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Synthesis, Spectroscopic Analysis and Antidiabetic Properties of Copper (II) Complex of *Mangifera indica* Leaf Crude Extract

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Abstract

Many applied conventional drugs in treating diabetes have been reported to possess some drawbacks which necessitate a search for alternative therapies. In order to search for a more active antidiabetic agent, this study synthesized and evaluated antidiabetic properties of Mangifera indica crude extract and its Cu (II) complex in alloxan-induced diabetic albino rats. The leaf crude extract and its metal complex were characterized using percentage metal analysis and IR spectroscopic data. Experimental animals were induced by a single intraperitoneal injection of Alloxan monohydrate at a single dose of 140 mg/kg body weight and animals with fasting blood glucose level (BGL) > 200 mg/dL were considered diabetic. Metformin was used as a standard drug. Fasting blood glucose level and body weight were used to assess the antidiabetic activity. One-way ANOVA was used to determine the level of statistically significant at $p< 0.05$. The crude extract was found to coordinate with the metal ion through O donor atom of C=O and O-H of phenol and ketone respectively. The Cu (II) complex of the crude extracts at tested dose of 600mg/kg demonstrated more antidiabetic activity without weight gain than the standard drug. It is concluded that the Cu (II) complex could be a potential material in the development of more active and negative-side-effect-free antidiabetic drug.

Keywords: Albino rats; Body weight; Blood glucose; Metal complex.

INTRODUCTION

The increase in mortality caused by diabetes has become one of the biggest health challenges of the 21st century. According to (WHO, 2016), diabetes is responsible for about 0.02% of mortality worldwide in 2015 and about 8.8% of adults were evaluated by International Diabetes Federation (IDF) to possess diabetes. Diabetes has become rampant and steadily increasing worldwide. However, the widespread of this disease can harshly impact the finances of individuals and their families, and the economies of nations. Many individuals with Type 2 diabetes which is typified by hyperglycemia and abnormal carbohydrate metabolism, who are dependent on insulin for survival, suffer due to a dearth of affordable insulin (WHO 2016; Upadhyay et al., 2018). The number of mortality caused by diabetes is envisaged to reach about 10.4% unless effective prevention is accessible (Patarakijavanic eta al., 2019).

Many synthetic drugs have been applied to combat diabetes and these drugs are reported to act in different ways by lowering the level of blood glucose, for example, increased insulin secretion (sulfonylureas and meglitinides), decreased insulin resistance (biguanides and thiazolidinediones), increased prandial insulin secretion (DPP-4 inhibitors), reduced carbohydrate absorption (αglucosidase inhibitors), and inhibiting glucose reabsorption in the proximal renal tubule, resulting in increased renal glucose excretion and lower blood glucose levels (SGLT2 inhibitors) (Chao and Henry, 2010). Many of the present antihyperglycemic agents have been reported to be less effective, expensive and many drawbacks such as hypoglycemic episodes, gastrointestinal disturbances, skin reactions, lactic acidosis, fluid retention, and weight gain are associated with them (Krentz and Bailey, 2005).

Since many orthodox drugs and other conventional therapies are less effective or ineffective with several shortcomings in treating diabetes, there is an increase in the study of medicinal plants with hypoglycemic properties to replace the conventional drugs. Recently, there is a rapid evolution of studies in the area of herbal medicine and an upsurge in the use of medicinal plants in treating many ailments both in developing and developed countries. Their use is attributed to their natural origin,

fewer side effects, accessibility, effectiveness, and affordability (Bandaranayake, 2006).

The leaves of *Mangifera indica Linn*. commonly called mango have been applied for the treatment of fever, diarrhea, fainting, abnormality of lymph nodes, diabetes, and many ailments (Aderibigbe et al., 2001; Dineshkumar et al., 2010; Andrew et al., 2013; Garrido-Suarez et al., 2014; Ganogpichayagrai et al., 2017). The bioactivities of the leaf are attributed to the presence of secondary metabolites which include flavonoids, tannins, alkaloids, terpenoids, anthraquinones, saponins, cardiac glycosides, and steroids. Mangiferin, $C_{19}H_{18}O_{11}$ (Figure 1), a glucosyl xanthone (1, 3, 6, 7-tetrahydroxyxanthone-C2-β-D-glucoside) has been reported to be a prominent polyphenolic constituent found in *mongo* and it is mainly found in mango leaves, barks, and fruit peels (Patarakijavanic et al. 2019).

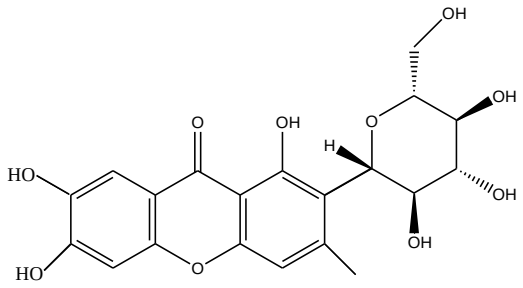

Figure 1:Mangiferin

Figure 1. Mangiferin.

The discovery of secondary metabolites from plants for clinical applications has provided a model for drug synthesis. In the last few years, there is a developing interdisciplinary field in the development of metal-based drugs using natural products. Transition metals show different oxidation states and can coordinate with molecules containing donor atoms or anions. These properties of transition metals are responsible for the development of metal-based drugs. The pharmacological properties and bioavailability of natural products can be modified by metal coordination (Jurca et al. 2017). The dearth of access to affordable insulin and synthetic drugs has become a fundamental barrier to the successful treatment of diabetes and results in unnecessary problems and premature deaths in many developing and underdeveloped countries. Therefore, in order to overcome the spread of diabetes through the use of inexpensive and readily available drugs, this work investigated the antidiabetic activity of *Mangifera indica leaf* crude extract and its metal complex.

EXPERIMENTAL

Reagents and Equipment

All the chemicals and solvents employed are of analytical grade and they are used without further purification. They include n-hexane, ethyl acetate, methanol, distilled water, normal saline, alloxan monohydrate, calcium chloride, copper (II) acetate, sawdust, pelletized rat feed, and metformin Hydrochloride.

The Infrared Spectra of the leaves crude extract and its metal complexes were recorded on Agilent FTIR Spectrophotometer while percentage metal analysis was carried out using Atomic Absorption spectrometer model PG990.

Collection and Identification of Plant leaves

Mangifera Indica leaves were collected from the surroundings of the department of Pure and applied chemistry, Ladoke Akintola University of Technology, Nigeria. The plant specimen was properly identified and authenticated at the Herbarium of the Department of pure and applied biology, Ladoke Akintola University of Technology (LAUTECH). The collected leaves were washed, shade dried at room temperature, and powdered in a grinder mill.

Extract Preparation

About 1.2 kg of the washed, dried, and powdered leaves of *Mangifera Indica* were extracted by cold maceration method using 96 % n-hexane and ethyl acetate solvents for 72 h (Pepato et al. 2005). After 72 h of maceration, the ethyl acetate soluble portion was cautiously decanted and concentrated using a rotary evaporator. The residual solvent in the crude extract obtained after concentration was allowed to evaporate at room temperature to avoid the decomposition of the natural metabolites. The dark green colored *Mangifera Indica* leaves crude extract obtained was kept in an air-tight desiccator over calcium chloride. The crude extract was weighed and a fresh stock for treating the diabetic animals was daily prepared (Wadood et al. 2013; Mohammed et al. 2015)

Preparation of Copper (II) acetate Complex of the Crude Extract

A solution of 0.5 g (2.75×10^{-3} mol) of copper (II) acetate was dissolved in distilled water. The resulting metal solution was added drop wisely to a solution of 1g mango crude extract in methanol. The mixture was stirred on a magnetic stirrer for 1 hr at room temperature. The metal complex formed was filtered in a vacuum system, washed with water, and dried in a desiccator over Calcium Chloride ($CaCl_2$). A pure green solid was obtained and weighed.

Experimental Animals

Male albino rats weighing 120-150 g body were used and acclimatized at a closely maintained temperature of 25 ± 2 °C with a standard relative humidity under photo-periodicity of 12 Light:12 Dark cycles for 4 weeks with free access to standard rodent pellet diet and water *ad*

libitum. Adult male albino rats weighing around 180-220 g were selected for the study.

Induction of Experimental Diabetes

The male Albino rats were allowed to fast overnight with their blood glucose levels and body weight recorded prior to the induction of the alloxan prepared in normal saline. The animals were chemically induced by intraperitoneal injection of freshly prepared alloxan monohydrate in saline at a dose of 160 mg/kg b.wt. The animals were kept under observation and after 48 hr of alloxan, the blood glucose was confirmed using an Accuchek glucometer. All animals with plasma glucose levels > 200mg/DL were separated and considered diabetic for the study and the rats having a blood glucose lesser than 200mg/DL glucose level were rejected (Sabu and Subburaj, 2002; Mohammed et al. 2015).

Administration of the Leaf Extract and its Metal Complex

The diabetic rats were divided into seven groups for the experimental study with four rats in each group as shown below:

a) Normal (Non induced) rats
b) Diabetic control (untreated rats)
c) Diabetic rats treated with Metformin Hydrochloride (500mg/kgb.wt)
d) Diabetic rats treated with aqueous Mango leaf extract (400mg/kgb.wt),
e) Diabetic rats treated with aqueous Mango leaf extract (600mg/kgb.wt)
f) Diabetic rats treated with an aqueous Complex of Mango leaf extract with copper (II) (400mg/kgb.wt),
g) Diabetic rats treated with an aqueous Complex of Mango leaf extract with copper (II) (600mg/kgb.wt)

The blood glucose and body weight of rats in each group were measured and evaluated on day 0, day 5, day 10, and day 15 using Accuchek Glucometer with disposable test strips and digital weighing balance respectively

Monitoring of Blood Glucose Concentration and Animal Weight

The Accuchek glucometer with disposable test strips was used to determine the blood glucose level in rats. Blood samples were collected through the tail of the animals. The tail in each case was first wiped with ethanol and then nibbled with a set of new blades. A test strip was fully inserted into the glucometer before applying a drop of blood to fully cover the test area inside the grey target (Sharma et al. 2003). After the collection of blood, the nibbled side of the tail was rubbed with cotton wool soaked in ethanol in order to protect the animal from infection and to arrest further bleeding. Blood glucose levels and weight of each animal were monitored and taken three times in three weeks using the Accuchek and Digital weighing balance respectively (Shastr, 1980).

Statistical Analysis

The results from the number of experiments were expressed in mean and standard deviation, and subjected to the analysis of variance (one-way ANOVA) to determine the significant levels difference between the groups using a t-test. The values with $P < 0.05$ were considered significant.

RESULTS AND DISCUSSION

The *Mangifera indica* leaves crude extract and its metal complex were characterized using Infrared spectroscopic and AAS analyses. The physical and chemical properties of the mango leaf crude extract, its metal complex, IR, and effects of the leaf extract, its metal complex, and the standard drug on diabetics are presented in Tables 1, 2, 3, and 4 respectively. The anti-diabetic property of the mango leaves crude extract and its metal (II) complex was evaluated through the body weight and blood glucose level. The body weight and blood glucose level before and after inducing diabetes were compared to obtain the anti-diabetic efficacy of the complex.

The induction of alloxan monohydrate produced hyperglycemia in albino rats. The mango leaf extract and its metal complex caused a significant ($p < 0.05$) decrease in the fasting blood glucose of treated rats (Table 3) when compared with the diabetic control and the metformin-treated group. This decrease was comparable with that of the normal control on certain days. Also, extract-treated groups showed statistically significant ($p < 0.05$) increases in weight gain at the end of 15 days when compared with diabetic control (Table 5).

Table 1. The Physical and Chemical Properties of the *Mangifera indica* Leaf Crude Extract and its Cu (II) complex.

Compound	Color	(%wt) Cu (II)
Mangifera indica leaf crude extract	Dark green	0.0052
[Cu(II) crude extract]	Dark green	0.0071

Table 2. Important IR spectra of the *Mangifera indica* Leaf Crude Extract and its Cu (II) complex.

Compounds	O- H (cm^{-1})	C=O (cm^{-1})	C-O (cm^{-1})	M-O (cm^{-1})
Mangifera indica leaf crude extract	3308 b	1701 s	1194 s	-
[Crude extract Cu]	3276 b	1640 m	1190 m	800-1000 m

b-broad, s-strong, m-medium

Table 3. Effects of *Mangifera indica* Leaf crude Extract and its Cu (II) Complex on Blood Glucose level of Diabetic Albino.

Group	Pre-Treatment(mg/dl)	Post Treatment(mg/dl)		
	DAY 0	DAY 5	DAY 10	DAY 15
Normal control	76.4 ± 8.76	73.25 ± 7.63	78 ± 24.52	82.3 ± 22.60
Diabetic control	225.7 ± 67.52	244.1± 120.59	304.2 ± 45.80	269.9 ± 11.81
Metformin Hcl (500mg/kgb.wt)	337.2 ± 51.33	289.3 ± 99.78	244 ± 82.65	197.9 ± 7.69
Leaf crude extract (400mg/kgb.wt)	333.5 ± 40.99	278 ± 106.02	263 ± 23.39	206.1 ± 4.00
Leaf crude extract (600mg/kgb.wt)	301.4 ± 129.06	103.7± 107.19	93 ± 90.93**	77.2 ± 5.68 **
[Crude extract Cu] (400mg/kgb.wt)	269.2 ± 28.18	296.2± 115.59	187.4±98.19	102.3 ± 9.55
[Mango extract Cu] (600mg/kgb.wt)	305 ± 123.89	264.2± 130.47	128 ± 53.82**	80.4 ± 5.25**

The values are expressed as means ± SEM; n = 4. Values are statistically significant at **P < 0.05 compare to diabetic control group (ANOVA).

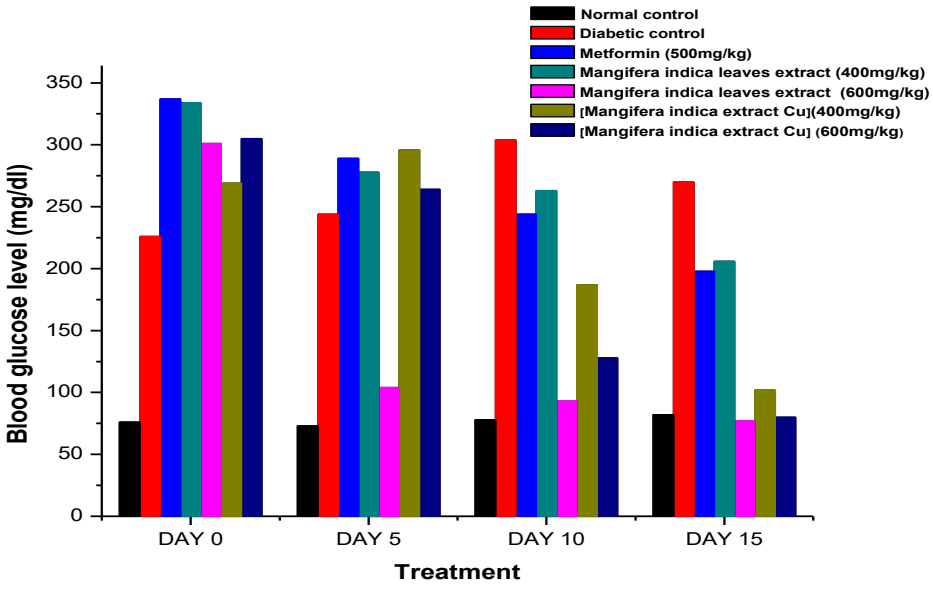

Figure 2. Histogram representation of effects of *Mangifera indica* leaf crude extract and its metal complex on blood glucose level.

Table 4. Effect of Standard rug, *Mangifera indica* Leaf Crude Extract and its Cu (II) Complex on body weight of Diabetic Albino rats.

Group(n=4)	Average body weight (g) ±SEM			
	DAY 0	DAY 5	DAY 10	DAY 15
Normal control	186.5 ± 47.96	185.71 ± 60.58	186.25 ± 46.95	190.2 ± 45.96
Diabetic control	225.86 ± 17.61	214.85 ± 18.95	201 ± 11.18	181.32 ± 18.20
Metformin Hcl (500 mg/kgb.wt)	202.71 ± 11.31	186.83 ± 21.38	197 ± 19.98	212.5 ± 7.64
Leaf crude extract (400 mg/kgb.wt)	189.86 + 14.36	184 ± 28.35	205 ± 30.35	213.31 ± 27.19**
Leaf crude extract (600 mg/kgb.wt)	209.71 ± 26.94	217 ± 41.17	217.29 ± 25.33	227 ± 11.43 **
[Crude extract Cu] (400 mg/kgb.wt)	204.6 ± 15.85	211.4 ± 36.10	226.14 ± 26.37	247.56 ± 1.57 **
[Crude extract Cu] (600 mg/kgb.wt)	176.86 ± 24.71	186.2 ± 33.62	169.6 ± 17.77	188.21 ± 2.88**

Values are expressed as mean±SEM. **P<0.05 as compared to diabetic control group; n= number of animals (One-way ANOVA)

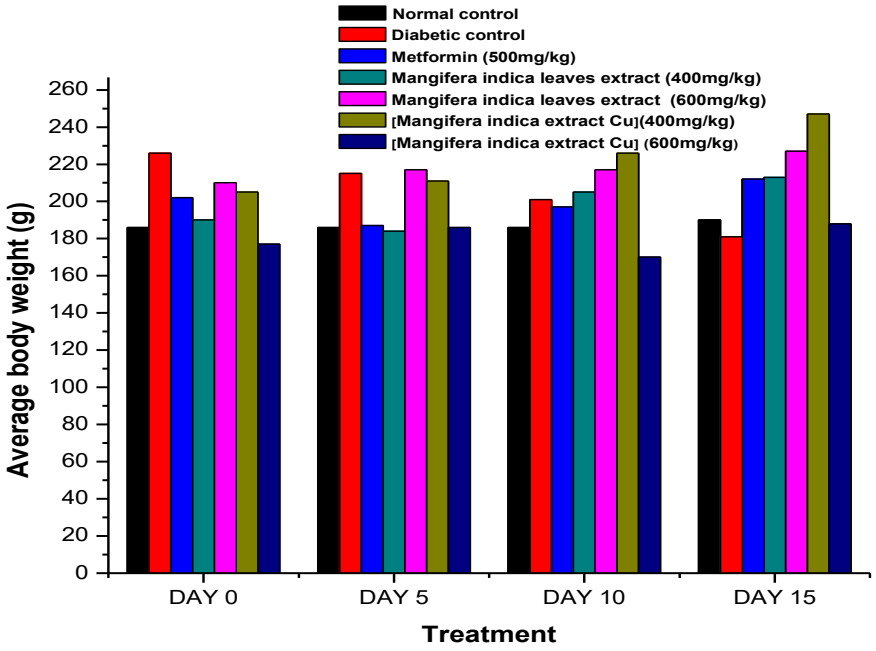

Figure 3. Histogram representation of effects of standard drug, *Mangifera indica* leaf crude extract and its Cu (II) complex on body weight of diabetic albino rats.

Discussion

The *Mangifera indica* leaves crude extract and its metal complex after complexation reaction were dark green in color and the percentage of Cu (II) presents in the crude extract and its metal complex are 0.0052 and 0.0071 respectively as shown in Table 1. The important IR bands were shown in Table 2. The spectrum of mango leaves crude extract showed two bands at 3308 cm^{-1} and1701 cm^{-1}. The bands were assigned to O-H of phenol and C=O of ketone respectively (Coate, 2001). These were found to shift to 3276 cm^{-1} and 1640 cm^{-1} upon coordination with the metal ion. The shift of the bands to the lower frequency and appearance of bands between 1000-800 cm^{-1} confirmed the formation of the complex and binding of the extract to Cu (II) through O-H and C=O of ketone, respectively as shown in Figure 4.

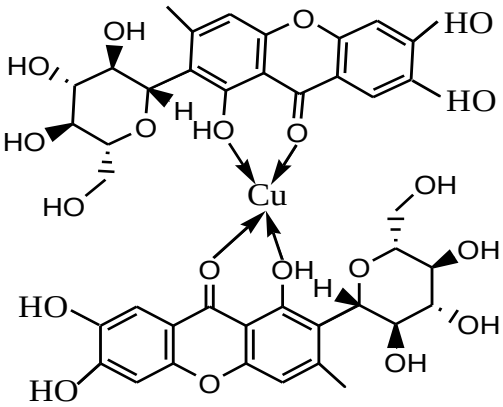

Figure 4. Proposed structure for Cu (II) complex of *Mangifera indica* leaf crude extract.

The rate of change in the blood glucose levels in the animals was shown in Table 3 and was represented by a histogram (Figure 2). No significant variation was observed in the blood glucose level of normal control within 15 days while a significant increase in the glucose level of blood was observed in the diabetic group during the study period compared to the normal control group. The standard drug, the crude extract, and Cu (II) complex of the extract when administered to an alloxan-induced diabetic rat produced a significant reduction in the blood glucose level on the 10th and 15th days of the treatment as compared to control rats. The standard drug at a dose of 500 mg/kgb.wt and *Mangifera indica* leaf crude extract and its Cu (II) complex were administered orally at doses of 400 mg/kgb.wt and 600 mg/kgb.wt for 15 days. On the 15th day, the blood glucose levels of the rats treated with the crude extract and its metal complex at doses of 400 mg/kgb.wt and 600 mg/kgb.wt were observed to significantly decrease ($p < 0.05$) when compared with the diabetic group. The decrease was found to be more significant in rats treated with the Cu (II) complex of the crude extract at a dose of 400 mg/kgb.wt while the crude extract was more effective at 600 mg/kgb.wt.

A change in body weight was observed in the treated rats as shown in Table 4 and represented by Figure 3. No significant change in the body weight of normal animals from the 0^{th} to the 15th day of treatment was observed. However, the body weight levels of the diabetic control group decreased gradually when compared to the normal non-diabetic rats. The weight levels of the rats treated with the crude extract at doses of 400 mg/kgb.wt and 600

mg/kgb.wt groups increased significantly at ($p < 0.05$) when compared to the diabetic control group on day 15th. Also, there was a significant increase in the body weight levels of the rats treated with Cu (II) complex at doses of 400 mg/kgb.wt and 600 mg/kgb.wt groups at ($p < 0.05$) when compared with the diabetic control group on day 15th. However, Cu (II) complex at a dose of 600 mg/kgb.wt produced a normal weight gain in the treated rat as compared to the normal control group. The standard drug was observed to produce abnormal weight gain which could be ascribed to the negative side effect associated with the drug. Therefore, the problem of the negative side effect was overcome with the administration of Cu (II) complex at a dose of 600 mg/kgb.wt.

In this study, the Cu (II) of the crude extract was found to be more effective than the leaf crude extract. The more increase in body weight and decrease in blood glucose level exhibited by the metal complex of the crude extract could be attributed to the ability of the metal ion to modify the bioavailability and pharmacological properties of the *Mangifera* indica leaf crude extract. The weight gain which was comparable with that of the normal control on the 15th day of administration of Cu (II) complex could be attributed to improved glycaemic control (Atangwho et al. 2007; Adeoye et al. 2017).

CONCLUSION AND RECOMMENDATION

In this study, the Cu (II) complex of *Mangifera indica* leaf crude extract showed more diabetic activity than the standard drug. Our results provide information on the antidiabetic activity of the metal complex and therefore the metal complex could be considered a potential antidiabetic drug without abnormal-weight-gain side effects. It is recommended that further studies such as Nuclear Magnetic Resonance Spectroscopy, Magnetic moment susceptibility, and X-ray should be conducted to establish the real structure of the complex. Also, the toxicity of the extract and its metal complex should be examined in order to establish its ideality as a potential antidiabetic drug.

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***Ethical Considerations*:** All experimental procedures were in agreement with the Ladoke Akintola University of Technology Ethics Committee on Research in Animals and internationally approved principles for laboratory animal upkeep and use.

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

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