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Syntheses, Characterizations and Antimicrobial Activity of Three New Mixed Ligand Fe(III) Ce(IV) and Th(IV) Schiff Base Chelates

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ABSTRACT

In the present study, a Schiff base (HL1) derived from the condensation reaction of L-alanine with o-hydroxyacetophenone in 1:1 molar ratio was synthesized. The Schiff base and ethylenediamine compound were used to form three mixed ligand chelates with iron(III), cerium(IV) and thorium(IV) ions. The compounds were characterized using several analytical and spectroscopic tools [IR, electronic, NMR and mass]. The results showed the bonding behavior between the ligands and metal ions. The antimicrobial activities of the ligands and mixed ligand chelates were investigated against *Staphylococcus aureus*, *Serratia marcescens*, *Acinetobacter baumannii* and *Candida albicans* using the agar disk diffusion method. The results indicated that the antimicrobial activity of the tested compounds exhibited a fairly good inhibitory effect on the pathogenic microbe's species. In contrast, the L-alanine showed no antimicrobial activity against *A.baumannii*. Schiff base, L-alanine, o-hydroxy acetophenone , [Fe(L1) (L3)(OH)] .H_2O, [Ce(L1) (L3)(SO_4)(H_2O)].H_2O and [Th(L1)(L3)(NO_3)$_2$]. 2H_2O showed bacteriostatic activity against the highly susceptible species of pathogenic bacteria (*S. aureus* and *S. marcescens*) with MIC reached 50 mg/ml. while ethylenediamine suppressed bacterial growth of these species at a concentration of 25 mg/ml.

Keywords: Schiff base, Ethylenediamine, Mixed ligand chelates, Antimicrobial activity, MIC.

INTRODUCTION

When two ligands or more were bonded to the metal ion, a mixed ligand complex is formed, where, each ligand has active atoms donated electrons to metal ion. This kind of complexes led the scientists to work on

them (Srivastava & Newman, 1972). The pharmacological activity of coordination metal compounds depend on the nature of the ligands and metal ions, in addition to the geometrical structures of the compounds, the mentioned factors are much important to determine the ability of the coordination compounds to locate the suitable target site of the their activity and as a consequence their pharmacological activity (Selvaganapathy & Raman, 2016; Anitha *et al.*, 2012). As we known that certain metal ions penetrate through bacteria cell walls, in the form of complexes to kill the bacteria (Slavin *et al.*, 2017; Chohan *et al.*, 2002). The Schiff base moieties at the poly functional donor site were widely used in the analytical, biocidal, agrochemical, enzyme models, catalysis, food, chemical and dye sectors (Kan *et al.*, 2013; Patel *et al.*, 2006). Schiff bases and their complexes are adaptable compounds made by precipitating a primary amines or an amino acids with a carbonyl group. It has been stated that Schiff base complexes show virtuoso incentive activities exemplified by copious reactions (AL-Garawi *et al.*, 2012). Lakshmi and Geetha (2016) prepared four mixed ligand complexes of Cu(II), Ni(II), Zn(II) and Co(II) ions with Schiff base formed by the condensation of L-tryptophan and 2-hydroxyacetophenone as primary ligand (HL1) and temn-N,N,N,N-tetramethylethylenediamine as secondary ligand(L2). The ligands and their mixed ligand complexes were characterized by using analytical and spectral tools. All the compounds were examined for their biological activity against some pathogenic organisms (bacteria and fungi). Hossian *et al.* (2019) synthesized and characterized two mixed ligand complexes of Ni(II) and Cu(II) ions with a Schiff base resulted from the reaction of isoniazid and p-anisaldehyde as main ligand and 2,2-Bipyridine as co-ligand. The ligands and their mixed ligand complexes were studied using several spectral techniques[IR, UV-Vis, NMR, mass] (Ghosh & Pal, 2015). Moreover, Prashanthi and Kiranmai (2012) have synthesized and examined Ni(II) mixed ligand complexes of some Schiff bases and 1,10-phenanthroline. The antimicrobial activity of these complexes have been screened against different microorganisms. It was found that the activity of tertiary complexes is more as compared to their corresponding binary complexes.

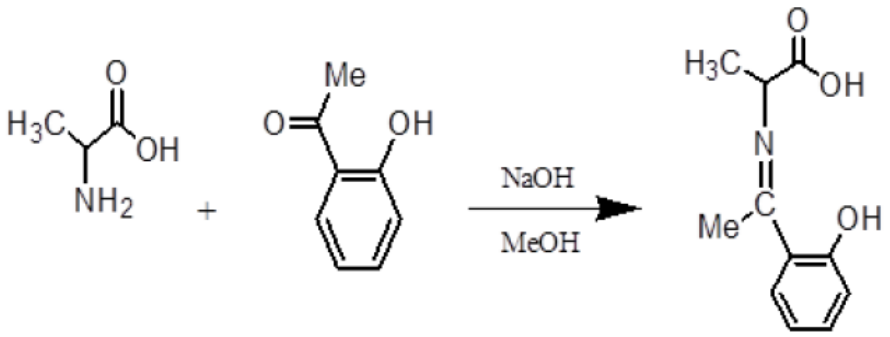
The aim of this study, therefore was to synthesis and characterize three mixed ligand chelates using a Schiff base derived from o-hydroxyacetophenone and L-alanine (HL1) as primary ligand and ethylenediamine ligand(L2) as secondary ligand. Moreover, this research was conducted to study the antimicrobial activity of all tested compounds against some pathogenic microbes species.

MATERIALS AND METHODS

All the chemicals and solvents used in this work were analytical Analar (BDH , Aldrich, Fluka and Merck), and used without purification. Chemicals and reagents which used to prepare the Schiff base and mixed ligand chelates were determined by ethylenediamine, o-hydroxyacetophenone, amino acid (L-alanine), acetic acid, $FeCl_3.6H_2O$ $Ce(SO_4)_2.4H_2O$ and $Th(NO_3)_4.4H_2O$, DMF, DMSO, NaOH, methanol, ethanol and distilled water.

Preparation of amino acid Schiff base (HL1)

The present amino acid Schiff base(scheme-1) was prepared as follows: NaOH (0.4g, 0.01 mol) was dissolved in 25mL methanol and L-alanine (0.90 g, 0.01 mol) was added to it. The mixture was stirred at room temperature for 5 minutes. When the mixture becomes homogeneous, o-hydroxyacetophenone (1.36g, 0.01mole) was added. After 2 minutes, the mixture was evaporated to 20% of its original volume and 1mL of acetic acid was added immediately. After 2 hours, a yellow product was formed. The obtained product was filtered, washed, dried and recrystallized from hot methanol to provide an excellent yield(85%) of pure crystals(El-ajaily *et al*.,2010).



L-alanine o-hydroxyacetophenone Schiff base HL1

Scheme 1: Preparation of amino acid Schiff base

Preparation of mixed ligand chelates

0.01mole;(2.70, 4.04 and 5.52g) of hydrated metal salts [$FeCl_3$, $Ce(SO_4)_2.4H_2OW$, $Th(NO_3)_4.4H_2O$], respectively, were dissolved in 25mL of absolute methanol solution and added to the same volume of a methanolic solution of HL1; (0.01mole;2.07g). Slowly, few drops of sodium hydroxide solution were added to adjust the P^H value at 8 until the chelates isolated. Subsequently, the mixtures were stirred and refluxed for one hour, then the secondary ligand ethylenediamine (0.01mole,0.60g) was added to the mixtures dropwise. The obtained mixtures were again refluxed for extra 3-4 hours. The obtained crystals were filtered, washed several times with hot ethanol until the filtrates become clear. The resulting pure crystals were dried in desecrator under calcium chloride(Sari & Gurkan, 2004) .

Test microbes

Four pathogenic microbial species, including three species of bacteria Staphylococcus *aureus* , *Serratia marcescens* and *Acinetobacter baumannii* and unicellular fungi *Candida albicans*.

Preparation of culture media

Mueller Hinton Agar (MHA) was prepared by suspending 38 g in 1000 ml of distilled water. The media was sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50 °C, then pour into sterile Petri plates.

Assay of anti-microbial activity

The ligand and its complexes were tested against the bacterial species and the fungal species using the agar disk diffusion method (Ashraf *et al.*, 2011; Matar *et al.*, 2015). The dimethylformamide solvent (DMF) was used as negative control. Ciprofloxacin, Imipenem, Colistin (antibacterial) and Clotrimazole (antifungal) were used as positive controls for comparison with the ligand and the synthesized metal complexes. Whatman filter paper (no. 1) discs of 5 mm diameter were sterilized in an autoclave and then soaked in the desired concentration (100mg/ml) of the tested compounds. Petri dishes were poured with (MHA) medium and allowed to solidify to make a base layer. The paper discs were placed on the top of Mueller-Hinton agar plates seeded with tested microbial species separately and then the plates incubated at 37°C. After the incubation the plates were observed for formation of clear inhibition zone around the disk indicated the presence of antimicrobial activity. The diameter of inhibition zone around each disc was measured after 24 hours in the case of bacteria and 48 hours in the case of fungi. The experiment was performed in triplicate for each microbe and tested compounds and the mean zone of inhibition was calculated for each compound and standard antibiotic.

Determination of minimum inhibitory concentration (MIC) of the tested compounds

The MIC test was prepared according to the method of Mostafa *et al.*, (2018), with some modifications. Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial agent that will inhibit the visible growth of a microorganism after overnight incubation. The MIC of the tested compounds was determined using disk diffusion method and evaluate their efficacy in controlling microbial species causing diseases. Various concentrations of the synthesized compounds [L-alanine, o-hydroxyacetophenone, ethylenediamine, mixed ligand chelates] (3.1, 6.25, 12.5, 25, 50, and 100 mg/ml) were prepared separately by dissolving 300mg in 3 ml of dimethylformamide (DMF) and loaded their requisite amount over sterilized filter paper discs (5 mm in diameter). The loaded filter paper discs

with various concentrations of the synthesized compounds were placed on the top of the Mueller-Hinton agar plates. The plates were incubated in the incubator at 37 °C for 24 hours. The inhibition zones (IZ) were measured with a ruler and recorded against the concentrations of the synthesized compounds.

RESULTS AND DISCUSSION

Physical properties, microanalysis and molar conductivity

The amino acid Schiff base is soluble in alcohols, DMSO or DMF solvents, but not in water, meanwhile, the synthesized mixed ligand chelates are not soluble in alcohols, but they are partially soluble in DMF and DMSO solvents. All the compounds are stable in air. The carbon, hydrogen, nitrogen and sulfur elemental analysis data of the Schiff base and mixed ligand chelates show a good agreement between the calculated and found values indicating the formation of the compounds under investigation as presented in (Table 1). Also, the molar conductivity values of the mixed ligand chelates displayed the existence of non-electrolytic nature (Tamiru *et al.*, 2019), confirming the absence of any inorganic anions outside the coordination sphere.

Table 1: Analytical, some related properties and molar conductivity

Compounds	M. wt.	Color	Elemental analyses Calcd. **Found**				^m; $\Omega^{-1}cm^2mol^{-1}$	µ B.M
			C%	H%	N%	S%		
HL1; ($C_{11}H_{13}NO_3$)	207	Daffodil	63.77 **64.20**	6.28 **5.21**	6.76 **6.25**	----	_____	-------
[Fe((L1)(L2)(OH)].H_2O	356	Merlot	43.82 **43.87**	6.74 **6.68**	11.80 **11.98**	----	0.00033	6.11
[Ce(L1)(L2)(SO_4)(H_2O)].H_2O	477	Tortilla	32.70 **31.89**	5.24 **5.00**	8.81 **8.50**	6.71 **6.25**	0.001187	0.00
[Th((L1)(L2)(NO_3)$_2$].$2H_2O$	657	White Dove	23.74 **24.10**	3.81 **3.52**	6.39 **6.80**		0.000848	0.00

Infrared spectrum of the Schiff base (HL1)

The IR spectral results of Schiff base compound HL1; ($C_{11}H_{13}NO_3$) is shown in Table 2 and its spectrum is shown in Figure 1. The strong broad band is appeared at 3441cm^{-1} due to -O-H vibration of the phenolic (Thakur *et al.*, 2007). This band vanished due to complexation with metal ion. The spectrum shows a band at 1605cm^{-1}due to the formation of azomethine group (C=N) (Karipcin & Kabalcilar, 2007). The same spectrum exhibits a band at 3094 cm^{-1} assigned to υCOOH vibration (Ejidike & Ajibade, 2015).

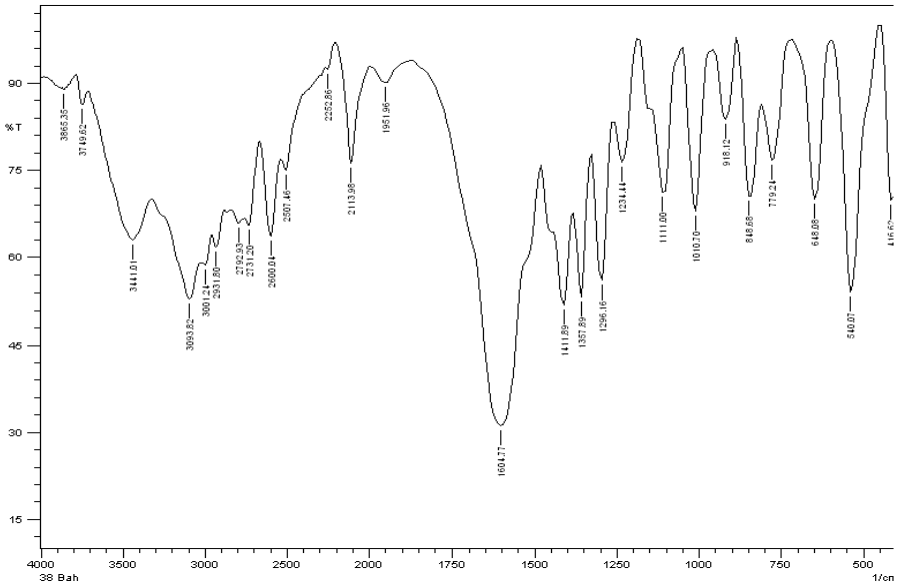


Figure 1: Infrared spectrum of the Schiff base HL1

IR spectra of the mixed ligand chelates

The infrared spectra of the [Fe(III), [Ce(IV) and [Th(IV) mixed ligand chelates are shown in Figures 2-4 and their bands assignments are listed in Table 2. In the free Schiff base, the band at 1605 cm^{-1} is analogous to the azomethine υ(-C=N) group, this band is changed (1620-1558 cm^{-1}) indicating the participation of this group through nitrogen atom in chelation process (Palanimurugan *et al.*, 2019). The band at 3441 cm^{-1} is due to the phenolic group of the free Schiff base ligand which was absent in the spectrum of the chelates, indicating coordination of phenolic oxygen after deprotonation (Gobara, 2017). Other bands seen at 1381, 1219, 1055 and 733 cm^{-1} corresponding to vibrations of the two bidentate nitrate groups in the Th(IV) mixed ligand chelate (Thakur & Shaikh, 2006). In IR spectrum of the Ce(IV) mixed ligand chelate exhibits three bands at 1303, 1134 and 1034 cm^{-1}, these bands are attributed to the spilt bands of bidentate coordinated sulphate group (Nakamoto, 1978). The band due to carboxylic (O–H) stretching that observed in the free Schiff base is absent in the spectra of the mixed ligand chelates confirming the involvement of this group in complexation through oxygen atom (Fugu *et al*., 2013). New bands in the regions 733-617 and 540-439 cm^{-1} refer to υ(M-O) and υ(M-N) vibrations (Thakkar & Thakkar, 2000). The band owing to–NH_2 group is overlapped with the bands of crystal water molecules present in the mixed ligand chelates(Derebe et al., 2002). Also, the band of Ce(IV) mixed ligand chelate at 825cm^{-1} vibration agrees with frequency reported for coordinated water molecule (Alassbaly *et al.*, 2016).

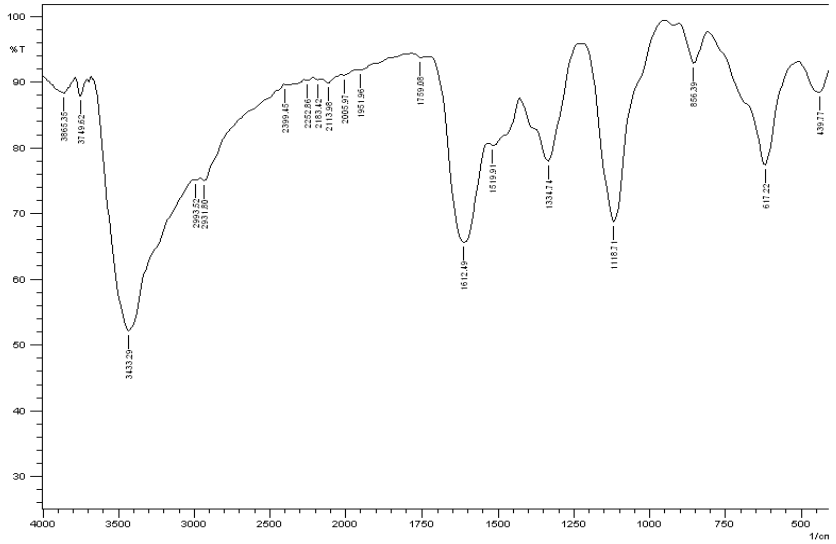


Figure 2: Infrared spectrum of [Fe(L1)(L2)(OH)].H_2O mixed ligand chelate**.**

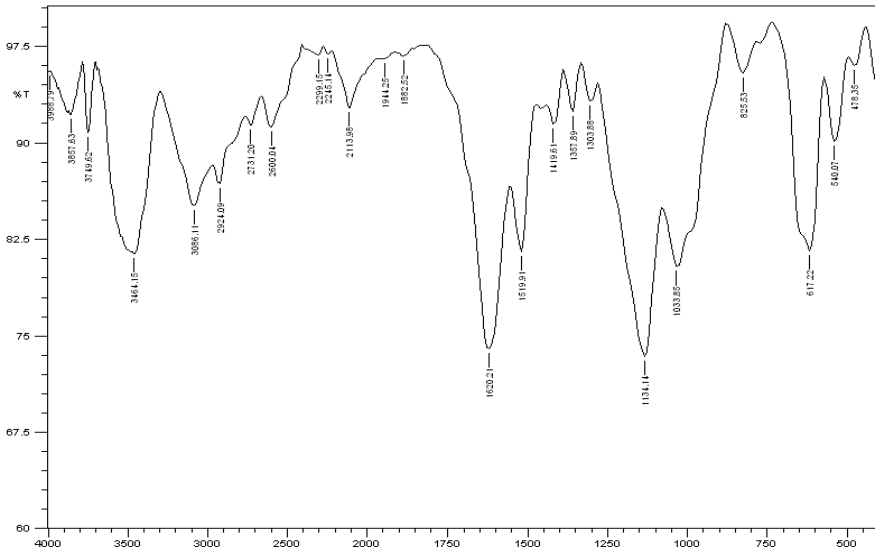


Figure 3: Infrared spectrum of [Ce(L1)(L2)(SO_4)(H_2O)].H_2O mixed ligand chelate.

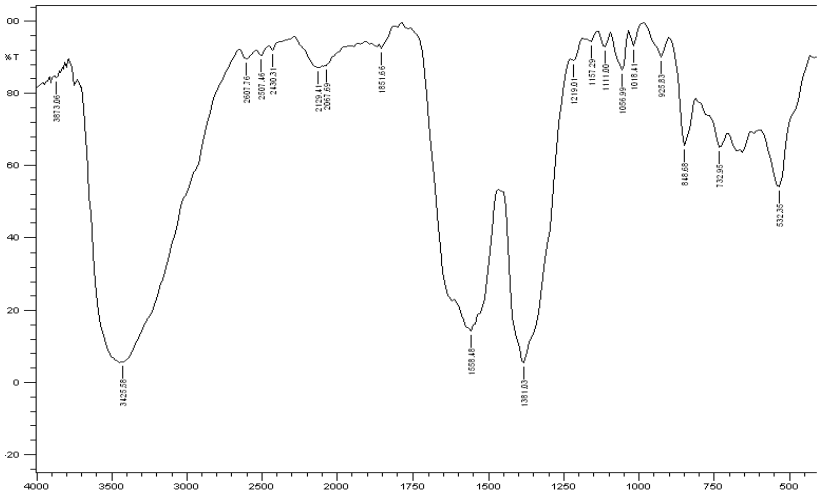


Figure 4: Infrared spectra of [Th(L1L2)(NO_3)$_2$].$2H_2O$ mixed ligand chelate**.**

Table 2: Characteristic infrared absorption frequencies ($cm^{-1)}$ of the Schiff base and mixed ligand chelates

Compounds	υ (OH) (H_2O) υ	ʊ (C=N)	ʊ (M-O)	ʊ (M-N)
HL1; ($C_{11}H_{13}NO_3$)	3441	1605	---	---
[Fe(L1)(L2)(OH)].H_2O	3433	1612	617	439
[Ce(L1)(L2)(SO_4)(H_2O)].H_2O	3462	1620	617	540 476
[Th(L1)(L2)(NO_3)$_2$].2H_2O	3426	1558	733	532

Electronic spectra and magnetic moments

The electronic spectral assignments(**nm**.cm^{-1}) of the Schiff base and mixed ligand chelates are listed in Table 3 and their spectra are shown in figures 5-7. The spectrum of the Schiff base (HL1), (figure 5) exhibits absorption bands at 260 nm (38711cm^{-1}) and 313 nm(31949 cm^{-1}) corresponding to $\pi \rightarrow \pi^*$ (phenyl ring) and $n \rightarrow \pi^*$ transition, respectively (Al-Jeboori *et al.*, 2014).

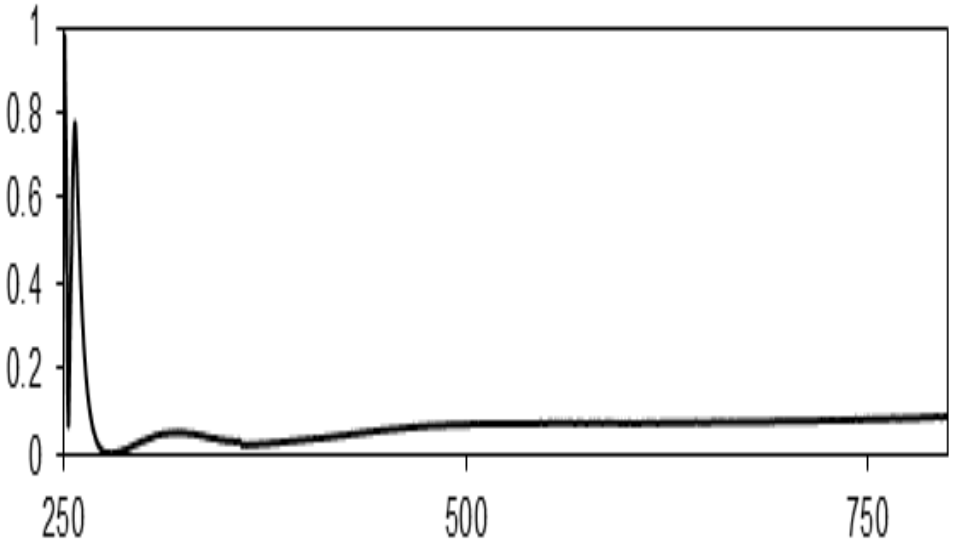


Figure 5: Electronic spectrum of Schiff base (HL1)

Whereas, the Fe(III) chelate spectrum exhibits two bands at 323nm (30959 cm^{-1}) and 600-700nm (16667-14286 cm^{-1}) which are due to M→L charge transfer excitation and d-d transitions (Hamil *et al.*, 2012; EL-zweay *et al.*, 2013; Hosny & El-Dossoki, 2008). Both magnetic moment value (6.11B.M) and the intensity of the bands suggest an octahedral geometry around Fe(III) mixed ligand chelate (Al-Noor *et al.* 2017). As mentioned above, the UV spectrum of the Schiff base ligand shows two bands at

260(38462 cm^{-1}) and 313 nm(31949 cm^{-1}) which are attributed to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions. These bands were slightly red shifted to 266-318 nm (37539-31446 cm^{-1}) in the spectrum of cerium(IV) mixed ligand chelate which can be attributed to the binding of these coordination centers to the central metal ion (Amani *et al.*, 2007). A new absorption band at 419 nm(23866 cm^{-1}) which is appeared in the spectrum of Ce(IV) mixed ligand chelate is due to charge transfer transition (Alghool *et al.*, 2013). The electronic spectral data of the Th(IV) mixed ligand chelate reveal a band approximately at 310 nm (32258cm^{-1}) that is ascribed to the $n \rightarrow \pi^*$ transition. This band shifts in comparison to the position band in the free Schiff base, revealing changes in the location of lone pair electrons of the azomethine chromosphore's after chelation with the metal ion (Klamm *et al.*, 2018). Due to the ligand-metal charge transfer, an absorption band at 451nm(22173 cm^{-1}) appears in the spectrum of Th(IV) mixed ligand chelate (Liu *et al.*, 2009). The absence of any absorption bands in the electronic spectra of the Ce(IV) and Th(IV) above 460 nm denotes the absence of d-d transition, indicating the d^0 configuration of cerium and thorium ions. Also, the spectra of Ce(IV) and Th(IV)) mixed ligand chelates exhibit no clear transitions in the range of 500-700 nm(20000-14286 cm^{-1}), because the f-f bands are line or sharp. This is attributed to the shielding of the f -orbitals by 6s, 6p or 7s, 7p orbital's (Humelnicu *et al.*, 2020).

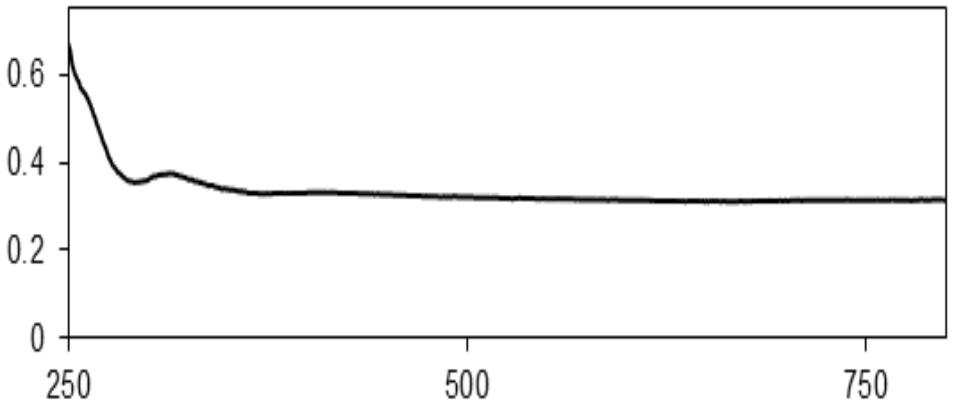


Figure 6: Electronic spectrum of [Fe(L1)(L2)(OH)].H_2O mixed ligand chelate

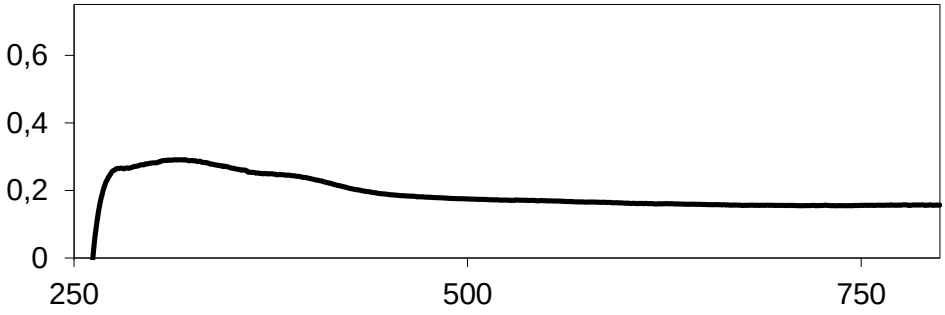


Figure 7: Electronic spectrum of $[Ce(L1)(L2)(SO_4)(H_2O)].H_2O$ mixed ligand chelate

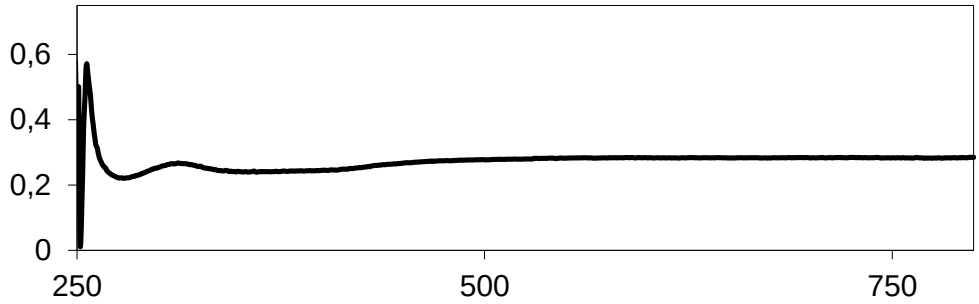


Figure 8: Electronic spectrum of $[Th(L1)(L2)(NO_3)_2].2H_2O$ mixed ligand chelate

Table 3: Electronic spectral data**(nm**, cm^{-1}) of the Schiff base and mixed ligand chelates

Compounds	**nm** (cm^{-1})	**assignment**
HL1; ($C_{11}H_{13}NO_3$)	260**nm(**38711cm^{-1})	$\pi^{*}\rightarrow \pi$
	313 **nm**(31949 cm^{-1}	$n\rightarrow \pi^{*}$
$[Fe(L1)(L2)(OH)].\ H_2O$	323**nm** (30960 cm-^{1})	MLCT
	600-700**nm**(16667-14286cm^{-1})	d-d transition
$[Ce(L1)(L2)(SO_4)(H_2O)].H_2O$	266-318 **nm**(37539-31446cm^{-1})	$\pi\rightarrow \pi^{*}$ $n\rightarrow \pi^{*}$
	419 **nm**(23866 cm^{-1})	CT
	500-700**nm**	f-f transitions
	(16667-14286cm^{-1})	
$[Th(L1)(L2)(NO_3)_2].2H_2O$	310**nm** (32258 cm-^{1})	$n\rightarrow \pi^{*}$
	451**nm**(22173cm-^{1})	CT
	500-700 **nm**(16667-14286cm^{-1})	f-f transitions

^{1}HNMR spectrum of Schiff base

The ^{1}HNMR spectra of the compounds were obtained in dimethylsulphixide (d^6 DMSO solution as a solvent at room temperature using tetramethylsilane(TMS) as an internal reference. In the present study, the ^{1}HNMR spectral signals are depicted in Figure 9. The resonance of protons has been assigned on the basis of their integration and multiplicity pattern. The spectrum shows two signals at 4.1 and 7.73 ppm, which are due to the existence of –OH proton in phenyl ring and –COOH in the amino acid. Also, the signals at 1.2, 2.3, 3.2 and 2.5 ppm are due to the presence of methyl groups and DMSO solvent, respectively (McCleverty & Meyer, 2003; El-ajaily *et al.*, 2016). The aromatic protons have been resonated in the region at 6.4-7.5 ppm (Al-barki *et al.*, 2016; Sobola & Watkins, 2013).

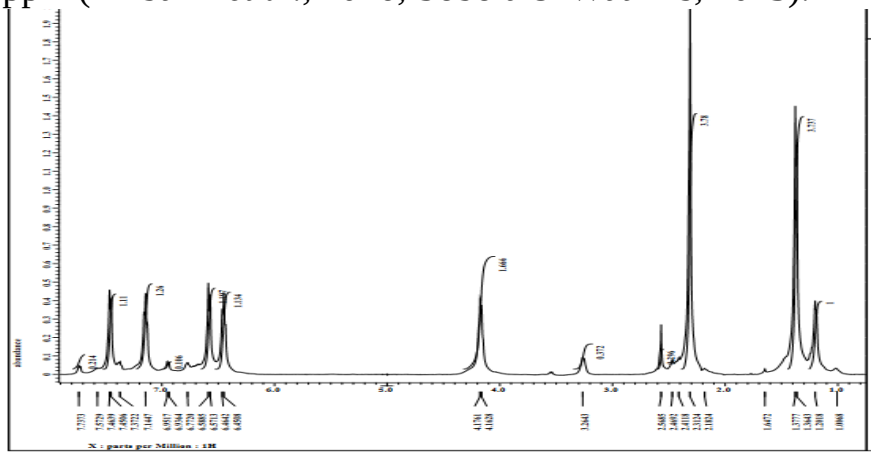


Figure 9: ^{1}HNMR spectrum of Schiff base HL1

Mass spectra

The mass spectral fragmentation data of Schiff base HL1(Figure 10) and [Fe(L1)(L2)(OH)] .H_2O (Figure 11) mixed ligand chelate are listed in Table 4.

Table 4: Mass spectral fragmentation of the Schiff base and mixed ligand chelate

Compound	Fragmented ion	m\z$^+$ Value
HL1; ($C_{11}H_{13}NO_3$)	$C_{11}H_{13}NO_3$	207
	$C_{11}H_{10}NO_2$	188
	$C_8H_9NO_2$	151
	C_7H_9O	109
	C_7H_7	91

Compound	Fragmented ion	m\z$^+$ Value
[Fe(L1)(L2)(OH).H_2O	$C_{13}H_{22}N_3O_5Fe$	356
	$C_{13}H_{22}N_3O_4Fe$	340
	$C_{12}H_{20}N_3O_2Fe$	294
	$C_{11}H_{18}N_3Fe$	248
	C_8H_8N	118
	C_8H_8N	

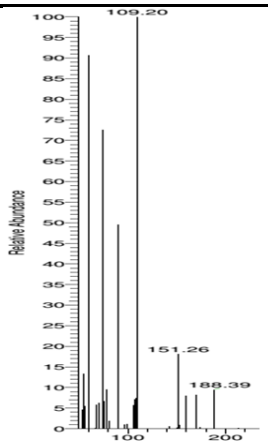


Figure 10: Mass spectrum of the Schiff base (HL1)

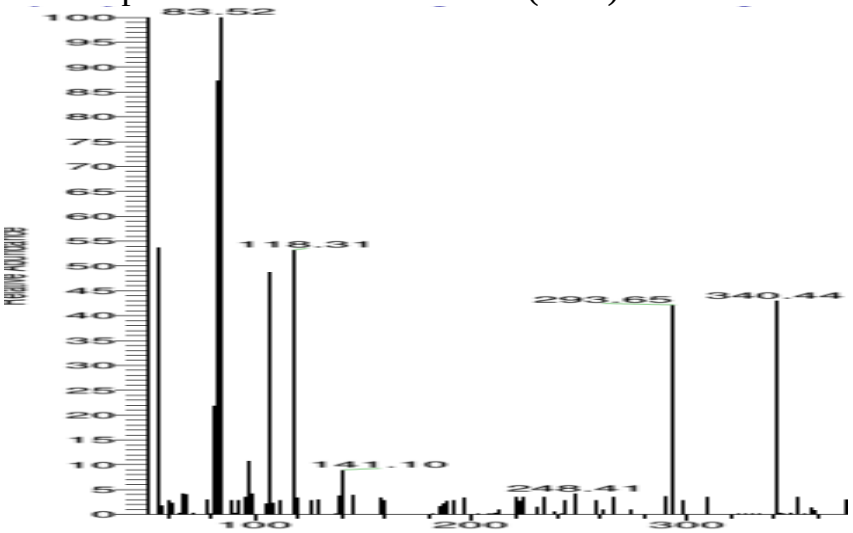


Figure 11: Mass spectrum of [Fe(L1)(L2)(OH)].H_2O-mixed ligand chelate

Carbon nuclear magnetic resonance spectrum of Schiff base

The carbon nuclear magnetic resonance spectrum for the Schiff base has been recorded in d^6-DMSO as a solvent. (Figure 12) exhibits a chemical shift at 170.82 ppm refers to imine carbon atom (C=N) group (Abdel Rahman *et al.,* 2015). While the other chemical shift at 173.19 ppm is

attributed to the carboxylic carbon atom COOH group (Salama *et al*., 2017), and signals at 57.62, 19.94 and 14.23 ppm assigned to carbons of methyl groups (Lateef *et al.,* 2016). Signals that are associated with the aromatic carbons have been detected at a range of 113.02-133.40 ppm and signal at 169.96 ppm may be due to carbon of C-OH phenolic group (Ossowicz *et al*., 2013; Pervaiz *et al*., 2019; Alothman *et al*., 2021). The signal observed at 39.50 ppm leads to d^6-DMSO (Atiyah *et al*., 2020).

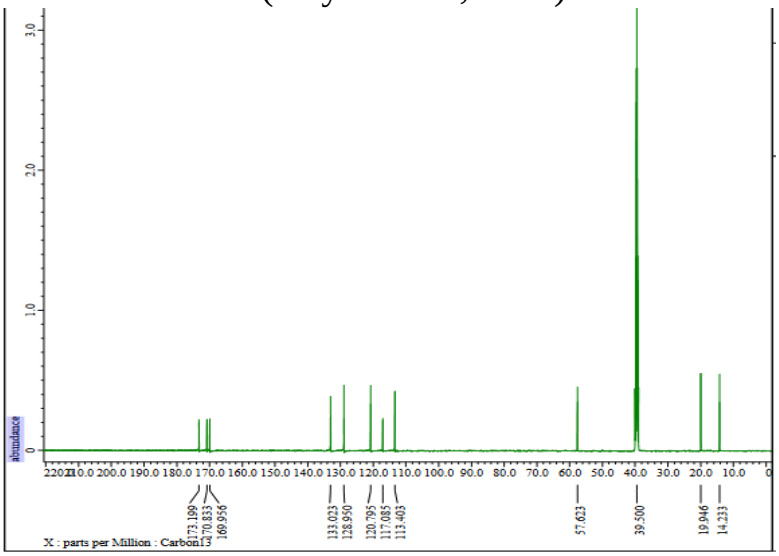


Figure 12: ^{13}CNMR spectrum of Schiff base

Assay of anti-microbial activity

The antibacterial and antifungal screening results are given in Table 5 and illustrated in (Figures 12-16). DMF is used as negative control and Ciprofloxacin (CIP), Imipenem (IPM) and Colistin (CT) are used as positive standard for antibacterial and Clotrimazole (CLT) for antifungal activities. The antimicrobial activity of the tested compounds was evaluated by agar disk diffusion method. The tested compounds exhibited a fairly good inhibitory effect on the pathogenic microbes species with inhibition zones of 7 to 14 mm. In contrast, the L-alanine showed no antimicrobial activity against *A. baumannii*. The tested compounds showed less antimicrobial activity when compared to the standard antibiotics used in this study. The MIC results have been reported in Table 6 and Figures 17-18. The inhibitory effect of Schiff base, L-alanine, o-hydroxyacetophenone, [Fe(L1)(L3)(OH)].H2O, [Ce(L1)(L3)(SO4)(H2O)]. H2O and [Th(L1)(L3)(NO3)2]. 2H2O reached 50 mg/ml with an inhibition zones of 9 to 12 mm against *S. aureus* and 9 to 11against *S. marcescens*. Whereas the inhibitory effect of ethylenediamine was 25 mg/ml with an inhibitory zones of 10 and 9 mm against *S. aureus* and *S. marcescens*. These results showed

in Table 5 can be attributed to the structure of the tested compounds, which seemed to be the principal factor influencing the antimicrobial activity. This property is certainly correlated with the ability of a compound to diffuse through the biological membranes, in turn reaching its site of action. The membrane of Gram-negative bacteria contains lipopolysaccharides, therefore the reported Schiff base and their metallic complexes could be combined with these lipoic layers to enhance the membrane permeability of the Gram-negative bacteria (Gaballa *et al.*, 2007). Accordingly, the lipid membrane surrounding the cell favors the passage of any lipid soluble materials like lipophilicity is an important factor controlling antimicrobial activity. The increased lipophilicities of Schiff bases and complexes permit easy penetration into lipid membranes and seizing the growth of the organism (Abu-Dief & Mohamed, 2015). In general, the complexes can inhibit a series of enzymes that play important roles in different metabolic processes that affect the development and growth of microorganisms (El-Sherif & Eldebss, 2011).

Table 5: Antimicrobial activity results of tested compounds on pathogenic microbes species

No.	Compounds			The zone of inhibition is measured in millimeter			
				Concentration 100 mg/ml			
				bacterial species			fungal pecies
				S. aureus	*S. marcescens*	*A. baumannii*	*C. albicans*
1	Schiff base			11	13	12	9
2	L-alanine			12	11	R	12
3	Ethylenediamine			14	13	13	10
4	o-hydroxyacetophenone			12	11	13	9
5	$[Fe(L1)(L3)(OH)].H_2O$			13	12	9	10
6	$[Ce(L1)(L3)(SO_4)(H_2O)].H_2O$			11	11	7	10
7	$[Th(L1)(L3)(NO_3)_2].2H_2O$			13	11	7	8
8	C^+	Ciprofloxacin	Anti-bacterial	23	R	R	R
		Imipenem		R	21	R	R
		Colistin		R	R	13	R
		Clotrimazole	Antifungal	R	R	R	26
9	C^- DMF			R	R	R	R

R: Resistant

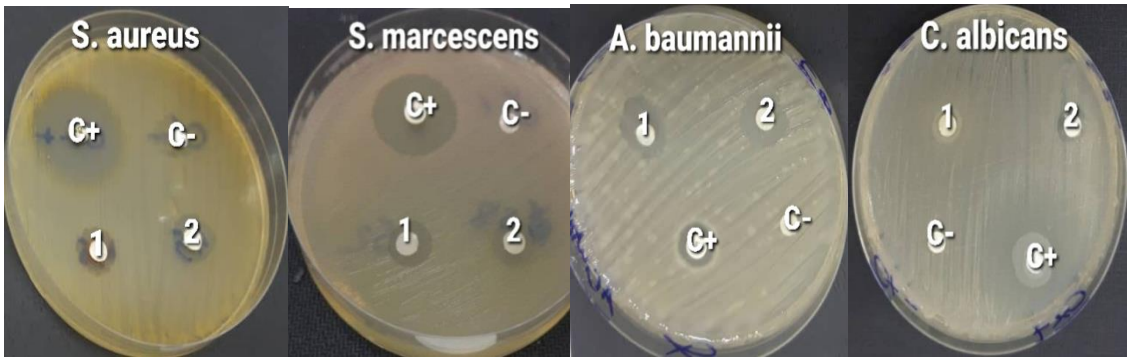


Figure 12: Effect of **1-** Schiff base and **2-** o-hydroxyacetophenone on microbes species at 100 mg/ml concentration, compared with a positive and a negative controls.

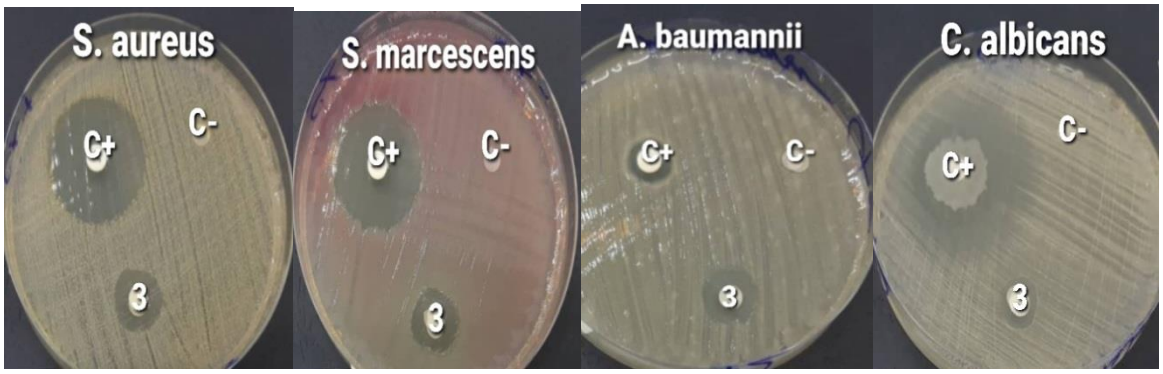


Figure 13: Effect of **3-** Ethylenediamine on microbes species at 100 mg/ml concentration, compared with a positive and a negative controls.

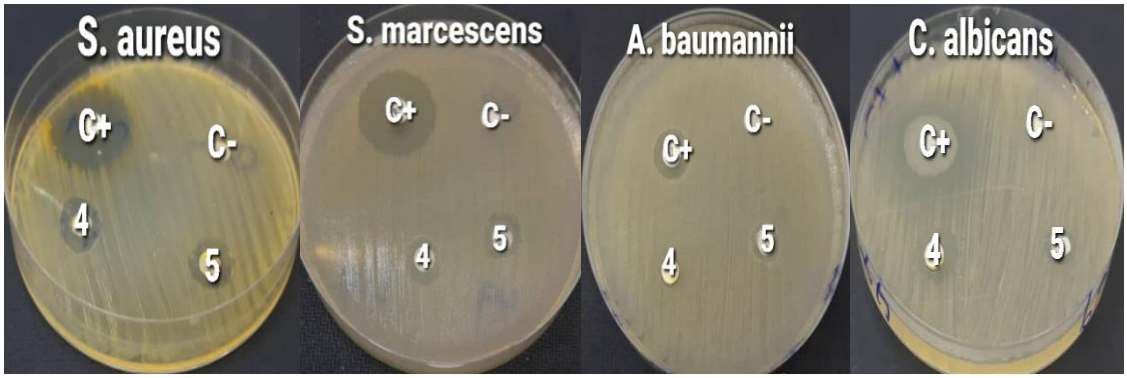


Figure 14: Effect of **4-** L-alanine and **5-** $[Ce(L1)(L3)(SO_4)(H_2O)].H_2O$ on microbes species at 100 mg/ml concentration, compared with a positive and a negative controls.

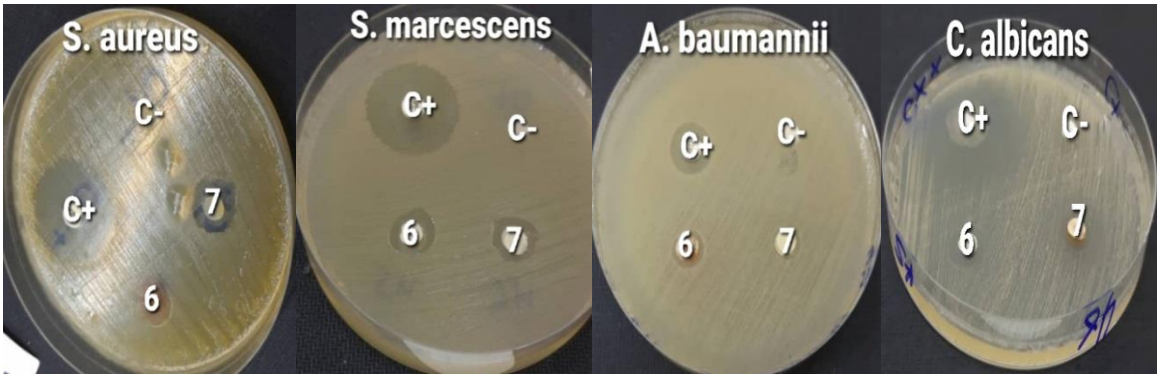


Figure 15: Effect of **6-** [Fe(L1)(L3)(OH)].H_2O and **7-** [Th(L1)(L3)$(NO_3)_2$]. $2H_2O$ on microbes species at 100 mg/ml concentration, compared with a positive and a negative controls.

Table 6: MIC of the tested compounds against *S. aureus* and *S. marcescens*.

S.No.	Compounds	Concentration in mg/ml											
		Gram (ve^+) bacteria						Gram (ve^-) bacteria					
		S. aureus						*S. marcescens*					
		3.1	6.2	12.5	25	50	100	3.1	6.2	12.5	25	50	100
1	Schiff base	-	-	-	-	10	11	-	-	-	-	10	13
2	L-alanine	-	-	-	-	10	12	-	-	-	-	9	11
3	Ethylenediamine	-	-	-	10	12	14	-	-	-	9	10	13
4	o-hydroxyacetophenone	-	-	-	-	9	12	-	-	-	-	10	11
5	[Fe(L1)(L3)(OH)].H_2O	-	-	-	-	11	13	-	-	-	-	11	12
6	[Ce(L1)(L3)$(SO_4)(H_2O)$].H_2O	-	-	-	-	9	11	-	-	-	-	9	11
7	[Th(L1)(L3)$(NO_3)_2$].$2H_2O$	-	-	-	-	10	13	-	+	-	-	9	11

-No activity (there was no zone of inhibition)

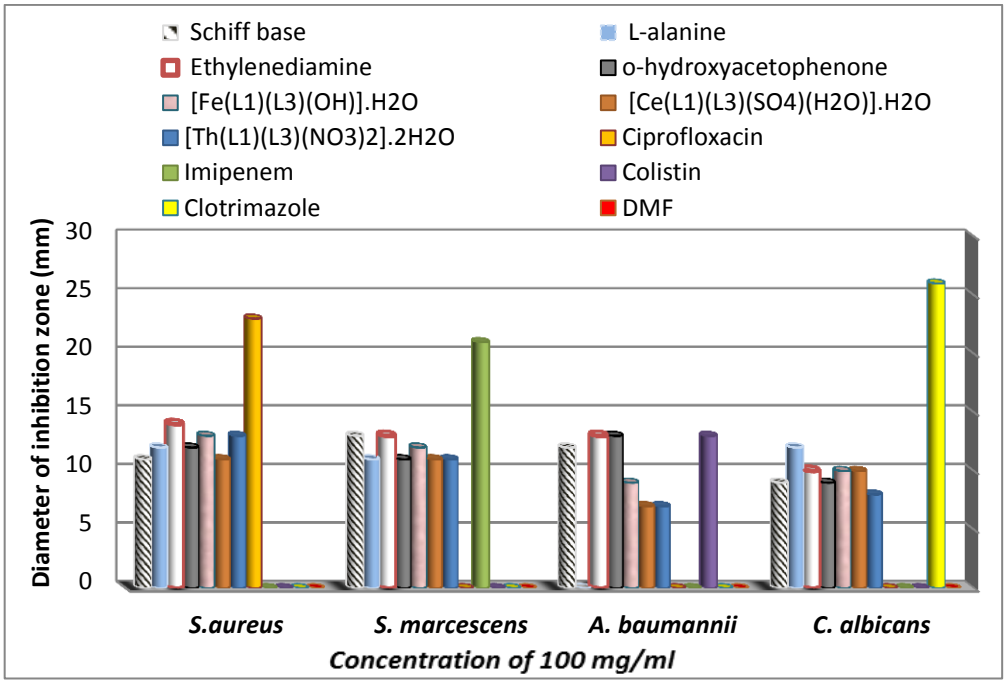


Figure 16: Effect of tested compounds and standard antibiotics on microbes species

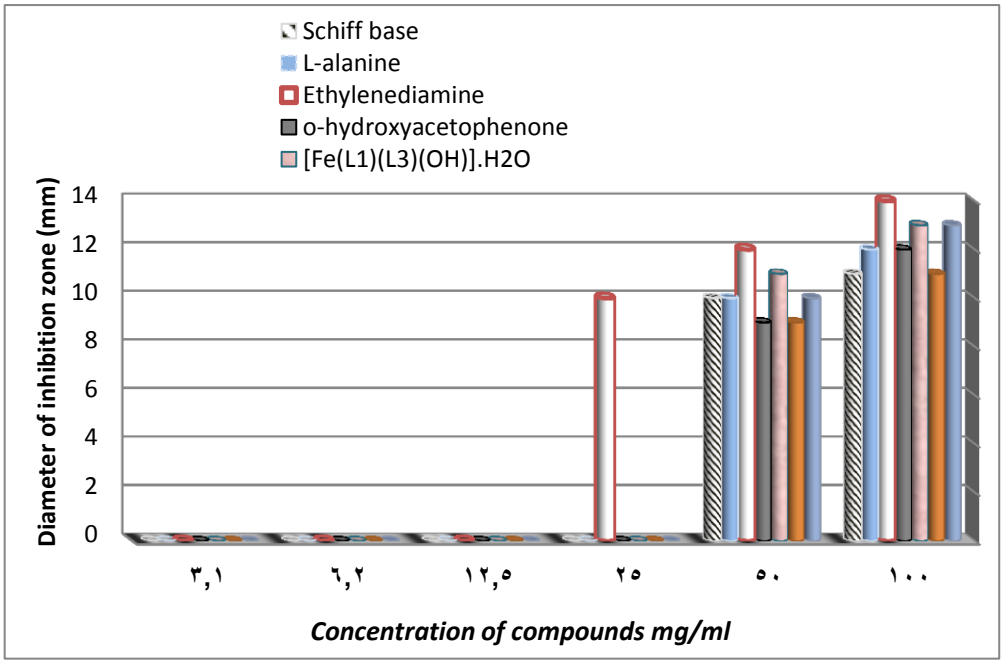


Figure 17: MIC of the tested compounds against *S. aureus*

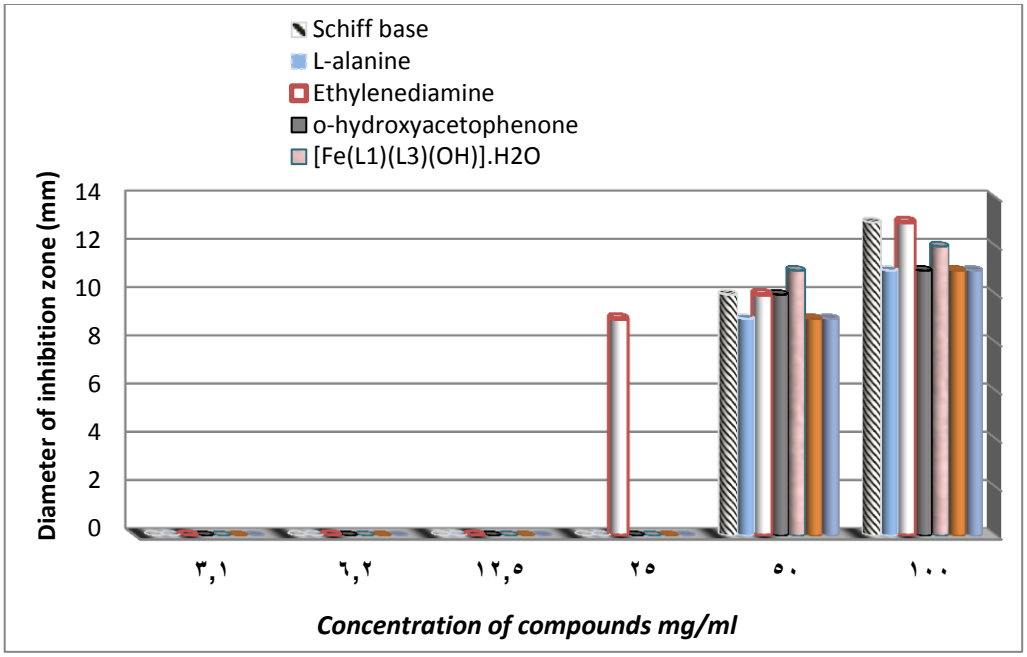
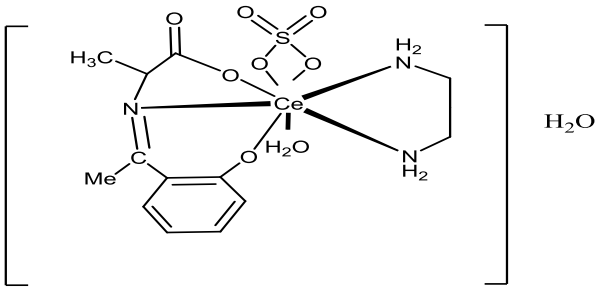
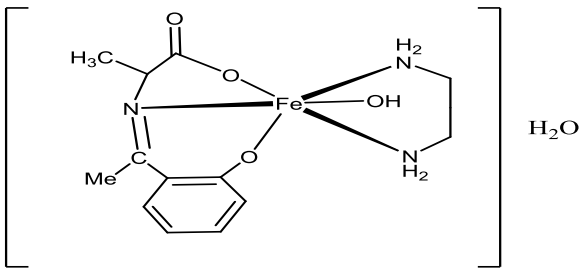


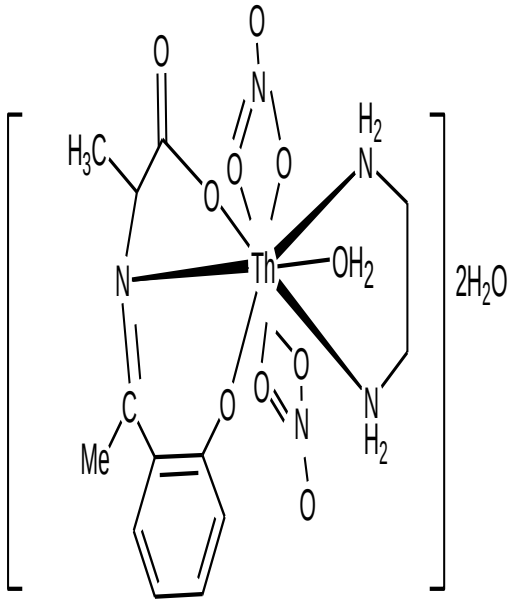
Figure 18: MIC of the tested compounds against *S. marcescens*

Conclusion

The analytical and physical techniques showed the formation of the mixed ligand chelates in 1:1:1[M:L1L2] ratio and non-electrolytic in nature was observed for all chelates . Also their electronic transitions were reported. The antimicrobial activity of Schiff base, ethylenediamine and mixed ligand chelates were screened against some pathogenic microbial species. The chemical structures of the chelates were listed below:







References

Abu-Dief, A. M., & Mohamed, I. M. (2015). A review on versatile applications of transition metal complexes incorporating Schiff bases, *Beni-Suef University Journal of Basic and Applied Sciences*, 4(2), 119-133. https://doi.org/10.1016/j.bjbas.2015.05.004

Alassbaly, F.S., Maihub, A.A., Ben-Gweirif, S.F., El-Ajaily, M.M. & Al-Noor, T.H., (2016). Chelation trends and antibacterial activity of some mixed ligand chelates, *Saudi Journal of Pathology and Microbiology*, 1(2), 29-35. https://doi.org/10.21276/sjpm.2016.1.2.1

Al-Barki, N.S., Maihub, A.A., El-Ajaily, M.M. & Al-Noor, T.H. (2016). Synthesis and Physiochemical Studies of Some Mixed Schiff Bases Complexes. *Academic Journal of Chemistry*, 1(3), 66-75.

AL-Garawi, S., Tomi, I & AL-Daraji, A. (2012). Synthesis and characterization of new amino acid Schiff bases and studies their effects on the activity of ACP, PAP and NPA enzymes (In Vitro), *E-Journal of Chemistry*, 9(2), 962-969. https://doi.org/10.1155/2012/218675

Alghool, S., Abd El-Halim, H.F., Abd El-sadek, M.S., Yahia, I.S. & Wahab, L.A. (2013). Synthesis, thermal characterization, and antimicrobial activity of lanthanum, cerium, and thorium complexes of amino acid Schiff base ligand, *Journal of thermal analysis and calorimetry*, 112(2), 671-681. https://doi.org/10.1007/s10973-012-2628-4

Al-Jeboori, F.H., Al-Shimiesawi, T.A.M., Abd Oun, M.A., Abd ul-Ridha, A. & Abdulla, A.Y (2014). Synthesis and characterization of amino acid (phenylalanine) schiff bases and their metal complexes, *Journal of Chemical and Pharmaceutical Research*, 6(8), 44-53.

Al-Noor, T.H., Al-barki, N.S., Maihub, A.A. & El-ajaily, M.M. (2017). Synthesis and Spectroscopic Characterization of some Mixed Schiff Base Complexes, *International Journal of Science and Research*, 6(3), 2421-2426.https://doi.org./10.21275/ART20172143

Alothman, A. A., Albaqami, M. D. & Alshgari, R. A. (2021). Synthesis, spectral characterization, quantum chemical calculations, thermal studies and biological screening of nitrogen and oxygen donor atoms containing Azo-dye Cu(II), Ni(II) and Co(II) complexes, *Journal of Molecula Structure*, 1223, 128984. https://doi.org/10.1016/j.molstruc.2020.128984

Amani, V., Safari, N., Khavasi, H.R. & Mirzaei, P. (2007). Iron (III) mixed-ligand complexes: Synthesis, characterization and crystal structure determination of iron (III) hetero-ligand complexes containing 1, 10-phenanthroline, 2, 2′-bipyridine, chloride and dimethyl sulfoxide,[Fe (phen) Cl3 (DMSO)] and [Fe (bipy) Cl3 (DMSO)], *Polyhedron*, 26(17), 4908-4914. https://doi.org/10.1016/j.poly.2007.06.038

Anitha, C., Sumathi, S., Tharmaraj, P. & Sheela, C.D. (2012). Synthesis, characterization, and biological activity of some transition metal complexes derived from novel hydrazone azo Schiff base ligand, *International Journal of Inorganic Chemistry*, 2011. https://doi.org/10.1155/2011/493942

Ashraf, M.A., Mahmood, K., Wajid, A., Maah, M.J. & Yusoff, I. (2011). Synthesis, characterization and biological activity of Schiff bases, *IPCBEE*, 10(1), 185.

Atiyah, E. M., Alwan, W. M. & Alkam, H. H. (2020). New mixed ligand complexes of New Schiff base 4,4'-((naphthalen-1-ylimino) methylene) dibenzene-1,3-diol and 8-hydroxy quinoline: Synthesis, Spectral Characterization, Thermal studies and Biological Activities, *Systematic Reviews in Pharmacy*, 11(8), 725-735. https://doi.org/10.31838/srp.2020.8.104

Chohan, Z.H., Farooq, M.A., Scozzafava, A. & Supuran, C.T. (2002). Antibacterial schiff bases of oxalyl-hydrazine/diamide incorporating pyrrolyl and salicylyl moieties and of their zinc (II) complexes, *Journal of Enzyme Inhibition and Medicinal Chemistry*, 17(1), 1-7. https://doi.org/10.1080/14756360290005598

Derebe, M.G., Raju, V.J.T. & Retta, N. (2002). Synthesis and characterization of some metal complexes of a Schiff base derived from ninhydrin and α, L-alanine, *Bulletin of the Chemical Society of Ethiopia*, 16(1), 53-64. https://doi.org/10.4314/bcse.v16i1.20948

Ejidike, I.P. & Ajibade, P.A. (2015). Synthesis, characterization and biological studies of metal (II) complexes of (3E)-3-[(2-{(E)-[1-(2, 4-dihydroxyphenyl) ethylidene] amino} ethyl) imino]-1-phenylbutan-1-one

Schiff base, *Molecules*, 20(6), 9788-9802. https://doi.org/10.3390/molecules20069788

El-Ajaily, M.M., Al-Barki, N.S. & Maihub, A.A., (2016). Mixed Schiff Bases Chelates: Synthesis and Spectroscopic Investigation, *Asian Journal of Advanced Basic Sciences*, 4(2), 123-130.

M. El-ajaily,M.M., Sarangi, A.K., Mohapatra,R.K., Hassan, S.S., Eldaghare, R.N., Mohapatra, P.K., Raval, M.K., Das, D. Mahal, A., Cipurkovic, A.[i] & Al-Noor, T.H.(2019). transition metal complexes of (E)-2((2-hydroxylbenzylidene) amino- 3-mercaptopropanoic acid: XRD, anticancer, molecular modeling and Molecular docking studies, *Chemistry Select.*, *4*, 9999 –10005. **DOI: 10.1002/slct.201902306**

El-Sherif, A.A. & Eldebss, T.M., (2011). Synthesis, spectral characterization, solution equilibria, in vitro antibacterial and cytotoxic activities of Cu (II), Ni (II), Mn (II), Co (II) and Zn (II) complexes with Schiff base derived from 5-bromosalicylaldehyde and 2- aminomethylthiophene, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 79(5), 1803-1814. https://doi.org/10.1016/j.saa.2011.05.062

EL-zweay, R .S., EL-ajaily, M. M., Ben-Gweirif, S. F., & Maihub, A.A. (2013). Preparation, characterization and antibacterial activity of some mixed ligand chelates, *Journal of the Chemical Society of Pakistan*, 35(1),67-71.

Fugu, M. B., Ndahi, N. P., Paul, B. B & Mustapha, A. N. (2013) Synthesis, characterization and antimicrobial studies of some vanillin Schiff base metal(II) complexes, *Journal of Chemical and Pharmaceutical Research*, 5(4), 22-28.

Gaballa, A. S., Asker, M. S., Barakat, A. S., & Teleb, S. M. (2007). Synthesis, characterization and biological activity of some platinum(II) complexes with Schiff bases derived from salicylaldehyde, -2 furaldehyde and phenylenediamine, *Spectrochimica acta. Part A, Molecular and Biomolecular Spectroscopy*, 67(1), 114 - 121. https://doi.org/10.1016/j.saa.2006.06.031

Ghosh, T. & Pal, S. (2015). Synthesis and characterization of dodecahedral cerium (IV) and gadolinium (III) complexes with a tetradentate Schiff Base, *Journal of Chemical Sciences*, 127(7), 1201-1209. https://doi.org/10.1007/s12039-015-0887-x

Gobara, A.G.H. (2017), Synthesis, Characterization and Biological Activity of Some Thiosemicarbazone Ligands and their Complexes with Some M (II) ions, (PhD diss., University of Gezira.

Hamil, A.M., Abdelkarem, M., Hemmet, M. & El-ajaily, M.M. (2012). Synthesis of a New Schiff Base: 2-[2-(E)-(2-hydroxyphenyl)-ethylidene] aminoethyl) ethanimidoyl] phen, *International Journal of ChemTech Research*, 4(2), 682-685.

Hosny, N.M. & El-Dossoki, F.I. (2008). Schiff base complexes derived from 2- acetylpyridine, leucine, and some metal chlorides: their preparation, characterization,and physical properties, *Journal of Chemical & Engineering Data*,53(11), 2567-2572. https://doi.org./10.1021/je800415n

Hossian, M.S., Banu, L.A, El-Zahan, M.K, Haque, M.M. (2019). Synthesis, Characterization and Biological Activity Studies of mixed ligand complexes with Schiff base and 2,2 Bipyridine, *International Journal of Applied Science-Research and* Review, 6 (1:2). https://doi.org./10.21767/2394-9988.100086

Humelnicu, D., Pui, A., Malutan, C., Malutan, T. & Humelnicu, I. (2020). Synthesis, characterization and theoretical investigations of new uranium (VI) and thorium (IV) complexes with 1-furfurylaldehyde-derived Schiff bases as ligands, *Journal of Saudi Chemical Society*, 24(6), 451-460. https://doi.org/10.1016/j.jscs.2020.04.001

Kan, J.Y., Hsu, Y.L., Chen, Y.H., Chen, T.C., Wang, J.Y. & Kuo, P.L. (2013). Gemifloxacin, a fluoroquinolone antimicrobial drug, inhibits migration and invasion of human colon cancer cells, *BioMed research international*, 2013. https://doi.org/10.1155/2013/159786

Karipcin, F. & Kabalcilar, E. (2007). Spectroscopic and Thermal Studies on Solid Complexes of 4-(2-pyridylazo) resorcinol with Some Transition Metals, *Acta Chimica Slovenica*, 54(2), 242-247.

Klamm, B.E., Windorff, C.J., Marsh, M.L., Meeker, D.S. & Albrecht-Schmitt, T.E. (2018). Schiff-base coordination complexes with plutonium (IV) and cerium (IV), *Chemical Communications*, 54(62), 8634-8636. https://doi.org/10.1039/C8CC03571G

Lakshmi, S. S. & Geetha, K. (2016). Synthesis, characterization and biological studies of amino acid (L-tryptophan) Schiff base transition complexes, *Journal of Chemical and pharmaceutical research*, 8(1), 668-674.

Lateef, S. M., Sarhan, B.M. & Al-Saedi, W. A. J. (2016). Synthesis, Characterization and Biological Activity for Complexes VO(II), Mn(II), Co(II) and Ni(II) With New Multidentate Ligand [2-((E)-3-(2-hydroxyphenylimino)-1,5-dimethyl-2-phenyl-2,3- dihydro-1H-pyrazol-4-ylimino) acetic Acid][H2L] type (N2), *Diyala Journal for pure Sciences*, 12(1), 10-27.

Liu, T., Duan, G. & Zeng, Z. (2009). Synthesis and characterization of cerium, thorium, and uranyl complexes with (E)-4-(4-methoxyphenoxy)-4-oxobut-2-enoic acid, *Journal of Coordination Chemistry*, 62(13), 2203-2211. https://doi.org/10.1080/00958970902769799

Matar, S.A., Talib, W.H., Mustafa, M.S., Mubarak, M.S. & AlDamen, M.A.

(2015). Synthesis, characterization, and antimicrobial activity of Schiff bases derived from benzaldehyde and 3, 3′-diaminodipropylamine, *Arabian Journal of Chemistry*, 8(6), 850- 857. https://doi.org/10.1016/j.arabjc.2012.12.039

McCleverty J. A, Meyer T. J. (2003) Comprehensive Coordination Chemistry II: From Biology to Nanotechnology. 2nd ed, 232- 236, (*Elsevier, Amsterdam. Netherlands*).

Mostafa, A.A., Al-Askar, A.A., Almaary, K.S., Dawoud, T.M., Sholkamy, E.N. & Bakri, M.M. (2018). Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases, *Saudi Journal of Biological Sciences*, 25, 361–366. https://doi.org/10.1016/j.sjbs.2017.02.004

Nakamoto, K. (1978). Infrared and Raman Spectra of Inorganic and Coordination Compounds 3rd ed, (Wiley-Interscience, New York).

Ossowicz, P., Janus, E., Schroeder, G. & Rozwadowski, Z. (2013). Spectroscopic studies of amino acid ionic liquid-supported Schiff bases, *Molecules*, 18(5),4986-5004. https://doi.org/10.3390/molecules18054986

Palanimurugan, A., Dhanalakshmi, A., Selvapandian, P. & Kulandaisamy, A., (2019). Electrochemical behavior, structural, morphological, Calf Thymus-DNA interaction and in-vitro antimicrobial studies of synthesized Schiff base transition metal complexes, *Heliyon*, 5(7), e02039. https://doi.org/10.1016/j.heliyon.2019.e02039

Patel, R.N., Singh, N., Shukla, K.K., Gundla, V.L.N. & Chauhan, U.K. (2006). Synthesis, characterization and biological activity of ternary copper (II) complexes containing polypyridyl ligands, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 63(1),.21-26. https://doi.org/10.1016/j.saa.2005.04.030

Pervaiz, M., Ahmad, I., Yousaf, M., Kirn, S., Munawar, A., Saeed, Z., Adnan, A., Gulzar, T., Kamal, T., Ahmad, A. & Rashid, A. (2019). Synthesis, spectral and antimicrobial studies of amino acid derivative Schiff base metal (Co, Mn, Cu, and Cd) complexes, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 206, 642-649. https://doi.org/10.1016/j.saa.2018.05.057

Prashanthi, Y. & Kiranmai, K. (2012). Spectroscopic characterization and biological activity of mixed ligand complexes of Ni (II) with 1, 10-phenanthroline and heterocyclic Schiff bases, *Bioinorganic chemistry and applications*, 2012. https://doi.org/10.1155/2012/948534

Rahman, L.A., Abu-Dief, A.M., Hashem, N.A. & Seleem, A.A. (2015). Recent advances in synthesis, characterization and biological activity of nano sized Schiff base amino acid M (II) complexes, *International Journal of Nanomaterials and Chemistry*, 1(2), 79-95. https://doi.org/10.12785/ijnc/010205

Salama, M.M., Ahmed, S.G. & Hassan, S.S. (2017). Synthesis, characterizations, biological, and molecular docking studies of some amino acid Schiff bases with their cobalt (II) complexes, *Advances in Biological Chemistry*, 7(05), 182. https://doi.org/10.4236/abc.2017.75013

Sarı, N. & G¨urkan, P., 2004. Some novel amino acid-Schiff bases and their complexes synthesis, characterization, solid state conductivity behaviors and potentiometric studies, Z. Naturforsch. 59b, 692 – 698. DOI:10.1515/znb-2004-0610

Selvaganapathy, M. & Raman, N. (2016). Pharmacological activity of a few transition metal complexes: A Short Review, *Journal of Chemical Biology and Therapeutics*, 1(2), 1-17. https://doi.org10.4172/2572-0406.1000108

Slavin, Y.N., Asnis, J., Häfeli, U.O. & Bach, H. (2017). Metal nanoparticles: understanding the mechanisms behind antibacterial activity, *Journal of nanobiotechnology*, 15(1),1- 20. doi.org/10.1186/s12951-017-0308-z

Sobola, A. O & Watkins, G. M. (2013). Antimicrobial activity and Cu(II) complexes of Schiff bases derived from ortho-aminophenol and salicylaldehyde derivatives, *Journal of Chemical and Pharmaceutical Research*, 5(10), 147-154.

Srivastava, S.C. & Newman, L. (1972). Mixed ligand complexes of palladium (II) with chloride and iodide, *Inorganic Chemistry*, 11(12), 2855-2859. https://doi.org/10.1021/ic50118a001

Tamiru, G., Abebe,A., Abebe, M. & Liyew, M. (2019). Synthesis, structural investigation and biological application of new mono- and binuclear cobalt (II) mixed-ligand complexes containing 1,10-phenanthroline, acetamide and ethylenediamine, *Ethiopian Journal of Science and Technology*, 12(1), 69-91. https://doi.org./10.4314/ejst.v12i1.4

Thakkar, N.V. & Thakkar, J.R. (2000). Synthesis and characterization of chiral mixed ligand Co(II) complexes of isonitrosopropiophenone and amino acids. Synthesis and Reactivity in Inorganic and Metal-Organic Chemistry, 30(10),1871- 1887. doi.org/10.1080/00945710009351875

Thakur, G.A. & Shaikh, M.M. (2006). Synthesis, characterization, antibacterial and cytotoxicity studies on some mixed ligand Th (IV) complexes, *Acta poloniae pharmaceutica*, 63(2), 95-100.

Thakur, G.A., Athlekar, S.V., Dharwadkar, S.R. & Shaikh, M.M. (2007). Synthesis and biological activity of mixed ligand dioxouranium (VI) and thorium (IV) complexes, *Acta poloniae pharmaceutica*, 64(1), 9-15.