the fallowness of modern life style. They forgot their basic ethics and moral. Therefore elderly of rural society can be neglected from society and family.

10) Unatraction of NGO's and social organizations: –

Many social organizations are known in urban society but they ignore to come at villages. Lack of government control negligence and avoidance to elderly is very common in rural society.

Suggestion for the improvement of status and role of old person in rural society:-

There is a need of time to maintain a suitable situation for elderly for their convenient survival. Following suggestions should be important for elderly.

1) Healthy and positive role of family members.
2) Participation of elderly in cultural, religious and social programs
3) As a human beings, consider to them.
4) Economical support to be provided to elderly.
5) Role of counseling and motivation.
6) Involvement of elderly in farming work for guidance.
7) Medical facilities and focus on their balance diet.
8) Role of peer groups, life partner and grandson or daughters.
9) Entertainment sources and some time shifting to them are also helpful for them.
10) Spirituality and religious work attachment can provide mentally peace to elderly.

SITUATION OF ELDERLY IN RURAL SOCIETY

1. In rural society, old person are the symbol of decline economical, health and social indicator. They have poor housing condition and financially weak and dependent on others.
2. Elderly are the carrier of various disorders.
3. Ignorance and negligence
4. Hygenic and cleanness related problems

Rural old person face the following problems:-

1) Migration.
2) Environmental challenges.
3) Remoteness.
4) Health issues.
5) Rural elderly and mental problems.
6) Elderly and rural social security.
7) Age factors and related contents in rural society.
8) Social economic factors and poverty in rural society.
9) Population distribution and rural society.
10) Role of government, administration policies, NGO and other co-operative societies for elderly.
11) Self-care, support, social status and decision making.
12) Preparation of garment plan strategy and empowerment commission.
13) Old age home, care centers and help desk.
14) Subsidies and involvement of other activities for awareness in society.

Rural elderly tend to experience more functional limitations. In particular, rural non-farm elders are found to report higher numbers of medical conditions, more functional limitations, and greater difficulty in performing tasks of daily living than all other residential categories.

The children of rural non-farm residents, on the other hand, are most likely to have moved some distance away from their parents, minimizing opportunities for interaction. Supporting from grandsons and daughters is the need of time to improve the status of elderly.

This disparity in services adds to the stress and burden experienced by caregivers of rural elders, contributing to their need to turn to outside family resources when they deplete their own physical and emotional resources, or experience extreme stress. However, there are many factors that have changed the social support networks available in rural areas decreasing their size and availability. The availability of adequate support networks of friends and family are an important factor in the primary caregiver being able to cope.

The rural elderly who are comfortable with accepting assistance from friends and neighbors set boundaries as to what amount of assistance is acceptable. They generally are more comfortable if there has been some ongoing reciprocal relationship where they had been of assistance. Older persons who are new to the area may find it difficult since they have had no time to develop these reciprocal relationships that have been built over lifetimes.

The healthy relationship is needed for overall improvement in rural society. Elderly creative in facilitating the integration of family and friends into the care system. Make services available to address short-term and emergency needs for them.

Relationship in a daily life of elderly in rural society:-

To identify the relationship between the elderly and rural community, we need to examine whom they have connection with and the meaning of this connection for them. Female elderly have more interaction with their relatives and neighbors than the male elderly have. On the other hand, female elderly have a tendency to be

more social in the case of social relationships. In other words, female elderly have quite strong network in rural community, which is valuable in order to sustain the daily life of rural community. As we reveal these facts, the social position and role of female elderly should be reconsidered through the relationship structure

Following are the some of the recommendations, suggestions and implement strategy for improve the status of elderly in rural society:.-

1. Focus on the values and goal system in society-
2. Rural social progress and development-
3. Role of NGOs and social organizations
4. Particiaption of family members and others supplementary persons for elderly-
5. Immovability of urban to rural society-
6. Government and administration role for rural elderly-
7. The Ministry of Justice and Empowerment has announced national council for old person called age well foundation
8. School education about elderly people-

CONCLUSION

There is urgent need to amend the Constitution for the special provision to protection of aged person and bring it in the periphery of fundamental right. The rural atmosphere in less of population and therefore definitely the age structure of elderly can be raised. But other factors are most needed to provide them from various agencies.

To improve the status of elderly, there is a need of cumulative efforts from all the stages. They should treated properly from family members, relatives and society. Elderly is an important stage in human life. It needs psychological support from all side for satisfaction of them. Elderly should be treated properly and handle carefully. Old is gold is the feather of Indian rural culture therefore old person needs behavior as per their expectation.

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Anabaena sp.	+	+	+	+	+	+	+	+	+	+	+	+
Nostoc sp.	+	+	+	+	+	+	+	+	+	+	+	+
Oscillatori sp.	+	+	+	+	+	+	+	+	+	+	+	+
Microcystis sp.	+	+	+	+	-	-	-	-	-	-	-	-
C) Bacillariophyceae												
Diatom sp.	+	+	+	+	+	+	+	+	+	+	+	+

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EFFECTS OF AZADIRACHTIN ON SOME FERTILITY ASPECTS OF MALE ALBINO RAT, *RATTUS NORVEGICUS*

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Abstract

The present study aimed to elucidate the effect of azadirachtin, a neem product on some fertility aspects of male albino rat, Rattus norvegicus. The rats were treated with azadirachtin for 8, 16 and 24 days and a significant alterations in the weight of testes, cauda and caput epididymis and in the diameter of testes and seminiferous tubules were noticed in the experimental animals. Similarly, the treatment was also responsible to brought adverse effects on the counting of spermatogonia, spermatids, sperms, sperm motility and abnormal sperm counting. Our findings suggested the antifertility effect of azadirachtin on the male reproductive organs in albino rat.

Key words: - Azadirachtin, Fertility, Albino rat

Introduction

Chemical pesticides, once considered as a boon for farmers to increase the yield of food crops, now become a seriously environmental problem owing to their numerous pesticidal hazards. Thus, it is now become mandatory to search for alternative pesticides, which are safe, cheap and eco-friendly to replace the present harmful chemical pesticides. A neem, *Azadirachta indica*, A. juss, is the evergreen and multipurpose tropical tree is now emerged as a suitable substitute for chemical pesticides because its products have strong pesticidal property. Apart from this pesticidal property, neem tree is considered to be a source of medicines, fodder, furniture, neem cake, timber, neem oil and fungicides etc.

Several investigators suggested the contraceptive potential of various preparations of neem in female rats, mouse, monkey and human. On the contraty, investigation about the effect of neem on the male reproductive organs is relatively few and also controversial. The inhibition of spermatogenesis was reported by Joshi et al (1996) in rat when treated with

aqueous solution of neem leaf powder. On the contrary, Prashad et al (1997) failed to show any adverse effect of extract of neem leaf on spermatogenesis, litter size and fertility in Wister rats. According to Choudhary et al (1990), effect of ethanolic leaf extract of neem failed to alter the morphology and in number of spermatozoa in the cauda epididymis of rat but marked alteration in sperm parameters have been reported in rat treated with aqueous suspension of neem leaf powder in the same experiment. Neem seed extract have proven effective in the control of agricultural pests in an environmentally benign manner (Immaraju, 1998; Allan et al, 1999). The administration of neem leaf extract had adverse effect on motility, morphology and on number of spermatozoa in the cauda epididymis of mice (Rajlakshami, 1992).

More than 300 compounds have been isolated from the various parts of neem tree but major active compounds are highly oxidized triterpenoids called, Limnoids. The azadirachtin (Aza), the chief substance is accumulated in seed kernels of neem tree. There are many pests destroying the crops of farmer causing loss in agricultural production. Among these agricultural pest, a rodent pest, rat is the more dangerous destroying agricultural crop in large quantities. Because of its high fertility rate this rodent pest becomes a hurdle for farmers for more food production. Hence, it becomes mandatory to control the rat community in the interest of agriculturalists keeping this view in mind, the present study was undertaken to investigate the effect of azadirachtin on fertility and reproductive organs of male albino rat, *Rattus norvegicus*.

Materials and Methods

Azadirachtin, a neem product was purchased from the local market of Amravati city which was used for the experimentation. Adult male albino rats, weighing 200-230 gm. were maintained and acclimatized in well ventilated animals cages. They were provided daily with the pellet diet and water ad libitum as per the direction of the "Institutional Animal Ethical Committee" of Sant Gadge Baba Amravati University, Amravati. The animals were divided into two groups such as control and experimental. The dose response to azadirachtin for 24 days was designed. The control rats were treated by intramuscular administration of 0.19 ml. of 70% ethanol and experimental rats were treated by intramuscular administration of 0.19 ml. of azadirachtin. Both control and experimental rats were sacrificed at the end of the each experimental period i.e. 8, 16 and 24 days and the testes, cauda epididymis and caput epididymis were taken out quickly. The tissues were blotted and weighed on the electronic digital balance. The testis was fixed in Bouin's fluid for the histological study and cauda and caput epididymis were used for the study of number, motility and morphology of sperms. The sperm counting was carried out as per the method of Freud's and Carol (1964). The motility of sperm from the sample was visually examined at magnification of 400X under microscopes. The motility was defined as the percentage of spermatozoa that had any form of motility (Didion et al, 1989). The sperm morphology was studied by teasing the cauda epididymis in a known volume of normal saline at 37°c for morphological changes in head and tail to assess the sperm abnormality under microscope. Spermatogenic elements such as spermatogonia, spermatocytes and spermatids were counted at 5µ thick cross sections of testis from 10 seminiferous tubules of each group experimental rat. All raw counts were transferred to true counts by an adaptation of ambercrombie formula (Ambercrombie, 1946) from germ cell diameter measurement. The diameter of seminiferous tubule was measured by using occulometer under microscope. Statistical analysis was done for above counts.

Results and Discussion

Table 1 – Effect of azadirachtin on weight of testis, cauda epididymis, caput epididymis and diameter of seminiferous tubules of albino rats.

Duration	Group	Wt. of Testis (mg.)	Wt. of Cauda epididymis (mg.)	Wt. of Caput epididymis (mg.)	Diameter of Testis (mm.)
8Day	Control	893.3±15.69nNS	213±1.1 NS	227±2.1 NS	9.6±0.08 NS
	Experimental	837.4±9.89 (-6.25)	211±1.6 (-0.93)	217±1.16 (-4.40)	3.4±0.05 (-5.55)
16Day	Control	860.3±8.89 *	218±1.52 **	224±2.3 NS	3.2±0.08 **
	Experimental	789.1±25.73 (-8.27)	209±0.81 (-4.1)	215±1.73 (-4.01)	2.7±0.21(-15.52)
24Day	Control	930.1±25.73 **	217±1.25 **	227±4.4 NS	3.8±0.14 NS
	Experimental	682.8±14.59 (-26.58)	192±3.2 (-11.52)	209±1.63 (-7.92)	1.8±0.13 (-52.63)

Values are mean ± of three observations * $p<0.05$, ** $p<0.01$ and NS = Not significant. Figures in paranthesis indicate the percent change over control.

Table 2 – Effect of azadirachtin on diameter of seminiferous tubule, counting of spermatogonia, spermatocytes, spermatids, study of sperm motility and normal and abnormal sperm counting.

Duration	Group	Diameter of seminiferous tubule (um.)	Sperma-togonia %	Sperma-tocytes %	Sperm-atids %	Normal sperm Counting %	Sperm motility %	Ab. sperm counting %
8Day	Control	153.1±4.5 NS	15.52±0.31 NS	20.2±0.75 NS	62.57±2.5 NS	8.60±0.65 NS	76.33±3.5 **	4±0.6 **
	Experimental	143.8±3.69 (-14.23)	16.81±1.04 (+8.31)	16.41±0.67 (-18.76)	64.43±2.29 (-4.64)	7.3±0.49 (-15.11)	62.5±4.5 (18.11)	7±0.52 (+75)
16Day	Control	169.9±1.58 **	15.65±0.75 NS	19.40±0.76 **	68.39±3.6 *	8.40±0.45 *	76±4.2 **	4±0.49 **
	Experimental	137.6±3.32 (-16.04)	14.79±0.42 (-4.36)	15.96±0.74 (-17.73)	57.18±1.07 (-16.39)	6.79±0.49 (-19.16)	52±3.9 (-31.57)	11±0.58 (+17.5)
24Day	Control	158.6±1.64 **	17.64±0.96 *	19.33±1.10 **	64.28±1.96 **	8.05±0.23 **	75±3.4 **	5±052 **
	Experimental	129.2±1.89 (-18.53)	14.75±0.67 (-16.38)	14.45±0.61 (-25.24)	36.92±3.7-42.56)	4.81±0.42 (-40.24)	36±2.0 (-52)	27±1.17 (+44.0)

Values are mean ± of three observations * $p<0.05$, **$p<0.01$ and NS = Not significant. Figures in paranthesis indicate the percent change over control.

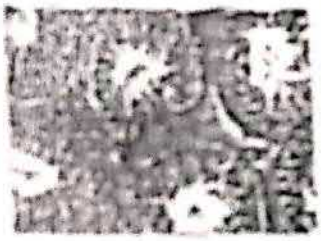

Fig. No. 1.0 T.S. Testis of control rat showing normal seminiferous tubules, interstitial cells, spermatogonia, primary and secondary spermatocytes, spermatids and sperms.

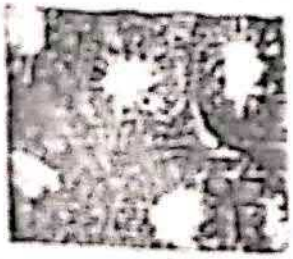

Fig. No. 1.1 T.S. Testis after 8 days administration of azadirachtin showing shrinking of interstitial cells, degeneration of secondary spermatocytes and sperms.

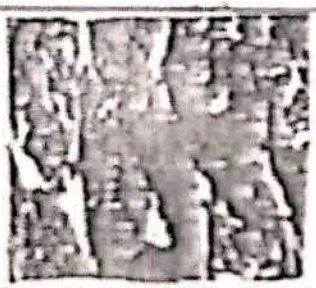

Fig. No. 1.2 T.S. Testis after 16 days administration of azadirachtin showing disarrangement of the germinal epithelial cells of seminiferous tubule.

Fig. No. 1.3 T.S. Testis after 24 days administration of azadirachtin showing degeneration of the connective tissue and decrease in the number of interstitial cells.

The weight of testis was not significantly decreased after the treatment of azadirachtin for 8 days but a moderate decrease and significant decrease was found after treatment for 16 and 24 days respectively (Table-1). This decrease in the testes weight may be due to muscular atrophy as a stress effect of azadirachtin. Patil et al. (1988) studied the effect of pethidine on spermatogenesis in albino rat and recorded a significant reduction in the weight of testis due to the reduced availability of androgen, low protein content of the testis and retarded growth of testis caused due to the stress of toxicant. A significant decrease in the weight of testis of albino rat was found when treated with the dry leaves powder of *Andrographis paniculata*, which was resulted due to the antispermatogenic effect of the treatment (Akhertha et al. 1990).

The weight of cauda epididymis was not significantly changed after 8 day's treatment but a significant decrease was found in 16 & 24 day's treatment (Table-1). Such decrease in the weight of cauda and caput epididymis reflects in decrease of the normal sperm count and increase in the abnormal sperms indicates the action of azadirachtin (Table-2). The weight of epididymis was decreased after the low dose of

treatment of nicotine in albino mice but a high dose of the same caused a significant reduction in the weight of epididymis which might be due to the lack of availability of androgens, as the synthesis of androgen might have been decreased due to the treatment of nicotine (Prabhakarrao & Patil, 1992). The effect of prenatal treatment with busulfan on the hypothalamo-pituitary axis, genital tract and testicular histology of prepubartal male rats was studied by Marie-Claude Viguier et al, (1984) and they found a decrease in the weight of cauda and caput epididymis.

A significant decrease, a moderate decrease and significant decrease in the diameter of testis were observed after 8, 16 and 24 day's treatment respectively (Table-1). Such decrease in the diameter of testis may be due to the degeneration of primary and secondary spermatocytes of seminiferous tubules of testis (Fig. 1.1). Nicotine treatment reduced the diameter and weight of testis which may be due to the decrease in the number of spermatogonia and primary spermatocytes with increase in the spermatocytes and spermatids conversion process due to low level of primary gonadotrophins under the stress of nicotine (Prabhakarrao & Patil 1992). The investigation of above cited worker also supports our findings.

No significant change was observed in diameter of seminiferous tubule after 8 day's treatment but a significant decrease was noted in 16 and 24 day's treated rats. Such a decrease in the diameter of seminiferous tubule may be due to the disarrangement and decrease in size of germinal epithelial cells of seminiferous tubule of testis caused by the stress of azadirachtin (Fig.1.2). Effect of endosulfan on testis of rats, which interfere in the process of spermatogenesis and disrupted the germ cells of seminiferous tubule (Sinha et al, 1997). The antifertility effect of dry leaf powder of *Andrographis peniculata* in male rats observed by Akbarsha (1990) which resulted into the reduction in diameter of seminiferous tubules which may be exerted due to the invasion of genital elements into the lumen of seminiferous tubules. In our present study, a decrease in the diameter of seminiferous tubule was noted which may be exerted by above referred reasons due to the action of azadirachtin.

No significant change in spermatogonia counting was found in 8 and 16 day's but a significant decrease was noted in 24 day's treatment. In spermatocytes counting, no significant decrease was found after 8 day's but significant decrease was found in 16 and 24 day's treatment. Similarly in spermatids counting, a no significant decrease, a moderate decrease and a significant decrease was observed in 8, 16 and 24 day's treatment (Table- 2). Such decrease in spermatogonia, spermatocytes and spermatids may be due to the arrest in the process of spermatogenesis and degeneration of the germinal cells of seminiferous tubule (Fig.1.3). The effect of 80 KDa human semen glycoprotein on albino rat resulted into decrease in the number of spermatogonia, spermatocytes and spermatids, which may be due to the disorganization of the tubular elements, and alteration in the cell association between Sertoli cells and germ cells (Bhandivdekar et al, 1992). Similar finding was also documented by Mandavarao et al, (1998) when they administered a medoxy-progesterone acetate (MPA) and dihydrotestosterone (DHT) in rat, *Rattus norvegicus* that resulted into the histological significant loss in spermatogenic elements, which may be probably due to reduction in level of androgen binding protein, which brought androgen deprivation. In our present study, a significant decrease in counting of spermatogonia, spermatocytes and spermatids was observed which may be exerted due to the disorganization of tubular elements under the action of azadirachtin.

The sperm motility in azadirachtin treated male rats was moderately decreased after 8 days but significant decrease was noted after 16 & 24 days (Table-2). The sperm motility was adversely affected in male mice when administred with leaf extract of neem and this resulted due to disturbances in epididymal function (Mishra and Singh, 2000). The decrease in sperm motility may be exerted due to the resulted alterations in the microenvironment of seminiferous tubule. The endocrine approach to male fertility control by steroid hormone combination in rat and the significant reduction in the seminiferous tubule was found which was caused due to the disturbances in epididymal microenvironment (Madhavarao and Shah, 1998). In our present study, a decrease in sperm motility may be exerted due to the changes in microenvironment of seminiferous tubule by the action of azadirachtin.

No significant decrease, a moderate decrease and a significant decrease in normal sperm counting was found after the treatment for 8, 16 and 24 days respectively. A significant increase in the abnormal sperm counting was found in all treated rats (Table-2). Mishra and Singh (2005) also recorded a similar type of reduction in sperm count in male mice when treated with aqueous leaf extract of neem and according to them this happened due to the suppressive effect of neem on spermatogenesis. The decrease in normal sperm counting and increase in abnormal sperm

counting may be exerted due to the disarrangement and decrease in size of germinal epithelium which resulted into the reduction in the number of spermatids which was responsible for decrease in daily sperm production in the testis. Such low sperm production which further led to decrease sperm release in the epididymis of the treated rats which then caused a significant sperm abnormality in all treated groups. The feeding impact of ethanolic extract of *Achyranthes aspera* in reproductive function in male rats led into the reduction in sperm count which may caused due to the repression of epeididymal epithelium and a decline in the number of spermatozoa (Sandhyakumary, 2002).

In our investigation, all parameter of male reproduction in albino rat were affected adversely under azadirachtin stress and our finding is parallel with the findings of some workers cited above.

Thus, it can be concluded that azadirachtin play an important antifertility role in male reproduction of albino rat, *Rattus norvegicus*.

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Short Communication

Study of Stability Constants of Cu (II), Co (II), Ni (II), Mn (II) Complexes with Substituted Δ^2 Pyrazole in DMF Solvent using pH-Meter

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Abstract

The stability constant of substituted 2pyrazole (P1) with Cu(II), Co(II), Ni(II), Mn(II) complexes using pH metric titration technique in 90% DMF-water mixture at an ionic strength of 0.1M KNO3 were studied. The data obtained showed that Cu (II) has highest stability while Mn(II) has lowest stability.

Keywords: Stability constants, Pyrazole, DMF, pH-meter study.

Introduction

A pH meter is used to find out the stability constant which is useful as equilibrium constant for the formation of a complex in solution. In recent years, most of the co-workers have focused their studies on the pH-meter for stability constants in order to obtain accurate results.

R.K. Tada ***et al*** have investigated Evaluation of stability constant of Thiosemicarbazide (TRM-1) with copper (II), cobalt (II) and nickel (II) complexes using pH-meter[1]. A.B. Naik ***et al*** have investigated pH-meteric studies on the substituted pyrozoles with some lanthanides metal ions and the influence of ionic strength on complex equillibria in a 70% dioxane-water mixture[2]. G.H. Murhekar ***et al*** have investigated formation constants of lanthanides metal ion chelates with some substituted pyrozoles in different solvent compositions[3]. K.T. Kiranpure ***et al*** have studied proton ligand and metal ligand stability constant by effect of dielectric constants of methanol-water and acetone-water mixtures on Cu (II)-salicyclic acid complex[4]. M. M. Rathore ***et al*** have investigated the effect of dielectric constants of 1, 3 diphenyl thiazines with Cu (II) complexes in dioxane-water mixtureusing pH-meter at 0.1 M ionic strength[5]. Y.K. Meshram ***et al*** have reported the stability constants of transition metal complexes with substituted ketones and simple ketones at 0.1 M ionic strength using pH-meter[6]. A. Ramteke ***et al*** have studied stability constants of the complexes of chlorosubtituted pyrazoles and pyrazolines with Cu (II), Ni (II), Co (II) and Nd (II) metal ions in 70 % dioxane-water mixture at 0.1 M ionic strength[7].

The present work described interaction of Cu (II),Co(II), Ni(II), Mn(II) complexes with 3-(2-hydroxyl-3,5-dicholorphenyl)-4-benzoyl-5-(2'-furyl)-1-phenylpyrazole (P_1) as ligand in DMF (N, N-dimethyl formamide) solvent. The ligands are insoluble in water hence 90 % DMF-water mixture is used as a solvent.

Materials and Methods

The lignads were synthesized by known literature method .The stock solutions of the lignads (0.01 M) were prepared by dissolving the requisite quantity of the lignad in a 90% DMF-water solvent and diluted to the final volume. The solution of sodium hydroxide (0.2 N) was prepared by making it free from carbonate and standardized by titration against standard oxalic acid. 0.1M HNO_3 acid was used for the preparation of a stok solution. Its exact normality was calculated by titrating against standard sodium hydroxide. 0.1M KNO_3 solution which was prepared from carbonates free double distilled water.

Metal Chlorides: Present investigation focused on the study of transition elements: (1) Copper chlorides ($CuCl_2.2H_2O$), (2) Cobalt chlorides ($CoCl_2.6H_2O$), (3) Nickel chlorides ($NiCl_2$) and (4) Magnese chlorides ($MnCl_2.4H_2O$). Double distilled water was used for the preparation of 0.02M metal solutions.

Instruments: All the pH measurements and titrations were carried out on ELICO-L1-10 pH meter with accuracy 0.01by using a glass and calomel electrode assembly. The instrument could read the pH in 0.0 to 14 in a step of 0.005. Firstly the electrodes were washed with distilled water and dried with filter paper for any pH measurement. The pH meter was standardized before each titration with a buffer solution of pH 4.00, 7.00 and 9.20. The qualigens buffer tablets used for standardization of pH meter at pH 4.00, 7.00 and 9.20.

The determination of metal-ligand stability constants carried from three kinds of titrations: i. Acid titration: - 5ml 0.1M

(HNO_3) +5ml (0.1M) KNO_3+ 35 ml DMF+5ml water. .ii. Acid + Ligand titration: 5 ml HNO_3 (o.1M) + 5 ml KNO_3 (0.1M) + 10 ml ligand (in DMF) + 25 ml DMF + 5 ml water. iii. Acid+ Ligand+ Metal titration:- 5 ml HNO_3 (0.1M) + 5 ml KNO_3 (o.1M) + 10 ml ligand (in DMF) + 25 ml DMF 2 ml metal ion solution + 3 ml water.

0.2 N NaOH (alkali) solution used for all titrations and titration data used to draw the curves between volume of NaOH added and pH values. Readings related to estimate the value of pH and volume of alkali added presented in Table-1. Metal-ligand formation curves and acid-ligand formation curve represented in Figure-1.

Results and Discussion

The dissociation of OH^- clearly indicated by the titrations (acid + ligand) curves deviated from acid curves at pH 4.60 and continued up to pH 12.58.

Metal ion hydrolysis: The pH at which metal ion start association (hydroxylation) with OH^- group showed its co-relation with the process of complex formation with the ligand. The formation of hydroxide M (OH) is given by the equation,

$$M + H_2O \rightleftharpoons M(OH^-) + H^+$$

At the time of departure of metal complexes titration curve was observed always at lowest pH values than the pH of hydrolysis of metal ion.

Formation of Curves: The deviation of metal titration curves from ligand curve (metal+ ligand) were found in between 4.50 to and continue up to 12.58. This shows the formation of complexes with respect to change in colour.

Conclusion

From the graphical data it was conclude that the formation of strong metal complexes due to higest pH values of ligand titration curves as compare to metal titration curves. The higher co-ordination achieved by large metal ion, hence Cu (II) shows highest stability and Mn(II) shows lowest stability.

Table-1
The pH titration reading of acid, acid + Ligand, acid + Ligand 0.1M, T = 25^{0}C, solvent DMF-water (90:10) + Metal, Ionic Strength μ =

Vol. of Alkali Adade	Acid	Acid + Ligand (P_1)	Acid + Ligand (P_1) + Cu (II)	Acid + Ligand (P_1) + Co (II)	Acid + Ligand (P_1) + Ni (II)	Acid + Ligand (P_1) + Mn (II)
0.00	3.37	3.42	3.51	3.49	3.40	3.41
0.10	3.40	3.54	3.52	3.50	3.49	3.50
0.20	3.51	3.56	3.58	3.56	3.55	3.54
0.30	3.57	3.60	3.62	3.60	3.58	3.56
0.40	3.62	3.69	3.70	3.68	3.67	3.65
0.50	3.70	3.75	3.73	3.72	3.70	3.71
0.60	3.82	3.82	3.80	3.78	3.79	3.76
0.70	4.05	4.17	4.10	4.03	4.02	4.01
0.80	4.58	4.60	4.58	4.56	4.55	4.54
0.90	11.30	10.88	10.76	10.64	10.56	10.52
1.00	12.35	11.68	11.55	11.02	10.88	10.60
1.10	12.56	11.50	11.12	11.00	10.98	10.90
1.20	12.60	12.55	12.52	12.40	12.38	12.36
1.30	12.40	12.20	12.00	11.90	11.12	11.10
1.40	12.68	12.58	12.48	12.02	12.79	11.70

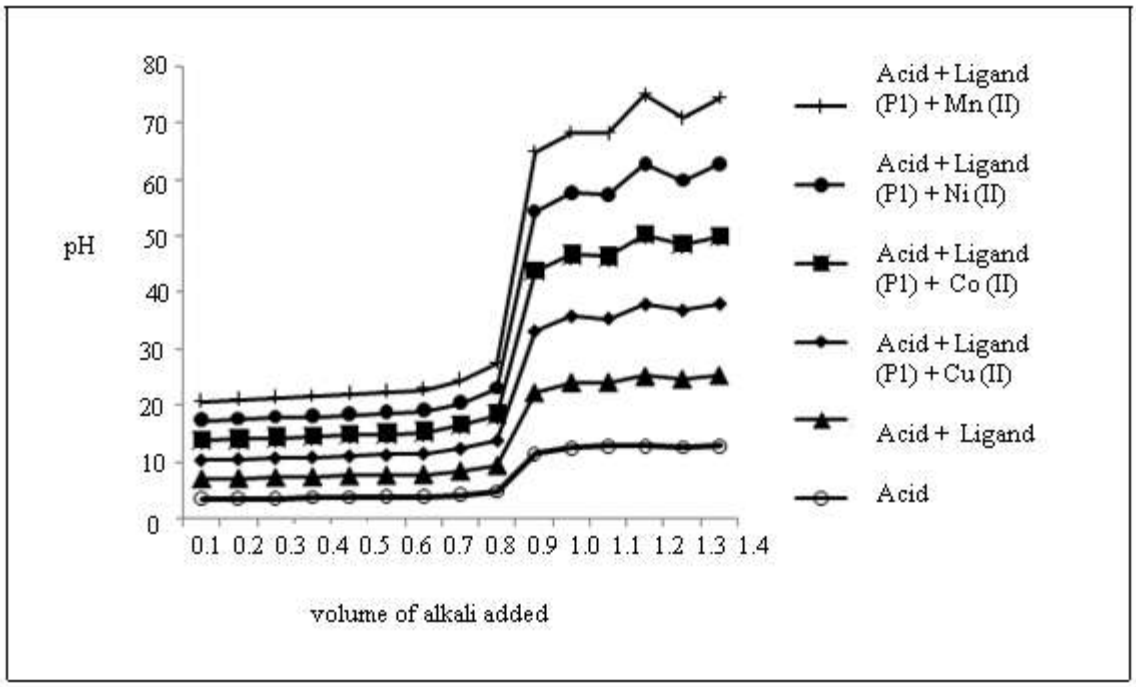

Figure-1
Experimental curve of acid, acid+ligand and acid + ligand +metal

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Synthesis, Characterisation And Screening Of Some New Chlorosubstituted Imidazolo-Pyrazolines With Special Reference To Their Growth Promoting And Curative Impact On *Oyster Mushroom* Crop

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Abstract

Five membered heterocycles with an additional hetero-atom called azoles are well known for their pharmacological, agricultural and industrial applications. The chemotherapeutic agents such as orisul (bacterostatic), antipyrine (antipyretic), butazolidine (anti-inflammatory) contain pyrazoline nucleus. They have remarkable insecticidal activity against the insects like lepidopteran and coleopteran. Imidazole derivatives of pyrazoline substrates were also reported as main constituents of many pesticides used in agriculture. Some of their derivatives show the fungicidal and plant growth regulatory activities. Owing to their applications, a significant amount of research activity has been directed towards synthesis of this class of compounds. In this context, synthesis of some new chlorosubstituted 1-phenyl-3-(2-substitiuted-5-chlorophenyl)-4-benzoyl-5-[2-mercapto-4-(2'-hydroxy-5'-chlorophenyl)-imidazolo]-4,5-dihydro-Δ^2-pyrazolines were undertaken from 1-phenyl-3-(2-hydroxy-5-chlorophenyl)-4-benzoyl-5-amino-4,5-dihydro-Δ^2-pyrazoline, which was prepared by the reaction of substituted-3-benzoyl-6-chloroflavanone with phenylhydrazine hydrochloride in 1,4-dioxane containing a little piperidine. The structures of newly synthesised compounds were determined on the basis of elemental analysis and spectral characterization. The newly synthesised compounds were assayed for their antimicrobial activity against some fungi viz. Gliocladium roseum (Link) Bainier, Verticillium fungicola and some bacteria viz. Pseudomonas stutzeri, Pseudomonas alcaligens, Pseudomonas fluorescene, Burkholderia gladioli which are mainly responsible for the damage of mushroom crop. So also the titled compounds were screened for their impact on phytotic growth of Oyster mushroom spp.

Keywords: Chlorosubstituted pyrazolines, α-amino ketone of pyrazolines, imidazolo-pyrazolines, acetyl analogues of imidazolo-pyrazolines and antimicrobial activity.

I. INTRODUCTION

Heterocyclic compounds play an important role in mediating many biological processes[1-5]. One of the important reasons for the widespread applicability of heterocyclic compounds is the flexibility of their structure towards modification to incorporate functional moieties either as substituent or as a part of the ring system. Owing to this property, an organic chemist is enabled to tailor a structure to meet a particular need by modifying the heterocyclic component. It is, therefore, not surprising that lot of efforts have been extended in studying their chemistry and applicability.

Literature survey reveals that medicinal as well as agricultural chemists have become interested in the synthesis of pyrazoline analogues possessing active moieties for the development of bioactive agents having greater and improved properties towards respective fields. Kalirajan *et al.*[6] reported the formation of some pyrazoline substituted benzimidazoles and studied their biological activities.

Rajora *et al.*[7] transformed 1-benzimidazolyl-3-aroyl-prop-2-ene-1-one into N-substituted pyrazoline derivatives by the treatment of phenylhydrazine, thiosemicarbazide and hydrazinehydrate in presence of formic acid and reported their antimicrobial properties.

Literature survey also reveals that, mushrooms species easily fall prey to infections caused by pathogens and thus become a serious problem in the mushroom crop cultivation. The diseases like *white cottony growth* and fruiting body covered with the *green spots* are reported to cause by *Gliocladium sp.* In addition to this, diseases like powdery white growth on stipe, fluffy growth on substrate, dry bubble disease, brown spot disease are also reported to be caused by the infection of *Cladobotrym apiculatum, Arthrobotrys pleuroli, Velricillium fungicola* and *Pseudomonas stulzeri* respectively.

Thus it was thought significant to explore the properties of titled compounds against mushroom crop pathogens *viz* fungi *Gliocladium roseum (Link) Bainier, Verticillium fungicola* and bacteria *Pseudomonas stutzeri, Pseudomonas alcaligens, Pseudomonas fluorescene, Burkholderia gladioli* and also their growth promoting and curative impact on *Oyster mushroom* crop.

In tune with the literature survey, we, herein, report the synthesis of 1-phenyl-3-(2-hydroxy-5-chlorophenyl)-4-benzoyl-5-amino-4,5-dihydro-Δ^2-pyrazoline (1), 1-phenyl-3-(2-hydroxy-5-chloro phenyl)-4-benzoyl-5-N-[(2'-hydroxy-5'-chlorophenyl)ethanonylamino]-4,5-dihydro-Δ^2-pyrazoline (2), 1-phenyl-3-(2-hydroxy-5-chlorophenyl)-4-benzoyl-5-[2-mercapto-4-(2'-hydroxy-5'-chloro phenyl)imidazolo]-4,5-dihydro-Δ^2-pyrazoline (3), 1-phenyl-3-(2-acetyloxy-5-chlorophenyl)-4-benzoyl -5-[2-mercapto-4-(2'-acetyloxy-5'-chloro-phenyl)imidazolo]-4,5-dihydro-Δ^2-pyrazoline (4).

II. EXPERIMENTAL

The structures of all the newly synthesised compounds, reported herein, were confirmed on the basis of their chemical properties, elemental analysis and spectral data. UV-Vis spectra were recorded in ethanol solvent. IR spectra were recorded on Perkin-Elmer spectrophotometer in the range 4000-400 cm^{-1} in KBr pellets. ^1H NMR spectra were recorded on Bruker Avance-II 400 NMR spectrophotometer in $CDCl_3$ using TMS as an internal standard. The melting points were recorded by capillary method in paraffin using Thiele's apparatus and all are uncorrected. Chemicals used were of A.R. Grade. The purity of newly synthesized compounds was checked by TLC using solvent combination.

Preparation of 1-phenyl-3-(2-hydroxy-5-chlorophenyl)-4-benzoyl-5-N-[(2'-hydroxy-5'-chloro phenyl)ethanonylamino]-4,5-dihydro-Δ^2-pyrazoline (2):

1-Phenyl-3-(2-hydroxy-5-chlorophenyl)-4-benzoyl-5-amino-4,5-dihydro-Δ^2-pyrazoline (1) (0.01M) was refluxed with 1-(2-hydroxy-5-chlorophenyl)-2-bromoethanone (1a) (0.01M) in absolute ethanol for about 1 hour. After cooling, the reaction mixture was decomposed in ice-cold water. The product, thus separated, was filtered and crystallized from ethanol to get the compound 2.

M.F. $C_{30}H_{23}N_3O_4Cl_2$ (2): Brown crystalline solid, m.p.64 ^oC, yield 76 %, Elemental analysis (%): **C** 64.21/64.29; **H** 4.09/4.14; **N** 7.42/7.50; **O** 11.29/11.42; **Cl** 12.58/12.65. **UV** (ethanol): λ_{max} 670 nm, $n \rightarrow \pi^*$ transition. **IR** (KBr) (cm^{-1}): 3600-2400 (-OH stret.), 3085.52 (Ar. C-H stret.), 2918.54 (Ali. C-H stret.), 1650.16 (C=O stret.), 1566.35 (C=N stret.), 1354.30 (C-N stret.), 1209.32 (C-O stret.). 772.32 (C-Cl stret.). **^{1}H NMR** (δ ppm): 2.6 (s, 2H, -C**H**$_2$), 1.6 (s, 1H, -N**H**), 1.2 (d, 1H, C**H**-CH-CO-Ph), 1.61 (d, 1H, CH-C**H**-CO-Ph), 6.9-8.2 (m, 16H, Ar-**H**), 12.13 (s, 1H, H-bonded -O**H**).

Preparation of 1-phenyl-3-(2-hydroxy-5-chlorophenyl)-4-benzoyl-5-[2-mercapto-4-(2'-hydroxy-5'-chlorophenyl)imidazolo]-4,5-dihydro-Δ^2-pyrazoline (3):

1-Phenyl-3-(2-hydroxy-5-chlorophenyl)-4-benzoyl-5-N-[(2'-hydroxy-5'-chloro-phenyl) ethanonylamino]-4,5-dihydro-Δ^2-pyrazoline (2) (0.01M) was refluxed with potassium thiocyanate (0.01M) for 4 hours in glacial acetic acid. After cooling, the reaction mixture was poured into ice-cold

water and the product, thus separated, was crystallized from ethanol-acetic acid mixture to get the compounds 3.

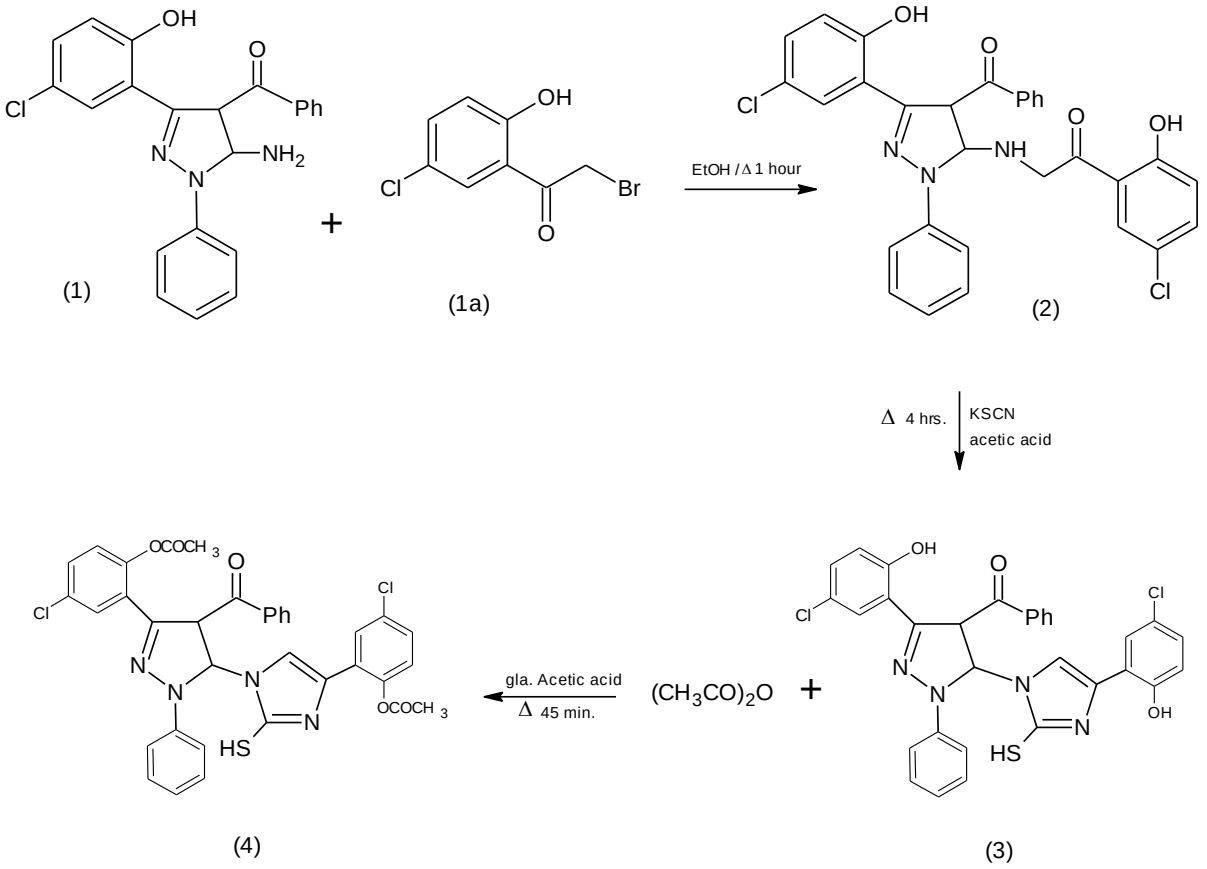
M.F. $C_{31}H_{22}N_4O_3SCl_2$ (3): brown crystalline shining solid, m.p.108 ^oC, yield 77 %, Elemental analysis (%): **C** 61.83/61.90; **H** 3.57/3.69, **N** 9.26/9.31, **O** 7.86/7.98, **S** 5.27/5.33, **Cl** 11.67/11.79. **UV** (ethanol): λ_{max} 540 nm, n→ π* transition. **IR** (KBr) (cm^{-1}): 3500-2400 (O-H stret.), 3084.45 (Aro.C-H stret.), 2916.44 (Ali. C-H stret.), 2532 (S-H stret.), 1647.26 (C=O stret.), 1633.33 (C=N stret.), 1600.33 (C=C stret.), 771.31 (C-Cl stret.).**^{1}H NMR** (δ ppm): 6.7 (s, 2H, C-**H**), 1.5 (d, 1H, C**H**-CH-CO-Ph), 2.5 (d, 1H, CH-C**H**-CO-Ph), 6.87 (s, 1H, N-C**H**=C), 7.1-8.1 (m, 16H, Ar-**H**), 7.86 (s, 1H, -C**H**=CH), 12.06 (s, 1H, O-**H**).

Preparation of 1-phenyl-3-(2-acetyloxy-5-chlorophenyl)-4-benzoyl-5-[2-mercapto-4-(2'-acetyl-oxy-5'-chlorophenyl)imidazolo]-4,5-dihydro-Δ^2-pyrazoline (4):

1-Phenyl-3-(2-hydroxy-5-chlorophenyl)-4-benzoyl-5-[2-mercapto-4-(2'-hydroxy-5'-chloro phenyl)imidazolo]-4,5-dihydro-Δ^2-pyrazoline (3) (0.01M) was refluxed with acetic anhydride for 45 min. in glacial acetic acid. After cooling, the reaction mixture was decomposed in water and the product, thus separated, was crystallized from ethanol-acetic acid mixture to get the compound 4.

M.F. $C_{35}H_{26}N_4O_5SCl_2$ (4): Brown solid, m.p. 89 ^oC, yield 69 %, Elemental analysis (%): **C** 61.25/61.32, **H** 3.74/3.82, **N** 8.13/8.17, **O** 11.53/11.67, **S** 4.64/4.68, **Cl** 10.28/10.34. **UV** (ethanol): λ_{max} 570 nm, n→ π* transition. **IR** (KBr) (cm^{-1}): 3084.39 (Ali. C-H stret.), 1640.25 (C=O stret.), 1645.78 (C=N stret.), 773.29 (C-Cl stret.). **^{1}H NMR** (δ ppm): 6.8 (s, 2H, C-**H**), 6.80 (d, 1H, C**H**-CH-CO-Ph), 6.86 (d, 1H, CH-C**H**-CO-Ph), 7.1-8.1 (m, 16H, Ar-**H**).

SCHEME:



III. ANTIMICROBIAL SCREENING

The compounds 1, 2, 3 and 4 were assayed against *Mushroom* crop pathogens using cup plate diffusion method. The inhibitory effects of compounds against these organisms are given in Table 1.The screening results indicate that the compound 1, 2, 3 and 4 showed good to moderate antifungal and antibacterial activities against fungi *Gliocladium roseum (Link) Bainier*, *Verticillium fungicola* and Bacteria *Pseudomonas stutzeri*, *Pseudomonas alcaligens, Pseudomonas fluorescene, Burkholderia gladioli.*

In this method, potato carrot agar and nutrient agar were melted, cooled and poured into sterile petri-plates and allowed for solidification. After solidification, by using Lawn method the fungal organisms were inoculated on the petri-plates having potato carrot agar and the bacterial organisms were inoculated on the petri-plates having nutrient agar. After some time, the cups of about 10 mm diameter were cut with the help of sterile borer. The drops of melted agar were added to seal the bottom of the cups and the wells were filled by pipetting 0.1 ml solution of test compounds 100 µg ml^{-1}.

The discs of *Cabendizum* (10mcg/disc) and *Gentamycine* (10mcg/disc), were used as positive controls. The zones of inhibitions were recorded in millimetres by using Himedia Zone Reader Scale.

the results obtained in the antimicrobial study are given in table no. 1

Table 1: Antimicrobial screening of titled compounds against Oyster mushroom crop pathogens.

S.N.	Compounds	Zone of inhibition (mm)					
		Fungal pathogens		Bacterial pathogens			
		Gliocladium roseum	*Verticillium fungicola*	*Pseudomonas stutzeri*	*Pseudomonas alcaligens*	*Pseudomonas fluorescene*	*Burkholderia gladioli*
1.	1	08	08	09	07	09	09
2.	2	06	07	05	06	08	04
3.	3	11	16	13	13	15	09
4.	4	09	04	07	08	11	07
5.	*Carbendizium*	09	09	NA	NA	NA	NA
6.	*Gentamycine*	NA	NA	08	08	08	08

IV. GROWTH PROMOTING IMPACT OF TEST COMPOUNDS

The spawns of experimental species *P. sajor-caju* ie *P. pulmonarius* were procured from genuine agricultural agencies and cultivated in the culture house of the ICAR affiliated Krushi Vidyan Kendra, Durgapur (Badnera) Dist. Amravati.

The experimental setup was divided into two parts ie 'A'-control group plants and 'B'-treated group plants. The spawns were inoculated and cultivated by the conventional methods.

The soyabean straw was used as a substrate for the cultivation of *Pleurotus sajor-caju* and it was firstly chopped into smaller pieces up to 3-5 cm and soaked in water tank for 12-15 hours. This was subjected for sterilization using hot water treatment maintained at 60-80 ^{o}C for 1 hour. The sterilised substrate was taken out and allowed to lower down the temperature.

The uniform size beds were prepared in sterilized polythene bags filled with alternate layers of sterilized soybean straw and spawns treated with the solution of test compounds. The mouth of packets (beds) were plugged and tightened with threads and 20-25 pin-holes were made on all sides of the packets. Similarly the untreated spawns were filled in control group beds (bags).

After proper labelling, the packets were hanged to iron racks and incubated in cultivation room on or below 25^{o}C. for mycelium running for 25-30 days. During this incubation period, appropriate temperature of the incubation room was maintained.

After the complete development of mycelium, the packets were taken out of the incubation room and shifted to growing room, where the packets were hanged to bamboo frame. During the harvesting of mushroom beds were irrigated according to need.

When the first primordial initiation was observed, the test compounds were sprayed on the mushroom with specific intervals. Mushroom crop was harvested before the fruiting body showed any splitting on the edges. The yields of mushroom crop from various bags with different parameters *viz* length, diameter, weight and colour were recorded.

The results of field experiments with test compounds are tabulated in table no. 2 and also shown in fig. no. 1 and 2:

Table 2: Effect of titled compounds on Oyster mushroom: Pleurotus sajor-caju spp.

Treated bags	Compo-unds	D (cm)	T (cm)	L (cm)	Weight of Dry Bags (gm) (After Harvesting)	Total Weight (gm)		Colour
						Fresh	Dry	
1.	1	8.0	0.5	5.8	0.930	219	20.45	White
2.	2	8.7	0.4	5.8	0.992	198	17.61	White
3.	3	11.7	0.6	6.9	0.983	227	21.35	Creamy
4.	4	11.6	0.5	6.3	0.955	207	18.93	Creamy
5.	1,4-Dioxane	6.0	0.4	6.1	0.990	176	19.13	White
6.	Control	6.8	0.3	5.5	0.853	204	20.00	White
D = Diameter ; T = Thickness ; L = Length								

V. ANALYSIS OF MUSHROOM SAMPLES TREATED WITH TEST COMPOUNDS

The samples of *P. sajor-caju* collected during the experimental study of growth promoting impact were sun-dried and immediately proceeded for analysis of % crude fibre, % crude protein and elemental detection with special reference to N, P, K and S.

The analysis of crude fibre percentage of the samples was carried out at Food Testing Laboratory, Krishi Vigyan Kendra, Durgapur (Badnera) Dist. Amravati using Pelicans FBS-06 (P) Laboratory Manuals & AOAC Method, whereas percentage of crude protein and element detection were determined at Analytical Lab, using Leaf method of analysis. The Kjeldahls method, UV spectrophotometer and Flame photometer were used for the analysis of N, P, K and S elements.

The results of analysis obtained for treated mushroom samples are tabulated in table no. 3:

Table 3: Analytical results of dry Oyster mushroom: P. sajor-caju spp. treated with titled compounds.

S.N.	Sample	% of Crude Fibre	% of Crude Protein	% N	% P	% K	% S
1.	1	8.00	15.15	2.425	0.3042	2.560	0.1267
2.	2	8.73	16.02	2.564	0.3115	2.937	0.1369
3.	3	10.05	20.30	3.248	0.3238	2.710	0.1325
4.	4	9.85	18.93	3.029	0.2980	2.444	0.1317
5.	1,4-Dioxane	8.06	13.29	2.127	0.275	2.346	0.1358
6.	Control	5.64	15.98	2.558	0.367	2.747	0.1412

VI. RESULTS AND DISCUSSION

In the present study newly synthesized 1-phenyl-3-(2-hydroxy-5-chlorophenyl)-4-benzoyl-5-amino-4,5-dihydro-Δ^2-pyrazoline (1), 1-phenyl-3-(2-hydroxy-5-chlorophenyl)-4-benzoyl-5-N-[(2'-hydroxy-5'-chlorophenyl)ethanonylamino]-4,5-dihydro-Δ^2-pyrazoline (2), 1-phenyl-3-(2-hydroxy-5-chlorophenyl)-4-benzoyl-5-[2-mercapto-4-(2'-hydroxy-5'-chloro-phenyl)imidazolo]-4,5-dihydro-Δ^2-pyrazoline (3) and 1-phenyl-3-(2-acetyloxy-5-chlorophenyl)-4-benzoyl-5-[2-mercapto-4-(2'-acetyloxy-5'-chloro-phenyl)imidazolo]-4,5-dihydro-Δ^2-pyrazoline (4) were screened for their antimicrobial activity against some *Mushroom* crop damaging pathogens includes fungi *viz. Gliocladium roseum (Link) Bainier*, *Verticillium fungicola* and bacteria *viz. Pseudomonas stutzeri*, *Pseudomonas alcaligens*, *Pseudomonas fluorescene*, *Burkholderia gladioli*. From the results, it has been observed that the titled compounds showed good to moderate amount of antibacterial activity.

Pleurotus sajor-caju, a species of *Oyster mushroom* was treated with test compounds to examine the efficacy of the newly synthesised compounds on the morphology of treated mushroom species with inclusion of analysis of treated samples.

When the treated and control species of mushroom were compared with reference to their morphological characters, it was interesting to note that the treated species exhibited significant growth in diameter and thickness of caps as well as lengthening of stipes. In addition to this, there was remarkable increase in the yields because of that healthy growth and disease free environment.

The analytical results obtained for all the treated mushroom samples clearly show the increase in the value of crude fibre percentage as well as the crude protein percentage. The presence of elements like N, P, K and S were also analysed in the treated mushroom samples. The more vigorous observations revealed that the mushroom crop treated with imidazole blends of azoles were found more effective in the enhancement of crude fibre percentage compared to other treated compounds.

However, further investigation and a systematic approach in the light of agricultural science would certainly prove to be a potential tool for the growth promoting and creating ecofriendly environment for mushroom cultivation.

VIII. CONCLUSION

On the basis of chemical analysis and spectral data, it is concluded that, the synthesis of titled compounds was achieved successfully. The antimicrobial screening of these compounds showed good to moderate antifungal and antibacterial activities. From the Table-1 it can be noticed that the imidazolo-pyrazoline have great potential towards mushroom pathogens.

The treated species ie. *P. sajor-caju* showed significant growth with respect to diameter, thickness and lengthening of stipe that reflects the curative and growth promoting properties of the titled compounds. Besides this, enhancement of the yields reveals the healthy growth due disease free environment.

The newly synthesised compounds also showed noticeable enhancement in the nutritive values ie increase in crude fibre percentage and crude protein percentage. In this regard, the imidazole blends of azoles were found more effective in the enhancement of nutritive value.

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SYNTHESIS OF SOME CHLOROSUBSTITUTED THIAZOLES, IMIDAZOLO-THIAZOLES-AS EFFICIENT ANTIBACTERIAL AGENTS

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ABSTRACT
In the past 2,3 decades, the literature survey is enriched with progressive finding about the synthesis and pharmacological evaluation of fused heterocycles containing imidazole and thiazole moeities. Due to their vital role in biological activities, it was thought interesting to synthesize some chlorosubstitutedthiazoles and their imidazole containing derivatives. The newly synthesized compounds when screened for antibacterial activities against some plant pathogens showed good to excellent activity.

KEYWORDS: thioureas, thiazoles, imidazolo-thiazoles, antibacterial agent.

INTRODUCTION

Literature survey reveals that many heterocyclic[1] compounds containing fused ring system have a broad spectrum of biological[2-3] as well as physiological activities. It is also revealed that thiazole moieties have attracted considerable attention of medicinal chemists as they are endowed with a wide range of diverse biological activities[4] such as anti-inflammatory[5], analgesic, antifungal[6], antimicrobial[7], anti-oxidant activity.[8] Imidazole is also one of the most fascinating classes of compounds possessing variety of biological activities[9-14] such as anti-HIV, anti-histamine, antibacterial, tranquillizer *etc*. Morever imidazole[15] motif is found in number of chemo-therapeutic agents such as etomidate, zolpidem, nafimidone, cimetidine, clodine, pilocarpine and metronidazole. Encouraged by the earlier reports, we have designed and synthesized some new chlorosubstituted thiazoles and their imidazolo-thiazole blends. These titled compounds were screened for their antibacterial[16-19] assay against some *ornamental plant pathogens* viz. *Staphylococcus aureus, Staphylococcus epidermis, Pseudomonas aeruginosa and Salmonella typhi* by using Agar disc diffusion method.

EXPERIMENTAL

Physical characterization data of all the compounds are given in Table-1.

TABLE-1: Characterization data of newly synthesized compounds

Compound	Molecular Formula	Melting Point (^oC)	Yield (%)	R_f
1a	$C_8H_6Cl_2O_2$	221(B.P)	75	0.81
2a	$C_8H_6Cl_2O_2$	53	74	0.84
3a	$C_{15}H_8Cl_4O_2$	130	78	0.74
4a	$C_{15}H_6Cl_4O_2$	122	75	0.86
5a	$C_{15}H_8Cl_4O_3$	141	59	0.77
6a	$C_{15}H_7BrCl_4O_3$	100	60	0.79
7a	$C_{16}H_8Cl_4N_2O_2S$	150	59	0.88
7b	$C_{22}H_{12}Cl_4N_2O_2S$	141	56	0.86
8a	$C_{24}H_{12}Cl_4N_2O_4S$	111	65	0.80
9a	$C_{25}H_{11}Cl_6N_3O_3S_2$	103	70	0.75

The synthetic routes which furnished the target compounds are shown below along with their IR and NMR data.

Scheme-I

Preparation of 2,4-dichlorophenyl acetate (1a): 2,4-Dichlorophenol (0.01M) was mixed with acetic anhydride (0.01M) and anhydrous sodium acetate (5g). The mixture was refluxed for about an hour. It was then cooled and poured into cold water. Acetate layer thus separated was washed with water for several times. Finally it was purified by distillation and the distillate of compound (1a) was collected at about 221^{o}C; yield: 75%, b.p: 221^{o}C.

Preparation of 1-(3,5-dichloro-2-hydroxyphenyl)ethanone (2a): The compound (1a) (50ml) was mixed with anhydrous aluminum trichloride (120 g) and heated at 120^{o}C for 45 minutes on sand bath. The reaction mixture was decomposed by ice cold water containing a little HCl to get the crude product. It was then purified by recrystalization using ethanol to get a greenish white solid as compound(2a); yield:74%; m.p.:53^{o}C.

IR(KBr ν_{max})=3423cm^{-1}(-OH str),1664cm^{-1}(C=Ostr), 1300cm^{-1}(C-Ostr), 766cm^{-1}(C-Clstr).

NMR: δ12.69(s,1H,Ar-H) , δ7.25 to 7.63 (m,2H,Ar-H) , δ2.60,(s,3H,-CH_3).

Preparation of 1-(3,5-dichloro-2-hydroxyphenyl)-3-(2,3-dichlorophenyl)prop-2-en-1-one (3a): The compound (2a)(0.01M) dissolved in ethanol and 2,3-dichloro benzaldehyde (0.01M) was added to it, the mixture was heated to boiling, aqueous sodium hydroxide solution 40% (10ml) was added to it dropwise with constant stirring. The mixture was mechanically stirred for 30 minutes at room temperature and kept overnight. Then the mixture was acidified with HCl (10%). The solid product thus obtained was filtered, washed with sodium bicarbonate (10%) followed by washing with water to get the crude product. It was crystallized from ethanol-acetic acid mixture to get the compound (3a); yield:78%; m.p.:130^{0}C.

IR(KBr ν_{max})=3445cm^{-1}(O-Hstr),1649(C=Ostr),3068cm^{-1}(ArC-Hstr),1608cm^{-1} (C=Cstr), 780cm^{-1}(C-Clstr).

NMR: δ13.16(s,1H,Ar-OH),δ8.39(d,1H,-CH), δ8.35(d,1H,-CH), δ7.26-7.78(m,5H,Ar-H).

Preparation of 6,8-dichloro-2-(2,3-dichlorophenyl)-4*H*-chromen-4-one(4a): The compound (3a)(0.01) suspended in 10 ml DMSO refluxed with crystals of iodine for 45 minutes. After cooling the reaction mixture was diluted with water. The solid thus obtained was filtered, washed with 20% sodium thiosulphate solution and finally crystallised from ethyl alcohol to get the compound (4a);yield:75%, m.p.:122^{o}C.

IR(KBr ν_{max})=1664cm (C=Ostr), 1176cm^{-1} (C-Osrt), 780cm^{-1} (C-Clstr).

NMR: δ7.8-8.1(m,5H,Ar-H), δ6.7(s,1H,=CH).

Preparation of 1-(3,5-dichloro-2-hydroxyphenyl)-3-(2,3-dichlorophenyl)propane-1,3-dione (5a): The compound (4a) (0.01M) dissolved in ethanol (25ml) treated with HCl solution (25ml) (20%). The reaction mixture was then refluxed for 45 minutes, cooled and diluted with cold water. The product thus separated was filtered and washed with water and finally recrystallised from ethanol to get the compound (5a); yield:59%, m. p.:141^{o}C.

IR(KBr ν_{max})=3364cm^{-1}(O-Hstr),1690cm^{-1} (C=Ostr),1310cm^{-1}(C-Osrt),798cm^{-1}(C-Clstr).

NMR: δ10.45(s,1H,Ar-OH), δ7.2-7.9(m,5H,Ar-H), δ3,9(s,2H,CH_2).

Preparation of 2-bromo-1-(3,5-dichloro-2-hydroxyphenyl)-3-(2,3-dichlorophenyl)propane-1,3-dione (6a): The compound (5a) (0.01M) dissolved in acetic acid (10 ml), treated with bromine in acetic acid reagent (0.01M) (0.5ml). The mixture was allowed to stand for 1 h. at room temperature. The reaction mixture was decomposed with ice cold water to get the compound 6a; yield:60%, m.p.:100^{o}C.

IR(KBr ν_{max})= 3367cm^{-1}(O-Hstr),1692cm^{-1} (C=Ostr),1162cm^{-1}(C-Osrt),782cm^{-1}(C-Clstr), 630cm^{-1} (C-Br str).

NMR: δ12.9(s,1H,Ar-OH), δ7.2-7.9(m,5H,Ar-H), δ3.3(s,1H,C-H).

Preparation of 2-amino-4-(2,3-dichlorobenzoyl)-5-(2-hydroxy-3,5-dichlorophenyl)-1,3-thiazole (7a) and **2-aminophenyl-4-(2,3-dichlorobenzoyl)-5-(2-hydroxy-3,5-dichlorophenyl)-1,3-thiazole (7b):** The compound (6a) (0.01M) refluxed separately with thiourea (0.01M) and phenyl thiourea(0.01M) in presence of aqueous KOH solution (25ml, 0.02M) in ethanol (25ml) to obtain the compounds (7a); yield 59%; m.p. 150^{o}C and (7b); yield 56%; m.p. 141^{o}C respectively.

IR(KBr ν_{max})= 3510cm^{-1} (O-Hstr), 3340cm^{-1} (sharp,NH_2str), 1660cm^{-1} (C=Nstr), 1600cm^{-1} (C=Ostr),1310cm^{-1} (C-Ostr),780cm^{-1}(C-Clstr).

NMR: δ10.20(s,1H,Ar-OH), δ7.2-7.9(m,5H, Ar-H), δ4.0(s,2H,-NH_2).

Preparation of 2-({5-(3,5-dichloro-2-hydroxyphenyl)-4-[(2,3-dichlorophenyl)carbonyl]-1,3-thiazol-2-yl}amino)-1-(2,3-dichlorophenyl) ethanone (8a)

The compound (7a) (0.01M) was refluxed with 2-bromo-1-(3,5-dichloro-2-hydroxyphenyl) ethanone (0.01M) in absolute alcohol for 1 h. On cooling, the mixture was decomposed in ice cold water. The product thus separated was filtered and crystallized from alcohol to get the compound (8a); yield:65%; m.p.:111^{o}C.

IR(KBr ν_{max})=3460cm^{-1} (O-Hstr), 3367cm^{-1} (sharp,NH_2str), 1622cm^{-1} (C=Ostr), 1583cm^{-1} (C=Nstr),1310cm^{-1} (C-Ostr),796cm^{-1}(C-Clstr).

NMR: δ12.72(s,2H,Ar-OH), δ7.25-7.65(m,7H, Ar-H), δ2.65(s,2H,-CH_2), δ1.8(s,2H,-NH_2).

Preparation of 2-[2-mercapto-4-(2-hydroxy-3,5-dichlorophenyl) imidazolo]-4-(2,3-dichlorobenzoyl)-5-(2-hydroxy-2,3-dichlorophenyl)-1,3-thiazol (9a)

The compound (8a) (0.01M) was refluxed with KSCN in glacial acetic acid (20 ml) for about 4 h. On cooling, the reaction mixture was poured into ice cold water. The product thus separated was filtered and recrystallised from ethanol to get the compound (9a); yield:70%; m.pt.:103^{o}C.

IR(KBr ν_{max})=3341cm^{-1} (O-Hstr), 3068cm^{-1} (sharp,NH_2str), 2548cm^{-1} (S-Hstr), 1662cm^{-1} (C=Ostr),1641cm^{-1} (C=Nstr),796cm^{-1}(C-Clstr).

NMR: Δ12.61-13.0(S,2H,AR-OH), Δ7.25-7.92(M,8H, AR-H), Δ2.5(S,1H,-SH).

ANTIBACTERIAL ACTIVITY

The test compounds were screened for their antibacterial assay against *ornamental plant pathogens* viz. *Staphylococcus aureus, Staphylococcus epidermis, Pseudomonas aeruginosa and Salmonella typhi* by using Agar disc diffusion method. The zones of inhibition formed were measured in mm and are shown in table-2.

Table No.2: Impact of newly synthesized chlorosubstituted heterocycles against plant pathogens.

Sample Code	*Pseudomonas aeruginosa* MTCC-424 (Gram Negative)				*Salmonella typhi* ATCC-25812 (Gram Negative)				*Staphylococcus aureus* ATCC-33591 (Gram Positive)				*Staphylococcus epidermidis* MTCC-3086 (Gram Positive)			
	AB	SP	ABSP	CL	AB	SP	ABSP	CL	AB	SP	ABSP	CL	AB	SP	ABSP	CL
7a	23	16	25	00	27	22	31	00	15	17	17	00	26	16	27	00
7b	23	16	25	00	26	21	32	00	14	18	16	00	26	17	27	00
8a	23	17	24	00	26	19	33	00	14	21	17	00	28	16	28	00
9a	23	16	24	00	27	18	31	00	13	21	17	00	27	16	27	00

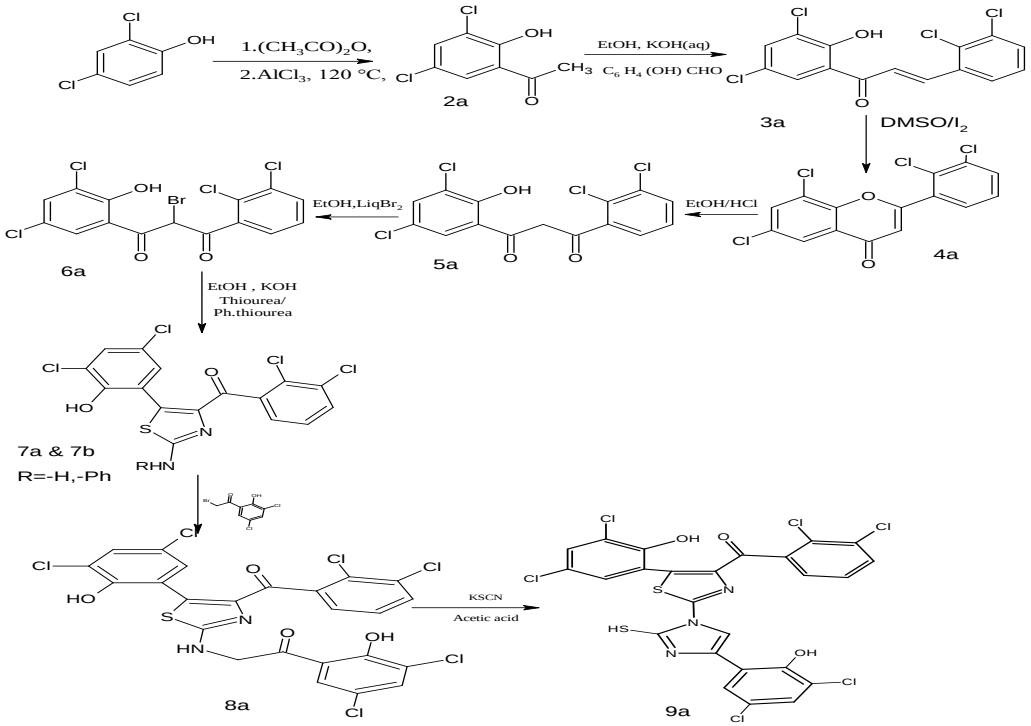
Diameter of inhibition zone (mm)

AB- Antibiotic Disc (Chloramphenicol-10), SP- Sample, ABSP- Antibiotic+Sample, CL- Control (DMSO).

RESULTS AND DISCUSSION

The newly synthesized compounds (7a,7b,8a and 9a) showed good to excellent activity against test pathogens. A further detailed study in the light of Plant pathology is advised.

Scheme-1



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DESIGN AND SYNTHESIS OF SOME IMIDAZOLE DERIVATIVES CONTAINING 4-(3,5-DICHLORO-2-HYDROXYPHENYL) IMIDAZOLE MOIETY AS ANTIBACTERIAL AGENTS

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Abstract
In the last few decades, the compounds bearing heterocyclic nuclei have received much attention due to their chemotherapeutic values in the development of novel antimicrobials. The literature survey is enriched with the synthesis and pharmacological evaluation of fused heterocycles containing imidazole moieties. Due to their vital role in biological activities, it was thought interesting to synthesize some imidazole derivatives containing 4-(3,5-dichloro-2-hydroxyphenyl) moiety. The newly synthesized compounds were screened for antibacterial activities against some plant pathogens.
Keywords- chlorosubstituted, fused heterocycles, imidazole, antibacterial activity, plant pathogens.

I. INTRODUCTION

Certain small fused heterocyclic[1] molecules act as highly functionalized scaffolds and are known pharmacophores of a number of biologically[2,3] active and medicinally useful molecules. The literature contains several reports on the incorporation of imidazole moiety[4,5] with substituted pyrazole[6-12] ring resulting in compounds with potent bioactivities[13-15]. The five-membered imidazole[16-19] ring is a structural unit found in many biologically active[20,21] compounds. The strong therapeutic properties of imidazole containing drugs have encouraged medicinal chemists to synthesize a large number of novel chemotherapeutic agents comprising this entity. Amongst others, imidazole core structures are found in different carboxypeptidase, hemeoxygenase and lactamase inhibitors showing anti-inflammatory[22-24], anticancer[25], antifungal[26], antibacterial[27,28], antitubercular[29], anti-diabetic, anticonvulsant[30], antiamoebial[31,32], anti-hyperlipidemic[33], antiviral, antiasthmatic, cardioprotective, alpha-blocker, CNS-depressants, antiprotozoal and antihelmintics [34] activities. Keeping in view the advantages of imidazole and pyrazole moieties, we had planned for synthesis of some imidazole derivatives containing 4-(3,5-dichloro-2-hydroxyphenyl) moiety and assayed them for antibacterial activity.

II. MATERIALS AND METHODS

The synthetic routes which furnished the target compounds are as under along with their IR and NMR data.

Preparation of 2,4-dichlorophenyl acetate (1a): 2,4-Dichlorophenol (0.01M) was mixed with acetic anhydride (0.01M) and anhydrous sodium acetate (5g). The mixture was refluxed for about an hour. It was then cooled and poured into cold water. Acetate layer thus separated was washed with water for several times. Finally it was purified by distillation and the distillate of compound (1a) was collected at about 221^{o}C; yield: 75%, b.p: 221^{o}C.

Preparation of 1-(3,5-dichloro-2-hydroxyphenyl)ethanone (2a): The compound (1a) (50ml) was mixed with anhydrous aluminum trichloride (120 g) and heated at 120^{o}C for 45 minutes on sand bath. The reaction mixture was decomposed by ice cold water containing a little HCl to get the crude product. It was then purified by recrystalization using ethanol to get a greenish white solid as compound(2a); yield:74% ; m.p.:53^{o}C.

IR(KBr ν_{max})=3423cm^{-1}(-OH str),1664cm^{-1}(C=Ostr), 1300cm^{-1}(C-Ostr), 766cm^{-1}(C-Clstr)

NMR: δ12.69(s,1H,Ar-OH) , δ7.25 to 7.63 (m,2H,Ar-H) , δ2.60,(s,3H,-CH_3).

Preparation of 1-(2-hydroxy-3,5-dichlorophenyl)-2-bromoethanone (3a): 1-(3,5-dichloro-2-hydroxypheny d…….l)ethanone (2a) (0.01M) dissolved in acetic acid (0.02M) treated with bromine in acetic acid (0.02M) reagent in cold condition with occasional shaking for 30 minutes. It was then poured into ice cold water. The solid thus separated was filtered, washed with sodium bisulphate and finally with water to get the crude product. It was then recrystallised from ethanol to get the compound (3a); yield:65%; m.p.:97^{0}C.

IR(KBr ν_{max})=3336cm^{-1}(-OH str),1692cm^{-1}(C=O str), 756cm^{-1}(C-Br str), 732cm^{-1}(C-Cl str).

NMR: δ12.34(s,1H,Ar-OH) , δ7.7 to 7.4 (m,2H,Ar-H) , δ3.9,(s,2H,-CH_2Br).

Preparation of 1H-2-one-4-(2-hydroxy-3,5-dichlorophenyl)-5*H*-imidazole (4a) and **1-H-2-imine-4-(2-hydroxy-3,5-dichloro-phenyl)-5*H*-imidazole (4b):**

1-(2-Hydroxy-3,5-dichlorophenyl)-2-bromoethanone (3a)(0.01M) dissolved in ethanol refluxed with aqueous urea (0.01M) and aqueous guanidine(0.01M) independently for 3 h. using TEBA (Triethyl Benzyl Ammonium Chloride) catalyst. After cooling the reaction mixtures were diluted with water to get the compound (4a); yield:65%; m.p.:110^{o}C and the compound (4b) yield:63%; m.pt.:115^{o}C respectively.

IR(KBr ν_{max})=3522cm^{-1}(-OH str),3423cm^{-1}(N-H str), 1610cm^{-1}(C=O str),1568cm^{-1}(C=N str).

NMR: δ12.59(s,1H,Ar-OH) , δ7.7 to 7.9(m,2H,Ar-H) , δ3.3,(s,1H,-NH), δ2.7,(s,2H,-CH_2).

Preparation of 6-(2-hydroxy-3,5-dichlorophenyl)-2,5-dihydro-imidazo[1,2-a]imidazol-3-one (5a) and **6-(2-hydroxy-3,5-dichlorophenyl)-2,5-dihydro-imidazo[1,2-a]imidazol-3-one (5b):**

The compound (4a) (0.01) dissolved in ethanol (5 ml) refluxed with aqueous glycine (0.02 M) and aqueous alanine (0.02 M) separately in presence of TEBA (0.05 M) catalyst for 2 h. After cooling the reaction mixtures were triturated until the solid gets separated. The products thus obtained were filtered, washed with water and recrystallized from ethanol to get the compound (5a); yield:73%; m.p.: 95^{o}C and the compound (5b); yield:71%; m.pt.:83^{0}C.

IR(KBr ν_{max})=3366cm^{-1}(-OH str),1600cm^{-1}(C=O str),1522cm^{-1}(C=N str).

NMR: δ12.32(s,1H,Ar-OH) , δ7.7 to 7.3(m,2H,Ar-H) , δ3.3,(s,1H,-NH), δ2.9,(s,2H,-CH_2).

Preparation of 1-acetyl-4-(3,5-dichloro-2-hydroxyphenyl)-1,5-dihydro-2H-imidazol-2-one (6a): A mixture of compound (5a)(0.01M) and acetyl chloride(0.01M) dissolved in THF (5 ml) refluxed with aqueous NaOH (0.03M) for 2 h. After cooling the reaction mixture was triturated until the solid gets separated. The product thus obtained was filtered, washed with water and recrystallized from ethanol to get the compound (6a);yield:65%; m.p.:102^{o}C.

IR(KBr ν_{max})=3367cm^{-1}(-OH str),1692cm^{-1}(C=O str),1649cm^{-1}(C=O str),1588cm^{-1}(C=N str).

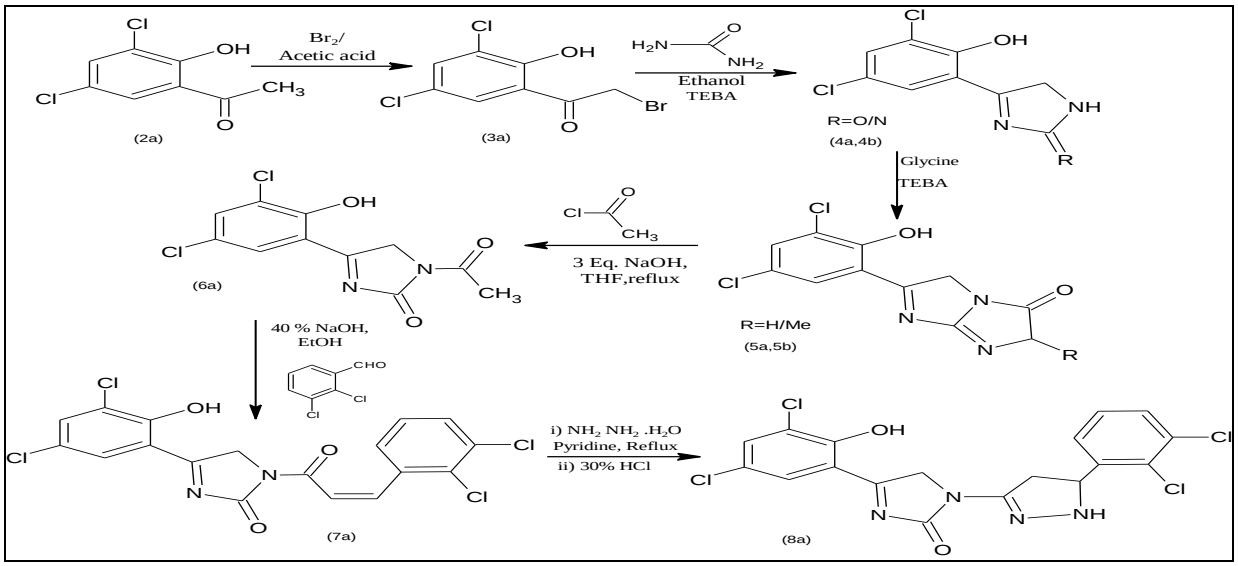
NMR: δ12.11(s,1H,Ar-OH) , δ7.7 to 7.3(m,2H,Ar-H) , δ2.8,(s,3H,-CH_3), δ2.3,(s,2H,-CH_2).

Preparation of 4-(3,5-dichloro-2-hydroxyphenyl)-3-(2,3-dichlorophenyl)prop-2-enoyl]-1,5-dihydro-2*H*-imidazol-2-one (7a): The compound (6a) (0.01M) dissolved in ethanol treated with 2,3-dichlorobenzaldehyde (0.01M) at its boiling temperature. Aqueous sodium hydroxide solution 40% (10ml) was added to it dropwise with constant stirring. The mixture was mechanically stirred for 30 minutes at room temperature and kept for overnight. It was then acidified with HCl (10%). The solid product thus obtained was filtered, washed with sodium bicarbonate (10%) followed by washing with water to get the crude product. It was crystallized from ethanol to get the compound (7a); yield:75%; m.p.:124^{o}C.

IR(KBr ν_{max})=3367cm^{-1}(-OH str),1693cm^{-1}(C=O str),1650cm^{-1}(C=C-H str),1563cm^{-1}(C=N str).
NMR: δ12.65(s,1H,Ar-OH) , δ7.7 to 7.3(m,2H,Ar-H) , δ7.6,(dd,2H,CH-CH), δ2.7,(s,2H,-CH_2).

Preparation of 4-(3,5-dichloro-2-hydroxyphenyl)-1-[5-(2,3-dichloro-phenyl)-4,5-dihydro-1*H*-pyrazol-3-yl]-1,5-dihydro-2*H*-imidazol-2-one (8a): A reaction mixture of the compound (7a) (0.01M) and hydrazine hydrate (0.01M) in pyridine (10ml) was refluxed on oil bath using magnetic stirrer for 2.5h. On cooling the reaction mixture was acidified with HCl (30%). The solid product thus obtained was filtered, washed with sodium bicarbonate (10%) followed by washing with water to get the crude product. It was crystallized from ethanol to get the compound (XaIII); yield: 64%; m.p.:103^{o}C.
IR(KBr ν_{max})=3550cm^{-1}(-OH str),3423cm^{-1}(C-N str),1693cm^{-1}(C=Ostr),1588cm^{-1}(C=N str).
NMR: δ12.23(s,1H,Ar-OH) , δ7.8 to 7.3(m,5H,Ar-H) , δ6.1,(s,1H,N-H), δ3.9,(s,1H,N-C-H), δ3.0,(s,2H,-CH_2), δ1.7,(dd,1H,-C-H), δ1.4,(dd,1H,-C-H).

Scheme-



III. ANTIBACTERIAL ACTIVITY

The test compounds showed good to excellent antiabacterial activities when screened against some ornamental plant pathogens viz. Staphylococcus aureus, Staphylococcus epidermis, Pseudomonas aeruginosa and Salmonella typhi by using Agar disc diffusion method. The zones of inhibition formed were measured in mm and are shown in table-1.

"Table No.1- Impact of test compounds against plant pathogens"

	Pseudomonas aeruginosa MTCC-424 (Gram Negative)				*Salmonella typhi* ATCC-25812 (Gram Negative)				*Staphylococcus aureus* ATCC-33591 (Gram Positive)				*Staphylococcus epidermidis* MTCC-3086 (Gram Positive)			
Sample Code	AB	SP	ABSP	CL	AB	SP	ABSP	CL	AB	SP	ABSP	CL	AB	SP	ABSP	CL
4a	23	16	26	00	26	19	32	00	16	18	18	00	27	16	28	00
4b	23	16	26	00	27	18	33	00	17	19	17	00	27	15	29	00
5a	23	17	26	00	27	17	33	00	17	20	18	00	27	15	29	00
5b	23	16	25	00	27	18	32	00	17	20	18	00	27	16	28	00
6a	23	12	24	00	27	16	29	00	17	17	18	00	27	15	28	00
7a	22	11	23	00	27	16	30	00	17	16	19	00	27	13	27	00
8a	22	10	23	00	27	15	28	00	16	15	16	00	27	12	28	00

Diameter of inhibition zone (mm) AB-Antibiotic Disc (Chloramphenicol-10), SP- Sample, ABSP- Antibiotic+Sample, CL-Control (DMSO),Values were represented as the mean.

IV. RESULTS AND DISCUSSION

The newly synthesized compounds (4a-8a) showed good to excellent activity against test pathogens. A further detailed study in the light of Plant pathology is advised.

V. ACKNOWLEDGEMENTS

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Synthesis And Study Of Substituted 1,3-Thiazoles And Their Nanoparticles On Phytotic Growth Of Some Vegetable Crops

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Abstract

The synthesis, spectral analysis and biological activities of 5-phenyl-2-hydroxy-chlorosubstituted-2-amino-1,3 thiazoles have been carried out. In this case 5-(2'-hydroxy-3',5'-dichlorophenyl)-4-(heptan-1-one)-2- amino-1,3-thiazole(J), 5-(2'-hydroxy-3',5'-dichlorophenyl)-4-(heptan-1-one)-2-phenyl amino-1,3-thiazole (K), and 5-(2'-hydroxy-3',5'-dichlorophenyl)-4-(heptan-1-one)-2-diphenyl amino-1,3-thiazole (L) have been screened. The compounds J, K and L were synthesized from1-(2'-hydroxy-3',5'-dichlorophenyl)-2-bromo-1,3-nonanedione(a_4) by the action of thiourea, phenylthiourea, diphenylthiourea. The nanoparticles of the compounds J,K and L have been prepared by using ultrasonic technique. The titled compounds and their nanoparticles were screened for growth promoting activity on some vegetable crop plants viz.. Momordica charantia-L-Bitter guard (Karela), Lagneria siceraria-snake guard (Lavki), Luffa cylindrica L-sponge guard (Gilke) and Benincasa hispida-Pumpkin (Kohle).

Keywords : Chalcone, thiazole, thiourea, phenyl thiourea, diphenyl thiourea, growth promoting activities.

I. INTRODUCTION

Heterocyclic nucleus plays an important role in medicinal chemistry and it is a key template for the growth of various therapeutic agents. Thiazole is a heterocyclic compound featuring both a nitrogen atom and sulfur atom as part of the aromatic five-membered ring. Thiazoles and related compounds are called 1,3-azoles (nitrogen and one other hetero atom in a five-membered ring.) They are isomeric with the 1,2-azoles, the nitrogen and sulphur containing compound being called isothiazoles. Thiazoles are found naturally in the essential vitamins. Molecules that possess sulfur atoms are important in living organisms. The researchers[1-6] have reported the synthesis of several thiazoles and also their potent biological activities such as antimicrobial[7], antibacterial[8-11], antifungal[12-13], fungicidal[14] and insecticidal agent[15]. Chalcones and their analogues having α, β-unsaturated carbonyl system are very versatile substrates for the evolution of various reactions and physiologically active compounds.

In the present study, various 5-phenyl-2-hydroxy-chlorosubstituted-2-amino-1,3-thiazoles have been synthesized from 1,3-propanediones by using thiourea, phenyl thiourea and diphenyl thiourea. The synthesized compounds along with their nanoparticles were evaluated for their growth promoting activity on some vegetable crop plants viz. *Momordica charantia-L-Bitter guard (Karela), Lagneria siceraria-snake guard (Lavki), Luffa cylindrica L-sponge guard (Gilke) and Benincasa hispida-Pumpkin (Kohle).*

II. EXPERIMENTAL

All the glasswares used in the present work were of pyrex quality. Melting points were determined in hot paraffin bath and are uncorrected. The purity of compounds was monitored on silica gel coated TLC plate. IR spectra were recorded on Perkin-Elmer spectrophotometer in KBr

pelletes, H^1 NMR spectra on spectrophotometer in $CDCl_3$ with TMS as internal standard. UV spectra were recorded in nujol medium. The analytical data of the titled compounds was highly satisfactory. All the chemicals used were of analytical grade. All the solvents used were purified by standard methods. Physical characterisation data of all the compounds is given in Table 1.

2'-Hydroxy 3',5'-dichloroacetophenone :

2-Hydroxy-5-chloroacetophenone was dissolved in acetic acid (5 ml), Sodium acetate (3g) was added to the reaction mixture and then chlorine in acetic acid reagent (40 ml; 7.5 w/v) was added dropwise with stirring. The temperature of the reaction mixture was maintained below 20^0C. The mixture was allowed to stand for 30 minutes. It was poured into cold water with stirring. A pale yellow solid then obtained was filtered, dried and crystallized from ethanol to get the compound 2'-hydroxy 3',5'-dichloroacetophenone.

Preparation of 2'-hydroxy-3',5'-dichlorophenyl-4-hexylchalcone (a) :

2-Hydroxy-3,5-dichloroacetophenone (0.01 mol) dissolved in ethanol (50 ml) treated with heptanaldehyde (0.1 M) at its boiling temperature. Aqueous sodium hydroxide solution [40%, 40 ml] was added dropwise and the the mixture was stirred mechanically at room temperature for about 1 hour. It is then kept for 6 to 8 hours followed by decomposition with ice cold HCl [1:1]. The yellow granules thus obtained were filtered, washed with 10% $NaHCO_3$ solution and finally crystallized from ethanol-acetic acid solvent mixture to get the compound (a).

Preparation of 1-(2'-hydroxy-3'-5'-dichlorophenyl)-2,3-dibromononan-1-one (a_1)

2'-Hydroxy-3',5'-dichlorophenyl-4-hexylchalcone (a) (0.01 M) was suspended in bromine–glacial acid reagent [25% w/v] [6.4 ml]. The reagent was added dropwise with constant stirring. After complete addition of reagent the reaction mixture was kept at room temperature for about 30 minutes. The solid product, thus separated, was filtered and washed with a little petroleum ether to get the compound (**a_1**).

Preparation of 2-(4"-hexyl)- 6,8-dichloroflavone (a_2) :

1-(2'-Hydroxy-3',5'-dichlorophenyl)-2,3-dibromo-nonan-1-one (a_1) (0.01 mol) was dissolved in ethanol (25 ml). To this, aqueous solution of KOH (25 ml) was added. The reaction mixture was refluxed for 1 hour, cooled and diluted with water. The product, thus separated, was filtered and crystallized from ethanol to get the compound (a_2).

Preparation of 1-(2'-hydroxy-3',5'-dichlorophenyl)-1,3-nonanedione (a_3) :

2-(4"-Hexyl)-6,8-dichloroflavone (a_2) (0.01 mol) was dissolved in ethanol (25 ml). To this, aqueous solution of HCl (25 ml) was added. The reaction mixture was then refluxed for one hour, cooled and diluted with water. The solid product, thus obtained, filtered and crystallized from ethanol to get the compound (a_3).

Preparation of 1-(2'-hydroxy-3',5'-dichlorophenyl)-2-bromo-1,3-nonanedione (a_4) :

1-(2'-Hydroxy-3',5'-dichlorophenyl)-1,3-nonanedione (a_3) (0.01 mol) was dissolved in a mixture of ethanol (10 ml) and dioxane (10 ml). To this, calculated amount of liquid bromine (0.5 ml) was added. The product was not separated even after standing for one hour. It was then diluted with water and washed with water several times and extracted with ether. The solvent was removed under reduced pressure to get the white solid of the compound (a_4).

Preparation of 5-(2'-hydroxy-3',5'-dichlorophenyl)-4-(heptan-1-one)-2- amino-1,3-thiazole (J) :

1-(2'-Hydroxy-3',5'-dichlorophenyl)-2-bromo-1,3-nonanedione (a_4) (0.01 mol) and thiourea (0.01 mol) were dissolved in ethanol (25 ml). To this, aqueous KOH solution (0.01 mol) was added. The reaction mixture was then refluxed for three hours, cooled, diluted with water and acidified with

conc HCl. The product, thus separated, was filtered and crystallized from ethanol to get the compound (J).

Preparatrion of 5-(2'-hydroxy-3',5'-dichlorophenyl)-4-(heptan-1-one)-2-phenyl amino-1,3-thiazole (K) :

1-(2'-Hydroxy-3',5'-dichlorophenyl)-2-bromo-1,3-nonanedione (a_4) (0.01 mol) and phenylthiourea (0.01 mol) were dissolved in ethanol (25 ml). To this, aq. KOH solution (0.02 mol) was added. The reaction mixture was refluxed for three hours, cooled, diluted with water and acidified with conc. HCl. The product, thus separated, was filtered and crystallized from ethanol to get the compound (K).

Preparation of 5-(2'-hydroxy-3',5'-dichlorophenyl)-4-(heptan-1-one)-2-diphenyl amino-1,3-thiazole (L) :

1-(2'-Hydroxy-3',5'-dichlorophenyl)-2-bromo-1,3-nonanedione (a_4) (0.01 mol) and diphenylthiourea (0.01 mol) were dissolved in ethanol (25 ml). To this, aq solution of KOH (0.02 mol) was added. The reaction mixture was refluxed for three hours, cooled, diluted with water and acidified with conc. HCl. The product, thus separated, was filtered, crystallized from ethanol to get the compound (L).

The newly synthesized compounds were characterised on the basis of elemental analysis, molecular determination, UV, IR, NMR. spectral data.

The UV, IR, and NMR spectral data :-

Compound (J) :

UV : Spectrum No. 1

The UV-Vis spectrum of the compound (J) reported in dioxane showed λ_{max} value 410 nm corresponding to $n \rightarrow \pi^*$ transition.

IR (KBr) :- Spectrum No. 2

3036.60 cm^{-1}(-OH phenolic), 2955.55 cm^{-1} (aliphatic -C-H stretching), 3208.58 cm^{-1} (aromatic -C-H stretching), 3797.72 cm^{-1} (-N-H stretching), 1228.56 cm^{-1} (-C=N- stretching), 756.57 cm^{-1} (-C-Cl stretching in aliphatic), 1073.66 cm^{-1} (C-Cl stretching in aromatic).

PMR :- Spectrum No. 3

∂ 5.2 (hump, 2H, (-N-**H**) ; ∂ 6.7 (d, 1H, -CH=C-**H**-) ; ∂ 6.8 (d, 1H, -C**H**=C-H-) ; ∂ 7.0 to 7.8 (m, 2H, Ar-**H**) ; ∂ offset, (region not observed, observed, O-**H**)

Compound (K) :

UV : Spectrum No. 4

The UV-Vis spectrum of the compound (K) reported in dioxane showed λ_{max} value 392 nm corresponding to $n \rightarrow \pi^*$ transition.

IR KBr : Spectrum No. 5

3078.56 cm^{-1} (O-H phenolic) , 2956.24 cm^{-1} (aliphatic -C-H stretching) , 3305.55 cm^{-1} (aromatic C-H stretching) , 3786.79 cm^{-1} (-NH stretching), 1552.51 cm^{-1} (-C=N-stretching) , 754.50 cm^{-1} [C-Cl stretching in aliphatic) , 1174.48 cm^{-1} [C-Cl stretching in aromatic].

PMR :- Spectrum No. 6

∂ 1.06 (t, 3H, -CH_2-C**H**$_3$) ; ∂ 1.25 [Envelope of –CH_2 , 8H, -(C**H**$_2$)$_4$-CH_3], ∂ 3.32 (hump, 2H, -N**H**) ; ∂ 4.15 (d 1H, -CH=C-**H**-) ; ∂ 4.16 (d, 1H, -C**H**=C-H-); ∂ 7.01 to 7.72 (m, 7H, Ar-**H**).

Compound (L) :

UV : Spectrum No. 7

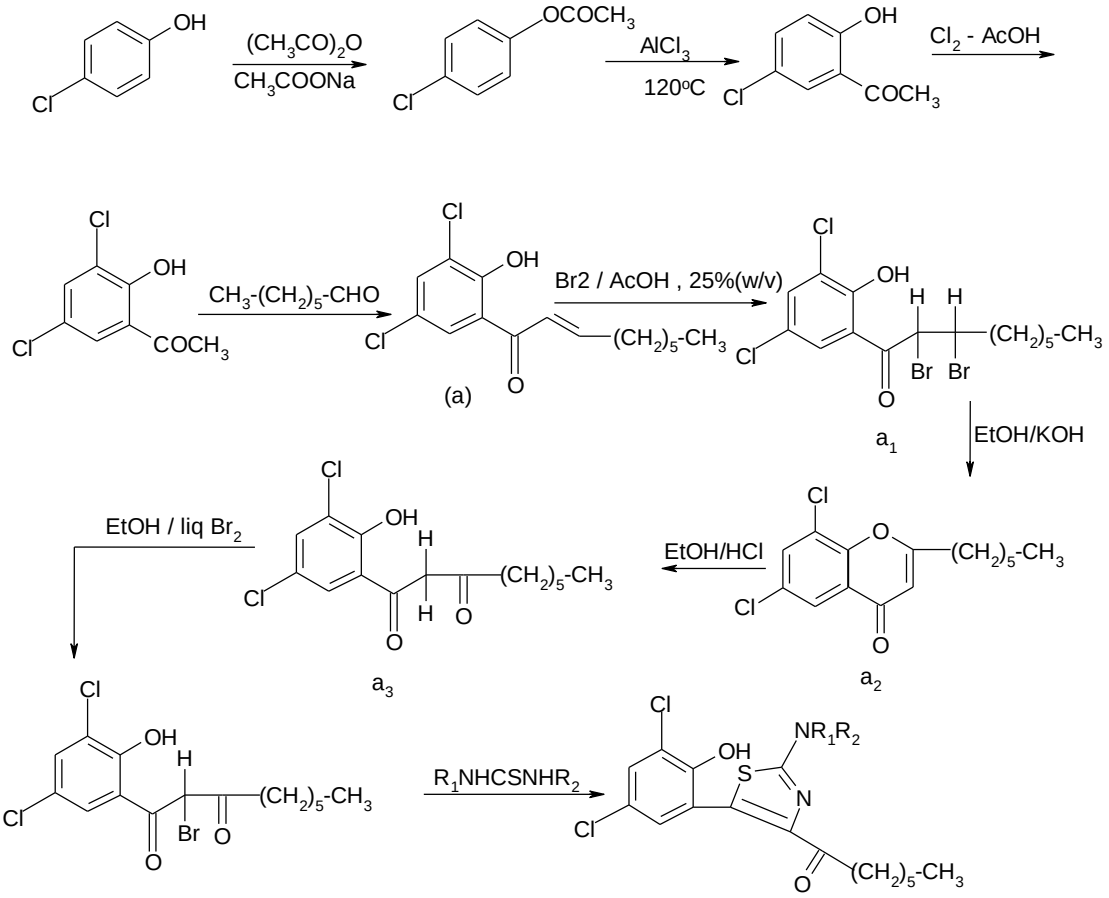
The UV-Vis spectrum of the compound (L) reported in dioxane showed λ_{max} value 386 nm corresponding to n→π* transition.

IR KBr :- Spectrum No. 8

3431.6 cm^{-1} (-OH phenolic) , 3057.11cm^{-1} (aromatic C-H stretching), 1543.2 cm^{-1} (-C=N stretching), 772.13 cm^{-1} (C-Cl stretching in aliphatic), 1051.11 cm^{-1} (C-Cl stretching in aromatic).

PMR :- Spectrum No. 9

∂ 6.72 (d 1H, -CH=C-**H**-) ; ∂ 6.70 (d,1H, -C**H**=C-H-) ; ∂ 11.85 (m, 12H, Ar-**H**) ; ∂ 10.3 (s, 1H, O-**H**).



Scheme:

Where :

1) R_1 = -H, $-C_6H_5$
2) R_2 = -H, $-C_6H_5$

Preparation of nanoparticles of the titled compounds:

Ultrasonic Processor Sonapros PR-250MP was used to produce nanoparticles of the test compounds. The test compounds were dissolved in dioxane to prepare 0.1 M solutions. These solutions were taken in beakers and the probe of the sonapras 250 MP was dipped in solution. These solutions were exposed to sonopros MP 250 for 10 minutes separately. The test compounds were converted to nanoparticles. The solvent dioxane was evaporated by conventional heating method. The size of nanoparticles of the test compounds was confirmed by X-ray diffraction studies using Benchtop x-ray diffraction (XRD) instrument **(**Miniflex).

The thin film of the nanoparticles of the test compounds was prepared on glass slide. This slide was introduced to the X-ray diffraction instrument to get graphical information which was used for the calculation of the crystal size of test compounds.

Characterisation of size of nanoparticles of the test compounds :-

The crystal size of nanoparticles of the test compounds calculated by using Debye-Scherrer equation.

$$D = \frac{0.94\,\lambda}{\beta.\,\mathrm{Cos}\,\theta}$$

Where,

D = The average crystalline size.

0.94 = The particle shape factor which depends on the shape and size of the particle.

λ = is the wavelength.

β = is the full width at half maximum [FWHM] of the selected diffraction peaks ($\beta = 0.545$)

θ = is the Bragg's angle obtained from 2θ values which was corresponding to the maximum intensity peak in XRD pattern ($\theta = 0.7501$ rad).

Growth Promoting Effect on some Vegetable crop Plants :-

The experimental set up of the study was devided into two parts:
(i) Seed treatment (ii) Field experiment.

(i) Seed treatment :-

With a view to safeguard dormant seed's potential from harmful external agencies, the seeds of the test plants were treated by solution of test compounds (0.01dilution) prepared in dioxane before sowing.

(ii) Field experiment :-

Pregerminated quality seeds of *Momordica charantia L-Bitter guard (Karela), Lagneria siceraria -snake guard (Lavki), Luffa cylindrica L-Sponge guard-(Gilke) and Benincasa hispida - Pumpkin (Kohle*) were procured from Department of Horticulture, Dr. PDKV, Akola.

The beds of cotton soil, 2.5 x 2.5 m size were prepared in an open field. The sowing of seeds of all four test vegetable crop plants were done in separate beds and irrigated periodically.

The plants from each bed were devided into two groups i.e. A and B and designated as "Control" and "Treated" group plants respectively.

The plants from group B were sprayed with the solution of test compounds at weekly intervals. The field experiments were conducted to compare the treated plants of group B with untreated plants of controlled group A. In this context, the observations were recorded on 7, 14, 21, 28, 35, 42, 45, 56, 63, 70, 77, 84, 91 days after sowing corresponding to early vegetative, late vegetative, flowering, fruitification and fruit maturation, with special reference to number of leaves and height of shoots.

The results of field's experiments are tabulated in the tables 2, 3 and 4.

III. RESULT AND DISCUSSION

The titled compounds and their nanoparticles were screened for their growth promoting activity on test vegetable crop plants viz, *Momordica charantia-L-Bitter guard (Karela), Lagneria siceraria-snake guard (Lavki), Luffa cylindrica L-sponge guard (Gilke) and Benincasa hispida-Pumpkin (Kohle).*

When a comparison of morphological characters was made between those of treated and control group plants, it was interesting to note that all the treated plants exhibited significant shoot growth and considerable increase in the number of leaves as compared to those of untreated ones. Also it was observed that the yield of treated plants enhances to a remarkable extent than control group plants.

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.

Table 1 : Characterisation data of newly synthesized compounds :

Compounds	Molecular formula	M.P. in ^{0}C	% of yield	% of element					
				C	H	N	S	Cl	Br
	$C_8H_6O_2Cl_2$	54	80	47.90/48	2.95/3			34.15/34.58	
a	$C_{15}H_{18}O_2Cl_2$	103	70	52.20/53.35	53.10/53.21			23.25/23.27	
a_1	$C_{15}H_{18}O_2Cl_2Br_2$	67	50	39.01/39.04	3.85/3.90			15.20/15.40	34.18/34.70
a_2	$C_{15}H_{16}O_2Cl_2$	73	50	60.10/60.20	5.25/5.35			23.70/23.74	
a_3	$C_{15}H_{18}O_3Cl_2$	118	60	56.60/56.78	5.60/5.67			22.33/22.39	
a_4	$C_{15}H_{17}O_3Cl_2Br$	84	50	45.40/45.45	4.20/4.29			17.90/17.92	20.15/20.20
J	$C_{16}H_{20}O_2N_2Cl_2S$	96	60	51.10/51.20	5.30/5.33	7.40/7.46	8.50/8.53	18.90/18.93	
K	$C_{22}H_{23}O_2N_2Cl_2S$	135	60	58.46/58.66	5.07/5.11	6.18/6.22	7.00/7.11	15.60/15.77	
L	$C_{28}H_{26}O_2Cl_2N_2S$	122	50	63.92/64.0	4.90/4.95	5.30/5.33	6.0/6.09	13.50/13.52	

Activity of the test compounds J, K and L :

Table No. (02)

5-(2'-Hydroxy-3',5'-dichlorophenyl)-4-(heptan-1-one)-2-amino-1,3-thiazole (J)

Periodicity of Observations [in days]	*Momordica charantia* (Bitter guard) (Karela)				*Lageneria siceraria* (Snake guard) (Lavki)				*Luffa cylindrica* (Sponge guard) (Gilke)				*Benincasa hispida* (Pumpkin) (Kohle)			
	Shoot height		No. of leaves		Shoot height		No. of leaves		Shoot height		No. of leaves		Shoot height		No. of leaves	
	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T
7	2.5	1.5	2	2	2.5	2.5	2	2	4.5	11.5	2	2	20	17.5	2	2
14	7	7.2	2	2	7.5	2.5	2	2	10	16	2	2	20	23	2	2
21	25	17	7	13	8	5	2	3	15	18	3	4	23	28	3	5
28	35	40	9	14	9	9.2	3	4	16	22	4	5	25	29	4	6
35	47	48	10	15	11	12.5	4	4	20	26	5	7	27	36	5	8
42	51	74	12	25	17	21	5	5	25	29	7	9	30	42	6	10
49	55	82	14	28	25	39	6	7	30	32	8	11	35	50	8	12
56	60	100	16	30	28	45	7	9	35	40	10	13	38	62	10	16
63	67	108	18	34	31	51	8	10	40	51	12	15	42	71	12	18
70	72	112	20	38	34	55	9	12	45	58	14	16	46	79	14	20
77	75	114	22	40	36	59	10	15	50	62	16	18	49	78	16	22
84	80	115	24	43	38	64	11	17	55	68	18	20	53	65	18	24
91	82	118	26	45	40	68	12	19	57	72	20	24	56	68	20	26

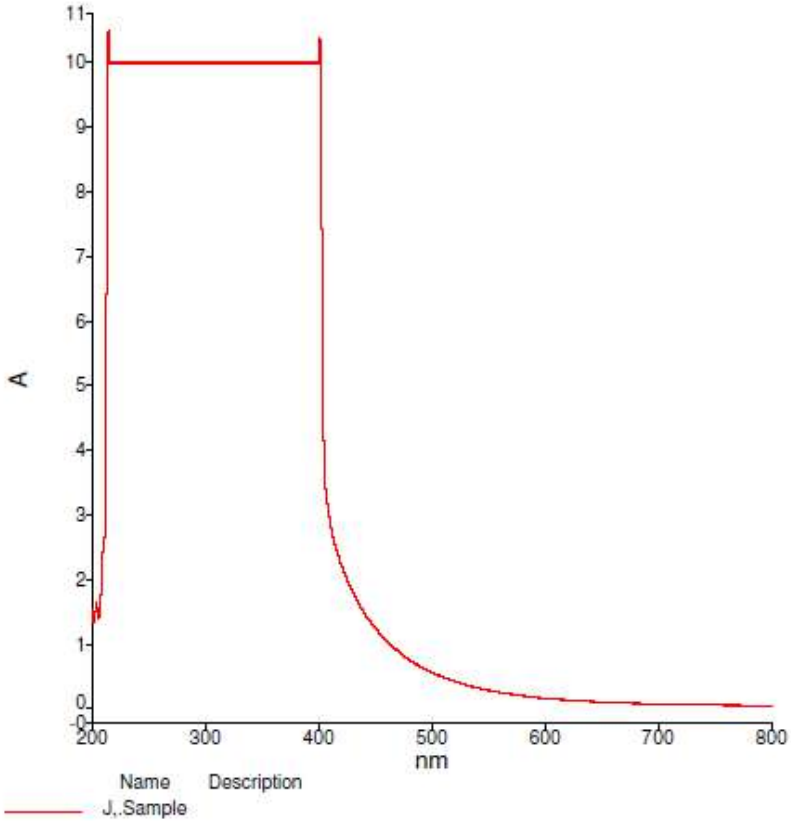
Table No. (03)

5-(2'-Hydroxy-3',5'-dichlorophenyl)-4-(heptan-1-one)-2-phenylamino-1,3-thiazole (K)

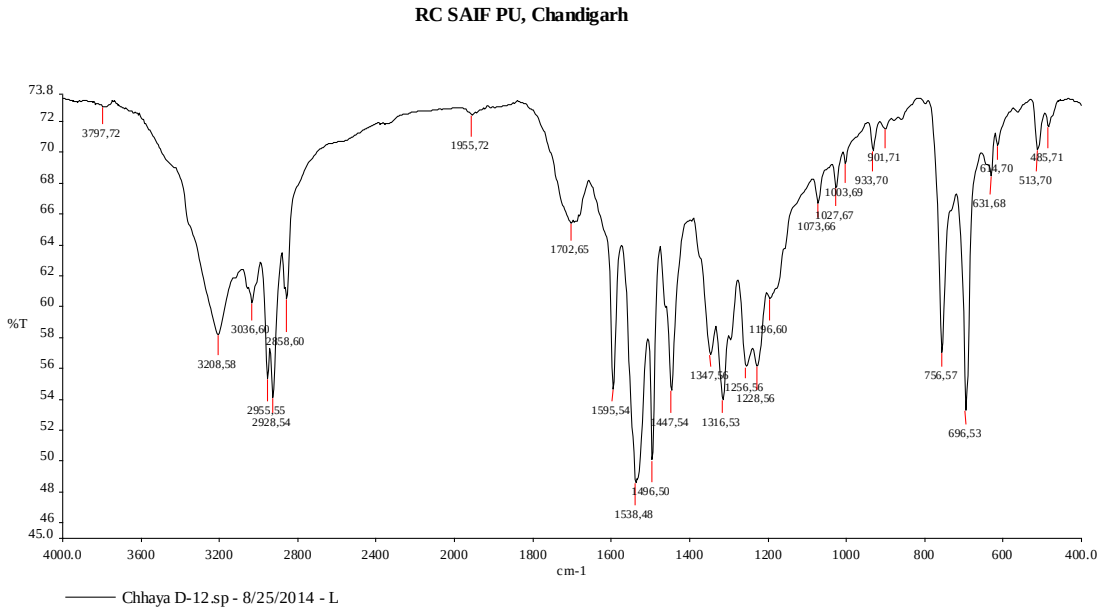
Periodicity of Observations [in days]	*Momordica charantia* (Bitter guard) (Karela)				*Lageneria siceraria* (Snake guard) (Lavki)				*Luffa cylindrica* (Sponge guard) (Gilke)				*Benincasa hispida* (Pumpkin) (Kohle)			
	Shoot height		No. of leaves		Shoot height		No. of leaves		Shoot height		No. of leaves		Shoot height		No. of leaves	
	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T
7	2.5	1.5	2	2	2.5	1.5	2	2	4.5	11.5	2	2	20	17.5	2	2
14	7	7.5	2	4	7.2	5	2	2	10	16	2	2	20	23	2	2
21	25	17	7	11	8	7.5	2	3	15	18	3	4	23	28	3	5
28	35	44	9	13	9	10	3	4	16	22	4	5	25	29	4	6
35	47	54	10	19	11	15	4	6	20	26	5	7	27	36	5	8
42	51	62	12	22	17	30	5	7	25	29	7	9	30	42	6	10
49	55	92	14	25	25	39	6	7	30	32	8	11	35	50	8	12
56	60	105	16	32	28	45	7	9	35	40	10	13	38	62	10	16
63	67	127	18	35	31	58	8	10	40	51	12	15	42	71	12	18
70	72	132	20	40	34	62	9	12	45	58	14	18	46	79	14	20
77	75	135	22	42	36	66	10	13	50	64	16	19	49	87	16	22
84	80	140	24	45	38	69	11	15	55	70	18	21	53	94	18	24
91	82	142	26	47	40	71	12	17	57	74	20	23	56	98	20	28

Table No. (04)
5-(2'-Hydroxy-3',5'-dichlorophenyl)-4-(heptan-1-one)-2-diphenylamino-1,3-thiazole (L)

Periodicity of Observations [in days]	*Momordica charantia* (Bitter guard) (Karela)				*Lageneria siceraria* (Snake guard) (Lavki)				*Luffa cylindrica* (Sponge guard) (Gilke)				*Benincasa hispida* (Pumpkin) (Kohle)			
	Shoot height		No. of leaves		Shoot height		No. of leaves		Shoot height		No. of leaves		Shoot height		No. of leaves	
	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T
7	2.5	1.5	2	2	2.5	1.5	2	2	4.5	13.5	2	2	20	21	2	2
4	7	7	2	4	7.5	5	2	2	10	16	2	2	20	23	2	2
21	25	18	7	10	8	7.5	2	3	15	18	3	4	23	25	3	5
28	35	40	9	12	9	9.8	3	4	16	21	4	5	25	27	4	6
35	47	48	10	13	11	18	4	7	20	25	5	7	27	32	5	8
42	51	60	12	17	17	31	5	8	25	32	7	9	30	41	6	10
47	55	78	14	20	25	41	6	9	30	35	8	11	35	50	8	12
56	60	92	16	26	28	56	7	10	35	45	10	13	38	61	10	16
63	67	105	18	28	31	63	8	11	40	60	12	15	42	70	12	18
70	72	111	20	32	34	70	9	13	45	68	14	17	46	78	14	20
77	75	114	22	34	36	75	10	14	50	75	16	19	49	86	16	22
84	80	118	24	36	38	79	11	16	55	81	18	21	53	91	18	24
91	82	120	26	38	40	81	12	18	57	84	20	23	56	95	20	26



Spectrum No. 1



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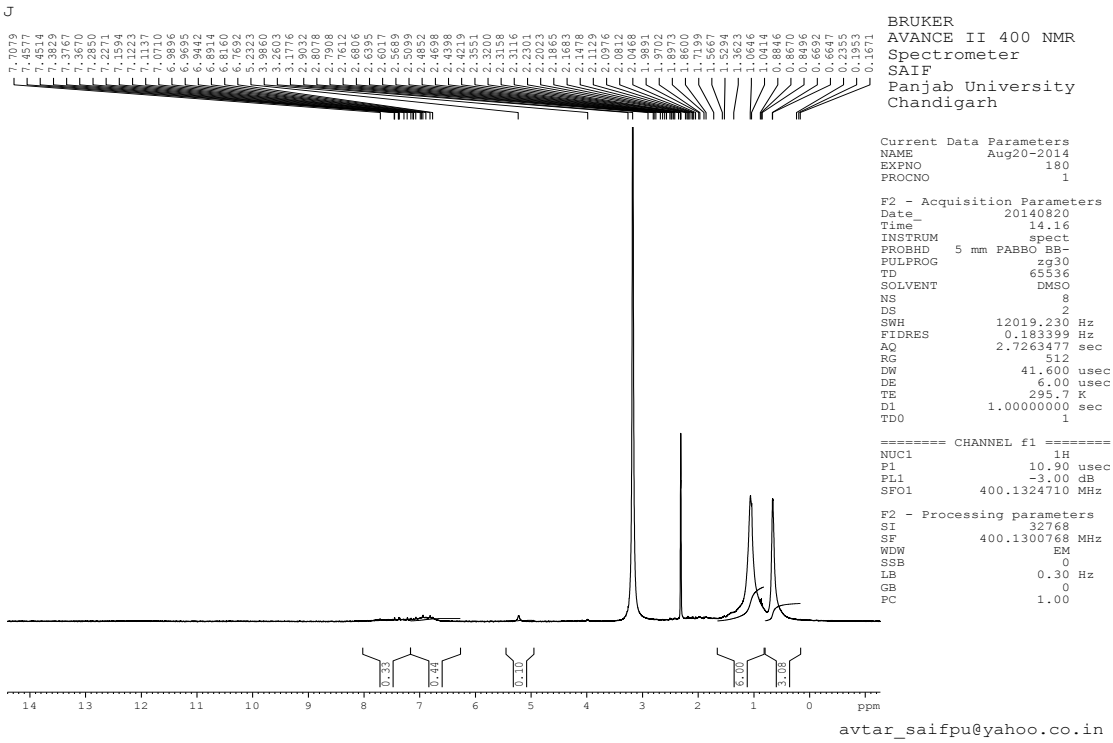

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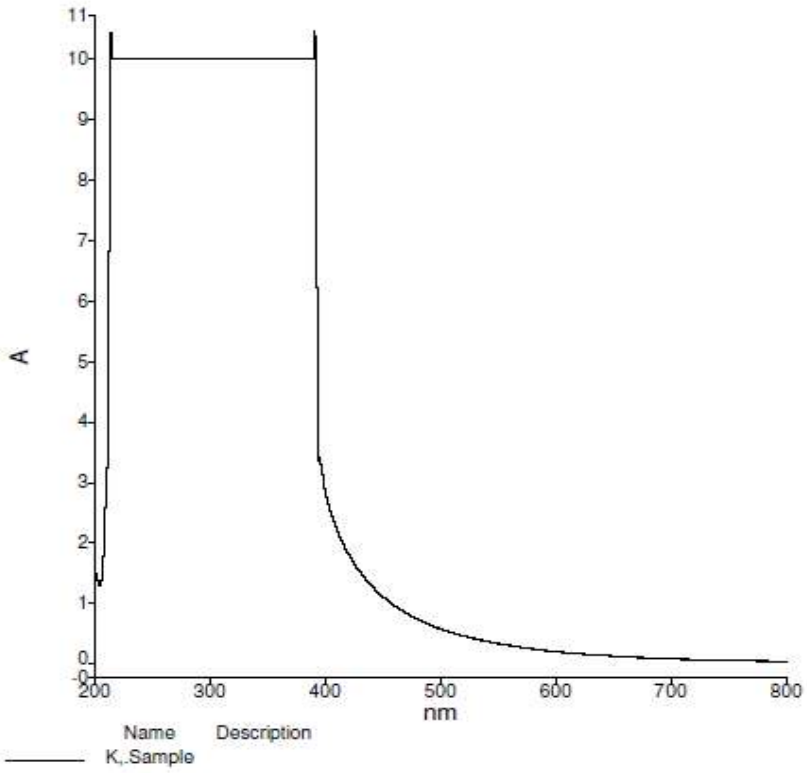

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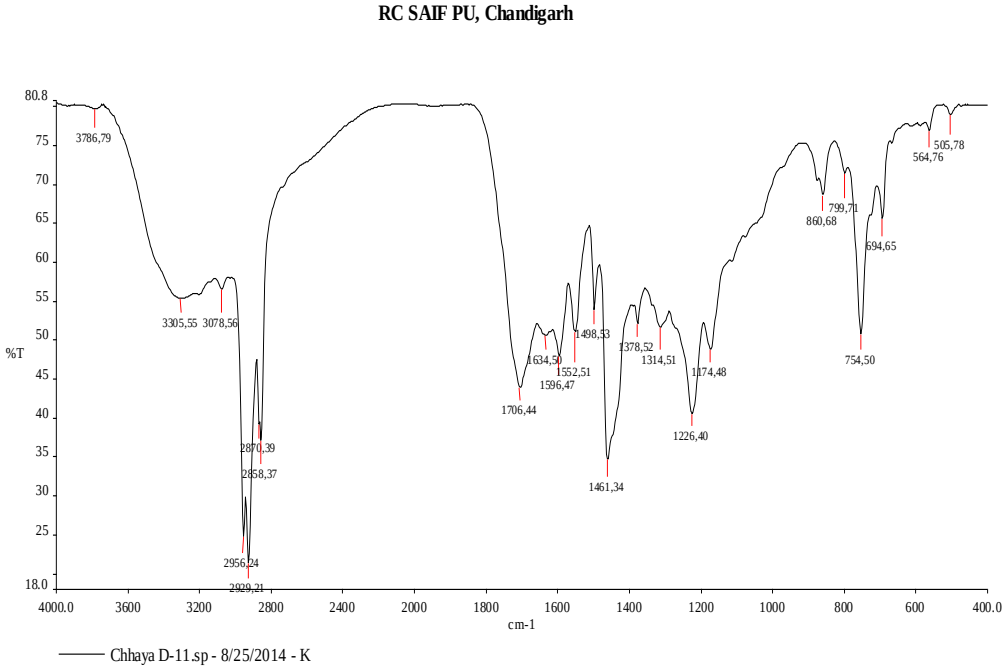

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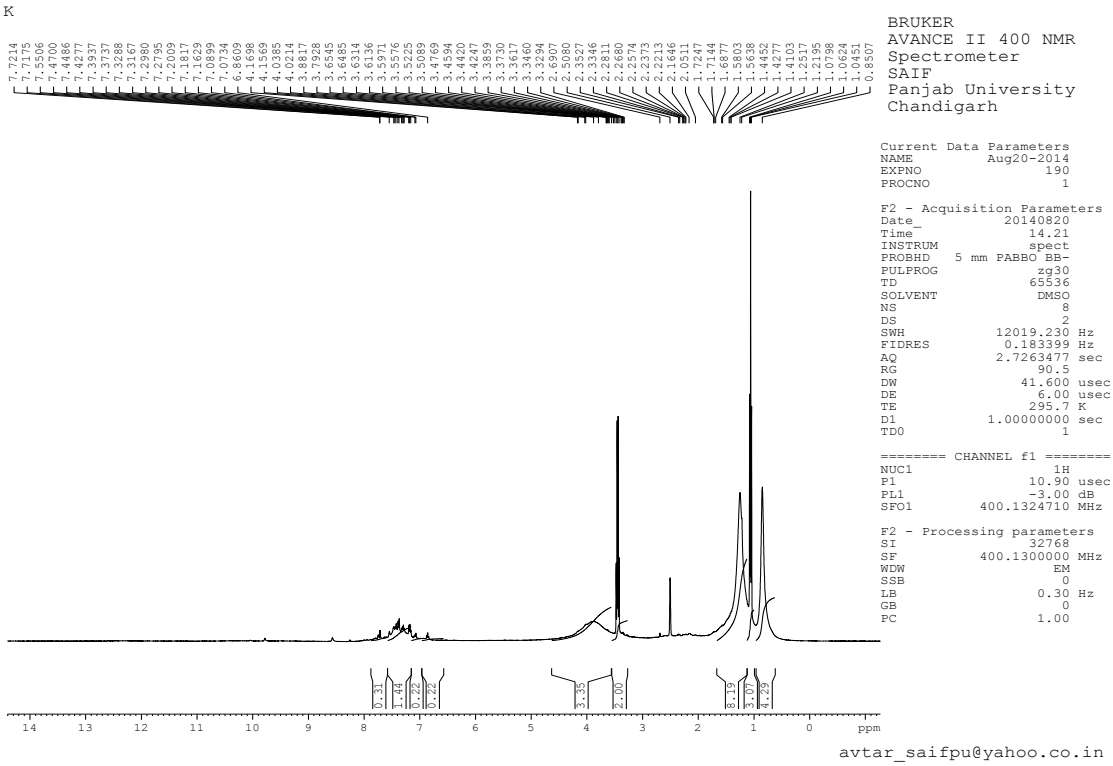

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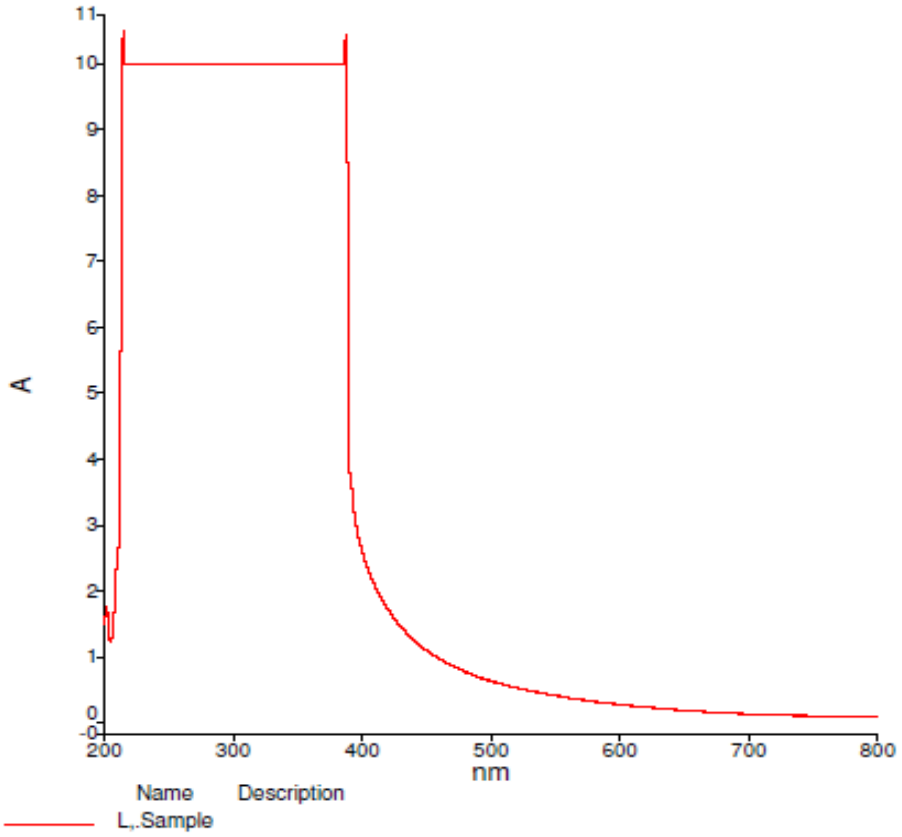

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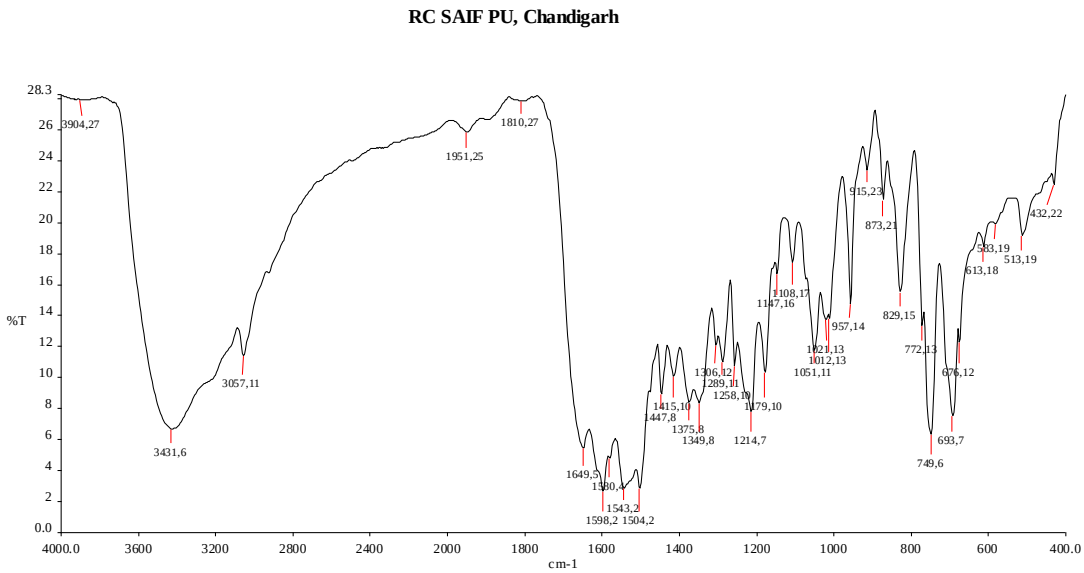
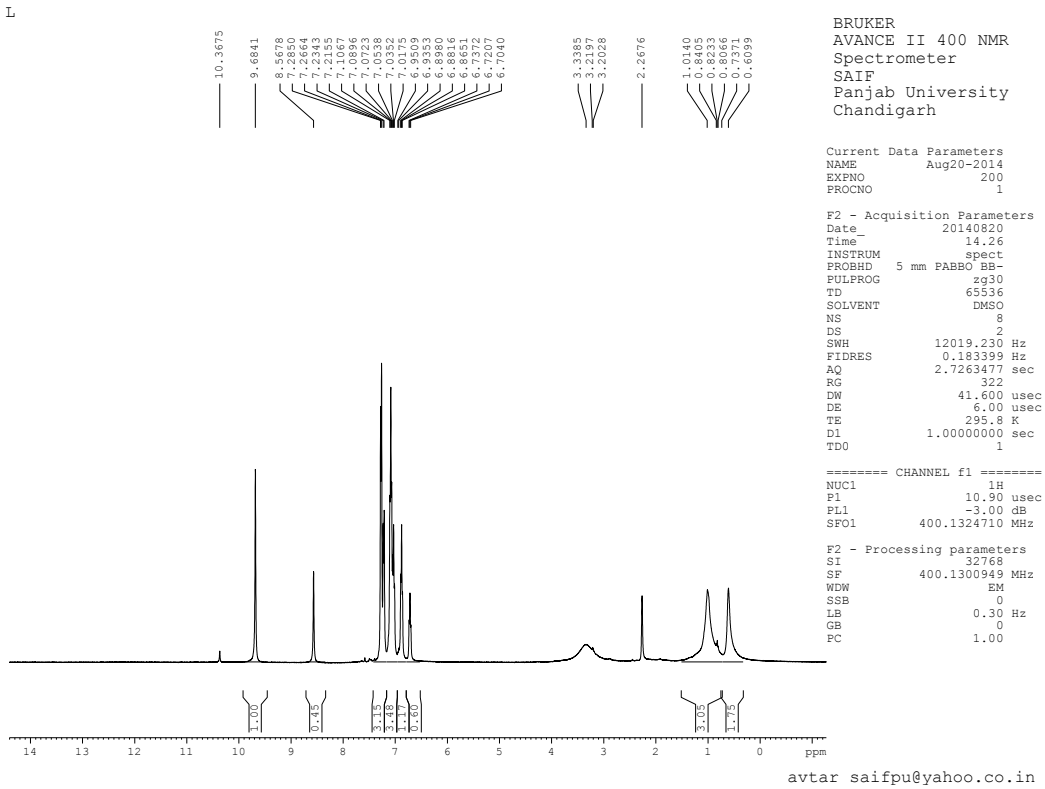
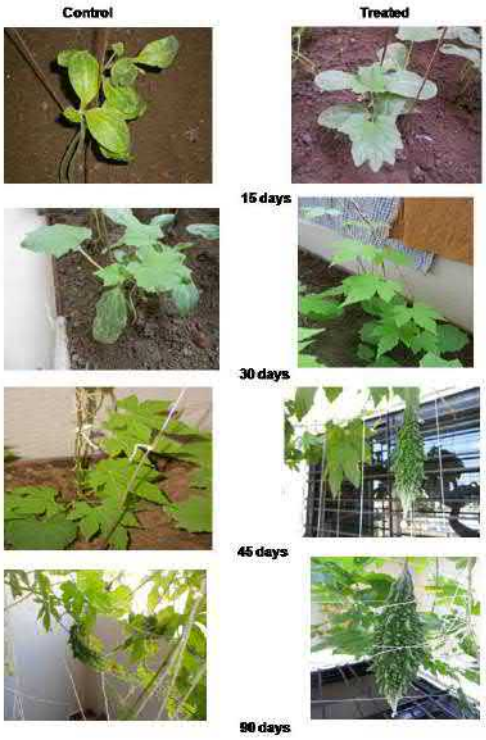


Spectrum No. 8

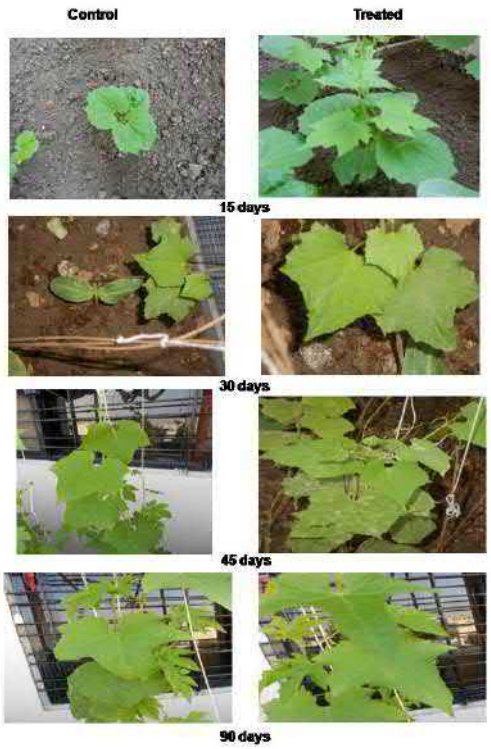


Spectrum No. 9

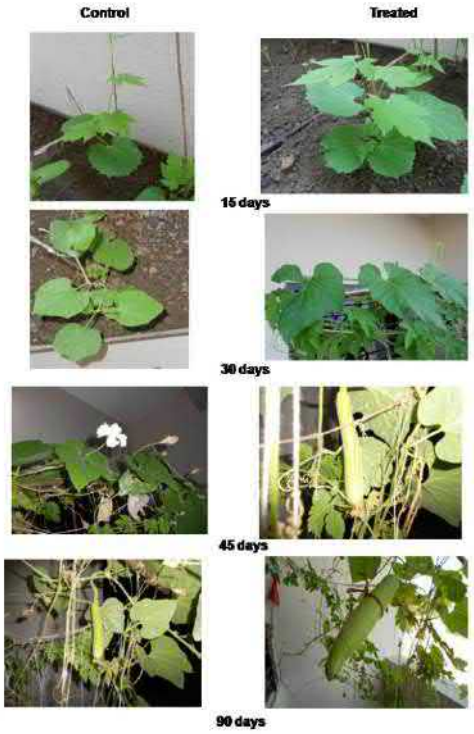
Impact of compound 5-(2'-hydroxy-3',5'-dichlorophenyl)-4-(heptan-1-one)-2- amino-1,3-thiazole (J) on phytotic growth of *Momordica charntia*



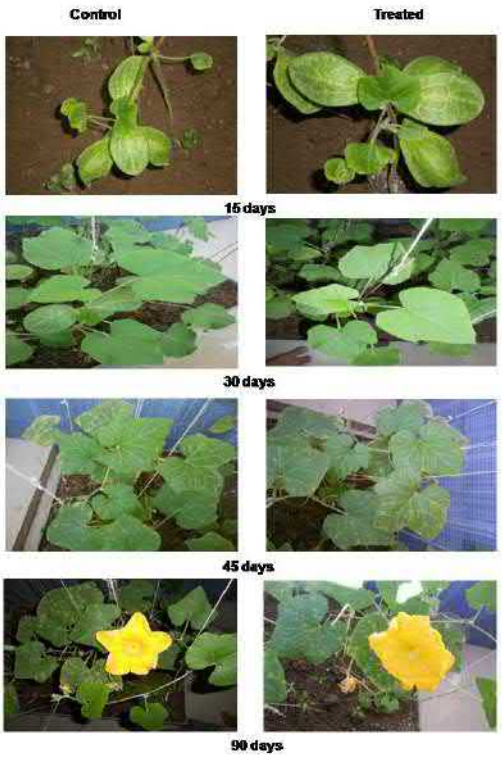
Impact of compound 5-(2'-hydroxy-3',5'-dichlorophenyl)-4-(heptan-1-one)-2- amino-1,3-thiazole (J) on phytotic growth of *Luffa cylindirca*



Impact of compound 5-(2'-hydroxy-3',5'-dichlorophenyl)-4-(heptan-1-one)-2- amino-1,3-thiazole (J) on phytotic growth of *Lageneria siceraria*



Impact of compound 5-(2'-hydroxy-3',5'-dichlorophenyl)-4-(heptan-1-one)-2- amino-1,3-thiazole (J) on phytotic growth of *Benincasa hispida*



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Research Article

SYNTHESIS AND STUDY OF SUBSTITUTED 1,3-THIAZOLES AND THEIR NANOPARTICLES ON PHYTOTIC GROWTH OF SOME VEGETABLE CROPS

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ABSTRACT
The synthesis, spectral analysis and biological activities of 5-phenyl-2-hydroxy-chlorosubstituted-2-amino-1,3 thiazoles have been carried out. In this case 5-(2'-hydroxy-3',5'-dichlorophenyl)-4-(4"-nitrobenzoyl)-2-amine-1,3-thiazole (D), 5-(2'-hydroxy-3',5'-dichlorophenyl)-4-(4"-nitrobenzoyl)-2-phenyl-amino-1,3-thiazole (E), and 5-(2'-hydroxy-3',5'-dichlorophenyl)-4-(4"-nitrobenzoyl)-2-diphenyl-amino-1,3-thiazole (F) have been screened. The compounds D, E and F were synthesized from 1-(2'-hydroxy-3',5'-dichlorophenyl)-2-bromo-3-(4"-nitrophenyl)-1,3 propanedione (a_4) by the action of thiourea, phenylthiourea, diphenylthiourea. The nanoparticles of the compounds D, E and F have been prepared by using ultrasonic technique. The titled compounds and their nanoparticles were screened for growth promoting activity on some vegetable crop plants viz.. *Momordica charantia-L-Bitter guard (Karela), Lagneria siceraria-snake guard (Lavki), Luffa cylindrica L-sponge guard (Gilke) and Benincasa hispida-Pumpkin (Kohle).*

Keywords: *Chalcone, Thiazole, Thiourea, Phenyl Thiourea, Diphenyl Thiourea, Growth Promoting Activities*

INTRODUCTION
Heterocyclic nucleus plays an important role in medicinal chemistry and it is a key template for the growth of various therapeutic agents. Thiazole is a heterocyclic compound featuring both a nitrogen atom and sulfur atom as part of the aromatic five-membered ring. Thiazoles and related compounds are called 1,3-azoles (nitrogen and one other hetero atom in a five-membered ring). They are isomeric with the 1,2-azoles, the nitrogen and sulphur containing compound being called isothiazoles. Thiazoles are found naturally in the essential vitamins. Molecules that possess sulfur atoms are important in living organisms. The researchers Patterson and Capell (1940), Pullman A and Metzger (1948), Schwarz (1945), Alajarin *et al.,* (2006), Kumar and Kumar (2011-12), Patton *et al.,* (2009) have reported the synthesis of several thiazoles and also their potent biological activities such as antimicrobial (Jain *et al.,* 2011), antibacterial (Kaspady *et al.,* 2009; Sanz-Cervera *et al.,* 2009; Patel and Mehta, 2006; Shakeel *et al.,* 2010), antifungal (Kopnarr *et al.,* 2004; Logu *et al.,* 2005), fungicidal (Liu *et al.,* 2004) and insecticidal agent (Pattanaik *et al.,* 1998). Chalcones and their analogues having α, β-unsaturated carbonyl system are very versatile substrates for the evolution of various reactions and physiologically active compounds.
In the present study, various 5-phenyl-2-hydroxy-chlorosubstituted-2-amino-1,3 thiazoles have been synthesized from 1,3 propanediones by using thiourea, phenyl thiourea and diphenyl thiourea. The synthesized compounds along with their nanoparticles were evaluated for their growth promoting activity on some vegetable crop plants viz. *Momordica charantia-L-Bitter guard (Karela), Lagneria siceraria-snake guard (Lavki), Luffa cylindrica L-sponge guard (Gilke) and Benincasa hispida-Pumpkin (Kohle).*

MATERIALS AND METHODS
All the glasswares used in the present work were of pyrex quality. Melting points were determined in hot paraffin bath and are uncorrected. The purity of compounds was monitored on silica gel coated TLC plate. IR spectra were recorded on Perkin-Elmer spectrophotometer in KBr pelletes, H^1 NMR spectra on

spectrophotometer in $CDCl_3$ with TMS as internal standard. UV spectra were recorded in nujol medium. The analytical data of the titled compounds was highly satisfactory. All the chemicals used were of analytical grade. All the solvents used were purified by standard methods. Physical characterisation data of all the compounds is given in Table 1.

2'-Hydroxy 3',5'-Dichloroacetophenone:

2-Hydroxy-5-chloroacetophenone was dissolved in acetic acid (5 ml), Sodium acetate (3g) was added to the reaction mixture and then chlorine in acetic acid reagent (40 ml; 7.5 w/v) was added dropwise with stirring. The temperature of the reaction mixture was maintained below 20^0C. The mixture was allowed to stand for 30 minutes. It was poured into cold water with stirring. A pale yellow solid then obtained was filtered, dried and crystallized from ethanol to get the compound 2'-hydroxy 3',5'-dichloroacetophenone.

Preparation of 2''-hydroxy-3'',5''-dichlorophenyl-4-(4'''-nitrophenyl) chalcone (a):

To the boiling solution of the 2-hydroxy-3,5-dichloroacetophenone (0.01 mol) and p-nitrobenzaldehyde (0.01 mol) in ethanol (20 ml) a 40% solution of NaOH was added gradually. The reaction mixture was stirred mechanically at room temperature for 1 hour and kept steady for 6 to 8 hours, followed by decomposition with ice cold HCl (1:1). The yellow granules thus obtained were filtered, washed with 10% $NaHCO_3$ solution and then crystallized from ethanol-acetic acid mixture to obtain the compound (a).

Preparation of 1-(2''-hydroxy-3'',5''-dichlorophenyl)-2,3-dibromo-3-(4'''-nitrophenyl)-propan-1-one (a_1):

2'-Hydroxy-3',5'-dichlorophenyl-4-(4"-nitrophenyl) chalcone (a) (0.001 M) was suspended in bromine–glacial acetic acid reagent (25% w/v) (6.4 ml).

The reagent was added dropwise with constant stirring and the reaction mixture was kept at room temperature for about 30 minutes. The solid product, thus separated, was filtered and washed with a little petroleum ether to get the compound (a_1).

Preparation of 2-(4'''-nitrophenyl)-6,8-dichloroflavone (a_2):

1-(2'-Hydroxy-3',5'-dichlorophenyl)-2,3-dibromo-3-(4"-nitrophenyl)-propan-1-one (a_1) (0.01 mol) was dissolved in ethanol (25ml). To this, aqueous KOH solution (25 ml) was added. The reaction mixture was refluxed for 1 hour, cooled and diluted with water. The product thus separated was filtered and crystallized from ethanol to get the compound (a_2).

Preparation of 1-(2''-hydroxy-3'',5''-dichlorophenyl)-3-(4'''-nitrophenyl)-1,3-propandione (a_3):

2-(4"-Nitrophenyl)-6,8-dichloroflavone (a_2) (0.01 mol) was dissolved in ethanol (25ml). To this, aqueous solution of HCl (25 ml) was added. The reaction mixture was then refluxed for 1 hour, cooled, and diluted with water. The product, thus separated, was filtered, and crystallized from ethanol to get the compound (a_3).

Preparation of 1-(2''-hydroxy-3'',5''-dichlorophenyl)-2-bromo-3-(4'''-nitrophenyl)-1,3 propanedione (a_4):

1-(2'-Hydroxy-3',5'-dichlorophenyl)-3-(4"-nitrophenyl)-1,3-propandione (a_3) (0.01 mol) was dissolved in a mixture of ethanol and dioxane. To this, calculated amount of liquid bromine was added. The product was not separated even after standing for one hour. It was then diluted with water, washed with water several times and extracted with ether. The solvent was removed under reduced pressure to get the white solid of the compound (a_4).

Preparation of 5-(2''-hydroxy-3'',5''-dichlorophenyl)-4-(4'''-nitrobenzoyl)-2-amine-1,3-thiazole (D):

1-(2'-Hydroxy-3',5'-dichlorophenyl)-2-bromo-3-(4"-nitrophenyl)-1,3-propandione (a_4) (0.01 mol) and thiourea (0.01 mol) was dissolved in ethanol (25 ml). To this, aqueous KOH solution (0.02 mol) was added. The reaction mixture was then refluxed for 3 hours, cooled, diluted with water and acidified with conc. HCl. The product thus separated was filtered and crystallized from ethanol to get the compound (D).

Preparation of 5-(2''-hydroxy-3'',5''-dichlorophenyl)-4-(4'''-nitrobenzoyl)-2-phenyl- amino-1,3-thiazole (E):

1-(2'-Hydroxy-3',5'-dichlorophenyl)-2-bromo-3-(4"-nitrophenyl)-1,3-propandione (a_4) (0.01 mol) and phenyl thiourea (0.01 mol) were dissolved in ethanol. To this, aqueous KOH solution (0.02 mol) was added.

The reaction mixture was refluxed for 3 hours, cooled, diluted with water and acidified with conc. HCl. The product, thus separated, was filtered and crystallized from ethanol to get the compound (E).

Preparation of 5-(2'-hydroxy-3',5'-dichlorophenyl)-4-(4''-nitrobenzoyl)-2-diphenyl-amino-1,3-thiazole (F):

1-(2'-Hydroxy-3',5'-dichlorophenyl)-2-bromo-3-(4''-nitrophenyl)-1,3-propandione (a_4) (0.01 mol) and diphenyl thiourea (0.01 mol) were dissolved in ethanol. To this aqueous solution of KOH (0.02 mol) was added. The reaction mixture was then refluxed for three hours, cooled, diluted with water and acidified with conc. HCl. The product, thus separated, was filtered and crystallized from ethanol to get the compound (F).

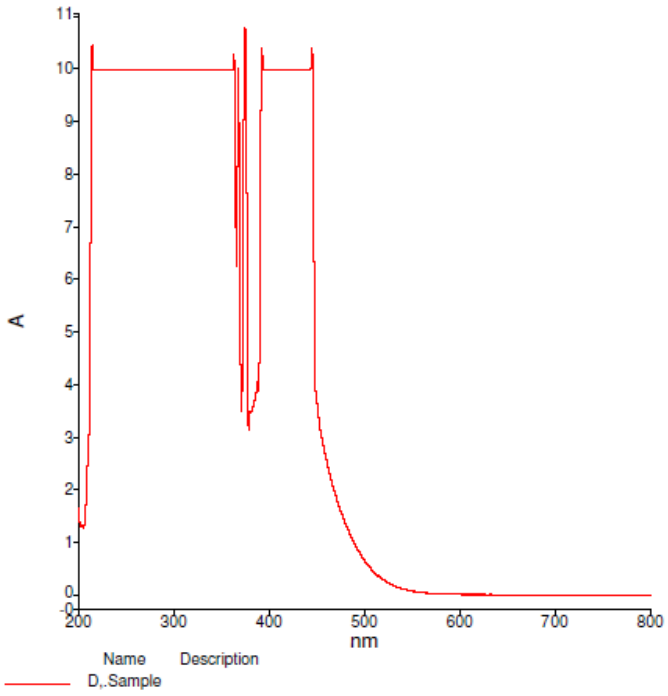
The newly synthesized compounds were characterized on the basis of elemental analysis, molecular determination, UV, IR, NMR. spectral data.

The UV, IR, and NMR Spectral Data:

Compound (D):

UV: Spectrum No. 1

The UV-Vis spectrum of the compound (D) reported in dioxane showed λ_{max} value 475 nm corresponding to n→π* transition.



Spectrum No. 1

IR (KBr): Spectrum No. 2

3335.23 cm^{-1}(-OH phenolic), 2923.23 cm^{-1} (aliphatic -C-H stretching), 3074.22 cm^{-1} (aromatic -C-H stretching), 3788.41 cm^{-1} (-N-H stretching), 1229.14 cm^{-1} (-C=N- stretching), 740.22 cm^{-1} (-C-Cl stretching in aliphatic), 1053.26 cm^{-1} (C-Cl stretching in aromatic).

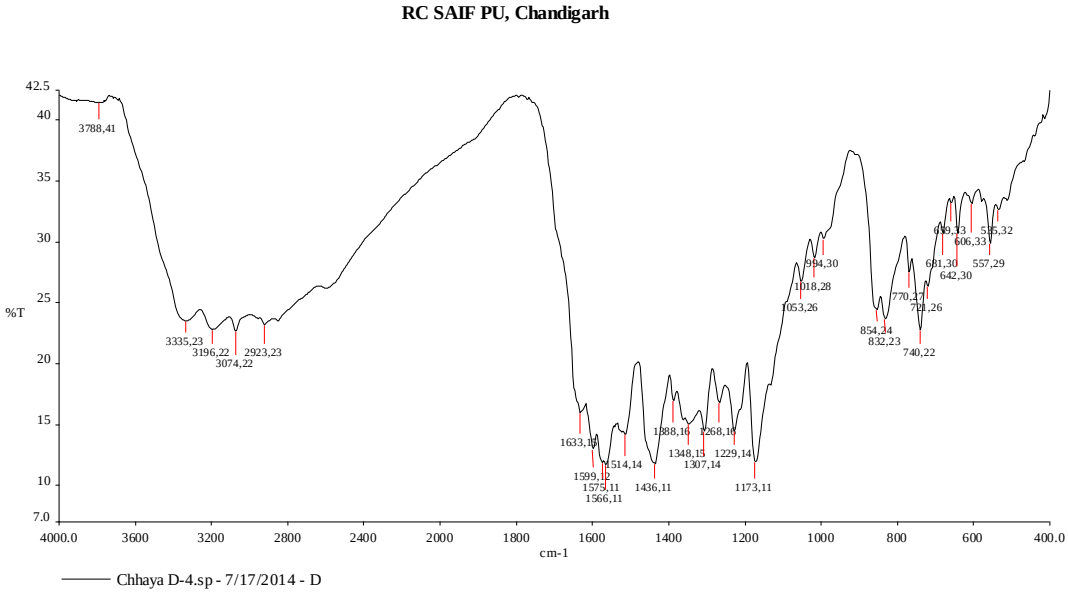
PMR: Spectrum No. 3

∂ 3.4 (hump, 2H, (-N-**H**); ∂ 6.7 (d, 1H, -CH=C-**H**-); ∂ 6.8 (d, 1H, -C**H**=C-H-); ∂ 7.1 to 8.3 (m, 6H, Ar-**H**); ∂ 12.6 (s, 1H, O-**H**)

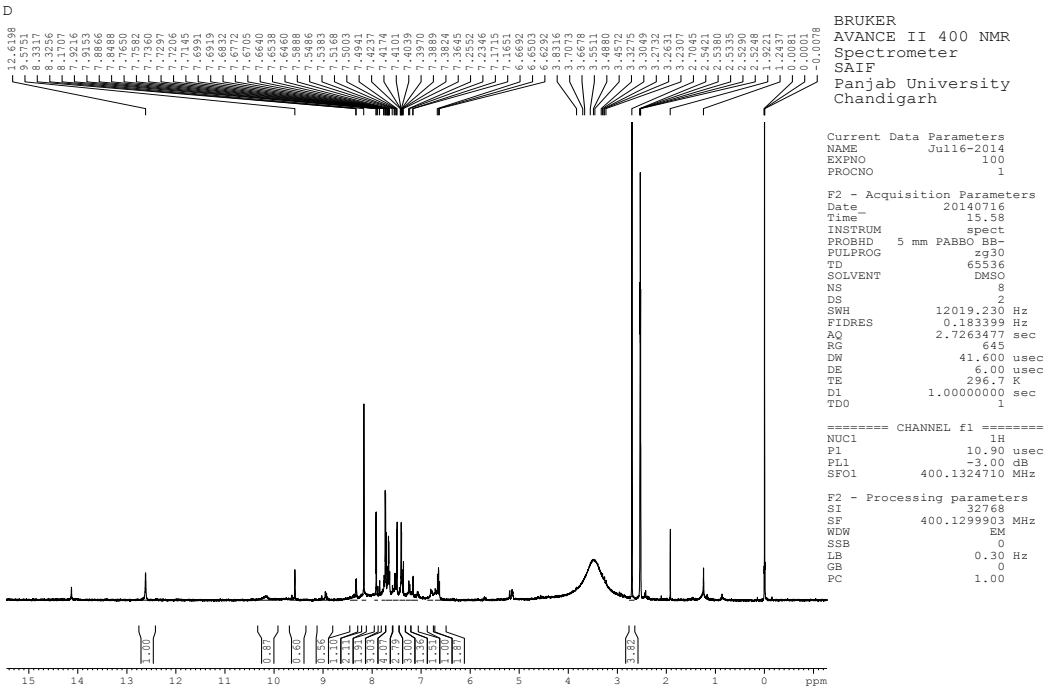
Compound (E):

UV: Spectrum No. 4

The UV-Vis spectrum of the compound (E) reported in dioxane showed λ_{max} value 490 nm corresponding to n→π* transition.



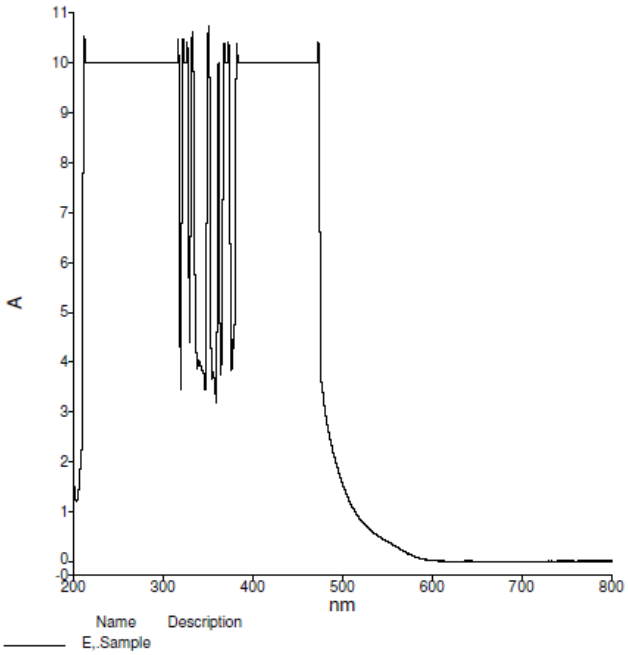
Spectrum No. 2



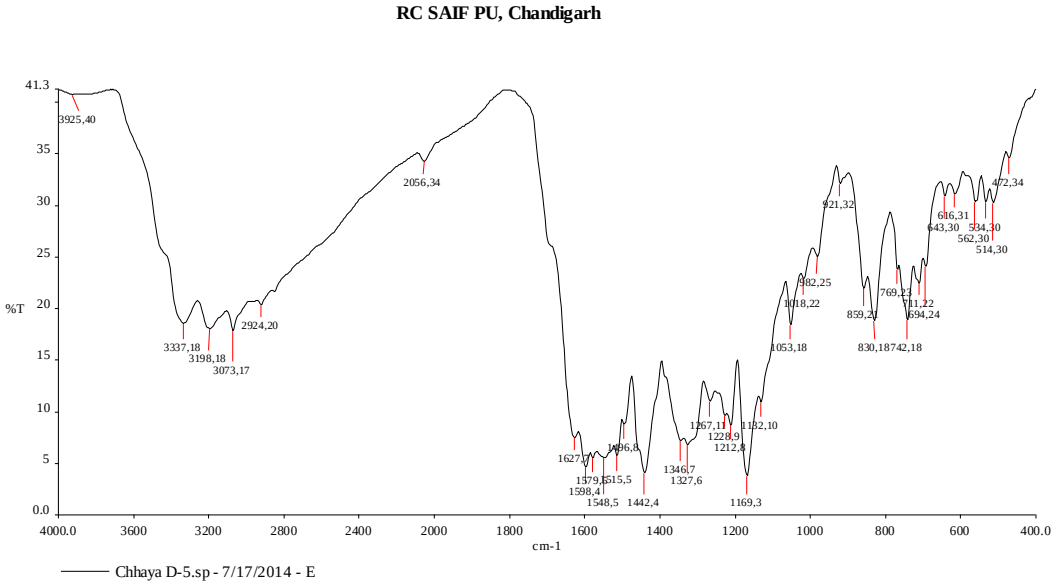
Spectrum No. 3

IR KBr: Spectrum No. 5
3337.18 cm^{-1} (O-H phenolic), 2923.20 cm^{-1} (aliphatic -C-H stretching), 3073.17 cm^{-1} (aromatic C-H stretching), 3925.40 cm^{-1} (-NH stretching), 1218.9 cm^{-1} (-C=N-stretching), 769.23 cm^{-1} [C-Cl stretching in aliphatic), 1053.18 cm^{-1} [C-Cl stretching in aromatic].
PMR: Spectrum No. 6

∂ 3.58 (hump, 1H, =N**H**); ∂ 6.64 (d 1H, -CH=C-**H**-); ∂ 6.69 (d, 1H, -C**H**=C-H-); ∂ 7.1 to 8.3 (m, 11H, Ar-**H**); ∂ 12.5 (s, 1H, O-**H**).



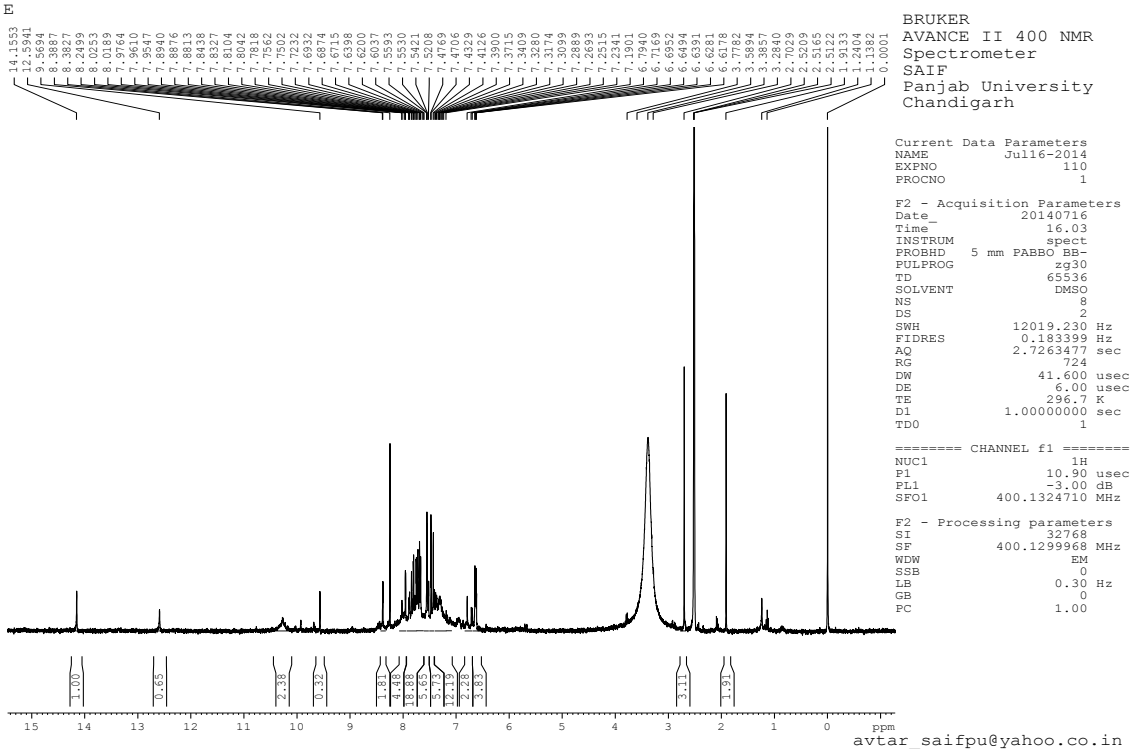
Spectrum No. 4



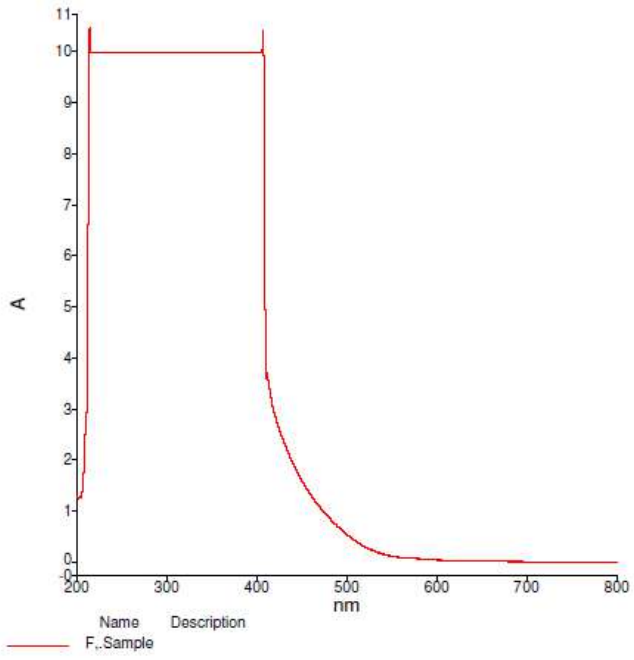
Spectrum No. 5

Compound (F):
UV: Spectrum No. 7
The UV-Vis spectrum of the compound (F) reported in dioxane showed λ_{max} value 405 nm corresponding to n→π* transition.
IR KBr: Spectrum No. 8

3025.30 cm^{-1} (-OH phenolic), 3035.51cm^{-1} (aromatic C-H stretching), 1548.5 cm^{-1} (-C=N stretching), 755.8 cm^{-1} (C-Cl stretching in aliphatic), 1072.15 cm^{-1} (C-Cl stretching in aromatic), 1344.6 (C=N stretching).



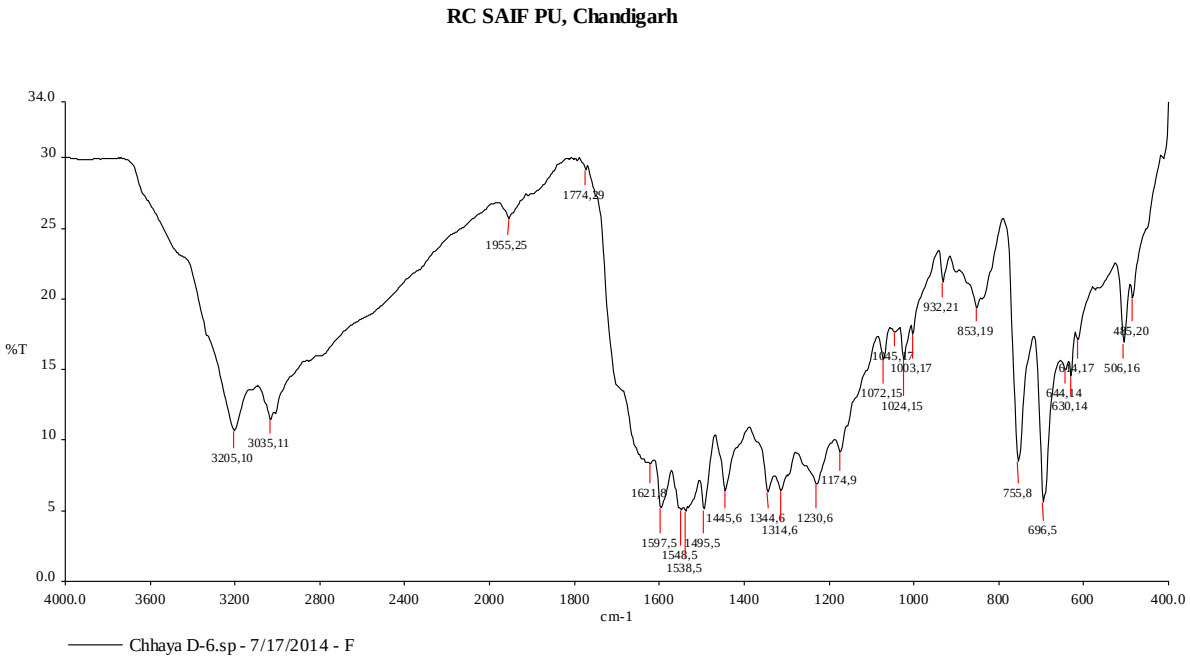
Spectrum No. 6



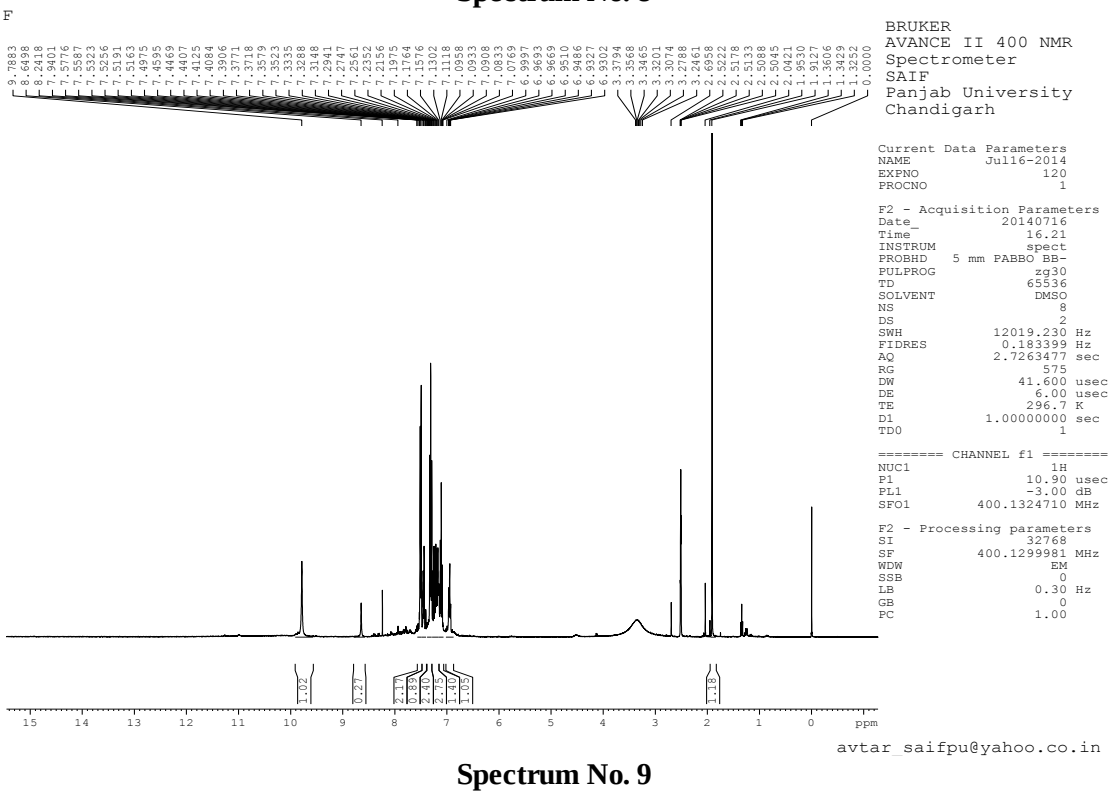
Spectrum No. 7

PMR: Spectrum No. 9

∂ 6.93 (d, 1H, d 1H, -CH=C-**H**-); ∂ 6.93 (d,1H, -C**H**=C-H-); ∂ 7.07 to 8.6 (m, 16H, Ar-**H**); ∂ 9.7 (s, 1H, O-**H**).



Spectrum No. 8



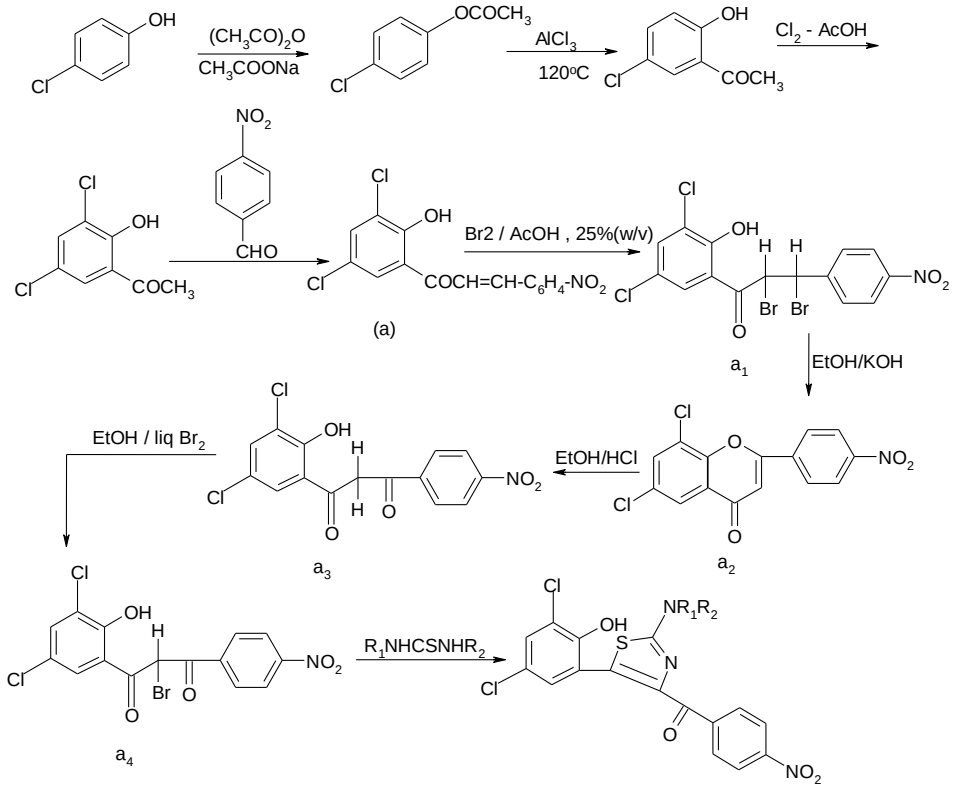
Spectrum No. 9

Preparation of Nanoparticles of the Titled Compounds

Ultrasonic Processor Sonapros PR-250MP was used to produce nanoparticles of the test compounds. The test compounds were dissolved in dioxane to prepare 0.1 M solutions. These solutions were taken in beakers and the probe of the sonapras 250 MP was dipped in solution. These solutions were exposed to sonopros MP 250 for 10 minutes separately. The test compounds were converted to nanoparticles. The solvent dioxane was evaporated by conventional heating method. The size of nanoparticles of the test compounds was confirmed by X-ray diffraction studies using Benchtop x-ray diffraction (XRD) instrument **(**Miniflex).

The thin film of the nanoparticles of the test compounds was prepared on glass slide. This slide was introduced to the X-ray diffraction instrument to get graphical information which was used for the calculation of the crystal size of test compounds.

Scheme:



Where:

1) R_1 = -H, $-C_6H_5$
2) R_2 = -H, $-C_6H_5$

Characterisation of Size of Nanoparticles of the Test Compounds:

The crystal size of nanoparticles of the test compounds calculated by using Debye-Scherrer equation.

$$D = \frac{0.94\,\lambda}{\beta.\,\cos\theta}$$

Where,

D = The average crystalline size.

0.94 = The particle shape factor which depends on the shape and size of the particle.

λ = is the wavelength.

β = is the full width at half maximum [FWHM] of the selected diffraction peaks ($\beta = 0.545$)

θ = is the Bragg's angle obtained from 2θ values which was corresponding to the maximum intensity peak in XRD pattern ($\theta = 0.7501$ rad).

Table 1: Characterisation Data of Newly Synthesized Compounds

Compounds	Molecular Formula	M.P. in ^{0}C	% of Yield	% of Element					
				C	H	N	S	Cl	Br
	$C_8H_6O_2Cl_2$	54	80	47.90/48	2.95/3			34.15/34.58	
a	$C_{15}H_9O_4NCl_2$	250	70	53.10/53.25	2.40/2.66	3.98/4.18		21/21.77	
a_1	$C_{15}H_9O_4NCl_2Br_2$	72	70	36.01/36.14	1.78/1.80	2.78/2.81		14.20/14.25	32.08/32.12
a_2	$C_{15}H_7O_4Cl_2N$	132	60	53.14/53.57	2,07/2.08	4.13/4.16		21.03/21.13	
a_3	$C_{15}H_9O_5Cl_2N$	117	50	50.74/50.84	2.45/2.54	3.90/3.95		20.03/20.05	
a_4	$C_{15}H_8O_5Cl_2BrN$	78	60	41.12/41.57	1.78/1.84	3.20/3.23		16.08/16.39	18.34/18.47
D	$C_{16}H_{11}O_4N_3Cl_2S$	170	70	46.50/46.60	2.56/2.66	10.05/10.19	7.67/7.76	17.20/17.23	
E	$C_{22}H_{15}O_4N_3Cl_2S$	168	70	54/54.09	3.0/3.07	8.56/8.60	6.50/6.55	14.50/14.54	
F	$C_{28}H_{17}O_4Cl_2N_3S$	180	75	59/59.78	3/3.02	7.4/7.47	5.6/5,69	12.6/12.63	

Activity of the Test Compounds D, E and F:

Table 2: 5-(2'-Hydroxy-3',5'-Dichlorophenyl)-4-(4"-Nitrobenzoyl)-2-Amine-1,3-Thiazole (D)

Periodicity of Observations [in Days]	*Momordica Charantia* (Bitter Guard) (Karela)				*Lageneria Siceraria* (Snake Guard) (Lavki)				*Luffa Cylindrica* (Sponge Guard) (Gilke)				*Benincasa Hispida* (Pumpkin) (Kohle)			
	Shoot Height		No. of Leaves		Shoot Height		No. of Leaves		Shoot Height		No. of Leaves		Shoot Height		No. of Leaves	
	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T
7	2.5	1.5	2	2	2.5	1.5	2	2	4.5	5	2	2	20	23	2	3
14	7	7.2	2	2	7.5	7.2	2	2	10	8.2	2	2	20	24	2	3
21	25	20	7	6	8	13	2	4	15	13	3	6	23	24.5	3	4
28	35	40	9	10	9	20	3	6	16	38	4	9	25	25	4	5
35	47	47	10	12	11	32	4	7	20	52	5	12	32	27	5	7
42	51	51	12	15	17	59	5	9	25	83	7	17	30	32	6	9
49	55	62	14	17	25	71	6	11	30	89	8	20	35	38	8	11
56	60	80	16	19	28	90	7	13	35	103	10	26	38	52	10	15
63	67	134	18	22	31	102	8	14	40	138	12	29	42	60	12	16
70	72	142	20	28	34	109	9	16	45	145	14	32	46	65	14	18
77	75	148	22	30	36	114	10	18	50	150	16	35	49	71	16	20
84	80	152	24	32	38	120	11	20	55	154	18	38	53	76	18	22
91	82	155	26	35	40	122	12	22	57	156	20	40	56	78	20	24

Table 3: 5-(2'-Hydroxy-3',5'-Dichlorophenyl)-4-(4"-Nitrobenzoyl)-2(Phenylamino)-1,3-Thiazole (E)

Periodicity of Observations [in Days]	*Momordica Charantia* (Bitter Guard) (Karela)				*Lageneria Siceraria* (Snake Guard) (Lavki)				*Luffa Cylindrica* (Sponge Guard) (Gilke)				*Benincasa Hispida* (Pumpkin) (Kohle)			
	Shoot Height		No. of Leaves		Shoot Height		No. of Leaves		Shoot Height		No. of Leaves		Shoot Height		No. of Leaves	
	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T
7	2.5	1.5	2	2	2.5	1.5	2	2	4.5	6	2	2	20	22.5	2	3
14	7	10	2	4	7.5	7	2	2	10	8.5	2	2	20	23	2	3
21	25	30	7	6	8	12	2	3	15	12	3	4	23	25	3	5
28	35	48	9	10	9	16	3	4	16	20	4	6	25	27	4	6
35	47	70	10	12	11	24	4	5	20	29	5	7	27	34	5	7
42	51	83	12	14	17	40	5	6	25	34	7	9	30	40	6	10
49	55	86	14	16	25	45	6	7	30	37	8	11	35	45	8	11
56	60	120	16	19	28	52	7	9	35	45	10	13	38	55	10	16
63	67	132	18	35	31	58	8	10	40	51	12	15	42	60	12	18
70	72	140	20	38	34	62	9	12	45	56	14	18	46	66	14	20
77	75	143	22	30	36	64	10	14	50	70	16	19	49	70	16	22
84	80	149	24	32	38	67	11	16	55	74	18	21	53	75	18	24
91	82	151	26	34	40	69	12	18	57	78	20	23	56	78	20	26

Table 4: 5-(2'-Hydroxy-3',5'-Dichlorophenyl)-4-(4"-Nitrobenzoyl)-2-Diphenylamino-1,3-Thiazole (F)

Periodicity of Observations [in Days]	*Momordica Charantia* (Bitter Guard) (Karela)				*Lageneria Siceraria* (Snake Guard) (Lavki)				*Luffa Cylindrica* (Sponge Guard) (Gilke)				*Benincasa Hispida* (Pumpkin) (Kohle)			
	Shoot Height		No. of Leaves		Shoot Height		No. of Leaves		Shoot Height		No. of Leaves		Shoot Height		No. of Leaves	
	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T
7	2.5	1.5	2	2	2.5	1.5	2	2	4.5	6	2	2	20	21.5	2	3
14	7	7	2	4	7.5	7	2	2	10	8.5	2	2	20	23	2	3
21	25	15	7	6	8	12	2	4	15	12	3	4	23	24	3	5
28	35	42	9	10	9	16	3	4	16	20	4	6	25	26	4	5
35	47	45	10	12	11	24	4	5	20	29	5	7	27	32	5	7
42	51	54	12	14	17	40	5	6	25	34	7	9	30	40	6	9
49	55	60	14	16	25	45	6	7	30	37	8	11	35	45	8	12
56	60	99	16	19	28	52	7	9	35	45	10	13	38	52	10	15
63	67	104	18	25	31	58	8	10	40	57	12	15	42	61	12	17
70	72	110	20	28	34	62	9	12	45	56	14	17	46	68	14	19
77	75	114	22	30	36	64	10	14	50	59	16	18	49	75	16	20
84	80	118	24	32	38	67	11	16	55	62	18	20	53	81	18	22
91	82	121	26	34	40	69	12	18	57	65	20	23	56	84	20	24

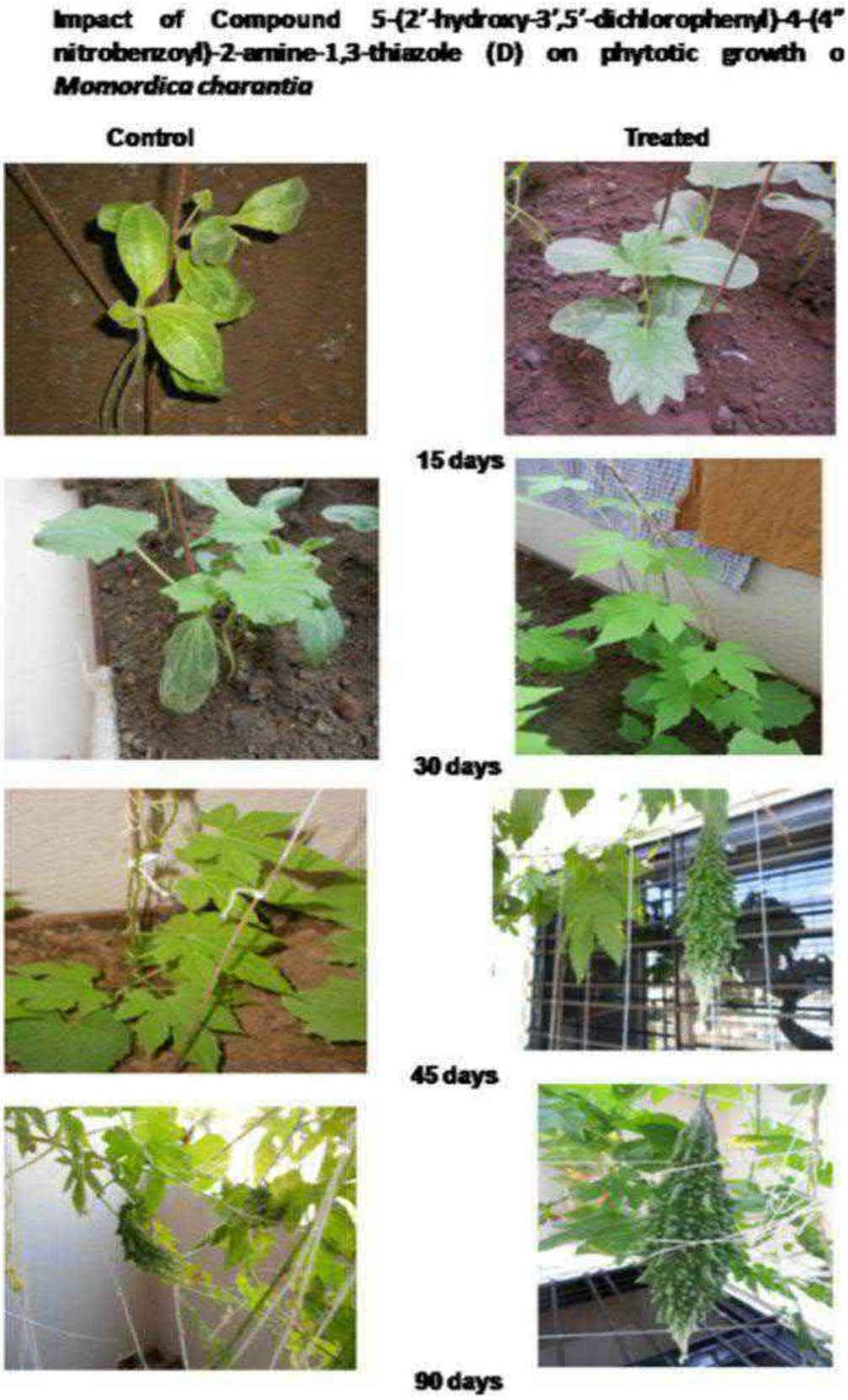

Impact of Compound 5-(2'-hydroxy-3',5'-dichlorophenyl)-4-(4" nitrobenzoyl)-2-amine-1,3-thiazole (D) on phytotic growth o Momordica charantia
Control
Treated
15 days
30 days
45 days
90 days

Figure 1

Impact of Compound 5-(2'-hydroxy-3',5'-dichlorophenyl)-4-(4"-nitrobenzoyl)-2-amine-1,3-thiazole (D) on phytotic growth of *Lageneria siceraria*

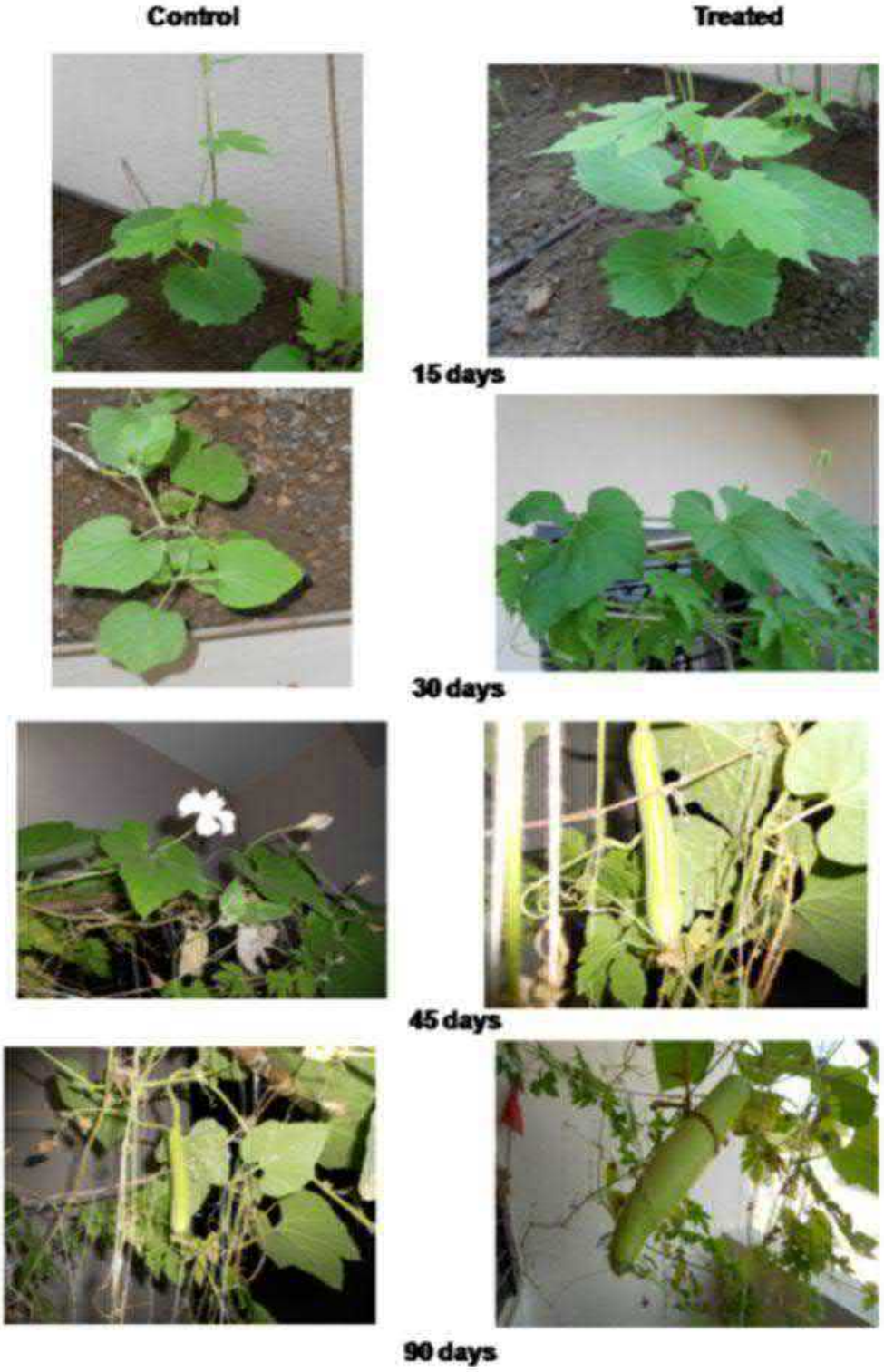


Figure 2

Impact of Compound 5-(2'-hydroxy-3',5'-dichlorophenyl)-4-(4"-nitrobenzoyl)-2-amine-1,3-thiazole (D) on phytotic growth of *Luffa cylindrica*

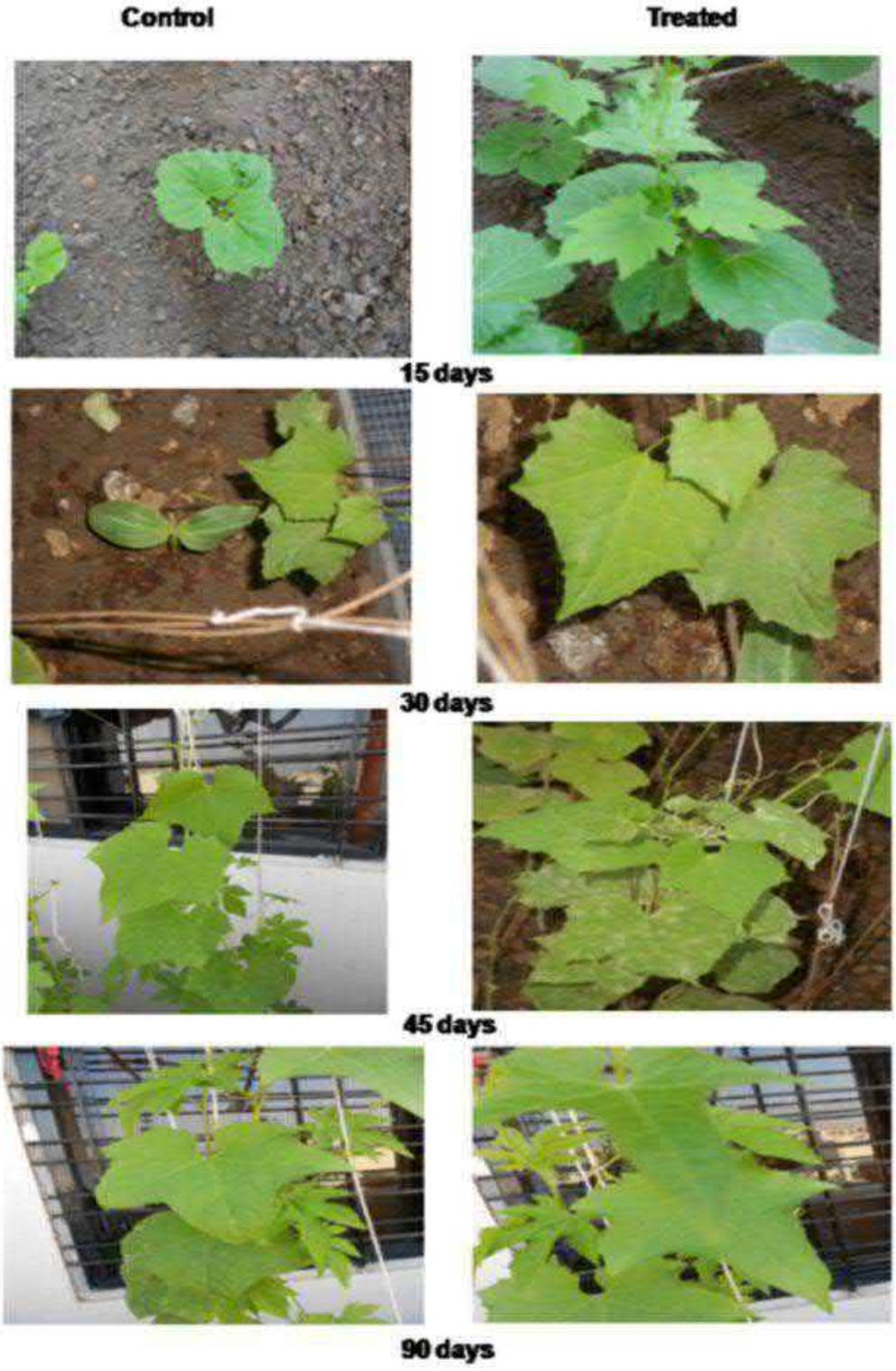


Figure 3

Impact of Compound 5-(2'-hydroxy-3',5'-dichlorophenyl)-4-(4"-nitrobenzoyl)-2-amine-1,3-thiazole (D) on phytotic growth of *Benincasa hispida*

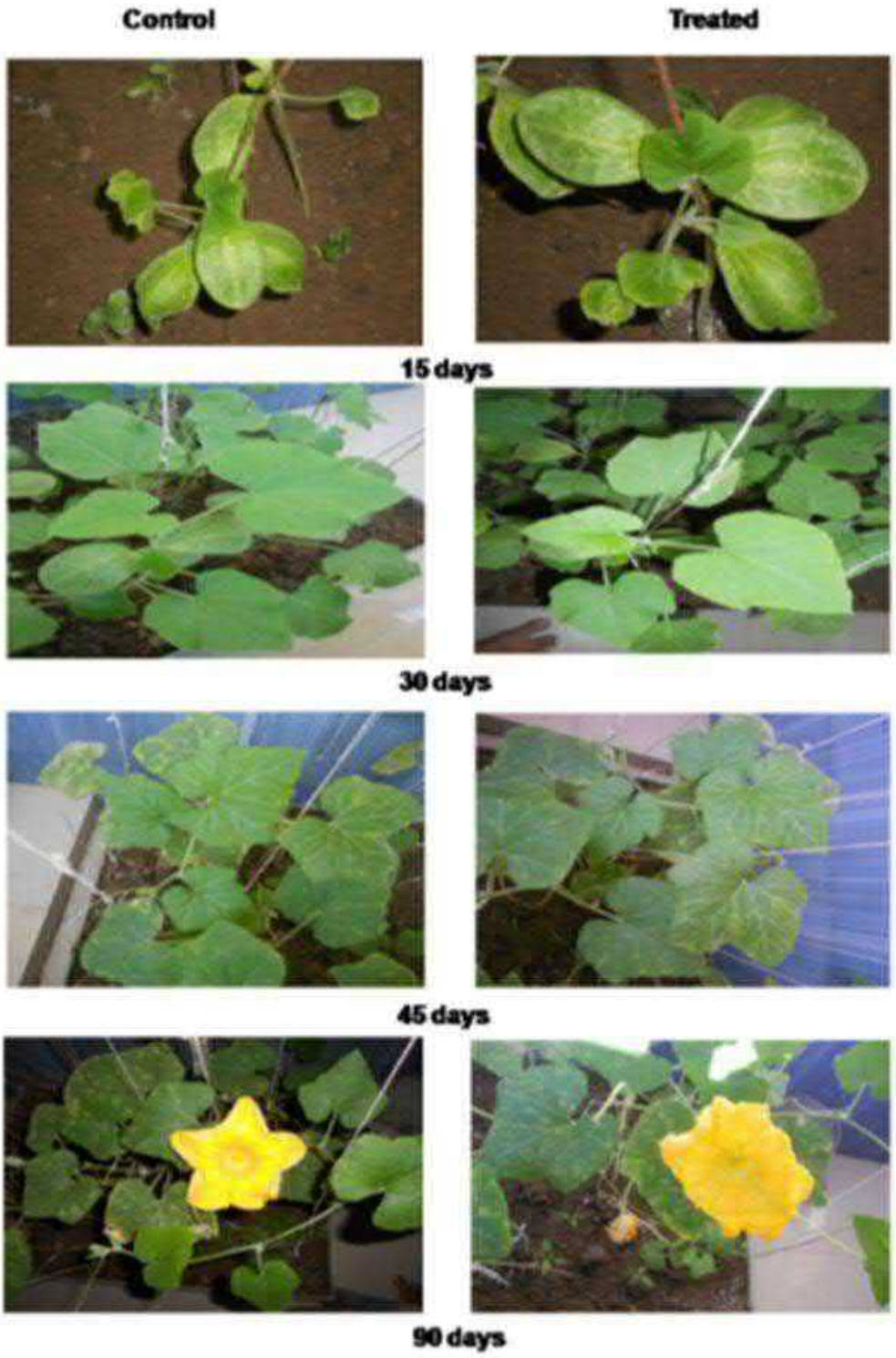


Figure 4

***Growth Promoting Effect on some Vegetable Crop Plants*:**
The experimental set up of the study was divided into two parts:
(i) Seed treatment (ii) Field experiment.
(i) Seed Treatment:
With a view to safeguard dormant seed's potential from harmful external agencies, the seeds of the test plants were treated by solution of test compounds (0.01dilution) prepared in dioxane before sowing.
(ii) Field Experiment:
Pregerminated quality seeds of *Momordica charantia L-Bitter guard (Karela), Lagneria siceraria -snake guard (Lavki), Luffa cylindrica L-Sponge guard-(Gilke) and Benincasa hispida -Pumpkin (Kohle*) were procured from Department of Horticulture, Dr. PDKV, Akola.
The beds of cotton soil, 2.5 x 2.5 m size were prepared in an open field. The sowing of seeds of all four test vegetable crop plants were done in separate beds and irrigated periodically.
The plants from each bed were divided into two groups i.e. A and B and designated as "Control" and "Treated" group plants respectively.
The plants from group B were sprayed with the solution of test compounds at weekly intervals. The field experiments were conducted to compare the treated plants of group B with untreated plants of controlled group A. In this context, the observations were recorded on 7, 14, 21, 28, 35, 42, 45, 56, 63, 70, 77, 84, 91 days after sowing corresponding to early vegetative, late vegetative, flowering, fruitification and fruit maturation, with special reference to number of leaves and height of shoots.
The results of field's experiments are tabulated in the tables 2, 3 and 4.

RESULTS AND DISCUSSION

The titled compounds and their nanoparticles were screened for their growth promoting activity on test vegetable crop plants viz, *Momordica charantia-L-Bitter guard (Karela), Lagneria siceraria-snake guard (Lavki), Luffa cylindrica L-sponge guard (Gilke) and Benincasa hispida-Pumpkin (Kohle).*
When a comparison of morphological characters was made between those of treated and control group plants, it was interesting to note that all the treated plants exhibited significant shoot growth and considerable increase in the number of leaves as compared to those of untreated ones. Also it was observed that the yield of treated plants enhances to a remarkable extent than control group plants.

ACKNOWLEDGEMENTS

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Phytochemical screening, Antimicrobial and Antioxidant activity of whole extract of *Cardiospermum halicacabum* Linn. (*Sapindaceae*)

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ABSTRACT

Man's existence on this earth has been made possible only because of the vital role played by the plant kingdom in sustaining his life. The three important necessities of life – food, clothing and shelter and a host of other useful products are supplied to a great extent by the plant kingdom. *Cardiospermum halicacabum* is one of the medicinally potential plants which is used in the treatment of rheumatism, lumbago, cough, hyperthermia, and nervous diseases. The present investigation was undertaken to screen the phytochemical analysis, antimicrobial and antioxidant activity of *Cardiospermum halicacabum* whole extract.

Keywords: Antimicrobial, antioxidant, *Cardiospermum halicacabum,* whole extract.

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INTRODUCTION

The modern system of medicine has made a tremendous progress and synthetic drugs and antibiotics have revolutionized the complete scenario. But their toxic side effects are also being realized which has attracted the attention of whole world towards natural system of medicine. Man has made use of plants in the treatment of diseases. A large number of drugs are of plant origin. Plants keep the body in tune with nature as nature intended and maintain proper balance. Natural sources help people to build their good health[1]. Recently there has been a shift in universal trend from synthetic to herbal medicine, which we can say 'Return to Nature'. Medicinal plants have been known for 'millennia' and are highly esteemed all over the world as a rich source of therapeutic agents for the prevention of diseases and ailments. Nature has bestowed our country with an enormous wealth of medicinal plants; therefore, India has often been referred to as the medicinal garden of the world[2]. *Cardiospermum halicacabum* is a deciduous climber growing up to 3meters. The ground stem carries alternate double triad leaves 3 to 6 cm long, the tiny radiate flowers. Stems are 5 to 10cm in length. The fruits are tiny green balloon shaped; spherical capsule containing the characteristics. Seeds with their heart shaped white markings. The plant has been used in the treatment of rheumatism, stiffness of limbs, snake bite; its roots for nervous diseases, as a diaphoretic, diuretic, emetic, laxative, its leaves used in the treatment of diarrhoea, dysentery and headache. Phytochemical constituents such as flavones, aglycones, triterpenoids, glycosides and a range of fatty acids and volatile ester have been reported from the various extracts of this plant[3-5].

MATERIALS AND METHOD

The plant material of *Cardiospermum halicacabum* were collected seasonally and authenticated by the taxonomists Dr. S. P. Rothe from the Department of Botany, Shri Shivaji College Akola.

Chemicals

All the chemicals used in the study were obtained commercially and of analytical grade.

Microorganisms

The test organisms *Escherichia coli, Staphylococcus aureus, Bacillus subtilis,* and *Pseudomonas aeruginosa* were procured from the National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory, Pune 411 008.

Phytochemical Screening

The chemical tests were performed for testing different chemical groups present in ethanol extract of test plant.

1) **Test for Sugar**

a) Molisch's Test: - Positive

b) Iodine Test: - Positive

2) **Test for Flavonoids**

a) Shinoda test: - Positive

b) H_2SO_4 test: - Positive

3) **Test for Sterols**

a) Salkowaski test:- Negative

b) Vanillin test:- Negative

4) **Test for Alkaloids**

a) Wagner's reagent test: - Positive

b) Mayer's reagent test: - Positive

5) **Test for Tannin**

a) $FeCl_3$ test: - Positive

b) Lead acetate test: - Negative

6) Test for Protein and Amino acid

a) Biuret test: - Positive

b) Xanthoprotein test: - Positive

7) **Test for Resin**

a) NaOH test:- Positive

MATERIALS AND METHOD

Soxhlet extraction method is used for the preparation of extracts of *Cardiospermum halicacabum* plant material. The coarse powders of *Cardiospermum halicacabum* plant material were extracted with water, ethanol and acetone solvents by using soxhlet apparatus. These extracts were concentrated at 40 ^oC using rotary evaporator. The extracts thus obtained were stored separately in air tight bottles for further study.

Study of Antioxidant Activity by DPPH[6]

The antioxidant activity of the ethanol extract was assessed on the basis 1, 1-diphenyl-2-picrylhydrazyl (DPPH). The diluted working solutions of the test extract was prepared in ethanol. 0.002% of DPPH was prepared in methanol and 1 ml of this solution was mixed with 1 ml of sample solution. These solution mixtures were kept in dark for 30 min and optical density was measured at 517 nm using UV Visible spectrophotometer. Methanol (1 ml) with DPPH solution (0.002%, 1 ml) was used as blank. The optical density was recorded and % inhibition was

calculated using the formula given below

$$\text{PERCENTAGE (\%) INHIBITION OF DPPH (\%AA)} = \frac{A-B}{A} \times 100$$

Where A = Optical density of the blank and B = Optical density of the sample

RESULTS AND DISCUSSION

The stock solution 1 mg/ml of ethanol extract was prepared. The required dilutions 0.11 mg/ml to 0.19 mg/ml were prepared by appropriate dilutions. The optical density and percent antioxidant activity was calculated and reported in table 1.

Table 1. Optical density and percent antioxidant activity for ethanol extract. O. D. of blank DPPH = 0.565

CONC. mg/ml	1 mg/ml	1.1	1.2	1.3	1.4	1.5	1.6	1.7	1.8	1.9	2.0
O.D. OF SAMPLE	0.387	0.385	0.380	0.375	0.370	0.367	0.365	0.363	0.360	0.358	0.356
%AA	19.37	19.79	20.83	21.87	22.91	23.54	23.95	24.37	25.00	25.41	25.83

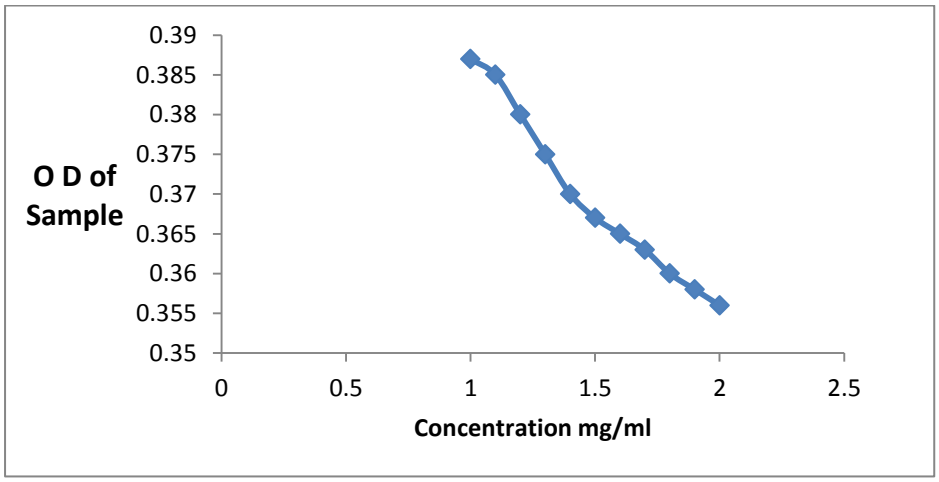

Figure 1: Decrease in O. D. of sample with increase in concentration of Ethanol Extracts

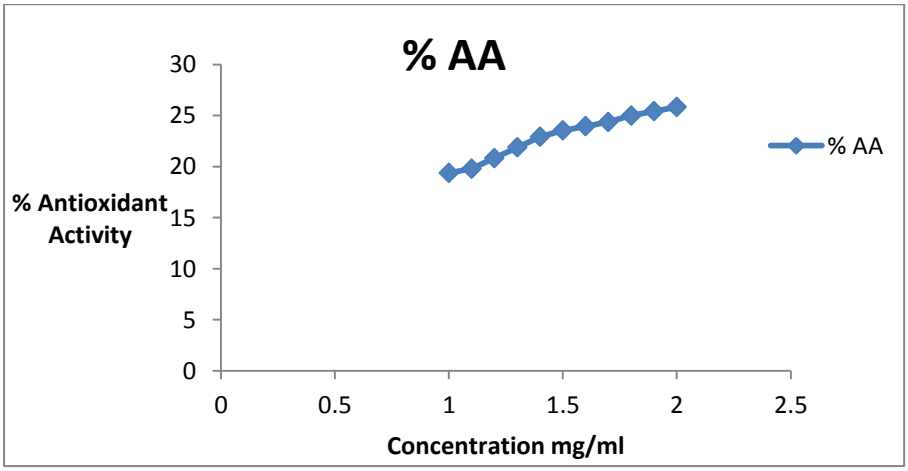

Figure 2: Increase in percent antioxidant activity with increase in concentration of Ethanol Extracts

Antimicrobial Assay[7]

The whole extracts of plant material in aqueous, ethanol and acetone solvents were screened for their antimicrobial potency by cup plate agar method against microbial species *viz. Escherichia coli, Staphylococcus aureus, Bacillus subtilis,* and *Pseudomonas aeruginosa*. The petriplates were prepared with 25ml sterile Mueller Hinton Agar. A sterile cork borer (8 mm) was used to make wells in each plate. 1 ml inoculums' suspension was swabbed uniformly over the agar medium to get uniform distribution of bacteria. After labelling the plates 100µl of each test compound (at concentration of 0.01 mol) was added aseptically into the wells. The petriplates were then incubated at 37°C for 24 hrs during which the activity was evidenced by the presence of zone of inhibition surrounding the well. The negative control was prepared using respective solvent. *Ampicilin disc* (10 mcg/disc) and *Vancomycin disc* (30 mcg/disc) were used as positive control. The zones of inhibition were recorded in millimetres by using Himedia Zone Reader Scale.

Table 2: Antimicrobial activity of different extracts of the test plant

Sr. No.	Extracts	Concentration	Inhibitory zones in mm			
			Escherichia coli	***Staphylococcus aureus***	***Bacillus subtilis***	***Pseudomonas aeruginosa***
1)	Aqueous extract	100 mg/ml	12 mm	12 mm	10 mm	11 mm
2)	Ethanol extract	100 mg/ml	13 mm	11 mm	11 mm	12 mm
3)	Acetone extract	100 mg/ml	12 mm	10 mm	11 mm	13 mm
4)	*Ampicilin disc*	(10 mcg/disc)	14 mm	13 mm	14 mm	12 mm
5)	*Vancomycin*	(30 mcg/disc)	14 mm	16 mm	12 mm	13 mm

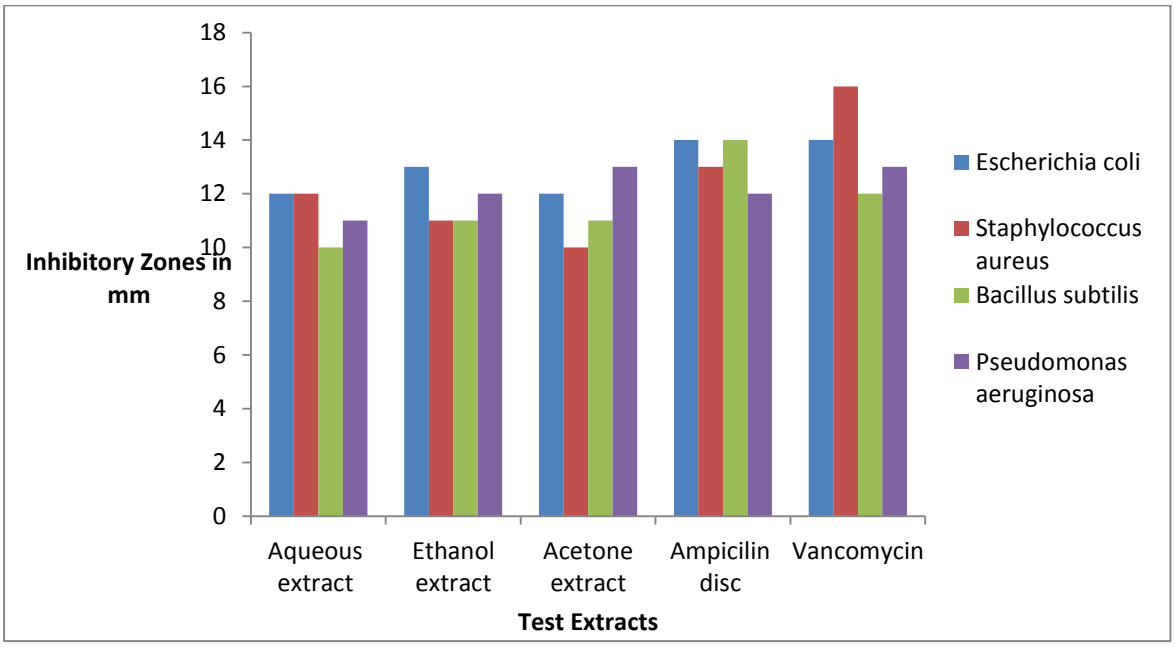

Figure 3: Antimicrobial activity of different extracts of the test plant

CONCLUSION

Remarkable decrease in the O. D. values of sample for three different solvent extract were observed indicating antioxidant activity of the fractions. Ethanol extract of the whole test plant showed good to moderate antioxidant activity which is evident from the graph. The IC_{50} value for ethanol extract is calculated from the figure 2 which is 1.4 mg/ml. The whole extracts of plant material in aqueous, ethanol and acetone solvents were screened for their antimicrobial potency which shows moderate to good activity as shown in figure 3.

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Quantitative structure–activity relationships (QSARs) and pharmacophore modeling for human African trypanosomiasis (HAT) activity of pyridyl benzamides and 3-(oxazolo[4,5-b]pyridin-2-yl)anilides

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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Quantitative structure–activity relationships (QSARs) and pharmacophore modeling for human African trypanosomiasis (HAT) activity of pyridyl benzamides and 3-(oxazolo[4,5-b]pyridin-2-yl)anilides

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Abstract In the present work, quantitative structure–activity relationship and pharmacophore modeling analysis were performed for human African trypanosomiasis healing activity of pyridyl benzamides (dataset-1) and 3-(oxazolo [4,5-b]pyridin-2-yl)anilides (dataset-2). For quantitative structure–activity relationship analysis, a pool of descriptors (mono-dimensional to three-dimensional) was generated, followed by descriptor reduction using objective and subjective feature selection. Multiple splitting was employed for the generation of multiple quantitative structure–activity relationship models to get maximum information about the descriptors that have correlation with the HAT activity of pyridyl benzamides and 3-(oxazolo[4,5-b]pyridin-2-yl)anilides. The genetic algorithm-multilinear regression quantitative structure–activity relationship models have excellent statistical robustness with good external predictive ability. The pharmacophore model and quantitative structure–activity relationship analyses furnished complementary and consensus results to each other.

Keywords QSAR · Pharmacophore model · Human African trypanosomiasis (HAT) · Pyridyl benzamides · 3-(Oxazolo[4,5-b]pyridin-2-yl)anilides

Introduction

Human African trypanosomiasis, also known as sleeping sickness, is a vector-borne parasitic disease usually transmitted by the bite of an infected tsetse fly (*Glossina* genus) and caused by *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense* (Ferrins et al., 2014; Sykes et al., 2012). This neglected disease has high occurrence in Africa continent, except North Africa. However, the genome of the parasite has been sequenced and many proteins have been recognized as prospective targets for drug development. Analysis of the genome revealed that *T. brucei* has over 800 genes that make proteins the parasite 'mixes and matches' to evade immune system detection, consequently, developing a vaccine for this disease is very difficult (Barrett et al., 2011; Lutje et al., 2013; Simarro et al., 2012). Moreover, the type of treatment depends on the stage of the disease. The drugs used in the first stage of the disease have lower toxicity and easy to administer. The earlier the disease is identified, the better the probabilities of a cure. But, in majority of cases, the disease is identified in second stage (Barrett et al., 2011; Carvalho et al., 2014; Ferrins et al., 2014; Lutje et al., 2013; Simarro et al., 2012; Sykes et al., 2012). A drug that can cross the blood–brain barrier to reach the parasite is crucial for treatment success in the second stage. Such drugs are mostly toxic and problematical to administer (Barrett et al., 2011; Lutje et al., 2013; Simarro et al., 2012). In Fig. 1, the marketed drugs

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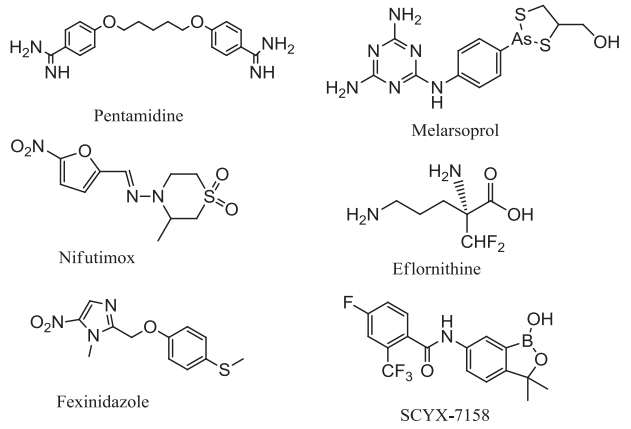


Fig. 1 Marketed and clinical stage drugs for HAT

and drugs in clinical stages have been depicted (Barrett et al., 2011; Carvalho et al., 2014; Ferrins et al., 2014; Gilbert, 2014; Lutje et al., 2013; Nagle et al., 2014; Simarro et al., 2012; Sykes et al., 2012).

To overcome these difficulties, Sykes et al. (2012) employed high-throughput screening (HTS) against *T. brucei* using a library of 87,296 compounds, and discovered a good number of classes with encouraging activity. Unfortunately, some inhibitor series like pyridyl benzamide and its derivatives were excluded in the initial HTS work because they did not satisfied the predefined selection criteria. Later studies (Ferrins et al., 2014) revealed that derivatives of pyridyl benzamide possess HAT activity in nM range with the possibility to treat the disease in second stage. Despite the efforts executed till this date, the search for specific biological target or targets of this series of compounds still persists (Ferrins et al., 2014). In such a situation, computer-aided drug design is considered an attractive tool for lead optimization.

In the past decades, computer-based techniques have emerged as attractive alternatives to conventional 'trial and error' methodology of drug design/discovery to map the mysterious ways of bio-chemistry of drug action, optimization and toxicity reduction (Bukhari et al., 2014, 2015; Ebalunode et al., 2011; Rastija and Masand, 2014; Yoon et al., 2013). Computer-aided drug designing (CADD) is a faster, cheaper and highly result oriented thriving modern technique involving amalgamation of different fields with major emphasis on understanding how different biological molecules interact, to probe the mechanisms of disease and to determine the effective structural features linked with activity/toxicity (Alafeefy et al., 2012; Bandgar et al., 2012; Chavan et al., 2013; Mahajan et al., 2012; Masand et al., 2013; Patil et al., 2012; Pourbasheer et al., 2015). Quantitative structure–activity relationship (QSAR), molecular docking, pharmacophore modeling, etc. are popular CADD methods, which when used in chorus results in highly successful analysis giving maximal of information required for lead (and drug) optimization. These methods provide information about the structural features that govern the specific activity/toxicity of drug candidate and better insight in mechanism of drug action (Barakat et al., 2014; Bukhari et al., 2014, 2015; Huang and Fan, 2011; Masand et al., 2014c, 2015; Rastija and Masand, 2014; Rastija et al., 2013; Yang et al., 2014; Yoon et al., 2013).

Molecular docking is especially more promising when enough information is available about the target receptor (protein or bio-polymer) with which the drug interacts (Mahajan et al., 2012). The receptor with which pyridyl benzamides and 3-(oxazolo[4,5-b]pyridin-2-yl)anilides interact is unknown (Ferrins et al., 2014), hence, QSAR and pharmacophore modeling were performed to determine the structural features that have good correlation with the HAT activity of pyridyl benzamides and 3-(oxazolo[4,5-b]pyridin-2-yl)anilides.

Experimental methodology

Experimental datasets

In the present work, HAT activities of two different datasets comprising different heterocyclic scaffolds were subjected to QSAR and pharmacophore modeling. The reported IC_{50} (μM) values for HAT activity were converted to pIC_{50} ($-\log_{10}IC_{50}$) before QSAR analysis.

Dataset-1

The dataset-1 consists of 32 pyridyl benzamides screened against *T. brucei* with a variety of substituents at diverse positions (Ferrins et al., 2014). The IC_{50}, pIC_{50} along with the substituents are tabulated in Table 1.

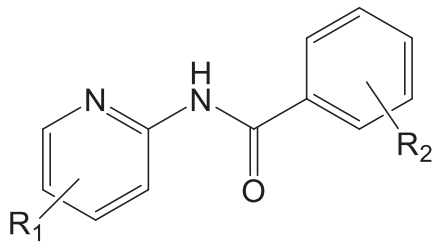
Dataset-2

The datset-2 selected for the present study comprises 31 derivatives of 3-(oxazolo[4,5-b]pyridin-2-yl)anilide previously reported to have HAT activity (Ferrins et al., 2013). The molecules possess different types of substituents present at different positions. The IC_{50}, pIC_{50} along with the substituents are tabulated in Table 2.

Modeling and molecular descriptors calculation

In the present work, for QSAR analysis the procedure reported in the literature has been followed (Mahajan et al., 2012; Masand et al., 2013, 2015; Rastija and Masand, 2014; Rastija et al., 2013). The structures were drawn using ChemSketch 12 freeware followed by energy minimization

Table 1 IC_{50}, pIC_{50} along with the substituents for dataset-1



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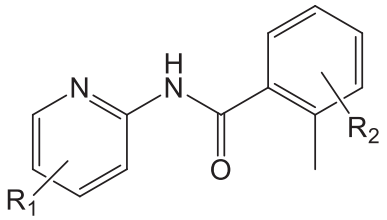
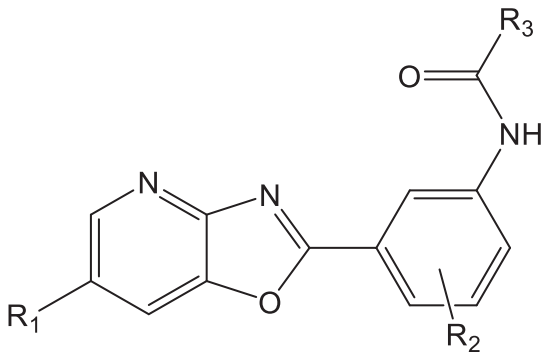


24-32

S. no.	R_1	R_2	Exp. IC_{50}	Exp. pIC_{50}
1	H	2-Me	3.03	5.5190
2	4-CN	2-Me	2.3	5.6380
3	4-Me	2-Me	2.1	5.6780
4	4-Cl	2-Me	2.1	5.6780
5	4-Br	2-Me	1.8	5.7450
6	4-F	2-Me	0.51	6.2920
7	4-C≡CPh	2-Me	1.7	5.7700
8	4-C≡CCH_2-*i*Pr	2-Me	1.1	5.9590
9	4-Ph	2-Me	2	5.6990
10	5-OMe	2-Me	3.8	5.4200
11	6-CH=$CH(CH_2)_2CH_3$	2-Me	6	5.2220
12	6-NH_2	2-Me	4.6	5.3370
13	H	H	9.07	5.0420
14	H	2-Et	2.74	5.5620
15	H	2-Me, 3-F	0.89	6.0510
16	H	3-F	7.33	5.1350
17	H	2-Me, 3-Cl	0.63	6.2010
18	H	2-Me, 3-Br	5.7	5.2440
19	H	2,3-diMe	3.5	5.4560
20	H	2-Me, 4-F	1.9	5.7210
21	H	2-Me, 4-Cl	1.1	5.9590
22	H	2-Me, 4-Br	1.1	5.9590
23	H	2,4-diMe	0.98	6.0090
24	H	3-F, 4-F	0.41	6.3870
25	4-Me	3-F	0.83	6.0810
26	4-Me	3-F, 4-F	0.9	6.0460
27	4-Cl	3-F	0.47	6.3280
28	4-Cl	3-F, 4-F	0.53	6.2760
29	4-F	3-F	0.1	7.0000
30	4-F	3-F, 4-F	0.19	6.7210
31	5-OMe	3-F	2.3	5.6380
32	5-OMe	4-F	2.6	5.5850

using MMFF94 force field in TINKER. The optimized structures were used as input for calculation of myriad number of descriptors using PaDEL 2.21 as a descriptor calculator. The descriptor pool of more than 16,000 descriptors consists of mono-dimensional (1D) to three-dimensional (3D), electro-topological, finger-prints and other descriptors. As all the calculated descriptors do not contain valuable information, hence, objective feature selection was employed to reduce the number of descriptors. As a rule, constant, near constant (>85 %) and highly correlated ($|R| > 85$ %) descriptors were eliminated before subjective feature selection (SFS) using QSARINS-Chem 2.2.1. This resulted in a reduced cluster of 264 and 181 descriptors for dataset-1 and 2, respectively, with a wide set

Table 2 IC_{50}, pIC_{50} along with the substituents for dataset-2



S. no.	R_1	R_2	R_3	IC_{50}	pIC_{50}
1	H	4-Cl	2-Furanyl	0.22	6.658
2	H	4-Cl	3-Me-2-furanyl	0.65	6.187
3	H	4-Cl	5-Isoxazolyl	3.2	5.495
4	H	4-Cl	5-Oxazolyl	1.5	5.824
5	H	4-Cl	3-Furanyl	0.3	6.523
6	H	4-Cl		1.8	5.745
10	H	4-Cl	Phenyl	1.5	5.824
13	H	4-Cl	2-Pyridinyl	5.7	5.244
14	H	4-Cl	4-Pyridinyl	3.4	5.469
17	H	H	2-Furanyl	0.34	6.469
18	H	H	3-Me-2-furanyl	0.17	6.77
19	H	H	5-Oxazolyl	2.9	5.538
20	H	H	3-Furanyl	0.6	6.222
21	H	H	4-Thiazolyl	1.3	5.886
26	H	4-OMe	2-Furanyl	1.1	5.959
27	H	4-Me	2-Furanyl	6.2	5.208
28	H	4-F	2-Furanyl	6.3	5.201
32	H	6-Me	2-Furanyl	0.94	6.027
33	H	6-Me	3-Me-2-furanyl	0.57	6.244
35	H	6-Me	3-Furanyl	0.31	6.509
36	H	6-Me	4-Thiazolyl	2.6	5.585
37	H	6-F	2-Furanyl	0.29	6.538
38	H	6-F	3-Me-2-furanyl	0.1	7
39	H	6-F	5-Oxazolyl	2	5.699
40	H	6-F	3-Furanyl	0.12	6.921
41	H	6-F	4-Thiazolyl	0.76	6.119
42	Ph	H	2-Furanyl	0.12	6.921
43	4-OMe-Ph	H	2-Furanyl	0.63	6.201
44	4-Cl-Ph	H	2-Furanyl	0.55	6.26
45	H	H	2-Furanyl	0.78	6.108
46	H	H	3-Furanyl	1	6

of theoretical molecular descriptors that takes into account different structural features, viz. constitutional (0D), 1D, bi-dimensional (2D), and 3D, capturing and magnifying distinct aspects of chemical structures.

Model development

QSAR models are developed (1) to determine the structural features that are correlated with the desired activity, and (2) to predict for a compound before its actual synthesis and/or biological screening (Masand et al., 2014a, b 2015). The predictive ability of a QSAR model can be defined as the ability of a model to correctly predict the biological activity of compounds that were not used for model development.

To get maximal information, models were developed using divided and undivided whole datasets. The datasets were randomly divided into training (80 %) and prediction (20 %) set before descriptor selection. Multiple splitting was performed to develop multiple QSAR models. A molecule in the training set of a splitting may or may not be in the training set of another splitting. The Genetic Algorithm (GA) module of QSARINS-Chem 2.1.1 (Gramatica et al., 2013, 2014) was used for selection of optimum number and set of descriptors. For the sake of simplicity and to avoid the problem of over-fitting, the heuristic search of descriptors was limited to three (for dataset-1) and four (for dataset-2) descriptors using the defaults settings in QSARINS-Chem 2.1.1. Q^2_{loo} was used as a fitness function to avoid the problem of naive Q^2. The strategy used in QSAR model development has been summarized in Fig. 2.

Model validation

Appropriate model validation is an important aspect of QSAR model development. The statistical qualities and validity of the genetic algorithm-multilinear regression (GA-MLR) equations were established by means of: (a) internal validation or cross-validation (CV) by leave-one-out (LOO) and leave-many-out (LMO) procedure; (b) using the prediction set; (c) data randomization, i.e., Y-scrambling; and (d) examining if the following conditions are satisfied (Masand et al., 2015): $R^2_{tr} \geq 0.6$, $Q^2_{loo} \geq 0.5$, $Q^2_{LMO} \geq 0.6$, $R^2 > Q^2$, $R^2_{ex} \geq 0.6$, $RMSE_{tr} < RMSE_{cv}$, $\Delta K \geq 0.05$, $CCC \geq 0.80$, $Q^2\text{-}F^n \geq 0.60$, $r^2_m \geq 0.6$, $(1-r^2/r_o^2) < 0.1$, $0.9 \leq k \leq 1.1$ or $(1-r^2/r'^2_o) < 0.1$, $0.9 \leq k' \leq 1.1$, $|r_o^2 - r'^2_o| < 0.3$ with RMSE and MAE close to zero. The threshold values of these parameters ensure robustness and external predictive ability of a GA-MLR model. Thus, all the models having low internal and external predictive ability were subsequently rejected.

Pharmacophore model development

For pharmacophore modeling, all the molecules in the dataset were aligned using the lowest energy conformer of the most active compounds in the datasets. PyMOL 1.7 and

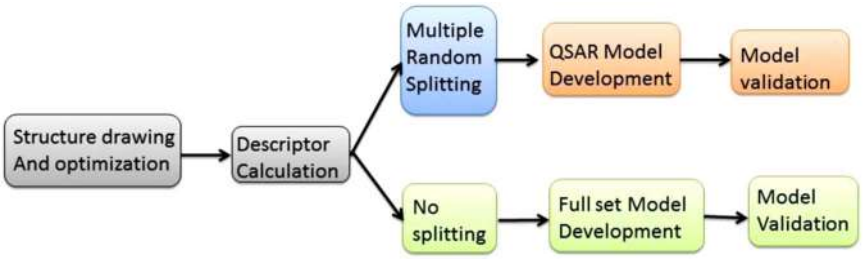


Fig. 2 Strategy used in QSAR model development

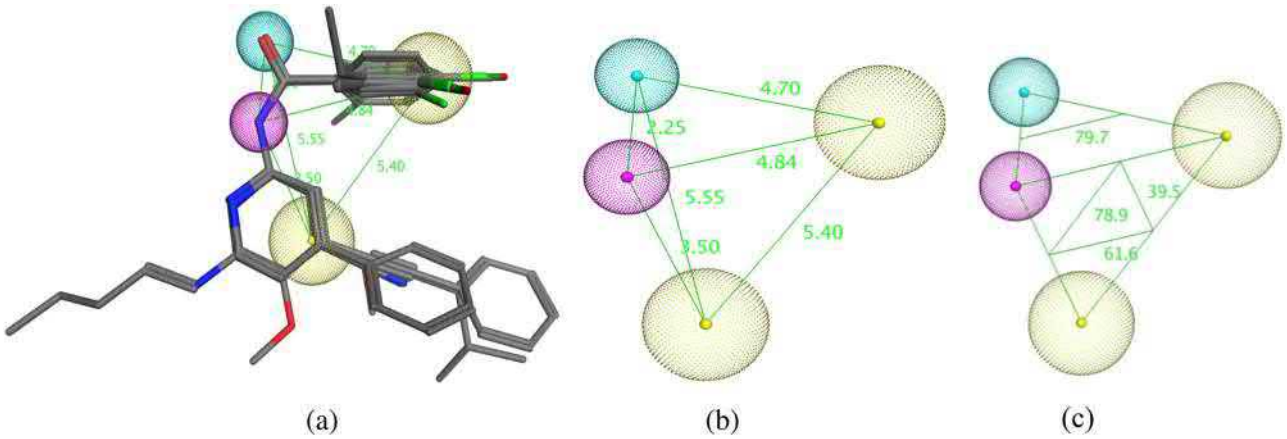


Fig. 3 Consensus pharmacophore model for dataset 1 (*cyan*: H-acceptor, *magenta*: H-donor, *yellow*: hydrophobic). **a** Pharmacophore model with aligned ligands; **b** pharmacophore model with distances (Å) between different features; **c** pharmacophore model with angles between different features

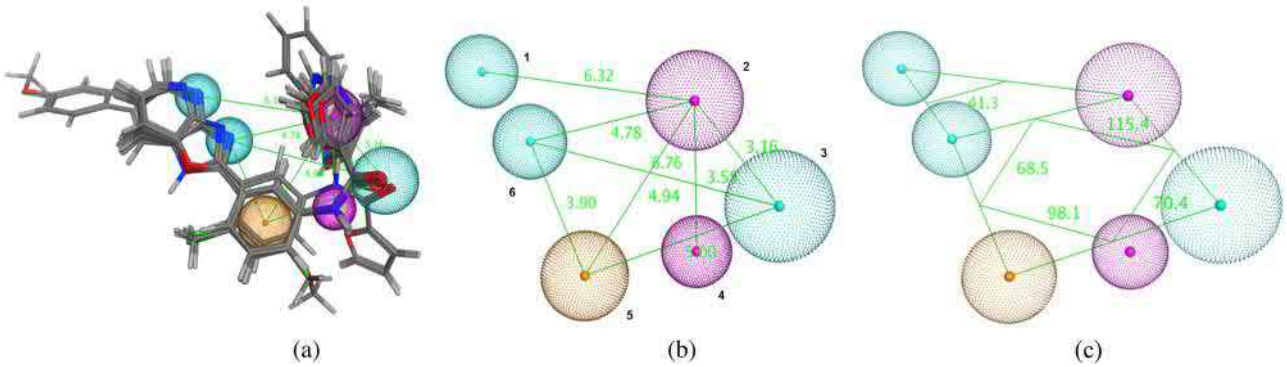


Fig. 4 Consensus pharmacophore model for dataset 2 (*cyan*: H-acceptor, *magenta*: H-donor, *yellow*: hydrophobic). a Pharmacophore model with aligned ligands; **b** pharmacophore model with distances (Å) between different features; **c** pharmacophore model with angles between different features

LIQUID 1.0 were used for the generation of consensus pharmacophore model using default settings.

Results and discussion

Consensus pharmacophore model for dataset-1

The consensus pharmacophore model reveals that the pharmacophoric pattern (see Fig. 3) for dataset-1 consists of two hydrophobic regions at a distance of 5.40 Å from each other, one H-bond acceptor at distance of 4.70 and 5.55 Å from the two hydrophobic regions, and a H-bond donor region at a distance of 4.84 and 3.50 Å from the two hydrophobic regions.

Consensus pharmacophore model for dataset-2

The consensus pharmacophore model for dataset-2 consists of six pharmacophoric features (see Fig. 4). The H-bond

Table 3 Statistical parameters for various GA-MLR QSAR models for dataset-1

S. no	Statistical parameter	Model-1.1	Model-1.2	Model-1.3	Model-1.4
1	N_{tr}	32	26	26	26
2	N_{ex}	--	6	6	6
3	Number of descriptors	3	3	3	3
4	R^2_{tr}	0.8284	0.8155	0.8142	0.8139
5	$R^2_{adj.}$	0.8100	0.7903	0.7889	0.7886
6	LOF	0.0509	0.0628	0.0466	0.0467
7	Kxx	0.2148	0.1665	0.2122	0.2358
8	ΔK	0.1547	0.1717	0.1569	0.2031
9	$RMSE_{tr}$	0.1833	0.1928	0.1785	0.1786
10	MAE_{tr}	0.1572	0.1597	0.1161	0.1115
11	CCC_{tr}	0.9061	0.8983	0.8976	0.8974
12	S	0.1960	0.2096	0.1941	0.1942
13	F	45.0488	32.4047	32.1362	32.0824
14	R^2_{cv} (Q^2_{loo})	0.7719	0.7342	0.7707	0.7672
15	$RMSE_{cv}$	0.2114	0.2314	0.1983	0.1998
16	MAE_{cv}	0.1806	0.1923	0.1319	0.1303
17	CCC_{cv}	0.8746	0.8556	0.8741	0.8754
18	Q^2_{LMO}	0.7343	0.7425	0.7456	0.7240
19	R^2_{Yscr}	0.1072	0.1092	0.1236	0.1100
20	$RMSE_{ex}$	–	0.1504	0.2283	0.2235
21	MAE_{ex}	–	0.1266	0.1929	0.1931
22	R^2_{ex}	–	0.8298	0.8271	0.8271
23	Q^2_{F1}	–	0.8770	0.8289	0.8359
24	Q^2_{F2}	–	0.8092	0.8186	0.8260
25	Q^2_{F3}	–	0.8878	0.6962	0.7086
26	CCC_{ex}	–	0.8871	0.9074	0.9021

donor feature **2** is at a distance of 4.94, 6.32, and 4.78 Å from hydrophobic **5**, H-bond acceptor **1** and **6** features, respectively. The H-bond acceptor region **3** is at a distance of 3.16, 6.76, and 5.00 Å from H-bond donor region **2**, H-bond acceptor region **6**, and hydrophobic region **5**, respectively.

QSAR analysis

The datasets used in the present study are small but the molecules are either positional or functional isomers, thereby, cover significant chemical space. For small datasets, we have previously demonstrated (Masand et al., 2015) that at least one QSAR model must be built using undivided whole dataset to capture maximum relevant descriptors, which in turn not only provides useful information for future modification but is useful for comparison and assessment of QSAR models constructed using divided datasets, also. Therefore, models were derived using divided and undivided datasets.

While performing the QSAR analysis, generally stepwise regression, GA, etc. algorithms are employed for SFS. This results in a good number of MLR models with nearly comparable statistical characters embracing different descriptors. Mostly, in such a situation, only one MLR model is selected on the basis of its statistical performance. The drawbacks, however, of this 'first among equals' approach (Masand et al., 2014a, b) are (1) accurate and rational association of descriptors with correct structural features is challenging for a QSAR model involving complex/esoteric descriptors only, (2) a single QSAR model may be influenced by (i) the method of splitting, composition of training and prediction sets, procedure used for descriptor pruning and selection, and (ii) some molecules in the training/prediction dataset. An easy and possible solution to overcome the different drawbacks of 'first among equals' approach is to build multiple models using multiple splitting (Masand et al., 2014a, 2015). This also helps in capturing less privileged yet useful structural features that govern the activity. Hence, in the present study, multiple models were built for both the sets.

Dataset-1: For dataset-1, the various GA-MLR QSAR models are as follow:

Table 4 Experimental and predicted pIC_{50} values along with the status of the molecules in different models

S. no.	Exp. pIC_{50}	Status model-1.1	Pred. pIC_{50} model-1.1	Status model-1.2	Pred. pIC_{50} model-1.2	Status model-1.3	Pred. pIC_{50} model-1.3	Status model-1.4	Pred. pIC_{50} model-1.4
1	5.5190	Training	5.4457	Training	5.4674	Prediction	5.4166	Prediction	5.4850
2	5.6380	Training	5.5113	Training	5.5605	Training	5.5774	Training	5.6531
3	5.6780	Training	5.6936	Training	5.4025	Training	5.3535	Training	5.4856
4	5.6780	Training	5.7937	Training	5.7839	Training	5.7267	Training	5.7525
5	5.7450	Training	5.6184	Prediction	5.6472	Training	5.6961	Training	5.7507
6	6.2920	Training	6.0440	Training	6.1053	Training	6.2829	Training	6.1439
7	5.7700	Training	5.5688	Training	5.9221	Training	5.8677	Training	5.8572
8	5.9590	Training	5.8578	Training	5.6516	Prediction	5.5349	Prediction	5.5589
9	5.6990	Training	5.5577	Training	5.7362	Training	5.7246	Training	5.6937
10	5.4200	Training	5.5854	Training	5.6094	Training	5.5644	Training	5.5986
11	5.2220	Training	5.3502	Prediction	5.4240	Training	5.3370	Training	5.3955
12	5.3370	Training	5.2989	Training	5.4585	Prediction	5.3818	Prediction	5.5919
13	5.0420	Training	5.1085	Training	5.1915	Training	5.0538	Training	5.0516
14	5.5620	Training	5.4327	Training	5.6515	Training	5.6200	Training	5.4868
15	6.0510	Training	6.0293	Prediction	6.0860	Training	6.0680	Training	6.0254
16	5.1350	Training	5.1857	Prediction	5.3721	Training	5.3065	Training	5.1638
17	6.2010	Training	5.9086	Training	5.9231	Training	5.6231	Training	5.6841
18	5.2440	Training	5.6305	Training	5.6857	Training	5.6521	Training	5.7911
19	5.4560	Training	5.6239	Training	5.6531	Training	5.6474	Training	5.5023
20	5.7210	Training	5.8896	Training	5.7213	Prediction	5.9763	Prediction	5.8707
21	5.9590	Training	6.0595	Prediction	5.9813	Training	5.9427	Training	5.9459
22	5.9590	Training	5.7816	Training	5.7378	Training	5.9660	Training	5.9743
23	6.0090	Training	5.7663	Training	5.7348	Training	5.7544	Training	5.9328
24	6.3870	Training	6.1195	Training	6.0564	Training	6.2657	Training	6.1155
25	6.0810	Training	6.3177	Training	6.0790	Training	5.9855	Training	6.0830
26	6.0460	Training	6.3863	Training	6.0855	Prediction	6.2253	Prediction	6.1972
27	6.3280	Training	6.2824	Training	6.4306	Training	6.3806	Training	6.2962
28	6.2760	Training	6.3741	Training	6.3844	Training	6.3129	Training	6.3017
29	7.0000	Training	6.7187	Training	6.9288	Prediction	6.8487	Prediction	6.8315
30	6.7210	Training	6.7775	Training	6.8455	Training	6.7595	Training	6.8521
31	5.6380	Training	5.9028	Training	5.8545	Training	5.7107	Training	5.7471
32	5.5850	Training	5.7374	Prediction	5.4196	Training	5.5969	Training	5.4917

Model-1.1 (undivided dataset): $pIC_{50} = 4.8523(\pm 0.3676) - 0.9802(\pm 0.4082) * GATS8c + 0.1240(\pm 0.0734) * RDF40p + 0.0552(\pm 0.0099) * RDF55s$

Model-1.2: $pIC_{50} = 6.4521(\pm 1.2282) - 3.1455(\pm 2.3630) * E1s + 0.0545(\pm 0.0492) * RDF40m + 0.0489(\pm 0.0111) * RDF55s$

Model-1.3: $pIC_{50} = 5.2245(\pm 0.9946) - 3.4904(\pm 1.8452) * E1s + 0.2540(\pm 0.1731) * AD2D423 + 0.9151(\pm 0.2562) * cbrtRDF55s$

Model-1.4: $pIC_{50} = 7.4582(\pm 0.9582) - 0.8339(\pm 0.5028) * GATS6m + 0.0425(\pm 0.0128) * RDF55s - 3.2008(\pm 1.8707) * E1s$

The various parameters calculated for models 1.1–1.4 have been tabulated in Table 3. From Table 3, it is clear that the developed models satisfy threshold values for most of the internal and external validation parameters. For dataset-1, GA analysis for undivided whole dataset resulted in many QSAR models, but, only one model (model-1) was found to satisfy the threshold values for various statistical parameters. A simple comparison of different statistical parameters for different models (see Table 3) reveals that the models 1.1–1.4 are statistically robust with good external predictive ability. For the model 1.2, $Q^2_{F1} > R^2$, this leads to the contrasting inference that the model

Table 5 Statistical parameters for various GA-MLR QSAR models for dataset-2

S. no.	Statistical parameter	Model-2.1	Model-2.2	Model-2.3	Model-2.4
1	N_{tr}	31	31	25	25
2	N_{ex}	--	--	6	6
3	Number of descriptors	4	4	4	4
4	R^2_{tr}	0.8176	0.7834	0.8292	0.8650
5	$R^2_{adj.}$	0.7895	0.7500	0.7951	0.8380
6	LOF	0.0840	0.0997	0.0948	0.0750
7	Kxx	0.2711	0.2185	0.2343	0.2425
8	ΔK	0.0590	0.1123	0.0738	0.0593
9	$RMSE_{tr}$	0.2150	0.2343	0.2094	0.1862
10	MAE_{tr}	0.1753	0.1968	0.1741	0.1663
11	CCC_{tr}	0.8996	0.8785	0.9067	0.9276
12	*s*	0.2348	0.2558	0.2341	0.2082
13	*F*	29.1302	23.5052	24.2815	32.0406
14	R^2_{cv} (Q^2_{loo})	0.7480	0.6889	0.7348	0.7864
15	$RMSE_{cv}$	0.2527	0.2808	0.2610	0.2343
16	MAE_{cv}	0.2076	0.2357	0.2175	0.2094
17	CCC_{cv}	0.8604	0.8267	0.8539	0.8841
18	Q^2_{LMO}	0.7441	0.7246	0.7420	0.8083
19	R^2_{Yscr}	0.1426	0.1495	0.1479	0.1690
20	$RMSE_{ex}$	–	–	0.2378	0.2456
21	MAE_{ex}	–	–	0.1828	0.1874
22	R^2_{ex}	–	–	0.7574	0.7417
23	Q^2_{F1}	–	–	0.7670	0.7515
24	Q^2_{F2}	–	–	0.7478	0.7310
25	Q^2_{F3}	–	–	0.7798	0.7651
26	CCC_{ex}	–	–	0.8477	0.8302

is able to predict better for the prediction set than the training set.

In all the models, RDF55s (radial distribution function-055/weighted by relative I-state, a 3D-descriptor) is commonly present with a positive coefficient. Therefore, RDF55s has a positive correlation with the activity. Another descriptor with positive relation with the activity is AD2D423 (presence of oxygen-halogen at a topological distance of 6). E1s (1st component accessibility directional WHIM index/weighted by relative I-state, a 3D-descriptor) is present in the models 2–4 with a negative coefficient, indicating its negative relation with the activity.

Model-1.1

The high value of R^2_{tr}, CCC_{tr}, R^2_{cv}, CCC_{cv} and *F* with close value of R^2_{adj} indicates that the model is statistically robust with adequate number of descriptors, i.e., it is free from over-fitting. This is further confirmed by the low value of LOF, $RMSE_{tr}$, *s*, $RMSE_{cv}$, and R^2_{Yscr}. The correlation, residual, and applicability domain plots further confirm the statistical quality of the model. In addition, the model is based on descriptors having very low inter-correlation (see the supplementary material).

Models 1.2–1.4

For models 1.2–1.4, R^2_{tr}, CCC_{tr}, R^2_{cv}, CCC_{cv}, R^2_{adj} and *F* satisfy the recommended threshold value, which indicates that the models are statistically robust with acceptable number of descriptors. The CV parameters R^2_{cv}, $RMSE_{cv}$, MAE_{cv}, CCC_{cv}, and Q^2_{LMO} vindicate the statistical robustness of the models. The external predictive ability of the models is confirmed by the high values of R^2_{ex}, Q^2_{F1}, Q^2_{F2}, Q^2_{F3}, and CCC_{ex}.

In Table 4, the experimental and predicted pIC_{50} values along with the status of the molecules in different models have been tabulated.

Dataset-2: For dataset-2, the statistical parameters for various GA-MLR QSAR models are tabulated in Table 5.

Table 6 Experimental and predicted pIC_{50} values along with the status of the molecules in different models

S. no.	Exp. pIC_{50}	Status model-1	Pred. pIC_{50} model-1	Status model-2	Pred. pIC_{50} model-2	Status model-3	Pred. pIC_{50} model-3	Status model-4	Pred. pIC_{50} model-4
1	6.6580	Training	6.5467	Training	6.4296	Training	6.5480	Training	6.4840
2	6.1870	Training	6.2442	Training	6.1332	Training	6.2457	Training	6.3019
3	5.4950	Training	5.6939	Training	5.4748	Training	5.7024	Training	5.7097
4	5.8240	Training	5.6856	Training	5.7094	Training	5.6941	Training	5.7602
5	6.5230	Training	6.5996	Training	6.4762	Training	6.6009	Training	6.5297
6	5.7450	Training	5.7470	Training	5.6548	Training	5.7577	Training	5.8656
7	5.8240	Training	5.5625	Training	5.5603	Training	5.5795	Training	5.5277
8	5.2440	Training	5.3701	Training	5.3789	Prediction	5.3871	Prediction	5.4355
9	5.4690	Training	5.8982	Training	5.8452	Training	5.9079	Training	5.7716
10	6.4690	Training	6.3096	Training	6.1833	Training	6.3113	Training	6.3235
11	6.7700	Training	6.3267	Training	6.2714	Prediction	6.3271	Prediction	6.2664
12	5.5380	Training	5.4968	Training	5.5203	Training	5.5056	Training	5.4149
13	6.2220	Training	6.4666	Training	6.3659	Prediction	6.4676	Prediction	6.3956
14	5.8860	Training	5.7585	Training	5.7135	Training	5.7683	Training	5.6735
15	5.9590	Training	5.6389	Training	5.7195	Training	5.6374	Training	5.7643
16	5.2080	Training	5.3692	Training	5.4802	Training	5.3752	Training	5.2679
17	5.2010	Training	5.3822	Training	5.7036	Training	5.3890	Training	5.4388
18	6.0270	Training	6.3231	Training	6.2825	Training	6.3310	Training	6.2565
19	6.2440	Training	6.0928	Training	6.0622	Training	6.1008	Training	6.0480
20	6.5090	Training	6.5092	Training	6.4943	Prediction	6.5165	Prediction	6.4971
21	5.5850	Training	5.4202	Training	5.4028	Training	5.4376	Training	5.4607
22	6.5380	Training	6.6790	Training	6.7607	Training	6.6888	Training	6.7277
23	7.0000	Training	6.6736	Training	6.7892	Training	6.6827	Training	6.6325
24	5.6990	Training	5.6207	Training	5.8055	Training	5.6388	Training	5.7560
25	6.9210	Training	6.9418	Training	7.0711	Training	6.9505	Training	7.0062
26	6.1190	Training	6.1746	Training	6.3624	Training	6.1920	Training	6.2441
27	6.9210	Training	6.5269	Training	6.5242	Training	6.5261	Training	6.7398
28	6.2010	Training	6.3307	Training	6.3314	Training	6.3300	Training	6.3100
29	6.2600	Training	6.2680	Training	6.2274	Prediction	6.2681	Prediction	6.4581
30	6.1080	Training	6.3574	Training	6.3239	Prediction	6.3573	Prediction	6.1536
31	6.0000	Training	6.3396	Training	6.2961	Training	6.3397	Training	6.2262

Model-2.1 (undivided dataset): $pIC_{50} = 3.6176(\pm1.1170) + 4.7639(\pm1.2164) * MATS7s - 0.3082(\pm 0.1906) * FP144 + 0.9061(\pm0.2021) * KRFPC3159 - 1.2136(\pm0.9844) * AM1\_LUMO$

Model-2.2 (undivided dataset): $pIC_{50} = 3.4617(\pm1.2086) + 3.9877(\pm1.2580) * MATS7s - 0.2446(\pm0.2206) * ExtFP474 + 0.8142(\pm0.2232) * KRFPC3159 - 1.4583(\pm1.0627) * AM1\_LUMO$

Model-2.3: $pIC_{50} = 3.6318(\pm1.2369) + 4.7733(\pm1.2560) * MATS7s - 0.3012(\pm0.2150) * FP144 + 0.8978(\pm0.2222) * KRFPC3159 - 1.2077(\pm1.0827) * AM1\_LUMO$

Model-2.4: $pIC_{50} = 3.6897(\pm0.8080) + 4.7111(\pm1.1005) * MATS7s - 0.2938(\pm0.1905) * FP144 + 0.9284(\pm0.1977) * KRFPC3159 + 0.0221(\pm0.0133) * PEOE\_VSA\_POL$

From Table 5, it is clear that all the models satisfy the threshold value for many parameters that are used to judge the external predictive ability of a QSAR model. MATS7s (Moran autocorrelation–lag 7/weighted by I-state, a 3D-descriptor) and KRFPC3159 (a finger print descriptor which stands for presence of furan ring) are present in all the models with positive coefficient. This indicates that furan ring has good correlation with the activity and it must be retained in future developments. A plausible explanation of molecular descriptor MATS7s could be that the compounds should have atoms at a topological distance of 7 with different I-state as a tendency. This means that one atom *i* should have I-state higher than the average I-state of the molecule and the other atom *j* should display the opposite.

From models 2.1–2.3, it appears that AM1_LUMO (least unoccupied molecular orbital calculated by AM1 method) has negative correlation with the activity. AM1_LUMO signifies the overall eletrophilicity of a molecule. To enhance the activity, the value of MATS7s and KRFPC3159 must be as high as possible, whereas reverse is true for AM1_LUMO. PEOE_VSA_POL is a descriptor that corresponds to van de Waal's polar surface area. The positive coefficient for PEOE_VSA_POL indicates its positive correlation with the activity.

In Table 6, the experimental and predicted pIC_{50} values along with the status of the molecules in different models have been presented.

Conclusions

In conclusion, robust QSAR models with good external predictive ability have been developed. The developed models, since, satisfy the threshold values for many statistical parameters that are essential to ascertain the quality and utility of a QSAR model, the developed models could be useful for future optimization of the activity profile of the molecules used in dataset 1 and 2. The pharmacophore models revealed the characteristic features that are essential for activity. Both pharmacophore and QSAR models provide consensus and complementary prospective ideas about the structural features that must be considered.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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Computational Strategies to Explore Antimalarial Thiazine Alkaloid Lead Compounds Based on an Australian Marine Sponge *Plakortis Lita*

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ABSTRACT

In this work, an attempt was made to propose new leads based on the natural scaffold Thiaplakortone-A active against malaria. The 2D QSAR studies suggested that three descriptors correlate with the anti-malarial activity with an R^2 value of 0.814. Robustness, reliability and predictive power of the model were tested by internal validation, external validation, Y-scrambling and Applicability domain analysis. HQSAR studies were carried out as an additional tool to find the sub-structural fingerprints. The CoMFA and CoMSIA models gave Q^2 values of 0.813 and 0.647, and R^2_{ncv} values of 0.994 and 0.984, respectively. Using the 2D-QSAR equation, the activity values of the seven modified compounds were calculated and it was found that three molecules showed good anti-malarial activity. Molecular docking of the 42 Thiaplakortone-A derivatives with *P. falciparum* calcium-dependent protein kinase 1 (*Pf*CDPK1) was carried out to find out protein-ligand interactions. Data mining of the bioassay dataset AID: 504850 using the classifier based on Random Forest (RF) of Weka suggested that all of the eight molecules selected and three out of the seven virtual molecules were anti-malarial active. Both the virtual molecules and drug molecules were docked with CYP3A4, indicating that the virtual molecules could metabolize easily. Toxicity studies using Osiris shows that three molecules showed no toxic characters.

Keywords: Malaria, 2D-QSAR, HQSAR, 3D-QSAR, Molecular docking

Introduction

Malaria is a devastating global health threat. Approximately half of the world population is at the risk of being infected by the disease (White et al., 2014). Malaria is caused by five *Plasmodium* species: *P. falciparum, P. ovale*, *P. vivax*, *P. malarie* and *P. knowlesi*, of which *P. falciparum*, sources cerebral malaria and is the primary cause of mortality. Because of the emergence of multi drug-resistant pathogens, there is an urgent necessity for the development of new drugs. Many of the newly synthesized medicines do possess many side effects. Many multinational drug companies keep unethical business and profit-oriented mindset which leaves, insufficient input in promoting research. Thus research and development, leading to the discovery of medicines for diseases which affect the third world countries, are meager. The increase and spread of artemisinin-resistant parasites is alarming, since no drugs are available to replace the ACTs as the frontline anti-malarial treatment. This necessitates the importance of modification of the existing drugs or of designing new drugs.

Calcium-dependent protein kinases are lacking in human beings, but their presence is reported in plants and Alveolates (Harper and Harmon, 2005). *Pf*CDPK1 is a calcium-dependent calmodulin-independent protein kinase which is expressed in the asexual blood stages of the parasite responsible for disease pathology (Zhao et al., 1993). It is concerned in parasite motility and host cell invasion, where it is able to phosphorylate components of the molecular motor that drives parasite invasion of red blood cells (Green et al., 2008; Holder et al., 2012). The prevention of this invasion process could break the parasite lifecycle, causing the parasites to die. Pf CDPK1 therefore represents a novel target for the potential treatment of malaria. Earlier workers have reported inhibitors of *Pf*CDPK1 and inhibitors of the CDPK1 enzymes from the

related Apicomplexan protozoa, Toxoplasma gondii and Cryptosporidium parvum (Kato et al. 2008; Lemercier et al. 2009).

The effectiveness of natural products has stimulated scientists to search for new paths in drug design (Newman et al., 2012)**.** Due to the ease of accessibility, pharmaceutical synthesis has drawn greatest consideration towards herbal medicines. One fourth of the drugs prescribed worldwide at present comes from plants and 60% of anti-infectious drugs already on the market or under clinical investigations are of natural origin (Sala et al., 2011). This highlights the importance of developing natural products based drugs for diseases like malaria (López et al., 2009).

Many scientists have put in their efforts to conduct studies to understand the anti-malarial activities of plant based products. Researchers have reported the *in vivo* and *in vitro* studies of the *Aspidosperma* (Apocynaceae) plant against malarial parasite and concluded that the plant species is likely to be useful in the further development of an anti-malarial drug (Aguiar et al., 2015). Mojarrab et al., studied the anti-malarial activity of the three Iranian *Artemisia* species, *A. ciniformis*, *A. biennis* and *A. turanica* by invitro β-hematin formation assay. They observed that the dichloromethane (DCM) extract of *A. ciniformis* and ethyl acetate (EtOAc) extracts of *A. biennis* and *A. turanica* showed significant anti-malarial activities (Mojarrab et al., 2015). But it can be seen that many of the phytochemicals as such are not so active against the malarial parasites. Possibility of another derivative with more biological activity is not probed. This could result in the loss of vital information regarding the formulation of any active derivatives out of the chosen phytochemicals. Therefore it is wise to develop derivatives out of the phytochemicals and examine their activities.

Thiaplakortones A-D displayed significant growth inhibition against chloroquine-sensitive (3D7) and chloroquine-resistant (Dd2) *P. falciparum* (IC_{50} values <651 nM) and showed only moderate cytotoxicity against HEK293 cells (IC_{50} values >3.9 μM). Thiaplakortone-A, is the most active natural product found in the Australian marine sponge *Plakortis lita* and shows IC_{50} values of 51.0 and 6.6 nM against chloroquine-sensitive (3D7) and multidrug resistant (Dd2) *P. falciparum* parasite lines, respectively (Davis et al., 2013). These studies suggest that none of the modification resulted in getting derivatives with activity higher than that of Thiaplakortone-A.

Synthesis and structural characterization of new compounds and the subsequent understanding of their biological activity are rather cumbersome procedures which demand time, expense and hard work. This is where computational drug design comes for our help and understanding. Quantitative Structure-Activity Relationship (QSAR) study can be used to demonstrate how the structural features of molecules relate to the biological activity using chemometric methods. Thus we can replace the expensive biological tests or experiments, since QSAR studies can be used to predict the responses to the new compounds. The QSAR technique mathematically correlates the biological activity of the molecules with their various structural features called molecular descriptors. Accordingly, the QSAR technique has been widely reformed by different groups of researchers for identifying novel chemical entities. We have earlier reported the QSAR and molecular docking studies for a series of 7-substituted-4-aminoquinoline derivatives to study their anti-malarial activity (Shibi et al., 2015). Sainy and Sharma have reported the anti-malarial activity studies of thiolactone derivatives using QSAR and molecular docking studies (Sainy and Sharma, 2015). Masand et al., 2015 reported the application of QSAR studies to find out the anti-proliferative activity of substituted Phenyl 4-(2-Oxoimidazolidin-1-yl) benzenesulfonates. In an attempt for finding out new potent anti-malarial agents, we have performed 2D- and 3D-QSAR studies on Thiaplakortone-A derivatives for quantifying the necessary structural and physicochemical requirements for designing some leads as potent anti-malarial drugs.

The present study is focused on understanding the theoretical aspects of the structural features of the natural scaffold Thiaplakortone-A and the derivatives for designing new drug molecules. For the purpose, more than 15000 molecular descriptors which represent the structural characterization of Thiaplakortone-A and its derivatives are generated to understand a logical correlation between structure and activity. We also constructed highly predictive and acceptable HQSAR model (Moda et al., 2007). The comparative molecular field analysis (CoMFA) was carried out to find the correlation of steric and electrostatic properties with that of their biological activity (Cramer et al., 1988). The role of steric, electrostatic, hydrophobic and H-bond donor in properly curating the molecules is of utmost importance. Hence we carried out comparative molecular similarity indices analysis (CoMSIA) studies (Klebe et al., 1994).

Molecular docking also plays an important role in drug design and elucidation of protein-ligand complex structures (Amtul et al., 2007). It can be used prior to experimental screening and

can be considered as a dominant tool to reduce the effort and cost needed for the development of new drug molecules. The interactions of drug molecules with the receptor protein were effectively understood by a molecular docking analysis. Several studies have revealed the pivotal role played by the drug candidates in interacting with the protein *P. falciparum* calcium-dependent protein kinase 1 (*Pf*CDPK1), which prompted us to select the same proteins for our study (Bansal et al., 2013; Holder et al., 2012). A series of 42 analogues of Thiaplakortone-A were selected for the present study.(Schwartz et al., 2015). All the 42 compounds were docked with both the two protein targets which aided the filtering of eight molecules. Based on the structural information gathered from the above studies seven new virtual molecules were created. Using the 2D-QSAR model developed, the biological activity of the new virtual molecules were computed and were found to be very much promising. Before further analysis another screening was carried out by artificial intelligence method based on Random Forest (RF) algorithm of Weka. Based on the RF model virtual screening of all the 42 Thiaplakortone-A derivatives and seven virtual molecules was conducted and it was found that 18 molecules from the Thiaplakortone-A bunch and four of the new virtual molecules possess anti-malarial properties. It is also interesting to note that those molecules screened by Weka and possessing high docking score are also validated by Osiris software which exemplifies their lead like properties. Several studies found out that the metabolic action with respect to Cytochrome P450 gives a proper indication regarding the toxicity of the selected molecules.

Materials and Methods

Datasets

The 3D-structures of the 42 Thiaplakortone-A derivatives were drawn in Molecular Operating Environment (MOE) software (MOE Version 2007.09, Chemical Computing Group, Inc.). Each compound was energy minimized and optimized by using Merck Molecular Force Field (Halgren, 1996). Structures of the selected Thiaplakortone-A derivatives and their respective inhibitory activities are given in **Table 1.**

2D-QSAR

2D-QSAR studies require the calculation and selection of suitable descriptors. The energy-minimized geometry of each molecule was used for the calculation of the various descriptors. The MOE and PaDEL-Descriptor programs were used for the calculation of 15533

different descriptors. Descriptor selection is carried out by the software PHAKISO. “General” and “CORCHOP” are the main descriptor filtration algorithms in PHAKISO. “General” method is used to remove descriptors which have the same values and with missing values. And “CORCHOP” method was used to remove the repeated, inter-correlated and correlated descriptors. After the selection, the pool of descriptors was reduced to 214. These descriptors were selected as independent variables and IC_{50} as the dependent variable. The IC_{50} (nM) values were converted into pIC_{50} (-log IC_{50}), which can be used as dependent variable for subsequent 2D-QSAR analysis. The dataset was split into 85% training set and 15% test set for model development and subsequent validation. The software QSARINS was employed to search the optimum number and set of descriptors using multiple linear regression (MLR) models. (Gramatica et al., 2013)

2D-QSAR model validation

The quality of the built model is evaluated by internal validation, using squared cross validated corrrelation coefficient (Q^2).

$$Q^2 = 1 - \frac{\sum_{i=1}^{n}(Y_{pred} - Y_{exp})^2}{\sum_{i=1}^{n}(Y_{pred} - Y_{mean})^2} \quad (1)$$

where Y_{pred}, Y_{exp} and Y_{mean} are the predicted, experimental and mean values of the pIC_{50}, respectively.

The predictive ability of the developed QSAR model is estimated using correlation coefficient (R), standard deviation (s), Fischer’s test (F), level of confidence (p), predicted residual sums of squares standard deviation (S_{PRESS}) and standard deviation error in prediction (S_{DEP}).

The model is further validated by external validation, Y-randomization and applicability domain calculation. The external validation of the built model is tested with the following parameters: r^2_{ext} (external determination coefficient), Q^2_{F1}, Q^2_{F2}, Q^2_{F3}, Concordance Correlation Coefficient (CCC), CCC_{ext}, r^2_m, and Δr^2_m. Δr^2_m estimated the closeness between the values of the predicted and the corresponding observed activity values. Y-randomization experiment is performed to detect the chance correlations between the dependent variable and the descriptors. (Rücker et al., 2007)

Other parameters such as R_0^2, $R_0'^2$, k, and k' are also calculated. R_0^2 (Predicted vs. observed activities) and $R_0'^2$(observed versus predicted activities) are the coefficients of correlation obtained by the regression lines through the origin, with the intercept set to zero. Generally for a model with good predictive ability, R_0^2 or $R_0'^2$ must be equal to or lower than R^2. For the slope value k (predicted versus observed activities) or k' (observed versus predicted activities), suitable range is $0.85 \leq k \leq 1.15$ or $0.85 \leq k' \leq 1.15$. (Golbraikh & Tropsha, 2002; Zhang et al., 2006)

Applicability domain (AD) calculation is applied to investigate the possible presence of outliers in the whole data set. The AD of the developed QSAR models is evaluated using the standardization approach. (Roy et al., 2015)

HQSAR

The structures of Thiaplakortone-A derivatives were converted into fragments initially utilizing the default fragment size of 4-7 atoms per fragment. All fragments were assigned in characterized molecular hologram lengths (53, 59, 61, 71, 83, 97, 151, 199, 257, 307, 353, 401 bins) and fragment distinction examination was performed in terms of atoms, bonds, connectivity, hydrogen and donor/acceptor atoms. During the HQSAR runs, different combinations of these parameters were considered. After the partial least-squares (PLS) analysis, several QSAR models were generated for each distinguishing fragment. The internal validation was performed by leave-one-out (LOO) cross-validation. An external validation was performed with the test set of 7 compounds, which was not considered in the HQSAR model development.

3D-QSAR

All the selected Thiaplakortone-A derivatives and their inhibition zone were used to construct CoMFA and CoMSIA models using the program SYBYL (Tripos International, Missouri, USA). For both CoMFA and CoMSIA analysis, the standard Tripos force field was employed. The total set of 42 molecules was divided into two subsets: the training set and the test set comprising 35 and 7 compounds, respectively, in such a way that both sets cover the entire range of biological activity (Table 1). The training set was used to generate the models and the test set was used to validate the quality of the models. The partial atomic charges of the compounds were calculated by the Gasteiger-Hückel method and energy minimizations were performed using the Tripos force field (Gasteiger and Marsili, 1980) with 1000 iterations, a

distance-dependent dielectric and the Powell conjugate gradient algorithm convergence criterion of 0.01 kcal mol^{-1} Å (Clark et al., 1989).

3D-QSAR model construction

The steric (S) and electrostatic (E) CoMFA potential fields were computed at every lattice intersection of a regularly spaced grid of 2.0Å. The grid pattern was generated automatically by the SYBYL/CoMFA routine. The steric (Lennard-Jones potentials) and electrostatic (Coulombic potentials) fields were ascertained utilizing the standard Tripos force fields in 3D grid with a spacing of 2.0 Å and extended at least 4.0 Å beyond the van der Waals around aligned compounds in all directions. A distance-dependent dielectric constant of 1.00 was used. A sp^3 hybridized carbon atom with +1 charge served as probe atom to calculate steric and electrostatic fields. The steric and electrostatic contributions were reduced to +30.0 kcal/mol and electrostatic contributions were ignored at the lattice intersections with maximal steric interactions. The minimum sigma (column filtering) was set to 2.0 kcal/mol to increase the efficiency of the predictive models and to decrease the noise so that the columns possessing less energy variance in contrast to this value were excluded from analysis. To derive appropriate results, the "StDev*coeff" (the standard deviation and the coefficient) values as different weighing factors in addition to the grid spacing were performed.

For CoMSIA examination, five similarity indices comprising of steric (S), electrostatic (E), hydrophobic (H), hydrogen bond donor (D) and hydrogen bond acceptor (A) fields were computed for each lattice with a grid of 2Å. The CoMSIA descriptors were acquired utilizing the settings of sp^3 carbon atom as a probe atom with a +1 charge, +1 hydrophobicity, +1 hydrogen bond donor and +1 hydrogen bond acceptor at each lattice. A default value of 0.3 was used as the attenuation factor.

3D-QSAR Statistical Results

Partial least squares (PLS) methodology for cross-validation with leave-one-out (LOO) method is used in 3D-QSAR to produce a series of coefficients (Bush and Nachbar, 1993; Wold et al., 2001). The most active molecule tpk1 was selected as the template for alignment. Different statistical parameters such as the cross-validated correlation coefficient (Q^2), non-cross-validated correlation coefficient (R^2_{ncv}), the predicted correlation coefficient (R^2_{pred}), standard error of

estimate (SEE), the optimum number of components (ONC) and F values were taken into consideration to validate the model.

The LOO cross-validation was performed to identify the cross-validated correlation coefficient (Q^2) values. Then the ONC perceived in the LOO cross-validation process was utilized in the final non-cross-validated PLS run with a column filter value of 2.0 kcal/mol. In CoMSIA, five descriptors available are considered. In all cases it has been established that the five different descriptor fields are not totally independent of each other and that such dependency among individual field usually decrease the statistical significance of the models (Bringmann and Rummey, 2003). We have generated 14 different models in order to build the optimal 3D-QSAR models with the highest Q^2 values and other statistical results for each class.

To evaluate the predictive power of 3D-QSAR models, the biological activities of the external test set were predicted. The R^2_{pred}, is calculated using the equation

$$R^2_{pred} = 1 - \frac{\sum(Y_{predicted} - Y_{observed})^2}{\sum(Y_{observed} - Y_{mean})^2} \quad (2)$$

where $Y_{predicted}$, $Y_{observed}$ and Y_{mean} are predicted, actual and mean values of the activity, respectively. $\sum(Y_{predicted} - Y_{observed})^2$ is the predictive sum of squares (PRESS).

To further validate the predictive ability of the constructed models, the following equation was used (Rännar et al., 1994):

$$R^2_{m\,(overall)} = R^2 \times \left(1 - \sqrt{R^2 - R_0^2}\right) \quad (3)$$

Molecular docking

Molecular docking studies were performed using MOE (Chemical Computing GroupInc, Montreal, Quebec, Canada), in order to investigate the interaction mechanism and probable binding mode of Thiaplakortone-A derivatives at the active site of Plasmodium falciparum calcium-dependent protein kinase 1 (*Pf*CDPK1). The 3D crystal structures of *Pf*CDPK1 (PDB ID: 3I79 & 3HZT) were retrieved from the protein data bank (Berman et al., 2007). This structure was protonated and energy minimized in MMFF94x force field to a gradient of 0.0001 kcal/mol/Å (Halgren, T.A. 1996). The "Site Finder" module in MOE was used to identify and place dummy atoms into the active site pocket. The default settings for all of the parameters including Ligand Placement (Triangle Matcher) and Rescoring (London dG) were used. The top scoring ligand pose from each docking run was used for calculation of binding energy.

The physicochemical properties of the selected protein molecules 3I79 and 3HZT were determined using Expasy ProtParam tool (Gasteiger et al., 2003). ProtParam in Expasy Proteomics Server computes various physicochemical properties that can be deduced from a protein sequence. The properties such as theoretical isoelectric point (pI), molecular weight, total number of positive and negative residues in the query, its value extinction coefficient, instability index (II), aliphatic index (AI) and grand average hydropathy (GRAVY) were computed.

Data mining

Data mining was carried out using Weka software, using RF algorithm. The RF algorithm creates multiple decision trees using bootstrap samples from the original training data set. Then the set of trees is used for classification. The RF algorithm is an ensemble classifier. It gives high accuracy and time efficiency for predictive data modeling. Classifier performance is evaluated via k-fold cross-validation. 10-fold cross-validation (CV) was used here as it gives higher bias in the expected prediction error but lower variance. The result from the test set is presented by a confusion matrix which consists of true positives (TP), false positives (FP), false negatives (FN) and true negatives (TN). It gives an idea to understand the results of the classification method. TP (TN) represents the total number of correctly classified instances and FN (FP) refers to the total number of respectively misclassified instances.

Prediction of metabolic behavior of the virtual molecules

SMARTCyp is used for predicting the reactivity at C, S, N, and P positions in a given ligand. (Rydberg et al., 2010) The results are obtained in the form of a structure and a table for each molecule. The 3 top ranking atoms are highlighted in the structure and table. From this the site with the highest probability of metabolism can be understood. If a given substrate had more than one known site of metabolism (SOM), only the highest predicted SOMs were considered.

Results and Discussion

2D-QSAR

In the present study, the natural scaffold Thiaplakortone-A based derivatives were taken to understand a logical correlation between structure and activity. Using the MLR analysis method the following 2D-QSAR model was obtained.

$\mathbf{pIC_{50}}$ = - 0.0072 (± 0.0033) **SMR_VSA1** - 0.0713 (± 0.0324) **MNDO_LUMO** - 6.4747 (± 3.4349) **maxHssNH** + 6.1505 (± 0.0429) .(4)

$R^2 = 0.814$; $R^2_{Adj.} = 0.790$; s = 0.1258; F = 33.825; $Q^2 = 0.757$; $p < 0.0001$

where R^2_{Adj} refers to the adjusted coefficient of determination, s is standard deviation, F indicates F-ratio test, Q^2 indicates cross validated correlation coefficient.

The three physicochemical descriptors (SMR_VSA1, MNDO_LUMO and maxHssNH) were involved in this QSAR equation. All the descriptors were found to contribute negatively to the biological activity of the compounds which indicates that decrease in their values would result in an increase in activity. The negative coefficient of the three descriptors indicates that the values should be kept as low as possible to have acceptable activity. The model has good capacity to explain the observed values of biological activity because it possesses high cross validated correlation coefficient ($Q^2 = 0.757$) and is further confirmed by the low standard deviation (s = 0.1258). The model explains 81.4% of the variance in the antimalarial activity.

The predictive ability of the model is very high, which is reflected by the external validation parameter values $RMSE_{ext} = 0.0981$, $Q^2_{F1} = 0.9311$, $Q^2_{F2} = 0.9244$, $Q^2_{F3} = 0.8684$ and $CCC_{ext} = 0.9524$, $r^2_{m\ aver} = 0.7117$, $\Delta r^2_m = 0.0954$. Other statistical parameters, i.e., R_0^2 and $R_0'^2$ were 0.758 and 0.700, respectively, whereas k and k' were 1.00 and 0.99, respectively. All the parameters were within acceptable range, and a small residual difference between the observed and predicted activities proved that the developed model is good.

SMR_VSA1 is the sum of v_i such that R_i is in (0.11,0.26). It belongs to the category generally called as the Subdivided Surface Areas which are descriptors based on an approximate accessible van der Waals surface area (in $Å^2$) calculated for each atom, v_i along with some other atomic property, p_i. If n_i is the number of occurrences of atomic number i in the molecule, then $p_i = n_i / n$ where n is the sum of the n_i. The v_i values are calculated using a connection table approximation. Each descriptor in a series is defined to be the sum of the v_i over all atoms i such that p_i is in a specified range (a,b). R_i denotes the contribution to Molar Refractivity for atom i as calculated in the SMR descriptor.

MNDO_LUMO is a potential energy based descriptor. It denotes the energy (eV) of the Lowest Unoccupied Molecular Orbital calculated using the MNDO Hamiltonian (Stewart, 1993). maxHssNH is a 2D descriptors which means Maximum atom-type H E-State of -NH-. Table 2 gives the values for observed, predicted antimalarial activities and residual values.

In the present work, only three descriptors have been incorporated in the final 2D-QSAR model, as adding more descriptors in the model resulted in negligible improvements only in the value of R^2. Hence, the heuristic search was limited to three descriptors to avoid over-fitting and to keep the model as simple as possible. Thus the model predicts the anti-malarial activities of the set molecules with greater predictability using the selected parameters.

Y-randomization test

Y-randomization experiment is performed to detect the chance correlations between the dependent variable and the descriptors. (Rücker et al., 2007) In the Y-randomization test, the dependent variable is scrambled and new QSAR models are developed using the same set of independent variables as present in the un-randomized model. The models obtained should be of poor quality. The statistics of the randomized models (Q^2 and R^2) should be poor, otherwise, each resulting model may be based on pure numerical effects. For an acceptable QSAR model, the average correlation coefficient (R_r) of randomized models should be less than the correlation coefficient (R) of non-randomized model. The extent of difference in the values of the mean squared correlation coefficients of the randomized (R_r^2) and that of the non-randomized (R^2) models is reflected in the value of ${}^cR_p^2$ parameter. (Todeschini, 2010) The value of ${}^cR_p^2$should be more than 0.5 for passing Y-randomization test.

$$ {}^cR_p^2 = R \times \sqrt{R^2 \times R_r^2} \qquad (5) $$

The biological activity, which is the dependent variable, is randomized keeping the others descriptors as such. In the present study, fifty randomized models were constructed and the model parameters obtained are presented in table 3. Since the ${}^cR_p^2$ value obtained is 0.792, the Y-randomization test was passed for our model.

Applicability domain (AD) analysis

In addition to Y randomization, AD of the developed QSAR model was analyzed by employing basic theory of standardization approach. It helps to define the model limitations with respect to its structural domain and response space. Applicability domain is the area of the physicochemical, structural, or biological space where the model is expected to be exploitable and the predictions are assumed to be trustable. The training set compounds define the boundaries of the chemical space where predictions could be considered as the result of data

interpolation. The algorithm and methodology proposed by Roy et al was used to define outliers (in the case of the training set) and to identify the compounds residing outside the AD to cross-examine and substantiate the QSAR model (Roy et al., 2015). No-X outliers were identified either in the training or in the test set which eliminates the chances of uncertainty in the predictions. This also proves that compounds in the training set are similar and their modelled 3D descriptors and modelled response reside within the AD.

HQSAR

It is not clear which substitution position and what kind of substituents could provide the most significant contribution to the *Pf*CDPK1 inhibitory activity. So HQSAR study was conducted to obtain more inclusive information. HQSAR is a 2D-QSAR method which creates structure activity relationship based on the fragments (Salum and Andricopulo, 2009). The extended form of the fingerprint used by it is known as a molecular hologram which contains all the possible molecular fragments within a molecule. The advantage of HQSAR technique is that it does not require the alignment of dataset on common template. HQSAR requires only 2D structure which employs specialized fingerprints as predicted variable of pharmacological activity, which is considered as dependent variable. Even though this method is 2D in nature, it utilizes 3D information such as chirality and molecular hybridization.

The best HQSAR model for *Pf*CDPK1 inhibitors was found to be the model with atoms, bonds, connectivity and hydrogen atoms (A/B/C/H) as the fragment distinction parameters. The results of the HQSAR analysis are reported in Table 4. The model shows good predictive capacity (R^2_{cv} = 0.682), high data fitting (R^2_{ncv} = 0.958) and low cross-validated standard error (SEP = 0.198).

Considering only the HQSAR contribution maps of the most active compound tpk1, (Fig. 1a), the generated model is able to identify fragments which increase the biological activity which are colored in yellow and green). However, in the case of least active compound tpk22, (Fig. 1b), the model identifies the fragments that decrease the activity which are coloured in red and orange in the model. The fragments colored in white (neutral contribution) highlights fragments without correlation with the biological activity variation.

The fragment CH_2 adjacent to the pyrrole ring exerts maximum contribution. Besides this, the hydrogen atom attached to the nitrogen of the pyrrole ring has also been marked green. The fragments contributing moderately to the activity profile of the molecules constitute N atom of the pyrrole ring bearing the R1 substituent. Thus, compounds bearing all of the essential molecular fragments exhibit potent anti-malarial activity, while those lacking the essential fragments lie down in the lower activity range.

Even though the above model explains how various alkyl groups affect the activity of a compound, the fragment collision is the limitation of HQSAR technique. Also the predictive quality of an HQSAR model is dependent on the setting of the atoms, bonds, connections, hydrogens and chirality parameters. So, 3D-QSAR studies have been carried out to find out how the presence of certain groups affects the anti-malarial activity of the compounds.

3D-QSAR

CoMFA statistical analysis

CoMFA and CoMSIA were generally used for understanding the QSAR at the 3D level. In 3D-QSAR methods the active conformer and superposition rule for a set of molecules were employed to clarify how the molecular structures of the compounds and bio-activity are related whereby the potency of the molecules could be predicted. The CoMFA methodology assumes that a suitable sampling of the steric and electrostatic fields around a set of aligned molecules provides all the information needed for understanding their biological properties. The statistical parameters of standard CoMFA models constructed for Thiaplakortone-A derivatives with steric and electrostatic fields are given in Table 6. PLS analysis of the training set showed a high Q^2 value of 0.813 with seven ONC. The non-cross-validated PLS analysis with the ONC results in an R^2_{ncv} of 0. 994, F = 216 and a SEE of 0.027.

Considering both steric and electrostatic field contributions, the former accounts for 59%, while the latter contributes 41%, indicating that the steric factor contributes slightly more to the binding affinities of the ligands to the target. The SEP (0.149) suggest a high degree of confidence in the analysis.

To further evaluate the reliability and predictability of the built model, the external test set of 7 compounds was used. The statistic parameters, R^2_{pred} = 0.892 and $R^2_{m\ (overall)}$ = 0.788,

suggested a good predictive ability of our CoMFA model. Table 7 lists the experimental, predicted activities and the residual values of the dataset.

CoMFA contour map analysis

The CoMFA contour map of steric field is revealed in Fig. 2a. The green contour recommends where increased steric bulk is connected with enhanced *Pf*CDPK1 inhibitory activity, whereas the yellow contour indicates the regions where sterically bulky groups would decrease the *Pf*CDPK1 inhibitory activity.

Near the R2 region, there is one big green contour which demonstrates the favorable impact of bulky groups in increasing the inhibitory activity of compounds. This is illustrated by comparing the activities of molecules 11 and 6 where the presence of bulky groups like i-Butyl, (> Ethyl) as R2 substituent would bring about higher pIC_{50} values (6.270 > 6.153), respectively.

This is additionally seen between compounds 12 and 8 where compound 12 possessed Cyclopentylmethyl group and displayed higher inhibitory activity (pIC_{50} = 6.219) and bulky characteristics than compound 8 which contained c-Propyl group as the R2 substituent (pIC_{50} = 5.943). By means of the bulky substituents (−Chloromethoxyphenyl > −Fluorophenyl) as R2 substituent would prompt pIC_{50} values in the compounds: 23 (pIC_{50} = 6.034) and 17 (pIC_{50} = 5.889).

This outcome can also be observed between compounds 36 and 27 where the higher activities can be related to the presence of – N(H)-adamantyl and – Furanyl substituents., The increase in the bulky behavior of the substituent (−Furanyl < – N(H)-adamantyl), increases the inhibitory activities also. (5.967 < 6.200).

The contour map of the electrostatic field of CoMFA model is shown in Fig. 2b. Increasing positive charge is favored in blue regions (contribution level of 80%) and increasing negative charge is favored in red regions (contribution level of 20%). The electrostatic contours map shows red contour at the R2 substituent. The presence of red contours illustrates the favorable effect of negative charge in some series of compounds. The electrostatic contour plot has a blue contour near R1 group and red contours around R2 group. The red contour above the R2 group can be explained by comparing compounds 14 and 13 where replacing of phenyl with

2-Fluorophenyl increases the negative charge behavior of this substituent. Consequently, the pIC_{50} values also increase (6.235 < 6.352). Further, the influence of the substituents can be understood by comparing compounds 19 (having −2, 6-Difluorophenyl group with pIC_{50} = 5.962) and 17 (having −4-Fluorophenyl group with pIC_{50} = 5.889) where the use of the negative charge group as R2 would lead to the increase of the inhibitory activities. The red contours observed near R2 substituent can be described considering compounds 23 and 16.; When the negative charge features increase (3-Chloro-4-methoxyphenyl > −3-Bromophenyl), the inhibitory activities (6.034 > 5.847) decrease. The blue contour above the R1 group can be explained by comparing compounds 41 and 42 where Benzyl is replaced with 4-Chlorobenzyl resulting in the negative charge behavior of this substituent, which was confirmed by the increased inhibitory activity (6.356 > 6.197).

CoMSIA statistical analysis

CoMSIA uses Gaussian approximation for calculating similarity indices at various grid points. But in CoMFA analysis the lattice points located inside the molecules are not taken into account in the statistical correlation analysis. Partial least square analysis with the CoMSIA descriptors resulted in satisfactory Q^2 and R^2_{ncv} values. Table 5 shows the summary of the statistical analysis of CoMSIA. In the CoMSIA, the LOO cross-validated value Q^2 with combined steric (S), electrostatic (E), hydrophobic (H) and hydrogen bond donor (D) fields was 0.647 with six ONC for training set. The non-cross-validated PLS analysis with the ONC results in an R^2_{ncv} of 0. 984, F = 292.970 and a SEE of 0.042. The percentage of the variance explained by S, E, H and D descriptors are 0.151, 0.313, 0.188 and 0.349, respectively.

The predicted pIC_{50} values with the CoMSIA model is found to be in good agreement with the experimental data within a statistically tolerable error range, with R^2_{pred} value of 0.718.

CoMSIA contour map analysis

The electrostatic contour map of the CoMSIA model is shown in the Fig. 3a, where increasing positive charge is favored in blue regions (contribution level of 80%) and increasing negative charge is favored in red regions (contribution level of 20%). The large blue contour around R2 group is explained by comparing compounds 11 and 32 where replacing i-Butyl with Ot-Bu increases the positive charge behavior of this substituent and accordingly, the pIC_{50} values

are increased (compound 11; pIC_{50} = 6.270 < compound 32; pIC_{50} = 6.123). Comparison of compounds 36 and 34 suggests that the negative charge groups (–N(H)-adamantyl> –N(Me) OMe) impart higher biological activities (6.200 > 6.130), to compound 36. As shown by the pIC_{50} values, the presence of groups such as –Difluorophenyl (compound 19 with pIC_{50} = 5.963) in contrast to –Phenyl (compound 13 with pIC_{50} = 6.235) resulted in the decrease of activity.

Fig. 3b describes the CoMFA steric contour map. In the CoMSIA steric contour map, green colour represents areas were more bulk groups are favorable, while the unfavorable steric areas are indicated by yellow contours. A large green contour is present near the R2 group which demonstrates that the presence of bulk group will increase the activity of the molecules. This is explained by considering the molecules 23 and 16 where replacing 3-Chloro-4-methoxyphenyl with 3-Bromophenyl increases the steric behavior of the substituent and consequently, the pIC_{50} value is increased (compound 23; pIC_{50} = 6.034 < compound 16; pIC_{50} = 5.847). The presence of Cyclopentylmethyl ring in the compound 12 exhibits (pIC_{50} = 6.219) higher inhibitory activity compared to the compound 8 (pIC_{50} = 5.943), having a c-Propyl group in the R2 position.

The magenta region of the CoMSIA hydrophobic contour plot in Fig. 3c, reveals that the hydrophobic substituents in this region could enhance the inhibitory activity while the orange contour represents the enhancement of the inhibitory activity by the hydrophilic substituents in this region.

In the CoMSIA hydrogen bond donor contour maps, as observed in Fig. 3d, the cyan contour indicates that hydrogen bond donor substituents in this region will improve the activity while the purple contour indicates that hydrogen bond donor substituents in this region will lead to decrease in activity.

The steric and electrostatic field contributions to the CoMFA model were 59% and 41%, respectively. The field graphics obtained from CoMSIA analysis elucidated the relationships between differences in the fields and variations in the dependent variable. The contributions of steric, electrostatic, hydrophobic, H-bond donor and acceptor fields to CoMSIA were 15.1, 31.3, 18.8 and 34.9%, respectively.

Comparison of the QSAR models

A consensus details regarding the prediction of activities from all the models is assessed by finding the correlation between the predicted and experimental pIC_{50} values for 2D QSAR, HQSAR, CoMFA and CoMSIA models and are presented in Fig.4. The trend lines with respect to 2D QSAR and CoMFA are very close with R^2 values of 0.843 and 0.757, respectively. The CoMSIA model shows an R^2 value of 0.851.The plot also identifies the existence of outliers of the HQSAR model. The HQSAR plot shows an R^2 value of 0.657. All the methods have provided consensus and complementary results.

Protein validation and Molecular docking

P. falciparum calcium-dependent protein kinase 1 (*Pf*CDPK1) is responsible for phosphorylation of components of the molecular motor that drive parasite invasion of red blood cells. If this invasion process can be prevented the parasite life cycle will be broken, leading to the destruction of the parasites. This could potentially prevent the infection. Therefore *Pf*CDPK1 represents a novel target for the potential treatment of malaria and offers promise for achieving selectivity over the kinases of the human host (Ahmed et al., 2012). The primary features of the proteins 3I79 and 3HZT were studied using Expasy proteomics server (Wilkins et al., 1999). By using ProtParam tool, the physicochemical characters were analyzed. The molecular weight of 3I79 obtained is 55289.55 Da and the theoretical pI is 5.70. The II of the protein is calculated by considering all the residues in the protein. This index has been used to predict whether a protein is stable (II<40; proteins that have an *in vivo* half-life of >16 h) or unstable (II >40; proteins that have an *in vivo* half-life of <5 h. (Guruprasad et al., 1990). The instability index is found to be 28.87 which classifies the protein as stable. The negative value of GRAVY (-0.423) illustrates that the protein molecule is hydrophilic. Similarly, the molecular weight of 3HZT obtained is 54603.45 Da, theoretical pI is 5.55. The instability index is found to be 31.96 which classifies the protein as stable. The AI of proteins can serve as a measure of thermostability of proteins (Ikai., 1980). The AI of the proteins 3I79 and 3HZT are 85.19 and 87.84, respectively, which shows that both the proteins are thermally stable. The negative value of GRAVY (-0.419) illustrates that the protein molecule is hydrophilic. 3I79 has the extinction coefficient 49070 $M^{-1}cm^{-1}$ in 280 nm solution assuming all pairs of Cys residues form cystines, while 3HZT shows 51840 $M^{-1}cm^{-1}$. The half-life estimated for both the proteins was 30 hours (unit measured based on mammalian reticulocyte *in vitro* studies).

The quality of the proteins 3I79 and 3HZT (Fig 5) were evaluated using Ramachandran plot obtained from MOE 2007.09 (Lovell et al., 2003). The output of the result shows that the protein 3I79 has three outlier amino acids Asn 370, Ala 413 and Asp 415. The number of residues in the core region is 94.01%. The number of residues in the allowed region is 5.54% and the number of residues in the outlier region is only 0.66%. 3HZT has no outlier amino acids. The number of residues in the core region is 93.64%. The number of residues in the allowed region is 6.36%. Ramachandran's plot predicts that the final structure is highly reliable for molecular docking.

The molecular docking of 42 ligands with the proteins 3I79 and 3HZT were carried out using MOE 2007.09. The protein was made rigid and ligand was left free to rotate (flexible docking). This was necessary to ensure that the docking process was in accordance with lock and key mechanism. The 'Site Finder' tool of the program is used to find the active sites of the proteins. The results of the molecular docking are summarized in Table 8 and Fig. 6 and 7.

Molecular docking interaction studies of the selected ligands with 3I79

The molecular docking results of the eight active compounds with the protein **3I79** were shown in the Table 8. The binding interactions of the ligands and the proteins were studied using LigPlot. The 2D and molecular docking pose of the 8 active compounds with the protein **3I79** are shown in the Fig. 6.

Molecular docking of compound tpk1 (Fig. 6) with molecular docking score -13.6612 kcal/mole showed four side chain hydrogen bonding interactions between the residue Lys 59 and the groups $-NH_2$, carbonyl and sulfonyl group.

Compound tpk17 shows three side chain hydrogen bonding interactions (Fig. 6). The carbonyl group shows a side chain hydrogen bonding interaction with the polar residue Lys 59 (2.63 Å, 28%). One of the oxygen atoms of the sulfonyl group show two side chain hydrogen bonding interactions with the polar residues Lys 176 and Ser 61 (2.91 Å, 28%, 2.99 Å, 22%). It shows a molecular docking score of -13.2110 kcal/mole.

The binding mode observed (Fig. 6) for the compound tpk20 shows that the residue Lys 176 forms side chain hydrogen bonding interactions with the oxygen atoms of both the carbonyl

and sulfonyl groups (2.44 Å, 56%, 2.57 Å, 58%). The proximity contour shows that the molecule is very closely hemmed in by the active site. A side chain hydrogen bonding interaction is observed between the –NH group and the residue Ser 83. The molecular docking score is found to be -13.2729 kcal/mole.

Compound tpk28 shows interactions like side chain hydrogen bonding interaction and backbone hydrogen bonding interaction (Fig. 6). The oxygen atom of the sulfonyl group shows a back bone hydrogen bonding interaction with the amino acid Ser 205 (2.6 Å, 86%). The carbonyl group forms a side chain hydrogen bonding interaction with the residue Ser 83 (3.0 Å, 12%). This compound shows a molecular docking score of -13.9943 kcal/mole.

Compound tpk34 shows a molecular docking score of -14.0290 kcal/mole. The -NH functional group of the ring shows a side chain hydrogen bonding interaction with the residue Asn 210 (1.98 Å, 17%). The proximity contour shows that the molecule as a whole is very closely hemmed in by the active site.

Molecular docking of compound tpk36 with molecular docking score -13.6347 kcal/mole showed a side chain hydrogen bonding interaction between the Oxygen atom of the S=O group and the residue Lys 209 (2.83Å, 18%). The carbonyl group shows a side chain hydrogen bonding interaction with the residue Lys 59 (2.51Å, 35%). The carbonyl group at the other end shows a backbone hydrogen bonding interaction with the residue Phe 62 (2.50Å, 22%).

The binding mode observed for the compound tpk41 shows that the carbonyl group of the forms a side chain hydrogen bonding interactions with residue Lys 59 (2.29 Å, 46%). The molecular docking score is found to be -15.0210 kcal/mole.

Compound tpk42 shows interactions with the active site of the protein molecule. The carbonyl group shows a side chain hydrogen bonding interaction with the amino acid Lys 59 (2.54 Å, 51%). The oxygen atom of the sulfonyl group present at the opposite side of the molecule shows a side chain hydrogen bonding interaction with the amino acid Lys 391 (2.64 Å, 84%). The benzene ring shows an arene-cation interaction with the residue Arg 442. This compound shows a molecular docking score of -11.2184 kcal/mole.

Molecular docking interaction studies of the selected ligands with 3HZT

The molecular docking results of the 8 active compounds with the protein 3HZT are shown in the Fig. 7. Compound tpk1 showed two direct interactions with the residues of the binding cavity of the protein (Fig. 7). The binding site residues involved in these prominent interactions are Gln 367 and Asn 204. Gln 367 showed a side chain hydrogen bonding interaction with the carbonyl group at 2.55 Å. Asn 204 shows a side chain interaction with the carbonyl group 2.40 Å (21%). Asp 220 and Lys 201 were also incorporated in the linkage due to the presence of water molecules. The molecular docking score of the compound is -10.5007 kcal/mole.

In Compound tpk17, only one prominent interaction is observed and the binding site residue is Glu 160 (Fig. 7). Glu 160 showed a side chain hydrogen bonding interaction at 1.74 Å with the -NH group. The compound shows a docking score of -10.9846 kcal/mole.

Compound tpk20 showed indirect hydrogen bonding interaction with oxygen of morpholine ring with the amino acid residues Gly 159 and Tyr 156 (Fig. 7). The molecular docking score is -11.9554 kcal/mole.

Compound tpk28 showed three prominent interactions with the residues Asn 204 and Tyr 156 (Fig. 7) with a molecular docking score of -10.2702 kcal/mole. Asn 204 forms a side chain hydrogen bonding interaction with the carbonyl group at 2.78 Å. The amino acid residue Tyr 156 forms a back bone hydrogen bonding interaction with the carbonyl group of the pyrrolidinone ring at 3.06 Å. Asp 220 and Lys 201 were also involved in the interactions because of bridging water molecule.

In Compound tpk34, only one prominent interaction was observed (-11.0446 kcal/mole). Glu 160 showed a side chain hydrogen bonding interaction with the N atom of the pyrrolidine ring (1.30Å, 41%). It shows an indirect hydrogen bonding interaction with the oxygen atom of the methoxy group.

Compound tpk36 shows two prominent interactions with the residues Glu 160 and Ser 84 (-10.7451 kcal/mole). Ser 84 shows a backbone hydrogen bonding interaction with the -NH group of the pyrrole ring at 2.01 Å. The residue, Glu 160 shows a sidechain hydrogen bonding interaction with the -NH group of the compound at 1.38 Å.

Compound tpk41 shows a polar side chain hydrogen bonding through amino acid residue Glu 160 with the –NH group of the compound at 1.87 Å. It shows a side chain hydrogen bonding interaction with the residue Asn 204 with the oxygen atom of the sulfonyl group (1.87 Å, 18%). The molecular docking sacore of the compound is -10.8624 kcal/mole.

The Compound tpk42 shows a prominent side chain hydrogen bonding interaction through the amino acid residue Ser 84 with the carbonyl group (2.76 Å, 18%). The residue Ser 84 is also interacted with the compound through the water molecule (-11.6409 kcal/mole).

Results of the molecular docking of 42 Thiaplakortone-A derivatives with 3I79 and 3HZT revealed that eight compounds namely, tpk1, tpk17, tpk20, tpk28, tpk34, tpk36, tpk41 and tpk42 exhibited good docking scores.

Design of new virtual molecules

In order to design new virtual molecules we have used information obtained from 2D-QSAR, HQSAR, CoMFA and CoMSIA models. The primary objectives of building a 2D-QSAR model are to find out the structural features correlated with activity and to predict the activity of a molecule. We have used the equation obtained from 2D-QSAR, to find out the activity of the modified compounds. The results of HQSAR model demonstrates that the fragment -CH_2- adjacent to the pyrrole ring positively contribute to the biological activity. So we have retained the methylene group in the modified molecules. Moreover, the HQSAR contribution plot shows that, the hydrogen atom attached to the nitrogen of the pyrrole ring also shows favourable contribution. Considering this fact, we have retained H atom in the four virtual molecules.

CoMFA and CoMSIA models derived for Thiaplakortone-A derivatives throw light on how to design novel molecules with improved anti-malarial activity. A remarkable elucidation of the CoMFA and CoMSIA contour maps gave us valuable information for amending the structure of the main scaffold. In CoMFA and CoMSIA electrostatic contour maps, the existence of a red contour near R2 position indicates that electronegative groups are favorable for increasing the anti-malarial activity. The CoMSIA hydrogen bond donor contour map also indicates that at the R2 position of the Thiaplakortone-A scaffold, a hydrogen bond donor group is favorable for anti-malarial activity. Based on these, we introduced electronegative and hydrogen bond donor

groups at the R2 position and created 7 virtual molecules tpk(1a), tpk(1b), tpk(1c), tpk(1d), tpk(1e), tpk(1f) and tpk(1g). The suggested molecules are thus the result of harmonious use of CoMFA and CoMSIA with 2D-QSAR and HQSAR models.

The descriptors of the modified virtual molecules were calculated using PaDEL-Descriptor and MOE softwares. The descriptors used to find out the activity of the modified molecules using the equation obtained from 2D-QSAR are listed in the table 9. From the calculated activities, it is clear that out of the seven modified virtual molecules, tpk(1a), tpk(1b), tpk(1c), tpk(1d), tpk(1e), tpk(1f) and tpk(1g), three molecules tpk(1c), tpk(1d) and tpk(1e) showed good anti-malarial activity values of 6.159, 6.542 and 6.391, respectively.

Data Mining Using WEKA

Machine Learning (ML) method was employed in order to confirm whether molecules were active against malarial parasites or not. Using WEKA tools, binary classifiers for the molecules were created, based on their bio activity. For the purpose, the bioassay dataset AID: 504850 which contained compounds having the potential to inhibit apicoplast formation in the malarial parasite *Plasmodium* is taken. The assay was based on a Luciferase reporter assay and the compounds that cause inhibition of apicoplast formation was assayed by a delayed death response. Growth inhibition is detected by a decrease in luciferase activity. The dataset AID: 504850 contained a total of 2304 tested compounds. Compounds in AID: 504850 were characterized based on a compound ranking system called 'PubChem Activity Score'. Compounds having an activity score between 40 and 100 were considered as active (1172), all compounds with a score of 0 were considered as inactive (344) and the ones having a score between 1 and 39 were labeled as inconclusive (788).

PowerMV software was used to generate 179 molecular descriptors for each dataset, which includes 147 pharmacophore fingerprints, 24 weighted burden number and 8 property descriptors. The dataset was split into 20% test set and 80% training-cum-validation set. The selected compounds after the molecular docking studies were tested using the classifier based on RF. Earlier workers have observed that RF provides the best classifier (Sajeev et al., 2013; Seal et al., 2012; Periwal et al., 2011). The algorithm tries to find as good a distinction as possible between active compounds and others, on the basis of a set of molecular descriptors. It identifies

features shared by different subsets of active compounds and accordingly filters out compounds within the target data set in which these combinations are lacking. It is the most accurate classifiers available. For evaluation of the RF classifier, we employed the 10-fold cross validation. With RF model, the selected 8 molecules were screened using AID dataset 504850 and the confusion matrix shows that all the selected 8 compounds; tpk1, tpk17, tpk20, tpk28, tpk34, tpk36, tpk41 and tpk42, were found to be active. The specificity and sensitivity of the built model was 62.7% and 76.8 %, respectively. The accuracy of the generated model was determined by the overall effectiveness of a classifier. The accuracy (Q) of the model was found to be 76.82 %, which depicts that the results are accurate. ROC area was 69.4%.

Data mining study was then performed to find out whether the modified molecules were active or not. The virtual screening carried out using the RF model based on the AID: 504850 shows that out of the seven modified molecules the compounds tpk(1e), tpk(1f) and tpk(1g) are anti-malarial active and can be considered to be the lead molecules (Fig. 8).

Prediction of toxicity risks and drug score assessment

The molecules screened will be safe for use only if they are not toxic to humans. The present study therefore further relied upon theoretical *in silico* methods to assess the toxicity of the molecules. Depending on the chemical structure of the molecules their pharmaceutical actions may vary. The pharmacokinetics of the molecules within the body and its action and performance rely upon absorption, distribution, metabolism and excretion.

The cytochromes P450, which contain heme, metabolize a wide range of therapeutic agents (Bezirtzoglou, 2012). The isoforms of CYP enzymes are CYP1A2, CYP2C9 and CYP2C19, CYP2D6 and CYP3A4. (Williams et al., 2004) Out of these CYP3A4 is the most abundant human hepatic P450 isoform which is responsible for the metabolism of about 50% of known drugs (Guengerich, 1999). The metabolite prediction of CYP3A4 substrates by MetaSite shows an accuracy of 78%. (Zhou et al., 2006). A better understanding of molecular interactions between CYP3A4 and drugs are useful perceptions for the development of new medications. The size and shape of the active site cavity of the CYP3A4 structures are remarkable. (Scott and Halpert, 2005)

In order to find out whether the modified molecules will be metabolized or not, molecular docking of the commercially available drug molecules and the modified molecules were performed into the active site of the enzyme CYP3A4. (Kjellander et al., 2007; Oda et al., 2004). If these molecules are not metabolized by the enzyme they will accumulate and ultimately will be converted to toxic compounds. The shape and size of the active site present in the CYP3A4 shows that, it can metabolize several bulky substrates (Rendic, 2002).

The human microsomal cytochrome P450 3A4 protein structures, 1WOE and 1WOF, downloaded from Protein Data Bank were subjected to 3D protonation and energy minimization utilizing the MOE program. Using the processed CYP3A4 crystal structures, the 'Site Finder' tool of the program is used to find out its active site. The modified molecules were subjected to molecular docking. The energy data showed a rough correlation between the docking score values of the compounds and their interactions. The saved pose for the ligand-enzyme complex of each modified molecule was subjected to detailed 3D analysis for its interactions at the enzyme active site.

In order to correctly predict the CYP3A4 derived SOM, SMARTCyp was used. The prediction of the seven virtual molecules by SMARTCyp to identify the best three SOMs, are presented in the Fig. 9. The SOMs with first three ranks are represented with rings. The methylene group (-CH_2-) adjacent to amino group of compounds tpk(1a), tpk(1b), tpk(1c) and tpk(1e) are ranked as the best SOM. This is followed by amino group in rank two position. For the compound tpk (1e), the double bond adjacent to the -SO_2 group is regarded as the SOM. In compound tpk (1f), the methyl group and methylene group attached to the oxygen atom, are considered as SOM with highest ranks as shown in Fig. 9. In compound tpk (1g), the -CH- group connected to the hydroxyl group is identified as the SOM. The modified compounds bind tightly to CYP3A4, and they showed good docking scores than the drug molecules, consistent with the observation that they probably interacts with CYP3A4 through the active site. The present study therefore gives the metabolic nature of the lead like molecules. To summarize, we have shown that the modified compounds if taken by patients with malaria interact well with CYP3A4. These molecules also obeyed Lipinski's Rule of Five.

Osiris Property Explorer was used to predict the toxicity and drug likeness of the compounds. The database of this online program contains *in vitro* and *in vivo* validated compounds and the predictions were based on the functional group similarity for the query molecule. The results were visualized using the color codes. Green color shows low toxic tendency, yellow shows the midcore and red color shows high tendency of toxicity. It is used to calculate toxicity risk parameters such as mutagenicity, tumorigenicity, irritation, and reproductive toxicity of all the modified compounds, and it was found that compounds tpk(1b) and tpk(1c) showed midcore and high tendency of irritation, respectively. Compound tpk(1d) indicated risk of mutagenicity and tumorigenicity. In order to assess the compound's overall potential to qualify for a drug, overall drug score (DS) was calculated, which combines drug-likeness, hydrophobicity (LogP), aqueous solubility (LogS), MW, and toxicity risk parameters. All the calculated values were given in Table 10.

The DS for tpk(1e) [(2-{1,1,5,9-tetraoxo-4H,5H,9H-1λ^6-[1,4]thiazino[2,3-f]indol-8-yl}ethyl)urea], tpk(1f) [2-methoxy-N-(2-{1,1,5,9-tetraoxo-4H,5H,9H-1λ^6-[1,4]thiazino[2,3-f]indol-8-yl}ethyl)acetamide] and tpk(1g) [2-hydroxy-N-(2-{1,1,5,9-tetraoxo-4H,5H,9H-1λ^6-[1,4]thiazino[2,3-f]indol-8-yl}ethyl)propanamide] show comparatively high values suggesting that they can be developed as potential anti-malarial drug. All these compounds have no effect on mutagenicity, tumorigenicity, irritation, and reproductive toxicity. Other drug-like properties are under the acceptable limits.

Conclusion

The analysis of 42 Thiaplakortone-A derivatives by 2D-QSAR, HQSAR, 3D-QSAR, molecular docking and Data mining studies provides a new path for the design of new series of anti-malarial drugs. In this study, 2D-QSAR models are generated by MLR method, and the robustness of the model is validated by internal, external, Y-randomization and Applicability domain analysis. The CoMFA and CoMSIA analysis have provided distinguishing key structural features affecting the anti-malarial activity of these inhibitors. We identified and confirmed the residues that play key role in the hydrogen bond donors, hydrophobic, steric and electrostatic interactions. All the 42 Thiaplakortone-A derivatives were subjected to molecular docking and the compounds with good docking score were selected and subjected to data mining. Data

mining shows that 4 of the selected compounds with good docking score are anti-malarial active. Based on 3D-QSAR, seven virtual compounds were designed. These molecules were subjected to data mining and drug-likeness studies. The result shows that all the molecules obey Lipinski's RO5 and machine learning study reveals that out of the seven modified molecules three molecules are anti-malarial. Thus the generated models through modified approach provide a functional guideline to design and predict the activity of virtual compounds with enhanced inhibitory activities and thus help rational design of novel anti-malarial drugs.

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Figure captions

Fig. 1. Individual atomic contributions for the activity of the most potent compound tpk 1 (a) and the least potent compound tpk 22 (b). The colours at the red end of the spectrum reflect unfavourable contributions in the order of red>red-orange>orange colour, while colours at the green end indicate favourable (positive) contributions in the order of green>green-blue>yellow. Atoms with intermediate contributions are coloured in white.

Fig. 2 CoMFA field contour maps for active compound tpk 1. Electrostatic fields (a): Blue fields indicate electropositive groups favored, red fields indicate electronegative groups favored. Steric fields (b): Green fields indicate steric bulk favored, yellow fields indicate steric bulk disfavored

Fig. 3. CoMSIA field contour maps for active compound tpk 1. Electrostatic fields (a): Blue fields indicate electropositive groups favored, red fields indicate electronegative groups favored. Steric fields (b): Green fields indicate steric bulk favored, yellow fields indicate steric bulk disfavored. Hydrophobic fields (c): Magenta and orange represent favored and disfavored regions respectively for hydrophobic interaction. H-bond donor fields (d): cyan and purple represent regions for favored and disfavored for H bond donor groups respectively.

Fig. 4. Predicted anti-malarial activities with experimental values of 2D-QSAR, HQSAR, CoMFA and CoMSIA.

Fig. 5. Ramachandran Plot of the proteins.

Fig. 6. 2D interaction and molecular docking pose of the active compounds tpk1, tpk17, tpk20 and tpk28 in the binding pocket of the protein 3I79

Fig. 7: 2D interaction and molecular docking pose of the active compounds tpk1, tpk17, tpk20 and tpk28 in the binding pocket of the protein 3HZT

Fig. 8: Representation of SOMs by SMARTCyp.

Table captions

Table 1. Representative skeletons and molecular structures of Thiaplakortone-A derivatives and their activity values

Table 2: Numerical values of the Observed Activity, Predicted Activity and Residual values of 2D-QSAR

Table 3: Statistical parameters after Y-randomization test

Table 4: Summary of the HQSAR statistical indices for various fragment distinction (FD) parameters using the default fragment size (4-7 atoms) for the Thiaplakortone-A derivatives (N=42).

Table.5: Statistical parameters of CoMSIA models

Table 6. Statistical results of CoMFA and the best CoMSIA models

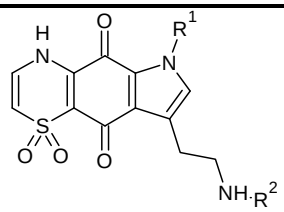
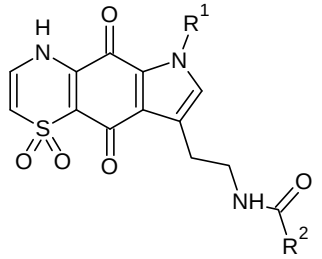
Table 7: Experimental and predicted activities (pIC_{50}) with residual values

Table 8: Data of protein-ligand docking.

Table 9: Descriptors used to find out the anti-malarial activity value

Table 10: Predicted molecular parameters of the modified compounds

Table 1. Representative skeletons and molecular structures of Thiaplakortone-A derivatives and their activity values

Compounds	R1	R2	IC_{50}	pIC_{50}
				
tpk1	H	H	104	6.983
tpk2	CH3	H	128	6.892
tpk3	CH3	CH3	137	6.863
				
tpk4	H	CH_3	160	6.795
tpk5	H	Methyl	472	6.326
tpk6	H	Ethyl	703	6.153
tpk7	H	n-Propyl	439	6.357
tpk8	H	c-Propyl	1139	5.943
tpk9	H	i-Propyl	537	6.270
tpk10	H	n-Butyl	644	6.191
tpk11	H	i-Butyl	537	6.270
tpk12	H	Cyclopentylmethyl	604	6.219
tpk13	H	Phenyl	582	6.235
tpk14	H	2-Fluorophenyl	445	6.351
tpk15	H	3-Fluorophenyl	1260	5.899
tpk16	H	3-Bromophenyl	1423	5.846
tpk17	H	4-Fluorophenyl	1290	5.889
tpk18	H	2,5-Difluorophenyl	1300	5.886
tpk19	H	2,6-Difluorophenyl	1090	5.962
tpk20	H	5-Fluoro-2-morpholinophenyl	1210	5.917
tpk21	H	2-(2-Fluorophenyl)acetyl	1300	5.886
tpk22	H	2-Fluoro-5-(trifluoromethyl)phenyl	1957	5.708
tpk23	H	3-Chloro-4-methoxyphenyl	925	6.033
tpk24	H	2-Hydroxy-4-methoxyphenyl	1412	5.850

tpk25	H	3-(4-Methoxyphenyl)propyl	1633	5.787
tpk26	H	Isoquinolin-1-yl	824	6.084
tpk27	H	Furan-3-yl	1079	5.967
tpk28	H	(S)-5-Oxopyrrolidin-2-yl	1120	5.950
tpk29	H	1H-Indol-2-yl	886	6.052
tpk30	H	N(H)n-Bu	709	6.149
tpk31	H	N(H)t-Bu	685	6.164
tpk32	H	Ot-Bu	753	6.123
tpk33	H	N(H)-3,4-dimethoxybenzyl	1110	5.954
tpk34	H	N(Me)OMe	741	6.130
tpk35	H	$N(H)CH_2CO_2Et$	663	6.178
tpk36	H	N(H)-adamant-2-yl	631	6.200
tpk37	Methyl	CH_3	684	6.164
tpk38	Isopropyl	CH_3	937	6.028
tpk39	Cyclopentyl	CH_3	933	6.030
tpk40	(3-Methyloxetan-3-yl)methyl	CH_3	822	6.085
tpk41	Benzyl	CH_3	441	6.355
tpk42	4-Chlorobenzyl	CH_3	635	6.197

Table 2: Numerical values of the Observed Activity, Predicted Activity and Residual

Compounds	Observed pIC_{50}	Predicted pIC_{50}	Residual	HAT i/i (h*=0.3571)	St Dev Res
tpk1*	6.9830	6.8039	-0.1791	0.3164	-1.7214
tpk2	6.8928	6.7100	-0.1828	0.2405	-1.6669
tpk3	6.8633	6.7997	-0.0636	0.3176	-0.6121
tpk4	6.7959	6.8615	0.0656	0.3398	0.6418
tpk5	6.3261	6.3036	-0.0225	0.2575	-0.2073
tpk6	6.1530	6.1714	0.0184	0.1030	0.1544
tpk7*	6.3575	6.3263	-0.0312	0.0741	-0.2576
tpk8	5.9435	5.9029	-0.0406	0.5683	-0.4906
tpk9	6.2700	6.1755	-0.0945	0.0733	-0.7801
tpk10	6.1911	6.2200	0.0288	0.0531	0.2355
tpk11	6.2700	6.2084	-0.0616	0.0568	-0.5044
tpk12	6.2190	6.1347	-0.0843	0.0865	-0.7006
tpk13	6.2351	6.2299	-0.0052	0.0520	-0.0426
tpk14	6.3516	5.9874	-0.3643	0.0924	-3.0387
tpk15*	5.8996	5.9689	0.0693	0.0899	0.5773
tpk16	5.8468	6.0483	0.2015	0.1028	1.6909
tpk17	5.8894	6.0140	0.1245	0.1000	1.0433
tpk18	5.8861	6.0510	0.1650	0.1336	1.4085
tpk19	5.9626	5.8392	-0.1234	0.1541	-1.0661
tpk20	5.9172	5.9937	0.0765	0.0934	0.6384
tpk21	5.8861	5.7658	-0.1202	0.1123	-1.0143
tpk22	5.7084	5.7471	0.0387	0.5009	0.4350
tpk23	6.0339	6.0199	-0.0140	0.0878	-0.1163
tpk24	5.8502	5.7790	-0.0711	0.2030	-0.6333
tpk25	5.7870	5.8311	0.0441	0.1003	0.3698
tpk26*	6.0841	6.0535	-0.0305	0.1002	-0.2557
tpk27	5.9670	6.1030	0.1360	0.0994	1.1388
tpk28	5.9508	6.0576	0.1068	0.1026	0.8963
tpk29	6.0526	6.0464	-0.0062	0.1030	-0.0518
tpk30	6.1494	6.0879	-0.0615	0.0977	-0.5145
tpk31	6.1643	6.1753	0.0110	0.1054	0.0927
tpk32*	6.1232	6.1423	0.0191	0.0890	0.1591
tpk33	5.9547	6.0826	0.1279	0.0787	1.0589
tpk34	6.1302	6.1884	0.0582	0.0435	0.4728
tpk35	6.1785	6.2311	0.0526	0.0522	0.4295
tpk36	6.2000	6.1232	-0.0768	0.0837	-0.6378
tpk37	6.1649	6.2184	0.0535	0.0585	0.4382
tpk38*	6.0283	6.1647	0.1365	0.0634	1.1205
tpk39	6.0301	6.2500	0.2199	0.0629	1.8054
tpk40	6.0851	6.1873	0.1022	0.1213	0.8663
tpk41	6.3556	6.1549	-0.2006	0.0976	-1.6784
tpk42	6.1972	6.1591	-0.0382	0.0645	-0.3135

*-Test set compounds

Table 3: Statistical parameters after Y-randomization test

Random Models Parameters	
R_r :	0.315
R_r^2 :	0.109
Q_r^2 :	-0.159
$^cR_p^2$:	0.792

R_r - average correlation coefficient
R_r^2-mean squared correlation coefficients of the randomized
Q_r^2- average cross-validated correlation coefficient

Table 4: Summary of the HQSAR statistical indices for various fragment distinction (FD) parameters using the default fragment size (4-7 atoms) for the Thiaplakortone-A derivatives (N=42).

Fragment	R^2_{cv}	SEP	R^2_{ncv}	HL	N
A/B/C	0.681	0.191	0.917	307	6
A/B/C/H	**0.682**	**0.198**	**0.958**	**151**	**8**
A/B/C/H/Ch	0.649	0.200	0.928	401	6
A/C/H/Ch/DA	0.468	0.238	0.803	401	4
A/B/C/H/Ch/DA	0.547	0.227	0.906	307	6
A/B/Ch/DA	0.398	0.262	0.897	61	6
A/B/C/Ch/DA	0.569	0.218	0.868	307	5
A/C/Ch/DA	0.407	0.247	0.655	71	3
A/B/H/Ch/DA	0.437	0.253	0.853	83	6

SEP: standard error of prediction, R^2_{cv}: cross-validated correlation coefficient, R^2_{ncv}: non cross-validated correlation coefficient, HL: hologram length, N: optimal number of components, Fragment distinction; A: atom, B: bond, C: connection, H: hydrogen, Ch: chirality, DA: donor and acceptor. The best model is in bold.

Table.5: Statistical parameters of CoMSIA models

Model	Descriptors	$R^2_{LOO}(Q^2)$/ONC	R^2_{ncv}/ SEE_{ncv}	F value	$R^2_{m\ (overall)}$	R^2_{pred}
1	S and E	0.658/7	0.979/0.050	175.779	0.760	0.672
2	D and A	0.501/5	0.854/0.126	33.826	0.648	0.393
3	S, E and H	0.595/7	0.987/0.039	299.385	0.768	0.792
4	S, E and A	0.687/7	0.983/0.045	221.859	0.789	0.405
5	S, E and D	0.721/6	0.972/0.057	160.663	0.768	0.470
6	D, A and H	0.534/4	0.895/0.106	63.705	0.621	0.892
7	D, A and E	0.566/6	0.963/0.065	122.082	0.728	0.573
8	S, D and H	0.593/4	0.926/0.089	94.388	0.685	0.722
9	S, E, D and A	0.682/6	0.977/0.051	201.207	0.773	0.400
10	**S, E, D and H**	**0.647/6**	**0.984/0.042**	**292.970**	**0.770**	**0.718**
11	S, E, A and H	0.637/7	0.987/0.040	283.145	0.784	0.598
12	D, A, H and S	0.612/6	0.968/0.061	139.470	0.764	0.524
13	D, A, H and E	0.549/6	0.966/0.062	134.057	0.727	0.779
14	S, E, D, A and H	0.565/6	0.927/0.085	74.046	0.860	0.606

$R^2_{LOO}(Q^2)$- cross-validated correlation coefficient
ONC- Optimum Number of Components
R^2_{ncv}- Non cross-validated correlation coefficient
SEE_{ncv}- Standard Error of Estimate
R^2_{pred}-Predicted correlation coefficient
S-Steric, E-Electrostatic, D-Hydrogen bond donor, A- Hydrogen bond acceptor and H-Hydrophobic.
The best model is in bold.

Table 6. Statistical results of CoMFA and the best CoMSIA models

	CoMFA	**CoMSIA (Model 10)**
Q^2/ONC	0.813/7	0.647/6
R^2_{ncv}	0.994	0.984
SEP	0.149	0.201
SEE	0.027	0.042
R^2_{pred}	0.891	0.718
F value	216	292.970
Field contribution		
steric	59	15.1
electrostatic	41	31.3
hydrophobic	-	18.8
H-bond donor	-	34.9

Q^2-cross-validated correlation coefficient.

ONC-Optimum number of components

R^2_{ncv} -non cross-validated correlation coefficient.

SEP- Standard error of prediction.

SEE-Standard errors of estimate.

R^2_{pred}- Predicted correlation coefficient for the test set

Table 7: Experimental and predicted activities (pIC_{50}) with residual values

Compound id	**Actual** pIC_{50}	**HQSAR** predicted	residual	**CoMFA** Predicted	Residual	**CoMSIA** Predicted	Residual
tpk1	6.983	6.609	0.373	6.861	0.122	6.914	0.069
tpk2	6.892	6.764	0.128	6.841	0.051	6.916	-0.023
tpk3	6.863	6.855	0.007	6.767	0.096	6.822	0.041
tpk4	6.795	6.763	0.032	6.879	-0.083	6.880	-0.084
tpk5	6.326	6.393	-0.067	6.262	0.064	6.319	0.007
tpk6*	6.153	6.288	-0.135	6.220	-0.067	6.215	-0.062
tpk7	6.357	6.235	0.121	6.258	0.099	6.358	-0.000
tpk8	5.943	6.177	-0.233	6.008	-0.064	5.963	-0.019
tpk9	6.270	6.207	0.062	6.278	-0.008	6.300	-0.030
tpk10	6.191	6.258	-0.067	6.002	0.189	6.132	0.059
tpk11	6.270	6.175	0.094	6.339	-0.069	6.274	-0.004
tpk12	6.219	6.169	0.049	6.169	0.050	6.192	0.027
tpk13*	6.235	6.028	0.207	6.189	0.046	6.192	0.043
tpk14*	6.351	5.973	0.378	5.789	0.562	5.853	0.498
tpk15*	5.899	5.954	-0.054	5.900	-0.000	5.943	-0.043
tpk16	5.846	6.000	-0.153	5.854	-0.007	5.847	-0.000
tpk17	5.889	5.901	-0.011	5.945	-0.055	5.905	-0.015
tpk18	5.886	5.848	0.037	5.928	-0.041	5.894	-0.007
tpk19*	5.962	5.918	0.044	6.105	-0.142	6.007	-0.044
tpk20	5.917	5.592	0.325	6.055	-0.137	5.904	0.013
tpk21*	5.886	6.077	-0.190	5.960	-0.073	6.097	-0.210
tpk22	5.708	5.667	0.040	5.652	0.056	5.696	0.012
tpk23	6.033	5.783	0.249	5.863	0.170	6.000	0.033
tpk24	5.850	5.911	-0.061	6.054	-0.203	5.868	-0.017
tpk25	5.787	5.951	-0.164	5.645	0.142	5.822	-0.035
tpk26	6.084	5.897	0.186	6.149	-0.064	6.039	0.045
tpk27	5.967	6.230	-0.263	6.094	-0.127	6.065	-0.098
tpk28*	5.950	5.770	0.180	6.376	-0.425	6.373	-0.422
tpk29	6.052	5.882	0.170	5.966	0.086	6.047	0.005
tpk30	6.149	6.248	-0.099	6.237	-0.087	6.159	-0.009
tpk31	6.164	6.006	0.157	6.147	0.017	6.126	0.038
tpk32	6.123	6.284	-0.161	6.090	0.033	6.130	-0.006
tpk33	5.954	6.043	-0.089	6.061	-0.106	5.981	-0.026
tpk34	6.130	6.334	-0.203	6.085	0.045	6.076	0.054
tpk35	6.178	6.194	-0.015	6.186	-0.007	6.197	-0.018
tpk36	6.200	6.062	0.137	6.206	-0.006	6.231	-0.031
tpk37	6.164	6.606	-0.441	6.188	-0.023	6.211	-0.046
tpk38	6.028	6.130	-0.102	6.182	-0.153	6.062	-0.033
tpk39	6.030	5.891	0.139	6.104	-0.073	6.020	0.010
tpk40	6.085	6.176	-0.091	6.177	-0.091	6.036	0.049

tpk41	6.355	6.151	0.204	6.168	0.187	6.365	-0.009
tpk42	6.197	6.260	-0.063	6.196	0.001	6.143	0.054

*-Test set compounds

Table 8: Data of protein-ligand docking.

Compounds	**3I79 (kcal/mole)**	**3HZT (kcal/mole)**
tpk1	-13.6612	-10.5007
tpk17	-13.2110	-10.9846
tpk20	-13.2729	-11.9554
tpk28	-13.9943	-10.2702
tpk34	-14.0290	-11.0446
tpk36	-13.6347	-10.7451
tpk41	-15.0210	-10.8624
tpk42	-11.2184	-11.6409

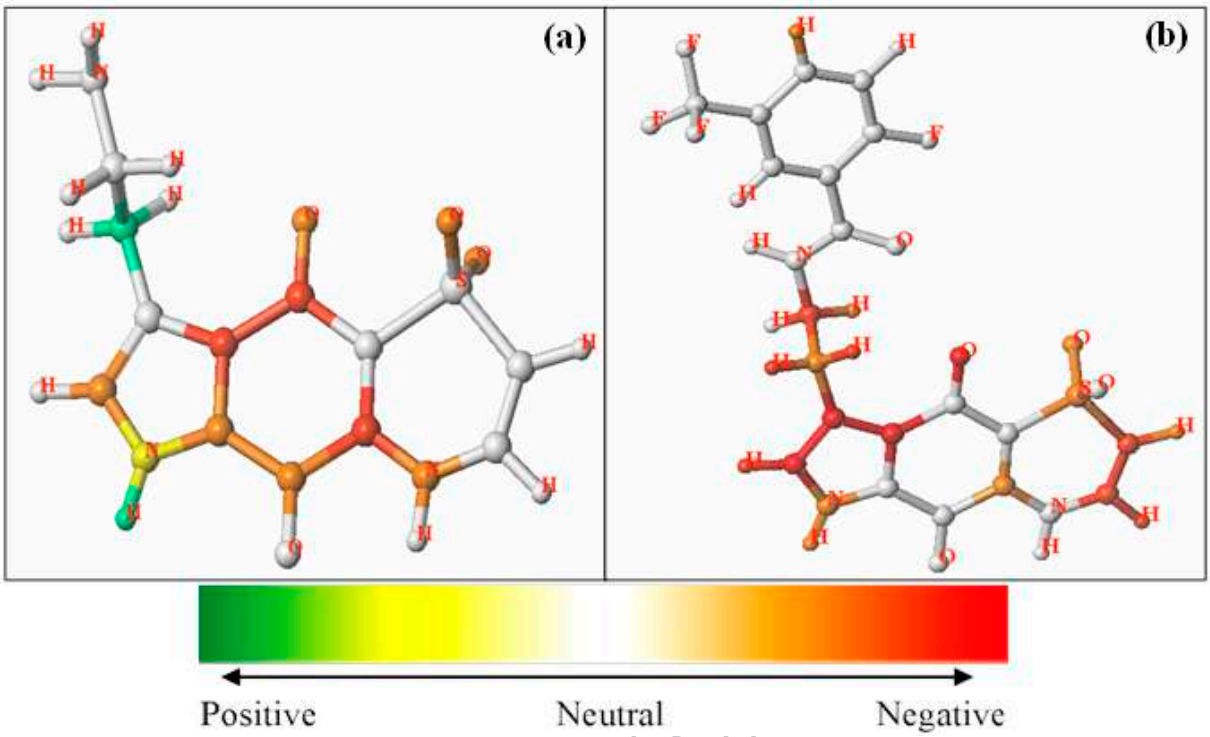
Table 9: Descriptors used to find out the anti-malarial activity value

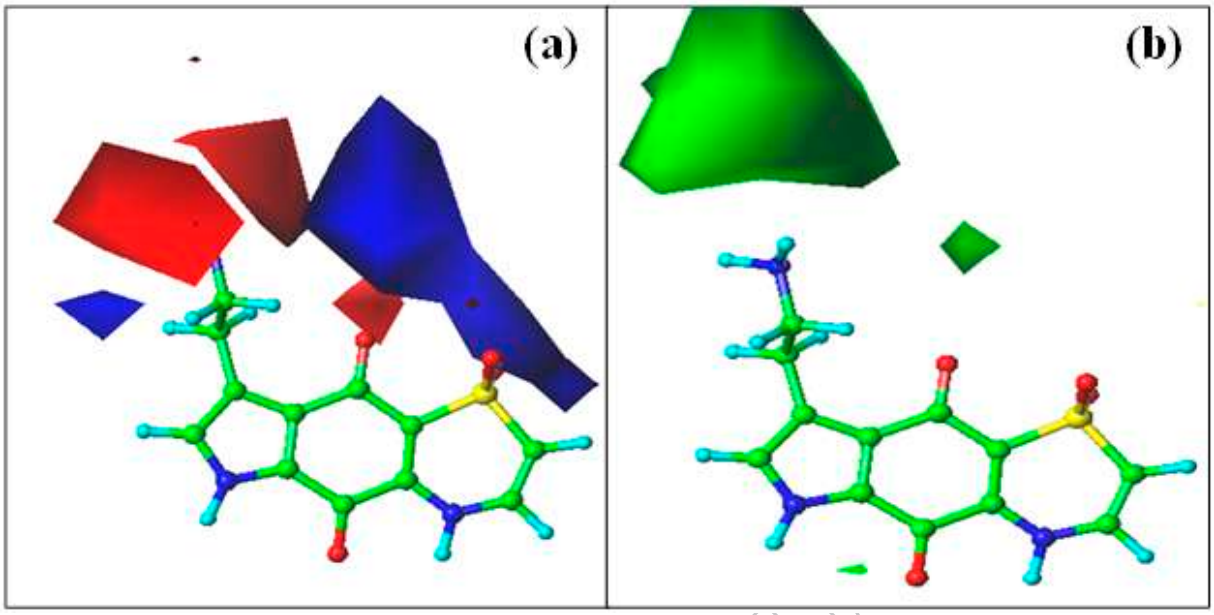
Modified molecules	**SMR_VSA1**	**MNDO_LUMO**	**maxHssNH**	**pIC_{50}**
tpk(1a)	11.9848	-0.3257	0.0188	5.872
tpk(1b)	26.9449	-0.1881	0.0392	5.498
tpk(1c)	13.1239	-0.0132	-0.0141	6.154
tpk(1d)	-17.9354	0.2459	-0.0231	6.543
tpk(1e)	-19.8345	0.0739	-0.0043	6.394
tpk(1f)	-19.8345	0.1285	-0.0079	6.425
tpk(1g)	5.5507	0.0787	-0.0084	6.168

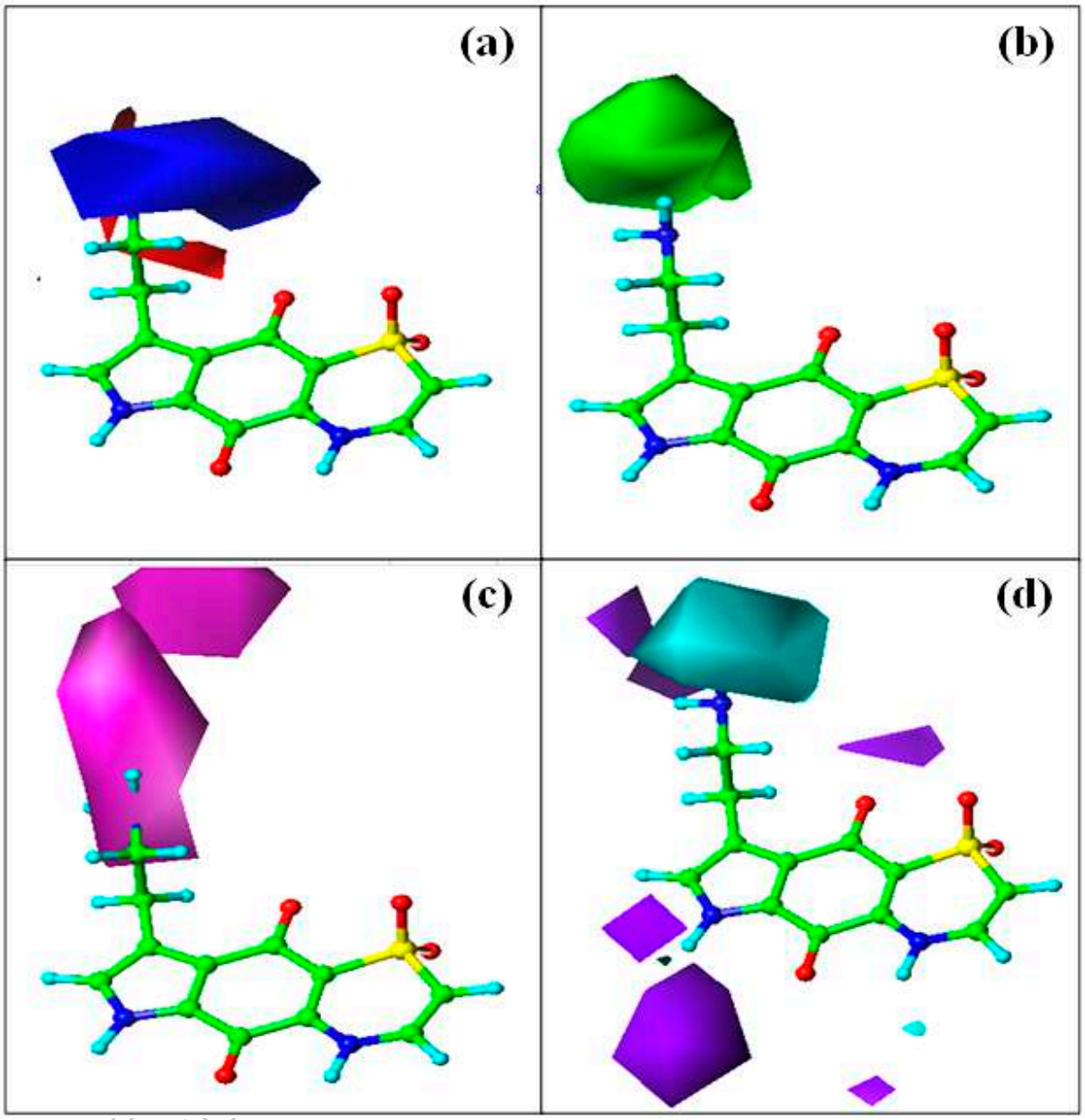
SMR_VSA1- Molecular refractivity based descriptor
MNDO_LUMO- potential energy based descriptor
maxHssNH- Maximum atom-type H E-State of -NH-

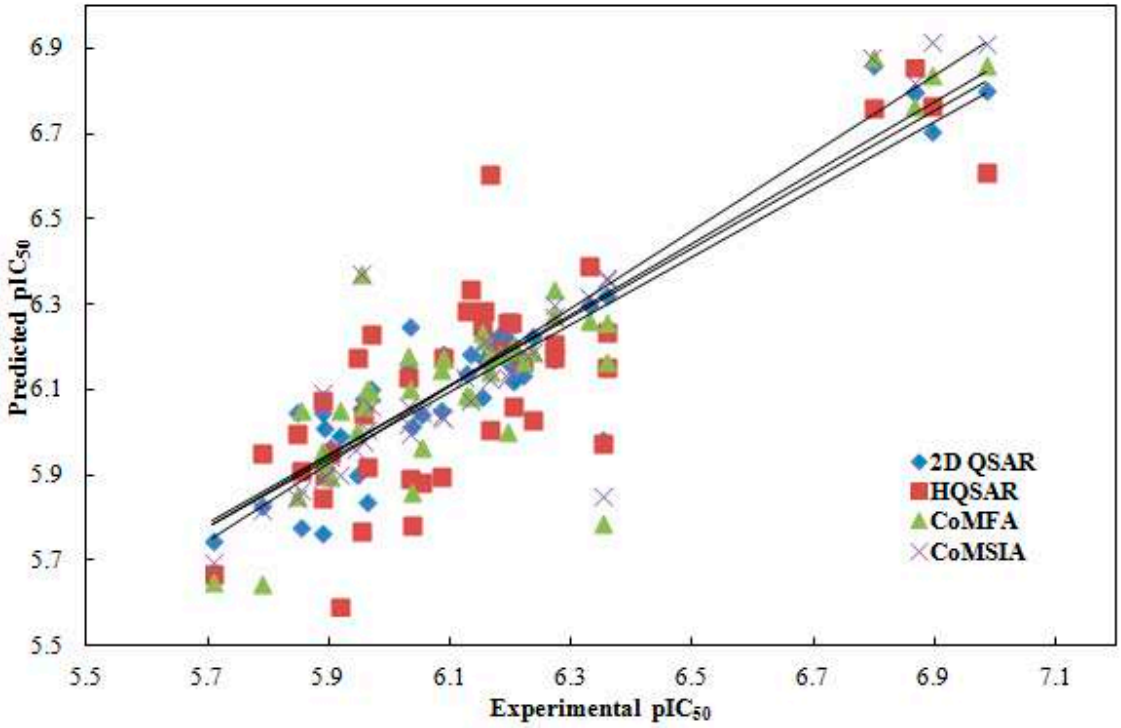
Table 10: Predicted molecular parameters of the modified compounds

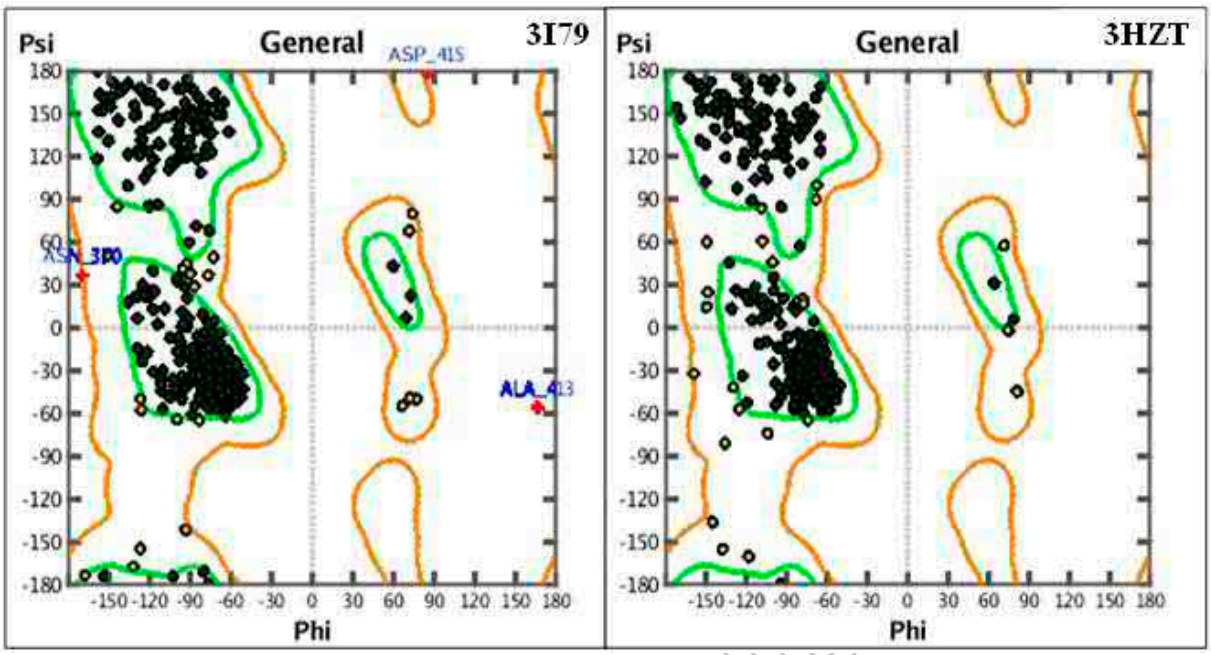
Compounds	ClogP	Solubility	Mol wt	Drug likeness	DS
tpk(1a)	0.19	-3.30	343	-0.37	0.604
tpk(1b)	0.82	-3.78	361	-3.12	0.337
tpk(1c)	-1.89	-2.28	332	-0.66	0.366
tpk(1d)	-1.74	-2.06	349	3.73	0.572
tpk(1e)	-1.74	-2.93	336	4.00	0.877
tpk(1f)	-1.62	-2.29	365	4.01	0.880
tpk(1g)	-1.68	-2.54	365	1.16	0.776

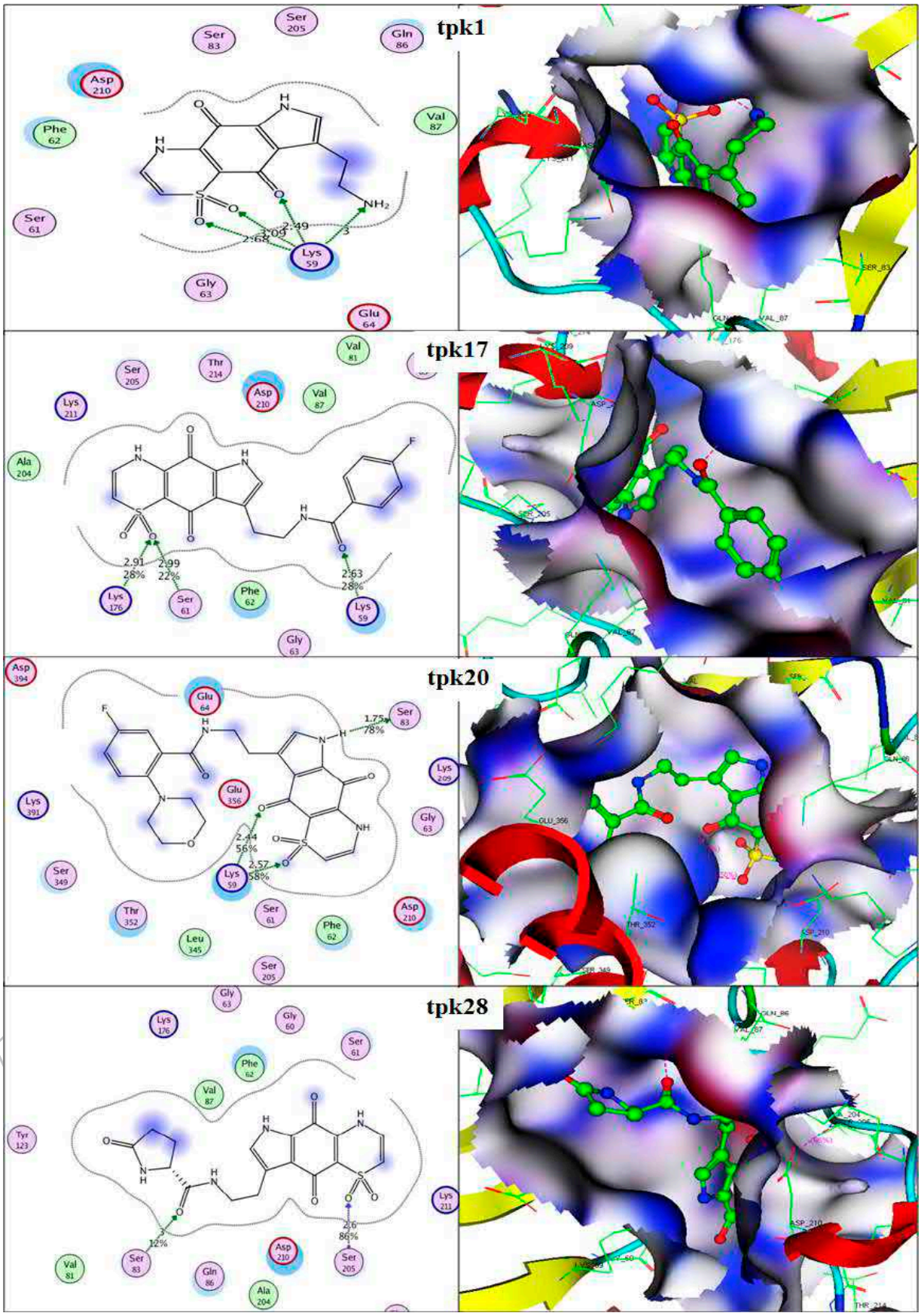


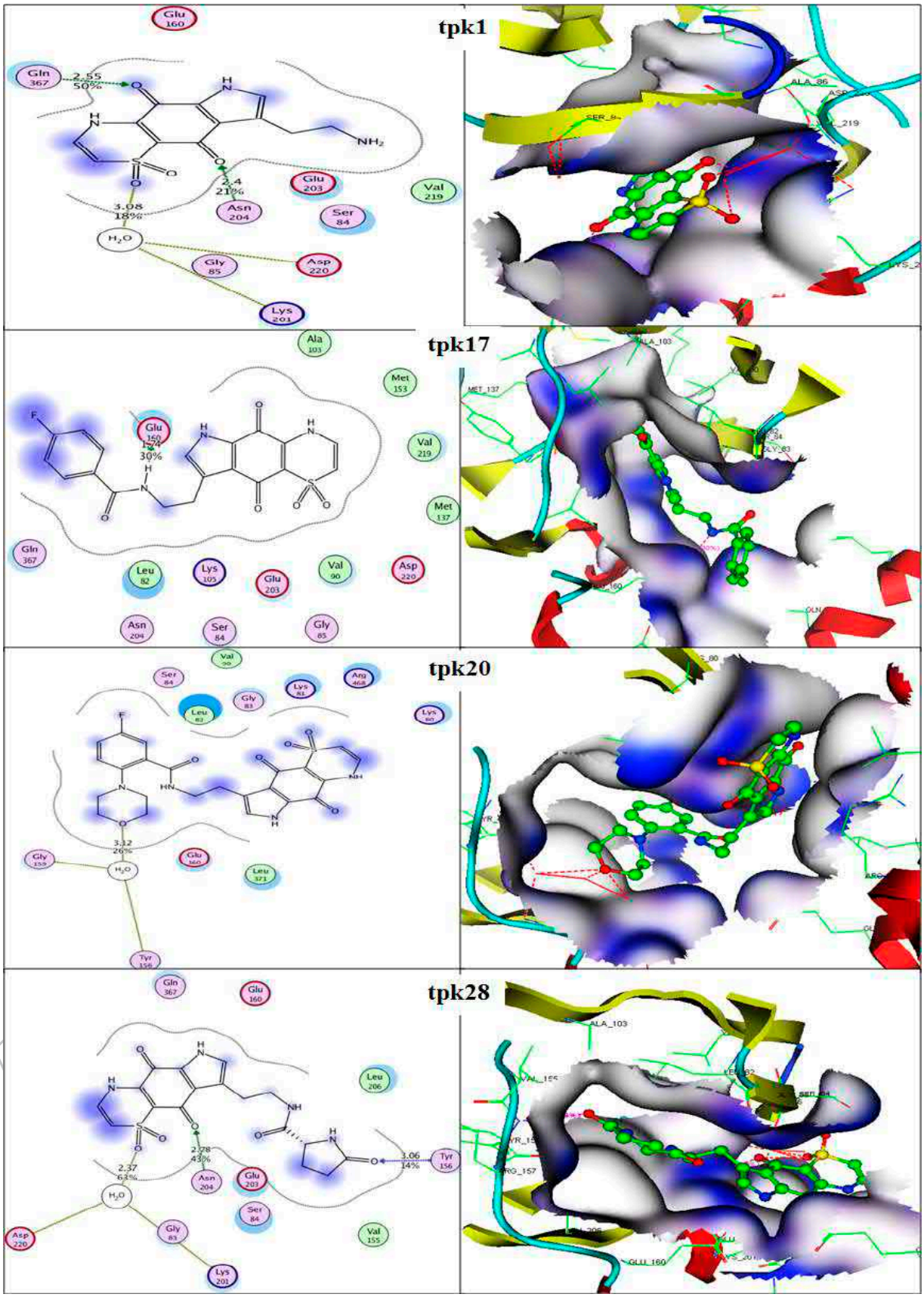


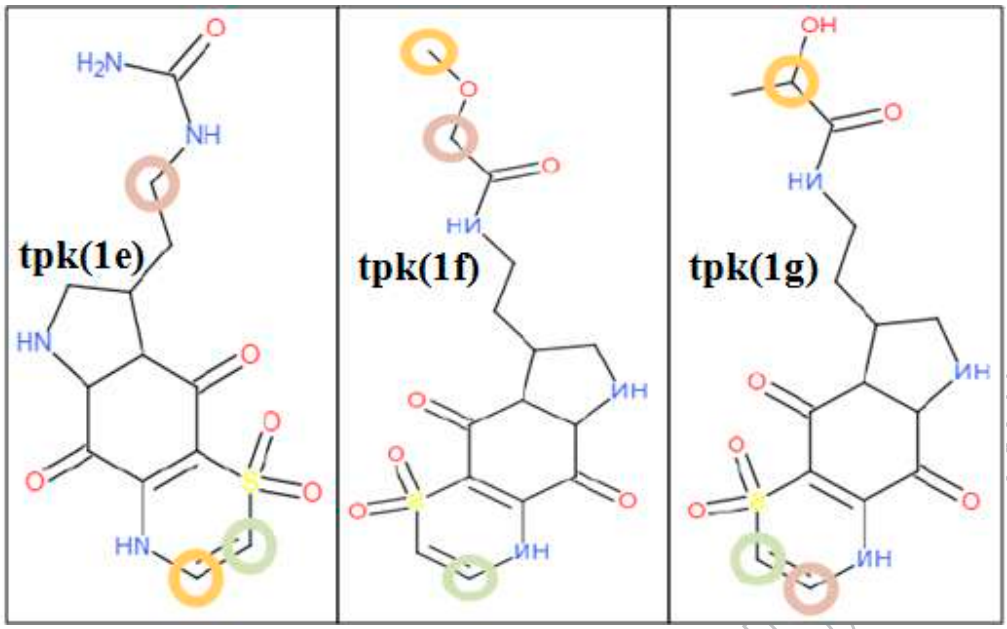












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QSAR modeling for anti-human African trypanosomiasis activity of substituted 2-Phenylimidazopyridines

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ABSTRACT

In the present work, sixty substituted 2-Phenylimidazopyridines previously reported with potent anti-human African trypanosomiasis (HAT) activity were selected to build genetic algorithm (GA) based QSAR models to determine the structural features that have significant correlation with the activity. Multiple QSAR models were built using easily interpretable descriptors that are directly associated with the presence or the absence of a structural scaffold, or a specific atom. All the QSAR models have been thoroughly validated according to the OECD principles. All the QSAR models are statistically very robust (R^2 = 0.80–0.87) with high external predictive ability (CCC_{ex} = 0.81–0.92). The QSAR analysis reveals that the HAT activity has good correlation with the presence of five membered rings in the molecule.

1. Introduction

Sleeping sickness or human African trypanosomiasis (HAT), transmitted by tsetse flies (genus *Glossina*), has a major occurrence in rural populations in sub-Saharan Africa. HAT, considered as a neglected tropical disease, was nearly eradicated in the mid-1960s. The resurgence in the late 1990s, due to poor sanitation and suitable habitats for its vector in the Democratic Republic of the Congo (DRC), Angola, Central African Republic, southern Sudan, and Uganda, received considerable attention of the researchers to develop better diagnosis and treatment for the disease [1–3]. Recent reports indicate that in humans the disease is thought to be mainly caused by *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense*, however the non-human-pathogenic trypanosome species *Trypanosoma brucei brucei*, *Trypanosoma congolense*, and *Trypanosoma evansi* are also responsible in some instances [1]. The disease has two stages; in stage 1 (hemolymphatic) the peripheral infection with non-specific clinical symptoms occur; and in stage 2 the parasite crosses the blood brain barrier (BBB) and intrudes the central nervous system (CNS) [1–3].

Suramin and pentamidine are the recommended drugs for stage 1 infection, whereas for stage 2, the therapeutic options are melarsoprol, eflornithine and the currently used combination therapy NECT (nifurtimox and eflornithine combination therapy). Unfortunately, vaccine cannot be developed due to a high degree of antigenic variation. In addition, the treatment is parasite- and stage-specific, depending on the ability of the compound to cross the BBB. For BBB clearance the drug must be sufficiently lipophilic, which results in poor water solubility, hence, such drugs are mostly toxic and problematical to administer. Consequently, the available drugs for stage 2 of the disease exhibit high toxicity, involve the complexity of administration procedures and progressive loss of efficacy in some geographical regions. Recent efforts identified Fexinidazole, furamidine, DB289 (parafuramidine), CPD-0802 (an aza analogue of parafuramidine) and SCYX-7158 (a boron based compound) as attractive lead/targets in the drug pipeline for

Abbreviations: HAT, Human African Trypanosomiasis; GA, Genetic algorithm; MLR, Multiple linear Regression; QSAR, Quantitative structure-activity analysis; WHO, World health organization; ADMET, Absorption, Distribution, Metabolism, Excretion and Toxicity; OLS, Ordinary Least Square; QSARINS-Chem, QSAR Insubria-Chemistry; OECD, Organization for Economic Co-operation and Development.

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developing better therapeutics for HAT (see Fig. 1). Despite the previous efforts executed high toxicity, poor oral bioavailability and blood–brain barrier penetration are the major obstacles ahead for these clinical candidates. Thence, the search for a drug candidate with adequate activity, ADME and toxicity profile still persists [1–4].

Recently, Tatipaka et al. [5] identified substituted oxazolopyridine **1** (see Fig. 2) as an attractive lead due to good whole-cell activity on *T. brucei*, no cytotoxicity on mammalian cell lines, acceptable exposure in the central nervous system, and satisfactory aqueous solubility. But, its poor metabolic stability in liver microsomes appeared as a severe liability. Later, to design a better analogue of **1** with the desired profile, they synthesized and screened a series of substituted 2-Phenylimidazopyridines. Since, the mechanism of action and the specific target with which these analogues interact is unknown [5]; in such a situation, a good strategy for lead optimization is to employ computer aided drug design (CADD) using the available information. Hence, in the present work, ligand based drug design technique, viz. QSAR (2D- and 3D-) has been executed to determine the structural features having a significant correlation with the HAT activity profile of substituted 2-Phenylimidazopyridines.

In the past decades, CADD has appeared as a thriving option to conventional 'trial and error' methodology of drug design/discovery to unknot the mysteries of structural patterns that govern the activity, pharmacokinetics, pharmacodynamics and toxicity profiles of a drug candidate. CADD is relatively fast, economical and significantly result oriented successful *in-silico* technique [6–10]. It encompasses a combination of different ideas, algorithms, tools and techniques of various scientific fields like computer, mathematics, statistics, etc. Its major emphasis is on simulation of interactions of different molecules, to determine the reasons behind the specific interactions of different molecules and identification of effective structural features associated with activity/toxicity. QSAR, molecular docking, pharmacophore modeling, etc. are established CADD methods, which when used in harmony provide significant and unrivalled information essential for lead/drug optimization [4,11–15]. These methods have been widely used for identification of the structural patterns that govern the specific activity/toxicity of drug candidates and provide better insight into the mechanism of drug action.

The main objective of the present work is to develop statistically robust and easily interpretable, in terms of structural fragments or specific atom, QSAR models with high external predictive ability.

2. Experimental methodology

2.1. Experimental datasets

In the present work, HAT inhibition activities of sixty substituted 2-Phenylimidazopyridines comprising different heterocyclic scaffolds and diverse substituents at various positions covering a meaningful portion of the chemical space were subjected to QSAR modeling [5]. The reported EC_{50} (μM) values for HAT activity were converted to pEC_{50} ($-\log_{10}EC_{50}$) before QSAR analysis. The EC_{50}, pEC_{50} and the substituents on 2-Phenylimidazopyridine moiety have been listed in Table 1.

2.2. Modeling and molecular descriptors calculation

In the present work, a QSAR analysis following the standard procedure recommended by OECD and different researchers was exercised [16–26]. The chemical structures were drawn using ChemSketch 12 freeware followed by energy minimization using MMFF94 force field in TINKER [4,12,15]. The optimized structures were used as input for the calculation of a good number of 1-3D, electro-topological, fingerprints and other descriptors. Two descriptor calculating softwares were used: PaDEL 2.21 and e-Dragon. Since, all the calculated descriptors (>18,000) do not contain significant information; objective feature selection was employed to reduce the descriptor pool. Nearly constant (>95%), constant, and highly correlated (|R| > 95%) descriptors were eliminated before subjective feature selection (SFS) using QSARINS-Chem 2.2.1 [16,17,20]. This resulted in a reduced cluster of 345 descriptors only. The very next step involved the elimination of highly esoteric descriptors, the descriptors for which an exact explanation is not available or it is difficult to interpret it in terms of structural features [26]. This led to a set of only 253 easily interpretable descriptors. The reduced set still consists a wide range of theoretical molecular descriptors that takes into account different structural features, viz. constitutional (0D-), mono-dimensional (1D-), bi-dimensional (2D-) and three-dimensional (3D-), capturing and magnifying the diverse aspects of the chemical structures.

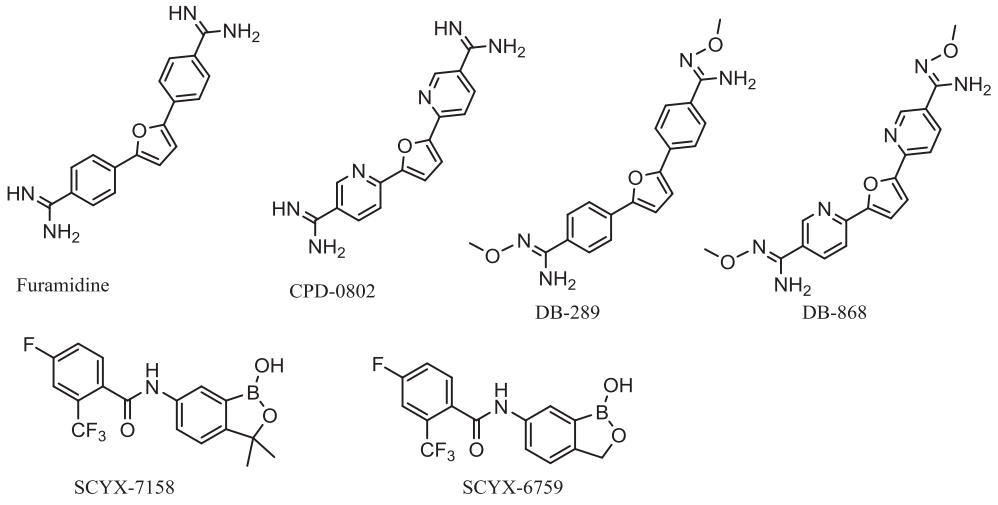
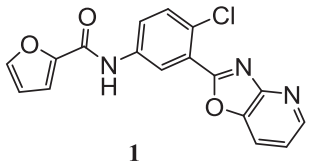


Fig. 1. Chemical structures of clinical drug candidates against HAT.



1

Ease of synthesis,
No stereogenic carbons,
Low MW and Lipinski rule-of-five compliance,
T. brucei in vitro EC_{50} = 220 nM,
Acceptable aqueous solubility,
Not cytotoxic to mammalian cells (L6, CRL-8155 and HepG2 cells),
Poor metabolic stability in liver microsomes

Fig. 2. Chemical structure and profile of substituted oxazolopyridine **1**.

2.3. QSAR model development

2.3.1. QSAR model

The very first principles and applications of QSAR analysis are to gain maximal information of activity related structural features and to predict the desired activity of a molecule before its actual synthesis and bio-screening. Hence, in order to achieve these goals easily interpretable descriptors were considered during model generation and multiple QSAR models were developed using divided and undivided datasets [19,27,28]. The dataset was divided into a training (80%) and a prediction (or test) (20%) sets in random fashion before descriptor selection. Multiple splitting were employed to develop multiple QSAR models [12,15], in this way a molecule in the training set of a splitting may or may not be in the training set of another splitting. Therefore, the multiple QSAR modeling approach ensured that maximum number and information is gained for molecular descriptors that govern the biological profile of the molecules. GA (Genetic Algorithm) module of QSARINS-Chem 2.2.1 was utilized for the selection of optimum number and set of descriptors. For the sake of simplicity and to avoid the problem of over-fitting, the heuristic search of descriptors was limited to four descriptors using the default settings in QSARINS-Chem 2.2.1. Q^2_{loo} was used as a fitness function to avoid the problem of naïve Q^2. The strategy used in QSAR model development has been summarized in Fig. 3 [12,15,24].

For development of QSAR models 6–10, HeuristicLab 3 was employed using different operand using the default settings.

2.4. Model validation

All QSAR models need to be appropriately validated to ascertain its predictive ability and utility. The statistical qualities and validity of the QSAR models were established by means of: (a) internal validation or cross-validation (CV) by leave-one-out (LOO) and leave-many-out (LMO) procedure; (b) using the prediction set; (c) data randomization i.e. Y-scrambling and (d) examining if the following conditions are satisfied [12,15]: $R^2_{tr} \geq 0.6$, $Q^2_{loo} \geq 0.5$, $Q^2_{LMO} \geq 0.6$, $R^2 > Q^2$, $R^2_{ex} \geq 0.6$, $RMSE_{tr} < RMSE_{cv}$, $\Delta K \geq 0.05$, $CCC \geq 0.80$, $Q^2 - F^n \geq 0.60$, $r^2_m \geq 0.6$, $(1 - r^2/r^2_0) < 0.1$, $0.9 \leq k \leq 1.1$ or $(1 - r^2/r'^2_0) < 0.1$, $0.9 \leq k' \leq 1.1$, $|r^2_0 - r'^2_0| < 0.3$ with *RMSE* and *MAE* close to zero. The threshold values of these parameters confirm the robustness and good external predictive ability of a GA-MLR model. Thus, all the models having low internal and external predictive ability were subsequently rejected.

3. Results and discussion

3.1. QSAR models

The GA analysis resulted in the generation of a good number of MLR models with nearly similar statistical performance but encompassing different descriptors. In such a situation, the usual practice followed by a QSAR modeller is to select only one MLR model on the basis of its statistical performance. However, this 'first among equals' approach is with following drawbacks [12,15] (1) a QSAR model consisting of only esoteric descriptors, suitable and realistic description in terms of structural features is highly problematic and challenging, (2) The single QSAR model may not be based on (i) appropriate composition of training and test sets, (ii) sufficient chemical and biologic space i.e. appropriate applicability domain, (3) the single QSAR model might have high predictive on a particular prediction set, but poor predictivity on another prediction set. To overcome these drawbacks of 'first among equals' approach, building and reporting multiple models or consensus modeling are two easy, practicable and efficient solutions. Recently, it has been established that developing multiple QSAR models based on divided and undivided dataset enhance the efficacy of QSAR in determining the dominant and concealed structural features that have significant correlation with the activity [12,15]. Therefore, in the present study, multiple QSAR models have been built following the OECD principles for acceptable QSAR models. This approach led to generation of ten QSAR models possessing excellent statistical performance. The GA-MLR QSAR models along with different statistical parameters, tabulated in Table 2, are as following:

3.2. Model-1 (Undivided dataset)

$$pEC_{50} = 6.486\ (\pm 0.378) - 0.214\ (\pm 0.067)\ ^{*}\ \text{C-024} + 0.756\ (\pm 0.273)\ ^{*}\ \text{nR05} - 1.087\ (\pm 0.318)\ ^{*}\text{F09[N–F]} + 0.897\ ((\pm 0.169)\ ^{*}\text{F10[C–F]}.$$

3.3. Model-2 (Undivided dataset)

$$pEC_{50} = 4.218\ (\pm 0.514) + 0.449\ (\pm 0.141)\ ^{*}\text{F02[N–N]} + 0.842\ (\pm 0.265)\ ^{*}\ \text{nR05} - 0.570\ (\pm 0.305)\ ^{*}\text{F09[N–F]} + 0.453\ (\pm 0.147)\ ^{*}\text{F10[C–F]}.$$

3.4. Model-3 (Divided dataset)

$$pEC_{50} = 5.148\ (\pm 0.537) + 0.518\ (\pm 0.399)\ ^{*}\text{B10[C–F]} + 0.624\ (\pm 0.292)\ ^{*}\ \text{nR05} - 0.558\ (\pm 0.476)\ ^{*}\text{B09[N–F]} + 1.178\ (\pm 0.404)\ ^{*}\text{B07[N–F]}.$$

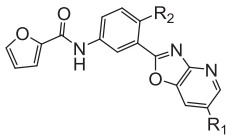
3.5. Model-4 (Divided dataset)

$$pEC_{50} = 5.729\ (\pm 0.258) - 1.328\ (\pm 0.724)\ ^{*}\ \text{nArNR2} + 1.450\ (\pm 0.337)\ ^{*}\ \text{nPyrrolidines} - 0.507\ (\pm 0.254)\ ^{*}\text{F10[N–F]} + 0.308\ (\pm 0.100)\ ^{*}\text{F09[C–F]}.$$

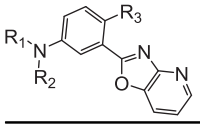
3.6. Model-5 (Divided dataset)

$$pEC_{50} = 5.789\ (\pm 0.258) - 1.375\ (\pm 0.743)\ ^{*}\ \text{nArNR2} + 1.322\ (\pm 0.331)\ ^{*}\ \text{nPyrrolidines} - 0.015\ (\pm 0.008)\ ^{*}\text{G(N..F)} + 0.381\ (\pm 0.121)\ ^{*}\text{F09[C–F]}.$$

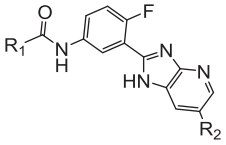
Table 1
Different substituted 2-Phenylimidazopyridines along with reported IC_{50} and pIC_{50}.



S.No.	R_1	R_2	EC_{50} (μm)	pEC_{50}(M)
1.	H	Cl	0.22	6.658
2.	H	F	0.12	6.921
3.	H	Me	0.43	6.367
4.	H	CN	0.4	6.398
5.	Br	F	0.23	6.638
6.	CN	F	0.38	6.42
7.	phenyl	F	0.04	7.398
8.	4-fluorophenyl	F	0.05	7.301
9.	3-chlorophenyl	F	0.05	7.301
10.	4-MeO-phenyl	F	0.17	6.77
11.	4-phenylphenyl	F	0.42	6.377

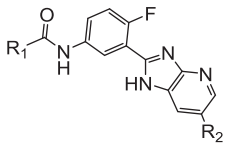


	R_1	R_2	R_3	EC_{50} (μM)	pEC_{50}(M)
12.	5-methylfuran-2-carbonyl	H	F	0.2	6.699
13.	3-methylfuran-2-carbonyl	H	F	0.1	7
14.	3-furanoyl	H	F	0.15	6.824
15.	benzoyl	H	Cl	7.1	5.149
16.	oxazole-5-carbonyl-	H	F	1.9	5.721
17.	2-thiophenoyl	H	Cl	1.5	5.824
18.	3-pyridinecarbonyl-	H	F	7	5.155
19.	pyrazine-2-carbonyl-	H	F	0.9	6.046
20.	N-methylpyrrole-2-carbonyl-	H	F	1.1	5.959
21.	methylsulfonyl	H	Cl	6.1	5.215
22.	2-furancarbothioyl-	H	F	0.41	6.387
23.	2-furanoyl	2-acetyl	F	0.5	6.301
24.	2-furanoyl	2-furanoyl	F	0.12	6.921
25.	benzyl	benzyl	F	1.1	5.959
26.	methylcarbamoyl	H	F	3.8	5.42
27.	isopropylcarbamoyl-	H	Cl	1	6
28.	phenylcarbamoyl-	H	Cl	12	4.921
29.	dimethylcarbamoyl-	H	Cl	0.4	6.398
30.	1-pyrrolidinoyl-	H	Cl	0.09	7.046
31.	1-piperidinoyl-	H	Cl	1.9	5.721



	R_1	R_2	EC_{50} (μM)	pEC_{50}(M)
32.	2-furanyl	H	0.2	6.699
33.	2-furanyl	Cl	0.07	7.155
34.	2-furanyl	F	0.2	6.699
35.	2-furanyl	5-Cl	10	5
36.	2-furanyl	7-Cl	0.12	6.921
37.	N-pyrrolidinyl	Cl	0.05	7.301
38.	N-pyrrolidinyl	phenyl	0.002	8.699
39.	N-pyrrolidinyl	3-methoxyphenyl	0.01	8
40.	N-pyrrolidinyl	2-methoxyphenyl	0.005	8.301
41.	N-pyrrolidinyl	3-Cl-phenyl	0.002	8.699
42.	N-pyrrolidinyl	2-chlorophenyl	0.005	8.301
43.	N-pyrrolidinyl	3-acetylphenyl	0.003	8.523
44.	N-pyrrolidinyl	3-Me-phenyl	0.002	8.699
45.	N-pyrrolidinyl	3-trifluoromethoxyphenyl	0.03	7.523
46.	N-pyrrolidinyl	3-methyl-4-fluorophenyl	0.02	7.699
47.	N-pyrrolidinyl	3-NH_2-phenyl	0.01	8
48.	N-pyrrolidinyl	3-furanyl	0.004	8.398
49.	N-pyrrolidinyl	3-thiophenyl	0.002	8.699
50.	N-pyrrolidinyl	2-thiophenyl	0.004	8.398
51.	N-pyrrolidinyl	3-pyridyl	0.005	8.301

Table 1 (*continued*)



	R_1	R_2	EC_{50} (μM)	pEC_{50}(M)
52.	N-pyrrolidinyl	5-(2-chloropyridyl)	0.01	8
53.	N-pyrrolidinyl	4-(2-chloropyridyl)	0.004	8.398
54.	N-pyrrolidinyl	5-(3-methylpyridyl)	0.01	8
55.	N-pyrrolidinyl	5-(2-methoxypyridyl)	0.005	8.301
56.	N-pyrrolidinyl	5-(3-pyrrolidino)	0.02	7.699
57.	N-pyrrolidinyl	5-(3-chloropyrimidinyl)	0.005	8.301
58.	N-pyrrolidinyl	5-pyrimidinyl	0.03	7.523
59.	N-pyrrolidinyl	5-(2-methoxypyrimidinyl)	0.06	7.222
60.	N-pyrrolidinyl	5-(2-chloropyrimidinyl)	0.04	7.398

3.7. Model-6 (Undivided dataset)

pEC_{50} = 8.455 (±0.292)−0.013 (±0.006) *G(N..F)−1.410 (±0.392) * invsqr-nR05−1.440 (±0.273) *B03[N−O] + 0.318 (±0.134) *F10 [C−F].

3.8. Model-7 (Undivided dataset)

pEC_{50} = 9.122 (±0.407)−0.013 (±0.006) *G(N..F)−1.217 (±0.341) * invsqr-nR05−0.846 (±0.159) * expB03[N−O] + 0.317 (±0.135) *F10 [C−F].

3.9. Model-8 (Divided dataset)

pEC_{50} = 8.162 (±1.455)−0.022 (±0.021) * Ss−2.053 (±0.442) * invcube-nR05 + 0.948 (±0.263) * C-041 + 0.901 (±0.325) *B10 [C−F].

3.10. Model-9 (Divided dataset)

pEC_{50} = 8.540 (±0.458)−0.015 (±0.011) *G(N..F)−1.603 (±0.450) * invsqr-nR05−1.416 (±0.355) *B03[N−O] + 0.340 (±0.160) *F10 [C−F].

3.11. Model-10 (Divided dataset)

pEC_{50} = 7.675 (±0.331) + 0.388 (±0.308) * Cl-089−1.485 (±0.412) * invcube-nR05−1.118 (±0.316) *B03[N−O] + 0.672 (±0.308) *B10[C−F].

The statistical symbols have their usual meaning [12,16,17,20,23] and are available in the supplementary material, also. From statistical analysis (Table 2), it is clear that all the developed QSAR models are statistically robust and possess good external predictive ability, especially, the models 6−10. The models 6−10 outperform their counterparts apropos of fitting, internal validation and external predictivity criteria. As expected, establishing the models 6−10 helped in the identification of less dominant descriptors like Ss (sum of Kier-Hall electrotopological indices), G(N..F) (sum of geometrical distances between N and F), C-041 (an atom-centered fragment representing X−C(=X)−X), and Cl-089 (an atom-centered fragment representing Cl attached to sp^2 hybridized C) with correlation with the activity, which were not discovered while building the models 1−5. For all the developed models, the value of $R^2_{adj.}$ is quite close to R^2_{tr} suggesting that the number of descriptors in the models are not too high, thereby, indicating that the models are free from over-fitting [26]. This is further supported by the low value of LOF (Lack of fit) for all the models. The low Kxx

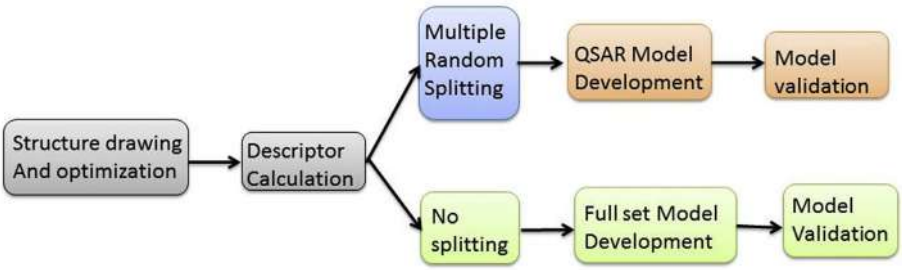


Fig. 3. Strategy used for developing the QSAR models.

Table 2
Statistical performance of different QSAR models.

S.No.	Statistical Parameter	Model-1	Model-2	Model-3	Model-4	Model-5	Model-6	Model-7	Model-8	Model-9	Model-10
1.	N_{tr}	60	60	48	48	48	60	60	48	48	48
2.	N_{ex}	–	–	12	12	12	–	–	12	12	12
3.	Number of Descriptors	4	4	4	4	4	4	4	4	4	4
Fitting Criteria											
4.	R^2_{tr}	0.807	0.806	0.822	0.823	0.812	0.863	0.862	0.839	0.876	0.874
5.	$R^2_{adj.}$	0.793	0.792	0.806	0.806	0.794	0.853	0.852	0.824	0.865	0.863
6.	R^2_{tr} - $R^2_{adj.}$	0.014	0.014	0.016	0.017	0.018	0.010	0.010	0.015	0.012	0.012
7.	LOF	0.288	0.289	0.292	0.301	0.312	0.205	0.206	0.246	0.214	0.217
8.	Kxx	0.255	0.300	0.398	0.365	0.406	0.441	0.440	0.306	0.478	0.374
9.	ΔK	0.119	0.139	0.119	0.064	0.058	0.064	0.063	0.131	0.080	0.104
10.	$RMSE_{tr}$	0.465	0.466	0.450	0.457	0.466	0.392	0.394	0.413	0.385	0.389
11.	MAE_{tr}	0.354	0.358	0.349	0.362	0.383	0.312	0.313	0.320	0.307	0.314
12.	RSS_{tr}	12.981	13.028	9.733	10.034	10.414	9.232	9.302	8.190	7.119	7.248
13.	CCC_{tr}	0.893	0.893	0.903	0.903	0.896	0.926	0.926	0.913	0.934	0.933
14.	s	0.486	0.487	0.476	0.483	0.492	0.410	0.411	0.436	0.407	0.411
15.	F	57.446	57.191	49.749	49.841	46.345	86.363	85.610	56.177	76.220	74.679
Internal Validation Criteria											
16.	R^2_{cv} (Q^2loo)	0.776	0.770	0.784	0.777	0.753	0.840	0.839	0.800	0.847	0.844
17.	R^2-R^2_{cv}	0.031	0.036	0.038	0.046	0.059	0.022	0.023	0.039	0.029	0.030
18.	$RMSE_{cv}$	0.501	0.508	0.496	0.513	0.533	0.423	0.425	0.461	0.428	0.432
19.	MAE_{cv}	0.382	0.394	0.389	0.411	0.442	0.340	0.341	0.358	0.344	0.351
20.	$PRESS_{cv}$	15.041	15.472	11.824	12.629	13.650	10.740	10.837	10.190	8.809	8.974
21.	CCC_{cv}	0.877	0.874	0.882	0.881	0.867	0.914	0.914	0.892	0.919	0.917
22.	Q^2_{LMO}	–	–	0.774	0.792	0.771	0.830	0.827	0.787	0.842	0.841
23.	R^2_{Yscr}	–	–	0.081	0.086	0.087	0.069	0.069	0.086	0.087	0.082
24.	Q^2_{Yscr}	–	–	−0.151	−0.178	−0.231	−0.137	−0.137	−0.144	−0.144	−0.144
External Validation Criteria											
25.	θ*	–	–	−10.983	3.116	2.325	–	–	−2.183	−2.328	1.261
26.	$RMSE_{ex}$	–	–	0.570	0.479	0.417	–	–	0.512	0.441	0.483
27.	MAE_{ex}	–	–	0.421	0.351	0.310	–	–	0.442	0.350	0.402
28.	$PRESS_{ext}$	–	–	3.894	2.756	2.085	–	–	3.148	2.330	2.801
29.	R^2_{ex}	–	–	0.673	0.838	0.924	–	–	0.819	0.779	0.787
30.	Q^2-F^1	–	–	0.690	0.748	0.830	–	–	0.807	0.759	0.711
31.	Q^2-F^2	–	–	0.673	0.713	0.800	–	–	0.804	0.752	0.702
32.	Q^2-F^3	–	–	0.716	0.805	0.849	–	–	0.753	0.838	0.806
33.	CCC_{ex}	–	–	0.805	0.886	0.916	–	–	0.905	0.882	0.875
34.	r^2m aver.	–	–	0.541	0.654	0.776	–	–	0.741	0.687	0.657
35.	r^2m delta	–	–	0.257	0.180	0.094	–	–	0.040	0.069	0.197
36.	R^2-ExPy	–	–	0.785	0.780	0.756	–	–	0.801	0.848	0.845
37.	R'^2_o	–	–	0.740	0.747	0.712	–	–	0.765	0.829	0.823
38.	k'	–	–	0.996	0.997	0.993	–	–	0.996	0.998	0.997
39.	1-(R^2/R'^2_o)	–	–	0.057	0.042	0.059	–	–	0.044	0.022	0.025
40.	r'^2m	–	–	0.413	0.639	0.597	–	–	0.650	0.731	0.722
41.	R^2_o	–	–	0.784	0.777	0.753	–	–	0.800	0.847	0.844
42.	k	–	–	0.999	0.998	1.002	–	–	0.999	0.999	0.999
43.	1-(R^2-ExPy/R^2_o)	–	–	0.001	0.004	0.004	–	–	0.001	0.001	0.001
44.	r^2m	–	–	0.767	0.737	0.716	–	–	0.780	0.829	0.829

R^2 – correlation coefficient, Q^2 – leave-one-out 'cross validated R^2', R^2_{adj} - adjusted R^2, SEE – standard error of estimates, RMSE - root mean squared error, MAE - mean absolute error, CCC - concordance correlation coefficient, for the training (tr), and test (ex) sets; LOF - lack of fit, F – Fischer's value; R^2_{LMO} and Q^2_{LMO} – leave many-out correlation coefficient and cross-validation coefficients; R^2_{Ycr} and Q^2_{Ycr} – Y–- scramble correlation and cross-validation coefficients.

(representing inter-correlation among descriptors) value in all the models indicates that low correlation among the descriptors used in a model [16,17,20]. The condition $RMSE_{tr} < RMSE_{cv}$ is satisfied by all the developed models. The values of cross validation parameters Q^2, Q^2_{LMO} and CCC_{CV} for the developed models are high, thereby, indicating the statistical robustness of the models. The low value of R^2_{Yscr} and Q^2_{Yscr} for all the models indicates that the models have not been developed by chance. For the models 3–5 and 8–10, the

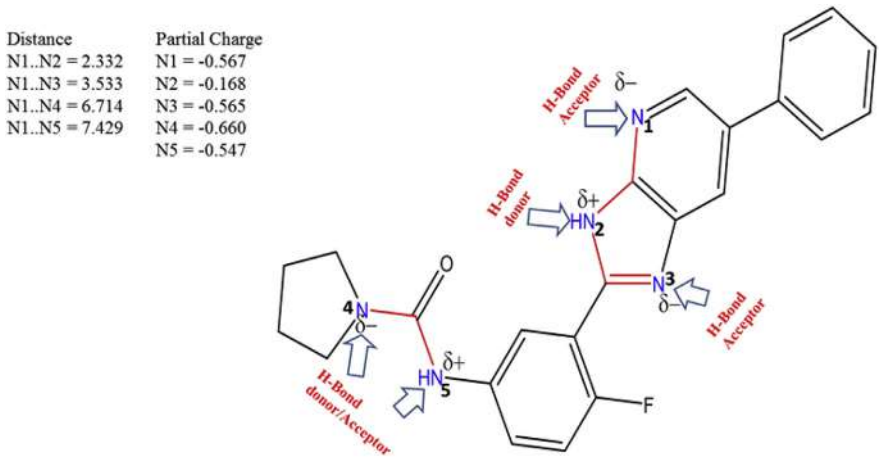


Fig. 4. Exemplification of F02[N–N] descriptor and H-bond donor/acceptor pattern associated with F02[N–N] using molecule **38** as a representative.

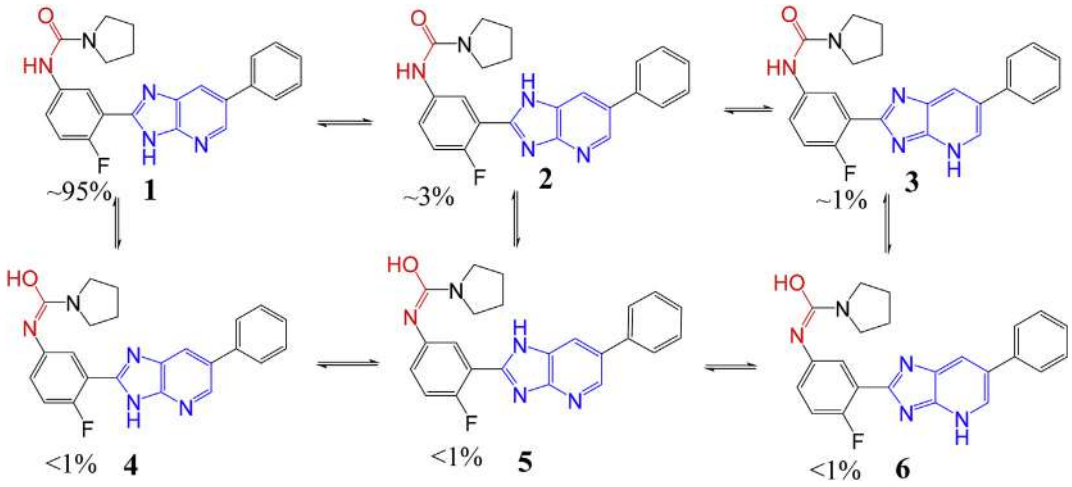


Fig. 5. Different possible tautomeric forms of **38**.

high value of various statistical parameters like R^2_{ex}, Q^2-F^1, Q^2-F^2, Q^2-F^3, CCC_{ex}, etc. indicate the models possess high external predictivity. This is further supported by the low value of $RMSE_{ex}$, MAE_{ex}, and $PRESS_{ext}$. In short, the developed models satisfy the recommended interrelations and threshold values for various statistical parameters suggested by different researchers. Interestingly, for model 5 a very rare observation is identified, the value of Q^2-F^1 (= 0.830) is greater than R^2 (= 0.812), thus, the model is able to predict new data better than fitting available ones, indicating that the molecules for which this model fits better are present in the prediction set [15,29,30]. This is again supported by the higher value of R^2_{ex} than R^2.

A commonly encountered problem associated with QSAR analysis is the interpretation or correlation of descriptors with specific structural fragment or atom. Therefore, in the present work, easily interpretable descriptors that are directly associated with the presence or the absence of a structural scaffold, or a specific atom were considered. This could be highly beneficial to synthetic chemists for future synthetic strategies.

In models 1–3, the 2D-constitutional type of descriptor 'nR05', representing the presence of a five membered ring, is present with a positive coefficient. The descriptors 'invsqr-nR05' and 'invcube-nR05' represent the inverse of square or cube of 'nR05', respectively, and have negative coefficients in models 6–10. Therefore, it appears that the presence of five membered rings in the molecules increases the activity. This observation is vindicated by comparing the activity of **1** with **15**, and **2** with **18**, as representatives. Therefore, in future modifications five membered ring must be retained to increase the activity. The 2D-descriptors, F09[N–F] and B09[N–F], which correspond to the frequency and presence/absence of N and F at a topological distance of 9, respectively, have a negative correlation with the activity. Interestingly, in models 1–3, the positive correlation of F10[C–F] and B10[C–F], which correspond to frequency and presence/absence of C and F at a topological distance of 10, respectively, with HAT activity indicates that the presence of F favors the activity. This is again supported by the positive correlation of B07[N–F], which represents the presence/absence of N and F at topological distance of 7, in model 3.

The descriptor C-024, an atom centered descriptor corresponds to R–CH–R fragment, has a negative coefficient in model-1, hence, its value must be kept as low as possible. The descriptor 'nPyrrolidines' indicates the presence of number of pyrrolidine rings in the molecule, its presence is beneficial for activity, as evident from its positive coefficient in model 4 and 5. The descriptor 'nArNR2' which stands for the presence of number of tertiary amines (aromatic) in the molecule has a negative association with the activity, hence such groups must be avoided in future modifications. F02[N–N] is a finger print descriptor that represents the frequency of

the presence of two nitrogen atoms at a topological distance of 2, its positive coefficient in the model 2 points out its positive influence on the activity. This descriptor has been depicted by red bonds in Fig. 4 using the molecule **38** as a representative. This descriptor is associated with an interesting pharmacophoric pattern of H-bond donor and acceptor nitrogen atoms present in a specific arrangement, in which a H-bond donor (like N2) is at a topological distance of 2 from at least one H-bond acceptor N (like N1 or N3).

The importance of this structural pattern is further supported by the fact that the presence of 5-chloropyridin-2-yl, as in *N*-(3-((5-chloropyridin-2-yl)carbamoyl)-4-fluorophenyl)furan-2-carboxamide, has negative influence on the activity. The compound *N*-(3-((5-chloropyridin-2-yl)carbamoyl)-4-fluorophenyl)furan-2-carboxamide ($EC_{50} = 7\ \mu M$) was not incorporated during the QSAR and pharmacophore model building, as the aim of the present work was to analyze the bicyclic derivatives only.

This interesting pattern of H-bond donor/acceptor nitrogen atoms also helps in attaining various tautomeric forms, thereby, providing additional flexibility to the molecules to acquire bioactive tautomeric form(s) while interacting with the target receptor. It has been established that a less stable tautomeric form of a molecule could be the true bioactive tautomeric form [12]. Thus, tautomeric transformations could be a possible reason behind higher activity of the molecules bearing imidazopyridine ring. Such a useful flexibility is diminished when an oxazolopyridine ring is present instead of an imidazopyridine ring. The various tautomeric forms along with their calculated distribution (using the software MarvinSketch 5.0) for the molecule **38**, as a representative, have been depicted in Fig. 5.

The descriptor B03[N–O], which represents the presence/absence of N and O at a topological distance of 3, or its exponential expB03[N–O] have negative coefficients in the model 6,7, 9, and 10. Therefore, the presence/absence of N and O at a topological distance of 3 has negative influence on the activity. Hence, such a combination of N and O must be avoided for better activity profile. This is supported by the observation that in the present series the molecules bearing oxazolopyridine ring, in general, are less active than those having an imidazopyridine ring.

4. Conclusions

The QSAR analysis revealed important information and observations about the structural features that steer the HAT activity of substituted 2-Phenylimidazopyridines. The developed models are statistically robust with high external predictive ability. The development of multiple QSAR models helped in identifying less dominant, but very useful descriptors, which have significant correlation with the activity. The QSAR models pointed out that the presence of five membered rings, especially the pyrrolidine ring, is beneficial for the HAT activity of the present series molecules.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.molstruc.2016.11.012.

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Synthesis, Antiphospholipase A_2, Antiprotease, Antibacterial Evaluation and Molecular Docking Analysis of Certain Novel Hydrazones

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Abstract: Some novel hydrazone derivatives **6a–o** were synthesized from the key intermediate 4-Chloro-*N*-(2-hydrazinocarbonyl-phenyl)-benzamide **5** and characterized using IR, ^{1}H-NMR, ^{13}C-NMR, mass spectroscopy and elemental analysis. The inhibitory potential against two secretory phospholipase A_2 ($sPLA_2$), three protease enzymes and eleven bacterial strains were evaluated. The results revealed that all compounds showed preferential inhibition towards *h*GIIA isoform of $sPLA_2$ rather than DrG-IB with compounds **6l** and **6e** being the most active. The tested compounds exhibited excellent antiprotease activity against proteinase K and protease from *Bacillus* sp. with compound **6l** being the most active against both enzymes. Furthermore, the maximum zones of inhibition against bacterial growth were exhibited by compounds; **6a**, **6m**, and **6o** against *P. aeruginosa*; **6a**, **6b**, **6d**, **6f**, **6l**, **6m**, **6n**, and **6o** against *Serratia*; **6k** against *S. mutans*; and compounds **6a**, **6d**, **6e**, **6m**, and **6n** against *E. feacalis.* The docking simulations of hydrazones **6a–o** with GIIA $sPLA_2$, proteinase K and hydrazones **6a–e** with glutamine-fructose-6-phosphate transaminase were performed to obtain information regarding the mechanism of action.

Keywords: benzo[*d*][1,3]oxazin-4-one; aroylhydrazides; *N*-acylhydrazones; hydrazones; benzylidene hydrazides; phospholipases; proteases; antimicrobial evaluation; molecular docking

1. Introduction

Hydrazones bearing azomethine moiety can be formed by condensation of hydrazides or aroyl hydrazides with aldehydes [1–4]. These compounds are most widely used as building blocks for the synthesis of 3-acetyl-2,5-disubstituted-2,3-dihydro-1,3,4-oxadiazoles [5], different mono-, di- and

trisubstituted hydrazines [6,7], azetidin-2-ones, thiazolidin-4-ones and methyl thiazolidines [8,9], and formazan derivatives [10,11]. Hydrazones are well known to exhibit a wide spectrum of biological activities [12] and have been utilized as useful candidates for development of antimalarial [13], antitumor [14], antiviral [15,16], antimicrobial [17], antioxidant [18], analgesic, anti-inflammatory and anti-ulcerogenic agents [19,20].

Secreted phospholipases A_2 (sPLA$_2$s) are enzymes found in mammals and animal venoms that catalyze hydrolysis of glycerophospholipids at the *sn*-2 ester position to release a free fatty acid and a lysophospholipid [21]. Mammalian phospholipases have been categorized into ten groups IB, IIA, IIC, IID, IIE, IIF, III, V, X and XIIA [22,23]. It has been reported that sPLA$_2$s play a key role in a good number of bio-chemical processes, thus, on one the hand, group IB sPLA$_2$ has been proposed to be involved in various physiological and pathophysiological processes such as cellular proliferation, cell migration, hormone release [24] and apoptosis in neuronal cells [25]. In addition, higher levels of this enzyme were detected in the serum of patient with chronic renal failure [26] and acute lung injury [27] compared to healthy controls.

On the other hand, the GIIA sPLA$_2$ has been correlated to inflammatory diseases, which is due to elevated levels of this enzyme were detected in the fluids of patients suffering from various inflammatory diseases such as rheumatoid arthritis [28], acute lung injury-acute respiratory distress syndrome (ALI-ARDS) [29] and pancreatitis-associated adrenal injury in acute necrotizing pancreatitis [30], as well as in various cancers [31]. Therefore, seeking potent and selective inhibitors to this sPLA$_2$ may be considered an effective approach to develop novel anti-inflammatory and antitumor agents.

Proteases or proteinases enzymes are proteolytic enzymes that break down proteins into peptides and amino acids. They are classified into different classes depending on their source and functions [32] such as proteinase K, protease from *Bacillus* and protease-esperase. Biologically, proteases are involved in controlling many biological pathways in the life cycle of human, plants, animals, insects and pathogens such as fungi, bacteria and viruses. Many studies reported their involvement in the pathogenic processes of human diseases such as bleeding disorders [33], inflammation [34], cancer [35], hypertension [36] and AIDS [37]. Therefore, proteases inhibitors' can be considered as targets in drug design for developing therapeutics and prevention of diseases [38].

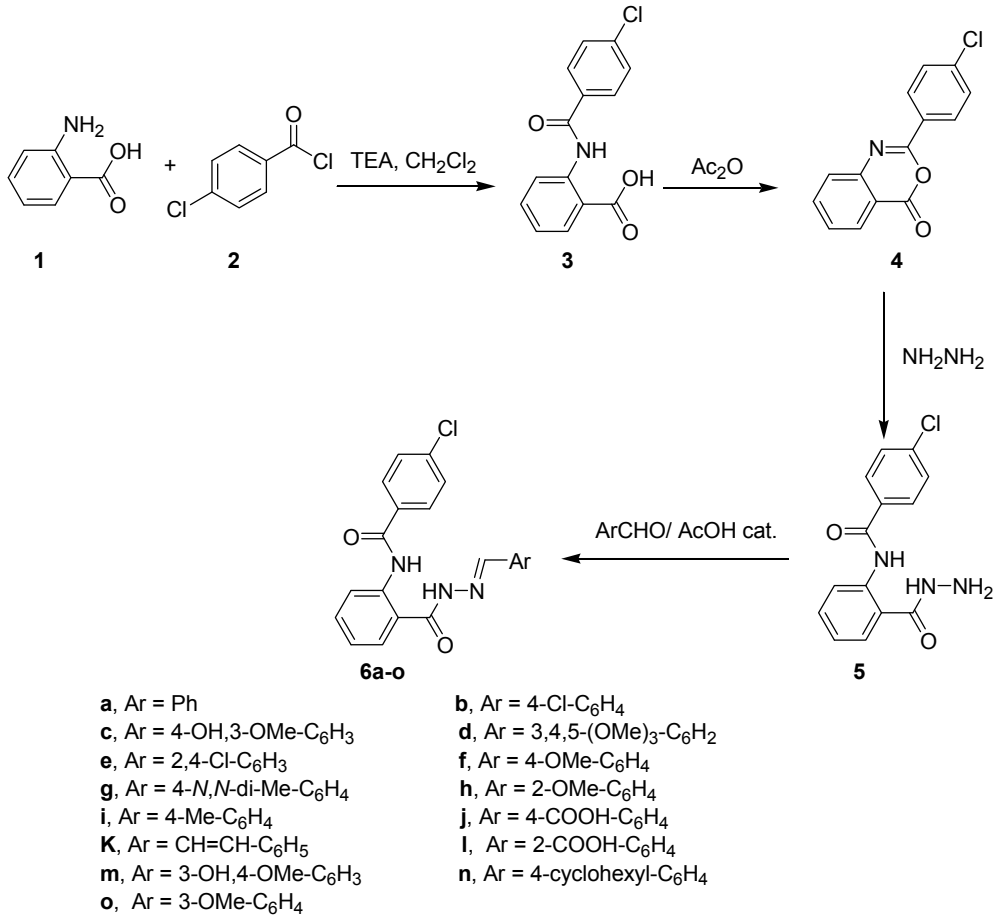
In continuation to our previous efforts towards synthesis of novel biologically active compounds [39–41], we report herein the synthesis of novel hydrazones **6a**–**o** and evaluation of their phospholipases, proteases and bacterial inhibitory activities. The docking simulations of hydrazones **6a**–**o** against GIIA sPLA$_2$, proteinase K and hydrazones **6a**–**e** against glutamine-fructose-6-phosphate transaminase were performed in order to obtain information regarding the mechanism of action.

2. Results and Discussion

2.1. Chemistry

Benzoylation of anthranillic acid **1** with 4-chlorobenzoyl chloride **2** in methylene chloride in the presence of triethylamine afforded the corresponding amido acid **3** which upon boiling with excess of acetic anhydride underwent intramolecular cyclization and afforded benzo[*d*][1,3]oxazin-4-one derivative **4**. Nucleophilic attack by the amino group of hydrazine hydrate on the carbonyl group of the latter benzoxazinone, and subsequent ring opening gives the key intermediate 4-Chloro-*N*-(2-hydrazinocarbonyl-phenyl)-benzamide **5**. Condensation of hydrazino derivative **5** with a variety of aromatic aldehydes yielded the desired hydrazones **6a**–**o** (Scheme 1). The structures of these products were confirmed based on their spectral data and elemental analyses. In general, the IR spectra of compounds **6a**–**o** displayed two absorption bands at ν_{max} 3421–3292 cm^{-1}, which are attributed to the two (NH) groups, and two absorption bands at ν_{max} 1684–1657 cm^{-1} due to the two carbonyl groups. In addition, the (C=N) groups appeared around 1598–1579 cm^{-1}. Moreover, the ^{1}H-NMR spectra of these compounds indicated the disappearance of the spectral line due to NH$_2$

group of the starting hydrazide **5** and appearance of two broad singlet signals at δ 12.37–11.08 ppm corresponding to 2NH groups, singlet signals at δ 8.84–8.30 ppm due to the characteristic azomethines' protons (N=CH) in addition to signals attributed to the aliphatic and aromatic protons at the expected chemical shift values. Analogously, the ^{13}C-NMR spectra proved the presence the two C=O groups at δ_C 165.61–163.86 ppm, azomethine carbons' at 151.46–145.10 ppm and the aliphatic and aromatic carbons at the expected chemical shift values. The mass spectra of all of the synthesized compounds revealed the existence of the parent ion peaks. Finally, the C, H, N elemental analyses of all of the synthesized compounds were in agreement with the proposed structures.



Scheme 1. The synthesis of Schiff bases **6a**–**o**.

2.2. Biological Evaluation

2.2.1. Phospholipases Inhibitory Activity

The phospholipase inhibitory activity of hydrazones **6a**–**o** have been detected against two different classes of sPLA$_2$s, namely human group IIA sPLA$_2$ (hG-IIA) and dromedary group IB sPLA$_2$ (DrG-IB). The obtained results revealed that compounds **6c**, **6d**, **6e** and **6l** exhibited selective inhibition against *h*GIIA rather than DrG-IB by 50.67% ± 4.04%, 55.67% ± 4.72%, 72.67% ± 2.51% and 74.33% ± 3.21%, respectively, as shown in Figure 1 and Table S1.

Conversely, the lowest inhibitory activity against this enzyme (9% ± 2%) was displayed by compound **6n**. These results are in agreement with the data obtained in our previous work [42].

As many clinical studies revealed that the levels of sPLA$_2$s are increased in different inflammatory conditions such as rheumatoid arthritis [28], these active hydrazones can be proposed as potential candidates to explore their utility for such ailment.

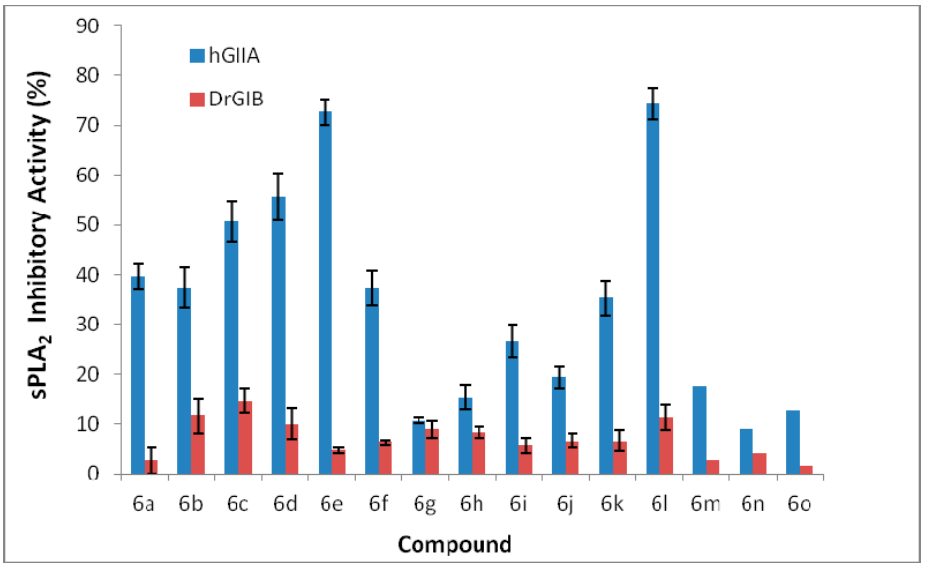


Figure 1. Inhibitory activity (%) of compounds **6a–o** against $sPLA_2$ (hG-IIA and DrG-IB).

2.2.2. Proteases Inhibitory Activity

The synthesized compounds were also screened for in vitro anti-proteases activity against the commercially available protease-esperase, proteinase K and protease obtained from *Bacillus* sp. The screening results shown in Figure 2 and Table S2 revealed that the tested compounds displayed varied degrees of proteases inhibition. The maximum inhibitory activities against proteinase K (86 ± 2.64), protease from *Bacillus* (74.66 ± 2.88) and protease-esperase (39.33 ± 3.21) were exhibited by compound **6l**.

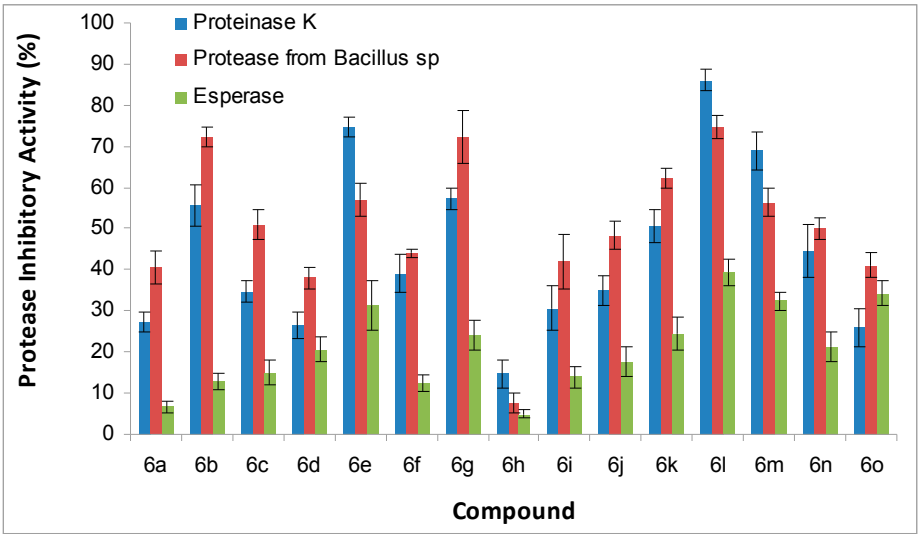


Figure 2. Antiprotease activity (%) of compounds **6a–o** against different protease enzymes.

Furthermore, the next highest inhibitory activities against proteinase K were exhibited by compounds **6e** (74.66% ± 2.51%) and **6m** (69% ± 4.58%), while the next highest inhibitory activities against protease from *Bacillus* sp. were exhibited by compounds **6b** (72.33% ± 2.51%) and **6g** (72.33% ± 6.42%).

It is worth mentioning that most of the compounds showed good antiprotease inhibition against proteinase K and protease from *Bacillus* sp., as depicted in Figure 2. It was also noticed that there was alignment in inhibitory activity against these two enzymes, as any single compound that showed high inhibitory potential against one of them also reacted in a similar fashion towards the other one.

As the anti-inflammatory activities of chemical compounds can be expressed by phospholipase A_2 (hGIIA) and/or through protease inhibitor potentials [42], compound **6l**, which was found to be the most active candidate against both phospholipases A_2 ($sPLA_2$) and protease enzymes under investigation, may be proposed as promising potential anti-inflammatory agent for treatment of ulcerative colitis.

2.2.3. Antibacterial Screening

Finally, compounds **6a**–**o** were further examined for in vitro antibacterial activity. Preliminary screening against eleven strains of Gram-positive and Gram-negative bacteria was performed by adopting standard protocol [43]. The antibacterial potency was determined by measuring the inhibition zones. All tests were performed in duplicates and means of inhibition zones were recorded in mm as presented in Table 1.

Analysis of the screening data revealed that the inhibition activity produced by some of the tested compounds was found to be good to excellent compared to the used reference drug tetracycline. Among the eleven strains that would be considered more susceptible to inhibition by one or more of the synthetic compounds were; *P. aeruginosa*, *Serratia*, *S. aureus*, *S. mutans* and *E. feacalis* with genus *Serratia* being the most sensitive one which was inhibited by nine hydrazones. The highest inhibition towards this pathogen was displayed by compounds **6a**, **6b**, **6d**, **6e**, **6f**, **6l**, **6m**, **6n**, and **6o** with inhibition zones of 15.5 ± 0.0, 12.5 ± 0.0, 12 ± 0.0, 14 ± 1.0, 14 ± 1.0, 13 ± 0.01, 14.5 ± 1.5, 14.5 ± 0.5 and 12.5 ± 0.5, respectively. The highest inhibitory activity against *P. aeruginosa* was exerted by compounds **6a**, **6m**, and **6o** with inhibition zones of 17 ± 1.0, 18.5 ± 0.5 and 18.5 ± 0.5, respectively. Although compounds **6j**, **6l** and **6n** produced the highest inhibition zones against *S. aureus* strain, they were considered to be less effective compared to the reference drug tetracycline. Moreover, the maximum zone of inhibition (24.5 ± 0.5) was exhibited by compound **6k** against *S. mutans*. Finally, the reference drug tetracycline was found to be completely inactive against *E. feacalis*, while compounds **6a**, **6d**, **6e**, **6m**, and **6n** exhibited the maximum inhibition zones of 18.5 ± 0.5, 18.0. ± 1.0, 18.5 ± 0.5, 13.5 ± 1.5 and 18.5 ± 0.5, respectively. The rest of the compounds showed moderate inhibition against all other bacterial strains.

2.3. Molecular Docking Analysis

Based on the data obtained from different biological evaluations, compounds **6a**–**o** were docked against proteinase K, GIIA $sPLA_2$, and compounds **6a**–**e** against glutamine-fructose-6-phosphate transaminase (GlcN6P) synthase (GlmS, L-glutamine: D-fructose-6P amidotransferase, EC 2.6.1.16) in order to provide a conceivable rationale for the observed activities.

2.3.1. Docking Simulations for Compounds **6a**–**o** in Active site of $GIIAsPLA_2$

$GIIAsPLA_2$ is a low molecular weight enzyme (14 kDa) with seven disulfide bonds with a highly conserved Ca^{2+}-binding loop and a catalytic dyad consisting of His47/Asp91 along with active a hydrophobic region lined near the *N*-terminal helix [44].

Table 1. Antibacterial activities of the synthesized hydrazones **6a**–**o**.

Comp #	*Gram-Negative Bacteria*					*Gram-Positive Bacteria*					
	E. coli	***p. aeruginosa***	***K. Pneumoniae***	***Salmonella***	***Serratia***	***S. aureus***	***S. aureus ALA1***	***MRSA ATCC3***	***S. mutans***	***E. feacalis***	***B. subtilis***
6a	0 ± 0	17 ± 1	0 ± 0	8 ± 1	15.5 ± 0	0 ± 0	10 ± 0	12 ± 0	12 ± 0.5	18.5 ± 0.5	0 ± 0
6b	14.5 ± 1.5	12 ± 0	0 ± 0	12.5 ± 0.5	12.5 ± 0.5	8 ± 0.5	0 ± 0	6 ± 0	0 ± 0	9.5 ± 0.5	0 ± 0
6c	1 ± 0	1.5 ± 0	0 ± 0	10.25 ± 1	1.4 ± 1	0 ± 0	0 ± 0	8 ± 0.5	0.6 ± 1.2	1.3 ± 1	0 ± 0
6d	14.5 ± 0.5	10 ± 0	12.2 ± 1	6.5 ± 0	12 ± 0	8.5 ± 0.5	0 ± 0	0 ± 0	10 ± 0.0	18 ± 1	0 ± 0
6e	10 ± 0	12.5 ± 0.1	12.5 ± 0.5	4 ± 0	14 ± 1	8.5 ± 0.5	0 ± 0	14 ± 1	8 ± 1	18.5 ± 0.5	0 ± 0
6f	0 ± 0	10.5 ± 0.1	4.5 ± 0.1	10 ± 0	14 ± 0.1	0 ± 0	0 ± 0	0 ± 0	8 ± 1	0 ± 0	0 ± 0
6g	0 ± 0	10.5 ± 0.5	8 ± 1	0 ± 0	10.5 ± 0.5	8.5 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
6h	8 ± 0	12.5 ± 0.7	0 ± 0	0 ± 0	10 ± 0	8.5 ± 0.7	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
6i	8.5 ± 0.5	10.5 ± 0.5	0 ± 0	0 ± 0	8.5 ± 0.5	10.5 ± 1.5	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
6j	8 ± 1	10 ± 0	0 ± 0	0 ± 0	8.5 ± 0.5	19.5 ± 1.5	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
6k	10 ± 0	13.5 ± 1.5	14.5 ± 1.5	0 ± 0	10 ± 0	10.5 ± 1.5	0 ± 0	0 ± 0	24.5 ± 0.5	0 ± 0	8.5 ± 0.5
6l	14 ± 1	11 ± 0	11 ± 0	8 ± 1	13 ± 0.1	18.5 ± 0.5	0 ± 0	0 ± 0	14 ± 2	0 ± 0	0 ± 0
6m	10 ± 1	18.5 ± 0.5	0 ± 0	4 ± 0	14.5 ± 1.5	8.5 ± 0.5	0 ± 0	8.5 ± 0.5	0 ± 0	13.5 ± 1.5	0 ± 0
6n	10 ± 0	0 ± 0	13 ± 1	0 ± 0	12.5 ± 0.5	14 ± 1	0 ± 0	10 ± 0	15 ± 1	18.5 ± 0.5	0 ± 0
6o	10 ± 0	18.5 ± 0.5	10 ± 0	0 ± 0	12.5 ± 0.5	0 ± 0	0 ± 0	0 ± 0	9 ± 0.6	0 ± 0	0 ± 0
TCN	19 ± 0.1	17 ± 0.2	17 ± 0.5	16 ± 0.1	12 ± 0.3	31 ± 0.6	29 ± 0.4	18 ± 0.3	27 ± 1.2	0 ± 0	15 ± 0.5

Comp # = Compound number; TCN = Tetracycline.

The docking simulations for **6a**–**o** in active site of GIIAsPLA$_2$ are presented in Supplementary Materials, Figure S1. Results of docking simulations for compound **6l**, the most active anti-GIIAsPLA$_2$ enzyme, depicted here as a representative in Figure 3, revealed that it interacts with hydrophobic and polar residues of active site of GIIAsPLA$_2$. Compound **6l** has adopted "U" shape with its central benzene ring oriented towards the polar residues viz. His6, Gly22, Asp48, and Val30. The –CONH–N– moiety is responsible for interactions with Phe5 and His47. The –CO–NH–N= moiety bridging the two benzene rings as a polar linker has incorporated good flexibility to the ligand, hence such a flexible moiety is beneficial for further amendments. The –Cl and –COOH groups are oriented toward Leu2 and Val3 due to polar interactions. The –CO– group of –CONH– group is close to Gly22. Hence, –CONH– group is important group for retention in future optimizations.

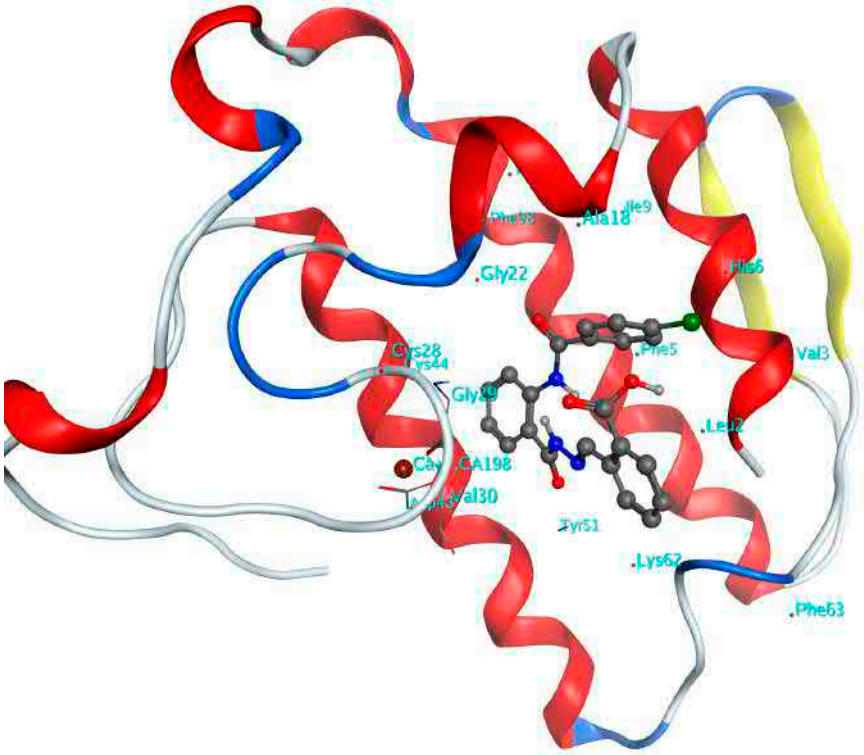


Figure 3. Docking pose for compound **6l** as a representative, in the active site of GIIAsPLA$_2$.

2.3.2. Docking Simulations for Compounds **6a**–**o** in Active Site of Proteinase K

Proteinase K (EC 3.4.21.64, protease K, endopeptidase K, Tritirachium alkaline proteinase, Tritirachium album serine proteinase, and Tritirachium album proteinase K) is a broad-spectrum serine protease belonging to Peptidase family S8 with ability to digest proteins. It is used for the destruction of proteins in cell lysates (tissue, and cell culture cells) and for the release of nucleic acids [45].

The docking simulations for hydrazones **6a**–**o** in active site of proteinase K are presented in Supplementary Materials, Figure S2. Results of docking simulations for compound **6l**, the most active antiproteinase K, depicted here as a representative in Figure 4, revealed that it interacts with hydrophobic and polar residues of active site of proteinase K. Compound **6l** is unable to occupy the complete space of active site of the enzyme. It interacts with polar residues viz. Asn161, Asn162, Ser224 and His69. In addition, it has H-bond formation with H_2O (at distance of 2.86 A°), present inside the active site of Proteinase K, due to the –COOH group present on benzene ring. Hence, the –COOH group is beneficial. Another important interaction is arene-cation interaction between the benzene ring possessing –Cl atom with H_2O (at distance of 4.08 A°), present inside the active site of proteinase K. Interestingly, the compound possesses intramolecular H-bond formation (distance 2.09 A°) between

the –NH– part of –CONH– group with –CO– part of –CONH–N– group. This H-bond is probably responsible for the specific orientation and shape of the molecule inside the active site. The compound **6l** has acquired weird "J" or "L" shape inside the active site. The benzene ring possessing –COOH group is closer to the active site residues. This indicates that the –CONH– and –CONH–N– groups are important for future modifications.

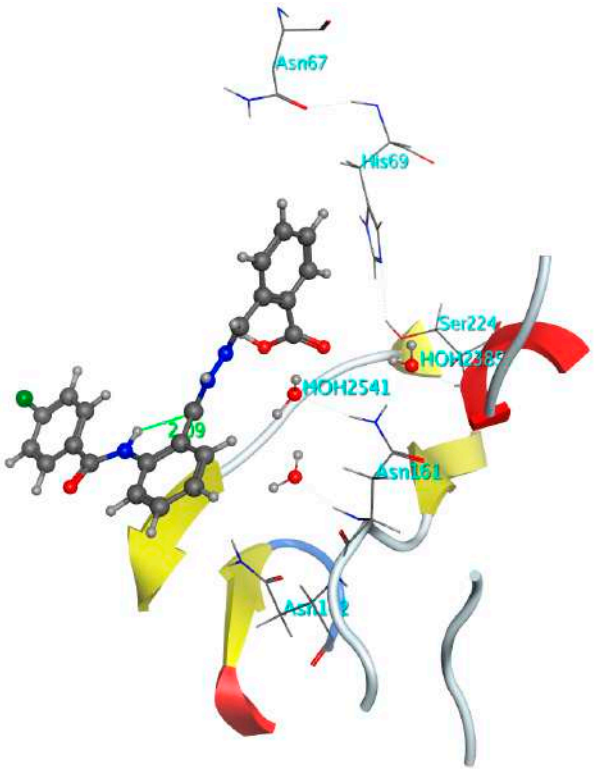


Figure 4. Docking pose for compound **6l** as a representative, in the active site of proteinase K.

2.3.3. Docking Simulations for Compounds **6a**–**e** in Active Site of Glutamine-Fructose-6-Phosphate Transaminase

The molecular mechanism of multifarious reaction catalyzed by glucosamine-6-phosphate (GlcN6P) synthase (GlmS, L-glutamine: D-fructose-6P amidotransferase, EC 2.6.1.16) enzyme comprises both ammonia transfer (L-glutamine to Fru-6-P) and sugar isomerization (fructosamine-6-phosphate to glucosamine-6-phosphate). This reaction is the initiation of the pathway leading to the eventual production of uridine 5′-diphospho-*N*-acetyl-D-glucosamine (UDP-GlcNAc), a product that is present in all class of organisms, but in fungi and bacteria it is utilized to construct macromolecules essential for the cell wall assembly, such as a number of amino sugar-containing macromolecules, comprising chitin and mannoproteins in fungi, and peptidoglycan and lipopolisaccharides in bacteria. In the prokaryotic cell, the inhibition of GlcN6P synthase even for a small time is fatal. Fortunately, because of the longer lifespan of human, the running down of amino sugar pool for a short time is not deadly. Consequently, it has been proposed as a possible target for developing antibacterial and antifungal agents [46–49].

It is evident that recognition of a molecule by a particular enzyme is dependent on the structural properties of that molecule and its distribution of the molecular electrostatic potential (MEP). Furturmore, analysis of ligand–receptor interactions for GlcN-6-P synthase revealed that ligands having primary amido groups can form stable hydrogen bonds with the amino acids residues present in the binding site of the fungal enzyme thus they may serve as "anchors" to lock the inhibitor in the binding pocket of the enzyme. In addition, previous structure–activity relationship experiments

revealed that presence of active electrophilic center at a proper position is required for inactivation of the enzyme to take place [49]. By analogy, hydrazones **6a**–**o** having the same structural features represented by two primary amido groups and electrophilic double bond may serve as inhibitors for bacterial GlcN6P synthase. This hypothesis is examined by docking hydrazones **6a**–**e** in the active site of the bacterial enzyme (these results are presented in Supplementary Materials, Figure S3).

Docking simulations for the most active candidate **6a** which inhibits collectively three bacterial strains and exhibiting inhibition zones larger than those produced by the reference drug tetracycline against two bacterial strains, *Serratia* and *E. feacalis*, depicted here as a representative in Figure 5, revealed that it interacts with a good number of residues of active site of glucosamine-6-phosphate (GlcN6P) synthase. It mainly interacts with polar and hydrophobic residues of the active site. It has adopted weird "J" or "L" shape in the active site. The oxygen atoms of the two amide groups are responsible for the formation of H-bonding with the polar residues Ser A401 (distance 2.61 A°), Gln A348 (distance 1.94 A°) and Ser A349 (distance 1.71 A°). Hence, the amide groups are beneficial for future development. The arene-cation interaction of benzene ring of ligand with the polar residue Arg A26 has strengthened the binding of ligand with the receptor. The chlorine atom on the benzene ring of the ligand has hydrophobic interactions with the hydrophobic residues Trp A74, Ala A602 and Val A399 that constitute the lipophilic region of the cavity. The –CO-NH-N– moiety present between the two benzene rings as a polar linker has furnished high flexibility to the ligand, hence such a flexible moiety is advantageous for further modifications. The benzene ring, which is acting as a bridge between the two –CONH– groups, is oriented toward polar region of the active site, which comprises polar residues such as Ser A401, Cys A300, Ser A303, Ser A347, Thr A352, Gln A348 and Ser A349.

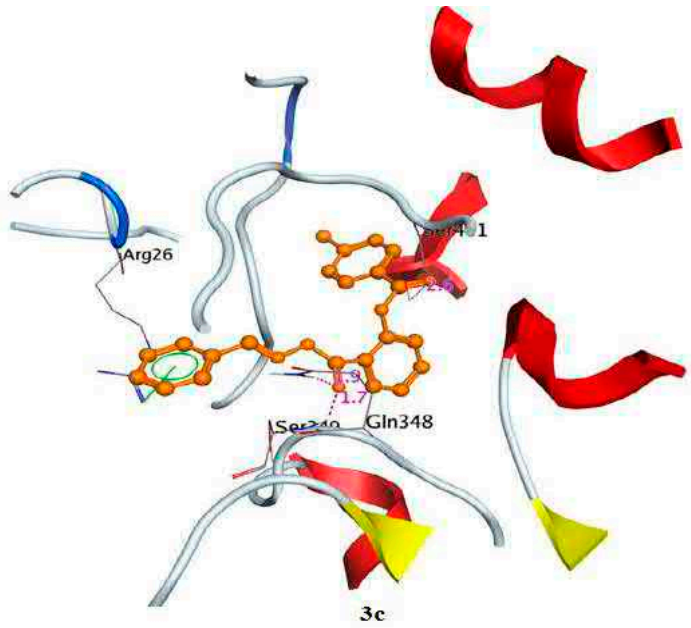


Figure 5. Docking pose for compound **6a**, as a representative, in the active site of glucosamine-6-phosphate (GlcN6P) synthase.

3. Experimental Section

3.1. Chemistry

3.1.1. General

All the chemicals were purchased from various suppliers, and were used without further purification, unless otherwise stated. Melting points were measured on a Gallenkamp melting point

apparatus (Sanyo Gallenkamp, South borough, UK) in open glass capillaries and are uncorrected. Infrared spectra (IR) were recorded using the KBr disc technique using a Perkin Elmer FT-IR spectrophotometer 1000 (PerkinElmer, Waltham, MA, USA). ^{1}H- and ^{13}C-NMR spectra were recorded on a BRUCKER-PLUS NMR (Billerica, MA, USA) operating at 500 MHz in deuterated dimethyl sulfoxide (DMSO-d_6). Chemical shifts are referred to in terms of ppm and *J*-coupling constants are given in Hz. Mass Spectra were recorded on a Shimadzu GCMS-QP 5000 instrument (Shimadzu, Tokyo, Japan). Elemental analysis was done to evaluate the presence of C, H, and N by Perkin Elmer-series-II and the found results were within ±0.4% of the theoretical values. The biological evaluations of the products were carried out at King Saud University, Riyadh, KSA.

3.1.2. Synthetic Procedures

Compounds **3** and **4** were prepared according to reported procedures [20].

4-Chloro-*N*-(2-(hydrazinecarbonyl)phenyl)benzamide **5**

A mixture of 2-(4-chlorophenyl)-4*H*-benzoyl [*d*][1,3]oxazin-4-one **4** (2.57 g, 10 mmol) and 10 mL of hydrazine hydrate (80%) was refluxed for 1 h. Evaporation of the excess hydrazine under reduced pressure and washing of the remaining solid product with plenty of water afford the title compound in pure form.

Yield (95%); shiny white powder; m.p. 175–177 °C. IR (KBr) ν_{max}: 3316–3128 (NH_2 and 2NH), 1673, 1635 (2 C=O), 1592 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 12.04 (s, 1H, NHCO), 11.92 (s, 1H, NHCO), 8.51 (d, 1H, Ar-H, *J* = 8.3 Hz), 7.95 (d, 2H, 2 × Ar-H, *J* = 8.4 Hz), 7.90 (d, 1H, Ar-H, *J* = 7.6 Hz), 7.76–7.62 (m, 3H, 3 × Ar-H), 7.28 (t, 1H, Ar-H, *J* = 7.5 Hz), 4.52 (s, 2H, NH_2); ^{13}C-NMR (DMSO-d_6) δ: 165.27 (CO), 164.16 (CO), 138.2, 137.10, 132.43, 129.11, 128.72, 127.80, 125.445, 124.32, 120.93 (12 × Ar-C); MS (ESI): 290 [M^+ + 1], Anal. Calcd. For $C_{14}H_{12}ClN_3O_2$: C (58.04%); H (4.17%); N (14.50%); Found: C (58.08%); H (4.21%); N (14.53%).

Synthesis of Compounds **6a**–**o**

General procedures: A mixture of 4-chloro-*N*-(2-(hydrazinecarbonyl)phenyl)benzamide **5** (2.89 g, 10 mmol) and the appropriate aldehyde (10 mmol) was refluxed in absolute ethanol (15 mL) in presence of few drops of glacial acetic acid as a catalyst for about 4 hour and monitored with TLC (*n*-hexane:EtOAc, 80:20) until the reaction was completed as observed by appearance of a single new spot. The resultant reaction mixture was cooled to room temperature and the solid product obtained in each experiment was collected by filtration and recrystallized from ethanol to afford the desired products **6a**–**o**

N-(2-(2-benzylidenehydrazine-1-carbonyl)phenyl)-4-chlorobenzamide (**6a**) Yield (89%); white crystals; m.p. 244–246 °C; IR (KBr) ν_{max}: 3336–3197 (2 NH), 1673, 1635 (2 C=O), 1592 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 12.14 (s, 1H, NHCO), 11.94 (s, 1H, NHCO), 8.51 (d, 1H, Ar-H, *J* = 8.3 Hz), 8.47 (s, 1H, N=CH), 7.96 (d, 2H, 2 × Ar-H, *J* = 8.4 Hz), 7.92 (d, 1H, Ar-H, *J* = 7.6 Hz), 7.76–7.47 (m, 8H, 8 × Ar-H), 7.30 (t, 1H, Ar-H, *J* = 7.5 Hz); ^{13}C-NMR (DMSO-d_6) δ: 165.39 (CO), 164.00 (CO), 149.53 (N=CH), 139.53, 137.42, 134.49, 133.63, 133.06, 130.86, 129.48, 129.36, 129.10, 127.75, 123.85, 121.85, 121.75, 121.31 (18 × Ar-C); MS (ESI): 379 ($C_{21}H_{16}ClN_3O_2$, [M^+ + 1]; Anal. Calcd. for $C_{21}H_{16}ClN_3O_2$: C (66.76%); H (4.27%); N (11.12%); Found: C (66.72%); H (4.24%); N (11.14%).

4-Chloro-N-(2-(2-(4-chlorobenzylidene)hydrazine-1-carbonyl)phenyl)benzamide (**6b**) Yield (95%); white powder; m.p. 252–256 °C; IR (KBr) ν_{max}: 3333–3201 (2 NH), 1675, 1639 (2 C=O), 1598 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 12.19 (s, 1H, NHCO), 11.95 (s, 1H, NHCO), 8.52 (d, 1H, Ar-H, *J* = 8.3 Hz), 8.45 (s, 1H, N=CH), 7.96 (d, 2H, 2 × Ar-H, *J* = 8.4 Hz), 7.90 (d, 1H, Ar-H, *J* = 7.8 Hz), 7.77 (d, 2H, 2 × Ar-H, *J* = 8.4 Hz), 7.65–7.61 (m, 3H, 3 × Ar-H), 7.52 (d, 2H, 2 × Ar-H, *J* = 8.4 Hz), 7.28 (t, 1H, Ar-H, *J* = 7.6 Hz); ^{13}C-NMR (DMSO-d_6) δ: 165.46 (CO), 163.96 (CO), 148.13 (N=CH), 139.62, 137.43, 135.32, 133.58, 133.42, 133.10, 129.67, 129.44, 129.33, 129.07, 123.76, 121.71, 121.08 (18 × Ar-C); MS (ESI): 413

[M^+ + 1], Anal. Calcd. for $C_{21}H_{15}Cl_2N_3O_2$: C (61.18%); H (3.67%); N (10.19%); Found: C (61.20%); H (3.64%); N (10.21%).

4-Chloro-N-(2-(2-(4-hydroxy-3-methoxybenzylidene)hydrazine-1-carbonyl)phenyl)benzamide (**6c**) Yield (85%); white crystals; m.p. 236–238 °C; IR (KBr) ν_{max}: 3341–3201 (2 NH and OH), 1675, 1639 (2 C=O), 1588 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 11.93 (s, 2H, NH and OH), 9.59 (br. s, 1H, NH), 8.46 (d, 1H, Ar-H, *J* = 8.3 Hz), 8.30 (s, 1H, N=CH), 7.90 (d, 2H, 2 × Ar-H, *J* = 8.4 Hz), 7.83 (d, 1H, Ar-H, *J* = 7.7 Hz), 7.58 (d, 2H, 2 × Ar-H, *J* = 8.4 Hz), 7.54 (d, 1H, Ar-H, *J* = 7.7 Hz), 7.29 (d, 1H, Ar-H *J* = 1.0 Hz), 7.21 (t, 1H, Ar-H, *J* = 7.7 Hz), 7.06 (dd, 1H, Ar-H, *J* = 8.1, 1.0 Hz), 6.80 (d, 1H, Ar-H, *J* = 8.1 Hz), 3.78 (s, 3H, OCH_3); ^{13}C-NMR (DMSO-d_6) δ 165.13 (CO), 163.98 (CO), 150.24, 149.81, 148.55, 139.52, 137.41, 133.65, 132.90, 129.46, 129.01, 125.84, 123.78, 123.06, 121.63, 121.31, 115.93, 109.49 (CH=N and 18 × Ar-C), 56.04 (OCH_3); MS (ESI): 425 [M^+ + 1], Anal. Calcd. for $C_{22}H_{18}ClN_3O_4$: C (62.34%); H (4.28%); N (9.91%); Found: C (62.32%); H (4.24%); N (9.93%).

4-Chloro-N-(2-(2-(3,4,5-trimethoxybenzylidene)hydrazine-1-carbonyl)phenyl)benzamide (**6d**) Yield (93%); white crystals; m.p. 247–248 °C; IR (KBr) ν_{max}: 3345–3208 (2 NH), 1671, 1644 (2 C=O), 1578 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 12.11 (s, 1H, NHCO), 11.81 (s, 1H, NHCO), 8.46 (d, 1H, Ar-H, *J* = 8.2 Hz), 8.37 (s, 1H, N=CH), 7.96 (d, 2H, 2 × Ar-H, *J* = 8.4 Hz), 7.88 (d, 1H, Ar-H, *J* = 7.6 Hz), 7.67 (d, 2H, 2 × Ar-H, *J* = 8.4 Hz), 7.63 (t, 1H, Ar-H, *J* = 8.2 Hz), 7.31 (t, 1H, Ar-H, *J* = 7.6 Hz), 7.04 (s, 2H, 2 × Ar-H), 3.85 (s, 6H, 2 × OCH_3), 3.74 (s, 3H, OCH_3); ^{13}C-NMR (DMSO-d_6) δ: 165.25 (CO), 164.12 (CO), 153.69 (3 × $\underline{C}$-OCH_3), 146.48 (N=CH), 139.32, 137.39, 133.68, 129.97, 129.52, 129.47, 129.15, 123.96, 121.96, 104.96 (15 × Ar-C), 60.60 (OCH_3), 56.50 (OCH_3), 56.46 (OCH_3). MS (ESI): 469 [M^+ + 1], Anal. Calcd. for $C_{24}H_{22}ClN_3O_5$: C (61.61%); H (4.74%); N (8.98%); Found: C (61.32%); H (4.71%); N (9.02%).

4-Chloro-N-(2-(2-(2,4-dichlorobenzylidene)hydrazine-1-carbonyl)phenyl)benzamide (**6e**) Yield (93%); creamish powder; m.p. 262–264 °C; IR (KBr) ν_{max}: 3421–3268 (2 NH), 1680, 1656 (2 C=O), 1592 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 12.36 (s, 1H, NHCO), 11.79 (s, 1H, NHCO), 8.82 (s, 1H, N=CH), 8.47 (d, 1H, Ar-H, *J* = 8.3 Hz), 8.04 (d, 1H, Ar-H, *J* = 8.5 Hz), 7.95 (d, 2H, 2 × Ar-H, *J* = 8.4 Hz), 7.91 (d, 1H, Ar-H, *J* = 7.8 Hz), 7.75 (s, 1H, CH-Ar), 7.69–7.64 (m, 3H, 3 × Ar-H), 7.55 (d, 1H, Ar-H, *J* = 8.5 Hz), 7.31 (t, 1H, Ar-H, *J* = 7.6 Hz); ^{13}C-NMR (DMSO-d_6) δ: 165.31 (CO), 164.42 (CO), 148.38 (N=CH), 139.64, 137.93, 135.26, 133.45, 133.39, 133.10, 129.51, 129.49, 129.27, 129.11, 123.80, 121.71, 121.88 (18 × Ar-C). MS (ESI): 447 [M^+ + 1], Anal. Calcd. for $C_{21}H_{14}Cl_3N_3O_2$: C (56.46%); H (3.16%); N (9.41%); Found: C (56.48%); H (3.20%); N (9.42%).

4-Chloro-N-[2-(4-methoxy-benzylidene-hydrazinocarbonyl)-phenyl]-benzamide (**6f**) Yield (88%); white powder; m.p. 260–262 °C; IR (KBr) v_{max}: 3374–3242 (2 NH), 1682, 1646 (2 C=O), 1589 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 12.06 (s, 1H, NHCO), 12.03 (s, 1H, NHCO), 8.55 (d, 1H, Ar-H, *J* = 8.3 Hz), 8.42 (s, 1H, CH=N), 7.96 (d, 2H, 2 × Ar-H, *J* = 8.5 Hz), 7.91 (d, 1H, Ar-H, *J* = 7.6 Hz), 7.69 (d, 2H, 2 × Ar-H, *J* = 8.7 Hz), 7.64 (d, 2H, 2 × Ar-H, *J* = 8.5 Hz), 7.60 (t, 1H, Ar-H, *J* = 7.5 Hz), 7.27 (t, 1H, Ar-H, *J* = 7.5 Hz), 7.02 (d, 2H, 2 × Ar-H, *J* = 8.7 Hz), 3.80 (s, 3H, OCH_3); ^{13}C-NMR (DMSO-d_6) δ: 165.25 (CO), 163.91 (CO), 161.55 ($\underline{C}$-OCH_3), 149.54 (N=CH), 139.65, 137.42, 133.60, 132.96, 129.45, 129.42, 128.99, 127.01, 123.69, 121.53, 121.02, 114.82 (17 × Ar-C), 55.75 (OCH_3). MS (ESI): 409 [M^+ + 1], Anal. Calcd. for $C_{22}H_{18}ClN_3O_3$: C (64.79%); H (4.45%); N (10.30%); Found: C (64.78%); H (4.42%); N (10.32%).

4-Chloro-N-(2-(2-(4-(dimethylamino)benzylidene)hydrazine-1-carbonyl)phenyl)benzamide (**6g**) Yield (82%); yellow powder; m.p. 249–251 °C; IR (KBr) ν_{max}: 3364–3212 ((2 NH), 1679, 1640 (2 C=O), 1582 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 12.11 (s, 1H, NHCO), 11.85 (s, 1H, NHCO), 8.56 (d, 1H, Ar-H, *J* = 8.2 Hz), 8.32 (s, 1H, CH=N), 7.96 (d, 2H, 2 × Ar-H, *J* = 8.5 Hz), 7.90 (d, 1H, Ar-H, *J* = 7.2 Hz), 7.68 (d, 2H, 2 × Ar-H, *J* = 8.5 Hz), 7.62 (t, 1H, Ar-H, *J* = 7.4 Hz), 7.56 (d, 2H, 2 × Ar-H, *J* = 8.8 Hz), 7.28 (t, 1H, Ar-H, *J* = 7.4 Hz), 6.77 (d, 2H, 2 × Ar-H, *J* = 8.8 Hz), 2.99 (s, 6H, 2 × N-CH_3); ^{13}C-NMR (DMSO-d_6) δ: 165.31 (CO), 164.13 (CO), 152.4 ($\underline{C}$-NCH_3), 148.27 (N=CH), 138.2, 137.4, 134.84, 129.53, 129.44, 129.18, 128.8, 127.7, 124.2, 123.74, 119.8, 112.3 (17 × Ar-C), 45.2 (2 × CH_3). MS (ESI): 422 [M^+ + 1], Anal. Calcd. for $C_{23}H_{21}ClN_4O_2$: C (65.63%); H (5.03%); N (13.31%); Found: C (65.59%); H (5.02%); N (13.33%).

4-Chloro-N-(2-(2-(2-methoxybenzylidene)hydrazine-1-carbonyl)phenyl)benzamide (**6h**) Yield (98%); white powder; m.p. 236–238 °C; IR (KBr) ν_{max}: 3383–3251 ((2 NH), 1680, 1652 (2 C=O), 1589 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 12.06 (s, 1H, NHCO), 11.97 (s, 1H, NHCO), 8.77 (s, 1H, N=CH), 8.47 (d, 1H, Ar-H, *J* = 8.3 Hz), 7.90 (d, 2H, 2 × Ar-H, *J* = 8.5 Hz), 7.88–7.83 (m, 2H, 2 × Ar-H), 7.60 (d, 2H, 2 × Ar-H, *J* = 8.5 Hz), 7.58–7.20 (m, 3H, 3 × Ar-H, *J* = 7.5 Hz), 7.05 (d, 1H, Ar-H, *J* = 8.4 Hz), 6.98 (t, 1H, Ar-H, *J* = 7.7 Hz), 3.80 (s, 3H, O-CH_3); ^{13}C-NMR (DMSO-d_6) δ: 165.28 (CO), 163.96 (CO), 158.39 (C-OCH_3), 145.11 (N=CH), 139.63, 137.42, 133.64, 133.05, 132.40, 129.49, 129.46, 129.09, 126.09, 123.77, 122.47, 121.58, 121.23, 120.99, 112.37 (17 × Ar-C), 56.18 (OCH_3). MS (ESI): 409 [M^+ + 1], Anal. Calcd. for $C_{22}H_{18}ClN_3O_3$: C (64.79%); H (4.45%); N (10.30%); Found: C (64.82%); H (4.46%); N (10.33%).

4-Chloro-N-(2-(2-(4-methylbenzylidene)hydrazine-1-carbonyl)phenyl)benzamide (**6i**) Yield (91%); white crystals; m.p. 256–258 °C; IR (KBr) ν_{max}: 3372-3264 ((2 NH), 1684, 1657 (2 C=O), 1579 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 12.08 (s, 1H, NHCO), 11.97 (s, 1H, NHCO), 8.52 (d, 1H, Ar-H, *J* = 8.3 Hz), 8.43 (s, 1H, N=CH), 7.98 (d, 1H, Ar-H, *J* = 8.5 Hz), 7.91 (d, 2H, 2 × Ar-H, *J* = 7.7 Hz), 8.68–7.62 (m, 5H, 5 × Ar-H), 7.31–7.28 (m, 3H, 3 × Ar-H), 2.34 (s, 3H, CH_3); ^{13}C-NMR (DMSO-d_6) δ (ppm): 165.31 (CO), 163.98 (CO), 149.61 (N=CH), 140.77, 139.55, 137.42, 133.62, 133.62, 133.02, 131.78, 129.97, 129.49, 129.46, 129.06, 127.74, 123.81, 121.67, 121.24 (18 × Ar-C), 21.52 (CH_3). MS (ESI): 393 [M^+ + 1], Anal. Calcd. for $C_{22}H_{18}ClN_3O_2$: C (67.43%); H (4.63%); N (10.72%); Found: C (64.42%); H (4.66%); N (10.73%).

4-((2-(2-(4-chlorobenzamido)benzoyl)hydrazono)methyl)benzoic acid (**6j**) Yield (85%); creamish powder; m.p. 252–254 °C; IR (KBr) ν_{max}: 3373–3292 (2 NH, OH), 1759, 1680, 1652 (3 C=O), 1581 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 12.25 (s, 1H, NHCO), 11.92 (s, 1H, NHCO), 8.51 (s, 1H, N=CH), 8.04–7.25 (m, 12H, 12 × Ar-H); ^{13}C-NMR (DMSO-d_6) δ: 167.37 (COOH), 165.58 (CONH), 163.98 (CO), 148.24 (N=CH), 139.63, 138.49, 137.44, 133.56, 133.13, 132.42, 130.26, 129.40, 129.30, 129.09, 127.72, 123.75, 121.75, 121.05 (18 × Ar-C). MS: *m*/*z* 423 [M^+ + 1], Anal. Calcd. for $C_{22}H_{16}ClN_3O_4$: C (62.64%); H (3.82%); N (9.96%); Found: C (62.63%); H (3.86%); N (9.97%).

Chloro-N-(2-(2-(3-phenylallylidene)hydrazine-1-carbonyl)phenyl)benzamide (**6k**) Yield (93%); creamish powder; m.p. 248–250 °C; IR (KBr) ν_{max}: 3391–3271 (2 NH), 1680, 1644 (2 C=O), 1594 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 12.04 (s, 1H, NHCO), 11.97 (s, 1H, NHCO), 8.51 (d, 1H, Ar-H, *J* = 7.8 Hz), 8.25 (d, 1H, N=CH, *J* = 8.3 Hz), 7.95 (d, 2H, 2 × Ar-H, *J* = 8.5 Hz), 7.88 (d, 1H, *J* = 7.8 Hz, Ar-H), 7.68 (d, 2H, 2 × Ar-H, *J* = 8.5 Hz), 7.64–7.62 (m, 3H, 3 × Ar-H, *J* = 7.6 Hz), 7.41 (t, 2H, 2 × Ar-H, *J* = 7.3 Hz), 7.36–7.08 (m, 4H, 4 × Ar-H); ^{13}C-NMR (DMSO-d_6) δ: 165.29 (CO), 163.92 (CO), 151.46, 140.39, 139.49, 137.44, 136.26, 129.50, 129.45, 129.34, 129.05, 127.67, 125.84, 123.84, 121.28 (N=C<u>H</u>-C<u>H</u>=C<u>H</u> and 18 × Ar-C). MS: *m*/*z* 405 [M^+ + 1], Anal. Calcd. for $C_{23}H_{18}ClN_3O_2$: C (68.40%); H (4.49%); N (10.40%); Found: C (68.42%); H (4.51%); N (10.44%).

2-((2-(2-(4-chlorobenzamido)benzoyl)hydrazono)methyl)benzoic acid (**6l**) Yield (87%); white powder; m.p. 251–253 °C; IR (KBr) ν_{max}: 3368–3278 (2 NH and OH), 1758 (CO), 1680, 1656 (2 C=O), 1579 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 12.37 (s, 1H, NHCO), 11.95 (s, 1H, NHCO), 8.74 (s, 1H, N=CH), 8.52 (d, 1H, Ar-H, *J* = 8.3 Hz), 8.09 (d, 1H, Ar-H, *J* = 7.8 Hz) 7.97 (d, 2H, 2 × Ar-H, *J* = 8.5 Hz), 7.95–7.62 (m, 6H, 6 × Ar-H), 7.56 (t, 1H, Ar-H, *J* = 7.4 Hz), 7.28 (t, 1H, Ar-H, *J* = 7.4 Hz); ^{13}C-NMR (DMSO-d_6) δ: 168.51 (COOH), 165.61 (CO), 164.02 (CO), 148.38 (N=CH), 139.56, 137.39, 134.82, 133.66, 133.09, 132.49, 131.29, 130.35, 129.47, 129.25, 127.23, 123.78, 121.70, 121.19 (18 × Ar-C). MS (ESI): 423 [M^+ + 1], Anal. Calcd. for $C_{22}H_{16}ClN_3O_4$: C (62.64%); H (3.82%); N (9.96%); Found: C (62.67%); H (3.85%); N (9.88%).

4-Chloro-N-(2-(2-(3-hydroxy-4-methoxybenzylidene)hydrazine-1-carbonyl)phenyl)benzamide (**6m**) Yield (94%); white powder; m.p. 239–241 °C; IR (KBr) ν_{max}: 3383–3256 (2 NH), 1680, 1657 (2 C=O), 1582 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 12.04 (s, 1H, NHCO), 11.97 (s, 1H, NHCO), 8.56 (d, 1H, Ar-H, *J* = 8.3 Hz), 8.34 (s, 1H, N=CH), 7.96 (d, 2H, 2 × Ar-H, *J* = 8.5 Hz), 7.90 (d, 1H, *J* = 7.7 Hz), 7.67 (d, 2H, 2 × Ar-H, *J* = 8.5 Hz), 7.62 (t, 1H, Ar-H, *J* = 7.5 Hz), 7.31 (d, 1H, Ar-H, *J* = 1.6 Hz), 7.28 (t, 1H, Ar-H, *J* = 7.6 Hz), 7.08 (dd, 1H, Ar-H, *J* = 8.3, 1.6 Hz), 6.98 (d, 1H, Ar-H, *J* = 8.3 Hz), 3.82 (s, 3H, O-CH_3); ^{13}C-NMR (DMSO-d_6) δ: 165.16 (CO), 163.93 (CO), 150.53, (C-OCH_3), 149.81 (C-OH), 147.37 (N=CH),

139.58, 137.44, 133.62, 132.62, 129.49, 129.43, 129.01, 127.29, 123.77, 121.55, 121.13, 112.90, 112.29 (16 × Ar-C), 56.04 (OCH_3). MS (ESI): 425 [M^+ + 1], Anal. Calcd. for $C_{22}H_{18}ClN_3O_4$: C (62.34%); H (4.28%); N 00(9.91%); Found: C (62.38%); H (4.26%); N (9.93%).

4-Chloro-N-(2-(2-(4-cyclohexylbenzylidene)hydrazine-1-carbonyl)phenyl)benzamide (**6n**) Yield (85%); white crystals; m.p. 257–259 °C; IR (KBr) ν_{max}: 3386–3260 (2 NH), 1682, 1654 (2 C=O), 1589 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 12.03 (s, 1H, NHCO), 11.08 (s, 1H, NHCO), 8.51 (d, 1H, Ar-H, *J* = 8.2 Hz), 8.36 (s, 1H, N=CH), 7.95 (d, 2H, 2 × Ar-H, *J* = 8.5 Hz), 7.88 (d, 1H, Ar-H, *J* = 7.7 Hz), 7.65 (d, 2H, 2 × Ar-H, *J* = 8.3 Hz), 7.59 (t, 1H, Ar-H, *J* = 7. 7Hz), 7.31–7.18 (m, 5H, 5 × Ar-H), 3.09–1.55 (m, 11H, cyclohexyl-H); ^{13}C-NMR (DMSO-d_6) δ: 165.54 (CO), 163.86 (CO), 146.14 (N=CH), 139.23, 137.44, 133.55, 132.61, 129.47, 129.39, 128.84, 127.16, 126.60, 123.76, 121.72, 121.49 (18 × Ar-C), 42.85, 41.23, 35.32, 34.39, 33.43, 28.39 (cyclohexyl-C). MS (ESI): 461 [M^+ + 1], Anal. Calcd. For $C_{27}H_{26}ClN_3O_2$: C (70.50%); H (5.70%); N (9.14%); Found: C (70.51%); H (5.71%); N (9.13%).

(Z)-4-chloro-N-(2-(2-(3-hydroxybenzylidene)hydrazine-1-carbonyl)phenyl)benzamide (**6o**) Yield (95%); white powder; m.p. 262–264 °C; IR (KBr) ν_{max}: 3386–3258 (2 NH, OH), 1680, 1652 (2 C=O), 1579 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 12.12 (s, 1H, NHCO), 12.04 (s, 1H, NHCO), 8.84 (s, 1H, N=CH), 8.55 (d, 1H, Ar-H, *J* = 8.3 Hz), 7.97 (d, 2H, 2 × Ar-H, *J* = 8.5 Hz), 7.95–7.90 (m, 2H, 2 × Ar-H), 7.67 (d, 2H, 2 × Ar-H, *J* = 8.5 Hz), 7.63 (t, 1H, Ar-H, *J* = 7.5 Hz), 7.46–7.03 (m, 4H, 4 × Ar-H); ^{13}C-NMR (DMSO-d_6) δ: 165.27 (CO), 163.96 (CO), 158.40 (C-OCH_3), 145.10 (N=CH), 139.64, 137.42, 133.64, 133.05, 132.40, 129.50, 129.46, 129.09, 126.09, 123.77, 122.47, 121.48, 121.57, 121.23, 120.99 (17 × Ar-C). MS (ESI): 395 [M^+ + 1], Anal. Calcd. For $C_{21}H_{16}ClN_3O_3$: C (64.05%); H (4.09%); N (10.67%); Found: C (64.07%); H (4.12%); N (10.63%).

3.2. Biological Evalustion

3.2.1. Inhibition of $sPLA_2$ Activity

The test of inhibitory activity of secretory phospholipase A_2 ($sPLA_2$) was performed as described by De Aranjo and Radvany [50]. Briefly, the substrate consisted of 3.5 mM lecithin in a mixture of 3 mM NaTDC, 100 mM NaCl, 10 mM $CaCl_2$ and 0.055 mM red phenol as colorimetric indicator in 100 mL H_2O. The pH of the reaction mixture was adjusted to 7.6 using phosphate buffer. The Human group IIA (hG-IIA) and dromedary group IB (DrG-IIA) $sPLA_2$ were solubilized in 10% acetonitrile at a concentration of 0.05 μg/μL. A volume of 10 μL of these PLA_2 solutions was incubated with 10 μL containing 10 μg of each compound for 20 min at room temperature. Then, 1 mL of the PLA_2 substrate was added, and the kinetic of hydrolysis was followed during 5 min by reading the optical density at 558 nm. The inhibition percentage was calculated by comparison with a control experiment (absence of compound).

3.2.2. Protease Inhibitor Assay

Three commercially available proteases; proteinase K (Sigma-Aldrich, St. Louis, MO, USA, P2308), esperase (Novozyme, Sigma-Aldrich, P5860) and that obtained from *Bacillus* sp. (Sigma-Aldrich, P3111) were evaluated for the effect of the studied compounds on their activities. Protease assays were carried out by adopting Kunitz caseinolytic method [51] using Hammerstein casein as substrate. Respective protease inhibitor activities were assayed under the same conditions with the addition of the inhibitor (0.1 mg/mL) to the respective reaction mixture and pre incubation for 10 min at 37 °C. The assay of the residual enzyme activity was followed by the addition of 2 mL of 1% casein and the resulting mixture was allowed to stand for 30 min at 37 °C. The reaction was stopped by the addition of 2.5 mL of 5% TCA solution. After centrifugation of the reaction mixture (12,000 rpm, 15 min), the absorbance was measured at 280 nm. Protease inhibitor unit is defined as the amount of protease inhibitor that inhibited one unit of respective enzyme activity. The protease inhibitor activity was expressed in

terms of percent inhibition. Appropriate blanks for the enzyme, inhibitor, and the substrate were also included in the assay along with the test.

3.2.3. Antibacterial Activity

Culture of Microbial Strains Preparation

Pure standard microbial isolates collected from King Khaled University Hospital were tested in this study; including *Staphylococcus aureus* ATCC 25923, Methicillin resistant *Staphylococus aureus* ATCC 12498, *Bacillus subtilis* ATCC 6633, *Enterococcus faecalis* ATCC 29212 as Gram-positive and *Escherichia coli* ATCC 25966, *Pseudomonas aeruginosa* ATCC 27853 and *Serratia marcescens* as Gram-negative bacteria. Fresh cultures of each microorganism were grown on nutrient agar plates (Oxoid, Thermo Scientific, Basingstoke, UK); of which small inoculums were suspended in 5 mL nutrient broth for bacterial suspension preparation of 0.5 MacFarland.

Antibacterial Assay

Antimicrobial activity was assayed using well diffusion technique according to given literature [43]. Briefly, small inoculums of each of the microbial suspension prepared were loaded on sterile Muller Hinton agar plates surface (Oxoid) with sterile cotton swabs. Loaded plates were then perforated equidistantly with a sterile 6 mm diameter cork borer, and 70 µL of each compound solution (1 mg/mL) were loaded in their respective wells. DMSO and Tetracycline were used as negative and positive controls, respectively. Plates were kept to rest for 30 min at room temperature and then incubated at 37 °C for 18–24 h. Antimicrobial activity was determined by measuring the inhibition zone. All tests were performed in duplicates and means of inhibition zones were recorded in mm.

3.3. Molecular Docking

In the present work, AutoDock 4.0 (The Scripps Research Institute, La Jolla, CA, USA) was used for molecular docking. The software is freely available and can be used with different molecular viewers like PMV, PyMol, etc. For docking simulations, the structures of the molecules were drawn using ChemSketch 12.0 (Advanced Chemistry Development, Inc., Toronto, ON, Canada) freeware and optimized using the inbuilt methodology. The optimized structures were saved in 3D-format in .mol file format. The structures of proteins GIIA $sPLA_2$ (pdb: 1DB4), proteinase K (pdb: 2PWB) and glutamine-fructose-6-phosphate transaminase (1JXA) were retrieved from www.rcsb.org. The pdb 1DB4, 2PWB, and 1JXA for the proteins were selected on the basis of reasonable X-ray resolution and sequence completion. The proteins structures were optimized before actual docking simulations. Following parameters were set for getting better docking results: Algorithm: genetic Algorithm; Number of runs: 10 GA runs; Number of evaluations: 250,000 evaluation/run; population size: 150.

4. Conclusions

In conclusion, fifteen novel hydrazone derivatives **6a–o** were synthesized, fully characterized and examined to evaluate their inhibitory activity against two phospholipases A_2, three protease enzymes and a panel of Gram-negative and Gram-positive bacterial strains. Among the investigated compounds; **6e** and **6l** selectively exhibited $sPLA_2$ the highest inhibitory activity against *h*G-IIA isoform. It is quite interesting to report that compound **6l** also displayed the highest antiprotease inhibition against all used protease enzymes.

Moreover, the series under investigation showed varied antibacterial inhibitory activity. Thus, while similar inhibition to that produced by the reference drug tetracycline was displayed by compound **6a** against *P. aeruginosa*, compounds **6m** and **6o** exhibited higher inhibition. In addition, the most susceptible bacterial strain to the synthesized compounds was genus *Serratia*, which was inhibited by nine of them, with compound **6a** being the most powerful inhibitory agent. Furthermore, the maximum inhibitory activities were exhibited by compounds **6j**, **6l**, and **6n** against *S. aureus* and by compound

6k against *S. mutans*. Finally, compounds **6a**, **6d**, **6e**, **6m** and **6n** were found to be more potent than the used reference drug against *E. feacalis*. The docking simulations of compounds **6a–o** with GIIA $sPLA_2$, proteinase K and compounds **6a–e** GlcN6P were carried out in order to investigate the mode of action.

Supplementary Materials: Supplementary materials can be accessed at: http://www.mdpi.com/1420-3049/21/12/1664/s1.

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"A STUDY OF IMPACT OF SAVINGS ON INVESTMENT PREFERENCES OF INVESTORS" A CASE STUDY OF BHMS DOCTORS OF AMRAVATI CITY, MAHARASHTRA, INDIA"

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ABSTRACT

Savings and investment which are promoted by the capital market are the basis of capital formation and economic growth in the country so that it is essential to highlight the relationship between saving objectives of investors and their investment preferences among the various alternatives of investments in the market. Savings are invested in assets depending upon their risk and return characteristics. But the main object of investor is to minimize the risk involved in investment and maximize the return. Researcher has taken into consideration all the available investment avenues and its relevant factors by conducting survey among BHMS Doctors in Amravati city. In the said study the convenience random sampling technique was used for the selection of respondents. Primary data has been collected from the respondent's personal interview and also with the help of structured questionnaire, which were distributed among them. The collected data has been interpreted and analyzed by using tables, percentage & chi-square test. Awareness about all the available investment avenues in the financial market is more in male respondents than the female respondents. Most of the respondents preferred various investment avenues according to the availability of the excess funds/savings made by them. This research paper highlights the impact of objectives of savings on preference of the investors towards various investment avenues and also provides valuable suggestions for the investors.

KEYWORDS: *Investment avenues, Investment Preference, Savings, BHMS Doctors.*

INTRODUCTION :

Savings are the foundation on which we can build our dreams, so keep saving! Explore new investment avenues to aim for potential wealth creation. Our approach towards saving our hard earned money is often shaped by our various aspects of routine life. Sometimes we save affects how we lead our life. It varies hand to hand. So

discover our unique take on savings and determine our Savings Avatar. And accordingly," while we work to save, let our savings work." If someone who looks beyond savings and wants his money to grow then that person has to think towards making smart investments by choosing better avenues among various alternatives available in India.

Investment means an acquisition of some assets. It also means the conversion of money into claims of money and use of funds for productive and income generating assets. Simply, we can say that the use of available funds i.e. savings utilized for the following productive purposes like Earning of income, appreciation of capital, wealth creation or for further production of goods and services with the intention of securing profits. Investment activity involves the use of funds or savings for further creation of assets or acquisition of existing assets.

Surplus money or savings and information are two basic pillars towards investment. The first requirement of investment is the surplus availability of money i.e. savings. But money is not enough, as investments are generally made on the basis of information of companies, instruments, industry, and economy. Both savings of money and information flow provides assistance in effective investment management.

Savings are the surplus funds left after incurring expenditure from the income. Savings are sometimes autonomous coming from households as a matter of Habits. There are some specific objectives of savings as follows:-

I. For education of children,
ii. To fulfill future needs and safety,
iii. To meet contingencies,
iv. For precautionary purposes,
v. For wealth creation like purchase of house, assets etc.,
vi. For improving standard of living.

REVIEW OF LITERATURES:-

1. Sushant Nagpal and B. S. Bodla (2009), on impact of investors' lifestyle on their investment pattern: an empirical study states that the modern investor is a mature and adequately groomed person. Occasions of blind investments are scarce, as a majority of investors are found to be using some source and reference groups for taking decisions.

2. Chaturvedi Meenakshi & Khare Shruti (2012) carried out study on the Saving Pattern and Investment Preferences of Individual Household in India. They found that most investors give the preference to Bank Deposit as the first choice of investment and next to bank deposits they prefer small saving schemes constituting the second choice of investment.

3. Geetha N, & Ramesh M. (2012) studied the importance of Demographic Factors in Investment Decision. They concluded by saying that there is significant relationship between the demographic factors such as gender, age, education, occupation, annual income and annual savings with the sources of awareness obtained by the investors.

4. Ramprasath .S and Dr. B. Karthikeyan (2013), on individual investors' behavior towards select investments, states that the majority of the investors are giving much importance for the factor "safety". Similarly investment avenues such as Bank deposits, LIC polices and Bullion has been preferred by the individual investors. Similarly the majority of the investors are periodically evaluating the performance of their investment avenues.

5. Sunil Kumari (2013), studies on investment attitude of rural investor states that all of the rural investors consider the risk and return on investment and most of them are also dependent on financial

advisor's opinion because of lacking the depth knowledge of market. But generalization of the study is subject to its limitations like unwillingness of respondents, limited period of time, lack of literacy of rural investors etc. It is concluded that psychological theory planned behavior reflects in rural people's investment decisions along with a finance theory is concepts i.e. risk and return equilibrium/ trade off.

6. Pandey Priyanka (2014) carried out a study of saving and investment pattern of investors of Haridwar District. She revealed that the awareness of investment knowledge and investment opportunities among investors of Haridwar District was quite high. Even having sound knowledge of financial market, investors need an assistance of financial planners. Most of the investors rely on fixed deposit and PPF as a best investment avenue. It also revealed that preference to invest in a specific investment avenue is strongly affected by objective of saving.

RESEARCH PROBLEM:

To study the relationship between saving objectives of BHMS Doctors and their investment priorities/preferences among various available investment avenues in India.

OBJECTIVES OF THE STUDY:

1.To know the preferences of BHMS Doctors towards various investment avenues.
2.To study about the preferred time period of investment among B.H.M.S Doctors.
3.To know the purpose behind their savings and the investment in a particular investment avenue.
4.To study the impact of saving objectives on the preferred investment avenues of BHMS Doctors in Amravati city.

HYPOTHESIS OF THE STUDY:-

1. There is no significant impact of saving objectives of investors on their preferred investment avenues.
2. There is no significant relationship between age and saving objectives of investors.

RESEARCH METHODOLOGY:-

The said study is based on primary as well as secondary data. Primary data has been collected by survey method for which suitable questionnaire was structured and distributed among 50 BHMS Doctors of Amravati city with the help of convenience random sampling technique.

Secondary data has been collected from books, journals, review of literatures, relevant articles, reports and related websites.

LIMITATIONS OF THE STUDY:-

1. Sample size is of only 50 respondents.
2. Conclusions and suggestions drawn on the basis of information provided by BHMS Doctors.
3. This study is restricted to BHMS Doctors of Amravati city only.

DATA ANALYSIS AND INTERPRETATION:

After collection of data, it has been arranged in a tabular form for its suitable analysis and interpretation so that the true and fair results may be drawn towards the stated study.

Table 1: Gender wise distribution of B.H.M.S doctors:-

Sr. No.	Particular	No. of Respondents	Percentage (%)
1	Male	39	78%
2	Female	11	22%
	TOTAL	**50**	**100%**

Analysis: From the above table it is revealed that most of the respondents are male i.e. 78% and the balance respondents are female i.e. 22%.

Table 2: Age wise distribution of B.H.M.S. doctors:-

Sr. No.	Age Groups	No. Of Respondents	Percentage (%)
1	Up to 30 years	15	30%
2	30 to 40 years	15	30%
3	40 to 50 years	16	32%
4	Above 50 years	04	08%
	Total	**50**	**100%**

Analysis: From the above table it is revealed that majority 32% respondents are from the age group 40 to 50 years, 30% respondents are from the age group up to 30 years, 30% respondents are from the age group 30 to 40 years and the least respondents i.e. 8% are from the age group of above 50 years.

Table 3: Income wise distribution of B.H.M.S doctors:-

Sr. No.	Income Group	No. Of Respondents	Percentage (%)
1	Below Rs. 2,50,000	13	26%
2	Rs. 2,50,000 to Rs. 5,00,000	27	54%
3	Rs. 5,00,000 to Rs. 7,50,000	08	16%
4	Above Rs. 7,50,000	02	04%
	TOTAL	**50**	**100%**

Analysis: From the above table it revealed that majority of the respondents i.e. 54% are from the income group of Rs.2,50,000 to Rs.5,00,000, 26% respondents are from the income group of below Rs.2,50,000, 16% respondents are from the income group of Rs.5,00,000 to Rs.7,50,000 and the least 4% respondents are from the income group of above Rs.7,50,000.

Table 4: Believe of BHMS Doctors in Savings which further leads towards investments:-

Sr. No.	Particulars	No. Of Respondents	Percentage (%)
1	**YES**	**46**	**92%**
2	**NO**	**04**	**08%**
	TOTAL	**50**	**100%**

Analysis: From the above table it is revealed that 92% of the respondents believe in savings which leads towards their further investments and only 8% of the respondents not believe in savings but they still does the investments as per their necessity.

Table 5: Saving objectives of BHMS Doctors:-

Sr. No.	Saving Objectives	No. Of Respondents	Percentage (%)
1	For Future Safety of family	**07**	**14%**
2	For Tax Savings/Benefits	**19**	**38%**
3	For Children education & welfare	**13**	**26%**
4	To meet contingencies	**03**	**06%**
5	For Purchase of house & assets (wealth creation)	**08**	**16%**
	TOTAL	**50**	**100%**

Analysis: From the above table it is revealed that 14% respondent's saving objective is for future safety of family, 38% respondent's saving objective is for tax savings, 26% respondent's saving objective is for children education & welfare, 06% respondent's saving objective is to meet contingencies and 16% respondent's saving objective is for purchase of house & assets i.e. for wealth creation.

Table 6: Awareness about various Investment Alternatives available in India:-

Sr. No.	Awareness about Investment Alternatives	No. Of Respondents	Percentage (%)
1	YES	38	76%
2	NO	12	24%
	TOTAL	50	100%

Analysis: From the above table it revealed that maximum respondents i.e. 76% have awareness about various investment alternatives available in the market whereas 24% of respondents have not awareness about the various investment alternatives available in the financial market.

Table 7: Striking factors among BHMS Doctors before choosing a particular investment avenue:-

Sr. No.	Think before investment	No. Of Respondents	Percentage (%)
1	About Return from it	19	38%
2	About Risk involved in it	06	12%
3	About Safety of invested money	08	16%
4	About liquidity	02	04%
5	Hedge against inflation	03	06%
6	All the above factors	12	24%
	TOTAL	50	100%

Analysis: From the above table it is analyzed that 38% respondents think about return from investments, 12% respondents think about risk involved in investment, 16% respondents think about safety of invested money, 04% respondents think about liquidity, 06% respondents think about hedge against inflation and 24% respondents think about all the above stated factors before making their investment in a particular investment avenue.

Table 8: Preferences of investment avenues given by BHMS Doctors:-

Sr. No.	Investment Avenues	No. Of Respondents	Percentage (%)
1	Insurance policy	18	36%
2	Fixed deposits	10	20%
3	Equities / shares	01	02%
4	Real estate	08	16%
5	Mutual funds	04	08%
6	Gold / Precious metal	09	18%
	TOTAL	50	100%

Analysis: From the above table it is revealed that 36% respondents preferred to invest in insurance policy, 20% respondents preferred to invest in fixed deposits of banks, 02% respondents preferred to invest in equities/shares, 16% respondents preferred to invest in real estate, 08% respondents preferred to invest in mutual funds and 18% respondents preferred to invest in gold/precious metals.

Table 9: Tenure of investment:-

Sr. No.	Tenure of Investments	No. Of Respondents	Percentage (%)
1	For Short term (Up to 1 year)	10	20%
2	For Medium term (Above 1 to 3 years)	24	48%
3	For Long term (Above 3 years)	16	32%
	Total	50	100%

Analysis: From the above table it is revealed that 20% respondents invest their money for short term period, 48% respondents invest their money for medium term period and 32% respondents invest their money for the long term period.

Testing of hypothesis:-

I."There is no significant impact of saving objectives of investors on their preferred investment avenues."

To test this hypothesis the researcher has applied chi-square test:-

Table: Observed Frequency and Expected Frequency (Primary Data) Showing relationship between preferred investment avenues and savings objectives of investors:-

Investment Avenues /Saving Objectives	Insurance Policy	Fixed Deposits	Equities/ shares	Real Estate	Mutual Fund	Gold/Precious metals	Total
For future safety of family	03(2.52)	01(1.4)	00 (0.14)	02(1.12)	01(0.56)	00 (1.26)	07
For Tax Savings/Benefits	13 (6.84)	04 (3.8)	00 (0.38)	00 (3.04)	02 (1.52)	00 (3.42)	19
For Children education & welfare	02 (4.68)	03 (2.6)	00 (0.26)	00 (2.08)	01(1.04)	07 (2.34)	13
To meet future contingencies	00 (1.08)	00 (0.6)	01 (0.06)	00 (0.48)	00 (0.24)	02 (0.54)	03
For Purchase of house & assets	00 (2.88)	02(1.6)	00 (0.16)	06(1.28)	00 (0.64)	00 (1.44)	08
TOTAL	18	10	01	08	04	09	50

Table:- Chi-square test:-

O	E	(O – E)	$(O - E)^2$	$(O - E)^2 / E$
3	2.52	0.48	0.230	0.091
1	1.4	- 0.4	0.160	0.114
0	0.14	- 0.14	0.020	0.143
2	1.12	0.88	0.774	0.691
1	0.56	0.44	0.194	0.346
0	1.26	- 1.26	1.588	1.260
13	6.84	6.16	37.946	5.548
4	3.8	0.2	0.04	0.010
0	0.38	- 0.38	0.144	0.379
0	3.04	- 3.04	9.242	3.040
2	1.52	0.48	0.230	0.151
0	3.42	- 3.42	11.696	3.420

2	4.68	- 2.68	7.182	1.535
3	2.6	0.4	0.16	0.062
0	0.26	- 0.26	0.068	0.262
0	2.08	- 2.08	4.326	2.080
1	1.04	- 0.04	0.002	0.002
7	2.34	4.66	21.716	9.280
0	1.08	- 1.08	1.166	1.080
0	0.6	- 0.6	0.36	0.600
1	0.06	0.94	0.884	14.733
0	0.48	- 0.48	0.230	0.479
0	0.24	- 0.24	0.058	0.242
2	0.54	1.46	2.132	3.948
0	2.88	- 2.88	8.294	2.880
2	1.6	0.4	0.16	0.100
0	0.16	- 0.16	0.026	0.163
6	1.28	4.72	22.278	17.405
0	0.64	- 0.64	0.410	0.641
0	1.44	- 1.44	2.074	1.440
			TOTAL	**72.125**

Degree Of Freedom (V):-

V = (r-1) *(c-1)
= (5-1) * (6-1)
= 4 * 5
V = 20

CONCLUSION:-

The table value of chi-square at 5% significance level with 20 degrees of freedom is 31.410, which is less than 72.125 the calculated value of chi- square. Hence the stated null hypothesis is rejected and accordingly we can state that there is a significant impact of saving objectives of investors on their preferred investment avenues.

Testing of hypothesis:-

II."There is no significant relationship between age and saving objectives of investors."

To test this hypothesis the researcher has applied chi-square test:-

Table: Observed Frequency and Expected Frequency (Primary Data) showing relationship between Age and Saving Objectives of investors:-

SR. NO.	Age Group	For future safety	For tax savings	For children education	To meet contingencies	For purchase of assets	Total
1.	Up to 30 years	02 (2.1)	04 (5.7)	02 (3.9)	00 (0.9)	07 (2.4)	15
2.	30 to 40 years	03 (2.1)	06 (5.7)	06 (3.9)	00 (0.9)	00 (2.4)	15
3.	40 to 50 years	02 (2.24)	07 (6.08)	05 (4.16)	01 (0.96)	01 (2.56)	16
4.	Above 50 years	00 (0.56)	02 (1.52)	00 (1.04)	02 (0.24)	00 (0.64)	04
Total		07	19	13	03	08	50

Table:- Chi-square test:-

O	E	(O – E)	$(O - E)^2$	$(O - E)^2 / E$
02	2.1	- 0.1	0.01	0.005
04	5.7	- 1.7	2.89	0.507
02	3.9	- 1.9	3.61	0.926
00	0.9	- 0.9	0.81	0.900
07	2.4	4.6	21.16	8.817
03	2.1	0.9	0.81	0.386
06	5.7	0.3	0.09	0.016
06	3.9	2.1	4.41	1.131
00	0.9	- 0.9	0.81	0.900
00	2.4	- 2.4	5.76	2.400
02	2.24	- 0.24	0.06	0.027
07	6.08	0.92	0.85	0.140
05	4.16	0.84	0.71	0.171
01	0.96	0.04	0.00	0.000
01	2.56	- 1.56	2.43	0.949
00	0.56	-0.56	0.31	0.554
02	1.52	0.48	0.23	0.151
00	1.04	-1.04	1.08	1.038
02	0.24	-1.76	3.10	12.917
00	0.64	-0.64	0.41	0.641
				32.576

Degree Of Freedom (v):-

V = (r-1) * (c-1)
= (4-1) * (5-1)
= 3 * 4
V = 12.

CONCLUSION:-

The table value of chi-square at 5% significance level with 12 degrees of freedom is 21.026, which is less than 32.576 the calculated value of chi- square. Hence the stated null hypothesis is rejected and accordingly we can state that there is a significant relationship between age and saving objectives of the investors.

FINDINGS:

1.In the stated study researcher has investigated 50 respondents/ BHMS Doctors of Amravati city and in it revealed that most of the respondents are male i.e. 78% whereas 22% respondents are female.
2.It is revealed that majority 32% respondents are from the age group of 40 to 50 years, 30% respondents are from the age group of up to 30 years, 30% respondents are from the age group of 30 to 40 years and the least respondents i.e. 8% are from the age group of above 50 years.
3.It is revealed that majority of the respondents i.e. 54% are from the income group of Rs.2,50,000 to Rs.5,00,000 and the least 4% respondents are from the income group of above Rs.7,50,000.
4.For testing of hypothesis researcher has used chi-square test and by way of which it is revealed that there is a significant impact of saving objectives of investors on their preferred investment avenues.
5.By using chi-square test for testing of hypothesis, it is revealed that there is a significant relationship between age and saving objectives of the investors.
6. It is revealed that majority i.e. 92% of the respondents believe in savings which leads towards their further investments and only 8% of the respondents not believe in savings but they still do investments

as per their convenience or availability of surplus funds.
7.It is revealed that majority 38% respondent's saving objective is for tax savings and thereafter 26% respondent's saving objective is for children's education & welfare and only 06% respondent's saving objective is to meet future contingencies.
8.It is revealed that maximum respondents i.e. 76% have awareness about various investment alternatives available in the financial market whereas only 24% have not awareness.
9.It is revealed that maximum i.e. 38% respondents think about return from investments there after 24% respondents think about all the stated factors before making their investment in a particular investment avenue and only 04% respondents think about liquidity.
10.It is revealed that majority 36% respondents preferred to invest in insurance policy thereafter 20% respondents preferred to invest in fixed deposits of banks and only 02% respondents preferred to invest in equities/shares.
11.It is revealed that maximum 48% respondents invest their money for medium term period thereafter 32% respondents invest their money for the long term period.

CONCLUSIONS:

After analysis and interpretation of the collected data it is concluded that most of the respondents believe in savings which leads towards their further investments and it further concludes that there is a significant impact of saving objectives of investors on their preferred investment avenues and there is a significant relationship between age and saving objectives of the investors. Most of the respondents preferred to invest in insurance policy for the safety of their family and thereafter preferred to invest in fixed deposits of banks for the sake of safety of their invested money in which their conservative ideology or traditional approach towards investment seems and it also resembles that they don't aware about the concept of hedging against the inflation rate.

SUGGESTIONS/RECOMMENDATIONS:

1.On the basis of said study I can say that least of the respondents invest their money in equities/shares, mutual funds and in other latest investment avenues so that they should be benefitted by the effective guidelines towards such avenues due to which such investors gets attracted towards latest investment options as well their awareness plays key role in the economic development of the nation as well.
2.Most of the respondents have not awareness regarding various mutual fund schemes available in the financial market so that it is also recommended to the broking agencies of mutual funds to provide effective guidelines among investors to highlight the benefits of mutual fund investments.
3.Most of the respondents are not aware about the concept of hedging against inflation due to which they still believe in old traditional investment avenues even if they have not fulfilled their wants so that they should invest their savings in modern investment avenues.

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PRELIMINARY PHYTOCHEMICAL ANALYSIS OF *MILIUSA TOMENTOSA* (ROXB.) J. SINCLAIR BY USING VARIOUS ORGANIC SOLVENTS

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ABSTRACT
The present study was to evaluate the phytochemical compound from six different solvent of dried leaves and stem of *Miliusa tomentosa* belongs to family Annonaceae. Qualitative preliminary phytochemical analysis of this plant contains carbohydrate, protein, cardiac glycoside, glycosides, alkaloids, flavonoids, saponins, steroids, anthraquinones, tannins, quinines and inorganic compound. The presence of these phytochemicals can be correlated with medicinal potential of these plants.

KEYWORDS: *Miliusa tomentosa*, Phytochemical screening; Quantitative analysis.

INTRODUCTION
Higher and aromatics plants have traditionally been used in folk medicine as well as to extend the shelf life of foods, medicinal plant use for inhibition against bacteria, fungi and yeasts. Annonaceae is a pantropical family of shrubs, trees and lianas. The family consists of about 130 genera and 2300 species. Although the position of Annonaceae within the Angiosperms and order Magnoliales and its family circumscription is clear and undisputed.[1] The plants belonging to family Annonaceae are used as antibacterial, anticancer, anthelmintic, antiparasitic and pesticidal agents.[2] The genus *Miliusa* (Annonaceae family) consists about 40 species which grows in tropical rainforest of India, Thailand, South China and North Australia.[3] The different species of *Miliusa* are invariably small to large trees and are found in a wide range of rainforest communities. Only three species of Genus *Miliusa* occur in Australia, which are endemic to there and contain two essential oils.[4] The plant is used in folk medicine for different symptom such as gastropathy and glomerulonephropathy.[5] In Chinese traditional medicine *Miliusa tomentosa* oil has been found to have both antibacterial and analgesic properties.[6]

Knowledge of the chemical constituents of plants is desirable because such information will be valuable for synthesis of complex chemical substances.[7] Two new isoquinoline alkaloids, 2,10- dimethoxy-3,11-dihydroxy-5,6-dihydroprotober -berine and 1,9-dihydroxy-2,11 -dimethoxy-4,5- dihydro-7-oxoaporphine, together with thirteen known alkaloids, were isolated from the ethanolic extracts of the stem and leaves of *M. cuneata* (Graib).[8] Since *Miliusa tomentosa* (Roxb.) J Sinclair is one of them, its traditional uses are not reported but its fruits are eaten in some parts of India and its tree yields a pale yellow gum known as karee gum.[9] Thus, main objective of this research work is to consider the photochemical screening of the content which is present in different crude extracts.

MATERIAL AND METHODS
Collection of plant materials
Fresh plant parts of *Miliusa tomentosa* (Roxb.) J. Sinclair were collected from naturally growing populations located in Melghat region Maharashtra, India, during February-March 2016. The samples were identified at the herbarium section of the Department of Botany, Vidya Bharati Mahavidyalaya, Amravati. The fresh plant part samples were washed by tap water, air dried in shadow at room temperature milled well into a fine powder in a mixer grinder and stored until for further analysis.

Preparation of plant extracts in aqueous and different organic solvent
5 gm of dried powder was macerated with 100 ml of aqueous, petroleum ether, chloroform, toluene, butanol and benzene in a conical flask and shaken at room temperature for 24 hours and filtered through Whatman No.1 filter paper. The concentrated extracts were taken in colour amber bottles and kept in refrigerator for further analysis using the standard methods.[10]

Calculation of Extraction Yield (% Yield)
The yield (%, w/w) from dried extract was calculated as:
Yield (%) = $(W_1 \times 100) / W_2$

W_1 is the weight of the extract after lyophilization of solvent and W_2 is the weight of the powdered material.

Phytochemical screening

Phytochemical screening tests for the identification of protein, sugar, amino acid, alkaloids, flavonoids, steroids, cardiac glycosides, tannins, phenols, quinines & inorganic tests were carried out for all the extracts by the standard methods.[11][12]

Tests for carbohydrates

Fehling's Test

1ml Fehling's A solution and 1 ml of Fehling's B solution were mixed and boiled for one minute. Now the equal volume of test solution was added to the above mixture. The solution was heated in boiling water bath for 5-10 minutes. First a yellow, the brick red precipitate was observed.

Benedict's reagent

Equal volumes of Benedict's reagent and test solution were mixed in a test tube. The mixture was heated in boiling water bath for 5 minutes. Solution appeared green showing the presence of reducing sugar.

Molisch's test

Equal volumes of Molisch's reagent and test solution were mixed in a test tube. The mixture was heated in boiling water bath for 5 minutes. Appearance of violet or purple colour ring showing the presence of reducing sugar.

Standard Test for combine sugar

In 1 ml extract 5 ml conc. Hcl added and hydrolyzed by boiling and neutralized by adding NaOH and repeat the fehling test, Brick red ppt indicate result.

Keton's test

2 ml extract add with few crystal of resorcinol and equal voloume of conc. Hcl and heat over sprit lamp observed rose coloration indicate presence of keton.

Test for proteins

Burette Test

To the small quantity of extract 1-2 drops of burette reagent was added. Formation of violet colour precipitate showed presence of proteins.

Million's Test

To the small quantity of extract 1-2 drops of Million's reagent was added Formation of white colour precipitate showed presence of proteins.

Test for Xanthoprotein

In 2ml of test solution a few drops of conc. nitric acid and 3ml of ammonia were added appearance of red precipitate indicate the presence of xanthoprotien.

Tests for Anthroquinone glycosides

Borntrager's Test

To the 3 ml of extracts, dil. H_2SO_4 was added. The solution was then boiled and filtered. The filtrate was cooled and to it equal volume of benzene was added. The solution was shaken well and the organic layer was separated. Equal volume of dilute ammonia solution was added to the organic layer. The ammonia layer turned pink showing the presence of glycosides.

Tests for Cardiac glycosides

Keller-Killiani test

5ml of extract, 1ml of conc.H_2SO_4, 2 ml of Glacial acetic acid and 1 drop of $FeCl_3$ solution was added. Appearance of brown ring shows the presence of cardiac glycosides.

Test for steroids

Salkowski test

To 2 ml of extract, 2ml of chloroform and 2ml of conc.H_2SO_4 was added. The solution was shaken well. As a result chloroform layer turned red and acid layer showed greenish yellow fluorescence.

Test for alkaloids

Hager's test

2-3 ml of filtrate, few drops of dil.HCl and Hager's reagent was added and shake well. Yellow precipitate was formed showing the presence of alkaloids.

Mayer's test

2-3 ml of filtrate, dil. HCl and Mayer's reagent was added and shake well. Yellow precipitate was formed showing the presence of alkaloids.

Dragendroff's test

2-3 ml of filtrate, few drops of dil.HCl and Dragendroff's reagent was added and shake well. Formation of orange-brown precipitate showed the presence of alkaloids.

Wagners test

2 ml extract add the reagent reddish brown ppt show indicates the presence of alkaloids.

Tests for flavonoids

Lead Acetate test

To the small quantity of extract lead acetate solution was added. Formation of yellow precipitate showed the presence of flavonoids.

Alkaline test

To the test solution add few drops o NaOH solution formation of intence yellow color which turn to colorless solution after addition of dil. Acetic acid indicate presence of flavonoids.

NaOH test

Small amount of extract was treated with NaOh and Hcl observe formation of yellow Orange color.

H_2SO_4
Fraction of extract treated with Conc.H_2SO_4 and observe formation of orange color.

Test for Tannins and Phenolics compound
$FeCl_3$ solution Test
On addition of 5% $FeCl_3$ solution to the extract, deep blue black colour appeared.

Lead Acetate test
On addition of lead acetate solution to the extract white ppt appeared.

Phlobotannins
2 ml extract add dil.HCl observe red ppt.

Gelatin test
To the test solution add few drops of 10% gelatin solution white ppt indicate the result.

Test for Saponin
Foam Test
1 ml extract 20 ml distilled water was added and shakes well in measuring cylinder for 15 min. Then 1 cm layer of foam was formed.

Frothing test
3 ml extract with 10 ml D/W in test tube and plug the Stoppard and shake vigorously for 5 min. it allow to stand 30 min observe the honey comb forth indicate result.

Test for Glycosides
The extract was mixed with a little anthrons on a watch glass. One drop of conc. sulphuric acid was added and made into a paste and warmed gentle over the water bath. Dark green coloration indicates the presence of glycosides.

Test for Quinone
2 ml test solution was treated with a few drops of conc. H_2SO_4 or aq. NaOH solution. Colour formation indicates the presence of quinoid compound.

Test for fixed oil
A small quantity of powder was pressed between the filter paper. Formation of grease spot indicates the presence of fixed oil and fats.

RESULT
The preliminary phytochemical analysis of leaf and stem of *Miliusa tomentosa* in this study revealed the presence of glycosides, steroid, saponins, flavonoids, tannins, alkaloids, phenolic compounds, quinine, anthraquinone and inorganic test (Table-1 and Table-2). Using different solvent (aqueous, benzene, butanol, chloroform, toluene, and petroleum ether) extract. This study revealed the presence of glycosides maximum in benzene, butanol and petroleum ether, where as steroids were present in all solvents except water. Alkaloids presence maximum in water and chloroform. Flavonoids minimum presence in benzene, butanol, toluene and water while phenolic compounds found in butanol and water extracts only but tannins were absent in all extracts. Saponins were maximum present in chloroform and anthraquinone absent in all extracts while as inorganic tests showed positive results in chloroform extracts. In contrast to all this carbohydrate are maximum in butanol extract and protein in aqueous solvent extracts of leaf.

The phytochemical analysis of stem extracts show the presence of glycosides, saponins maximum in chloroform extract while alkaloids, phenol, inorganic test in butanol extract. Flavonoids and steroid recorded in petroleum extract only. Interestingly, tannin and anthraquinone absent in all extract (Table-1 and Table-2). Extractive values of leaf and stem extract by various solvents shown in (Table -3). Extractive values are varies according to solvents systems.

Table 1: Phytochemicals analysis in the stem extracted by various solvents

Sr.No	Phytochemicals	Test Name	Benzene	Butanol	Chloroform	Toluene	Petroleum Ether	Water
1	Carbohydrate	Fehling	-	-	+	-	+	-
		Benedict	-	-	-	-	-	+
		Molisch	-	-	-	-	-	-
		Combine Sugar	-	+	-	-	-	+
		Keton	+	+	-	-	-	+
2	Proteins	Biurett	-	-	-	-	-	-
		Millions	-	-	+	-	-	-
		Xanthoprotein	-	+	-	-	-	+
3	Glycosides	Cardiac	+	+	+	+	+	+
		Keller-Killani	-	-	+	-	-	-
5	Steroid	Salkowski test	-	-	-	+	+	+
		Lieberman-Buchnard	-	-	-	-	-	-
		H_2SO_4	-	-	+	-	-	-
6	Alkaloids	Hager's	-	-	-	-	-	+
		Mayer's	-	+	+	+	-	-

		Dragendroff's	+	+	-	-	-	+
		Wagner's	+	-	-	+	+	+
7	Flavonoids 8	Lead acetate	+	+	+	+	+	+
		Alkalin	+	-	-	+	+	-
		NaOH	-	-	-	-	-	-
		H_2SO_4	-	+	+	-	+	+
8	Phenolic	$FeCl_3$	-	+	-	-	-	+
		Lead acetate	+	+	+	+	+	+
9	Tannin	Gelatin	-	-	-	-	-	-
		Phlobotannin	-	-	-	-	-	-
10	Saponin	Foam	-	-	+	+	+	+
		Frothing	-	-	+	-	-	+
11	Quinone	quinone	+	+	+	+	-	-
12	Anthraquinone	Anthraquinone	-	-	-	-	-	-
13	Inorganic	Sulphate test	+	+	+	+	-	-
		Carbonate test	+	+	+	+	+	-

(+) Present (-) Not detected

Table 2: Phytochemicals analysis in the Leaf extracted by various solvents

Sr.No	Phytochemicals	Test Name	Benzene	Butanol	Chloroform	Toluene	Petroleum Ether	Water
1	Carbohydrate	Fehling	+ +	+ +	+ +	- -	+ +	+ +
		Benedict	-	+	-	-	-	+
		Molisch	-	-	-	-	-	-
		Combine Sugar	-	+	-	-	-	+
		Keton	-	+	-	-	-	+
2	Proteins	Biurett	-	-	-	-	-	-
		Millions	-	-	-	-	-	+
		Xanthoprotein	-	+	-	+	+	+
3	Glycosides	Cardiac	+	+	-	-	+	+
		KellerKillaiani	+	+	-	+	+	-
5	Steroid	Salkowski	+	+	+	+	+	-
		Lieberman-Buchnard	+	+	+	+	+	-
		H_2SO_4	+	+	+	-	+	-
6	Alkaloids	Hager's	-	-	-	-	-	-
		Mayer's	-	-	+	-	-	-
		Dragendroff's	-	-	-	-	-	+
		Wagner's	+	+	+	+	+	+
7	Flavonoids	Lead acetate	+	+	+	+	+	+
		Alkalin	-	-	-	-	-	-
		NaOH	-	-	-	-	-	-
		H_2SO_4	+	+	-	+	-	+
8	Phenolic	$FeCl_3$	-	+	-	-	-	+
		Lead acetate	+	+	+	+	+	+
9	Tannin	Gelatin	-	-	-	-	-	-
		Phlobotannin	-	-	-	-	-	-
10	Saponin	Foam	+	-	+	-	-	+
		Fothing	-	-	+	-	-	-
11	Quinone	quinone	+	+	+	+	+	-
12	Anthraquinone	Anthraquinone	-	-	-	-	-	-
13	Inorganic	Sulphte test	-	-	+	+	+	-
		carbonate test	-	+	+	-	+	-

(+) Present (-) Not detected

Table 3: Extractive values of leaf and stem extract by various solvents

Sr. No.	Solvent Name	Leaf extract colour	Extractive of Leaf	Stem extract colour	Extractive value of Stem
1	Benzene	Blackish green	0.13	Green	0.07
2	Butanol	Green	0.04	Light green	0.45
3	Chloroform	Dark Green	0.08	Light green	0.012
4	Toluene	Yellowish Green	0.09	Light green	0.01
5	Pet. Ether	Yellowish Green	0.07	Light green	0.11
6	Water	Orange	0.19	Red Orange	0.11

DISCUSSION

The phytochemical screening revealed the presence glycosides, steroids, alkaloids, flavonoids, phenolics, tannins, saponins, quinines, anthraquinones and inorganic compounds. The leaf extract detected the phytochemicals including Flavonoids, Steroids, Saponins and Alkaloids which have been supported by some other workers also.[13] The crude extract of leaf and stem showed the absence of phytochemical constituents such as tannins and anthraquinones. Similar results have been found by other workers[14] hence supporting present work. Alkaloids tend to be organic and natural ingredients that have nitrogen and are also physiologically active together with sedative and analgesic roles.[15] Phenolic compounds are some of the most widespread molecules among plant secondary metabolites, are known to act as natural antioxidants.[16] Cardiac glycosides are the compounds used to treat congestive heart failure and cardiac arrhythmea. These compounds work by inhibiting the Na+/K+ pump.[17] Saponins are high molecular weight compounds in which a sugar molecule is combined with triterpene or steroid aglycon, so there are two major groups of saponins; triterpene saponins and steroid saponins. These are therapeutically important as they show hypolipidemic and anticancer activity of cardiac glycosides.[18] Flavonoids enhance the effects of Vitamin C and function as antioxidants. They are also known to be biologically active against liver toxins, tumors, viruses and other microbes.[19] The yield of extracts depending on the solvent and plant material used.[20] Hence the preliminary phytochemical investigation are actually obliging in finding chemical ingredients in the plant that may help to their quantitative evaluation and also in locating the source of pharmacologically active principle.

CONCLUSION

The results of the phytochemical analysis showed the leaves and stem extracts indicate their potential as a source of bioactive principles that may supply drugs for modern medicines. Further studies are therefore required to validate their antimicrobial, antihyperglycemic, anti-inflammatory and anthelminthic activities. In addition, isolation, purification and characterization of the active chemicals are necessary to ensure that the plant has novel chemicals which will be helpful in future studies.

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GOODS AND SERVICE TAX

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Introduction

In the year 2000, for the first time the idea of initiating the GST was made by the BJP Government under the leadership of Atal Behari Vajpayee. An empowered committee was also formed for that, headed by Asim Dasgupta (the then Finance Minister of the West Bengal Government). The committee was formed to design the model of the GST and at the same time inspect the preparation of the IT department for its rollout. In 2011, the previous United Progressive Alliance (UPA) Government also introduced a Constitution Amendment Bill to facilitate the introduction of the GST in the Lok Sabha but it was rejected by many States.

The Constitution Amendment Bill for Goods and Services Tax (GST) has been approved by The President of India post its passage in the Parliament (Rajya Sabha on 3 August 2016 and Lok Sabha on 8 August 2016) and ratification by more than 50 percent of state legislatures. The Government of India is committed to replace all the indirect taxes levied on goods and services by the Centre and States and implement GST by April 2017.

With GST, it is anticipated that the tax base will be comprehensive, as virtually all goods and services will be taxable, with minimum exemptions. GST will be a game changing reform for the Indian economy by creating a common Indian market and reducing the cascading effect of tax on the cost of goods and services. It will impact the tax structure, tax incidence, tax computation, tax payment, compliance, credit utilization and reporting, leading to a complete overhaul of the current indirect tax system. GST will have a far-reaching impact on almost all the aspects of the business operations in the country, for instance, pricing of products and services, supply chain optimization, IT, accounting, and tax compliance systems.

Object of study

To know the GST in detail and find out advantages and disadvantages of it.

What is GST?

The GST is basically an indirect tax that brings most of the taxes imposed on most goods and services, on manufacture, sale and consumption of goods and services, under a single domain at the national level. In the present system, taxes are levied separately on goods and services. The GST is a consolidated tax based on a uniform rate of tax fixed for both goods and services and it is payable at the final point of consumption. At each stage of sale or purchase in the supply chain, this tax is collected on value-added goods and services, through a tax credit mechanism.

The proposed model of GST and the rate

A dual GST system is planned to be implemented in India as proposed by the Empowered Committee under which the GST will be divided into two parts:

- State Goods and Services Tax (SGST)
- Central Goods and Services Tax (CGST)

Both SGST and CGST will be levied on the taxable value of a transaction. All goods and services, leaving aside a few, will be brought into the GST and there will be no difference between goods and services. The GST system will combine Central excise duty, additional excise duty, services tax, State VAT entertainment tax etc. under one banner.

The GST rate is expected to be around 14-16 per cent. After the combined GST rate is fixed, the States and the Centre will decide on the SGST and CGST rates. At present, 10 per cent is levied on services and the indirect taxes on most goods is around 20 per cent.

Status of implementation of GST

To be fully viable by law in all the States, the GST Bill needs to be passed by a two-thirds majority in both Houses of Parliament and by the legislatures of half of the 29 States. In December 2014, Finance Minister Arun Jaitley introduced the constitutional amendment Bill of the GST in the Lok Sabha. He announced that the GST would be a major reform in India's taxation system since 1947, which would reduce transaction costs for business and boost the economy.

Earlier, the Bill was rejected by a few States saying that it does not include the issues of compensation, entry tax and the tax on petroleum products. Jaitley while introducing the Bill said that all efforts have been taken to make sure that the States do not suffer any loss of revenue with the implementation of the GST. The States will receive Rs 11,000 crore this fiscal year so that it would compensate the losses suffered by them for decline in Central sales tax (CST) and subsequently financial assistance would be provided for a five-year period.

All said and done, the GST Bill which was conceived way back in the year 2000 has not seen the light of the day as yet. If everything goes well, most likely the Bill will be legislated by April 2016. According to a study by the National Council of Applied Economic Research (NCAER), full implementation of the GST could expand India's growth of gross domestic product by 0.9-1.7 percentage points. By removing the system of multiple Central and State taxes, the GST can help in reducing taxation and filing costs and expand business profitability, thereby attracting investments and promoting GDP growth. Simplification of tax norms can help in improving tax compliance and increasing tax revenues.

Advantages of GST Bill

Introduction of a GST is very much essential in the emerging environment of the Indian economy.

- There is no doubt that in production and distribution of goods, services are increasingly used or consumed and vice versa. Separate taxes for goods and services, which is the present taxation system, requires division of transaction values into value of goods and services for taxation, leading to greater complications, administration, including compliances costs. In the GST system, when all the taxes are integrated, it would make possible the taxation burden to be split equitably between manufacturing and services.
- GST will be levied only at the final destination of consumption based on VAT principle and not at various points (from manufacturing to retail outlets). This will help in removing economic distortions and bring about development of a common national market.
- It will also help to build a transparent and corruption-free tax administration. Presently, a tax is levied on when a finished product moves out from a factory, which is paid by the manufacturer, and it is again levied at the retail outlet when sold.

Apart from full allowance of credit, there are several other advantages of introducing a GST in India:

- **Possible reduction in prices**: Due to full and seamless credit, manufacturers or traders do not have to include taxes as a part of their cost of production, which is a very big reason to say that we can see a reduction in prices. However, if the

government seeks to introduce GST with a higher rate, this might be lost.

- **Increase in Government Revenues:** This might seem to be a little vague. However, even at the time of introduction of VAT, the public revenues actually went up instead of falling because many people resorted to paying taxes rather than evading the same. However, the government may wish to introduce GST at a Revenue Neutral Rate, in which case the revenues might not see a significant increase in the short run.
- **Less compliance and procedural cost:** Instead of maintaining big records, returns and reporting under various different statutes, all assesses will find comfortable under GST as the compliance cost will be reduced. It should be noted that the assessees are, nevertheless, required to keep record of CGST, SGST and IGST separately

Benefits of GST Bill

For the Centre and the States

According to experts, by implementing the GST, India will gain $15 billion a year. This is because, it will promote more exports, create more employment opportunities and boost growth. It will divide the burden of tax between manufacturing and services.

For individuals and companies

In the GST system, taxes for both Centre and State will be collected at the point of sale. Both will be charged on the manufacturing cost. Individuals will be benefited by this as prices are likely to come down and lower prices mean more consumption, and more consumption means more production, thereby helping in the growth of the companies.

Items not under GST

Alcohol, tobacco, petroleum products

Disadvantages of GST Bill in India

- The Service Tax in India is now 15% but the proposed GST is about 18-20%. All the services will be Costlier and this one of the Disadvantages of GST Bill on Common Person.
- There are some retail products where the Tax rate is only 4 percent but with GST it will be costlier like Garments and cloths.
- The control on business will be of state and central government so it may be some complex for businessman.
- All credits will be online and some penalties are like criminal activity. So it is threatening for small businessman who is now free from Taxes.
- GST is also having three types of taxes and all have to be maintained and this not going too easy for small Businessman.

Above are the advantages and disadvantages of GST Bill in India and as per me it will be more comfortable than the existing Tax Process in India.

Conclusion

- In the GST system, when all the taxes are integrated, it would make possible the taxation burden to be split equitably between manufacturing and services. This will help in removing economic distortions and bring about development of a common national market. GST at a Revenue Neutral Rate, in which case the revenues might not see a significant increase in the short run. Instead of maintaining big records, returns and reporting under various different statutes, all assesses will find comfortable under GST as the compliance cost will be reduced.
- Reduce the cascading effect of taxes on the final price of the product. Eliminate tax-on-tax effect. ...
- Moderate prices and increase consumption.
- Uniform and Stable Tax Regime. One-Country-One-Tax.
- Simplify Tax Structure. ...
- Increase GDP, tax-GDP ratio and revenue surplus.
- Will eliminate multiple taxes.
- Will reduce logistics cost for firms.
- Would reduce black money; need for financial documentation will increase.

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VIDYABHARATI SHAIKSHANIK MANDAL AMRAVATI'S

S.S.S.K.R. INNANI MAHAVIDYALAYA

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REACCRIDATED BY NAAC AT LEVEL 'A'(CGPA3.24)

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ज्ञान-विज्ञान विमुक्तये

U.G.C. sponsored National Conference on

FOREIGN DIRECT INVESTMENT RELATED GROWTH OF INDIAN ECONOMY

(NCFDI-2017) 28 February 2017

CERTIFICATE

Prof./Dr./Mr./Mrs. S.B.Kadu **of**

Vidya Bharati Mahavidyalaya,Amravati

has Participated/Presented a paper entitled RURAL DEVELOPMENT AND DIGITAL VILLAGE

in UGC sponsored National Conference on Foreign Direct Investment Related Growth of Indian Economy, organized by Departments of Economics & Commerce S.S.S.K.R. Innani Mahavidyalaya Karanja (Lad) on 28th February 2017.

Mr.R.H. Mathurkar	Dr. K.G. Rajput	Dr. P. R. Rajput
Organizing Secretary	Convener	Chairman

1. **Proper land reforms** to make sure land is held, owned, cultivated, irrigated to make the most efficient use and maximum output.
2. **Rural credit** – Banking services need to be popularized and credit should be available for basic services like agriculture.
3. **Electrification** – Many villages still receive only 2 to 6 hours of electricity per day which needs to drastically improve to empower the villages of India.

Mobiles have empowered rural India

Basically, what we need is to empower the rural people by providing them education and proper health care. They need to have infrastructure like electricity and water so that they are free from the cycle of droughts and floods. We need to give them self-employment so that they want to stay in villages instead of migrating in cities. There is a need to empower the villagers, and not just supporting them by food subsidies, loan waivers which end up crippling them. India will grow only when rural India marches hand in hand with cities in the twenty first century.

What is a Digital Village?

A Digital Village is a space where a community expresses their identity though ICT and Digital Media. This may be from an artistic, heritage, or economic perspective or a mixture of all three. This can be done through poetry, digital stories, community newspapers online, image collections (old and new), audio (Internet radio, oral history), animations, video, and text. To engage in the activities the participants need to learn new skills and so the Digital Village also becomes a learning community. At it's simplest a Digital Village is a community website. The term "village" need not apply to an actual village (although in many cases it does) but to a cluster of villages, a geographical area or a group of participants in a town. On Teesside there is even an example of a Digital Cemetery! A Digital Village becomes a vehicle for participant led learning where the interests of the learning community set the agenda for what they learn. This is done using Community Media and innovative use of ICT, particularly open source software and web2 applications. The activity is informal and workshop based. Some technical support is required but the process becomes "flexible replication". Assistance from ICT animateurs is needed for these workshops but we have also observed peer learning taking place in these informal workshops. It is also possible to introduce an element of e-learning into the Digital Village concept. There is currently a great deal of interest in Virtual Learning Environments (VLE's) and e-portfolios. It is also very simple to consider the Digital Village as both VLE and e-portfolio. Whatever the participants want to learn e.g. digital images, creative writing, family or local history etc... can be supported on the Digital Village website VLE style and it is also presented on the DV website e-portfolio style.

Need for Digital Villages

The village communities are little republics, having nearly everything that they want within themselves, and almost independent of any foreign relations. In the development process, there will be many changes in the demand and supply of various needs, as rural population will pass through the process of change. At present, one of the major challenges in India is growing population and rapid urbanization. This urban growth to certain extent is unavoidable, as the economic pursuits and aspirations of the population do change and evolve. This needs to be reversed and suitably managed through a balance between rural and urban quality of life. The concept of "Digital Village" will address the multiple challenges faced for sustainable development of rural India.

A "Digital Village" will provide long-term social, economic, and environmental welfare activity for village community which will enable and empower enhanced participation in local governance processes, promote entrepreneurship and build more resilient communities. At the same time, a "Smart Village" will ensure proper sanitation facility, good education, better infrastructure, clean drinking water, health facilities, environment protection, resource use efficiency, waste management, renewable energy etc.

There is an urgent need for designing and developing "Smart Village", which are independent in providing the services and employment and yet well connected to the rest of the world. Based on various programs undertaken taken by Central and state governments along with further technological initiatives, the Smart Village can achieve SMART infrastructure, SMART service delivery, SMART technology and innovation, SMART institutions along with optimal mobilization and utilization of available resources, leading to faster and more inclusive growth. A 'Smart Village' will encompass a sustainable and inclusive development of all sections of the village community, so as they enjoy a high standard of living.

Objectives of Digital Village

- To prevent distress migration from rural to urban areas, which is a common phenomenon in India's villages due to lack of opportunities and facilities that guarantee a decent standard of living.
- To make the model village a "hub" that could attract resources for the development of other villages in its vicinity.
- To Provide easier, faster and cheaper access to urban markets for agricultural produce or other marketable commodities produced in such villages
- To contribute towards social empowerment by engaging all sections of the community in the task of village development.
- To Create and sustain a culture of cooperative living for inclusive and rapid development.
- To connect villagers to main stream of development.
- To make villagers smart by providing digital knowledge

Conclusion

Smart Villages are the need of the hour as development is needed for both rural and urban areas for better livelihood and Information technology will offer effective solution. There are successful technologies available, which have been implemented in urban areas. There is tremendous pressure on urban landscapes due to migration of rural people for livelihood. Smart Villages will not only reduce this migration but also irrigate the population flow from urban to rural area. ICT/ IT and GIS are the unbreakable pillars to support the whole process of village development. Smart village

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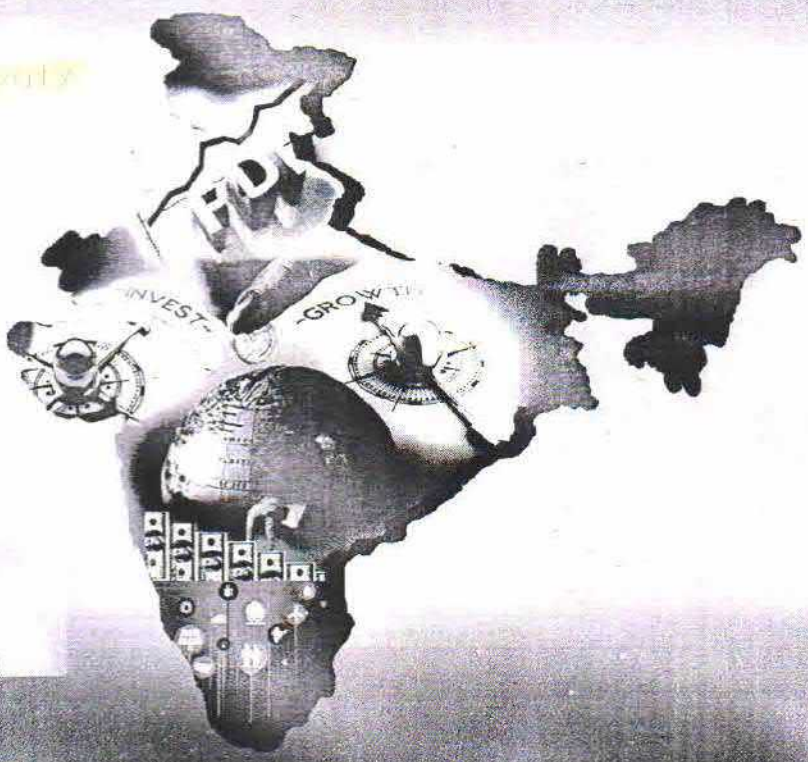

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RURAL DEVELOPMENT AND DIGITAL VILLAGE

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Introduction

Human society is developing with rapid momentum and achieved various successes for making its livelihood better. The civilization is witness for various changes related to it's the development through different catalysts like industrial development, green revaluation, science and technology, etc. The present era is augmented on Information and Communication Technology. This technology has proved its potential in various sectors of development in urban and rural landscapes. Urban areas are seems to more inclined to accept and adopt Information and Communication Technology due to advantages of literacy and better infrastructure as compared to rural areas. Due to such suitable situations of urban landscapes good amount of success of this technology is visible in the form of smart cities and better livelihood of residing human beings. But the problems, consequences and opportunities in urban areas are different for effective utilization of Information and Communication Technology for sustainable development of rural masses. The present research article discusses about rural development in developing world for the up-liftment of livelihood of the rural masses and to take a 'look ahead' at scientific developments and technologies that might be influential over the next 10-20 years. The driving motivation behind the concept on "Digital Village " is that the technology should acts as a catalyst for development, enabling education and local business opportunities, improving health and welfare, enhancing democratic engagement and overall enhancement of rural village dwellers. The "Digital Village " concept aims to realize its goal through providing policymakers with insightful, bottom–up analyses of the challenges of village development.

Improvement in the quality of life of rural people is the important agenda of rural development programme. In India – a country where the number of people living in rural areas, rural development programme is necessary aspect. Rural development implies both the economic betterment of people as well as greater social transformation. The basic objective of all rural development endeavors / programmes has been the welfare of the millions. In order to achieve this, planned attempts have been made to eliminate poverty, ignorance and inequality of opportunities. A wide spectrum of programmes has been undertaken so far, to alleviate rural poverty and ensure improved quality of life for the rural population especially those below the poverty line. In the initial phase of planned rural development, the concentration was on sectors of agriculture industry, communication, education and health. The Ministry of Rural Development places importance now on health, education, drinking water, housing and road so that the quality of life in rural areas improves and the fruit of economic reform are shared by all sections of the society.

With time and experience, it is realized that accelerated and meaningful development can be achieved only if people of the grass root are involved, "people's participation" has become the keyword in rural development programmes. The participation of the people is necessary to provide the rural people with better prospects for economic development

Why India's rural development is important for the nation?

India lives in its villages, and while the cities have grown immensely over the last 20 years, rural areas have not seen that kind of development. For India's economy to be strong, the rural economy needs to grow. Rural areas are still plagued by problems of malnourishment, illiteracy, unemployment and lack of basic infrastructure like schools, colleges, hospitals, sanitation, etc. This has led to youth moving out of villages to work in cities. This could be compared to the brain drain from India to US. Our villages need to grow in tandem with cities and standard of life has to improve there for inclusive growth to happen. If rural India is poor, India is poor.

Poverty in Rural India

India lives in many generations, and visiting rural areas very easily shows that they lag behind cities by decades. While we have latest services and products available in our cities now, villagers are still coping with age old products. It is easy to see the rising disconnect between cities and villages. Some examples are –

1. While we have international fully air conditioned schools in our cities, the schools in villages still don't have benches and chairs, leave alone computers. We have a huge shortage of teachers in rural areas, and the school drop out rate is huge.
2. In cities, we have wide roads, flyovers and underpasses while many villages still don't have proper roads. Urban-rural road links can play a vital role in rural growth.
3. Employment opportunities are hardly there in villages which forces youth to move to cities creating imbalance in the ecosystem and leaving the villages deprived.
4. While we may have numerous hospitals, nursing homes and medical facilities in cities, villages neither have health awareness nor health facilities. See the condition of major hospitals like AIIMS to know how many villagers have to flock to cities for even basic treatments.
5. Women fetching water from kilometers away Apart from the above options, villages need to have –

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INVESTIGATION OF SODIUM HYALURONATE SKIN SERUM BY USING NANATECHNOLOGY

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ABSTRACT

Nanotechnology is widely used in cosmetics. This technique is safe for targeted drug delivery. Nanoparticulate delivery system is more prominent and exhaustive. Basically, Nanoparticles are colloidal drug delivery systems. Nanotechnology can be used in products like lipstick soap, anti wrinkle cream, perfumes, toothpaste, etc. Serums are lightweight moisturisers that penetrate deeper to deliver active ingredients into your skin. This study presents methods, characterization of sodium hyaluronate nanoparticles further formulation of skin serum and its evaluation at different parameters.

Keywords: Nanotechnology, Sodium hyaluronate, skin serum, Stability testing.

INTRODUCTION

Nanotechnology is fastest growing area for the maintenance of skin health as well as for the diagnosis and management of cutaneous disease. It enriches the study of particles smaller than 100 nm in size. The prefix "nano" from nanotechnology it is a Greek word, in which "nano" means small or little [1]. Nanoparticle is type of colloidal drug delivery system where the particle size ranges from 10—1000 nm in diameter. The sub particles are prepared from a variety of material and synthetic polymers that include gelatine, poly methacarylate some biopolymers etc. Drugs can be dissolved, entrapped, or encapsulated into the nanoparticles, on simply absorbed on their surface. Nano sphere consists of a dense polymeric matrix in which the drug can be dispersed, whereas, Nanocapsules are constituted of a liquid core surrounded by a polymeric shell. Nanoparticles are formed by single layered shell and are filled with oil which tends themselves ideally as carriers for lipophilic agents [2]. Nanoparticles in cosmetic preparations are found to improve stability of various cosmetic ingredients such as unsaturated fatty acids, vitamins or antioxidants by encapsulating them, increase the efficacy and tolerance of UV filters on skin surface, make the product more aesthetically pleasing and enhance the penetration of certain active ingredients to the epidermis [3].

Nanoparticles under the skin in cosmetics

The important route is through dermal exposure. The dermis has a rich supply of blood and tissue macrophages, lymph vessels, dendritic cells, and five different types of sensory nerve endings. An increased inflammatory activity and epithelial translocation of manmade 20 and 30 nm solid particles was observed already 20 years ago. Broken skin represents a readily available entry even for larger (0.5-7 micro meter) particles, as evidenced by reports about accumulation of large amounts of soil particles in inguinal lymph nodes from people who runs or walks bare feet. However report shows that broken skin is not necessary for uptake of nanoparticles. Tinkle et al hypothesized that skin when flexed- as in wrist movements- can make the epidermis more permeable to nanoparticles and then favour uptake into lymphatic system and regional lymph nodes [4].

ADVANTAGES

1. Large scale production is possible.
2. Long term stability
3. Controlled and sustained release of active drug can be achieved.
4. Organic solvents can be avoided.
5. It can be lyophilized.
6. It can be freeze dried to form powder formulation.
7. By autoclaving and gamma radiation sterilization is possible.
8. It improves skin protection with organic compound.

DISADVANTAGES

1. Poor drug loading capacity.
2. High water content dispersion.

3. The low capacity to load hydrophilic drugs.

OBJECTIVE: The aim of my work was to prepare and investigate sodium hyaluronate in skin serum by using nanotechnology.

In the first part of the investigation, nanoparticles were prepared with a method described below and further synthesizing its size.

The main steps are as follows:

- Reactions under the same conditions and with concentration of Hyaluronic acid, oxalic acid, sodium monostearate, 1-(3- dimethylaminoproyl)-3-ethylcarbodiimide hydrochloride. The standard solutions were prepared with appropriate concentrations, pH was adjusted, the preparation of hyaluronic acid, oxalic acid, sodium monostearate, 1-(3- dimethylaminoproyl)-3-ethylcarbodiimide hydrochloride solutions respectively, the mixing and stirring time the temperatures applied, maintain storage conditions.
- Confirmation of nanoparticles by LM 20 nanosight.

In the second part of the investigation, the formulation and evaluation of skin serum was studies as follows:

- Formula was set for the formulation of skin serum.
- Physical appearance and Stability testing of the batches was studied with different concentrations of active. Serum was checked at different parameters and also microbial growth studies were done.

METHOD OF PREPARATION

1. High pressure homogenization.
   1.1 Hot homogenization.
   1.2 Cold homogenization.
2. Micro emulsion technique.
3. Ultra sonication or high speed homogenization.
4. Double emulsion method.
5. Spray drying method.

1. **High pressure homogenization.** In high pressure homogenization liquid is pushed at high pressure 100-2000 bar through a narrow gap. The fluid accelerates at very high velocity (1000 km/h). In this typical lipid contents in the range of 5-10% which represents no problem to the homogenizer. Higher lipid concentrations up to 40% have been also homogenized to lipid nano dispersions. It is widely used than any other method, because it is advantageous than other method. Following are some of the advantages of this method are that it is easy scale up and powerful techniques, short production times and more feasible [1].

1.1 Hot Homogenization. This method is similar to homogenization of an emulsion, because this is also carried out at temperature above the melting point of lipid. In the hot homogenization method the drug is dissolved or dispersed in melted solid lipid for SLN or in a mixture of liquid lipid (oil) and melted solid lipid for nano structured lipid carrier. This lipid melt containing drug is then mixed by high speed stirring in a solution of the hot surfactant at same

temperature (5– 10 °C) above the melting point of the solid lipid or lipid blend). This pre-emulsion is then passed through a high pressure. Homogenizer adjusted to the same temperature, generally applying three cycles at 500 bar or two cycles at 800 bars. This technique can be used for lipophilic and insoluble drugs as well as for the heat sensitive drugs because the exposure time to high temperature is comparatively short. The technique is not suitable for inclusion of hydrophilic drugs into solid lipid nanoparticle because of larger portion of drugs is in water during homogenization which leads to low entrapment capacity [5].

1.2 Cold homogenization This technique is developed to overcome the problems which are associated with hot homogenization like temperature induced drug degradation and drug distribution into the aqueous phase during homogenization [1]. In the cold homogenization method, the lipid micro particles are obtained by melting and subsequent cooling of drug containing lipid melt followed by crushing, grounding and diffusing in cold surfactant to obtain a cold pre-suspension of micronized lipid particles. This suspension is then forced to pass through a high pressure homogenizer at room temperature applying typically 5–10 cycles at 1500 bar. This method is the first choice for hydrophilic drugs with good as well as low solubility (surfactants are added to improve solubility). This technique avoids and shortens melting process of lipid and hence it is appropriate for thermo sensitive and thermo labile drugs [5].

2. **Micro emulsion technique** This method is based on the dilution of micro emulsions. As micro-emulsions are two-phase systems composed of an inner and outer phase. Micro emulsions are clear, thermodynamically stable system composed of a lipophilic phase, water, surfactant and co-surfactant. Micro emulsions are produced at a temperature above the melting point of the lipids, so the lipid should have melting point above room temperature [1]. Solid lipid nanoparticles can also be prepared by micro emulsification of inner molten lipids phase (oil) which is preloaded with drug (at 65-70 °C), followed by dispersion in cold aqueous phase with mechanical stirring (at 2-3 °C). The dispersion is washed two times with distilled water by ultra filtration. After washing, the suspension is freeze dried. The diameter of the disperse phase droplet should be always below 100nm. There is no need of energy for this preparation [5].

3. **Ultra sonication or High speed homogenization** Solid lipid nanoparticles were also developed by high speed stirring or sonication. The most advantage of this method is that, the Equipments that are used here are very common in every lab [1]. Solid lipid nanoparticles can also be prepared by sonication or high speed stirring. This is very general and simple technique and can be beneficial over other methods like hot and cold homogenization but with drawback of distribution of larger particle size ranging between micrometer range leading to physical instability such as particle growth upon storage and also metal contamination due to ultra sonication [5].

4. **Double emulsion method** It is a novel method of preparation of solid lipid nanoparticles loaded hydrophilic drug moiety and is based on solvent emulsification evaporation by drug

encapsulation in the outer water phase of w/o/w double emulsion along with a stabilizer to avoid partitioning of the drug to outer water phase during solvent evaporation [5]. For the preparation of hydrophilic loaded SLNs, a novel method based on solvent Emulsification-evaporation has been used. In double emulsion technique hydrophilic drugs was dissolved in aqueous solution, and then was emulsified in melted lipid. In this method the drug is encapsulated with a stabilizer to prevent drug partitioning to external water phase during solvent evaporation in the external water phase of w/o/w double emulsion. Stabilized primary emulsion was dispersed in aqueous phase which contains hydrophilic emulsifier after that the double emulsion was stirred and was isolated by filtration [1].

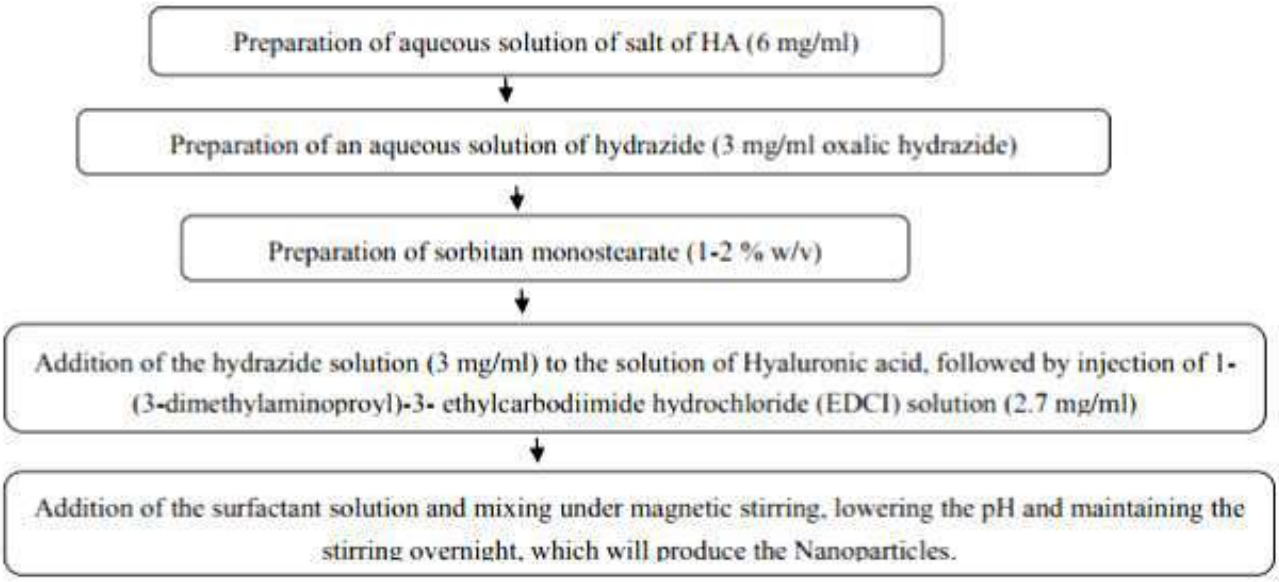
5. **Spray drying method** It is an alternative procedure to lyophilisation in order to transform an aqueous SLN dispersion into a drug product. This method is cheaper than lyophilisation. This method cause particle aggregation due to high temperature, shear forces and partial melting of the particle. In this method short drying time and consequently fast stabilization of feed material at moderate temperatures make spray drying method suitable for producing nanoparticles of drugs that are thermo labile. The 20% trehalose in ethanol-water mixtures (10/90 v/v). Due to high temperature and shear force it may cause aggregation of particle [1].

Materials and Method

Hyaluronic acid Hyaluronic acid (HA) is a high molecular weight biopolysacharide, discovered in 1934, by Karl Meyer and his assistant, John Palmer in the vitreous of bovine eyes. Hyaluronic acid is a naturally occurring biopolymer, which has important biological functions in bacteria and higher animals including humans. It is found in most connective tissues and is particularly concentrated in synovial fluid, the vitreous fluid of the eye, umbilical cords and chicken combs. It is naturally synthesized by a class of integral membrane proteins called hyaluronan synthases, and degraded by a family of enzymes called hyaluronidases [6]. Following are important points about hyaluronic acid. Hyaluronic acid derives from the Greek "hyalos", glossy vitreous and uronic acid. The molecule binds water and functions as lubricant between the collagen and the elastic fibre networks in dermis during skin movement. Effect on skin is that it hydrates viscoelastic film on the skin. The polymer may also be injected to obtain a smoother surface and reduce the depth of wrinkles.Properties: Most powerful moisturiser and humectants known so far provide smoothness and softening to the skin, reduce appearance of wrinkles. Ideal ingredient after skin peels.UsageTypically used at 0.1-2%. Hyaluronic acid is not readily soluble in water as it binds water very quickly forming a gel [7].

Method of preparation of hyaluronic acid nanoparticles The present study relates to the development of a hyaluronic acid nanoparticles for the administration of active molecules. These nanoparticles are made up of hyaluronic acid in salt form, preferentially the sodium salt of the polymers. In a typical experiment, the procedure comprises the following stages:

Flow chart 1: Method of hyaluronic acid nanoparticles

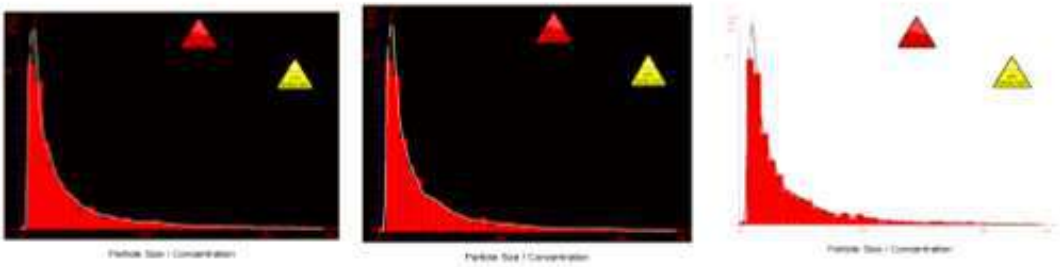


The work-up of the Nanoparticles was as follows: pH was increased to the range of 8-9, followed by the addition of alcohol to precipitate the Nanoparticles. The precipitated Nanoparticles were kept in drying oven at 25°C for six hours to dry. The resulting Nanoparticles can be kept in the refrigerator for storage [8].

CHARACTERIZATION OF NANOPARTICLES

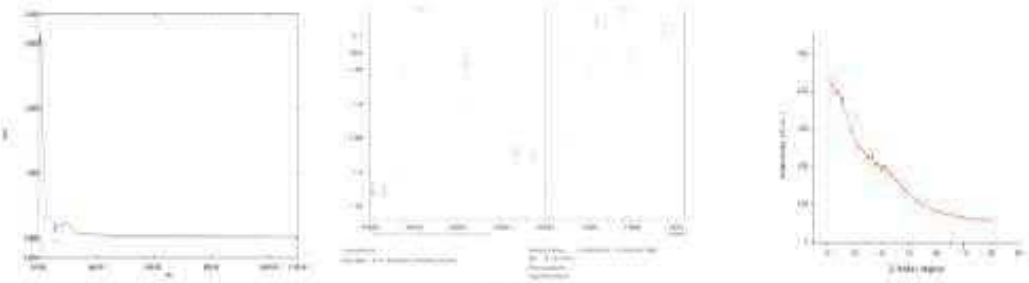
Estimation of particle size by LM 20 Nano Sight

Nanoparticle tracking analysis divulges size of nanoparticles by tracking the Brownian motion of particles freely suspended in colloidal solution. Mean size of nanoparticles was calculated by tracking minimum of 1000 nanoparticles active in Brownian motion. The size histograms of sodium hyaluronate are evident from Fig. 1 (a, b and c) respectively [9].



(a) (b) (c)

U.V. Spectrophotometry Ultraviolet (UV) and visible radiation comprise only a small part of the electromagnetic spectrum, which includes such other forms of radiation as radio, infrared (IR), cosmic, and X rays. Figure 2 (a), (b), (c) respectively [9].



(a) (b) (c)

Fourier Transform Electron Microscopy: A mathematical operation known as Fourier transform (FT) can separate the individual absorption frequencies from the interferogram, producing a spectrum virtually identical to that obtained with a dispersive spectrometer. This type of instrument is known as Fourier transforms infrared spectrometer [10].

X-ray Diffraction: X-ray diffraction is based on constructive interference of monochromatic X-rays and a crystalline sample. These X-rays are generated by a cathode ray tube, filtered to produce monochromatic radiation, collimated to concentrate, and directed toward the sample. The interaction of the incident rays with the sample produces constructive interference (and a diffracted ray) when conditions satisfy Bragg's Law ($n\lambda=2d \sin\theta$). This law relates the wavelength of electromagnetic radiation to the diffraction angle and the lattice spacing in a crystalline sample. These diffracted X-rays are then detected, processed and counted. By scanning the sample through a range of 2θangles, all possible diffraction directions of the lattice should be attained due to the random orientation of the powdered material. Conversion of the diffraction peaks to d-spacing allows identification of the mineral because each mineral has a set of unique d-spacing. Typically, this is achieved by comparison of d-spacing with standard reference patterns. All diffraction methods are based on generation of X-rays in an X-ray tube. These X-rays are directed at the sample, and the diffracted rays are collected. A key component of all diffraction is the angle between the incident and diffracted rays. Powder and single crystal diffraction vary in instrumentation beyond this [11].

Results and discussion

The particle size mean of sodium hyaluronate found to be 44nm whereas the mode is 11nm and standard deviation is 57nm. While the U.V. visible spectrophotometry shows that figure 2(a) absorbance is shown at peak of 257 nm. The XRD (*X-ray Diffraction)* result in figure 2(c) shows that the sample is of amorphous nature.

Skin Serum

A serum is a product typified by its rapid absorption and ability to penetrate into the deeper layers of the skin, together with its non-greasy finish and intensive formula with a very high concentration of active substances. Like many other skin products, serums are designed to focus on different actions – anti-ageing, brightening, acne prevention, etc. Because of the high concentrations of the active elements, it is common for cosmetic serums to contain only a few

active ingredients which provide intensive nutrition for the deeper layers of your skin. The oil-free finish doesn't leave your skin feeling tight after use: instead it should feel velvety smooth because of the serum's intensive and deep-layer action. Since the active ingredients are so highly concentrated, a serum will produce more visible results in less time than a simple moisturiser or other skin product. Sometime the high concentration of active ingredients can irritate sensitive skin [12].

Material and Methods

Sr. No.	Ingredients	Quantity for 100 gm			Uses
		F1	F2	F3	
1	Carbopol 940	0.5 gm	0.5 gm	0.5 gm	Gelling agent
2	Olive oil	1 ml	1ml	1ml	Emollient
3	Almond oil	1 ml	1 ml	1 ml	Emollient
4	Tween 80	1 ml	1.2 ml	1.3 ml	Emulsifier
5	Propylene glycol	2 ml	2.5 ml	2.8 ml	Humectant
6	Poly sorbate 60	1 ml	1.5 ml	1.8 ml	Solubuliser
7	Ethylenediaminetetraacetic acid	0.1 gm	0.1 gm	0.1 gm	Chellating agent
8	Triethanaloamine	0.3 ml	0.5 ml	0.6 ml	Stabiliser
9	Iso propyl alcohol	q.s	q.s	q.s	Solubuliser
10	Glycerine	0.7 ml	0.8 ml	0.9 ml	Humectant
11	Perfume	q.s	q.s	q.s	Perfume
12	DM DM Hyadantoin	q.s	q.s	q.s	Preservative
13	Sodium Hyaluronate	0.8 gm	0.5 gm	1 gm	Active
14	DM water	To make 100 ml	To make 100 ml	To make 100 ml	Aqueous phase

Observation: From the above observation formula F2 was selected as it was stable and it shows consistency, spreadability and feel and active was added with different concentration and evaluated for in vitro study as per IS and in vivo study with human volunteers.

Procedure for base formula: Take clean apparatus. Weigh Carbopol 940 disperses in distilled water containing EDTA, DM DM Hyadantoin and glycerine. After proper mixing add TEA drop by drop to form a gel, then take another beaker to this add almond oil, olive oil, rose oil, Polysorbate

60, Tween 80, propylene glycol, stir it well and then pour it into gel under stirring slowly, allow it to stir for some more time and then fill it into suitable container.

Table 1: Optimization of serum base

Sr. No.	PARAMETER	F1	F2	F3
1	Appearance	**	***	***
2	Colour	**	***	**
3	Odour	*	**	**
4	Consistency	**	**	*
5	Feel	*	***	**
6	Spread ability	**	***	**

Good= * Better = ** Best = ***

Evaluation of skin serum

Determination of physical parameters

In physical parameters, appearance, consistency, colour, odour, and spreadability was taken into consideration. The Physical Parameters are determined by visual observation by taking small amounts of sample. The Serum and lotion samples were kept at various temperatures such as room temp, at 45°C and at the elevated temp (freeze temp.).The formulations were checked after every 10 days for parameters such as colour, odour, consistency, spreadability and appearance [13].

Determination of pH (IS: 6608 - 1978)

For oil-in-water emulsion Serum Accurately 5±0.01 g of the Serum was weighted in a 100ml beaker. 45ml of water was added and the Serum was dispersed in it. The pH of the suspension at 270 C was determined using the pH meter [13].

Determination of total fatty substance content: (Indian Standard skin creams — specification, 2004)

For this the emulsion is broken up with dilute mineral acid and the fatty matter is extracted with petroleum ether. It is weighed after removal of the solvent. Accurately about 2g of sample was weighted into a conical flask, about 25ml of dilute HCl was added, reflux condenser was fitted into the flask and the content of the flask was boiled until the oil and water phases have separated. The content of the flask was poured into 300 ml separating funnel and it was allowed to cool to 20°C. The conical flask was rinsed with 50ml of ethyl ether in portions of 10ml. The ether rinsing was poured into separating funnel. The separating funnel was shaking well and leave until layers separate. Separate out with 50ml portions of ether twice. All the ether extracts was combined and washed them with water until free of acid. The ether extracts was filtered through a filter paper

containing sodium sulphate into a conical flask which had been previously dried at temperature of 6°C ±2°C and then weighed. The sodium sulphate was washed on the filter paper with ether and the material remaining in the flask was dried at a temperature of 6°C ±2°C to constant mass [13].

Total fatty substance % by mass =100x M1/M2.
Where, M1 is Mass in g of residue and M2 is Mass in g of material taken for the test.

Determination of Thermal Stability:

A 20 mm broad and 5 mm thick strip was spreaded from the material to be tested on the internal wall of a beaker of 100ml capacity in its total height. The beaker was kept for 8 hrs. in the humidity chamber at 60 to 70% relative humidity and temperature 37± 1°C.

Microbial examination of Serum: T.

The test consist of pleating a known dilution of the sample on soya bean casein digest agar medium suitable for the total count of aerobic bacteria and fungi after incubating them for a specified period to permit the development of visual colonies for counting Pre-treatment of sample: To 10 ml of sodium chloride solution pH 7 or any other suitable medium add 1gm or 1ml of sample
Total bacterial count: Pipette out in duplicate 1ml of pre-treated sample aseptically into 5 sterile Petri dishes. Pour 15 to 20 ml of molten soya bean casein digest agar maintained at about 45°C. Mix the content of the plate by swirling. Allowing the incubate the plates at 37°C +1°C in inverted position for three days Count the number of colonies in each plate. Determine the average number of colonies on plates and multiply by dilution factor. This will be the number of microorganisms per gm of the sample. If no colony was recovered from any of the plate it can be stated as less than 50 microorganisms per gm.
Total fungal count: Pipette out in duplicate 1ml of pre treated sample aseptically into 5 sterile petridishes. Pour 15 to 20 ml of molten sabouraud's chloranphenicol agar (SCA) maintained at about 45°C mixes the content of the plate by swirling. Allowing the plates to solidify, invert and incubated at 23+2°C for three days. Count the number of colonies in each plate.

Stability studies of Serum: Stability studies for Serums were carried out according to ICH guidelines. The Serum samples were kept on the 5°C, room temperature, and 40°C. The changes in the physical appearance, colour, odour etc and chemical changes such as change in pH, viscosity, pH separation were checked and thus. The formulation of Serum was optimized.

Table2:

Sr. No.	PARAMETERS	F1	F2	F3
	(A) Physical appearance			
1	Appearance	serum like	serum like	serum like
2	Colour	white opaque	white opaque	white opaqu
3	Odour	pleasant	pleasant	pleasant
4	Consistency	semi liquid	semi liquid	semi liquid
5	Spread ability	good	good	very good
6	Oily/tacky feel	No	No	No

Accelerated stability studies: To ensure that a cosmetic remain stable till the consumers has used the entire cosmetic or has stopped using it, a number of special accelerated test procedures have been developed. The evaluation employs a combination of tests. This method of evaluation not only indicates stability of Base formulation but also indicates the stability of functional ingredient [13].

Freeze thaw cycle: These tests are not carried out at fixed temperature and humidity. In these tests, temperature was changed cyclically every day e.g. Low-high-low-high-low-high, to simulate changes in temperature daily [13].

Table 3:

Sr. No.	PARAMETERS	F1	F2	F3
1	Freeze thaw cycle	Stable	Stable	Stable

RESULT AND DISCUSSION

In Vitro-Study

Table No. 4: Determination of physical parameter of a Skin Serum containing Sodium hyaluronate Active. (Stability study after 10 days).

Sr. No.	PARAMETERS	F1	F2	F3
	Physical appearance	**	**	**
1	Appearance	**	**	**
2	Colour	**	**	**
3	Odour	**	**	**
4	Consistency	**	**	**
5	Spread ability	**	**	**
6	Oily/tacky feel	**	**	**

Change = * No change= **

Table No. 5: Stability study after 20 days

Sr. No.	PARAMETERS	F1	F2	F3
	Physical appearance	**	**	**
1	Appearance	**	**	**
2	Colour	**	**	**
3	Odour	**	**	**
4	Consistency	**	**	**
5	Spread ability	**	**	**
6	Oily/tacky feel	**	**	**

Table No. 6: Stability study after 30 days

Sr. No.	PARAMETERS	F1	F2	F3
	Physical appearance	**	**	**
1	Appearance	**	**	**
2	Colour	**	**	**
3	Odour	**	**	**
4	Consistency	**	**	**
5	Spread ability	**	**	**
6	Oily/tacky feel	**	**	**

Table No. 7: Determination of pH of serum containing sodium hyaluronate as active. Standard value 5 to 9

Sr. No.	Time Interval	F1	F2	F3
1	0 Day	6.23	6.21	6.19
2	8th Day	6.21	6.2	6.18
3	16th Day	6.23	6.19	6.15
4	24th Day	6.22	6.17	6.11
5	30th day	6.23	6.16	6.12

Graphical Representation of data:

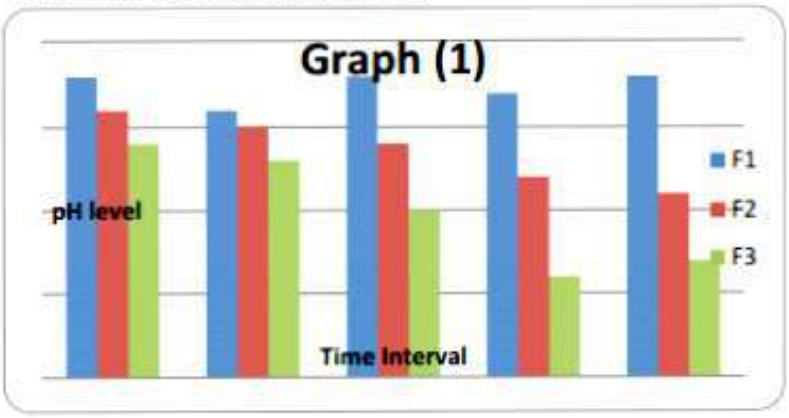


Table 8: Determination of Total fatty matter of serum containing sodium hyaluronate as active (Standard value 5.0%)

Sr. No	Parameter	F1	F2	F3
1	TFM % by mass	4.55%	4.65%	4.60%

Graphical Representation of data:

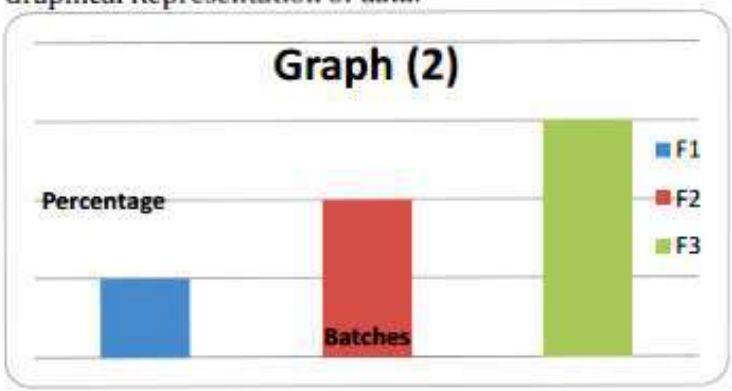


Table 9: Determination of Thermal Stability serum containing sodium hyaluronate as active.

Sr. No	Parameter	F1	F2	F3
1	Thermal Stability	passed	passed	passed

Microbial examination of a Serum

Table No. 10: Microbial examination of a serum containing sodium hyaluronate as active

Sr. No	Name of the test	Result	Specification	Unit
1	Total bacterial count	10 CFU/gm	NMT100 CFU/gm	CFU/gm
2	Fungal count	Nil	NMT10CFU/gm	CFU/gm

Conclusion

Nanoparticles are one of the promising drug delivery systems, which can be of potential use in controlling and targeting drug delivery. They possess better stability when compared with liposomes. They have various applications such as ophthalmic drug delivery, intravenous delivery as carriers for radio nucleotides in nuclear medicine, as cosmetics for the skin and hair care, sustained release formulations and many more. Nanoparticles formulated as amorphous spheres offer higher solubility than standard crystalline formulations, thus improving the poor aqueous solubility of the drug and hence its bioavailability. While serum on the other side, is a concentrated product widely used in cosmetology. The term itself comes from professional cosmetology. Cosmetic skin serum is a highly concentrated product based on water or all as any other cream. Serums are concentrates containing approximately 10 times more of biologically active substances than creams, therefore quicker and more effectively coping with cosmetic problems. The effect of serum when concentrates are that it immediately gets the necessary amount of active substances such form which assimilates easier. The active substances in high concentration act in the same way as cream they moisturise, rejuvenate, lift up, etc. The only difference is that in case concentrates are used correctly the noticeable result will be reached quickly.

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LRS Bianchi Type –I Magnetized Anisotropic Dark Energy Models with Variable Equation of State

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ABSTRACT

We discuss two dark energy models on LRS Bianchi Type-I magnetized anisotropic space –time with a variable equation of state (EoS). The EoS for dark energy ω is found to be time dependent and its existing range for derive models is in good agreement with the recent observations. Using the suitable condition, the anisotropic models approach to isotropic scenario. We also find that during the evolution of the universe, the EoS parameter for DE changes from $\omega > -1$ to $\omega = -1$ in first model whereas from $\omega > -1$ to $\omega < -1$ in second model which is consistent with recent observations. The cosmological constant Λ is found to be a positive decreasing function of time and it approaches a small positive value at late time (i.e. the present epoch) which is corroborated by results from recent supernovae Ia observations. The physical and geometric properties are also discussed.

Introduction

The nature of the dark energy component of the universe [1-3] remains one of the deepest mysteries of cosmology. There is certainly no lack of candidates: cosmological constant, quintessence [4-6], k-essence [7-9], phantom energy [10-12]. Modifications of the Friedmann equation such as Cardassian expansion [13,14] as well as what might be derived from brane cosmology [15-17] have also been used to explain the acceleration of the universe. A particular case of the linear Equation of state has used in the cosmological context by Xanthopuolos [18], he considered space-times with two hypersurface orthogonal, space-like, commuting killing fields.

The current standard model of cosmology implies the existence of dark energy which accounts for about 70% of the total energetic content of the universe, which ac-cording to the observations is spatially flat [19]. Several models have been proposed to explain dark energy [20-26]. An alternative consists of to consider a phenomenological decaying dark energy density with continuous creation of matter [26] or photons [27,28].The dark energy might decay slowly in the course of the cosmic evolution and thus provide the source term for matter and radiation. Different such models have been discussed and strong constraints come from accurate measurements of the CMB. Although some authors [29] have suggested cosmological model with anisotropic and viscous dark energy in order to explain an anomalous cosmological observation in the cosmic microwave background (CMB) at the largest angles.

Bianchi type models have been studied by several authors in an attempt to understand better the observed small amount of anisotropy in the universe. The same models have also been used to examine the role of certain anisotropic sources during the formation of the large-scale structures we see in the universe today. Some Bianchi cosmologies, for example, are natural hosts of large-scale magnetic fields and therefore, their study can shed light on the implications of cosmic magnetism for galaxy formation. The simplest Bianchi family that contains the flat FRW universe as a special case are the type-I space-times.

Measuring the equation of state for dark energy is one of the biggest efforts in observational cosmology today. The DE model has been characterized in a conventional manner by the equation of state (EoS) parameter $\omega = \frac{p}{\rho}$ which is not necessarily constant, where ρ is the energy density and p is the fluid pressure [9]. The present data seem to slightly favour an evolving dark energy with EoS $\omega < -1$ around the present epoch and $\omega > -1$ in the near past. Obviously, ω cannot cross -1 for quintessence or phantom alone. Some efforts have been made to build a dark energy model whose EoS can cross the phantom divide. The simplest DE candidate is the vacuum energy ($\omega = -1$), which is mathematically equivalent to the cosmological constant (Λ). The other conventional alternatives, which can be described by minimally coupled scalar fields, are quintessence ($\omega > -1$) [30], phantom energy ($\omega < -1$) [31] and quintom (that can across from phantom region to quintessence region as evolved) and have time dependent EoS parameter. Some other limits obtained from observational results coming from SNe Ia data [32] and combination of SNe Ia data with CMBR anisotropy and galaxy clustering statistics [33] are $-1.67 < \omega < -0.62$ and $-1.33 < \omega - 0.79$, respectively. The latest results in 2009, obtained after a combination of cosmological datasets coming from CMB anisotropies,

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luminosity distances of high redshift type Ia supernovae and galaxy clustering, constrain the dark energy EoS to $-1.44 < \omega < -0.92$ at 68% confidence level [34,35], However, it is not at all obligatory to use a constant value of ω. Due to lack of the observational evidence in making a distinction between constant and variable ω, usually the equation of state parameter is considered as a constant [36,37,4] with phase wise value $-1, 0, -\frac{1}{3}, and +1$ for vacuum fluid, dust fluid, radiation and stiff dominated universe respectively. But in general, ω is a function of time or redshift [38,39,40]. Some literature are also available on models with varying fields, such as cosmological models with variable EoS parameter in Kaluza-Klein metric and wormhole [41,42]. In recent years various form of time dependent ω have been used for variable Λ models by Mukhopadhyay et al. [43]. In well –known reviews on modified gravity [44,45],it is clearly indicated that any modified gravity may be represented as effective fluid with time dependent ω.

In Principle, once the metric is generalized to Bianchi types, the EoS parameter of the fluid can be generalized in a way conveniently to wield anisotropy with the considered metric . In such model, where both the metric and EoS parameter of the fluid are allowed to exhibit an anisotropic character, the universe can exhibit non-trivial isotropization histories and it can be examined whether the metric and/or the EoS parameter of fluid evolve toward isotropy. We may say about two main classes of such models; according to whether this anisotropization occurs at an early time or at late times of the universe, The former class can be related with the inflation field , which drives the inflation, which drives the late time acceleration of the universe. In the context of the former class, the generic inflationary model can be modified in a way to end inflation with a slightly anisotropic spatial geometry.

Bianchi type-I universe is the prime candidate for studying the possible effects of an anisotropy in the early universe on present-day observations, there are few other models (for example, $B-VI_0$), which describe an anisotropic space-time and generate interest among physicists.[46-49]. Pradhan and Bali [50] and Bali et al. [51] have studied $B-VI_0$ space time in connection with massive strings. Recently Amirhashchi et al.[52] presented dark energy models in an anisotropic $B-VI_0$ space –time by considering constant deceleration parameter. In this paper, we have investigated two new LRS Bianchi type-I magnetized dark energy models with variable equation of state . The discussion of the paper is as follows:

In section.2, the metric and the field equations are described. In section 3 deals with the solutions of the field equations in two different cases .Section 4 deals with physical and geometric behavior of the models. In section 5, we describe an other dark energy model and its physical aspects. Finally, conclusions are summarized in the last Section 6.

2. Metric and Field Equations:

We consider the LRS Bianchi Type-I metric in the following form

$$ds^2 = -dt^2 + a^2 dx^2 + b^2 (dy^2 + dz^2) \tag{1}$$

where the scale factors a and b are functions of cosmic time only.

The energy momentum tensor for anisotropic dark energy with magnetic field is given by

$$T_j^i = {}^{DE}T_j^i + {}^{EM}T_j^i \tag{2}$$

where

$${}^{DE}T_j^i = (\rho_{ED} + p_{ED}) u_i u^j + p g_j^{\ i} \tag{3}$$

where u^i is the flow vector satisfying

$$g_{ij} u^i u^j = -1 \tag{4}$$

${}^{EM}T_j^i$ is the electromagnetic field tensor which is given by

$${}^{EM}T_{ij} = \frac{1}{4\pi}\left[F_{i\alpha} F_{j\beta} g^{\alpha\beta} - \frac{1}{4} g_{ij} F^{\alpha\beta} F_{\alpha\beta} \right] \tag{5}$$

where F_{ij} is the electromagnetic field tensor which satisfies the Maxwell equations

$$F_{[ij;\alpha]} = 0, \qquad (F^{ij}\sqrt{-g}) = 0 \tag{6}$$

In commoving coordinates, the incident magnetic field is taken along x-axis, with the help of Maxwell equations (6), the only non-vanishing component of F_{ij} is

$$F_{23} = const = M. \tag{7}$$

By preserving the diagonal form of the energy momentum tensor in a consistent way with the above metric, the simplest generalization of EoS parameter of perfect fluid may be to determine it separately on each spatial axis. Therefore the energy momentum tensor of perfect fluid is taken as

$$T_i^j = diag[T_0^0, T_1^1, T_2^2, T_3^3] \tag{8}$$

Thus, one may parameterize it as follows

$$T_i^j = dia[-\rho + \frac{M^2}{8\pi b^4}, p_x - \frac{M^2}{8\pi b^4}, p_y + \frac{M^2}{8\pi b^4}, p_z + \frac{M^2}{8\pi b^4}]$$

$$= dia[-(\rho + \frac{M^2}{8\pi b^4}), (\omega_x \rho - \frac{M^2}{8\pi b^4}), (\omega_y \rho + \frac{M^2}{8\pi b^4}), (\omega_z \rho + \frac{M^2}{8\pi b^4})]$$
$$= dia[-(\rho + \frac{M^2}{8\pi b^4}), (\omega\rho - \frac{M^2}{8\pi b^4}), (\omega + \delta)\rho + \frac{M^2}{8\pi b^4}, (\omega + \delta)\rho + \frac{M^2}{8\pi b^4}] \quad (9)$$

where ρ is the energy density of the fluid p_x, p_y, p_z are the pressures and $\omega_x, \omega_y, \omega_z$ are the directional EoS parameters along the x, y, z respectively, $\omega(t) = p/\rho$ is the deviation free EoS parameter of the fluid. We have parameterized the deviation from isotropy by setting $\omega_x = \omega$ and then introducing skewness parameter δ which is the deviation from ω along both *y* and *z*-axes. ω and δ are not necessarily constants and might be function of the cosmic time t.

The Einstein's field equations are

$$R_{ij} - \frac{1}{2} R g_{ij} = -8\pi T_{ij} \quad (10)$$

where the symbols have their usual meaning.

By adopting commoving coordinates, Einstein's field equation (10), for the Kontowski-Sachs space-time, the field equations take the form

$$\frac{2a_4 b_4}{a\,b} + \frac{b_4^{\,2}}{b^2} = 8\pi\rho + \frac{M^2}{b^4} \quad (11)$$

$$\frac{a_{44}}{a} + \frac{b_{44}}{b} + \frac{a_4 b_4}{ab} = -8\pi(\omega + \delta)\rho - \frac{M^2}{b^4} \quad (12)$$

$$\frac{2b_{44}}{b} + \frac{b_4^{\,2}}{b^2} = -8\pi\omega\rho + \frac{M^2}{b^4} \quad (13)$$

where a subscript 4 indicates differentiation with respect to t.

3. Solutions of the field equations

The spatial volume for the model (1) is given by

$$V = R^3 = ab^2 \quad (14)$$

where *R* is the mean scale factor. The mean Hubble parameter *H* is given as

$$H = \frac{R_4}{R} = \frac{1}{3}\frac{V_4}{V} = \frac{1}{3}\left(\frac{a_4}{a} + 2\frac{b_4}{b}\right) \quad (15)$$

The directional Hubble parameters in the directions of *x,y and z* respectively may be defined as

$$H_x = \frac{a_4}{a} \qquad and \quad H_y = H_z = \frac{b_4}{b} \quad (16)$$

The volumetric deceleration parameter q, the scalar expansion θ, shear scalar σ^2 and the average anisotropy parameter A_m are defined by

$$q = -RR_{44}/R_4^{\,2} \quad (17)$$

$$\theta = \frac{a_4}{a} + 2\frac{b_4}{b} \quad (18)$$

$$\sigma^2 = \frac{1}{3}\left(\frac{a_4}{a} - \frac{b_4}{b}\right)^2 \quad (19)$$

$$A_m = \frac{1}{3}\sum_{i=1}^{3}\left(\frac{H_i - H}{H}\right)^2 \quad (20)$$

where $H_i (i = x, y, z)$ represents the directional Hubble parameter in the direction of $x,y\,and\,z$, respectively. $A_m = 0$ corresponds to isotropic expansion.

Initially we apply the law of variation for Hubble parameter for the LRS Bianchi Type –I metric may be given by

$$H = D(ab^2)^{-\frac{n}{3}} \quad (21)$$

where $D > 0$ *and* $n \geq 0$ are constants. Such type of relations have firstly been considered by Berman [53], Berman and Gomide [54] for solving FRW models. Letter on many authors have used this law to study FRW and Bianchi type models.

On integrating, after equating (15) and (21), we obtain

$$ab^2 = (nDt + c_1)^{\frac{3}{n}} \qquad for \quad n \neq 0 \tag{22}$$

here c_1 is positive constants of integration. The values for the deceleration parameter for the mean scale factor as:

$$q = n - 1 \tag{23}$$

which is constant. The sign of q indicates where the model inflates or not. The positive sign of q i.e. $0 \leq n < 1$ indicates inflation. It is remarkable to mention here that through the current observations of SNe Ia and CMBR favors accelerating models $(q < 0)$, but both do not altogether rule out the deceleration ones which are also consistent with these observations [55]

Now we assume that the expansion (θ) is proportional to shear (σ), this condition lead to

$$\frac{a_4}{a} - \frac{b_4}{b} = \alpha_0 \sqrt{3}\left(\frac{a_4}{a} + 2\frac{b_4}{b}\right)$$

which yields to

$$\frac{a_4}{a} = m\frac{b_4}{b}$$

where $m = \dfrac{2\alpha_0\sqrt{3}+1}{1-\alpha_0\sqrt{3}}$ and α_0 are arbitrary constants. Above equation, after integration, reduces to

$$a = \beta b^m$$

where β is an integrating constant. Here , for simplicity and without any loss of generality, we assume $\beta = 1$. Hence we have

$$a = b^m \tag{24}$$

The motivation behind assuming this condition is explained with reference to Thorne[56], the observations of the velocity-red-shift relation for extragalactic sources suggest that Hubble expansion of the universe is isotropic today within ≈ 30 percent [57,58]. To put more precisely, red- shift studies place the limit

$$\frac{\sigma}{H} \leq 0.3,$$

On the ratio of shear σ to Hubble constant *H* in the neighborhood of our Galaxy today. Collins et al. [59] have pointed out that for spatially homogeneous metric, the normal congruence to the homogeneous expansion satisfies that the condition $\dfrac{\sigma}{\theta}$ is constant.

Using (24) (14) in (15), we obtain the expressions for metric function as follows

$$b = (nDt + c_1)^{\frac{1}{ml}} \tag{25}$$

$$a = (nDt + c_1)^{\frac{1}{l}} \tag{26}$$

where c_1 is an integrating constant

Hence the model (1) reduces to

$$ds^2 = -dt^2 + (nDt + c_1)^{\frac{2}{l}} dx^2 + (nDt + c_1)^{\frac{2}{ml}} (dy^2 + dz^2) \tag{27}$$

4. Physical aspects of dark energy model

The expressions for the Hubble parameter H, scalar of expansion θ ,shear scalar σ and the average anisotropy parameter A_m for the model (27) are given by

$$\theta = 3H = \frac{3L}{(nDt + c_1)} \tag{28}$$

$$\sigma^2 = \frac{1}{3}\left[\frac{nD(m-1)}{ml}\right]^2 (nDt + c_1)^{-2} \tag{29}$$

$$A_m = \frac{(m^2+2)(nD)^2 - 2nDLml(m+2)}{3m^2l^2L^2} + 1 \tag{30}$$

Using equation (11) , the energy density of the fluid is obtain as

$$8\pi\rho = (2m+1)\frac{(nD)^2}{(ml)^2}(nDt+c_1)^{-2} - M^2(nDt+c_1)^{-4/ml} \tag{31}$$

Using equation (13) , the EoS parameter is obtained as

$$\omega = \frac{(2ml-3)\left(\frac{nD}{ml}\right)^2(nDt+c_1)^{-2} + M^2(nDt+c_1)^{-4/ml}}{(2m+1)\left(\frac{nD}{ml}\right)^2(nDt+c_1)^{-2} - M^2(nDt+c_1)^{-4/ml}} \tag{32}$$

Using equation (12), the skewness parameter δ are computed as

$$\delta = \frac{\left[2+l(m-1)-m(m+1)\right]\left(\frac{nD}{ml}\right)^2(nDt+c_1)^{-2} - 2M^2(nDt+c_1)^{\frac{-4}{ml}}}{(2m+1)\left(\frac{nD}{ml}\right)^2(nDt+c_1)^{-2} - M^2(nDt+c_1)^{-4/ml}} \tag{33}$$

From (32), it is observed that the equation of state parameter ω is time dependent, it can be function of redshift z or scale factor R as well. The redshift dependence of ω can be linear like

$$\omega(z) = \omega_0 + \omega' z \tag{34}$$

with $\omega' = \left.\frac{d\omega}{dz}\right|_{z=0}$ (see [60,61]) or nonlinear as

$$\omega(z) = \omega_0 + \frac{\omega_1 z}{1+z} \tag{35}$$

[62,63]. So as for as the scale factor dependence of ω is concern. The parametrization

$$\omega(R) = \omega_0 + \omega_R(1-R) \tag{36}$$

where ω_0 is the present value $(R=1)$ and ω_R is the measure of the time variation ω' is widely used in the literature [64]

So, if the present work is compare with experimental results [31,32,33,34], then one can conclude hat the limit of ω provided by (32) may accommodated with the acceptable range of EoS parameter . Also it is observed that at $t = t_c$, ω vanishes, where t_c is a critical time given by

$$t_c = \frac{1}{nD}\left(\frac{nD}{ml}\sqrt{\frac{2ml-3}{M^2}}\right)^{\frac{ml}{ml-2}} - \frac{c_1}{nD} \tag{37}$$

Thus, for this particular time , our model represents a dusty universe. We also note that earlier real matter at $t \le t_c$, where $\omega \ge 0$ later on at $t \ge t_c$, where $\omega > 0$ converted to the dark energy dominated phase of universe.

For the value of ω to be in consistent with observation [31], we have the following general condition

$t_1 < t < t_2$,

where

$$t_1 = \frac{1}{nD}\left(\frac{nD}{ml}\sqrt{\frac{3\left[m\left(\frac{2}{3}l+3.34\right)+0.67\right]}{2.67M^2}}\right)^{\frac{ml}{ml-2}} - \frac{c_1}{nD} \tag{38}$$

$$t_2 = \frac{1}{nD}\left(\frac{nD}{ml}\sqrt{\frac{3\left[m\left(\frac{2}{3}l+1.24\right)-0.38\right]}{1.62M^2}}\right)^{\frac{ml}{ml-2}} - \frac{c_1}{nD} \tag{39}$$

For this constrain, we obtain $-1.67 < \omega < -0.62$ which is in good agreement with the limit obtained from observational results coming from SNe Ia data [31].

For the value of ω to be in consistent with observation [12] , we have the following general condition

$$t_3 < t < t_4, \tag{40}$$

where

$$t_3 = \frac{1}{nD}\left(\frac{nD}{ml}\sqrt{\frac{3\left[m\left(\frac{2}{3}l+2.66\right)+0.33\right]}{2.33M^2}}\right)^{\frac{ml}{ml-2}} - \frac{c_1}{nD} \tag{41}$$

$$t_4 = \frac{1}{nD}\left(\frac{nD}{ml}\sqrt{\frac{3\left[m\left(\frac{2}{3}l+1.58\right)-0.21\right]}{1.79M^2}}\right)^{\frac{ml}{ml-2}} - \frac{c_1}{nD} \tag{42}$$

For this constrain, we obtain $-1.33 < \omega < -0.79$ which is in good agreement with the limit obtained from observational results coming from SNe Ia data [31] with CMB anisotropy and galaxy clustering statistics [32].

For the value of ω to be in consistent with observation [33,34] , we have the following general condition

$$t_5 < t < t_6,$$

$$t_5 = \frac{1}{nD}\left(\frac{nD}{ml}\sqrt{\frac{3\left[m\left(\frac{2}{3}l+2.88\right)+0.44\right]}{2.44M^2}}\right)^{\frac{ml}{ml-2}} - \frac{c_1}{nD} \tag{43}$$

$$t_6 = \frac{1}{nD}\left(\frac{nD}{ml}\sqrt{\frac{3\left[m\left(\frac{2}{3}l+1.84\right)-0.08\right]}{1.92M^2}}\right)^{\frac{ml}{ml-2}} - \frac{c_1}{nD} \tag{44}$$

For this constrain, we obtain $-1.33 < \omega < -0.79$ which is in good agreement with the limit obtained from observational results [33,34].

From (31), we note that energy density of the fluid $\rho(t)$ is a decreasing function of time and $\rho \geq 0$ when

$$t \leq \frac{1}{nD}\left(\frac{nD}{ml}\sqrt{\frac{2m+1}{M^2}}\right)^{\frac{ml}{ml-2}} - \frac{c_1}{nD} \tag{45}$$

Here ρ is a positive decreasing function of time and it approaches to zero as $t \to \infty$.

In absence of any curvature, matter energy density Ω_m and dark energy Ω_Λ are related by the equation

$$\Omega_m + \Omega_\Lambda = 1 \tag{46}$$

Where $\Omega_m = \frac{\rho}{3H^2}$ and $\Omega_\Lambda = \frac{\Lambda}{3H^2}$. Thus , (46) reduces to

$$\frac{\rho}{3H^2} + \frac{\Lambda}{3H^2} = 1 \tag{47}$$

Using (28) and (31) in (47), the cosmological constant is obtained as

$$\Lambda = \left[3L^2 - \frac{1}{8\pi}(2m+1)\left(\frac{nD}{ml}\right)^2\right](nDt+c_1)^{-2} + \frac{M^2}{8\pi}(nDt+c_1)^{-\frac{4}{ml}} \tag{48}$$

From (48), we observe that Λ is decreasing function of time and it is always positive when

$$t > \frac{1}{nD}\left[M^2(24\pi L^2 - (2m+1)\left(\frac{nD}{ml}\right)^2)\right]^{\frac{ml}{2(ml-2)}} + \frac{c_1}{nD} \tag{43}$$

We observe that cosmological parameter is decreasing function of time and it approaches a small positive value at late time (i.e. At present epoch). Recent cosmological observations[65,2]; [3,66,67] suggest the existence of a positive cosmological constant Λ with the magnitude $\Lambda\left(Gh/c^3\right) \approx 10^{-123}$. These observations on magnitude and red-shift of type Ia supernova suggest that our universe may be an accelerating one with induced cosmological density through the cosmological Λ - term. Thus, the nature of Λ in our derive DE model is supported by recent observations.

From (28)-(29) , it can be seen that all the kinematical parameters $H, \theta, and\ \sigma$ diverge at the initial singularity. There is a Point Type singularity [48] at $t = -\frac{c_1}{nD}$ in the model . The mean anisotropic parameter is constant and it increases. Thus, the dynamics of the mean anisotropy parameter depends on the value of n . Since $\frac{\sigma^2}{\theta^2} = cons\tan t,$ the models does not approach isotropy through the whole evolution of the universe.

5. Other dark energy model

Now we take the following ansatz for the scale factor, where the increase in terms of time evolution is

$$R(t) = te^t \tag{50}$$

By the above choice of scale factor yields a time dependent deceleration parameter. We define the deceleration parameter q as usual,

$$q = -\frac{R_{44}R}{R_4^{\,2}} = -\frac{R_{44}}{RH^2} \tag{51}$$

Using (50) into (51), we find

$$q = -1 + \frac{1}{(1+t^2)} \tag{52}$$

Using (24) and (51) in (15), we obtain the expressions for metric functions as follows

$$a = \left(te^t\right)^{\frac{3m}{2+m}} \tag{53}$$

$$b = (te^t)^{3/2+m} \tag{54}$$

Hence the model (1) reduces to

$$ds^2 = -dt^2 + (te^t)^{\frac{6m}{m+2}}dx^2 + (te^t)^{\frac{6}{m+2}}(dy^2 + dz^2) \tag{55}$$

The expressions for the Hubble parameter H , scalar of expansion θ, shear σ and the average anisotropy parameter A_m for the model (55) are given by

$$\theta = 3H = 3\left(\frac{R_4}{R}\right) = 3\left(\frac{t+1}{t}\right) \tag{56}$$

$$\sigma^2 = \frac{(m-1)^2}{m^2r^2}\left(\frac{t+1}{t}\right)^2 \tag{57}$$

$$A_m = \frac{(m^2+2) - 2mr(m+2)}{3mr^2} + 1 \tag{58}$$

Where $r = \frac{m+2}{3m}$. Since $\frac{\sigma^2}{\theta^2} \neq 0$ for all values of m except for $m = 1$, hence the model is anisotropic except for $m = 1$.

The dynamics of the mean anisotropic parameter depends on the value of m. The mean anisotropic parameter is constant. We observed that when $m = 0,\ A_m \to \infty$ and for $m = 1,\ A_m = 0$. Thus, the observed isotropy of the universe can be achieved in phantom model.

The energy density of the fluid can be find by using (53) and (54) in (11)

$$8\pi\rho = \frac{(2m+1)}{m^2r^2}\left(\frac{t+1}{t}\right)^2 - M^2(te^t)^{\frac{-4}{rm}} \tag{59}$$

Using (53) , (54) and (59) in (13) , the EoS parameter ω is obtained as

$$\omega = -\frac{\left[\frac{3(t+1)^2 - 2mr}{t^2 m^2 r^2} - M^2 (te^t)^{-\frac{4}{rm}}\right]}{\frac{(2m+1)}{r^2 m^2}\left(\frac{t+1}{t}\right)^2 - M^2 (te^t)^{-\frac{4}{rm}}} \tag{60}$$

Using (53), (54), (59) and (60) in (12), the skew parameter δ are computed as

$$\delta = -\frac{\left[\frac{(m^2+m-2)}{m^2 r^2}\left(\frac{t+1}{t}\right)^2 + \frac{(1-m)}{mrt^2} + 2M^2 (te^t)^{\frac{-4}{rm}}\right]}{\frac{(2m+1)}{r^2 m^2}\left(\frac{t+1}{t}\right)^2 - M^2 \left(te^t\right)^{-\frac{4}{rm}}} \tag{61}$$

So, if the present work is compared with experimental results [31,32,33,34], then one can conclude that the limit of ω provided by (60) may accommodated with the acceptable range of EoS parameter. Also it is observed that at $t = t_c$, ω *vanishes* , where t_c is a critical time given by the following relation

$$\frac{3(t+1)^2 - 2mr}{m^2 r^2 t_c^{\,2}} - M^2 (t_c e^{t_c})^{-\frac{4}{rm}} = 0 \tag{62}$$

Thus for this particular time, our model represents a dusty universe, We also note that the earlier real matter at $t \le t_c$, where $\omega \ge 0$ later on at $t > t_c$, where $\omega < 0$ converted to the dark energy dominated phase of universe.

From (59), we note that energy density of the fluid $\rho(t)$ is a decreasing function of time and $\rho \ge 0$ when

$$(te^t)^{4/rm}\left(\frac{t+1}{t}\right)^2 \ge \frac{M^2 m^2 r^2}{2m+1} \tag{63}$$

Here ρ is a positive decreasing function of time and it approaches to zero as $t \to \infty$.

Using (56),(59) in (47), the cosmological constant is obtained as

$$\Lambda = \left[3 - \frac{9(2m+1)}{8\pi(m+2)^2}\right]\left(\frac{t+1}{t}\right)^2 - \frac{M^2}{8\pi}(te^t)^{\frac{-4}{rm}} \tag{64}$$

From (64), we observe that Λ is a decreasing function of time and it is always positive when

$$\left(\frac{t+1}{t}\right)\left(te^t\right)^{\frac{12}{m+2}} \ge \frac{M^2 (m+2)^2}{24\pi(m-1)^2} \tag{65}$$

We observe that cosmological parameter is decreasing function of time and it approaches a small positive value at late time. Thus ,the nature of Λ in this derived DE model is also in good agreement with recent observations [1,2,68,69,70].

6. Conclusion

On getting motivated from increasing evidence for the need of a geometry that resembles Bianchi morphology to explain the observed anisotropy in the Wilkinson microwave anisotropy probe (WMAP) data, we have discussed some features of the Bianchi type- VI_0 universes in the presence of a fluid that wields an anisotropic EoS parameter in general relativity. A new class of anisotropic LRS Bianchi type –I magnetized Dark energy models with variable EoS parameter ω has been investigated by using time dependent deceleration parameter. In literature it is a plebeian practice to consider constant deceleration parameter. Now for a universe which was decelerating in past and acceleration at present epoch, the DP must show signature flipping as already discussed in Section 2 . Therefore our consideration of DP to be variable is physically justified.

Our Power law solution represents the singular model where the spatial scale factors and volume scalar vanish at $t = -\frac{c_2}{nD}$. All the physical parameters are infinite at this initial epoch and tend to zero as $t \to \infty$. There is a point Type singularity [48] at $t = -\frac{c_2}{nD}$ in the model.

It is observed that, in early stage, the EoS parameter ω is positive i.e. the universe was matter dominated in early stage but in late time, the universe is evolving with negative values i. e. the present epoch . DE model presents the dynamics of EoS parameter ω provided by (32) whose range is in good agreement with the acceptable range by the recent observations [31-34].

The existence of DE, in whatever form , is needed to reconcile the measured geometry of space with the total amount of matter in the universe, DE models present the dynamics of EoS parameter ω provided by (32) and (60) whose range is in good agreement with the acceptable range by the recent observations [31-34] .It can be easily seen that in both DE models . Thus , our both anisotropic parameter vanishes at $m = 1.$ Thus, our both anisotropic models approach to isotropy at $m = 1.$ It is already

discussed in previous section , we obtain cosmological constant dominated universe, quintessence and phantom fluid dominated universe [45], representing different phases of the universe through- out the evolving process.

Our DE model is of great importance in the sense that the nature of decaying vacuum energy $\Lambda(t)$ is supported by recent cosmological observations. Though there are many suspects (candidates) such as cosmological constant, vacuum energy, scalar field, brane world, cosmological nuclear-energy, etc. as reported in the vast literature for DE, the proposed model in this paper favors EoS parameter as a possible suspect for the DE.

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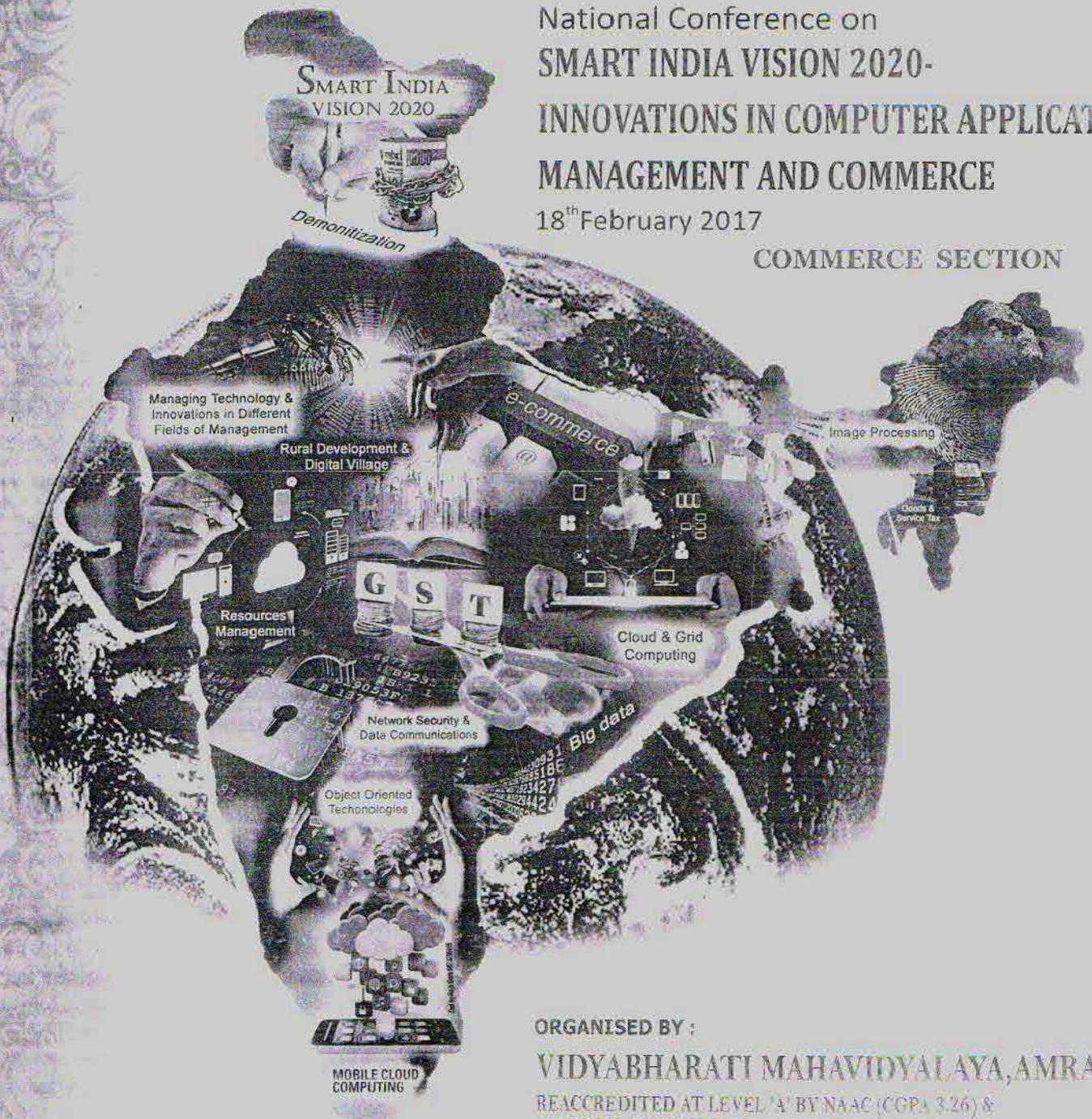

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CONTENTS

CHALLENGES IN RURAL DEVELOPMENT AND DIGITAL VILLAGE

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Introduction

This paper aims at discussion of few of challenges of rural life and utilization existing technologies for mitigating few of challenges of rural life.
The rural setup in India is mainly characterized by agriculture based life style with availability of few salaried jobs and access to unorganized small scale economy with limited access to much of infrastructure including digital infrastructure.
The digital divide prevents the population in villages to reap the socio-economic fruits of the technology. This paper discusses the existing solutions. in that direction. This paper should provide some inputs for aspiring social entrepreneur to use the technology for solving some the challenges of rural and agrarian life.
We see many social entrepreneursare riding the wave of technology to facilitates the rural population with means to tackle these challenges. Customizing established models to Indian scenarios seems to be the key. Building scalable models that benefit the population at large is a challenge that needs to be tackled. Hence the solutions may not completely be dependent on technology but need to address the human aspect of the endeavor as well for it to be successful.

Advantages of rural life style

Low pollution: Pollution industry and vehicular pollutions have increased levels of pollution in cities. This has given rise to increase in health issues in such polluted and congested cities where city dwellers try to go out of this pollution on it on every holiday they get.
Far from choking traffic: The unplanned urban growth has given rise to many problems the city dwellers face. The lack of planned public transport infrastructure in city areas is causing huge traffic jams and causing wastage of man hours and rise in pollution levels
More natural and tranquil setup:Far from madding crowd; hustle bustle of city life. People aspiring for peaceful life and with creative pursuits would certainly aspire to live at places offering such tranquil setting.

Challenges of rural life

The following section describes the challenges in rural life and use of technology to improve the situation.

Digital Divide

Large section of population does not have access to Internet and communication technologies hence may not be directly be using the different services that have come up using these technologies that can be of much help in tackling challenges of life and providing new opportunities. Apart from this lack of availability of computer application supporting regional languages also hamper their adaptability in rural scenario. The current demonetization drive has shown that those without the means of online transfer of money had even more difficulty in making and receiving payments. The availability of the payments application with linkage to Aadhar number have been an after-thought in such drives. The proper planning in the roll out of such applications would certainly have reduced some pain faced by population.
Aadhar based payment using thumb impression is a significant step that can facilitate getting the rural and semi urban populations to do digital transaction without getting into complexities of -e-wallets and banking debit/credit cards.
The overall apathy of successive governments regarding bridging this digital divide leaves open a vast space for social entrepreneur to implement initiatives that help rural and semi-urban population.

Use of technology in agriculture:
Many attempts have been made to find the root causes of farmer's suicides in India and especially Vidarbha. Following are some of causes cited in well-known reports and here some solutions have been proposed for it. [6][7]

Growing expenditure, especially on bought inputs, low productivity:
Recent times have seen resurgence of organic farming practice. Use of organic farming practices. Spreading this awareness about organic farming through use of current social media can be done. In fact, the mobile applications regarding the organic farming techniques are available now [7]. Government should facilitate organic food certifications of farmer group that follow these practices and connect them to the market. The social media technologies can help organize the farmers and connect them to the market. Health conscious customers are ready to pay higher price for organic grains and vegetables.

Challenge: There have been movements like "silent spring" in Unites states against usage of chemical pesticides. We have traditional self-sufficient techniques regarding this as being propagated by zero budget organic farming initiatives rooted in this very part of Vidarbha. Still surprisingly the following for these techniques seem to be more in other part of India than Vidarbha. Can the social media be used to raise awareness among farming community regarding this issue.

Inadequate prices of agriculture produce Difficulties in marketing and marketing hazards:
The solution lies in connecting farmers to market by online platforms and Creation of Agent-free. The private initiative in this direction is e-chaupal initiative. Government initiative in this regard is e-NAM.e-National Agriculture Market (NAM) is a pan-India e-trading platform. It is designed to create a unified national market for agricultural commodities It is designed to create a unified national market for agricultural commodities. Farmers can showcase their produce online from their nearest market and traders can quote price from anywhere. commodities including onion, wheat, pulses, coarse grains, and cotton, have been identified for online trading. [1]

Challenge: Can we find the direct market for organic cotton from Vidarbha?
There has been rising awareness regarding usage of the organic cotton and many people trying to buy cloths made up of organic cotton. The challenges are to navigate through the hurdles to make direct contact of this market for farmers in Vidarbha. The challenges are at multiple levels but can technology help in making this connection? The social entrepreneur and decision makers can try to tackle this issue.

Natural hazards causedby drought:
Proper tracking of sowing patterns and exact database of the farmers can help government reach out to the farmers who are affected by draught. Satellite imaging techniques can be used to have some estimates on effect of draught. Absence of proper crop planning: use of organic farming and pushing of correct information content to farmer through means like kiosks

Unsatisfactory agriculture credit, accumulated burden of debt:
Apart from government backed initiatives Crowd funding and microcredit provide way out of this situation by government wavers of loans and migrating to lower interest loans, crowd funding and microcredit.

Microcredits and Crowdfunding: These are the sites that provide small loans to needy. The loans can be funded by individuals who want to support the cause. There two aspects to it.
The investor funding small loan has satisfaction of supporting social cause and the borrower paying back means that it imbibes the cultures of working towards paying back and no free loans. As small interest is charged so this not charity but small help from one human to other.
The timely availability of fund without any collateral and mortgage is the key. The borrowers can borrow for education, their small business endeavors, etc.

Challenge: Main issue is how to establish the credibility of the genuine cases with donor.
As we see many NGOs which seem to have dedicated call centers to pursue with donors to make donations can repeatedly following up with them. This creates doubt even in case of genuine cases. This might prevent lot of donors from helping people in need.
The challenge social entrepreneur is to devise a way to build the confidence in mind of the donor to contribute to such social causes

Amongst the social challenges:
The drinking habit which affects the productivity of the farmers: Government if not able ban the sale of liquor if it monitors purchase of liquor through use of Aadhar based technology can really monitor or regulate serious drinking cases. This is possible by expensing the retail sale only through Aadhar based thumb readers. Keeping such measure of consumption by individual may help the individual and government. One of the steps in this regard is restricting sale of liquor only through Aadhar based payments using thumbprint authentication.

Extravagantexpenditure on marriages: Government incentives for "samudayik vivah" where many couple are married in common marriage ceremony. The usage of social media can play a role in this as to spread the awareness about it. The marriageexpenditure is sometimes made out as a prestige point and many

people fall prey to this false social prestige and incur debtsorganizing huge pompous ceremonies.The existing online matrimony sites can as well promote such marriages.

Challenge: Getting rid of social evils as practice of dowry and caste biases and building a progressive mind set without losing key values of the Indian culture that are essential for sustenance of community living and functional families.

Bad health and illness and inability to meet the necessary expenditure on medicine and health services:

There has been number of online medical ecommerce site that have come up in recent days where medicines can be ordered online. For affordability government, has started facilitating availabilityof Generic medicine Goods delivery mechanism need to incentivized for delivery of medicine for rural population to increase affordability of the medicine.The large e-commerce sites operating in India have built huge logistics and delivery channels but are normally operating in pockets where consumer's concentration is high. There is an opportunity to expand these channels to rural area.

Hospitals with expert staff: Government incentive for hospitals in rural area and the staff as well.

Using technologies as Telemedicine, Remote Diagnostics and cloud based healthcare solutions can alleviate the situation to some extent but are not complete replacement for good medical facilities in rural area.[2]

Lack of opportunities for employment and visibility of the job market

Because of absence of employment opportunities in rural areas there is large migration to industrialized area of the nation. There are some employments that can be easily replicated in the rural areas.

Some of the industry that can be moved to rural area from digital perspective are discussed below.

Migrating such industry to rural area has advantage for entrepreneurs and employment seekers in rural in rural area both. Also, availability of the collaborative tools which facilitate remotes teams to work together make it possible to have team members at different geographies to work together.

- Support call support centers for companies: This does require the communication infrastructure and especially the ones in regional language are easier to target. This model is also more suitable for towns and semi-urban places.
- Medical transcription: The trainings for these are available online and there are opportunities for remote working. So as an individual or company these can be done from the rural area as well.
- Online tutoring: This involves tutoring students remotely. There are many such platform available for teachers who want to conduct online coaching.
- Graphics design, animations: These jobs can be done in semi urban and rural setup.
- Software Development centers that deal with mass prevalent and easily available skillset. Mainly the developments with easily available skillsets can be targeted for development centers in rural area. The current technologies of audio video conferencing could be easily used to interact with remote sites and customers.
- Providing platform to artisans: There have been few of the companies that provide platform forming artisans to highlighttheir artifacts and sell them [9].

Rural employment exchange

Either government or social entrepreneurs can create rural employment exchange to connect job seekers to the employers.

Lack of social progression/access to fruits modern civilization.

Low cost kiosks which can pull relevant content. Community library can host these kiosks where people can access relevant content for them. There can be push of contents to such kiosk from government to disseminate different useful policy decision for rural population.

Need to have mobile application that address the problems which are specific to rural scenario.

Regional language support in mobile apps and software as such. Availability of such application in regional languages will certainly make them more adaptable for rural users. Also, this is a design challenge to have interface that is intuitive and very easy to use so people accept it easily and get their work done.

Rural Computing

There have been some very interesting initiatives for building Indian language based programming language. This idea seems to have big potential as can spread the skill of programming to commonly used languages. So the individuals with high creativity can express through their mother tongues and not hampered by the

lack of proficiency in English. This idea is also more suitable for rural setup and create a new wave of "rural computing"

Source of uninterruptedsustainable Electric power supply

Use of technology to circumvent power issues

India is blessed with sunshine for most of the time of the year hence usage of solar power should increase in India. Usage of gobar gas,bio gas for power generation can be explored. The temperature in most part of India isconducive for process of gas production throughout the year. This provides clean economic fuel. [5]

Key Recommendations:

1. Laying digital infrastructure: Either government provides it and incentivize private entities interested in providing this infrastructure.
2. Government incentives for building logistics channel for rural area that facilitated moving of goods to and from the rural setup.
3. Support for multilingual apps to connect to rural population. Making computer programming in Indian languages possible.
4. Getting involved more people into the social entrepreneurship through government initiatives
5. Facilitate easier regulations for such entrepreneurship to remove autocratic hurdles for more entities to compete to provide services in rural area.
6. Usage of telemedicine for providing health care.
7. Improving access to market for rural populations through initiatives like e-nam and other initiatives.

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Short Communication

Potentiometric titration of complexes with flavones and metal

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Abstract

The dissociation constant and equivalence point in 70% Dioxane-Water and end point in different concentration with flavones at different temprature. Dissociation constant equivalence point has also calculated by potentiometer. Ligand had been studied using potentiometric method using calomel and platinum electrode various temperature for 0.1M ionic strength.

Keywords: Dioxane, Potentiometer, Metal solutions Ni(II), Copper (II), NAOH.

Introduction

A number of investigations on potentiometric titrations of complexes have been ade to explain their prominent features in the solutions. However, the extensive literature dealing with the potentiometric titrations of complexes has been restricted to globular protines as well as synthetic polymeric acids, bases. As the part of general program on complex formation between metal and acid ions[1]. These paper presents the results on the flavones of benzaldehyde and crotonaldehyde complex of copper and Ni(II) using the method of potentiometry[2].

Complex formation between copper(II) and Ni(II) and unsubstituted acid[3]. Many quantitative studies were carried out with flavones to find its conformational change in neutralization and to estimate the molecular parameters from analysis of the potentiometric titration[4]. Experimental study carrid out on calomel electrode under both static and dynamic conditions presence of other ions. With ligands and metal for the determination of the relevant complex formation constant from potentiometric data[5].

Materials and methods

In potentiometric titration prepare electrolytic solution of given concentrations of and the ligands flavones of p-chloro benzaldehyde, flavone of chrotonaldehyde and flavones of benzaldeyde and metal solution of copper and nickel. Also prepare 0.1 N NAOH soutations. These NAOH solutation standerised oxalic acid. the std. metal solutions were prepared in dioxane-water. These NAOH soutions tritre against metal solutions.

Result and discussion

The graphical data showed that increasing volume of NaOH with also increasing pH value clearly indicate disociation constant at point of interaction.

Table-1: The titration reading of Copper(II) and volume of NaOH 0.1 M,at temp 25^0C

Ml	pH
0.5	1
1	1.147
1.5	1.308
2	1.499
2.5	1.757
3	0.243
3.5	7
4	1.624
4.5	12.103

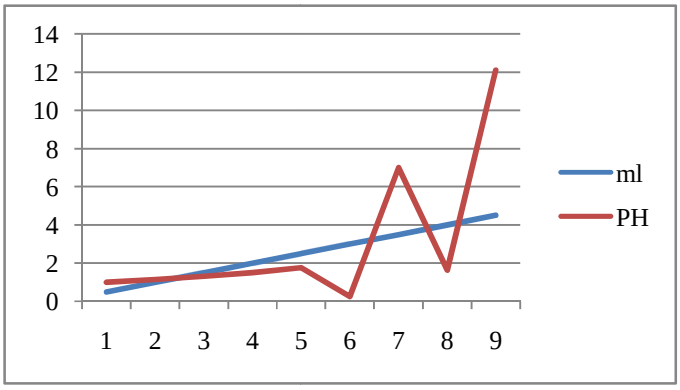

Figure-1: Volume of NaOH Vs pH on flavones of Benzaldehyde

Table-2: The titration reading of Ni(II), and volume of NaOH 0.1 M, at temp 25^0C

Ml	pH
0.5	1.678
1	1.986
1.5	2.89
2	1.789
2.5	3.678
3	2.456
3.5	2.678
4	1.896
4.5	4.789

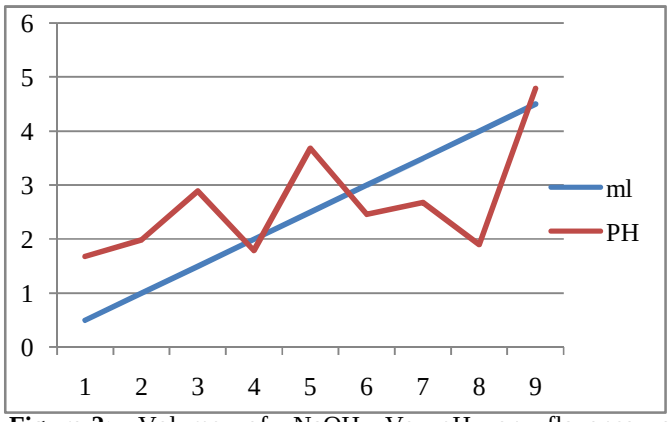

Figure-2: Volume of NaOH Vs pH on flavones of Crotonaldehyde

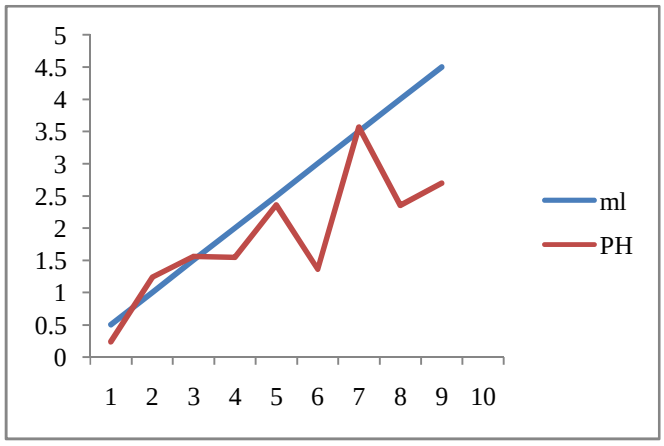

Figure-3: Volume of NaOH Vs pH on flavones of P-Chlorobenzaldehyde

Table-3: The titration reading of Ni(II) and volume of NaOH 0.1 M,at temp 25^0C

Ml	pH
0.5	0.236
1	1.235
1.5	1.563
2	1.548
2.5	2.362
3	1.365
3.5	3.569
4	2.356
4.5	2.698

Conclusion

The dissociation co nstant and equivalence point were determine potentiometrically. It is concluded that the dissociation constant and equivalence point different at different concentration.

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QSAR analysis for 6-arylpyrazine-2-carboxamides as Trypanosoma brucei inhibitors

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QSAR analysis for 6-arylpyrazine-2-carboxamides as *Trypanosoma brucei* inhibitors

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ABSTRACT

Human African trypanosomiasis (HAT) is prevalent in African countries, covering 37 countries, mostly sub-Saharan. A limited number of drugs are available to cure this neglected disease. In the present work, quantitative structure–activity (toxicity) relationships (QSA(T)R) analysis has been performed for a dataset of 54 6-arylpyrazine-2-carboxamides as *Trypanosoma brucei* inhibitors to identify the important structural features required for future optimization of lead candidates. The QSA(T)R models satisfy OECD guidelines and have high statistical robustness. The QSA(T)R models are based on easily interpretable molecular descriptors. The QSA(T)R models indicate that *Trypanosoma brucei* inhibitory activity of 6-arylpyrazine-2-carboxamides has correlation with the presence of *N-sec*-butylformamide and substituted benzene. The results could be beneficial for further optimization of 6-arylpyrazine-2-carboxamides as *Trypanosoma brucei* inhibitors. Some potential candidate molecules have been proposed.

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KEYWORDS
QSAR; 6-arylpyrazine-2-carboxamides; *Trypanosoma brucei*; neglected disease; toxicity

Introduction

Sleeping sickness, also known as Human African trypanosomiasis (HAT), is a neglected disease, which is mostly transmitted by the bite of the blood-sucking tsetse fly of the genus *Glossina* [1–3]. According to a report from the World Health Organization this disease has high occurrence in sub-Saharan countries, with 70 million people at risk of contracting HAT and about 8000 new HAT cases each year [1–4]. This vector-borne parasitic disease, caused by protozoan parasites of the genus *Trypanosoma*, has two main stages: (a) the peripheral infection, which has non-specific symptoms, and (b) central nervous system invasion [1–6]. If untreated, this disease is eventually incurable in practically all cases, and due to toxicity and poor efficacy, limited drugs are presently available for its effective treatment [1–6]. Fortunately, the genome sequence of the parasite has been determined and various proteins have been identified as potential targets for drug development. But further investigation of the genome sequencing has disclosed that *Trypanosoma brucei* has over 800 genes that

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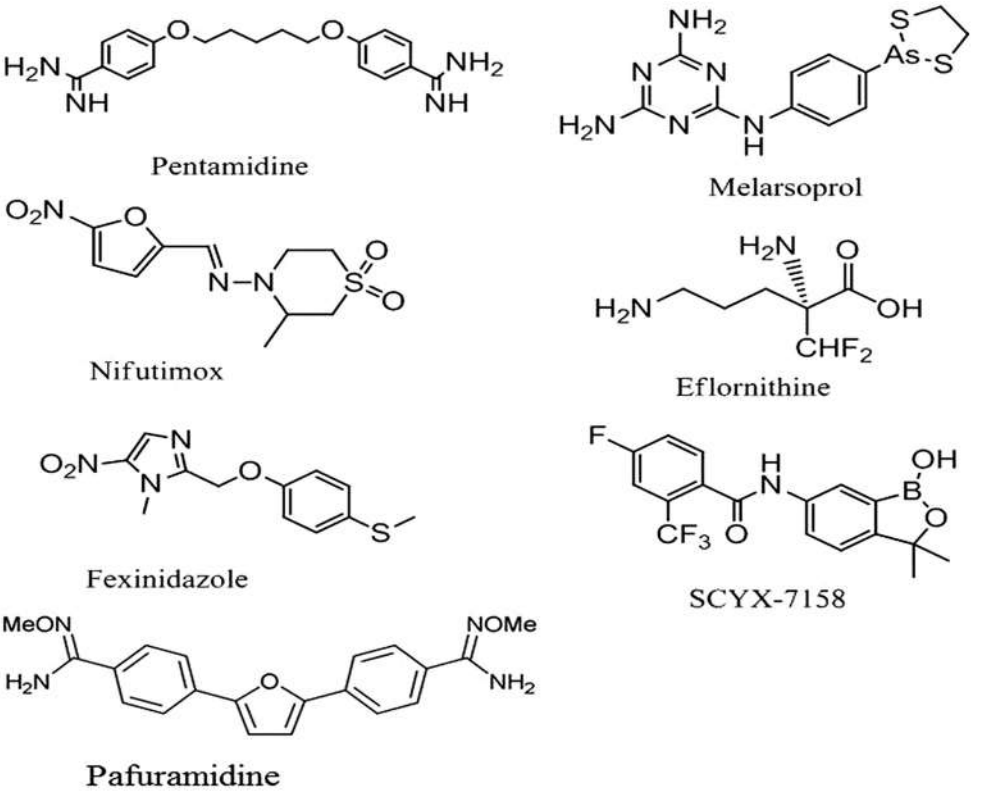


Figure 1. Some commercial and clinical stage drugs for treating HAT.

encode variant surface glycoproteins, resulting in parasite protein 'mixes and matches' to evade immune system detection; subsequently, developing a vaccine for this disease is rightly challenging [1–7]. In addition, the type of treatment varies with the stage of the disease. The drugs employed in the first stage of the disease have little toxicity and are easy to administer. The prospects of a cure are better if the disease is identified in its earlier stage. But, in most of the cases, the disease is identified at a later stage. The success of treatment in the second stage relies significantly on a drug that can cross the blood–brain barrier to reach the parasite. Unfortunately, such drugs are mostly toxic and difficult to administer. In Figure 1, the commercial drugs and drugs in clinical stages have been illustrated [1–10].

To overcome these problems, Rahmani et al. [3] used high-throughput screening against *T. brucei* using a library of 87,296 compounds, and identified 6-arylpyrazine-2-carboxamide as a promising scaffold for developing therapeutics against *T. brucei*. Further, they synthesized a good number of 6-arylpyrazine-2-carboxamide derivatives and screened for *in vitro* activity, microsome stability, solubility, etc. The analysis revealed that the compounds possess activity in the μM range with low microsome stability. Hence, further improvements are essential to achieve the goals with retention or augmentation of HAT healing activity and metabolic profiles. In this scenario, computer-aided drug design (CADD) is an attractive tool for further lead optimization.

In past decades, computer-aided techniques have appeared as promising alternatives to the conventional 'trial and error' methodology of drug design/discovery to unveil the secrets

of the biochemistry of drug action with absorption, distribution, metabolism, excretion and toxicity (ADMET) optimization. CADD is a rapid, cost-effective and impressively successful result-oriented contemporary technique comprising unification of various fields, with a main emphasis on mapping how diverse biological important molecules interact, to understand the mechanisms of disease and to find the pharmacophoric patterns linked with activity/toxicity [7,11–15]. The commonly employed CADD methods – quantitative structure–activity relationships (QSAR), molecular docking, pharmacophore modelling – when used concomitantly result in particularly effective analysis, providing maximal information desired for lead (and drug) optimization. These methods offer in-depth knowledge about the pharmacophoric patterns that control the specific activity/toxicity of a drug candidate and enhanced intuition for the mechanism of drug action.

Molecular docking is generally preferred when sufficient knowledge and evidence are available about the target protein (receptor or bio-polymer) with which the drug interacts. As the target protein with which 6-arylpyrazine-2-carboxamides interact is unidentified [3], QSAR (a ligand-based drug design technique) was performed to identify the structural features that have good correlation with the HAT healing activity of 6-arylpyrazine-2-carboxamides.

Experimental methodology

Experimental dataset

In the present work, anti-HAT activity values of a diverse dataset of 54 6-arylpyrazine-2-carboxamide derivatives encompassing different heterocyclic rings and a variety of substituents, previously reported by Rahmani et al. [3], were subjected to QSAR analysis (see Figure 2 and Table 1). The substituents are highly varied; consequently, the present dataset comprises isomers such as positional, functional, etc. Thus, the dataset covers a good chemical space.

A separate quantitative structure–toxicity relationships (QSTR) model has been developed for the cytotoxicity data of 13 arylpyrazine-2-carboxamide derivatives. The reported EC_{50} (μM) activity values for anti-HAT and rat skeletal myoblast cell L-6 strain were converted to

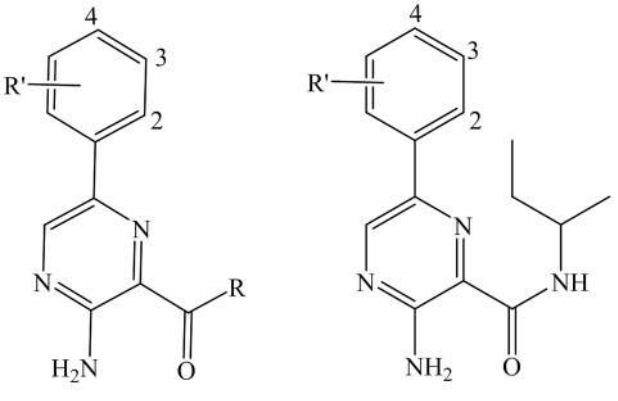
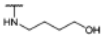


Figure 2. 6-arylpyrazine-2-carboxamide derivatives used in the present work.

Table 1. Substituents, experimental EC_{50} and pEC_{50} for anti-HAT activity of 6-Arylpyrazine-2-carboxamide derivatives.

S.N.		R	R′	EC_{50} (μM) Anti-HAT	pEC_{50} (M) anti-HAT	EC_{50} (μM)L-6 strain	pEC_{50} (M)L-6 strain
1	1a-9		H	0.49	5.690	18	7.744
2	1b-10		H	2.6	6.415	–	–
3	1c-11		H	0.26	5.415	20	7.699
4	1d-12		H	0.32	5.505	–	–
5	1e-14		H	1.7	6.230	–	–
6	1f-15		H	0.53	5.724	–	–
7	1g-16		H	7.3	6.863	–	–
8	1h-21		H	2.6	6.415	–	–
9	1i-22		H	3	6.477	–	–
10	1j-24		H	6.1	6.785	–	–
11	1k-25		H	4.6	6.663	–	–
12	1l-27	2-F	H	0.75	5.875	–	–
13	1m-28	2-Cl	H	1.1	6.041	–	–
14	1n-30	2-OCH_3	H	5.4	6.732	–	–
15	1p-31	2-NO_2	H	0.89	5.949	–	–
16	1o-33	2-$COCH_3$	H	10	7.000	–	–
17	1q-34	2-Me	H	1.2	6.079	–	–
18	1r-35	2-CN	H	0.35	5.544	18	7.744
19	1s-36	3-F	H	0.82	5.914	–	–
20	1t-37	3-Cl	H	0.48	5.681	39	7.409
21	1u-38	3-Br	H	0.57	5.756	–	–
22	1v-39	3-OCH_3	H	0.41	5.613	–	–
23	1w-40	3-NO_2	H	0.45	5.653	–	–
24	1x-41	3-NH_2	H	1.1	6.041	–	–
25	1y-43	3-CH_3	H	0.17	5.230	43	7.366
26	1z-44	3-CN	H	2.7	6.431	–	–
27	2a-45	4-F	H	0.4	5.602	17	7.769
28	2b-46	4-Cl	H	0.68	5.833	–	–
29	2c-47	4-Br	H	2.2	6.342	–	–
30	2d-48	4-OCH_3	H	1.3	6.114	–	–
31	2e-49	4-NO_2	H	4.5	6.653	–	–
32	2f-50	4-NH_2	H	2.9	6.462	–	–
33	2g-51	4-$COCH_3$	H	4.7	6.672	–	–
34	2h-52	4-CH_3	H	3.74	6.573	–	–
35	2i-53	4-CN	H	2.7	6.431	–	–
36	2j-54		2-CN	0.17	5.230	10	8
37	2k-55		3-Me	0.11	5.041	20	7.699
38	2l-56		3-Me and 5-Me	0.48	5.681	–	–

(*Continued*)

Table 1. (*Continued*)

S.N.		R	R′	EC_{50} (μM) Anti-HAT	pEC_{50} (M) anti-HAT	EC_{50} (μM)L-6 strain	pEC_{50} (M)L-6 strain
39	2m-57		4-F	0.08	4.903	28	7.553
40	2n-58		3-Me and 4-F	0.025	4.398	24	7.620
41	2o-59		2-CN and 4-F	0.1	5.000	20	7.699
42	2p-60		3-CF_3	0.26	5.415	–	–
43	2q-61		3-Cl	0.36	5.556	–	–
44	2r-62		3-Cl and 5-Me	0.77	5.886	–	–
45	2s-63		3-Cl and 4-F	0.22	5.342	–	–
46	2t-64		2-CN	0.2	5.301	11	7.958
47	2u-65		3-Me	0.035	4.544	42	7.376
48	2v-66		4-F	0.071	4.851	–	–
49	2w-67		3-Me and 4-F	0.024	4.380	–	–
50	2x-68		2-CN and 4-F	0.057	4.756	–	–
51	2y-69		3-CF_3	0.15	5.176	–	–
52	2z-70		3-Cl	0.24	5.380	–	–
53	3a-71		3-Cl and 5-Me	0.5	5.699	–	–
54	3b-72		3-Cl and 4-F	0.11	5.041	–	–

pEC_{50} ($pEC_{50} = -\log_{10}EC_{50}$) before quantitative structure–activity (toxicity) Relationships (QSA(T)R) analysis. The EC_{50}, pEC_{50} along with the substituents are shown in Table 1.

Modelling and molecular descriptors' calculation

In the present work, for successful QSA(T)R analysis, OECD guidelines and a standard protocol recommended and used by different researchers have been followed [12,16–25]. The structure drawing was accomplished using ChemSketch 12 freeware, followed by energy

minimization by means of MMFF94 force field available in TINKER. The energy-minimized 3D structures were used for calculation of a large number of descriptors (>29,000) using PaDEL 2.21 and *PyDescriptor* as a molecular descriptor calculator. This huge molecular descriptor pool of more than 29,000 descriptors covers mono-dimensional (1D) to three-dimensional (3D), electro-topological, finger-prints and other molecular descriptors. Since all the calculated descriptors do not contain relevant information, henceforward, objective feature selection (OFS) was performed using QSARINS-Chem 2.2.1 [24,26] to eliminate a large number of redundant molecular descriptors. Prior to subjective feature selection (SFS) performed using QSARINS-Chem 2.2.1 [24,26], constant, near constant (>98%) and highly correlated ($|R|$ >90%) descriptors were rejected. OFS caused significant reduction of descriptor pool to a cluster of 1200 and 865 molecular descriptors only for QSAR and QSTR models, respectively, still covering a wide structural and chemical space, including constitutional (0D), 1D, bi-dimensional (2D) and 3D [7,11,12,20–25,27,28].

Model development

The main objectives for QSA(T)R models analysis are (1) to identify the structural features that influence the activity/toxicity profile of a congeneric series of molecules, and (2) to predict the activity/toxicity for a compound prior to its actual synthesis and/or biological screening. To obtain maximum information about pharmacophoric features, QSAR models were derived using a divided and undivided dataset. The dataset was randomly split into training (80%) and prediction (20%) set earlier to descriptor selection. Multiple splitting was carried out to derive multiple QSAR models. A compound may or may not be in the training set of two different splittings. The genetic algorithm (GA) module of QSARINS-Chem 2.2.1 [24] was employed for selection of optimum number and cluster of molecular descriptors. The heuristic search of molecular descriptors was restricted to four descriptors using the defaults settings in QSARINS-Chem 2.2.1 to avoid over-fitting and have simplicity in understanding the developed QSAR models. Q^2_{loo} was considered as a fitness function to circumvent the problem of naïve Q^2.

As the dataset for toxicity is small, for QSTR model development the complete dataset was used. The heuristic search of molecular descriptors was constrained to two descriptors using the defaults settings in QSARINS-Chem 2.2.1 to circumvent the problem of over-fitting and complexity.

Model validation

QSA(T)R model development is incomplete without appropriate model validation. Appropriate QSA(T)R model validation ensures the external predictive ability of a model. Hence, the statistical qualities and strength of the genetic algorithm–multiple linear regression (GA–MLR) equations were assessed on the basis of: (a) cross-validation (CV) by leave-one-out (LOO) and leave-many-out (LMO) procedures (i.e. internal validation); (b) using the prediction set; (c) data randomization, i.e. Y-scrambling, and (d) observing if the following criteria are fulfilled: $r^2_{tr} \geq 0.6$, $Q^2_{loo} \geq 0.5$, $Q^2_{LMO} \geq 0.6$, $r^2 > Q^2$, $r^2_{ex} \geq 0.6$, $RMSE_{tr} < RMSE_{cv}$, $\Delta K \geq 0.05$, CCC ≥ 0.80, $Q^2 - F^n \geq 0.60$, $r^2_m \geq 0.6$, $(1-r^2/r_o^2) < 0.1$, $0.9 \leq k \leq 1.1$ or $(1-r^2/r'_o{}^2) < 0.1$, $0.9 \leq k' \leq 1.1$, $|r_o^2 - r'_o{}^2| < 0.3$ with RMSE and MAE close to zero [7,11–13,28]. A GA–MLR model that satisfies the threshold values of these parameters possesses statistical robustness and

external predictive ability. Therefore, the models that do not satisfy above mentioned criteria were consequently excluded [7].

Results and discussion

QSAR models

The selected dataset considered in the present study is of moderate size with the molecules either being positional or constitutional isomers, consequently covering a good chemical space. We have earlier proved that for small or moderate-size datasets at least one QSAR model must be constructed using an undivided whole dataset to determine maximal significant descriptors, which in turn not only affords valuable information for future variations but is also beneficial for comparison and evaluation of QSAR models built using divided datasets. Hence, in the present work, QSAR models were developed using divided and undivided datasets.

During the QSAR analysis, normally stepwise regression, GA, etc. algorithms are considered for SFS. SFS gives rise to a good number of MLR models with almost equivalent statistical performance but often comprising different descriptors. Generally, in such a situation, only one MLR model is chosen on the basis of its statistical robustness. The weaknesses, however, of this 'first among equals' approach are (1) if the chosen model comprises complex/esoteric descriptors then simple and straightforward correlation of descriptors with proper structural features is relatively difficult, (2) a single QSAR model may be swayed by (i) the method of splitting, constitution of training and prediction sets, method adopted for descriptor selection, and (ii) some more influencing molecules in the training/prediction dataset [7,11,12,14]. A simple and potential answer to outwit the shortcomings of the 'first among equals' approach is to construct multiple QSAR models using multiple splitting. A further advantage of this solution lies in identification of underprivileged yet valuable pharmacophoric features that control the anti-HAT activity [7,11,12,14]. Therefore, in the present study, multiple QSAR models were constructed. Interestingly, univariate statistical analysis revealed that anti-HAT activity has good but negative correlation with $R = 0.771$ with a finger print descriptor KRFPC3662, which signifies the presence of SMILES notation CCC(C)NC=O that corresponds to *N-sec*-butylformamide fragment. The developed multi-linear GA–MLR QSAR models are as follows:

Full set model Model 1:

pEC_{50} = + 5.598 (± 1.92) + 8.333 (± 0.343) * KRFPC1609 + 0.608 (± 0.128) * KRFPC3662 – 0.021 (± 0.009) * byring allplus_AbSA + 0.221 (± 0.075) * fHF4B

Full set model Model 2:

pEC_{50} = + 5.505 (± 0.184) – 0.460 (± 0.222) * AD2D627 + 0.701 (± 0.357) * KRFPC1609 + 0.514 (± 0.133) * KRFPC3662 + 0.202 (± 0.078) * fHF4B

Model 3:

pEC_{50} = + 5.539 (± 0.202) + 0.826 (± 0.340) * KRFPC1609 + 0.595 (± 0.135) * KRFPC3662 – 0.016 (± 0.010) * byring allplus_AbSA + 0.226 (± 0.075) * fHF4B

Model 4:

pEC_{50} = + 5.994 (± 0.473) + 0.773 (± 0.372) * KRFPC1609 + 0.484 (± 0.165) * KRFPC3662 + 1.934 (± 1.294) * O_all_3Bc + 0.219 (± 0.084) * fHF4B

From the derived models it is clear that KRFPC1609 (a Klekota–Roth finger print descriptor that corresponds to di-substituted benzene with one of the substituents at the meta position), KRFPC3662 (a Klekota–Roth finger print descriptor that corresponds to *N-sec*-butylformamide fragment) and fHF4B (frequency of occurrence of hydrogen within 4 Å from fluorine atom) are common in all the newly derived MLR–QSAR models. Their coefficients are positive in all the models; hence, the higher the values of these fragments, the higher the activity. In models 1 and 3, the descriptor byring allplus_AbSA, which represents absolute surface area of positively charged atoms of rings, has negative coefficient, therefore, its value must be as low as possible. For this, electron-withdrawing groups on rings and heterocyclic rings must be avoided.

The descriptor KRFPC3662 (representing the fragment *N-sec*-butylformamide) has encouraging correlation with the activity, which is supported by comparing the activity of following pairs of compounds: **1** (IC_{50} = 0.49 μM) and **2** (IC_{50} = 2.6 μM), **15** (IC_{50} = 0.53 μM) with **14** (IC_{50} = 1.7 μM), and **1** (IC_{50} = 0.49 μM) with **9** (IC_{50} = 3 μM). In general, molecules lacking the *N-sec*-butylformamide fragment have lower activity profile compared with their analogues with the *N-sec*-butylformamide fragment. The descriptor KRFPC1609 has significant influence in deciding the activity, as evident from the comparison of activity values of the following pairs of compounds: **13** (IC_{50} = 1.1 μM) and **20** (IC_{50} = 0.49 μM), **30** (IC_{50} = 5.4 μM) and **22** (IC_{50} = 0.41 μM), **17** (IC_{50} = 1.2 μM) and **25** (IC_{50} = 0.17 μM), and **15** (IC_{50} = 0.89 μM) and **23** (IC_{50} = 0.45 μM). Surprisingly, the compound number **19** (IC_{50} = 0.82 μM) even though it possesses –F at the meta position on the benzene ring, it has lower activity than **12** (IC_{50} = 0.75 μM) and **27** (IC_{50} = 0.40 μM). A plausible explanation for this abnormal observation was obtained after derivation of above QSAR models. The above QSAR models revealed that the descriptor fHF4B has positive correlation with the activity. In the case of compound **12** and **27**, the value of the descriptor fHF4B is higher than compound number **19** (see the supporting information, available via the Supplementary Content tab on the article's online page at ####). Hence, in future optimizations of 6-arylpyrazine-2-carboxamides as *T. brucei* inhibitors with –F as one of the substituents, the value of the descriptor fHF4B must be given significant importance.

The descriptor AD2D627, which stands for the presence of carbon and oxygen atoms at a topological distance of 9 from each other, has negative coefficient in model 2. Hence, such a combination of C and O must be avoided to have better activity. This is supported by the difference in the activity of compound **5** (IC_{50} = 1.7 μM) and **7** (IC_{50} = 7.8 μM), **30** (IC_{50} = 1.3 μM) and **14** (IC_{50} = 5.4 μM), and **18** (IC_{50} = 0.35 μM) and **35** (IC_{50} = 2.7 μM).

The statistical parameters for all the derived models have been tabulated in Table 2. In Table 3, experimental and predicted pEC_{50} by various models along with the status of the molecule are shown.

The GA heuristic search for undivided whole dataset resulted in many QSAR models, but only two models (models 1 and 2) were observed to accomplish the threshold values for different statistical parameters. From Table 2, it is evident that the derived QSAR models fulfil threshold values for many of the internal and external validation parameters. A comparative analysis of various statistical parameters for different models points out that the models are statistically acceptable because of good external predictive ability. For all the derived models, the close value of r^2_{adj} to r^2_{tr} indicates that the models consist of a sufficient number of descriptors and the problem of over-fitting is absent. The low value of RMSE and MAE (fitting,

Table 2. Different statistical parameters for goodness of fit, internal validation and external predictive ability for models 1–4.

S. No.	Statistical parameter	Model-1	Model-2	Model-3	Model-4
1.	N_{tr}	54	54	44	44
2.	N_{ex}	00	00	10	10
3.	Number of descriptors	04	04	04	04
Fitting criteria					
4.	r^2_{tr}	0.8351	0.8218	0.8459	0.8141
5.	r^2_{adj}	0.8216	0.8072	0.8301	0.7950
6.	$r^2_{tr} - r^2_{adj}$	0.0135	0.0145	0.0158	0.0191
7.	*LOF*	0.0967	0.1045	0.0973	0.1159
8.	K_{xx}	0.2004	0.1973	0.2133	0.2042
9.	ΔK	0.1458	0.1926	0.1395	0.1902
10.	$RMSE_{tr}$	0.2648	0.2753	0.2552	0.2786
11.	MAE_{tr}	0.2120	0.2216	0.2032	0.2228
12.	RSS_{tr}	3.7875	4.0931	2.8660	3.4143
13.	CCC_{tr}	0.9101	0.9022	0.9165	0.8975
14.	*s*	0.2780	0.2890	0.2711	0.2959
15.	*F*	62.0275	56.4818	53.5289	42.6884
Internal validation criteria					
16.	r^2_{cv} (Q^2loo)	0.8067	0.7854	0.8057	0.7809
17.	$r^2 - r^2_{cv}$	0.0284	0.0363	0.0402	0.0331
18.	$RMSE_{cv}$	0.2867	0.3021	0.2866	0.3024
19.	MAE_{cv}	0.2321	0.2435	0.2290	0.2461
20.	$PRESS_{cv}$	4.4393	4.9275	3.6144	4.0227
21.	CCC_{cv}	0.8943	0.8821	0.8941	0.8788
22.	Q^2_{LMO}	–	–	0.7980	0.7822
23.	r^2_{Yscr}	–	–	0.0924	0.0958
24.	Q^2_{Yscr}	–	–	-0.1713	-0.1686
External validation criteria					
25.	θ^*	–	–	-11.1670°	-9.0760°
26.	$RMSE_{ex}$	–	–	0.3190	0.3897
27.	MAE_{ex}	–	–	0.2716	0.2823
28.	$PRESS_{ext}$	–	–	1.0175	1.5185
29.	r^2_{ex}	–	–	0.7473	0.7231
30.	Q^2_{F1}	–	–	0.7724	0.6757
31.	Q^2_{F2}	–	–	0.7387	0.6430
32.	Q^2_{F3}	–	–	0.7593	0.6362
33.	CCC_{ex}	–	–	0.8369	0.8023
34.	r^2-*ExPy*	0.8068	0.7858	0.8060	0.7811
35.	r'^2_o	0.7671	0.7391	0.7678	0.7292
36.	k'	0.9980	0.9978	0.9983	0.9978
37.	$1 - (r^2/r'^2_o)$	0.0493	0.0595	0.0474	0.0665
38.	r'^2m	0.6460	0.6159	0.6484	0.6031
39.	R^2_o	0.8067	0.7854	0.8057	0.7809
40.	k	0.9999	0.9999	0.9996	0.9999
41.	$1 - (r^2 - ExPy/r^2_o)$	0.0002	0.0005	0.0003	0.0003
42.	r^2m	0.7972	0.7706	0.7930	0.7697

CV and external validation) indicates that the models have statistical acceptability. The high value of internal validation parameters r^2_{cv} (Q^2loo) and Q^2_{LMO} indicates that the derived models are statistically robust. Models 3 and 4 possess high external predictive ability as indicated by high value of CCC_{ex} (> 0.80); this is further supported by high values of Q^2_{F1}, Q^2_{F2}, and Q^2_{F3} (> 0.60). The high predictive ability is vindicated by a high value of r^2_{ex} for Models 3 and 4.

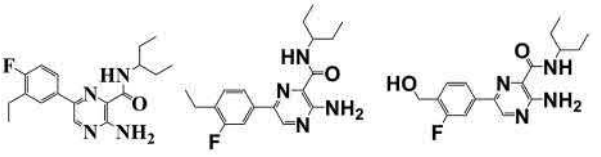
Table 3. Experimental and predicted pEC_{50} by various models along with the status of the molecule.

S.N.	Exp. pEC_{50}	Status for model 1 and 2	Pred. model 1	Pred. model 2	Status model 3	Pred. model 3	Status model 4	Pred. model 4
1	6.3100	Training	5.9519	6.0187	Training	5.9383	Training	5.9561
2	5.5850	Training	5.5679	5.5052	Training	5.5152	Prediction	5.4923
3	6.5850	Training	6.6599	6.5323	Training	6.6100	Training	6.4552
4	6.4950	Training	6.6220	6.5323	Training	6.5810	Training	6.4337
5	5.7700	Training	5.4075	5.5052	Training	5.3920	Training	5.4370
6	6.2760	Training	6.0342	6.0187	Prediction	6.0014	Training	5.9615
7	5.1370	Training	5.5607	5.0451	Training	5.5096	Prediction	5.2072
8	5.5850	Training	5.3093	5.5052	Training	5.3166	Training	5.5381
9	5.5230	Training	5.5121	5.5052	Training	5.4723	Training	5.5824
10	5.2150	Training	5.4076	5.5052	Training	5.3921	Training	5.1697
11	5.3370	Training	5.2272	5.5052	Prediction	5.2536	Prediction	5.1611
12	6.1250	Training	6.3324	6.4226	Training	6.3420	Training	6.4218
13	5.9590	Training	5.8078	6.0187	Training	5.8276	Training	5.9913
14	5.2680	Training	5.6895	5.5586	Prediction	5.7368	Training	5.4268
15	6.0510	Training	5.5574	5.5586	Training	5.6354	Training	5.6678
16	5.0000	Training	5.3445	5.5586	Training	5.4720	Training	5.7388
17	5.9210	Training	5.6280	6.0187	Training	5.6895	Training	6.0005
18	6.4560	Training	6.0655	6.0187	Prediction	6.0255	Prediction	6.0356
19	6.0860	Training	6.2159	6.2206	Training	6.1967	Prediction	6.1824
20	6.3190	Training	5.9965	6.0187	Prediction	5.9724	Training	5.9669
21	6.2440	Training	5.9618	6.0187	Training	5.9458	Training	5.9711
22	6.3870	Training	5.9697	5.5586	Training	5.9519	Training	5.7853
23	6.3470	Training	5.9905	6.0187	Training	5.9679	Prediction	5.3715
24	5.9590	Training	6.1657	6.0187	Training	6.1024	Training	5.9481
25	6.7700	Training	6.6703	6.7193	Training	6.6757	Training	6.7215
26	5.5690	Training	5.5158	6.0187	Prediction	5.6035	Training	5.9775
27	6.3980	Training	6.5026	6.4226	Training	6.4727	Training	6.4026
28	6.1670	Training	5.9992	6.0187	Training	5.9745	Training	5.9535
29	5.6580	Training	5.9637	6.0187	Training	5.9473	Training	5.9717
30	5.8860	Training	5.9366	6.0187	Prediction	5.9265	Training	5.7398
31	5.3470	Training	5.9654	5.5586	Training	5.9486	Training	5.3967
32	5.5380	Training	6.1523	6.0187	Training	6.0921	Training	5.9454
33	5.3280	Training	5.8321	5.5586	Prediction	5.8463	Prediction	5.5336
34	5.4270	Training	5.8992	5.5586	Training	5.8978	Training	5.9472
35	5.5690	Training	5.5359	5.5586	Training	5.6189	Training	5.9876
36	6.7700	Training	6.5957	6.5323	Training	6.5607	Training	6.4788
37	6.9590	Training	7.2413	7.2328	Training	7.2423	Training	7.2173
38	6.3190	Training	6.1893	6.5323	Training	6.2488	Prediction	6.4349
39	7.0970	Training	6.9895	6.9361	Training	6.9748	Training	6.8834
40	7.6020	Training	7.5257	7.5418	Training	7.5540	Training	7.5331
41	7.0000	Training	6.9134	6.7342	Training	6.8605	Training	6.7013
42	6.5850	Training	6.7637	6.9361	Prediction	6.8015	Training	6.9037
43	6.4440	Training	6.4919	6.5323	Training	6.4811	Training	6.4471
44	6.1140	Training	6.2654	6.5323	Training	6.3072	Training	6.4492
45	6.6580	Training	6.8804	6.9361	Training	6.8910	Training	6.8946
46	6.6990	Training	6.7132	6.5323	Training	6.6509	Prediction	6.5316
47	7.4560	Training	7.2735	7.2328	Training	7.2670	Training	7.2462
48	7.1490	Training	7.0749	6.9361	Training	7.0404	Training	6.9132
49	7.6200	Training	7.5350	7.5418	Training	7.5612	Training	7.5651
50	7.2440	Training	7.0186	6.7342	Prediction	6.9413	Prediction	6.7414
51	6.8240	Training	6.8358	6.9361	Training	6.8568	Training	6.9291
52	6.6200	Training	6.5221	6.5323	Training	6.5043	Training	6.4587
53	6.3010	Training	6.3190	6.5323	Training	6.3483	Training	6.4570
54	6.9590	Training	6.9099	6.9361	Training	6.9137	Training	6.9055

QSTR model

The derived QSTR model along with its statistical parameters is as follows:

$$pEC_{50} = + 1.266\ (\pm\ 1.401) + 0.268\ (\pm\ 0.107)\ *\ C\_don\_7A - 0.305\ (\pm\ 0.153)\ *\ RDF135s$$



pEC_{50}: Model-3 (μM):	0.052	0.088	0.094
Model-4 (μM):	0.048	0.078	0.096
QSTR Model (μM):	309.72	79.23	1499.85

Figure 3. Plausible 6-arylpyrazine-2-carboxamide derivatives with low toxicity (predicted by the QSTR model) and high activity (predicted by models 3 and 4).

N_{tr} = 13, Q^2_{loo} = 0.675, r^2_{tr} = 0.824, r^2_{adj} = 0.789, K_{xx} = 0.089, ΔK = 0.366, $RMSE_{tr}$ = 0.081, $RMSE_{cv}$ = 0.109, F = 23.399, CCC_{tr} = 0.904, CCC_{cv} = 0.832, MAE_{tr} = 0.064, MAE_{cv} = 0.087, RSS_{tr} = 0.084, $PRESS_{cv}$ = 0.155, s = 0.092

From the various statistical parameters, it is clear that the developed QSTR model is statistically robust with acceptable predictive ability, and satisfies the threshold values for many statistical parameters. The descriptor C_don_7A stands for the presence of a donor atom and carbon atom within 7 Å of each other. The positive coefficient for C_don_7A indicates that the presence of the donor atom and carbon atom within 7 Å of each other has strong correlation with the toxicity. RDF135s, a radial distribution function descriptor, which stands for radial distribution function – 135 / weighted by relative I-state, has negative correlation with toxicity. Its value must be lowered to reduce the cytotoxicity. The molecular descriptor values have been tabulated in Table S3 in the online supplementary material.

To summarize, QSA(T)R analysis reveals that for better *T. brucei* inhibitory activity and reduced toxicity the following 6-arylpyrazine-2-carboxamide derivatives could be plausible synthetic targets (see Figure 3).

Conclusions

In conclusion, the QSAR analysis reveals that the anti-HAT activity of 6-arylpyrazine-2-carboxamides has correlation with the frequency of occurrence of C and O at a topological distance of nine and the *N-sec*-butylformamide fragment. The QSA(T)R models are statistically robust with high external predictive ability. In addition, the idea of developing multiple QSAR models helped in identifying more significant descriptors. The developed models could be useful for future optimization of lead candidates.

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Short Communication

Eco-Friendly Synthesize and Biological Evaluation of 2-Amino-5-substituted-1,3,4-thiadiazoles

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Abstract

In the present work, we synthesized the 2-amino-5-substituted-1,3,4-thiadiazole moiety and its different derivatives. The preparation of above 1,3,4-thiadiazole involves cyclisation of aromatic acid with thiosemicarbazide in presence of few drops of $POCl_3$ as dehydrating agent. The derivatives, mostly Schiff' bases, were synthesized using 'Green Chemistry' approach. The reactions are simple one step reactions.The purity of derivatives confirmed by Thin Layer Chromatography. IR spectra was recorded on FT-IR SHIMADAZU, and X-ray Diffraction by RIGAKUMINIFLEXII. The synthesized compounds were tested for their antimicrobial activity against three microorganisms namely E-coli, S. Aureus and P. Seudomonas, and the minimum inhibitory concentrations (MICs) of the tested compounds were determined by the dilution method using Ampicillin, Chloramphenicol, Tetrecyclin.

Keywords: 1, 3, 4-thiadiazole, Synthesis, Antibacterial, Antifungal activities.

Introduction

Heterocyclic[1] are very important in biological activity and in industries[2]. One such important heterocyclic ring is 1, 3, 4-thiadiazole ring. It is a 5-membered heterocyclic ring in which nitrogen are at 3rd and 4th position and the sulphur is at 1st position.

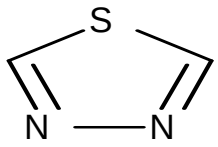


Figure-1
General structure of 1,3,4,-thiadiazole

1, 3, 4-thiadiazole and its derivatives are widely applied in medicine[3] and agriculture[4] as pesticides[5].

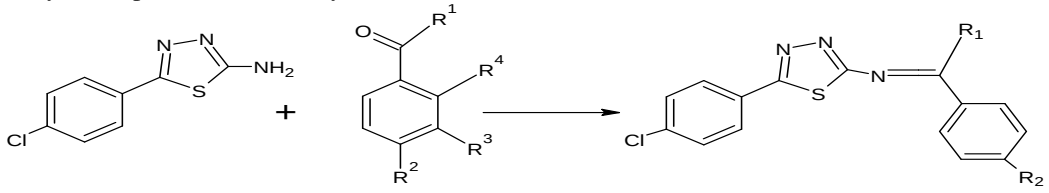
Nowadays, the research field dealing with Schiff base coordinationchemistry has expanded enormously[6].

Schiff bases resulted from aromatic aldehydes. Schiff bases have been reported in their biological properties, such as, antibacterial[7], antifungal activities[8-10].

Experimental data: In experimental study the melting point were taken in capillary tube at a room temperature which are incorrected. All derivatives are pure by crystallization process and the purity of derivatives confirmed by TLC. Using solvent system of Glacial acetic acid and Ethylacetate 1:1 ratio using iodine as a visualizing agent. FT-IR (SHIMADAZU), X-ray Diffraction- RIGAKUMINIFLEXII,

Methodology

General method for synthesis of Schiff bases from Mas131 to Mas135: A mixture of substituted thiadiazole and substituted aromatic aldehyde in glacial acetic acid was refluxed for two hours, cooled and poured cold water with stirring till precipitation was complete.



Where R^1 = H, CH_3 R^2 = Cl , -$N(CH_3)_2$, H R^3 = NO_2,H R^4 = OH,H

Scheme-1
General method for synthesis of Schiff bases

Results and Discussion

The data of physical properties of synthesized Schiff bases are given in Table-1. All compounds are studied by IR, NMR, Mass spectrometry, X-ray diffraction.

Characterization- FT-IR Spectra in cm^{-1}: Mas 131-1662.64(C=N bond), 1595.13(N-H bend), 1438.90(C-H bend), 812.03(para substitution), 729.09(C-Cl bond). Mas 132 - 3736.12 (-OH bond), 3088.03(C-H stretch), 831.32 (meta substitution-OH), 669.30(C-Cl stretch). Mas 134 - 3282.84(N-H stretch), 1508.33(Ar C=C stretch), 775.38(C-Cl stretch)

H^1NMR (DMSO, 400MH),: 7.3 (d. 1H, J=7.31), 7.5 (d, 1H, J=7.48), 7.9 (d,1H, J=7.93) 6.8 (s, J=6.83).

Table-1
Characterization

Sr no.	Molecular formula	M. Pt. ^{0}C	R1	R2	R3	R4	Yield (%)	Mol.wtgm/mole
S1	$C_8H_6N_3SCl$	128						221.5
Mas131	$C_{17}H_{14}N_4SCl$	90	H	$N(CH3)_2$	H	H	25	341.5
Mas132	$C_{15}H_9N_3SClO$	219	H	H	H	OH	20	314.5
Mas133	$C_{15}H_8N_3SCl_2$	160	H	Cl	H	H	30	333
Mas134	$C_{15}H_8N_4SClO_2$	110	H	H	NO_2	H	20	333
Mas135	$C_{16}H_{12}N_3SClO$	180	CH_3	H	H	OH	35	343.5

Table-2
Crystal data and structure refinement for SA1 Molecule

Empirical formula	C6H8NCl
Temperature	293K
Formula weight	179.2
Crystal system	Centro symmetric
Unit cell dimensions	a = 9.3, b = 7.25A˚, 91.5^0,c = 11.0A˚

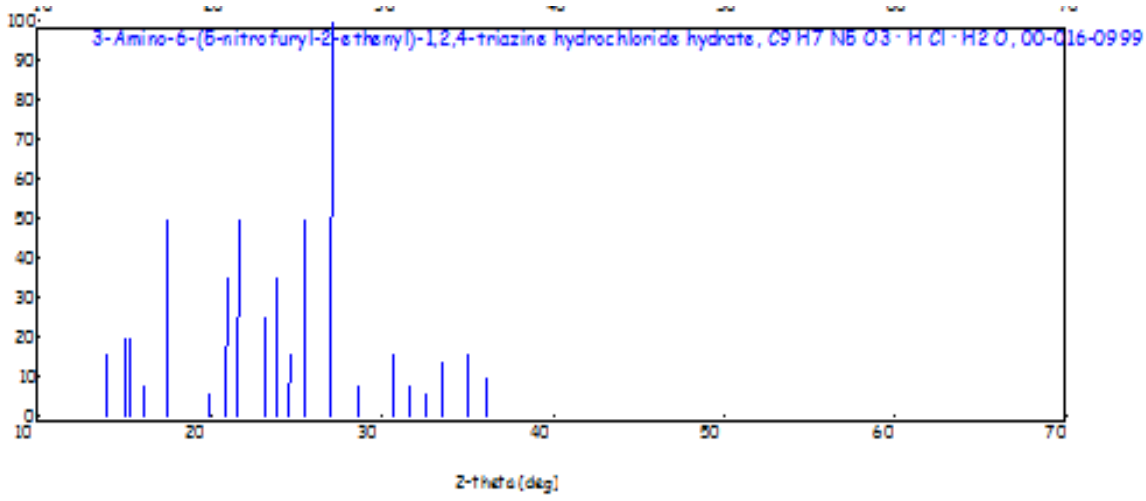


Figure-2
Characterization of X-ray diffraction

Table-3
Biological assay: Antibacterial study of Schiff base

Sr.No.	*S.aureus*	*P.Seudomonas*	*E. coli*
S1	15	17	15
Mas1	19	16	19
Mas2	15	16	19
Mas3	17	13	20

Conclusion

An environmental benign method was adopted to synthesize thiadiazole and its derivatives. The method is economical and very efficient[9]. The yield is quite high with good purity of the molecules. The molecules have good anti-microbial activity[10].

In the present work, various derivatives of 1,3,4-thiadiazole were synthesized by using aromatic carboxylic acid as starting material with moderate to good yield. The method is atom economic, easy and efficient and eco –friendly. The method has advantages of cheaper chemicals and safely too. The method has additional advantage of easy work up and the compounds are obtained in high purity without any tedious separation. Thus, the method has good number of advantages. The Rf values, determined for two molecules viz. compound number 1 and its derivatives, are close to 0.5. The structure confirmed by the FT-IR spectroscopy and X-ray diffraction studies gives the crystalline nature of the compounds.

The biological assay indicates high antimicrobial activity against ***E. coli***, ***S. Aureus*** and ***P. Seudomonas***[5]. For some compounds the activity is better than the reference drugs. This indicates that the molecules are good candidates for lead optimization.

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Synthesis of 3-Aroyl Flavanones by Using Microwave Irradiation and Study of Its Antibacterial Activity

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Abstract: Newly substituted 3 Aroyl flavanones were synthesised by using microwave irradiation. The structure of compounds was elucidate by spectral analysis (IR and NMR). The synthesised compounds were screened for their antibacterial activity.

Keywords:-3-aroyl flavanone, β- Diketone, antibacterial activity.

INTRODUCTION

In the resent years there is much biomedical interest in flavanoids because of their lot of beneficial effects on human being. Flavanoids are naturally occurring polyphenolic compounds with flavones nucleus having anti-oxidant, anti-tumour, anti-ulcer, anti-inflammatory activities. They are available as flavanone, flavanol, isoflavane, flavones and their derivatives. The flavanoides basically possess 15 carbon skeletons (C6-C3-C6). Flavanoides are mainly found in onions, apples, red wine, blueberries, grapes and tea. Flavanones are the important class of flavanoids containing a 2-phenyl-benzopyran-4-one skeleton, Flavanones are commonly found in various citrus fruits and vegetables[1-3].Synthetic flavanones have attracted considerable attention because of their various pharmacological properties including antifungal[4,5], antibacterial[4,6,7],analgesic[7], antioxidant[7].Flavanones are antioxidants, preventing heart disease. They are used as a potential cancer chemopreventive agents[8, 9].

MATERIALS AND METHODS

All the laboratory chemicals and solvents required for the study were of highest purity commercially available. Melting points of all synthesised compounds were determined by melting point apparatus. The purity of synthesised compounds was checked by thin layer chromatography on silica –G layers.

IR spectra were recorded on FTIR spectrophotometer using KBR pallets. NMR spectra were recorded on Bruker Avance II 400 NMR spectrometer.

Preparation of 5-chloro-2-hydroxy-4-methyl acetophenone(1): p-chloro-m-cresyl acetate was prepared by acetylation of p-chloro-m-cresol. Then by Fries migration 5-chloro-2-hydroxy-4-methyl acetophenone was obtained.

Preparation of substituted 2-benzoyloxy acetophenones (2 a-b):5-chloro-2-hydroxy-4-methyl acetophenone (1) (0.04 mol) and aromatic carboxylic acid (0.05 mol) were dissolved in pyridine and $POCl_3$ is added drop by drop with constant stirring till the viscous mass is obtained. Maintain the temperature below 10^0c during the addition of $pocl_3$ to the reaction mixture. The reaction mixture is allow to stand for overnight at room temperature .The reaction mixture is decomposed by 10% HCl. The product thus separated was filtered, washed with water followed by sodium bicarbonate (10% solution) and then again washed with water. The solid product was crystallised from ethanol to obtained corresponding 2-benzoyloxy acetophenones.

5-chloro-2-(4’ chloro benzoyloxy)-4-methyl acetophenone (2a) was prepared by above method by using 4-chloro benzoic acid. Similarly 5 -chloro -2-(2’-4’dichloro benzoyloxy) -4-methyl acetophenone (2b) was prepared by using 2-4 dichloro benzoic acid.

Preparation of 1-(2’-hydroxy aryl)-3-aryl propane-1, 3-diones (3 a-b): 2-substituted benzoyloxy acetophenones (2a-b) (0.05 mol) was dissolved in dry pyridine (40) ml. The solution was warmed up to about 60^0c and pulverised KOH (0.15 mol) was added slowly with constant stirring. After four hours the reaction mixture was acidified by adding ice cold dilute HCl (1:1). The solid product thus separated was filtered, washed with sodium bicarbonate solution (10%) and finally with water. It is then crystallised from ethanol acetic acid mixture to get 1-(2’-hydroxy aryl)-3-aryl propane-1, 3-diones (3 a-b).

Preparation of some new substituted 3-aroyl flavanones (4 a-c):

***Under microwave irradiation*:** Synthesis of flavanone was carried out by mixing appropriate amount of 1-(2’-hydroxy aryl)-3-aryl propane-1, 3-diones (3 a-b) (2.69 m. mol) and aromatic aldehydes (2.69 m. mol) in presence of catalytic amount of aqueous KOH in methanol and irradiating in microwave at 100 W for two minutes. The reaction mixture was cooled, poured into crushed ice and then conc. HCl was added. The mixture was left to stay at 2-3^0c overnight and the separated solid was collected by filtration, washed with water and recrystalized from methanol to give desired product.

2 (2’ chloro)-3-(4’-chloro benzoyl)-6-chloro-7-methyl flavanone (4a) was prepared by above method by using 1 -(2’hydroxy-4’methyl-5’ chloro phenyl)-3(4’ chloro phenyl) propane 1-3-dione (3a) and 0-chloro benzaldehyde. Similarly 2 (4’ chloro)-3-(2’4’-dichloro benzoyl)-6-chloro-7-methyl flavanone (4b) and 2 (2’ chloro)-3-(2’4’-dichloro benzoyl)-6-chloro-7-methyl flavanone (4c) were prepared by using 1-(2’hydroxy -4’methyl-5’ chloro phenyl)-3(2’4’di chloro phenyl) propane 1-3-dione (3b) and p-chloro benzaldehyde and o-chloro benzaldehyde respectively.

Table 1: Chemical data of the compound:

Compound no.	R_1	R_2	Mol. Formulae	M.P.(^{0} C)	Yield (%)
2a	Cl	H	$C_{16}H_{12}Cl_2O_3$	120	63%
2b	Cl	Cl	$C_{16}H_{11}Cl_3O_3$	105	67%
3a	Cl	H	$C_{16}H_{12}Cl_2O_3$	162	72%
3b	Cl	Cl	$C_{16}H_{11}Cl_3O_3$	125	69%

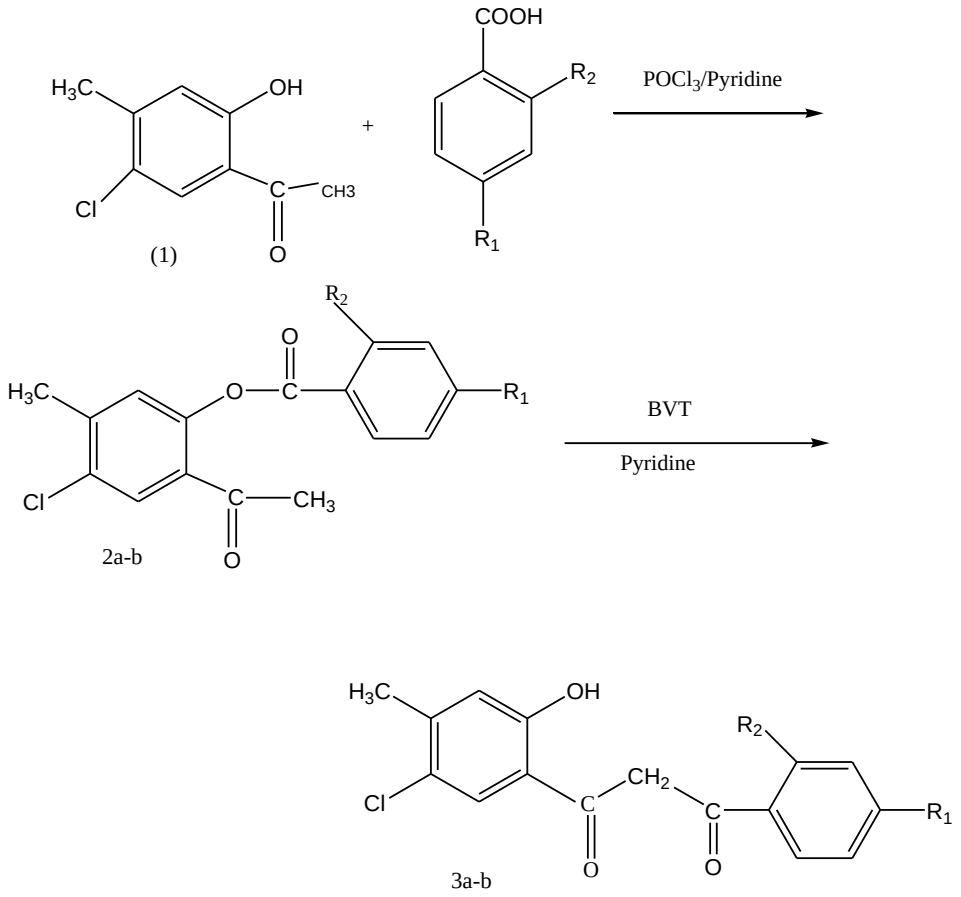

Fig.1: Experimental scheme for the synthesis of 1-(2'-hydroxy aryl)-3-aryl propane-1,3-diones(3 a-b)

Table 2: Chemical data of flavanones:

Compound codes	Compounds names	Mol. Formula	M.P (^{0}c)	% Yield
4a	2 (2' chloro)-3-(4'-chloro benzoyl)-6-chloro-7-methyl flavanone	$C_{23}H_{15}O_3Cl_3$	115	69%
4b	2 (4' chloro)-3(2'4'dichlorobenzoyl)-6-chloro-7-methyl flavanone	$C_{23}H_{14}O_3Cl_4$	110	65%
4c	2 (2' chloro)-3(2'4'dichlorobenzoyl)-6-chloro-7-methyl flavanone	$C_{23}H_{14}O_3Cl_4$	124	73%

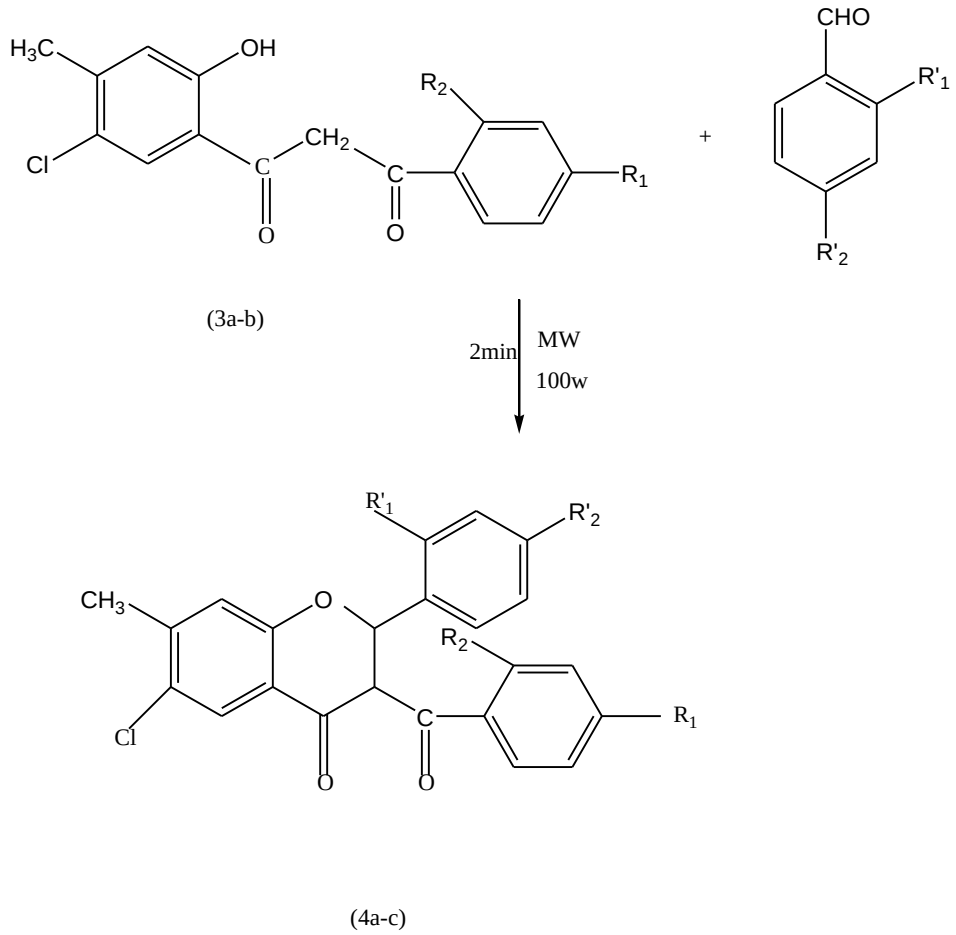

Fig.3: Experimental scheme for the synthesis of substituted 3-aroyl flavanones (4 a-c).

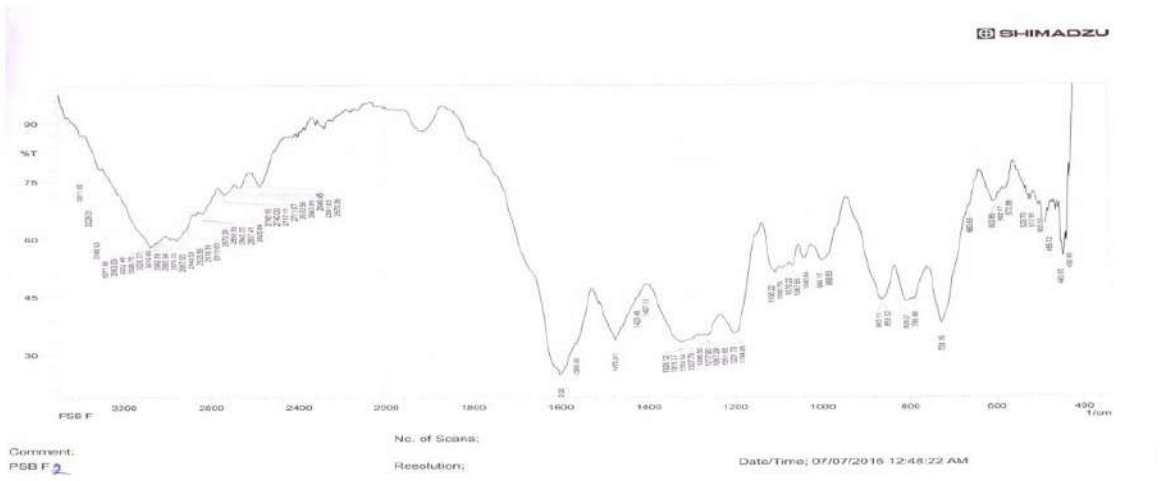

Fig.4: IR spectra of 2 (2’ chloro)-3-(4’-chloro benzoyl)-6-chloro-7-methyl flavanone

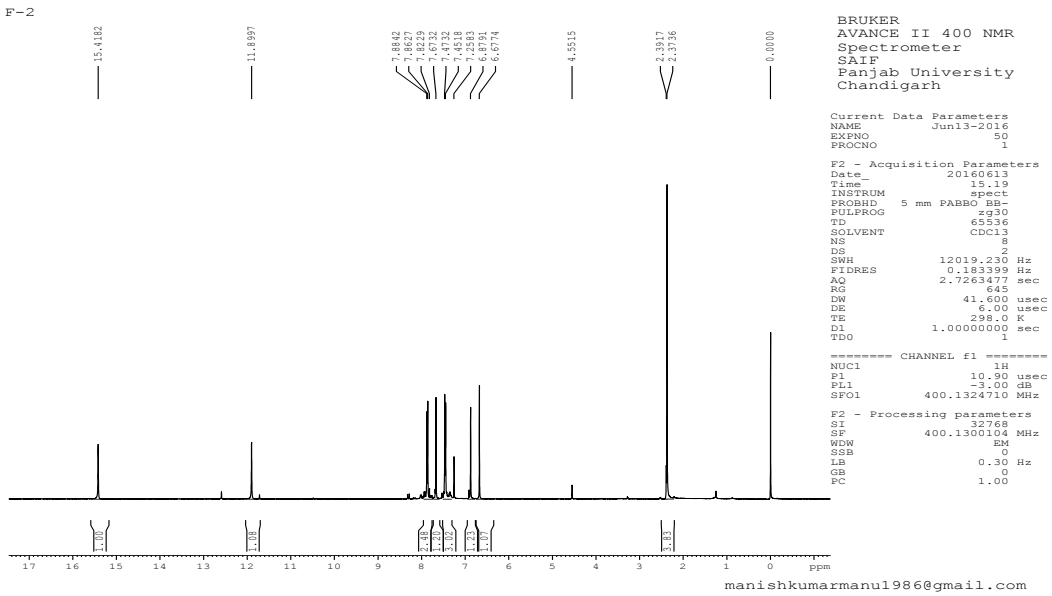

Fig.5: ^{1}HNMR spectra of 2 (2’ chloro)-3-(4’-chloro benzoyl)-6-chloro-7-methyl flavanone

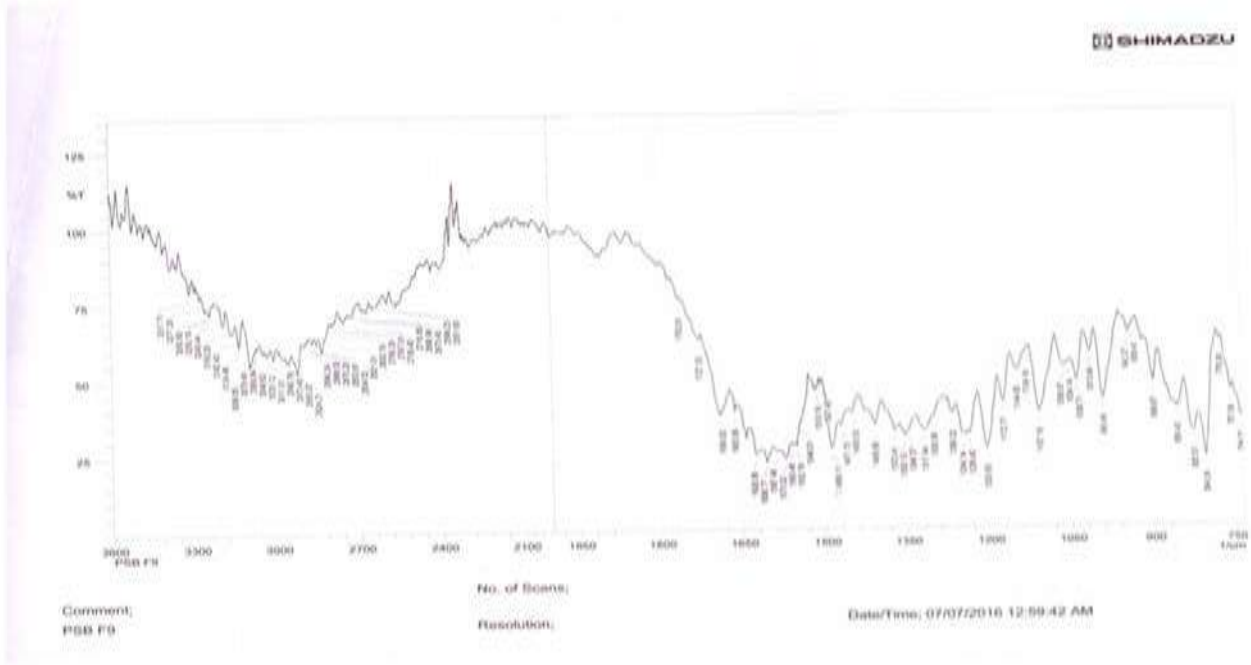

Fig.6: IR spectra of 2 (4’ chloro)-3(2’4’dichlorobenzoyl)-6-chloro-7-methyl flavanone.

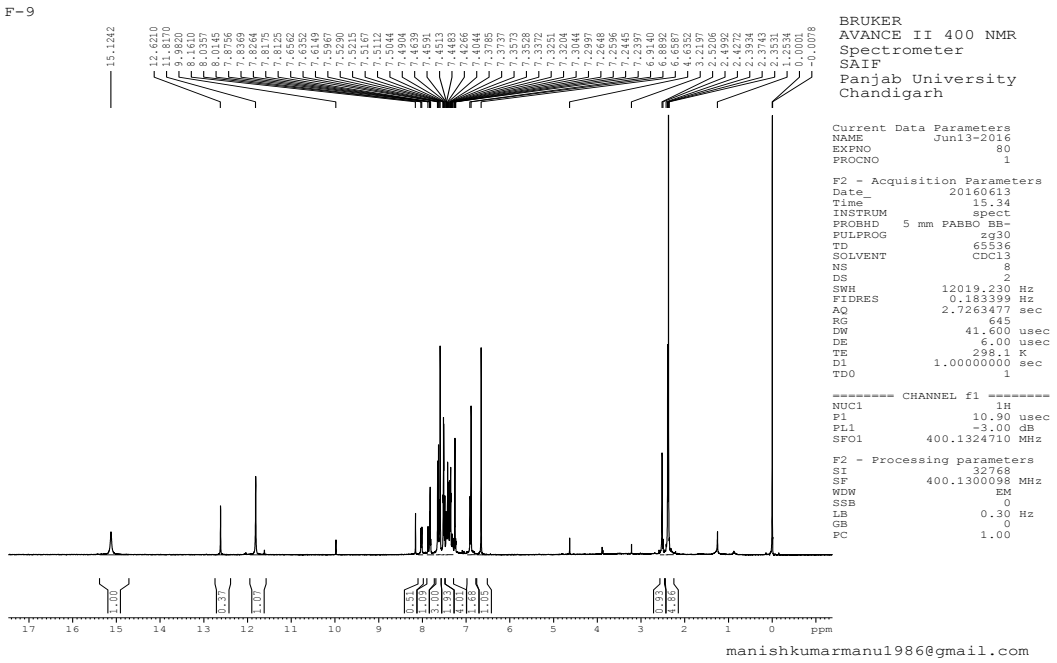

Fig.7:[1]HNMR spectra of 2 (4’ chloro)-3(2’4’dichlorobenzoyl)-6-chloro-7-methyl flavanone.

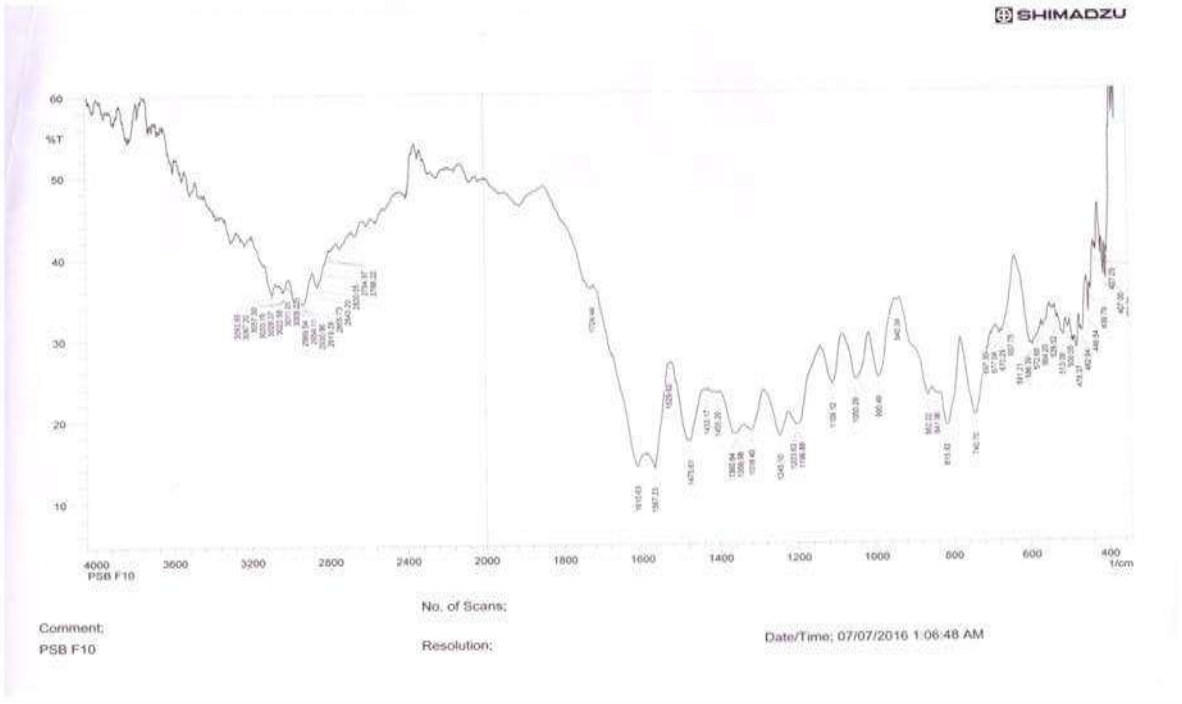

Fig.8: IR spectra of2 (2’ chloro)-3(2’4’dichlorobenzoyl)-6-chloro-7-methyl flavanone

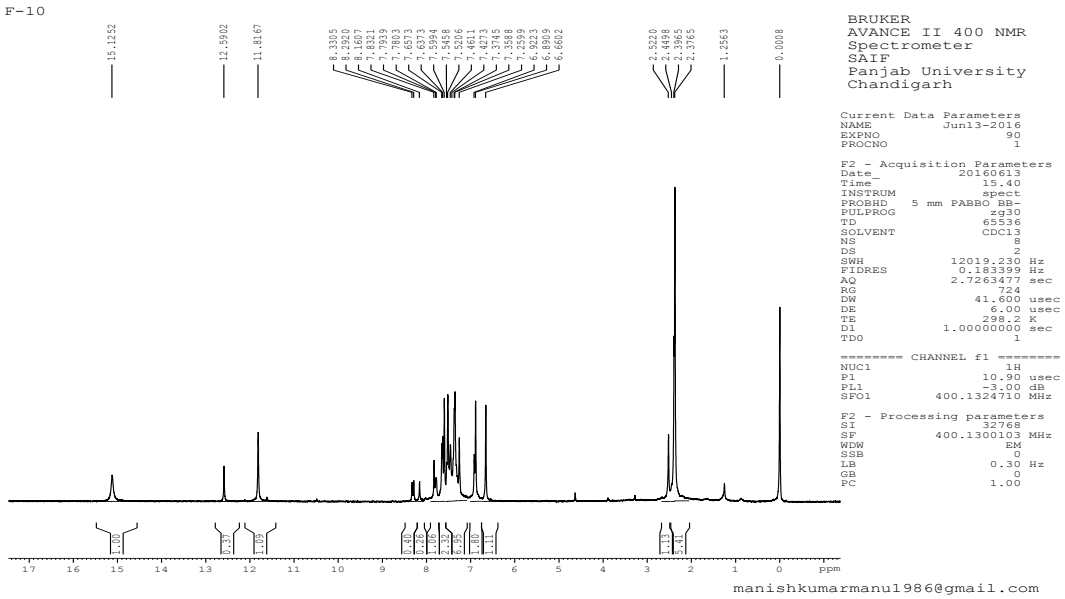

Fig.9: [1]HNMR spectra of 2 (2’ chloro)-3(2’4’dichlorobenzoyl)-6-chloro-7-methyl flavanone

Antibacterial activity: All the synthesised flavanones were screened for their antibacterial activity against S. Aureus, E. Coli and S. typhi using ciprofloxacin as a standard drug. Agar diffusion method was employed to study the activity. Initially, the stock culture of bacteria were revived by inoculating on broth media and grown at 37^0c for 18 hrs. The agar plates of the above media were prepared and wells were made in the plate. Each plate was inoculated with 18 hr. old culture and spread evenly on the plate. After 20 min, the wells were filled with the compound and antibiotic at different concentrations. All the plates were incubated at 37^0c for 24 hr. and the diameter of inhibition zone were noted.

Table 3: Antimicrobial activity of synthesised flavanones.

Sr. NO.	Compounds	Antibacterial activity inhibition zone (mm)		
		S.aureus	E.coli	S.typhi
1	4a	9	4	8
2	4b	9	6	6
3	4c	13	9	9

RESULT AND DISCUSSION

In this work, total three flavanones namely 4a-c are synthesised from different aromatic aldehydes by using microwave irradiation. All the synthesised flavanones were screened for their antibacterial activity against *S. Aureus*, *E. coli* and *S. Typhi*. From the data on Antibacterial activities given in table 3, it was observed that, compound 4a showed moderate activity against S. Aureus. while week activity against E. Coli and S. Typhi. Compound 4b also showed moderate activity against S. Aureus and week activity against *E. coli* and *S.Typhi*. Compound 4c showed moderate activity against all the three bacteria.

CONCLUSION

In the present study synthesis of flavanone using microwave provide practical alternative to the existing method for the synthesis of flavanone. This method is fast and clean so found to be beneficial according to time, improved yield and environmentally sound. From the antibacterial screening it is observed that all the synthesised flavanones exhibit activity against bacteria. All the synthesised flavanones show good activity against S.aureus gram positive bacteria than gram negative bacteria.

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Synthesis, Characterization and Antimicrobial Activity of Newly Substituted 3-Aroyl Flavanones

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ABSTRACT

One of the intention of medicinal chemistry research is to develop compounds that show desirable biological activity and easily accessible. Newly substituted 3 Aroyl flavanones were synthesised by using β- Diketones. The structure of compounds were elucided by specral analysis (IR and NMR). The synthesised compounds were screened for their amtimicrobial activity.

Keywords: 3-aroyl flavanone, β- Diketone, antimicrobial activity.

INTRODUCTION

Flavanoids are naturally occurring polyphenolic compounds with flavones nucleus having anti-oxidant, anti-tumor, anti-ulcer, anti-inflammatory activities. They are available as flavanone, flavanol, isoflavane ,flavones and their derivatives. The flavanoides basically possess 15 carbon skeleton (C6-C3-C6). Flavanoides are mainly found in onions, apples, red wine, blueberries, grapes and tea (Bylka *et al*., 2004). Flavanones are the important class of flavanoids containing a 2-phenyl-benzopyran-4-one skeleton, Flavanones are commonly found in various citrus fruits and vegetables.[1-3]Synthetic flavanones have attracted considerable attention because of their various pharmacological properties including antifungal[4,5], antibacterial[4,6,7], analgesic[7], antioxidant[7].

MATERIALS AND METHODS

All the laboratory chemicals and solvents required for the study were of highest purity commercially available. Melting points of all synthesised compounds were determined by melting point apparatus. The purity of synthesised compounds was checked by Thin layer chromatography on silica –G layers. IR spectra were recorded on FTIR spectrophotometer using KBR pallets. NMR spectra were recorded on Bruker Avance II 400 NMR spectrometer.

Preparation of 5-chloro-2-hydroxy-4-methyl acetophenone (1)

p-chloro -m-cresyl acetate was prepared by acetylation of p-chloro-m-cresol. Then by Fries migration 5-chloro-2-hydroxy-4-methyl acetophenone was obtained.

Preparation of substituted 2-benzoyloxy acetophenones (2 a-b)

5-chloro- 2-hydroxy-4-methyl acetophenone (1) (0.04 mol) and aromatic carboxylic acid (0.05 mol) were dissolved in pyridine and $pocl_3$ is added drop by drop with constant stirring till the viscous mass is obtained. Maintain the temperature below 10^0c during the addition of $pocl_3$ to the reaction mixture. The reaction mixture is allow to stand for overnight at room temperature .The reaction mixture is decomposed by 10% HCl. The product thus separated was filtered, washed with water followed by sodium bicarbonate (10% solution) and then again washed with water. The solid product was crystallised from ethanol to obtained corresponding substituted 2-benzoyloxy acetophenones.

5-chloro-2-(4' chloro benzoyloxy)-4-methyl acetophenone (2a) was prepared by above method by using 4-chloro benzoic acid. Similarly 5-chloro-2-(4' methoxy benzoyloxy) -4-methyl acetophenone (2b) was prepared by using p-methoxy benzoic acid.

Preparation of 1-(2'-hydroxy phenyl)-3-phenyl propan-1,3-diones (3 a-b)

2-substituted benzoyloxy acetophenones (2a-b) (0.05 mol) was dissolved in dry pyridine (40 ml) . The solution was warmed up to about 60^0c and pulverised KOH (0.15 mol) was added slowly with constant stirring. After four hours the reaction mixture was acidified by adding ice cold dilute HCl (1:1). The solid product thus separated was filtered, washed with sodium bicarbonate solution (10%) and finally with water. It is then crystallised from ethanol acetic acid mixture to get 1-(2'-hydroxy phenyl)-3-phenyl propane 1,3--diones (3 a-b)

Preparation of some new substituted 3-aroyl flavanones (4 a-d)

Under microwave irradiation

Synthesis of flavanone was carried out by mixing appropriate amount of 1-(2'-hydroxy phenyl)-3-phenyl propane-1,3-diones (3 a-b) (2.69 m. mol) and aromatic aldehydes (2.69 m. mol) in presence of catalytic amount of aqueous KOH in methanol and irradiating in microwave at 100 W for two minutes. The reaction mixture was cooled, poured into crushed ice and then conc. HCl was added. The mixture was left to stay at 2-3^0c overnight and the separated solid was collected by filtration, washed with water and recrystalized from methanol to give desired product.

2 (4' chloro)-3-(4'-chloro benzoyl)-6-chloro-7-methyl flavanone (4a) was prepared by above method by using 1 -(2'hydroxy-4'methyl-5' chloro phenyl)-3(4' chloro phenyl) propane 1-3-dione (3a) and p-chloro benzaldehyde. 2 (4' methoxy)-3-(4'-chloro benzoyl)-6-chloro-7-methyl flavanone (4b) was prepared by above method by using (3b) and p-methoxy benzaldehyde. Similarly 2 (4'chloro)-3(4'-methoxy benzoyl)-6-chloro-7-methyl flavanone (4c) and 2(2'chloro)-3(4'methoxy benzoyl)-6-chloro-7 methyl flavanone (4d)were prepared

by using 1-(2'hydroxy -4'methyl-5' chloro phenyl)-3(4'methoxy phenyl) propane 1-3-dione (3b) and p-chloro benzaldehyde and o-chloro benzaldehyde respectively.

Table 1: Chemical data of substituted acetophenones and diones

Compound code	R_1	R_2	Mol. Formulae	M.P.(^{0} C)	Yield (%)
2a	Cl	H	$C_{16}H_{12}Cl_2O_3$	120	63%
2b	OCH_3	H	$C_{17}H_{15}ClO_4$	80	50%
3a	Cl	H	$C_{16}H_{12}Cl_2O_3$	162	72%
3b	OCH_3	H	$C_{17}H_{15}ClO_4$	142	57%

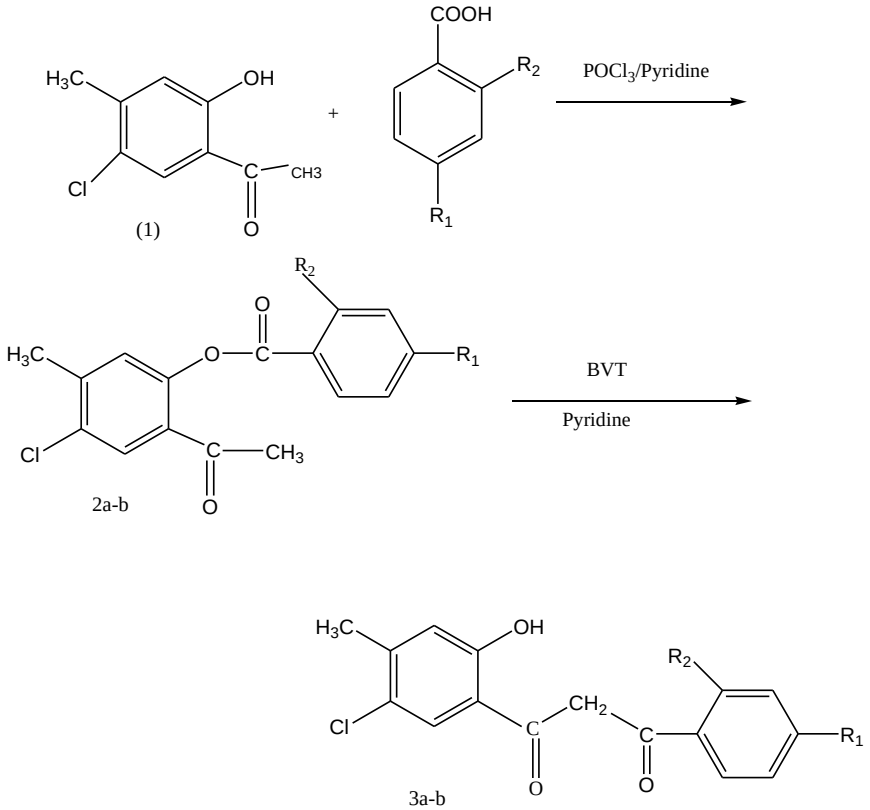


Fig.1: Experimental scheme for the synthesis of 1-(2'-hydroxy phenyl)-3-phenyl propane-1,3-diones (3 a-b)

Table 2: Chemical data of newly synthesised flavanones

Compound code	Name of compounds	Mol. Formulae	M.P (^{0}c)	% Yield
4a	2 (4' chloro)-3-(4'-chloro benzoyl)-6-chloro-7-methyl flavanone	$C_{23}H_{15}O_3Cl_3$	120	71%
4b	2 (4' methoxy)-3-(4'-chloro benzoyl)-6-chloro-7-methyl flavanone	$C_{24}H_{18}O_4Cl_2$	130	61%
4c	2 (4'chloro)-3(4'-methoxy benzoyl)-6-chloro-7-methyl flavanone	$C_{24}H_{18}O_4Cl_2$	145	55%
4d	2(2'chloro)-3(4'methoxy benzoyl)-6-chloro-7 methyl flavanone	$C_{24}H_{18}O_4Cl_2$	152	59%

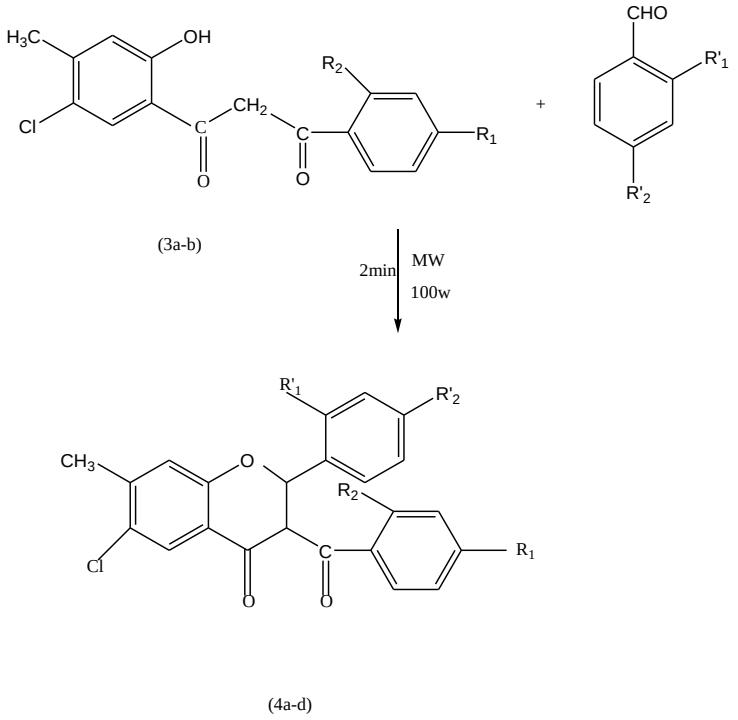


Fig.2: Experimental scheme for the synthesis of substituted 3-aroyl flavanones (4 a-d).

Spectral data of flavanones

Spectral data of 4a:

IR: 3071 cm^{-1} (Aromtic C-H stretch), 1748 cm^{-1}(C=O stretch), 1498 cm^{-1} (Aromatic C=C stretch), 2948 cm^{-1}(C-H stretch of methyl group), 1197 cm^{-1}(C-O-C stretch), 796 cm^{-1} (Aromatic substitution C-Cl), H NMR: δ 2.38 (S, 3H of CH_3), δ 4.5 (S, 1H of CH attached to C=O), δ 1.2(S,1H of CH attached to O), δ 6.8-8.0 (m,10H of Ar-H), δ 11.9 (tautomerism).

Spectral data of 4b:

IR: 3053 cm^{-1} (Aromtic C-H stretch), 1691 cm^{-1}(C=O stretch), 1567 cm^{-1} (Aromatic C=C stretch), 2970 cm^{-1}(C-H stretch of methyl group), 1196 cm^{-1}(C-O-C stretch), 795 cm^{-1} (Aromatic substitution C-Cl), H NMR: δ 2.38 (S, 3H of CH_3), δ 4.6 (S, 1H of CH attached to C=O), δ 1.3(S,1H of CH attached to O), δ 3.8 (S,3H of O CH_3), δ 6.6-7.8 (m,10H of Ar-H), δ 11.8 (tautomerism).

Spectral data of 4c:

IR: 3017 cm^{-1} (Aromtic C-H stretch), 1748 cm^{-1}(C=O stretch), 1565 cm^{-1} (Aromatic C=C stretch), 2840 cm^{-1}(C-H stretch of methyl group), 1205 cm^{-1}(C-O-C stretch), 794 cm^{-1} (Aromatic substitution C-Cl), H NMR: δ 2.38 (S, 3H of CH_3), δ 4.5 (S, 1H of CH attached to C=O), δ 2.4(S,1H of CH attached to O), δ 3.8 (S,3H of O CH_3), δ 6.6-7.9 (m,10H of Ar-H), δ 11.8 (tautomerism).

Spectral data of 4d:

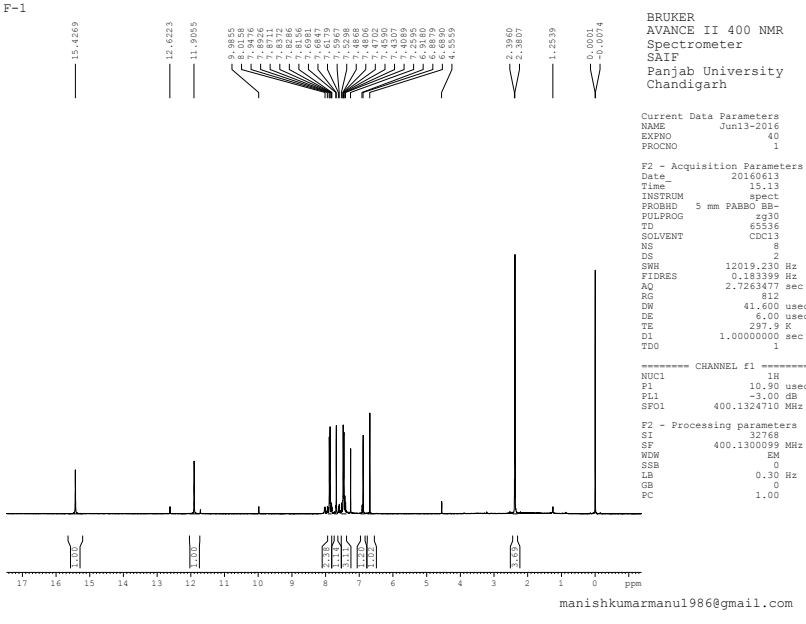
IR: 3065 cm^{-1} (Aromtic C-H stretch), 1737 cm^{-1}(C=O stretch), 1612cm^{-1} (Aromatic C=C stretch), 2995cm^{-1}(C-H stretch of methyl group), 1179 cm^{-1}(C-O-C stretch), 729 cm^{-1} (Aromatic substitution C-Cl), H NMR: δ 2.39 (S, 3H of CH_3), δ 4.5 (S, 1H of CH attached to C=O), δ 3.1(S,1H of CH attached to O), δ 3.8 (S,3H of O CH_3), δ 6.6-7.9 (m,10H of Ar-H), δ 12.5 (tautomerism).

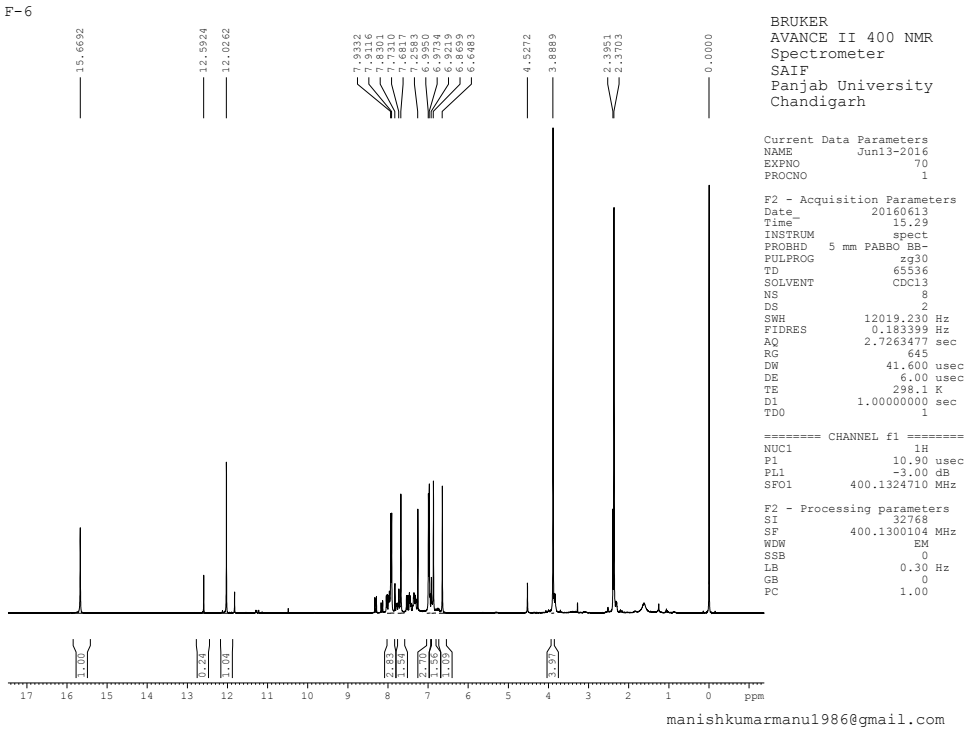
Antibacterial activity

All the synthesised flavanones were screened for their antibacterial activity against S. Aureus, E. Coli and S. typhi using ciprofloxacin as a standard drug. Agar diffusion method was employed to study the activity. Initially, the stock culture of bacteria were revived by inoculating on broth media and grown at 37^0c for 18 hrs. The agar plates of the above media were prepared and wells were made in the plate. Each plate was inoculated with 18 hr. old culture and spread evenly on the plate. After 20 min, the wells were filled with the compound and antibiotic at different concentrations. All the plates were incubated at 37^0c for 24 hr. and the diameter of inhibition zone were noted.

Table 3: Antimicrobial activity of newly synthesised flavanones(4a-d)

Sr. NO.	Compound codes	Antibacterial activity inhibition zone (mm)		
		S.aureus	E.coli	S.typhi
1	4a	7	10	7
2	4b	5	8	6
3	4c	5	5	6
4	4d	6	8	11





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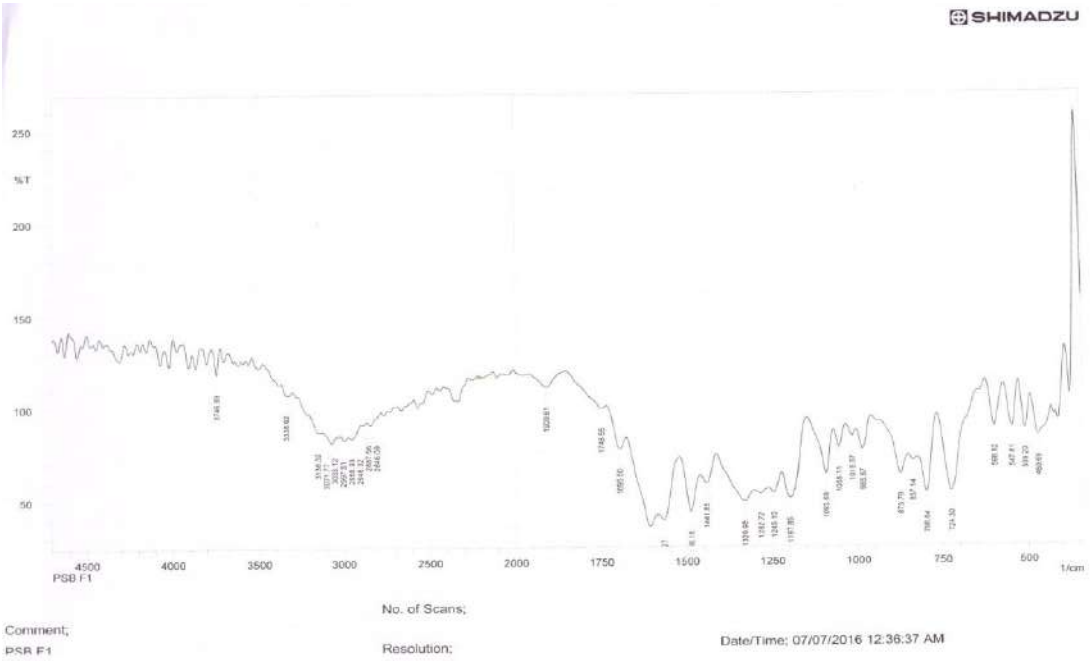

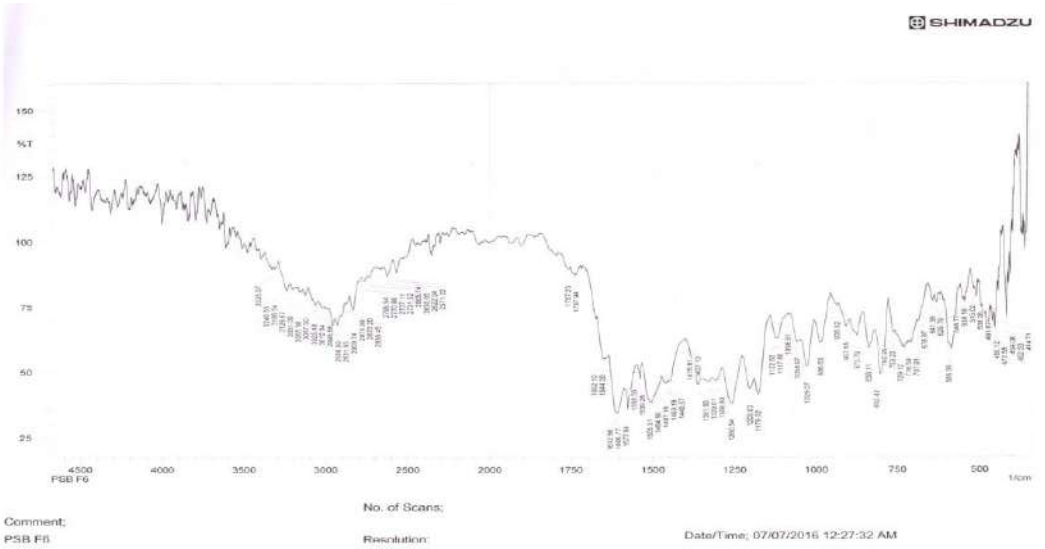

RESULT AND DISCUSSION

In this work, total four flavanones namely 4a-d are synthesised from different aromatic aldehydes. All the synthesised flavanones were screened for their antibacterial activity against S. Aureus, E. Coli and S. Typhi. From the data on Antibacterial activities given in table 3, it was observed that, compound 4a showed moderate activity against E. Coli while week activity against S. Aureus and S. Typhi. Compound 4b showed moderate activity against E. Coli and week activity against S. Aureus and S.Typhi. Compound 4c showed comparatively week activity against all the three bacteria. Compound 4d showed moderate activity towards S.Typhi. While week activity against S. Aureus, E. Coli.

CONCLUSION

From the above data it is conclude that 4a is found to be lead compound against E. Coli. And 4d is found to be lead compound against S. Typhi, as these compounds showed moderate antibacterial activity against E. Coli and S. Typhi respectively.

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pH Metric Study of Synthesised Substituted Propane -1,3-Diones with CU(II), CO(II) and NI(II) Cataions at 0.1 M Ionic Strength

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ABSTRACT

A newly synthesised propane-1,3- dione (β- Diketones) are synthesised from substituted 2-hydroxy acetophenone and different sustituted benzoic acids and are characterised by IR and NMR spectra. The stability constants of ligand complexes with transition metal ions Cu(II), Co(II) and Ni(II) were determined pH metrically. The stability constants of ligands and metals have been calculated.

Keywords: IR and NMR spectra, ions Cu(II), Co(II) and Ni(II), pH metrically.

INTRODUCTION

pH metric technique is simple, rapid, most versatile, cheap and do not need any sophisticated instruments, which is very much used for investigating the physical properties such as proton ligand and metal ligand stability constants of organic drugs. This technique has been extensively used by J. Bjerrum[1] and Irving-Rossotti[2]. Propane-1,3- dione are very important compound in organic synthesis, because they exhibits some biological activity such as antioxidant[3], antitumor[4], antibacterial[5] activities. B- Diketones have gained a lot of interest due to their importance as good ligands[6] and its complexes have been widely used in diverse area.

MATERIALS AND METHODS

All the laboratory chemicals and solvents required for the study were of highest purity commercially available. Metal ion concentration were determined by using EDTA with suitable indicator and used as a chelating reagent. B- Diketones were synthesised by using Baker Venkatraman reaction. The purity of synthesised compounds was checked by thin layer chromatography on silica –G layers. IR spectra were recorded on FTIR spectrophotometer using KBR powder. NMR spectra were recorded on Bruker Avance II 400 NMR spectrometer.

SYNTHESIS OF B-DIKETONE

The synthesis involves following steps:

Preparation of 5-chloro-2-hydroxy-4-methyl acetophenone(1):

p-chloro-m-cresyl acetate was prepared by acetylation of p-chloro-m-cresol.

Then prepared p-chloro-m-cresyl acetate (50ml) and anhydrous aluminium chloride (120 g) were heated at 120^0c for 60 min. in an oil bath. The reaction mixture was cooled and decomposed with ice-cold water containing a little HCl (10%) to get Ketone.

Preparation of substituted 2-benzoyloxy acetophenones (2a,b,c) :

5-chloro-2-hydroxy-4-methyl acetophenone (1)(0.04 mol) and aromatic carboxylic acid (0.05 mol) were dissolved in pyridine and $pocl_3$ is added drop by drop with constant stirring till the viscous mass is obtained. Maintain the temperature below 10^0c during the addition of $pocl_3$ to the reaction mixture. The reaction mixture is allowed to stand for overnight at room temperature. The reaction mixture is decomposed by 10% HCl. The product thus separated was filtered, washed with water followed by sodium bicarbonate (10% solution) and then again washed with water. The solid product was crystallised from ethanol to obtained corresponding substituted 2-benzoyloxy acetophenones.

5-chloro-2-(4' chloro benzoyloxy)-4-methyl acetophenone (2a) was prepared by above method by using 4-chloro benzoic acid. 5 chloro- 2-(2'-4'dichloro benzoyloxy) -4-methyl acetophenone (2b) was prepared by using 2-4 dichloro benzoic acid. Similarly 5 chloro -2-(4' methoxy benzoyloxy) -4-methyl acetophenone (2c) was prepared by using p-methoxy benzoic acid.

Preparation of 1-(2'-hydroxy phenyl)-3-phenyl propan-1,3-diones (3 a,b,c) :-

2-substituted benzoyloxy acetophenones (2a-b) (0.05 mol) was dissolved in dry pyridine (40 ml). The solution was warmed up to about 60^0c and pulverised KOH (0.15 mol) was added slowly with constant stirring. After four hours the reaction mixture was acidified by adding ice cold dilute HCl (1:1). The solid product thus separated was filtered, washed with sodium bicarbonate solution (10%) and finally with water. It is then crystallised from ethanol acetic acid mixture to get 1-(2'-hydroxy phenyl)-3-phenyl propan-1,3-diones (3 a,b,c) .

Spectral data of β- diketones:

Spectral data of 3a (L_1):

IR: 3424 cm^{-1} (Phenolic -OH stretch), 3093 cm^{-1} (Aromtic C-H srretch), 1685 cm^{-1} (C=O stretch), 1611 cm^{-1} (Aromatic C=C stretch), 2927 cm^{-1}(C-H stretch of methyl group), 761 cm^{-1}(Aromatic substitution C-Cl), H NMR: δ 3.54 (S, 1H of OH), δ 2.49 (S, 3H of CH_3), δ 2.61 (S, 2H of CH_2), δ 6.8-8.1 (m,6H of Ar-H), δ 11.94 (tautomerism).

Spectral data of 3b (L_2):

IR: 3444 cm^{-1} (Phenolic -OH stretch), 3095 cm^{-1} (Aromtic C-H srretch), 1749 cm^{-1}(C=O stretch), 1610 cm^{-1} (Aromatic C=C stretch), 2924 cm^{-1}(C-H stretch of methyl group),734 cm^{-1}

(Aromatic substitution C-Cl), H NMR: δ 4.52 (S, 1H of OH), δ 2.37 (S, 3H of CH_3), δ 2.52 (S, 2H of CH_2), δ 6.6-8.1 (m,5H of Ar-H), δ 11.8 (tautomerism).

Spectral data of (L_3):

IR: 3470 cm^{-1} (Phenolic -OH stretch), 3005 cm^{-1} (Aromtic C-H srretch), 1686 cm^{-1}(C=O stretch), 1605 cm^{-1} (Aromatic C=C stretch), 2939 cm^{-1}(C-H stretch of methyl group),794 cm^{-1} (Aromatic substitution C-Cl), H NMR: δ 4.68 (S, 1H of OH), δ 2.44 (S, 3H of CH_3), δ 3.80 (S, 2H of CH_2), δ 3.89(S,3H of O CH_3), δ 6.8-8.0 (m,6H of Ar-H), δ 11.56 (tautomerism).

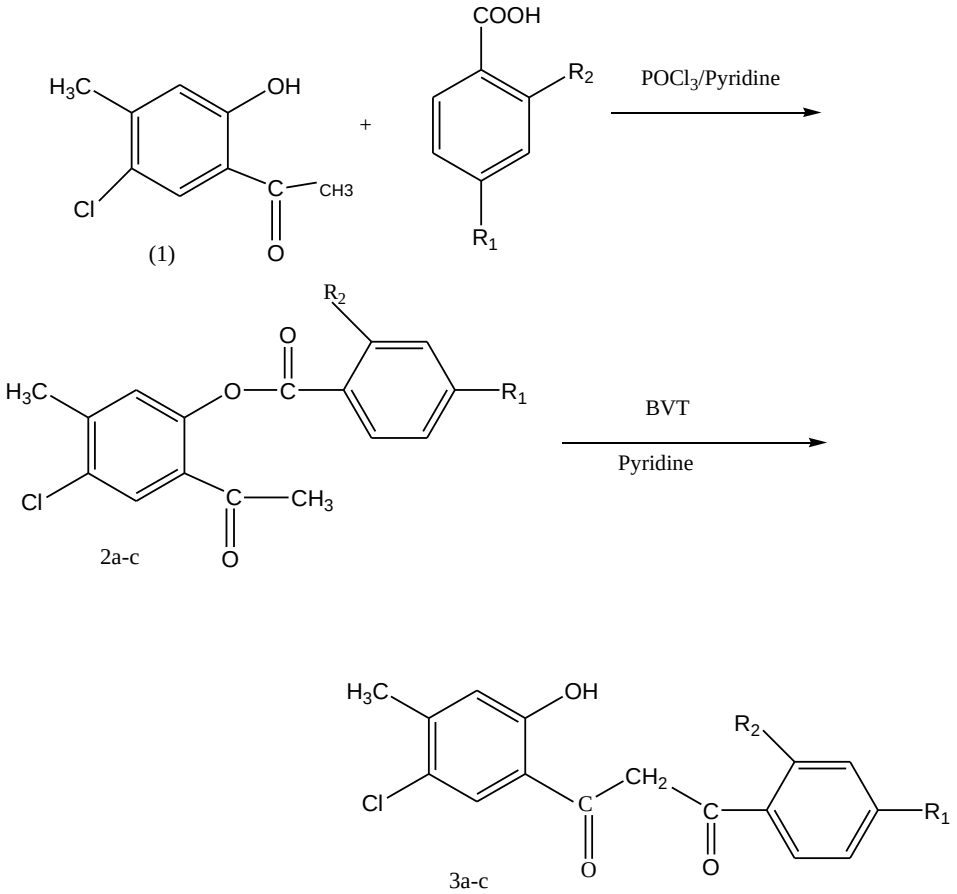

Fig.1: Experimental scheme for the synthesis of 1-(2'-hydroxy phenyl)-3-phenyl propan-1,3-diones (3 a-c)

Table 1: Physical data of β-diketones

Ligand	R_1	R_2	M.F	M.P °c	colour	%Yield
3a	Cl	H	$C_{16}H_{12}Cl_2O_3$	162-164	Yellow	72
3b	Cl	Cl	$C_{16}H_{11}Cl_3O_3$	125-126	Yellow	69
3c	OCH_3	H	$C_{17}H_{15}ClO_4$	142-144	Yellow	57

pH metric analysis :

The potentiometric titrations are performed by using systronic digital pH meter with accuracy in 0.01 unit with combined glass electrode. The electrode was activated by immersing 24 hours in 0.1 N hydrochloric acid and then 24 hours in distilled water. For smooth handling of electrodes all the precautions suggested by Albert and Sergent[7], Bates[8] were adopted. All titrations were carried out under inert atmosphere by bubbling nitrogen gas through an assembly. Instrument was calibrated with buffer solution of pH 7.00 and 9.20 at 30^0c before titration. The ligand solutions were prepared in 70% methanol-water which was used for further titrations i.e. without and with transition metals Cu(II),Co(II) and Ni(II). The 1-(2'-hydroxy phenyl)-3-phenyl propan-1,3-diones was monobasic acids having only one dissociable H^+ ion from OH group.

pH metric titration was utilized for determination of proton ligand stability constant and metal ligand stability constant. The respective pH metric data given in table 2 and table 3. The plot of pH vs. Volume of NaOH given in fig.2

RESULT AND DISCUSSION:

The dissociation constant of substituted diketones were determined pH-metrically. The ligands used in present work are monobasic acid having only one dissociable H^+ ion from –OH group and therefore represented as,

$$HL \rightleftharpoons H^+ + L^-$$

Proton ligand stability constant:

The titration curve of acid and ligand deviates at about pH- 3. The deviation between acid curve from ligand for all system showed the dissociation of H^+ ion from –OH group of ligands. The proton ligand formation number ($\bar{n}_A$)

$$\bar{n}_A = \gamma - \frac{(E°+N)\Delta V}{(V°+V1)T°L}$$

Where,

V^0 - Initial volume of solution

N - Normality of sodium hydroxide

T^0_L - Concentration of ligand

γ – Number of dissociable proton s from ligand

ΔV - volume of alkali consumed by acid and ligand on the same pH.

The pK values calculated from the formation curves between pH V_s $\bar{n}_A$. The half integral method was employed for the determination of proton ligand stability constant. The proton ligand stability constant were calculated from formation curve. The value of pH at $\bar{n}_A$ =0.5 corresponding to the value of pK.

Metal ligand stability constant:

Metal ligand formation number ($\bar{n}$) was calculated by following expression.

$$\bar{n} = \frac{(E+N)\Delta V}{(V°+V2)T°M}$$

The metal ligand formation curve were plotted between pH V_s $\bar{n}$. The metal ligand stability constant were determined by half integral methd.log K_1 and K_2 values are calculated from formation curves.

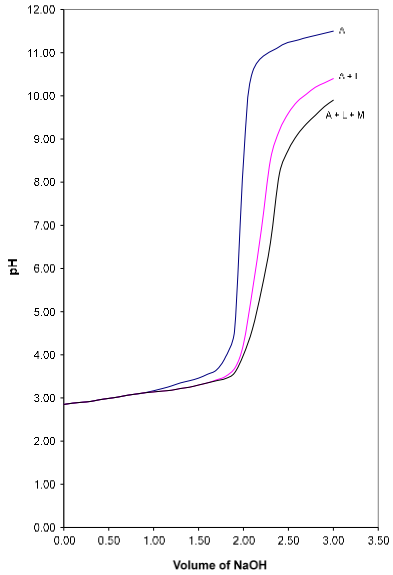

Fig. 2: Titration curve

Table 2: Proton-Ligand Stability Constants (pK)

No.	Ligands	pK Value
L_1	1-(5'-Chloro-2'-hydroxy-4'-methylphenyl)-3-(4'-chloro phenyl)-propane-1-3-dione	7.72
L_2	1-(5'-Chloro-2'-hydroxy-4'-methylphenyl)-3-(2',4'-dichloro phenyl)-propane-1-3-dione	7.58
L_3	1-(5'-Chloro-2'-hydroxy-4'-methylphenyl)-3-(4'-methoxy phenyl)-propane-1-3-dione	7.76

Table 3: Metal-Ligand Stability Constants (Log K)

System	Metal-Ligand Stability Constants (Log K)	
	Log K_1	Log K_2
L_1-Cu(II)	6.49	3.17
L_1-Co(II)	6.48	3.15
L_1-Ni(II)	7.03	4.17
L_2-Cu(II)	4.83	2.01
L_2-Co(II)	5.48	3.01
L_2-Ni(II)	6.34	3.50
L_3-Cu(II)	5.01	3.13
L_3-Co(II)	5.99	2.67
L_3-Ni(II)	6.52	1.68

CONCLUSION

The titration curve for all the system started from pH=3. This indicated the complex formation. Also change in the colour from yellow to red in the range 3to 10 during titration showed complex formation between metal and ligand. It is observed from the table- 3 that the difference between the values of log K_1 and K_2 is sufficiently large. This indicates stepwise complex formation between ligand and metal ion. For ligand-1,the difference between the values of log K_1 and K_2 is higher in Co(II) complex than Cu(II) and Ni(II).This indicated that Co(II) forms more stable complex with ligand-1 than Cu(II) and Ni(II).For ligand-2 the difference between the values of log K_1 and K_2 is higher in Ni(II) than Cu(II) and Co(II).And for ligand-3 also the difference between the values of log K_1 and K_2 is higher in Ni(II) than Cu(II) and Co(II).This indicates that ligand-2 and ligand-3 form more stable complex with Ni(II) than Cu(II) and Co(II).

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Synthesis, Characterization and Antimicrobial Screening of Azo Compounds containing 4-hydroxybenzaldehyde Moiety

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Abstract

Some azo compounds (1a-e) were synthesized by simple diazotization reaction of five different substituted aromatic amines using sodium nitrite and hydrochloric acid followed by coupling with 4-hydroxybenzaldehyde in alkaline medium. Synthesized azo compounds have been confirmed by UV, IR and ^{1}H NMR spectral data and also screened for their antibacterial activity using disc diffusion method.

Keywords: Azo compounds, 4-hydroxybenzaldehyde, Diazotization, Antimicrobial screening.

Introduction

Out of the different classes of dyes, azo dyes constitutes one of the largest and important class of synthetic organic compounds containing an azo N=N group generally connected to aromatic rings. Mostly, synthesis of azo compounds involves diazotization of substituted primary aromatic amines followed by coupling with nucleophiles.They do not occur naturally but synthesized only through chemical synthesis[1] and have been extensively used in different applications such as dying textile fibres, colouring variety of materials, biomedicinal studies and in advanced organic synthesis as well as shows excellent antibacterial and pesticidal properties[2]. Azo compounds and its derivatives are also known for their use as antifungal, antidiabetics, antineoplastics, anti-inflammatory, antiseptic and other useful chemotheraptic agents[3-6]. A number of azo compounds particularly synthesized from β-naphthol, m-cresol, resorcinol, tyrosine, aspirin, paracetamol etc have been frequently reported and exhibited impressive biocidal effects. Since compounds with an azo moiety and 4-hydroxybenzaldehyde moiety have been extensively used as dyes but their antimicrobial activity are less reported and hence in the present work, we have prepared five different substituted azo derivatives of 4-hydroxybenzaldehyde namely 1a-e, characterized and also screened for their antibacterial activity at different concentrations by using disc diffusion method.

Materials and Methods

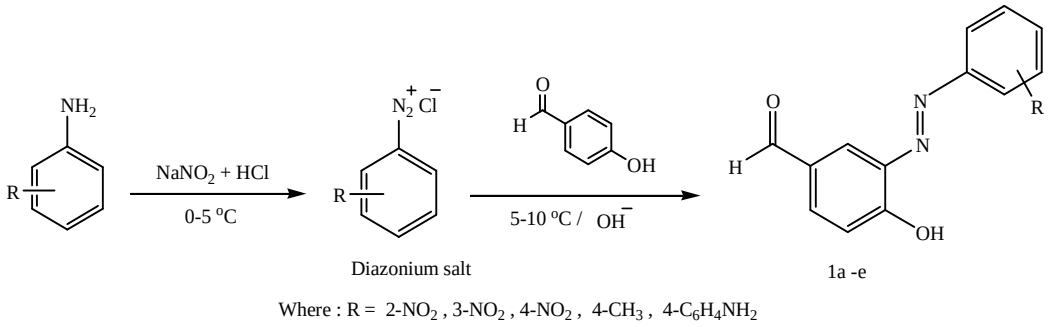
In present work, chemicals and reagents used were of analytical grade (Merck and Alfa Aesar Company Ltd). Melting points were determined by open capillary method and are uncorrected. The UV spectra of azo compound was determined by Lab India 3000+ spectrophotometer at Vidyabharati Mahavidyalaya, Amravati. IR spectra was recorded in KBr pellets from Shimadzu DRS-8000 IR spectrophotometer at Shri Shivaji Science College, Amravati and ^{1}H NMR spectra from Bruker Avance II 400 MHz NMR spectrometer using DMSO as a solvent and TMS as an internal standard at SAIF, Punjab University, Chandigarh.

Experimental procedure for the synthesis of azo compounds[7]: 4-Nitroaniline (0.01 m) was dissolved in 2.5 ml of conc. HCl and 2.5 ml (4N) cold solution of $NaNO_2$ was added with constant stirring. During addition, temperature of the reaction mixture was maintained up to 0-5^{o}C. Diazonium salt solution prepared above was added drop by drop to the solution of 4-hydroxybenzaldehyde in 10% NaOH with stirring for 30 to 40 minutes by adjusting the temperature between 5-10^{o}C. The crude product precipitate was filtered, washed with distilled water, dried and recrystalised from hot ethanol to yield brown coloured crystalline solid of 4-hydroxy-3-((4-nitrophenyl) diazenyl) benzaldehyde (1c). M.f. = $C_{13}H_9N_3O_4$; m.w. = 271.23; m.p. = 94-95^{o}C. Its alcoholic solution shows positive test of ferric chloride, showing the presence of phenolic –OH group. Similarly other compounds are prepared by same method and are shown in Scheme-1.

Spectral data of representative azo compound 1c: IR: 3707 cm^{-1} (Phenolic –OH stretch), 3032 cm^{-1} (Aromatic C-H stretch), 1716 cm^{-1} (C=O stretch of Ar-CHO), 1539 cm^{-1} (Aromatic C=C stretch), 1492 cm^{-1} (N=N stretch).^{1}H NMR: δ 3.35 (s, 1H of -OH), δ 11.7 (s, 1H of -CHO), δ 6.9-8.4 (m, 7H of Ar-H). UV (λ_{max}): 360 nm.

Antimicrobial screening: All the synthesized azo compounds were screened for their antibacterial activities against three microorganisms viz. ***Escherichia coli, Staphylococcus aureus and Salmonella typhi*** by adopting disc diffusion method[8] at Microbial Section, FTL, Krishi Vigyan Kendra, Durgapur (Badnera), Dist. Amravati. The azo compounds were dissolved in ethanol to get solutions of 50 and 100 mcg/ml concentrations.

Sterile discs were dipped in these solutions, dried and placed on nutrient agar plates spreaded with the bacteria. After incubation at 37°C for 24 hr, the zones of inhibition produced by azo compounds were measured in mm and were compared with streptomycin as a standard.



Where : R = 2-NO_2 , 3-NO_2 , 4-NO_2 , 4-CH_3 , 4-$C_6H_4NH_2$

Scheme-1
The general reaction scheme for synthesis of azo compounds 1a-e

Table-1
Compounds codes, names, mol. formulae, mol. weights, melting points and percentage yields of synthesized azo compounds 1a-e

Compds. codes	Compounds names	Mol. formulae	Mol. Wt.	M.p. (°C)	% Yield
1a	***(E)-4-hydroxy-3-((2-nitrophenyl)diazenyl) benzaldehyde***	$C_{13}H_9N_3O_4$	271.23	120-122	50%
1b	***(E)-4-hydroxy-3-((3-nitrophenyl)diazenyl) benzaldehyde***	$C_{13}H_9N_3O_4$	271.23	123-124	62%
1c	***(E)-4-hydroxy-3-((4-nitrophenyl)diazenyl) benzaldehyde***	$C_{13}H_9N_3O_4$	271.23	94-95	71%
1d	***(E)-4-hydroxy-3-(p-tolyldiazenyl) benzaldehyde***	$C_{14}H_{12}N_2O_2$	240.26	98-100	56%
1e	***(E)-4-hydroxy-3-(4-(4-anilinyl)phenyldiazenyl) benzaldehyde***	$C_{19}H_{15}N_3O_2$	317.34	102-104	52%

Table-2
Antimicrobial activity of synthesized azo compounds 1a-e

Compds. codes	Conc. (mcg/ml)	Zones of inhibition in mm		
		E.coli	***S.aureus***	***S.typhi***
1a	50	NI	NI	NI
	100	NI	NI	NI
1b	50	NI	NI	NI
	100	NI	NI	NI
1c	50	I(6)	I(13)	I(7)
	100	I(8)	I(14)	I(8)
1d	50	NI	NI	NI
	100	NI	NI	NI
1e	50	NI	NI	NI
	100	NI	NI	NI
Std.	25 mcg/disc	20	26	25

NI=No Inhibition, I=Inhibition (zones of inhibition are given in parenthesis)

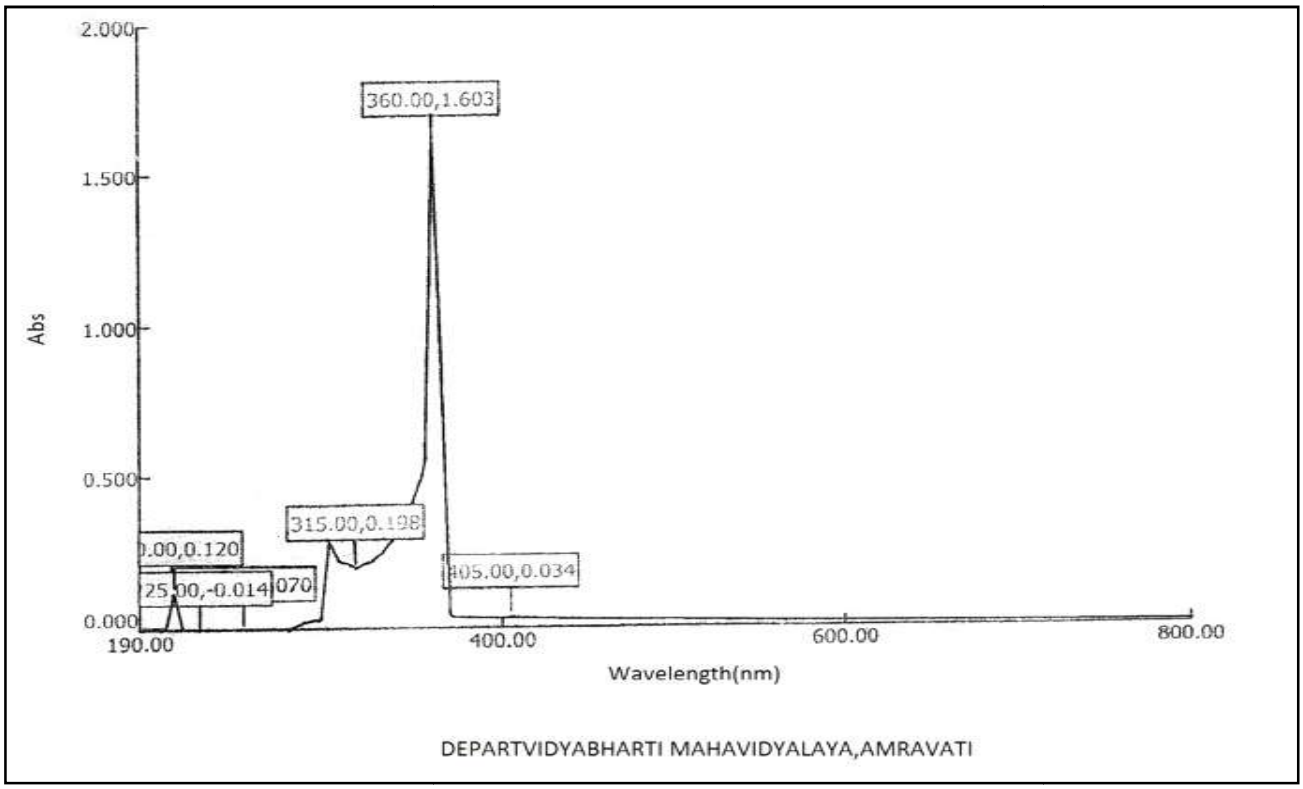


Figure-1
UV spectra of azo compound 1c

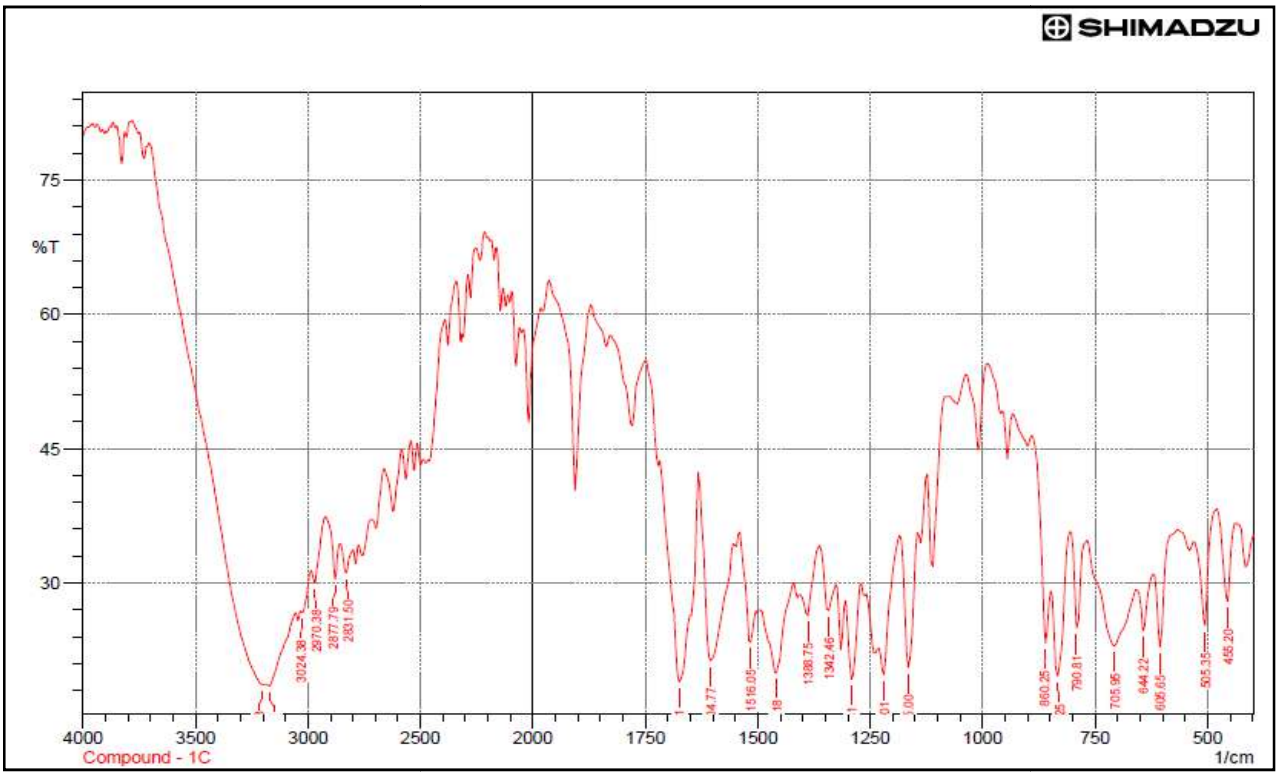


Figure-2
IR spectra of azo compound 1c

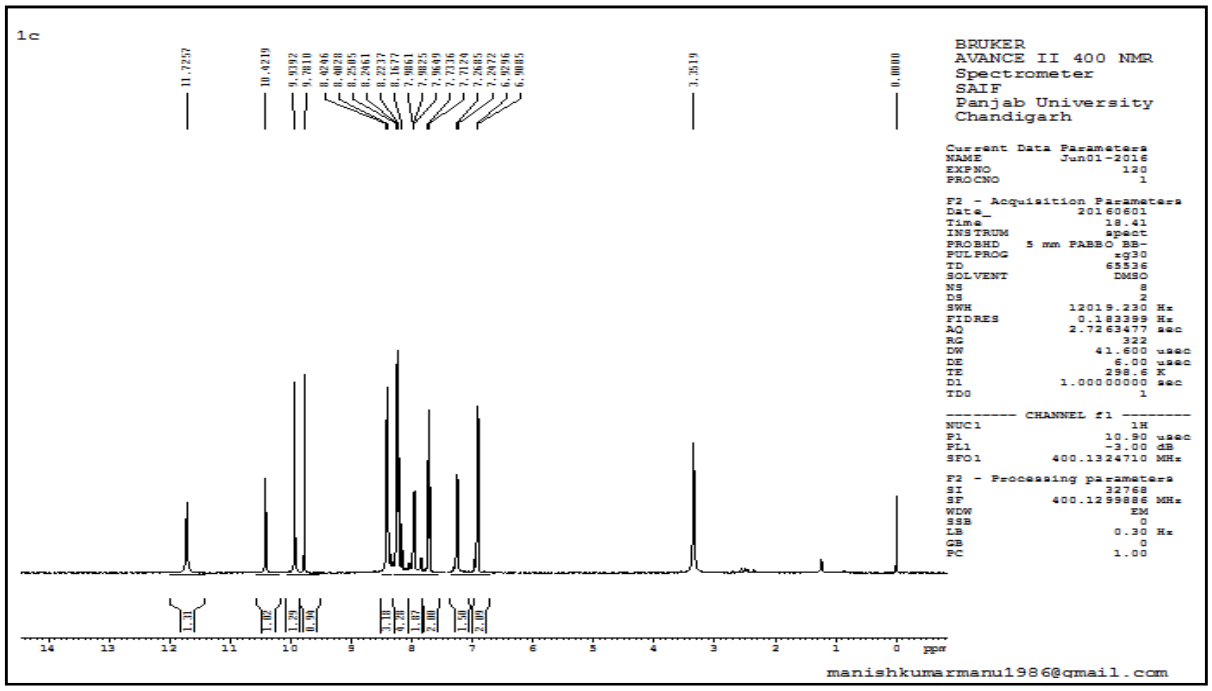


Figure-3
^{1}H NMR spectra of azo compound 1c

Results and Discussion

In this work, total five substituted azo compounds namely 1a-e were successfully synthesized from five different aromatic amines by simple diazotization-coupling reactions, recrystalised and two different concentrations of each compounds were prepared and further used individually to test their antibacterial activities against three microorganisms viz. ***Escherichia coli, Staphylococcus aureus and Salmonella typhi***. From the data on antimicrobial activities of azo compounds given in Table-2, it was observed that the azo compounds 1a, 1b, 1d and1e do not showed any inhibitory action and were found to be inactive against all the tested microorganisms at both the concentrations. Only the compound 1c showed inhibitory action against ***E. coli***, ***S. aureus*** and ***S.typhi*** at both concentrations. At 50 mcg/ml concentration, compound 1c showed 6, 13 and 7 mm of zones of inhibition and at 100 mcg/ml concentrations showed 8, 14 and 8 mm of zones of inhibition against ***E. coli***, ***S. aureus*** and ***S.typhi*** respectively. The maximum 13 and 14 mm zones of inhibition of compound 1c was found to be against ***S. aureus*** species at 50 mcg/ml and 100 mcg/ml concentrations respectively. Also Streptomycin at the concentration of 25 mcg/disc showed 20, 26 and 25 mm zones of inhibition against the microorganisms ***E. coli***, ***S. aureus*** and ***S.typhi*** respectively.

Conclusion

Out of the five laboratory synthesized substituted azo compounds, the compound 1c is found to be active and showed low to moderate antibacterial activity against all the microorganisms tested. The inhibitory effect of azo compounds 1c recorded especially against ***S. aureus*** was most satisfactory at both the concentrations and hence it can be used as lead compound against microorganisms ***S.aureus***.

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We are thankful to Principal, Vidyabharati Mahavidyalaya, Amravati for providing laboratory facilities and also thankful to Microbial Section, FTL, Krishi Vigyan Kendra, Durgapur (Badnera), Dist.Amravati for their support.

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RESEARCH ARTICLE

Spectrophotometric determination of p*K*a of schiff base ligand

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ABSTRACT

Schiff bases are a significant group of organic compounds that have biological activities and miscellaneous applications because of their antibacterial, antivirus activities, metal complexation and other Pharmacological activities. The simple and meticulous method used for the determination of the stability constants of the complexes by means of UV-visible spectrophotometry depending on the theoretical interpretation of the stoichiometry, Jobs and Yoe-Jones, methods was proposed. In present work, proton ligand stability constant (pKa) of substituted hydroxy Schiff base ligand 2,2'-((ethane-1,2-diylbis(azanylylidene))bis(ethan-1-yl-1-ylidene))bis(4-methylphenol) (H_2L) have been investigated by spectrophotometric method. Determination of p*K*a values has studied at different concentrations of ligand solution. p*K*a values are calculated at pH 4.82,5.12,5.40,5.70 & 5.90 by using standard literature method. The ruggedness of the determined result was also validated in this study for producing exact pKa value. The results obtained from this work were compared with those obtained using the potentiometric method. The difference between the results found with these two methods was approximately 0.04 units.

KEYWORDS

Schiff base, Spectrophotometric method, p*K*a determination.

INTRODUCTION

The Schiff bases and their metal complexes have got more importance recently [1-6] because of their application as biological, biochemical, analytical, antimicrobial, anticancer, antibacterial, antifungal and anti tumor activity. They have been studied as a class of ligands [7,8]. Recently there has been a considerable interest in the chemistry of Schiff base compounds because of their potential pharmacological applications [9]. Schiff base complexes showed catalytic activity in carbonylation of alcohols and alkenes at low pressure to produce arylpropionic acids and their esters [10, 11], which are used as non-steroidal anti-inflammatory

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In addition to monometallic, the bimetallic Schiff base complexes also showed catalytic activity in carbonylation reactions. The Heck reaction[12], an industrially useful process to synthesize fine chemicals and pharmaceutical was successfully catalyzed using Schiff base complexes.

The study of proton ligand stability constant [13](pKa) has gained much importance as it affects solubility, absorption across biological membranses, distribution to the site of action, renal elimination, metabolisms and binding of the drug with protein and receptor. The *pKa* plays very important role in drug discovery. The determination of p*K*a of drug is of interest from the stand point of its influence on pharmaceutical compound synthesis. The FDA of all countries has made it mandatory to estimate *pKa* value of new drug molecules[14]. The *pKa* of the drug is cited in literature [15, 16] by

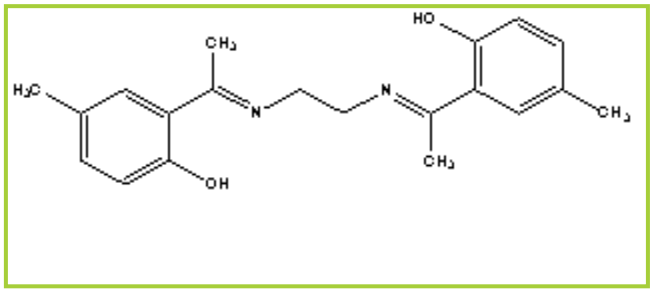
several authors. The first real report [17] on the determination of *pKa* value of drug appeared in which the dissociation constant was determined by a potentiometric method employing mixed solvent techniques using methanol–water mixtures. But *pKa* of Schiff base ligand at different concentration by spectroscopic method have not been studied yet. So in this report we determined the p*K*a of Schiff base ligand spectrophotometrically. Spectrophotometry is one of the most powerful methods for the investigation of solution equilibria, although potentiometric pH titrations are more convenient and more commonly used due to the simplicity of the equipment and minimum time requirement. Spectrophotometric titrations are useful for determining the ionization constants of acids and bases even below pH 2 and above pH 11, where potentiometry becomes inconvenient. Spectrophotometric titrations also yield additional spectral information about the species formed during the titration and requires very less amount of the compound [18], therefore we prefer this method in our experimental work.

An extra aspect of our study is the validation of p*K*a value. This study was done by S. Singh et al for pKa determination of nimesulide[19]. For this, the result was validated from determination of p*K*a by using different concentration of H_2L ligand on same spectrophotometer.

EXPERIMENTAL AND MATERIALS

All chemicals were used as obtained from Rankem and Sd fine chemicals. Anal grade solvent from Sd Fine chemicals was used without further purification. Freshly prepared triple distilled water was used for making solutions.

The Schiff base ligand was synthesized by condensation of the acetophenone with ethylene diamine in suitable stoichiometric proportion as per the literature method [20]. The pH of the solutions were measured on a pH meter (Systronics Ltd. India) equipped with a combined glass electrode. It was standardised at27°C using standard buffers. The UV-Visible absorption spectra were recorded on Systronics UV-Visible spectrophotometer-119.



2, 2'-((ethane-1, 2-diylbis (azanylylidene)) bis (ethan-1-yl-1-ylidene)) bis (4-methylphenol)

H_2L ligand

Method

The determination of pKa by spectrophotometric method is based on the principle that ionisation of the acidic or basic compound is pH dependent. Hence with change in pH of the solution is the ratio of ionised form to the unionised form changes. At definite wavelength the ionised and unionised forms have different absorptions. The UV-Visible absorption spectra were recorded on Model Systronics UV-Visible Spectrophotometer -119 operated at a wavelength range of 200-400 nm. The dissociation constant (pKa) of H_2L ligand were determined at room temp, according to the experimental method reported by Albert and Serjeant [21]. The pH values of all solutions were measured on a Systronics digital pH meter 335 equipped with combined glass electrode which was calibrated at 27^0 using standard buffers at pH 4.0 and 7.0.

The synthesized H_2L ligand was used after recrystallisation, DMF was purified by distillation process which was given in Vogel's TextBook of Practical Organic Chemistry [22]. Buffer chemicals and all other reagents were of analytical grade. Freshly prepared double distilled deionised water were used throughout. All of the experiments were carried out at room temperature; 27 ± 1^0C. The procedure reported by Albert & Serjeant was used for the determination and calculation of pKa. For the experiments at different pH, the acetate buffers

were used. Aliquots of five different sets were prepared by varying volume of buffer sol^{n}. 0.01M solution of H_2L ligand in DMF was prepared.To each set 10µL of this stock solutions was added to the buffer solutions of varying pH to get the final conc.of the ligand as 10µML^{-1}.The buffer solutions of varying pH were prepared by mixing suitable proportions of 0.04M sodium acetate, 0.02M Acetic acid, 0.1M HCl and 0.1M NaOH. Relevant quantity of sodium chloride was added in order to maintain the ionic strength of all solutions to 0.1.The solutions were mixed and the all five sets were placed in a High precision water bath set at 27^{0}c. The absorbance variation was illustrating this result for each solution at 294 nm.

The absorbances of neutral and ionised species of the ligand at 0.1M ionic strength of NaCl were measured. For validation of the determination of p*ka* value the total study of measurement of absorbance was repeated. This ruggedness study was done by absorbance measurements in three studies were done by using different conc. of ligand solution on same spectrophotometer.

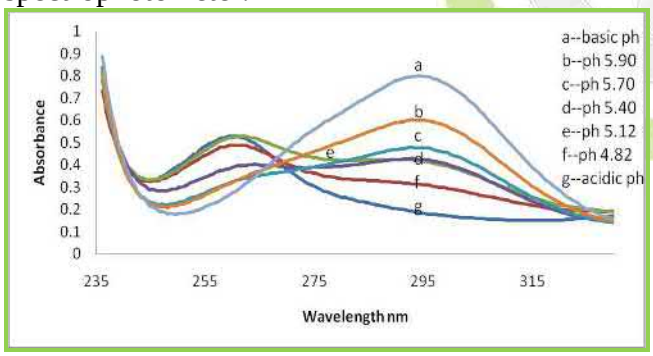


Fig 1: UV Spectrum of H_2L at Various pH.

RESULTS AND DISCUSSION

Fig. 1 shows a schematic representation of UV spectrum of H_2L ligand with pH. Table 1 shows variation in the UV spectrum of one of the determination in buffer solution of various pH.

Table 1: Analysis Data of Calculations of pKa from Absorbance at Aarious pH

pH	**Absorbance (d)**	**pKa = pH + log (d_i – d / d –d_m)**
4.82	0.30469	5.3573
5.12	0.4182	5.4224
5.40	0.4255	5.6384
5.70	0.4752	5.7586
5.90	0.6020	5.6055

Average: p*K*a = 5.5564

d_m = 0.1875 (in 0.1M HCl) ; d_i = 0.7996 (in 0.1M NaOH).

Table 2: Validation of Ruggedness of the p*K*a Value

Instrument 1			
Person 1,day 1,ligand Strength= 5µML^{-1}	Person 1,day 1,ligand Strength= 10µML^{-1}	Person 1,day 2,ligand Strength= 15µML^{-1}	Person 1,day 2,ligand strength = 20µML^{-1}
5.5533	5.5564	5.5578	5.5585

In fig.1 we observed that the overlapping spectra of all the sample solution which were taken and absorbance at 294 nm was recorded. In this spectrum clearly two isobestic points were observed at 241 and 269 nm. The sharp isobestic points were observed which only used for calculation of p*K*a. The absorbance data was used to estimate pKa values by three methods of calculation. Table 1 shows the pH dependance of absorption spectra of H_2L.

(i) The distinct p*k*a value was calculated by using following equation:

$$pKa = pH + log\ (d_i - d / d - d_m)$$

Where d_i is the absorbance of ionized species, d is the absorbance of respective buffers tested solution and d_m is absorbance of unionized species. The average pKa was calculated by taking antilogarithm of each individual calculated *pKa* value, by taking average of all the antilogarithmic values, and taking logarithm of the averaged value. This calculation method gives more precise *pka* value.

(ii) The plot of pH Vs log di-d / d-dm gave a straight line with intercept as pKa equal to 5.5545. It is a good agreement with these determinations by equation.

(iii) The values of degree of ionization (α) are determined from the absorption data. The plot of α Vs pH, gives the value of pKa equal to 5.7502 at α = 0.5.

Table 3: pKa Calculation by Various Methods of Calculation.

pKa Calculation from Equation	pKa by Intercept	pKa from Calculation of α
5.5564	5.5545	5.7502

To establish validation of ruggedness of the result the same process was repeated for all other working solutions of different concentration of ligand. The validation result of p*k*a was listed in Table 2. This result of the validation study were obtained very close, that means it indicate the accuracy of result and technique. The final determined p*k*a value calculated from our experiment is 5.55. the lower value of pka as compared to standaed value of 10.0 for phenolic –OH group, might be due to presence of unsaturated –N==C— linkage at ortho position.

In this work, the p*k*a value for H_2L ligand determined spectrophotometrically was found to be 5.55 which is about 0.04 unit different from 5.51 obtained by Calvin- Bjerrum method using potentiometric technique. The observed differences between the p*k*a values obtained by both method might be due to different experimental methods.

Finally we report that the close agreement of p*K*a value among spectrophotometric method and with potentiometric method shows robustness of the spectrophotometric method.

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PH -METRIC ANALYSIS OF COMPLEX FORMATION OF CU(II),CO(III) AND FE(III) METAL IONS AND SUBSTITUTED HYDROXY SCHIFF'S BASES IN 70% MIXED SOLVENT MEDIA.

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ABSTRACT : In the present work, The complex formation between Cu(II),Co(III) and Fe(III) metal ions and 6, 6'-((ethane1, 2-diylbis (azanylylidene)) bis (ethan-1-yl-1-ylidene)) bis(4-chloro-2-nitrophenol) [$\mathbf{L_1}$] and 2-((2-(1-(2-hydroxy-5-methylphenyl) ethylidene) amino) ethyl) ethanimidoyl)(4-methyl-6-nitrophenol [$\mathbf{L_2}$] have been investigated using pH metric titration technique in 70% DMF-Water mixture at 27^{0}C & at 0.1M ionic strength. The method of Calvin and Bjerrum as adopted by Irving and Rossotti has been employed to determine metal-ligand stability constant. It is observed that Cu (II), Co (III) and Fe (III) metal ions form 1:1 and 1:2 complexes with ligands (L_1 & L_2). The data obtained were used to estimate and compare the values of proton-ligand stability constant (pK) and metal-ligand stability constants (log k).

Key words: Schiff's bases, Metals ions Cu(II), Co(III) and Fe(III), DMF-Water mixture , Stability constant.

INTRODUCTION:

Schiff bases are an important class of ligands in coordination chemistry and find extensive applications in different fields. The chemistry of the carbon-nitrogen double bond plays a vital role in the progresses of chemistry science (Patai, 1970). Schiff bases have been known since 1864 when Hugo Schiff reported the condensation of primary amines with carbonyl compounds (Cimerman *et al*, 2000). Schiff-bases have been widely used as ligands because of high stability of the coordination compound, of them and their good solubility in common solvents such as ethanol, methanol, chloroform, dimethyl formamide . The active and well-designed Schiff base ligands are considered as "privileged ligands".

Considerable research work has been done in the past, on the study of complexes (Martell *et al* 1962). The stability constants for the metal complexes are widely used in various fields such as biological processes, pharmaceuticals, analytical processes, separations techniques, etc. Metal complexes play a vital role in nature, they have been extensively used in clinical applications as enzyme inhibitors (Shankarwar *et al*, 2013). Metal complexation not only brings the reacting molecules together to give activated complex but also polarized electrons from the ligand towards the metal (Floreneand Attwood 1981). The relation between stability and basicity of ligands is indicated by the formation constant and free energy change value bulkier group increases the basicity of ligands as well as stability. Thakur *et al* have studies the influence of dielectric constants of medium on the complex equilibrium of substituted hydroxyl-1,3- propandiones with Cr(II) metal ions and studies on interaction between Cu(II), Cr(II) and Ni(II) metal ions at 0.1M ionic strength pH metrically (Thakur *et al* ,2012). Metal complexes of the Schiff bases have occupied a central role in the development of coordination chemistry (Schiff H 1864). Many attempts have been made to evaluate different factors affecting the stability of the metal chelates along with their stability constants (Martell, 1982) ,(Mayadeo ,1980) and (Kiranmai *et a*l ,2010). Palaskar has studied the effect of ionic strength and dielectric constant of Cu(II) -3-nitrophthalic acid potentiometrically at 0.02,0.04,0.06,0.08 and 1.0M ionic strength in aqueous medium at 30^{o}C (Palaskar,N.G,Samyak, 1988). Recently Jadhav *etal* have investigated the stability constant of Mn(II), CO(II), Ni(II), Cu(II) and Zn(II) complexes with synthesis Schiff base compound N-[2-hydroxy-1-napthalydene]-2-ethoxyanilline *in 60*% Dioxane - water medium at 0.1M ionic strength potentiometrically (Jadhav *et al*, 2015).

The present paper deals with the complexation reactions between Cu(II),Co(III) and Fe(III) meta ions with substituted hydroxy ligands at 0.1M ionic strength, pH metrically in 70% DMF-water mixture.

MATERIALS AND METHODS :

Substituted Schiff's base ligand 6, 6'-((ethane1, 2-diylbis (azanylylidene)) bis (ethan-1-yl-1-ylidene)) bis(4-chloro-2-nitrophenol) [L_1] and 2-((2-(1-(2-hydroxy-5-methylphenyl) ethylidene) amino) ethyl) ethanimidoyl)(4-methyl-6-nitrophenol [L_2] were synthesized in the laboratory by known literature method & their purity was checked by TLC on microscopic slides with silica gel-G layer thickness 0.3mm. The structure was confirmed by IR , NMR spectra and melting point. All the chemicals used were of AR grade. The solution of ligands was prepared in DMF. DMF (A.R.) was purified by the method described by Vogel (Vogel,1989). All solutions were prepared in double distilled, CO_2 free water. Metal ion solutions were prepared by dissolving the requisite quantities of metal nitrate in double distilled water and standardized by using conventional procedures. Sodium hydroxide solution was prepared in deionised water and solution were standardized before use by known methods & used as afresh.

Measurements

All the pH measurements and titrations were carried out with Systronic microprocessor based pH meter with magnetic stirrer (accuracy ± 0.01 units) using glass electrode & calomel electrode at 27 ± 0.1 ^{0}C. pH meter was calibrated by standard buffer solution (pH 4.0,7.00 & 9.2).

The experimental procedure involved the titrations of –

(i) Free acid HNO_3 (0.01 mol.dm^{-3})
(ii) Free acid HNO_3 (0.01 mol.dm^{-3}) and ligand (2 x 10^{-3} mol.dm^{-3})
iii) Free acid + ligand (2 x 10^{-3}M) + metal ion titration (4 x 10^{-4}M) with standard NaOH solution (0.13 mol.dm^{-3}) in presence of an inert atmosphere by bubbling a constant flow of nitrogen gas. The ionic strength of all these systems was maintained constant 0.1M by adding appropriate amount of KNO_3 solution.

RESULTS AND DISCUSSION :

The substituted Schiff bases L_1 and L_2 having replaceable H^+ ion in phenolic hydroxy group may be ionized as i.e.

$$HL \text{-------} > H^+ + L^-$$

The titration data were obtained used to constuct the formation curves [acid curve (A), acid + ligand curve (A + L) and acid + ligand + metal ion curve (A + L + M)] by plotting pH Vs volume of NaOH . It is observed from titration curves that for all systems ligand start deviating from the free acid curves at pH = 2.9 and deviation continuous up to pH = 12. The deviation presents dissociation of proton in Schiff base. The average number of proton dissociated from the ligand (metal ion curve (A + L + M)] by plotting pH Vs volume of NaOH . It is observed from titration curves that for all systems ligand start deviating from the free acid curves at pH = 2.9 and deviation continuous up to pH = 12. The deviation presents dissociation of proton in Schiff base. The average number of proton dissociated from the ligand (*n*a) was determined from free acid and acid - ligand titration curves by employing the equation (1) proposed by Irving and Rossotti .(Table 1)

$$\bar{n}_A = \gamma - (E^\circ + N) X (V_2 - V_1) / (V^\circ + V1) x T^\circ_L \quad \text{..... (1)}$$

Where, γ is the number of replaceable H^+ ions, V° is the total volume of the solution , E° & T°_L are the total concentrations of Nitric acid & ligand respectively, V_1 &V_2 are volume of alkali added to reach the same pH reading during titration of free acid and free acid plus ligand respectively.V_2-V_1 is the horizontal difference in the volume at the given pH and N is the concentration of sodium hydroxide (0.13 mol.dm^{-3})
The pK_1 and pK_2values were determined from formation curves by half integral method (*n*a V/s pH) by noting the pH at which *n*a = 1.5 and *n*a = 0.5 respectively. The accurate values of pK_1 and pK_2 were calculated by point wise calculations which are presented in Table 1.

Determination of Metal-Ligand Stability Constant (Logk) of Schiff base at 0.1 M ionic Strength

Metal-ligand stability constant of metal chelates with Schiff bases of L_1 and L_2 were determined by employing Calvin - Bjerrum pH- metric titration method as adopted equation (2) by Irving and Rossotti . The formation of chelate between metal ions with Schiff bases L_1 and L_2 were indicated by the significant separation starting from pH =2.9 for all complexes.

$$\bar{n} = (E^\circ + N) X (V_3 - V_2) / (V^\circ + V_2) x T^\circ m \quad \text{----------(2)}$$

Where E° is the concentration of acid, N is the normality of the NaOH, T°m is the concentration of metal, V° is the total volume and V_3- V_2 is the horizontal difference in the volume at the given pH.

The metal ligand stability constants for CuIII),Co(III) and Fe(III) with Schiff base ligands L_1 and L_2 in 70% DMF-water can be found by the half integral method and pointwise calculation method (Table -2) . the formation curves were constructed by plotting the values of *n* against pH of the solution and are shown in fig. 1 to 6.

CONCLUSION :

In the present study, it is observed from titration curve that the extent of deviations confirms complete dissociation of –OH group .The deviation between acid+ligand curve and acid+ligand+metal curve indicates the commencement of complex formation. The change in colors during titration with respect to pH also indicates the complex formation between ligand and metal ions.

It could be observed from Table No. 2 that there is good agreement of Proton-liand stability constants between half integral method and pointwise calculation method.. The pK values for ligand depend on structures and resonance stabilization of ligands. The ligand L_2 shows higher pK values than L_1. The more pK values of ligand (L_2) is attributed because (L_2) has electron realizing methyl group which form the more stable complex ,in the DMF-water system. Symmetrical and asymmetrical structures for ligands are also responsible for the pK values for the ligand.

Determination of metal-ligand stability constants requires the accurate value of proton-ligand stability constant. Higher values of log K_1 and log K_2 showed that ligand are stronger chelating agents and vice versa. In the present investigation values of log K_1 and log K_2 have been calculated with half integral and pointwise calculation methods. The values obtained are in good agreement (shows in Table No. 2). The difference between log K_1 and log K_2 values are studied for all the systems and is represented in Table No.3.

It is observed from table-3 the difference between log K_1 and log K_2 values are less, it indicated the simultaneous formation of 1:1 and 1:2 complexes in the solution. They showed the linear relationships between log K and pK values of ligands suggesting identical binding sites in all ligands .

The higher value of ratio (Log K_1/ Log K_2) for Fe(III)-- L_1 & Cu(II)--L_1 complex indicates the more stable simultaneous complex formation as compare to Co(III)--L_1 & L_2 ,Cu(II)--L_2 and Fe(III)-L_2 complexes (Tayade and Wadekar,2017). Stability constants depend upon the size of cation.

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Table 1: Determination of proton-ligand stability constant (pK) of Schiff bases .

Medium : 70% DMF -water | **μ** =0.1M | **T ^{0}L** = 2 x 10 $^{-3}$M
T ^{0}M = 4 x 10 $^{-4}$M | **N** = 0.13N | **V_0** = 25 ml
E^o = 1 x 10^{-2} M | **Temp.** = 27 ± 0.1 ^{0}C

System	Constants (pK)			
	Half integral method		Pointwise calculation	
Schiff Base L_1	pK_1	pK_2	pK_1	pK_2
	7.18	12.42	7.07	12.32
Schiff Base L_2	8.51	12.05	8.49	11.98

Table 2 : Determination of $LogK_1$ & $LogK_2$ Values

System	Metal ligand stability constants (log K)			
	Half Integral		Pointwise Calculation	
	Log K_1	Log K_2	Log K_1	Log K_2
Cu(II)-L_1 Complex	5.25	3.48	5.13	3.39
Co(III)-L_1 Complex	4.85	3.24	4.95	3.10
Fe(III)-L_1 Complex	5.38	3.22	5.31	3.20
Cu(II)-L_2 Complex	6.68	5.51	6.59	5.49
Co(III)-L_2 Complex	6.25	5.18	6.21	5.14
Fe(III)-L_2 Complex	6.81	5.28	6.78	5.20

Table 3 : Metal-Ligand Stability constants (Log K).

System	Log K_1	Log K_2	Log K_1-LogK_2	Log K_1/Log K_2
Cu(II)-L_1 Complex	5.25	3.48	1.77	1.508
Co(III)-L_1 Complex	4.85	3.24	1.61	1.496
Fe(III)-L_1 Complex	5.38	3.22	2.16	1.670
Cu(II)-L_2 Complex	6.68	5.51	1.17	1.212
Co(III)-L_2 Complex	6.25	5.18	1.07	1.206
Fe(III)-L_2 Complex	6.81	5.28	1.53	1.289

Fig. 1
Plot between n vs pH

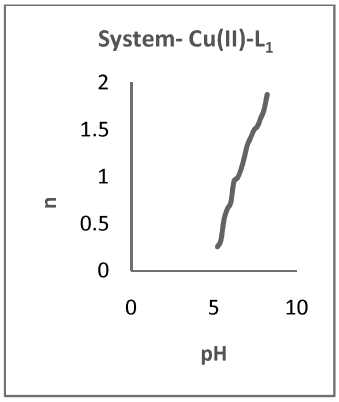


Fig. 2
Plot between n vs pH

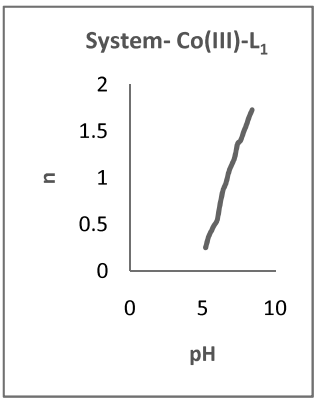


Fig. 3
Plot between n vs pH

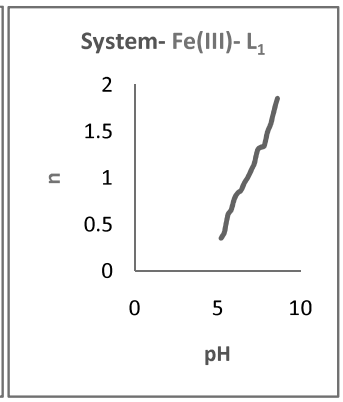


Fig. 4
Plot between n vs pH

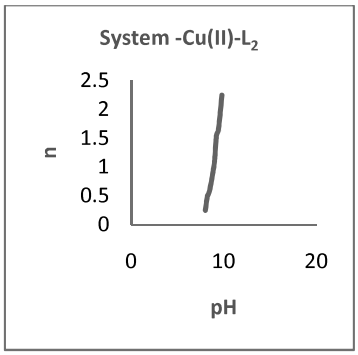


Fig. 5
Plot between n vs pH

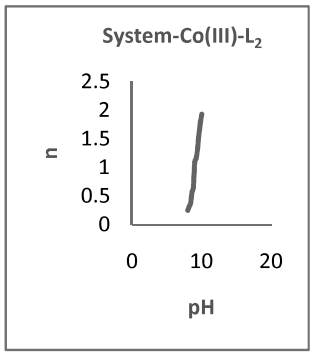
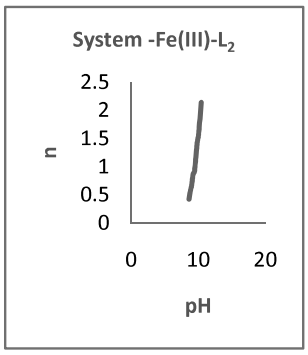


Fig. 6
Plot between n vs pH



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STUDY OF METAL-LIGAND STABILITY CONSTANTS OF La (III), Sm(III) & Nd(III) METAL ION COMPLEXES WITH SUBSTITUTED SCHIFF'S BASES AT 0.1 M IONIC STRENGTH PH- METRICALLY

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ABSTRACTS

In present work, the interaction of **La(III), Sm(III)** and **Nd(III)** metal ion with 6,6'((ethane1,2diylbis(azanylylidene))bis(ethan-1-yl-1-ylidene))bis(4-methyl-2-nitrophenol) [$\mathbf{L_1}$] and 6,6'((ethane1,2diylbis(azanylylidene))bis(ethan-1-yl-1-ylidene))bis (4-chloro -2-nitrophenol [$\mathbf{L_2}$] was investigated at 0.1 M ionic strength (27 ± 0.1 ^{0}C) in 70% DMF- water mixture by Bjerrum method as adopted by Calvin and Wilson . It is observed that La (III), Sm(III) and Nd(III) metal ions forms 1:1 & 1:2 complexes with Schiff base ligand . The substituted Schiff bases show formation of simultaneous complexes. From estimated data (pK & log K), the effect of substituents were studied.

KEYWORDS: Schiff base, Metals ions La (III), Sm(III) and Nd(III) , DMF-Water mixture , Stability constant.

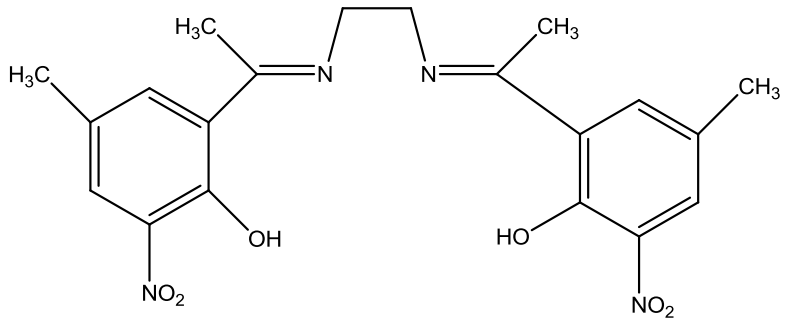
INTRODUCTION

The stability of metal complexes with medicinal drugs plays a most important role in the biological & chemical activity.[1-2] Generally metal complexes play a very major role in biological process such as metalloproteins, metalloenzymes, storage, transport, detoxification etc. The physico-chemical properties of organic reagents are necessary to understand their nature, reactivity and analytical applicability. Schiff bases are versatile complexing reagents. Studies on the metal complexes of the Schiff bases require the knowledge of their ionization constants. The ionization constants in particular are essential for the metal-ligand stability constants and to have knowledge of selectivity of the relevant analytical reactions. Schiff's Bases and their derivatives possess effective antibacterial[3], antitumour[4], antimicrobial[5] properties. Schiff bases and their complexes shows a high variety of applications in biological clinical and analytical fields.[6-8] Narwade *et al*[9] have investigated the equilibrium constants of Cu(II) complexes with some substituted chalcones at 0.1M ionic strength pH metrically. Deshmukh has studied proton –ligands stability constants with dichlrosubstituted pyrazolines, is oxazolines, pyrazoles and isoxazoles.[10] Recently Khambre and Narwade have studied stability constant of Cu(II),Ni(II),Co(II) complexes of substituted schiff's bases.[11] Mandakmare *et al* have investigated the interaction between UO2 (II) and substituted coumarins at 0.1M ionic strength potentiometrically and spectrophotometrically.[12] Looking into wide range of activities of Schiff bases and their metal complexes, the present work describe the interaction between La(III), Sm(III) and Nd(III) metal ions with substituted Schiff base complexes in mixed solvent at 0.1M ionic strength that are studied pH-metrically by Calvin- Bjerrum titration technique.

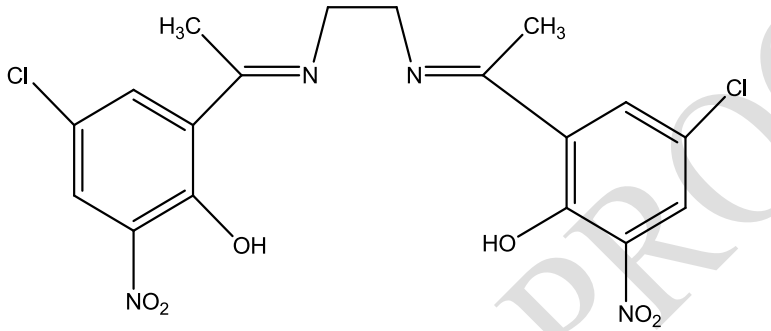
EXPERIMENTAL

MATERIALS AND METHOD

Substituted Schiff's base ligand 6,6'((ethane1,2diylbis(azanylylidene))bis(ethan-1-yl-1-ylidene))bis(4-methyl-2-nitrophenol [L_1] and 6,6'((ethane1,2diylbis(azanylylidene))bis(ethan-1-yl-1-ylidene))bis (4-chloro -2-nitrophenol [L_2] were synthesized in the laboratory by known literature method & their purity was checked by TLC on microscopic slides with silica gel-G layer thickness 0.3mm. The structure was confirmed by IR, NMR spectra and melting point. All the chemicals used were of AR grade. The solution of ligands was prepared in DMF. DMF (A.R.) was purified by the method described by Vogel.[13] All solutions were prepared in double distilled, CO_2 free water. Metal ion solutions were prepared by dissolving the requisite quantities of metal nitrate in double distilled water and standardized by using conventional procedures. Sodium hydroxide solution was prepared in deionised water and solution were standardized before use by known methods & used as afresh.



L_1 : 6,6'-((ethane-1,2-diylbis(azanylylidene))bis(ethan-1-yl-1-ylidene))bis(4-methyl-2-nitrophenol)



L_2 : 6,6'-((ethane-1,2-diylbis(azanylylidene))bis(ethan-1-yl-1-ylidene))bis(4-chloro-2-nitrophenol)

Measurements

All the pH measurements and titrations were carried out with Systronic microprocessor based pH meter with magnetic stirrer (accuracy ± 0.01 units) using glass electrode & calomel electrode at 27 ± 0.1 ^{0} C. pH meter was calibrated by standard buffer solution (pH 4.0,7.00 & 9.2).

The experimental procedure involved the titrations of

(i) Free acid HNO_3 (0.01 mol.dm^{-3})

(ii) Free acid HNO_3 (0.01 mol.dm^{-3}) and ligand (2 x 10 $^{-3}$ mol.dm^{-3})

(iii) Free acid + ligand (2 x 10 $^{-3}$ M) + metal ion titration (4 x 10 $^{-4}$M) with standard NaOH solution (0.13 mol.dm^{-3}) in presence of an inert atmosphere by bubbling a constant flow of nitrogen gas. The ionic strength of all these systems was maintained constant 0.1M by adding appropriate amount of KNO_3 solution.

RESULTS AND DISCUSSION

The substituted Schiff bases L_1 and L_2 having replaceable H^+ ion in phenolic hydroxy group may be ionized as i.e.

$$HL \text{ -------> } H^+ + L^-$$

The titration data were obtained used to constuct the formation curves [acid curve (A), acid + ligand curve (A + L) and acid + ligand + metal ion curve (A + L + M)] by plotting pH Vs volume of NaOH . It is observed from titration curves that for all systems ligand start deviating from the free acid curves at pH = 2.9 and deviation continuous up to pH = 12. The deviation presents dissociation of proton in Schiff base. The average number of proton dissociated from the ligand (n**A**) was determined from free acid and acid - ligand titration curves by employing the equation (1) proposed by Irving and Rossotti .(Table 1)

$$n_A = \gamma - \frac{(E^\circ + N) \times (V_2 - V_1)}{(V^\circ + V1) \times T^\circ_L} \quad \ldots\ldots\ldots\ldots (1)$$

Where, γ is the number of replaceable H^+ ions, V° is the total volume of the solution, E° & T°_L are the total concentrations of Nitric acid & ligand respectively, V_1 &V_2 are volume of alkali added to reach the same pH reading during titration of free acid and free acid plus ligand respectively.V_2-V_1 is the horizontal difference in the volume at the given pH and N is the concentration of sodium hydroxide (0.13 mol.dm^{-3}).

The pK_1 and pK_2values were determined from formation curves by half integral method (n**A** V/s pH) by noting the pH at which n**A** = 1.5 and n**A**= 0.5 respectively. The accurate values of pK_1 and pK_2 were calculated by point wise calculations which are presented in Table 1.

Table 1: Proton ligand formation number at (27 ± 0.1 ^{0} C) and at ionic strength μ = 0.1 mol dm-3

Medium: 70% DMF -water	**μ** =0.1M	**T ^{0}L** = 2 x 10 $^{-3}$M
T ^{0}M = 4 x 10 $^{-4}$M	**N** = 0.13N	**V_0** = 25 ml
E^o = 1 x 10^{-2}	**Temp.** = 27 ± 0.1 ^{0}C	

System: Ligand 1

pH	V_1	V_2	ΔV	nA
5.6	3.41	3.49	0.08	1.845125
5.8	3.45	3.56	0.11	1.797346
6	3.49	3.61	0.12	1.75834
6.2	3.5	3.69	0.19	1.633333
6.4	3.51	3.75	0.24	1.537005
6.6	3.54	3.82	0.28	1.460406
6.8	3.55	3.88	0.33	1.394273
7	3.58	3.93	0.35	1.356452
7.2	3.6	3.96	0.36	1.307692
7.4	3.63	4	0.37	1.289207
7.6	3.66	4.04	0.38	1.270761
7.8	3.69	4.08	0.39	1.252353
8	3.7	4.1	0.4	1.233449
8.2	3.74	4.15	0.41	1.215379
8.4	3.76	4.18	0.42	1.196801
8.6	3.79	4.22	0.43	1.178534
8.8	3.8	4.25	0.45	1.140625
9	3.83	4.29	0.46	1.122442
9.2	3.86	4.33	0.47	1.104297
9.4	3.88	4.36	0.48	1.085873
9.6	3.91	4.43	0.52	1.050723
9.8	3.95	4.48	0.53	0.999092
10	4	4.54	0.54	0.975862
10.2	4.03	4.58	0.55	0.957975
10.4	4.08	4.64	0.56	0.940853
10.6	4.12	4.69	0.57	0.92342
10.8	4.16	4.75	0.59	0.887174
11	4.21	4.83	0.62	0.832592
11.2	4.26	4.91	0.65	0.778195
11.4	4.35	5.05	0.7	0.688245
11.6	4.44	5.24	0.8	0.505435
11.8	4.5	5.43	0.93	0.266102
12	4.6	5.55	0.95	0.234797

System: Ligand 2

pH	V_1	V_2	ΔV	nA
5.2	3.35	3.4	0.05	1.902998
5.4	3.39	3.48	0.09	1.815643
5.6	3.41	3.53	0.12	1.767687
5.8	3.45	3.58	0.13	1.748682
6	3.48	3.62	0.14	1.729635
6.2	3.5	3.65	0.15	1.710526
6.4	3.52	3.7	0.18	1.652875
6.6	3.54	3.74	0.2	1.614576
6.8	3.55	3.8	0.25	1.538389
7	3.57	3.83	0.26	1.499475
7.2	3.6	3.87	0.27	1.480769
7.4	3.62	3.9	0.28	1.461915
7.6	3.65	3.95	0.3	1.424084
7.8	3.69	4	0.31	1.400716
8	3.7	4.05	0.35	1.349268
8.2	3.73	4.09	0.36	1.310825
8.4	3.76	4.15	0.39	1.269172
8.6	3.79	4.2	0.41	1.226742
8.8	3.82	4.24	0.42	1.198473
9	3.85	4.28	0.43	1.180243

9.2	3.86	4.31	0.45	1.142412
9.4	3.9	4.37	0.47	1.105536
9.6	3.93	4.42	0.49	1.068441
9.8	3.96	4.47	0.51	1.031423
10	4	4.52	0.52	1.013793
10.2	4.05	4.58	0.53	0.996558
10.4	4.09	4.65	0.56	0.951217
10.6	4.13	4.7	0.57	0.92379
10.8	4.17	4.76	0.59	0.887556
11	4.23	4.85	0.62	0.83339
11.2	4.27	4.95	0.68	0.732241
11.4	4.35	5.05	0.7	0.688245
11.6	4.43	5.14	0.71	0.673123
11.8	4.5	5.23	0.73	0.638983
12	4.57	5.35	0.78	0.566921
12.2	4.7	5.49	0.79	0.537037
12.4	4.8	5.65	0.85	0.431208

Table 2: Determination of proton-ligand stability constant (pK) of Schiff base at 0.1 M ionic Strength

System	Constants (pK)			
	Half integral method		Pointwise calculation	
Schiff Base L1	pK_1	pK_2	pK_1	pK_2
	6.50	11.52	6.61	11.49
Schiff Base L2	7.18	12.42	7.07	12.32

Determination of Metal-Ligand Stability Constant (Log k) of Schiff base at 0.1 M ionic Strength

Metal-ligand stability constant of metal chelates with Schiff bases of L_1 and L_2 were determined by employing Calvin -Bjerrum pH- metric titration method as adopted equation (2) by Irving and Rossotti . The formation of chelate between metal ions with Schiff bases L_1 and L_2 were indicated by the significant separation starting from pH =2.9 for all complexes.

$$n = \frac{(E°+ N) X (V_3 - V_2)}{(V°+ V_2) x T°m} \quad \text{---------- (2)}$$

Where E° is the concentration of acid, N is the normality of the NaOH, T°m is the concentration of metal, V° is the total volume and V_3- V_2 is the horizontal difference in the volume at the given pH.

The metal ligand stability constants for La(III), Sm(III) and Nd(III) with Schiff base ligands L_1 and L_2 in 70% DMF-water can be found by the half integral method and pointwise calculation method (Table -3).

Table 3: Determination of LogK1 & LogK2 Value

Medium: 70% DMF-water **μ** = 0.1M **T ^{0}L** = 2 x $^{-3}$M
T ^{0}M = 4 x 10^{-4}M **N** = 0.13N **V ^{0}** = 25 ml
E ^{0} = 1 x 10^{-2} M **Temp.** = 27 ± 0.1^{0}C

System	Metal ligand stability constants (log K)			
	Half Integral		Pointwise Calculation	
	Log K1	Log K2	Log K1	Log K2
La(III)-L1 Complex	9.38	7.39	9.18	7.29
Sm(III)-L1 Complex	9.12	7.52	8.908	7.379
Nd(III)-L1 Complex	9.48	7.53	9.383	7.390
La(III)-L2 Complex	3.39	3.11	3.610	3.047
Sm(III)-L2 Complex	3.62	3.01	2.816	2.748
Nd(III)-L2 Complex	4.68	3.41	4.307	3.494

Table: 4 Metal-Ligand Stability constants (Log K).

System	Log K1	Log K2	Log K1-Log K2	Log K1/Log K2
La(III)-L1 Complex	9.38	7.39	1.99	1.269
Sm(III)-L1 Complex	9.12	7.52	1.6	1.212
Nd(III)-L1 Complex	9.48	7.53	1.95	1.258

La(III)-L2 Complex	3.39	3.11	0.28	1.090
Sm(III)-L2 Complex	3.62	3.01	0.61	1.202
Nd(III)-L2 Complex	4.68	3.41	1.27	1.372

CONCLUSION

In the present study, it is observed from titration curve that the extent of deviations confirms complete dissociation of –OH group .The deviation between acid+ligand curve and acid+ligand+metal curve indicates the commencement of complex formation. The change in colors during titration with respect to pH also indicates the complex formation between ligand and metal ions.

Proton-Ligand stability constant (pK)

It could be observed from Table No. 2 that there is good agreement of Proton-liand stability constants between half integral method and pointwise calculation method. The pK values for ligand depend on structures and resonance stabilization of ligands. The ligand L_2 shows higher pK values than L_1.This indicates that the chlorine atom present at para position to the phenolic hydroxy group, is exhibiting strong electron donating positive resonance effect than the electron donating hyperconjugation effect of methyl group in ligand L_1,in the DMF-water system. Symmetrical and asymmetrical structures for ligands are also responsible for the pK values for the ligands.

Metal-Ligand stability constant(Log K)

Determination of metal-ligand stability constants requires the accurate value of proton-ligand stability constant. Higher values of log K_1 and log K_2 showed that ligand are stronger chelating agents and vice versa. In the present investigation values of log K_1 and log K_2 have been calculated with half integral and pointwise calculation methods. The values obtained are in good agreement (shows in Table No. 3). The difference between log K_1 and log K_2 values are studied for all the systems and is represented in Table No.4.

The difference between log K_1 and log K_2 values are less. The difference between log K_1 and log K_2 values indicated the simultaneous formation of 1:1 and 1:2 complexes in the solution. They showed the linear relationships between log K and pK values of ligands suggesting identical binding sites in all ligands.

The higher value of ratio (Log K_1/ Log K_2) for La(III)-Ligand- L_1 & Nd(III)-ligand- L_1 & L_2 complex indicates the more stable simultaneous complex formation as compare to Sm(III) –Ligand-L_1 & L_2 and La(III)-Ligand L_2 complexes. Stability constants depend upon the size of cation.

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A STUDY OF IMPACT ON ECONOMIC EMPOWERMENT OF WOMEN THROUGH SELF HELP GROUPS WITH SPECIAL REFERENCE ON AMRAVATI DISTRICT

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ABSTRACT

Women empowerment is a process in which women challenge the existing norms and culture, to effectively promote their well being. The participation of women in Self Help Groups (SHGs) made a significant impact on their empowerment both in social and economical aspects therefore this study addresses impact of Economic empowerment of women through self help groups in Amravati District of Maharashtra. The information required for the study has been collected from primary sources through structured questionnaire and personal interview and secondary sources. A Non Probability Convenience Sampling' Technique has been followed; Average and percentage analysis was carried out to draw meaningful interpretation of the results. chi-square test was used to find the reasons for joining the Self help group. Factor analysis was used to measure and determine the relationship between the observed variables. Women participation in Self Help Groups have obviously created tremendous impact upon the life pattern and style of poor women and have empowered them at various levels not only as individuals but also as members of the family members of the community and the society as whole. The results of the study revealed that the SHGs have had greater impact on both economic and social aspects of the beneficiaries.

KEYWORDS: *Self-Help Groups, Economic Empowerment, Empowerment of Women.*

1.INTRODUCTION

Self Help groups are nonprofessional organization formed by people with a common problem or situation, for the purpose of pooling resources, gathering information and offering mutual support, services, or care. Women constitute around half of the total human resources in our economy. Yet women are the more poor and under privileged than men as they are subject to many socio-economic and cultural constraints. They have no such place in society likes men .The situation is more severe in the rural and backward areas. Women development activities must be given importance to eradicate poverty, increase the economic growth and for better standard of living. The benefits Include mobilisation of savings and credit facilities and pursuit of group enterprise activities. The group-based approach not only enables the poor to accumulate capital by way of small savings but also helps them to get access to formal credit facilities.

In the words of ex. President Dr. A.P. J. Abdul Kalam, "Empowering women is a prerequisite for creating a good nation, when women are empowered, society with stability is assured". Empowerment of women is essential because their thoughts and value systems leads to the development of a good family, good society and ultimately a good nation.

Facilitating the participation of women in economic

life is seen to provide financial gain at both household and national level, as well as having long-term impacts upon poverty reduction through creating changes in the intergenerational transmission of poverty processes. However, enabling women to participate in economic life is subject to both formal and informal constraints: women face various institutional barriers, as well as discrimination played out within social relations. SHG's is a initiative for removing these barriers, and actively creating mechanisms through which women are able to add value to the economy, access to jobs, access to credit and financial services; land and property rights and; agricultural inputs and technology.

In short SHG's Objectives are:

• To help women to mobilize the resources of the individual members for their collective economic development,
• To uplift the living conditions and status in society
• Create a habit of savings, utilization of local resources,
• To gain mutual understanding, develop trust, Decision making skills, leadership skills and self-confidence.

Thus the SHGs function on the principle of the five 'p's.7

i) Propagator of voluntarism
ii) Practioner of mutual help
iii) Provider of timely emergency loan
iv) Promoter of thrift and savings, and
v) Purveyor of credit.

2. REVIEW OF LITERATURE

Rekha Goankar(2001)in her study concluded that the movement of SHGs can significantly contribute towards the reduction of poverty and unemployment in the rural sector of the economy and the SHGs can lead to social transformation in terms of economic development and the social change.

Naila kabeer (2005) in a study apparently concludes that while access to financial services can make important contributions to the economic productivity and social wellbeing of poor women and their households, it does not "automatically" empower women – any more than do education, political quotas, access to waged work or any of the other interventions.

M.Anjugam (2007) has observed that socially backward, landless and marginal farm house holds participate more in the self help group programme. Possession of livestock and consumer goods by the member households has been found to deter the joining of group.

Gladis Mary John(2008) found that membership in SHG inculcated a great confidence in the mind of majority of women to succeed in day to day life. Positive change was found in the attitude of relatives and friends towards the women in self help groups.

Dr.vasanthakumari (2012)in his study recognized that By organizing poor women into groups, they not only expand options available to them for their development but also provide them with opportunities to develop their confidence and skills to improve their status and to bring about a change in the attitude of the society towards women.

According to Dr. Dasarathi Bhuyan(2006) in his article discussed that the Indian women have cast of their age old shackles of serfdom and male domination. She has come to her own and started scaling the ladders of social advance with proud and dignity and the most important measure of their success should be the extent to which they enable woman to interpret, apply and enforce laws of their own making, incorporating their own voices, values and concerns.

According to Y.B. Shambharkar1, U.V. Jadhav2 and D.M. Mankar3 noticed that among majority women members of SHG despite of having higher level of knowledge about functioning of SHG and favourable attitude towards SHG, the level of outcome is not commendable. This tends to recommends that there is a scope to increase the impact of SHG on empowerment of women member specially in the Rural and tribal groups.

According to Reena , Rajdeep Kaur , Nikita who studies a Comparative Analysis of Women's Economic Empowerment through Self Help Groups to evaluate the level of Women's economic empowerment through SHG i.e. income, expenditure and saving of the member after joining SHG.

According to Rajeev Thomas (2015) who assessed the socio-economic profile of the Neighbourhood groups (NHG) members under microfinance programmes and their participation in NHG activities which will indirectly have a significant effect in their monthly saving habits and ensure this unique model of participation and empowerment

Vikrant Sharma, Preeti Sharma who studied the impact of self help groups on women empowerment and the results of the study revealed that the SHGs have had greater impact on economic, social and political aspects of the beneficiaries.

Pradnya Likhite in her research article evaluated the impact of SHG on the social empowerment of women in Maharashtra that the Self Help Groups are helpful to their family not only from the economic aspect but from the social status aspect as well. The movement of women empowerment is also marching ahead in the light of this strong confidence and the sense of self-realisation

Prof Archana Ajit Borde, Prof Ajit Kumar Borde studied on study on empowerment of women's self help groups and rural women entrepreneurs in Maharashtra. The study focuses on various opportunities received by women self help groups in participating various programmes.

3. RESEARCH PROBLEM

The fundamental aim of promoting SHGs is poverty alleviation and to achieve empowerment of women. This study is undertaken to analyse whether self help groups have a favorable impact on the economic empowerment of women in Amravati District, Maharashtra.

4. OBJECTIVES OF STUDY

The objective of present research study is to study:

1. The extent to which the SHG's are conducive for pushing the spirit of economic self-reliance among women
2. The role of SHG for the economic empowerment of women
3. The awareness & effectiveness of SHG for economic empowerment of women
4. To study the performance and impact of self help groups among women

5. RESEARCH METHODOLOGY:

The said study is based on primary as well as secondary data. Primary Data has been collected through well structured questionnaire and survey method wherein the questionnaire was distributed among 50 members of different self help groups in Amravati district with the help of 'Non Probability Convenience Sampling' Technique.

6. LIMITATION OF STUDY:

Limitations of the study are as follows:-

1. The study will be limited to Amravati city. As such the finding of the study may not be totally applicable to other cities.
2. The study will be limited for empowerment of Women's SHG only.
3. Conclusions and suggestions are drawn on the basis of information provided by SHG women beneficiaries only.
4. Sample size is of 50 only

7. DATA ANALYSIS AND INTERPRETATION:

The data after collection has been analyzed, arranged in tabular form followed by Analysis and Interpretation of data in a general way involves a number of closely related operations, which are performed with the purpose of summarizing the data that fulfill the research objective.

Table No. 1 Education-Wise Details of SHG Members:-

Sr. No.	Particulars	Responses	%
A	10th class	23	45%
B	12th class	15	30%
C	Graduate	7	15%
D	Post Graduate	1	02%
E	Illiterate	4	08%
Total		50	100%

Analysis

From the above table it can be revealed that 10th class education is 45%. 30% members of SHG pass their 12th class.15% member done graduation.2% member have complete the PG. And 8% members of SHG were illiterate.

Table No.2 Age-wise Distribution of Self Group Beneficiaries

Sr.No.	Particulars	Responses	%
A	Below 30 years	15	30%
B	30-40 Years	24	48%
C	Above 40 years	11	22%
Total		50	100%

Analysis

From the above table it can be interpreted that majority of the respondents 48% are from the age group 30-40 years, 30% beneficiaries are from the age group below 30 years and only 22 % of beneficiaries are from age group above 40 years

Table No. 3. Respondents Monthly Income

Sr.No.	Particulars	Responses	%
A	Rs. Less than 1500	11	22
B	Rs. 1500 - Rs. 3000	24	48
C	Rs. 3000 - Rs. 4500	9	17
D	Rs. 4500 - & Above	6	13
Total		50	100

Analysis

From the above table it can be revealed that the majority of the beneficiaries of SHG 48% have earned Rs. 1500 - 3000. 22% member earned less than Rs.1500. 17% women's earned Rs.3000 – 4500. 13% member earned Rs.4500 – & above.

Table No. 5. Reasons for Joining the SHG

Sr.No.	Particulars	Responses	%
A	To attain the economic independence	11	22
B	To get recognition from the society	7	14
C	For Savings and covering expenses	16	32
D	To show the talents	5	10
E	For children's education	7	14
F	For meeting emergency needs(health/medicines)	4	8
Total		50	100

Analysis:

32% of the respondents were of opinion regarding SHG enriching the saving, the second importance for to attain the economic independence 22%, and 14% to recognition from the society , 10% show the talents, 14% joined for supporting their children's education and others and Only 4% of the members opinion reveals that for meeting emergency needs.

Table No. 8. Status of SHG member in family

Sr.No.	Particulars	Responses	%
A	Live Your Life With Respect	20	40
B	You Make Your Own Decision	11	22
C	Have Financial Freedom	7	14
D	Complete Control of Your Life At House	2	4
E	Improvement in standard in living	10	20
Total		50	100

Analysis

From the above table we observed that 40% members of SHG live their life with respect. 22% members of SHG make their own decision. The 20% members have financial freedom. 5% members of SHG have complete control on their life.

Table No. 9. Members are satisfied after joining SHG

Sr.No.	Particulars	Responses	%
A	Yes	47	95
B	No	3	5
Total		50	100

Analysis: From the above table we observed that 95% members of SHG are satisfied and 5% members are not satisfied after joining SHG

Table No. 11. After becoming the member of SHG which objectives are satisfied

Sr.No.	Particulars	Responses	%
A	Habit of Saving	10	20
B	Short Term Financial Help	08	16
C	Long Term Financial Help	07	15
D	Medical Treatment	6	12
E	Higher Education for children	7	14
F	Start Small Enterprise	12	23
Total		50	100

Analysis: From the above table we can interpret that 23% members started their own small enterprise20% members start their habit of saving.16% member get short term financial help, 15% get access to long term financial needs,.14% members were able to achieve higher education for their children's, 12% members spend on medical treatment.

Table No. Problem face by the members after joining the SHG

Sr.No.	Particulars	Responses	%
A	Documentation	11	22
B	Getting Loan	14	28
C	Working Condition	8	15
D	With Member	17	35
Total		50	100

Analysis: From the above table we can interpret that 28% members found problem in lengthy procedure of getting loan 22% members face the problem of documentation process .15% member face the problem of working condition, 35% had the problems with members.

Table no: Advantages of the SHGs viewed by the women.(Multiple Choice)

Sr.No.	Particulars	Responses	%
A	Improved financial stability	45	90
B	Got knowledge about banking systems & else	32	64
C	Could meet each other frequently	15	30
D	Improved status in the family	5	9
E	Could know the other women's problems	7	14
F	Improved self confidence and self esteem	12	24
G	Women could organize and improved decision making	14	28
H	They do not have to take loans from the money lenders	3	6
Total		50	100

Analysis : From the above table we observed that 90% members of SHG believed that SHG improves the financial stability, 64% got knowledge of banking system, 30% members said that SHG help them to meet each other more frequently, 9% members said it improved their status in the family, 14% said that it helped to know other women's problems, 24% improved their self confidence and self esteem, 28% members could organize and improved decision making and 6% were of the opinion that they do not have to take loans from money lenders.

Table No: After the joining of SHG there are changes in your expenses and savings

Sr. No.	Particulars	Respondent	%
1	Strongly Agree	68	68
2	Agree	32	32
3	Strongly Disagree		
4	Disagree		
5	Can't Say		
Total		100	100

Analysis: From the table we can analyze that all the members are happy after joining the SHG. 68% are strongly satisfied with the SHG. And remaining 32% members are satisfied.

8.HYPOTHESIS TESTING

Statement:

1."There is no significant relationship between Education of SHG members for Joining the SHG".

For testing the hypothesis the researcher has applied Chi-Square test:-

Table: Observed Frequency and expected frequency Showing Relationship between Education of SHG members and reasons for Joining the SHG.

Reasons/Education	To attain the economic independence	Recognition from Society	Saving & Covering Expenses	Show talent/skill	For Children's Education	For meeting Emergency Needs	Total
10th class	05(5.06)	04(3.22)	07(7.36)	01(2.3)	04(3.22)	02(1.84)	23
12th class	05(3.3)	03(2.1)	03(4.8)	02(1.5)	01(2.1)	01(1.2)	15
Graduate	00(1.54)	00(0.98)	05(2.24)	02(0.7)	00(0.98)	00(0.56)	7
Post Graduate	00(0.22)	00(0.14)	01(0.32)	00(0.1)	00(0.14)	00(0.08)	1
Illiterate	01(0.88)	00(0.56)	00(1.28)	00(0.4)	02(0.56)	01(0.32)	4
Total	11	7	16	5	7	4	50

Table: Chi-Square Test

O	E	(O-E)	$(O-E)^2$	$(O-E)^2/E$
05	(5.06)	-0.06	0.0036	0.0007
04	(3.22)	0.78	0.6084	0.189
07	(7.36)	-0.36	0.1296	0.0176
01	(2.3)	-1.3	1.69	0.7347
04	(3.22)	0.78	0.6084	0.1889
02	(1.84)	0.16	0.0256	0.0139
05	(3.3)	1.7	2.89	0.8757
03	(2.1)	0.9	0.81	0.3857
03	(4.8)	-1.8	3.24	0.675
02	(1.5)	0.5	0.25	0.1667
01	(2.1)	-1.1	1.21	0.577
01	(1.2)	-0.2	0.04	0.0333
00	(1.54)	-1.54	2.3716	1.54
00	(0.98)	-0.98	0.9604	0.98
05	(2.24)	2.76	7.6176	3.400

02	(0.7)	1.3	1.69	2.414
00	(0.98)	-0.98	0.9604	0.98
00	(0.56)	-0.56	0.3136	0.56
00	(0.22)	-0.22	0.0484	0.22
00	(0.14)	-0.14	0.0196	0.14
01	(0.32)	0.68	0.4624	1.445
00	(0.1)	-0.1	0.01	0.1
00	(0.14)	-0.14	0.0196	0.14
00	(0.08)	-0.08	0.0064	0.08
01	(0.88)	0.12	0.0144	0.0163
00	(0.56)	-0.56	0.3136	0.56
00	(1.28)	-1.28	1.6384	1.28
00	(0.4)	-0.4	0.16	0.4
02	(0.56)	1.44	2.0736	3.70
01	(0.32)	0.68	0.4624	1.445
			TOTAL	23.2585

Degree of Freedom (V):-

V= (r-1)*(c-1)=(5-1)*(6-1)= 20

Conclusion: The table value of Chi-Square test at 5% significance level with 20 degrees of freedom is 31.410, which is greater than 23.258 the calculated value of chi-Square. Hence the stated null hypothesis is accepted and hence we can say that there is no significant relationship between education of SHG members and reasons for Joining the SHG.

9. FINDINGS

Following Are the Findings of the Present Study

1) The 10th pass level in the SHG is relatively high. Only 15% women's are done Graduation.
2) Most of members earn Rs. 1500 - 3000. This gap is because of priorities of job i.e. some members do full time job or some are part time.
3) Most of women joining and becoming the members of SHG fall in age category 30-40 Years.
4) Maximum of the women joined the SHG for saving and covering expenses and most of the member of SHG joined to attain the economic independence and very few of them joined for meeting emergency needs.
5) Majority of members are in favor of that SHG provide them improved financial stability , improvement in standard of living, decision making skills and many members also got trained and acquainted with the banking system and technology.
6) After becoming the member of SHG women have good position in the house, society and live her life with respect and few of them also improved with their decision making skills.
7) Most of SHG members satisfied their objectives for which they become the member of SHG.
8) Majority of the member of SHG start their own business which is very much helpful for their development and for self dependent.
9) Some member of SHG has faced the problem of working condition but most of the members of SHG have problem with the members.
10) Every women of SHG strongly agrees that after joining the SHG there are prominent changes in member's expenses and savings pattern as they increase the savings and also most of women make expenses on business growth.

10. SUGGESTIONS

1) The educated women should participate in SHG.
2) The members of SHG who are not get full time participation in SHG they should Take full time participation in SHG.
3) The training programme should be organised by the SHG member for better development.

4) The member of SHG should help the other member to solve problems within the group for self as well as group development.
5) More schemes can be introduced by the government and it has to be properly communicated and advertised to reach the Self Help Groups. Various Non Government Organizations and other support agencies should effectively participate and deals with Self Help Group.
6) It is suggested that motivational campaign may be conducted for increase in saving habit in the minds of the members. The campaign should give importance to savings for future benefits.
7) The number of member should be increase in SHG.
8) The programmes should be designed on the basis of needs of women at the micro level. Planning for self-employment for women needs a multi-pronged strategy.

11. CONCLUSIONS

• After Analysis and interpretation of the collected data it can be easily concluded that most of the women who have passed SSC are willing to join SHG for fetching various benefits of joining the SHG and gaining economic independence, financial stability and an improved mode of saving the incomes and covering expenses. Hence, when women's participation in the labor force grew fastest, the economy will experience the largest reduction in poverty rates.
• When women farmers can access the resources they need, their production increases, making it less likely that their families are hungry and malnourished.
• When women have access to time-saving technologies & Skill training programmes they increase their productivity, capacity and as well as launch income-generating pursuits and entrepreneurial ventures. Those kinds of outcomes empower women to become stronger leaders and to more effectively contribute financially to their families, communities and countries.
• Increased income controlled by women gives them self confidence, which helps them obtain a voice and vote in for empowerment
• Economic empowerment makes conducive the Economic decisions of acquiring, allocating, and selling assets.
• Increasing the role of women in the economy is part of the solution to the financial and economic crises and critical for economic resilience and growth.

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MANAGEMENT SECTION

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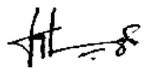
From the editor's desk…

It is matter of great honor and privilege for me to extend warm regards to everyone who has extended direct or indirect support and well wishes for this academic event, National Conference on "Smart India Vision 2020 – Innovations in Computer Applications Management and Commerce". This conference, I welcome all the dignitaries, invitees, eminent scholars, resource persons, research scholars and research students. We have organized this national conference with the view to provide an excellent opportunity to discuss various relevant aspects of innovations in Management, Commerce and Computer Applications.

India vision 2020 is aimed at transforming India into a developed country by giving priority to Education, Information and Communication Technology, Agriculture and Infrastructure. Dr. Kalam has said that we should use technology for the betterment of India. There are only three years left to achieve Dr. Kalam's vision of a developed India in 2020. Everyone has to work in coordination with every other industry in order to effectively support the Vision 2020. Dr. Kalam encouraged the youth of India to understand the importance of conservation and to lead the way towards sustainable development. The Youth should learn to take responsibility and work in the direction of development with honesty and integrity. In order to discuss "Smart India Vision 2020", the role of innovations in Computer Applications, Management and Commerce, this Conference will prove immensely beneficial to researchers, students and teachers.

I am extremely grateful to Hon'ble Dr. Devisinghji Shekhawat, the Founder President of Vidyabharati Shaikshanik Mandal, Amravati and Chief Patron of this conference and to Hon'ble Smt. Pratibhatai Patil, former President of India, for their valuable guidance and inspiring suggestions. I take this opportunity to express my gratitude to Hon'ble Raosaheb Shekhawat, President of Vidyabharati Shaikshanik Mandal, Amravati and all the other members of the Management for their cooperation, moral support and to go ahead with this academic event.

At the same time, I would like to express appreciation for the efforts done by faculty members to bring laurels for our college by organizing this event successfully. I express my sincere thanks to all Researchers, Resource persons, Delegates and Participants who have taken pains attend and grace the occasion with their research attempts.



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National Conference on

"Smart India Vision 2020- Innovations in Computer Applications, Management and Commerce"

On 18th February 2017

Programme Schedule for Commerce & Management (Teachers)

Inaugural Function- 10:00 am to 11:30 am

Venue- AV Theatre, 3rd Floor, Old Building

Chairperson: Shri Raosaheb Shekhawat

President, Vidya Bharati Shaikshanik Mandal, Amravati

Inaugurator: Hon'ble Dr. Murlidhar Chandekar

Vice Chancellor, Sant Gadge Baba Amravati University, Amravati

Dr. F.C. Raghuwanshi

Principal, Vidya Bharati Mahavidyalaya, Amravati

Keynote Address- 11:30 am to 12:30 pm

(Venue- Seminar Hall, Ground Floor, New Building)

Dr. Aditya Lunawat

Director, Swami Vivekanand Careeer Guidance Scheme with Govt of MP,
Higher Education, Bhopal

Chairperson: Dr. S.B. Sadar

Head, Department of Business Administration & Management,
Chairman, Board of Studies in Business Management, SGB Amravati University

Plenary Session I- 12:30 pm to 01:30 pm

(Venue- Seminar Hall, Ground Floor, New Building)

Chairperson: Dr. Kailash Rajput,

Head, Department of Commerce, Vidya Bharati Mahavidyalaya, Karanja Lad

Co Chairperson: Dr. Subhash Jadhao,

R.A. College, Washim, Chairman, Board of Studies in Commerce, SGB Amravati University

Lunch- 01:30 pm to 02:00 pm

(Venue- New Building)

Plenary Session II- 02:00 pm to 04:15 pm

(Venue- AV Theatre, 3rd Floor, Old Building)

Chairperson: Dr. A.K. Mishra

Bhilai, Chhattisgarh

Co Chairperson: Dr. P.N. Ladhe

Janta College, Malkapur, Chairman, Board of Studies in Accounts & Statistics, SGB Amravati University

Tea- 04:15 pm to 04:30 pm

Venue- Old Building

Valedictory Function- 04:30 pm

(Venue- AV Theatre, 3rd Floor, Old Building)

Chief Guest: Dr. P.T. Choudhary

Dean, North Maharashtra University, Jalgaon

Chairperson: Dr. F.C. Raghuwanshi

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Head, Department of Business Administration & Management,
Sant Gadge Baba Amravati University, Amravati

Plenary Session I- 12:30 pm to 01:30 pm

(Venue- Seminar Hall, Ground Floor, New Building)

Chairperson: Dr. M.R. Ingle

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Chairman, Board of Studies in Business Economics, SGB Amravati University

Co Chairperson: Prof. N.A. Dhawle

Head, Department of Business Administration, Sipna College of Engineering & Technology, Amravati

Lunch- 01:30 pm to 02:00 pm

(Venue- New Building)

Plenary Session II- 02:00 pm to 04:15 pm

(Venue- AV Theatre, 3rd Floor, Old Building)

Chairperson: Dr. Vivek Dandekar

Government MMR College, Champa, Chhattisgarh

Co Chairperson: Prof. A.V. Deshmukh

Head, Department of Business Administration, PRMITR, Badnera

Tea- 04:15 pm to 04:30 pm

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improvement of India in some of the international rankings, prevent corruption practices etc and these are some of the reasons why people although are going through difficulties are lauding this measure of the government.

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DIGITAL VILLAGE: BREAKING THE TRADITIONAL PERCEPTION TOWARDS RURAL DEVELOPMENT

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ABSTRACT

Any country whose rural population is still mired in poverty and unemployment, and faces high rural-urban economic equality cannot be termed as a developed country. In fact, the economic status of the rural population of any country provides a more realistic picture of the development quotient. Using this framework, countries such as India, China, South Africa, Vietnam, Brazil, Indonesia, and Egypt are far from being developed, even though they may be witnessing a high GDP growth rate. Thus, development of rural economies is the real challenge for developing countries where large sections of people reside in villages and smaller towns. And this is the area where development interventions either fail to penetrate or bounce back without creating a significant impact, and there are more reasons than one for this.

There is increasing realization that India cannot be a developed nation as long as we do not improve the basic amenities in villages. India's rural market is booming, but e-commerce in India is associated almost exclusively with urban distribution. We need rural e-commerce for Indian Digital Villages to overcome their logistical disadvantages. Indian villages desperately need low-end manufacturing to create jobs for youngsters who have no interest in farming. Large industries cannot do the job. What's needed is infrastructure plus marketing and financial linkages that enable rural entrepreneurs to start small-scale industries.

Introduction

The concept of smart village is very interesting because Indian villages in general have poor basic amenities as compared to the urban area. According to the 2011 census of India, 68.84% of Indians (around 833.1 million people) live in 640,867 different villages. The size of these villages varies considerably. 236,004 Indian villages have a population of fewer than 500, while 3,976 villages have a population of 10,000+.

Rural areas need not be and should not be characterized solely as agricultural belts and agrarian population. Instead, they should be considered as a human resource: a resource of young people, of skilled artisans, of innovators, and of entrepreneurs, that is waiting to be tapped to accelerate local and national development. A rural youth may be a son or daughter of a farmer but need not be a farmer herself/himself. Instead s(he) can be a software developer, a call-centre employee, an e-entrepreneur, a lawyer preparing cases for foreign firms, or a potential employee for new forms of employment which have not yet emerged. Once we start thinking from this angle, we will realize that we need to correct our development-oriented policies and actions. A case to point out is that of the ICT intervention. The impact of ICT in rural areas and particularly on rural poverty is very limited despite its penetration into urban areas. And even when ICT projects are taken up in rural areas, they are limited to agricultural sector.

The vision for Digital India unveiled by the Prime Minister is path breaking and has the potential to create a transformational change in various sections of the society with rural India poised for being the biggest beneficiary of this change. The plan to provide universal phone connectivity and access to broadband in 2.5 lakh villages by 2019 is the clarion call for

entrepreneurs and policy planners to take advantage of the opportunity to build new solutions for rural markets. Mobile telephony is expected to play the lead role in delivering the advantages of information access and digital empowerment to the rural population. India has close to 960 million mobile subscribers as of end March 2015 as per Telecom Regulatory Authority of India (Trai) and now service providers are turning their attention to rural India for their expansion. Urban mobile subscriber share stands at 58% as compared 42% of rural subscribers. Rural mobile subscriber base is growing twice as faster compared to urban subscriber base. While the national teledensity stands at 79% as of March 2015, rural teledensity is 46.5% thus indicating a yawning gap to be addressed.

What is encouraging is the fact that in order for digital revolution to take off in rural India, the supportive pillars namely the processes, the banking system, digital literacy and the willingness of people to accept the change in view of the benefit accruing to them—all of which are time consuming to conceptualize and implement, have already been thought through diligently and are being addressed in parallel. In order to realize the full potential of the digital vision articulated, investments envisaged will have to take place within the timeframes outlined, further reforms in the interface between the government and citizens need to be implemented expeditiously, better alignment between various agencies would have to be ensured and digital literacy would have to gain further momentum.

Latest Initiatives and Developments

1) Digital "Town Squares"

Digital Village Concept can best be done by creating Digital "Town Squares" – which will be tower-based sites that enable the Smart Village and would become the focal point for the providing information, social, in-site physical infrastructure, each with its own ecosystem of energy, security ,e-learning and e-governance etc., services to villages. This can become the spring board for rapid economic growth in the rural areas. Global case-studies have demonstrated how wireless broadband plays a key role in rural society, impacting GDP, productivity and employment. These towers can extend significant benefits to the village's economy, typical benefits like 24x7 backup energy at site premises, additional services such as mobile charging and recharging points, entrepreneurial opportunity to set up cyber cafes and other small businesses that require internet connectivity, uninterrupted e-education, mini ATMs, E-Government and other value added services

2) Godavari district of Seemandhra , village Mori

This tiny cashew-exporting village located near the Bay of Bengal in the East Godavari district has gone fully digital with all the households enjoying WiFI, Internet connectivity and cable TV while making all transactions cashless. It has also achieved the status of complete cashless transaction village, besides being open defecation free (ODF) village

3) In July 2015 that Akodara village,

Akodara village located a little over an hour away from Ahmedabad, in Sabarkantha district in Gujarat, has become the first digital village of India with operating ATMs, shops opposite the CCTV-monitored anganwadi with provision to pay through mobile phone to pay. The village has wifi too. The village of 1,200 people has been adopted by ICICI Bank, helped by the local administration, so that it can be showcased as an example of the bank's vision of the digital future that awaits India's hinterland. Almost every adult in Akodara now has a savings bank account with ICICI, which he or she can access through the local bank branch, or the village ATM, or through mobile phones via SMS. The villagers' most important transactions — selling agri-produce at the local mandi or selling milk at the co-operative society — have been digitised and made cashless. The system has made them automatically less susceptible to corruption and fraud. Also, their accounts are linked to their Aadhar cards, which mean that government benefits are now transferred directly into their savings accounts.

4) Provision of Urban Amenities to Rural Areas (PURA) is a strategy for rural development in India PURA proposes that urban infrastructure and services be provided in rural hubs to create economic opportunities outside of cities.

5) National Institute of Rural Development (NIRD)

Seven pilot projects were implemented and An evaluation study of these pilot projects was carried out by which identified the necessity of community and private sector participation as essential factors and the need for factoring infrastructure development with lead economic activities and livelihoods creation, requirement of project site selection on the basis of growth potential and need for convergence with other schemes of the government.

6) Dhasai village in Thane district of Maharashtra.

The idea was sown by Swatantryaveer Savarkar Rashtriya Smarak—a Mumbai-based NGO—which approached the Bank of Baroda with a request to

provide necessary infrastructure in the village of 10,000 people, including a significant tribal population.

On the similar grounds there were welcome initiatives and successful penetration in Punsari village Gujarat , Hiware-Bazaar, Maharashtra , Ankapoor, Telangana , Kumbalangi village, Kerala – a model for eco-tourism

7) Digital India Initiative for women empowerment.

W2E2 (India): Women for Empowerment and Entrepreneurship, in short W2E2 is helping rural women with digital tools, e-learning, internet connection. Women tend to use the Internet for their own projects in fields like sustainable agriculture and rural health.

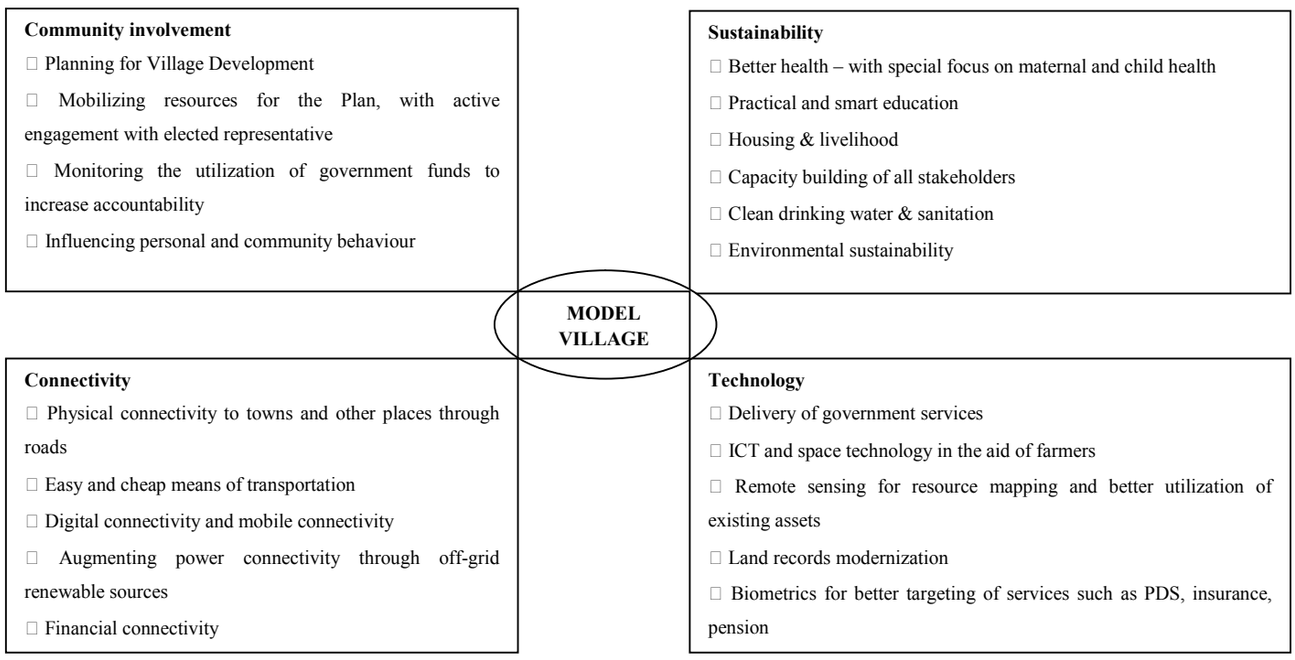
Similarly, **National e-Governance Plan** gives a chance to the rural entrepreneurs to provide citizen-centric services including access to land records and utility bill payments. The other similar pillars for women empowerment are: - Broadband Highways, Universal Access to Mobile Connectivity, Public Internet Access Program, e-Governance, e-Kranti – Electronic Delivery of Services, Electronics Manufacturing, IT for Jobs, Early Harvest Program, Internet Saathi etc.,A few years back, these were dream only. But now we're proud to say that Indian women are developing, so does India.

8) **PUBLIC PRIVATE PARTNERSHIPS**

Currently, India has a total of 450,000 telecom towers, only 60% of which are located in rural areas. These tracts have a poor tele-density of 46% (according to TRAI). We need another 60,000 telecom towers in order to achieve the goals of the 'Digital India' programme. Rural telecom remains expensive and the only way, to get broadband penetration in rural India is through Public Private Partnerships (PPP). PPP committees need to be set up at the highest level in each state to facilitate the rollout and provision of these services. Despite the tediousness, scale of the venture, and time required to medium-term, and associated social and economic implications for India in the long-term, make Public Private Partnerships an absolute necessity for the cause of 'Digital Villages'. To add value, our Indian subsidiary would be happy to participate in a few PPP pilot projects, by providing the Telecom Infrastructure that is essential to making these projects a success.

Key elements of a model village

A 21st century model village in India needs to incorporate certain key themes which would be essential for its success. The figure below highlights these broad thematic focus areas, and also mentions the important elements under each such theme.



An intervention under one of these areas could have an effect across other areas as well. For example, technology could be used to improve the quality and delivery of other services such as health and education, which in turn contributes to sustainable development. A number of these initiatives have already been taken in

different parts of the country, but most of them have been attempted in isolation. The urgent need is to bring about a convergence of all such initiatives, for which 2 things would be essential – a) grassroots level planning; and b) mobilization of resources.

Conclusion

The telecom infrastructure companies can play a major role in the eventual success of 'Digital India'. A well-orchestrated collaboration between the Government, policy makers, mobile network operators, and telecom infrastructure companies is crucial to the success of this venture.. However, rather than imposing taxes, levies, charges, and licence fees on the telecom sector, the government must provide 'gap funding' and other incentives to the Industry for expanding into rural locations; they also need to form a public-private partnership (PPP) to initiate and manage wireless broadband pilot projects in districts with government provided fibre backhaul (NOFN) aimed at creating smart villages.

Thus the next decade could catapult India to a new league of prosperity which will pave the way for elevating the living standards of sizeable number of rural poor and empower them as never before if only we seize the opportunity that digital mission provides.There is a need to transform schemes and subsidies being given to rural areas. New schemes and projects need to be created which promote rural e-Entrepreneurship. Bank loans and credit lines should be extended to provide funds for rural e-commerce and other e-Services.

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EMPLOYEE ENGAGEMENT: AN OVERVIEW

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ABSTRACT

The employee engagements a business buzzword has generated research and steam since the new economy service industries like Information Technology (IT) and Information Technology Enables Services (ITES) have taken off, the origins of engagement are as old as mankind itself. Employee engagement is the conditions for all members of an organization to give of their best each day, committed to their organization's goals and values, motivated to contribute to organizational success, with an enhanced sense of their own well-being. It is based on trust, integrity, two way commitment and communication between an organization and its members. Employee engagement is an approach that increases the chances of business success, contributing to organizational and individual performance, productivity and well-being. It is a property of the relationship between an organization and its employees. An engaged employee is one who is fully absorbed by and enthusiastic about his/her work and so takes positive action to further the organization's reputation and interests.In other words engaged employees are those who are emotionally connected to the organization and cognitively vigilant. In this papers an attempt is made to study overview of employee engagement.

***Keywords*:** *Employee Engagement, Information Technology, Work engagement.*

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development. Hence GST may usher in the possibility of a collective gain for industry, trade, agriculture and common consumers as well as for the Central Government and the State Government.

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INDIA ON ITS URGE TOWARDS CASHLESS ECONOMY

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ABSTRACT

India hopes to create a cleaner, more transparent economy via digitalization that will lead to an improved climate for foreign investment, boost economic growth, and ultimately propel the country to the next chapter of its emerging markets story.Debit and credit cards have started to take the place of cash all around the globe and the time is not so far when the term 'cashless society' becomes a reality. Everyday products and services for which one used to pay using cash are now being paid for using cards. In fact, your next festive purchase or that long overdue vacation can now be possible without having to worry about carrying or using cash.But even if India were to accomplish this rather incredible and, in the short-term at least, improbably feat, there is still a marked downside, "The long term impact will be a paradigm shift to the digital fintech platforms. It will be a surveillance, panopticon-led society of a new breed."

Keywords: *Demonatisation, Cashless economy, Digitilasation, Online banking, Mobile banking, Online shopping, e-wallets,*

Introduction

India's demonetization scheme was a unilateral initiative that was planned in secret — in a back room of Prime Minister Modi's home, in fact — by a small group of insiders tied-in with the upper echelons of India's government. The strategy was to instantly nullify all 500 and 1,000 rupee banknotes, the most common currency denominations in the country, and then eventually replace them with newly designed, more secure 500 and 2,000 rupee notes. This endeavor instantaneously became policy when the prime minister announced it via a surprise television address at 10:15 PM on November 8.

"Ever since Prime Minister Narendra Modi's demonetization announcement, we have suddenly seen a spike in both app downloads & merchant registrations. This spike is now coming from all cities, big and small, pan-India, consisting of small merchants like vegetable vendors, Kirana shopkeepers [small convenience stores], street vendors, rickshaw drivers, taxi's etc., who've signed onto our Oxigen Wallet app for the merchant payments service," said Pramod Saxena, the founder and CMD of Oxigen Services.

It was a move that could have brought India's economy to a shuddering halt. Indeed, the seemingly endless queues outside banks, and the difficulty of spending cash at shops and stalls may have seemed like it did. But the decision to demonetise the 500 and 1,000 rupee notes was just one in a series of moves that will push India towards a digital economy.

The demonetisation was implemented with the aim of eliminating societal corruption and counterfeit currency. But the move was sudden, happening overnight. The

two notes accounted for 86% of the bank notes in circulation in India, and retailers and consumers were forced to look immediately for options. Many turned to digital paying systems.

The digital money strategy has been laid out by Prime Minister Modi's government from its early days in power, via a string of major decisions. The Jan Dhan scheme, for example, saw more 220m new bank accounts being opened for the poorest in society. The Reserve Bank of India also decommissioned all currency notes issued before 2005.

Until recently, cash was used for more than two-thirds of transactions in India. However, just over a month into the demonetisation and the country had already started to see the benefits of digital transactions. Government figures show a 268% increase in year-on-year tax collection from 47 Indian cities for November 2016.

Perhaps the most significant development since the announcement of demonatisation is the shift in the legitimizing narrative around the note ban. What was touted as a 'surgical strike' on black money, fake currency and terror funding has now become a radical 'reform' to transform India into a cashless economy. A series of measures, not least a high-decibel advertising campaign, are already in place to build national consensus in favour of this transformation.

WHY CASHLESS???

What explains this urgent drive towards a cashless society? One way to answer this is to consider the likely outcomes of a cashless society, and read back from it the intent behind such a move. One immediate outcome of a cashless India would be a sharp rise in indirect taxes compliance. Traders, small businesses, shopkeepers, and consumers routinely use cash as a means to avoid paying service tax, sales tax, VAT, and any number of indirect taxes and fees. This mindset needs to change if the imminent Goods and Services Tax (GST) regime is to actually work. Brutally enforcing a cashless payments system — by sucking out 86 per cent of paper money and letting people flounder for a period in a condition of acute paper money scarcity — is perhaps the quickest means way to get there.

Cashless societies are generally corruption free. There are lots of benefits for being cashless(doesn't mean being poor). Cost of handling cash is high, it is in the favor of economies to go cashless. Cashless transfer is nothing but electronic /digital transaction of your capital with the help of netbanking, credit card, etc. You can shop, pay your bills, schedule transactions and manage all your finances from home, office or wherever you are with your smart phone. It not only ease our life but also authenticate and formalise our transactions. Electronic transactions also help in curbing corruption and black money flow which in result ameliorate economic growth. Digital payments indirectly reduces expenditure in manufacturing currency notes and its transportation. It's ingeniosity is only questionable only when it comes to cyber fraud and hacking. In a third world country, cashless transaction system is not that widespread due to lack of education and technology gap which is actually a matter of concern and must be addressed by government or financial institutions.

The moves like digilatisation are pushing the economy in the right direction….

Since the removal of the notes, the government has been working hard to promote digital payment systems to consumers, proactively offering different incentives and rewards. So far, it seems to be working: the government has reported a 400-1,000% increase in digital transactions since the demonetisation.

The changes have created perfect market conditions for alternative digital payment systems, in addition to existing e-wallets and debit/credit cards. These are not just the basic banking apps or websites either. The National Payments Corporation of India, together with the RBI, has launched UPI ("united payment interface"), which powers multiple accounts from participating banks, and offers several banking services all in a single mobile application.

A step in the right direction certainly, but not one without its problems. Although India has around 220m smartphone users as of February 2016, there is still a long way to go until 100% of the population has mobile internet access.

Nevertheless, the banks have made sure that smartphone ownership is not a barrier to accessing mobile payments, providing a USSD option on older, "non-smart" phones which users call up for. In addition, the "Digital India Initiative" has been set up to provide internet access and comprehensive mobile phone coverage across India, helping over a billion people to get online and utilise digital payment techniques.Furthermore, the RBI has been promoting a biometric authentication system for banking. The Aadhar Enabled Payment System (AEPS) can be used to open a bank account, withdraw or deposit cash, and transfer funds using just an identification number and fingerprint. AEPS was created to serve remote towns and villages where cash machines cannot be provided. It has the potential to be the cornerstone of the government's vision of a cashless digital society – if it can penetrate deeply enough into rural India.

The transaction costs are coming down and will further go down. Once a substantial part of transactions are cashless, it would bring down the cost of printing, managing and moving money around. Further, the cashless economy automatically solves the problems of cash out on long holidays, risk of carrying currency notes etc. Further, the lesser use of cash strangulates the grey economy, prevents money laundering and increase tax compliance. Increased tax base would result in greater revenue for state and greater amount available to fund the welfare programmes. Lastly, Cash being material, can be prevented from circulation but electronic channels alleviate this friction and increase circulation of currency. Enabling access to banking is a pre requisite to promote cashless economy. So the success of initiatives such as Jan Dhan accounts linked to Aadhaar data will be very important. A robust payments mechanism to settle a digital transaction is also needed, though the National Electronic Funds Transfer and Real Time Gross Settlement services. The Indian central bank will also have to shed some of its conservatism, part of which is because it has often seen itself as the protector of banking interests rather than overall financial development.

The expansion of telecom and smart phones would provide a digital shift to the economy in near future. The private sector the driver of this change. Government is also mulling to provide incentives for electronic payments for example waiver of tax when electronic settlements are used. The private sector has to come forward to drive the change. Apart from this government should also give incentives for electronic transactions.

Conclusion

Cashless is now the big buzzword in India, and the ball is rolling as the world's largest cash economy begins going digital. Seeing the digital landscape of other developing countries, we can be certain that in setting a huge goalpost for digital finance, we are not pursuing a chimerical dream. But the pace of this journey will have to be determined by the ability of our citizens to cope with it. We should not take to a highway that leaves millions of it citizens below.

What we need for any revolution to succeed is humility. When we design solutions that recognise everyone as equal partners, we have a real chance to achieve our national goals. This logic comes from the power of empathy — not a form of empathy that comes from superiority, but one born from a profound humility.

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