

Full Length Research Paper

Synthesis and antibacterial activity of metal complexes of barbituric acid

Ikotun A. A.^{1*}, Ojo Y.¹, Obafemi C. A.² and Egharevba G. O.²

¹Department of Chemistry and Biochemistry, Bowen University, Iwo, Osun State, Nigeria.

²Department of Chemistry, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria.

Accepted 30 September, 2010

Cobalt (II), copper (II), iron (III), manganese (II) and nickel (II) complexes of barbituric acid were synthesized and characterized by infrared and ultraviolet spectroscopy. The ligand and the complexes have been screened for *in vitro* antibacterial activity against a range of Gram-positive and Gram-negative bacteria (*Bacillus anthracis*, *Bacillus cereus*, *Bacillus stearothermophilus*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Staphylococcus aureus*, *Streptococcus faecalis*). Cobalt (II) complex displayed a better antibacterial activity than the ligand, showing a broad-spectrum activity against all the six Gram-positive and four Gram-negative bacterial strains, while the other complexes showed only selective activity against the organisms in a manner similar to the starting ligand.

Key words: Antibacterial activity, barbituric acid, complexes, ligand.

INTRODUCTION

Barbituric acid belongs to the family of 2, 4-pyrimidione derivatives which play an important role in nature and in technical or pharmaceutical applications (Hansel and Umlauff, 1993). The study of barbituric acid and its derivatives has received a lot of attention over the years. Recent studies revealed that the Schiff bases derived from 5-aminobarbituric acid have shown multifunctional properties like acidochromism, solvatochromism and adjustable hydrogen-bonding pattern for molecular recognition (Bolz et al., 2010).

Some transition metal complexes of barbituric acid have also been synthesized and characterized (Refat et al., 2008). Two new azo-dyes were prepared by linking 8-aminoquinoline to barbituric acid and 1, 3-dimethylbarbituric acid through diazo-coupling reactions (Gup et al., 2007). Metal complexes of these ligands were also prepared and characterized. All spectral data indicated that the azo-dyes acted as monobasic O,N,N'-tridentate ligands.

In addition, derivatives of 5-arylidene barbituric acid have been prepared by grinding a mixture of aromatic aldehydes, barbituric acid and aminosulfonic acid (an environmentally friendly catalyst) at room temperature

(Ji-Tai et al., 2006). The condensation of barbituric acid with aldehydes under microwave irradiation in dry media using a variety of catalysts has also been investigated (Dewan and Singh, 2003).

Cobalt (II), nickel (II) and copper (II) complexes of barbituric acid have also been synthesized at room temperature (Masoud et al., 1983). These complexes have also been characterized with the infrared and electronic spectra, as well as magnetic susceptibility measurements. Their elemental analyses suggested a range of stoichiometries 1:1, 1:2, 1:3. Their structures were also determined (Masoud et al., 1983).

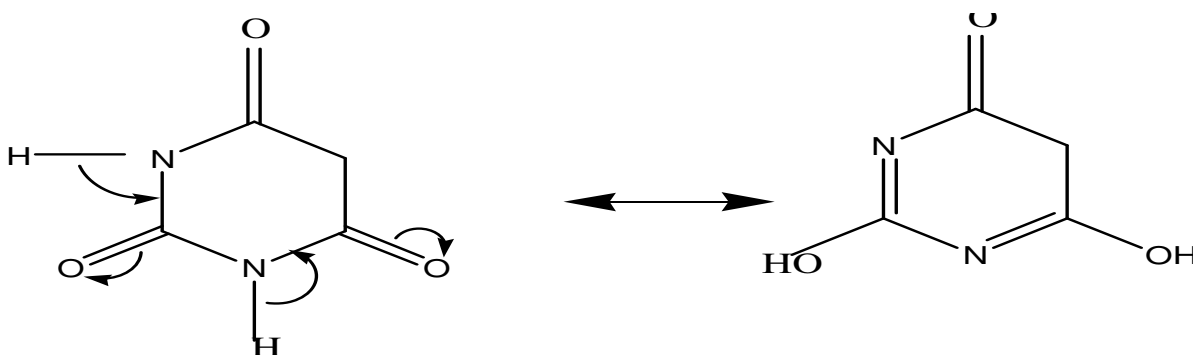
Early studies revealed the syntheses of barbiturates by condensing barbituric acid with aldehydes under conventional refluxing conditions in aqueous medium (Vvedenskii, 1970). Barbituric acid derivatives are associated with a number of biological activities such as anticonvulsant, antiplasmodic, sedatives, hypnotic and even local anaesthetic agents (Bojarski et al., 1985; Jursic, 2001). However, the antimicrobial study and some other biological importance of the transition metal complexes of barbituric acid are yet to be explored.

All these reports have spurred us to the syntheses of cobalt (II), copper (II), iron (III), manganese (II) and nickel (II) complexes of barbituric acid using the conventional refluxing method with the view to studying the antibacterial activities of these complexes.

*Corresponding author. E-mail: lhbcards@yahoo.com.

Table 1. Colour and melting points of synthesized complexes of barbituric acid (H₂L).

Compound	Colour	Melting point with observations
Co (II) complex	Blue with patches of pink	> 320°C
Cu (II) complex	Blue	> 320°C
Fe (III) complex	Dark brown	Turned off white at 169.2°C, turned light brown at 200.5°C, turned dark brown at 210.1°C, didn't melt at 320°C
Mn (II) complex	Light brown	> 320°C
Ni (II) complex	Green	Turned dark brown at 228.0°C, Didn't melt at 320°C

**Figure 1.** The keto and enol tautomeric forms of the ligand.

MATERIALS AND METHODS

Barbituric acid, NiCl₂·6H₂O, CoCl₂·6H₂O, MnCl₂·4H₂O, Cu(NO₃)₂·3H₂O and FeCl₃·6H₂O were purchased from Aldrich. All solvents used were purchased as analytical grades.

The melting points were determined using a Gallen Kemp Variable heater apparatus. The infrared spectra of complexes were measured as KBr discs on a Nicolet Avatar 330 FTIR spectrophotometer in the range 4000 to 400 cm⁻¹. The electronic spectra of the compounds were recorded on a Genesys 10s V1.2 UV-Visible spectrophotometer.

Synthesis of complexes

The ligand (1 g, 0.00781 mol) in ethanol (30 ml) was mixed with each of the metal salts (0.00781 mol) in ethanol (10 ml) and in the presence of concentrated ammonia (3.5 to 4.0 ml). The mixture was refluxed for about 90 min at a temperature of 78°C in an oil bath. The precipitate was cooled, filtered under suction and washed with ethanol. The coloured products were dried in the oven (60°C) for a few minutes, weighted and stored in the desiccator.

The physical properties of the metal complexes have been presented in Table 1.

Antimicrobial screening

Media used

Nutrient broth (Oxoid Ltd.) and nutrient agar (Oxoid Ltd.) were used

for sub culturing the bacterial isolates, while diagnostic sensitivity test agar (Oxoid Ltd.) was used for sensitivity test.

Microorganisms used

The following microorganisms were used: *Bacillus anthracis*, *B. cereus*, *B. stearothermophilus*, *B. subtilis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *P. fluorescens*, *Staphylococcus aureus*, *Streptococcus faecalis*.

Sensitivity testing

The sensitivity testing of the synthesized metal complexes with the ligand were determined using agar-well diffusion method. The bacterial isolates were first grown in nutrient broth (Oxoid Ltd). The plates were observed for zones of inhibition after 24 h incubation at 37°C. The effects were compared with that of streptomycin standard antibiotic at a concentration of 1 mg/ml (Irobi et al., 1996).

RESULTS AND DISCUSSION

Barbituric acid (H₂L) is capable of existing in tautomeric forms (Masoud et al 1983) Figure 1. This is due to the hydrolysable hydrogens in its structure, as well as the delocalisable electrons within its ring. The keto- and enol-tautomeric forms are presented as follows:

Table 2. Relevant infra red spectra data of the ligand and complexes.

COMPOUND	$\sqrt{(\text{OH})^{\text{as}} (\text{cm}^{-1})}$	$\sqrt{(\text{OH})^{\text{s}} (\text{cm}^{-1})}$	$\sqrt{(\text{NH}) (\text{cm}^{-1})}$	$\sqrt{(\text{C}=\text{O}) (\text{cm}^{-1})}$	$\sqrt{(\text{C}=\text{N}) (\text{cm}^{-1})}$	$\sqrt{(\text{CH}_2)\text{bend} (\text{cm}^{-1})}$	$\sqrt{(\text{C}-\text{N}+\text{C}-\text{O}) (\text{cm}^{-1})}$	$\sqrt{(\text{M}-\text{N}) (\text{cm}^{-1})}$	$\sqrt{(\text{M}-\text{O}) (\text{cm}^{-1})}$
Ligand (H ₂ L)	3532s	3480s 3441s	3370m	1743s 1717s	1617s	1440m	1394m 1058m 1350m	-	-
Co (II) complex	3543s	3326m	3237m	1701s 1647s	1613s	1427m	1384w 1077m	542s	480m 434m
Cu (II) complex	3429m	3357m	3277m 3200m	1681s	1609s	1460m	1387m 1064m 1064m	547s	462m
Fe (III) complex	3427b,m	3357m	3272b 3194m	1753m 1681s	1608s	1454m	1387m 1352m 1064m	547s	462m
Mn (II) complex	-	-	3160m	1713s 1616m	1598m	1434m	1394m 1067m	544s 547s	420m
Ni (II) complex	3552s	-	3163m	1712s 1612m	1590s	1431m	1394m 1067m	544s	490m 422m

s, strong; m, medium; b, broad; w, weak; as, asymmetric and s, symmetric.

Analysis of the I.R. spectra

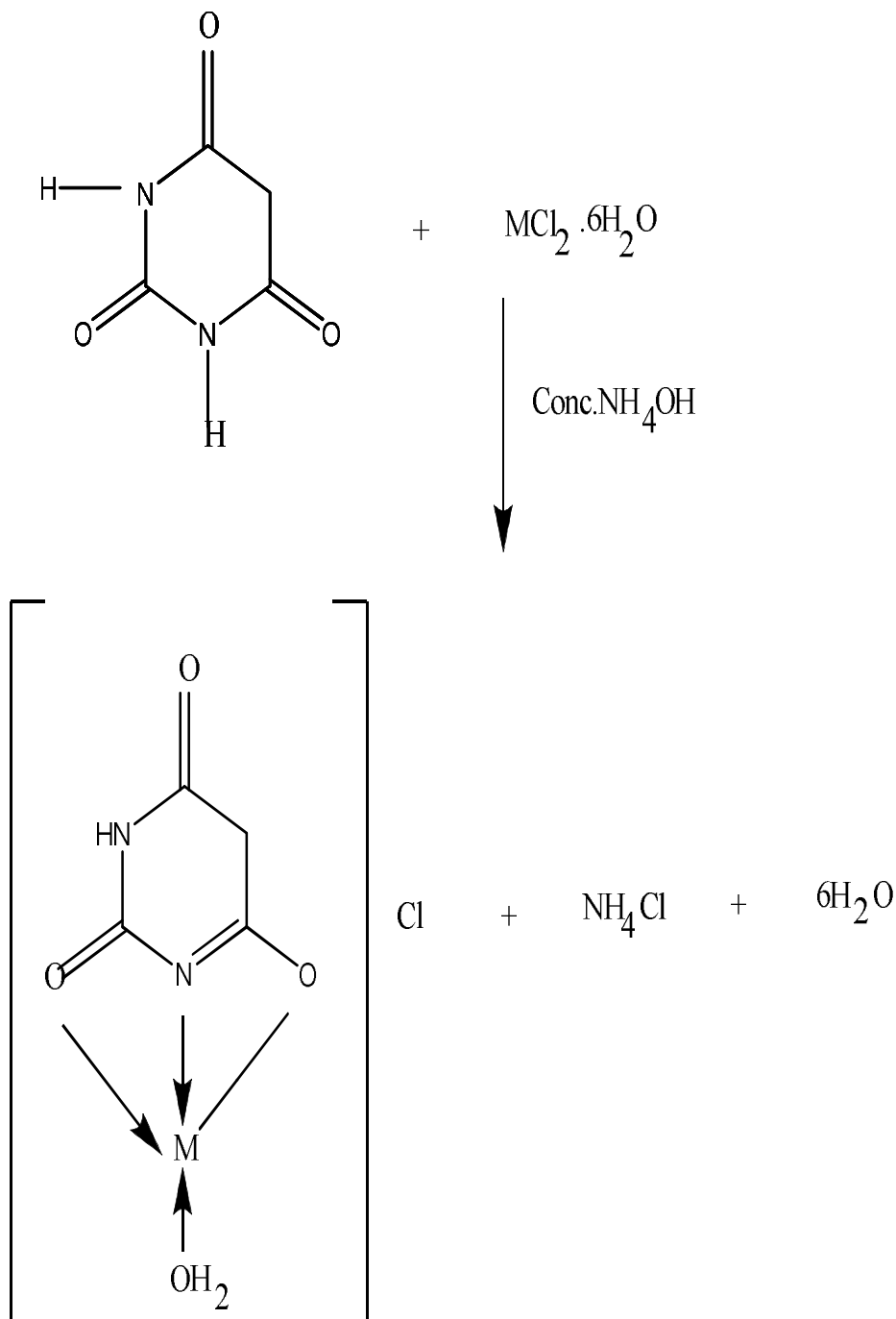
The solid reflectances of the compounds have been considered and the relevant IR spectral data of the ligand and its metal complexes is summarized in Table 2. The strong bands due to the asymmetric and symmetric $\sqrt{(\text{OH})}$ stretching of the ligand are observed at 3532 and 3480 cm^{-1} (also 3441 cm^{-1} , but weak) respectively. The $\sqrt{(\text{NH})}$ band appears at 3370 cm^{-1} . The strong bands at 1743 and 1717 cm^{-1} were attributed to $\sqrt{(\text{C}=\text{O})}$. While the strong band at 1617 cm^{-1} is for the $\sqrt{(\text{C}=\text{N})}$.

This data suggests the existence of barbituric

acid in the tautomeric forms. The mode of complexation is supported by the following observations; the disappearance of the $\sqrt{(\text{OH})}$ symmetric band in Co (II) complex and the disappearance of both $\sqrt{(\text{OH})}$ asymmetric and symmetric in Mn (II) complex. These signify the coordination of the oxygen atoms of the hydroxyl to the metals. Also, the $\sqrt{(\text{NH})}$ band moved to a lower wave number in all the complexes, thus, the nitrogen is involved in coordination. The strong $\sqrt{(\text{C}=\text{O})}$ bands at 1743 and 1717 cm^{-1} have also shifted appreciably downward in Co (II), Cu (II) and Mn (II) complexes signifying the coordination of the oxygen of the carbonyl group just as in

Structure 1. Whereas, the strong $\sqrt{(\text{C}=\text{O})}$ band at 1743 cm^{-1} rather moved upward (by 10 cm^{-1}) with Fe (III) and Ni (II) suggesting that no coordination occurs through the oxygen of the carbonyl group here, just as in Structure 2. Also, the $\sqrt{(\text{C}=\text{N})}$ band moved significantly downward with Co (II) and Mn (II) signifying the involvement of the nitrogen of the imine bond in coordination as in Structure 2.

The $\sqrt{(\text{CH}_2)}$ bend band has also been either moved to a lower or higher wave number. The $\sqrt{(\text{C}-\text{N})} + \sqrt{(\text{C}-\text{O})}$ band shifts in all the complexes, do not suggest any coordination through the nitrogen or oxygen atom. But rather, may suggest

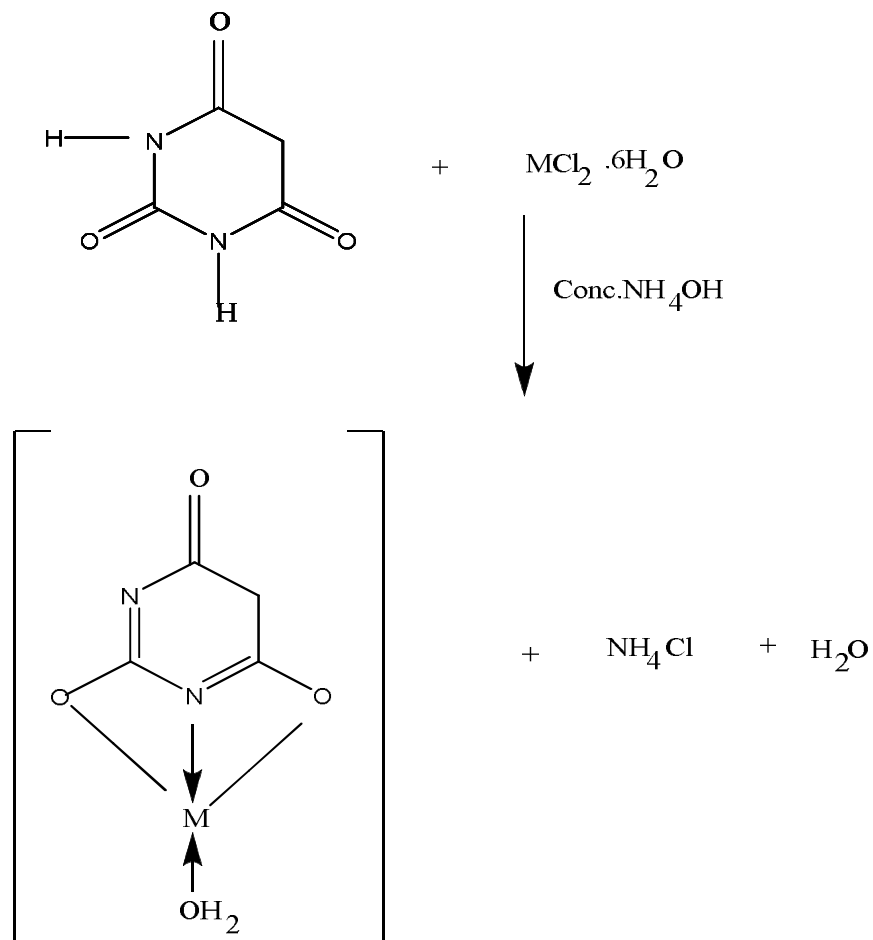


Structure 1. The general equation of the reaction with the ligand reacting in the keto form.

that, either is only satisfying the primary valency of the metal as predicted in Structures 1 and 2. The strong new bands appearing between 527 to 547 cm^{-1} and 420 to 480 cm^{-1} (except 490 cm^{-1} weak band in Co (II) complex) have been attributed to ν (M-N) and ν (M-O) bands respectively (Abd-Elzar, 2001; Raman et al., 2001; Singh et al., 2001; Thangadura and Natarajan, 2001; Chohan, 2001).

Electronic spectra analyses

The electronic spectra for the ligand and the complexes have been presented in Table 3. The electronic spectrum of the ligand showed absorption bands at 211 , 243 and 256 nm attributed to $\pi - \pi^*$ transitions, while the band at 340 nm is attributed to $n - \pi^*$ transition. The interpretations of ultraviolet spectra of metal complexes revealed



Structure 2. The general equation of the reaction with the ligand reacting in the enol form.

Table 3. Electronic spectra data analyses.

Compound	Band position (nm)	Band assignment
Ligand (H_2L)	211; 243; 256	$\pi \longrightarrow \pi^*$
	340	$n \longrightarrow \pi^*$
Co (II) complex	208; 232; 253	$\pi \longrightarrow \pi^*$
	337	$n \longrightarrow \pi^*$
	520; 620	$d \longrightarrow d$
Cu (II) complex	231; 258	$\pi \longrightarrow \pi^*$
	480; 850	$d \longrightarrow d$
Fe (III) complex	208; 232; 255	$\pi \longrightarrow \pi^*$
	329	$n \longrightarrow \pi^*$
	750; 870	$d \longrightarrow d$
Mn (II) complex	211; 256, 260	$\pi \longrightarrow \pi^*$
	420	$d \longrightarrow d$
Ni (II) complex	214; 239; 253, 261	$\pi \longrightarrow \pi^*$
	342	$n \longrightarrow \pi^*$
	410; 715	$d \longrightarrow d$

Table 4. Antibacterial activity of barbituric acid (H₂L) complexes and streptomycin (stm) as determined by dilution techniques (at 1 mg/ml).

Microorganism	Gram	H ₂ L	Co (II) complex	Cu (II) complex	Fe (II) complex	Mn (II) complex	Ni (II) complex	Stm	DMSO
<i>Bacillus anthracis</i> (LIO)	+	0	15	0	0	0	0	0	0
<i>Bacillus cereus</i> (NCIB 6349)	+	16	14	18	16	15	12	28	0
<i>Bacillus stearothermophilus</i> (NCIB 3610)	+	20	28	21	23	28	22	23	0
<i>Bacillus subtilis</i> (NCIB 3610)	+	20	23	18	23	18	21	20	0
<i>Escherichia coli</i> (NCIB 86)	-	0	18	0	0	0	0	0	0
<i>Klebsiella Pneumonia</i> (NCIB 418)	-	0	17	0	0	0	0	0	0
<i>Pseudomonas aeruginosa</i>	-	17	22	16	18	15	16	ND	0
<i>Pseudomonas fluorescens</i> (NCIB 3756)		0	21	0	0	0	0	30	0
<i>Staphylococcus aureus</i> (NCIB 8588)	+	0	16	0	0	0	0	21	0
<i>Streptococcus faecalis</i> (NCIB 755)	+	16	18	22	14	18	13	24	0

LIO = Locally Isolated Organism; NCIB = National Collection of Industrial Bacteria; ND = Not determined; 0 = No activity; The respective values for the zones of inhibition presented in Table 4 were recorded in mm.

that charge transfer bands occur in the same region with $\pi - \pi^*$ transitions.

The Cobalt (II) complex absorption bands at 208, 232 and 253 nm have been assigned to $\pi - \pi^*$ transitions. The absorption band at 337nm is due to $n - \pi^*$ transition. The spectrum of the Iron (III) complex revealed absorption bands at 208, 232 and 255 nm assigned to $\pi - \pi^*$ transitions. The absorption band at 329 nm has been assigned as $n - \pi^*$ transitions, those at 750 and 870 nm are due to $d - d$ transitions.

The absorption bands at 211, 256 and 260 nm in the Manganese (II) complex have been assigned as $\pi - \pi^*$ transitions. The band at 420 nm is due to $d - d$ transition.

The spectrum of the Nickel (II) complex showed absorption bands at 214, 239, 253 and 261 nm. These have been assigned as $\pi - \pi^*$ transitions. The absorption band at 342 nm has been assigned as $\pi - \pi^*$ transition, while those at 410 and 715 nm. Also, the absorption bands at 520 and 620 nm are due to $d - d$ transitions.

The spectrum of the Copper (II) complex showed absorption bands at 231 and 258 nm

assigned to $\pi - \pi^*$ transitions. The bands at 480 and 850 nm are due to $d - d$ transitions have been assigned as $d - d$ transitions.

Antibacterial activity

The results of antibacterial activity of the metal complexes, ligand (H₂L) and streptomycin (a reference clinical antibiotic used at 1 mg/ml) against the various bacteria were summarized in Table 4. Generally, metal (II) complexes have been shown to be, in most cases, more effective than the free ligands. Tweedy's chelation theory (Thangadura and Natarajan, 2001) predicts that chelation reduces the polarity of the metal atom mainly because of partial sharing of its positive charge with donor groups and possible π -electron delocalization over the whole ring. This consequently increases lipophilic character of the chelates, favouring its permeation through lipid layers of the bacterial membrane.

These results show that the ligand (H₂L) had activity against four Gram-positive bacteria strains

which Cu (II), Fe (III), Mn (II) and Ni (II) complexes also showed activity. Co (II) complex showed activity against all the six gram-positive bacteria strains. Co (II) and Fe (III) complexes had the largest (even greater than streptomycin) zones of inhibition against *Bacillus subtilis*. Also, Co (II) and Mn (II) complexes had the largest zones of inhibition against *B. stearothermophilus* (greater than that of streptomycin).

For the Gram-negative bacteria strains, the ligand (H₂L) had activity against only one which Cu (II), Fe (III), Mn (II) and Ni (II) complexes were also active against. But Co (II) complex showed activity against all the four Gram-negative bacteria strains. And only Co (II) complex had activity against *Escherichia coli* and *Klebsiella Pneumonia* which even streptomycin had no activity against.

REFERENCES

- Abd-Elzar MM (2001). Spectroscopic characterization of some tetradentate Schiff bases and their complexes with nickel, copper and zinc. J. Chin. Chem. Soc., 48: 153.
Bojarski JT, Mokrosz JL, Barton HJ, Paluchowska MH (1985).

- Recent progress in babituric acid chemistry. *Adv. Heterocycl. Chem.*, 38: 229-297 and reference therein.
- Bolz I, Bauer M, Rollberg A, Spange S (2010). Chromophoric barbituric acid derivatives with adjustable Hydrogen-bonding patterns as component for supramolecular structures. *Macromol. Symp.*, 287(1): 8-15.
- Chohan ZH (2001). Synthesis, characterization and biological properties of bivalent transition metal complexes of Co (II), Cu (II), Ni (II) and Zn with some acylhydrazine derived from furanyl and thienyl ONO and SNO donor Schiff base ligands. *Synth. React. Inorg. Met.-Org. Chem.*, 31(1): 1-16.
- Dewan SK, Singh R (2003). One pot synthesis of Babiturates on reaction of babituric acid with aldehydes under microwave irradiation using a variety of catalysts. *Synthetic Commun.*, 33(17): 3081-3084.
- Gup R, Giziroglu E, Kirkan B (2007). Synthesis and spectroscopic properties of new azo-metal complexes derived from barbituric acid and aminoquinoline. *Dyes Pigments*, 73(1): 40-46.
- Hansel W, Umlauff S (1993). [Barbiturate analysis with heavy metal ions from the beginning to the present]. *Pharm. Unserer Zeit.*, 22(4): 207-213.
- Irobi ON, Moo-Young M, Daramola SO (1996). Antimicrobial activity of *Annato bixoovellana* extra. *Int. J. Pharm.*, 34: 87-90.
- Ji-Tai L, Hong-Guang D, Da L, Tong-Shuang Li (2006). Efficient Method for synthesis of the derivatives of 5-Arylidene barbituric acid catalyzed by amininosulfonic acid with grinding. *Synthetic Commun.*, 36: 789-794.
- Jursic BS (2001). A simple method for knoevenagel condensation of α , β - conjugated and aromatic aldehydes with barbituric acid. *J. Heterocycl. Chem.*, 38: 655-657.
- Masoud MS, Heiba AM, Ashmawy FM (1983). Synthesis and characterization of babituric and thiobabituric acid complexes. *Trans. Met. Chem.*, 8: 124-126.
- Raman N, Kulandaisamy A, Jeyasubramanian K (2001). Synthesis, spectroscopic characterization, redox and biological screening of some Schiff base transition metal (II) complexes derived from salicylidene-4-aminoantipyrine and 2-aminophenol/2-aminothiophenol. *Synth. React. Inorg. Met.-Org. Chem.*, 31(7): 1249-1270
- Refat MS, El-Korash SA, Ahmed AS (2008). A convenient method for the preparation of barbituric and thiobarbituric acid transition metal complexes. *Spectrochim. Acta A Mol. Biomol. Spectrosc.*, 71(3): 1084-1094.
- Singh KN, Singh DK, Singh SB (2001). Synthesis, Characterization and biological studies of Co (II), Ni (II), Cu (II) and Zn (II) complexes with N-picolinoyl-N-thiobenzoyl hydrazine. *Synth. React. Inorg. Met.-org. Chem.*, 32 (4): 703-720.
- Thangadurai TD, Natarajan K (2002). Mixed ligand complexes of Ruthenium (II) containing α , β -unsaturated- β -ketoamines and their antibacterial activity. *Trans. Met. Chem.*, 26: 500-504.
- Vvedenskii VM (1970). Synthesis of 5-(arylmethylene)hexahydro-*pyrimidine-2,4,6-triones*. *Khim. Geterotsikl. Soedin*, 6: 1092-1095.