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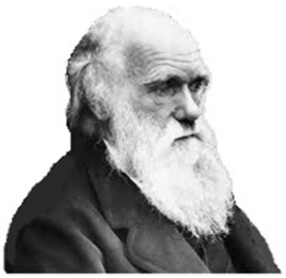

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In Vitro Evaluation of Alpha-Glucosidase and Alpha-Amylase Inhibitory Activity of Copper(II) Complex of King of Bitters Crude Extract

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ABSTRACT

Diabetes mellitus is a chronic disorder which characterized by high concentration of blood glucose. It is known as one of the major deadly diseases that require a serious attention. Many conventional drugs which have been employing in treating the disease are reported to less effective, expensive and some are not locally available. Therefore, in order to search for more effective, inexpensive and locally available drug, this study investigated antidiabetic activity of Cu(II) complex of King of bitters leaves crude extract. The crude extract of the plant was obtained in n-hexane. The metal complex of the crude extract was synthesized and characterized using solubility tests, Infrared and Ultraviolet-Visible spectroscopic analyses. The antidiabetic activities of the crude extract and its metal complex were examined against α-amylase and α-glucosidase enzymes while acarbose drug was employed as a standard drug. The metal complex showed different degrees of solubility in different solvents. Infrared analysis suggested coordination of the crude extract to the metal ion through oxygen donor atom while the formation of the complex was affirmed through the occurrence of d-d transitions in the visible region of the metal complex. The crude extract and its metal complex displayed good activities against α-amylase and α-glucosidase enzymes. It is suggested that the compounds are promising candidates to inhibit α-amylase and α-glucosidase.

***Keywords*:** Coordination chemistry, Diabetes mellitus, Medicinal plant, Metal complex

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1. INTRODUCTION

Diabetes milletus (DM) has been recognized as a deadly disease which has caused high mortality in the world. Diabetes mellitus is a metabolic disorder and is described as a chronic hyperglycemia which produces more or less damage in the metabolism of carbohydrates, lipids and proteins. It always incorporates a deficiency in either insulin secretion or response, or in both, at some point in the course of the disease. The widespread nature of DM, its particular consequences, and the existence of additional disorders that frequently coexist with DM make this illness one of the most important social and public health issues of the present [1].

Type 2 diabetes mellitus is rampant, and elevated blood glucose levels are associated with it, which could also cause major side effects such as nephropathy, neuropathy, retinopathy, and cardiovascular disease [2, 3]. 90% of all occurrences of diabetes are caused by type 2, which continues to be one of the most prevalent health problems in the world [4]. Inhibiting the digestion of dietary carbohydrates is one of the treatment methods for managing postprandial hyperglycemia in type 2. An essential digestive enzyme called pancreatic -amylase converts dietary carbohydrates like starch into simple monosaccharides. By α-glucosidases, they are further broken down into glucose, which, upon absorption, enters the bloodstream. Therefore, blocking the enzymes α -amylase and α-glucosidase can impede the digestion of carbohydrates, postpone the uptake of glucose, and subsequently diminish blood sugar levels [5]. Although α-glucosidase and α-amylase are inhibited by medications like acarbose, voglibose, and miglitol in practice, they produce adverse effects such as bloating, abdominal pain, diarrhea, and gas, which necessitate other drugs with less or no side effects [6, 7] while many are less effective, expensive, and not locally available in developing countries. Therefore, in order to overcome the inherent problems of conventional drugs, the application of medicinal plants has increasingly become the focus of research in recent years. The use of herbs as a substitute has been attributed to the presence of certain bioactive components that possess unique mechanisms of action, perceived efficacy, and limited side effects.Medicinal plants have been recognized for many years as essential materials possessing healing power. They have been applied to cure many diseases in many local communities around the world. Currently, they are considered the primary source of many synthetic drugs. The presence of essential components with therapeutic properties in many plants has led to the investigation of the biological importance of a great number of useful plants and their application in synthesizing many drugs [8, 9]. The World Health Organization's (WHO) strategy, 2014–2023, advocates the importance of enhancing the impact of traditional medicine by promoting the application of medicinal plants in the health systems of its member countries [10]. The application of medicinal plants to treat many diseases has resuscitated attention in many developed countries and signifies the basic therapeutic plan for many developing countries. Traditional herbal medicine is used in many developed and developing countries to cure health difficulties [11-13]. King of Bitters (Andrographis paniculata) has been reported to be of great importance in the management of disease and infection. The plant contains a variety of active chemical substances, including kaempferol, andrographolide, 14-deoxyandrographolide, 14-deoxy-11,12-didehydro andrographolide, quercetin, and other secondary metabolites while at andrographolide (Figure 1) was reported to be major active component of the plant [14]. Numerous researchers have carried out studies on the plant, the plant's primary active ingredient, and its potential medical value in treating a variety of disorders. [15] studied *Andrographis paniculata's* antioxidant and anti-diabetic properties. The plant was discovered to have strong anti-oxidant and anti-diabetic properties.

Inhibitory activity of sambiloto and Andrographis paniculata's aqueous and ethanolic extracts against the α-amylase enzyme was investigated by [16]. The plant leaf's ethanol extract had greater activity than the aqueous extract did. [17] examined the α-amylase enzyme inhibitory activity of *Andrographis paniculata* in aqueous methanol, crude methanol extract, and n-hexane fraction. Compared to the study's standard medicine, the plant extract showed more activity. The maximum activity was reported in the crude methanol extract.

Transition metals possess unique properties that increase the biological activity of many ligands. The properties have been taken into account when synthesizing novel compounds with anti-diabetic, anti-cancer, anti-inflammatory, antifungal, and antibacterial effects. As the pharmacological and pharmacotechnical behaviours of many organic therapeutic agents are observed to increase upon coordination with transition metal ions, progress has been made in the field of medicinal inorganic chemistry in developing various novel organic therapeutic agents [18, 19].

There are numerous metal complexes that have been found to enhance the efficacy of organic medicinal agents and speed up medication activity. The biological properties of King of Bitters and its metal complexes, particularly in the treatment of diabetes mellitus, have not received as much attention. Few studies have examined only the efficiency of this organic medicinal agent, but no study has examined its copper ion complex. The study of novel compounds with antidiabetic properties is clearly necessary. Therefore, this work examined the antidiabetic efficacy of the Cu(II) complex of the crude extra of the plant to find a more potent, affordable, and locally accessible medication.

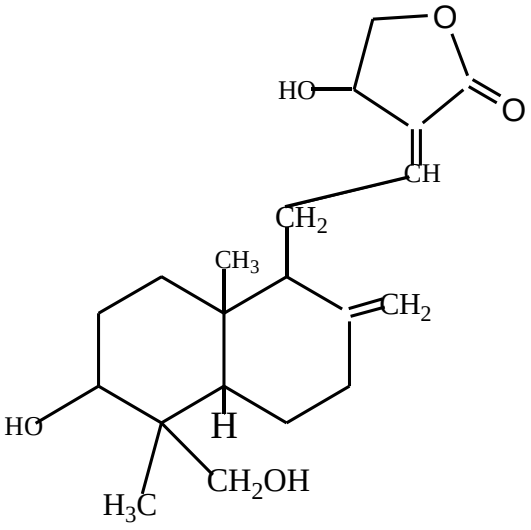

Figure 1. Structure of andrographolide

2. MATERIALS AND METHODS

2. 1. Materials

All chemicals used were of analytical grade, and they include copper acetate, sodium hydroxide, petroleum ether, hexane, ethanol, methanol, acetone, chloroform, tetra-chloro-methane, and distilled water.

2. 2. Methods

2. 2. 1. Plant Collection and Crude Extract Preparation

At the Oyo State College of Agriculture in Igboora, Oyo State, mature leaves of the Andrograhis paniculata plant were gathered. The plant was identified at the Department of Botany, University of Ibadan, Ibadan, with the voucher number UIH-23122. The leaves were collected, twice cleaned under running water, and then placed in distilled water to remove any remaining dirt.

The plant underwent room-temperature air drying. It was then broken down into tiny pieces and ground into powder. The oils, lipids, waxes, and terpenes were eliminated to produce the crude extract of the dried leaves in n-hexane at 60 to 80 °C. Following soxhlet extraction with 95 percent ethanol, the extract was concentrated using a rotary evaporator [20].

2. 2. 2. Synthesis of Metal Complex

The mixture of 10 g of the crude extract and 5 g of copper salt in ethanol was refluxed for approximately 4 hours. When precipitate had formed, the mixture was once again heated at 80 °C. Under vacuum, the precipitate was filtered and dried in a dessicator.

2. 2. 3. Characterization of the Metal Complex

The solubility tests in solvents such as water, ethanol, methanol, chloroform, acetone, and diethyl ether were used to characterize the metal complexes. UV-visible spectrophotometers (CE 2021, CECIL) and FTIR spectrophotometers (530M, BUCK) were used to characterize the plant crude extract and its metal complex. IR was measured within the range of 600 – 4000 cm^{-1} while UV-Visible measurement was performed within the range of 190 nm – 900 nm.

2. 2. 4. Effect of the Crude Extract and its Metal Complex on α-amylase Enzyme

The mixture of crude extract (100 µL), 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) was incubated at 25 °C for 10 min. Then, 500 µL of a 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 minutes, and cooled to room temperature. The reaction mixture was then diluted by adding distilled water (10 ml), and absorbance of each sample was measured at 540 nm. A complete reaction mixture without acarbose or extract was used as the control [21, 25-27]. The α-amylase inhibitory activity is expressed as percentage inhibition and calculated using the formula:

$$\text{Inhibition (\%)} = \frac{A_C - A_S}{A_C} \times 100$$

Ac = absorbance of the control (containing all reagents except extracts or acarbose)
As = absorbance of the sample (extract or acarbose).

For the purpose of determining the IC_{50} values, four diluted solutions of the crude extract and its metal complexes (40–100 mg/l) were gathered. The IC_{50} AAT Bioquest calculator [22] was used to determine the concentration of the extract needed to inhibit the enzyme's activity.

2. 2. 5. Effect of the Crude Extract and its Metal Complex on α-glucosidase Enzyme

Using acarbose as the standard, the method employed by [21] was used to test the effect of the extract on α-glucosidase activity. 100 mL of the α -glucosidase solution and 50 mL of the crude extract with its metal complexes were incubated at 25 °C for 10 min. 50 L of 5 M p-nitrophenyl-D-glucopyranoside solutions in 0.1 M phosphate buffer (pH 6.9) were then added, and the mixture was then incubated at 25 °C for 5 min. At 405 nm, the absorbance was then measured. The percentage inhibition used to express the α-glucosidase inhibitory activity is determined as follows:

$$\text{Inhibition (\%)} = \frac{A_C - A_S}{A_C} \times 100$$

A_c = absorbance of the 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 complex (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 (IC_{50}) was calculated using the AAT Bioquest calculator [22].

3. RESULTS AND DISCUSSION

Table 1 includes the physical features of the crude extract of King of Bitters and its metal (II) complex while Tables 2 and 3 highlight significant IR and UV-visible bands of the crude extract and its metal (II) complex. Table 4 displays the compounds' IC_{50} values. Figures 3 and 4 depict histograms of the plant's crude extract and its metal complexes having been tested for their antidiabetic effects on α-amylase and α-glucosidase enzymes.

Table 1. Physical Characteristic of the Plant Crude extract and its Metal Complex

Compound	**Colour**
Crude Extract	Green
Cu (II) complex	Green

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

Solvents	Crude Extract	Cu(II) Complex
Water	IN	IN
Methanol	VS	VS

Ethanol	VS	VS
Chloroform	SS	S
Acetone	VS	VS
Diethylether	SS	SS

IN = Insoluble, VS = Very Soluble, S = Soluble, SS = Slightly Soluble

Table 3. Important Infra-red Spectra (cm^{-1}) of the Plant Crude Extract and its Metal (II) Complex.

Compound	υC-H	υC-O	υC=O	υ-OH
Crude extract	2935 s	1165	1704	3426
Cu (II) complex	2936 s	1140	1710	3428

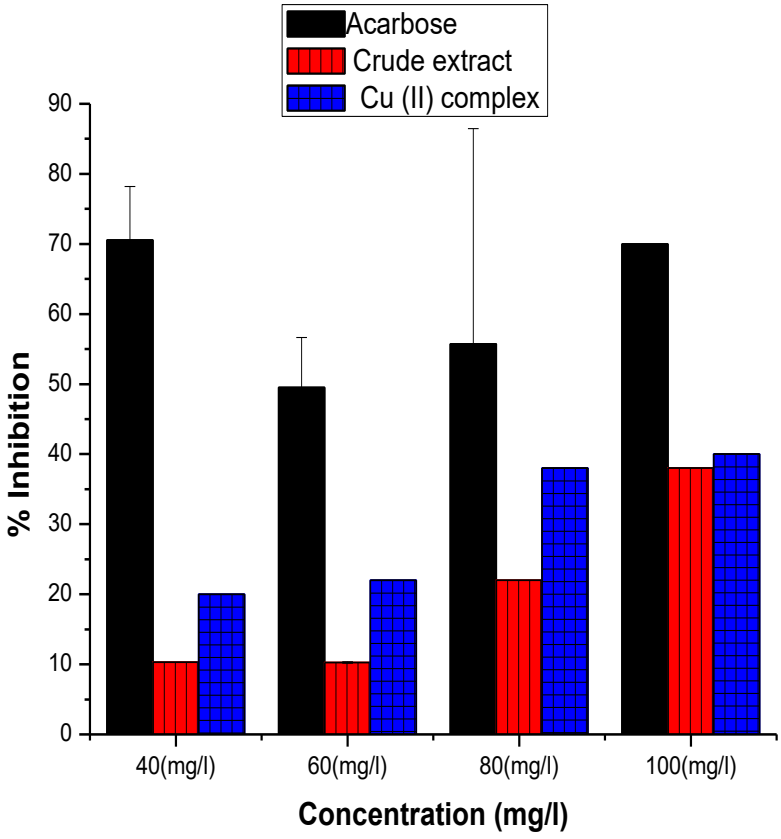

Figure 2. Histogram representation of inhibitory effects of King of bitters crude extract, its metal complex and standard drug on α-amylase

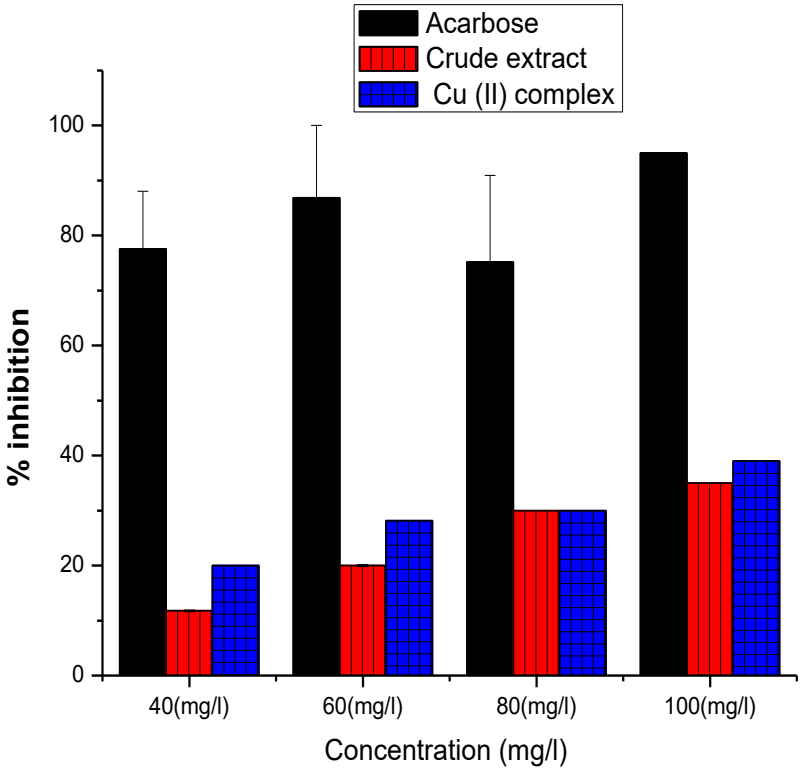

Figure 3. Histogram representation of inhibitory effects of King of bitters crude extract, its metal complex and standard drug on α–glucosidase

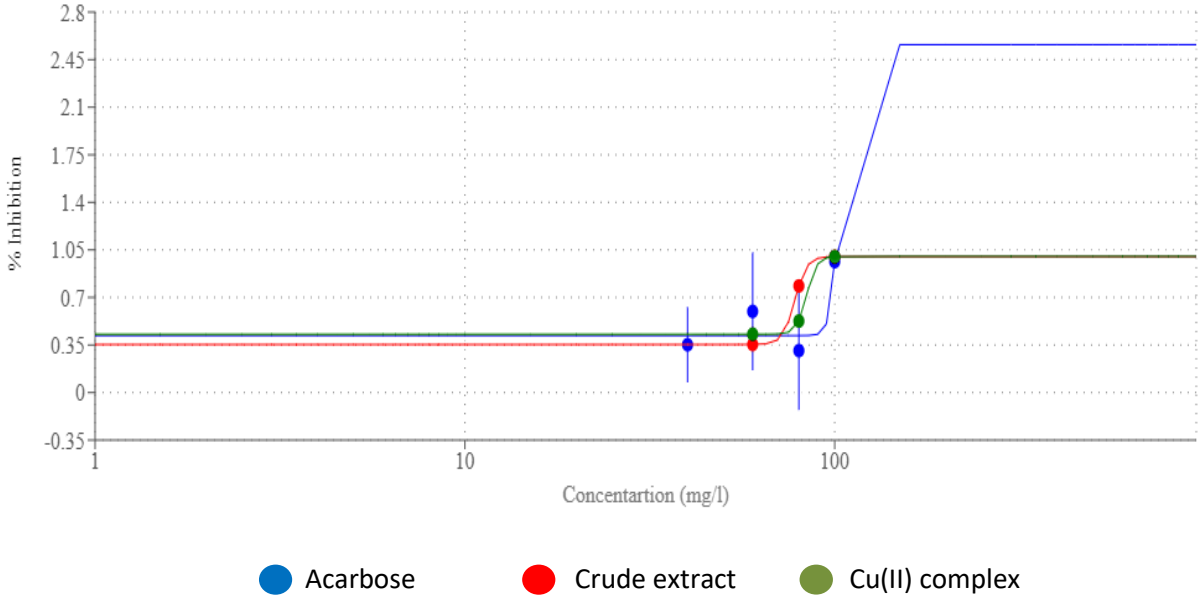

Figure 4. Plot of inhibitory effects of the king of bitters crude extract, its metal complex and standard drug on α–glucosidase at different concentrations

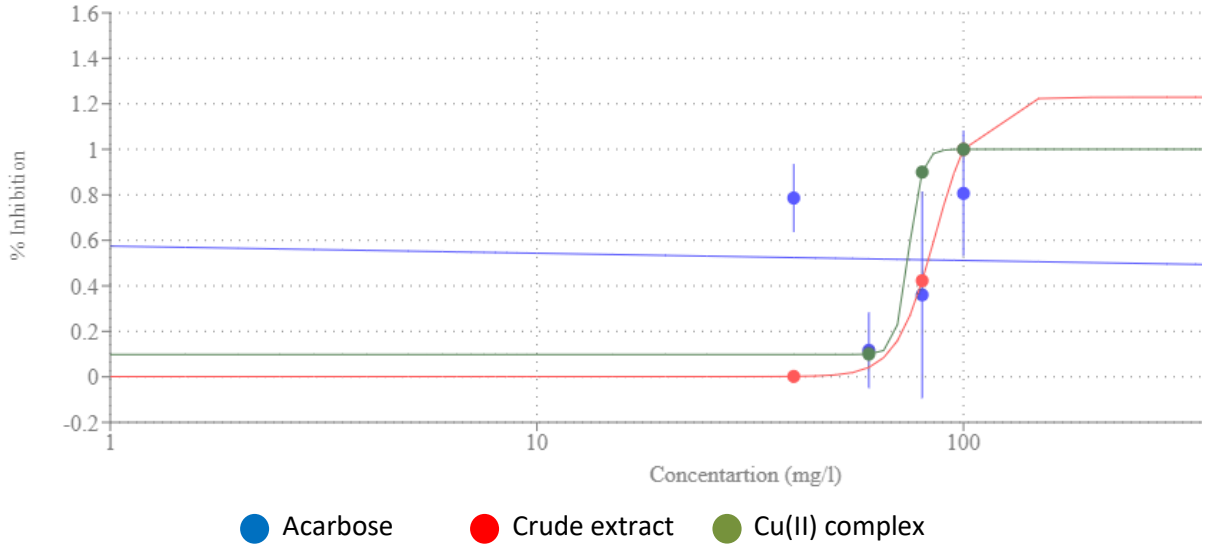

Figure 5. Plot of inhibitory effects of bitters crude extract, its metal complex and standard drug on α-amylase at different concentrations

Table 4. Electronic Spectra of the Plant Crude Extract and its Metal Complex

Compound	Band (cm^{-1})	Transition
Crude extract	28169 42553	$n \rightarrow \pi^*$ $\pi \rightarrow \pi^*$
Cu (II) complex	14641	d-d

Table 5. α-Amylase and α-glucosidase inhibitory activities of the plant crude rxtract and its metal complex

	IC_{50}	
Compound	α–amylase	α–glucosidase
Acarbose	55.49	102.66
Crude extract	85.65	77.98
Cu (II) complex	74.44	84.03

4. DISCUSSION

The crude extract and its metal complex are greenish in colour as shown in Table 1. They showed varying degrees of solubility in the solvents. The crude extract and its metal complex

were very soluble in ethanol, methanol and acetone while they were slightly soluble in diethylether, as shown in Table 2. The copper complex was only soluble in chloroform, while the crude extract slightly soluble in diethyl-ether. However, the metal complex is soluble in chloroform, while the extract is found to be slightly soluble. The extract and its metal complex are insoluble in water.

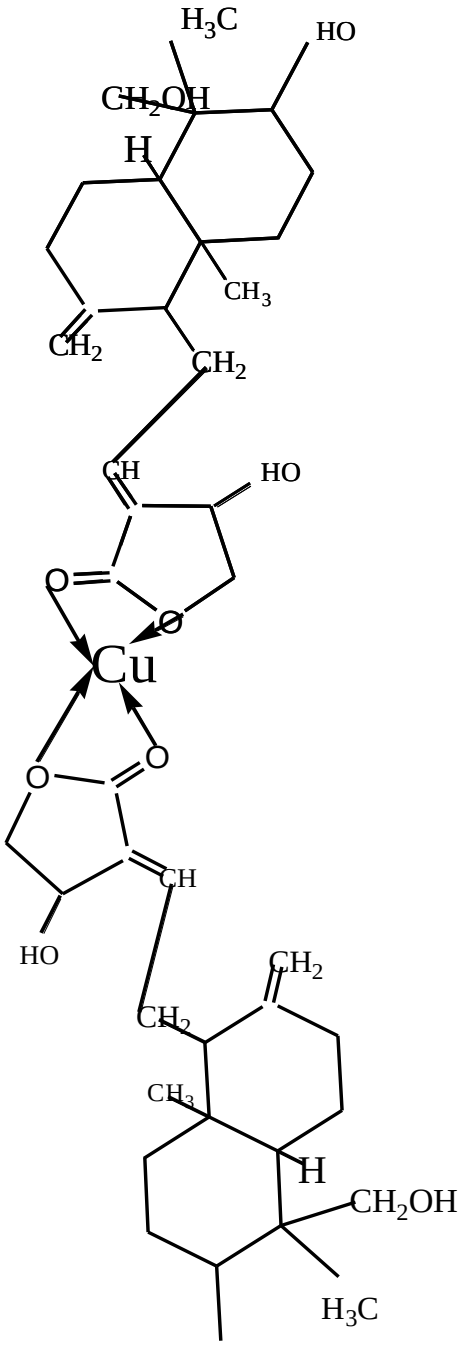

Figure 6. Proposed structure for Cu(II) complex of King of Bitters crude extract

Table 3 displays the pertinent infrared spectra of the crude extract and its metal complex. Four significant bands may be seen in the crude extract's spectrum at 2935 cm^{-1}, 1165 cm^{-1}, 1704 cm^{-1}, and 3426 cm^{-1}, which are attributed to the stretching vibrations of υ(C-H), υ (C-O), υ (C=O), and υ (C-OH), respectively. When coordinated with Cu (II), the bands at 1165 cm^{-1} and 1704 cm^{-1}, that are assigned to υ (C-O) and υ (C=O) underwent hyperchromic and hypsochromic shifts, respectively, to become 1140 cm^{-1} and 1710 cm^{-1}. The coordination of the crude extract with the metal ion through oxygen atom was confirmed by band shifts and the emergence of a new band in the area 400-800 cm^{-1} in the complex [23].

As indicated in Table 4, bands at 28169 cm^{-1} and 42553 cm^{-1} in the visible range of the spectrum of the crude extract are attributable to n- and - electronic transitions, respectively. Band 14641 cm^{-1}, seen in the spectrum of the Cu(II) complex, was attributed to a d-d electronic transition [24]. The most likely geometry could resemble that in Figure 6.

The percentage inhibitory effect of standard drug (acarbose), king of bitters crude extract against α-amylase and α-glucosidase enzymes at different concentrations (40, 60, 80, and 100 mg/l) was represented by histograms in Figures 2 and 3, respectively. At the concentrations tested, the standard drug showed more inhibitory effects against the enzymes. At the concentrations, the Cu(II) complex exhibited a more inhibitory effect against α-amylase than the crude extract, as shown in Figure 2. More inhibitory effect against α-glucosidase enzyme was displayed by the Cu(II) complex than the crude extract at concentrations of 40, 60, and 80 mg/l, while the crude extract showed more activity than the metal complex at concentrations of 100 mg/l (Figure 3).

Inhibitory activities of the standard drug (acarbose), King of Bitters crude extract, and its metal complex against α-amylase and α-glucosidase were estimated from Figures 4 and 5, respectively using the IC_{50} AAT Bonquest calculator and reported as half-maximal inhibitory concentration (IC_{50}) as shown in Table 5. The standard drug displayed the highest inhibitory strength against α -amylase enzyme with an IC_{50} value of 55.49, while the lowest inhibitory potency against the enzyme was observed to be exhibited by the crude extract with an IC_{50} value of 85.65. The α-amylase inhibitory IC_{50} values of the crude extracts, its metal complex, and acarbose are in the order of acarbose > Cu(II) complex > crude extract.

The crude extract showed the best inhibitory efficacy against α - glucosidase enzyme with an IC_{50} value of 77.98, while an IC_{50} value of 84.03 was obtained for Cu(II), and an IC_{50} value of 102.66 mg/l was obtained for the standard. The inhibitory effects against α–glucosidase enzyme were found in the order of King of Bitters crude extract > Cu(II) complex > acarbose.

The greater activity displayed by the metal complex than the crude extract against α-amylase could be attributed to the capacity of the metal ion to transform the bioavailability and pharmacological behaviour of the king of bitters crude extract.

5. CONCLUSION

The standard drug (acarbose), king of bitters crude extract and its metal complex were tested for their inhibitory activities against α-amylase, and α-glucosidase at different concentrations. Compared to the crude extract, the complex had stronger inhibitory effect against α-amylase. The crude extract and its metal complex could be readily available and inexpensive potential anti-diabetic medications. The crude extract and its metal complex should

be the subject of pharmacological and toxicological investigations to determine their viability as anti-diabetic drugs.

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