



## Synthesis, Antibacterial and Antifungal Activities of Some Transition Metal (II) Chelates Derived from Benzoyl Acid Hydrazide

Salawu, O.W.

Department of Chemistry, Federal University Lokoja, Kogi State, Nigeria.

### ABSTRACT

The reaction of benzoylacylhydrazine with hydrazinemonohydrate gave the hydrazide derivative. The hydrazide reacted with some transition metal (II) ions to give chelated complexes. These complexes were characterized by elemental analysis, FT-IR and molar conductance. All the FT-IR bands of the newly prepared complexes are lowered between 15-50 $\text{cm}^{-1}$  compared with the FT-IR band of the hydrazide. The complexes are coloured and stable in air. Analytical data showed that all the complexes exhibited 1:2 (metal:ligand) ratio. The hydrazide act as neutral bidentate donors which is reflected through the non-electrolytes properties in ethanol. The new complexes are relatively high in melting points. The synthesized compounds were screened using two plant pathogens and two human pathogens. It was observed that the complexes exhibited significantly high activity at concentration of 200 $\mu\text{g/ml}$  and moderate activity at 100 $\mu\text{g/ml}$  in human pathogen compared to plant pathogens. It was also observed that  $[\text{Co}(\text{BAH})_2]\text{SO}_4 \cdot 2\text{H}_2\text{O}$  showed higher activity compared to the other two complexes.

**Keywords:** synthesis, antifungal, antibacterial, chelate, ligand, *E.coli*, hydrazide

### INTRODUCTION

Though transition metals occupy many key positions in biological processes (Abram and Murrer, 1993), metal based drugs are traditionally undervalued by the pharmaceutical industry, which is dominated by organic chemistry (Chohan *et al*, 2006). Nevertheless, a number of coordination compounds have been applied in the therapy of various diseases (Bergamaschi *et al*, 1997) e.g., historically Salvarsan against syphilis (Bottari *et al*, 2000), gold complexes against arthritis (Chohan, 1997), bismuth compounds as antiulcer drugs (Dianu *et al*, 2009), or platinum compounds against cancer (Heslop and Jones, 1976).

It is evident that the compounds containing hydrazine moiety (Amin and Cryer, 2016), have a strong ability to form metal complexes and they exhibit a wide range of biological activities (Seibin *et al*, 2012). In an hydrazide group there are two potential binding sites, *i.e.*, oxygen and nitrogen for complexation (Kaymakcioglu *et al*, 2015), also a transition metal complexes which usually contain nitrogen, sulphur or oxygen as ligands atoms are becoming increasingly important because these ligands can bind with different metal centres involving various coordination sites and allow successful synthesis

of metallic complexes with interesting stereochemistry (Sinn, 2012).

Acid hydrazides have frequently been investigated for testing their potentiality as tuberculostats (Rollas and Gulerman, 2014). Hydrazides and their condensation products have displayed diverse range of biological properties such as bactericidal, anti-fungal (Nwabueze and Salawu, 2012), anti-convulsant, anti-helminthic, anti-tumor, anti-leprotic, anti-malarial, anti-cancer (Salawu and Aliyu, 2012) anti-depressant, anti-HIV, analgesic-anti-inflammatory, leishmanicidal, vasodilator activities (Abdelkarim *et al*, 1999).

In the present work, the antibacterial and antifungal activities of newly prepared complexes from benzoyl acid hydrazide using two plants and human pathogens is reported.

### METHODOLOGY

Melting points were determined on an Electrothermal melting point apparatus

Received 21 November, 2016

Accepted 02 March, 2017

Address Correspondence to:

[o.wsalawu@yahoo.com](mailto:o.wsalawu@yahoo.com)

(Electrothermal 9100) and are uncorrected. IR spectra were recorded for KBr. Conductivity measurement was made in  $10^{-3}$ M solution of the complexes and ligand in DMSO at ambient temperature using conductivity meter Jenway PMC3. Elemental analysis was performed on a Perkin Elmer 2400 instrument at leather research institute Zaria, Kaduna State, Nigeria.

The antimicrobial and antifungal screening was performed using paper disc method. Nutrient agar was taken as media for cultivation of bacteria. The inhibitory effects of the samples were measured against the bacteria after incubation for 24 hours at  $37^{\circ}\text{C}$ .

All other chemicals and solvents used were of analytical grade. The metal (II) salts used for the preparation of the ligand and complexes were obtained from Sigma Aldrich.

#### Preparation of the ligand (benzoyl acid hydrazide) [BAH]

The ligand was prepared using standard method (Salawu *et al*, 2011). The ligand benzoyl acid hydrazide [BAH] was prepared from the mixtures of butyl benzoate (25ml) and hydrazine hydrate (10ml) in 100 ml absolute ethanol for 6 hours in a refluxing machine in a 500ml; round bottom flask. The isolated compound were filtered off as milky crystals, washed with ether, recrystallised from absolute ethanol and finally dried and weighed. (yield = 15g, 73%). The equation for the reaction is shown as equation 1.

#### Preparation of the Cu(II), Co(II) and Ni (II) complexes

A sample of ligand [BAH] (2.27g, 0.01mol) each was dissolved in dimethyl sulphoxide (DMSO) (100 ml). A solution of sulphate salts

of metal (II) [ $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  (1.00g, 0.02mol),  $\text{CoSO}_4 \cdot 6\text{H}_2\text{O}$  (1.00g, 0.002mol) and  $\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$  (1.00g, 0.002mol)] in 100 ml of distilled water was added drop wise to the ligand solution with continuous stirring. The complex started to form immediately upon addition of the metal (II) salts solution. The precipitated complex was filtered off, washed with water and cold ethanol several time, dried under reduced pressure over anhydrous  $\text{CaCl}_2$  in a desiccator. The equation for the reaction is shown as equation 2.

#### Antibacterial and antifungal activities

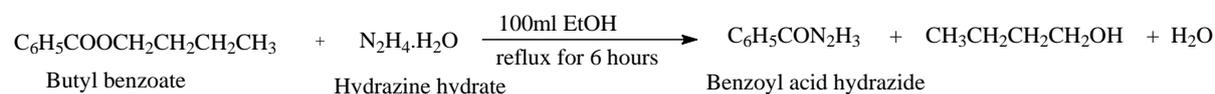
Antibacterial activity of the hydrazide [BAH] and its metal (II) complexes were screened against two plants pathogens *Xanthomonas citri* (Xc) and *Erwinia carotovora* (Ec) and human pathogen *Escherichia coli* (E. coli) and *Rhizopus stolonifer* (Rs).

The activity was studied by using cup plate agar diffusion method (NCCLS, 2010) by measuring diameter of zone of inhibition in mm. The compounds were tested at the concentration of 50,100 and 200  $\mu\text{g/ml}$  in 5% DMSO. The solution was poured in the cup/well of bacteria seeded agar plates.

The plates were incubated at  $37^{\circ}\text{C}$  for 24 hours for *E. coli* whereas the plates of other three bacteria were incubated at  $27 \pm 2^{\circ}\text{C}$  for 24 hours. The activity is reported by measuring the diameter for zone of inhibition in mm. The standard antibiotic used was streptomycin. The results are presented in Table 3.

#### RESULTS AND DISCUSSION

The preparation of the hydrazide from the ester and hydrazine hydrate was represented by the following equations below:



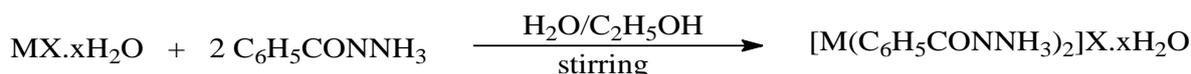
#### Equation 1. Preparation of Acid hydrazide

The hydrazide obtained were white crystals which gave moderately yield 73%. The ligand are quite stable in air. It could be stored for months without any appreciable change. The result of the melting point/ decomposition of the ligand and the metal complexes is presented in Table 1. The result showed that the melting

point/decomposition is high ( $190\text{-}203^{\circ}\text{C}$ ) which indicate non-hygroscopic nature of the ligand and the complexes (Seibin *et al*, 2012).

#### Metal Complexes

The reaction of metal (II) salts and the hydrazide from to form metal (II) complexes can be represented in Fig. 2 below:



M = Co, Cu and Ni while X = SO<sub>4</sub><sup>2-</sup> x = 1 - 6

## Equation 2. Preparation of Metal (II) complexes

**Table I: Physical and Analytical Data of Compounds**

S/N	Compounds	M.P °C	Yield	Mol. Wt. gmol <sup>-1</sup>	Crystal appearance	Molecular Formular	Elemental Analysis Found (calculated)			
							C	H	N	M
1.	[BAH]	190	73	270.32	White	C <sub>14</sub> H <sub>14</sub> O <sub>2</sub> N <sub>4</sub>	61.90 (62.20)	5.20 (5.23)	20.63 (20.73)	- -
2.	[Co(BAH) <sub>2</sub> ].SO <sub>4</sub> .2H <sub>2</sub> O	220	85	461.36	Dark green	C <sub>14</sub> H <sub>18</sub> O <sub>8</sub> N <sub>4</sub> S Co	36.04 (36.44)	4.20 (3.94)	12.14 (12.15)	13.67 (13.77)
3.	[Ni(BAH) <sub>2</sub> ].SO <sub>4</sub> .H <sub>2</sub> O	251	82	443.1	Greenish	C <sub>14</sub> H <sub>16</sub> O <sub>7</sub> N <sub>4</sub> S Ni	37.95 (37.94)	3.65 (3.64)	12.64 (12.65)	13.07 (13.25)
4.	[Cu(BAH) <sub>2</sub> ].SO <sub>4</sub> .H <sub>2</sub> O	203	77	447.96	Bluish	C <sub>14</sub> H <sub>16</sub> O <sub>7</sub> N <sub>4</sub> S Cu	36.99 (37.53)	3.20 (3.61)	12.52 (12.51)	14.19 (14.18)

## Infrared spectra

The relevant IR spectra bands of the ligand and the complexes are given in Table 2. Benzoyl acid hydrazide as a ligand, provides two potential donor sites, *viz* O and N, owing to the presence of lone pair electrons on the nitrogen atom of the azomethine group.[1-2] The infrared spectra of the [BAH] ligand and its complexes, as shown in Table 2, have been assigned mainly for those specific frequencies directly involved in complex formation. The vibrational band at the range of 3165-3187cm<sup>-1</sup> assigned to  $\nu$ N-H in the ligand shifted to a lower wavelength (3142-3163cm<sup>-1</sup>) in the three complexes. This is probably due to coordination of the metal *via* nitrogen atom of the N-H group in the ligand[3]. The band

observed at the 1105cm<sup>-1</sup> and 1350cm<sup>-1</sup> corresponds to the  $\nu$ (N-N) and  $\nu$ (C-N) stretching[3-4]. The band corresponding to  $\nu$ (C=O) at 1581cm<sup>-1</sup> is shifted by about 50cm<sup>-1</sup> to a lower frequency, supporting the coordination of the carbonyl oxygen[2]. However the vibrational frequencies of the ring  $\nu$ (N-H),  $\nu$ (C=C) (aromatic) and  $\nu$ (C-N) remains almost unaffected, indicative of non participation of these groups in coordination[4]. The presence of water of crystallization in the complexes is revealed by a broad band at between 3465, 3488 and 3499 cm<sup>-1</sup>[2-4] and the results of elemental analysis also supported the presence of water of crystallization.

**Table 2. Relevant IR data for the ligand and complexes**

Compounds	$\nu$ C=C(aromatic)	$\nu$ C=O	$\nu$ C=O	$\nu$ NH	$\nu$ NH	$\nu$ OH
	cm <sup>-1</sup>	cm <sup>-1</sup>	$\Delta$ cm <sup>-1</sup>	cm <sup>-1</sup>	cm <sup>-1</sup>	cm <sup>-1</sup>
[BAH]	1450	1580		3177		
[Co(BAH) <sub>2</sub> ].SO <sub>4</sub> .2H <sub>2</sub> O	1530	1530	-50	3145	-32	3465
[Ni(BAH) <sub>2</sub> ].SO <sub>4</sub> .H <sub>2</sub> O	1520	1525	-55	3155	-22	3488
[Cu(BAH) <sub>2</sub> ].SO <sub>4</sub> .H <sub>2</sub> O	1542	1535	-45	3162	-15	3499

## Antimicrobial activity

A result of the antimicrobial activity of the complexes and ligand against the growth of micro-organism is summarized in Table 3 while

the graphical plot is shown Fig 3 – Fig 6. From the result, inhibition is found to increase with increase in concentration of metal complexes Fig 3 – Fig 6. The result also indicates that metal

complexes have a high activity against each class of organism Fig. 4 – Fig. 6[8]. The activity is related to the nature and structure of the complex. However, all the complexes show higher activity against the human pathogens than the plant pathogens compared to the ligand Fig 3 – Fig 6. Antibacterial activity increase in the following ordered as [BAH] < [Ni(BAH)<sub>2</sub>]SO<sub>4</sub>.H<sub>2</sub>O < [Cu(BAH)<sub>2</sub>]SO<sub>4</sub>.H<sub>2</sub>O < [Co(BAH)<sub>2</sub>]SO<sub>4</sub>.2H<sub>2</sub>O. It is also observed that the complexes showed higher activity on plant pathogens than human pathogens except on *e.coli* Fig 4 – Fig. 6. This could be as a result of Tweedy chelation theory. These complexes do not show strong concentration dependence of antibacterial activity, as compared to antifungal activities of the same complexes. The relation between chelation and toxicity is very complex and expected to be a function of steric, electronic and pharma-kinetic factor along with mechanistic pathway (Silvaea and Costa, 2014). A comparative study of the ligand and its complexes indicates that metal complexes

exhibits higher antimicrobial activity than the free ligand Fig. 3 – Fig. 6. Such increased activity of the complexes can be explained on the basis of the overtone concept and Tweedy chelation theory (Silvaea and Costa, 2012). According to the overtone concept of cell permeability, the lipid membrane surrounding the cell favours the passage of only lipid soluble materials; due to which liposolubility is an important factor controlling the microbial activity. On chelation, the polarity of the metal ion will be reduced to a greater extent due to the overlap of the ligand orbital and partial sharing of the positive charge of the metal ion with donor groups. Furthermore, the mode of action of the compound may involve formation of a hydrogen bond through the azomethine group with the active centre of the cell constituents resulting in interference with normal cell processes (Nwabueze and Salawu, 2012).

**Table 3: Antibacterial and Antifungal results of the compounds**

S/No.	Diameter of zones showing complete inhibition(mm)					
	Compounds	Concentration (µg/ml)	<i>E.coli</i>	<i>R.s</i>	<i>X.c</i>	<i>E.c</i>
1.	[BAH]	50	05	06	05	02
		100	07	08	08	04
		200	22	12	12	10
2.	[Co(BAH) <sub>2</sub> ]SO <sub>4</sub> .2H <sub>2</sub> O	50	03	02	06	05
		100	08	05	10	08
		200	64	18	20	43
3.	[Ni(BAH) <sub>2</sub> ]SO <sub>4</sub> .H <sub>2</sub> O	50	05	10	06	08
		100	12	12	08	12
		200	40	20	35	32
4.	[Cu(BAH) <sub>2</sub> ]SO <sub>4</sub> .H <sub>2</sub> O	50	05	03	10	08
		100	12	08	12	10
		200	50	25	38	44
6.	<b>Streptomycin</b>	200	32	16	15	30

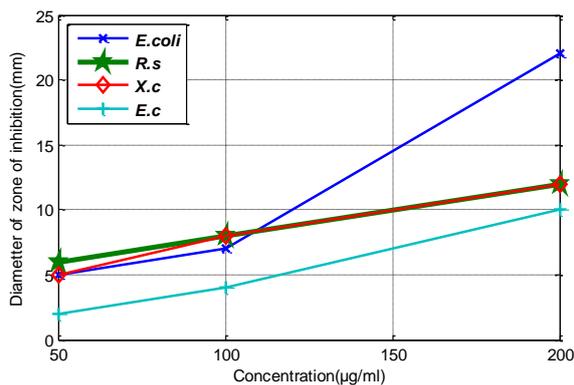


Fig. 3. Graphical representation of activity of the ligand [BAH] with pathogens

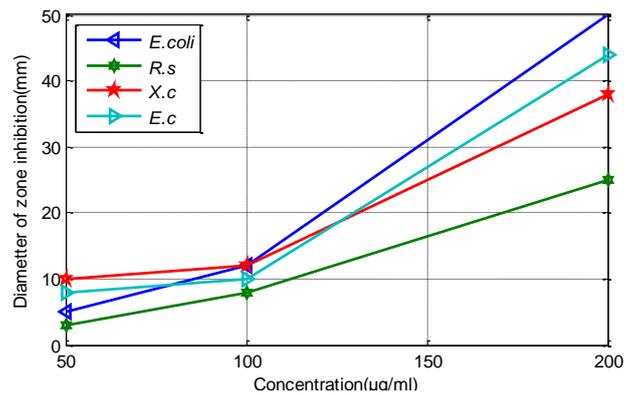


Fig. 6. Graphical representation of activity of the [Cu(BAH)<sub>2</sub>]SO<sub>4</sub>.H<sub>2</sub>O with pathogens

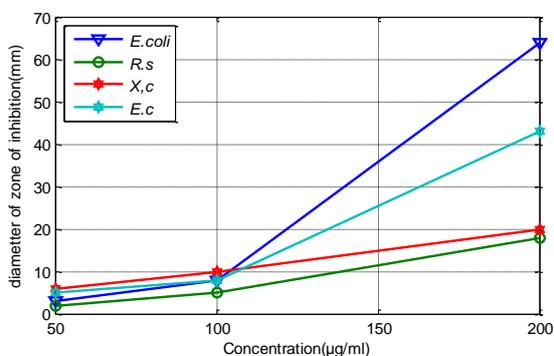


Fig. 4. Graphical representation of activity of the [Co(BAH)<sub>2</sub>]SO<sub>4</sub>.2H<sub>2</sub>O with pathogens

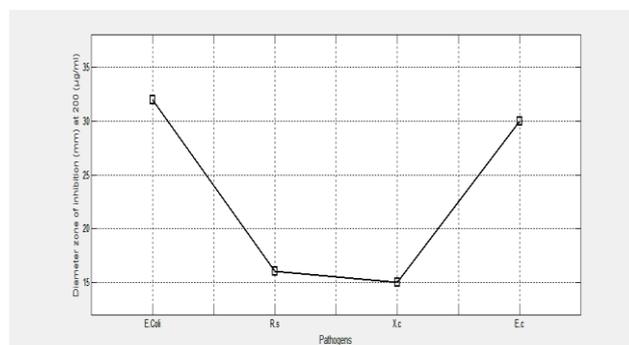


Fig. 7. Graphical representation of activity of the Standard with pathogens

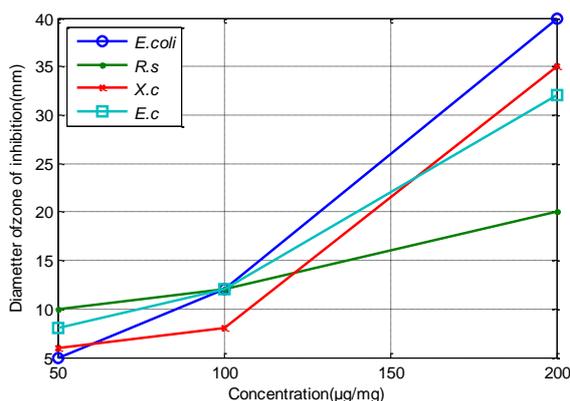


Fig. 5. Graphical representation of activity of the [Ni(BAH)<sub>2</sub>]SO<sub>4</sub>.H<sub>2</sub>O with pathogens

**Conclusion**

Co (II), Ni (II) and Cu (II) benzoyl acid hydrazide are newly synthesized complexes. The complexes were all obtained as coloured powdery substances soluble in ethanol and dimethylsulphoxide, but sparingly soluble in methanol and other organic solvents. These complexes are more or equally active with that of standard antibiotic used for comparison. Though, the ligand was found moderately active but the complexes showed high significant activity against all bacteria. Considering the significant activity of this complexes, further biological testing like cytotoxicity studies of the ligand and the complexes is under study.

**Acknowledgement**

Author is grateful to management of Federal medical Centre (FMC) Lokoja, Kogi State for the support during the research work.

## REFERENCES

- Abdelkarim, A. T., Gailo, M. N., Suid, S., and Abdal, H. (2015). Spectroscopic characterization of novel Cu(II) mixed-ligand complexes involving tridentate hydrazide ligand and some amino acids as antibacterial and antioxidant agents. *International Journal of Pharma Sciences*, 5(1): 839-851.
- Abram, M. J., Murrer, B. A. (1993). Metal compounds in therapy and diagnosis *Science*, 261, 725-730.
- Amin.K., and Cryer.K.K., (2016), Preparation and physical properties of hydrazide – hydrazone derivatives, *Inorganic Chemistry*. 19, 40.
- Bergamaschi, G., Bonardi, A., Leporati, E., and Mazza, P. (1997). Organotin complexes of hydrazide: Synthesis, spectroscopic properties, antimicrobial activity, and genotoxicity. *Journal of Inorganic Biochemistry*, 68(4) 295-305.
- Bottari, B., Maccari, E., Monfor, F., and Rottondoe, E., (2000). Isoniazid-related Copper(II) and Nickel(II) complexes with antimycobacterial in vitro activity. *Bioorganic and Medicinal Chemistry Letters*, 10(7): 657-660.
- Chohan, S. A., Afor, M., and Akhora, J.T. (2006). Metal-based antibacterial and antifungal agents: Synthesis, characterization and in vitro evaluation of Co (II), Cu (II), Ni (II) and Zn (II) complexes with amino acid-derived compounds. *Bioinorganic Chemistry and Applications*, 83, 131-141.
- Chohon, Z. H. (1997). Antibacterial and antifungal ferrocene incorporated dithione and dithioketone compounds. *Applied organometallic Chemistry*, 20(2), 112-116.
- Dianu, M. L., Angela, K., Dragahi, A., Stanvae, S. and Popoiu, S. B. (2009). Synthesis and characterization of some transition metals complexes with glyoxal bis-isonicotinoyl hydrazone. *Revista De Chime Bucharest*, 60(6), 555-560
- Heslop F. G. and Jones, K. L. (1976). *Inorganic Chemistry. A guide to advanced study*, Elsevier scientific publishing company, Amsterdam, 110-141.
- Kaymakcioglu.K.B., Shevts.N.; Rollas.S.; and Anatholy.D. (2015), Synthesis and characterization of novel hydrazide and the study of thier structure –antituberculosis activity. *European. Journal Medicinal Chemistry* 30, 12– 22.
- National Committee for Clinical Laboratory Standard, NCCLS: (2010), *Methods for antimicrobial dilution and disk susceptibility testing of infrequently isolated or fastidious bacteria; Approved guideline, Document M45-A*, 26(19), Willanova, PA., USA.
- Nwabueze, J. N and Salawu, O.W. (2012), complexes of Ni(II) and Cu(II) sulphates with and enol forms of Isobutryl acetic acid, 4-aminobenzoic acid and 4-cyano benzoic acid hydrazides, *Advances in Pure and Applied Chemistry (APAC)*, 1(2), 40-48.
- Nwabueze, J. N and Salawu, O.W. (2012), complexes of Zn(II) and VO(IV) sulphates with keto and enol forms of Isobutryl acetic acid, 4-aminobenzoic acid and 4-cyano benzoic acid hydrazides, *Advances in Pure and Applied Chemistry (APAC)*, 1(2), 20-30.
- Rollas, A. B. and Gulerman.M. (2014), Synthesis and antimicrobial activity of some new hydrazides of 4-fluorobenzoic acid hydrazide and 3-acetyl -2,5- disubstituted-1,3,4-oxadiazolines , *Farmaco*. 57 , 171-174
- Salawu,O.W., Kolawole, S. and Akpah,F. A. (2011), Preparation and characterization of some metal ion complexes. *Pelagia Research library*, 2 (2): 1-8.
- Salawu, O. W. and Aliyu, O.C. (2012), synthesis, spectral studies of metal(II) complexes. *Advances in Pure and Applied Chemistry (APAC)*, 1(1), 12-17.
- Seibin.J., Manning.H.R., and Cessac.G., (2012), Preparation of various complexes in *Inorganic Chemistry, Journal Inorganic Nuclear Chemistry*. 10, 135.
- Silvaea, G. A. and Costa, B. M. (2014), New class of antitumor and antifungal activity and insecticides against multidrug – resistant, *Bioorg.Med.Chem*. 9, 435-450.
- Sinn, E. H. (2012); Schiff base metal complexes as ligands. *Coordination Chemistry Reviews*. 4(4):391–422.