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Synthesis, Spectral Characterization and Biological Evaluation of Glutaric Acid based Macrocyclic Complexes

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In the presence of divalent transition metal ions, glutaric acid and *o*-phenylenediamine were used to design and synthesize a unique series of macrocyclic complexes. The UV-visible, IR, ESR and ESI-MS spectroscopic methods were used to analyze these newly designed complexes. In order to evaluate their thermal behaviour, TGA analysis was employed. The general formula for the synthesized complexes was $[M(L)X_2]$ (L = ligand, $X = Cl^-$ and $M = Co^{2+}$, Ni^{2+} and Cu^{2+}). The central metal ions are surrounded by octahedral environment. These macrocyclic complexes were tested for their antimicrobial effectiveness against the selected bacterial strains (*Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Escherichia coli*), as well as fungi strains (*Aspergillus niger* and *Candida albicans*) and their efficacy was compared to that of standard drugs streptomycin and itraconazole, respectively. Moreover, the antioxidant activity of the macrocyclic complexes were also assessed using the DPPH assay.

Keywords: Antifungal, Antibacterial, Glutaric acid, Tetraaza macrocyclic complexes, DPPH assay.

INTRODUCTION

Macrocycles have acquired great deal of attention as they offer a wide variety of donor atoms, geometry, ionic charges and also due to their distinct behaviour [1]. Tetraaza macrocycles were of prompt interest to chemists as of the structural resemblance with that of porphyrins, phthalocyanines and corrins complexes as discovered in the nature [2]. These tetraaza macrocyclic ligands having four nitrogen atoms are broadly known in the literature. Ligands with donor nitrogen atoms have a high affinity to coordinate with transition metals. In recent study, the characteristics of these complexes are examined as the analogues of broadly recognized family of crown ethers [3,4]. Now a days, tetraaza macrocyclic complexes are ubiquitous across all disciplines in the core sciences and can be easily altered by using the well-established methods.

The literature generally attributes the cyclic tetraaza framework's overwhelming and consistent pervasiveness to the promiscuous behaviour of its metal-binding nature [5-10]. For instance, transition metal complexes, such as cyclam, cyclen are investigated as potential contrast agents. Cyclen have also been studied as a ligand in C-C coupling reactions and oxidation processes that are catalyzed by transition metal ions [11,12]. Significantly, the usage of tetraaza macrocyclic complex (like cyclen) furnished promising building blocks, which mimics the biological structure [13,14] for the synthesis of biomimetics and potent therapeutic agents [15-18]. Tetraaza macrocycles have a wide range of uses, which are primarily due to (i) changes in C-C bridges between N-atoms and (ii) increase in the properties of complexes that are modulated through modifications in size of complex ring and donor atoms of macrocycles [19-26]. Hence, the design and synthesis of novel scaffolds, which may be tuned for multifarious applications, having a potency to endow a wide ranging field of pharmacological activities are required.

Prompted from the above applications of *o*-phenylenediamine, a series of tetraaza macroccylic complexes has been synthesized by one pot method, which has been characterized by several spectroscopic techniques. Further these macrocyclic complexes were screened for their antimicrobial and antioxidant activity.

EXPERIMENTAL

All chemicals employed in the synthesis were of the highest quality grade for analysis. Sigma-Aldrich was used to purchase glutaric acid, methanol, *o*-phenylenediamine and metal (II) chlorides. The use of solvents does not require further purification. The Fourier-transform infrared (FT-IR) spectra were captured using KBr pellets on a Perkin-Elmer spectrum, BX II, in the 4000-400 cm^{-1} range. Digital Conductivity Meter was used to calculate the values of molar conductance (HPG System, G-3001). The ESR spectra were captured in the X and Q bands using a Bruker A300. TGA study has been determined by Thermogravimetric analyzer SDT650 in temperature range of 50-800 ºC with a heating rate of 10 ºC/min. The UV-Vis-NIR was recorded on Cary 5000 spectrophotometer in the wavelength range of 180-3300 nm. The mass spectrum was measured on SCIEX Triple TOF 5600 mass spectrometer.

Synthesis: The molar ratio of 2:1:2 was used in the one pot template process for the synthesis of metal complexes. Refluxing was carried out for 0.5-1 h using methanolic solutions of 50 mL *o*-phenylenediamine (10 mmol) and 20 mL divalent metal salt (5 mmol). Due to the coordination of the metal ion and amine, a little change in the colour can be observed as the reaction proceeds. Furthermore, glutaric acid (10 mmol) was added to this heated mixture and refluxed further for 7-8 h. This mixture was kept aside overnight on room temperature for cooling, resulting into the formation of the coloured precipitates at the bottom of flask which imparts indication of completion of reaction and cyclization of the complex. The product was subsequently isolated, after which the precipitates were washed meticulously with solvent and vacuum-dried (**Scheme-I**). The complexes were soluble in methanol, isopropyl alcohol, acetone, DMF and DMSO.

Cobalt complex: Yield: ~68%, light purple, *m.w*. 537.93, *m.f.*: $[Co(C_{22}H_{24}N_4O_4)Cl_2]$; μ_{eff} (B.M.): 3.8; UV-visible [DMSO, λ (nm)]: ~210 nm, ~1500 nm, 1700 nm and ~1900 nm.

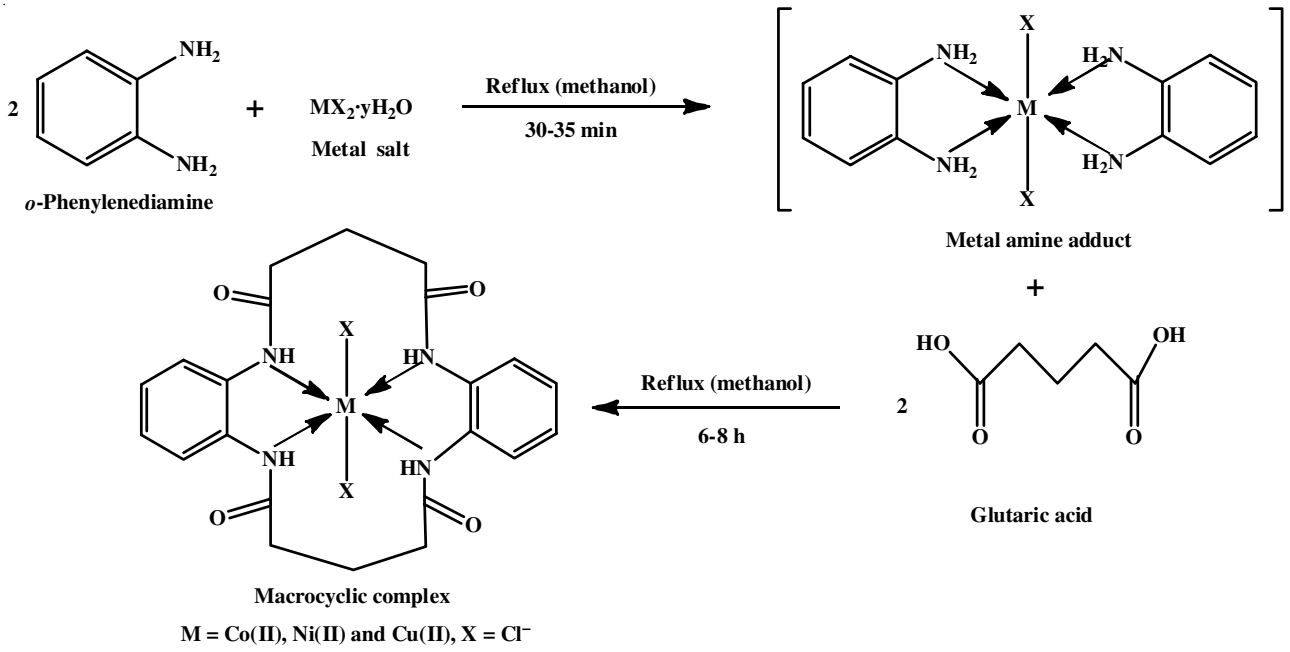
Nickel complex: Yield: ~64%, light blue, *m.w.* 537.69, *m.f.*: $[Ni(C_{22}H_{24}N_4O_4)Cl_2]$; μ_{eff} (B.M.): 2.83; UV-visible [DMSO, λ (nm)]: ~220 nm, ~300 nm, ~1600 nm, ~1700 nm and ~1950 nm.

Copper complex: Yield: ~76%, black colour, *m.w.* 542.54, *m.f.*: $[Cu(C_{22}H_{24}N_4O_4)Cl_2]$; μ_{eff} (B.M.): 1.73, UV-visible [DMSO, λ (nm)]: ~200 nm, ~400 nm, ~1400 nm, ~1700 nm and ~1900 nm.

Biological assays: Agar well diffusion method has been adopted for evaluating *in vitro* antimicrobial activity of all the synthesized macrocyclic complexes. For this evaluation two Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and two Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) were selected. The synthesized metal complexes were also assessed for antifungal activity against two fungal species (*Aspergillus niger* and *Candida albicans*).

***In vitro* antibacterial activity:** The antibacterial activity of the synthesized macrocyclic complexes has been studied using the Agar well plate diffusion technique. For this, a solution of 11.5 g of Muller Hinton powder in 300 mL of water was prepared and then sterilized by autoclaving it at 121 ºC for 20 min. The pH of the medium is intentionally kept close to 7.4; after cooling around 30 mL of media were aseptically applied to the sterilized petri dishes (100 mm × 15 mm). Stock solutions (2.5 mg/mL, 5.0 mg/mL and 7.5 mg/mL) were generated after the complexes were dissolved in DMSO and 50 µL of each of these solutions was used in each well. In order to grow the chosen antibacterial strains, agar plates were prepared. The incorporated Petri plates having bacterial strains were then incubated at 37 ºC for about 24 h. Further, the bacterial potential of all three synthesized macrocyclic complexes was evaluated and contrasted with that of the reference drug streptomycin [27,28].

***In vitro* antifungal activity:** The antifungal potency of macrocyclic complexes was assessed using the agar well plate



Scheme-I: Schematic representation of synthesized macrocyclic complexes

diffusion method. An agar medium was prepared by mixing 19.5 g of Saboraud dextrose agar powder with 300 mL of water and sterilizing at 121 ºC for 20 min. A specific pH of 7.2 was kept for the medium. The medium were ice-cold and then 30 mL were aseptically poured into each of the 100 mm × 15 mm Petri dishes [29,30]. The chosen bacteria were evenly dispersed around the plates using a sterile brush. Thereafter, 50 µL of macrocyclic complexes at concentrations of 2.5 mg/mL, 5.0 mg/mL and 7.5 mg/mL were introduced to 6 mm diameter wells. Petri plates with Gram-positive and Gram-negative bacterial cultures were incubated at 37 ºC for 24 h and 28 ºC for 48 h, respectively, to optimize the conditions for the development of the bacteria and fungus. Following incubation, the average diameter of the inhibition zone at 6 mm wells was measured. Based on the triplicates with DMSO as solvent, the mean and the standard deviations of all macrocyclic complexes were calculated. All the synthesized macrocyclic complexes were evaluated against the standard drug itraconazole [27].

Antioxidant activity: The ability of the complexes to scavenge free radicals was assessed through the utilization of the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay and the results were subsequently compared to those obtained from the standard.

DPPH assay: The capacity of macrocyclic complexes to scavenge free radicals was assessed using the DPPH assay [31]. The different concentrations of the synthesized macrocyclic complexes have been prepared at 3, 6, 9, 12, 15 and 18 µg/mL. With the use of ethanol, ascorbic acid (standard) was diluted to 3 mL and 1 mL of ethanolic DPPH solution of 0.1 mM was added to the macrocyclic complexes and then incubated for around 30 min in dark at 30 ºC. The standard administration of antioxidants resulted in a statistically significant decrease in DPPH absorbance at 517 nm compared to the control compound. Ascorbic acid (10 mg/mL DMSO) serves as a reference. The colour of DPPH, a free radical solution with a purplish red tint, changes to yellow when it is scavenged. Radical scavenging capability (%) was used to test the capacity of the synthesized complexes to scavenge DPPH free radicals at various concentrations and in the standard solution and is calculated by following formula:

$$\text{Radical scavenging capability (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where $A_{control}$ is the absorbance of DPPH radical and ethanol; A_{sample} is the absorbance of the DPPH radical and macrocyclic complex.

RESULTS AND DISCUSSION

By using template condensation of *o*-phenylenediamine and glutaric acid, the biologically active coloured solid macrocyclic tetraaza complexes of cobalt, nickel and copper have been synthesized (**Scheme-I**). All the complexes are soluble in majority of the common organic solvents. All the synthesized macrocyclic complexes are found to be non-electrolytic in nature.

Infrared spectral studies: The analysis of FT-IR spectra of the synthesized macrocyclic metal complexes obtained from dicarboxylic acid and diamine complexes has been done. The absence of peak of OH group in carboxylic acid and free amino acids -NH_2 group in spectra indicated the formation of macrocyclic complexes. The NH stretching vibration is represented by the absorption band, which was observed at 3275-3262 cm^{-1} while a strong absorption band at 1640-1637 cm^{-1} belongs to the carbonyl group [32] indicating the cyclization of complexes. A medium intensity band in the region between 3200 and 2900 cm^{-1} are due to the (C-H) methyl groups of complexes [33,34]. The symmetric and asymmetric vibrations are appeared at 1416-1400 cm^{-1} and 1346-1309 cm^{-1}, respectively. Bands between 1272 and 1241 cm^{-1} are due to the (N-H) amide groups of the compounds whereas the C-N stretching occured in the region 1340-1000 cm^{-1} [35]. Finally, the peaks at 418-480 cm^{-1} appeared due to the (M-N) vibration, which corresponds to metal coordination with nitrogen [36,37].

Thermal studies: Macrocyclic complexes were studied using thermogravimetric (TG) analysis at a flow rate of 20 mL/min of N_2 and a heating rate of 10 ºC/min between the temperature ranging from 50-800 ºC. All of the thermal characteristics have been observed in the temperature range of 0 ºC to 800 ºC. TG curves for each synthesized macrocyclic complex are represented in Fig. 1.

Cobalt complexes are thermally stable up to 240 ºC. This complex degrades thermally in three stages. First there is a weight loss of 19% at a temperature between 240 and 290 ºC. Around 300-780 ºC, there is a second phase of decomposition involving 29% weight loss, while in the third stage a weight loss occurs at the temperature range of 790 and 900 ºC with a minimal 1% loss. The thermogravimetric curve of the nickel complex exhibited three stages of thermal breakdown with good thermal stability up to 250 ºC. The TG curve provides information that the first 55% weight loss occurred between

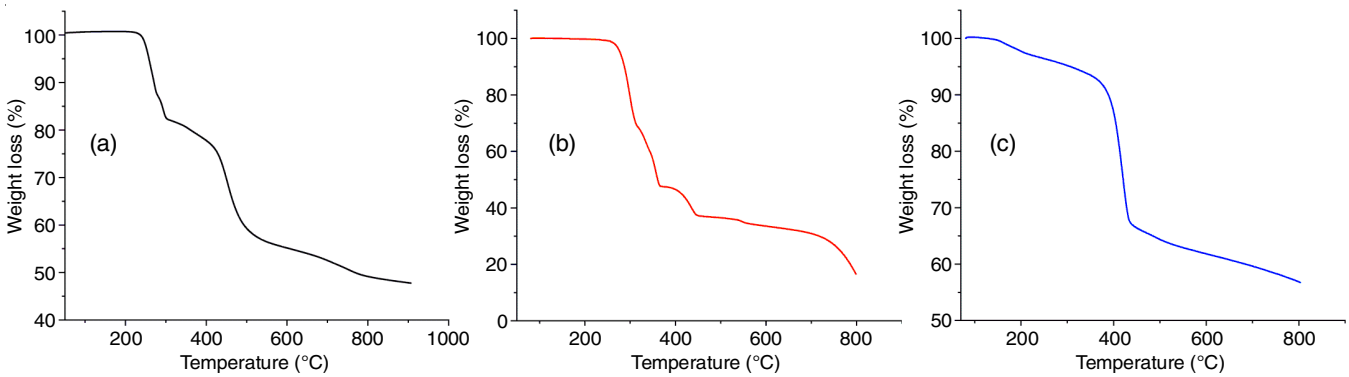


Fig. 1. Thermogravimetric curve of macrocyclic complexes of (a) cobalt (b) nickel and (c) copper

250-370 ºC; second breakdown between 371- 440 ºC represented by 7% of weight loss. Around 500-800 ºC, a third weight loss of 24% was identified. The copper complex displayed the thermal stability up to 130 ºC in the TGA analysis, which indicates the lack of coordinated water molecules in the coordination sphere. A complete disintegration of the copper complex occurred in three phases on the TGA curve. At 130-350 ºC, the initial 8% weight loss was observed. The weight of the complex exhibits a loss of 27% within the temperature range of 350-420 ºC; while the third decomposition was recorded between 430-800 °C that resulted in a 10% weight loss [38].

ESR studies: At room temperature, the electron spin resonance spectrum of copper tetraaza macrocyclic complex was captured on the X-band (frequency 8.75-9.65 GHz) (Fig. 2). A 3,000 G magnetic field with a field centre of 336.791 mT is employed for the analysis. At room temperature, the spectra of copper macrocyclic complexes showed an anisotropic signal and the complexes showed a small hyperfine splitting. Hence, the values of $g_{\parallel}$ and $g_{\perp}$ were determined to be 2.114 and 2.092, respectively. The result unveils the presence of an unpaired electron in the d_{xy} orbital of the complex with a distorted octahedral structure [39].

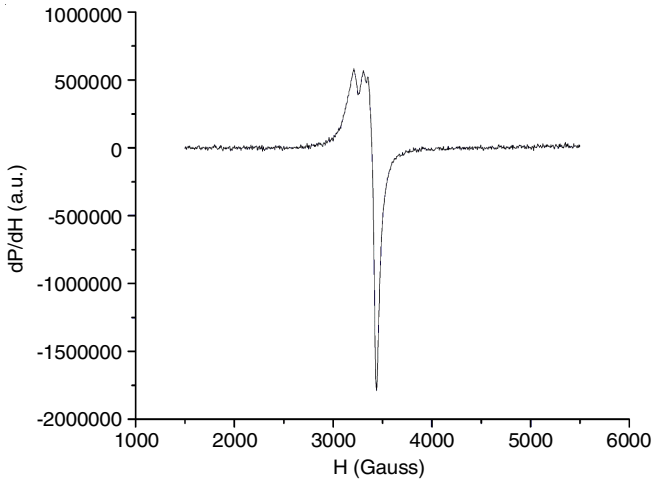


Fig. 2. EPR spectrum of copper complex

Mass spectral studies: In order to elucidate the monomeric or polymeric nature of the synthesized macrocyclic complexes, ESI-MS analysis was carried out. The mass data of the cobalt complex imparts molecular ion peak at *m/z* 540.64 that may corresponds to $[M+3H]^+$ complex (Fig. 3a). In nickel complex, the molecular ion peak is present at *m/z* 538.42, which can be designated to the $[M + H]^+$ while the other peaks at higher molecular mass may be because of addition of more cations from ionization source (Fig. 3b). In the similar way, copper complex exhibits a molecular ion peak at *m/z* 542.53 which corresponds to its molecular weight (542.54) (Fig. 3c).

Electronic spectral studies: At room temperature, the electronic spectra of all the three synthesized macrocyclic complexes were recorded in DMSO solvent.

The Co(II) complex has a magnetic moment of 3.8 B.M. at room temperature, which denotes the existence of three unpaired electrons. The cobalt complex electronic spectra exhibited various bands at 210-270 nm and three broad bands at ~1500 nm, ~1700 nm, ~1900 nm (Fig. 4a), which correspond to the $\pi\rightarrow\pi^*$ and *d–d* transitions respectively. The distortion of the octahedral geometry is compatible with this absorption maxima [40]. In case of nickel macrocyclic complex, an effective magnetic moment (μ_{eff}) was found to be 2.83 B.M. at room temperature and exhibited two bands at ~220 nm and ~300 nm, which are allocated to $\pi\rightarrow\pi^*$ and $n\rightarrow\pi^*$, respectively (Fig. 4b). Three broad bands at ~1600 nm, ~1700 nm and ~1950 nm are due to *d-d* transition. Thus, effective symmetry was found to be octahedral around nickel complex [41].

At room temperature, the copper complex exhibited a magnetic moment (μ_{eff}) of 1.73 B.M. Two bands appeared at ~280 nm and ~400 nm were observed for $\pi\rightarrow\pi^*$ and $n\rightarrow\pi^*$ transition, respectively [42]. It displayed broad bands at the wavelengths of ~1400 nm, 1700 nm and 1900 nm which corresponds to *d–d* transitions (Fig. 4c). These transitions are in good consistency with the octahedral geometry [43,44].

Biological studies

Antimicrobial activity: The zone of inhibition of the synthesized macrocyclic complexes has been studied for their ability to suppress bacterial growth and their effectiveness has been compared to that of standard streptomycin. The majority of the investigated macrocycles exhibit antibacterial action against the selected bacterial species (Table-1). Among all the synthesized complexes, nickel complex exhibited good antimicrobial potency against *E. coli*, *P. aeruginosa* and *S. aureus* while copper complex proved efficient against the bacterial colony *of B. subtilis.* Based on Tweedy's chelation theory, it was revealed that the core metal ions polarity decreased with chelation as the positive charge on metal ion is shared with the donor groups [45], which further increases the lipophilicity of

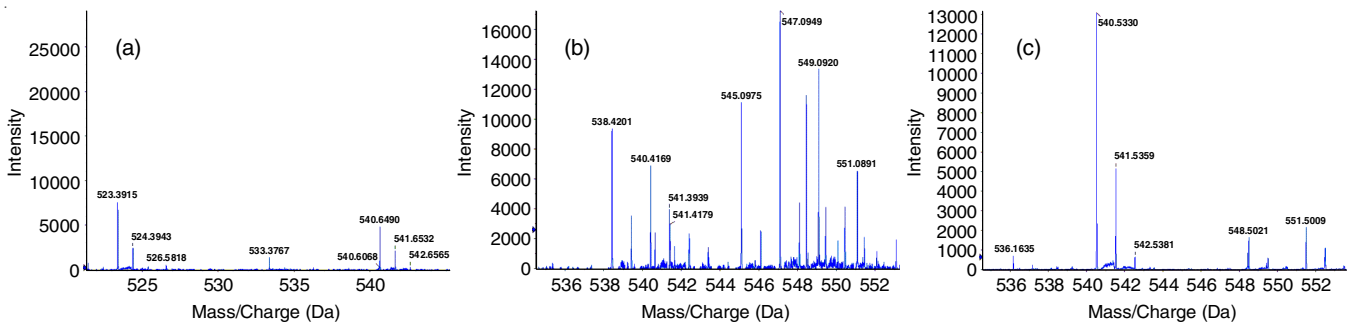


Fig. 3. Mass spectra of macrocyclic complexes of (a) cobalt (b) nickel and (c) copper

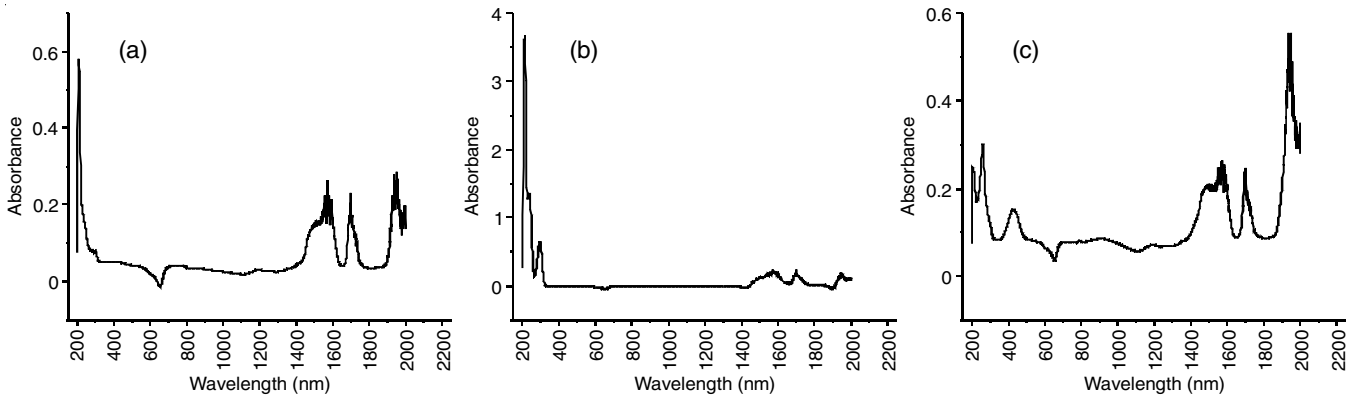


Fig. 4. UV-VIS spectra of macrocyclic complexes (a) cobalt (b) nickel and (c) copper

TABLE-1
ANTIBACTERIAL AND ANTIFUNGAL STUDIES OF MACROCYCLIC COMPLEXES

Compound	Zone of inhibition (mm) at the concentration (µg/mL)					
	2.5	5.0	7.5	2.5	5.0	7.5
	Antibacterial activity					
	E. coli			*P. aeruginosa*		
Cobalt	6.6 ± 0.12	7.5 ± 0.25	9.0 ± 0.15	5.8 ± 0.10	6.7 ± 0.32	7.9 ± 0.10
Nickel	7.6 ± 0.15	8.6 ± 0.20	9.5 ± 0.15	7.5 ± 0.30	8.6 ± 0.15	9.6 ± 0.15
Copper	6.7 ± 0.26	7.5 ± 0.30	8.8 ± 0.32	6.8 ± 0.15	8.4 ± 0.20	9.6 ± 0.25
Streptomycin	21 ± 0.13	25 ± 0.18	25 ± 0.18	24 ± 0.01	28 ± 0.01	28 ± 0.02
	B. subtilis			*S. aureus*		
Cobalt	6.8 ± 0.10	7.8 ± 0.06	9.0 ± 0.10	5.8 ± 0.10	7.8 ± 0.10	8.8 ± 0.17
Nickel	7.6 ± 0.25	8.9 ± 0.06	9.9 ± 0.06	7.7 ± 0.15	8.7 ± 0.20	9.7 ± 0.06
Copper	8.6 ± 0.21	9.7 ± 0.21	10.07 ± 0.21	7.1 ± 0.15	8.3 ± 0.20	9.1 ± 0.15
Streptomycin	12 ± 0.03	16 ± 0.01	18 ± 0.01	13 ± 0.02	18 ± 0.01	18 ± 0.11
	Antifungal activity					
	C. albicans			*A. niger*		
Cobalt	5.7 ± 0.20	6.8 ± 0.06	7.9 ± 0.10	5.9 ± 0.20	6.9 ± 0.10	7.9 ± 0.06
Nickel	6.9 ± 0.10	7.7 ± 0.21	9.0 ± 0.15	6.4 ± 0.26	7.9 ± 0.10	9.5 ± 0.15
Copper	6.5 ± 0.32	7.9 ± 0.06	9.8 ± 0.15	6.5 ± 0.40	7.9 ± 0.10	8.8 ± 0.10
Itraconazole	11 ± 0.01	14 ± 0.02	15 ± 0.01	11 ± 0.06	13 ± 0.07	13 ± 0.10

the macrocyclic complexes core metal ion. This favours their easy passage across the lipid layer of the cell membrane of microorganisms.

The results of *in vitro* antifungal activity displayed that nickel complex exhibited a highest zone of inhibition against *C. albicans* and *A. niger,* which divulges the good antifungal potency (Table-1).

Antioxidant activity: The macrocyclic complexes DPPH scavenging capabilities were represented as IC_{50}, whose concentration was sufficient to achieve 50% of the maximal scavenging activity. The IC_{50} (µM) values of all the synthesized macrocyclic complexes are displayed in Table-2. Results demonstrated that all macrocyclic complexes have high antioxidant activity, but nickel exhibited best antioxidant activity and its scavenging ability was more than that of the standard ascorbic acid [46]. According to their IC_{50} values against the standard ascorbic acid solution, the macrocyclic complexes antioxidant activity was in the following order: nickel > copper > cobalt.

TABLE-2
IC_{50} VALUES (µg/mL) OF SYNTHESIZED MACROCYCLIC COMPLEXES

Compounds	IC_{50} (µg/mL)
Cobalt	65.18
Nickel	8.46
Copper	58.59
Ascorbic acid	12.22

Conclusion

The synthesis of three transition metal macrocyclic complexes using glutaric acid and *o*-phenylenediamine has been sucessfully achieved. All the macrocyclic complexes have been characterized by several spectroscopic techniques such as IR, ESR, mass, electronic spectra and TGA, which supports their octahedral geometry. The antimicrobial and antioxidant activity profiles of the investigated macrocyclic complexes indicated increased biological activity, which might be attributed due to the presence of metal ions in the coordination sphere of macrocyclic complexes.

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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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