BIOLOGICAL APPLICATIONS OF SCHIFF BASE TRANSITON METAL COMPLEXES- A REVIEW

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ABSTRACT

Schiff bases and their metal complexes plays a very important role in modern co-ordination chemistry. It possesses novel structural features and find number of applications in various fields including food industries,dyes, agricultural ,catalysis, polymer science. The most important feature of Schiff bases is complexation with various metals which lead to the formation of new structures which exhibit wide range of biological activities. Transition metal complexes acts as antimicrobial, antifungal, antiulcer, anticancer agents. This review mainly focuses on the research contributing to the preclinical screenings.

Keywords : *Metal complexes , antimicrobial ,antifulgal ,biological activities*

Introduction

Schiff bases are formed by condensation of carbonyl compounds and amines under specific conditions.Schiff bases contain a azomethine group (-C=N-) Which has considerable biological significance and found tobe responsible for biological activities.

Schiff base containimg azomethine nitroge not only binds the metal ion but also attached to the macrobiomolecules like proteins ,amino acids or also to the DNA molecules.Some of the Schiff base complexes and their biological activities are summerised below.

A number of complexes of Co(II), Ni(II), Cu(II), Zn(II), Cd(II), Au(III) and Hg(II) of the Schiff bases prepared by the condensation of 2,6-diformyl-4-methylphenol and 5-aminouracil, ((5-{[(3-{[(2,4-dioxopyrimidin-5(1*H*,3*H*)-yl)imino]methyl}-2-hydroxy-5-methylphenyl)methylene]amino}pyrimidine-2,4(1*H*,3*H*)-dione in 1:2 ratio were synthesized. It was also found that these Schiff base complexes showed significant inhibitory activity against A. niger ,P.aurenosa a d C. albicans.[1]

Metal complexes, ML_2Cl_2, where M is Fe(II), Co(II), Ni(II), Cu(II), Zn(II), or Cd(II), and L is the Schiff base formed by condensation of 2-thiophenecarboxaldehyde with 2-aminopyridine, N-(2-thienylmethylidene)-2-aminopyridine (TNAPY) were prepared and characterized. The Schiff base and its metal chelates have been screened for their in vitro antibacterial activity against Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa. The metal chelates were shown to possess more antibacterial activity than the uncomplexed Schiff-base. [2]

A series of first complexes of Co(II), Ni(II), Cu(II), Mn(II) and Fe(III) have been synthesized with Schiff base derived from isatin monohydrazone and fluvastatin.. The Schiff bases and their complexes have been screened for their in-vitro antibacterial (Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa and Bacillus subtilis) and antifungal (Aspergillus niger and Pencillium Chrysogenum) activities by minimum inhibitory concentration (MIC) method.[3]

Schiff base (L) ligand is prepared via condensation of pyridine-2,6-dicarboxaldehyde with -2-aminopyridine. The synthesized ligand, in comparison to their metal complexes also was screened for its antibacterial activity against bacterial species, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus pyogones and Fungi (Candida). The activity data shows that the metal complexes to be more potent/antibacterial than the parent Schiff base ligand against one or more bacterial species. [4]

Neutral and cationic copper bis (thiosemicarbazone) complexes bearing methyl, phenyl, and hydrogen, on the diketo-backbone of the ligand have been synthesized. In vitro cytotoxicity studies revealed that they are cytotoxic unlike the corresponding zinc

complexes. Copper complexes synthesized from glyoxal-bis(4-methyl-4-phenyl-3-thiosemicarbazone) (GTSCH2) are the most cytotoxic complexes against various human cancer cell lines, with a potency similar to that of the anticancer drug adriamycin and up to 1000 fold higher than that of the corresponding zinc complex. [5]

Microwave assisted synthesis of Schiff base ligand and their metal complexes, was obtained from the condensation of Riluzole (6-(trifluoromethoxy)benzothiozole-2-amine) and 2-hydroxyacetophenone with high atom economy of 95% and metal complexes of the type MLn=1,2.(H2O)n=1,2Cln=1.2, where M is the metal ion and L is the ligand The Schiff base behaves as a bidentate ligand and it coordinates through the oxygen atom of the deprotonated phenolic group and the nitrogen of imine group. All these complexes were screened for their antibacterial activity by agar cup-plate method against various organisms, and the results were compared. [6]

Co(II), Ni(II), Cu(II) and Cd(II) complexes of bidentate Schiff bases derived from the condensation of 4-amino-5-benzyl-4H-1,2,4-triazoles-3-thione with pyridine-2-carboxyaldehyde, pyridine-3-carboxya-ldehyde and pyridine-4-carboxyaldehyde were synthesized. The Schiff bases act as bidentate ligands coordinating via the azomethine nitrogen and thiolate sulfur atoms. The Schiff bases and their metal complexes have been screened for antimicrobial activity against six bacteria, namely Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus cereus, Micrococcus luteus, Escherichia coli and Serratia marcescens and six fungi, namely Candida albicans, Geotrichum candidum, Fusarium oxysporum, Scopulariopsis brevicaulis, Aspergillus flavus, and Trichophyton rubrum.[7]

A condensation reaction between 1,2-diphenylethane-1,2-dione dihydrazone (DPEDDH) and dimethyl or diethyloxalate in methanol resulted in a novel Schiff base octaazamacrocyclic ligand, (L). Subsequently metal complexes of the type [MLX 2] and [CuL]X 2 ; (M = Mn(II), Co(II), Ni(II) and Zn(II); X = Cl or NO 3) were synthesized by the reaction of the free macrocyclic ligand (L) with the corresponding metal salts in 1:1 molar ratio. The ligand and its complexes were screened for their antibacterial activity in vitro against Gram-positive (Staphylococcus aureus) and Gram-negative (Escherichia coli) bacteria and were also studied for their anticancer activity against the human cancer cells lines: HeLa (Human cervical carcinoma), MCF7 (Human breast adenocarcinoma) and Hep3B (Human Hepatocellular carcinoma). The recorded IC 50 values for the tested compounds show moderate to good cytotoxicity against these cancer cell lines. The copper complex, [CuL]Cl 2 , showed excellent antimicrobial activity against tested microorganisms which is almost equivalent to the standard drug ciprofloxacin.[8]

Co(II), Ni(II), Cu(II) and Zn(II) mixed ligand complexes have been synthesized from bis(4-nitrophenyl)quinoxaline-2,3-diamine and 1,10-phenanthroline. The mixed ligand metal complexes were screened for antimicrobial activity against bacterial species Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa and Staphylococcus aureus; fungal species Aspergillus niger, and Candida albicans by disc diffusion method. The DNA binding and DNA cleavage activities of the compounds were determined using electronic absorption titration and agarose gel electrophoresis respectively. The superoxide radical scavenging and free radical scavenging activities of the Cu(II) complex was also evaluated. Molecular docking studies of the synthesized mixed ligand metal complexes were carried out against B-DNA dodecamer and the protein Plasmodium falciparum dihydrofolate reductase [9]

Mn(III) and Cu(II) complexes based on bis(N-(3-methoxy-salicylidene)-4-amino-phenyl)ether (H2L) have been successfully synthesized and characterized The antioxidant activity of the ligand and its metal complexes have been determined by DPPH, superoxide, hydroxyl and ABTS radical scavenging methods in vitro, suggesting that the Cu(II) complex exhibits greater antioxidant activity against DPPH, superoxide, hydroxyl and ABTS radicals than those of the ligand and the Mn(III) complex. Furthermore, the

antimicrobial effects of the tested compounds have been tested against the growth of bacteria in vitro to assess their bactericidal properties. It has been found that the Cu(II) complex has lower MIC values and exhibits better antimicrobial effects than the free ligand and the Mn(III) complex.[10]

A novel Schiff base ligand of type HL was prepared by the condensation of amoxicillin trihydrate and nicotinaldehyde. The metal complexes of Co+2, Ni+2, Cu+2, and Zn+2 were characterized The in vitro antibacterial activity of all the compounds, at their two different concentrations, was screened against four bacterial pathogens, namely, E. coli, P. vulgaris, K. pneumoniae, and S. aureus, and showed better activity compared to parent drug and control drug.[11]

Conclusion

Transiton metal complexes prepared form Schiff base ligands have attracted attention due to its versatile applications in various fields. But still some simple and ecofriendly procedures are needed for their synthesis and also there is a need to explore the biological activities of this complexes.

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SYNTHESIS, SPECTRAL INVESTIGATION AND ANTIMICROBIAL STUDIES OF SCHIFF BASE COMPLEXES OF (E)-4-CHLORO-2-((PHENETHYLIMINO) (PHENYL) METHYL) PHENOl

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ABSTRACT

Six complexes of Mn(II), Co(II), Ni(II), Cu(II), Zn(II) and Cd(II) with Schiff base ligand (E)-4-chloro-2-((phenethylimino)(phenyl)methyl)phenol have been prepared under reflux in DMF. The ligand was synthesized by the condensation of 2-phenylethylamine with 5-chloro-2-hydcoxybenzophonone in ethanol. All the synthesized compounds were coloured solids, investigated by elemental analysis, FT-IR, ^{1}H NMR and diffuse reflectance spectra. The ligand and complexes have been screened for antibacterial activity against E. coli, P. aeruginosa, P. vulgaris, S. aureus, K. pneumonia. The antifungal activity carried out against two strains A. niger and C. albicans. Moderate to strong activity of the compounds was observed against most of the microorganisms under study.

Keywords: *Schiff base, antibacterial, antifugal activities, benzophenone, complexes.*

1. Introduction

The Schiff bases and their metal complexes are very important class of compounds in inorganic chemistry. Because of their versatile nature they have not only the interesting spectral and magnetic properties but also exhibit a broad range of biological activities[1-3] like antibacterial, antifungal, anti-tumour, anti-inflammatory etc. The Schiff bases behave as chelating agents and can coordinated with many transition and non-transition metal ions[4,5]. There are many reports about use of Schiff base complexes as model for biological systems[6]. Many reports show excellent catalytic activities[7] of metal complexes used in catalytic reactions. Because of many unusual properties the Schiff base metal complexes are extensively used for industrial purposes.

In the recent past, it has been observed that the N, O-chelating Schiff base metal complexes have considerable stability, biological activity and many applications in different areas [8-12]. So attention has been given by chemists about synthesis and study of metal complexes of Schiff bases containing nitrogen and oxygen donor atoms.

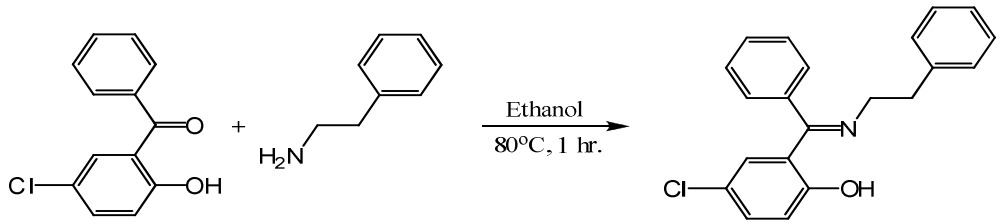
In the present work synthesis, characterization antimicrobial activities of Schiff base ligand derived by the condensation of 5-chloro-2-hydroxybenzophonone with 2-phenylethylamine and its Mn(II), Co(II), Ni(II), Cu(II), Zn(II) and Cd(II) complexes have been reported.

2. Experimental

The metal acetate of Mn(II), Co(II), Ni(II), Cu(II), Zn(II) and Cd(II) used in present work were of Merck. The organic solvents such as ethanol, methanol, dimethyl formamide (DMF), dimethyl sulfoxide (DMSO) etc. used were of AR grade. The nutrient agar medium was used to assess antibacterial activities and antifungal activity by using potato dextrose agar medium.

2.1 Preparation of Schiff base:

The Schiff base ligand was synthesized by taking equimolar quantities 5-chloro-2-hydroxybenzophonone and 2-phenylethylamine in ethanol. The reaction mixture was then refluxed for one hour on a water bath.



5-chloro-2-hydroxy benzophenone | 2-phenylethyamine | (*E*)-4-chloro-2-((phenethylimino)(phenyl)methyl)phenol

Scheme 1: Synthesis of Schiff base ligand (CHBPE)

2.2 Preparation of metal complexes

The metal complexes of Mn(II), Co(II), Ni(II), Cu(II), Zn(II) and Cd(II) were prepared by mixing solutions of CHBPE and metal acetates in DMF in 2:1 molar ratio. The reaction mixtures were refluxed for 5-6 hours on a sand bath. The solid products obtained on cooling the reaction mixture were filtered and washed several times with petroleum ether, dried in desiccators over anhydrous calcium chloride.

3. Results and Discussion

The synthesized complexes were coloured solids, stable in air and soluble in DMSO. The ligand was characterized by elemental analysis, ^{1}H NMR and FT-IR spectra. The metal complexes have been characterized by elemental analysis, FT-IR and diffuse reflectance spectra. All the synthesized compounds were screened for their antimicrobial activities. The analytical data of CHBPPD and its metal complexes are given in Table 1.

3.1 ^{1}H NMR Spectra of CHBPE (300 MHz, $CdCl_3$, δ in ppm): The ^{1}H NMR spectrum of ligand was recorded in $CdCl_3$ which indicated that different non-equivalent proton resonates at different values of applied field [13-16]. The δ-values in ppm are- δ 7.402 – 7.486 (5H, m, Ar-H); δ 7.174 – 7.291 (5H, m, Ar-H); δ 7.565 (1H, s, Ar-H); δ 7.055 – 7.104 (1H, d, Ar-H); δ 6.887 – 6.935 (1H, d, Ar-H); δ 6.660 – 6.668 (1H, d, Ar-H); 5.349 (1H, s, (broad)-OH); δ 3.553 – 3.599 (2H, t, -CH_2); δ 2.937 – 2.983 (2H, t, -CH_2).

Table 1: Analytical data of CHBPE and its metal complexes

Schiff Base/ Complex	Colour	Reflux Time (Hrs.)	Elemental Analysis % Found (Calculated)				
			M %	%C	%H	%N	%Cl
CHBPE	Pale Yellow	1	--	75.24 (75.11)	05.38 (05.40)	04.19 (04.17)	10.42 (10.56)
$[Mn(CHBPE)_2(H_2O)_2].H_2O$	Dark Pink	5	7.14 (7.06)	64.72 (64.79)	2.28 (5.18)	3.68 (3.60)	9.24 (9.11)
$[Co(CHBPE)_2(H_2O)_2]$	Light Pink	3	7.86 (7.71)	65.94 (65.98)	5.14 (5.01)	3.61 (3.66)	9.38 (9.27)
$[Ni(CHBPE)_2(H_2O)_2]. H_2O$	Light Green	4	7.59 (7.50)	64.53 (64.48)	5.23 (5.15)	3.66 (3.58)	9.19 (9.06)
$[Cu(CHBPE)_2].H_2O$	Copper Leaf	6	8.53 (8.46)	67.08 (67.15)	4.92 (4.83)	3.65 (3.73)	9.59 (9.44)
$[Zn(CHBPE)_2].H_2O$	White	5	8.77 (8.69)	66.91 (66.99)	4.94 (4.82)	3.76 (3.72)	9.56 (9.42)
$[Cd(CHBPE)_2]$	Yellow	6	14.48 (14.37)	64.56 (64.50)	4.447 (4.38)	3.67 (3.58)	9.18 (9.07)

3.2 FT-IR Spectra (KBr, cm^{-1}): In order to determine the coordinating atom of the ligand to metal ion FT-IR spectrum of ligand was compared with its metal complexes [17]. The spectrum of CHBPE show strong sharp band at 1612 cm^{-1} assigned to C=N stretching which is shifted to lower frequencies by 6-36 cm^{-1} in all complexes indicating the coordination of azomethine nitrogen to the metal ion [18, 19]. The ligand spectrum shows broad band at 3427 cm^{-1} assigned to intramolecular hydrogen bonded phenolic O-H stretching which is absent in the spectra of complexes. The characteristic medium intensity band of free ligand at 1265 cm^{-1} assigned to phenolic C-O stretching was shifted to higher frequency in all complexes further suggesting coordination through deprotonated phenolic oxygen [20-22]. The

appearance of new bands in the spectra of complexes in region 576-626 and 456-498 cm^{-1} assigned to the M-O and M-N stretching [23, 24]. Appearance of broad bands in the complexes in the range 3466-3515 cm^{-1} indicating hydrated complexes [25]. The coordinated water molecules are confirmed by the bands 1586-1603 and 827-876 cm^{-1} assigned to H_2O shuttle –OH rocking vibrations. The FT-IR data of compounds is given in Table 2.

Table 2: FT-IR spectra of CHBPE and its complexes

Sr. No.	Compound	υ(O-H)	υ(C=N)	υ(C-O)	υ(M-O)	υ(M-N)	υ(H_2O)
1.	CHBPE	3427	1612	1265	--	--	--
2.	$[Mn(CHBPE)_2(H_2O)_2].H_2O$	--	1595	1305	615	473	3515, 1586, 833
3.	$[Co(CHBPE)_2(H_2O)_2]$	--	1603	1334	597	469	3487, 1593, 876
4.	$[Ni(CHBPE)_2(H_2O)_2]. H_2O$	--	1587	1321	576	487	3498, 1603, 827
5.	$[Cu(CHBPE)_2].H_2O$	--	1576	1354	607	463	3476
6.	$[Zn(CHBPE)_2].H_2O$	--	1597	1316	626	498	3466
7.	$[Cd(CHBPE)_2]$	--	1606	1343	589	456	--

3.3 Diffuse Reflectance Spectra: The assignments of diffuse reflectance spectra [26-33] of the metal complexes of CHBPE are given in Table 3.

Table 3: Assignments of diffuse reflectance spectra of CHBPE and its metal complexes

Complex	Absorption band		Assignments
	(nm)	(cm^{-1})	
$[Mn(CHBPE)_2(H_2O)_2].H_2O$	582	17182	$^6A_{1g} \rightarrow {}^4T_{1g}(^4G)$
	447	22371	$^6A_{1g} \rightarrow {}^4T_{2g}(^4G)$
	354	28248	$^6A_{1g} \rightarrow {}^4E_g$
$[Co(CHBPE)_2(H_2O)_2]$	892	11210	$^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$
	626	15974	$^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$
	482	20746	$^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$
$[Ni(CHBPE)_2(H_2O)_2]. H_2O$	892	11185	$^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$
	668	14970	$^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$
	396	25252	$^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$
$[Cu(CHBPE)_2].H_2O$	635	15748	$^2B_{1g} \rightarrow {}^2A_{1g}$
	558	17921	$^2B_{1g} \rightarrow {}^2E_g$
	355	28169	C. T.
$[Zn(CHBPE)_2].H_2O$	--	--	--
$[Cd(CHBPE)_2]$	--	--	--

3.4 Antimicrobial activity: The ligand CHBPE and its transition metal complexes have been tested for antimicrobial activity against bacterial strains *E. coli, P. aeruginosa, P. vulgaris, S. aureus* and *K. pneumoniae* and fungi *A. niger* and *C. albicans* by Mueller-Hinton agar plates method [35]. The results are given in Table 4.

Table 4: Antimicrobial activities of ligand CHBPE and its metal complexes

Compound	Antibacterial					Antifungal	
	E. coli	*P. aeruginosa*	*P. vulgaris*	*S. aureus*	*K. pneumoniae*	*A. niger*	*C. albicans*
CHBPE	17	13	12	19	18	17	14
Mn-CHBPE	13	12	19	14	13	21	22
Co-CHBPE	16	17	14	15	17	15	19
Ni- CHBPE	16	15	08	24	14	13	13
Cu-CHBPE	14	11	15	18	11	21	R
Zn- CHBPE	16	R	17	16	22	16	20
Cd-CHBPE	R	20	22	14	16	24	18
Amikacin	28	25	27	24	26	--	--
Fluconazole	--	--	--	--	--	25	26

It has been observed that CHBPE exhibited moderate to weak activity against bacteria *E. coli, P. aeruginosa, P. vulgaris* and *K. pneumoniae* and fungi *A. niger* and *C. albicans,* while it showed strong activity against *S. aureus*. Mn(II) complex has strong activity against *P. vulgaris*, *A. niger* and *C. albicans,* while moderate to low activity against remaining microorganisms. Co(II) complex showed strong activity against *C. albicans* while moderate activity against remaining microorganisms. The Ni(II) complex exhibited strong activity against *S. aureus* and moderate to weak activity against others. Cu(II) complex showed moderate to weak activity against all pathogens under study. Zn(II) complexes was resistant against *P. aeruginosa* while good activity against remaining all microorganisms. The Cd(II) complex was resistant against *E. coli*, while showed strong to moderate activity against remaining microorganisms [35, 36].

E. coli **S. aureus**

Figure 1: Plates showing the Antibacterial Activity of CHBPE and its Metal Complexes.

4. Conclusion

The ligand CHBPE and its transition metal complexes have been synthesized and characterized by analytical and spectral studies. The results show that CHBPE is a bidentate ligand and its complexes have 1:2 (Metal:Ligand) stoichiometry. The analytical and spectral studies suggests octahedral geometry for Mn(II), Co(II) and Ni(II) complexes; square planar geometry for Cu(II) complex while tetrahedral geometry for Zn(II) and Cd(II) complexes. Antimicrobial activity of CHBPE and its metal complexes shows that most of the synthesized compounds have strong to moderate activity against most of the bacterial and fungal strains.

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DEGRADATION OF 3,7-BIS (DIMETHYL AMINO)-PHENOTHIAZIN-5-IUM CHLORIDEIN AQUEOUS SOLUTION BY ULTRASONIC CAVITATIOn

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ABSTRACT

Degradation of 3,7-bis (Dimethyl amino)-phenothiazin-5-ium chloride(methylene blue) in aqueous solution using ultrasonic cavitation was investigated under variety of operating condition. It is found that the degradation of methylene blue in aqueous solution follows pseudo first order reaction kinetics. The degradation rate is dependent on the initial concentration, temperature and acidity of the methylene blue solution. The effects of Fe^{2+}, Fenton reagent and NaCl addition on the sonochemical degradationof methylene blue were also investigated. The pseudo-first-order rate constants of methylene blue degradation in aqueous solution increases from$1.43x10^{-3}$ min^{-1} to $6.44x10^{-3}$, $7.13x10^{-3}$ $min^{-1}$with 2.5 and 5µmol/L Fe^{2+} addition respectively.The sonochemical degradation rate constants of methylene blue were 6.21 $\times10^{-3}$ min^{-1}7.36$\times$ $10^{-3}min^{-1}$, and 8.75 $\times10^{-3}$ min^{-1} at NaCl concentration 0.6, 1.2, and 1 mol/L respectively.The results obtained indicate that the degradation rate of methylene blue in aqueous solution was substantially accelerated by Fe^{2+}, NaCl and Fenton reagent addition.

Keywords: Degradation; Methylene Blue; Cavitation; Sonochemistry.

1. Introduction

Textile wastewaters contain usually a considerable amount of unfixed dyes, many of which are azodyes. It is estimated that 15% of the total world dye production is lost during dyeing process and it is released in textile effluents. The colours produced by minute amount of dye accidentally released in water during dyeing process are consider topose serious problems because they have considerable environmental effects on the water and make them visually unpleasant [1]. The ultrasonic degradation ina aqueous solutions of a large number of chemical compounds of environmental interest such as 1,1,1-trichloro-ethane[2],chlorinated hydrocarbons[3,4], polychlorinated biphenyls[5],phenols[6,7] and surface active reagent [8].

Ultrasonic irradiation provides a way of onsite·OH generation by such way that the dissolved vapours and gases in the liquid are entrapped by cavitation bubbles which release very high temperatures during their adiabatic collapse[11,12]. In sonolysis of water containing hydrophilic compounds such as textile dyes hydroxyl radicals are generated. Only by water fragmentation in threw collapsing bubbles and oxidative dye destruction is possible if the radicals are effectively ejected into the solution bulk the efficiency of ·OH diffusion in to the aqueous phase is related to system parameters such as frequency, reactor, geometry, presence of cavitation nuclei and the ambient condition[9,10].

During sonication of a liquid, water vapour and dissolved gases are entrapped by cavitation bubbles, which expand at rarefaction cycles of the bubbles and release extreme temperatures upon adiabatic collapse [13,14]. Under these conditions, bubble contents are pyrolytically fragmented into radical species, some of which diffuse in to the aqueous phase to initiate a series of oxidation reaction called "Sonochemistry" [15]. The main chain reactions occurring during collapse ofa bubble are shown below

$$H_2O +))) \longrightarrow {\cdot}OH + {\cdot}H \text{(Pyrolysis)} \quad (1)$$

$$\cdot OH + \cdot H \longrightarrow H_2O \quad (2)$$

$$\cdot OH + \cdot 0H \longrightarrow H_2O_2 \quad (3)$$

$$2\cdot OH \longrightarrow H_2O + \cdot O \quad (4)$$

In water and wastewater treatment practices, organic pollutants may be destroyed either (i) in the cavitation bubble itself by pyrolytics decomposition if the compounds are hydrophobic (ii) at the interfacial sheath between the gaseous bubble and the surrounding liquid, or (iii) in the solution bulk via oxidative degradation by hydroxyl radicals, the extent of oxidation in bulk liquid is limited by the quantity of hydroxyl radicals diffused in the water.

3,7-bis (Dimethyl amino)-phenothiazin-5-ium chloride that is methylene blue (MB) is a member of the thiazine class of dye. Methylene blue is an organic dye usually uses to dye cotton, wool,acrylic fibres, andsilk. When it is used recklessly,it can cause serious illnesses such as vomiting, hard breathing, mental disorder and sweating. MB^+ is widely used in textile dyeing plants which is used as a model dye for removal of basic dyes by adsorption [16,17, 18]. In the present paper, attempt has been made to employ ultrasonic cavitation for the degradation of methylene blue. The effects of dye concentration, temperature, $Fe^{2+}$and Fenton reagent on the degradation rate of methylene blue were also investigated.

2.Experimental

2.1 Materials and apparatus

3,7-bis (Dimethyl amino) phenothizin-5-ium chloride(methylene blue) is a cationic azo dye (Molecular formula $C_{16}H_{18}N_3SCl$).The dye was obtained from LobaChemie.$FeSO_4$, $H_2O_2$and NaCl were of analytical grade and used without further purification. Concentrations of the dye solution were estimated using absorbance recorded on UV-VISspectrophotometerat the wavelength 670 nm.Sonication was performed with ultrasonic Probe Sonicator-20 KHz, 150 W.(Dakshin Ultrasound, Mumbai, India).

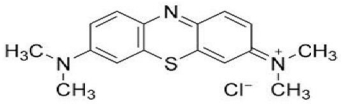

Fig1: structure of methylene blue dye

2.2 Procedure

100 mL aqueous solution of methylene blue was sonicated. The aqueous solution was saturated with pure air before and during the sonication. The reactor was sealed and connected to a gas burette to ensure a constant pressure for 1atm. The reactor cell was provided with cryostat in order to maintain the desired reaction temperature.

3. Result and discussion

3.1 Degradation Kinetics

The air saturated aqueous solution of methylene blue (10 mg/L) were sonicated for 240 min. during sonication the dye sample were analysed at regular interval of 30 minutes with UV-VIS spectrophotometer for their concentration. It was found that the concentration of methylene blue in aqueous solution decrease with increase in sonication time (fig. 2) In order to study the order of the ultrasonic degradation of methylene bluein aqueous solution, the degradation kinetics was investigated and the regression coefficients for zero. First and second order reactions were calculated. The result indicated that the degradation of methylene blue in aqueous solution can be described by first order kinetics (fig. 3). The degradation rate constant is found to be $2.2x10^{-3}$ with regression coefficient is r = 0.987 for 10 mg/L methylene blue in aqueous solution at temperature 26°C and pH 10.

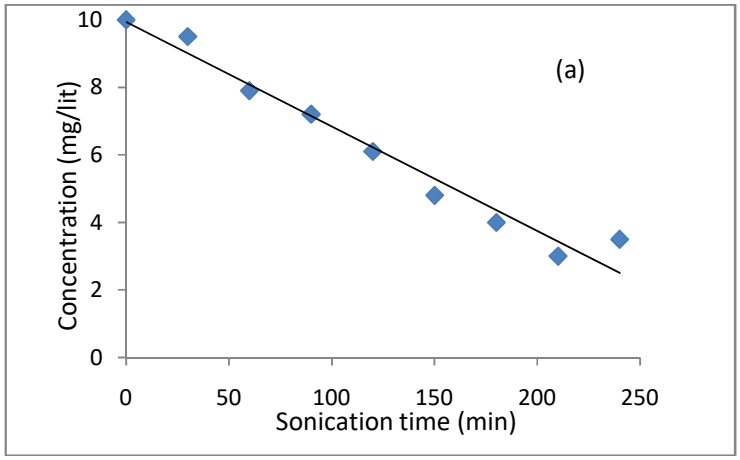

Fig.2. Concentration Vs sonication time profiles dependency of methylene blue at temperature 26°C and pH10.

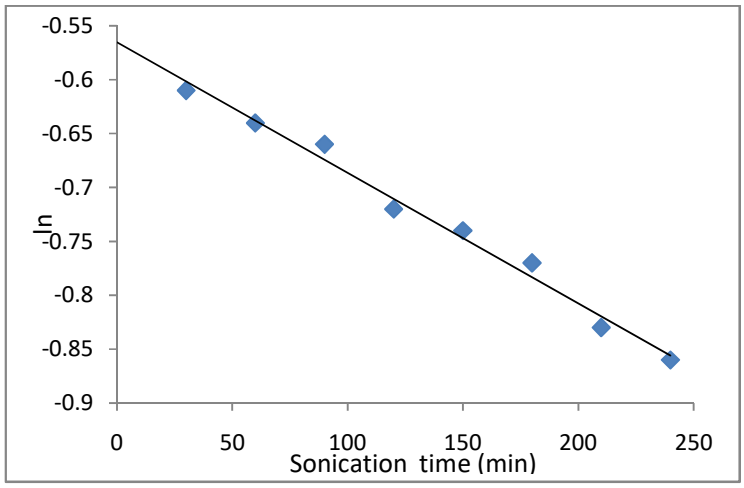

Fig.3. Degradation Vs time dependency of methylene blue at temperature 26°C and pH10.

3.2 Effect of Initial dye concentration

The effect of initial dye concentration on the sonochemical degradation rate of methylene blue was investigated at temperature 26°Cand pH10. Fig.4 shows the degradation kinetics of methylene blue for different initial concentrations. It was found that with increasing initial concentrations, the degradation rate constants were decreased. The ultrasonic degradation rate constant of methylene blue were $8.06x10^{-3}$, $5.06x10^{-3}$, $2.99x10^{-3}$, and $1.38x10^{-3}$ min^{-1} at initial concentration 5, 8, 10 and 15mg/Lrespectively. The results are correspondent closely withprevious works [19, 20]. Visscher et al. reported a result of sonochemical degradation of ethyl benzene in water and found that degradation rate was higher at lower initial concentration [21].

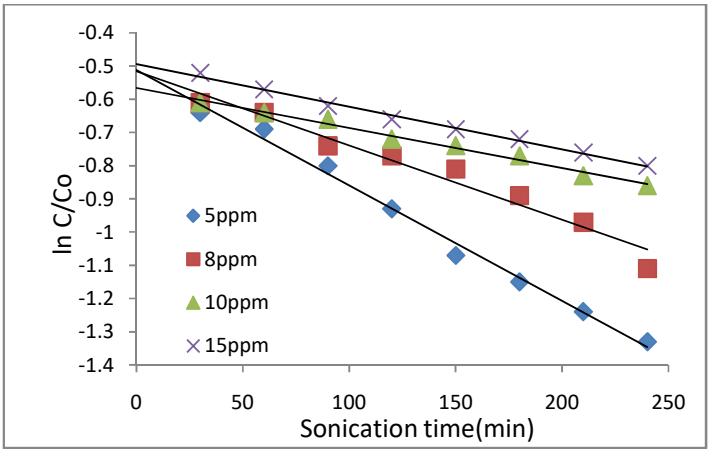

Fig.4. Effect of initial concentration on the degradation rate of methylene blue at temperature 26°C and pH10.

3.3 Effect of temperatures:

The influence of the temperature of reaction solution on the ultrasonic degradation of methylene blue was investigated and results are shown in figure 5. It was found that ultrasonic degradation rate constant of methylene blue were $5.06x10^{-3}$,$2.99x10^{-3}$, $2.55x10^{-3}$and $1.38x10^{-3}$ min^{-1} at temperature 30, 40, 50 and 60°Crespectively. The result shows that, with increasing reaction temperature from 30 to 60°C the rate of degradation was decreased. It has been reported that three different regions are formed in the aqueous sonochemical process [22], (i) The gas phase within the cavitation bubble where elevated temperature and high pressure are produced.

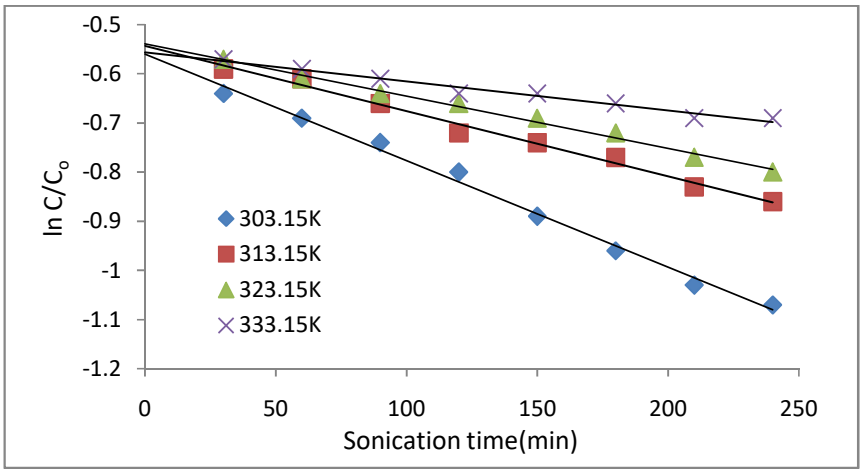

Fig.5.Theeffect of temperature on the degradation rate of methylene blueinitial concentration 10 mg/L,pH10.

(ii) The interfacial zone between the bubble and the bulk solution where the temperature is lower than that inside the bubble out still high enough for a sonochemical reaction.(iii) The bulk solution at ambient temperature where the reaction still takes place. Of the mentioned three regions, we prefer the interfacial zone of the region where methylene blue was destructed because of the low vapour pressure of the compound as the bulk temperature of water increased. The collapse of cavity is thus cushioned more than that at a lower bulk temperature. This result is more moderate conditions and a lower sonochemical degradation rate.

The removal of hazardous dyes methylene blue was highly influencedby the pH factor due to the structural modifications of dyemolecules. The effect of pH was studied in the pH range from 2 to12 using the 10 mg/L dyesolutions and temperature 26^{o}Cis shown in Fig. 6. The pH ofthe dye solutions were adjusted by using 0.1 N NaOH and 0.1 NHCl. The pH of the solutions was measured by pH meter.The % degradation of methylene blue increased gradually withincrease of pH and reach maximum at pH 10. The low degradation atlow pH may be due to the protonation of amino groups present inthe methylene blue dyes. Hence, the degradationdecreased with decrease of pH.

3.4 Effect of pH

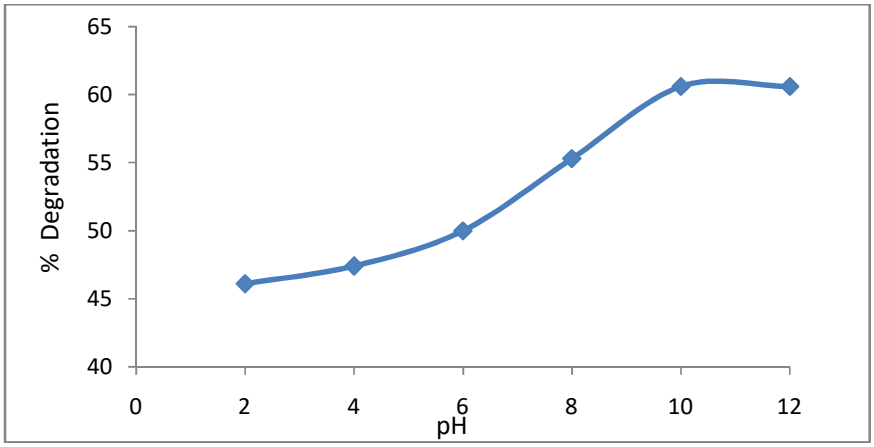

Figure 6: Effect of initial pH of the aqueous solution on the % degradation of methylene blue (initial concentration = 10 mg/L and temperature26^o C)

3.5. Effect of Fe^{2+} and Fenton reagent addition

When an aqueous solution undergoes ultrasonic irradiation certain chemical effect takes place due to the propagation of a pressure wave.This pressure wave leads to the occurrence of cavitation phenomenon that is nucleation growth and the eventual collapse of the bubble. The split second collapse of the bubble creates localized supercritical conditions with extremely high temperature and pressure electrical discharge and plasma effect. These extreme conditions create hydroxyl ($^{\cdot}OH$), hydrogen ($^{\cdot}H$) hydroperoxyl ($HO_2^{\cdot}$) radicals and hydrogen peroxide from the aqueous solution [22]. According to the Fenton reaction, Fe^{2+}can react with H_2O_2 to produce $^{\cdot}OH$ in weakly acidic media (p^H 2-3) so Fe^{2+} can accelerate the sonolytic degradation of pollutants.

$$H_2O \longrightarrow {}^{\cdot}OH + {}^{\cdot}H \quad (1)$$

$$^{\cdot}OH + {}^{\cdot}OH \longrightarrow H_2O \quad (2)$$

$$Fe^{2+} + H_2O_2 \longrightarrow FeOH^{2+} + {}^{\cdot}OH \quad (3)$$

$$FeOH^{2+} + H_2O_2 \longrightarrow Fe(OH_2)^{2+} \quad (4)$$

It has been reported that the sonochemical degradation of phenols can be enhanced by the addition of Fe^{2+} [23,24]. The effect of Fe^{2+} addition on the sonochemical degradation of methylene blue (initial concentration 10mg/L, pH10,temperature 26^{o}Cwas investigated and the result is shown in Figure 7. The results reveal that much higher degradation rate was observed when $Fe^{2+}$was added in the dye aqueous solution during sonication. The pseudo-first-order rate constants of methylene blue degradation in aqueous solution increases from$1.43x10^{-3}$ min^{-1} to $6.44x10^{-3}$, $7.13x10^{-3}min^{-1}$with 2.5 µmol/L and 5µmol/L Fe^{2+} addition respectively. This increment was mainly due to the higher $^{\cdot}OH$ radical concentration produced from the reaction. Similarly the addition of Fenton reagent can increase the yield of $^{\cdot}OH$ radical and accelerate the sonochemical reactions. Many researchers have reported the improved efficiency of sonolysis processes by employing sonolysis couple Fenton reagent [25, 26]. Here the enhancement effect of Fenton reagent on the sonochemical degradation of methylene bluewas also examined. Figure 8 shows the degradation of methylene blue in aqueous solution at pH10 with H_2O_2oxidation, Fenton reagent oxidationand sonication only and sonication coupled.

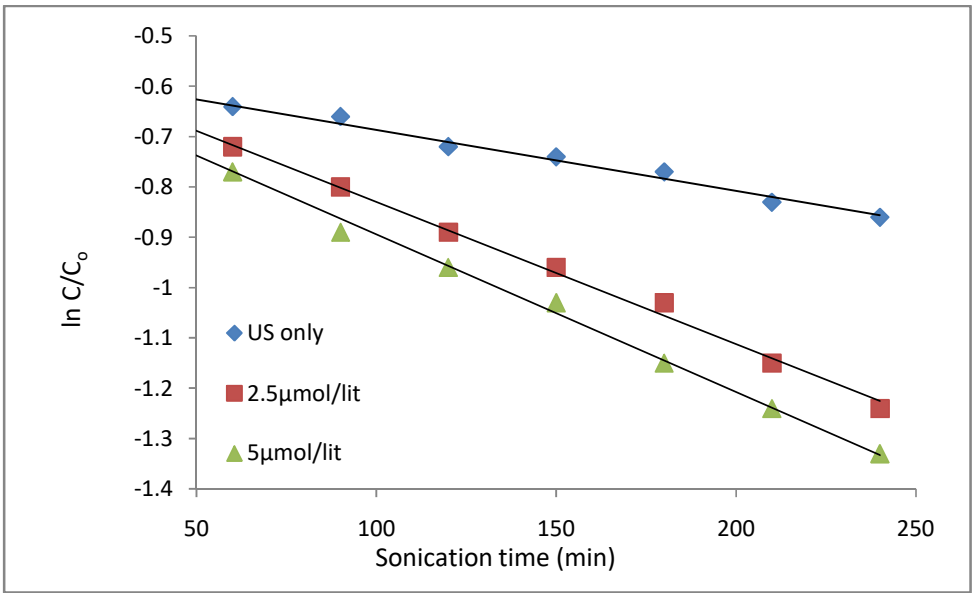

Fig.7. Effect of Fe^{2+}addition on the degradation rate of methylene blue at initial concentration 10 mg/L, temperature 26^{o}C and pH10.

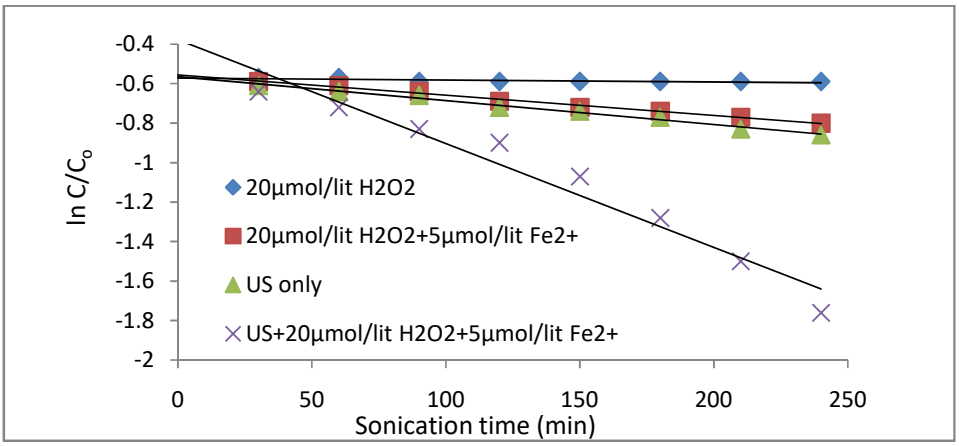

Fig.8.Effect of H_2O_2addition on the degradation rate of methylene blue at initial concentration 10 mg/L, temperature 26°C and pH10.

Fenton reagent,It was found that the oxidation of methylene blue with 20µmol/L H_2O_2 (26°C, pH10) for 240 min is less than 2% so the degradation of the dye by H_2O_2 oxidation can be neglected. From the result it can be seen that the degradation rate methylene blue was substantially enhanced by the addition of Fentonreagent to the ultrasound system. The degradation rate constants are found to be 12.20×10^{-3} min^{-1} with 5µmol/L $Fe^{2+}$and 20µmol/lit H_2O_2addition but the degradation rate constant are only 2.2×10^{-3} and 2.0×10^{-3} min^{-1} with sonication and Fenton reagent (5µmol/L Fe^{+2} and 20µmol/LH_2O_2) can improve the degradation rate by about 4 and 5 times compared to that of direct sonication or Fenton reagent oxidation the enhancement in the degradation rate constant of methylene blue could be mainly due to the contribution of additional ·OH radicals produced from the Fenton reagent.

3.6 Effect of NaCl Addition

Many common ions present in dye wastewater are Fe^{2+},Zn^{2+},Ag^{+},Na^{+},Cl^{-},BrO_3^{-},HCO_3^{-}andper sulphate ions. Each of these added ions causes a certain increase or decrease in percentage degradation of dye solution.It is expected that the sonochemical degradation of non-volatile pollutants in aqueous solution takes place in the bubble-bulk interface area due to the exposure to free radicals and high temperature and pressure. The aim of NaCl addition to the aqueous solution is to push methylene blue molecule from the bulk aqueous phase to the interface addition of NaCl can increase the hydrophilicity the surface tension and ionic strength of the aqueous phase and decrease the vapour pressure [27]. All these factors help in collapsing of the bubbles more violently and resulting in high degradation degrees of pollutant.

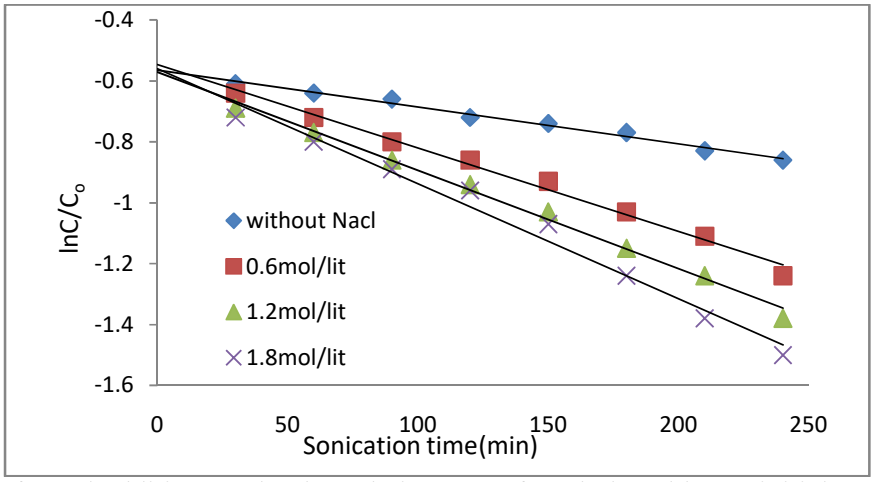

Fig.9. Effect of NaCl addition on the degradation rate of methylene blue at initial concentration 10 mg/L, temperature 26°C and pH10.

The effects of the addition of NaCl with different concentration (0.6,1.2,1.8 mol/L) on the degradation rate were instigated with 10mg/Lmethylene blue in aqueous solution at 26^{o}C andpH10 and the result are shown figure 9. It can be seen from the results that the degradation rates increase with increasing concentration of NaCl. The sonochemical degradation rate constants of methylene bluein aqueous solution with the addition of NaCl at different concentrations are higher than that without NaCl and the degradation rates increase with increasing concentration of NaCl. The sonochemical degradation rate constants of methylene blue were 6.21 $\times 10^{-3}$, 7.36$\times$ 10^{-}, and 8.75 $\times 10^{-3}$ min^{-1} at NaCl concentration 0.6, 1.2, and 1 mol/L respectively.

4. Conclusion

In present study the degradation of methylene blue in aqueous solution operated at various parameters. It is found that degradation of methylene blue in aqueous solution follows pseudo first order reaction kinetics and the degradation constant is found to be 2.27 $\times$ 10^{-3} min^{-1} for 10 mg/L. methylene blue in aqueous solution at temperature 26 ^{o}C and pH10. The degradation rate of methylene bluestrongly dependent on the initial concentration of the dye solution,temperatureand pH of aqueous medium. The effect of Fe^{2+}, NaCl and Fenton reagent addition on the sonochemical degradation of methylene blue also investigated. The result obtained indicate that degradation rate of methylene blue in aqueous solution was substantially accelerated by Fe^{2+},NaCl or Fenton Reagent addition.

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REMEDIATION OF WATER POLLUTION USING NANOTECHNOLOGY

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ABSTRACT

Nanotechnology is an upcoming field which has several environmental applications including bioremediation. It can be considered asgood clean up technology wherein various methods can be involved to form new products, improve existing instruments and help in reformulating new materials and chemicals which has better performance. It is therefore referred as green technology in terms of energy conservation and reduces the release of toxic compounds into environment. Nanotechnology in future holds great promises in remediating problems associated with water pollution in a sustainable way. The following review article reveals the recent trends in the ongoing research related to environment remediation by nanotechnology.

Keywords: Nanotechnology, bioremediation, water pollution, environment, energy conservation

1. Introduction

Bioremediation is the process by which various biological agents such as bacteria, fungi and enzymes are used to degrade contaminants to less toxic forms [1]. It has added advantage over conventional approaches in terms of competence, metal selection, metal recovery etc. The common techniques in bioremediation include bioventing, bioleaching, bioaugumentation[2]. Bioremediation is a sustainable solution for the treatment of contaminated waters but bio- based approaches are time consuming, becomes toxic at high concentration to the organism involved [3]. The emergence of nanotechnology recently has invoked many researchers to explore remediation of water bodies.Nanotechnology focuses on synthesis, design, characterization and application of nanomaterials and nanodevices. It involves the study of phenonmenon and manipulation of materials atnanoscale[4, 5]. The nanomaterials exhibits significant changes in physical, chemical, and biological properties due to their size, structure, surface ratio etc. opening new avenue in material sciences[6]. These properties allow developing highly miniature, accurate and sensitive pollution monitoring devices called nanosensors. Thus with challenging issues due to complexicity of the mixture of different compounds with low reactivity, recent studies have focused on the use of nanoscience based projects for the development of environmental remediation technologies [7]. Hence there is a need to monitor, recognize and treat the contaminants that are found in parts per million (ppm)in water bodies. In this context nanotechnology offers many thrust areas to reduce and treat environmental contaminants in manipulating at atomic scale and molecular level. The technology helps in fabricating with specific properties that can recognize particular types of pollutants within a mixture.

2. Nanotechnology- Treatment of water bodies

Contamination of various water bodies including groundwater from industries is a matter of great concern. The affected areas such as lakes, rivers in their vicinity, underground storage tank leakages, landfills etc. shows the presence of heavy metals[8]such as pollutants (eg: mercury, lead, cadmium,) and organic compounds (eg: benzene, chlorinated solvents, creosote). Currently conventional methods which are employed to remove toxic contaminants are laborious, time consuming and expensive techniques. Also during removal of thesecontaminants from affected area, ecosystem may also bedisturbed.

Nanotechnology offers technologies that can perform *in situ* remediation and reach inaccessible areas such as crevices and aquifersand eliminatesthe use of costly pumps and treatment operations. Working at nanoscale can be used to develop remediation tools thatare specific for certain pollutants (eg: metal), enhances affinity and selectivity as well as improving the sensitivity of the technique.Another aspect includes drinking water and its contamination from pollutants.

Two toxic metals that possess very high health risks are mercury and arsenic.Again nanoscience offers technology which can introduce new methods for the treatment and purification of water from pollutants as well as new techniques for waste water management and water desalinization [9].

3. *In situ* treatment using nanoparticles:

Use of nanotechnology in the treatment of contaminated groundwater is well shown by iron nanoparticles for remediation. Iron in presence of air oxidizes easily to rust. But in presence of contaminants such as CCl4, dioxins, parachlorobenzenes, when iron oxidizes, these organic contaminants are broken down into simpler forms. The practice of using iron powders (being non toxic and abundant) to clean up was found to be less effective as it partially treats the industrial wastes and produces toxic by products.

3.1 Iron nanoparticles: Nanotechnology offers a solution to this problem in the form of iron nanoparticles which are more reactive than iron powders[7]. Nanosizediron powders are injected at contaminated sites and are transported effectively by the flow of ground water. The properties of these nanoparticles do not change by soil conditions such as acidity, temperature and nutrient levels. It thus results in complete transformation and detoxification of environmental contaminants without formation of any toxic by-products and reduces contaminant level. It is reported that the iron nanoparticles remain active in a site for 8 weeks (approximately) before they become dispersed completely in the ground water and become less concentrated.Experimental results collected have shown that a nanoscale iron particle are very effective against chlorinated organic solvents, organochlorine pesticides as contaminants and brings about complete transformation and detoxification.Preliminary research is going on to use this technique for remediation of aquifers and for immobilization of heavy metals and radionucleotides.Bimetallic iron nanoparticles such as iron palladium [10] is also shown to be more active, which can further enhance the improvement in remediation technology by anchoring these on solid support such as activated carbon or silica.

4. Solar photocatalysis remediation:

A preliminary investigation for use of light in activating nanoparticles for their capability of removing contaminant is also another area of application of nanotechnology.It was found that nanosizedtitanium oxide and zinc oxide which are activated by light are considered to remove water contaminantsbecause they available easily and are inexpensive and also helps in conversion of toxic contaminants (chlorinated detergents) into non toxic products[11].Thus photodegradation of numerous toxic compounds by these semiconductors is possible but requires an improvement in terms of efficiency as titanium oxide or zinc oxide adsorb UV light. Herein nanotechnology can bring an improvement by modifying surfaces with organic and inorganic dyes so that these oxides will have improved photoresponsefrom UV to visible light.[12]. Recently it has been shown that ZnO nanoparticles can act as a sensor of chlorinated phenols and as photocatalyticdegradation tool also.

5. Dendrimers:

Dendrimers are polymers which are comprised of smaller molecules linked together. They are relatively monodipersed and highly branched macromolecules. Dendrimers-nanoparticle composites owing to have enhanced catalytic activity can be used in water treatment such as polyamidoaminedendrimers. The researchers also developed simple filtration unit for removal of organic pollutants by utilizing TiO2 porous ceramic filters of which pores was impregnated with dendrimers offering high mechanical strength and high surface area[13]. These can be designed so to able to act as cages and trap metal ions and zero valent metals, making them soluble in media or able to bind to certain surfaces. In near future dendrimers holds place to be used as chelating agents for polymers supported as ultra-filtration.

6. Magnetic nanoparticles:

Magnetic property of rust nanoparticles can be used to remove arsenic from polluted water[14]. Arsenic pollutant sticks to rust which essentially is iron oxide (magnetic nature), thus this metal can be removed from water using magnet. Nano sized rust (10nm in

diameter) providing high surface area,was found to improve removal of contaminants with high efficiency while reducing the amount of materials used. When compared to centrifugation orfiltration systems, this technology has added advantage of not requiring electricity. This is very important especially in remote areas with limited or no access to electricity.Magnetic nanoparticles modified with specific functional groups are also used for the detection of bacteria in water samples [9].

7. Nanomembranes and nanofilters:

Nanotechnology is employed for the fabrication of nanofilters, nanoadsorbents, nanomembranes with specific properties used for decontaminating water and air. Nanotrapsare designed for certain contaminant having specific pore size and surface reactivity[15, 16]. Also membranes are engineered to trap and chemically react with contaminant and convert it to a non- toxic products.

As freshwater resources are becoming scarce, researchershave begun to consider seawater as another source for drinking water. Scientists developed reverse osmosis membrane for sea water desalinization and waste water remediation[17]. Membrane made of cross linked matrix of polymers and engineered nanoparticles designed to draw in water ions but repel contaminants. This is possible due to nanosizedimension of holes forming the membrane which are tunnels accessible only to water molecules. Also nanomembranes has ability to repel organics and bacteria due to their pore size composition. These are thus less prone to clogging which increases the membrane lifetime with an economic benefit [18].

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8. Conclusions:

The small size of nanoparticles together with their high surface to volume ratio can lead to very sensitive detection. These properties will allow developing miniature, accurate and sensitive pollution monitoring devices. Nanomaterials can also be engineered to actively interact with a pollutant and decompose it in less toxic species. In future nanotechnology could be used in detecting and treating them.

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ANALYSIS OF SOME PHYSICO-CHEMICAL PARAMETERS OF WATER SAMPLES OF SOME VILLAGES OF BULDANA DISTRICT, MAHARASHTRA, INDIA

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ABSTRACT

Ground water plays a fundamental role in human living, and it forms a major foundation of drinking water in metropolitan as well as in countryside areas. It contains many chemicals like nitrates, sulphates, fluorides in dissolved state. So it becomes necessary to analyze the water excellence to be suitable for drinking and household purposes. Thus, the present paper portray analysis of ground water quality and some of its physico-chemical factors like pH, TDS, temperature, Alkalinity, Chloride, nitrate, total hardness etc. of water samples in five villages of Sindkhed Raja Taluka, district Buldana, Maharashtra. The water samples were sampled from different region of the villages and analyzed for the suitability of drinking purposes. The obtained values of each constraint were compared with the standard values set by the World Health Organization (WHO). The values of each factor were found to be within the safe limits set by the WHO.

Keywords: *Ground water quality, physico-chemical parameters, etc.*

Introduction

Due to increasing globalization, surface water is getting over unhygienic and more rigorous treatment would be required to make surface water drinkable. Therefore, it is required to supplementary sources for execute the necessity of water. Because the ground water sources are safe and potable for drinking and other useful purposes of human being. Hence studies of physico-chemical distinctiveness of underground water to find out whether it is fit for consumption or some other valuable uses. Water quality index is one of the most helpful tools to correspond information on the quality of water to the concerned citizens and policy makers. It, thus, becomes an important parameter for the estimation and supervision of groundwater. The greater part of the soluble constituents in ground water comes from soluble minerals in soils and sedimentary rocks.

Therefore, in view of the above, it is of great importance to study the quality of water, especially in those regions, where water level is declining due to less precipitation. The present paper focused on the analysis of some physico-chemical parameters and ground water quality of the 5 villages around Sakharkherda Village, Taluka Sindkhed Raja, district Buldana, Maharashtra, India. The water sample investigation involved pH, temperature, Alkalinity, Chloride, sulphate, nitrate, total hardness and fluoride. All of the drinking water samples were randomly taken from the tap water of residential and commercial areas of the selected five villages. The villages are Ratali, Saodad, Sendurjan, Pimpalgaon Sonara and Mohadi. All of the sampling location are open for public such as tea-breakfast stalls and private houses. According to Census 2011 information, the Geographical details of villages are as follows:

Name of the Village	Village Code	Distance from		Geographical Area (in Hectares)	Total Population
		Taluka Place (Sindkhed Raja) (in Km)	District Headquarters (Buldana) (in Km)		
Ratali	529392	55	60	737.32	1239
Mohadi	529393	55	60	713.02	1466
Saodad	529394	55	62	1067	2504
Pimpalgaon Sonara	529403	55	60	521.45	1334
Sendurjan	529416	40	60	1666.36	4866

Materials and Methods:

Sampling: All of the drinking water samples were randomly taken from the tap water of residential and commercial areas of the selected five villages. The villages were Ratali, Saodad, Sendurjan, Pimpalgaon Sonara and Mohadi. All of the sampling premises are open for public such as tea-breakfast stalls and private houses. All samples were collected and kept in Plastic bottles, which have been previously washed with 10% HNO_3 and 1:1 HCl for 36 hr. Temperature and pH of water samples were measured at the time of collection. The Plastic bottles were labeled and without delay few drops of HNO_3 were added in order to prevent loss of metals and the growth of any micro-organisms.

Chemical Analysis: The collected samples were estimated for Alkalinity, Chloride, sulphate, nitrate, total hardness and fluoride The method used for the determination of these physico-chemical parameters was described by A.O.A.C. International[4] and using standard procedures by APHA[5] and EPA[6].The chemicals and reagent used for analysis were of analytical grade.

Statistical Analysis: All data generated were analyzed statistically by calculating the mean and compare the mean value with the acceptable standards. pH meter Equiptronics model was used to determine the pH of the samples, Titrimetric procedures were followed for the analysis of total hardness, alkalinity, Chloride, sulphate, nitrate and Fluoride. Borosilicate Glassware was used for all the estimations.

Result and Discussions

The physico-chemical parameters obtained from analysis of water samples are presented in the **Table No. 1.** The various physico-chemical characteristics were analyzed for ground water from different sampling locations. The details of the average results were summarized in table 1.

pH: The pH value of water source determines the hydrogen ion concentration in water and indicates whether the water is acidic or alkaline[6]. Most of the biological and chemical reactions are influenced by the pH of water. If pH is not within the acceptable limit, it damages the mucous membrane of cells[7]. In the present reading all the ground water samples have pH values between 6.6 -7.3. Which lies within the tolerable limit laid down by WHO[7] (7.0-8.5).

Total dissolved solids (TDS): The total dissolved solids in water are due to presence of all inorganic and organic substances. The solids can be salts of manganese, magnesium, potassium, sodium, calcium, carbonates, bicarbonates, chlorides, phosphates and other minerals. The high values of TDS results in gastrointestinal irritation to the any person but long time use of water with high TDS can cause kidney stones and heart diseases[8]. In the present analysis, the TDS values were observed from 340 to 465 mg/l. The most desirable limit of TDS is 500 mg/l and maximum acceptable limit is 1500 mg/l. The TDS value for all the ground water samples fall within the acceptable limit.

Total alkalinity (TA): Alkalinity[9] of water is the measure of the ability to neutralize a strong acid. The bases like Carbonates, bicarbonates, hydroxides, phosphates, nitrates, silicates, borates etc are responsible for alkalinity of water. Alkalinity provides an information of natural salts present in water. Alkalinity is a constraint, which is not harmful to human beings. The alkalinity values were recorded below the acceptable limit (57-79mg/l). So, All samples are within the desirable limit for drinking water 100 mg/l (WHO).

Total hardness (TH): Hardness[10] of water is an artistic quality of water and is caused by salts like carbonates, bicarbonates, sulphates and chlorides of calcium and magnesium. Hardness more than 300 mg/l may cause heart and kidney problems. The total hardness in ground water samples listed in the present paper is beyond the desirable limit. All the ground water samples are very hard and hence require suitable treatments before use.

Chloride (Cl^-): Chloride in ground water can be caused by industrial or household waste. The chloride concentration serves as an indicator of pollution by sewage. High chloride content in water bodies, harms agricultural crops, metallic pipes and harmful to people

suffering due to cardiac and renal diseases. Most of the ground water samples show chloride concentration within the permissible limit (250 mg/l) of WHO.

Fluoride (F^-): Fluorine[11] exists combining with other substances as fluoride. The main source of fluoride in ground water is fluoride bearing rock such as fluorspar, fluorite, cryolite, and fluorapatite. High fluoride content in ground water causes serious damage to the teeth and bones of human body, diseases caused called dental fluorosis and skeletal fluorosis. The value of fluoride concentration in ground water samples lie between 0.59-0.84 mg/l. All the ground water samples have fluoride concentration within permissible limit (1.0 mg/l) of WHO

Nitrate (NO_3^-): Nitrate is an inorganic chemical that is highly soluble in water. Major sources of nitrate in drinking water include fertilizers, sewage and animal manure. Nitrates[12] also occur naturally in the environment, in mineral deposits, soil, seawater, freshwater systems, and the atmosphere. High nitrate content may lead into Irritability, lack of energy, headache, dizziness, vomiting, diarrhea . The Nitrate levels are found to be within the acceptable limit

Table No. 1: Analytical average results for different water quality parameters and comparison with WHO Standards

Sr. No	Spots	pH (1)	Temp.(^oC) (2)	TA (3)	Cl^- (4)	NO_3^- (5)	TH (6)	F^- (7)	TDS (8)
1	**Ratali**	7.1	32.1	78.5	182	34.4	524.35	0.61	390
2	**Mohadi**	6.7	32.5	57.5	174.5	41.5	545.12	0.59	340
3	**Saodad**	7.3	31.3	76.5	113.5	36.4	522.12	0.68	450
4	**Pimpalgaon Sonara**	6.6	33.4	78.9	98.6	38.1	534.11	0.84	465
5	**Sendurjan**	6.8	31.3	69.3	110.5	40.2	511.12	0.71	456
6	**WHO standard**	**6.5 - 8.5**	**30-34**	**100**	**200-600**	**20-45**	**100-500**	**1.2-1.5**	**500-1500**

*All the results in the entries from (3) to (8) are in mg/l

Conclusion

It is observed that from the above data, ground water quality of the village is not so good for drinking purpose without prior treatment as it contains more total hardness beyond the permissible limits as recommended by WHO and Indian standards. The conclusion derived from these results is that some physico-chemical parameters examined were consistent with World Health Organization standard for drinking water (WHO). And for such parameters that had mean values below the recommended WHO standard, water treatment plant should be built for these people to correct these anomalies. In addition, bacteriological determination of water from these different water sources be carried out to be sure if the water was safe for drinking and other domestic application.

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TOTAL PHENOLICS AND SCREENING OF ANTI BMPS ACTIVITY IN 95 MEDICINAL PLANT EXTRACTS

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ABSTRACT

Medicinal plants and their different constituents have been authenticated to use as drugs for the prevention and relief of various disorders. In present work we report the evaluation of total phenolics content and screening of 95 medicinal plantextracts for inhibitor activity against BMPs.Extraction of phenolics from various plant materials was carried out by using methanol as solvent system. Total phenolics in plant extracts were estimated by using Folin-Ciocalteau assay. Screening of methanolic extracts for anti-BMP activity was performed by using dot blot assay on X-ray film.Among all plant extracts the highest total phenolics(257.58 ± 0.75 mg GAE /g) was observed in Gloriosa superba fruit while the lowest amount of total phenolics (0.468 ± 0.003 mg GAE /g) was observed in Crataeva nurvala stem. In case of screening, out of 95 different plant materials 32 exhibited inhibitory activities against ChC while 27 exhibited inhibitory activities against BpM and that 25 plants exhibited both anti ChC and BpM inhibitory activity. The most of plant Barks were found to contain high amount of total phenolics as compared to other plant materials while the most of plant leaves were found to contain low amount of total phenolics. The most of different barks were found to exhibit inhibitory activity as compared to other parts. Only 6 different leaves were found to exhibit inhibitory activity out of 42 different leaves. None of the root extracts exhibited inhibitory activity. In conclusion, the BMPs inhibitory activity exhibiting plant extracts could be used as significant source for determination of novel drug.

Keywords: *Total phenolics, Clostridium histolyticum collagenase, Basillus polymyxa metalloprotease, Gloriosa superba, Syzygium cumini*

Abbreviations*: MMPs, Matrix metalloproteases; BMPs, Bacterial metalloproteases; GAE, Gallic acid equivalent; ChC, Clostridium histolyticum collagenase; BpM, Basillus polymyxa metalloprotease; MeOH, Methanol*

1. Introduction

Matrix metalloproteinases (MMPs), a family of zinc dependent endopeptidases, have been reported to exhibit a central role in various physiological conditions such as embryonic development, blastocyte implantation, nerve growth, ovulation, morphogenesis, angiogenesis, tissue resorption and tissue remodelling. The overexpression or dysregulation of MMPs is associated with several disorders such as tumor metastasis, arthritis, artherosclerosis, and aneurysm, breakdown of the blood-brain barrier, periodontal disease, skin ulcers, corneal ulcers, gastric ulcers, and liver fibrosis. Role of MMPs in various disorders has led to development of inhibitors as therapeutic agents in relevant diseases [1, 2]. Many synthetic inhibitors have been developed but they failed all clinical trials because of low oral bioavailability, poor stability in vivo, pharmacokinetic problems, and undesirable side effects. Naturally derived MMP inhibitors have been approved by medically and could be utilized successfully as therapeutic agents against MMP relevant diseases [3, 4].

Clostridium histolyticum collagenase (ChC) is a zinc containing metalloprotease having molecular weight 116 kDa and belongs to the M9 metalloproteinase family [5]. It is capable of hydrolyzing collagen mainly triple helical regions as well as completes range of synthetic peptide substrates [5, 6, 7, 8]. It is similar to the vertebrate MMPs [9, 10]. The zinc-binding motif HExxH is the conserved sequence of ChC. Two histidine residues (His 415 and His 419) acting as Zn (II) ligands and the third ligand appears to be Glu 447 and a water molecule/hydroxide ion acts as nucleophile in the hydrolytic cleavage [5, 6, 8, 9, 10].

Bacillus polymyxa metalloprotease (BpM) can cleave fibronectin, collagen IV and to a smaller extent collagen I, but it does not cleave collagen V or laminin [11]. It hydrolyzes peptide bonds at N-terminal side of non-polar amino acid residues [11]. It specially digests the intercellular proteins that exposed hydrophobic amino acid residues [11]. It

consists one zinc ion and four calcium ions per subunit. Therefore, we hypothesized that those, compounds which are capable of inhibiting ChC can also act as MMPs (collagenases and gelatinases) inhibitors and use this in screening for MMP-2 and MMP-9 inhibitors.

Plant derived phenolic compounds have received renewed interest as bioactive chemicalsand have diverse biological activities such as anti-cancer, anti-arthritis, anti-inflammatory, anti-bacterial, anti-oxidant [12], phytoalexins [13], antifeedants activities and protective agents against UV light [14]. Some plant derived crude phenolic extracts and purified compounds have been investigated to possess protease inhibitory activity [15, 16]. Thus, plant phenolics provide an approach to investigate their activity against various disorders as therapeutic agents. In present study 95 important medicinal plant materials were collected, pulverized into fine powders and were used for preparation of methanol extracts. Total phenolic contents in all plant extracts were estimated by using Folin-Ciocalteau assay. All extracts were exploited to screen inhibitory activity against BpM and ChC by using dot blot assay on X-ray film.

2. Materials and Methods

C. histolyticum collagenase (ChC), and *B. polymyxa* metalloprotease (BpM) were procured from Sigma Aldrich. Tris-hydroxymethyl amine, Calcium chloride, Zinc chloride, 1, 10-Phenanthroline, X-ray films and Trichloroacetic acid were brought from Spectrochem.

All chemicals used in this study were of analytical grade.

2.1 Sample collection

Medicinal plant materials were obtained from local market of Aurangabad and campus of Dr. Babasaheb Ambedkar Marathwada University, Aurangabad (MS), India. All plant materials were identified and authenticated by taxonomist, Department of botany Dr. Babasaheb Ambedkar Marathwada University, Aurangabad. Ninety five plant tissues (flowers, leaves, stem, root, bark etc.) believed to have medicinal properties were included in this study. Collected plant materials were completely dried at 37 ^{0}C in oven. Dried plant materials were pulverized into fine powders in a grinder mixture. Fine powders were stored in moisture free containers at room temperature for further experiments.

2.2 Preparation of crude extracts

For extraction fine powders were soaked in MeOH (1:6 w/v), stirred for 2 hrs at room temperature. The mixed suspensions were centrifuged at 6000 rpm for 15 min, supernatants were collected and preserved at -20ºC for further experiments [17].

2.3 Estimation of total phenolics

Amounts of total phenolics in all plant extracts were determined by using Folin-Ciocalteau assay with slight modification of method used by Ainsworth and Gillespie [18]. Gallic acid was used as a reference standard for plotting of calibration curve. Methanolic extract (10 µl) of each plant was diluted up to 1.5 ml with distilled water. To this extract 0.5 ml Folin-Ciocalteau reagent was added and incubated at room temperature for 3 min. Thereafter, each aliquot was neutralized by adding 1 ml sodium carbonate (20% w/v). Reaction mixtures were incubated at room temperature for few min with intermittent shaking for color development. Absorbance of resulting blue color was measured at 650 nm using double beam UV-VIS spectrophotometer. Amount of total phenolics was estimated from standard graph of gallic acid and expressed as GAE (mg/ml).

2.4 Screening of plant extracts for inhibitory activities against BMPs (Gelatinase-spot test)

Screening of inhibitory activities from methanolic extracts of 95 plants against ChC andBpM was carried out by using dot blot assay/spot test on X-ray film [19]. In a typical reaction mixture 5 µl BpM (100 µg/ml in 50 mM Tris-HCL buffer pH 7.5, 50 mM $CaCl_2$, 100 mM NaCl) was mixed with 5 µl buffer, 5 µl of each plant extract and incubated at 37^{0}C for 10 min. Simultaneously an aliquot was kept without plant extract as control. After incubation all samples were loaded on X-ray film and X-ray film was incubated at 37^{0}C for 15 min. Then X-ray film was washed under tap/warm water and wetted X-ray film was dried at room temperature. Inhibitory activity

was checked by comparing with control spot. Spots appeared as unhydrolyzed gelatin revealed the presence of inhibitory activity in extract. Films were visually assessed and scanned on EPSON scanner at 300 dpi. Same procedure was used for screening of inhibitory activity against ChC (200 µg/ml).

3. Results and Discussions

3.1 Total phenolic contents in plant methanolic extracts

It is reported that polyphenols synthesized by plants are utilized in defense against insects and pathogens [20]. They are extensively distributed and havebiogenetically developed from the shikimate-phenylpropanoids-flavonoids pathways [20]. They exist as simple phenolic acids, flavonoids and tannins [20, 21]. It has been reported that phenolic and non-phenolic compounds are capable of inhibiting the various MMPs, cancer growth and invasion of tumor [22]. In the present study, for the extraction of phenolics from fine powders of different plant materials, methanol was used as a solvent system. Methanol is a highly polar solvent and it has been reported that it exhibits better potential in the isolation of secondary metabolites such as flavonoids, tannins and phenolic acids from plant [23]. Also, methanol exhibits higher extractive efficiency of phenolic compounds [17]. The amounts of total phenolics in plant methanolic extracts were determined by well-known Folin-Ciocalteau assay and expressed as mg/g tissue. On the basis of plant tissue the 95 plants were categorized into five groups and each group was sorted according to amounts of total phenolics (Table 1 to 5). The highest amount of total phenolics (257.58 ± 0.75 mg/g) was observed in *Gloriosa superba* fruit while the lowest amount of total phenolics (0.468 ± 0.003 mg/g) was observed in *Crataeva nurvala* stem among all extracts.*Total phenolic contents in bark extracts*; The highest amount of total phenolics (108.96 ± 0.41 mg/g) was observed in *Acacia catechu*among all barks followed by *Sygyzium cumini* (96 ± 0.28 mg/g), *Terminalia arjuna*(78 ± 0.49 mg/g) and *Albezia lebbeck* (62.4 ± 2.97 mg/g). The moderate amount of total phenolics was observed in *Myristica fragrans* (30.78 ± 0.09 mg/g) and *Pithocelibium dulce* (25.38 ± 0.14 mg/g). The minimum amount of total phenolics was observed in *Terminalia argentea* (10.08 ± 0.64 mg/g) and *Mesua ferrea*(7.5 ± 0.040 mg/g) and the lowest amount of total phenolics was observed in *Bauhinia variegata* (1.5 ± 0.009 mg/g) among all barks followed by Erythrina strictavar. suberosa (1.8 ± 0.05 mg/g), *Ailanthus excelsa* (2.58 ± 0.15 mg/g), *Commiphora mukul* (3.54 ± 0.01 mg/g), *Diospyros melanoxylon* (3.84 ± 0.04 mg/g), *Limonia acidissium* (5.16± 0.09 mg/g) and *Terminalia chebula* (5.4 ± 0.04 mg/g) (Table 1).*Total phenolic contents in flower, fruit and fruit rind extracts*; The highest amount of total phenolics (257.58 ± 0.75 mg/g) was observed in *Gloriosa superba* fruit among all fruits, flowers and fruit rind as well as all plants. The maximum amount of total phenolics (143.16 ± 0.49 and 82.38 ± 0.67 mg/g) was observed in *Punica granatum*fruit rind and *Terminalia bellerica* fruit. The minimum amount of total phenolics (16.8 ± 0.75 mg/g) was observed in *Withania coagulans.* All flowers were found to be containing < 2 mg/g total phenolics. The lowest amount of total phenolics (1.56 ± 0.01 mg/g) was observed in *Datura metal* among all fruits followed by *Semicarpus anacardium* (3.96 ± 0.03 mg/g), *Pongamia pinnata* (4.2 ± 0.020 mg/g), *Grewia tiliaefolia* (4.68 ± 0.27 mg/g) and *Gordonia obtusa* (5.88 ± 0.04 mg/g) (Table 2).*Total phenolic contents in leaf extracts*; The highest amount of total phenolics (118.38 ± 0.37 mg/g) was found in *Terminalia catappa* among all leaves followed by *Shorea robusta* (29.76 ± 0.04 mg/g) and *Peltophorum pterocarpum* (20.76 ± 0.20 mg/g). Three plant leaves such as *Strychnos nux-vomica*, *Tribulus adscendens*and *Tridax procumbens* were found to be containing very low amount of total phenolics (<1 mg/g). Thirteen plant leaves were found to be containing low amount total phenolics (1 to 2 mg/g). Nineteen plant leaves were found to be contanining moderate amount of total phenolics (2 to 6.5 mg/g). The high amounts of phenolics were found in *Annona reticulate, Cedrus deodara, Cymbopogon citratus* and *Andrographis paniculata* as 17.94 ± 0.01, 14.88 ± 0.08, 9.48 ± 0.53 and 9.18 ± 0.062 mg/g respectively (Table 3).*Total phenolic contents in root, seed, rhizome and pod extracts*; The highest amount of total phenolics (33.96 ± 0.18 mg/g) was found in

Linum usitatissum seedamong all seeds, roots, rhizome and pod followed by *Cassia fistula* pod (15.78 ± 0.12 mg/g). Five roots were found to be containing moderate amounts of total phenolics (2 to 4.5 mg/g). The lowest amount of total phenolics (0.582 ± 0.03 mg/g) was found in *Trichosanthes labata* seed among all seeds, roots, rhizome and pod. The high amounts of total phenolics such as 9 ± 0.49 8.52 ± 0.01 and 9.36 ± 0.04 mg/g were observed in *Hedychium spicatum* Rhizome, *Cassia tora* root and *Vitex negundo* seed respectively (Table 4).*Total phenolic contents in stem extracts*; The highest amount of total phenolics (202.56 ± 0.47 mg/g) was observed in *Bergenia ligulata* while the lowest amount of total phenolics (0.468 ± 0.003 mg/g) was observed in *Crataeva nurvala* among all stems. High amounts of total phenolics were observed in *Plumbago indica* (18.84 ± 1.49 mg/g) and *Vinca rosea* (13.38 ± 0.062 mg/g). Eight plants were found to be containing moderate amounts of total phenolics (2 to 6.9 mg/g). Low amounts of total phenolics were observed in *Barleria strigosa* (1.74 ± 0.01 mg/g), *Berberis aristata* (1.2 ± 0.047 mg/g) and *Rubia cordifolia* (1.14 ± 0.06 mg/g) (Table 5). It is reported that occurrence of phenolic components depends upon the type of plant tissue, maturity at harvest, growing conditions, soil conditions and post-harvest treatment [24]. Different parts of the same plant can synthesize and store different compounds or different amount of a specific compound due to their differential gene expression, which in turn affects the different biological and antioxidant activities [24, 25].

3.2 Screening of inhibitory activities of plant methanolic extracts against BMPs

It was assumed that plant extract exhibits inhibitory activity of bacterial metalloproteases, this extract may be capable of inhibiting MMPs from other sources due to structural similarities [9, 10, 26]. Preliminary screening of methanolic extracts of different (95) plant resources for MMP inhibitor activity the bacterial metalloproteases (ChC and BpM) were used. Screening of inhibitory activities of metalloproteases from plant extracts was accomplished by using dot blot assay on X-ray film. Normally bacterial metalloproteases spotted on X-ray film hydrolyze the gelatin and after washing under tap water produce a clear spot due to hydrolysis of gelatin indicating protease activity. When spotted with plant extracts containing inhibitors, the digestion of gelatin is inhibited and hence clear spots are not seen. These spot tests indicated that 32 plants have inhibitory activities against ChC while 27 have inhibitory activities against BpM and that 25 plants exhibited inhibitory activity against ChC as well as BpM (Figur 1 and 2). This number would have increased if the amount of extract to be loaded for spot test has been higher. But then these plants would come under a new category of low inhibitory potential.*Screening of inhibitory activities of bark extracts against BMPs;* Fifteen bark extracts were screened for BMPs inhibitory activity out of which spot tests indicated that, 11 barks have inhibitory activity against ChC and BpM and 3 barks have no inhibitory activity against ChC and BpM. In case of screening, the anti-ChC and anti-BpM activities were found to be dependent upon amount of total phenolics present in bark extracts. Bark of *Limonia acidissium was* found to be contaning rich amount total phenolics (5.16 ± 0.09 mg/g) but not exhibited inhibitory activity against ChC and BpM. This indicates that different kinds of phenolics as compared to other bark phenolics are available in this plant. Bark extracts of *Diospyros melanoxylon, Commiphora mukul* and*Terminalia chebula* were found to contain low amounts of total phenolics but exhibited inhibitory activity against ChC and BpM (Table 1). Therefore, the phenolics of *Diospyros melanoxylon* and *Commiphora mukul* have high inhibitory potential or compounds other than phenolics may responsible for inhibitory activity.*Screening of inhibitory activities of flower, fruit and fruit rind extracts against BMPs;* Flowers extracts do not exhibit inhibitory activity against ChC and BpM. Out of 8 fruit extracts 3 fruit extracts exhibited inhibitory activity against ChC and BpM. Fruit extracts of *Gordonia obtusa* and *Pongamia pinnata*exhibited inhibitory activity against ChC but not against BpM. Fruit rind extract of *Punica granatum*exhibited inhibitory activity against ChC and BpM. The anti-ChC and anti-BpM activities were found to be

dependent upon amount of total phenolics present in fruit and fruit rind extracts. Fruit extracts of *Pongamia pinnata* and *Gordonia obtusa*were found to contain low amounts of total phenolics but exhibited inhibitory activity against ChC (Table 2).*Screening of inhibitory activities of leaf extracts against BMPs;* Out of 42 leaf extracts 4 leaf extracts exhibited inhibitory activity against ChC and BpM. Leaf extracts of *Cedrus deodara, Ficus benghalensis and Hyptis suaveolens* exhibited inhibitory activity against ChC but not against BpM. The anti-ChC and anti-BpM activities were found to be dependent upon amount of total phenolics present in leaves extracts. Leaf extract of *Caesalpinia pulcherrima* was found to contain low amount of total phenolics but exhibited inhibitory activity against ChC and BpM. Leaves extracts of *Hyptis suaveolens*and *Ficus benghalensis*were found to contain low amounts of total phenolics but exhibited inhibitory activity against ChC (Table 3).*Screening of inhibitory activities of pod, rhizome, root and seed extracts against BMPs;* Root extracts did not exhibit inhibitory activity against ChC and BpM. Out of 3 seeds, seed extract of *Linum usitatissum* exhibited inhibitory activity against ChC and BpM. Seed extract of *Vitex negundo* exhibited inhibitory activity against BpM but not against ChC. Pod extract of *Cassia fistula* exhibited inhibitory activity against ChC and BpM.Rhizomeand root extractsof *Hedychium spicatum*and *Cassia tora*were found to be contaninig high amount of total phenolics but did not exhibit inhibitory activity against ChC and BpM (Table 4). *Screening of inhibitory activities of stem extracts against BMPs;* Out of 15 stem extracts 4 stem extracts exhibited inhibitory activity against ChC and BpM. Stem extracts of *Glycyrrhiza glabra* and *Solanum xanthocarpum* exhibited inhibitory activity against ChC but not against BpM. The anti-ChC and anti-BpM activities were found to be dependent upon amount of total phenolics present in stem extracts. Stem extract of *Plumbago zeylanica* was found to contain low amount of total phenolics but exhibited inhibitory activity against ChC and BpM. Stem extract of *Solanum xanthocarpum*was found to contain low amount of total phenolics but exhibited inhibitory activity against ChC (Table 5).In case of screening, the anti-ChC and anti-BpM activities in general were found to be dependent upon amount of phenolics present in plant extract. Some plant extracts (*Pongamia pinnata*fruit, *Hyptis suaveolens*leaf,*Plumbago zeylanica* stem, *Solanum xanthocarpum* stems, *Ficus benghalensis* leaf, *Diospyros melanoxylon* bark, *Commiphora mukul* bark,*Caesalpinia pulcherrima*leaf, *Terminalia chebula bark* and *Gordonia obtusa* fruit) were found to contain low amounts of total phenolics but they exhibited anti-metalloproteases activities. This indicated that molecules other than phenolics or high potential of particular phenolics may be responsible for inhibitory activity.

Conclusions

In conclusion, the result of this study shows that among 95 different plant materials bark is a promising source of phenolic compounds. The highest amount of total phenolics is present in *Gloriosa superba*fruits. The anti-BMP activity of plant extracts significantly depend upon total phenolics content. Total 32 different plant materials exhibit inhibitory activities against ChC while 27 exhibit inhibitory activity against BpM. The extracts of inhibitory activity exhibiting plant materials could be utilized for isolation of novel therapeutic constituents as drug for controlling of MMPs relevant disorders.

Conflict of interest statement

We declare that we have no conflict of interest.

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Tables

Table 1: Total phenolics and inhibition of ChC and BpM activity in bark extracts of different plants. + Indicates presence of inhibitory activity, - Indicates absence of inhibitory activity.

Sr. No.	Name of plant	Part Used	Family	Phenolics content mg GAE/g tissue	ChC Inhibitor activity	BpM Inhibitor activity
1.	*Acacia catechu* Roxb. Willd.	Bark	*Fabaceae*	108.96 ± 0.41	+	+
2.	*Sygyzium cumini* L. Skeels.	Bark	*Myrtaceae*	96 ± 0.28	+	+
3.	*Terminalia arjuna* Roxb. Wight & Arn.	Bark	*Combretaceae*	78 ± 0.49	+	+
4.	*Albezia lebbeck* L.Benth.	Bark	*Fabaceae*	62.4 ± 2.97	+	+
5.	*Myristica fragrans* HOUTT.	Bark	*Myristicaceae*	30.78 ± 0.09	+	+
6.	*Pithocelibium dulce* Roxb.Benth.	Bark	*Fabaceae*	25.38 ± 0.14	+	+
7.	*Terminalia argentea* Mart.	Bark	*Combretaceae*	10.08 ± 0.64	+	+
8.	*Mesua ferrea*	Bark	*Clusiaceae*	7.5 ± 0.040	+	+
9.	*Terminalia chebula*Retz.	Bark	*Combretaceae*	5.4 ± 0.04	+	+
10.	*Limonia acidissium* L	Bark	*Rutaceae*	5.16 ± 0.09	-	-
11.	*Diospyros melanoxylon* Roxb.	Bark	*Ebenaceae*	3.84 ± 0.04	+	+
12.	*Commiphora mukul* Arn.Bhandari	Bark	*Burseraceae*	3.54 ± 0.01	+	+
13.	*Ailanthus excelsa* Roxb.	Bark	*Simaroubaceae*	2.58 ± 0.15	-	-
14.	Erythrina stricta var. suberosa (Roxb.)	Bark	*Fabaceae*	1.8 ± 0.05	-	-
15.	*Bauhinia variegata* L.	Bark	*Fabaceae*	1.5 ± 0.009	-	-

Table 2: Total phenolics and inhibition of ChC and BpM activity in flower, fruit and fruit rind extracts of different plants.

Sr. No.	Name of plant	Part Used	Family	Phenolics content mg GAE/g tissue	ChC Inhibitor activity	BpM Inhibitor activity
1.	*Macuna gigantea* Willd. DC.	Flower	*Fabaceae*	1.5 ± 0.004	-	-
2.	*Nerium indicum*Mill.	Flower	*Apocynaceae*	1.2 ± 0.06	-	-
3.	*Crocus sativus* L.	Flower	*Iridaceae*	0.576 ± 0.02	-	-
4.	*Gloriosa superba* L.	Fruit	*Liliaceae*	257.58 ± 0.75	+	+
5.	*Terminalia bellerica* (Gaertn.) Roxb.	Fruit	*Combretaceae*	82.38 ± 0.67	+	+
6.	*Withania coagulans* Dunal.	Fruit	*Solanaceae*	16.8 ± 0.75	+	+
7.	*Gordonia obtusa* Wallich ex	Fruit	*Theaceae*		+	-
8.	*Grewia tiliaefolia* L.	Fruit	*Tiliaceae*	4.68 ± 0.27	-	-
9.	*Pongamia pinnata*L. Panigrah.	Fruit	*Fabaceae*	4.2 ± 0.020	+	-
10.	*Semicarpus anacardium* L.	Fruit	*Anacardiaceae*	3.96 ± 0.03	-	-
11.	*Datura metal* L.	Fruit	*Solanaceae*	1.56 ± 0.01	-	-
12.	*Punica granatum* L.	Fruit rind	*Lythraceae*	143.16 ± 0.49	+	+

Table 3: Total phenolics and inhibition of ChC and BpM activity in leaves extracts of different plants.

Sr. No.	Name of plant	Part Used	Family	Phenolics content mg GAE/g tissue	ChC Inhibitor activity	BpM Inhibitor activity
1.	*Terminalia catappa* L.	Leaf	*Combretaceae*	118.38 ± 0.37	+	+
2.	*Shorea robusta*Roth.	Leaf	*Dipterocarpaceae*	29.76 ± 0.04	+	+
3.	*Peltophorum pterocarpum*DC. K. Heyne	Leaf	*Fabaceae*	20.76 ± 0.20	+	+
4.	*Annona reticulate* L.	Leaf	*Annonaceae*	17.94 ± 0.01	-	-
5.	*Cedrus deodara* Roxb.G.Don.	Leaf	*Pinaceae*	14.88 ± 0.08	+	-
6.	*Cymbopogon citratus* DC.Stapf.	Leaf	*Poaceae*	9.48 ± 0.53	-	-
7.	*Andrographis paniculata*Burm.f.Wall. ex Nees.	Leaf	*Acanthaceae*	9.18 ± 0.062	-	-
8.	*Madhuca indica* J F Gmel.	Leaf	*Sapotaceae*	6.3 ± 0.07	-	-
9.	*Anacardium occidentale* L.	Leaf	*Anacardiaceae*	5.40 ± 0.035	-	-
10.	*Ficus benghalensis* L.	Leaf	*Moraceae*	5.1 ± 0.03	+	-
11.	*Cannabis sativa* L.	Leaf	*Cannabinaceae*	4.98 ± 0.06	-	-
12.	*Cassia obstusifolia* L.	Leaf	*Fabaceae*	4.56 ± 0.10	-	-
13.	*Allium sativum* L.	Leaf	*Amaryllidaceae*	4.5 ± 0	-	-
14.	*Justicia gendarussa* Burm. f.	Leaf	*Acanthaceae*	4.44 ± 0.03	-	-
15.	*Marsdenia straminea*	Leaf	*Apocynaceae*	4.14 ± 0.22	-	-
16.	*Caesalpinia pulcherrima* (L.) Sw.	Leaf	*Fabaceae*	4.14 ± 0.008	+	+
17.	*Hyptis suaveolens* L.Poit.	Leaf	*Lamiaceae*	3.84 ± 0.042	+	-
18.	*Rauvolfia serpentina* L.Benth.exKurz.	Leaf	*Apocynaceae*	3.84 ± 0.011	-	-
19.	*Inula racemosa* Hook.f.	Leaf	*Asteraceae*	2.94 ± 0.02	-	-
20.	*Tribulus terestris* L.	Leaf	*Zygophyllacee*	2.76 ± 0.004	-	-
21.	*Gmelina arborea*Roxb.	Leaf	*Lamiaceae*	2.52 ± 0.025	-	-
22.	*Centella asiatica* L.Urban.	Leaf	*Mackinlayaceae*	2.52 ± 0.020	-	-
23.	*Jasminum sambac* L.Aiton.	Leaf	*Oleaceae*	2.46 ± 0.09	-	-
24.	*Baliospermum montanum* Willd. Muell.	Leaf	*Euphorbiaceae*	2.4 ± 0.09	-	-
25.	*Leucas aspera* L. aspera.	Leaf	*Lamiaceae*	2.34 ± 0.01	-	-
26.	*Nyctanthes arbortristis* L.	Leaf	*Oleaceae*	2.22 ± 0.01	-	-
27.	*Barleria obtusa*	Leaf	*Acanthaceae*	1.98 ± 0.10	-	-
28.	*Aspargus racemosus* Willd.	Leaf	*Asparagaceae*	1.92 ± 0.01	-	-
29.	*Agle marmelos* L.Correa.	Leaf	*Rutaceae*	1.86 ± 0.01	-	-
30.	*Ficus racemosa* Roxb.	Leaf	*Moraceae*	1.86 ± 0.01	-	-

31.	*Acorus calamus* L.	Leaf	*Acoraceae*	1.5 ± 0.008	-	-
32.	*Senseveria javanica* Thunb.and Dalm.	Leaf	*Asparagaceae*	1.32 ± 0.01	-	-
33.	*Sansevieria cylindrica* L.	Leaf	*Asparagaceae*	1.32 ± 0.01	-	-
34.	*Marsdenia acuta* Thunb.	Leaf	*Apocynaceae*	1.26 ± 0.01	-	-
35.	*Alstonia scholaris* L. R. Br.	Leaf	*Apocynaceae*	1.2 ± 0.04	-	-
36.	*Macuna pruriens* L.DC.	Leaf	*Fabaceae*	1.2 ± 0.009	-	-
37.	*Cucumis melo* L.	Leaf	*Cucurbitaceae*	1.14 ± 0.047	-	-
38.	*Gliricidia sepium* Jacq.Kunth ex Walp.	Leaf	*Fabaceae*	1.02 ± 0.01	-	-
39.	*Macuna urens*L. Fawcett & Rendle.	Leaf	*Fabaceae*	1.02 ± 0.01	-	-
40.	*Strychnos nux-vomica* L.	Leaf	*Loganiaceae*	0.9 ± 0.006	-	-
41.	*Tribulus adscendens*B.L.Rob.	Leaf	*Zygophyllaceae*	0.9 ± 0.040	-	-
42.	*Tridax procumbens* L.	Leaf	*Asteraceae*	0.78 ± 0.01	-	-

Table 4: Total phenolics and inhibition of ChC and BpM activity in pod, rhizome, root and seed extracts of different plants.

Sr. No.	Name of plant	Part Used	Family	Phenolics content mg GAE/g tissue	ChC Inhibitor activity	BpM Inhibitor activity
1.	*Cassia fistula* L.	Pod	*Fabaceae*		+	+
2.	*Hedychium spicatum*	Rhizome	*Zingiberaceae*	9 ± 0.49	-	-
3.	*Cassia tora* L. syn.	Root	*Fabaceae*	8.52 ± 0.01	-	-
4.	*Clematis* cyanea	Root	*Rannunculaceae*	4.02 ± 0.01	-	-
5.	*Barleria cristata* L.	Root	*Acanthaceae*	3 ± 0.009	-	-
6.	*Cassia grandis* L.	Root	*Fabaceae*	2.88 ± 0.04	-	-
7.	*Barleria Montana* DUN.	Root	*Acanthaceae*	2.76 ± 0.008	-	-
8.	*Crossandra infundibuliformis*L.Nees	Root	*Acanthaceae*	2.28 ± 0.01	-	-
9.	*Linum usitatissum*	Seed	*Linaceae*	33.96 ± 0.18	+	+
10.	*Vitex negundo* L.	Seed	*Lamiaceae*	9.36 ± 0.04	-	+
11.	*Trichosanthes labata* Roxb.	Seed	*Cucurbitaceae*	0.582 ± 0.03	-	-

Table 5: Total phenolics and inhibition of ChC and BpM activity in stemextracts of different plants.

Sr. No.	Name of plant	Part Used	Family	Phenolics content mg GAE/g tissue	ChC Inhibitor activity	BpM Inhibitor activity
1.	*Bergenia ligulata* Wall.Engl.	Stem	*Saxifragaceae*	202.56 ± 0.47	+	+
2.	*Plumbago indica* L.	Stem	*Plumbaginaceae*	18.84 ± 1.49	+	+
3.	*Vinca rosea* L.	Stem	*Apocynaceae*	13.38 ± 0.062	+	+
4.	*Glycyrrhiza glabra* L.	Stem	*Fabaceae*	6.9 ± 0.040	+	-
5.	*Cassia roxburghii* DC.	Stem	*Fabaceae*	6.36 ± 0.04	-	-
6.	*Solanum xanthocarpum* Schrad and Wendl.	Stem	*Solanaceae*	5.4 ± 0.02	+	-
7.	*Plumbago zeylanica* L.	Stem	*Plumbaginaceae*	4.98 ± 0.03	+	+
8.	*Crossandra pungens*	Stem	*Acanthaceae*	3.72 ± 0.009	-	-
9.	*Barleria prionitis* L.	Stem	*Acanthaceae*	3.06 ± 0.008	-	-
10.	*Cassia ferruginea* Schrad. DC.	Stem	*Fabaceae*	2.58 ± 0.01	-	-
11.	*Marsdenia tenacissima* Wight & Arn.	Stem	*Apocynaceae*	2.16 ± 0.01	-	-

12.	*Barleria strigosa* Willd.	Stem	*Acanthaceae*	1.74 ± 0.01	-	-
13.	*Berberis aristata* DC.	Stem	*Berberidaceae*	1.2 ± 0.047	-	+
14.	*Rubia cordifolia* L.	Stem	*Rubiaceae*	1.14 ± 0.06	-	-
15.	*Crataeva nurvala* Buch.-Ham.	Stem	*Capparidaceae*	0.468 ± 0.003	-	-

Figure 1: Screening of inhibitory activities of 95 different plant methanolic extracts against ChC.

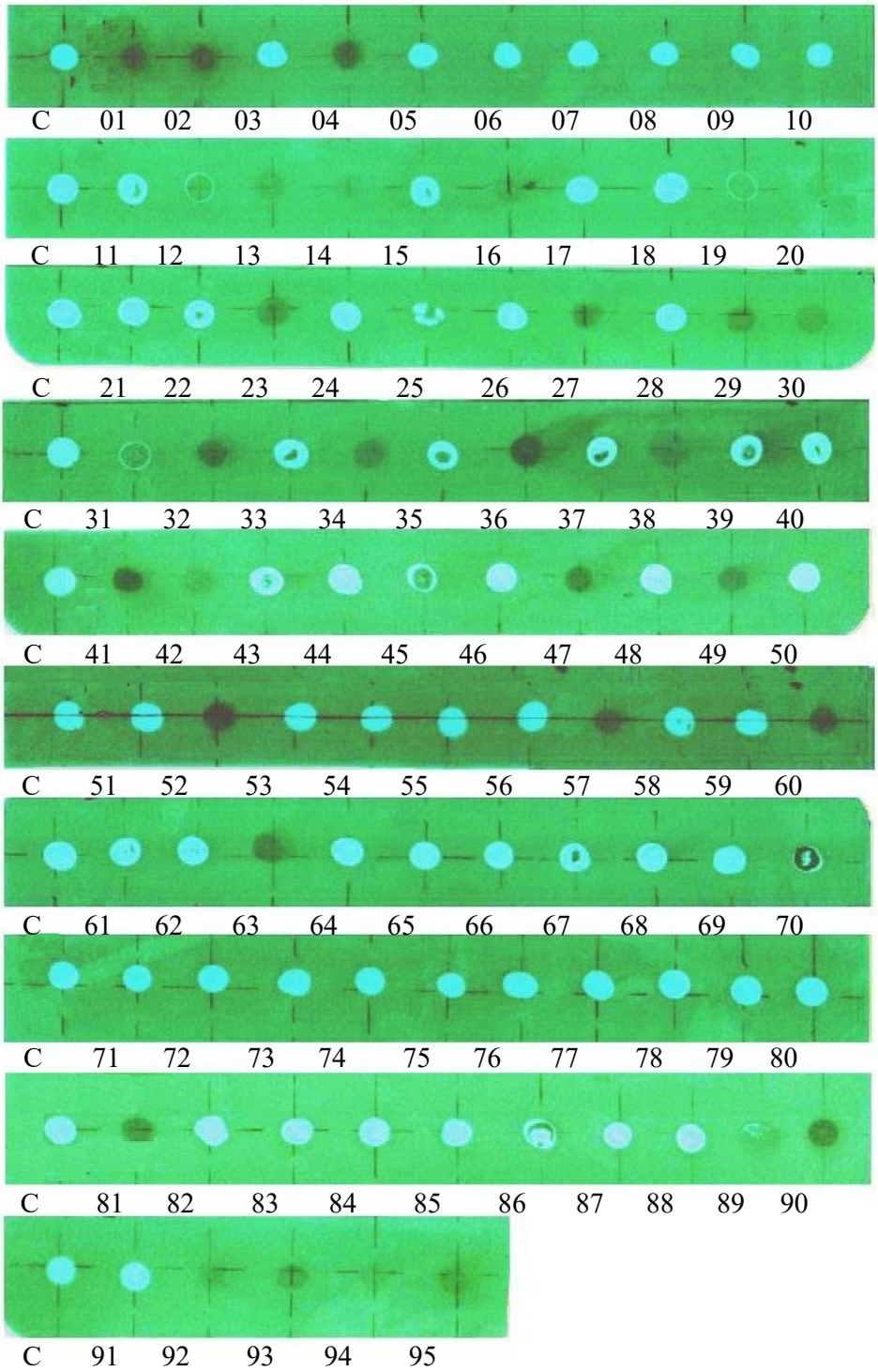
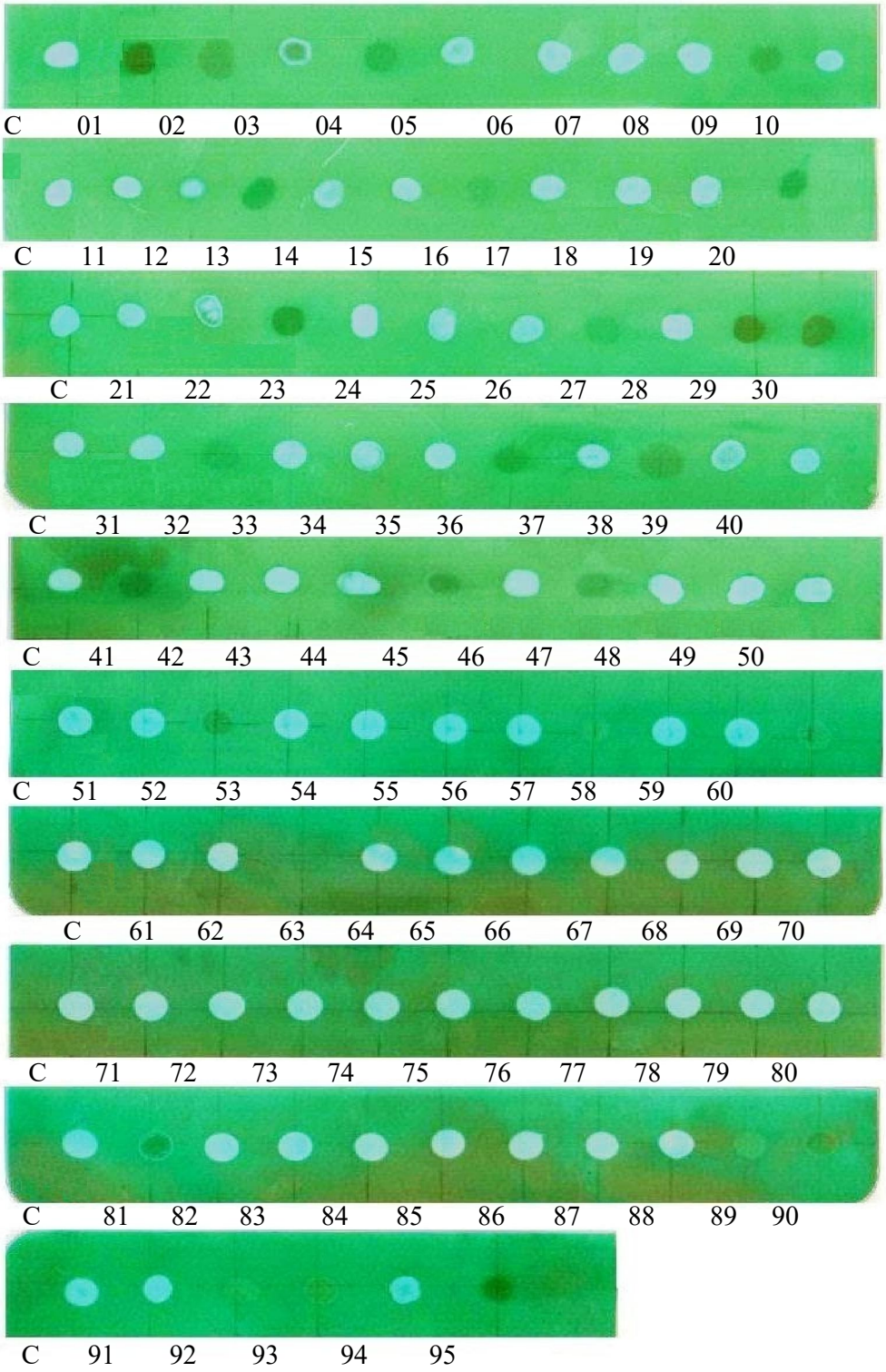


Figure 2: Screening of inhibitory activities of 95 different plant methanolic extracts against BpM.



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SYNTHESIS OF COMPLEX OF FE (III) WITH CINNMALDEHYDE & 4-CHLORO ANILINE SCHIFF BASE

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ABSTRACT

Metal complex of Schiff bases or imines have large application in organic synthesis and also show significant biological activity. The Schiff bases complexes combinations with a metal ion are used as insecticides, fungicides, herbicides. Properties of Schiff base and their metal complexes have been identified by various scientists in the past. So another attempt was made for formation of metal complex of Fe ion with Schiff base ligand namely (E)-4- Chloro – N (E)-3- phenylallylidene aniline derived from condensation reaction of Cinnamic aldehyde with 4- chloro aniline.
In this study, new Schiff base complex of Iron have been prepared by the condensation reaction of $Fecl_3$ and newly produced Schiff based. The complex have been characterized by IR spectroscopy technique and solubility test. The synthesized complexes have very poor solubility in water and carbon tetrachloride and completely soluble in DMSO (Dimethyl sulphoxide) and benzene. The yield is quite high with good purity of the molecule.

Keywords: *Schiff base, Metal complex of Schiff base, IR Spectroscopy, Cinnamic aldehyde, 4- chloro aniline*

Introduction

Coordination chemistry are these compounds in which metal ions are coordination by other atom's,anions or molecules, called as ligands The variety of Schiff base metal complex having much choice of ligands create interest in to research in this area. The Importance of coordination phenomenon in biological processes was realized, lots of metal containing macromolecules have been synthesized and studied to understand the role of these ligands in biological system, and they also contribute to the development of new metal-based chemotherapeutic agents. Coordination compounds have important roles as industrial field, agriculture and pharmaceutical fields. [1] Metal complex of schiff bases or imines have large application in organic synthesis and also show significant biological activity. [2]

A Schiff base name is given after Hugo Schiff, is a functional group that contain a carbon-nitrogen double bond. [3] cinnamaldehyde is a antimicrobial in nature and generally recognized as safe by U.S. food and drug Administration, since cinnamaldehyde forms stable Schiff base with amine compound. [4] Many kinds of amino have free amino groups that provide the possibility to generate cinnamaldehyde-amino Schiff base. [5] Many Schiff base complexes showed excellent catalytic activity in various reactions at high temperature (above 100° C) and in the presence of moisture. [6]

Some of the Schiff bases complexes combinations with a metal ion are used as insecticides, fungicides, herbicides. [7] Recently reported aldimines from benzaldehyde with different aromatic amines viz. aniline, 2- chloroaniline and 4- chloro aniline. Similarly, aldimine from Benzaldehyde with aniline have been reported their spectral behavior. [8] The coordination chemistry of the metal chelates of schiff base have been known for more than century. In 1840, Ettling prepared a copper (ll) complex with salicylideneimine. Since then large numbers of research article on Schiff bases and their metal complexes have appeared. [9]

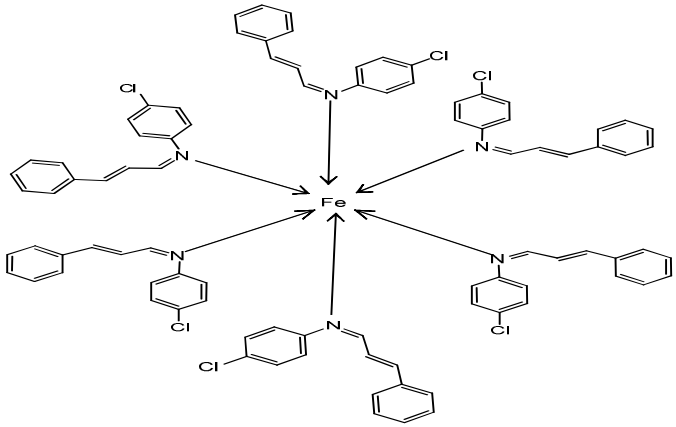
Methodology

All the chemicals and solvents were used of analytical reagent grade and used as commercially obtained without any further purification Cinnamaldeyde and 4-chloro aniline are the title compound in extra pure. Ethanol was used as solvent for the synthesis of complex. The metal salt of Fe^{3+} was used for the synthesis of metal complex. The apparatus used in this project are condenser, round bottom flask, beaker, measuring cylinder, water bath, suction pump, filter paper etc.

Synthesis of Schiff base: Cinnamaldehyde (10 ml) and 4-chloro aniline (5 gm) was taken in

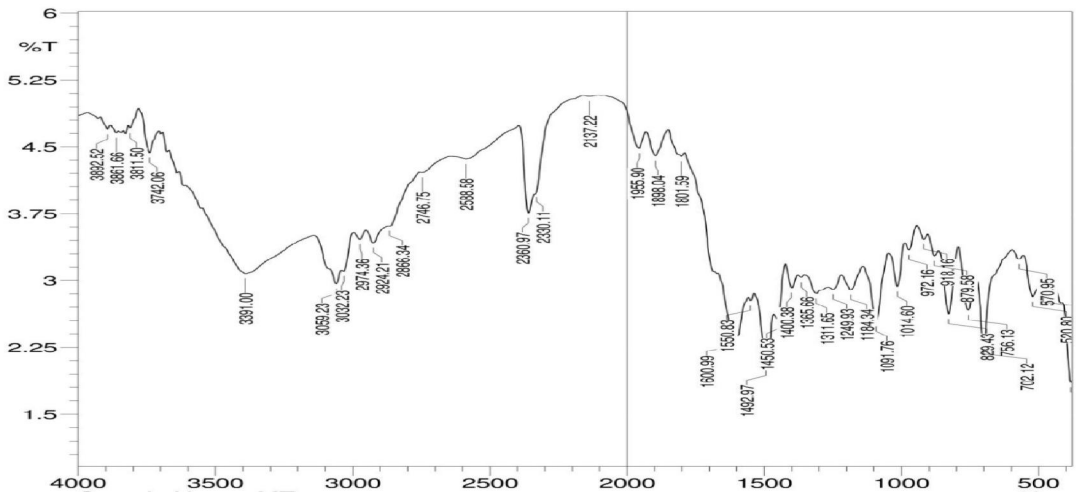
100 ml round bottom flask mixed completely and by adding 2-3 drops of concentrated sulfuric acid reflux the reaction for the 2 hours in water bath. The dark brown coloration occurs. This intermediate product gets poured into ice cold water in beaker. The solid product (Schiff base) form was filtrated using suction pump. The resulting precipitate gets dried and Schiff based is formed. It gives following type of reaction

Synthesis of Metal complex: The metal complex were prepared in 1:1 ratio (metal: ligand). The appropriate quantity of Schiff base ligand was dissolved in absolute ethanol (10 ml) to this solution was added a metal salt of ferric(III) chloride anhydrous. This mixture was reflux for 3 hours in water bath. Then cooled the product and precipitation occur in an ice cold water, a solid reddish brown color precipitated were obtained which were filtered of and dried. The precipitated was found to be soluble in DMSO(Dimethyl Sulphoxide) Partially soluble in benzene & Ccl_4(carbon tetrachloride) and insoluble in water. Then crystallized the precipitated using diethyl ether in a 100ml beaker and take the beaker overnight. It gives good percentage of yield.
The proposed structure of complex was shown in below:



Observation

Fourier transforms infrared (FTIR) spectra of the metal complex shown below.



In the metal complex of Fe the OH stretching is observed at 3742 cm^{-1} because of the ethanol is used as the solvent in the reaction. Afterwards the N-H stretching is seen at 3392 cm^{-1}. The broad & strong spectrum is observed. C-H stretching of aldehydic H is seen in between 3059-3032 cm^{-1} as doublet. Then C-H stretching of alkynes is seen at 2974 $cm^{-1}$and medium peak is observed. The Imine peak is observed at 1600 cm^{-1}.

(-OH) Stretching	-(NH) Stretching	-(CH) Aldehyde Stretching	-(CH) Alkyne Stretching	-(C=N) Imine
3742(s)	3391(s)	3059-3032(s)	2974(m)	1600(s)

s = small, m = medium

Result and Discussion

In the present work, derivative of (E)–4-Chloro-N (E)-3-phenylallylidene aniline Schiff base was synthesized by using Cimmaldehyde and 4-chloro aniline as starting material with moderate to good yield. The method is atom economic, easy and efficient. The method has advantages of cheaper chemicals and safely too. The crude solid product obtained was purified by recrystalizing with diethyl ether.

Conclusion

In this study, new Schiff base complex of Iron have been prepared by the condensation reaction of $Fecl_3$ and newly produced Schiff based. The complex has been characterized by IR spectroscopy technique. The synthesized complexes have very poor solubility in water and carbon tetrachloride and completely soluble in DMSO (Dimethyl sulphoxide) and benzene. The yield is quite high with good purity of the molecule.

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LEMON JUICE AS CATALYST FOR BIGINELLI REACTION: A GREENER PATHWAY FOR THE SYNTHESIS OF 3, 4-DIHYDROPYRIMIDIN-2-(1*H*)-ONES

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ABSTRACT

A greener pathway for the synthesis of Dihydropyrimidones (DHPM) using lemon juice as a naturally occurring acid catalyst is studied. The advantages of this method include one-pot synthesis of biologically very important DHPM with excellent yields in short time. The synthesized product is well characterized by IR, NMR and UV techniques.
The performed reaction for the synthesis of 3,4-dihydropyrimidin-2(1H)-ones using lemon juice as a catalyst and ethanol as a solvent, gives good yield having electron donating and withdrawing group. The reaction was performed by stirring at 90°C which gives excellent yields of desired product in 5 hour. The crude solid product obtained was purified by recrystalizing with ethanol.
In conclusion a clean, chromatography-free and ecological method has been developed for the synthesis of commercially very important DHPMs compounds. Utilization of naturally occuring lemon juice as an acid catalyst makes this protocol environmentally benign. Mild reaction condition, high yields of products, short reaction time are the keynote feaatures of this methods.

Keywords: *Biginelli Reaction, Dihydropyrimidones, Lemon Juice, Multi-Component Reaction, One-Pot Synthesis*

Introduction

In 1893 Pietro Biginelli firstly synthesized dihydropyrimidin-2(1H)-(thi)-one (4) (DHPM) derivatives by the cyclo-condensation three component i.e. aldehyde, β-keto ester and urea or thiourea in presence of Bronsted acid catalyst [1]. In the reported methods, toxic and polluting Lewis acid catalyst was used for the synthesis of DHPM, which was harmful for the environment [2]. In 1997, Milcent et al. described the synthesis of a series of 2 iminodihydropyrimidines in 42% yield by the reaction of guanidine hydrochloride with the Knoevenagel adducts derived from aryl aldehydes and benzoyl acetate in the presence of NaHCO [3]. In this case, yields were limited by the concomitant formation of double Biginelli adducts. In 2001, Kappe and co-workers described the first direct three-component Biginelli reaction with guanidine hydrochloride to give the corresponding 2-aminodihydropyrimidines. However, the method was limited to aromatic aldehydes and ethyl benzoylacetate as the β-dicarbonyl partner [4]. Cepanec also reports the synthesis of DHPM using antimony trichloride [5].

The DHPMs have major biological importance and pharmacological uses such as antitumor activity [6], anti-malarial [7], monastrol, calcium channel modulators, antihypertensive agents, α1a antagonists, antiviral, anti-inflammatory and antitumor agents, antibacterial, antifungal, anticonvulsant, antihistamines [8], anti-microbial [9], anti-HIV Agents [10].

A multi-component reaction (MCR) is a process in which three or more reactants combined together in a one pot to form a product. MCRs have played a central role in the development of modern synthetic methodology due to its selectivity, synthetic convergence and atom-economy for pharmaceutical and drug discovery research [11].

The main focus of this study is on catalyst which we have used. In this study reaction is carried out in presence of lemon juice which is naturally occurring acid catalyst, which is eco-friendly and in-expensive. By thinking environmental point of view, there is a serious need to replace toxic and polluting solvent with eco-friendly solvent like ethanol for the synthesis of DHPMs. As the reaction is one-pot, it reduces the number of time consuming and tedious steps of reaction thus, it is atom-economy.

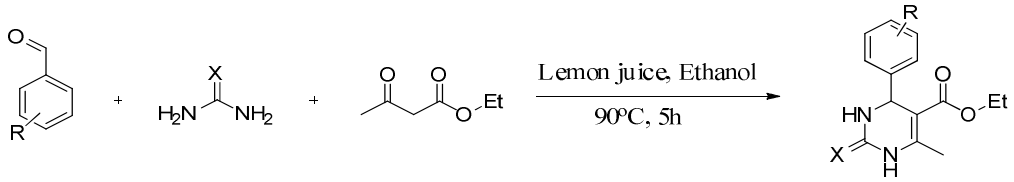
Methodology

Product obtained are all known compounds and were identified by comparing their physical and spectral data with those reported in literature. Melting points were determined in

open capillary and are uncorrected. The progress of the reaction was monitored by TLC [12]. The ^{1}H NMR spectra were recorded on spectrometer (400 MHz) using TMS as reference scale. The representive spectral analysis is given below.

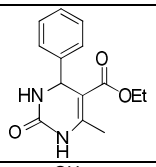
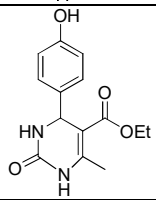
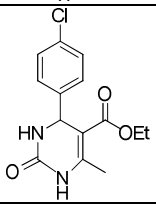
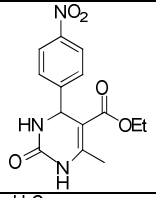
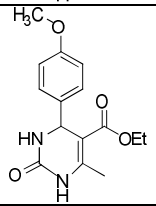
In a 100 ml round bottom flask, 20 ml Ethanol as a solvent was taken, then 1 ml of lemon juice i.e. catalyst was added then three components were sequentially added i.e. Benzaldehyde (1 eq.), urea (1.5 eq.), Ethylacetoacetate (1 eq.). The reaction mixture was continuously stirred at 90°C in oil-bath for 5 hr. After the completion of reaction as indicated by TLC, the reaction mixtured was kept open to evaporate the excess of ethanol. The obtained solid was recrystallized by ethanol to afford 3,4-dihydropyrimidin-2-(1*H*)-ones with excellent purity.

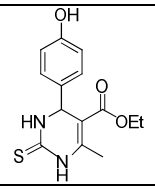
Scheme

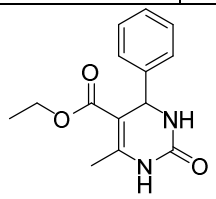


X = O, S

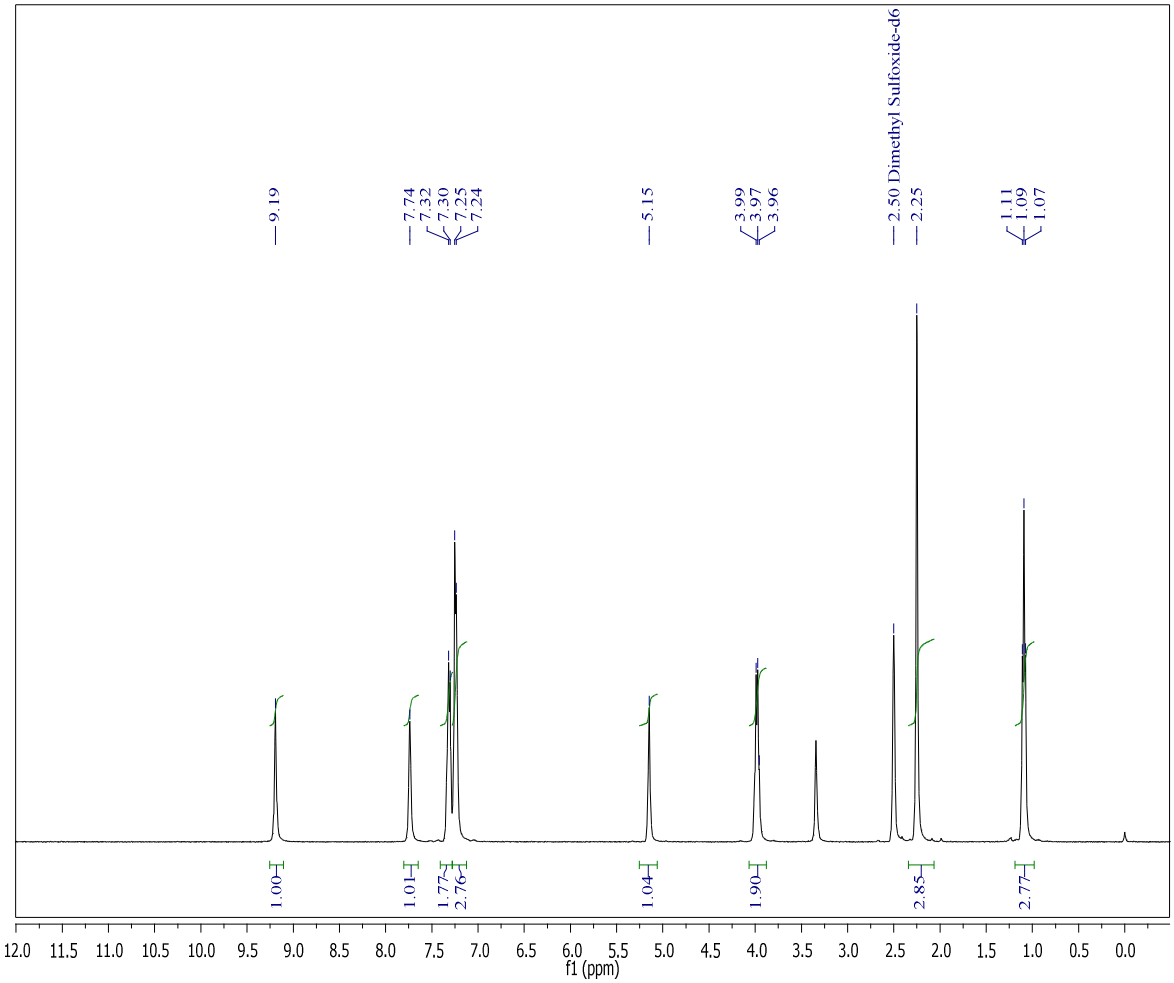
Observation

Sr. no.	Product	M.P.(°C)	colour	AE (%)	Yield(%)
1		201-202	White solid	87.83	80
2		228-229	White solid	88.40	94
3		211-212	White solid	89.0	84
4		206-207	Light brown	89.40	87
5		200-201	White solid	88.90	69

6		192-193	Off white solid	89.0	80



Ethyl 6-methyl-2-oxo-4-phenyl-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate



^{1}H NMR (400 MHz, dmso) δ 9.16 (s, 1H), 7.71 (s, 1H), 7.28 (d, *J* = 6.6 Hz, 2H), 7.21 (d, *J* = 6.2 Hz, 3H), 5.11 (s, 1H), 4.03 – 3.85 (m, 2H), 2.22 (s, 3H), 1.06 (t, *J* = 6.8 Hz, 3H).

Figure 1: ^{1}H NMR Spectra of Ethyl 6-methyl-2-oxo-4-phenyl-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate

Interpretation of ^{1}H NMR: White solid, M.P=201-203°C. ^{1}H NMR (400 MHz, DMSO-D_6) δ: 9.16 (s, 1H), 7.71 (s, 1H), 7.28 (d, *J* = 6.6 Hz, 2H), 7.21 (d, *J* = 6.2 Hz, 3H), 5.11 (s, 1H), 4.03 – 3.85 (m, 2H), 2.22 (s, 3H), 1.06 (t, *J* = 6.8 Hz, 3H); IR (Cm^{-1}): 3228 (N-H), 1703 (C=O), 1216,1091 (C-O).

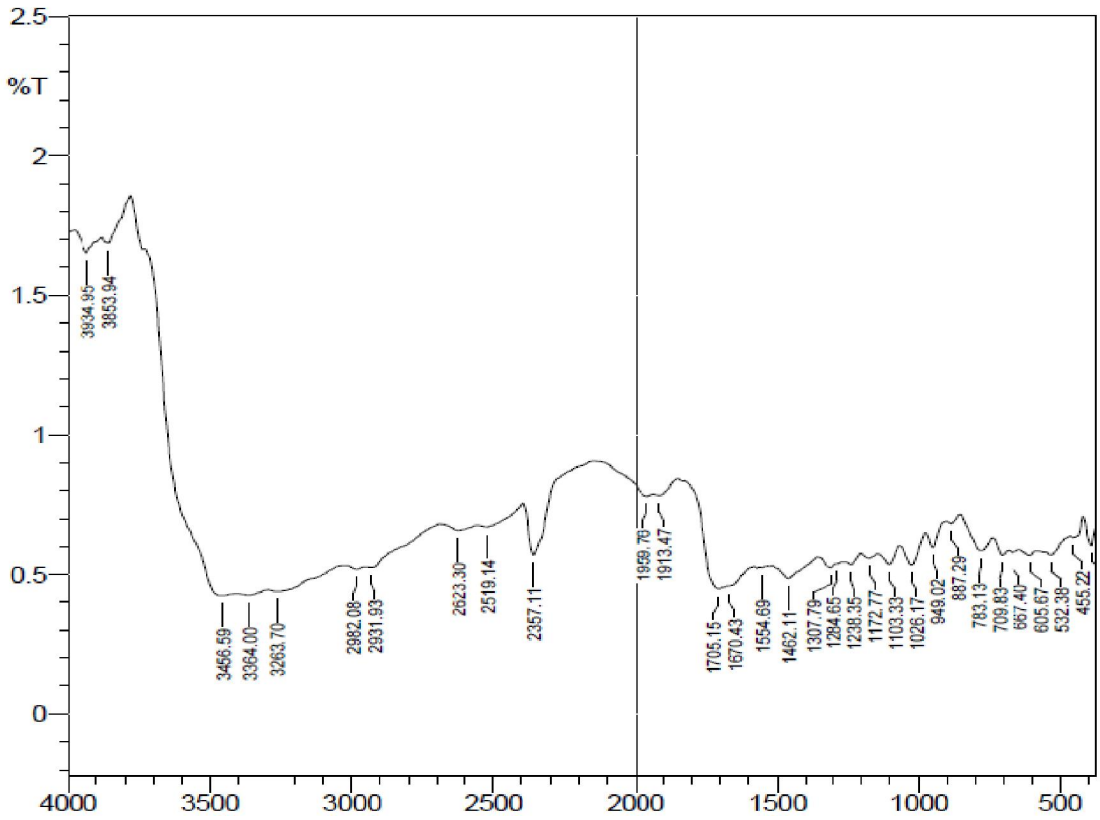


Figure 2: IR Spectra of Ethyl 6-methyl-2-oxo-4-phenyl-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate

Interpretation of IR: -NH stretch (3456.59 Cm^{-1}), H-C=C stretch (3364 Cm^{-1}), C-H stretch (3263.70 Cm^{-1}), $-CH_3$ stretch (2982.08 Cm^{-1}), C=O (Amide) stretch (1670.43 Cm^{-1}), C=C stretch (1462.11 Cm^{-1}), C=O stretch (1103.33 Cm^{-1}) –C-N stretch (1026.17 Cm^{-1}).

Results and Discussion

The performed reaction for the synthesis of 3,4-dihydropyrimidin-2(1H)-ones using lemon juice as a catalyst and ethanol as a solvent, gives good yield having electron donating and withdrawing group. The reaction was performed by stirring at 90°C which gives excellent yields of desired product in 5 Hour. The crude solid product obtained was purified by recrystalizing with ethanol.

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Conclusion

In conclusion a clean, Chromatography-free and ecological method has been developed for the synthesis of commercially very important DHPMs compounds. Utilization of naturally occuring lemon juice as an acid catalyst makes this protocol environmentally benign. Mild reaction condition, high yields of products, short reaction time are the keynote feaatures of this methods.

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EFFECTIVE REMOVAL OF Fe (II) IONS FROM AQUEOUS SOLUTION USING *ACASIA ARABICA* TREE BARK SUBSTRATE

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ABSTRACT

Intensive industrial and agricultural cultivate is the basic reason of enormous pollution of the environment with heavy metals. Such pollution is especially dangerous to open fresh water aquifer which is used in main as a source of drinking water. The removal of heavy metals from drinking water is a complicated task due to their low concentration and complexation of natural and organic matter. The present study aimed at effective arrangement and purification of industrial waste water using cheaper and locally available tree bark free removal of heavy metals of a substitute for conventional. The effect of tree bark ferrous ammonium sulphate on the metal content of industrial waste water was investigated in the pH range of 4 to 6. It is observed that, the process uptake followed first order adsorption rate expression and obeyed Langmuir Freundlich models of adsorption. Effect of variations in parameters such of pH, contact time and adsorption dosage.

Keywords: *Tree bark, Fe (II) metal ion solution, tree barksubstrate, spectrophotometer, pH meter*

Introduction

Heavy metals generally occur in water in low concentration of as a result of metal industries and partly through geological processes. But these causes direct toxicity to human and other living things due to their presence obeyed the specified limits [1].Heavy metals in wastewater have emerged focus of environmental remedies efforts of industrialization and urbanization with new technological advance metals.

The existing water resources are contaminated by discharging water containing organic color, heavy metal, etc. The groundwater contaminated, particularly by heavy metals from industrial effect and their persistence in food chain has been of major concern as it is poisoning a serious threat to aquatics culture including fisheries [3]. Hence the removal of toxic heavy metal contaminants from wastewater is one of the most important environmental and economic issue today. [4] The ever increasing demand for water of high quality has caused considerable attention to be focused towards recovery and reuse of wastewater. Particularly Fe (II) is one of the common elements on earth, making about 5 % of the earth crust. Iron is generally stored in the center of mettaloprotein, because free ions that bind nonspecifically to main cellular component can catalyze the production of toxic free radical. Excessive iron can be toxic because free ferrous ion reacts with peroxide to produce free radicals which are highly reactive and damage DNA, proteins, lipids and other cellular components. Iron uptake is tightly regulated by the human body which has no physiological; means of excreting iron so controlled iron level safely. The large amount of iron can cause excessive level of iron in blood because high level can damage the cell of gastrointestinal tract. High blood concentration of iron damages cell of heart and, liver, which can cause serious problem, including long term organ damage and even death.

Material and Methods

Preparation of Adsorbent: *Acacia arabica* tree bark substrate

Raw material used for preparation of adsorbent are collected from plant material. Dried cell of the plant Acacia arabica were collected and crush to small size in an electric grinder. The powder was shifted and 2 gram of powder were added to a mixture of 20 ml of 0.25 N H_2SO_4 AND 39% formaldehyde. It was kept in water bath at 50 ^{0}C for about 6 hr. Stir the powder was wash with distilled water for several times to remove sulphuric acid and used for removal of metal ions. The treatment of tree bark with acidic medium polymerize insolubilize in water. The bark substrate was prepared and used for further study.

For removal of metal from wastewater the general treatment method used adsorption is the best techniques which is broadly applied to remove metals. Adsorption is surface

phenomenon that may be define in the terms of unique operation.

Result and Discussion

The result and discussion are given in relevant paragraph for Fe (II) metal ions with the *Acasia arabica* tree bark substrate.

Batch Method:

1. Effect of pH:

1 gm of acacia Arabica bark substrate was agitated with 100 ml of Fe (II) solution for 1 hr at room temperature. The pH of metal solution was varied between 2 to 9, it is observed that the adsorption of Fe (II) ions gradually increases. The final pH found to be less than initial pH of the solution. The percentage removal of metal ions from the solution on the substrate is found to be 81.1% at pH 4.5. In all further investigation of Fe (II), the pH of the metal solution is maintained at 4, In order to prevent the possibility of metal oxide.

2. Contact Time/Agitated Time

100 ml of …… Fe (II) of metal solution is agitated with 1 gm of *Acasia arabica* tree bark substrate for different time interval varying from 5 min to 120 min. It is evident from the data that 80.42% of the metal ions removal from the solution occurred within 30 min showing that the metal ions adsorption on the substrate is very fast. The metal ions removal solution after contact time 30 min and value remain constant even after the contact time of 120 min. Hence approximately 1 hr contact time was fixed I further studies.

3. Adsorption dosage

100 ml of Fe (II) is adjusted with pH 4 and varying dosage of bark substrate from 0.5 gm to 4 gm, it is observed that the removal of metal ions increases with increasing dosage of bark substrate, however 1 gm is chosen for further studies for sake of convenience.

Using the data a Freundlich adsorption isotherm has been drawn BY PLOTTING LOG OF X/M Vs log CE, where X/M is concentration of Fe (II) (adsorbed per gm. of adsorbent) and CE is residual concentration of the metal ions.

4. Effect of Temperature

The metal ion solution were adsorbed on tree bark substrate at different temperature varying from 0 - 70°C in the step of 10°C temperature. It is evident that the Fe(II) ions removal decreases with increase in temperature. Hence all further studies were performed at 32°C (at room temperature).

Conclusion

For removal of metal ions from wastewater the general treatment method used, adsorption is the best technique, which is broadly applied to remove metal ions. Adsorption is a surface phenomenon that may be defined in terms of unit operation that utilizes surface forces. It is one of the most effective physical process for the removal of toxic metal ions from wastewater. It is based on the concept of partition of chemical species between a bulk phase and an interphase.

The retention capacity increases with contact time, pH and metal ion concentration but decreases with temperature. However, the present studies have been restricted to only such pH values, where the respective heavy metal ions do not get precipitate as hydroxide. Similarly, the effect of dosages of tree bark substrate also prove that the adsorption of heavy metal ions increases with increase in dosages.

Table 1 Effect of pH on adsorption of Fe(II)

Sr. No.	Initial pH	Final pH	Initial Conc. (ppm)	Final Conc. (ppm)	Cond. Adsorbed (ppm)	% Removal of Fe(II)
1	2.5	2.4	72.36	47.64	24.72	34.16
2	3.5	3.2	72.36	17.56	54.80	75.73
3	4.5	4.4	72.36	13.51	58.85	81.32
4	5.5	5.1	72.36	15.49	56.87	78.58
5	6.5	6.2	72.36	15.49	56.87	78.58
6	7.5	7.1	72.36	16.17	56.19	77.64
7	8.5	8.3	72.36	16.86	55.50	76.69
8	9.5	9.2	72.36	18.27	54.09	74.75

In each case 1 gm of substrate was agitated with 100 ml Fe(II) ion solution for 1 hr. at 30°C

Table 2 Effect of Contact time on adsorption of Fe(II)

Sr. No.	Time of Agitation (min)	Initial Conc. (ppm)	Final Conc. (ppm)	Cond. Adsorbed (ppm)	% Removal of Fe(II)
1	5	72.36	56.10	16.26	22.47
2	10	72.36	48.95	23.40	32.34
3	15	72.36	41.55	30.80	42.57
4	30	72.36	14.16	58.20	80.42
5	60	72.36	14.16	58.20	80.42
6	120	72.36	14.16	58.20	80.42
7	240	72.36	14.16	58.20	80.42

In each case 1 gm of substrate was agitated with 100 ml Fe(II) ion solution at pH 4.5

Table 3 Effect of Dosages of adsorbent on adsorption of Fe(II)

Sr. No.	Substrate Dosages (gm)	Initial Conc. (ppm)	Final Conc. (ppm)	Cond. Adsorbed (ppm)	% Removal of Fe(II)
1	0.5	72.36	19.71	52.64	72.75
2	1.0	72.36	14.16	58.20	80.42
3	1.5	72.36	13.51	58.85	81.32
4	2.0	72.36	12.86	59.49	82.21
5	2.5	72.36	11.60	60.75	83.96
6	3.0	72.36	9.77	62.59	86.49
7	3.5	72.36	8.00	64.36	88.94
8	4.0	72.36	6.85	65.50	90.52
9	4.5	72.36	3.57	68.79	95.06

In each case 100 ml Fe(II) ion solution at pH 4.5 was agitated for 30 min. at 30°C

Table 4 Freundlich Adsorption Isotherm for adsorption of Fe(II)

Sr. No.	Substrate Dosages (gm)	Initial Conc. (ppm)	x/m	log (x/m)	Equilibrium Conc. (C_e)	log C_e
1	1	72.36	58.20	1.76	14.16	1.15
2	1	76.84	58.57	1.76	18.27	1.26
3	1	81.77	61.31	1.78	20.45	1.31
4	1	87.25	63.71	1.80	23.53	1.37
5	1	90.23	65.08	1.81	25.15	1.40
6	1	93.41	66.59	1.82	26.82	1.42
7	1	96.81	68.26	1.83	28.55	1.45
8	1	100.41	70.11	1.84	30.34	1.48
9	1	104.40	71.24	1.85	33.15	1.52
10	1	108.68	72.54	1.86	36.14	1.55

Table 5 Effect of Temperature on adsorption of Fe(II)

Sr. No.	Temperature (°C)	Initial Conc. (ppm)	Final Conc. (ppm)	Cond. Adsorbed (ppm)	% Removal of Fe(II)
1	30	72.36	14.16	58.20	s
2	40	72.36	25.15	47.21	65.24
3	50	72.36	37.17	35.18	48.62
4	60	72.36	47.64	24.72	34.16
5	70	72.36	57.66	14.69	20.21

In each case 1 gm of substrate was agitated with 100 ml Fe(II) ion solution at pH 4.5 for 30 min.

Figures:

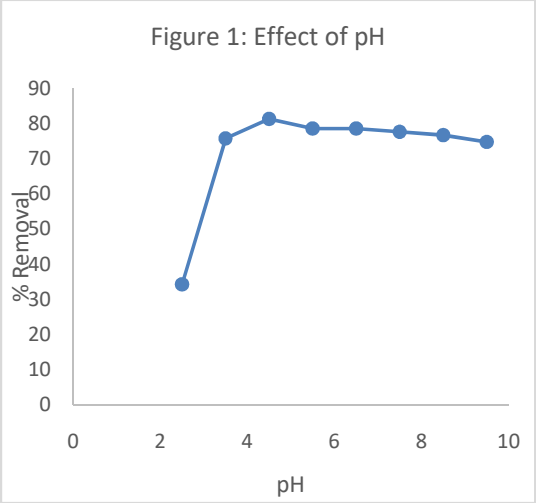
Figure 1: Effect of pH
% Removal
pH
0
10
20
30
40
50
60
70
80
90
0
2
4
6
8
10

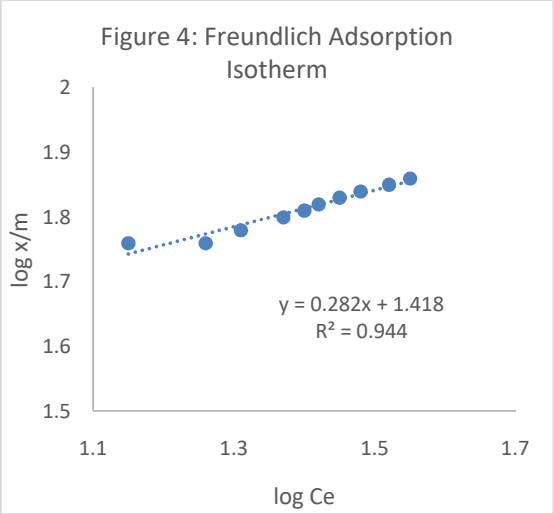
Figure 4: Freundlich Adsorption Isotherm
log x/m
log Ce
y = 0.282x + 1.418
R² = 0.944
1.5
1.6
1.7
1.8
1.9
2
1.1
1.3
1.5
1.7

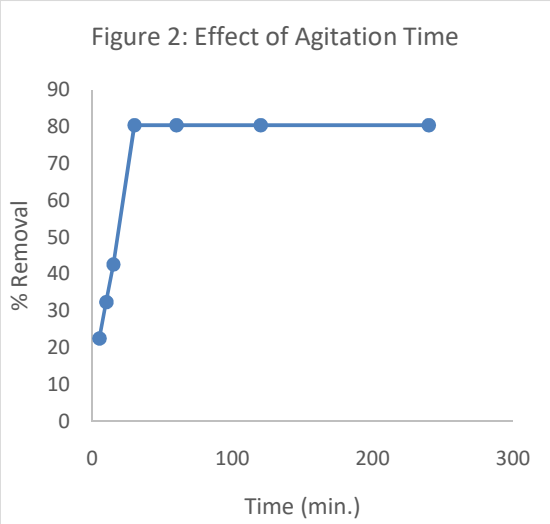
Figure 2: Effect of Agitation Time
% Removal
Time (min.)
0
10
20
30
40
50
60
70
80
90
0
100
200
300

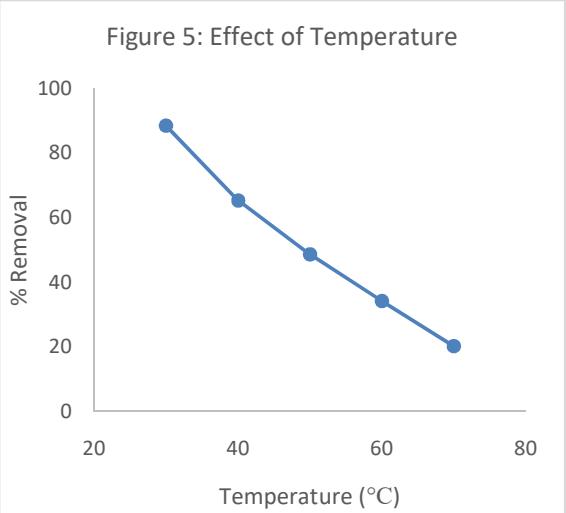
Figure 5: Effect of Temperature
% Removal
Temperature (°C)
0
20
40
60
80
100
20
40
60
80

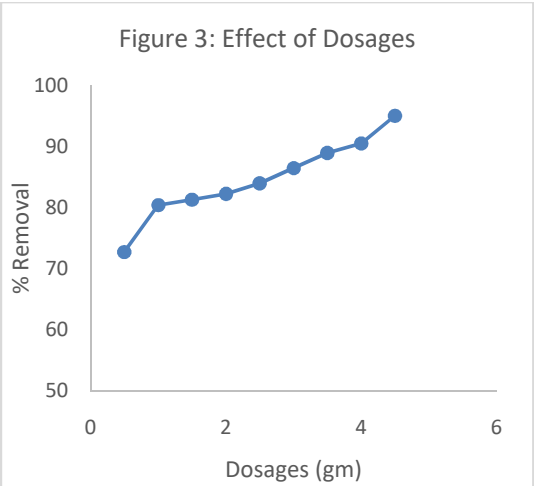
Figure 3: Effect of Dosages
% Removal
Dosages (gm)
50
60
70
80
90
100
0
2
4
6

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DETERMINATION OF ASCORBIC ACID BY SPECTROPHOTOMETRIC AND TITRIMETRIC METHOD

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ABSTRACT

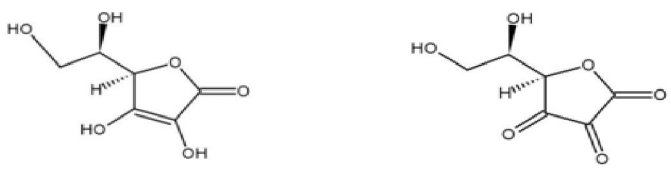
Among all vitamins, vitamin C plays a crucial role in maintaining good health. In present study five types of fruits were analysed to compare ascorbic acid level in different fruits. The fruits were collected from local market of Amravati. The ascorbic acid content of some fruits namely ripen lemon, unripen lemon, orange, ripen guava and unripen guava was determined by Spectrophotometric and titrimetric method using Potassium permanganate as a chromogenic agent. The absorbance was measured at 340 nm. Titrimetric method was carried out by iodimetric blank titration. The amount of Ascorbic acid present in Ripen Lemon, Unripen Lemon, Orange, Ripen Guava and Unripen Guava by Spectrophotometric method is found to be higher as compare to Trimetric Method. Spectrophotometric method of determination of Ascorbic acid is found to be simple.

Keywords - *Ascorbic Acid, Spectrophotometric method, Trimetric method, Ripen Lemon, Unripen Lemon, Orange, Ripen Guava and Unripen Guava*

Introduction

Vitamins helps the human to maintain a healthy diet. Vitamin C is known as ascorbic acid. It is important antioxidant for human body. It is soluble in water. [1] Ascorbic acid occurs in living tissues, fruits, vegetables, meat, etc. It is used in processed foodstuffs as an antioxidant. [2] Ascorbic acid helps in biosynthesis of collagen and protein metabolism. It is also required for neurotransmitters. It is also important for immune functions. [3] Low level of vitamin C in body causes scurvy. Vitamin improves absorption of inorganic iron, reduction of plasma cholesterol and reduce cardiovascular diseases and some form of cancer. [4] Vitamin C converts the inactivate form of folic acid to active form i.e. folonic acid which helps in calcium metabolism. [5] It is used in treatment of some diseases such as scurvy, anemia, hemorrhagic disorder, wound healing, infertility, etc. [6] Vitamin C is present in fruits such as lemon, orange, guava, grapefruit, watermelon, etc. and vegetables. [7]

The determination of ascorbic acid is important. For this there are numerous analytical methods are developed. These method include Titrimetric method, Spectrophotometric method [8-9], Chromatography, voltmeter, fluorometry, potentiometry [10], electrochemical method, polarographic method [11], HPLC [12]. Similarly, liquid chromatography [13], capillary electrophoresis [14] and gas Chromatography [15] were also used for determination of vitamin C. Some aspects of Polarographic wave of Ascorbic Acid and its determination from some synthetic and medicinal samples by Calibration and Standard Addition method was done. [16-19]



Ascorbic acid
(Reduced form) **(Oxidized form)**

In present study five types of fruits were analysed to compare ascorbic acid level in different fruits. The fruits were collected from local market of Amravati. The ascorbic acid content of some fruits namely ripen lemon, unripen lemon, orange, ripen guava and unripen guava was determined by Spectrophotometric and titrimetric method

using Potassium permanganate as a chromogenic agent. The absorbance was measured at 340 nm. Titrimetric method was carried out by iodimetric blank titration. The amount of Ascorbic acid present in Ripen Lemon, Unripen Lemon, Orange, Ripen Guava and Unripen Guava by Spectrophotometric method is found to be higher as compare to Trimetric Method. Spectrophotometric method of determination of Ascorbic acid is found to be simple.

Methodology

1. Determination of Ascorbic Acid by Spectrophotometric Method

0.1 N ascorbic acid solution is prepared by dissolving 1.7613 g ascorbic acid in 100 ml distilled water.0.1 N Potassium permanganate solution is prepared by dissolving 0.3160 g of Potassium permanganate in 100 ml distilled water. Spectrophotometer 166 is used for Determination of Ascorbic Acid by Spectrophotometric Method.

Preparation of Fruit juice sample: Ripen lemon, Unripen lemon, Orange and Ripen guava, Unripen guava Fruits are weighed and washed thoroughly and there Juice is prepared by using blender.Then volume of juice was made upto 100 ml with distilled water.

Solution of standard ascorbic acid 1ml, 2ml, 3ml, 3ml, 4ml, 5ml, 6ml is taken in six separate test tubes followed by addition of 1ml of KMnO4 in each test tube. Then distilled water of volume 8ml, 7ml, 6ml, 5ml, 4ml, 3ml is added in each test tube respectively. Then Test tubes are shaken well and allowed to stand for 5min. In another test tube 9ml juice sample is taken followed by addition of 1ml KMnO4 solution. Tubes are shaken well and allowed to stand for 5min.

Table 1: preparation of Standard Ascorbic Acid Solution

Volume of Ascorbic acid	Volume of KMnO4	Volume of Distilled water
1ml	1ml	8ml
2ml	1ml	7ml
3ml	1ml	6ml
4ml	1ml	5ml
5ml	1ml	4ml
6ml	1ml	3ml

Table 2: Preparation of Fruit juice sample

Fruits	Volume of juice sample	Volume of KMnO4
Ripen lemon	9ml	1ml
Unripen lemon	9ml	1ml
Orange	9ml	1ml
Ripen guava	9ml	1ml
Unripen guava	9ml	1ml

2. Determination of Ascorbic Acid by Titrimetric Method

0.1N Sodium thiosulfate, 0.1 N Iodine solution, 0.1 N Ascorbic acid, Starch solution is prepared. Preparation of 0.1 N Ascorbic acid solution is done by dissolving 1.7613 g Ascorbic acid in 100 ml distilled water. Preparation of 0.1 N Sodium thiosulfate solution is doneby dissolving 1.5811 g of sodium thiosulfate in 100 ml distilled water.

Preparation of Fruit juice sample: Unripen and fully ripen lemon, orange and green guava is collected from local market of Amravati during winter season. Fruits are weighed and washed thoroughly. Juice is prepared by using blender.Then volume of Ripen lemon, Unripen lemon, Orange, Ripen guava, Unripen guava juice was made upto 100 ml with distilled water.

Titration of Ascorbic acid solution with 0.1 N Sodium Thiosulfate: 10 ml 0.1 N ascorbic acid is pipetted out in conical flask followed by addition of 10 ml Iodine solution and 1-2 ml of Starch solution and shaken well. Then titrated with 0.1 N Na2S2O3 solution until colour changes from blue to colourless. This procedure is repeated for 3 times to obtain constant readings and end point was noted.

Blank Titration: 10 ml Iodine solution is pipette out in conical flask and followed by addition of 1ml starch solution.Then titrated with Sodium Thiosulfate solution until colour changes from blue to colourless. This procedure is repeated for 3 times to obtain constant readings and end point was noted.

Titration of fruit juice with 0.1 N Sodium Thiosulfate solution: 10 ml fruit juice is taken in 100 ml conical flask followed by addition of 10ml Iodine solution and 1ml of Starch solution. This mixture is titrated with 0.1 N Sodium Thiosulfate solution until colour changes from blue to colourless. This procedure is repeated for 3 times to obtain

constant readings. End point is noted.This procedure is performed for each juice sample.

Observation

1. Determination of Ascorbic Acid by Spectrophotometric Method

The absorbance of system no.1 at different wavelength is measured. Highest absorbance was obtained at wavelength 340 nm. Absorbance of remaining 5 systems is measured at wavelength 340 nm against water as a blank by Spectrophotometer.

Volume of Standard Ascorbic Acid Solution	Molar Concentration	Absorbance at 340 nm
1	0.01	0.820
2	0.02	0.940
3	0.03	1.036
4	0.04	1.124
5	0.05	1.266
6	0.06	1.367

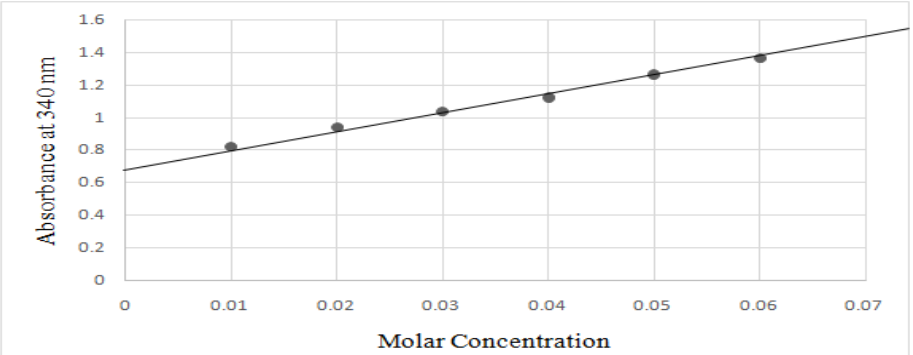


Figure 1. Calibration Graph for Determination of Ascorbic Acid by Spectrophotometric Method

The absorbance of all juice sample is measured at wavelength 340 nm against water as blank by Spectrophotometer.

Juices sample	Absorbance at 340 nm	Molar concentration (From graph)
Ripen lemon	1.671	0.0875
Unripen lemon	1.167	0.037
Orange	1.172	0.039
Ripen guava	1.321	0.0315
Unripen guava	1.321	0.0315

2. Determination of Ascorbic Acid by Trimetric Method

Titration of standard solution of ascorbic acid with 0.1N $Na_2S_2O_3$

Sr. No.	Volume of ascorbic acid	Volume of Iodine solution	Volume of $Na_2S_2O_3$	Mean (V1)
1.	10 ml	10 ml	12 ml	12 ml
2.	10 ml	10 ml	12 ml	
3.	10 ml	10 ml	12 ml	

Blank titration

Sr. No.	Volume of ascorbic acid	Volume of Na2S2O3	Mean (V2)
1.	10 ml	15 ml	15 ml
2.	10 ml	15 ml	

Titration of fruit juice sample with Na2S2O3 solution

Fruit juice sample	Volume of Na2S2O3 for titration			Mean volume
Ripen lemon	14.5ml	14ml	14ml	14.2
Unripen lemon	14ml	14ml	14ml	14.0
Orange	14ml	13.5ml	14ml	13.8
Ripen guava	13ml	13.5ml	13ml	13.3
Unripen guava	14.5ml	14.5ml	14.5ml	14.5

CALCULATIONS

1. Determination of Ascorbic Acid by Spectrophotometric Method

Normality of Ascorbic acid $= \frac{wt.perlit.}{molecularwt} = \frac{17.6}{176.13} = 0.1$ N

Molecular concentration of ascorbic acid present in each system

M1V1 = M2V2 0.1×1 = M2 × 10 M2 = 0.01
M1V1 = M2V2 0.1× 2 = M2 × 10 M2 = 0.02
M1V1 = M2V2 0.1× 3 = M2 × 10 M2 = 0.03
M1V1 = M2V2 0.1 × 4 = M2 × 10 M2 = 0.04
M1V1 = M2V2 0.1 × 5 = M2 × 10 M2 = 0.05
M1V1 = M2V2 0.1 × 6 = M2 × 10 M2 = 0.06

Amount of ascorbic acid present in fruit juice sample

Ripen lemon: Molar concentration from graph = 0.0875

Molarity = $\frac{wt.perlit.}{molecularwt}$; 0.0875 = $\frac{wt0.05.perlit.}{176.13}$; wt /lit. = 15.411375 g;

Wt /10 ml =0.1541 g ascorbic acid
9ml juice = 0.1541 g ascorbic acid; 100ml juice = $\frac{0.1541}{9} \times 100$ = 1.7122 g ascorbic acid
Hence amount of Ascorbic acid present in one fruit weighing .....g = 1.7122 g ascorbic acid

Unripen lemon: Molar concentration from graph = 0.037

Molarity = $\frac{wt.perlit.}{molecularwt}$; 0.037 = $\frac{wt.perlit.}{176.13}$; Wt./lit =6.51681 g;

wt./10 ml = $\frac{6.5168\ 1}{1000} \times 10$ = 0.0651 g ascorbic acid
9Ml juice = 0.0651 g ascorbic acid; 100ml = $\frac{0.0651}{9} \times 100$ = 0.7233 g ascorbic acid
Hence amount of Ascorbic acid present in one fruit weighing .....g = 0.7233 g ascorbic acid

Orange: Molar concentration from graph = 0.039

Molarity = $\frac{wt.perlit.}{molecularwt}$; 0.039 = $\frac{wt.perlit.}{176.13}$; Wt./lit = 6.86907 g

wt./10 ml = $\frac{6.8\ 6907}{1000} \times 10$ = 0.0686 g ascorbic acid
9ml = 0.0686 g ascorbic acid; 100 ml = $\frac{0.08\ 6}{9} \times 100$ = 0.7622 g ascorbic acid
Hence amount of Ascorbic acid present in one fruit weighing .....g = 0.7622 g ascorbic acid

Ripen guava: Molar concentration from graph =0.0315

Molarity = $\frac{wt.perlit.}{molecularwt}$; 0.0315= $\frac{wt.perlit.}{176.13}$; Wt./lit = 5.548095 g

wt./10 ml = $\frac{5.548\ 095}{1000} \times 10$ = 0.0554 g ascorbic acid

9ml = 0.0554 g ascorbic acid; 100 ml = $\frac{0.0554}{9} \times 100$= 0.6155 g ascorbic acid
Hence amount of Ascorbic acid present in one fruit weighing .....g = 0.6155 g ascorbic acid

Unripen guava: Molar concentration from graph =0.0315

Molarity = $\frac{wt.perlit.}{molecularwt}$; 0.0315= $\frac{wt.perlit.}{176.13}$; Wt./lit = 5.548095 g

wt./10 ml = $\frac{5.548\ 095}{1000} \times 10$ = 0.0554 g ascorbic acid

9ml = 0.0554 g ascorbic acid; 100 ml = $\frac{0.0554}{9} \times 100$= 0.6155 g ascorbic acid
Hence amount of Ascorbic acid present in one fruit weighing .....g = 0.6155 g ascorbic acid

2. Determination of Ascorbic Acid by Trimetric Method

Ascorbic acid:
Volume of Na2S2O3 required for titration of ascorbic acid (V1) = 12 ml
Volume of Na2S2O3 required for blank titration of ascorbic acid (V2) =15 ml
1ml of 0.1N Na2S2O3 ~ 0.0088 g of Ascorbic Acid
(V2 - V1)ml of 0.1N Na2S2O3 = 15-12 = 3ml
Amount of ascorbic acid in 10ml standard solution = 0.0088 × (V2-V1) = 0.0088 × 3 = 0.0264 g
Amount of ascorbic acid in 100ml = 0.264 g

Blank titration:
Volume of Na2S2O3 required for blank titration(V2) = 15ml

Amount of ascorbic acid present in fruit juice sample

Ripen lemon
Volume of Na2S2O3 required for titration of ripen lemon (V1) = 14.16 ml
Volume of Na2S2O3 required for blank titration of ascorbic acid (V2) = 15 ml
(V2 - V1)ml of 0.1N Na2S2O3= 15-14.16 = 0.84ml
Amount of ascorbic acid in 10ml standard solution = 0.0088 × (V2-V1) = 0.0088 × 0.84 = 0.007392 g
Amount of ascorbic acid in 100ml = 0.07392 g
Hence amount of Ascorbic acid present in one fruit weighing .....g = 0.07392 g ascorbic acid

Unripen lemon
Volume of Na2S2O3 required for titration of unripen lemon (V1) = 14 ml
Volume of Na2S2O3 required for blank titration of ascorbic acid (V2) = 15 ml
(V2 - V1)ml of 0.1N Na2S2O3 = 15-14 = 1ml
Amount of ascorbic acid in 10ml standard solution = 0.0088 × (V2-V1) = 0.0088 × 1 = 0.0088 g
Amount of ascorbic acid in 100ml = 0.088 g
Hence amount of Ascorbic acid present in one fruit weighing .....g = 0.088 g ascorbic acid

Orange
Volume of Na2S2O3 required for titration of orange (V1) = 13.83 ml
Volume of Na2S2O3 required for blank titration of ascorbic acid (V2) = 15 ml
(V2 - V1)ml of 0.1N Na2S2O3= 15-13.83 = 1.17 ml
Amount of ascorbic acid in 10ml standard solution = 0.0088 × (V2-V1) = 0.0088 × 1.17 = 0.01030 g
Amount of ascorbic acid in 100ml = 0.1030 g
Hence amount of Ascorbic acid present in one fruit weighing .....g = 0.1030 g ascorbic acid

Ripen guava
Volume of Na2S2O3 required for titration of ripen guava (V1) = 13.33 ml
Volume of Na2S2O3 required for blank titration of ascorbic acid (V2) = 15 ml
(V2 - V1)ml of 0.1N Na2S2O3= 15-13.33 = 1.67 ml
Amount of ascorbic acid in 10ml standard solution = 0.0088 × (V2-V1) = 0.0088 × 1.67 = 0.014696 g
Amount of ascorbic acid in 100ml = 0.1470 g
Hence amount of Ascorbic acid present in one fruit weighing .....g = 0.1470 g ascorbic acid

Unripen guava
Volume of Na2S2O3 required for titration of unripen guava (V1) = 14.5 ml
Volume of Na2S2O3 required for blank titration of ascorbic acid (V2) = 15 ml
(V2 - V1)ml of 0.1N Na2S2O3= 15-14.5 =0.5 ml
Amount of ascorbic acid in 10ml standard solution = 0.0088 × (V2-V1) = 0.0088 × 0.5 = 0.0044 g
Amount of ascorbic acid in 100ml = 0.044 g
Hence amount of Ascorbic acid present in one fruit weighing .....g = 0.044 g ascorbic acid

RESULTS AND DISCUSSION

The amount of Ascorbic acid present in different sample by Spectrophotometric and Trimetric Method were found to be as follows:

Sample	Weight of Fruit	Amount of Ascorbic acid present in per Fruit	
		Spectrophotometric Method	Titrimetric Method
Ripen Lemon		1.7122 g	0.0739 g
Unripen Lemon		0.7233 g	0.0880 g
Orange		0.7622 g	0.1030 g
Ripen Guava		0.6155 g	0.1470 g
Unripen Guava		0.6155 g	0.0440 g

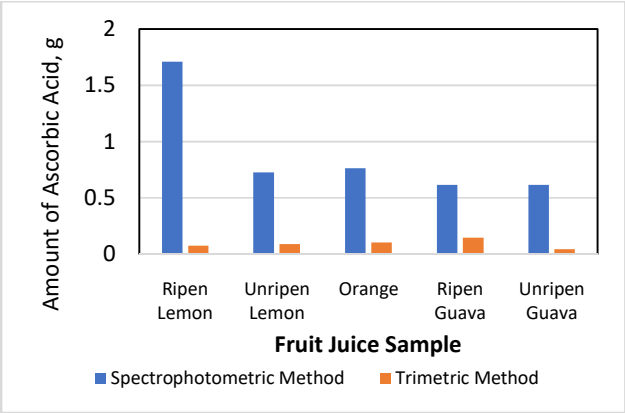


Figure 2. Amount of Ascorbic acid present in different sample by Spectrophotometric and Trimetric Method

CONCLUSION

The amount of Ascorbic acid present in Ripen Lemon, Unripen Lemon, Orange, Ripen Guava and Unripen Guava by Spectrophotometric method is found to be higher as compare to Trimetric Method. Spectrophotometric method of determination of Ascorbic acid is found to be simple.

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CATALYST - FREE SYNTHESIS OF MONO - SUBSTITUTED UREA FROM ANILINE USING ETHANOL AS A SOLVENT

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ABSTRACT

Mono-substituted urea is important class of natural products. They act as enzyme inhibitor, possess neuro protective activities, anti diabetic and herbicidal properties. It is also used for protection of amino group in peptide chemistry.
In present study, a simple catalyst-free method for synthesis of Mono-substituted urea from aniline and urea in ethanol as a solvent has been described. Short reaction time, good excellent yield of product and most important catalyst as well as Chromatography-free approach are the major advantages of this study. All the synthesized products are well characterized by ^1H NMR and FT-IR spectroscopic techniques.

Keywords: *Aniline, Catalyst-free reaction, Mono-substituted urea, Phenyl urea, Urea*

Introduction

Urea and its derivatives are important class of molecules. They have number of chemical and biological properties and hence used in chemicals, pharmaceutical and agrochemical industries. N-Substitutedureas are important class of urea derivatives. They are used in various important chemicals of high commercial interest. [1] Mono-substituted urea is important class of natural products. They act as enzyme inhibitor, possess neuro protective activities, antidiabetic and herbicidal properties. It is also used for protection of amino group in peptide chemistry. [2] Numerous method have been developed for synthesis of mono-substituted urea. Commercially ureas are prepared by using phosgenation of primary amine in which highly toxic phosgenwas used. [3] Mono-substituted ureas are also prepared from amines in organic and aqueous solvent by using 4-nitrophenyl-N-benzyl carbonate followed by hydrolysis gives corresponding ureas. [4] In 1958, Neville explain that silicone tetraisocynate and alone isocynateor in benzene solution with allylamine, diallylamine, aniline, benzylamine and o- and p-toulidiene to fine corresponding N-monosubstituted urea. [5] Mono substituted urea is also prepared by carboplatin of aliphatic amine with S,S-Dimethyl Dithiicarbonate. [6] Other method include the microwave-assisted reaction of sodium or potassium cynate with amine in presence of HCL [7], the reaction of alkyl- and arylamine with chlorocarbonylsulfenyl chloride [8], The conversion of thiourea into corresponding urea derivatives using bismuth (ll) nitrate pentahydrate. [9] Various Mono substituted ureas were synthesized by using Superparamagnetic Fe_3O_4 nanoparticles. [10]
Out of these all method some methods show some drawbacks such as expensive and hazardous chemicals, low yield, very high temperature, number of steps, long reaction time. In present study, a simple catalyst-free method for synthesis of Mono-substituted urea from aniline and urea in ethanol as a solvent has been described.

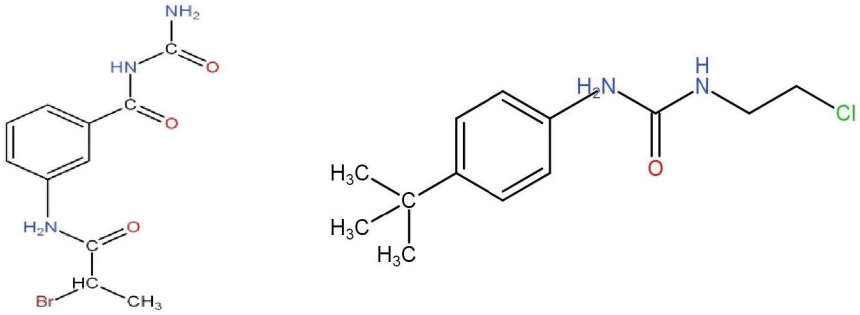
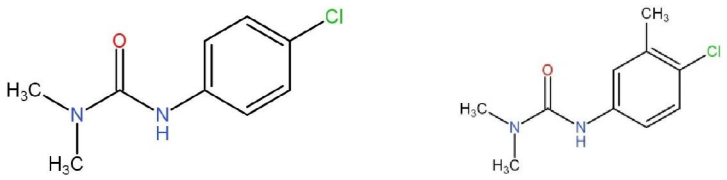


Figure 1: Some biologically important phenyl urea.



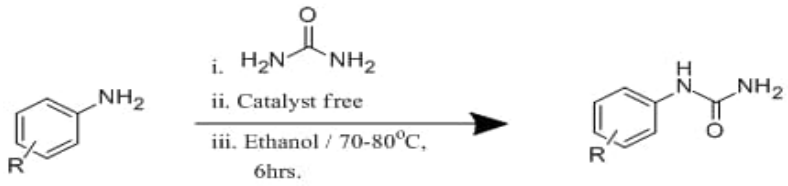
Monuron **Diuron**

Figure 2: Some phenylurea used in Herbicides

Methodilogy

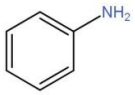
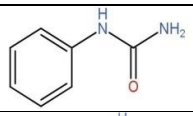
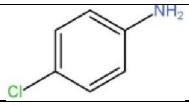
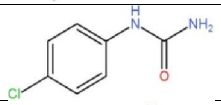
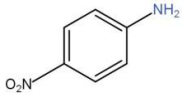
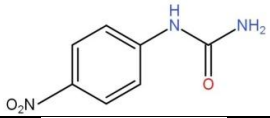
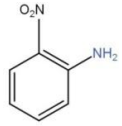
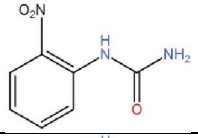
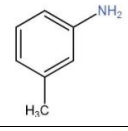
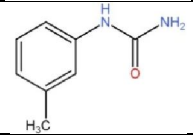
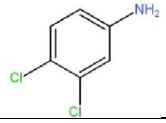
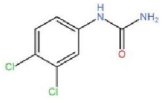
A mixture of aniline(1mol), urea(1mol) and ethanol taken in round bottom flask and heated at 80°C under reflux conditions for 6 hours. The progress of reaction was observed by Thin Layer Chromatography (TLC). After completion of reaction, product was kept to evaporate excess of ethanol to obtain crude solid product and purified by recrystallization.

The structure of products were confirmed by FT-IR, 1H NMR spectral data and by comparison with literature data.



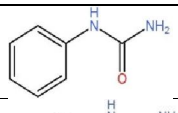
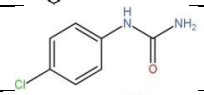
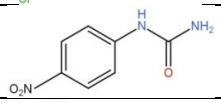
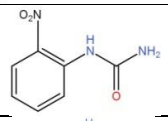
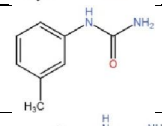
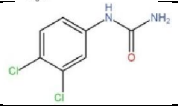
Scheme 1

Table 1: Preparation of phenylurea under catalyst free condition at 80°C

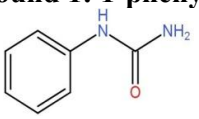
Sr. No.	RNH2	Product	Yield (%)	Time
1.			60.23%	6 hrs.
2.			81.18%	6 hrs.
3.			77.98%	6 hrs.
4.			77.7%	6 hrs.
5.			72.34 %	6 hrs.
6.			79%	6 hrs.

Reaction condition: aniline (1mol), urea (1mol), ethanol (2-3ml), 80°C

Analysis of Compounds

Sr. no.	Structure	Nature	Colour	Melting point
1.		Solid	Off White	210°C
2.		Solid	Brown	260°C
3.		Solid	Yellowish Brown	300°C
4.		Solid	Yellow	250°C
5.		Solid	Greenish	142°C
6.		Solid	Faint Brown	155°C

I R Spectral Analysis
Compound 1: 1-phenyl urea



1-phenyl urea

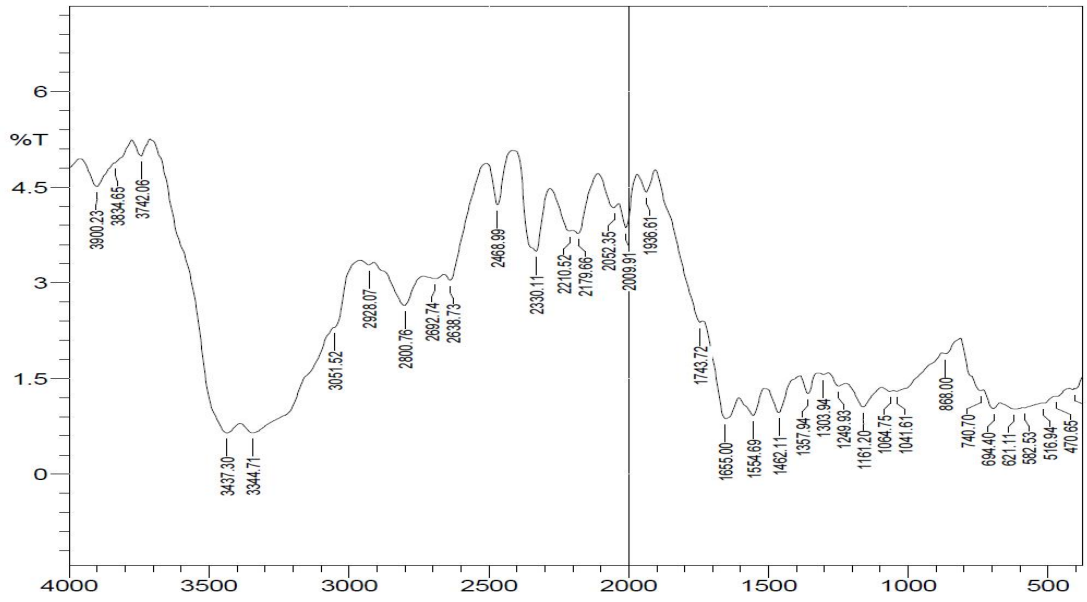
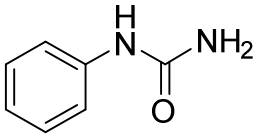


Figure 3: IR spectra of 1-phenyl urea

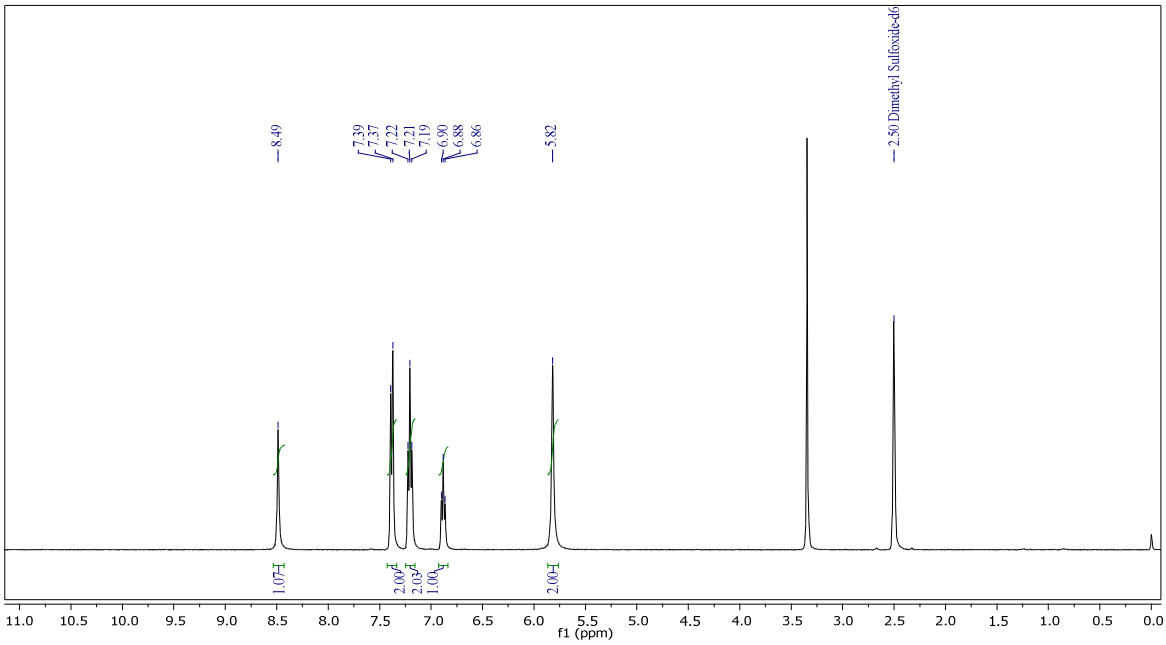
N-H stretch (3437.30 cm-1), Ar-H (3051.52 cm-1), C=O (Amide) (1655.00 cm-1), C=C (Aromatic) (1554.69 cm-1), Monosubstituted aromatic (5 adjacent H) (694.40 cm-1)

^{1}H NMR Spectral Analysis



1-Phenylurea

White solid. M.P=143-145°C. ^{1}H NMR (400 MHz, DMSO-D_6) δ: 8.49 (s, 1H), 7.38 (d, *J* = 8.4 Hz, 2H), 7.21 (t, *J* = 7.3 Hz, 2H), 6.88 (t, *J* = 6.9 Hz, 1H), 5.82 (s, 2H). ^{13}C NMR (126 MHz, DMSO-D_6) δ 156.48 (s), 141.03 (s), 129.09 (s), 121.53 (s), 118.20 (s).



^{1}H NMR (400 MHz, DMSO-D 6) δ 8.49 (s, 1H), 7.38 (d,*J* = 8.4 Hz, 2H), 7.21 (t,*J* = 7.3 Hz, 2H), 6.88 (t,*J* = 6.9 Hz, 1H), 5.82 (s, 2H).

Results and Discussion

Interpretation of IR Spectra

Spectral Region, Wave number, cm^{-1}	Bond Causing absorption
3437.30	N-H stretch
3051.52	Ar-H
1655.00	C=O (Amide)
1554.69	C=C (Aromatic)
694.40	Monosubstituted aromatic (5 adjacent H)

Interpretation of ^{1}H NMR Spectra

White solid. M.P=143-145°C. ^{1}H NMR (400 MHz, DMSO-D_6) δ: 8.49 (s, 1H), 7.38 (d, *J* = 8.4 Hz, 2H), 7.21 (t, *J* = 7.3 Hz, 2H), 6.88 (t, *J* = 6.9 Hz, 1H), 5.82 (s, 2H). ^{13}C NMR (126 MHz, DMSO-D_6) δ 156.48 (s), 141.03 (s), 129.09 (s), 121.53 (s), 118.20 (s).

Experimental results shows that electron donating group gives better yield as compare to electron withdrawing group. Among all anilines 4-Chloro aniline gives best yield i.e. 81.18 %.

Conclusion

In conclusion, a simple method is described for synthesis of Mono-substituted urea from aniline and urea in ethanol. The major advantages of this protocol is that it is catalyst and Chromatography-free, short reaction time and excellent yield. All the synthesized products are well purified by recrystallization method which provided good yield and purity.

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OZONE LAYER DEPLETION

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ABSTRACT

Widespread use of chlorofluorocarbons has led to about 3% ozone depletion by the end of the 80's. This fact has resulted in sharp decline in subsequent chlorofluorocarbon use and manufacture in conformation to Montreal Protocol. Turco and Whitten have reported dynamic solution to establish quantitative effect of chlorofluorocarbons. A one dimensional time dependant model was used and results were arrived at considering the prevalent increase of chlorofluorocarbon production and emission at that time. Miller et al described a far more detailed model and presented results for a wide variety of species with time and altitude. Taking cognizance of this fact, the ozone layer stability and the projected ultimate equilibrium condition has been studied in this paper.

Keywords : *ozone layer, irreversible thermodynamics*

Introduction

The steady state solution of the system outlined the fact that the ozone layer can reach a steady state for any pollutant concentration values. The natural limit of the ozone concentration, as well as the upper limits for the pollutant concentration was calculated. The procedure suggested a way of assessing the effect of anthropogenic activities on ozone layer. The transmission coefficients of the UV radiation were calculated establishing the lower limits of the "permitted" pollution. Ozone layer stability was studied in the framework of irreversible thermodynamics. The following 16 chemical reactions describing the ozone creation and destruction processes in the stratosphere were considered.

$O_2 + h\nu \rightarrow 2O$
$O + O_2 + M \rightarrow O_3 + M$
$O_3 + h\nu \rightarrow O_2 + O^*$,
$O_3 + O^* \rightarrow O_2$,
$HO + O_3 \rightarrow HO_2 + O_2$
$HO_2 + O \rightarrow OH + O_2$
$NO_2 + h\nu \rightarrow NO + O$
$NO + O_3 \rightarrow NO_2 + O_2$
$NO_2 + O \rightarrow NO + O_2$
$Cl + O_3 \rightarrow ClO + O_2$
$ClO + O \rightarrow Cl + O_2$
$ClO + NO \rightarrow NO_2 + Cl$
$HO_2 + Cl \rightarrow HCl + O_2$
$HCl + OH \rightarrow H_2O + Cl$
$CF_2Cl_2 + h\nu \rightarrow CF_2Cl + Cl$
$CFCl_3 + h\nu \rightarrow CFCl_2 + Cl$

In the above, M stands for a catalyst.

Species continuity equation was applied for 11 relevant species taking in to account vertical motion through eddy diffusion term.This gave set of 11 partial and coupled differential equations dependent on time and altitude. For various species, the basic species continuity equation is given as

$$\frac{dXi}{dt} = Ji\,Ci + \sum_{n=1}^{l} kn\,\pi\,C^{v}_{m}\,m$$

Where n any single reaction
i stoichiometric coefficient
m number of species in nth reaction
l number of reaction

The eleven species considered were $O, O_3, HO, Cl, ClO, NO, NO_2, HO_2$, F-11, F-12, HCl. The model of ozone layer lead to a system of non- linear differential equations. To solve these equations, numerical discretization method was employed using semi-implicit finite difference schemes. The simultaneous equations thus obtained were solved using matrix inversion technique for a tridigonal matrix wherein the MxM matrix is transformed to Mx_3 matrix reducing the computer storage memory and time. Steady state solution of the system indicated that ozone layer stability is independent of pollutant concentration viz Freon-11 and Freon-12 and is instead strongly dependent on the relation between HO_2 and NO_2 concentration in the stratosphere. Hypothetical fluctuations appearing in the system were also studied to get a better measure of the stability of the system. Real and

negative eigen values were obtained indicating good damping characteristics. Natural limits of pollutants have been calculated knowing ozone lifetime.Thereafter, putting a condition on change in UV transmission coefficient, which is consonant with the risk of skin cancer, the upper limits of pollutants were also obtained which indicated that current concentrations of Freons were well within the permitted range. Dynamic simulation has been carried out which gave predicted future concentrations of all species with altitude. Despite halt in production of CFCs, the concentration does not show much decline. Consequently, because of the photochemical dissociation of the molecules already in the stratosphere, free chlorine concentration keeps on increasing. However, the increasing free chlorine seems to take the HCl route rather than the depletion causing ClO route.

Conclusion

In this paper the ozone layer stability and ultimate equilibrium condition has been studied. Analysis in the form of ClO concentration, total column ozone depletion, $CFCl_3$ concentration has been studied.

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PHOTOCATAYTIC DEGRADATION OF METHYL ORANGE USING NANO FE_2O_3 AND CO DOPED FE_2O_3 OF DIFFERENT COMPOSITIONS

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ABSTRACT

Fe_2O_3 and Co doped Fe_2O_3 were synthesized by sol gel method. Structure and morphology of synthesized Co doped Fe_2O_3 nanocatalyst was investigated using Field emission scanning election microscopy, Electron dispersive X-ray spectroscopy and X-ray diffraction. The photocatalytic activity of Co doped Fe_2O_3 nanocatalyst was investigated for degradation of Methyl orange solution under visible light radiation. The effects of various experimental parameters such as the methyl orange concentration, catalyst dose, pH on the photocatalytic degradation were investigated. Among the different amounts of dopant that like 2, 5, and 10 wt. % Co-doped Fe_2O_3 nanocatalyst. It was observed that 10wt % Co doped Fe_2O_3 shows highest degradation with visible light radiation for methyl orange than pure Fe_2O_3 nanocatalyst. The particle size, morphology and separation of photo induced electron–hole pair are the main factors which influence photocatalytic activity and degradation extent.

Keywords: Methyl Orange, Fe_2O_3 and Co doped Fe_2O_3, photocatalysis

Introduction

Polluted waste water plays significant role in environmental pollution. Industrial effluents contain different chemicals especially synthetic dyes which are carcinogenic in nature1-3. Some dyes decompose aerobically and anaerobically resulting in the formation of carcinogenic compounds. In addition the coloured pollutants decrease light penetration & prevent photosynthesis [1]. This is the adverse consequences of the modern industrialization an environmental pollution. It is extremely necessary to seek efficient and more importantly green technologies to remove these pollutants [2]. Many techniques such as adsorption, electrochemical oxidation, ozonation, membrane filtration and biological treatment have been widely used to remove the dye components from polluted wastewater. These methods generally suffer from disadvantages including complicated processes, need for special operating conditions, high equipment cost, energy intensive time consuming operations, finite versatility and lower adaptability to the extensive range of dye waste waters [3]. The fast moving developments in the field of nanotechnology have stimulated considerable research efforts on the synthesis and manufacturing of novel devices for various high-technological potential applications. Nanocrystalline Iron oxide Hematite (Fe_2O_3) has important applications such as: photocatalytic splitting of water to hydrogen and oxygen, self-cleaning surfaces and degradation of environmental pollutants [5]. Iron oxides nanocrystals have attracted increasing attention for their outstanding new properties such as their biocompatibility, catalytic activity and low toxicity. Due to the stability of modern dyes, conventional biological treatment methods for industrial wastewater are ineffective and insufficient resulting often in an intensively colored discharge from the treatment facilities. Recently, a number of researches have dealt with heterogeneous photocatalytic decomposition of many kinds of azo-dyes [6,7] by UV, visible light and solar irradiation [8]. In addition, hematite has a relatively high band gap value of 3.2 eV. However for many applications it would be desirable to extend the band gap excitations towards the visible region, and also to prolong the lifetime of photogenerated charge carriers. Doping of titanium dioxide with transition metal ions provides a relatively well-studied and convenient way of solving both problems described above. Titanium dioxide doped with transition metal ions can demonstrate extended band gaps and significantly higher photocatalytic efficiencies [9,10]. The dopant

concentration is an important parameter to be considered, as the amount of dopant influences the processes of charge carrier trapping, separation, and recombination [11]. Therefore, the amount of transition metal introduced should be within a so-called optimum concentration, as too low a dopant content does not affect the process of charge carrier generation and too high a content of doping metal results in the formation of extra recombination sites and shortens the lifetime of photo generated electrons and holes. Consequently, defining the optimum concentration of doping metal is a key factor for successful doping. This optimum value may vary significantly and depends on several factors, such as the type of dopent chosen, the coating deposition technique, annealing conditions, *etc*. [12].

Present study involves the synthesis of Fe_2O_3 and Co doped Fe_2O_3 nanocatalyst which was characterized by scanning electron microscopy (SEM), Electron dispersive X-ray spectroscopy (EDS) and X-ray diffraction (XRD). The effect of various parameters like pH of dye solution, contact time, dose of catalyst in photocatalytic degradation Methyl Orange (MO) using Fe_2O_3 and Co doped Fe_2O_3 nanocatalyst were studied.

Experimental

Method

From stock solution of MO, different concentrations were prepared in distilled water and pH maintain to 7. The 50 mL MO solution containing Fe_2O_3 and Co doped Fe_2O_3 nanocatalyst taken in the photoreactor. The solution was stirred for 2 hours in the dark to allow to reach the equilibration of the system The dye sensitized Fe_2O_3 and Co doped Fe_2O_3 was subjected to visible light irradiation for the degradation of MO. The catalyst was separated from the solution by centrifugation and the solution was analyzed for determining concentration of dye at λ_{max} 465 nm.

Synthesis of α- Fe_2O_3 and Co doped α- Fe_2O_3

Pure and 2, 5 &10 % Co incorporated *α*-Fe2O3 were prepared by a simple and cost effective solution method. For the synthesis purpose, the preparation strategy of iron oxide from foam-based precursor is used. In a synthesis, iron nitrate and cobalt chloride was dissolved (Fe:Co, 98:2, 95:5 and 90:10; atomic ratio) in deionized water and heated the solution at 70^{o}C to obtain a gel like material [12,13]. The gel was formed after 2 h with continuous stirring of the precursor solution. On the other hand separately, a clear solution containing 4 wt% PVA was made by stirring and warming the solution at 40^{o}C [14]. After that an appropriate amount PVA solution (7 ml) was added slowly to the gel with vigorous stirring. The solution was found to transform into a gel after stirring and warming at 70˚C for an hour. The gel was kept at 100˚C in an air oven and obtained a foam-like material. In the next, the foam was heat-treated under air atmosphere at 600˚C for three hour to remove the organics and the product of cobalt incorporated *α*- Fe_2O_3 (AFC) was obtained. Similar procedure was also adopted for preparation of undoped*α*-Fe_2O_3 (AF) without the addition of hydrated cobaltous chloride in the precursor solution. It is important here that the foam formation depends upon the PVA weight percent Co (wt%). In this respect, initially the synthesis was made by adding 2 wt% PVA solution but there was no formation of the foam-like material

Results and discussion

SEM analysis

The SEM image of Fe_2O_3 and Co doped Fe_2O_3 nanocatalyst are shown in Fig. 1 (a, b, c and d). The FESEM image of Fe_2O_3 and Co doped Fe_2O_3 nanocatalyst shows irregular elongated spherical shape with crystal structure having irregular size and shape. It was observed that the particle size increases with increasing doping concentration of Co.

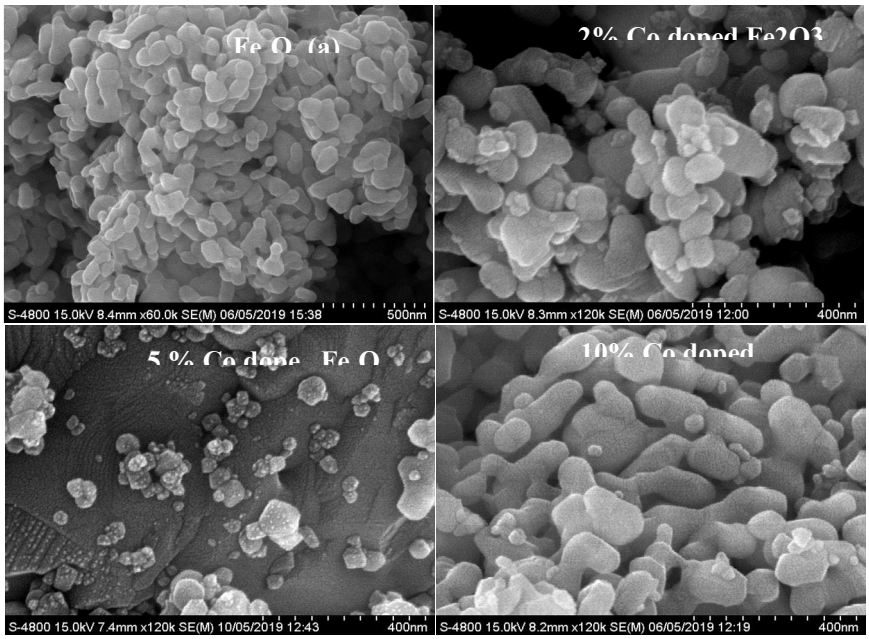

Fig. 1. (a, b, c and d). FESEM image of $Fe_2O_3$and Co doped Fe_2O_3 nanocatalyst

X-ray diffraction analysis

Fig.1 (a, b, c and d). The FESEM image of Fe_2O_3 and Co doped Fe_2O_3

The XRD patterns of the samples show well-defined peaks, indicating that the samples are crystalline. The pattern exhibits the characteristic XRD pattern of hematite (α-Fe_2O_3) in accordance with data from the ASTM (American Society of Testing Materials) cards**.** The Scans were performed over 2θ = 20-80° for each sample. Fig. 2 indicate different peaks at 24.10°, 33.26°, 36.30°, 42.23°, 51.37°, 42.23°**,** 54.00° and 63.54° corresponded to planes (012), (104), (110), (112), (024), (116) and (118). The diffraction peaks of the samples were found to be in a good agreement with those reported for hematite in the literature (JCPDS Card no. 89-0529).

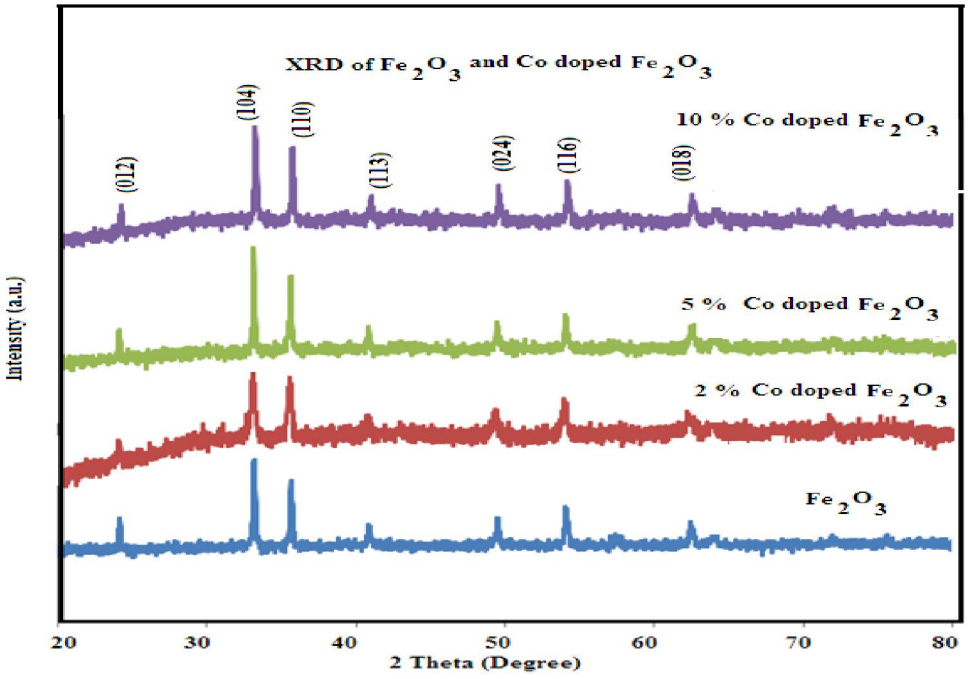

Fig. 2 XRD of Fe_2O_3 and Co doped Fe_2O_3

Effect of pH

The influence of pH on photocatalytic degradation of MO was performed. Degradation of MO is lower in basic media that is increase in pH, the percentage degradation decreases and higher degradation is observed to neutral medium. The pH 5 is suitable for degradation of MO in presence of Fe_2O_3 nanocatalyst.

Effect of catalyst on initial Dye Concentration

The effect of catalyst on initial dye concentration of MO was investigated by changing the doping amount Co in Fe_2O_3 as 2%, 5% and 10% using 2 g/L of Fe_2O_3 and Co doped Fe_2O_3 nanocatalyst at pH 5. The results showed that dye concentration decreases from 20 mg/L to 6.6 mg/L, 6.23 mg/L, 5.26 mg/L, 5.52 mg/L with increasing in doping concentrations from Fe_2O_3, 2 % Co doped Fe_2O_3, 5% Co doped Fe_2O_3, 10% Co doped Fe_2O_3 (Fig. 3). This was due to the reason as doping concentration increased the concentration of unabsorbed dye in the solution decreases which lead to more penetration of light through the solution on to the surface of Fe_2O_3 and Co doped Fe_2O_3 thereby increase the concentration of ·OH radicals on the surface and hence increases the percentage degradation [15].

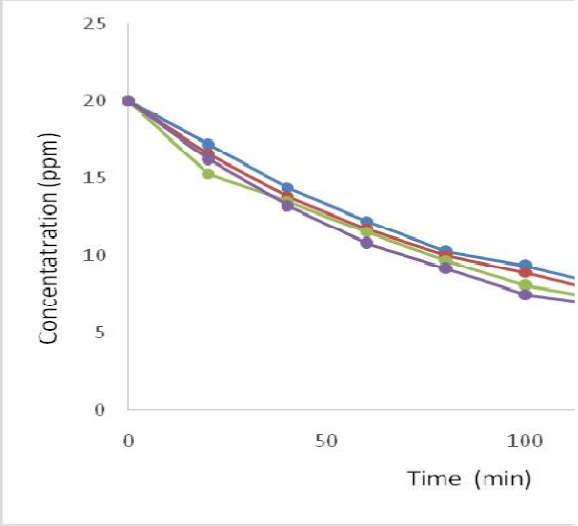

Fig. 3. Effect of catalyst on initial dye concentration

Effect of Dose

To avoid the excessive use of catalyst, the optimum dose was determined using concentrations of Fe_2O_3. It was found that rate of degradation initially increases with the increase in catalyst dose, but beyond a certain level it remained almost constant. From Fig. 4 it is observed that after 1g/L of catalyst dose percent degradation remains almost constant so in present case 1g/L was found to be the optimum catalyst concentration [16]. The increase in catalyst concentration has a positive influence on the number of photons absorbed and number of dye molecules adsorbed. This in turns enhances the rate of dye degradation. Above a certain catalyst concentration the numbers of substrate molecules are not sufficient to fill the surface active sites of Fe_2O_3. Hence, further addition of catalyst does not lead to the enhancement of degradation rate. The surplus addition of the catalyst makes the solution more turbid and light penetration is hindered from the sample observed that the increase in photocatalyst loading leads to availability of more number of catalytic sites for adsorption further increase in the dosage results in saturation and very high dosage results in increased turbidity of the suspension hindering the light penetration due to shielding effect thus reducing the efficiency of the process decreases. [17].

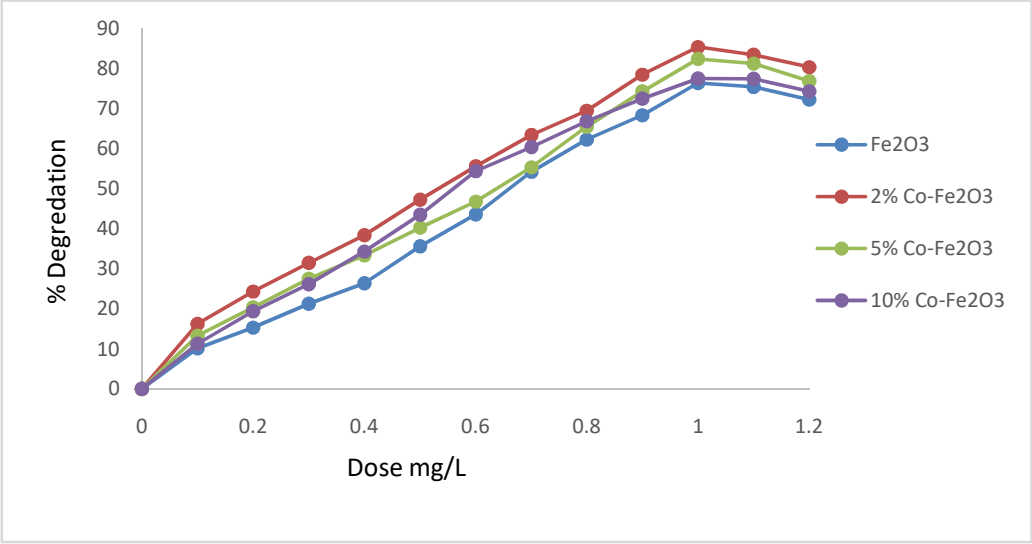

Fig. 4 Effect of dose on percentage degradation of MO (pH=5, MO conc.= 40 mg/L and reaction time 120 min.)

Effect of doping percentage

Fig. 5 shows that the effect of different doping ratios on the photocatalytic degradation of MO. The values of catalyst dose 1 g/L, pH 5 and the concentration of MO were 20 mg/L, respectively. As shown in Fig. 5, during the contact time, the MO degradation efficiency (%) was increased slightly with the increase of doping ratio from 2 to 10 %. Photocatalytic activity of doping concentration increases with decreasing the band gap energy [18]. In addition, rapid transfer of the electrons from the Fe_2O_3 to the Co may improve the photocatalytic activity and increase the efficiency of photodegradation [19].

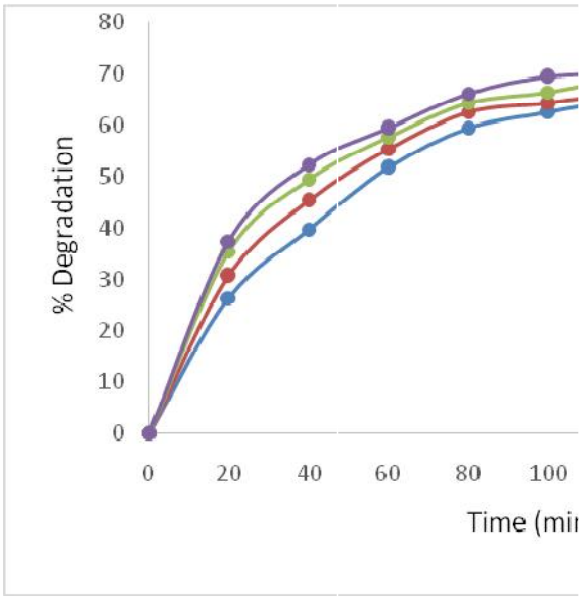

Fig. 5. Effect of doping percentage on percentage degradation of MO (Catalyst dose= 1.0 g/L, pH=5 MO conc. = 20 mg/L and reaction time 200 min)

Effect of initial dye concentration

The effect of initial dye concentration on photocatalytic degradation of MO was studied by varying the dye concentration from 10 to 40 mg/L (Fig. 6) at fixed catalyst concentration 5% Co doped Fe_2O_3. It can be observed that, as the dye concentration increases percent degradation decreases. As the concentration of dye increases the color of solution becomes

more intense due to more dye molecules which alters the light to reach the catalyst surface to produce active species responsible for degradation and thereby decreases the degradation efficiency of catalyst and secondly, less number of active site of catalyst is available due increase in adsorption which also lowers the catalyst efficiency.

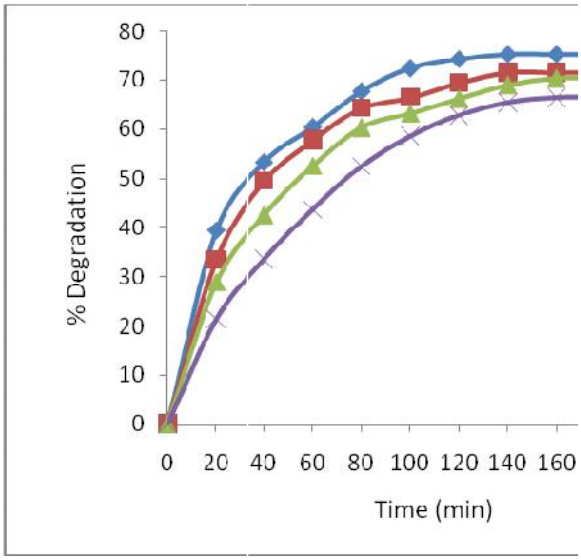

Fig.6. The effect of initial dye concentration on photocatalytic degradation of MO.

Recycle performance of Co doped Fe_2O_3 nanocatalyst

To find out the stability and efficiency of Co doped Fe_2O_3 nanocatalyst as well as cost effectiveness of the process, the reusability of Zn doped Fe_2O_3 nanocatalyst was investigated for the % degradation of MO. To study its reusability, the powdered nanocatalyst was centrifuged after completion of each photocatalytic experiment. The recovered sample was reused for 3 times under same experimental conditions. Fig. 7 shows % degradation of MO by Co doped Fe_2O_3 nanocatalyst after 1st run achieved up to 85.6% (100 min). After 4th run it decreases down to 81.55%. The catalytic activity was found to decrease marginally after 4th run. This decrease may be attributed to loss of reused catalyst during sampling each time and irreversible changes of the surface of the photo catalyst by pollutants. Fig. 7 shows that Co doped Fe_2O_3 have excellent stability and do not undergo photocorrosion.

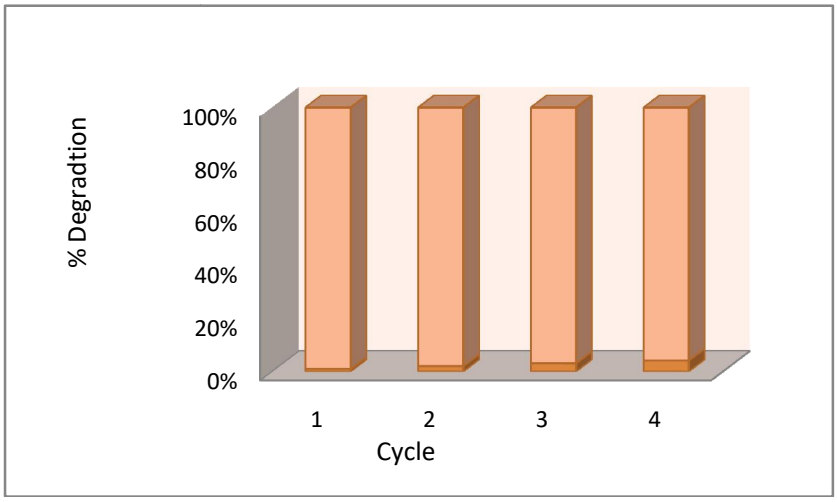

Fig. 7. Reusability study of Zn doped Fe_2O_3 nanocatalyst.

Efficiency study of catalyst by effluent treatment

For estimating the extent up to which the organic moieties degraded in the effluent after treatment, total organic carbon (TOC) analysis of the sample is the most appropriate method. As TOC is the measure of the level of organic molecules or contaminants in purified water [20]. To estimate the degradation level of organic contaminants TOC analysis of the sample before and after degradation was conducted along with other parameters like electrical conductivity, colour by absorbance at λ_{max}, associated with water quality by treatment with catalyst. For this purpose 4 % Zn doped CuO was used as it was found most efficient among all compositions of doped and undoped materials. 5 g/L of catalyst effluent was used for the treatment. The result is summarized in the Table.

Sample Parameters	Before treatment	After treatment
pH	7.9	7.6
Electrical conductivity	15.96 X 10-3 mhos	12.64 X 10-3 mhos
TOC	325.6 ppm	44.78ppm
Absorbance	0.96	0.41

The degradation results are near about the pollutants discharged by each plant are limited by a Central Pollution Control Board New Delhi [21].

Conclusions

The photocatalytic degradation of MO in the presence of Fe_2O_3 and Co doped Fe_2O_3 were show promising results towards degradation of MO. The percentage degradation of dye increased with an increase doping percentage of Co and decrease with increase in initial concentration of dye. The pH 5 found to be suitable for photocatalytic degradation of MO. A comparative study shows that 5% Co doped Fe_2O_3 nanophotocatalyst degradation of MO. The reusability study shows the stability of the Co doped Fe_2O_3 nanocatalyst.

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SYNTHESIS AND IN VITRO ANTIMICROBIAL ACTIVITY OF SUGAR HYDRAZINO BENZOTHIOZOLYL THIOCARBAMIDE

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ABSTRACT

Benzothiazoles are bicyclic ring system with multiple applications. The synthesis of novel glycosides derivatives and investigation of their chemical and biological behaviour have gained more importance in recent decades. Serial of 1-tetra-O-benzoyl-β-D-glucosyl-3-(2)-hydrazino-1, 3-substituted benzothiozolyl thiocarbamide has been synthesized by the interaction of two pharmocophores, tetra-O-benzoyl-β-D-glucosyl isothiocyanates and substituted 2-hydrazino-1,3-benzothiazoles in acetone medium. The reaction mixture was kept at room temp for 24 hrs. Acetone is evaporated then product is recrystallised by petroleum ether (60-80%). Benz-fused compounds have been employed in the synthesis of various compounds which show very potential pharmacological activities. Carbohydrate is the key element in variety of biological phenomena and its N-linked sugar derivatives also exhibit wide range of medicinal activities. Keeping in this view, when one biological active molecule is linked to another, the resultant molecule generally has increased potency. The identities of these newly synthesised 1-tetra-O-benzoyl-β-D-glucosyl-3-(2)-hydrazino-1, 3-substituted benzothiozolyl thiocarbamide have been established on the basis of usual chemical transformations and IR , ^{1}H NMR and Mass spectral studies. The antibacterial and antifungal activities of also reported. Some of these derivatives exhibit significant antimicrobial activity. These compounds show appreciable activity towards these microorganisms like Escherichia coli, Proteus vulgaris, Staphylococcus aureus, Salmonella typhimurium, Psudomonas aeruginosa, Aspergillus Niger and Candida albicance.

Keyword: *2-hydrazino-1,3 benzothiozole , substituted benzothiozolyl thiocarbamide, tetra-O-benzoyl-β-D-glucosyl isothiocyanates, Biological studies.*

Introduction

In spite of tremendous advance made in modern medicine, there are still a large number of aliments for which suitable drugs are yet to be found. Today, there is an need to develop safer drug for the treatment of pain. Hydrazino benzothiazole and isatin derivatives are an important class of organic heterocycles because of their potential activities are reported to be effective in CNS disorders such as convulsion[1] and depressions[2]. Indole and benzothiazoles its analogs constitute the active class of the compounds possessing wide spectrum of antimicrobial[3], anthelmintic[4], analgesic[5],anti-inflammatory[6] and anti-tuberculosis[7]activities.

Benzothiozoles constitute an importance's class of compounds. In recent year heterocyclic compound analogues and derivatives have attracted strong interest due to their useful biological and pharmacological properties. Benzothiazole, a multifaceted nucleus, has been under research for the last two decades. Being a heterocyclic compound, benzothiazole finds use in research as a starting material for the synthesis of larger, usually bioactive structures. Its aromaticity makes it relatively stable, although as a heterocycle, it has reactive sites which allow for functionalization. From the literature survey, it has been found that extensive work has been reported on 2-substituted benzothiazole derivatives in past and evaluated for different activities like antibacterial[8], anticancer[9], antiviral[10], antitumor[11], anticonvulsant[12], neuroprotective[13], a topical carbonic anhydrase inhibitor and an antihypoxic. Taking this into view, and in continuation of our search for biologically potential benzothiazole derivatives, a certain new derivatives were synthesized taking benzothiazole as the basic moiety. Different benzothiazoles react with hydrazine and this hydrazino benzothiazoles then focused to fuse with *N*-lactosylated compound[14]. Hence, in present work, different benzothiazoles react with hydrazine and this hydrazino benzothiazoles then focused to fuse with *N*-glucosylatd compound.

Results and discussion

Herein, we report the synthesis of various 1-tetra-O-benzoyl-β-D-glucosyl-3-(2)-hydrazino-1, 3-substituted benzothiozolyl thiocarbamide **III(a-d)** by interaction of tetra-O-benzoyl-β-D-

glucosyl isothiocyanates **(I)** and substituted 2-hydrazino-1,3-benzothiazole **II(a-d)** in acetone medium. All products were crystallized from ethanol before recording the physical data (Table-1). The purity of compounds was checked by TLC. The spectral analysis[15-17] IR, 1H NMR and Mass spectra of the product were observed.

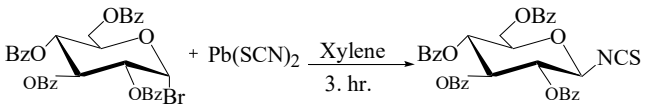
Experimental
Material and Methods

All chemicals were research grade. Melting points determined are uncorrected. IR spectra were recorded in KBr on a FT-IR Perkin-Elmer RXI(4000-450cm^{-1}) spectrophotometer. ^{1}H NMR measurements were performed on a Bruker DRX-300 (300 MHz FT NMR) NMR spectrometer in $CDCl_3$ solution with TMS as internal reference. The Mass spectra were recorded on a THERMO Finnigan LCQ Advantage max ion trap Mass spectrometer. Thin layer chromatography (TLC) was performed on silica Gel G and spots were visualized by iodine vapour. The compounds were screened for their antibacterial and antifungal activities by the disc diffusion assay method [18]. The compounds describe in this paper were first time synthesized by the multistep reaction protocol.

1] Preparation of tetra-O-benzoyl-β-D-glucosyl isothiocyanates: [19]

To a suspension of tetra-O-benzoyl-β-D-glucosyl bromide (21 g) in sodium dried xylene (80 ml) was added lead thiocyanate (5 g). The reaction mixture was refluxed gently for 3 hr.with frequent shaking .This solution was then cooled and liberated lead bromide was removed by filtration .The xylene filtrate was then treated with petrolium ether.(60-80) with stirring ,the pale yellow solid obtained (12 g).this solid was expected tetra–O-benzoyl-β-D-glucosylisothiocyanate .It was purified by dissolving it in a minimum quantity of chloroform and reprecipitating with petroleum ether.m.p 115-120^{0}C.

Scheme I



Tetra-O-Benzoyl-α-D-Glucosyl Bromide

Tetra-O-Benzoyl-β-D-Glucosyl Isithiocyanate

2] Preparation of 2- hydrazino-1,3-benzothiozole

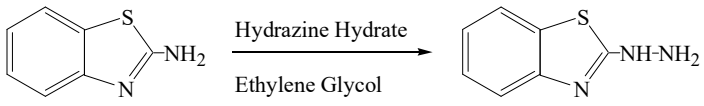
a) Preparation of benzothiozole

The required substituted benzothiazoles has been prepared by the oxidative cyclization of aryl thiocarbamide with the help of molecular bromine. To the chloroformic paste of phenyl thiocarbamide (5gm in 10ml) molecular bromine (20%) was added gradually with constant stirring until a slight excess of bromine was added as evident from an orange red colour. It was then allow to stand for 5 to 6 hrs. to resultant acidic solid was treated with cold ethanol, the solid dissolved in ethanol. On basification with dilute ice cold NH_4OH 2-aminobenzothiozole was separated out as a white solid (3gm). M.p. 130^{o}C.

b) Preparation of 2- hydrazino-1,3-benzothiozole

Concentrated HCl (1mL) was added drop wise to hydrazine hydrate (0.2 M, 1mL 80%) at 5-10^{o}C followed by ethylene glycol (20mL). To the above solution 2-aminobenzothiazole (0.01 M, 1.85g) was added in portions. It was then refluxed for 3 h, cooled and poured onto crushed ice. The separated solid was filtered, dried and recrystalized from ethanol. **II(a-g). (Scheme-II)**

Sceme 2:

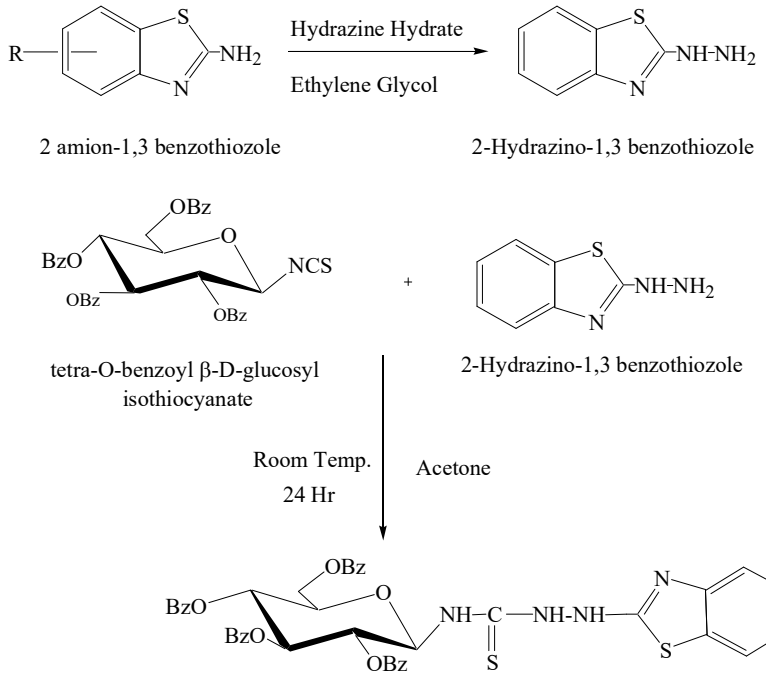


2 amion-1,3 benzothiozole

2-Hydrazino-1,3 benzothiozole

3] Preparation of 1-tetra-O-benzoyl-β-D-glucosyl-3-(2)-hydrazino-1, 3-substituted benzothiozolyl thiocarbamide

A acetone solution of tetra-O-benzoyl-β-D-glucosyl isothiocyanates (0.025M, 2.5g in 20mL) was mixed with acetone solution of 2-hydrazino-1,3-benzothiazole (0.025M, 0.37g in 10mL), and mixture after shaking for sometime was kept at room temperature for 24 hr. Acetone was distilled off to obtained sticky residue. This residue was triturated several times with petroleum ether to afford a light coloured solid. **III(a-d). (Scheme-III).**



1-tetra-O-benzoyl β-D-Glucosyl-3-(2)-hydrazino-1,3-benzothiazolyl Thiocarbamide

Where, R= (a) Phenyl, (b) *o*-tolyl, (c) *m*-tolyl , (d) *p*-tolyl, And Bz = Benzoyl

3a: IR (KBr):υ 3400.29 (N-H), 3066.82 (Ar-H), 1730.15 (C=O), 1598.99 (C=N), 1263.37 (C-N), 1140.07 (C=S), 920.05 (Characteristics of glucose), 709.80 (C-S), H NMR (δ in ppm, $CDCl_3$): δ 5.7 -5.21 (3H, s, N-H), δ 7.5 (24H, m, aromatic protons), δ 4.5 (7H, m, glucosyl proton). Mass (m/z): 801 (M^+), 723, 579, 475, 374, 305; Anal. Calcd for $C_{42}H_{33}O_9N_4S_2$.: C, 62.92; H, 4.11; N, 6.99; S, 7.99; Found: C, 62.90; H, 4.17; N, 7.01; S, 7.95.

On the basis of all above facts the product with m. p. 200°C was assigned the structure 1-tetra-O-benzoyl-β-D-glucosyl-3-(2)-hydrazino-1, 3-substituted Phenyl-benzothiozolyl thiocarbamide When the reaction of tetra-O-benzoyl-β-D-glucosyl isothiocyanates was extended to several other 2- hydrazino-1,3-benzothiozole corresponding 1-tetra-O-benzoyl-β-D-glucosyl-3-(2)-hydrazino-1, 3-substituted -benzothiozolyl thiocarbamide has been synthesized.

3b: : IR (KBr):υ 3433.29 (N-H), 3064.89 (Ar-H), 1730 (C=O), 1600.92 (C=N), 1140.07 (C=S), 939.33 (Characteristics of glucose), 1265.30 (C-N), 783.10 (C-S), H NMR (δ in ppm, $CDCl_3$): δ 5.7 -5.21 (3H, s, N-H), δ 7.5 (24H, m, aromatic protons), δ 4.5 (7H, m, glucosyl proton), δ 0.9 (3H, s, Methyl proton,). Mass (m/z): 815 (M^+), 723, 579, 475, 374, 305; Anal. Calcd for $C_{43}H_{35}O_9N_4S_2$.: C, 62.92; H, 4.11; N, 6.99; S, 7.99; Found: C, 62.90; H, 4.17; N, 7.01; S, 7.95.

On the basis of all above facts the product with m. p. 190°C was assigned the structure 1-tetra-O-benzoyl-β-D-glucosyl-3-(2)-hydrazino-1, 3-substituted o-tolyl-benzothiozolyl thiocarbamide

Table -1: Physical data for characterization of compounds (3a-d)

Compd	Yield %	R_f	M.P. ^0C	Analysis (%): Found (calcd)	
				N	S
3a	60.00	0.48	200	7.01(6.99)	7.95(7.99)
3b	75.00	0.69	190	6.60 (6.64)	7.80(7.87)
3c	80.00	0.52	185	6.70 (6.64)	7.85(7.87)
3d	64.00	0.60	173	6.69(6.64)	7.90(7.87)

C and H analysis was found satisfactory in all cases.

Antimicrobial Studies

All the compounds have been screen for both antimicrobial and antifungal activity using cup plate agar diffusion method18-20 by measuring the inhibition zone in mm. the compounds were taken at a concentration of 1 mg/mL using Dimethyl Sulphoxide (DMSO) as solvent. Amikacin (100 µg/mL) was used as standard for antibacterial activity and Fluconazole (100 µg/mL) as standard for antifungal activity. The compounds were screen for antibacterial activity against *Escherichia coli, Staphylococcus aureus, Proteus vulgaris, Pseudomonas aeruginosa* and *Klebsiyella species* by using Nutrient Agar medium and antifungal activity against *Thrichoderma harzianum* and *Verticillium species* was determined by using Potato Dextrose Agar medium. These sterilized agar media were poured into Petri dishes and allowed to solidify. On the surface of the media microbial suspensions were spread with the help of sterilized cotton swab. After inoculation the well was punched by using sterile stainless steel cork borer of 6mm diameter. In to these wells were added 0.1 mL portion of the test compounds in solvent. The drug solution was allowed to diffuse for an hour into the medium. The plate was incubated at 37°C for 24 h and 30°C for 48 h for antibacterial and for antifungal activities respectively. The zone of inhibition observed around the cups after respective incubation was measured. The results are presented in Table 2. Antibacterial studies of these compounds indicated that compounds exhibited most significant activity against All the other compounds exihibited low to moderate activity.

(Table2)

Sr. no	*E. c.*	S. *a.*	*P.v*	*P.a*	*S.t*	*K.p*	*A.n*	*C.a*
1(3a)	17	20	20	19	18	21	19	20
2(3b)	10	19	15	12	20	19	20	21
3(3c)	18	14	19	17	15	18	17	19
4(3d)	14	20	18	18	19	20	20	19
Amikacin	18	21	23	19	20	21		
Fluconazole							24	24

Sample	**Disc content**	**Resistant**	**Intermediate**	**Sensitive**
Amikacin	100ug/ml	≤ 15 mm	16-20 mm	≥ 21 mm
Fluconazole	100ug/ml	≤ 15 mm	16-20 mm	≥ 21 mm

Conclusion

Derivatives were synthesized and characterized for their structure elucidation. As outline in synthesis process, important novel -1,3-substituted benzothiozolyl thiocarbamide has been synthesized. All the structure of the above compounds was in good agreement with Spectral and Analytical data. Various chemical and spectral data supported the structures. Some of the compounds synthesized showed promising antimicrobial activities. The newly synthesized thiocarbamides exhibits comparable antibacterial and antifungal activities against the organisms tested. The method adopted in this investigation is simple,

efficient and inexpensive and is useful in synthesizing pharmacologically important molecules.

Acknowledgement

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CURRENT STATUS OF CHANNA SPECIES IN AKOLA DISTRICT

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ABSTRACT

Akola district is located in the central eastern part of the stateand important for the natural water resources and so for the production of fish species. In this area, 30 species indicate the high fish diversity. They have been recorded belonging to its 15 families. Among them, Channa species is commonly called as 'Snakeheads'. It is important species and fetch high price due to its flesh quality, taste, nutritive value, high protein and less spines as well as their hardy nature. In Akola district, the family Channidae is known as Maral, represented by 4 popular species i.e. Channa gachua, Channa marulius, Channa striatusand Channa punctatus which is found abundantly. The population of these economically important Channa species is declining due to overexploitation of brood and juveniles as well as due to destruction of breeding ground. Therefore, effective conservative measures are suggested to sustain Channa species in Akola district.

Keywords: Akola district, Conservation, Channa.

Introduction

Akola district is located in the central eastern part of the state. It has 221 ponds which include 165 seasonal and 56 permanent ponds. The district also has five major reservoirs having water area more than 200 hectors. So this area has favourable environmental conditions to cultivate the Channa fish species. The family Channidae was represented by 4 species, *Channa gachua*, *Channa marulius*, *Channa striatus*and *Channa punctatus*. These Fishes are known as important aquatic food item and are considered as major source of protein. Due to their high rated commercial value, all of these Channa species are conserved in priority base.

Material and Methods

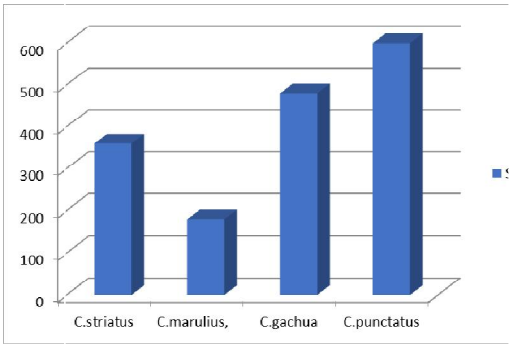
The field research on these species has been carried out during Junuary 2018 to May 2019. Every month, the freshly dead Channa species are collected from selected major reservoirs having water area more than 200 hectors with the support of local fishermen. These study sites are Katepurna, Morna, Dagadparava, Nirguna and Uma. On the filed visit, immediately, photographs are taken and also recorded number of fish samples found as per species. All collected specimens are preserved in formalin. Fishes having small size are directly preserved in 4% formalin solution, while large fishes are preserved in 10% formalin and also they have been given an incision in their abdomen. The researcher identifies the Channidae family fishes to the species level with the help of taxonomic keys and books.

Results and Discussion

The family Channidae is represented by 4 species, *Channa gachua*, *Channa marulius*, *Channa striatus* and *Channa punctatus.* Among these, *Channa punctatus* is found abundantly. While netting, it is found only 2 kg per 100 kg of the total fish fauna of each reservoir. This indicates low number of the species in Akola district.

Channa species is useful for implantation. So conservation strategies should be implemented such as making fishermen scientifically trained for fishing as well as to aware them to avoid brooders and immature fishing. Many scientists are involved in doing research on fish diversity for conservation of fish species [1-5].

Sr. No.	Family	Genus	Species	Local name	Total sample observed	status
1	Channidae	Channa	striatus	Pattewalimaral	360	+
		Channa	marulius,	Spotted maral	180	-
		Channa	gachua	Small dhok	480	++
		Channa	punctatus	Dhok	600	+++
+++ = most abundant ,++ = abundant, + =less, - = Rare						



Graphical presentation of Channa Species in Akola district.

Channa striata

Channa marulius

Channa gachua

Channa punctatus

Conclusion

Decline of Channa species population is marked due to brooders and immature fishing. In order to conserve these valuable resources, make fisherman scientifically trained. Furthermore, regular monitoring program should be implemented in major reservoirs in Akola district to determine and to document the population status of the Channa species.

Acknowledgement

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EVALUATION OF REMOVAL EFFICIENCY OF Mn (II) FROM AQUEOUS SOLUTION USING SURFACE MODIFIED ACTIVATED CHARCOAL

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ABSTRACT

The removal of Manganese (II) ions from aqueous solution by using activated carbon prepared from Phyllanthus emblica tree bark (AC-PETB) and activated carbon modified by Sodium lauroyl sarcosinate and 2-Acrylamido-2-methylpropane sulfonic acid was investigated. Adsorption studies were performed by batch experiments. The effect of contact time, pH, adsorbent dose and temperature were explored. From the experimental data, the isotherm parameters of Freundlich and Langmuir were calculated. The equilibrium was best represented by the Langmuir. Langmuir adsorption capacity (Qo) for AC-PETB-AMPSA was found to be 4.46 mg/g, for AC-PETB-SLS 2.55 mg/g and for AC-PETB 2.09 mg/g.

Keywords: *Adsorption, Manganese (II), Sodium lauroyl sarcosinate, 2-Acrylamido-2-methylpropane sulfonic acid*

Introduction

An industrial effluent contains many heavy metals in the form of pollutants. Ground water and surface water gets contaminated by heavy metal ions released from the industries such as metal plating, metal finishing, rubber processing, fertilizers, mining etc[1-2]. Rapid industrialization has led to increased disposal of heavy metals into the environment. The Groundwater that contains an appreciable amount of iron or manganese or both is always devoid of dissolved oxygen and high in carbon dioxide content[3]. As far as is known, humans suffer no harmful effects from drinking water containing manganese. However, manganese interferes with laundering operation, imparts objectionable stains to plumbing fixture, and causes trouble in distribution systems by supporting growths of iron bacteria[4]. According to W.H.O. the maximum permissible limit of manganese in drinking water is 0.5 mg/L. With better awareness of the problems associated with manganese came an increase in research studies related to methods of removing manganese from wastewater, for which a number of technologies have been developed over the years[5]. The most important technologies include chemical precipitation, electro flotation, ion exchange, reverse osmosis and adsorption onto activated carbon. These methods are not cost-effective in the Indian context. Low-cost and nonconventional adsorbents include agricultural wastes, such as natural compost, Irish peat, planer shell, walnut shell, and biomass[6].

Materials and Methods

Preparation of Solutions

Standard solution of manganese bromide was prepared in deionised water. The concentration of Mn (II) was analyzed by UV-Visible spectrophotometer (model-117) using periodate as the complexing agent at the wavelength of 460 nm. The Sodium lauroyl sarcosinate, 2-Acrylamido-2-methylpropane sulfonic acid (purchased from Merk), was used for surface modification of activated carbon.

Surface modification of AC

0.01 M concentration of each chelating agent is used for the modification of surface of activated charcoal. 200 ml solution of chelating agent and 0.5 gram of adsorbent (AC-PETB) in reagent bottle, shaken for 3 hours at 1000 rpm at room temperature, then dried in oven for surface modification. Activated charcoal loaded with Sodium lauroyl sarcosinate, 2-Acrylamido-2-methylpropane sulfonic acid designated as AC-PETB-SLS and AC-PETB-AMPSA

Results

Effect of pH

The effect of pH can be done experimentally by taking 0.5 gm of adsorbent with working volume of Mn (II) 200 ml having constant

initial metal ions concentration and the contact time of 3 hours with shaking speed 1000 rpm. The result indicates that maximum uptake capacity for Mn (II) was found to be at pH 4 with AC-PETB, AC-PETB-SLS and AC-PETB-AMPSA. The adsorption capacity of Mn (II) as a function of pH it was observed that percentage removal of Mn (II) is maximum of pH = 4 shown in fig.1.

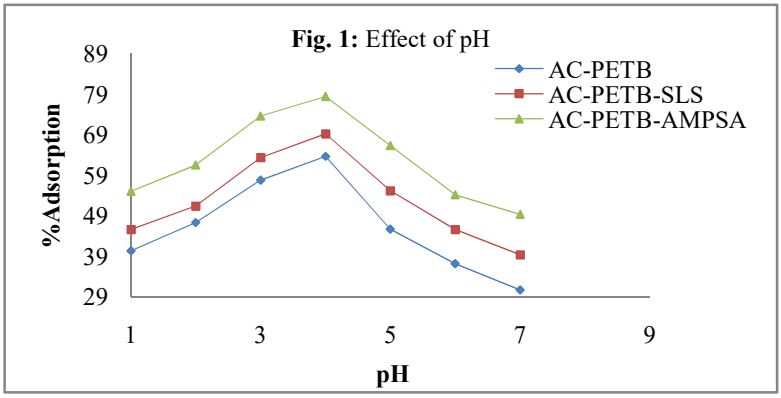

Fig. 1: Effect of pH

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Effect of Contact time

Study was carried out by taking 0.5 gm of adsorbent with working volume of Mn (II) 200 ml with known concentration of metal ions. It was observed that initially rate of adsorption is rapid up to 180 min with shaking speed 1000 rpm and then there was no further change in equilibrium concentration. Equilibrium time was found to be 300 minutes for this adsorption. The result indicates that maximum uptake capacity for Mn (II) at pH 4 and at 180 minute shown in fig.2.

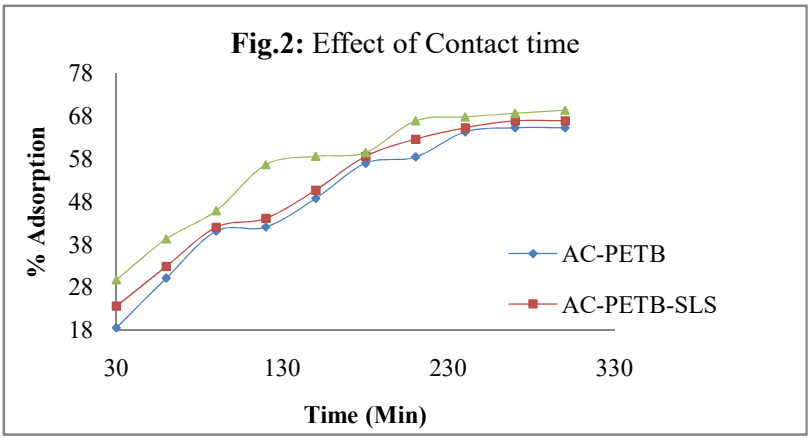

Fig.2: Effect of Contact time

Effect of Adsorbent dose

The effect of varying the adsorbent dosage (AC-PETB, AC- PETB-SLS and AC- PETB-AMPSA) from 0.2 – 1 gram for adsorption of Mn (II) from their aqueous solutions having known volume of initial concentration was studied at pH 4 It has been found that the percent removal of Mn (II) increases with increase in adsorbent dose up to some extent, thereafter further increase adsorbent dose shown in fig.3.

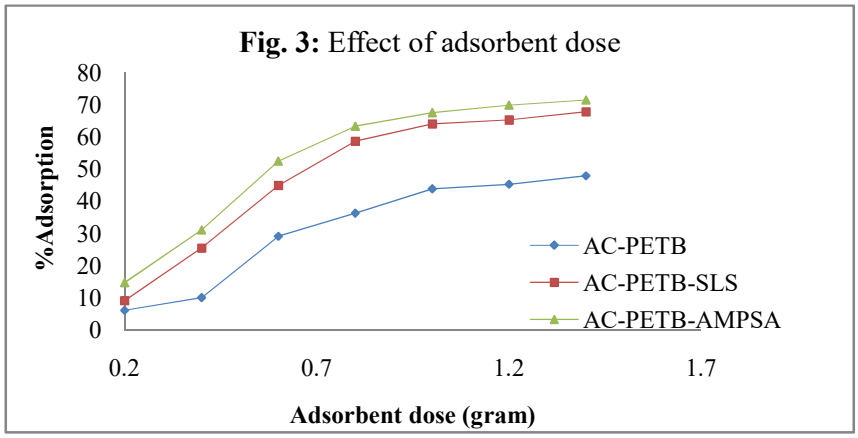

Fig. 3: Effect of adsorbent dose

Effect of Temperature

Effect of temperature was studied by varying the temperature from 30°C to 60°C with working volume 200 ml having known concentration. Study was carried out at pH 4 and at 1000 rpm with contact time 3 hours shown in fig.4. As the temperature increases porosity increases and percent of adsorption increases up to certain extent and then remains constant this is due to chemisorptions process. In chemisorptions as the temperature increases adsorption increases up to certain extent and then decreases while in physisorption process as the temperature increases adsorption decreases. From the study it was observed that the phenomenon was chemisorptions.

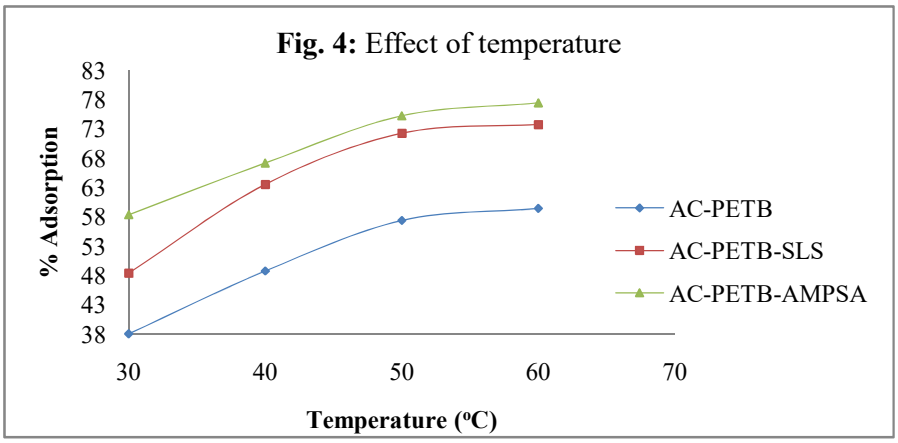

Fig. 4: Effect of temperature

Isotherm Modelling

***Langmuir Adsorption Isotherm*: -** The Langmuir isotherm model can be given as:

$$\frac{1}{q_e} = \frac{1}{Q^0 b} \times \frac{1}{Ce} + \frac{1}{Q^0}$$

The Langmuir constant Q^o is a measure of adsorption capacity and b is the measure of energy of adsorption. In order to observe whether the adsorption is favourable or not, a dimensionless parameter 'R_L' obtained from Langmuir Isotherm. The values of Q^o and b were evaluated from the intercept and slope of linear plots of $1/q_e$ vs. $1/C_e$ respectively.

$$R = (1 + b \times Cm)^{-1}$$

The value of R indicated the type of the isotherm to be either unfavourable (R>1), linear (R=1), favourable (0<R<1) or irreversible (R=0). Where, b is Langmuir adsorption constant and Cm is the maximum initial solute concentration used in the Langmuir isotherm. The adsorption of Mn (II) on Activated charcoal and loaded Granular Activated charcoal is a favourable process as "R_L" values lies between zero to one shown in table 1. The Langmuir adsorption isotherm shown in fig.5.

***Freundlich Adsorption isotherm*: -** It is most commonly used adsorption isotherm model which describes adsorption on heterogeneous surfaces with interactions among adsorbed molecules. It helps to investigate the nature of adsorption and the adsorption capacity of an adsorbent. The linear form of Freundlich isotherm model is

$$\log q_e = B.\log C_e + \log K_f$$

Where, B and K_f are Freundlich constant. These constants represent the adsorption capacity and the adsorption intensity respectively. q_e is the amount adsorbed at equilibrium(mg/g), Ce is the equilibrium concentration of adsorbate. Plot of log q_e Vs log C_e was also found to be linear. The values of B and K_f are calculated from the intercept and slope respectively shown in table 1. Freundlich plot for the adsorption of Mn (II) on AC-PETB, AC-PETB-SLS and AC-PETB-AMPSA is given in Table 1. It shows that the values of adsorption intensity 1/n < 1, reveal the applicability of Freundlich adsorption shown in fig.6.

Table 1: Adsorption Isotherm Constants

System	Langmuir Isotherm				Freundlich Isotherm		
	Q_o	b	R_L	R^2	K_f	1/n	R^2
AC-PETB-Mn(II)	2.0920	0.4434	0.1592	0.990	1.641	0.034	0.998
AC-PETB-SLS-Mn(II)	2.5575	0.6196	0.1193	0.995	1.778	0.094	0.998
AC-PETB-AMPSA-Mn(II)	4.4642	0.3758	0.1826	0.997	2.965	0.045	0.999

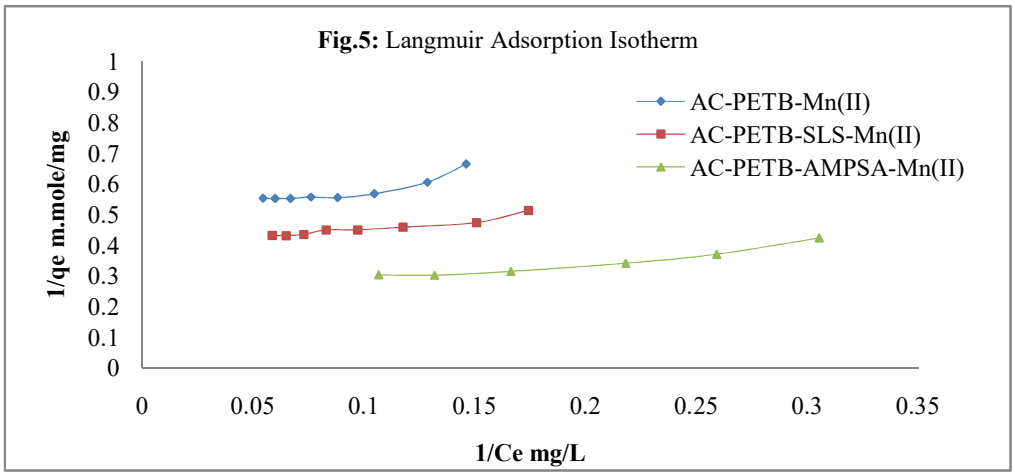

Fig.5: Langmuir Adsorption Isotherm

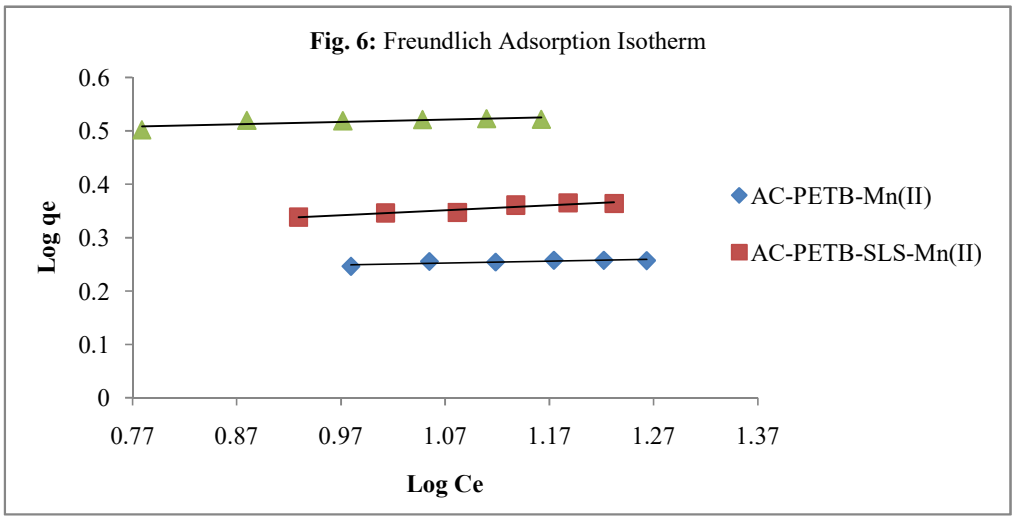

Fig. 6: Freundlich Adsorption Isotherm

Conclusion

Activated charcoal prepared from *Phyllanthus emblica* tree bark (AC-PETB) shows good adsorption efficiency for the removal of divalent manganese. The removal efficiency was found to be rapid at initial stage and then slow down because it is depend upon the concentration of metal ions and active sites on the adsorbent for the binding of metal ions. The adsorption isotherm data was best revealed by the Langmuir adsorption isotherm where maximum adsorption capacity for the removal of Mn (II) was found to be 4.46 mg/g by using Ac-PETB-AMPSA adsorbent which is higher as compared the AC-PETB-SLS and virgin AC-PETB. The maximum removal efficiency was found to be at pH 4. Batch study indicates that as the temperature, adsorption dose, contact time increases adsorption capacity increases. Activated charcoal loaded with 2-Acrylamido-2-methylpropane sulfonic acid shows higher adsorption capacity than Sodium lauroyl sarcosinate and virgin activated carbon.

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SCREENING AND COMPARISON OF ANTIOXIDANT ACTIVITY OF DIFFERENT TYPES OF MILK (COW, BUFFALO, GOAT AND DONKEY)

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ABSTRACT

Milk is nature's most complete food, and dairy products which are considered to be the most nutritious foods to all. Milk contains numerous nutrients which makes a remarkable contribution towards body's needs for calcium, magnesium, selenium, riboflavin, vitamin B_{12} and pantothenic acid (vitamin B_5). The present investigation was undertaken to screen and compare the antioxidant activity of cow, buffalo, goat and donkey milk to make aware the importance of these milk.

Keywords: *Antioxidant activity, Milk, DPPH.*

Introduction

Milk is nature's most complete food, and dairy products which are considered to be the most nutritious foods to all. Milk contains numerous nutrients which makes a remarkable contribution towards body's needs for calcium, magnesium, selenium, riboflavin, vitamin B_{12} and vitamin B_5 (pantothenic acid). The cow / buffalo become the main dairy animals associated with milk. The demand for milk in developing countries is expected to increase by 25 percent by 2025. So another source of milk such as goat, donkey and other must be considered in order to cope up the gap between demand and supply of milk. Cow milk generally contains 4 g of fat/100 g, although values as high as 5.5 g/100 g have been reported in raw milk. Most milk consumed contain a standardized fat content of around 3.5 g/100 g. Buffalo milk contains more fat than cow milk on average (5.5 g/100 g) and is therefore more energy dense. Goat milk has a smaller fat 3.0 g/100 g than cow milk which may make it more easily digestible. Donkey milks are renowned for their therapeutic properties. Their milks contain substantially lesser amounts of fat and protein than cow milk, and are nearest in composition to human milk because of their high lactose and low protein contents. So the present investigation was undertaken to screen and compare the antioxidant activity of *cow, buffalo, goat* and *donkey* milk to make aware the importance of these milk[1-3].

Chemicals

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

Materials and Methods

The various types of milk samples were collected from Dabaki road Akola and stored in air tight bottles at 4 ^{o}C for further study.

Study of Antioxidant Activity by Dpph[4]

The antioxidant activity of the milk was assessed on the basis of the radical scavenging effect of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH). The diluted working solutions of the different milks were prepared in ethanol. 0.004% of DPPH was prepared in ethyl alcohol and 3 ml of this solution was mixed with 3 ml of sample solutions. These solution mixtures were kept in dark for 30 min and optical density was measured at 517 nm using UV Visible spectrophotometer. Ethanol (3 ml) with DPPH solution (0.004%, 3 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 was prepared. The required dilutions 0.1 mg/ml to 0.9 mg/ml were prepared by appropriate dilutions. The optical density and percent antioxidant activity were calculated

. **Table No. 1 : Optical density and percent antioxidant activity for *Buffalo* milk**

(O.D of Blank DPPH = 0.591)

Conc.mg/ml	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09
O.D. of buffalo milk	0.301	0.282	0.283	0.271	0.272	0.261	0.251	0.240	0.221
%AA of buffalo milk	49.06	52.28	52.11	54.14	53.97	55.83	57.52	59.39	62.60

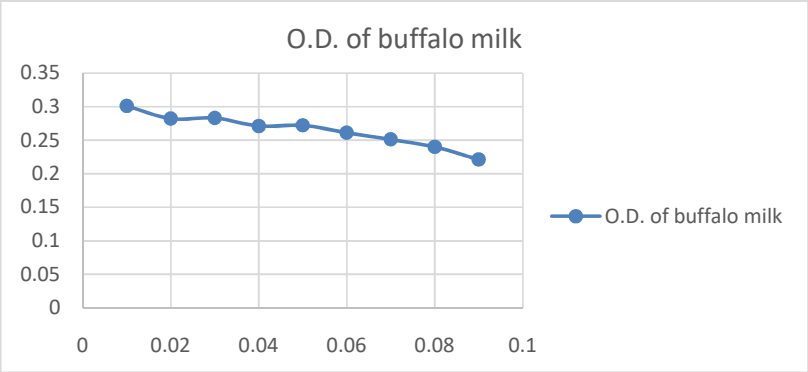

Figure 1: Decrease in optical density of sample with increase in concentration of *buffalo* milk

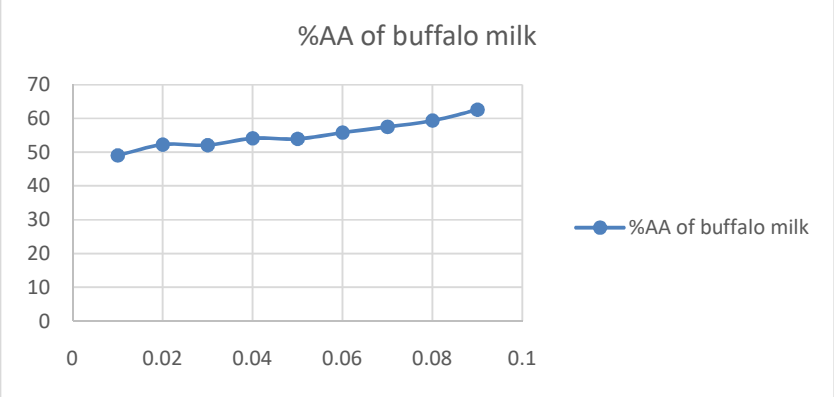

Figure 2: Increase in percent antioxidant activity with increase in concentration of *buffalo* milk

Calculations of Ic50 for buffalo milk = Max. – ½ (Max.-Min.)
=62.60-1/2(62.60-49.06)
=6.77mg/ml

Ic50 value corresponding to *buffalo* milk is = 0.041mg/ml.

Table no 2. Optical density and percent antioxidant activity for *Goat* milk

Conc.mg/ml	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09
O.D. of goat milk	0.28	0.24	0.24	0.23	0.20	0.19	0.15	0.10	0.8
%AA goat milk	52.62	59.39	59.39	61.08	66.15	67.85	74.61	83.07	84.01

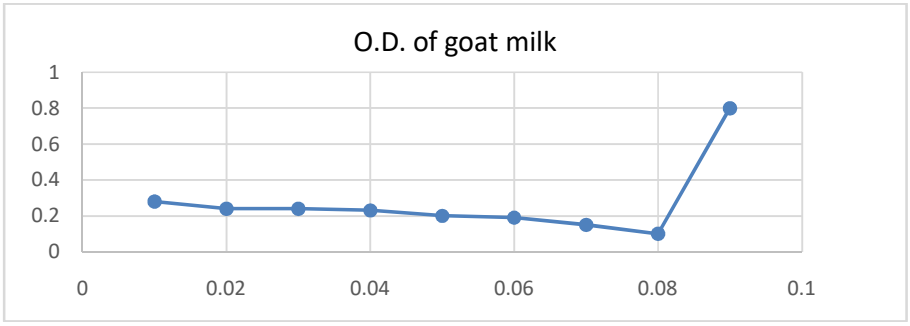

Figure 3: Decrease in optical density of sample with increase in concentration of *goat* milk

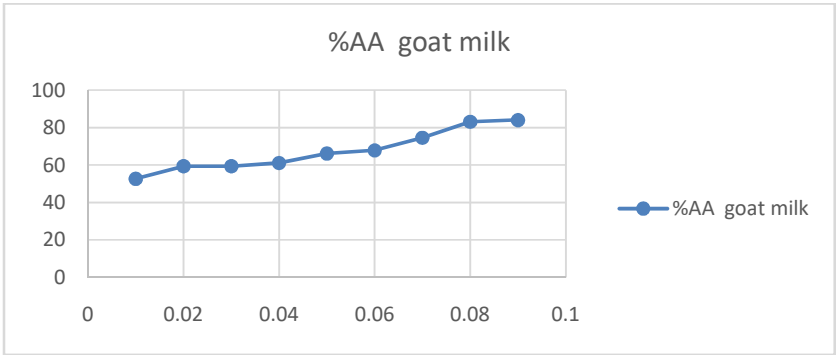

Figure 4: Increase in percent antioxidant activity with increase in concentration of *goat* milk

Calculations of Ic50 for goat milk = Max. – ½ (Max.-Min.)
=84.01-1/2(84.01-52.62)
=68.32mg/ml
Ic50 value corresponding to *goat* milk is = 0.064mg/ml.

Table No. 3 Optical density and percent antioxidant activity for *Cow* milk

(O.D. of Black DPPH = 0.591)

Conc.mg/ml	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09
O.D. of cow milk	0.27	0.25	0.20	0.19	0.18	0.12	0.10	0.8	0.8
%AAcow milk	54.31	57.69	66.15	67.85	69.54	79.69	83.07	82.01	82.01

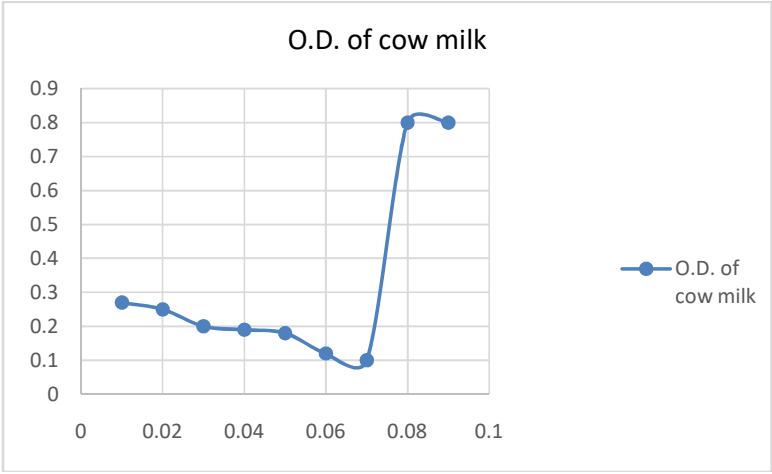

Figure 5: Decrease in optical density of sample with increase in concentration of *cow* milk

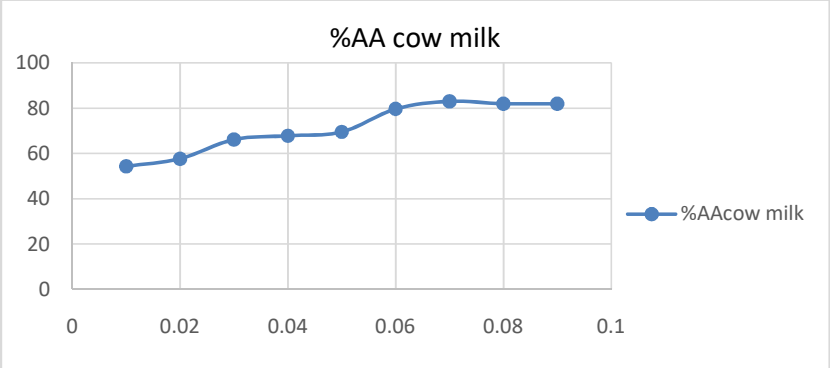

Figure 6: Increase in percent antioxidant activity with increase in concentration of *cow* milk

Calculations of Ic50 for cow milk = Max. – ½ (Max.-Min.)
= 82.01-1/2(82.01-54.31)
= 69.60mg/ml

Ic50 value corresponding to *cow* milk is = 0.068mg/ml.

Table No. 4: Optical density and percent antioxidant activity for *Donkey* milk

(O.D. of Black DPPH = 0.591)

Conc.mg/ml	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09
O.D. of donkey milk	0.35	0.32	0.30	0.29	0.28	0.26	0.25	0.24	0.22
%AA donkey milk	40.77	45.85	49.23	50.93	52.62	56.00	57.69	59.39	62.77

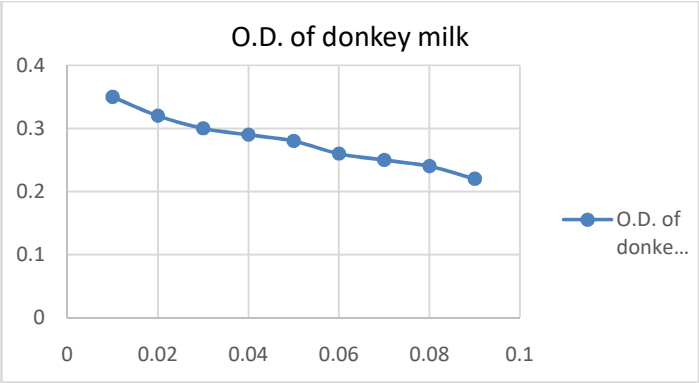

Figure 7: Decrease in optical density of sample with increase in concentration of *donkey* milk

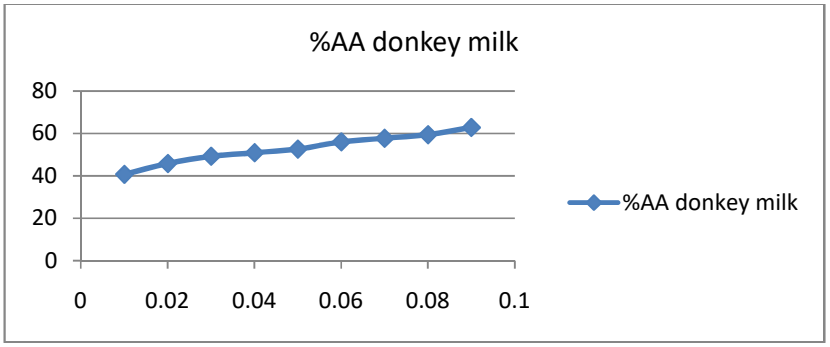

Figure 8: Increase in percent antioxidant activity with increase in concentration of *donkey* milk

Calculations of Ic50 for donkey milk= Max. – ½ (Max.-Min.)
=62.77-1/2(62.77-40.77)
=51.77mg/ml

Ic50 value corresponding to *donkey* milk is = 0.045mg/ml.

Conclusion

The results obtained for the antioxidant assay by DPPH for milk of cow, goat, buffalo and donkey are reported. Remarkable decrease in O. D. value of milk samples were observed from the graph, showed antioxidant activity. The IC_{50} value for milk of cow, goat, buffalo and donkey were found to be 0.041 mg/ml, 0.064 mg/ml 0.068 mg/ml and 0.045 mg/ml respectively.

Acknowledgement

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Refereces

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COMPARATIVE STUDY OF THE NEUTRALIZATION POWER OF ANTACIDS IN PHARMACEUTICAL SAMPLES BY ACIDIMETRY

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ABSTRACT

Increased level of acid in stomach (acidity) is the chief problem of human being all over the world. To decrease the acidity antacid either directly neutralize acidity by increasing the pH or reduce the secretion of acid by gastric cells. When gastric hydrochloric acid reaches the nerves in the gastrointestinal mucosa, they signal ache to the central nervous system, In addition to the lessening of acidity, antacids also changes the prostaglandins profile produced by gastroduodenal mucosa. Antacids are taken orally to relive heartburn. Although reactions to any drug may vary from person to person, those medicines that contain aluminium or calcium are the probable to cause constipation whereas those contain magnesium are likeliest to cause diarrhea. The aim of this work is to find out which antacid neutralize the most stomach acid from different pharmaceutical samples by acid base titrimetry, this method is simple and fast and can be used for comparison of neutralization power of antacids.

Keywords: *acidity, antacid, constipation, gastric cells, gastrointestinal mucosa, titrimetry.*

Introduction

Acidity is very common problem and often can be cured by using antacids [1]. The stomach has an acidic interior produced by dilute HCl, "stomach acid", which insures proper digestion. When the acid level of the stomach becomes high enough to cause discomfort, brought due the intake of certain types of food, an antacid formulation can be given to neutralize the excess stomach acid.Self-diagnosis of indigestion may carry some hazards, because causes can vary from a minor dietary indiscretion to a peptic ulcer. Misdiagnosis can be fatal. A bleeding ulcer can be life threatening. It is for this reason that the histamine H2 antagonists including cimetidine (Tagamate), famotidine (Pepcid), and ranitidine (Zantac), and the proton pump inhibitor(PPI) omeprazole (Prilosec) were made available over the world. These drugs stop the production of stomach acid and provide longer lasting relief but they do notneutralize acid already present in the stomach. Antacid tablets dissolved in water are also used as a temporary remedy to sooth pain induced by lachrymatory agents, commonly known as tear gas.

The antacids can be categorized into two main types, one is systemic antacids and another one is non-systemic antacids. Systemic antacid is the one that undergoes complete systemic absorption following oral ingestion. The non -systemic antacids are the one that does not undergo systemic absorption following the oral ingestion. The mostly used non systemic antacids are aluminium hydroxide, aluminium phosphate, magnesium trisilicate, magnesium hydroxide, magnesium carbonate and calcium carbonate[2, 3]. The systemic antacid most commonly used is sodium bicarbonate. There occurs no systemic absorption of the bases among non-systemic antacids, because the salt formed with combination of the gastric acid combines with the bicarbonate in the intestine to form the original base which will be excreted in the feces [4, 5]. Table 1shows some of the most common active ingredients in antacid tablets. These compounds neutralize stomach acid to produce salts and water [6]. Antacid tablet contains $NaHCO_3$, $MgCO_3$, $Mg(OH)_2$, $Al(OH)_3$, to neutralize the acid in the stomach, When required , alkali equivalent of the antacid tablet is determined by preparing a suitable solution of the antacid tablet and titrating it with a standard HCl solution. The acid neutralizing power of antacid tablet is the amount of acid that it can neutralize. This capacity can be determined by a simple experimental technique called neutralization reaction between acid and base. A known amount of antacid is dissolved in an excess of HCl, and then back titration of excess acid with standardized NaOH solution. When the reaction completes, the number of moles of

acid that was added to the antacid sample is equal to the number of moles of base present, NaOH plus the antacid. It is simple acid base neutralization. Alternatively the determination may be carried out by dissolving the definite weight of tablets in a measured quantity of HCl and back titration with standard alkali.

Oxalic acid is a primary standard acid, so standardization of an alkali solution with oxalic acid is possible. Sodium hydroxide is a strong base, so use of phenolphthalein as an indicator is possible and the color change is sharp. This is the advantage of this procedure. In our present study, we attempted to compare the activity of the different antacid tablets. Formulation by using acid-base neutralization reaction studies. Most of the antacid contains carbonates the neutralization reaction produces carbon dioxide. Because CO_2 dissolves in water to produce carbonic acid, H_2CO_3, it can cause results to be off. CO_2 is removed by heating the solution just below boiling for about 5 minutes to alleviate this problem [7].

Table 1: Common antacids and it's mechanism of action through neutralizing reaction with acid

Compound	Chemical Formula	Chemical Reaction
Sodium bicarbonate	$NaHCO_3$	$NaHCO_3$ (aq) + HCl (aq) = NaCl (aq) + H_2O (l) + CO_2 (g)
Magnesium hydroxide	$Mg(OH)_2$	$Mg(OH)_2$ (s) + 2HCl (aq) = $MgCl_2$ (aq) + $2H_2O$ (1)
Magnesium carbonate	$MgCO_3$	$MgCO_3$ (s) + 2HCl (aq) = $MgCl_2$ (aq) + H_2O (1) + CO_2 (g)
Calcium carbonate	$CaCO_3$	$CaCO_3$ (s) + 2HCl (aq) = $CaCl_2$ (aq) + H_2O (l) + CO_2 (g)
Aluminum hydroxide	$Al(OH)_3$	$Al(OH)_3$ (s) + 3HCl (aq) = $AlCl_3$ (aq) + $3(H_2O)$ (l)

Materials and Methods

Chemical Reagents: Analytical grade reagents were: 0.1 N HCl solution, 0.1N NaOH solution, 0.1 N Oxalic acid solution, Phenolphthalein indicator, Antacid sample solution, Distilled water.

Apparatus: Volumetric flask, funnel, beakers, Measuring cylinder, burette, pipette and stirrer used were of Pyrex type and were used by thorough washing with chromic acid and water.

Experimental

1. Commercially available conc. HCl was 11.2 N and prepared 1 litre of 0.1 N solution.
2. Prepared 0.1 N NaOH by Dissolving 4 gm. NaOH in 1 litre water.
3. Prepared standard 0.1 N oxalic acid solution by weighing 1.575 gm. of itand dissolved in water to get 250 ml solution.
4. Prepared phenolphthalein indicator.

Sample Information: The sixdifferent antacid tablets were purchased from a local drug shop[Drug Lic.No.20-343946] located atAkot in Akoladistrict. Table 2 shows labeled information of different brands and Table 3 shows content in each drug sample.

Experimental Design: This method is based on simple acid base neutralization reaction.

A) Standardization of NaOH Solution:25 ml of standard oxalic acid was pipetted out in a 250 ml of conical flask, 25 ml of distilled water was added and two drops of phenolphthalein indicator was added to it. It was then titrated with NaOH solution from a burette until the color of the solution turned pink; and strength of NaOH is calculatedwhich found to be 0.0625 N.

B) Standardization of HCl Solution: 25 ml ofHCl solution was pipetted out in a 250 ml of conical flask , 25 ml of distilled water and two drops indicator wasalso added to it and then titrated with NaOH solution until a pink color appeared; and strength of HCl is measuredand found to be 0.1375 N.

C) Antacids tablet was crushed in mortar and pestle, powdered form of various samples weighed 1 gm each of them. This 1 gmsample was transferred in titration flask. Then 50 ml standard HCl was added to the weighed sample taken in titration flask. The acid should be in excess so that it could neutralize all the alkaline compound of the tablet. The flask was warm to dissolve most of powder. Two drops of phenolphthalein was added and titrated each of this solution against standardized NaOH solution until end point is obtained.

Table 2: Label information of antacid tablets

S.N.	Brand name	Batch no.	Mfg. Lic. No	Mfg. Date	Exp. Date	Manufactured by
E	Aciloc	BN20100	G/1500	Feb.2020	Jul.2022	CadilaPharm.Ltd.
A	Gelusil	919080 M	PD/198-A	Nov.2019	Oct.2021	Pfizer Limited
C	OMEE	20280033	L/MNB/05/103	Jan.2020	Dec.2021	AlkemLab.Ltd.
B	PFANTO-D	RHC-045/19	MNB/18/1041	Apr.2019	Mar.2021	RHC Limited
D	RABEZ-D	GC19946A	405//DRmgf/2017	Nov.2019	Apr.2021	Hetero Healthcare Ltd.
F	Rantac	DR39200	G/25A/4124-A	Aug.2019	Jul.2021	J.B.Chemicals Ltd.

Table 3: Contents of sample of antacids

S.N.	Brand name	Contains
1	Aciloc	Ranitidine Hydrochloride I.P 167.4 mg
2	Gelusil	Activated dimethicone I.P.50 mg, Magnesium Hydroxide I.P. 250 mg, Dried Aluminium Hydroxide I.P.250 mg, Magnesium Aluminium Silicate Hydrate 50 mg
3	OMEE	Omeprazole I.P. 20 mg
4	PFANTO-D	Pantoprazole Sodium Sysquihydrate 40 mg , Domperidone I.P. 30 mg
5	RABEZ-D	Rabeprazole Sodium I.P. 20 mg , Domperidone I.P. 30 mg
6	Rantac	Ranitidine Hydrochloride I.P.150 mg

Results

Interactions between oral antacid neutralizing effects and gastric secretory function are eccentric.It was found that all the samples have different neutralization action. The most effective antacid out of the taken sample was Gelusil and less effective was Rantac. Table 4 shows the observations of different formulation. The trend of neutralization action is as follows:

Gelusil> PFANTO-D > OMEE > REBEZ-D > Aciloc > Rantac (Figure1)

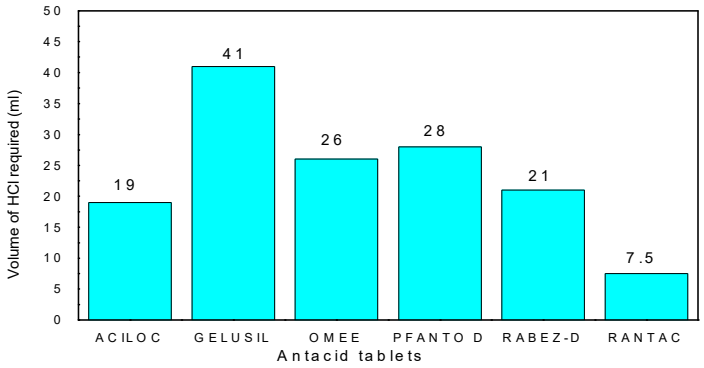

Figure 1: Acid neutralizing capacity of different antacid tablets

Table 4: Observation Table

S.N.	Sample	Weight of antacid powder (g)	Vol.of 0.1 N HCl Added (ml) = V ml	Vol. of 0.1 N NaOH used to neutralize unreacted acid (ml) =V1 ml	Volume of HCl used to neutralize antacid (V-V1) ml
1	Aciloc	1	50	31	50-31=19
2	Gelusil	1	50	9	50-9=41
3	OMEE	1	50	24	50-24=26
4	PFANTO-D	1	50	22	50-22=28
5	RABEZ-D	1	50	29	50-29=21
6	Rantac	1	50	42.5	50-42.5=7.5

Discussion

The key properties of antacid are; a slight dose of antacid should neutralize an sufficient volume of gastric acid, the maximum neutralizing outcome should be achieved quickly, the action should be maintained for a adequate length of time and an antacid should preferably produce prolonged neutralization when administered in tolerable amounts[8].The measure of the antacid "capability" of a preparation in terms of hydrochloric acid units with which physician is familiar and also in terms of pH values [9]. Theoretically the ideal neutralization is the maintenance of gastric contents at a pH of 3.0 to 5.0 at which level pepsin activity is significantly reduced [10].

Conclusion

Antacid are commonly used to help the neutralization of stomach acid. The action of antacids facts on that a base reacts with acid to form a salt and water.Antacids are takenby mouth to relieve heartburn, the major symptoms of gastrous, esophageal reflux disease or acid indigestion. Treatment with antacid alone is symptotic and only justified for minor symptoms. The information gained from this work will help people to know which antacid they should look for in the pharmacy stores. It will also let them know which antacid will give them most relief. This is in turn could also save money of patients and provide better health. This study may help in further research work such as design and discovery of new antacid formulation.

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The authors proudly acknowledge to the PG Department of Chemistry, Government Vidarbha Institute of Science and humanities, Amravati (MS) for valuable supports of instruments and equipment's during period of laboratory work.

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EXTRACTION OF AMINO ACIDS FROM DROPPINGS OF SILKWORM *BOMBYX MORI*

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ABSTRACT

Silkworm is a monophagous insect, which derives required nutrients from mulberry (Morus alba L., Moraceae) leaves. However, approximately 60% of the leaves are excreted without being digested. Silkworm droppings are therefore likely composed of undigested mulberry leaves and various bio-transformed constituents due to enzymes and microbes in the gut of the silkworm. These dropping therefore consider as one of the by-products of sericulture, containing, nutrients and biomolecules. In this paper, we tried to Separate amino acids from silkworm faeces and also from mulberry leaf extract by paper chromatography following the standard method. Calculating the Rf value we found that in the mulberry leaf extract glycine was 0.11, 0.12, 0.11, whilein silkworm droppings it was 0.13, 0.12 which is in maximum amount, followed by alanine, cystine, histidine, lysine, proline, methionine, serine, threonine, valine, tryptophan etc. About 13 amino acids were recorded both in mulberry leaf extract and in silkworm droppings. It is interesting to found that glycine is excreted in higher amount as it is involved in the biosynthesis of fibroin and abundantly accumulated in the silkworm body, which make the silkworm dropping as an important by-product of sericulture.

Keywords: *Silkwormdroppings, Monophagous, amino acid, Chromatography,Centrifugation*

Introduction

Sericulture is a silk producing agro-industry and India is a second largest silk producing country in the World next to China. Maximum quantity of silk, about 95%produced in the world is the mulberry silk which is obtained from mulberry silkworm (*Bombyx mori)* and can cultivated on mulberry plants. Every year 40-50%of the total silk and silk products are exported from India to Europe, USA and morethan 15 countries. Mulberry silkworm mainly feeds on leaves of mulberry plant *Letifera indicia*.The silkworm, Bombyx mori L. (Bombicidae: Lepidoptera), basically a nocturnal moth, is a native of China but has long been domesticated throughout the world largely in the temperate and sub-tropical regions for its fine cocoon filaments.It is a monophagous insect, which derives required nutrients from mulberry (Morus alba L., Moraceae) leaves. Supplementation of minerals such as Mg, Zn, Ca, P, K, Fe and Mn improve larval development and cocoon characters (Rathinam & Chetty, 1991; Sarkar et al., 1995). When the silkworm ingests the leaves of the mulberry, Morus alba, approximately 60% of the leaves are excreted without being digested Lee and Lee (1971). Silkworm droppings are therefore likely composed of both mulberry leave constituents and various bio-transformed constituents by enzymes or microbes in the intestine of the silkworm. In an exploratory study, we extracted mulberry leaves and silkworm droppings using paper chromatography by Tatsu Kawano *et al.*, (1989). Mulberry leaves are the primary diet ofsilkworms. In oriental medicine, parts of the mulberryplant are used to treat or protect against several diseases.Proper utilization of sericulture and silk waste adds a value of up to 40% to the silk industry. In the silkworm larva, the nitrogenous waste products of metabolism are mainly excreted as urine, together with faecal pellets.

Silkworm feces act as a raw material for variety of products such as paste chlorophyll, sodium copper chlorophyllin, pectin, phytol, carotene and triacontanol, which are used in the pharmaceutical and food industries (Raju, 1996). The feces have been found to contain solanesol, a highly valued precursor for many cardiac drugs (Babu, 1994). Chlorophyll extracted from the feces of silkworm is used as a medicine for gastric disorders such as ulcer and hepatitis. It is also used to treat liver and blood diseases (Rajiv & Vijaykumar, 1996;

Koul et al., 1994).Pectin from silkworm feces reduces blood triglyceride and blood cholesterol (Raju, 1996). Phytol extracted from silkworm feces is used in the preparation of vitamin E, K, and carotene as a source of vitamin A (Rajiv & Vijaykumar, 1996; Koul et al., 1994).

Material &Method

The healthy silkworms droppings were collected at the Vth instar stages and the fresh mulberry leaves from the local farmers in Akola district from villages as Babhulgaon, Adgaon, Shirla, Deulgaun,Wadegaon and Patur.The fresh mulberry leaves were washed with distilled water and crushed in the pestle mortal and filtered by the muslin cloth. The filtrate was diluted by distilled water 1: 2.5 proportion and centrifuged at 600 rpm for 2 mins. The supernatant was used for finding the content of amino acids by paper chromatographyBong-Hee Sohn et.al (2009).Amino acids from the silkworm faeces wereextracted as suggested by Tatsu Kawano *et al.*, (1989). For the extraction of amino acids, the 10 gms of silkworm faeces dissolved in 250ml of distilled water at 20℃ and centrifugation was done in four steps. While in every step the supernatant is used for fractionation of amino acids. Thefraction IV is used for paper chromatography, Tatsu Kawano *et.al*., (1989)

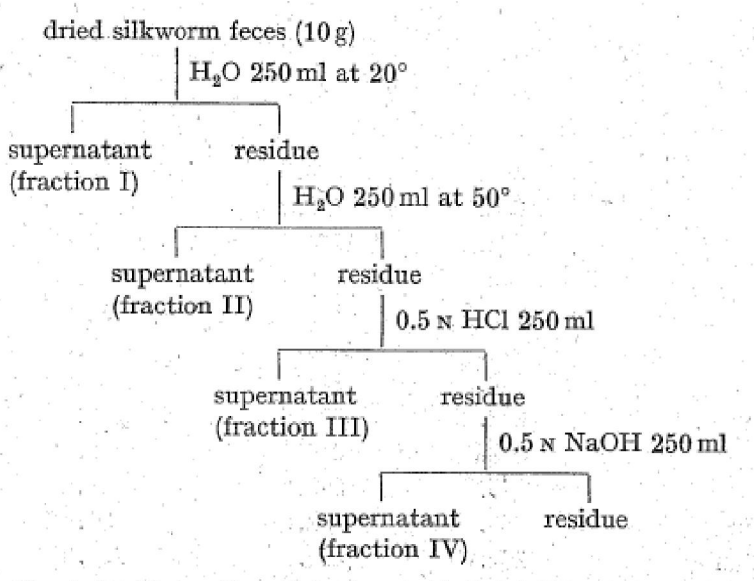
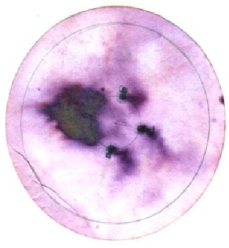


Chart 1. Extraction of Amino Acids from Silkworm Feces

Results& Discussion

Separation of amino acids from Mulberry Leaf Extract: Chromatography Photo plate -I

	
Many amino acids are recorded from leaf extract	**Glycine was recorded in maximum quantity**

Separation of different amino acids from extract of silkworm Droppings:
Photo Plate– II

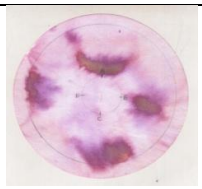
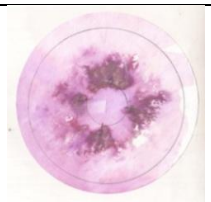
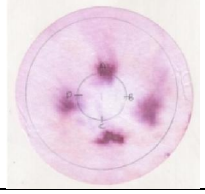
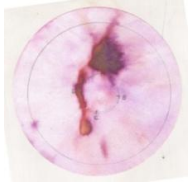
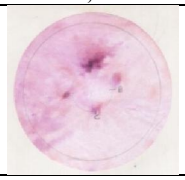
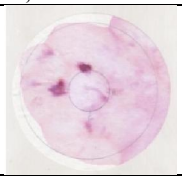
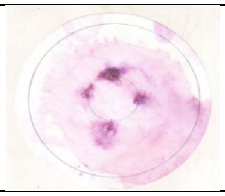
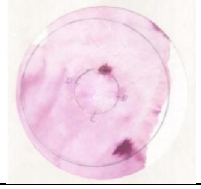
	
Valine,Glutamic acid, Proline are recorded	**Lysine, Valine, Prolineare recorded**
	
Valine, Aspartic acid, Proline are recorded	**Alanine, Valine are recorded**
	
Alanine	**Proline, Aspartic acid**
	
Lysine,methionine are recorded	**Glutamic acid, Glycine are recorded**

Table 1: The Amino Acids extracted from mulberry leaf and from Silkworm Droppings:

Sr. no.	Amino Acids	Standard RfValues	Rf values of leaf extract	Rf value of silkworm faeces	Amino acids recorded
1	L-Alanine	0.38	0.36	0.36	Present
2	L-Arginine	0.20	-	-	Absent
3	L-Aspartic Acid	0.50	0.50	0.48	Present
4	L-Cystine	0.24	0.23	0.21-	Present
5	L-Glutamic acid	0.40	0.2	0.4	Present
6	Glycine	0.13	0.11, 0.12,0.11	0.13, 0.12	obtained repeatedly
7	L-Histidine	0.30	0.36	0.33,0.31	obtained repeatedly
8	L-Isoleucine	0.26	-	-	Absent
9	L-Leucine	0.11	-	-	Absent
10	L-Lysine	0.72	0.58	0.72	Present
11	L-Methionine	0.73	0.58	0.72	Present
12	Phenylalanine	0.14	-	-	Absent
13	L-Proline	0.55	0.54,0.58, 0.54	0.54	obtained repeatedly
14	L-Serine	0.68	0.64	0.67	Present
15	L-Threonine	0.43	0.45	0.47	Present
16	L-Tyrosine	0.27	-	-	Absent
17	L-Tryptophan	0.35	0.36	0.31	Present
18	L-Valine	0.66	0.58	0.63, 0.63	obtained repeatedly

According to photo-plate 1 and table 1 from the mulberry leaf extract we recorded many amino acids were present after calculating the Rf valve we found that glycine (0.11,0.12,0.11) is present in maximum amount in the leaf extract,followed by alanine (0.36), cystine (0.23), histidine (0.36), lysine (0.58), proline (0.54,0.58), methionine (0.58), serine (0.64), threonine (0.45), valine (0.58), tryptophan (0.36). From the silkworm droppings extract we recorded many amino acidswere present as in photo-plate 2 and table 1. After calculating the Rf value we found that glycine(0.13,0.12), histidine (0.33,0.31), valine (0.63), proline (0.54) are found to be in maximum quantity in the faeces extract followed by alanine (0.36), aspartic acid (0.48), cystine (0.21), glutamicacid (0.4), lysine (0.72), methionine (0.72), serine (0.67), threonine (0.47), tryptophan (0.31).

Silkworm droppings are therefore likely composed of undigested mulberry leaves and various bio-transformed constituents due to enzymes and microbes in the gut of the silkworm. These dropping therefore consider as one of the by-products of sericulture, containing, nutrients and biomolecules.Excretion forms an important factor for the balance of nitrogen in the body. The excretionof nitrogenous waste products has been studied in a number of insects Gang (1997), According to Tatsu Kawano *et al.*, (1969)Eighteen kinds of the free L-amino acids found in extract and hydrolysates of fasted silkworm feces, and those in the extract of mulberry leaves were determined by microbiological assay.In the present study about 13 amino acids are recorded from the silkworm *B. mori* feces extract.The amount of Histidine and Serine in the extracts was equal to that in the hydrolysates and the amount of the others in the hydrolysates was more than that in the extractsLee (1994), Yen *et al.,* (1996).

It is interesting to found that Glycine is excreted in higher amount as it is involved in the biosynthesis of fibroin (silk protein) and abundantly accumulated in the silkworm haemolymph and whole body, reported by Tatsu Kawano *et al.*, (1969). Bong *et.al.*(2007) also reported Amino acid content from dried feces of silkworm. According to his report we also found Glycine, alanine, serine and other amino acids from silkworm droppings.Inthis study an attempt has been made to highlight the potential of silkworms as a medicinal insect. From the recorded results the content of amino acids in Seri-waste and faecal extract suggests that it has considerable potential for many industries and may be used as the raw material.

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SYNTHESIS, SPECTRAL CHARACTERIZATION, THERMAL AND BIOLOGICAL STUDIES OF Cr(III), Mn(III) Fe(III) and VO(IV) COMPLEXES WITH SCHIFF BASE (E)-4-BROMO-2-(1-((2,4-DIMETHYLPHENYL)IMINO)ETHYL)PHENOL

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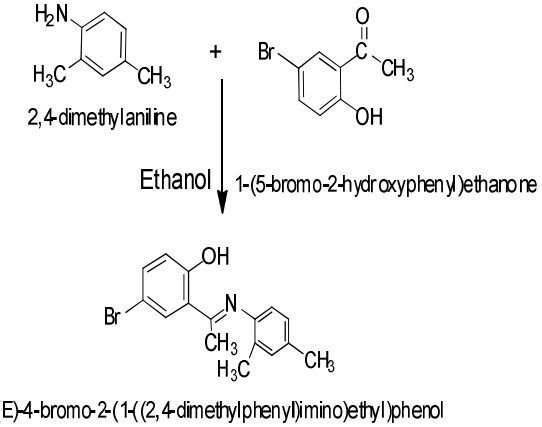
ABSTRACT

Schiff base complexes of Cr(III), Mn(III) Fe(III) and VO(IV) with 4-Bromo-2-(1-((2,4-dimethylphenyl) imino)ethyl) phenol have been prepared. Various IR, NMR, UV-visible and ESR techniques spectroscopically characterized the compounds. The molar conductance data of all the complexes indicated their non-electrolyte nature. The spectroscopic data point out the legating ability of the ligand towards different metal ions and the geometries of the synthesized complexes. Thermogravimetric analysis (TGA) of the compounds have been carried out, starting from room temperature to 800^{0}C at a linear rate of heating 10^{0}C per minute. The thermograms were analysed and from the change in mass, activation energy, frequency factor, entropy of activation and free energy parameters of decomposition were calculated. The synthesized Schiff base ligand and its complexes were tested for their antimicrobial activity against E. Coli, S. aureus, S. epidermis, and K. pneumoniea.

Keywords: *2,4-dimethylaniline, IR, UV-visible, ESR, Biological activities.*

1. Introduction

Schiff bases, the condensation products of amines with active carbonyl compounds, contain azomethine (>C=N) group as an active donor site for the transition and inner transition metals. These coordination complexes have created significant interest to chemist and pharmaceutical researchers for several decades due to their various biological and pharmaceutically applications. The synthesis of transition metal complexes with Schiff bases of nitrogen and oxygen donor has stimulated interest due to their vast variety of biological activities ranging from pharmacological, antitumour, fungicide, bactericide, anti-inflammatory, and antiviral activities[1-4]. As part of our investigation the present work describes the synthesis of Cr(III), Mn(III) Fe(III) and VO(IV) metal complexes with the Schiff base ligand LH derived from 2,4-dimethylaniline and 4-Bromoacetophenone **(Scheme.1)**. The coordinating behavior of the ligand towards transition metal ions was investigated by spectral and thermal techniques and their biological activities by antimicrobial screenings of compounds.



Scheme 1. Synthesis of the ligand (LH).

2. Materials And Methods

2.1. Materials and physical measurements

All the chemicals were obtained from S D Fine and Aldrich and were used without further purification. The solvents were of analytical grade and purified by standard methods. The C, H, N elemental analyses were carried out at Sophisticated Analytical Instrumentation Facility (Chandigarh). The IR spectra of the ligand and complexes were recorded in the 4000-400 cm^{-1} region in KBr disks. FT-IR instrument used during research work was of Perkin-Elmer spectrophotometer (L1280032). The electronic spectra of the compounds were

recorded on a Shimadzu UV-1800 Series UV/Vis spectrophotometer. Molar conductance of the synthesized complexes was recorded in DMF (10^{-3} M) by using Equip-tronic conductivity meter. ^{1}H NMR spectra of the ligand was recorded in DMSO-d_6 solution on a Bruker 300-FT-NMR spectrophotometer. ESR spectra were recorded on a JES - FA200 ESR Spectrometer at the IIT Mumbai. The magnetic susceptibilities were recorded at room temperature by the Gouy method. Thermal analysis of complexes was carried out by heating in air at a rate of 10^0C per minute on a Rijaku-Thermo plus EVO2 thermodilatometer.

2.2. Synthesis

2.2.1. Synthesis of the ligand (LH).

The mixture of 2,4-dimethylaniline (0.01mole, 1.23ml) and 4-Bromoacetophenone and (0.01mol, 2.15) in absolute ethanol (20 mL) was reflux for 2h. The reaction mixture was allowed to cool to room temperature for half an hour. A yellow precipitate obtained was filtered off and washed with absolute ethanol.

2.2.2. Synthesis of the Cr(III), Fe(III), Mn(III) and VO(IV) complexes of ligand (LH).

An ethanolic solution of the ligand (0.002 mol) was added to an ethanolic solution of $CrCl_3.6H_2O$, $Mn(CH_3COO)_3.2H_2O$, $FeCl_3.6H_2O$, and $VO(acac)_2$ (0.002 mol) separately. The resulting mixtures were refluxed for about 4 h on a water bath. The solid product obtained on cooling was filtered, washed thoroughly with ethanol and finally with petroleum ether to remove unreacted ligand and metal salts. Finally dried and stored in vacuum over fused calcium chloride (yield 72-76%).

2.3. Antimicrobial activity

Antimicrobial activity of each sample against the test species have been studied by measuring "zone of inhibition" near the respective spots[5]. The bacterial strains of *E. coli* (ATCC 14948), *K. pneumoniae* (MTCC 4030), *S. aureus* (ATCC 33591) and *S. epidermis* (MTCC 3086) were used in the study.

3. Results and Discussion

Condensation of 2,4-dimethylaniline and 4-Bromoacetophenone in ethanol yields the Schiff base (LH). All the complexes derived from LH are colored, non-hygroscopic solids and stable in air. They are insoluble in water, but soluble in coordinating solvents like DMF and DMSO. The molar conductance values of 10^{-3}M solutions of complexes range in the range 6-17 Ω^{-1} cm^2mol^{-1}, indicating the non-electrolytic nature of the complexes. The physical and analytical properties of the ligands and their complexes are depicted in Table 1.

Table 1.Analytical and physical data of the ligand and its metal complexes.

Compound	Colour	Yield (%)	%Found (calcd.)				Molecular Mass*
			C	N	H	M	
LH	Yellow	70	6.35(**60.39**)	4.35(**4.40**)	5.02(**5.07**)	-	317
$[Cr(L)_2Cl(H_2O)]$	Dark Brown	74	50.14(**51.95**)	3.16(**3.79**)	4.41(**4.36**)	7.01(**7.03**)	740
$[Mn(L)_2(CH_3COO)].H_2O$	Cherry	73	52.94(**53.28**)	3.62(**3.66**)	4.53(**4.60**)	7.12(**7.17**)	767
$[Fe(L)_2Cl(H_2O)]$	Brown	71	51.61(**51.68**)	3.74(**3.77**)	4.32(**4.34**)	7.48(**7.51**)	744
$[VO(L)_2].H_2O$	Green	72	53.39(**53.43**)	3.85(**3.89**)	4.46(**4.48**)	7.01(**7.08**)	720

* values obtained from mass spectrum of the compounds.

3.1. IR spectra

For studying the bonding mode of asymmetric Schiff base to the metal ion in the coordination complexes, the IR spectra of the complexes were interpreted by comparing with that of the free ligand. The important assignments are given in Table 2. A broad band at 2924 cm^{-1} in the spectrum of free ligand ascribed to ν(O-H) vibration, the lowered value was due to intramolecular O-H hydrogen bonding. The

other band at 1274 cm^{-1} is assignable to *ν*(C-O) (phenolic) stretching frequency. The free ligand exhibits IR band at 1641 cm^{-1} indicates C=N stretching frequency[6,7]. The band due to intramolecular hydrogen bonded O-H disappeared in the spectra of all the complexes indicating coordination of ligand to the metal ions via the oxygen of the hydroxyl group. The appearance of new bands in between 590 and 562 cm^{-1} also support the metal-oxygen (phenolic) coordination. The band due to azomethine group in the spectrum of free ligand shifts to lower frequencies by 20-40 cm^{-1} in the spectra of all the complexes indicating the coordination of metal ion with the ligand through azomethine nitrogen atom. The new band appeared in the region 466 - 431 cm^{-1} in all the spectra may probably due to the formation of ν(M-N). An additional band is observed in the IR spectrum of oxovanadium complex at 988 cm^{-1} assigned to (V=O)[8]. New broad band observed in the spectra of all the complexes in the region 3400 – 3000 cm^{-1} is ascribed may be due to lattice or coordinated water molecules.

Table 2. Infrared frequencies (cm^{-1}) of the ligand LH and its metal complexes

Compound	H bonded-OH stretching	Coordinated water υ(OH)	υ(C=N)	υ(C-O) phenolic	υ(M-O)	υ(M-N)
LH	2924	-	1641	1274	-	-
$[Cr(L)_2Cl(H_2O)]$	-	3488	1605	1219	567	452
$[Mn(L)_2(CH_3COO)].H_2O$	-	-	1616	1220	562	431
$[Fe(L)_2Cl(H_2O)]$	-	3371	1602	1230	583	436
$[VO(L)_2].H_2O$	-	-	1621	1233	590	466

3.3. Electronic *absorption* spectra and magnetic moments

The electronic spectrum of Cr(III) complex shows band in the region 628, 490 and 278 ascribe to $^4A_2g \rightarrow {}^3T_2g$, $^4A_2g \rightarrow {}^3T_1g(F)$ and $^4A_2g \rightarrow {}^3T_1g$ (P) respectively. These values predicted the octahedral geometry for Cr(III) complex. The magnetic moment recorded at 3.85 B.M. also suggests the octahedral geometry for Cr(III) complex[9]. Mn(III) complex exhibited broad bands around 715, 588, 490 and 395 assignable to $^5B_1 \rightarrow {}^5B_2$, $^5B_1 \rightarrow {}^5A_2$, $^5B_1 \rightarrow {}^5E$ and LMCT respectively, indicating that the complex possesses a square pyramidal geometry[10]. The electronic spectrum of Fe(III) complex displays three bands at 775, 540 and 310 nm assignable to $^6A_{1g} \rightarrow {}^4T_{1g}$, $^6A_{1g} \rightarrow {}^4T_{1g}$ and charge transfer transitions respectively, indicating that the complex possesses a high spin octahedral configuration. The magnetic moment recorded at 5.47 B.M. also suggests the octahedral geometry for Fe(III) complex[11]. The UV-Vis. spectra of $[VO(L)_2].H_2O$ complex exhibits three d-d transitions at 435 nm ($^2B_2g \rightarrow {}^2A_1g$), 622 nm ($^2B_2g \rightarrow {}^2B_1g$) and 855 nm ($^2B_2g \rightarrow {}^2Eg$) which are supporting the square pyramidal geometry of the oxovanadium complex[12,13].

3.4 Mass Spectral studies of Schiff base and its complexes

The mass spectra of newly synthesised compounds were recorded and investigated. The molecular ion peaks confirmed the proposed formulae of the compounds. The mass spectrum of the ligand shows the fragment at m/z = 317 was due to the original molecular weight of the free Schiff base (LH).

Table 3. Electronic and magnetic data of LH and its complexes

Compound	ν(nm)	d-d transition	μ_{eff}(BM)	Geometry	Molar cond. (Ω^{-1} cm^2mol^{-1})
LH	220 355	$\pi \rightarrow \pi^*$ $n \rightarrow \pi^*$	-	-	-
$[Cr(L)_2Cl(H_2O)]$	628 490 278	$^4A_2g \rightarrow {}^3T_2g$ $^4A_2g \rightarrow {}^3T_1g(F)$ $^4A_2g \rightarrow {}^3T_1g$ (P)	3.85	Octahedral	12.7
$[Mn(L)_2(CH_3COO)].H_2O$	715 588 490 395	$^5B_1 \rightarrow {}^5A_1$ $^5B_1 \rightarrow {}^5A_2$ $^5B_1 \rightarrow {}^5E$ LMCT	4.78	Square Pyramidal	11.2
$[Fe(L)_2Cl(H_2O)]$	795 555 460	$^6A_{1g} \rightarrow {}^4T_{1g}$ $^6A_{1g} \rightarrow {}^4T_{2g}$ LMCT	3.93	Octahedral	16.6
$[VO(L)_2].H_2O$	855 622 435	$^2B_2g \rightarrow {}^2Eg$ $^2B_2g \rightarrow {}^2B_1g$ $^2B_2g \rightarrow {}^2A_1g$	1.83	Square Pyramidal	6.75

3.5 ESR Spectra

ESR spectrum of $[VO(L)_2].H_2O$ complex was recorded in the DMF solution at 77 K as shown in Figure 1**.** ESR data provide valuable information regarding the electronic environment of the metal and orientation of the ligand. As $g_{\|}$ = 1.951, $g\perp$=1.987, $A_{\|}$=144.26 x .

10^{-4} cm^{-1} and $A\perp$= 46 x 10^{-4} cm^{-1}, hence the relationship $g\perp<g\perp$ and $A\perp>A\perp$.The study of the parameters of the spectra suggests square pyramidal geometry for VO(IV) complex with one unpaired electron in an orbital of mostly d_{xy} character[14-15]

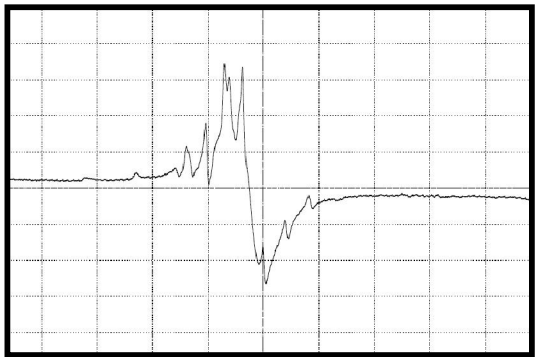


Figure 1. ESR spectra of VO(IV)complex (LNT)

3.6 *Thermogravimetric Analysis*

Thermogravimetric analysis (TGA) was performed in with heating rate 10°C min^{-1} and thermograms were recorded in the temperature range 40-800°C. The thermal data have been analyzed using Freeman Carroll and Sharp-Wentworth methods. Several kinetic parameters such as activation energy (Ea), order of reaction (n), entropy change (ΔS), apparent entropy change (ΔS*) and frequency factor (Z) were calculated[16]. The TGA curves of ligand and all the complexes are given in Figure 2. Analysis of thermograms shows one step decomposition pattern for the ligand while two to three steps for the complexes. The thermograms of the Cr(III) and Mn(III) complexes show two step decomposition pattern while Fe(III) and VO(IV) complexes decomposed in two steps. The weight loss in the temperature range 60-120°C in the thermograms of Mn(III) and VO(IV) complexes indicating the presence of one lattice water molecule. The Cr(III) and Fe(III) complexes show first decomposition step in

between 120 and 220°C with weight loss 2.12(calculated 3.04%) and 2.06(calculated 2.99%) respectively corresponding to one coordinated water molecule. The further weight losses in the thermograms of all the complexes is ascribed due to partial and complete decomposition of the organic moieties present in the complexes finally leading to the formation of stable metal oxides. The thermal stability order of the compounds on the basis of half decomposition temperatures is Fe(III) > VO(IV) > Mn(III) > Cr(III) > LH.

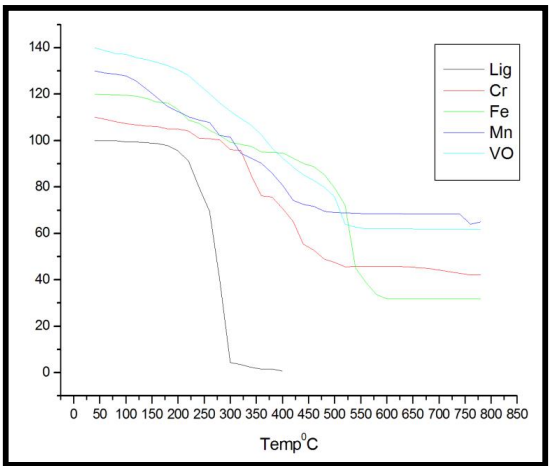


Figure 2. Thermograms of the ligand and its metal complexes.

Table 4. Thermal analytical data of the compounds

Compound	Half Decomposition Temp (^{0}C)	Ea(kj/mole^{-1})		ΔS (J)	ΔF (KJ)	Z (s^{-1})	ΔS*(KJ)	Order of Reaction (n)
		FC	SW					
LH	150	17.45	18.63	7.59	35.25	107	-10.10	0.92
$[Cr(L)_2Cl(H_2O)]$	345	17.90	18.75	7.52	71.30	115	-11.12	0.95
$[Mn(L)_2(CH_3COO)].H_2O$	350	19.45	18.71	7.67	66.15	120	-10.45	0.93
$[Fe(L)_2Cl(H_2O)]$	370	17.70	19.25	7.60	71.14	121	-11.47	0.95
$[VO(L)_2].H_2O$	360	13.27	14.24	7.57	76.54	119	-11.15	0.90

3.7Antibacterial Activity

The compounds were screened against antimicrobial activity against *E. Coli, K. pneumoniea, S. aureus* and *S. epidermis* (**Table-5)**. The study shows that the ligands have lower activity against *K. pneumoniae*. The values in mm of inhibition of bacteria growth show that the complexes were more active than their schiff base ligand. The Cr(III) and Fe(III) complexes were found to show almost high to moderate bacteriocidal (sensitive) nature against all the bacterial strains.

Table 5. Antibacterial activity of the ligand LH and its metal complexes

Compound	*E. coli* (ATCC 14948)	*K. pneumoniae* (MTCC 4030)	*S. aureus* (ATCC 33591)	*S. epidermis* (MTCC 3086)
LH	S09	S05	S10	S09
$[Cr(L)_2Cl(H_2O)]$	S10	R	S07	S11
$[Mn(L)_2(CH_3COO)].H_2O$	S12	S12	S12	S11
$[Fe(L)_2Cl(H_2O)]$	S10	R	S12	R
$[VO(L)_2].H_2O$	R	S11	R	S10

Conclusion

All the complexes are nonelectrolytic in nature with 1:2 [M:L] ratio. Coordination of ligand with the metal ion commonly through hydroxyl oxygen and azomethine nitrogen indicated its monobasic bidentate nature. The bonding of the ligand with metal was confirmed by spectroscopic measurements. Cr(III) and Fe(III) complexes exhibit octahedral geometry while Mn(III) and VO(IV) exhibits square pyramidal geometry. The activation energy calculated by the Freemann-Caroll and Sharp-Wentworth methods were in good agreement with each other. The thermal stability order of the compounds is determined from their half decomposition temperatures. The probable structures of the complexes are depicted in Fig. 3 and 4. The ligand as well as some metal complexes showed antibacterial activity against selected kind of bacteria.

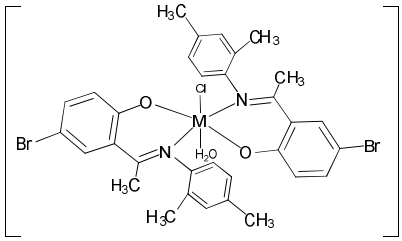
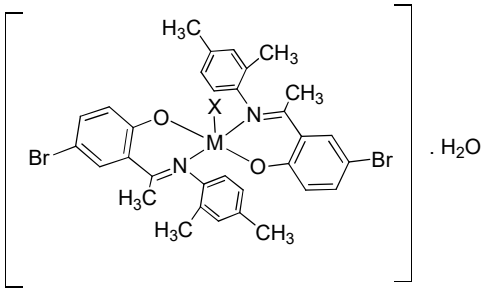


Figure 3. The probable structure for Cr(III) and Fe (III) metal complexes.



X= OAc for Mn(III)
X= =O for VO(IV)

Figure 4. The probable structure for Mn (III) and VO (IV) metal complex.

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