Antidiabetic effects of partitioned methanol extract of
*Boswellia dalzielii* (Frankincense tree) on rats

James Yakubu*, Umar Tanko Mamza, Victor Musa Balami, Asinamai Ndai Medugu, Fanna Inna Abdulrahman, Olufunke Adebola Sodipo

**ABSTRACT**

Medicinal plants have been the major source of bioactive phytochemicals employed for the treatment and management of disease since time immemorial. The present study was aimed at investigating the anti-diabetic potentials of various partitioned portions of crude methanol extract of *Boswellia dalzielii* in alloxan-induced diabetic rats. Fresh leaf of *Boswellia dalzielii* was air-dried, pulverized and extracted using cold maceration method with 85% methanol and concentrated to dryness. The crude methanol extract was partitioned using n-hexane, chloroform, ethylacetate and n-butanol to afford portions encoded BMENH, BMECM, BMEEA, BMENB respectively, and were screened for phytochemicals. The portions were evaluated for their anti-diabetic effects on alloxan-induced rats. The phytochemical studies of the crude methanol leaf, stem and root bark extracts revealed the presence alkaloids, cardiac glycoside, flavonoids, saponins, tannins and terpenoids. The partitioned crude methanol leaf extract yielded 14.12% (%w/w) n-hexane, 6.85% (%w/w) chloroform, 4.18% (%w/w) ethyl acetate and 36.40% (%w/w) n-butanol extracts respectively. Antidiabetic activity of fractions BMEH, BMEC, BMEEA, BMENB at doses of 200 and 400 mg/kg bd wt. produced significant (p<0.05) % inhibitions of glycaemia of 13.51, 18.91, 53.36 and 71.21 respectively. Asinamai Ndai Medugu

**INTRODUCTION**

Nature has been the source of medicine for thousands of years in the maintenance of human health since ancient time [1]. WHO supports the use of effective and safe remedies and accepts traditional medicine as a valuable resource for primary health care [2]. In addition, majority of the populations in developing countries still have limited access or no access, especially those in remote areas, to modern medicines. Instead traditional medicines were employed for a range of illness including diabetic complications [3]. As a result of renewed interest from western countries in herbal medicines and the increasingly urgent need to develop new effective drugs, traditionally used medicinal plants have recently received the attention of the pharmaceutical and scientific communities [4].

The management of diabetes is a global problem and successful treatment has not yet discovered. More than 50 % of all the drugs currently in use are of natural product origin [5]. Higher plants have been the source of medical agents since earliest time and continue to play a dominant role in the healthcare industry [6]. The physiological effect of medicinal plants lies within some of the chemical substances produced by the plants during secondary metabolism. These are called phytochemicals. These secondary metabolites are the compounds in responsible plants for their bioactive properties based on literatures [7].

Diabetes mellitus is a group of metabolic disorder characterized by hyperglycaemia resulting from a defect in insulin secretion, its action, or both. It is basically of two types, namely: Type I, which is an insulin dependent diabetes that affects only 5 % of the diabetic population, while a Type II, which is non-insulin dependent, usually develops in adults over the age of 40 [7] and covers about 95% of the remaining of the diabetic population. Currently, the available treatment for diabetes includes the use of insulin and various oral hypoglycaemic drugs such as sulfonylureas, metformin, glucosidase inhibitors, troglitazone, etc. These drugs have however been reported to produce serious side effects such as liver problems, lactic acidosis and diarrhoea [8]. Diabetes is currently the fifth leading cause of death and has affected around 422 million people [7] and the number of those affected is increasing daily. The World Health Organization...
identified and authenticated by Prof. S.S. Sanusi, a Plant Taxonomist in the Department of Biological Science, University of Maiduguri, Borno State, Nigeria to be of *Boswellia dalzielii*, given a voucher specimen of #341 and deposited at the herbarium of the Postgraduate Research Laboratory of Chemistry Department, University of Maiduguri, Maiduguri, Borno State. The fresh plant samples were cleaned and air-dried under shade at room temperature for ten (10) days and were rendered free of foreign material through manual picking. The air-dried plant materials were pulverized using a mortar and pestle and then subjected to these analyses below.

**Plant Extraction**

The powdered plant material (2 kg) each of the leaf was macerated using 7.5 litres of 85 % methanol for 72 hours with periodic shaking and allowing to stand at room temperature for a proper dissolution of soluble plant chemicals. The liquid mixture of the extract was filtered using a clean muslin followed by filtration using 200mm diameter of Watmann No. 1 filter paper. The crude extracts were concentrated to dryness by the use of rotary evaporator at 40°C. The crude methanol extract was weighed, coded BMLE - *Boswellia dalzielii* methanol leaf extract.

**Partitioning of Methanol Extract of *B. dalzielii***

Three hundred grammes (300g) of the crude leaf methanol extract was further partitioned exhaustively using n-hexane, chloroform and ethyl acetate sequentially. The residue was suspended in distilled water and then partitioned with n-butanol. The fractions obtained were evaporated to dryness at reduced pressure using a rotary evaporator and then coded BMENH, BMECM, BMEEA, BMENB, – as n-hexane, dichloromethane, ethyl acetate and n-butanol respectively. The percentage (%) yield, colour, texture and weight of each partitioned portion were noted, labelled and preserved in a dessicator until required for further studies.

**Preliminary Phytochemical Screening**

The partitioned fractions of the methanolic leaf extract of *Boswellia dalzielii* were screened qualitatively for phytochemical constituents using standard procedures [22]. These include Alkaloids, anthraquinones, cardiac glycosides, flavonoids, tannins, terpenes and saponins.

**Pharmacological Investigations of the Partitioned Portions of Crude Methanol Leaf Extract of *Boswellia dalzielii***

**Experimental Animals**

All the experiments performed on laboratory animals in this study followed standard procedure for the treatment of animals. The animals were handled according to the International Guiding Principle for Biomedical Research involving animals [23].

A total of one hundred and sixty-four (164) albino rats weighing (100-180) g of both sexes were obtained from the Animal House of the Faculty of Pharmacy, University of Maiduguri, Borno State. The study was conducted at the Department of Pharmacology, Faculty of Veterinary Medicine, University of Maiduguri, Maiduguri, Borno State. The animals were fed with standard feed and allowed water, at their will.

**Materials and Methods**

**Sample Collection, Identification and Preparation**

Fresh leaves stem and root barks of *Boswellia dalzielii* were collected from Gulantabar, Song Local Government Area, Adamawa State, Nigeria in the month of January, 2019. The plant materials were transported in a wool sacked-bag to the University of Maiduguri,
Ethical Approval

All experiments were conducted in accordance with the National Institute of Health Guidelines for the Care and use of Laboratory Animals (NIH Publications No.80-23) as revised in 1996.

Extract Fractions and Alloxan Preparation

The partitioned portions of crude methanol extract and alloxan (2 g) each were dissolved in 10 ml distilled water to give a stock solution of 200 mg/ml.

Volume to be administered = Dose x Body Weight in Kg
Concentration of the Extract in mg

Test for Hypoglycaemic Activity

The animals were fasted for 12 hr but were allowed access to water before and throughout the duration of the experiment. The blood of each rat was withdrawn from the tip of the tail of each rat under mild anesthesia and the fasting blood glucose (FBG) was estimated with a blood glucose meter (Accu-Check, Roche, Germany) at the end of their fasting period at a time taken of zero (0 hr).

Evaluation of Extracts Activity in Alloxan-induced hyperglycaemic Rats

Method described by Uzor et al. [24] and Ezeigbo [25] were adopted in this study, with little modification. The animals were fasted for 12 hrs with water ad libitum and injected intraperitoneally with freshly prepared alloxan monohydrate (150 mg/kg) in ice-cold 0.9% saline (NaCl) solution. They were given 5 ml of 10% dextrose solution to overcome the drug induced hypoglycaemia and were provided with standard laboratory diet ad libitum after one hour. The FBG was checked before and 72 hrs after alloxan injection by withdrawing blood from the tip of the tail of each rat under mild anesthesia. The FBG was measured as described above. The animals were considered diabetic when the FBG is raised beyond 200 mg/dl. They were segregated into five (5) groups of four animals in each. Group I served as the normal control and received vehicle (normal saline, 2 ml/kg, p.o.). Blood glucose concentration was measured after 0, 1 hr, 3 hrs, 5 hrs and 7 hrs of administration of single dose of each of the treatments.

Statistical Analysis

Results of pharmacological studies were analysed using GraphPad Prism, 2016 Model, Version 7.0 for windows. One-way Analysis of Variance (ANOVA) test followed by Dunnet’s Multiple Comparison test was used to analyse and compare the results at a 95% confidence level. Results were expressed as mean ± standard error of mean (SEM).

RESULTS

The Partitions Profile of Crude Methanol Leaf Extract of B. dalzielii

The crude methanol leaf extract of B. dalzielii which was partitioned with n-hexane, chloroform, ethyl acetate and n-butanol with colours ranging from dark green colour of the n-hexane fraction, to brown of the n-butanol, and were mostly gummy masses except for the n-butanol fraction which was powdery. The n-butanol fraction also had the highest yield (36.40 %), while n-hexane, chloroform and ethyl acetate had 14.12 %, 6.85 %, and 4.18 % respectively. The result of the extraction profile is shown on Table 1:

Table 1: The Partitioning Profile of B. dalzielii Methanol Leaf Extract

<table>
<thead>
<tr>
<th>S/N</th>
<th>Extract</th>
<th>Mass (g)</th>
<th>% Yield (%)</th>
<th>Colour</th>
<th>Texture</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>n-hexane</td>
<td>42.37</td>
<td>14.12</td>
<td>Dark green</td>
<td>Gummy</td>
</tr>
<tr>
<td>2</td>
<td>Chloroform</td>
<td>20.55</td>
<td>6.85</td>
<td>Dark green</td>
<td>Gummy</td>
</tr>
<tr>
<td>3</td>
<td>ethyl acetate</td>
<td>12.53</td>
<td>4.18</td>
<td>Greenish grey</td>
<td>Gummy</td>
</tr>
<tr>
<td>4</td>
<td>n-butanol</td>
<td>109.20</td>
<td>36.40</td>
<td>Reddish brown</td>
<td>Powdery</td>
</tr>
</tbody>
</table>

Phytochemical Constituents’ Analysis of Partitioned Portions of Methanol Leaf Extract of B. dalzielii

The phytochemical screening of the partitioned portions of the crude methanol leaf extract of B. dalzielii with n-hexane, chloroform, ethyl acetate and n-butanol revealed the presence of cardiac glycosides, flavonoids, saponins, tannins and terpenoids in the n-butanol fraction while n-hexane had the least phytochemicals present. The result of the phytochemical screening of the gradient extraction is shown in Table 2:

Table 2: Photochemical screening of partitioned portions (chloroform, n-hexane, ethyl acetate and n-butanol) of methanol crude leaf extract of B. dalzielii

<table>
<thead>
<tr>
<th>S/N</th>
<th>Phytochemical Test</th>
<th>BMEH</th>
<th>BMEC</th>
<th>BMEE</th>
<th>BMEB</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Test Tor Carbohydrates</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Test for Tannins</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Test for Steroids/Triterpenes</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Test for Flavonoids</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Test for Saponins</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Test for Steroidal Nucleus/cardiolides</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Test for Terpenoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Key: BMEH – n-hexane partitioned portion of methanol leaf extract of Boswellia dalzielii
BMEC – chloroform partitioned portion of methanol leaf extract of Boswellia dalzielii
BMEE – ethyl acetate partitioned portion of methanol leaf extract of Boswellia dalzielii
BMEB – n-butanol partitioned portion of methanol leaf extract of Boswellia dalzielii
Effect of n-Hexane, Chloroform, Ethylacetate and n-Butanol Partitioned Portions of crude Methanol Extract of B. dalzielii on Alloxan-induced Wistar Strain Rats

The wet partition method for fractionation of the methanol leaf extract of B. dalzielii afforded BMEH, BMEC, BMEE and BMEB which were tested for antidiabetic activity at two dose levels of 200 mg/kg and 400 mg/kg and the results shown in Table 4.17, Table 4.18, Table 4.19 and Table 4.20.

The fraction encoded BMEH at 200 mg/kg and 400 mg/kg produced a non-significant reduction (14.87% and 13.51% respectively) in the FBG of the animals with reductions of 15.84 and 18.91% at 200 mg/kg and 400 mg/kg doses respectively. 52.68%.

The BMEC produced a dose-dependent significant (P<0.05) reduction in the FBG of the animals with reductions of 15.84 and 18.91% at 200 mg/kg and 400 mg/kg doses respectively.

The antidiabetic activity of both BMEH and BMEC are however, lower than that of BMEE at the same doses of 200 mg/kg and 400 mg/kg which had a remarkable significant decrease of 50.25% and 53.36%, equaling the effect of the standard drug glibenclamide (52.68%) at 2.0 mg.

Similar pattern was also observed for the BMEB fraction, but had the most remarkable significant % reduction of FBS levels in alloxan induced diabetes in rats, which produced dose-dependent non-significant (P<0.05) reduction in the blood glucose (66.16% and 71.21% for 200 mg/kg and 400 mg/kg respectively) after 9 hrs when compared to the other fractions stated above, the -ve control (diabetic rats) (5.32%), and standard control drug glibenclamide (52.68%) used for this study at 2.0 mg concentration.

The results show that the various fractions possess antidiabetic activity, however, the activity resides more with the n-butanol fraction.

Table 4.17: Effect of n-hexane partitioned portion of crude methanol extract of Boswellia dalzielii on alloxan-induced Wistar strain rats

<table>
<thead>
<tr>
<th>S/N</th>
<th>Treatment (mg/kg)</th>
<th>Basal FBG (mg/dL)</th>
<th>Fasting Blood Glucose (FBG) concentration (mg/dL)</th>
<th>% Inhibition of Glycaemia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Time (hr) after treatment</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>Normal</td>
<td>73.50±5.24</td>
<td>73.00±4.92, 75.75±5.02, 72.75±4.33, 70.50±4.65, 78.00±2.74</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>D.C (150)</td>
<td>73.50±5.24</td>
<td>263.00±16.50, 286.50±25.18, 262.00±10.80, 251.80±4.33, 249.00±9.25</td>
<td>5.32</td>
</tr>
<tr>
<td>3</td>
<td>200</td>
<td>75.00±3.24</td>
<td>465.50±38.17, 419.00±38.76, 413.50±41.65, 402.80±42.06, 396.30±40.91*</td>
<td>14.87</td>
</tr>
<tr>
<td>4</td>
<td>400</td>
<td>72.00±4.08</td>
<td>503.00±4.73, 495.50±4.40, 486.00±36.22, 471.00±41.24, 435.30±42.85*</td>
<td>13.51</td>
</tr>
<tr>
<td>5</td>
<td>Glibenclamide (2.0mg)</td>
<td>305.70±32.69, 305.00±32.16, 240.30±46.48, 215.30±4.33, 145.50±28.93*</td>
<td>52.68</td>
<td></td>
</tr>
</tbody>
</table>

Results expressed as Mean ±SEM (n=4). *P<0.05, as compared with control group (One way, ANOVA followed by Dunnet's t-test, 2 sided). % Inhibition of glycaemia denote percentage reduction of blood glucose from 0 h. Basal FBG=FBG before induction of diabetes; D.C = diabetic control.

Table 4.18: Effect of chloroform partitioned portion of crude methanol extract of Boswellia dalzielii on alloxan-induced Wistar strain rats

<table>
<thead>
<tr>
<th>S/N</th>
<th>Treatment (mg/kg)</th>
<th>Basal FBG (mg/dL)</th>
<th>Fasting Blood Glucose (FBG) concentration (mg/dL)</th>
<th>% Inhibition of Glycaemia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Time (hr) meansSEM after treatment</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>Normal</td>
<td>73.50±5.24</td>
<td>73.00±4.92, 75.75±5.02, 72.75±4.33, 70.50±4.65, 78.00±2.74</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>D.C (150)</td>
<td>73.50±5.24</td>
<td>263.00±16.50, 286.50±25.18, 262.00±10.80, 251.80±4.33, 249.00±9.25</td>
<td>5.32</td>
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<tr>
<td>3</td>
<td>200</td>
<td>75.00±3.24</td>
<td>465.50±38.17, 419.00±38.76, 413.50±41.65, 402.80±42.06, 396.30±40.91*</td>
<td>14.87</td>
</tr>
<tr>
<td>4</td>
<td>400</td>
<td>72.00±4.08</td>
<td>503.00±4.73, 495.50±4.40, 486.00±36.22, 471.00±41.24, 435.30±42.85*</td>
<td>13.51</td>
</tr>
<tr>
<td>5</td>
<td>Glibenclamide (2.0mg)</td>
<td>305.70±32.69, 305.00±32.16, 240.30±46.48, 215.30±4.33, 145.50±28.93*</td>
<td>52.68</td>
<td></td>
</tr>
</tbody>
</table>

Results expressed as Mean ±SEM (n=4). *P<0.05, as compared with control group (One way, ANOVA followed by Dunnet's t-test, 2 sided). Figures in parenthesis denote percentage reduction of blood glucose from 0 h. Basal FBG=FBG before induction of diabetes; D.C = diabetic control.

Table 4.19: Effect of ethylacetate partitioned portion of crude methanol extract of Boswellia dalzielii on alloxan-induced Wistar strain rats

<table>
<thead>
<tr>
<th>S/N</th>
<th>Treatment (mg/kg)</th>
<th>Basal FBG (mg/dL)</th>
<th>Fasting Blood Glucose (FBG) concentration (mg/dL)</th>
<th>% Inhibition of Glycaemia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Time (hr) after treatment</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>Normal</td>
<td>73.50±5.24</td>
<td>73.00±4.92, 75.75±5.02, 72.75±4.33, 70.50±4.65, 78.00±2.74</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>D.C. (150)</td>
<td>73.50±5.24</td>
<td>263.00±16.50, 286.50±25.18, 262.00±10.80, 251.80±4.33, 249.00±9.25</td>
<td>5.32</td>
</tr>
<tr>
<td>3</td>
<td>200</td>
<td>75.00±3.24</td>
<td>260.30±15.97, 248.15±14.79, 243.00±13.86, 232.80±8.23, 220.00±6.17*</td>
<td>15.48</td>
</tr>
<tr>
<td>4</td>
<td>400</td>
<td>72.00±4.08</td>
<td>460.00±39.66, 435.00±44.05, 427.30±49.03, 432.80±8.23, 373.00±45.98*</td>
<td>18.91</td>
</tr>
<tr>
<td>5</td>
<td>Glibenclamide (2.0mg)</td>
<td>305.00±32.16, 305.00±32.69, 240.30±46.48, 215.30±4.33, 145.50±28.93*</td>
<td>52.68</td>
<td></td>
</tr>
</tbody>
</table>

Results expressed as Mean ±SEM (n=4). *P<0.05, as compared with control group (One way, ANOVA followed by Dunnet's t-test, 2 sided). % Inhibition of glycaemia denote percentage reduction of blood glucose from 0 h. Basal FBG=FBG before induction of diabetes; D.C = diabetic control.
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Table 4.20: Effect of n-butanol partitioned portion of crude methanol extract of *Boswellia dalzielii* on alloxxan-induced Wistar strain rats

<table>
<thead>
<tr>
<th>S/N</th>
<th>Treatment (mg/kg)</th>
<th>Basal FBG (mg/dL)</th>
<th>Fasting Blood Glucose (FBG) concentration (mg/dL)</th>
<th>% Inhibition of Glycaemia</th>
</tr>
</thead>
<tbody>
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<td>2</td>
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<td>1</td>
<td>Normal</td>
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<td>2</td>
<td>D.C (150)</td>
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</tr>
<tr>
<td>3</td>
<td>200</td>
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<tr>
<td>4</td>
<td>400</td>
<td></td>
<td></td>
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<tr>
<td>5</td>
<td>Glibenclamide</td>
<td>37.5±5.0</td>
<td>307.5±32.69</td>
<td>240.30±46.48</td>
</tr>
</tbody>
</table>

Results expressed as Mean ±SEM (n=4). *P<0.05, as compared with control group (One way, ANOVA followed by Dunnett’s test, 2 sided). % Inhibition of glycaemia denote percentage reduction of blood glucose from 0 h. Basal FBG=FBG before induction of diabetes; D.C=diabetic control.

DISCUSSION

The phytochemical studies of the methanol leaf, stem and root barks of *B. dalzielii* revealed chemical constituents such as alkaloids, cardiac glycosides, flavonoids, saponins, tannins and terpenoids. The presence of these phytochemicals in the plant parts are in agreement with the reports of Manza et al. [21] and Balogun et al. [28]. The reported hypoglycemic activity of the plant extract could be associated with these phyto-compounds present. These compounds are also known to exert pharmacological and antagonistic effects [27]. Terpenes have been reported to pose important biological activities, such as analgesic [27], anticonvulsant [28], cardiovascular [29], antimalarial and antibacterial [30]. Alkaloids have pharmaceutical applications as anesthetics and CNS stimulants [30].

The glucose lowering effect by the partitioned portions of the methanol crude extract in a dose-independent manner may reflect uptake of the bioactive chemicals through saturable active transport [31]. Although, the n-butanol partitioned portion had a more remarkable antidiabetic activity. This could be due to high content of phenolic compounds such as flavonoids, saponins and tannins present in the fraction. These class of compound have been reported to possess excellent antidiabetic properties [32, 33, 34].

The possible mechanisms underlying the hypoglycemic activity exhibited by *B. dalzielii* could be inhibition of intestinal absorption of glucose, facilitation of glucose-induced insulin release, enhancement of peripheral glucose uptake, promotion of the regeneration of β-cell of islets of Langerhans as well as amelioration of oxidative stress [35]. Thus the order of the activity in in increase order of polarity n-hexane<chloroform<ethylacetate<n-butanol.

Diabetes mellitus is one of the rapidly growing endocrine disorders with major complications affecting populations living throughout the world [36]. The pathophysiological mechanisms are being scrutinized and the knowledge on heterogeneity and complexity of this disease is being advanced. Accordingly, the search for more appropriate therapy is also being under way. In line with that, traditional medicines are used substantially by diabetic patients across the globe [37] and medicinal plants have been identified to be a target for scientists to come up with newer and better therapeutic options in the future.

Moreover, during the past few years many phytochemicals responsible for anti-diabetic effects have been isolated from the plants, such as alkaloids, glycosides, flavonoids, saponins polysaccharides, glycolipids, peptidoglycans, amino acids etc. Quite a number of plants has been used traditionally in treatment of diabetes and some have been proven scientifically to have hypoglycemic activity [39, 40, 41, 42] and these compounds are responsible for the antidiabetic activity.

CONCLUSION

In conclusion, the present study revealed that, the n-butanol partitioned portion of crude methanolic leaf extract of *Boswellia dalzielii* was more efficacious than the n-hexane, chloroform, and ethylacetate in respective, while the portions had activity in increasing order of polarity. Therefore, further antidiabetic study on the n-butanol of the leaf extract of *Boswellia dalzielii* should be carried out in order ascertain the actual bioactive constituent(s) responsible for this effect.

Conflict of Interest

The authors declare no conflict of interest.

REFERENCES

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