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Analysis of Biological Activities of Two Novel Metal (II) Complexes of *Andrographis Paniculata* Crude Extract

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Abstract

Many diseases in nature have led to the death of many young and old. Many bacteria have developed resistance to the available antibiotics on the market. And many drugs employed in treating many diseases such as diabetes mellitus are expensive and are not locally available. Therefore, in order to search for more effective, inexpensive, and locally available drugs, this study synthesized and investigated the biological activities of *Andrographis paniculata* crude extract and its Co (II) and Ni (II) complexes. The crude extract and synthesized complexes were characterized using a solubility test, Infrared, and Ultraviolet-Visible spectroscopic analysis. Their antibacterial potentials were investigated against two gram-negative bacteria (*Escherichia coli*,Staphylococcus *aureus*) and three gram-negative bacteria (*Proteus*, *Klebsiella*, *Pseudomonas*) while their antidiabetic activities were examined against α-amylase and α-glucosidase enzymes. Acarbose was employed as a standard drug. The crude extract and its metal complexes showed different degrees of solubility in the employed solvents. Infrared analysis suggested coordination of the crude extract to the metal ions through the oxygen donor atom while the formation of the complexes was affirmed through the occurrence of d-d transitions in the visible region of the metal complexes. The metal complexes were found to display more antibacterial activity than the crude extract. Co (II) and Ni (II) complexes of the crude extract were found to exhibit better activities against α-amylase and α-glucosidase enzymes, respectively than the crude extract and acarbose. It is concluded that the metal complexes could be considered potential antibacterial and antidiabetic agents.

Keywords: Anti-diabetes; Anti-bacteria; Medicinal plant; Metal complexes.

INTRODUCTION

High mortality in nature has been attributed to different diseases caused by bacteria, fungi, and viruses. Diabetes mellitus described by high blood glucose levels due to insulin deficiency and/or ineffective insulin action is also one of the major deadly diseases that require serious attention. Many drugs have been synthesized to combat the diseases. However, many pathogenic organisms have been reported to develop resistance to many of the available drugs while many of the drugs are not locally available. Many drugs such as metformin, sitagliptin glimepiride, and acarbose have been widely applied in treating diabetes mellitus. However, these drugs are discovered to possess negative side effects on the patient's health while some of them are expensive. Therefore, a search for more effective and inexpensive antibiotics and antidiabetic drugs without side effects becomes imperative. Medicinal plants have become a promising source of natural effective antimicrobial agents. These plants have been observed to possess many bioactive compounds capable of fighting different types of disease.

Medicinal plants have been widely applied for medical treatment as a result of their ability to exhibit known pharmacological actions for humans and animals. Medicinal plants have been the basis of the basic treatment of diseases in many developing countries like Africa. *Andrographis paniculata* has been reported to be of great importance in the management of disease and infection. *Andrographis paniculata* (Figure 1) also known as 'King of Bitters" in Yoruba is known as 'Mejemeje' is one of the medicinal plants used in treating many diseases such as cancer,ulcer, malaria, and urinary tract infections (Karmegam et al. 2015; Sachin and Kailasam, 2016). The plant has been reported to contain many active organic compounds such as kaempferol, andrographolide, 14-deoxy andrographolide 14- deoxy-11,12-didehydroandrographolide, quercetin, and other secondary metabolites (Subramanian et al. 2008). Andrographolide (Figure 2) is found to be the main active component of the plant and its medical importance in treating different ailments has been examined by many researchers (Flores et al., 2014; Daneman & Prat, 2015; Tao et al. 2018; Owoade et al. 2021)

The antioxidant and anti-diabetic activity of *Andrographis paniculate* investigated by Reddy et al. (2022). The plant was found to possess good antioxidant and anti-diabetic activity. Hartini et al. (2021) studied the inhibitory activity of aqueous extract and ethanolic extract of sambiloto *Andrographis paniculata* against the α-amylase enzyme. The ethanol extract of the plant leaf demonstrated higher activity than that of the aqueous extract. The α-amylase enzyme inhibitory activity of *Andrographis paniculata* in aqueous methanol, crude methanol extract, and n-hexane fraction was investigated by Ajayi et al. (2021). The plant extract demonstrated more activity than the standard drug employed for the study. The crude methanol extract was observed to demonstrate the highest activity. The contribution of *Andrographis paniculata* in the treatment of metastatic esophageal cancer was investigated by Lin et al. (2017). The anti-migratory and suppressive effects on metastasis-related factors of the absorbed *Andrographis paniculate* were verified. The diterpenes and flavonoid components of the plant component were observed to display esophageal anticancer activity.

Advancement has been made in the field of medicinal inorganic chemistry in developing different novel organic therapeutic agents as pharmacological and pharmacy technical behaviors of many organic therapeutic agents are observed to increase upon coordination with transition metal ions (Farrer and Sadler, 2013; Newman and Cragg, 2016). Therefore, there is an apparent need for the study of new metal complex of organic compounds endowed with antimicrobial activities which could be applied in combating multi drug-resistant microorganisms and other diseases. *Andrographis paniculata* has been reported to be of great importance in the management of diseases. However, there is no report on biological activity of metal complex of the plant. Therefore, this study synthesized metal (II) complexes of *Andrographis paniculata* crude extract and evaluated biological activity of the crude extract and its metal complexes against some bacteria and enzymes.

Figure 1. Image of *Andrograhis paniculata* plant.

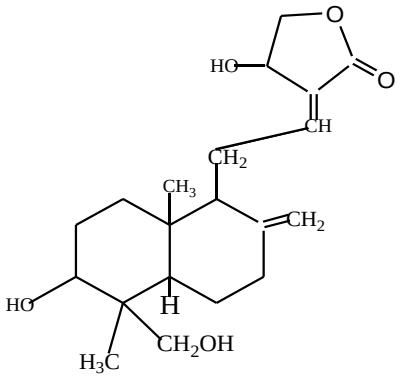

Figure 2. Structure of andrographolide.

MATERIALS AND METHODS

All chemicals used were of analytical grade and they include cobalt acetate, nickel acetate, sodium hydroxide, petroleum ether, hexane, ethanol, methanol, acetone, chloroform, tetra-chloro-methane, distilled water, positive gram strains (*Escherichia Coli, Staphylococcus aureus*) and negative gram strains (*Pseudomonas, Klebsiella, and Proteus*).

Collection and Preparation of the Plant Crude Extract

The mature leaves of *Andrograhis Paniculata were* collected from Oyo State College of Agriculture, Igboora, Oyo State. The plant was identified by a botanist in the Department of Botany the University of Ibadan, Ibadan with the voucher number UIH-23122.

The collected leaves were rinsed twice under running water and then put in distilled water to get rid of dirt particles. The plant was air-dried at room temperature. It was then crushed into small particles and then ground into powder. The crude extract of the dried leaves was obtained in n-hexane at 60-80^{o}C where oils, fats, waxes, and terpenes were removed. The extract was then subjected to soxhlet extraction with ethanol (95%) and the solution was concentrated using a rotary evaporator (Mousumi et al. 2014).

Preparation of Metal Complexes

Solutions of 10 g of the crude extract and 5 g of cobalt salt in ethanol were mixed together and refluxed for about 4 hr. The mixture was then heated at 80 ^{o}C until a precipitate was formed. The precipitate was filtered and dehydrated under a vacuum. The same procedure was repeated for the nickel complex (Mousumi et al. 2014).

Characterization of the Metal Complexes

The metal complexes were characterized by solubility test solvents in water, ethanol, methanol, chloroform, acetone, and diethyl ether. FTIR and UV spectroscopic analysis was carried out using a UV-Visible spectrophotometer (CE 2021, CECIL) and an FTIR

spectrophotometer (530M, BUCK) within the range of 600-4000cm-1.

Antibacterial Assay

Whatman No 1 filter paper was used to prepare a 6 mm diameter disc. The 6 mm diameter discs were sterilized inside an autoclave at 121ºC. The moisture discs were dried in a hot air oven at 50 ºC. The crude extract and its metal complexes disc and control were prepared. The tests were carried out using the original technique of Bauer *et al* (1996). Muller-Hinton agar was prepared and autoclaved at 15 bs pressure for 20 mins and cooled. The media was then poured into the sterilized petri dishes and allowed to solidify. The petri plate with the poured media was then seeded, after which the microbial was suspended with the aid of a sterile swab. The plant crude extract and its metal complexes were then placed on each of the plates as well as the control. The plates were then incubated at 37 ºC for 24 hr. Thereafter, the inhibition zone was measured and expressed in mm.

Effect of extracts on α- amylase activity

The extracts (100 µL) and 500 µL of 20 mM sodium phosphate buffer (pH 6.9 with 6 mM NaCl) containing pancreatic α-amylase (EC 3.2.1.1) (0.5 mg/ml) were incubated at 25 °C for 10 min. Then, 500 µL of 1% starch solution in the same phosphate buffer was added and incubated for another 10 min. 1.0 ml of di-nitrosalicylic acid (DNSA) was added, boiled for 5 min, and cooled to room temperature. The reaction mixture was then diluted by adding distilled water (10 ml), and the absorbance of each sample was measured at 540 nm. A complete reaction mixture without Acarbose or extract was used as the control. The α-amylase inhibitory activity is expressed as percentage inhibition and calculated using the formula;

$$\%\ inhibition\ = \frac{Ac - As}{Ac} X100$$

Ac : Absorbance of control (containing all reagents except extracts or Acarbose)

As : Absorbance of the sample (extract or Acarbose).

Four diluted solutions of the crude extract and its metal complexes (40 - 100 mg/l) were collected for the calculation of the IC_{50} values. The concentration of the extract required to inhibit the activity of the enzyme by 50% (IC_{50}) was evaluated by IC_{50} AAT calculator (www.aatbio.com).

Effects of extract on α – glucosidase activity i*n-vitro*

The effect of the extract on α – glucosidase activity was determined according to the procedure described by Apostolid *et al.* (2007), using acarbose as a reference. The crude extract and its metal complexes (50 µL) and 100 µL of α-glucosidase solution were incubated at 25°C for 10 min. Thereafter, 50 µL of 5 M p-nitrophenyl-α-D-glucopyranoside solutions in 0.1 M phosphate buffer (pH 6.9) was added and incubated at 25 °C for 5 min. The absorbance was then read at 405 nm. The α-glucosidase inhibitory activity is expressed as percentage inhibition and calculated as shown below:

$$\%\ inhibition\ = \frac{Ac - As}{Ac} X100$$

A_c : Absorbance of control (containing all reagents except extracts or Acarbose)

A_s : Absorbance of the extract or Acarbose

At least four serially diluted solutions of the crude extract and its metal complexes (40 - 100 mg/l) were taken for calculation of the IC_{50} values. The concentration of the extract required to inhibit the activity of the enzyme by 50% (IC_{50}) was calculated using the AAT calculator (www.aatbio.com).

RESULT AND DISCUSSION

The physical properties of *Andrograhis Paniculata* crude extract and its metal (II) complex are shown in Table 1 while Tables 2 and 3 depict important IR and Uv-visible bands of the crude extract and its metal (II) complexes. Antibacterial activities of the plant crude extract and its metal complexes were tested against two-gram-positive and three gram-negative bacteria. The zone of inhibitions of the plant crude extract and its metal complexes against the bacteria in mm are measured as shown in Figure 3. Figures 4 and 5 depict the compound's antibacterial activities against the bacteria.

Table 1. Physical Characteristics of the Plant Crude extract and its Meeta Complexes.

Compound	Color
Crude Extract	Green
Nickel (ll)Complexes	Green
Cobalt (ll) complexes	Pink

Table 2. Solubility Property of the Pant Crude Extract and its Metal Complexes.

Solvents	Crude Extract	Cobalt (II) Complex	Nickel (II) Complex
Water	IN	IN	IN
Methanol	VS	VS	SS
Ethanol	VS	VS	VS
Chloroform	SS	SS	SS
Acetone	VS	VS	VS
Diethyl ether	SS	SS	SS

Table 3. Important Infra-red data of the Plant Crude Extract and its Metal (II) Complexes.

Compound	υC-H	υC-O	υC=O	υ-OH
Plant crude extract	2935 s	1157 s	1717 s	3406 b
Co (II) complex	2935 s	1144 s	1702 s	3447
Ni (II) complex	2929 s	1143 s	1698 s	3440 b

Table 4. Electronic data of the Plant Crude Extract and its Metal Complexes.

Compound	Band (cm^{-1})	Transition
Plant crude extract	28169	$n \rightarrow \pi^*$
	42553	$\pi \rightarrow \pi^*$
Co (II) complex	17241	d-d
	14925	
Ni (II) complex	19685	d-d
	15408	

Figure 3. Measurement of the inhibitory zone of the plant crude extract and its metal complexes.

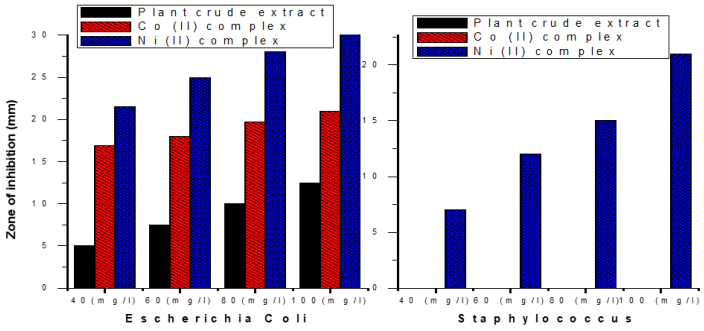

Figure 4. Histogram representation of antimicrobial activities of the crude extract and its metal complexes against gram-positive bacteria.

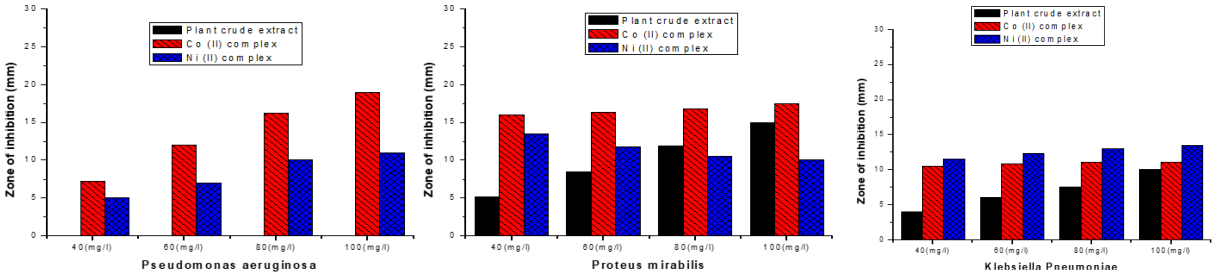

Figure 5. Histogram representation of antimicrobial activities of the crude extract and its metal complexes against gram-negative bacteria.

Table 5. α-Amylase and α-glucosidase inhibitory Activities of the Plant Crude Extract and its Metal Complexes.

Compound	IC_{50} (mg/l)	
	α –amylase	α –glucosidase
Acarbose	55.49	102.66
Plant crude extract	85.65	77.98
Co (II) complex	80.52	74.96
Ni (II) complex	42.03	100.79

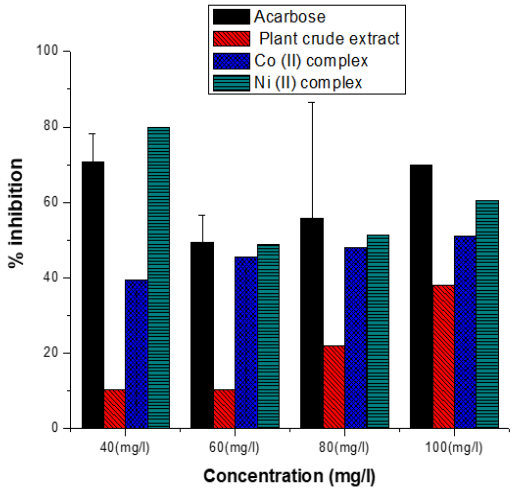

Figure 6. Percentage inhibitory effect of *Andrograhis Paniculata* crude extract, its metal complexes and standard drug on α-amylase.

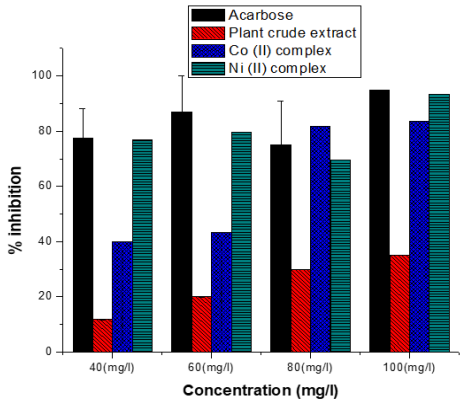

Figure 7. Percentage inhibitory effect of *Andrograhis Paniculata* crude extract, its metal complexes and standard drug on α-glucosidase.

Discussion

The various colors of the plant crude extract and its metal complexes are shown in Table 1. The plant crude extract and its Ni (II) complex are green in color while the Co (II) of the extract shows is dark pink in color. The plant crude extract and its metal complexes are all very soluble in ethanol, methanol, and acetone, insoluble in distilled water, and slightly soluble in chloroform and diethyl ether as shown in Table 2.

Infrared and Uv-Visible spectra

The relevant infrared spectra data for the crude extract (ligand) and its metal complexes are shown in Table 3. The spectrum of the crude extract shows four important bands at 2935 cm^{-1}, 1157 cm^{-1}, 1717 cm^{-1}, and 3406 cm^{-1} which are ascribed to $\nu_{(C-H)}$, $\nu_{(C-O)}$, $\nu_{(C=O)}$ and $\nu_{(C-OH)}$ stretching vibrations, respectively. The bands of $\nu_{(C-O)}$ and $\nu_{(C=O)}$ observed in the crude extract are observed, upon coordination with Co (II) and Ni (II), to undergo a hypsochromic shift to 1144 cm^{-1}, 1702 cm^{-1}, and 1143 cm^{-1}, 1698 cm^{-1}, respectively. The band shifts and appearance of the new band in the region 400-800 cm^{-1} in the complexes confirmed the coordination of the ligands to the metal ions through M-O **(Talavara et al. 2016;** Teleb et al. 2019) as shown in Figure 8.

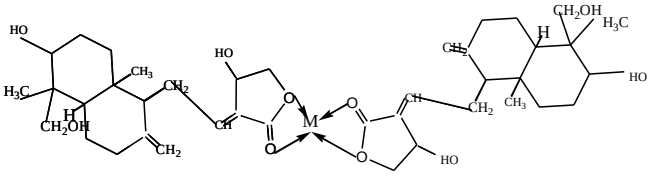

Figure 8. Proposed structure for M (II) complex of *Mangifera indica* leaf crude extract (M= Co (II) and Ni (II))

Bands at the visible region of 2817 cm^{-1} and 4255 cm^{-1} are in the spectrum of the crude extract and the bands are attributed to n– π* and π – π* electronic transitions, respectively as shown in Table 4. In the spectra of Ni(II) and Co(II) complexes, bands at 1724 cm^{-1}, 1494 cm^{-1} and 1969 cm^{-1}, and 1541 cm^{-1} are observed and are ascribed to d-d electronic transition (Table 4) (Rasool et al. 2014).

Antibacterial activities

Antibacterial activities of the plant crude extract and its metal complexes in ethanol were tested against two gram-negative and three positive bacteria at concentrations of 40 mg/l, 60 mg/l, 80 mg/l, and 1000 mg/l, and the zones of inhibition of the complexes against the bacteria are measured over the disc plates as shown in Figure 3. The antibacterial activities of the extract and the complexes at different concentrations as measured are represented in histograms (Figures 4 and 5).

The solvent employed showed no antibacterial activity at the concentrations. Ni (II) complex was found to show more activity against *Escherichia coli* at the concentrations of Co (II) while the complexes were more active than the plant crude extract. Also, only Ni (II) complex was observed to be effective against *Staphylococcus.* The metal complexes exhibited more activity against Pseudomonas, Proteus, and Klebsiella than the extract. Co (II) complex exhibited the highest activity against the *Pseudomonas* and *Proteus* while the plant crude extract was inactive against *Pseudomonas* at all the concentrations. However, Ni (II) complex was observed to display the most activity against K*lebsiella* as shown in Figure 5. The antibacterial activities against both gram-negative and positive bacteria increased with an increase in concentration.

Antidiabetic activities

The percentage inhibitory effect of the standard drug (acarbose), Andrograhis Paniculata crude extracts against α-amylase and α-glucosidase enzymes at different concentrations (40, 60, 80, and 100 mg/l) were represented in Figures 6 and 7, respectively. At a

concentration of 40 mg/l, Ni (II) complex showed more inhibitory effect against α-amylase even than the standard drug while the same activity was exhibited by Co (II) complex and acarbose at a concentration of 60 mg/l. However, the most activity was displayed by acarbose at 80 and 100 mg/l concentrations as shown in Figure 6. It is shown in Figure 7 that at a concentration of 40 mg/l, Ni (II) and acarbose displayed almost the same activity against α-glucosidase while acarbose exhibited the highest activity at a concentration of 60 mg/l. Co (II) complex and acarbose showed the highest activity at concentrations of 80 and 100 mg/l, respectively.

α-Amylase and α-glucosidase inhibitory activities of the standard drug (acarbose), Andrograhis Paniculata crude extract and its metal complexes were calculated and reported as half-maximal inhibitory concentration (IC50) as shown in Table 5. Ni (II) displayed the highest inhibitory strength against α -amylas enzyme with an IC50 value of 42.03 while the lowest inhibitory potency against the enzyme was observed to be exhibited by the plant crude extract with an IC_{50} value of 85.65. The calculated a-amylase inhibitory IC_{50} values of the crude extracts, its metal complexes, and acarbose in descending order were Ni (II) complex > acarbose > Co (II) complex > *Andrograhis Paniculata* crude extracts.

Conversely, Co (II) showed the highest inhibition against α -glucosidase with an IC_{50} value of 74.96 mg/l while the lowest activity was displayed by acarbose. The inhibitory effects against α –glucosidase enzyme was found in the order of Co (II) complex > Andrograhis Paniculata crude extracts > Co (II) complex > acarbose. Co (II) and Ni (II) complexes of the crude extract were found to exhibit better activities than the positive control, acarbose against α-amylase and α-glucosidase enzymes, respectively. The more antidiabetic activities displayed by the metal complexes than the crude extract and acarbose could be ascribed to the capacity of the metal ions to transform the bioavailability and pharmacological behavior of the Andrograhis paniculate crude extract.

CONCLUSION AND RECOMMENDATION

The potential inhibitory properties of Co (II) and Ni (II) complexes of *Andrograhis Paniculata* crude extract against some bacteria, α-amylase and α-glucosidase have been examined. The complexes showed more pronounced antibacterial and antidiabetic activities than the crude extract. The metal complexes could be considered potential antibacterial and antidiabetic agents. Further characterization analysis should be performed to ascertain the main structures of the complexes. Pharmacological and toxicology studies on the metal complexes should be conducted to establish their feasibility as antibacterial antidiabetic agents.

***Conflict of Interest*:** No conflict of interest.

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