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## Anti-Diabetic Property and Phytochemical Composition of Aqueous and Methanol Extracts of *Buchholzia coriacea* Seeds in Alloxan-Induced Diabetic Rats

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The present study was aimed at investigating the anti-diabetic properties and photochemical constituents of aqueous and methanol extracts of *Buchholzia coriacea* seeds in albino rats. Phytochemical screening was performed using standard procedures. Forty five adult male albino rats, placed in nine groups (A-I), of five in each group were used. Diabetes was induced intraperitoneally with a single dose of 100 mg kg<sup>-1</sup> b.wt., of alloxan monohydrate solution. After diabetes induction (in all the groups except I), groups A, B and C were orally administered 100, 200 and 400 mg kg<sup>-1</sup> b.wt., of aqueous extract respectively, D, E and F received 100, 200 and 400 mg kg<sup>-1</sup> b.wt., of methanol extract respectively, while G was given glibenclamide (antidiabetic drug) 2 mg kg<sup>-1</sup> b.wt., Groups H and I received distilled water only. Administration was done twice daily for fourteen days. Phytochemical screening of the extracts revealed the presence of alkaloids, flavonoids, tannins, saponins, anthraquinones and glycosides. The glucose concentration of all the groups treated with alloxan monohydrate was significantly higher (p<0.05) than the untreated one. After treatment with the extract and the drug (Glibenclamide), there was a significant reduction (p<0.05) in glucose concentration of the administered groups relative to the untreated. The effect was linearly dose-dependent. The difference between the groups given the extracts and the one treated with glibenclamide was significant (p<0.05). The effect of the aqueous extracts was not significantly different (p>0.05) from that of methanol extract. The result of this research indicates that aqueous and methanol extracts of *Buchholzia coriacea* seeds possess the ability to reduce blood glucose level and may be partly responsible for its application in treatment of diabetes.

**Key words:** Diabetes mellitus, alloxan monohydrate, *Buchholzia coriacea*, glibenclamide, photochemicals

## INTRODUCTION

Herbal medicine is the oldest form of health care known to mankind. The use of medicinal plants in the treatment of diseases has been in practice since ancient time in different parts of the world especially in Africa (Sofowora, 1993). Plants have always been the most vital source of drugs mainly because most plants are autotrophs and are able to synthesize a large variety of basic biochemical and organic substances such as carbohydrates, proteins, terpenes, steroids, alkaloids and glycosides (Nweze *et al.*, 2009).

The plant kingdom provides a tremendous reservoir of various chemical substances with potential therapeutic properties (Lawrence *et al.*, 2008). Generally, plants which produce constituents having medical values are designated as medicinal plants (Lawrence *et al.*, 2008). In addition, all plants that taste bitter are used as medicine (Sofowora, 1993).

Diabetes mellitus is characterized by insufficient blood levels of the hormone insulin. If the blood concentration of insulin is too low, muscle and liver cells do not absorb glucose, resulting to high blood glucose (hyperglycemia), impaired metabolism of fats and proteins, ketosis and possible diabetic coma (American Diabetes Association, 2007).

During the past 12 years, the World Health Organization has been collecting information on the prevalence of diabetes mellitus in adult communities worldwide. Within the age range of 30-64 years, diabetes was found to be absent or rare in some traditional communities in Melanesia, East Africa and South America. In communities of European origin, the prevalence of diabetes were in the range of 3-10% but migrant Indian, Chinese and Hispanic American groups were at higher risks (15-20%) (Lucas and Gilles, 2003).

Type 1 diabetes mellitus, Insulin-Dependent Diabetes Mellitus (IDDM) is an auto-immune disorder in which the insulin-producing  $\beta$ -cells of the pancreas are destroyed. Patients are prone to ketosis and dependent on insulin therapy (Rubenstein *et al.*, 2003; Merck and Co, 2010).

Type II diabetes mellitus Non Insulin Dependent Diabetes Mellitus (NIDDM) is the commonest form, accounting for 90% of patients with diabetes and affecting 5% of the world's population. Patients are typically obese adults. Insensitivity of the tissues to insulin (insulin-resistance) and inadequate pancreatic  $\beta$ -cell response to blood glucose are characteristic leading to overproduction of glucose by the liver and underutilization by the tissues. Although, initially controlled with diet and oral hypoglycemic, many patients eventually need supplemental insulin, making them insulin-requiring type II diabetics (Rubenstein *et al.*, 2003).

The long-term complications are steadily increasing the burden of the disease in some communities. For example, diabetes is now the commonest cause of new cases of irreversible blindness. Apart from the direct complications of diabetes, the disease is a risk factor for cardiovascular diseases (Lucas and Gilles, 2003).

The explosive increase in the prevalence of diabetes has been in the adult form of the disease, the Non Insulin Dependent Diabetes Mellitus (NIDDM). There is strong epidemiology evidence that this epidemic is related to the changing lifestyle: refined foods have replaced natural whole grain, high-fiber diets and there is lack of physical exercise (Lucas and Gilles, 2003).

A 2008 study completed in the U.S. found the number of American women entering pregnancy with pre-existing diabetes in expectant mothers has more than doubled in the past six years (Lawrence *et al.*, 2008). This is particularly problematic as diabetes raises the risk of complications during pregnancy as well as increasing the potential for the children of diabetic mothers to become diabetic in the future.

The use of insulin, glibenclamide, sulphonylurea and other hypoglycemic agents in the treatment of diabetes has lasted for years but there is an urgent need for new, effective and inexpensive drugs for the treatment of diabetes mellitus.

*Buchholzia coriacea*, commonly known as "Wonderful kola" is a perennial plant which grows as a tree. It belongs to the family *Capparaceae* and genus *Buchholzia* (Quattrocchi, 1999). The seeds gave the plant its common name because of its popular usage in traditional medicine (Keay, 1989) such as in the cure for diabetes. Previous researches carried out on this plant shows that it possess diverse medicinal potentials. Ezeja *et al.* (2011), reported the analgesic effects of *Buchholzia coriacea*. Okoli *et al.* (2010) investigated the hypoglycemic and anti-oxidant effects of the methanol of extract *Buchholzia coriacea* fruits. Fred-Jaiyesimi *et al.* (2011) reported the anthelmintic potentials of the chloroform and methanol extracts of *Buchholzia coriacea* seeds. Adjanohoun *et al.* (1996), reported that Cameroonians use *Buchhozia coriacea* as a remedy to relieve chest pain. The present study investigated the antidiabetic property and phytochemical composition of aqueous and methanol leaf extracts of the plant.

## MATERIALS AND METHODS

The equipment and reagents used were in good condition and of analytical grade respectively.

**Collection of plant material:** Fresh seeds of *Buchholzia coriacea* were plucked from a tree in March, 2013 from Aku, Igbo-etiti north local government area of Nsukka in Enugu state and was authenticated by Mr. A.O. Ozioko of the international centre for ethno medicine and drug development, Nsukka.

**Collection of animals:** The seeds were washed, cut into small bits and sun dried for 1 week. They were ground into powder with an electric mill machine and stored in airtight container until use. Forty five (45) adult male albino rats,

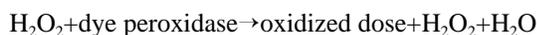
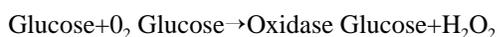
weighing 120-120 g, obtained from the department of pharmacology and veterinary medicine, university of Nigeria Nsukka were used.

**Preparation of extracts:** The seeds were dried under room temperature and ground into a powder. The 250 g of the seed powder was extracted with 1200 mL of distilled water. Similarly, 250 g of the seed powder was extracted with 1200 mL of methanol. They were stirred intermittently every 3-4 h for 48 h at room temperature. The mixtures were filtered using Whatman no. 1 filter paper. The filtrate obtained with distilled water was dried using rotor evaporator, while the filtrate from methanol was placed in a steel basin and allowed to evaporate in an atmosphere devoid of sunlight.

**Handling of animals:** Ethical approval was given by Research and Ethics Committee of Godfrey Okoye University, Enugu. All the animals were allowed free access to feed and water. The animals were divided into 9 groups of 5 rats in each. Hyperglycemia was induced by a single intraperitoneal injection of 100 mg kg<sup>-1</sup> b.wt., alloxan monohydrate (Qualikems, India) freshly dissolved in distilled water immediately before use to all the animals except those in group I. Animals with blood glucose level above 200 mg dL<sup>-1</sup> after 48 h were considered diabetic. They were treated with both methanol and aqueous extracts which were administered through oral intubation twice daily for 14 days. Groups A, B and C were administered 100, 200 and 400 mg kg<sup>-1</sup> b.wt., of aqueous extract respectively, D, E and F received 100, 200 and 400 mg kg<sup>-1</sup> b.wt., of methanol extract respectively, while G was given glibenclamide (antidiabetic drug) 2 mg kg<sup>-1</sup> b.wt., Groups H and I received distilled water only.

**Collection of samples:** After an overnight fasting, blood samples were collected by a snip-cut at the tip of the tail vein and blood sugar level was measured with an accu-check glucometer with strips. The strips were inserted one at a time in the glucometer.

**Principle:** Glucometer test strip is based on double sequential enzyme reaction in which an enzyme, Glucose Oxidase (GOD) converts glucose to hydrogen peroxide and glucuronic acid while peroxidase oxidizes the dye in the test strip to produce a color. The blood glucose level in mg dL<sup>-1</sup> will be displayed on the screen after 20 sec. The reduction equations are shown below:



**Phytochemical screening:** Chemical tests were carried out on the aqueous and methanol extracts using standard procedures

to identify the phytochemicals as described by Sofowora (1993), Trease and Evans (1989) and Harborne (1973).

**Test for tannins:** The 0.5 g of each sample was boiled in 20 mL of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration.

**Test for saponin:** The 2 g of the sample was boiled in 20 mL of distilled water in a water bath and filtered. The 10 mL of the filtrate was mixed with 5 mL of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

**Test for flavonoids:** The 5 g of the plant sample was heated with 10 mL of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 mL of dilute ammonia solution was added to the filtrate. A yellow coloration indicated presence of flavonoids.

**Test for cardiac glycosides (keller-killani test):** The 5 mL of the extracts was treated with 2 mL of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1 mL of concentrated sulphuric acid. A brown ring at the interface indicated positive result. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

**Test for anthraquinones:** The 0.5 g of the extract was boiled with 10 mL H<sub>2</sub>SO<sub>4</sub> and filtered while hot. The filtrate was shaken with 5 mL of chloroform. The chloroform layer was pipetted into another test tube and 1 mL of dilute ammonia was added. The resulting solution was observed for color change.

**Test for alkaloids:** The 0.5 g of the extracts was stirred in 5 mL of 1% HCl on a steam bath for 5 min. The mixture was then filtered using Whatman's no 1 filter paper. To 1 mL of the filtrate, 24 drops of Dragendoff's reagent was added. An orange color was observed indicating the presence of alkaloids.

**Statistical analysis:** The data obtained were subjected to statistical analysis. Means were compared using analysis of variance (ANOVA). Values having p<0.05 were considered significant.

## RESULTS AND DISCUSSION

The low percentage yields of the extractions (Table 1) could be as a result of the fact that most of the chemical

Table 1: Percentage yield of extraction

Mass of (g)	Mass of extract (g)	Volume of solvent (mL)	Yield of extract (%)	Color of sample extract
250	35	1200	14 (aqueous)	Dark brown
250	17	1200	6 (methanol)	Dark brown

Table 2: Phytochemical composition of the extracts

Phytochemical	Methanol	Aqueous
Alkaloids	+++	-
Flavonoids	+++	++
Tannins	++	+
Saponins	++++	+++
Cardiac glycosides	+	+
Anthraquinones	+	+

+++; Highly present, ++: Moderately present, +: Sparingly present, -: Not present

components of the seeds are not readily soluble in the solvents. The percentage of distilled water was significantly higher ( $p < 0.05$ ) than that of methanol. This is consistent with the result of methanol extraction of the plant seed by Ezeigbo (2011).

Phytochemical screening of the extracts revealed the presence of alkaloids, flavonoids, tannins, saponins, anthraquinones and cardiac glycosides (Table 2). The presence of the various identified phytochemicals may be responsible for the therapeutic usage of *Buchholzia coriacea* seeds in the treatment of various illnesses such as diabetes mellitus. Previous phytochemical analysis has reported the presence of alkaloids, saponins, cardiac glycosides and flavones glycosides (Adisa *et al.*, 2011). Saponins, anthraquinones, alkaloids, cyanogenic glycosides have been observed by Fred-Jaiyesimi *et al.* (2011). Tannins and cardiac glycosides were also reported by Mbata *et al.* (2009) in the seeds of *Buchholzia coriacea*.

The blood glucose level of the animals given alloxan monohydrate administration was significantly higher ( $p < 0.05$ ) than in the untreated ones and values were higher than 200 mg dL<sup>-1</sup>. This showed that alloxan monohydrate induced hyperglycemia in the albino rats. The induction of hyperglycemia on administration of alloxan in presence or absence of glucose has been attributed to the cytotoxic action of alloxan and the action of reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentrations (Szkudelski, 2001). Alloxan exerts its diabetogenic action when it is administered intravenously, intraperitoneally or subcutaneously (Williams, 1963). The dose of alloxan required to induce diabetes depends on the animal species, route of administration and nutritional status (Szkudelski, 2001). Fasted animal are more susceptible to alloxan-induced hyperglycemia (Szkudelski, 2001) (Table 3).

After treatment with the extract and the standard drug, glibenclamide, there was a significant reduction ( $p < 0.05$ ) in glucose concentration of the treated groups relative to the untreated. The difference between the groups given the extracts and the one treated with the drug, glibenclamide was

Table 3: Mean blood glucose level

Groups	Mean blood glucose level (mg dL <sup>-1</sup> )		
	Before induction	After induction	After treatment
A (aqueous, 100 mg kg <sup>-1</sup> )	103.24±1.25 <sup>a</sup>	261.60±4.05 <sup>b</sup>	157.41±2.25 <sup>a</sup>
B (aqueous, 200 mg kg <sup>-1</sup> )	95.60±1.08 <sup>a</sup>	249.45±4.10 <sup>b</sup>	120.27±2.05 <sup>a</sup>
C (aqueous, 400 mg kg <sup>-1</sup> )	106.02±1.20 <sup>a</sup>	258.60±3.99 <sup>b</sup>	104.80±1.89 <sup>a</sup>
D (methanol, 100 mg kg <sup>-1</sup> )	102.60±1.18 <sup>a</sup>	245.62±5.68 <sup>c</sup>	129.80±1.99 <sup>a</sup>
E (methanol, 200 mg kg <sup>-1</sup> )	100.16±2.04 <sup>a</sup>	264.40±3.44	135.65±2.09
F (methanol, 400 mg kg <sup>-1</sup> )	98.35±1.60 <sup>a</sup>	281.68±2.88	149.52±3.75
G (glibenclamide)	100.61±2.13 <sup>a</sup>	265.66±4.80	96.46±2.05
H (alloxan)	103.55±1.22 <sup>a</sup>	277.76±3.70	285.33±3.02
I (distilled water)	106.40±1.14 <sup>a</sup>	108.43±4.55	105.88±2.60

Effect of *Buchholzia coriacea* on the blood glucose level (mg dL<sup>-1</sup>) of the animals. Values are presented as mean±SD n = 5. Values having different superscripts differ significantly ( $p < 0.05$ )

significant ( $p < 0.05$ ). The effect of the aqueous extracts was not significantly different ( $p < 0.05$ ) from that of methanol extract.

## CONCLUSION

The ability of *Buchholzia coriacea* to reduce blood glucose level in the animals may partly explain its usage by traditional medical practitioners in the treatment and management of diabetes. This property may be attributed to the chemical constituents of the extracts. We are currently working on identification of the specific constituent(s) responsible for the antidiabetic action and their possible mechanism of action in our laboratory.

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