Investigation of Antidiabetic Activities of Cu(II) Complex of *Anacardium occidentale* Leaves Crude Extract

Mary Adelaide Oladipo¹, Folasade Omobolanle Ajao², Adewusi John Adepoju¹, Kayode Taiwo Ishola³ and Deborah Omowumi Afolabi¹

¹Department of Pure & Applied Chemistry, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria.
²Department of Physiology, Ladoke Akintola University of Technology, Oyo State, Nigeria.
³Department of Chemistry, Federal College of Education (Special), Oyo, Oyo State, Nigeria.

*Corresponding author, e-mail: Isholatk@gmail.com*

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**Abstract**

Many synthetic chemical drugs have been widely used for the treatment of diabetes. However, many of these drugs are not locally available, are less effective, and are unaffordable for many diabetic patients in developing and underdeveloped countries. Therefore, in order to search for a locally available, effective, and cost-effective antidiabetic agent, this study synthesized the Cu(II) complex of crude leaf extract of *Anacardium occidentale* and investigated its antidiabetic activity in alloxan induced albino rats. The leaf crude extract and its metal complex were characterized using atomic absorption spectroscopy (AAS) and infrared (IR) spectroscopy. Experimental diabetic animals were induced by a single intraperitoneal injection of alloxan monohydrate at a single dose of 140 mg/kg body weight (b.wt.), and animals with fasting blood glucose levels (BGL) > 200 mg/dl were considered diabetic. Metformin was used as a standard drug. Fasting blood glucose level (BGL) and body weight were examined in assessing the antidiabetic activities of the crude extract and its complex in the rats. One-way ANOVA was used to determine the antidiabetic activity at a statistical significance level of *p* < 0.05. The hypsochromic shifts of C=O and O-H bands in the Cu(II) complex and the high concentration of the metal ion in the metal complex established the coordination of the crude extract with the metal ion. A more significant reduction in the blood glucose level and increase in body weight in alloxan-induced diabetic albino rats was observed when treated with the leaves crude extract and its Cu(II) complex than when treated with the standard drug metformin. It can be concluded that the Cu(II) complex of *Anacardium occidentale* leaf extract at a dose of 400 mg/kg wt is more effective without abnormal weight gain and could be considered as a potential antidiabetic drug to replace some of the less effective and expensive conventional antidiabetic drugs.

**Keywords**: Albino rats, Crude extract, Diabetes mellitus, Medicinal plant, Metal complex.

**Introduction**

Diabetes mellitus, one of the universal chronic metabolic disorders with chronic hyperglycaemia and insulin resistance, is one of the major diseases responsible for high worldwide mortality (Policardo et al. 2015, Bragg et al. 2017). Diabetes can be classified as Type 1 & Type 2. All types can impair many parts of the body, thereby increasing the overall consequence of premature death. The widespread nature of this disease has made it a fast-growing global problem due to...
its consequences for health, society, and the economy. Diabetics and their families are subjected to considerable economic decline and deterioration in health as a result of direct medical costs and the efforts required in treating the patients. Also, the disease might lead to a loss of work and wages for the patients, thus exerting negative effects on their economic situation.

Many synthetic chemical drugs have been widely used for the treatment of different types of diseases, including diabetes. However, many of these drugs have been reported to show various shortcomings arising from negative side effects and the unaffordability of the drugs for many diabetics. Therefore, in order to overcome the shortcomings, medicinal plants have been considered as replacements. They are widely used to treat the disease in some parts of the world due to their effectiveness and lack of side effects. Many traditional medicinal plants are known to possess a diversity of active chemical compounds that are potential weapons for destroying many diseases occurring in nature. They have been widely applied for the treatment of numerous pathologies worldwide and have become a significant foundation for developing new chemotherapeutic agents for various applications (Onuh et al. 2017).

Cashew, *Anacardium occidentale* (Family Anacardiaceae), is a multipurpose tree of the tropics; its bark, nut, and leaf have been used medicinally in treating different types of diseases and in other industrial applications (Barbosa-Filho et al. 2014). *Anacardium occidentale* plants are known to exhibit some biological activities due to the presence of secondary metabolites (flavonoids, phenols, phenolic glycosides, saponins, and glycosides) in the plant (Arekemase et al. 2011, Fadeyi et al. 2015). The antimicrobial activities of cashew plants and their phytochemicals (alkaloids, tannins, flavonoids, phenolics, and other compounds) have been investigated.

Polyphenol and flavonoid components of the cashew plant and, in particular, 2-hydroxy-6-pentadecylbenzoic acid (Figure 1), a component of *Anacardium occidentale* leaf (Agedah et al. 2010), are considered compounds responsible for the plant's antidiabetic activity. The activity is attributed to the inhibition of α-glucosidase, a key enzyme linked to type II diabetes. The leaf of the plant has been considered a potential agent for effective regulation of glucose (Fadeyi et al. 2015, Damsud et al. 2018). As a result of its effectiveness, many traditional practitioners in African countries have been applying *Anacardium occidentale* L. (*Anacardiaceae*) leaf to combat diabetes mellitus.

![Figure 1: 2-hydroxy-6-pentadecylbenzoic acid.](image)

The effectiveness of *Anacardium occidentale* L. leaf against diabetes mellitus established its use in the indigenous system of medicine. Jaiswal et al. (2017) investigated the antidiabetic efficacy of *Anacardium occidentale* Linn. leaf extract in n-streptozotocin diabetic rats. The leaf extract was found to be more effective against diabetes mellitus than the standard drug pioglitazone. The α-glucosidase inhibitory activity and bioaccessibility of *Anacardium occidentale* shoot and leaf extracts were examined by Damsud et al. (2021). The methanolic extract of the leaves was observed to contain higher total phenolics and flavonoids than the shoots extract and displayed high α-glucosidase inhibitory activity. Encarnacao et al. (2022) investigated the antidiabetic activity of *Anacardium occidentale* bark in non-fasting and fasting glycemia states and compared its effect with that of the standard drug glibenclamide. The plant was found to show a greater glucose-lowering effect than the standard drug. Comparative effects of methanolic extracts of *Anacardium occidentale* Linn. nut, leaf, and stem bark on hyperglycaemia and associated
abnormalities in streptozotocin-induced diabetic rats were examined by Ajao et al. (2022). The nut extract was found to exhibit great antidiabetic efficacy.

Metal ions and metal complexes have been established to play significant roles in several processes, such as biochemical, agricultural, pharmaceutical, industrial, and chemical processes. Transition metal complexes have been widely applied in therapy as a result of their pharmacodynamic activities, toxicity reduction, and bioavailability (Farrer and Sadler 2013, Newman and Cragg 2016, Zhang 2017). Therefore, the significant roles played by metal complexes in different processes have led to investigations of the antimicrobial activity of metal complexes of natural products.

Natural products are reported to contain phenolic metabolites capable of binding to various metal ions. The relationship between plants and metal ions is evident in many biologically essential processes such as metalloenzymes, mineral nutrition, photosynthesis, prooxidant/antioxidant systems, etc. (Fedenko et al. 2022). Metal complexes of many natural products have been widely used as chemotherapeutic agents to treat several human diseases such as carcinomas, lymphomas, infection control, diabetes, anti-inflammatory, antimalarial, and neurological disorders (Pattan et al. 2012, Jurca et al. 2017, Heras et al. 2019).

The use of metal complexes of natural products in the design and development of metal-based drugs for the treatment of diabetes is becoming a fast-growing field because metal ions are discovered to possess the ability to modify the pharmacology of active components of natural products, thereby improving the efficacy and/or reducing the negative side effects of the active compounds (Muthusamy and Natarajan 2016).

The antidiabetic properties of different parts of Anacardium occidentale have been investigated by many researchers. However, there is a dearth of information on the antidiabetic activity of metal(II) complexes of cashew (Anacardium occidentale) leaf. Therefore, in order to search for a locally available, affordable, and effective antidiabetic agent for diabetics, the antidiabetic activity of a novel copper(II) complex of cashew leaf crude extract was investigated with a view to establishing its potential usefulness in preparing a drug capable of treating diabetes mellitus.

Materials and Methods

Reagents and Equipment

All the chemicals and solvents employed were of analytical grade and they were used without further purification. They included: n-hexane, ethyl acetate, methanol, distilled water, normal saline, alloxan monohydrate, calcium chloride, copper(II) acetate, sawdust, pelletized rat feed and metformin. The Infrared spectra of the leaves crude extract and its metal complex were recorded on FTIR Spectrophotometer while metal component of leaves extract and its metal complex was carried out using Atomic Absorption spectrometer model PG990.

Collection and extraction of the leaves

The Anacardium occidentale leaves were collected at Ladoke Akintola University of Technology, Ogbomoso, Oyo state, Nigeria and authenticated at the Herbarium of the Department of Pure and Applied Biology, Ladoke Akintola University of Technology (LAUTECH). The collected leaves were washed and shade dried at room temperature. The dried samples were crushed into fine particles using a milling machine. The powdered samples were extracted using cold maceration method. The powdered leaves sample was first soaked in 96% n-hexane solvent for 72 h and the n-hexane portion was cautiously decanted. The residual portion was obtained and allowed to dry at room temperature. The dried leaves were again soaked in 96% ethyl acetate solvent for 72 h with thorough shaking to ensure complete extraction of active component of the leaves. The ethyl acetate soluble fraction obtained after decantation was concentrated using a rotary evaporator. The dark green colour of Anacardium occidentale leaves crude extract was kept in an air tight desiccator over calcium chloride for 2 days. The fresh stock
for treating the diabetic animals was prepared from the crude extract (Bagdade et al. 1991).

**Preparation of metal complexes of Anacardium occidentale crude extract**

A solution of 0.5 g of copper(II) acetate was prepared in distilled water. The metal solution was added drop wisely to 1 g of Anacardium occidentale leaves crude extract solution in methanol. The reacting mixture was stirred on a magnetic stirrer for an hour at 30 °C. The resulting light and shining green complex was obtained, filtered, washed with water, and dried in a desiccator over calcium chloride. The probable equation for the reaction is given below:

\[
\text{Cu(CH}_3\text{COO)}_2 + 2\text{C}_{22}\text{H}_{39}\text{O}_3 \rightarrow [\text{Cu(C}_{22}\text{H}_{39}\text{O}_3)_2]
\]

**Experimental animals**

Male albino rats weighing 120–150 g were used for this study. The animals were housed in clean propylene cages (four rats per cage) with sawdust as bedding and acclimatized under controlled room temperature (25 °C) with standard relative humidity under a 12:12 h light and dark cycle for 4 weeks with free access to food and water ad libitum (Kumar et al. 2006, Garber et al. 2010). Wood shavings were used for their bedding, which was changed every 2 days to prevent odour and dirtiness. All animals were properly maintained and sacrificed according to ethical rules. The average weight of the rats in each cage was taken four times in four weeks to ensure the required weight of 200 g.

**Induction of experimental diabetes**

The male albino rats were allowed to fast overnight, with their blood glucose levels and body weight being recorded prior to the induction of the alloxan. All the male albino rats were grouped and induced (except the normal control group) by intraperitoneal injection of alloxan monohydrate dissolved in normal saline at a single dose of 140 mg/kg wt. The rats were given access to food and water ad libitum after 30 mins of diabetes induction. The presence of diabetes was confirmed in the rats 72 h after alloxan induction with an Accucheck glucometer. All animals with plasma glucose levels > 200 mg/dl were considered diabetic and used for the in vivo experiment (Ogbonnia et al. 2010).

**Administration of Anacardium occidentale leaves crude extract**

The experimental male albino rats were divided into 7 groups, each group containing 4 Albino rats, as shown below:

- Group 1: Normal rats.
- Group 2: Diabetic rats as control.
- Group 3: Diabetic rats treated with metformin (500 mg/kg b.wt).
- Group 4: Diabetic rats treated with Anacardium occidentale leaf crude extract (400 mg/kg b.wt).
- Group 5: Diabetic rats treated with Anacardium occidentale leaf crude extract (600 mg/kg b.wt).
- Group 6: Diabetic rats treated with Cu(II) complex of Anacardium occidentale leaf crude extract (400 mg/kg b.wt).
- Group 7: Diabetic rats treated with Cu(II) complex of Anacardium occidentale leaf crude extract (600 mg/kg b.wt).

The treatments of diabetic rats in Groups 4 to 7 were carried out according to the dosage designed for each group, with a single full dose daily. The Group 2 diabetic rats were treated with the metformin standard drug (reference drug). The crude extract, its metal complex, and metformin were administered orally to all the treated rats using the oral canula for 15 days with free access to feed and water. The rats in Group 1 (normal control) and Group 2 (diabetic control) were given access to feed and water ad libitum without the administration of any of the leaves crude extract, the complex of the leaves crude extract, or metformin.

A digital glucometer (Accucheck Advantage, Roche Diagnostic, Germany) with test strips was employed to determine the blood glucose level in the plasma of the rats by the glucose oxidase/peroxidase method. Blood samples were collected through the tails of the animals. The tail in each case was first wiped with ethanol and
then nibbled with a new blade. A test strip was fully inserted into the glucometer before applying a drop of blood to fully cover the test area inside the gray target. The test area of the strip is designed in such a way that when a drop of blood is placed on the top surface, colour change occurs, which is determined by the glucometer and is proportional to the concentration of glucose in the blood sample. After the collection of blood, the nibbled side of the tail was rubbed with cotton wool soaked in ethanol to protect the animal from infection and arrest further bleeding. The blood glucose and body weight of rats in each group were thoroughly monitored and measured every 5 day for 15 days. An Accucheck glucometer with disposable test strips and a digital weighing balance were used to determine variation in blood glucose level and body weight, respectively among the treated groups.

**Statistical analysis**

The values obtained from the number of experiments were evaluated as mean and standard deviation of mean. The data were subjected to the analysis of variance (one-way ANOVA) to determine the significant changes at a statistical significance level of $p < 0.05$.

**Results and Discussion**

The leaves crude extract and its metal complex were characterized using IR spectra and AAS analysis. The physicochemical properties of *Anacardium occidentale* leaves crude extract and its metal complex are shown in Table 1, while Table 2 showed the IR bands of the extract and its metal complex. The antidiabetic activities of the crude extract, its metal complex, and the standard drug are presented accordingly in Tables 3 and 4. Histogram representations of the crude leaf extract and its metal complex on the blood glucose level and body weight of alloxan-induced diabetic albino rats are displayed in Figures 2 and 3, respectively.

**Table 1:** Physico-chemical properties of *Anacardium occidentale* leaves crude extract and its Cu(II) complex

<table>
<thead>
<tr>
<th>Compound</th>
<th>Colour</th>
<th>Cu(II) (% wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaves crude extract</td>
<td>Dark green</td>
<td>0.023</td>
</tr>
<tr>
<td>Cu(II) leaves crude extract]</td>
<td>Shining dark green</td>
<td>0.035</td>
</tr>
</tbody>
</table>

**Table 2:** Important IR bands of *Anacardium occidentale* leaves crude extract and its Cu(II) complex

<table>
<thead>
<tr>
<th>Compound</th>
<th>O-H (cm$^{-1}$)</th>
<th>C=O (cm$^{-1}$)</th>
<th>M-O (cm$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaves crude extract</td>
<td>3388$^b$</td>
<td>1617$^s$</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1459$^s$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[Cu(II) leaves crude extract]</td>
<td>3388$^b$</td>
<td>1550$^s$</td>
<td>800–1000 m</td>
</tr>
<tr>
<td></td>
<td>1420$^s$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

b = broad, s = strong, m = medium.
Table 3: Effects of *Anacardium occidentale* leaves crude extract and its Cu(II) complex on blood glucose level

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day 0</th>
<th>Day 5</th>
<th>Day 10</th>
<th>Day 15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>76.4 ± 8.76</td>
<td>73.25 ± 7.63</td>
<td>78 ± 24.52</td>
<td>82.3 ± 22.60</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>225.7 ± 67.52</td>
<td>244.1 ± 120.59</td>
<td>304.2 ± 45.80</td>
<td>269.9 ± 11.81</td>
</tr>
<tr>
<td>Metformin 400 mg/kg.b.wt</td>
<td>337.2 ± 51.33</td>
<td>289.3 ± 99.78*</td>
<td>244 ± 82.65*</td>
<td>197.9 ± 7.69**</td>
</tr>
<tr>
<td>Leaves crude extract 400 mg/kg.b.wt</td>
<td>314.6 ±56.96</td>
<td>252 ±81.33*</td>
<td>195.6 ±85.02**</td>
<td>137.2 ± 4.15**</td>
</tr>
<tr>
<td>Leaves crude extract 600 mg/kg.b.wt</td>
<td>289 ±171.14</td>
<td>149.4 ±125.12*</td>
<td>129.8 ±100.41**</td>
<td>98.4 ± 3.85**</td>
</tr>
<tr>
<td>[Cu(II) leaves crude extract] 400 mg/kg.b.wt</td>
<td>266.6 ±46.96</td>
<td>241.6 ±132.61</td>
<td>170.4 ±141.41**</td>
<td>98.7 ± 3.56**</td>
</tr>
<tr>
<td>[Cu(II) leaves crude extract] 600 mg/kg.b.wt</td>
<td>294.8 ±44.12</td>
<td>288.75 ±84.26</td>
<td>105.6 ±48.46***</td>
<td>90.5 ± 5.50***</td>
</tr>
</tbody>
</table>

The values are expressed as means ± SEM; n = 4. Values are statistically significant at *P < 0.05, more significant at **P < 0.05, very significant at ***p < 0.05.

Table 4: Effects of standard drug, *Anacardium occidentale* leaves crude extract and its Cu(II) complex on body weight

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Average body weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
</tr>
<tr>
<td>Normal control</td>
<td>186.5 ± 47.96</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>225.86 ± 17.61</td>
</tr>
<tr>
<td>Metformin 500 mg/kg.b.wt</td>
<td>202.7 ± 11.31</td>
</tr>
<tr>
<td>Leaves crude extract 400 mg/kg.b.wt</td>
<td>192.85 ±10.48</td>
</tr>
<tr>
<td>Leaves crude extract 600 mg/kg.b.wt</td>
<td>209.86 ±19.41</td>
</tr>
<tr>
<td>[Cu(II) leaves crude extract] 400 mg/kg.b.wt</td>
<td>151.20 ±21.56</td>
</tr>
<tr>
<td>[Cu(II) leaves crude extract] 600 mg/kg.b.wt</td>
<td>194.50 ±19.39</td>
</tr>
</tbody>
</table>

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

The *Anacardium occidentale* leaves crude extract was dark green in colour and the colour changed to light, shining green upon coordination with Cu(II). The amount of Cu(II) obtained in the crude extract and its...
metal complex was found to be 0.023 mg/kg and 0.035 mg/kg, respectively, as shown in Table 1. The important IR bands for the extract and its metal complex were as shown in Table 2. The spectrum of the crude extract of the leaves showed three bands at 3388 cm\(^{-1}\), 1459 cm\(^{-1}\) and 1617 cm\(^{-1}\). The bands were assigned to the O-H of carboxylic acid and the C=O carboxylic acid vibrations, respectively (John 2000). The cashew leaf extract bands at 1617 cm\(^{-1}\) and 1459 cm\(^{-1}\) were found to shift to 1550 cm\(^{-1}\) and 1420 cm\(^{-1}\), respectively, upon coordination with the metal ion. The high proportion of the metal in the complex, the shifts of the bands, and the appearance of bands between 1000 and 800 cm\(^{-1}\) in the complex spectrum confirmed the formation of the complex and binding of the crude extract of the leaves to Cu(II) through O-H and C=O of carboxylic acid, respectively, as shown in Figure 4.

![Proposed structure for the complex.](image)

The effects of the standard drug (Metformin), Anacardium occidentale leaves crude extract, and its metal complex on blood glucose levels of alloxan-induced diabetic albino rats within an interval of 5 days for 15 days were depicted in Table 3. The values in the table are represented using a histogram (Figure 2). The standard drug at a dose of 500 mg/kgb.wt, and the leaves crude extract and its metal complex at doses of 400 mg/kgb.wt and 600 mg/kgb.wt were administered to the diabetic albino rats within the interval. A very significant variation in the blood glucose level of diabetes induced rats treated with the extract and its metal complex at the significance level of p < 0.05 was observed compared to the diabetic group within the intervals. The standard drug metformin on 5th and 10th days significantly lowered the glucose level and the action of the drug was found to give more significant results on 15th day (p < 0.05) as compared to the diabetic control. The dose 400 mg/kgb.wt of the leaves crude extract produced a significant reduction in the blood glucose level (p < 0.05) on 5\(^{th}\) day and a more significant reduction (p < 0.05) on 10th and 15th as compared to the diabetic control. Also, the leaves extract at a dose of 600 mg/kgb.wt as compared to diabetic control produced a significant and more significant (p < 0.05) reduction in the blood glucose level on 5th and 10th days respectively. However, a very significant reduction (p < 0.05) in the glucose level at the dose of 600 mg/kgb.wt was observed on the 15\(^{th}\) day with the glucose level almost close to normal.

The Cu(II) metal complex of the crude extract offered the most significant effect at 400 mg/kgb.wt on the 10\(^{th}\) (p < 0.05) and 15\(^{th}\) days (p < 0.05) of treatment as compared to the diabetic control. Similarly, the Cu(II) complex of the crude extract at administration of 600 mg/kgb.wt produced a very significant reduction (p < 0.05) in the blood glucose level of the rats on the 10\(^{th}\) and 15\(^{th}\) days as compared to diabetic control and close to the normal group. At the end of the 10\(^{th}\) and 15\(^{th}\) day treatments, the Cu(II) complex of the leaf crude extract was found to produce a very significant reduction in the blood glucose level compared to the leaf crude extract and the reference drug, metformin, at a dose of 600 mg/kg wt. The increase in complex activity in reducing the blood glucose level could be attributed to an increase in pharmacodynamic activity and bioavailability of the crude extract as a result of coordinated metal ion (Newman and Cragg 2016, Zhang 2017).

Hyperglycemia was accompanied by weight loss in all diabetic induced groups before the positive effects of the standard drug, Anacardium occidentale leaves crude extract and its metal complex as shown in Table 4 and represented by histogram (Figure
3. A reduction in body weight was observed in the untreated diabetic group from (225.86 ± 17.61 g) to (181.32 ± 18.20 g) after 5–15 days. However, no significant change was observed in the body weight of the normal animals from the beginning to the end of treatment.

The administration of the standard drug, metformin, to the diabetic albino rats led to a statistically significant increase in body weight as compared to diabetic control on the 15th day of treatment. However, the Cu(II) complex of the crude extract of the leaves at a dose of 400 mg/kg wt on the 15th day of treatment significantly increased (p < 0.05) body weight of the diabetic rats and produced a new body weight very close to that of the normal group. Therefore, the problem of abnormal weight gain associated with many conventional drugs has been overcome.

In this study, an increase in blood glucose level and decrease in body weight were observed in diabetes-induced albino rats as compared to normal rats. The increase could be attributed to the inability of cells in the rats to utilize glucose, lipolysis in adipose tissue, and protein breakdown, leading to skeletal muscle wasting. Conversely, the oral administration of Anacardium occidentale leaves crude extract and its metal complex was found to significantly lower the blood glucose level and increase body weight. Cu(II) complex of the crude extract is found to produce the same body weight in diabetic rats as in normal rats. The activities of the crude extract and its metal complex could be attributed to other possible mechanisms like stimulation of glycogenesis in the liver, enhanced tissue glucose utilization, and decreased gluconeogenesis (Sudha et al. 2011, Nabi et al. 2013, Tesfaye et al. 2016).

Conclusion and recommendation

Many conventional antidiabetic drugs are observed to produce abnormal body weight when they are applied to treat diabetes. In this study, the Cu(II) complex of Anacardium occidentale leaves crude extract was found to demonstrate a more significant anti-diabetic activity in diabetes-induced albino rats than the Anacardium occidentale leaves crude extract and the standard drug metformin hydrochloride. At a dose of 400 mg/kg wt, the Cu(II) complex of Anacardium occidentale leaves crude extract produced a pronounced antidiabetic effect without abnormal weight gain as compared to the leaves crude extract and the standard drug. This provides justification for the metal complex to be a promising material for the production of an effective antidiabetic agent in order to replace those conventional drugs that are less effective, expensive, and not locally available. It is recommended that the toxicity of the metal complex be investigated to establish its therapeutic ideality for a potential application in producing diabetes drugs.

Acknowledgement

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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.

Competing Interests: No competing interests.

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