In vivo antihyperglycaemic activity of crude and partitioned fractions of selected medicinal plants

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ABSTRACT

The incidence of diabetes mellitus is skyrocketing in sub-Saharan Africa and the world at large, with about 8.8% of the adult population currently affected and projected to rise to 9.9% by the year 2045. The currently used drugs are beset with serious side effects, reduced efficacy due to prolonged use and high cost. This study therefore aimed at exploring the use of medicinal plants for the development of cheaper and more effective herbal remedies/plant-derived anti-diabetic drugs. Ethanol extracts of the leaves of three (3) medicinal plants – Bauhinia reticulata (BR), Cassytha filiformis (CF) and Daniella ollieveri (DO) were subjected to in vivo anti-hyperglycaemic screening. The BR and CF crude extracts were partitioned because of their promising antihyperglycaemic potentials, using solvents of varying polarities. The partitioned fractions were thereafter evaluated for anti-hyperglycaemic activity. 100 and 200 mg/dL b.w. were the doses of extracts and fractions administered to the experimental animals. Crude ethanol extract of BR had the highest antihyperglycemic activity, as it lowered blood glucose level of experimental mice from 219.80±22.94 mg/dL to 105.40±10.87 mg/dL with percentage blood glucose lowering effect of 52.05±0.53 %, which was higher than that recorded by the standard drug-Metformin (50.88±0.86 %) at dose of 200 mg/kg b.w. There was no significant difference (p>0.05) in the percentage glucose lowering effect of the ethyl acetate and n-hexane partitioned fractions of BR (55.91±0.20 and 55.80±0.68 % respectively), although, the standard drug had a higher percentage glucose lowering effect (65.92±0.87 %) at dose of 200 mg/kg b.w. Further purification of the fractions could yield active compound(s) which could serve as leads in the development of novel anti-diabetic drugs.

Keywords: Medicinal plant, Bauhinia reticulata; Cassytha filiformis; Metformin; Fasting blood glucose

1.0 INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycaemia, resulting from defects in insulin secretion, insulin action, or both (Ottah et al., 2012). It is a major cause of disability and hospitalization, which results in a significant financial burden (Bommer et al., 2017; American Diabetes Association (ADA), 2018). Complications that result from the disease could be acute, sub-acute, or chronic, resulting from defects in metabolism of carbohydrates, fats, proteins, and electrolytes in the body (Ojezele and Abatan, 2011).
Characteristically, the symptoms include polyuria, polydipsia, polyphagia, pruritus, and unexpected weight loss. Hypoglycaemia, diabetic ketoacidosis, hyperosmolar, and hyperglycaemic nonketotic syndromes are amongst the acute complications, while sub-acute complications include thirst, lack of energy, polyuria, visual blurriness, and weight loss (Kumar and Clark, 2002). Chronic hyperglycaemia causes glycation of body proteins, which in turn leads to complications that may affect the eyes, kidneys, nerves, and arteries (Agarwal et al., 2012; Semba et al., 2014). Many of the acute effects of this disease can be controlled by insulin replacement therapy; however, it has long-term adverse effects on blood vessels, nerves, and other organs of the body (Ojezele and Abatan, 2011). Globally in 2017, an estimated 8.8 percent of the adult population worldwide had diabetes and this figure is projected to rise to 9.9 percent by the year 2045 (Elflein, 2019). About 90 % of this population are affected with T2DM. According to the International Diabetes Federation (IDF), an estimated 15.5 million and 19 million adults aged 20-79 years were living with diabetes in African Region in 2017 and 2019 respectively; this is projected to increase by 143 % by 2045 (IDF, 2020).

Plants are endowed with various phytochemical molecules such as vitamins, terpenoids, phenolics, lignins, stilbenes, tannins, flavonoids, quinones, coumarins, alkaloids, amines, betalains and other metabolites which are rich source of free radical scavengers (Gracelin et al., 2012). From ancient period, people have been using medicinal plants for the treatment of diabetes and WHO estimates that 80 % of the populations presently use herbal medicine for primary health care (Atmakuri and Dathi, 2010). Anti-diabetic plants have the ability of inhibiting the intestinal absorption of glucose and also restore the function of damaged pancreatic tissue brought about oxidative damage, thereby increasing the secretion of insulin (Malviya et al., 2010). Many studies have confirmed the benefits of medicinal plants with hypoglycaemic effects in the management of diabetes mellitus.

With the increasing incidence of diabetes in rural populations throughout the world, coupled with the inability of current therapies to control the metabolic defects of the disease and their pathological consequences, as well as the great expense of modern therapy, the demand by patients to use natural products with antidiabetic activity is increasing, since access to traditional medicines is less constraining and more affordable (Kamgang et al., 2008; Afolayan and Sunmonu (2010); Ocho-Anin et al., 2010). Furthermore, plant drugs are frequently considered to be less toxic and freer from side effects than synthetic ones. Integral components of drug development includes the selection of plants on the basis of traditional reputation for efficacy in treatment of diabetes or other diseases and then carry out biological and chemical analysis to produce pure compounds. This fact has encouraged the continuing search for new plant-derived anti-diabetic drugs. It is important therefore, to investigate the anti-diabetic activities of some of the plants used locally for diabetic treatment, isolate and characterize the active anti-diabetic constituents in them, which could then form basis for the development of novel drug for diabetes therapy.

2.0 Materials and Methods

2.1 Plant materials

The leaf parts of three (3) medicinal plants which have been shown to be used traditionally for the treatment of diabetes in Nigeria, were collected in Beiji and its environs, Niger State. The plants were identified by a Botanist in the Department of Biological Sciences, Federal University of Technology, Minna, Niger state. They were thereafter authenticated at National Institute for Pharmaceutical Research and Development (NIPRD), Abuja and voucher specimen number allotted. The plants were; Bauhinia reticulata (NIPRD/H/7048), Cassytha filiformis (NIPRD/H/7044) and Daniella oliveri (NIPRD/H/7051).
2.2 Experimental Animals

Albino mice used for the study were purchased from the Animal facility centre of the Department of Pharmacology and Toxicology, Ahmadu Bello University, Zaria, Nigeria. The animals were fed ad libitum with standard feed and had free access to water. They were also maintained under standard conditions of humidity, temperature and 12 hrs light/darkness cycles. The animals were allowed to acclimatize for two weeks before the commencement of the study. A standard protocol on Good Laboratory Practice (GLP) regulations (ENV/MC/CHEM (98) 17, 1998) for animal care was adopted. The principle of laboratory animal care (NIH Publication No. 85-23, 1985) was also followed in this study.

2.3 Reagents and Chemicals

Streptozotocin and Nicotinamide were purchased from Sigma Aldrich chemicals (St Louis U.S.A). All other chemicals and reagents used were of analytical grade.

2.4 Extraction of plant samples

The leaves of the plant samples were washed with distilled water, cut into small pieces, air-dried at room temperature, and pulverized into fine powder before extraction. Reflux extraction method was used, as described by Ogbadoyi et al. (2007), with slight modification. Fifty grams (50 g) of the dried powder of each plant sample was exhaustively extracted using ethanol for 2 h. Extracts were filtered hot using muslin cloth and whatmann filter paper. Solvent was evaporated and concentrated using water bath at 40 °C. The dried extracts were transferred into sterile sample bottles and stored in the refrigerator until required for use.

2.5 Partitioning of selected active crude plants’ extracts

Out of the three (3) ethanol crude plant extracts tested for in vivo anti-hyperglycaemic activity, Bauhinia reticulata (BR) and Cassytha filiformis (CF) showed promising antihyperglycemic activity. As such, both of them were partitioned for different components with solvents of graded polarities, using the methods described by Usman et al. (2013) and Abu et al. (2017), with slight modification. Partitioning was carried out using n-hexane, ethyl acetate and methanol. One gram (1 g) of the extract was dissolved in 100 mL of 50 % methanol. This was put in a 1000 mL separating funnel and then 100 mL of 100 % n-hexane was added. This was used for separating the nonpolar compounds. The separating funnel was shaken for 2 minutes before being allowed to settle down for 2 hours at room temperature. The lower phase which was the 50 % methanol layer was withdrawn from the funnel’s stopcock, while the n-hexane layer on the upper part was poured out from the funnel’s stopper. The procedure was repeated at least 3 times, until the n-hexane layer became very clear and the total hexane layer was collected. The collected n-hexane layers were filtered and concentrated under vacuum in rotary evaporator to give the n-hexane partitioned fraction. Ethyl acetate was added to the methanol layer obtained after the partitioning with n-hexane with 1:1 ratio (v/v) in the separating funnel. The funnel was shaken for 2 minutes and left at room temperature for 2 hours to settle down. The methanol layer settled at the bottom layer was withdrawn from the stopcock, while the ethyl acetate layer settled on the upper layer was collected from the funnel’s stopper.

The procedure was repeated for at least 3 times until the ethyl acetate layer became clear and total ethyl acetate layer was collected. The collected ethyl acetate layer was filtered and concentrated under vacuum in rotary evaporator to give the ethyl acetate partitioned fraction. The concentrated and dried 50 % methanol layer (methanol partitioned fraction) as well as the n-hexane and ethyl acetate partitioned fractions were kept in sterile sample bottles and tested for in vivo anti-hyperglycaemic activity.
2.6 Induction of experimental diabetes

Nicotinamide was prepared in NaCl, while Streptozotocin was dissolved in 0.1 M cold citrate buffer, pH 4.5 (Archana et al., 2001). The animals were fasted overnight; the initial body weight and blood glucose levels were taken before the commencement of induction. The mice (diabetic control and test groups) after an overnight fast were injected intra-peritoneally with streptozotocin solution at a dose of 60 mg per kg body weight, 30 minutes after the intraperitoneal administration of 80 mg/kg body weight of solution of Nicotinamide. 72 hours after the induction of experimental diabetes, Accu-chek® glucometer was used to check the blood glucose level and the animals with plasma glucose of more than 200 mg/dL were considered diabetic and were allowed for 1 week diabetic stabilization before the commencement of treatment.

2.7 Screening for in vivo anti-hyperglycaemic activity of the crude and partitioned fractions of plant extracts

Albino mice of both sexes (weighing between 23.60±0.60 to 29.97±1.87 g) were randomly allocated to negative/positive control and treatment groups of five animals each. Fasting blood glucose (FBG) concentration was first determined in overnight fasted diabetes induced mice by the enzymatic glucose oxidase method using a commercial glucometer (Accu-chek® Active, Roche diagnostic, Mannheim, Germany). Thereafter, following which the plants’ extracts and fractions (100 and 200 mg/kg b.w.) were administered. The negative control group (diabetic untreated) and the normal control group (not induced) received 0.2 mL 20 % Dimethyl sulfoxide (DMSO) in place of the extract/fraction. Similarly, a standard antidiabetic drug (Metformin) was administered orally (100 and 200 mg/kg b.w.) to the positive control group. The plants’ extracts, fractions, standard drug and 20 % DMSO were administered orally using gastric cannula. Treatment was carried out for a period of four weeks and blood glucose level was monitored on weekly basis throughout the period. The percentage decrease in fasting blood glucose (FBG) level was calculated using the following method:

\[
\% \text{ decrease in FBG} = \frac{\text{FBG 1 wk. stab.} - \text{FBG 4 wks trt.}}{\text{FBG 1 wk stab.}} \times 100
\]

Where; FBG 1 wk. stab. = FBG after 1 week of diabetes stabilisation (before commencement of treatment). FBG 4 wks trt. = FBG after 4 weeks of treatment

2.8 Statistical Analysis

Data were calculated as mean ± SEM and were analysed statistically using One-way ANOVA followed by Duncan multiple comparison test and values of p<0.05 were considered significant. Statistical Package for Social Sciences (SPSS), 20th version was used.

3.0 Results

3.1 In vivo Anti-hyperglycemic Activity of Crude Plant Extracts

The mean FBG levels of streptozotocin-induced diabetic mice treated with ethanol crude extracts of Bauhinia reticulata, Cassytha filiformis and Daniella oliveri are shown in Figures 1 and 2 at dose of 100 mg/kg b.w. and 200 mg/kg b.w. respectively. The crude extracts had moderate dose dependent anti-hyperglycaemic activity, with the highest percentage decrease in FBG (52.05±0.53 %) displayed by the group treated with 200 mg/kg b.w of crude extract of Bauhinia reticulata (Table 1).
Figure 1: Effect of ethanol extracts of *B. reticulata*, *C. filiformis* and *D. oliveri* leaves on FBG levels of streptozotocin-induced diabetic mice at dose of 100 mg/kg b.w. BR: *B. reticulata*, CF: *C. filiformis*, DO: *D. oliveri*, MET: Metformin (Standard drug), BI: Before induction, 1 wk stab.: After 1 week of stabilization.

Table 1: Percentage Decrease in FBG Levels of Diabetic Mice after 4 Weeks of Treatment with Ethanol Extracts of *B. reticulata*, *C. filiformis* and *D. oliveri* Leaves

<table>
<thead>
<tr>
<th>Crude plant extracts</th>
<th>Doses (mg/kg b.w.)</th>
<th>Percentage decrease in FBG levels (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. reticulata</em> (BR)</td>
<td>100</td>
<td>44.57±0.59&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>52.05±0.53&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>C. filiformis</em> (CF)</td>
<td>100</td>
<td>42.58±0.57&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>49.11±0.80&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>D. oliveri</em> (DO)</td>
<td>100</td>
<td>35.88±0.21&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>41.99±0.57&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Standard drug (Metformin)</td>
<td>100</td>
<td>49.73±0.69&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>50.88±0.85&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Negative control</td>
<td>0.2 mL 20 % DMSO</td>
<td>-33.14±0.11&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Normal control</td>
<td>0.2 mL 20 % DMSO</td>
<td></td>
</tr>
</tbody>
</table>

Values are presented in means ± standard error of five replicates, with the same superscript alphabets on the same column not significantly different at \( p>0.05 \)
Figure 2: Effect of ethanol extracts of *B. reticulata*, *C. filiformis* and *D. oliveri* leaves on FBG levels of streptozotocin - induced diabetic mice at dose of 200 mg/kg b.w. BR: *B. reticulata*, CF: *C. filiformis*, DO: *D. oliveri*, MET: Metformin (Standard drug), BI: Before induction, 1 wk stab.: After 1 week of stabilization

### 3.2 In vivo anti-hyperglycemic activity of partitioned fractions of selected crude plant extract

The mean FBG levels of streptozotocin-induced diabetic mice treated with partitioned fractions of *B. reticulata* are shown in figures 3 and 4 at dose of 100 mg/kg b.w. and 200 mg/kg b.w. respectively, while that of *C. filiformis* are shown in figures 5 and 6 at dose 100 mg/kg b.w. and 200 mg/kg b.w. The partitioned fractions generally had appreciable dose dependent anti-hyperglycaemic activity, with the highest percentage decrease in FBG (55.91±0.20 %) displayed by the group treated with 200 mg/kg b.w of ethyl acetate partitioned fraction, although, there was no significant difference at p>0.05 with the group treated with 200 mg/kg b.w of *n*-hexane partitioned fraction (55.80±0.68 %) (Table 2).
Figure 3: Effect of solvent partitioned fractions of crude ethanol extract of *B. reticulata* leaves on FBG levels of streptozotocin-induced diabetic mice at dose of 100 mg/kg b.w. *n*-hex: *n*-hexane fraction, EtAc: Ethylacetate fraction, Meth: Methanol fraction, MET: Metformin (Standard drug), BI: Before induction, 1 wk stab.: After 1 week of stabilization

Table 2: Percentage decrease in FBG levels of diabetic mice after 4 weeks of treatment with partitioned fractions of crude extract of *B. reticulata* leaves

<table>
<thead>
<tr>
<th>Solvent partitioned fractions</th>
<th>Doses (mg/kg b.w.)</th>
<th>Percentage decrease in FBG levels (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-hexane</td>
<td>100</td>
<td>45.03±0.84&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>55.80±0.68&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ethylacetate</td>
<td>100</td>
<td>48.15±0.53&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>55.91±0.20&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Methanol</td>
<td>100</td>
<td>42.07±0.70&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>44.50±0.15&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Metformin</td>
<td>100</td>
<td>60.62±0.81&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>65.90±0.87&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>Negative control</td>
<td>0.2 mL 20 % DMSO</td>
<td>-5.39±0.64&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Normal control</td>
<td>0.2 mL 20 % DMSO</td>
<td></td>
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</tbody>
</table>

Values are presented in means ± standard error of five replicates, with the same superscript alphabets on the same column not significantly different at p>0.05

Figure 4: Effect of solvent partitioned fractions of crude ethanol extract of B. reticulata leaves on FBG levels of streptozotocin-induced diabetic mice at dose of 200 mg/kg b.w. n-hex: n-hexane fraction, EtAc: Ethylacetate fraction, Meth: Methanol fraction, MET: Metformin (Standard drug), BI: Before induction, 1 wk stab: After 1 week of stabilization

Table 3: Percentage decrease in FBG levels of diabetic mice after 4 weeks of treatment with partitioned fractions of crude extract of C. filiformis leaves

<table>
<thead>
<tr>
<th>Solvent partitioned fractions</th>
<th>Doses (mg/kg b.w.)</th>
<th>Percentage decrease in FBG levels (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-hex</td>
<td>100</td>
<td>42.21±0.90b</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>42.07±0.49b</td>
</tr>
<tr>
<td>Ethylacetate</td>
<td>100</td>
<td>45.09±0.12c</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>47.64±0.19d</td>
</tr>
<tr>
<td>Methanol</td>
<td>100</td>
<td>42.97±0.38b</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>47.16±0.71d</td>
</tr>
<tr>
<td>Metformin</td>
<td>100</td>
<td>60.62±0.81e</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>65.90±0.87f</td>
</tr>
<tr>
<td>Negative control</td>
<td>0.2 mL 20 % DMSO</td>
<td>-5.39±0.064a</td>
</tr>
<tr>
<td>Normal control</td>
<td>0.2 mL 20 % DMSO</td>
<td></td>
</tr>
</tbody>
</table>

Values are presented in means ± standard error of five replicates, with the same superscript alphabets on the same column not significantly different at p>0.05
**Figure 5:** Effect of partitioned fractions of crude ethanol extract of *C. filiformis* leaves on FBG levels of streptozotocin-induced diabetic mice at dose of 100 mg/kg b.w. *n*-hex: *n*-hexane fraction, EtAc: Ethylacetate fraction, Meth: Methanol fraction, MET: Metformin (Standard drug), BI: Before induction, 1 wk stab.: After 1 week of stabilization

### 4.0 Discussion

Medicinal plants are among the most common sources and mainstay options of medicines for about 75–80 % of the World's population (Prabhakar and Doble, 2008; Molalegn *et al*., 2020). Modern pharmaceutical industry relies mainly on the diversity of phytochemicals in medicinal plants for the discovery of new therapeutic agent for the treatment of various ailments (Anand *et al*., 2019). Therefore, screening of extracts from medicinal plants are necessary steps for the synthesis of useful drugs (Altemimi *et al*., 2017). The results obtained in this study confirm the reports of the antidiabetic potentials of various parts of the three plants used in the study.
Glucose is a primary fuel source for humans and animals. The liver plays a pivotal role in regulating the uptake and storage of glucose through various metabolic pathways (Sharabi et al., 2015). Thus, the maintenance of glucose homeostasis is vital in the prevention of hyperglycemia, diabetes mellitus and its associated complications (Farzaei et al., 2015). Therefore, the elevation of fasting blood glucose level after the administration of streptozotocin in the present study suggests hyperglycaemic condition as a consequence of the accumulated glucose in the blood.

The reduction in the level of fasting blood glucose by the crude extract and the fraction of the plant evaluated in this study suggest the anti-hyperglycemic effect in streptozotocin induced hyperglycemia. The exhibited antidiabetic activities by the plant extracts could be by inhibiting renal glucose reabsorption (Eddouks et al., 2002), stimulation of insulin secretion from beta cells of islets or/and inhibition of insulin degradative processes, reduction in insulin resistance (Pulok et al., 2006), regenerating and/or repairing pancreatic beta cells (Mohamed et al., 2006; Oyagbemi et al., 2014).

Results of the present study are in agreement with the findings from previous studies; Etuk et al. (2010) and Oyagbemi et al. (2014) have both reported the antidiabetic potentials of B.
**In vivo antihyperglycaemic activity of crude and partitioned fractions of selected medicinal plants.**

**5.0 Conclusion**

This study has revealed that the crude ethanol extracts of *Cassytha filiformis*, *Bauhinia reticulata* and its n-hexane and ethylacetate fractions have been able to decrease the FBG levels of diabetic mice within the normal range which is less than 110 mg/dL. The anti-diabetic activity demonstrated by the crude extracts and partitioned fractions could be attributed to the presence of some plant secondary metabolites such as phenols, alkaloids, flavonoids, tannins, saponins and their derivatives. The long term goal of this study is to be able to develop novel antidiabetic drug(s) from medicinal plants. To complement the work done so far, it is recommended that further bioassay-guided purification be carried out using chromatographic techniques, in order to isolate and characterise the active compound(s), check their drugability and then channel them into drug discovery processes.

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**Author contributions**

Authors OEO and UMB conceived the study. OEO, KAY and UMB participated in the design and quality assessment of the study. Author MA and UMB partook in the selection and extraction of the plant samples used. Author UMB reconstituted the chemicals/reagents used, as well as carried out the experiment, with the assistance of the Laboratory Technologists. UMB carried out the statistical analysis and drafted the manuscript, with significant input from OEO. All authors proofread the manuscript and made inputs. All authors approved the final version of the article for publication.

**Conflicts of interest**

The authors declare that they have no competing interests.
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