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METAL COMPLEXES OF SALICYLHYDROXAMIC ACID AND 1,10-PHENANTHROLINE; EQUILIBRIUM AND ANTIMICROBIAL ACTIVITY STUDIES

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ABSTRACT. Salicylhydroxamic acid and its binary and ternary copper(II), nickel(II), and iron(III) complexes involving 1,10-phenanthroline were studied pH-potentiometrically in 0.15 mol.L^{-1} $NaNO_3$ aqueous solutions at 37 ^{o}C. The protonation constants of salicylhydroxamic acid and 1,10-phenanthroline as well their binary and mixed ligand complex species stability constants were determined based on 3 estimation models (Irving Rossetti, Bjerrum-Calvin and Hyperquad 2008). Also, six solid complexes were synthesized and their biological activities were evaluated against some pathogenic bacteria and fungi organisms.

KEY WORDS: Salicylhydroxamic acid, 1,10-Phenanthroline, Potentiometry, Antifungal and antibacterial activities

INTRODUCTION

Metal complexes which are formed in biological systems between a ligand and a metal ion are in dynamic equilibrium with the free metal ion in a more or less aqueous environment. All biologically important metal ions can form complexes and the number of different chemical species which can be coordinated with these metal ions is very large. During the past few decades, a lot of scientist research groups operated through specialization in the direction of drug discovery, by studying the simplest species that use metal ions and researching them as whole compound; for example, they suggested the addition of metal ion to antibiotics to facilitate their spread throughout the body [1].

The development of drug resistance as well as the appearance of undesirable side effects of certain antibiotics [2-3] has led to the search of new antimicrobial agents with the goal to discover new chemical structures which overcome the above disadvantages [4-6]. As a ligand with potential oxygen and nitrogen donors, hydroxamic acids are interesting and have gained special attention not only because of the structural chemistry of their coordination modes, but also because of their importance in medical chemistry. These materials have been used as drugs and they are reported to possess a wide range of biological activities against bacteria, fungi and certain types of tumors. In a continuation of our research work oriented towards studying the acid-base and complexation equilibria of many biological oxygen and nitrogen donor drugs with different chelating property [7-14], the present work concerns the equilibrium studies and synthesis of some binary and mixed ligand complexes involving salicylhydroxamic acid, 1,10-phenanthroline.Most interest in the design of binary and ternary complexes as drugs has been centered on the area of antimicrobial and anticancer agents. Hereby, in this paper, we report the results of the equilibrium studies and synthesis of binary and ternary complexes of salicylhydroxamic acid (SHAM) and 1,10-phenanthroline (PHEN) (Scheme 1) as well as investigated their antibacterial and antifungal activities in order to orient future investigations towards the finding of new potent and safe antimicrobial drugs.

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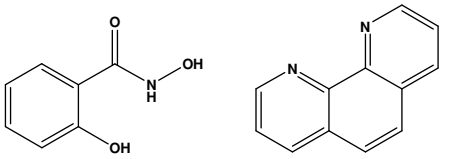


salicylhydroxamic acid (SHAM) **1,10-phenanthroline (PHEN)**

Scheme 1. Molecular structures of the studied ligands.

EXPERIMENTAL

pH-Potentiometric measurements

Materials and solutions

Salicylhydroxamic acid (SHAM) was commercially available pure chemical (Sigma, UK) and used without further purifications. 1,10 phenanthroline (Phen) was obtained from Alfa Aesar (UK). Metal salts [copper chloride dehydrate ($CuCl_2.2H_2O$), nickel chloride hexahydrate ($NiCl_2.6H_2O$) and iron chloride hexahydrate ($FeCl_3.6H_2O$)] used in this work were annular grades and purchased from Sigma-Aldrich, USA. Stock concentrated solutions of these metal salts were prepared in ultrapure water and the metal concentration was obtained by standard analytical methods [15]. A free carbonate sodium hydroxide pellet (titrant, prepared in 0.15 $mol.L^{-1}$ $NaNO_3$ solution) was standardized potentiometrically with potassium hydrogen phthalate solution (Merck AG). Nitric acid (HNO_3), sodium hydroxide pellets (NaOH), and sodium nitrate ($NaNO_3$) were supplied by Fisher Scientific (USA). All solutions were prepared freshly before each series experiments using ultra-pure water obtained from NANO Pure Ultrapure water 18.3 $M\Omega.cm^{-1}$. The solvents used during the synthesis of metal complexes were chemically pure, commercially available, and used without further purifications.

Apparatus and procedures

The pH-potentiometric titrations were performed using a Metrohm 702 SM titrator, equipped with 664 Dosimate, 728 magnetic stirrer and coupled with Dosino burette model 683. Prior to each titration, the pH-automatic titrinometer was calibrated with buffer standard solutions at three pH values ~ 4.00, 7.00 and 9.00. The burette's volume droplet calibration was also carried out daily to increase the experiment accuracy based on the scale of the U.S. National Bureau of Standards [16]. The electrode response can be read to the third decimal place in terms of pH units with a precision of ±0.001. The device was coupled to a personal computer and the titration software Tiamo^{TM}version 2.3 was used to control the titration process and record the data. The pH-potentiometric titrations were carried out in 150 mL commercial double-walled glass vessel. The ionic strength of the solutions was maintained at constant level (0.15 mol L^{-1}) using the desired concentration of $NaNO_3$ solution as supporting electrolyte, and the temperature was adjusted constant inside the titration cell at the desired temperature (37±0.05 ^{o}C), by circulating water using an oil-Thermo stated set-up. The potentiometric titration of the binary complexes were carried in pH range ~2.5 to 11, with more than 80 points of pH reading collected for each set of titration, and at two different molar ratio of metal to ligand (1:1, and 1:2 molar ratio) to fulfil all possible coordination numbers of the metal ions. Each set of titration was repeated at least 3 times under controlled experimental conditions with a reproducibility of ± 0.04 in pH unit. During the course of titrations, a stream of oxygen-free nitrogen was passed through the reaction cell to eliminate the adverse effect of the atmospheric carbon dioxide. For

determination of the protonation constant of ligands and the stability constant of binary and ternary complex between metal ions and ligands, the following solutions were prepared each with a total volume 50 mL: (a) 0.003 mol.L^{-1} HCl + 0.15 mol.L^{-1} NaCl, (b) solution a + 0.01 mol.L^{-1} SHAM, (c) solution a + 0.01 to 0.03 mol.L^{-1} SHAM + 0.01 mol.L^{-1} metal ion, (d) solution a + 0.01 mol.L^{-1} PHEN, (e) solution a + 0.01 to 0.03 mol.L^{-1} PHEN + 0.01 mol.L^{-1} metal ion, and (f) solution a + 0.010 mol.L^{-1} SHAM + 0.010 mol.L^{-1} metal ion + 0.010 mol.L^{-1} PHEN. The protonation constant of ligands (SHAM and PHEN) were determined by titrating solutions (b) and (d) potentiometrically against carbonate free NaOH while the stability constants of metal-ligand binary and mixed ligand complexes were determined by titrating mixtures (c), (e), and (f).

Equilibrium data analysis

To determine the protonation and stability constants of SHAM, PHEN and their metal complexes, Hyperquad2008 was used to make nonlinear least square curve fitting based on experimental pH-potentiometric titration data as described in our previous work [17, 7-14]. Various models with possible compositions of complex species and estimation of its stability constants were proposed to the program. Also, for estimation purpose, the protonation and stability constants of these complexes were calculated by using Bjerrum-Calvin half integral method [18, 19, 21] and Irving Rossetti method [22, 23]. Bjerrum-Calvin estimation was refined by adopting Gauss Newton Marquardt algorithm to minimize the nonlinear least squares sum of the difference between the calculated and experimental data of the titration curves. During all refinements, the ionization constant of water at chosen experimental condition was kept constant ($pK_w \approx 13.384$ at 37 ^oC and ionic strength of 0.15 mol.L^{-1} $NaNO_3$).

Metal complexes synthesis

The binary and ternary complexes were synthesized as following:

*$Cu(SHAM)_2.2H_2O$ (**I**).* Solid $CuCl_2.2H_2O$ (0.28 g, 1.63 mmol) was dissolved in a hot (80 ^oC) aqueous solution (45 mL) of SHAM (0.5 g, 3.26 mmol). The pH of the resulting solution was raised to 5.5 using 0.1 M NaOH solution where upon a copious pale green precipitate was obtained. After standing at room temperature for several hours this was suction filtered, washed with the warm water and dried over P_2O_5 in vacuum. Yield: 42.2%. Anal. found: C, 41.3; H, 4.0; N, 6.9; Cu, 15.7%. IR (KBr disc): 3020, 1610, 1390, 1270, 1045, 750, 520, 430 cm^{-1}. Melting point = 322 ^oC. Magnetic susceptibility ($\mu_{effective}$ = 1.78 BM).

*$Ni(SHAM)_2.2H_2O$ (**II**).* This was prepared as above using $NiCl_2.6H_2O$ (0.78 g, 3.2 mmol) and SHAM (1 g, 6.53 mmol).Yield: 62%; pale green. Anal. found: C, 41.6; H, 3.7; N, 7.0; Ni, 14.7%. IR (KBr disc): 3075, 1610, 1390, 1270, 1030, 750, 580, 434 cm^{-1}. Melting point = 264 ^oC. Magnetic susceptibility ($\mu_{effective}$ = 3.43 BM).

*$Fe(SHAM).2H_2O$ (**III**).* Also, this was prepared as above using $FeCl_3.2H_2O$ (0.59 g, 2.18 mmol) and SHAM (1 g, 6.53 mmol). Yield: 69%; maroon precipitate. Anal. found: C, 45.4; H, 3.8; N, 7.6; Fe, 10.6%. IR (KBr disc): 3063, 1610, 1370, 1260, 1040, 750, 538, 442 cm^{-1}. Melting point = 271^oC. Magnetic susceptibility ($\mu_{effective}$ = 5.74 BM).

*[Cu(Phen)(SHAM)]ternary complex (**IV**).* A solution of $CuCl_2.2H_2O$ (3.26 mmol) in methanol (30 mL) was added to a stirring solution of SHAM (3.26 mmol) in methanol (50 mL). Phen (6.57 mmol) in methanol (15 mL) was then added to the resulting solution which was left to stand in a refrigerator for several days which then blue green crystals are formed. The

precipitated crystals were filtered, washed several times with 50% (*v/v*) methanol-water to remove any traces of the unreacted starting materials. Finally, the complexes were washed with diethyl ether and dried in a vacuum desiccator over P_2O_5. Yield: 87%; blue green. Anal. found: C, 57.4; H, 3.5; N, 10.6; Cl, 10.6%. IR (KBr disc): 3067, 2115, 1750, 1640, 1410, 1250, 1070, 730, 540, 470 cm^{-1}. Melting point = 352 ^oC. Magnetic susceptibility ($\mu_{effective}$ = 1.76 BM).

[Ni(Phen)(SHAM)] (**V**) and [Fe(Phen)(SHAM)] (**VI**) ternary complexes were prepared as above. Yield: 38-62%; green. Anal. found: ≈ C, 57.4; H, 3.6; N, 10.8; Cl, 10.9%, for each of them. IR (KBr disc): 3027, 2050, 1850, 1650, 1450, 1270, 1150, 760, 555, 478 cm^{-1}. Melting point = 294, and 312 ^oC. Magnetic susceptibility, $\mu_{effective}$ = 3.25, and 5.34 BM for Ni(II), and Fe(III) complexes, respectively.

Elemental analysis and FT-IR spectra were performed in Micro Analytical Centre, Faculty of Science, Cairo University, Egypt. Magnetic susceptibility measurements of the powdered mixed ligand complexes were measured at room temperature with a Magway MSBMk1 magnetic susceptibility balance using $Hg[Co(NCS)_4]$ as the calibrant. Magnetic measurements were carried out according to the Gauy method. The calculations were evaluated by applying the following equations:

$$x_g = \frac{cl(R-R_0)}{10^9 M}, \quad x_m = x_g MW_t, \quad \mu_{eff} = 2.828\sqrt{x_m T}$$

where x is mass susceptibility per g of sample; c is the calibration constant; R is the balance reading for the sample and tube; R_0 is the balance reading for the empty tube; M is the weight of the sample in g. The metal content of the complexes was determined by a Buck Scientifics 210VGP Atomic Absorption Spectrophotometer.

Antimicrobial activity techniques

Preparation of test samples

For the antimicrobial assay; SHAM and their synthesized binary and ternary complexes (**I-VI**) were dissolved in a minimal amount of dimethyl sulfoxide (DMSO) with a concentration range from 2.0 to 8.0 μgmL^{-1}. Stock solutions before dilution were no more than 2.5% DMSO so that the final concentrations in the microwells were typically less than 1% DMSO and solvent controls were run at these concentrations.

Test microorganisms and media

SHAM and its binary and ternary complexes (**I-VI**) were screened for antibacterial activities against 6 bacterial strains; *Escherichia coli*, *Staphylococcus aureus*, *Enterobacter cloacae*, *Salmonella gallinarum*, *Bacillus Subtilis*, and *Pseudomonas aeruguinosa*. The six registered bacterial strains used were clinically isolated and obtained from the Microbial Centre of Ain Shams University, Egypt. The microorganisms (*Aspergillus fumigatus*, *Candida albicans*, *Alternaria alternate*, *Penicillium italicum*, *Sacharamyces cerevisiae*, and *Microsporum canis*) used for the fungistatic evaluation were clinical isolated kindly provided by National Research Centre and the Microbial Centre of Ain Shams University, Egypt. The following media were used: Mueller Hinton agar medium, tryptic soy browth (TSB), (ICN, biochemical Co., USA).

Antimicrobial bioassay

Methods used for analysing compounds for antibacterial and antifungal activities are varied. In this experiment, the analysis was carried out by the paper disc diffusion methods [23-27], which

is a simple and rapid method. The entire surface of the selective agar is inoculated with the bacteria or fungi and sterile filter paper discs of uniform size and thickness containing known amounts of the compounds is placed on the seeded agar. The tested compound diffuses into the agar and prevents the growth of the bacteria, indicated by a clearing zone around the disc if the compound does possess bactereocidal or bactereostatic effect. In most studies inhibition zones are compared with those determined for antibiotics. Briefly a lawn of the organism was prepared by spreading 50 mL of overnight cultures (conc. 10^6–10^7 CFU/mL) onto agar set in sterile glass petri dishes. Sterile filter paper discs (measuring 6 mm in diameter) containing the required concentrations of the compounds are placed at equal distances on the agar plate. Control experiments were performed where only equivalent volumes of the solvents without the added test compounds were applied to the paper discs. Ampicillin at a concentration of 2 μgmL^{-1} was used as the positive control. Plain dry discs were used as negative controls. The plates were incubated at 37 ^oC for 24 h (for bacteria) and at 30 ^oC for 72 h (for fungi) to check clear or inhibition zones around the discs. Bioactivity was determined by measuring diameter of inhibition zones (DIZ) in mm, each experiment was repeated three times and the mean of the diameter of the inhibition zones was calculated. The relative percentage inhibition with respect to standard was calculated by using the following formula: *Relative percentage inhibition of the test sample = 100×(a-b)/(c-b)*, where, *a*, total area of inhibition of the test sample; *b*, total area of inhibition of the solvent; *c*, total area of inhibition of the standard drug (Ampicillin). The total area of the inhibition was calculated by using area = πr^2 where, r = radius of zone of inhibition.

Determination of minimum inhibitory concentration (MIC)

Sterile 96-well microplates were used for the assay (0.5 mL volume, ICN Biochem. Co., USA). To the first row of wells in each of the 96 well microdilution plates, 100 µL of double strength TSB was added. The remainder of the wells was filled with single strength TSB. To the first row of wells, 100 µL of the appropriate dilution of the test compound was added. This combination was allocated and dispensed back into the well 5 times to insure the solution was mixed. From the first row of wells, 100 µL was removed and added to the second row of wells. This was again mixed. This procedure was repeated for each subsequent row until 23, where the combination was mixed and 100 µL was allocated and discarded. Each organism was performed in duplicate. The test was performed in triplicate. Positive and negative controls were prepared. Plates were inoculated by placing 96 prong inoculator into the inoculums and transferring to the prepared 96 well plates. A tape sealer was secured to the microdilution plates to prevent drying. The plates were incubated for 24 h, at appropriate temperatures, after which growth was recorded.

RESULTS AND DISCUSSIONS

Equilibrium studies

A representative pH-potentiometric titration curves for ligands (SHAM and PHEN), and their metal complexes involving copper(II)metal ion is shown in Figure 1. The data analysis of the pH-potentiometric titration curves were refined with HYPERQUAD 2008 software and by using Bjerrum-Calvin half integral and Irving Rossetti estimations. The protonation constants (pK_a) values of the studied ligands (L = salicylhydroxamic acid (SHAM), 1,10-phenanthroline (PHEN)) were determined and reported in Table 1. By comparing our calculated values (Table 1) with the literature values, we found a negligible variance, and could be explained in regard to the different in the experimental condition.

In this paper, the protonation constants of the ligands studied (SHAM and Phen) and their metal complex stability constants (Table 1) were determined carefully under the conditions (37±0.05 ^oC, I = 0.15 $mol.L^{-1}$ $NaNO_3$) described in the experimental section. Generally, it is

observed that the binary (Cu(II), Ni(II) and Fe(II)) metal complexes of salicylhydroxamic acid (SHAM) and 1,10-phenanthroline (PHEN) begin to form in the pH range 3.0-6.4. The complexes are quite stable up to high pH values. In all cases, no calculations have been performed beyond the precipitation point, hence, the hydroxyl species likely to be formed after this point could not be studied. With respect to the titration curves of the different metal-tertiary diimine (Phen) investigated, it is observed that the metal ions (Cu(II), Ni(II) and Fe(II)) show a tendency to form binary complexes with Phen where complete complex formation takes place at very low pH values. Accordingly, determination of the formation constant values of such complexes is quietly possible. The stabilization of the ternary system containing 1,10-phenanthroline can be understood by considering that this tertiary diimine not only induces a strong ligand to metal σ-bonding but also acts as a powerful π-acceptor (Scheme 2).

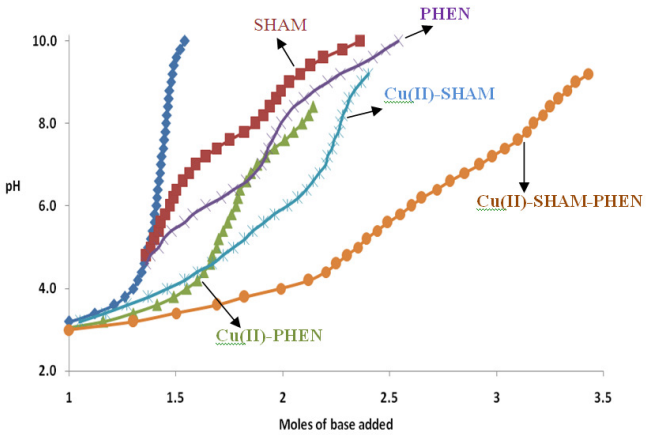
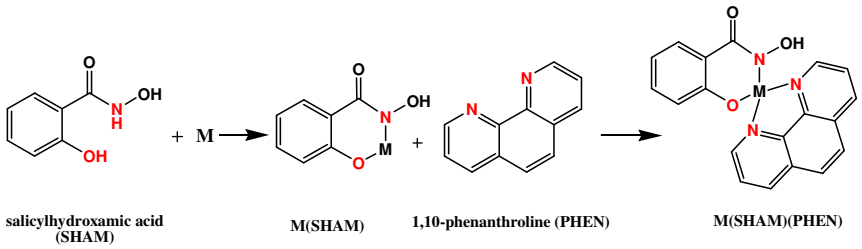


Figure 1. pH-potentiometric titration curves for salicylhydroxamic acid (SHAM), 1,10-phenanthroline (PHEN) and their copper (Cu(II)) complexes at T = 37 °C and I = 0.15 mol·L^{-1} $NaNO_3$.

Table 1. Protonation and stability constants data for the binary and ternary complexes of salicylhydroxamic acid (SHAM) and 1,10-phenanthorline (Phen) at 37±0.05 °C and I = 0.15 mol L^{-1} $NaNO_3$.

	pK_{a1}	pK_{a2}	$\log K_1$			$\log K_2$			$Log K^{M(SHAM)}_{M(SHAM)(PHEN)}$		
			Cu(II)	Ni(II)	Fe(III)	Cu(II)	Ni(II)	Fe(III)	Cu(II)	Ni(II)	Fe(III)
Irving Rossetti model											
SHAM	7.40± 0.03	9.70± 0.05	13.0± 0.04	6.02± 0.06	17.20± 0.03	3.40± 0.03	3.95± 0.04	12.03± 0.06	8.50± 0.05	6.64± 0.06	13.14± 0.04
Phen		4.37± 0.06	3.65± 0.03	3.21± 0.05	4.34± 0.05	4.97± 0.04	4.75± 0.07	5.92± 0.04			
Bjerrum-Calvin model											
SHAM	7.34± 0.06	9.58± 0.04	13.23± 0.05	5.92± 0.04	17.20± 0.06	3.44± 0.04	3.98± 0.06	12.54± 0.03	8.53± 0.04	6.81± 0.03	13.35± 0.02
Phen		4.25± 0.08	3.36± 0.05	3.63± 0.05	4.41± 0.07	4.56± 0.06	4.67± 0.03	5.94± 0.05			
Hyperquad 2008 model											
SHAM	7.76± 0.04	9.73± 0.03	13.52± 0.04	6.31± 0.06	17.20± 0.06	3.420± 0.02	4.04± 0.05	12.46± 0.06	8.64± 0.03	6.92± 0.05	13.52± 0.03
Phen		4.46± 0.02	3.49± 0.02	3.45± 0.07	4.71± 0.06	4.86± 0.04	4.80± 0.08	5.98± 0.05			



Scheme 2. Plausible mixed ligand complex formation equilibria.

Antimicrobial activities

The present study was conducted to evaluate *in vitro* antimicrobial activities of SHAM and PHEN bioligands and their synthesized metal complexes (**I-VI**) against representative pathogenic bacteria and fungi. The relative percentage inhibition and minimum inhibitory concentrations (MICs) were determined used the methods described in the experimental part. The MIC value was defined as the lowest concentration of the antibacterial, antifungal agents at which there showed optically clear. Quality control standardization of the bioassay described above was performed using Ampicillin as standard antibiotic. The assay was performed several times. The results are given in Tables 2, 3 and 4.

Based on the results of bactericidal and fungicidal screening listed in Tables 2, 3 and 4, the following conclusions could be drawn: (1) SHAM and most of their metal complexes (**I-VI**) showed significant *in vitro* antibacterial and antifungal activities against all tested organisms as shown in Figures 1 and 2. (2) SHAM exhibited to have strong antifungal and antibacterial activities with low MIC values and high relative % inhibition. The mode of action may involve the formation of a hydrogen bond through the nitrogen and oxygen atoms in SHAM with the active centers of the cell constituents, resulting in interference with the normal cell process [28]. (3) Most of the synthesized binary and ternary metal ion complexes showed that these complexes are less toxic against the bacterial and fungi organisms than the free SHAM ligand (Figures 1 and 2).

Table 2. Antimicrobial activities of SHAM, and its complexes (**I-VI**) and the reference drug (A) against 6 bacterial species and 6 fungi species in a gar disc diffusion method measured by diameter of inhibition zones (DIZ, mm).

	Antibacterial bioassay						Antifungal bioassay					
	Bacterial strain						Fungi strain					
Compd.	*S.a.*	*S.g.*	*B.s.*	*P.a.*	*E.c.*	*E.c.*	*A.f.*	*C.a.*	*A.a.*	*P.i.*	*S.c.*	*M.c.*
SHAM	++++	++++	++++	++++	+++	++++	++++	+++	++++	++++	++++	++++
I	+++	+++	+++	+++	++	++	++++	+++	+++	+++	++++	+++
II	++	++	++	++	+	+	++	+++	++	++	+	+
III	+++	+++	+++	+++	++	++	+++	++	+++	+++	++	+++
IV	++++	++++	++++	++++	+++	++++	++++	++++	+++	++	++++	+++
V	+++	+++	+++	+++	++	+++	++++	++	+++	++++	++	++
VI	++++	+++	++++	++++	++++	+++	++++	+++	+++	+++	++	+++
A	+++	+++	++	++	+++	++++	+++	++	++++	++++	++	++

+ (weak activity): DIZ = 7-9 mm; ++ (moderate): DIZ = 10-14 mm; +++ (good): DIZ = 15-18 mm; ++++ (significant): DIZ > 18. The bacterial species: (*S.a.*) *Staphylococcus aureus*, (*S.g.*) *Salmonella gallinarum*, (*B.s.*) *Bacillus subtilis*, (*P.a.*) *Pseudomonas aeruguinosa*, (*E.c.*) *Escherichia coli*, and (*E.c.*) *Enterobacter cloacae*. The fungi species: (*A.f.*) *Aspergillus fumigatus*, (*C.a.*) *Candida albicans*, (*A.a.*) *Alternaria alternate*, (*P.i.*) *Penicillium italicum*, (*S.c.*) *Sacharamyces cerevisiae*, and (*M.c.*) *Microsporum canis*. Reference drugs: (A) Ampicillin (H_2O).

Table 3. Antibacterial bioassay parameters (relative % inhibition and minimum inhibitory concentration, MIC) of SHAM, and its complexes (**I-VI**) and the reference drug (A) against 6 bacterial species; *in vitro* disc diffusion method and microtiter plate assay.

	Relative percentage inhibition						Minimum inhibitory concentration (MIC, µg/mL)					
	Strain						Strain					
Compd.	*S.a.*	*S.g.*	*B.s.*	*P.a.*	*E.c.*	*E.c.*	*S.a.*	*S.g.*	*B.s.*	*P.a.*	*E.c.*	*E.c.*
SHAM	88	86	75	84	69	88	73	73	73	66	66	66
I	61	64	60	58	42	62	78	79	79	72	71	72
II	32	33	34	33	18	18	101	101	99	95	96	96
III	65	61	49	36	34	35	78	78	78	72	70	70
IV	89	85	89	88	67	85	68	68	67	65	66	65
V	71	73	66	65	51	63	83	83	83	81	82	81
VI	92	77	84	83	89	71	69	69	67	68	68	67
A	93	59	41	37	69	88	79	78	79	76	77	77

MIC represents the mean from dose-response curves of at least three experiments. Bacterial species: (*S.a.*) *Staphylococcus aureus*, (*S.g.*) *Salmonella gallinarum*, (*B.s.*) *Bacillus subtilis*, (*P.a.*) *Pseudomonas aeruginosa*, (*E.c.*) *Escherichia coli*, and (*E.c.*) *Enterobacter cloacae*. Reference drugs: (A) Ampicillin (H_2O).

Table 4. Antifungal bioassay parameters (relative % inhibition and minimum inhibitory concentration, MIC) of SHAM, and its complexes (I-VI) and the reference drug (A) against 6 fungi organisms; *In Vitro* disc diffusion method and microtiter plate assay.

	Relative percentage inhibition						Minimum inhibitory concentration (MIC, µg/mL)					
	Strain						Strain					
Compd.	*A.f.*	*C.a.*	*A.a.*	*P.i.*	*S.c.*	*M.c.*	*A.f.*	*C.a.*	*A.a.*	*P.i.*	*S.c.*	*M.c.*
SHAM	90	89	78	88	73	93	85	84	84	81	81	81
I	72	77	65	64	51	66	87	87	83	83	83	83
II	43	37	42	39	23	27	105	105	106	102	102	103
III	69	66	59	43	37	36	83	83	83	79	79	78
IV	94	55	97	95	73	89	79	79	76	76	76	76
V	75	79	68	72	75	75	91	91	88	89	89	88
VI	93	87	89	89	89	85	79	79	76	76	76	76
A	98	67	48	43	69	98	89	89	88	88	87	87

MIC represents the mean from dose-response curves of at least three experiments. The fungi species: (*A.f.*) *Aspergillus fumigatus*, (*C.a.*) *Candida albicans*, (*A.a.*) *Alternaria alternate*, (*P.i.*) *Penicillium italicum*, (*S.c.*) *Sacharamyces cerevisiae*, and (*M.c.*) *Microsporum canis*. Reference drugs: (A) Ampicillin (H_2O).

This is due to the chelation therapy, chelation reduces the polarity of the metal atom mainly because of partial sharing of its positive charge with the donor groups and possible π-electron delocalization within the whole chelate ring, also the chelation increases the lipophilic nature of the central atom which subsequently favors its permeation through the lipid layer of the cell membrane [29]. (4) The binary (**I** and **III**) and ternary (**IV** and **VI**) metal ion complexes were found to be highly active towards bacteria and fungi organisms than the other binary(II) and ternary (**V**) metal ion complexes, this may be due to presence of copper and iron metal ions in the complexes (**I, III, IV, VI**). The nickel metal ion binary and ternary complexes (**II** and **V**) was found to be less active against tested bacteria and fungi organisms, this may be due to its ionic character and the nature of this metal ion. (5) The variation in the effectiveness of different compounds against different bacteria or fungi organisms depend either on the impermeability of the cells of the microbes or difference in ribosomes of microbial cells [30, 31]. (6) comparison of the biological activities of SHAM and its synthesized binary and ternary metal ion complexes with the standard antibiotic Ampicillin (**A**) shows that SHAM and some of their metal ion complexes exhibit almost the activity similar to the reference drug (**A**).

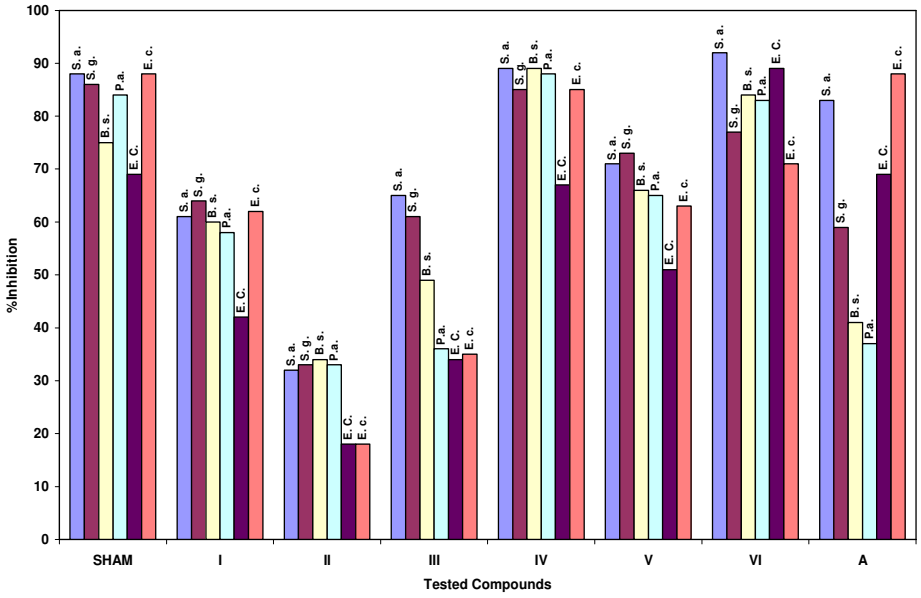


Figure 1. Comparison of relative % inhibition of the complexes against bacterial species; (*S.a.*) *Staphylococcus aureus*, (*S.g.*) *Salmonella gallinarum*, (*B.s.*) *Bacillus subtilis*, (*P.a.*) *Pseudomonas aeruguinosa*, (*E.c.*) *Escherichia coli*, and (*E.c.*) *Enterobacter cloacae*.

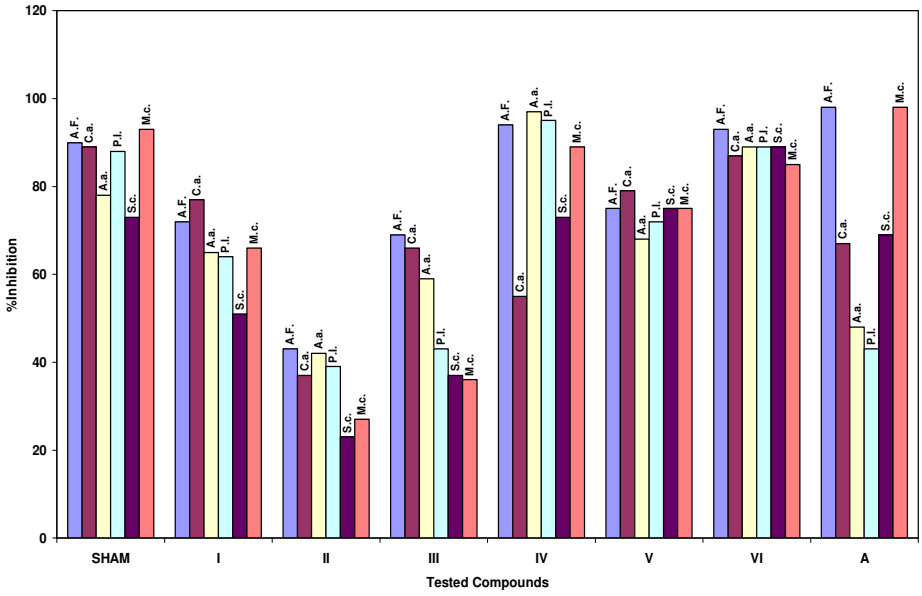


Figure 2. Comparison of relative % inhibition of the complexes against fungal species; *A.f.*) *Aspergillus fumigatus*, (*C.a.*) *Candida albicans*, (*A.a.*) *Alternaria alternate*, (*P.i.*) *Penicillium italicum*, (*S.c.*) *Sacharamyces cerevisiae*, (*M.c.*) *Microsporum canis*.

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