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Synthesis, Characterization and Biological Studies of Gemifloxacin Mesylate with Nickel(II) and Copper(II)

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Gemifloxacin mesylate, a fourth-generation fluoroquinolone-type antibiotic, is widely used for community treatment of acquired pneumonia and severe bacterial infections. The Cu(II) and Ni(II) complexes of gemifloxacin mesylate were synthesized by refluxing an aqueous solution of the ligand with the required amount of metal(II) salts in a round bottom flask with 2:1 (L:M) ratio in an oil bath at 90 ºC with a continuous stirring for 4 h. The mixture was kept overnight to obtain a coloured solid complex. The synthesized metal(II) complexes were characterized by physico-chemical parameters and spectral analyses, including UV-Vis, FT-IR and ^{1}H NMR. The structures of the complexes were further confirmed by elemental (CHNS) and TG-DTA analyses. The spectral findings revealed that metal-ligand interaction has successfully occurred where the ligand acted as a bidentate chelate in the synthesized metal(II) complexes. The IR studies showed that upon complexation the characteristic carboxylic stretching frequency at 1714 cm^{-1} disappeared and the pyridone stretching frequency shifted to a lower frequency region. The ^{1}H NMR data of the complexes confirm the effective interaction between the metal and the ligand *via* 3-carboxyl group and 4-oxo groups. The thermal and elemental analyses data were also in agreement with the proposed interaction. The ligand as well as its metal(II) complexes showed significant activity against all the studied 11 bacterial strains and one of the fungal strains, *Candida* sp.

Keywords: Gemifloxacin, Metal(II) complexes, Thermal analysis, Biological activity.

INTRODUCTION

Gemifloxacin mesylate is a synthetic antibiotic that belongs to the fluoroquinolone category. It has a long range of activity against both Gram-positive and Gram-negative bacterial strains. It is bactericidal and extensively used for community treatment of acquired pneumonia and severe bacterial infections allied with enduring bronchitis [1]. The inhibition of different enzymes such as topoisomerase II (DNA gyrase) and topoisomerase IV produces the bactericidal action of gemifloxacin necessary for bacterial growth [2].

It is well-established that several metal complexes are used as chemotherapeutic agents [3,4]. Metals due to their variable valences, coordination site, redox activity and reactivity towards organic ligands provide unique features and these features encourage medicinal chemists to develop new metal complexes as a drug with therapeutic importance [5]. Many drugs showed modified pharmacological and toxicological properties after complexation with transition metals [6,7]. Recent studies reported significant progress in the development of new drugs with improved bioactivity through the metalation of antibiotics. A literature survey revealed that bismuth complex of norfloxacin [8], Pd(II) complex of tetracycline [9] and Cu(II) complex of ciprofloxacin [10] exhibit higher antimicrobial activity than parent drugs. Several research works were carried out with different quinolone antibiotics like ciprofloxacin [10], gatifloxacin [11], levofloxacin [12] and lomefloxacin [13]. Sadeek *et al.* [14] and Shamim *et. al.* [15] synthesized few heavy metals (U, La, Ce, Pb) complexes of fluoroquinolone antibiotic, gemifloxacin investigate the alterations in physico-chemical and biological aspects.

According to the literature study, no report was found on essential and trace metal complexes of gemifloxacin. To continue our previous work on the metal complexation of different antibiotics with bivalent metals [16,17], the complexation of gemifloxacin mesylate with essential and trace metals (Cu and Ni) was reported with the aim to develop more potent antibacterial agent(s). The newly synthesized complexes were then charac-

terized by several spectral methods like UV-Vis, FT-IR, ^{1}H NMR as well as thermal analyses. Different physico-chemical characteristics like colour, solubility and melting temperature and % yields are reported. Finally, the antibacterial screening of the parent drug and corresponding metallodrug was done *in vitro* against a long range of bacteria including Gram-positive and Gram-negative. The antifungal study was also conducted against two species *Candida* sp. and *A. niger*.

EXPERIMENTAL

Analytical grade solvents and chemicals were procured from Merck, Germany and used without any further purification. Hydrated salts containing $CuSO_4 \cdot 5H_2O$ and $NiCl_2 \cdot 6H_2O$ were used as metal sources for complexation. Incepta Pharmaceuticals Ltd., Bangladesh provided gemifloxacin mesylate (potency 100%) as a gift sample.

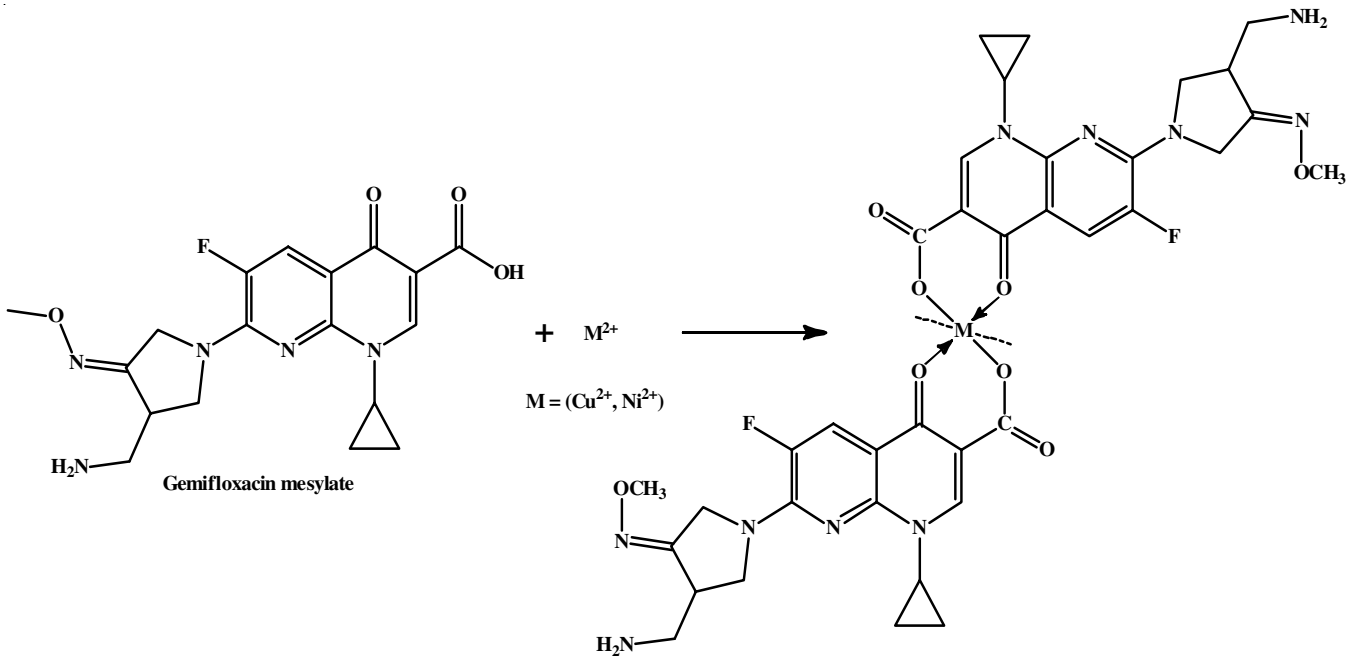
Characterization: The melting points of pure drug and corresponding complexes were determined using a digital melting point apparatus (Model: WRS-1B, China). The % of element composition was determined using an Elemental analyzer (varioMicro V1.6.1GmbH, Germany). The ultraviolet-visible spectral analysis was carried out in dimethyl sulfoxide (DMSO) in the region of 800-200 nm using a double-beam Shimadzu UV-visible spectrophotometer, model UV-1650 PC. The FT-IR studies of all samples were done by analyzing IR spectra taken as KBr pellets, scanning from 4000-400 cm^{-1} using an FT-IR spectrophotometer (Model: 8400s, Shimadzu, Japan). A Bruker AMX 500 MHz spectrophotometer was used to determine ^{1}H NMR spectra in DMSO-d_6 as an NMR solvent where TMS was mixed as an internal standard. A DTA-TGA thermal analyzer employing a simultaneous technique (TGA-50H, Shimadzu, Japan) was utilized. A platinum pan was used to keep 4-7 mg sample and the temperature was raised to 1000 ºC where a 10 ºC/min heating rate was maintained in a nitrogen atmosphere.

Synthesis of bivalent metal complexes of gemifloxacin mesylate: The metal(II) complexes of gemifloxacin mesylate were synthesized by mixing an aqueous solution of ligand (100 mg, 0.2 mmol) with the required amount of metal(II) (0.1 mmol) in a round bottom flask in a 2:1 (L:M) ratio. After that, refluxing was done in an oil bath at 90 ºC with a continuous stirring for about 4 h and left the mixture for overnight. In each case, a coloured solid complex was obtained. The solid complex was purified several times washing with distilled water and *n*-hexane, respectively. The solid complex was dried at 40 ºC in oven and stored in a desiccator at room temperature (**Scheme-I**). The solid products are air, moisture-stable and soluble in DMSO and methanol.

Biological strains: The studied strains were *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella typhi*, *Klebsiclla pneumoniae*, *Citrobacter freundii*, *Vibrio cholerae*, *Enterobacter faecalis*, *Enterobacter faecium* and two fungal species, *Candida* sp. and *A. niger* were chosen for antifungal screening. The antimicrobial activity were carried out in Centre for Advanced Research in Sciences, University of Dhaka, Dhaka, Bangladesh.

Biological activities: Kirby-Bauer paper disc diffusion technique [18,19] was employed to investigate the antimicrobial activities of synthesized metallo-antibiotics. In this method, paper discs (diameter 6 mm) saturated with the antimicrobial agent were placed in each of the previously seeded agar culture plates of tested microorganisms. For antibacterial screening, the plates were kept in an oven for 1 day at 37 ºC and a week at room temperature for antifungal screening. Measuring the diameter (in mm) of zone of inhibition, the activity was evaluated. DMSO (solvent) was selected as negative control and gemifloxacin itself acts as positive control.

Statistical analysis: The statistical analyses were carried out by using Microsoft Office Excel.



Scheme-I: Synthetic route of gemifloxacin-metal(II) complexes

RESULTS AND DISCUSSION

The elemental analyses data of metal(II) complexes of gemifloxacin mesylate are shown in Table-1, which were agreed well with a 1:2 metal to ligand stoichiometry for the metal(II) complexes.

UV-Vis studies: The shifting of absorption bands towards to lower (hypsochromic shift) or higher values (bathochromic shift) or variation of intensity of absorption bands, distinctive of ligand were indicative of complex formation. Moreover, appearance of new absorption bands and the disappearance of maxima observed for the parent drug revealed the formation of metal complexes. The UV spectra of gemifloxacin mesylate and its metal complexes are shown in Fig. 1. The absorption bands for free gemifloxacin appeared at 342, 270 and 296 nm, assigned to n-π* and π-π* transitions (Fig. 1a) while in Cu(II)-complex the bands were found at 341, 267 and 299 nm (Fig. 1b) and in Ni(II) complex at 366, 334 and 267 nm (Fig. 1c). The shifting of bands to lower or higher values are due to the complexation of metal ions to ligand molecules. Moreover, a new band appeared at 366 nm in Ni(II) complex is attributed to the charge transfer transition.

FT-IR studies: A close similarity is observed in the IR spectrum of metal complexes compared to the ligand because the same atoms have participated in complexation (Fig. 2). However, the complexes possess significant changes in the vibrational frequencies as compared to the parent drug, thus indicating the development of new metal complexes. Generally, in quinolone antibiotics, the 3-carboxyl and 4-oxo groups participate in the chelate formation where ligand acts as a bidentate chelator [20].

In case of free gemifloxacin mesylate, the bands observed at 1714 and 1633 cm^{-1} were attributed to carboxyl stretch $\nu(C=O)_c$ and the pyridone stretch $\nu(C=O)_p$. After complexation, deprotonation of the carboxylic group occurs as well as the characteristic peak for carboxylic stretch disappears, demonstrating the

TABLE-1
PHYSICO-ANALYTICAL DATA OF GEMIFLOXACIN MESYLATE AND ITS METAL(II) COMPLEXES

Compound	Colour	State	m.p. (°C)	Elemental analysis (%): Found (calcd.)			
				C	H	N	S
Gemifloxacin mesylate (ligand)	Whitish brown	Amorphous	210	–	–	–	–
$[Cu(GMX)_2(H_2O)_2]SO_4{\cdot}14H_2O$	Black	Crystalline	258	32.34 (32.16)	5.72 (5.68)	9.82 (9.87)	6.47 (6.77)
$[Ni(GMX)_2(H_2O)Cl]Cl{\cdot}11H_2O$	Light brown	Crystalline	313	34.39 (34.66)	5.24 (5.51)	10.97 (10.64)	4.90 (4.87)

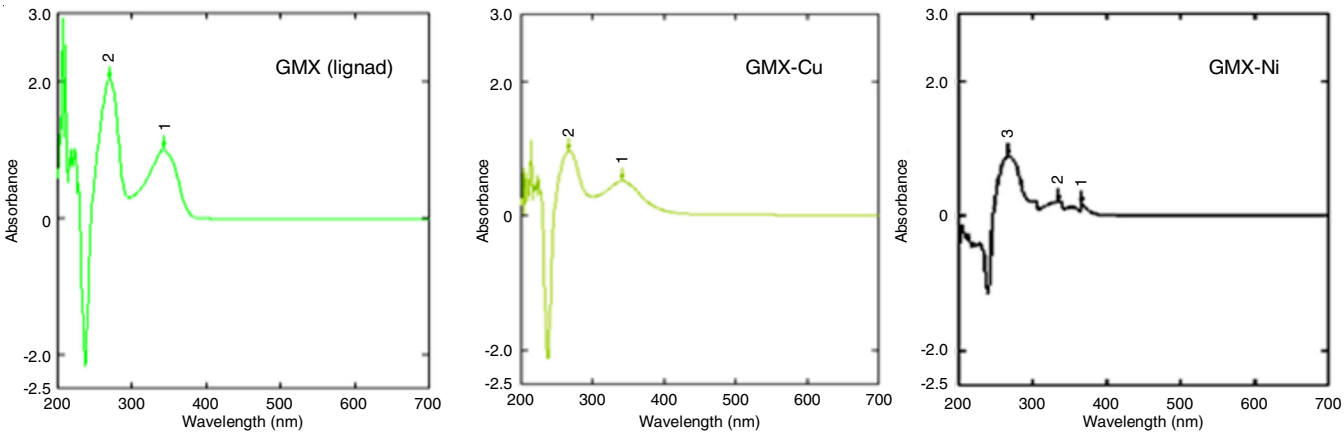


Fig. 1. UV spectra of gemifloxacin mesylate (ligand) and its metal(II) complexes

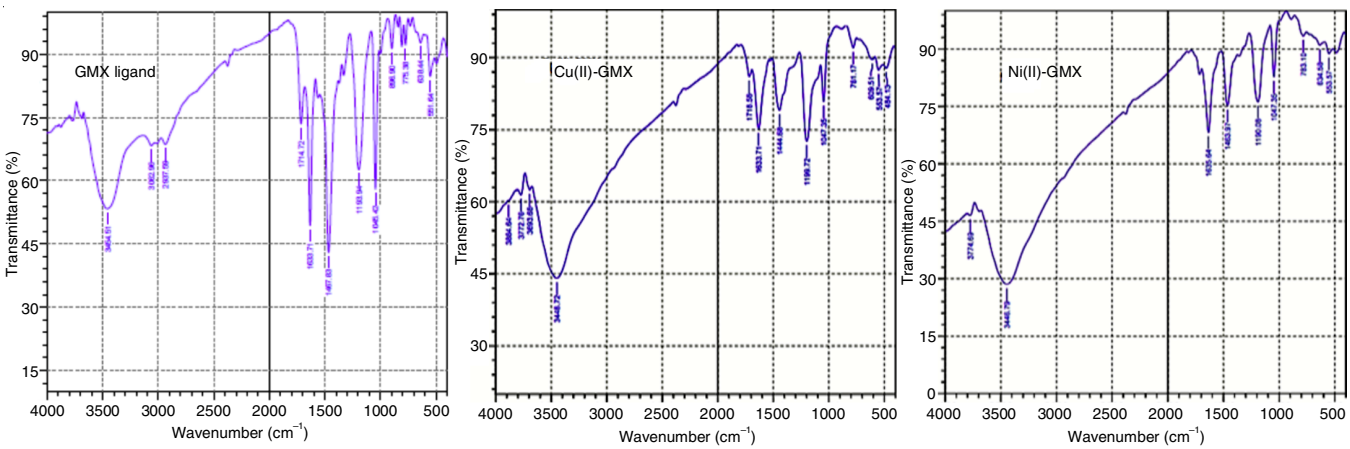


Fig. 2. FT-IR spectra of gemifloxacin mesylate (ligand) and its metal(II) complexes

participation of carboxyl group in the complex formation. In both complexes, a broad band ranging from 3600-3100 cm^{-1} with maxima at 3440 cm^{-1}, assigned to ν(O-H) vibration and giving evidence of water participated in complex formation [21]. Several researchers also reported the participation of water to form coordinated metal complexes of quinolone antibiotics [11-14]. The peaks at 1632 cm^{-1} and 1450 cm^{-1} in the complexes can be designated the asymmetric vibration, $\nu(O\text{-}C\text{-}O)_{asym}$ and symm-etric vibration, $\nu(O\text{-}C\text{-}O)_{sym}$, respectively. The mode of coordi-nation of carboxylate ion depends on the separation of bands (*i.e.* $\Delta\nu = \nu_{COOasym} - \nu_{COOsym}$) [22]. The monodentate coordination mode of the carboxylate group, a greater separation value of $\Delta\nu$ >180 cm^{-1} was observed due to the equivalence of the two C–O bonds. On the other hand, when uncoordinated carboxyl oxygen is involved in forming strong hydrogen bonds with water molecules, the value might be lowered [23].

In this work, the separation value, $\Delta\nu$ around 180 cm^{-1} was found in both complexes, suggesting the monodentate bonding of the carboxylate group. On the other hand, the characteristic band of the pyridone group, $\nu(C{=}O)_p$ is affected by interaction with metal and shifted towards a lower frequency region and appeared in the same region of symmetric vibration of the carboxylate group. Furthermore, several bands of different frequencies at 780-480 cm^{-1} were observed which are assigned to ν(M-O) [14].

NMR studies: The ^{1}H NMR spectra of gemifloxacin mesylate and its corresponding metal complexes were taken in DMSO-d_6. An investigation of spectral data revealed that the peaks that are found in the parent drug are also found in spectra of metal complexes with a slight change in chemical shift upon chelation. The ^{1}H NMR spectrum of the ligand molecule showed a duplet at 1.23 ppm corresponding to cyclopropyl 3-H; a multiplet at δ = 3.15-3.22 ppm for amino methyl-H (-CH_2); 3.38-3.42 ppm for pyrrolidinyl 2-H; a singlet at 8.60 ppm for 1,8-naphthyridine-2-H and a duplet at 8.05-8.08 ppm for 1,8-naphthyridine-5-H, respectively. In cases of metal complexes, there is no peak found in the region of δ 10-13 ppm, indicative of the absence of carboxylic proton (COOH). The absence of carboxylic proton (COOH) recommends that metal ion is coordinated *via* carboxylate oxygen atom [11,14]. However, the naphthyridine proton at C-2 and C-5 positions nearer to the coordination site showed a slight downfield shift after complexation. The complexes also possess an O-H proton peak at δ 4.1-4.5 ppm, suggesting the involvement of water to form coordinated complexes. These variations were indicative of complexation through 3-carboxyl and 4-oxo groups.

Thermal studies: Fig. 3 represents the thermograms of ligand (antibiotic) and its Cu^{2+} and Ni^{2+} complexes. For the TG curve, it is observed that a multistep degradation process has occurred in case of ligand. The complexes also followed a similar degradation profile. The newly synthesized metal complexes showed the greater stability than the parent ligand. The ligand decomposes 100% at around 800 ºC, whereas in Cu(II) complex and Ni(II) complex, 14.4% remained even at 1008 ºC and 25.1% remained at 1010 ºC, respectively. However, the decomposition of the metal complexes starts with the release of lattice water followed by the removal of coordinated water and then the fragmentation of organic moiety, finally leaving metal oxide as residue [24]. Table-2 depicts the thermoanalytical (TG/DTG/DTA) data of the antibiotic and its metal complexes. In TG curve of the Cu(II) complex of gemifloxacin mesylate, a weight loss of 16% was observed at around 200 ºC, attributed to the loss of lattice and coordinated water.

The DTG curve showed a short and broad merged peak at 80 and 160 ºC, indicating to release of uncoordinated and coordinated water. The DTG curve also showed two sharp peaks at around 335 and 510 ºC, assignable to the decomposition of the anhydrous complex. The DTA results were also well in agreement with the TG and DTG analytical results.

The Ni(II) complex was also found to be thermally more stable than the precursor antibiotic, GMX itself. The thermal degradation of Ni(II) complex occurred in four steps. The 1st step of degradation occurred at 25-158 ºC associated with T_{DTG} at 65 ºC and T_{DTA} at 56 ºC, which corresponds to 12% weight loss. This loss may be due to the loss of uncoordinated water. In 2nd step (158-230 ºC) of degradation in the TG curve, the rate of mass loss is slow and found 6.7%, associated with T_{DTG} at 194 ºC, which may be due to the loss of coordinated water. The 3rd step (229-501 ºC) of the TG curve, associated with T_{DTG} at 313, 364 ºC and T_{DTA} at 312, 470 and 366 ºC, corresponds to the degradation of ligand (29.2%). The final step of degradation also proceeded with a mass loss of 27%, leaving 25% residual mass as metal oxide.

Both spectral and thermal analyses provide strong evidence for the formation of new metal(II) complexes of gemifloxacin mesylate. Qualitative determination also confirmed the pres-

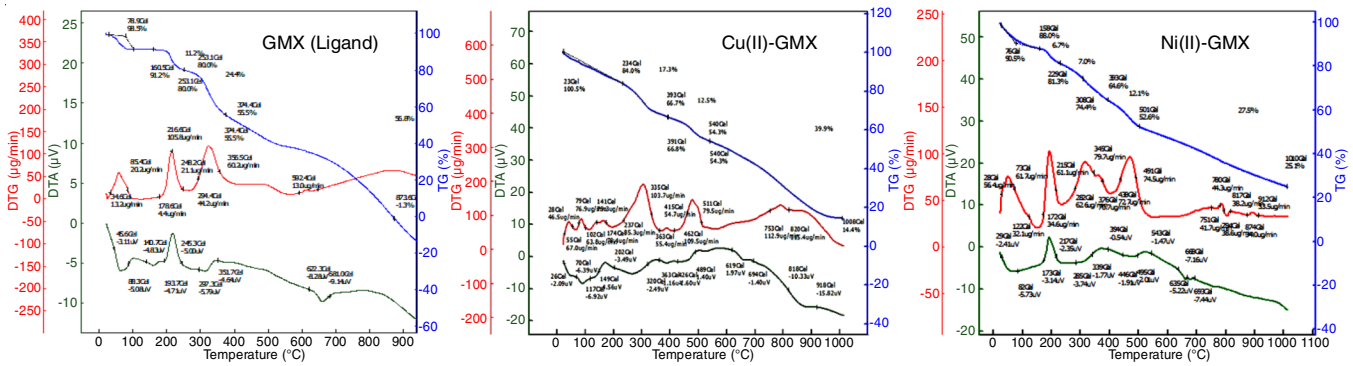


Fig. 3. TG/DTG/DTA thermograms of gemifloxacin mesylate (ligand) and its metal(II) complexes

TABLE-2
THERMO-ANALYTICAL (TG/DTG/DTA) DATA OF ANTIBIOTIC AND ITS COMPLEXES

Compound	Step	Temperature range (°C)	Weight loss (%)	DTG_{max} (T_{DTG})	DTA (T_{DTA})	
					Endo	Exo
Gemifloxacin mesylate (ligand)	1st	25-160	8.8	59.7	67	
	2nd	160-253	11.2	216.6	166.5	114.5
	3rd	255-374	24.4	356.5	324	219
	4th	374-850	56.8	592.4	651	
Total loss			100			
Residue			–			
$[Cu(GMX)_2(H_2O)_2]SO_4 \cdot 14H_2O$	1st	23-234	16	41.5, 90.5	48, 93.5	175.5
	2nd	234-393	17.3	286	261	341.5
	3rd	393-540	12.5	486	457	
	4th	541-1008	39.9	786.5	658, 868	
Total loss			85.6			
Residue			14.4			
$[Ni(GMX)_2(H_2O)Cl]Cl \cdot 11H_2O$	1st	25-158	12	64.5	55.5	
	2nd	158-229	6.7	193.5		
	3rd	229-501	29.2	313, 364	312, 470	366.5
	4th	501-1010	27.5	787, 893	651, 680	519
Total loss			75			
Residue			25			

TABLE-3
BIOLOGICAL SCREENING RESULTS OF THE LIGAND AND ITS METAL COMPLEXES REPRESENTINGINHIBITION ZONE DIAMETER VALUES (mm)

Compound	Bacterial strain												Fungal species	
	BC	EC 0157	EC	PA	SA	L	ST	VC	KP	CF	EF1	EF2	Cda	AN
GMX	34	32	34	34	45	33	30	25	28	26	28	32	26	No activity
Cu(II) complex	30	32	33	32	42	33	26	20	25	23	27	28	30	No activity
Ni(II) complex	32	36	34	32	43	33	26	24	28	25	26	30	NA	NA

GMX = Gemifloxacin mesylate, BC = *B. cereus*, EC 0157 = *E. coli* 0157, EC = *E. coli,* PA = *P. aeruginosa*, SA = *S. aureus*, L = *Listeria,* ST = *S. typhi*, VC = *V. cholerae*, KP = *K. pneumoniae*, CF = *C. freundii,* EF1 = *E. faecalis*, EF2 = *E. faecium, Cda* = *Candida* sp. and AN = *A. niger,* NA = Not assayed

ence of chloride and sulphate ions as negative counterparts [25-29].

Biological activities: The antimicrobial screening of gemifloxacin mesylate and its metal-coordinated complexes was done using the disk-diffusion method. A wide range of bacterial strains and two fungal strains named *Candida* sp. and *A. niger* were used. The activity of the parent antibiotic and its metal(II) complexes was determined by measuring the inhibition zone diameter (mm). The ligand and its metal(II) complexes exhibited considerable action against all bacterial strains and one fungus strain, *Candida* sp. The Cu(II) complex of gemifloxacin mesylate showed enhanced antifungal activity against *Candida* sp. compared to ligand. On the other hand, the Ni(II) complex of gemifloxacin mesylate showed increased activity against *E. coli* 0157 and similar activity like ligand against other bacterial strains (Table-3).

Conclusion

Drug metal interaction plays an important role not only in the enhancement of biological activity but also in mitigating the drug resistance issue. The synthesized two metals (Cu, Ni) complexes of gemifloxacin mesylate were characterized by UV-Vis, FT-IR, ^{1}H NMR, elemental (CHNS) and TG-DTA analyses. Based on the physico-chemical and spectral data, the ligand acts as bidentate in both metal complexes. Eleven bacterial species and two fungal strains were evaluated against the newly generated two complexes using gemifloxacin (30 µg/disc) as standard. The Cu(II)-complex of gemifloxacin mesylate showed enhanced antifungal activity against *Candida* sp. compared to the ligand.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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