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Metal Complexes of Gatifloxacin: Synthesis, Characterization and Evaluation of Biological Activity

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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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 Mn(II), Co(II), Ni(II), Cu(II) and Mg(II) complexes of gatifloxacin is reported. Ligand is itself prepared using o-phenylenediamine (OPD) and 3-acetyl coumarin. The structure of complexes has been investigated using some physiochemical and spectroscopic techniques. In vitro evaluation of these complexes was also carried out for antibacterial and antimicrobial activity. Some complexes exhibited promising anti-bacterial and antimicrobial activity compared to gatifloxacin drug.

Keywords: Gatifloxacin; drug-metal complexes; coumarine derivatives; antimicrobial activity; TGA.

1. INTRODUCTION

Transition metals are capable to form various compounds via coordinate covalent bond. These compounds are structurally complex and also known as coordination complexes or metal complexes. The study of these complexes is part of inorganic chemistry and specifically known as

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"coordination chemistry". 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. Though some metals can be proved toxic to human body and need to be excreted, trace amount of these toxic metals can enter the body via different routes and metal toxicity might be developed [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]. Metals are useful to perform number of cellular functions and are highly specific. For example haemoglobin: An Iron complex is popular and performs oxygen and electron transport. In fact the therapeutic effect of metals can be modified by drafting cellular processes which can identify metal molecule interactions. Study shows that even DNA and RNA can be altered through these metal-complexes with a high degree of regiochemistry involved. It is possible to develop new complexes which possess higher specificity in their conformations and sequence [5]. Metallopharmaceuticals is being developed globally by performing advanced clinical trials to identify their therapeutic effects [6]. For e.g.; clinical trials of Platinum based anticancer drug complexes [7] and Silver biotics have been carried out for variety of infections caused in human's body [8].

Quinolones are known for their wide ranging applications in pharmaceuticals and medical sciences.Structurally, they contains quinoline ring as a core and cyclopropane attached with its nitrogen atom. At position 2, 3, 7, 8 carboxylic acid, ketone, heterocyclic ring and nitrile group are attached consecutively. They are famous antimicrobials and possess complexation properties. The addition of fluorine atoms in quinolone moiety produce fluoroquinolones which possess improved antimicrobial activities than quinolone [9]. Fluoroquinolones are broad spectrum antibiotics used against various infections [10]. Fluoroquinolones bind to subunit of DNA gyrase enzyme and prevent binding of substrate to DNA gyrase, thus prevent the formation of the enzyme substrate complex. The mRNA synthesis can be inhibited followed by inhibition of protein synthesis in bacteria. The formation of metal complex also takes place as an intermediate. Several studies shows that this metal complex can transport ligands into cell [11-14].Several metal complexes of fluoroquinolones are well known for their antibacterial, antifungal and biomimetic activities.

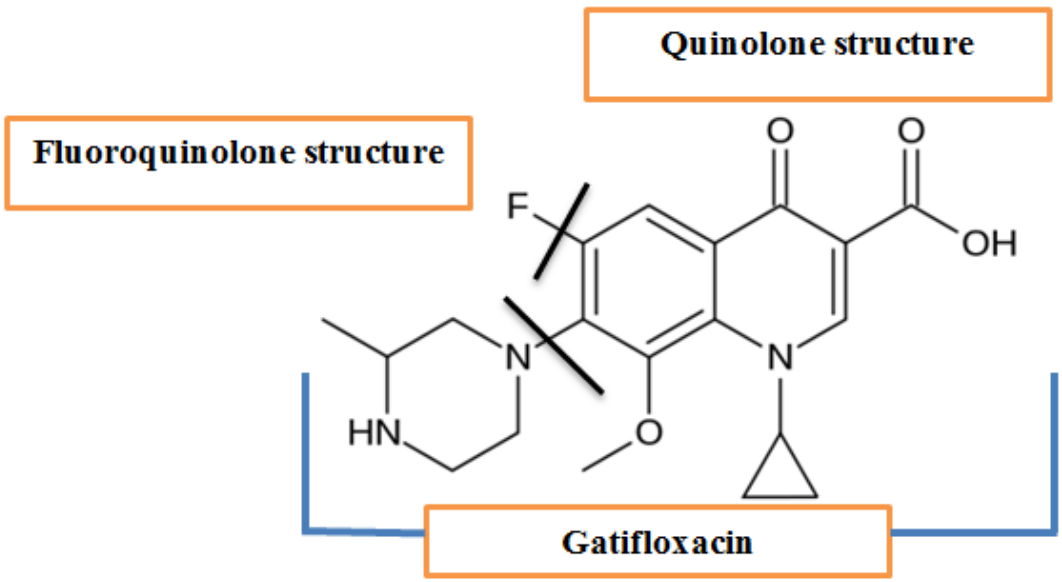


Fig. 1. Structure of Gatifloxacin which includes Fluoroquinolone and Quinolone

Gatifloxacin(1-Cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-(3-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic Acid) is a potent fluoroquinolone and fourth generation antibiotic. It possess core structure of quinolones with methoxy group at position 8. Gatifloxacin is used in ophthalmic solutions in minor to major conjunctivitis [15]. In this manuscript, synthesis and characterization of metal complexes of Gatifloxacin have explained. The aim of this work is to present synthesis of various Mg(II), Mn(II), Co(II), Ni(II), and Cu(II) complexes of gatifloxacin with neutral bidentate ligands of coumarine. Coumarines are class that possess alpha-pyrone ring fused with benzene ring and also known as their anti-inflammatory, antibacterial, antiviral properties. *In vitro* antimicrobial activities against pathogenic and non-pathogenic 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 and physicochemical studies like IR, Mass, NMR and TGA.

2. MATERIAL AND METHODS

All the chemicals are of analytical grade and used as received. Salicylaldehyde, Ethyl acetoacetate and Acetic acid purchased from Finar Chemicals. Licensed Ethanol was used as solvent and purchased from authorized domestic vendor. Piperidine and OPD were purchased from thomasbaker chemicals. Gatifloxacine in hydrated form was purchased from Tokyo Chemical Industry. Nitrates of Mg, Cu, Co, Ni and Mn were purchased from SD Finechem limited. Melting points were determined by open capillary tubes method. The reaction mixture were timely checked for TLC(Thin Layer Chromatography) to determine the purity and homogeneity. Solvent system used for TLC was Hexane: Ethyl acetate and DCM. Spots were visualized in UV light chamber.

Agar well diffusion method is widely used to evaluate the antimicrobial activity of newly discovered chemical compound. After autoclaving N-agar, molten is prepared by inoculating the *Escherichia coli* (MTCC 443) and then the plate is poured. After solidifying the plate, a hole with a diameter of 6 to 8 mm is punched aseptically with a sterile cork-borer and a volume (100 µL) of the newly discovered chemical compound (100 µg/mL) is poured in well. Then, agar plates are incubated for 24 hour at 37°C. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain is tested. Same procedure is performed for testing the antimicrobial activity of newly synthesized chemical compound against the various pathogenic microbial strains. Here we have to set one control, therefor in one well, in the place of newly discovered chemical compound,100µL sterile distilled water is poured. Incubation period is given to microorganism is as per optimum microbial growth condition as *Pseudomonas aeruginosa* (MTCC 1688) are incubated at 37 °C for 24 hours, *Staphylococcus aureus* (MTCC 96) are incubated at 37 °C for 24 hours, *Streptococcus pyogenes* (MTCC 442) are incubated at 37 °C for 48-72 hours. Here we want to compare antibacterial activity of our chemical compound ND1, ND2, ND4 with other antibiotic such as gentamycin, ampicillin, chloramphenicol, ciprofloxacin, norfloxacin. After incubation growth of the microbial strain is tested and diameter of the zone of inhibition is checked. The entire antibiotic is considered as the perfect solution to bacterial infection and bacteria show resistance to most of the currently available antibiotics. Since antibiotic resistance is significant trait to public health.

The entire antibiotic is considered as the perfect solution to bacterial infection and bacteria show resistance to most of the currently available antibiotics. Since antibiotic resistance is significant trait to public health. Researchers all over the world are trying to discover new antibacterial drugs. After identifying new antibiotic we have to determine its effective dose of its minimum inhibitory concentration. Pure bacterial culture of *Escherichia coli* (MTCC 443), *Pseudomonas aeruginosa* (MTCC 1688), *Staphylococcus aureus* (MTCC 96), *Streptococcus pyogenes* (MTCC 442) and N-broth is added to microtiter plate in equal volume. Then different concentration of the ND1, ND2, ND4 is added in microtiter plate, here the bacterial growth had been compared with control which has been prepared without adding any bacterial culture to check the microbial growth. Then it is incubated at organism's optimum growth conditions. This procedure is performed separately for each bacterial culture. Turbidity indicates bacterial growth in well. Clear well indicated effective drug concentration of compounds that prevent bacterial growth. To distinguish between live and dead bacteria, indicator dye resazurin is added to well. Then microtiter plate is incubated for 2 hours at 37 °C. Formation of pink colour represents live bacteria and blue colour represents dead bacteria. The

MIC of the drug is the concentration at which bacteria starts to die through observation of colour difference for quantification purpose. Colour can be measured by using the plate reader as well.

2.1 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.

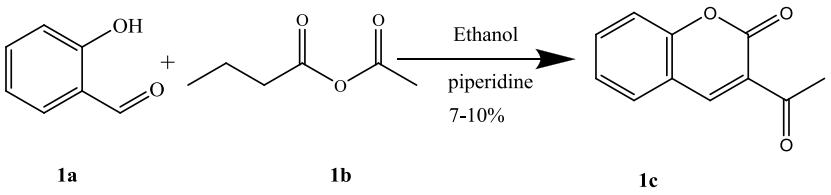
2.2 Synthesis of 1e (Ligand)

Ligand synthesis involves two steps:

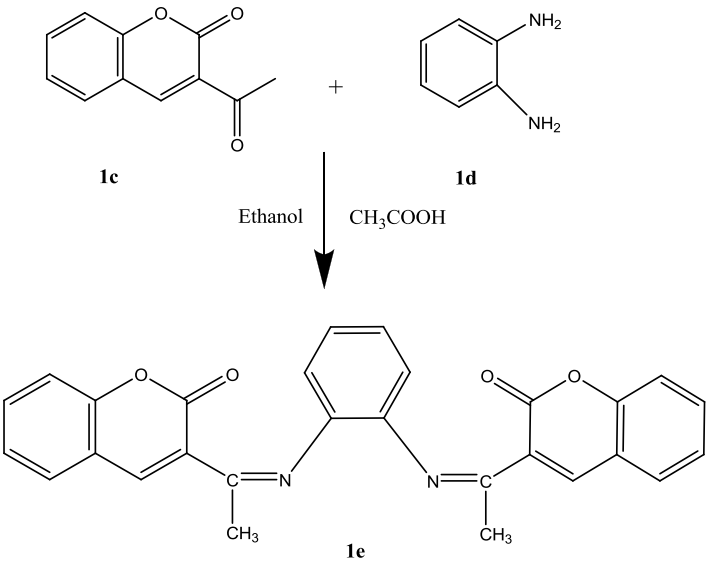
2.2.1 Preparation of 1c (acetyl coumarine) [16,17]:

In to a clean and dry round-bottomed flask, specific amount of salicylaldehyde **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.

Reaction Scheme [1e]



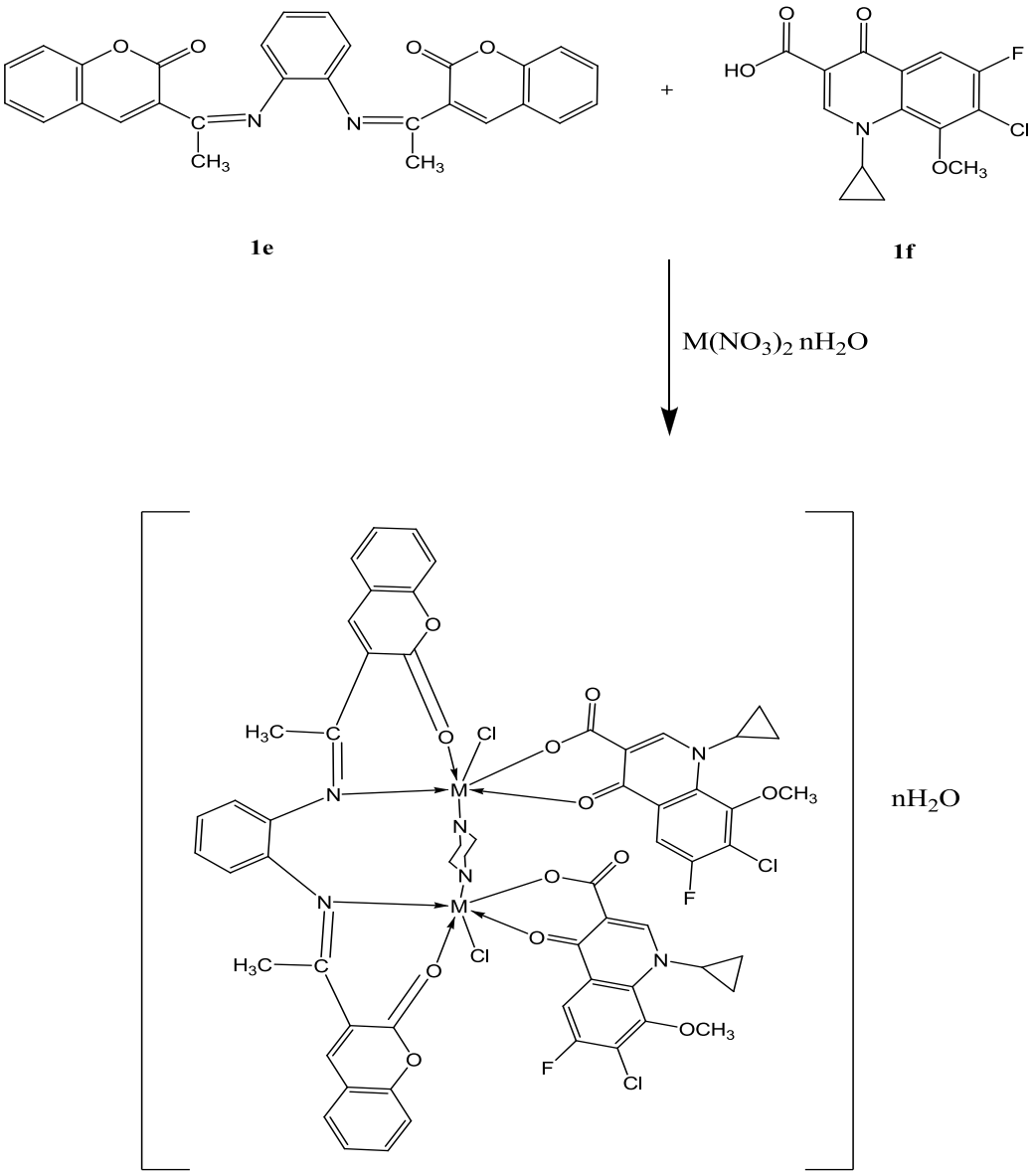
1a **1b** **1c**



1c **1d**

1e

Reaction Scheme [1g]



1g

Where, n= 3, 4, 5, 6, 7, M= Ni, Cu, Co, Mn, Mg

2.2.2 Synthesis of 3,3'-(1,2-phenylenebis (azanylylidene))bis(ethanylidene))bis (2H-chromen-2-one) 1e [18-20]:

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.

2.3 Synthesis of 1g (Metal Complex):

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 ethanol. The solid complex obtained was dried in desiccators over anhydrous calcium chloride.

3. 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*.

Mass spectra (FAB) of all complexes were obtained using 18 crown 6 ether as matrix. Figure IV represent the mass spectra of Ligand ND-1. Observed molecular peak is 453.65 m/z which clearly shows the molecular weight of ligand. IR spectra of ND-2 compound have been observed and in the IR spectra, carbonyl stretching vibrations observed near about 1600 cm-. Lactone carbonyl stretching observed near about 1700cm-. Stretching vibrations of C=N observed at 1550 cm-. Both symmetry and asymmetry stretching COO vibrations recorded at 1365 and 1580 consecutively. Metal Oxygen bond stretching observed at 535 cm-.C=N stretching observed at 1550 cm-. Broad band observed at longer wave number represent water molecule. For better understanding of structure, NMR data was observed for intermediate ligand ND-1. It is very necessary to know that the ligand has actually formed as per the assumed structure. Singlet with six protons confirms two methyl groups at near about 2.50 ppm. Fourteen aromatic protons can be observed in the range of 6.973-9.036 ppm. TGA (Thermogravimetric analysis) were applied to the metal complex ND-4. Temperature range was taken from 50 to 600 with the interval of 50 ℃. First decomposition of both the water molecules was observed at 100℃ with the mass loss of 8.56%. Piperazine ring removed in between 100 to 200℃ with 6.78 % mass loss. Decomposition of Ligand and drug gatifloxacin was observed at higher temperature.

3.1 *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. The minimum inhibitory concentration (MIC) values were compared with standard antibiotics. Well diffusion method resultant that the newly synthesized chemical molecules ND1, ND2, ND4 shows zone of inhibition to various pathogenic organisms. Its diameter is measured by caliper and it is mentioned in table 2. Newly synthesized chemical molecules ND1, ND2, ND4 inhibit pathogenic microbial growth at specific concentration and its minimum inhibitory concentration is mentioned in table 3.

Table 1. Characterization of synthesized compounds

Compounds	Colour	M.P. (^{o}C)	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	$C_{60}H_{60}C_{l4}Ni_2N_2F_6O_{18}$	78
Magnesium Gatiflox Ligand Complex (ND-3)	Yellow	>300	$C_{60}H_{60}C_{l4}Mg_2N_2F_6O_{18}$	67
Copper Gatiflox Ligand Complex (ND-4)	Sky Blue	>300	$C_{60}H_{60}C_{l4}Cu_2N_2F_6O_{18}$	63
Cobalt Gatiflox Ligand Complex (ND-5)	Greenish Yellow	>300	$C_{60}H_{60}C_{l4}Co_2N_2F_6O_{18}$	66
Manganese Gatiflox Ligand Complex (ND-6)	Brown	>300	$C_{60}H_{60}C_{l4}Mn_2N_2F_6O_{18}$	59

Solvent system for thin layer chromatography technique is:
Hexane: Ethylacetate: DCM (7.5:2.0:0.5)

Table 2. Antimicrobial screening results of diffusion method

	Zone of inhibition in millimètre			
Compounds	***E.Coli.***	***P. Aeruginosa***	***S. Aureus***	***S. Pyogenes***
ND-1	3.4	3.2	5.0	3.1
ND-2	4.0	3.0	5.0	4.9
ND-4	3.4	2.5	2.0	3.0

Table 3. Antibacterial activity (Minimal Inhibition Concentration), MIC Value, µg/mL

CODE	***E.Coli***	***P. Aeruginosa***	***S. Aureus***	***S. Pyogenes***
NO. / Name	**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. Pyogenes(MTCC-442) ;

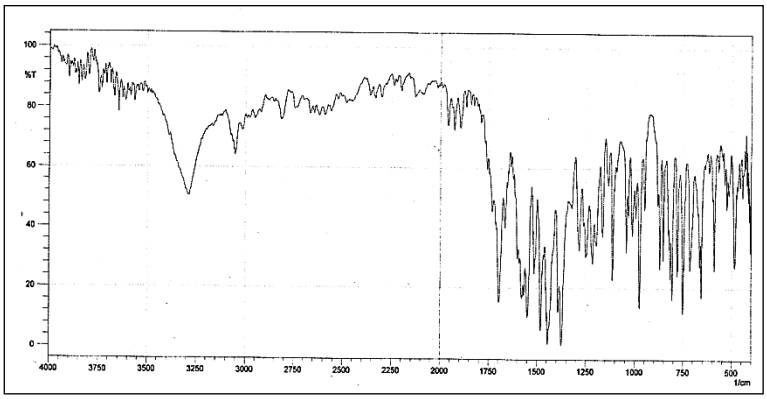


Fig. 2. Infrared spectrograph of ND-2

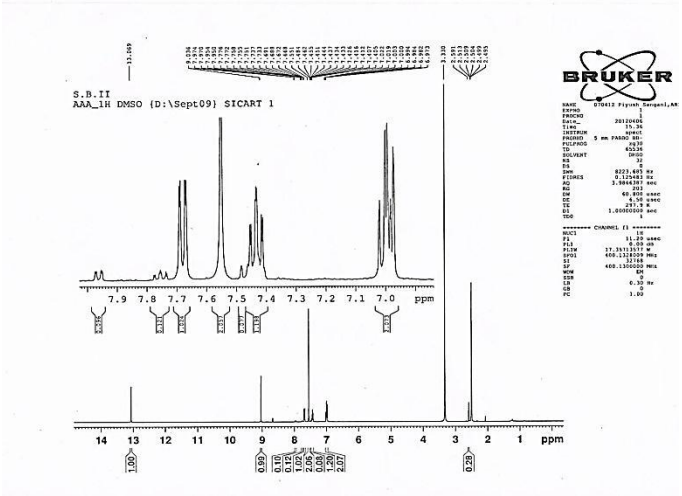


Fig. 3. ^{1}H Nuclear Magnetic Resonance spectrograph of ND-1

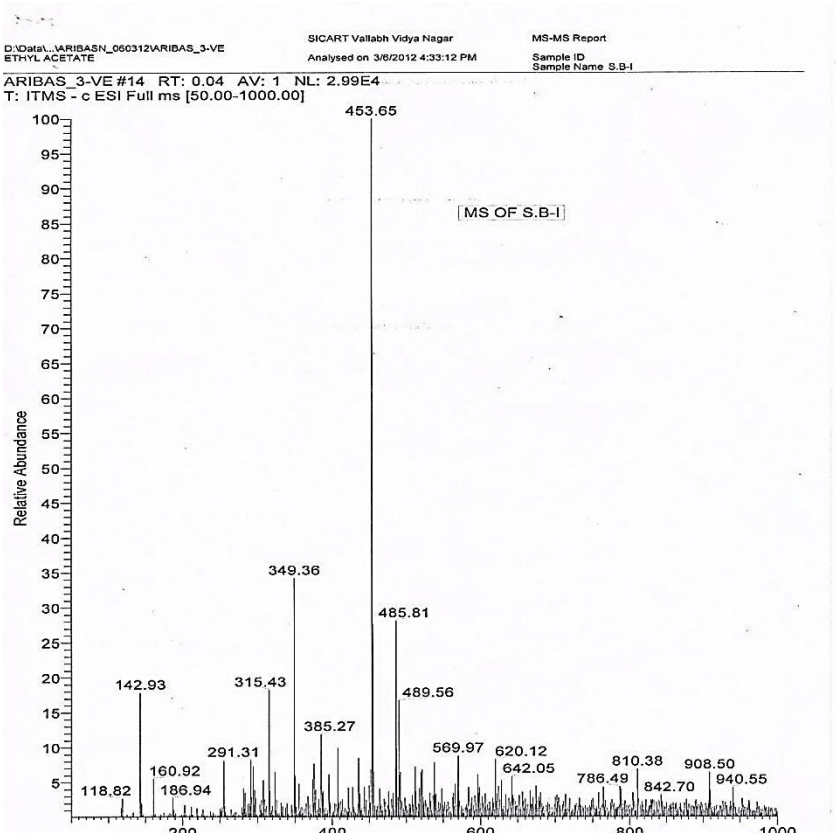
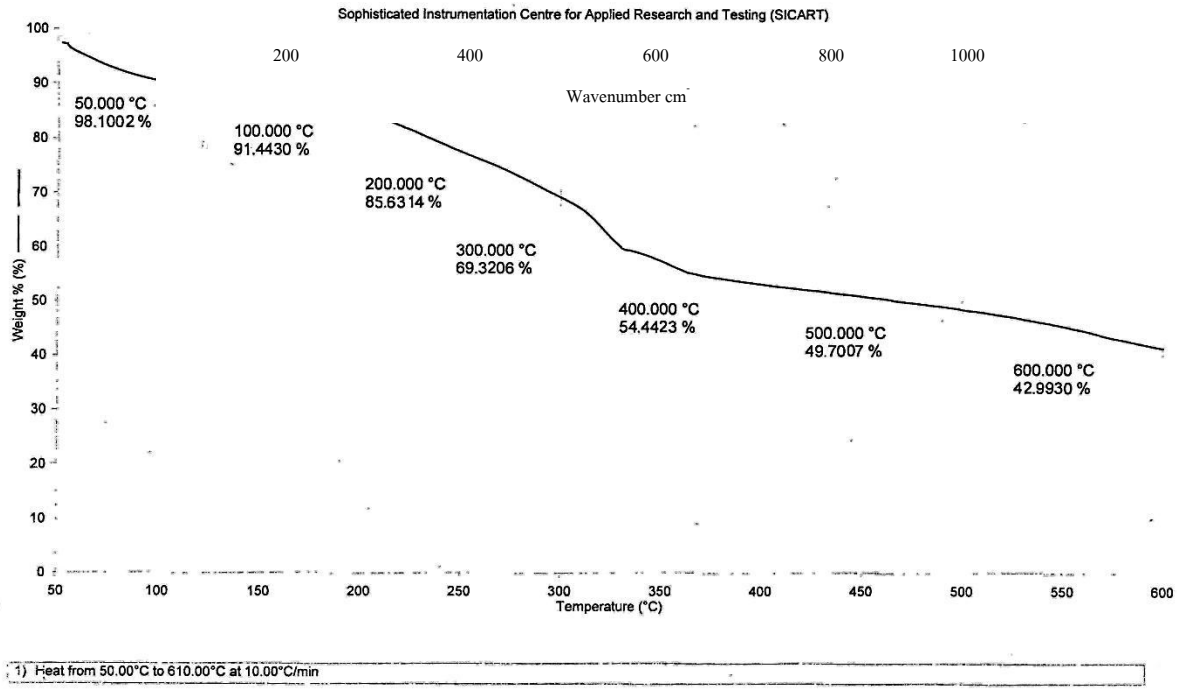


Fig. 4. Mass spectra of ND-1



Fig/ 5. Thermogravimetric analysis of ND-4

4. CONCLUSION

Variety of Drug-metal complexes were synthesized and characterized for their structure elucidation. In the synthesis of metal complexes, Nickel Gatifloxacine complex showed promising effects among all the complexes. Nickel complex also possess highest yield. Spectral data of ligand and metal complexes helped us to prove their structures. Antibacterial activity of the compound indicate that some of the compounds show comparable activity against some of the microbial strains while all the compounds were found to be promising antibacterial compared to respective standard drugs.

We can conclude that the complex ND1 can be the analogue molecule of ampicillin as it gives better results against *staphylococcus aureus* (MTCC 96) comparatively. ND2 gives similar inhibition of pathogens as it can be the analogue of ampicillin and also it gives more effective inhibition against *Staphylococcus aureus* (MTCC 96), *Streptococcus pyogenes* (MTCC 442). ND4 gives same result as ampicillin so it can be the optional drug molecule.

DISCLAIMER

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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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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