Original Research Article

METAL COMPLEXES OF GATIFLOXACINE: SYNTHESIS, CHARACTERIZATION AND EVALUATION OF BIOLOGICAL ACTIVITY

ABSTRACT

The antibiotic agent Gatifloxacin is well known for its drug design and coordinating ability towards metal ions. In this paper, synthesis and biological activity of Ni(II), Cu(II), Co(II), Mn(II) and Mg(II) complexes of gatifloxacin is reported. Ligand is itself prepared using o-phenylenediamine and 3-acetyl coumarin. The structure of complexes has been investigated using spectral and physiochemical properties. They were also evaluated for their in vitro antibacterial and antifungal activity. Some of the compounds displayed promising anti-bacterial and antifungal activity compared to standard drugs.

Keywords: Gatifloxacin, Drug-Metal Complexes, Coumarine derivatives, antimicrobial activity, TGA

INTRODUCTION

Metal complexes are also known as coordination complexes. The study of "coordination chemistry" is the study of "inorganic chemistry" of alkali and alkaline earth metals, transition metals, lanthanides, actinides and metalloids. Ligands are the ions or molecules surrounding the metal. Ligands are bound to the metal by coordination covalent bond. Many metal complexes plays important role in medicines. It is found that many drugs shows higher activity with metal. [1]. Many metals are essential in our diets in diverse amounts. However certain metals remain toxic in trace amounts, which can enter the body via verity of routes and often cannot be excreted leading to metal toxicity [2, 3].

Metal complexes possess some exceptional advantages which can be useful in the development and discovery of novel drugs. The centre of these metals is capable to generate pharmacophore [4]. Additionally the effect of metals can be highly specific and can be modulated by recruiting cellular processes that recognizes specific types of metal-macromolecule interactions. Metals can be useful probes of cellular functions. Metal-complexes can modify both DNA and RNA with a high degree of regiochemical, sequential and conformational specificity [5]. The current literature also shows that metallopharmaceuticals is an area of growing interest as is evident through clinical trials that are being conducted worldwide for the usage of metals in therapeutics [7]. For e.g.; clinical trials for Silver biotics have been carried out for assessing its efficacy in a wide diversity of human problems, including malaria, upper respiratory tract infections, urinary tract infections, sinusitis infections, vaginal yeast infections, eye, nose and ear infections, cuts and fungal skin infections and even for sexually transmitted diseases like gonorrhoea etc. proving it to be an antibiotic alternative at a convenient dosage [6].

Quinolones are known for their wide ranging applications in medical and life sciences. They are famous antimicrobials and possess complexation properties. The addition of fluorine atoms produce flouroquinolones which possess improved antimicrobial activities than quinolone [8]. The mechanism of fluroquinolones action involves intercalation of purine/pyrimidine of nucleic acids and inhibition of DNA gyrase which is important for DNA replication. The mechanism also involves the formation of metal complex as an intermediate. It has also been proposed that the transport of ligands into cell can be facilitated by the formation of metal complex. [9-12]

Gatifloxacin is a potent fluoroquinolone and fourth generation antibiotic. Several metal complexes of Fluoroquinolones are well known for their antibacterial, antifungal and biomimetic activities. In this menuscript, synthesis of metal complexes of Gatifloxacin and their characterization is explained. The aim of this work is to present synthesis of various Ni(II), Cu(II), Co(II), Mn(II) and Mg(II) complexes of gatifloxacin with neutral bidentate ligands of coumarine. *In vitro* antimicrobial activities against pathogenic and nonpathogenic microbial strains of synthesized compound were also evaluated. For better understanding of the interaction of drug and metal, the structures of the complexes were analysed with spectrometric studies like IR, Mass, NMR and TGA.

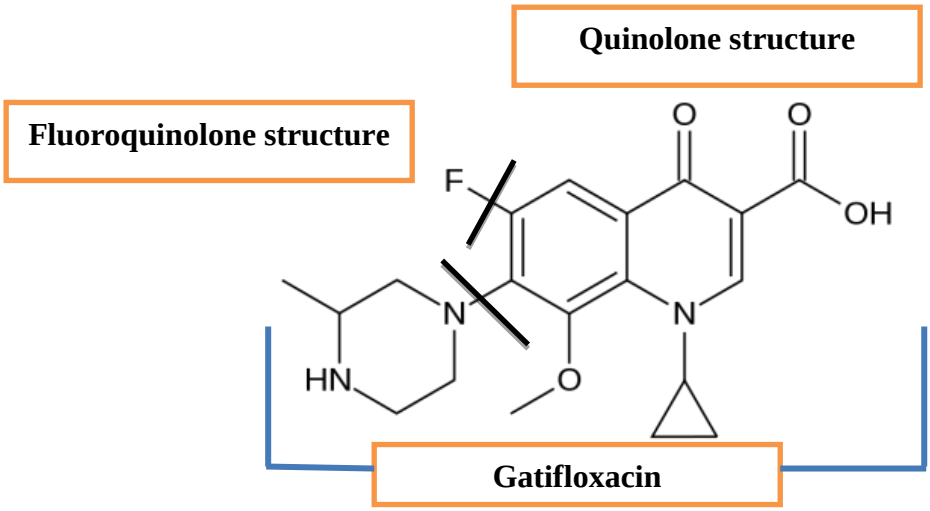


Figure I : Structure of Gatifloxacin which includes fluoroquinolone and quinolone

MATERIAL AND METHODS

All the chemicals are of analytical grade and were purified by the established methods. Melting points were determined by open capillary tubes method, purity and homogeneity of the compounds was routinely determined by thin layer chromatography on glass plates using silica gel Gas absorbent and solvent system Hexane: Ethyl acetate and DCM. Spots were visualized by iodine vapor by irradiation with UV light.

Instrumentation

The scale of the spectrum is usually marked in parts per million (ppm) of the applied field or in frequency units (Hz). ^{1}H and ^{13}C NMR spectra were recorded on Bruker DRX - 300 MHz NMR spectrometer using TMS as internal standard in CDCl3-DMSO-d6 solvent, IR spectra were recorded by Perkin Elmer spectrophotometer, Spectrum Instrument (Germany) with FTIR paragon 1000 PC software, Mass spectra were recorded on Mass spectra were scanned on a Shimadzu LCMS 2010 spectrometer using TMS as internal standard in DMSO-d6 solvent and thermogravimetric analysis or thermal gravimetric analysis (TGA) has been carried out at the Sophisticated and Instrumentation Centre for Applied Research and Training (SICART), Vallabh Vidyanagar-Gujarat. All the structures and reaction schemes are prepared with ChemBioDraw licenced version.

Synthesis of 1e (Ligand):

Materials:

Aromatic aldehyde, Ethyl aceto acetate, Pipiridine, 3-acetyl coumarin, O-Phenylin diamine, Acetic acid, and Ethanol

Method of Preparation:

Preparation of 1c (acetyl coumarine) [13, 14]:

In to a clean and dry round-bottomed flask, specific amount of aromatic aldehyde **1a,** ethylacetoacete **1b** and ethanol were taken. Piperidine was poured with constant stirring into the mixture at room temperature. Reaction progress was monitored using TLC. After

completion of the reaction, reaction mass was distilled off and poured in cool water, solid yellow precipitates separated and filtered, washed with ethanol and dried. Purification was done by crystallization from ethanol to get pure compound **1c**. Yield 85%, M.P. 129-130^{o}C.

Synthesis of 3,3'-(1,2-phenylenebis(azanylylidene))bis(ethanylidene))bis(2H-chromen-2-one) 1e [15-17]:

1c and Orthophenylene diamine **1d** was refluxed in ethanol for 4-5 hours. Reaction progress was monitored using TLC, excess ethanol was removed by distillation and suitable solvent was used to dissolve solid mass. **1c** is added into this solution and allowed to reflux for 9-10 hours. After consumption of reactant, reaction mass was distilled off and poured in cool water, precipitates fall out, filtered and dry. Purification was achieved by column chromatography using 15% methanol in chloroform to obtained pure **1e.** Yield 58%, M.P. 212-123°C.

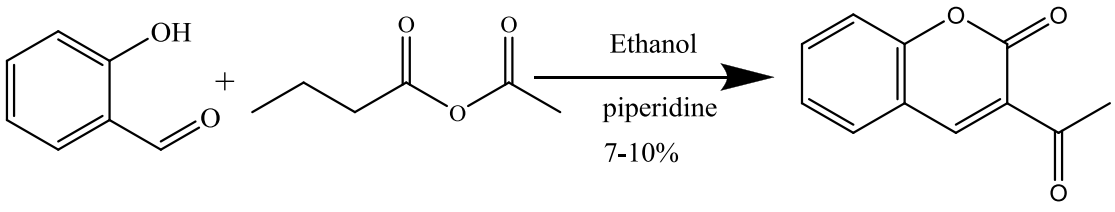
Synthesis of 1g (Metal Complex):

Materials:

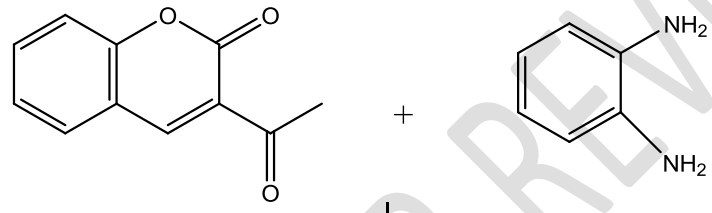
Ligand, $M(NO_3)_2$, [M=Ni,Cu,Co,Mn,Mg] and Gatifloxacine.

A hot solution of a respective metal salts in ethanol was added to a hot solution of **1e** in ethanol, the reaction mixture was refluxed. Thereafter, it was treated with sodium acetate and the resultant reaction mixture was further refluxed, and then decomposed by pouring to distilled water with stirring. The separated solid (metal complex) was allowed to settle and collected by filtration, washed several times with distilled water and then with hot ethanol. The solid complex obtained was dried in desiccators over anhydrous calcium chloride.

Reaction Scheme [1e]

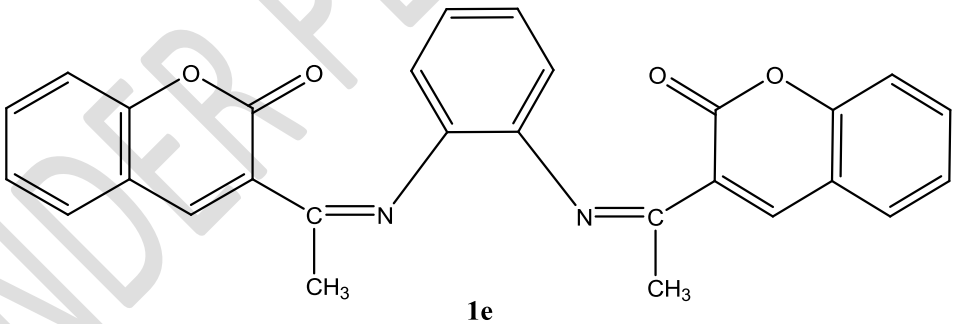


1a **1b** **1c**



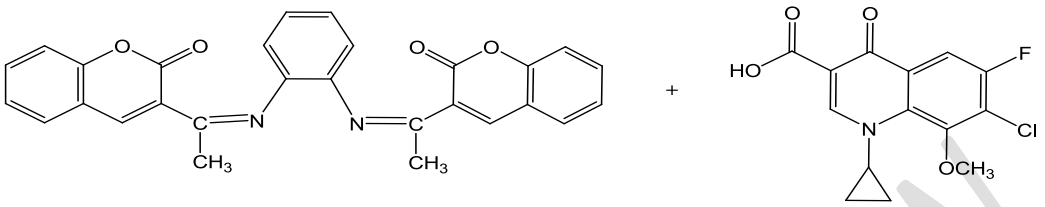
1c **1d**

Ethanol CH_3COOH



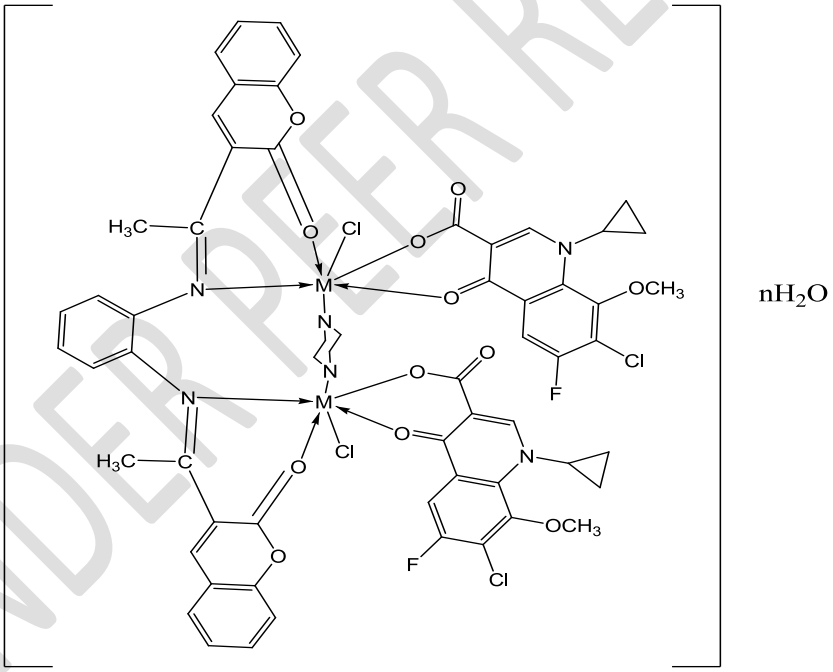
1e

Reaction Scheme [1g]



1e **1f**

$M(NO_3)_2\ nH_2O$



nH_2O

1g

Where, n= 3, 4, 5, 6, 7

M= Ni, Cu, Co, Mn, Mg

RESULTS AND DISCUSSION

Structures of all the newly synthesized ligand and metal complexes are found to be in good agreement with the spectral data like IR, ^{1}H-NMR and Mass Spectral study. Also from thermogravimetric study it is confirmed that compounds are thermostable at comparative higher temperature. While from *in-vitro* antibacterial activity, it is confirmed that compound ND-1, ND-2 and ND-4 is effective against *E.Coli* while they are showing promising activity against *S. aureus*. From antifungal data it was evaluated all the tested compounds are better antifungals except ND-2 against *A. Clavatus*.

Compounds	Colour	M.P. (^oC)	Molecular Formula	Yield (%)
Acetyl Coumarine	Light Yellow	121	$C_{11}H_8O_3$	92
Ligand (ND-1)	Brownish Yellow	>200	$C_{28}H_{20}N_2O_4$	81
Nickel Gatiflox Ligand Complex (ND-2)	Pale Green	>300	$C_{60}H_{60}Cl_4Ni_2N_2F_6O_{18}$	78
Magnesium Gatiflox Ligand Complex (ND-3)	Yellow	>300	$C_{60}H_{60}Cl_4Mg_2N_2F_6O_{18}$	67
Copper Gatiflox Ligand Complex (ND-4)	Sky Blue	>300	$C_{60}H_{60}Cl_4Cu_2N_2F_6O_{18}$	63
Cobalt Gatiflox Ligand Complex (ND-5)	Greenish Yellow	>300	$C_{60}H_{60}Cl_4Co_2N_2F_6O_{18}$	66
Manganese Gatiflox Ligand Complex (ND-6)	Brown	>300	$C_{60}H_{60}Cl_4Mn_2N_2F_6O_{18}$	59
Solvent system for thin layer chromatography technique is: Hexane: Ethylacetate: DCM (7.5:2.0:0.5)				

Table I : Characterization of synthesized compounds

In vitro antimicrobial activity

The targeted compounds were evaluated for their *in vitro* antibacterial activities against representative gram-positive and gram-negative organisms using standard techniques [18]. The minimum inhibitory concentration (MIC) values were compared with standard antibiotics. The antifungal activities of the compounds were studied against selected fungal strains; Nystatin and Greseofulvin were used as reference for inhibitory activity.

CODE NO. / Name	*E.c.*	*P.a*	*S.a.*	*S.py.*
	MTCC 443	**MTCC 1688**	**MTCC 96**	**MTCC 442**
ND-1	**100**	100	**62.5**	100
ND-2	**125**	100	**62.5**	**62.5**
ND-4	**100**	125	250	100
Gentamycin	0.05	1	0.25	0.5
Ampicillin	100	--	250	100
Chloramphenicol	50	50	50	50
Ciprofloxacin	25	25	50	50
Norfloxacin	10	10	10	10
E.c.=E. Coli(MTCC-443); *P.a.=P. Aeruginosa(MTCC-1688);* *S.a.=S. Aureus(MTCC-96);* *S.py.=S. Pyogenus(MTCC-442) ;*				

Table II: Antibacterial activity (Minimal Inhibition Concentration), MIC Value, µg/mL

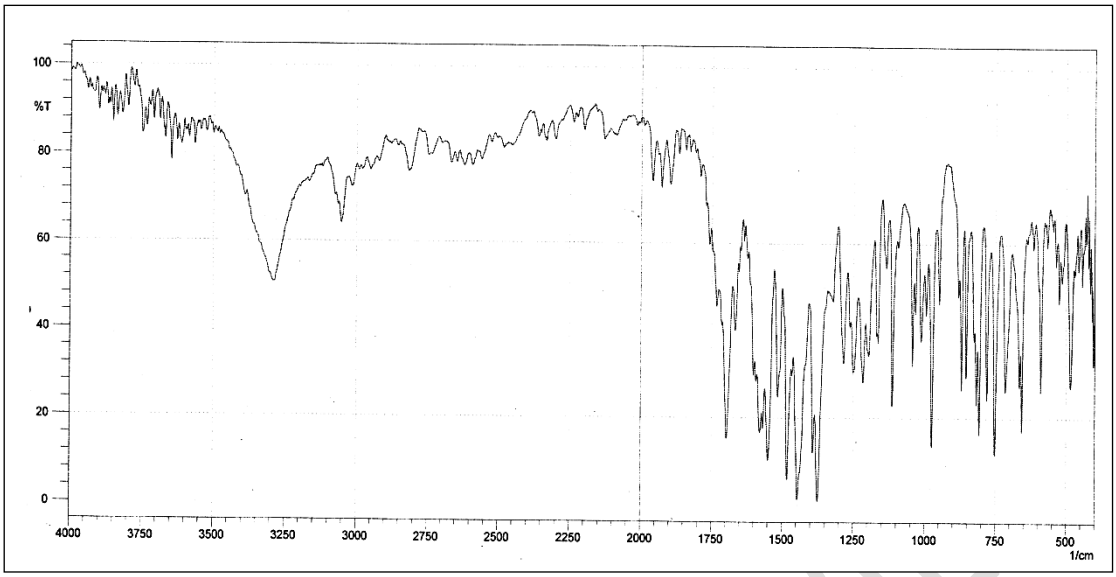


Figure II: Infrared spectrograph of ND-2

	Wavenumber cm^{-}
α,β-unsaturated C=O stretching	1600
lactone carbonyl C=O stretching	1700
C=N stretching	1550
COO(symmetry) stretching	1365
COO(asymmetry) stretching	1580
C-Cl stretching	1110
M-N stretching	750
M-O stretching	535

Table III: Analysis of Infrared spectrograph of ND-2

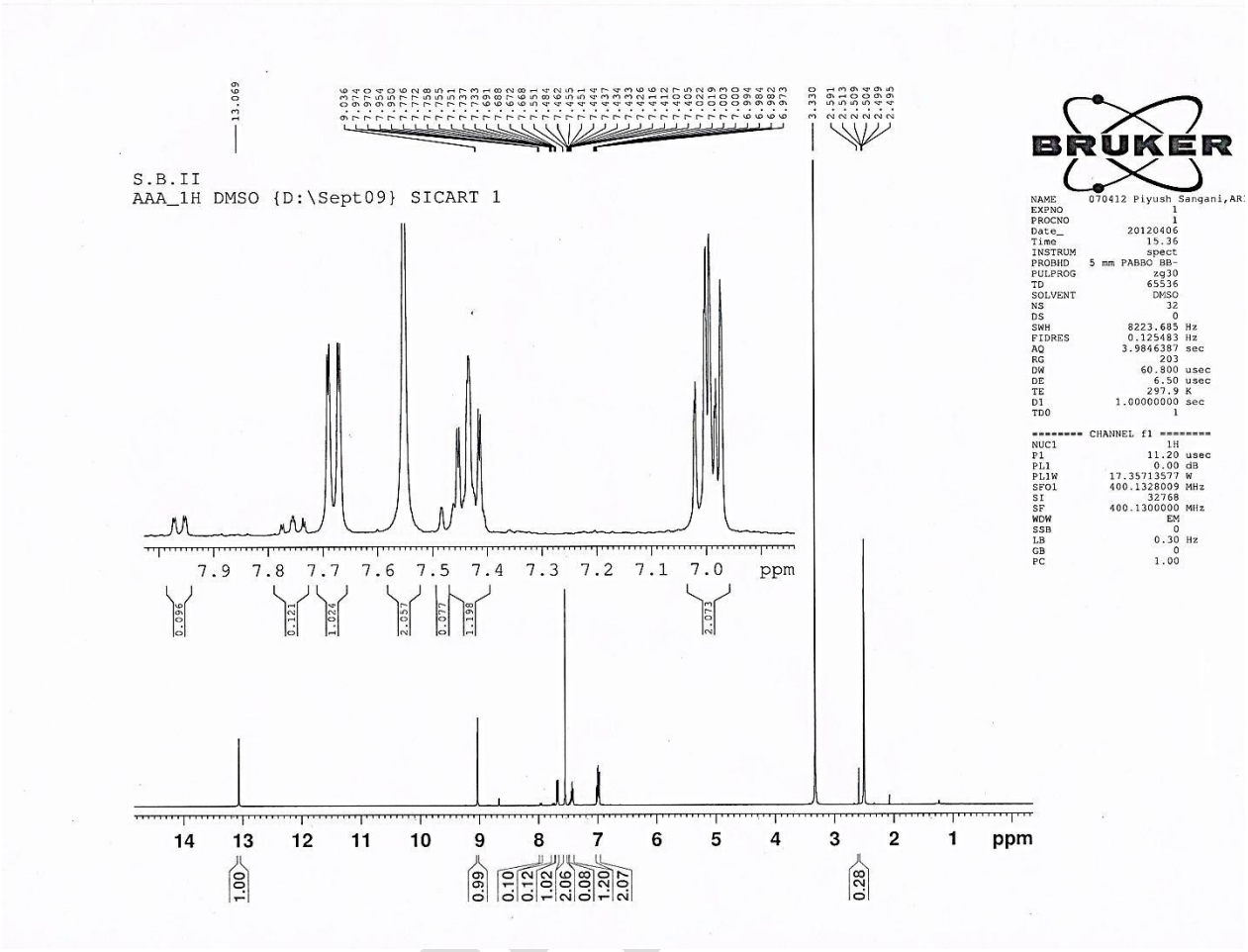


Figure III: ^{1}H Nuclear Magnetic Resonance spectrograph of ND-1

Signal Position (δ ppm)	Relative No. of Protons	Multiplicity	Inference
6.973-9.036	14	m	Ar-H
2.495-2.591	6	s	$2CH_3$

Table IV: ^{1}H NMR Spectra of Intermediate Ligand ND-1

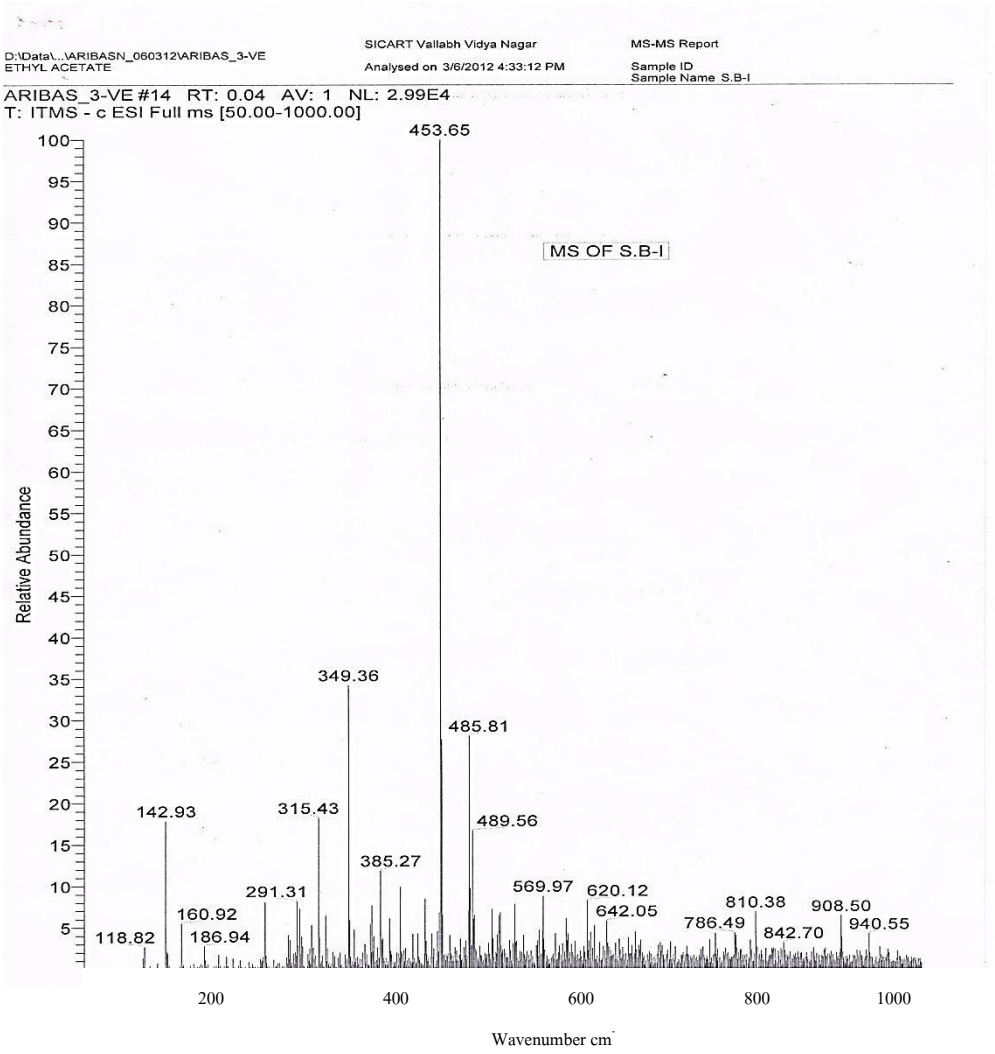


Figure IV: Mass spectra of ND-1

Observed Molecular Ion Peak: 453.65

Formula	TG range /˚c	Mass Loss% Calcd.(obs.)	Assignment
$[Cu_2(ND\text{-}1)(GF)_2(pep)]\ 6H_2O$	50-100	8.50 (8.56)	Removal of 6 mole of H_2O
ND-4	100-200	6.78 (5.81)	Removal of coordinated piperazine ring
	200-500	33.11 (35.93)	Removal of Ligand
	500-600	(6.70)	Removal of Gatigloxacine

Table V: Thermogravimetric Analysis of ND-4

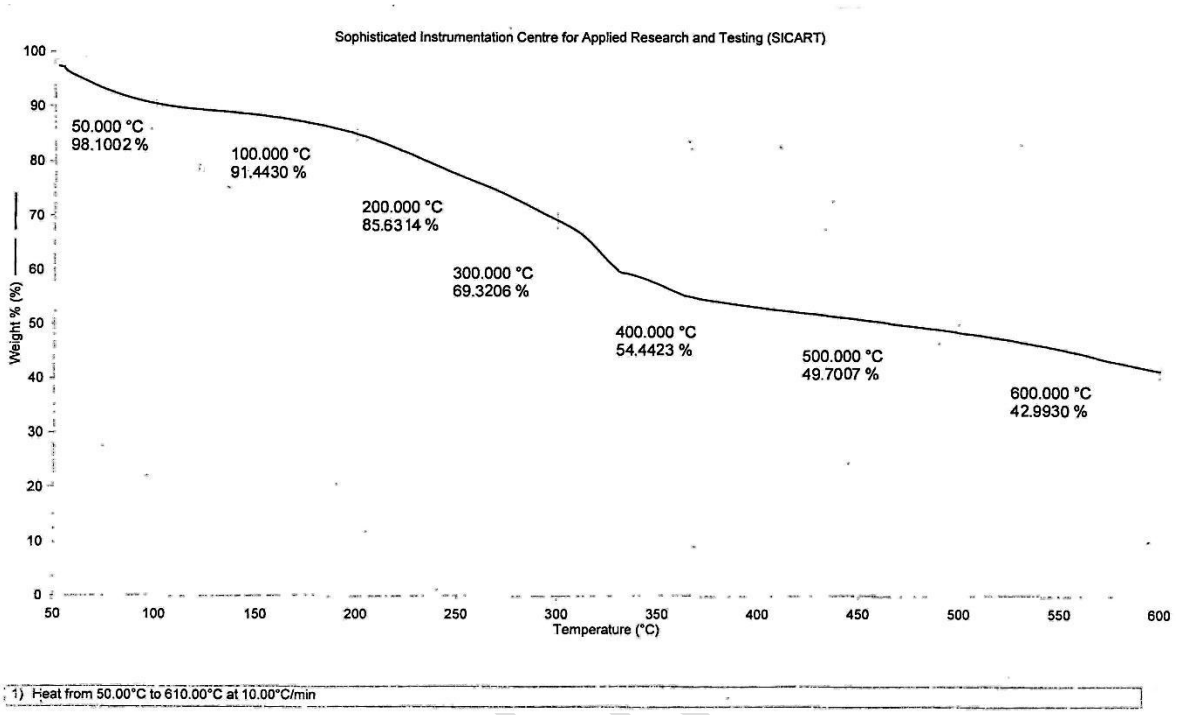


Fig V. Thermogravimetric analysis of ND-4

CONCLUSION

Variety of Drug-metal complexes were synthesized and characterized for their structure elucidation. Various chemical and spectral data supported the structures thought of. Antibacterial activity of the compound indicate that some of the compounds comparable activity against some of microbial strains while all the compounds were found to be the promising antifungals compared to respective standard drugs.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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