# Antibacterial Activities of Some Mixed Salicylic Acid- Vitamin C Metal Complexes

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## ABSTRACT

The increase of resistant bacteria was found to reduce the efficiency of antimicrobial therapies with the current antibiotics for more efficient drugs for the treatment of infections. Most studies have demonstrated an increase in antimicrobial activity following the interaction of several compounds with metal drug complexes. This study used a methodology adapted for antimicrobial bioassays for plant extracts, in compliance with the standards of the Clinical and Laboratory Standards Institute against Gram-positive and Gram-negative bacteria. Mixed metal drug complexes of Salicylic acid – Vitamin C were prepared and characterised by physico-chemical techniques such as melting point determination, Conductivity measurements and Infrared spectroscopy. The new alternative complexes possessed antimicrobial activity against some isolated organisms: *Eurobacter aerogenes, Escherichia coli, Staphylococcus aureus, Serrate marcereus, Bacillus megaterium, Bacillus subtilis, Pseudomonas aeruginosa* and *Proteus vulgaris*. The highest efficacy was shown by $CuSO_4$ complex against *Proteus vulgaris* (25 mm) while the least inhibition was shown by Salicyclic acid against *Pseudomonas Aeroginosa* (11 mm). Some of them showed significant activity compared to their parent ligands i.e. $NiCl_2$ against *E. coli*, and *Bacillus subtilis* (20 mm and 24 mm); $CdSO_4$ against *Serrate marcereus, Bacillus megaterium, Bacillus subtilis and Pseudomonas aeruginosa* (24 mm, 20 mm, 21 mm and 22 mm); $CuSO_4$ against *E. coli, Serrate marcereus,* and *Bacillus megaterium* (23 mm, and 24 mm); and $CoSO_4$ against *E. coli, Staphylococcus aureus and Proteus vulgaris* (20 mm).

**Keywords**: salicylic acid vitamin, spectroscopy, antimicrobial activity

## INTRODUCTION

It has been well documented that people die due to infections caused by microorganisms resistant to current antibiotics (WHO, 2012). When an antibiotic is discovered and commercially available, the appearance of resistant strains begins to reduce its clinical utility after a period of indiscriminate use, leading to future use restriction. The use of antibiotics with broad spectrum of action and low toxicity can reduce the efficacy of future antimicrobial therapies. (Willey *et al.*, 2008 and Rocha *et al.*, 2011). In the search for new antimicrobials, effective in the treatment of infections caused by multiresistant bacteria, due consideration ought to be given to the synthesis of drugs with new activation targets, as well as the potentialization of the activity of compounds with known antimicrobial activity (Schaechter et al., 2002 and Masunari and Tavares 2007). A new strategy in the production of drugs proposes the interaction of metal ions for antibiotics within three study fields: the first one aiming at creating a reversed mechanism of microbial resistance; the second one seeking to promote the development of new drugs with an action mechanism unknown to the pathogenic bacteria; and a third one aiming at reducing the toxicity of the metal ion in the form of a complex (Rocha *et al*., 2011).Therefore, the purpose of this research was to evaluate the antimicrobial activity of some metal complexes, and to compare their performance against free ligands. From previous research, metal drug complexes have played some important roles in the development of coordination chemistry (Adediji, *et al*., 2009). In the research of the new novel drug against resistant Micro-organism, the modification of existing drug by combination to a metal centre has gained attention in recent years [Siddiqui, *et al.*, 2010]. Complexation of metal cations with organic drugs is a promising strategy which has been successful in several ways with different pharmacological activities (Farrar, *et al.*, 2004). It is popularly known that the presence of metal ion in a synthesized complex increases the action of the drug and the efficacy of the organic therapeutic agent [Carballo, *et al.*, 2002]. It is evident that the metal complexes have a great function in biological activity of drugs [Teslyuk, *et al.*, 2007].

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In previous research, it is well known in many cases that metal complexes are more potent that their parent ligands [Obaleye, *et al.,* 1989; Mohammed, *et al.,* 2005). Increase in effectiveness of the complexes is due to reacting a parent drug with metal ions by analyzing them with some physico-chemical properties. (Tella, *et al.,* 2010). The pharmacological efficiencies of the synthesized complexes depend on the nature of the metal ions and the parent ligands [Paula-Atkins, *et al.,* 2009].

## MATERIALS AND METHODS

All metal salts, reagent and chemicals used were of analytical grade. They are used without further purification. Melting point of the complexes was carried out using Gallenkamp melting point apparatus. Conductivity measurement was obtained using Jenway 4510 conductivity meter.

The infrared spectra of both the complexes and the ligands were recorded on Thermo Scientific Nicolet 912A0712 iS5 FT-IR spectrophotometer at the Redeemer University, Ogun state Nigeria. Uv-vis spectra was obtained on Beckman coulter du 730-life science spectrophotometer at the Department of Chemistry, University of Ilorin, Ilorin Nigeria.

The antibacterial assay was carried out at the Department of Microbiology, University of Ilorin, Ilorin Nigeria. The species used were: *Eurobacter aerogenes, Escherichia coli, Staphylococcus aureus, Serratemarcereus, Bacillus megaterrium, bacillus subtilis, pseudomonas aeruginosa and proteus vulgaris.*

### Methodology

### Synthesis of metal complexes

The synthetic method described by (Tella, *et al.,*2010) was used in the synthesis of the complexes. Two ligands namely Salicyclic acid (1mmol) and Vitamin C (1 mmol) were dissolved in 20 ml of distilled water in separate 250mL beakers and later poured into a single 250mL beaker. A solution of the ($NiCl_2$) (1 mmol) previously dissolved in 20 ml of distilled water was then added to the mixture. The final mixture of each ligands and ($NiCl_2$) was reflux for 2 hours at a determined temperature. It was kept for two weeks. Precipitate products were filtered and washed with methanol and dried.

### Characterization of the complexes

Melting points of the two ligands and the complexes were determined using Gallenkamp Melting point apparatus. Conductivity measurements was carried out using Jenway 4510 Conductometer Bridge. The Infrared spectra of the ligands and the complexes were recorded in FT-IR spectrophotometer. The antibacterial assay of the ligands and their metal complexes were determined according to the procedure previously reported by Tella, *et al.,* 2010.

### Antibacterial assay

The antibacterial susceptibility of all the complexes were determined against the isolated organism. About 7 g of nutrient agar was weighed into 250 ml of sterilized water and mixed. The mixture was heated for 15 minutes and placed in an autoclave to sterilize at 121ºC. The agar was reheated and poured into the sterilized Petri-dishes which was allowed to set. Wells were drilled into the solidified agar by the use of sterilized cork borer. Cotton wool swabs was soaked in the pure broth culture of the organisms and was used to rub the surface of the solidified agar. The prepared solution complexes and ligands at different concentrations were introduced into the wells. The plates were incubated at 37ºC for 24 hrs. Clear zones around the well indicate the antibacterial activity of the complexes on the tested organisms.

## RESULTS AND DISCUSSION

The ligands, Salicylclic acid (SA) and Vitamin C (Vit. C) melted at 158-159oC and 191-192oC respectively. All metal complexes melted in the range of 201-215oC except for Co (II) complex which showed complete decomposition, this probably confirms coordination. All the metal complexes were coloured due to d-d transitions (Obaleye, et al., 1989). The melting points, molar conductivity, percentage metal, room temperature magnetic moments and elemental micro-analysis for the mixed drug metal complexes are presented in Table 1 and 2. Based on the conductivity measurement, it was observed that the complexes are electrolytic in nature. Magnetic susceptibility measurements were carried out on all the four (4) samples at room temperature. The effective magnetic moments, 7.12 B.M. for [Ni(SA)(Vit.C.) $SO_4$]; 5.60B.M. for [Cd(SA)(Vit.C.)$Cl_2$]; 3.19 B.M. for [Cu(SA)(Vit.C.)$SO_4$] and 3.78 B.M. for [Co(SA)(Vit. C.) $Cl_2$] are consistent with d7, d8, d9 and d10octahedral metal (II) complexes, respectively.

Table 1: Analytical data of the complexes

| Ligands/Complexes | Melting Point (°C) | C | H | O | S | Conductivity $\Omega^{-1}cm^{-1}$ $mol^{-1}$ |
|---|---|---|---|---|---|---|
| | | Calc/ Found | Calc/ Found | Calc/ Found | Calc/ Found | |
| Salicyclic Acid | 158-159 | - | - | - | - | - |
| Vitamin C | 191-192 | - | - | - | - | - |
| [Ni(SA)(Vit.C.)$SO_4$] | 201-202 | 33.33/ 33.96 | 2.99/ 2.24 | 44.44/ 44.39 | 6.84/6.00 | 20.49 |
| [Cd(SA)(Vit.C.)$Cl_2$] | 221-223 | 29.89/ 29.13 | 2.68/ 2.27 | 39.85/ 39.46 | 6.13/6.12 | 25.42 |
| [Cu(SA)(Vit.C.)$SO_4$] | 214-215 | 32.91/ 32.17 | 2.95/ 2.26 | 59.07/ 59.69 | 6.75/6.29 | 27.56 |
| [Co(SA)(Vit. C.)$Cl_2$] | Decompose | 33.26/ 33.27 | 2.99/ 2.04 | 44.35/ 44.56 | 6.82/6.31 | 22.39 |

Table 2: Physical properties of the complexes

| Ligands/Complexes | Molecular weight ( Calc/Found) | Metal content (Calc/Found) | Magnetic moment (BM) |
|---|---|---|---|
| Salicyclic Acid | 138 | - | - |
| Vitamin C | 176 | - | - |
| [Ni(SA)(Vit.C.)$SO_4$] | 468 | 12.39/12.23 | 7.12 |
| [Cd(SA)(Vit.C.)$Cl_2$] | 522 | 21.46/21.48 | 5.40 |
| [Cu(SA)(Vit.C.)$SO_4$] | 474 | 13.50/13.69 | 3.19 |
| [Co(SA)(Vit. C.)$Cl_2$] | 469 | 12.58/12.21 | 3.78 |

Table 3: Infrared spectra of the complexes

| Ligands/Complexes | √(O-H) | √(C-O) | √(C=O) | √(M-L) |
|---|---|---|---|---|
| Salicyclic Acid | 3214 | 1259 | 1621 | - |
| Vitamin C | 3300 | 1245 | 1641 | - |
| [Ni(SA)(Vit.C.)$SO_4$] | 3517 | 1246 | 1678 | 519 |
| [Cd(SA)(Vit.C.)$Cl_2$] | 3398 | 1254 | 1743 | 523 |
| [Cu(SA)(Vit.C.)$SO_4$] | 3374 | 1258 | 1696 | 538 |
| [Co(SA)(Vit. C.)$Cl_2$] | 3467 | 1241 | 1652 | 556 |

Table 3 shows the FT-IR spectrum of salicylic acid and Vit. C. and their metal complexes. The the FT-IR spectrum of salicylic acid and Vit. C. showed √C=O asymmetric stretching vibration from 1621 to 1641 cm-1 and 1652 to 1662 cm-1. These bands shifted to 1678 cm-1, 1743 cm-1, 1696 cm-1 and 1652 cm-1 in the metal complexes respectively, confirming coordination through the carbonyl oxygen atoms of the salicylic acid and Vit. C (Tella et al, 2010). The broad and medium bands at 3214 cm-1 and 3300 cm-1 in salicylic acid and Vit. C. were assigned as √OH band respectively. These bands were shifted in the metal complexes to 3317 cm-1, 3398 cm-1, 3374 cm-1, 3467 cm-1 respectively, due to hydrogen bonding.

Table 4: Antibacterial Activities of Some Mixed Salicyclic Acid- Vitamin C Metal Complexes.

| ***Ligand/ complexes*** | ***E. coli*** | ***S. aureus*** | ***S. marcereus*** | ***E. Aerogenes*** | ***B. megaterium*** | ***B. substillis*** | ***P. Aeroginosa*** | ***P. vulgaris*** |
|---|---|---|---|---|---|---|---|---|
| | Zone of Inhibition (mm) Concentration = 20 ppm | | | | | | | |
| Salicyclic Acid | R | R | 12 | 14 | 14 | R | 11 | 15 |
| Vitamin C | 15 | 12 | R | 13 | R | 15 | 10 | 17 |
| [Ni(SA)(Vit.C.)$SO_4$] | 20 | 16 | R | 15 | 19 | 24 | 16 | 18 |
| [Cd(SA)(Vit.C.)$Cl_2$] | R | R | 22 | R | 20 | 21 | 22 | 20 |
| [Cu(SA)(Vit.C.)$SO_4$] | 23 | 18 | 25 | 21 | 24 | 17 | 18 | 25 |
| [Co(SA)(Vit. C.)$Cl_2$] | 20 | 20 | R | 19 | 15 | 18 | 17 | 20 |

The reactions of Salicyclic acid (SA) and Vitamin C (Vit.C) with metal salts (M = Ni, Cd, Co, and Cu) are in accordance with equations 1 and 2.

$$MSO_4.xH_2O + SA + Vit.C \rightarrow [M(SA)(Vit.C)SO_4] + xH_2O \quad (1)$$
(M = Ni, Cd, Co, and Cu; x = 4 respectively)

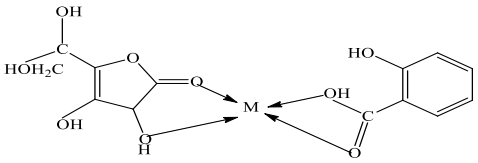
$$MCl_2{\cdot}xH_2O\ SA + Vit.C \rightarrow [M(SA)(Vit.C)Cl_2] + xH_2O \quad (2)$$
(M = Co and Ni; x = 6 )

The observance of √M-O in the metal complexes at 519 $cm^{-1}$, 523 $cm^{-1}$, 538 $cm^{-1}$ and 556 $cm^{-1}$, further confirmed coordination sites. However, √(M-Cl) was not observed because it was outside the range of the equipment.

Table 4 shows antibacterial activities of some mixed salicyclic acid- Vitamin C metal complexes. The antibacterial activities of the $NiSO_4$, $CdCl_2$, $CuSO_4$ and $CoCl_2$ complexes were proven to possess higher antibacterial activities than their parent ligands. Based on the data obtained, it was observed that the complexes have increased activity of metal ions upon coordination to these ligands [Mukamel, 2000]. The antibacterial activities of the ligands and their complexes are presented in the Table 4.0. The complexes prepared were screened and tested against some isolated organisms at the same concentration. The results obtained shows that the complexes possess higher activity than their parent ligands when compared. $CoCl_2$ complex has no effect in *S. marcereus*.

Salicylic acid inhibited the growth of *Serrate marcereus, Eurobacter aerogenes, Bacillus megaterium, Pseudomonas aeruginosa,* and *Proteus vulgaris.* Vitamin C showed good efficacy against all the tested organisms except for *Serrate marcereus* and *Bacillus megaterium*. $NiCl_2$, $CuSO_4$ and $CoCl_2$ complexes showed good antibacterial activity against all the tested organisms except for some organisms which are *Serrat emarcereus, Bacillus megaterium* and *Serrate marcereus* respectively. Based on the tested complexes, Salicyclic acid and $CdCl_2$ complex were not effective against *Escherichia coli* and *Staphylococcus aureus.* The highest effect was shown by $CuSO_4$ complex against *Proteus vulgaris* (25 mm) while the least inhibition was shown by Salicyclic acid against *Pseudomonas aeroginosa* (11 mm) (Aiyelabola *et al.,* 2012).

**Proposed Structure**




M = Ni, Cd, Cu, and Co

**CONCLUSION**

Measurement of inhibition zones of ligands and complexes indicated that the complexes possess enhanced antibacterial activity than the ligands. The complexes could be used as chemotherapy for

the treatment of disease caused by the organisms. It can be concluded thatmetal complexes show a wide range of antimicrobial activities.

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