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Antibacterial Activities of Benzoin Thiosemicarbazone and Its Complexes with Co(II) and Ni(II)

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ABSTRACT: The schiff base benzoin thiosemicarbazone and two of its complexes $Co(BTSC)_2$ and $Ni(BTSC)_2$ were used to study their antibacterial activities against some pathogenic bacterial strains by disc diffusion method. Benzoin thiosemicarbazone was synthesized by refluxed standard solutions of benzoin and thiosemicarbazone in 1:1 molar ratio for a period of 3-4 hours. The crystals of benzoin thiosemicarbazone were obtained after partial evaporation of the resulting solution and cooling to room temperature. The complexes were similarly obtained by refluxing the standard solutions of BTSC and metal acetates in 2:1 molar ratio. The antibacterial activities of all the compounds were studied in vitro by disc diffusion methods. The potency was justified by measuring the zone of inhibition in mm on a previously inoculated nutrient broth and incubated for 24 hours at 37^0C. All of the compounds were found to possess different degrees of antibacterial activity but Co(II)-Benzoin thiosemicarbazone complex showed a better antibacterial activity against both gram positive and gram negative bacteria. It showed 29 mm zone of inhibition against *E.Coli* bacteria at the dose of 600µg/disc whereas the standard drug Kanamycin showed 30 mm zone of inhibition against the same bacteria at the dose of 30µg/disc. All of these three compounds were found to possess cytotoxic effect. Minimum inhibitory concentration and minimum bactericidal concentration of these compounds were also determined.

Keywords: Antibacterial activity, Minimum inhibitory concentration, Minimum bactericidal concentration, Brine shrimp lethality

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INTRODUCTION

Antibacterial drugs are probably one of the most successful forms of chemotherapy in the history of medicine. They save countless lives since the beginning of the antibacterial era. But the problems of multi-drug resistant microorganisms have reached an alarming level in many countries around the world (Mitscher et al., 1999; Harbart et al., 2001; Berber et al., 2003). Bacterial resistance to antibacterial drugs has led to severe health and economic problems. The extensive use of antibacterial drugs and their resistance against bacterial infections is positively correlated with the use of antibacterial agents in clinical practice. That is why it is very much essential to find out safe, more effective and inexpensive new chemical compounds as antibacterial agents. In this context a series of researches with various compounds have been carried out by different workers (Cerhiaro et al., 2006; Jesmin et al., 2008; Islam et al., 2013; Shariar et al., 2014; Matar et al., 2015; Tabong et al., 2016). In the present paper, the antibacterial activities of a schiff base namely Benzoin ThioSemiCarbazone (BTSC) and two of its complexes with transition metal Co(II) and Ni(II) have been studied. Schiff bases play a very important role in many biological activities. They have been found to possess the pharmacological activities such as anticancer (Ali et al., 2013), anti HIV (Pandeya et al., 1999), anti-tubercular, anti-inflammatory, antiviral (Shipman et al., 1986) and antimalarial (Li et al., 2003) etc. Besides schiff base, complexes with transition metals have drawn special attention from many researchers. Because these types of metal complexes show diverse structural aspects viz. Metal ligands chelating agents having various functional groups and donor atoms are capable of exerting selective toxicity. The antimicrobial and toxicological activity of some mixed ligand transition metal complexes of Schiff bases were studied by **(**Chohan et al., 2006). In addition to the cytotoxic effects, Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) have also been evaluated.

MATERIALS AND METHOD

Chemicals

All the chemicals used throughout the research work were purchased from British drug house (England) and used without further purification. Solvents were distilled prior to use. All other reagents used were of reagent grade.

General procedure for the synthesis of the compounds

The compounds were synthesized according to the method as described in the literature (Chinnasamy et al., 2010; Pandey et al., 2011; El-Shahawi et al., 2013). For BTSC, saturated alcoholic solution of benzoin and thiosemicarbazide (1:1 molar ratio) were mixed together and refluxed for a period of 3-4 hours and then distilled to half of the total volume. Saturated solution of metal (II) acetate (Co/Ni) in ethanol was added to the condensed solution. Within a few minutes' crystals of metal (II)-benzoin thiosemicarbazone [grey crystals for Co(II) and black crystal for Ni(II)] were obtained. The crystals were then recrystallized twice, dried in an oven at 50°C and stored in a desiccator. Synthesis of metal (II)-benzoin thiosemicarbazone complexes is shown in figure 1.

Characterization of the synthesized compounds

The synthesized compound was characterized by taking melting point by using an electro thermal melting point apparatus. Elemental analytical data were determined by using Perkin Elmer 2400 CHNS/O elemental analyser at Bangladesh Council of Science and Industrial Research Laboratory, Dhaka. The amount of metal was determined by using Atomic Absorption Spectrometer at Dhaka University and IR spectra data were obtained from Rajshahi University central laboratory as KBr disc by using a Shimadzu FTIR spectrometer. The data are shown in tables 1, 2 and 3.

Table 1. Yield percentage and physical characteristics of the benzoin thiosemicarbazone and its complexes

Test compounds	Yield %	Melting point °C	Physical Form	Solubility
BTSC	60	150-153°c	Yellowish white crystalline	Ethanol, methanol, DMSO and acetone
Co(BTSC)2	45	Stable up to ~165°c	Grey crystalline	Ethanol, methanol, DMSO and acetone
Ni(BTSC)2	50	Stable up to ~155°c	Black crystalline	Ethanol, methanol, DMSO and acetone

Table 2. Elemental analytical data of the benzoin thiosemicarbazone and its complexes

Compounds		Elemental analytical data in %					
		C	H	N	O	S	Metal
BTSC	Found	62.78	5.27	14.50	5.30	10.07	
	Theoretical	63.13	5.30	14.72	5.33	10.67	
$Co(BTSC)_2$	Found	39.17	4.01	5.26	20.96	12.55	Co(17.02)
	Theoretical	39.75	4.05	5.15	21.60	13.25	Co(17.13)
$Ni(BTSC)_2$	Found	52.36	4.39	12.21	4.65	9.31	Ni(16.90)
	Theoretical	52.93	4.11	12.44	4.67	9.98	Ni(17.08)

Table 3. IR spectral data of the benzoin thiosemicarbazone and its complexes

Compounds	$\nu(NH_2)$	ν(N-H)	ν(C=N)	ν(C=S)	ν(NH-C=S)	ν(M-O)	ν(M-N)	ν(M-S=C)
BTSC	-------	3379 w	1682 s	1263 w	977 s	-------	-------	-------
$Co(BTSC)_2$	3339 w	-------	1567 w	1135 s	682-1024 Wbr	617 s	526 m	427 s
$Ni(BTSC)_2$	3415 s	------	1566 s	1078 s	679-1029 Wbr	617 s	524 w	481 s

[s= strong, w= weak, m= medium, br= broad]

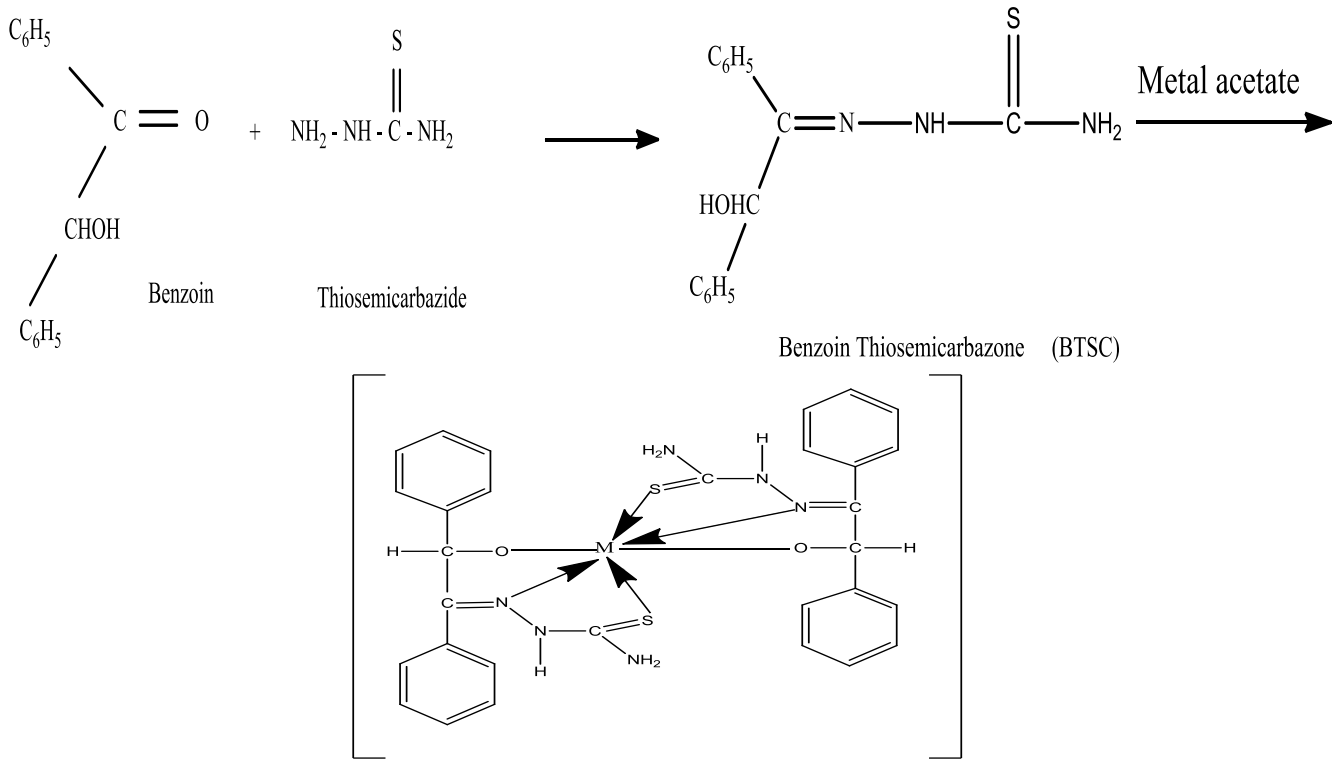

Figure:Structure of $[M(BTSC)_2]$ complex, Where M=Co/Ni

Figure 1. Synthesis of metal (II)-benzoin thiosemicarbazone complexes(II) stands for Co(II)/Ni(II)

Antibacterial screening

Antibacterial activities of the compounds were measured by observing the growth response of various microorganisms. The susceptibilities of such growth rate of microorganisms were measured *in vitro* by disc diffusion method (Bauer et al., 1966). A loop full of the given test strain was inoculated in 30 mL of nutrients broth and incubated for 24 hours in an incubator at 37 °C in order to activate the bacterial strain activity. 20 mL of the nutrients agar media was added in to 120 mm diameter Petri dishes. 0.1 mL of the activated strain was inoculated into the media when it reached the temperature of 37 °C. The media was allowed to solidify. After solidification of the media, a sterilized (BBL, Cocksrvile, U.S.A) filter paper disc (3 mm diameter) for sample and standard drug (30 µg/disc) disc were taken in the Petri dishes. The test samples were applied on the disc with the help of a micropipette in an aseptic condition, controls were run (for each bacterial strain and each solvent), where pure solvent was applied on the disc in the Petri dishes. The Petri dishes were incubated for 24 hours at 37°C.The inhibition zone formed by two compounds against the particular test bacterial strain determined the antibacterial activity. The diameter of zones showing complete inhibition (mm) were measured and the growth inhibition was calculated with reference to positive control.

Preparation of stock solution

Exactly 20mg, 40mg and 60mg of BTSC, $Co(BTSC)_2$ and $Ni(BTSC)_2$ were dissolved separately in 1ml of DMSO to get concentrations of 200,400 and 600 µg/disc respectively for antibacterial screening.

MIC and MBC of the test compounds

MIC of the test compounds were determined by serial tube dilution technique (Reiner, 1947; 1982 and 1982) against the same bacteria as used for antibacterial screening. Nutrient agar media was used for this purpose. Decreasing concentrations of test compounds were prepared in serial two-fold dilution using the stock solution. Bacterial suspension (10µL) containing 10^7 cells/mL was inoculated into all tubes. After incubation for

24 hours at 37 °C, the test tube with no visible growth of the microorganism was taken to represent the MIC value of the sample in µg/mL. MBC is the concentration in which no viable organism will be present. It was determined by keeping the test tubes which were used for MIC determination for four days. After four days' bacterial growth was observed and MBC was determined at the lowest concentrations where no bacterial growth was observed.

Brine shrimp lethality bioassay

The cytotoxic effect of the test compounds was studied by the method as described by (Attaur Rahman et al., 1999*)*. Brine shrimp (*Artemia salina*) eggs were hatched in artificial sea water (prepared by dissolving 38 g NaCl in one liter distilled water) at room temperature under constant aeration for 48 hours. Stock solutions of the schiff base and schiff base complexes (10 mg/mL) in DMSO were added to each vial, so that the final concentration of the compounds became 0, 10, 20, 40, 60, 80 and 100 µg/mL after diluting them to 5 mL with sea water. To each vials, 12 living shrimps were added and allowed to stay there for 24 hours. The survived nauplii in each vial were counted and the results were noted.

RESULTS AND DISCUSSION

The antibacterial activities of these compounds measured in terms of zone of inhibition are shown in table 4. From the table it is clear that the experimental compounds showed moderate antibacterial activity against a number of pathogenic bacteria. The results were compared with standard drug disc of kanamycin (30µg/disc). Antibacterial activities of $Co(BTSC)_2$ against *shigella sonnei* and *E.coli* were quite remarkable than the other compounds and standard drug kanamycin 30µg/disc. However, at still higher doses the activity was found to increase noticeably. On the other hand, the schiff base BTSC showed moderate antibacterial activity against *bacillus subtilis* at the dose of 600µg/disc. Another compound $Ni(BTSC)_2$ showed moderate activity. The solvent DMSO showed no activity against any bacterial strain. A brief comparison of the results obtained recently by different workers can be cited in table 5. It is evident that the present results are quit comparable with those studied earlier.

The cytotoxicity as well as a wide range of pharmacological activities (e.g. anticancer, antiviral, insecticidal, pesticidal, anti HIV, etc) of the compounds can be measured by Brine shrimp lethality bioassay test. Natural and synthetic products can be tested for their bioactivity by this method. Here *in vivo* lethality bioassay is conducted using simple zoological organism brine shrimp nauplii (*Artemia salina*). This bioassay is used as a convenient monitor for screening of compounds in the discovery of new bioactive principle (Perssone,1980; Meyer et al., 1982; Mc Laughlin et al., 1990). The test compounds showed positive results in brine shrimp lethality bioassay shown in figure 2. The median lethal concentration (LC_{50}) of BTSC, $Co(BTSC)_2$ and $Ni(BTSC)_2$ were found to be 27.1677,24.14772 and 33.78005 µg/mL respectively which was obtained from probit statistical analysis and measured from the plots of percentage of mortality versus concentration of the samples (Figure 7). LC_{50} values after probit transformations of the mortality data of standard bleomycin, gallic acid and experimental compounds are given in the table 7. Regression line of log dose of BTSC,$Co(BTSC)_2$ and $Ni(BTSC)_2$ against brine-shrimp nauplii and percent mortality of brine shrimp treated with BTSC, $Co(BTSC)_2$ and $Ni(BTSC)_2$ after 24 hours exposure are shown in figure 3a-5b respectively. From the results discussed above it is clear that the synthesized schiff base and schiff base complexes are biologically active and show significant activity against brine shrimp nauplii. In this bioassay, the mortality rate of brine shrimp was found to increase with the increase in concentration of the test sample. MIC and MBC values of the tested compounds were determined against 6 pathogenic bacteria as µg/mL and the results are cited in the table 6. MIC and MBC values of BTSC and $Co(BTSC)_2$ were found at 32 and 64µg/ml against *S.sonnei*, whereas the MIC and MBC values of $Ni(BTSC)_2$ were found 64 and 128µg/ml against the same bacteria. In case of *E. coli*, *B.subtilis* and *S. aureus* the MIC and MBC values of the test compounds were the same and found to be 256 and 512 µg/ml, 128 and 256 µg/ml and 256 and 512 µg/ml respectively. The MIC and MBC values of BTSC against *Pseudomonas aeroginosa* were found 128 and 256 µg/ml, whereas the MIC and MBC values of $Co(BTSC)_2$ were found 64 and 256 µg/ml. On the other hand, the MIC and MBC values of $Ni(BTSC)_2$ against *Pseudomonas aeroginosa* were found 128 and 512 µg/ml respectively. MIC and MBC values of BTSC and $Ni(BTSC)_2$ were found at 256 and 512µg/ml against *Sarcina lutea*, whereas the MIC and MBC values of $Co(BTSC)_2$ were found 64 and 128µg/ml against the same bacteria respectively.

Table 4. Results of antibacterial activities of benzoin thiosemicarbazone and complexes of benzoin thiosemicarbazone

Name of the compounds	Dose	Diameter of zone of inhibition (mm) of the following bacteria					
		Gram negative			Gram positive		
		Shigella sonnei	***E.coli***	***Pseudomonas arioginosa***	**Bacillus subtilis**	***Sarcina lutea***	***St. aureus.***
Standard (*Kanamycin*)	30µg/disc	31	30	30	26	25	27
BTSC	200µg/disc	12	11	9	10	7	9
	400µg/disc	17	15	13	14	11	12
	600µg/disc	21	22	18	20	17	18
$Co(BTSC)_2$	200µg/disc	15	13	12	12	10	11
	400µg/disc	18	17	16	16	15	16
	600µg/disc	25	29	22	21	19	22
$Ni(BTSC)_2$	200µg/disc	12	10	7	10	6	8
	400µg/disc	16	14	10	13	9	11
	600µg/disc	19	20	16	19	14	16

Table 5. Comparative data against three pathogenic bacteria obtained recently by different workers

Name of the bacterial stains	**BTSC** [200µg/disc]	**$Co(BTSC)_2$** [200µg/disc]	**$Ni(BTSC)_2$** [200µg/disc]	**VTS** [200µg/disc]	**BTS** [200µg/disc]	**ATS** [200µg/disc]	**$[Ni(HMTA)_2(NCS)_2(H_2O)_2]\ H_2O$** [50µg/disc]
Shigella sonnei	12	15	12	22	25	23	_
E.coli	11	13	10	20	23	22	11
St.aureus	9	11	8	18	26	24	9

BTSC: Benzoin thiosemicarbazone; $Co(BTSC)_2$ and $Ni(BTSC)_2$:Complexes of benzoin thiosemicarbazone with Co(II) and NI(II) metal; VTS:Vanillin thiosemicarbazone; BTS: Benzophenone thiosemicarbazone; ATS: Acetophenone thiosemicarbazide; $[Ni(HMTA)_2(NCS)_2(H_2O)_2]H_2O$: [Diaquabis(hexamethylenetetramine) diisothiocyanato-*k*N]nickel(II) complex

Table 6. Results of minimum inhibitory concentration and minimum bactericidal concentration of the compounds

Test organisms	**BTSC**		**$Co(BTSC)_2$**		**$Ni(BTSC)_2$**	
	MIC µg/mL	MBC µg/mL	MIC µg/mL	MBC µg/mL	MIC µg/mL	MBC µg/mL
S. sonnei	32	64	32	64	64	128
E. coli	256	512	256	512	256	512
Pseudomonas arioginosa	128	256	64	256	128	512
B. subtilis	128	256	128	256	128	256
Sarcina lutea	256	512	64	128	256	512
St. aureus.	256	512	256	512	256	512

BTSC: Benzoin thiosemicarbazone; $Co(BTSC)_2$ and $Ni(BTSC)_2$:Complexes of benzoin thiosemicarbazone with Co(II) and NI(II) metal; VTS: Vanillin thiosemicarbazone

Table 7. LC_{50} values after probit transformations of the mortality data of bleomycin, gallic acid and experimental compounds

Compounds	LC_{50} (µg/ml)	95%Confidense Limit(µg/ml) Lower-Upper		Regression equation	λ^2	df
Bleomycin	0.41	0.27	0.62	Y=3.16+2.99X	0.62	2
Gallic acid	4.53	3.33	6.15	Y=3.93+1.62X	1.25	2
BTSC	27.1677	18.27273	40.39267	Y = 2.09011 + 2.029137 X	0.9520188	4
$Co(BTSC)_2$	24.14772	15.48253	37.66258	Y = 2.37672 + 1.896974 X	0.200182	4
$Ni(BTSC)_2$	33.78005	23.65396	48.24105	Y = 1.777629 + 2.107971 X	2.018728	4

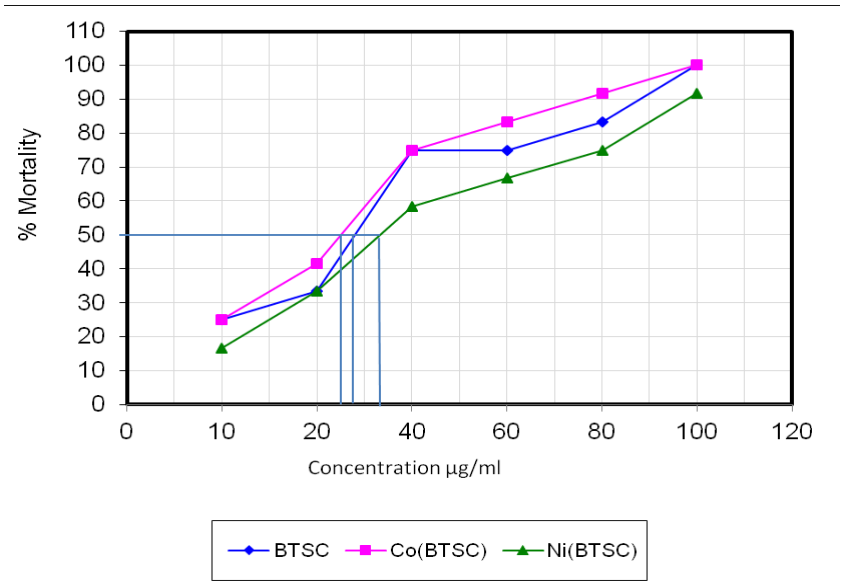

Figure 2. Brine shrimp lethality bioassay for measuring the bioactivity of the test compounds

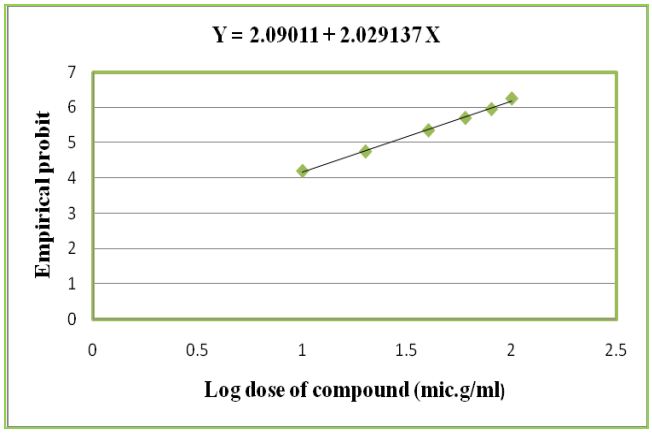

Figure 3a. Regression line of log dose of BTSC against brine-shrimp nauplii after 24h of exposure

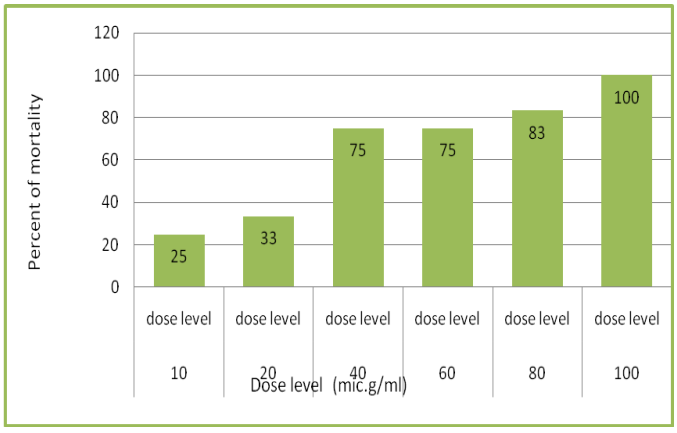

Figure 3b. Percent mortality of brine shrimp treated with BTSC after 24 hours' exposure

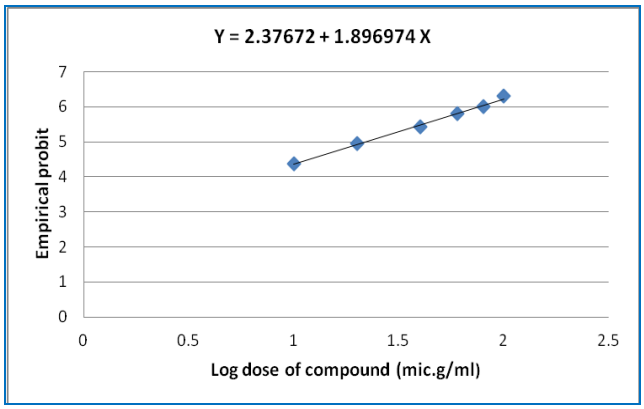

Figure 4a. Regression line of log dose of $Co(BTSC)_2$ against brine-shrimp nauplii after 24h of exposure

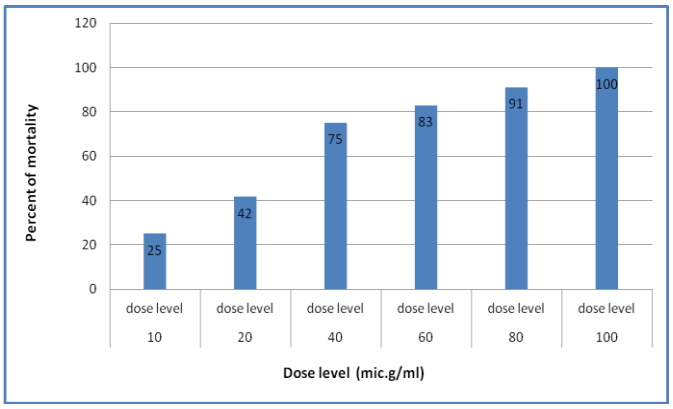

Figure 4b. Percent mortality of brine shrimp treated with $Co(BTSC)_2$ after 24 hours' exposure

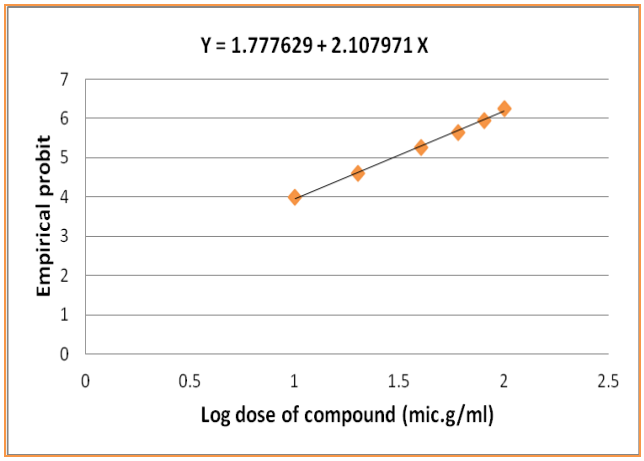

Figure 5a. Regression line of log dose of $Ni(BTSC)_2$ against brine-shrimp nauplii after 24h of exposure

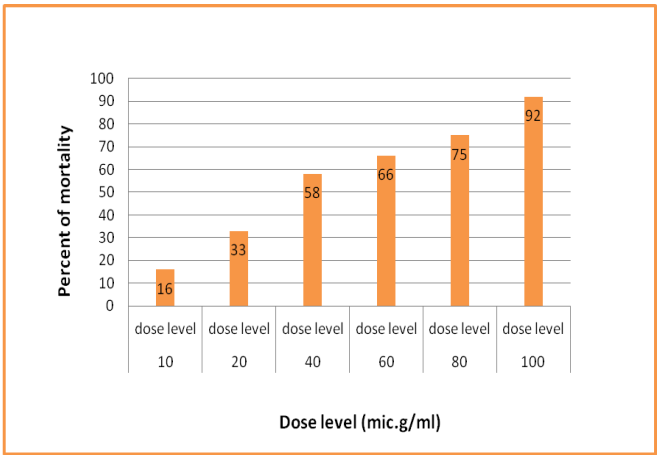

Figure 5b. Percent mortality of brine shrimp treated with $Ni(BTSC)_2$ after 24 hours' exposure

CONCLUSION

Antibacterial activities of the synthesized compounds have been investigated against some pathogenic bacteria by disc diffusion methods. Among the synthesized compounds, it is evident that $Co(BTSC)_2$ complex exhibit a significant antibacterial activity. On the other hand, BTSC and $Ni(BTSC)_2$ complex showed a moderate sensitivity even with higher doses. The compound $Co(BTSC)_2$ showed a prominent effect on brine shrimp lethality bioassay in comparison with BTSC and $Ni(BTSC)_2$. The positive response suggested that the experimental compounds possess cytotoxic effect. In the light of the above observations these three compounds may be used as new antibacterial drugs. But in order to ascertain these compounds as novel potential antibacterial drugs, it is necessary to carry out further experiments against more pathogenic bacteria with advanced techniques. These findings definitely give positive support to carry out further researches in a way to formulate novel antibacterial drugs.

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