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Original Article

Metal (II) Complexes of some Carboxylic group Drugs: Chelation, Characterization, Antibacterial, Analgesic, and Toxicology Studies

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Abstract

The present work focuses on the synthesis and biological evaluation of metal complexes of mixed acetylsalicylic acid and *para*-aminobenzoic acid in ratio 1:1:1 to give a complex type: $[M(ASA)(PABA)(H_2O)_n(Cl_2)_x](Cl_2)_y$ (where M = Cu(II), Cd(II), Ni(II), Fe(III), or Mn(II); ASA = Acetylsalicylic acid; PABA = *para*-aminobenzoic acid; n = 0 or 2; x = 0 or 1; y = 0 or 1). The metal complexes were obtained by a refluxing method and characterized by elemental analysis, melting point, conductivity measurements, ultraviolet-visible absorption, and infrared spectroscopy. The conductance measurement indicates the non-electrolytic nature of the complexes. The octahedral environment has been proposed for the complexes except for Cd (II) complex. The level of toxicity of the synthesized complexes was determined *in vivo*. $[Cu(ASA)(PABA)(H_2O)_2]$, $[Cd(ASA)(PABA)]Cl_2$, and $[Mn(ASA)(PABA)(H_2O)_2]$ exhibited higher enzymatic activities in the serum and kidney homogenates of the Wister rats investigated. The acetic acid-induced writhing model method was used in the evaluation of the analgesic activities of the prepared complexes. Metal complexes of Cu (II), Cd(II), Ni(II), Fe(III), and Mn(II) exhibited percentage writhing inhibition of 67.61, 43.87, 60.42, 70.45, and 52.34 % respectively. The complexes proved to be more effective than their parent-free ligands with Fe (III) possessing the highest analgesic potentials. The *in vitro* antimicrobial activity against bacterial strains was studied using the agar well diffusion procedure. It was also observed that the complexes exhibited higher bacteriostatic activities than the free ligands.

Keywords: Acetylsalicylic acid, Antibacterial, Enzymatic activities, Metal complexes, para-aminobenzoic acid Toxicity, Serum chemistry.

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1. Introduction

Acetylsalicylic acid is well known as the derivative of salicylic acid possessing analgesic and anti-inflammatory action [1, 2]. Previous studies have shown that chemotherapeutic agents act inhibiting metalloenzymes [2]. Researchers have reported the determination of how metal ions coordination affects the activities of biological systems [3, 4]. Increase in the effectiveness of these chelating agents is due to the ability of the free ligands to coordinate to the center metal ions, thus enhancing research quest for alternative compounds with wide activity spectrum [4, 5]. Synthesis of inorganic compounds is gaining ground in the study of novel drugs [5-7]. Metal ions play vital roles in the biological system as they display several reactivities in different physical and chemical processes [2, 4]. The great achievement in the development of cisplatin revealed that center metal ion can be used as a therapeutic agent [6]. Complexes have received great interest in structural studies due to different modes of bonds between ligands and transition metal ions [4].

Moreover, studies on bioactive complexes have been reported to decrease the mortality rate of infectious diseases [4-8]. Antimicrobial resistance possesses great concern, because of its worldwide threat [9-11]. The binding of mixed drugs with some metal ions is expected to improve the antimicrobial activities [4, 5, 7, 12]. Benzoic acid derivatives complexes continue to gain researchers interest due to their biochemical properties [13]. The benzoic acid and related derivatives are structural elements for some natural products that participate in different physiological processes in plants. *para*-aminobenzoic acid (PABA) has gained much interest in the area of biochemistry, medical and industries. It possessed a high biological significance found in the tissue of plants and animals. It is also known as vitamin H10, which acts as an antioxidant and antibacterial agent [14].

In line with the above properties, we report the preparation of mixed acetylsalicylic acid and *para*-aminobenzoic acid chelates. The compounds were characterized spectroscopically and their antibacterial, analgesic, and toxicity potentials in Wister rats were investigated

2. Materials and Methods

2.1. Materials

Acetylsalicylic acid and *para*-aminobenzoic acid, the metal salts: Cu (II), Cd (II), Ni (II), Fe (III), Mn (II), and solvents were received from Sigma-Aldrich chemical company and used without further purification. The melting points of the ligands and the complexes were obtained using the

Gallenkamp apparatus. HANNA apparatus conductivity meter at a cell constant of 1.24 was used to determine the molar conductance of the complexes. The elemental analysis (CHN) was performed using Control Equipment CE 440 Analyzer, Egham, United Kingdom. The ultraviolet-visible spectroscopy was performed on the free ligands and the complexes using Aquamate V4.60 spectrophotometer within the range of 200 – 800 nm. The infrared spectroscopy was performed using the Shimadzu Scientific model FT-IR Spectrophotometer.

2.2. Synthesis of the Complexes

The complexes were prepared following previously reported procedure [15]. *para*-aminobenzoic acid (0.14 g) and acetylsalicylic acid (0.18 g) were dissolved separately in 10 mL ethanol. The metal used: (M) = Cu^{2+} (0.064 g), Cd^{2+} (0.11 g), Ni^{2+} (0.059 g), Fe^{3+} (0.056 g) and Mn^{2+} (0.055 g) were dissolved in 10 mL distilled water. Each of the solutions of the metal salts was mixed with the solution of the mixed ligands. The observed coloured solutions were refluxed for 4 h, and allowed to cool to room temperature, and kept for days. Precipitates obtained were filtered off and washed with mixed cold ethanol and distilled water in the same volume. It was allowed to dry over silica gel in a desiccator for further analysis.

2.3. Antibacterial Activity

The prepared complexes and the free ligands were screened *in-vitro* to evaluate their antibacterial activity against selected organisms: *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Staphylococcus faecalis*, and *Pseudomonas aeruginosa* following agar diffusion techniques as described by Hoda *et al.* [16]. The test compounds were made ready by mixing each of the compound (20 mg) in 1 mL of dimethyl sulfoxide. The dispersing solvent was used as a negative control. Acetylsalicylic acid and *para*-aminobenzoic acid were used as the ligands. The bacterial culture was introduced to the Hinton agar plate with the use of sterile swab. Each paper disc (with 6 mm diameter) was impregnated with a constant quantity of 100 µg/mL of each test compound. The organized agar plates were then incubated at 37 °C for 24 h. The antimicrobial potentials of the investigated compounds were then measured by assessing the zone of inhibition in diameter. The activities of the as-synthesized complexes were compared with the free ligands [11]. The solvent used as a control showed no zone of inhibition.

2.4. Analgesic Activity

The analgesic assay of the compounds with the use of acetic acid-induced writhing model was performed as reported by Kundu *et al.* [17]. Acetic acid (0.7%) at a dose of 0.1 ml/ 10 g was administered intraperitoneally to generate pain sensation. The free ligands and the synthesized complexes were introduced to the test mice intraperitoneally after 15 min of injecting acetic acid. Then, after 5 min, the mice were observed for body contraction within 10 min.

2.5. Tail immersion Test

Tail (2 cm) was measured and deep inside warm water at 57-60 ℃. The reaction period was the period observed for the animals to deflect their tails. The time was noted and recorded as the average of the results. Half a minute of the latent time was observed and the experiment immediately was stopped in order not to injure the mice. This was evaluated at 0, 15, 30, and 45 min after administration of the ligands and the metal complexes, as previously reported [18].

2.6. Biochemical Analysis of the Compounds

2.6.1. Toxicological Activity

The toxicity studies were carried out to evaluate the toxic effect or toxicity level of the ligands and their metal complexes on Wister rat cellular systems. The ligands and their metal complex solutions were administered on the rats for fourteen days. The internal organs like the kidney and serum of the animals were collected after sacrificing, and evaluated to determine the level of toxicity of the ligands and the metal complexes.

2.6.2. Animal Handling and Administration of the Free Ligands and Metal Complexes

The free ligands and the prepared complexes were screened for their level of safety to the body in line with previous report [19]. The solution of the parent free ligands and their metal drug complexes were prepared in dimethylsulfoxide (DMSO). The Wister rats were obtained from the Biochemistry Department of the University of Ilorin, Ilorin, Nigeria. Forty Wister rats were grouped into eight (8) groups of five animals and each group was exposed to the prepared agents (20 mg/kg body weight). The experimental animals were handled following the international guidelines for the care and use of laboratory animals. They were allowed access to normal rat feeds (pelleted) and water *ad libitum* for two weeks throughout the experiment. The solutions of the parent free ligands and their metal drug complexes were orally administered to the Wister rats on daily basis for seven days. After the complete administration of the test agents, the rats were sacrificed following the international guidelines. The animals grouping and drugs administered are as follows:

Group 1 (control): was administered 2 % DMSO

Group 2: received 20 mg/kg body weight of Acetylsalicylic acid [ASA]

Group 3: received 20 mg/kg body weight of *para*-aminobenzoic acid [PABA]

Group 4: received 20 mg/kg body weight of $[Cu(ASA)(PABA)(H_2O)_2]$

Group 5: received 20 mg/kg body weight of $[Cd(ASA)(PABA)]Cl_2$

Group 6: received 20 mg/kg body weight of $[Ni(ASA)(PABA)Cl_2]$

Group 7: received 20 mg/kg body weight of $[Fe(ASA)(PABA)(H_2O)_2]$

Group 8: received 20 mg/kg body weight of $[Mn(ASA)(PABA)(H_2O)_2]$

2.6.3. Preparation of Serum

Blood samples were collected immediately when the Wister rats were sacrificed using

slight ether anaesthesia. Serum samples were collected by centrifuge for 10 min. at 1500 RPM and carefully pipetted out from the blood sample. They were kept in the freezer for further analysis.

2.6.4. Homogenization of the Kidney

The kidneys of the sacrificed animals were removed, weighed and grounded smoothly. They were then homogenized in ice-cold 0.25 M sucrose solution (1:4 w/v) using Teflon Homogenizer. After centrifugation for about 10 min at 1500 RPM, the samples were allowed to cool and pipetted out and frozen for further analysis.

3. Results and Discussion

3.1. Chemistry of the Ligands and their Complexes

The analytical properties of the ligands and their coordinated compounds are shown in Table 1. According to the data obtained, it was observed that the complexes showed a higher melting point than their ligands. This proved that coordination has happened between the ligands and the transition metal ions [20, 21]. The proposed structures for the complexes are shown in (Figure 1). Cu(II) and Cd(II) complexes yielded a high percentage of 75% and 70% products, respectively, when compared to other complexes. The isolated complexes were stable in air and insoluble in water and other common solvents but were easily soluble in polar coordinating solvents such as DMF and DMSO.

The value obtained from the molar conductance of the complexes with the exception of Cd (II) complex was in the range of 11 – 20 $\Omega^{-1}cm^2mol^{-1}$ confirming the complexes as non-electrolytic. [Cd (ASA) (PABA)]Cl_2 with 79 $\Omega^{-1}cm^2mol^{-1}$ is electrolytic in nature. Based on the result obtained from the theoretical and experimental values, the complexes were found to be good supportive of each other and the proposed structures [10, 11, 15]. The synthesis of the complexes may be represented by the equation below:

$$\text{[ASA] + [PABA]} + \text{M (II)/(III)} \xrightarrow[\text{EtOH/ distilled } H_2O]{\text{Reflux, 4 hrs}} \text{M(ASA)(PABA)}(H_2O)_n(Cl_2)_x](Cl_2)_y$$

Where M = Cu (II), n=2, x=0, y=0 M = Cd (II), n=0, x=0, y=1
M = Ni (II), n=0, x=1, y=0 M = Mn (II), n=2, x=0, y=0
M = Fe (III), n=2, x=0, y=0

3.2. Infrared Spectra of the Ligands and Complexes

The infrared spectra of the ligands and their complexes are presented in (Table 2). The spectra of the complexes were compared to the parent ligands in order to understand the sites of coordination present in the complexation matrix (Figure 3). The absorption bands at 3426 cm^{-1} and 3480 cm^{-1} in *para*-aminobenzoic acid and acetylsalicylic acid, respectively, attributable to υ(O-H), were shifted to a higher frequency in all the complexes in the range of 3504 to 3568 cm^{-1} [7-10, 15]. This shifting can be attributed to the deprotonation and involvement of the hydroxyl group, leading to the bond formation with the metal centre [4, 10]. The υ(C=O) group in the ligands occurred at 1672 cm^{-1} in

acetylsalicylic acid, and at a region of 1688 cm^{-1} in *para*-aminobenzoic acid (PABA). These bands shifted to higher wavenumber in all the synthesized complexes in the range of 1699 to 1757 cm^{-1} for [Cd (ASA) (PABA)] Cl2 to [Ni (ASA) (PABA) Cl2].

This shifting was an indication of the carbonyl **υ**(C=O) group participation in the coordination sphere of the complexes. The υ(C-N) in the PABA and complexes pitched at the region range of 1120 – 1140 cm^{-1}. In line with previous studies, the results obtained are in agreement with findings in the published works [11, 22]. It was observed that coordination occurred through the oxygen and carbonyl group of the carboxylic acid groups present in both the *para*-aminobenzoic acid (PABA) and acetylsalicylic acid (ASA) [4, 5, 7]. New bands due to metal-oxygen υ **(**M-O) in the complexes were observed within the regions 509 – 552 cm^{-1}, while bands at the region 415 – 429 cm^{-1} were attributed to υ (M-Cl) [7, 23, 24].

3.3. Ultraviolet-visible Spectra of the Ligands and Complexes

The ultraviolet-visible spectra of ligands and their complexes in 10^{-3} M DMSO at room temperature at a wavelength range of 200 – 800 nm are shown in (Table3) and (Figure 3). The absorption bands at 186 nm and 253 nm in PABA are attributed to π - π* and n - π*, respectively [7, 8, 15]. Two absorption bands at 229 nm and 246 nm observed in acetylsalicylic acid spectrum are attributed to π - π*. Cu(II) complex indicated three bands at 269 nm, 351 nm, and 604 nm which are assigned to n - π*, charge transfer, and $^{2}E_{g} \rightarrow {}^{2}T_{2g}$ with the magnetic moment of 4.28 BM confirming Cu(II) ion to be in an octahedral environment [10, 25]. The high-energy band in the region 258 nm was observed in the cadmium complex. This is assigned to intraligand charge transfer transitions as the *d-d* transition is not expected for Cd (II) complex. This complex possesses a magnetic moment of 0.31 BM indicating that Cd (II) is in the tetrahedral geometry [25, 26].

The nickel complex revealed three bands at 419 nm, 436 nm, and 494 nm which are attributed to $^{3}A_{2g}$ (F) → $^{3}T_{1g}$ (P), $^{3}A_{2g}$ (F) → $^{3}T_{1g}$ (F), and $^{3}A_{2g}$ (F) →$^{3}T_{2g}$ (F), respectively. These are in support of octahedral geometry with a magnetic moment of 2.16 BM [6, 7]. The electronic configuration for Fe (III) complex is d^{5} and ground state $^{6}A_{1g}$. The iron complex was found to possess two absorption bands at 300 nm and 341 nm. Therefore, it becomes difficult to categorize the type of the *d-d* transition. The weak band in the region 341 nm is assignable to Spin and Laporte forbidden $^{6}A_{1g} \rightarrow {}^{4}T_{2g}$ transitions with a magnetic moment of 5.04BM. The data suggested an octahedral geometry for the Fe (III) complex [27]. Mn (II) complex showed three bands at 323 nm, 359 nm, and 394 nm which are assigned to n - π*, charge transfer, and $^{6}A_{g} \rightarrow {}^{4}T_{1g}$ (D) with 3.80 BM magnetic moment. The complex was also confirmed to be in an octahedral geometry [28].

3.4. Analgesic Activity of the Ligands and the Metal Complexes

The analgesic activity of the ligands and their complexes are presented in (Table 4). The activities were carried out by taking compounds at a dosage of 20 mg/kg per body weight. The complexes possessed significant activity with inhibition percentage of acetic acid-induced writhing when compared to the standard (Acetylsalicylic acid). The flicking tail procedure can be used to evaluate the response of antinociceptive activity of the complexes. This procedure can be distinguished by responding to the nociceptive stimuli together with the neuronal as the deep tail mediates a spinal reflex to the stimuli [17, 18]. From previous work, it has been noted that narcotic analgesics showed the mechanism of pain. The metal drug complexes were administered at an equimolar dosage of acetylsalicylic acid at 20 mg/kg per body weight as indicated in (Table 5). The metal complexes of Cu(II), Cd(II), Ni(II), Fe(III), and Mn(II) possessed percentage writhing inhibition of 67.61, 43.87, 60.42, 70.45, and 52.34 %, respectively. The result showed good significant analgesic activity when compared to acetylsalicylic acid (standard) at an equimolar dosage of 20 mg/kg per body weight [18].

The order of the analgesic activity of the test compounds was found to be:
Standard (Acetylsalicylic acid) >
[Fe (ASA) (PABA) $(H_2O)_2$] >
[Cu (ASA) (PABA) $(H_2O)_2$] >
[Ni (ASA) (PABA) Cl_2] >
[Mn (ASA) (PABA) $(H_2O)_2$] > [Cd (ASA) (PABA)] Cl2.

3.5. Toxicity Activity of the Ligands and the Metal Complexes

It was observed that there was no significant increase in serum of the test agents when compared to the control. This is an indication that the integrity of the plasma membrane of the cell in the different organs might not be affected [19, 29]. The high increase in the enzyme activity in the serum and kidney of the Wister rats administered with the synthesized complexes may improve the activity of the enzymes by not destroying the plasma membrane of the organs [11, 19]. This may also be due to stress undergone in the organs by the complexes thereby resulting in loss of the enzyme molecule through space into an extracellular fluid, recognized in the serum as shown in (Figure 4) [11, 29]. $[Cu(ASA)(PABA)(H_2O)_2]$, $[Cd(ASA)(PABA)]Cl_2$ and $[Mn(ASA)(PABA)(H_2O)_2]$ exhibited higher enzymatic activities in the serum and kidney homogenates of the Wister rats examined.

The toxicity activity order of the test compounds is as follows:
For serum homogenate:
[Mn (ASA) (PABA) $(H_2O)_2$] >
[Cd (ASA) (PABA)] Cl_2 >
[Cu (ASA) (PABA) $(H_2O)_2$] >
[Ni (ASA) (PABA) Cl_2] >
[Fe (ASA) (PABA) $(H_2O)_2$] >
Acetylsalicylic acid [ASA] >
para-aminobenzoic acid [PABA] > Control
For kidney homogenate:

[Cu (ASA) (PABA) (H_2O)$_2$] >
[Cd (ASA) (PABA)]Cl_2 >
[Mn (ASA) (PABA) (H_2O)$_2$] >
[Ni (ASA) (PABA) Cl2] > [Fe (ASA) (PABA) (H_2O)$_2$] > Acetylsalicylic acid [ASA] > *para*-aminobenzoic acid [PABA] > Control

3.6. Antibacterial Activity of the Ligands and the Metal Complexes

The antibacterial studies of the complexes were measured as zone of inhibition, and compared with the parent ligands as shown in (Table 6). According to the data obtained, the synthesized metal complexes were capable of reducing the population rate of bacterial strains. This is in agreement with previous studies [7, 10, 11, 15, 19]. [Fe (ASA) (PABA) (H_2O)$_2$], [Ni (ASA) (PABA) Cl_2], [Mn (ASA) (PABA) (H_2O)$_2$], and [Cd (ASA) (PABA)]Cl_2 exhibited better antibacterial activities with zone of inhibitions: 36.30±0.82 mm, 35.54±0.29 mm, 34.77±0.58 mm, and 31.05±0.63 mm respectively against *S. aureus*; while [Cu (ASA) (PABA) (H_2O)$_2$] showed good activity of 27.30±0.55 mm; *para*-aminobenzoic acid [PABA]─10.36 ±0.31 mm and acetylsalicylic acid [ASA]─7.40±0.30 mm against same bacterial strain (*S. aureus*). It was observed that the complexes exhibited higher bacteriostatic activities than the ligands.

It is interesting to note that [Cd (ASA) (PABA)] Cl2 displayed the highest bacteriostatic activities (mm) against *S. faecalis*, *B. subtilis*, and *P. aeruginosa*. Consequently, coordination helps to improve the nature of lipophilic metal ions by assisting their permeation through the layer of lipid of the cell membrane [9, 10, 20]. Coordination helps to decrease the polarity of the metal ions due to the *partial* sharing of the positive charge (+ve) of metal with the donor group [7, 28]. Studies have shown that high rate of the lipophilic character of the complexes might be responsible for their improved antibacterial activity. This might be attributed to the deactivation of complexes by different cellular enzymes which are responsible for different pathways of metabolism in bacterial strains [19, 29]. The increased activity of the metal complexes as compared to the free ligands can be elucidated on the theory of Overtone's cell permeability and model of Tweedy's chelation [10, 24-26].

4. Conclusion

Five mixed complexes of acetylsalicylic acid – *para*-aminobenzoic acid were prepared in a ratio 1:1:1 and characterized using elemental analysis, conductivity measurements, FT-IR, and electronic spectra. Based on the data obtained, it was revealed that the ligand drugs exhibited chelating properties by coordinating to the metal ions through the oxygen of the carboxylic acid groups in both *para*-aminobenzoic acid and acetylsalicylic acid. Acetylsalicylic acid exhibited a good significant analgesic activity. The level of toxicity of the complexes was safe at the level of administered dosage for the management of diseases. The free ligands and the metal complexes were also screened against the selected bacterial strains, and the results showed that the synthesized complexes possessed better activities as compared to the

chelating ligands. Therefore, incorporation of the metal ions into the free ligands centers is one of the major metalloenzymes cofactors that enhances the inhibitory activities against infectious diseases. The biological properties exhibited by the synthesized complexes established their behaviour as good antimicrobial agents.

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Tables:

Table 1. Analytical properties of the ligands and their mixed complexes.

Compound	Melting Point (℃)	Yield (%)	Colour	Conductivity ($\Omega^{-1}cm^2mol^{-1}$)	Elemental analysis (%) (Theoretical/Experimental)			
					C	H	N	M
Acetylsalicylic acid [ASA]	135-137	68	White	-	-	-	-	-
Para-aminobenzoic acid [PABA]	187-189	65	White	-	-	-	-	-
$[Cu(ASA)(PABA)(H_2O)_2]$	205-207	75	Pale Blue	20	46.10/46.32	4.56/4.61	3.36/3.47	15.25/15.86
$[Cd(ASA)(PABA)]Cl_2$	214-216	70	White	79	38.40/38.47	3.00/3.45	2.80/2.53	22.40/22.01
$[Ni(ASA)(PABA)Cl_2]$	236-238	48	Green	15	41.32/41.76	3.66/3.80	3.01/3.75	12.63/12.59
$[Fe(ASA)(PABA)(H_2O)_2]$	258-260	50	Deep Brown	11	40.05/40.53	3.13/3.65	2.92/2.88	11.65/11.27
$[Mn(ASA)(PABA)(H_2O)_2]$	233-235	45	Light pink	18	43.44/43.00	3.39/3.45	3.16/3.02	12.40/12.63

Where C = Carbon, H = Hydrogen, N = Nitrogen, M = Metal.

Table 2. Infrared spectra of the ligands and their mixed complexes.

Ligands/complexes	ν(O-H)	ν(C=O)	ν(C-N)	ν(N-H)	ν(M-O)	ν(M-Cl)
Para-aminobenzoic acid [PABA]	3426	1688	1120	3365	-	-
Acetylsalicylic acid [ASA]	3480	1672	-	-	-	-
$[Cu(ASA)(PABA)(H_2O)_2]$	3531	1735	1140	3345	539	419
$[Cd(ASA)(PABA)]Cl_2$	3565	1699	1125	3324	509	-
$[Ni(ASA)(PABA)Cl_2]$	3504	1757	1121	3368	552	427
$[Fe(ASA)(PABA)(H_2O)_2]$	3526	1732	1128	3365	548	429
$[Mn(ASA)(PABA)(H_2O)_2]$	3568	1705	1134	3361	533	415

Table 3. Electronic spectra of the ligands and their mixed complexes.

Compounds	Wavelength (nm)	Tentative assignment	Magnetic moments (B.M)
Para-aminobenzoic acid [PABA]	186	π - π*	-
	253	n - π*	
Acetylsalicylic acid [ASA]	229	π - π*	-
	246	π - π*	
$[Cu(ASA)(PABA)(H_2O)_2]$	269	n - π*	4.28

	351	MLCT	
	604	$^2E_g \rightarrow {}^2T_{2g}$	
$[Cd(ASA)(PABA)]Cl_2$	234	n- π*	0.31
	258	MLCT	
$[Ni(ASA)(PABA)Cl_2]$	419	$^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$	2.16
	436	$^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$	
	494	$^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$	
$[Fe(ASA)(PABA)(H_2O)_2]$	300	n - π*	5.04
	341	$^6A_{1g} \rightarrow {}^4T_{2g}$	
$[Mn(ASA)(PABA)(H_2O)_2]$	323	n - π*	3.80
	359	MLCT	
	394	$^6A_{1g} \rightarrow {}^4T_{2g}(D)$	

Table 4. Immersion test of the ligands and their mixed complexes.

Groups	**Dosage (mg/kg)**	**Average latent period in seconds during administration of the compounds**				**Increase in latent period (%)**		
		0	**15 (min)**	**30 (min)**	**45 (min)**	**15 (min)**	**30 (min)**	**45 (min)**
Standard (Acetylsalicylic acid)	20	1.71±0.35	1.80±0.73	2.00±0.12	2.08±0.34	66.00	69.32	71.05
$[Cu(ASA)(PABA)(H_2O)_2]$	20	1.32±0.46	1.54±0.30	1.61±0.84	1.90±0.62	34.85	42.63	48.31
$[Cd(ASA)(PABA)]Cl_2$	20	1.45±0.73	1.53±0.32	1.75±0.19	1.90±0.75	23.57	29.41	33.76
$[Ni(ASA)(PABA)Cl_2]$	20	1.66±0.93	1.87±0.70	2.64±0.49	2.79±0.74	54.37	61.34	77.38
$[Fe(ASA)(PABA)(H_2O)_2]$	20	1.23±0.58	1.74 ±0.37	1.85±0.60	2.66±0.30	26.09	32.76	37.11
$[Mn(ASA)(PABA)(H_2O)_2]$	20	1.40±0.67	1.76±0.93	1.83±0.75	1.92±0.53	53.25	57.83	64.89
Control	20	0.64±0.42	0.73±0.25	0.96±0.38	1.54±0.63	-	-	-

Data obtained are identical as mean ± SEM. The significant values are $P<0.05$ when compared with the control.

Table 5. Activity of the ligands and their complexes on acetic acid induced writhing test in mice.

Groups	**Dosage(mg/kg)**	**Writhing Frequency (Mean±SEM)**	**Writhing Inhibition (%)**
Standard (Acetylsalicylic acid)	20	23.15±0.30	78.66
Control	-	64.25±0.21	-

$[Cu(ASA)(PABA)(H_2O)_2]$	20	31.42±0.72	67.61
$[Cd(ASA)(PABA)]Cl_2$	20	42.33±0.26	43.87
$[Ni(ASA)(PABA)Cl_2]$	20	35.06±0.43	60.42
$[Fe(ASA)(PABA)(H_2O)_2]$	20	55.69±0.85	70.45
$[Mn(ASA)(PABA)(H_2O)_2]$	20	40.32±0.37	52.34

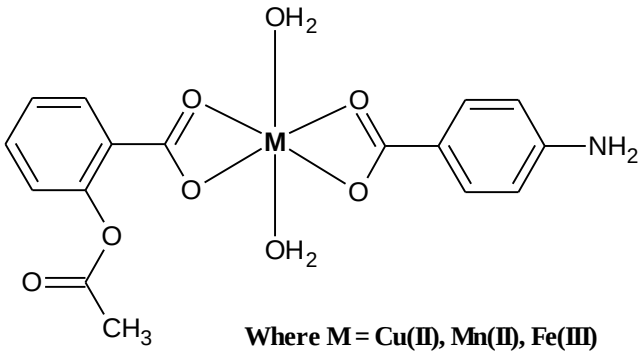
Values are expressed as mean ± standard deviation (mean ± SEM) of three replicates (n=3).

Table 6. Antibacterial activity of the ligands and their mixed complexes.

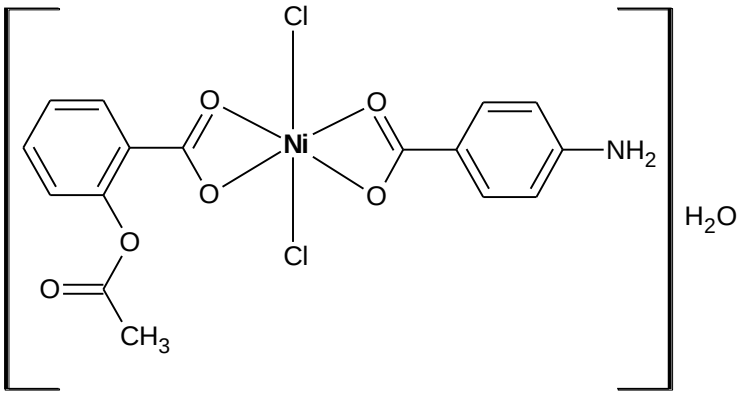
Ligand/Complexes	***S. aureus***	***S. faecalis***	***K. pneumoniae***	***E. coli***	***B. subtilis***	***P. aeruginosa***
	Zone of Inhibition (mm)					
Para-aminobenzoic acid [PABA]	10.36 ±0.31	7.15±0.14	5.57±0.33	6.73±0.42	11.74±0.18	13.03±0.63
Acetylsalicylic acid [ASA]	7.40±0.30	3.25±0.57	2.55±0.12	8.15±0.41	5.63±0.60	10.77±0.45
$[Cu(ASA)(PABA)(H_2O)_2]$	27.30±0.55	32.73±0.39	21.38±0.79	37.32±0.51	29.57±0.61	30.48±0.27
$[Cd(ASA)(PABA)]Cl_2$	31.05±0.63	34.77±0.55	36.34±0.94	25.30±0.44	36.74±0.47	38.50±0.56
$[Ni(ASA)(PABA)Cl_2]$	35.54±0.29	31.78±0.22	38.41±0.75	23.32±0.36	31.70±0.63	33.76±0.45
$[Fe(ASA)(PABA)(H_2O)_2]$	36.30±0.82	34.33±0.54	35.05±0.47	36.57±0.73	30.37±0.94	32.43±0.86
$[Mn(ASA)(PABA)(H_2O)_2]$	34.77±0.58	30.65±0.72	26.98±0.49	35.32±0.55	27.76±0.53	34.60±0.79

Values are expressed as mean ± standard deviation (mean ± SEM) of three replicates (n=3).

Figures:



Where M = Cu(II), Mn(II), Fe(III)



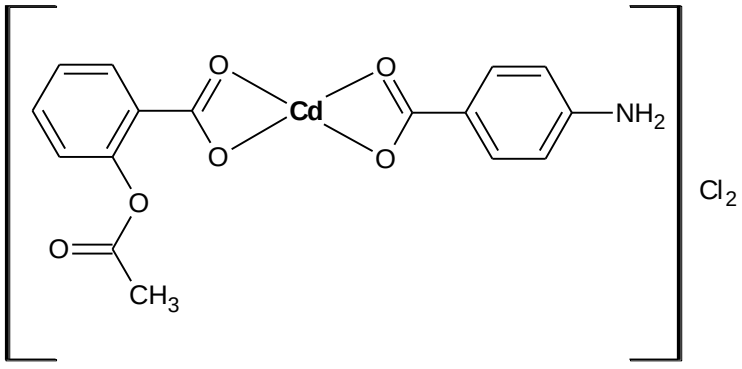


Figure 1. Proposed structures of the metal complexes.

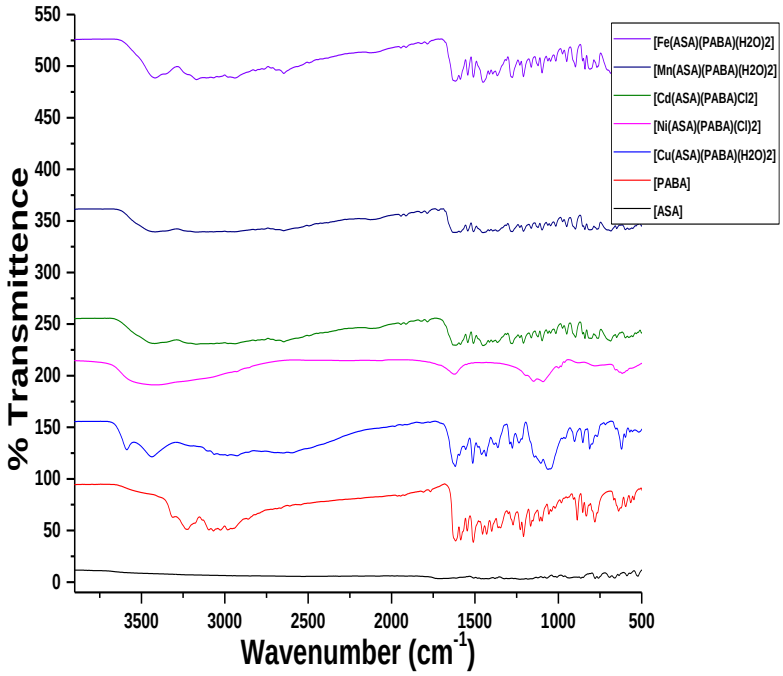


Figure 2. FT-IR spectra of the ligands and the metal complexes.

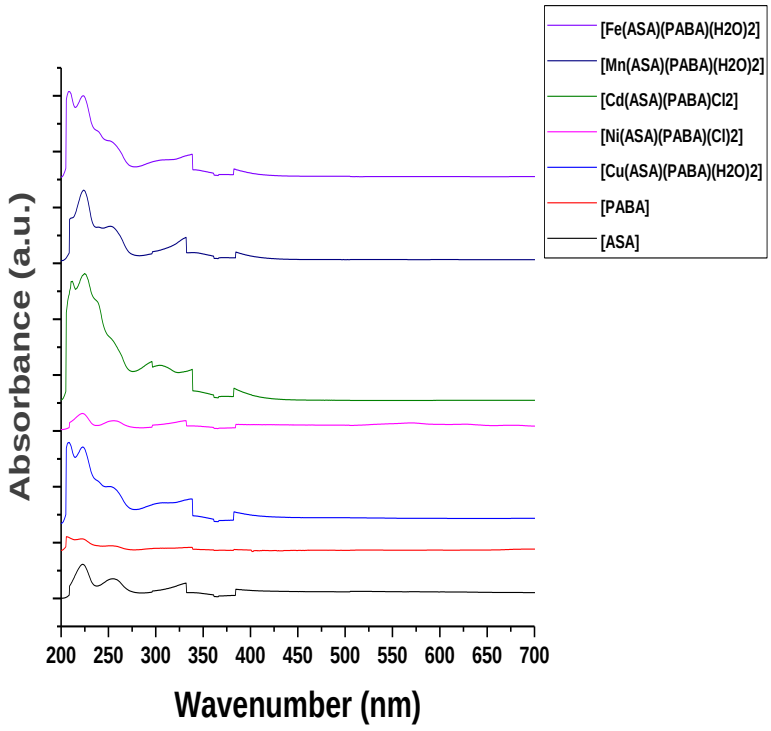


Figure 3. UV-Visible spectra of the ligands and the metal complexes.

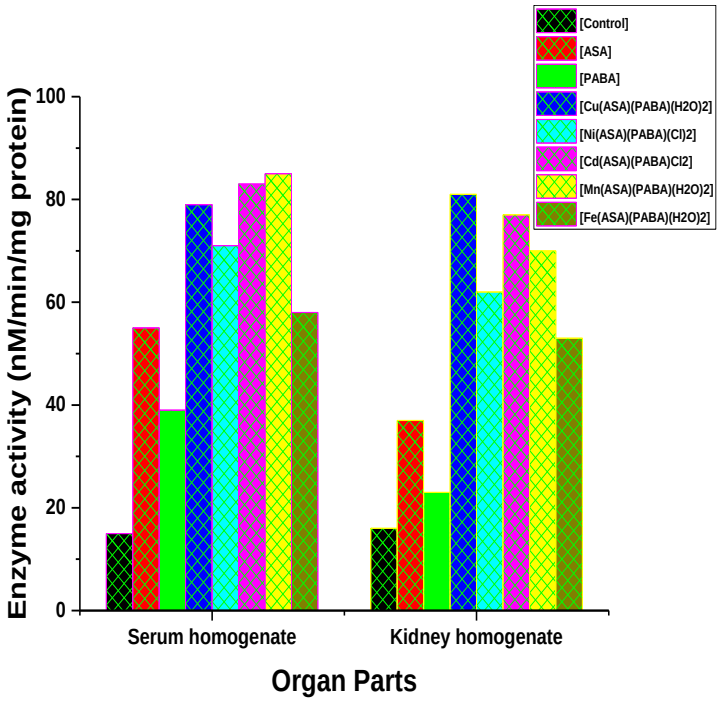


Figure 4. Toxicity screening of the ligands and their mixed complexes against serum homogenate and kidney homogenate.

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