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# The Effect of Aqueous Extract of *Buchholzia coriacea* Seeds on Some Biochemical Parameters in Normal and Alloxan-induced Diabetic Rats

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Author LJL designed the study. Author LCD supervised the work. Authors LSN and LJL carried out all laboratory work. Author LSN managed the analyses of the data and the literature searches. Author LJL wrote the first draft of the manuscript. Author LCD edited the manuscript. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aims:** To investigate the effect of oral administration of *Buchholzia coriacea* aqueous seed extract on blood glucose and other biochemical parameters in alloxan-induced diabetic rats and in normal rats.
**Study Design:** Twenty adult male rats were used, rats were divided into four groups of five rats each (Groups A, B, C and D) in which group A (Diabetic control) and group B (Diabetic treated) were induced with diabetes by intraperitoneal injection of alloxan monohydrate at a dose of 150 mg/kg body weight while group C (Normal control) and D (Normal treated) were normal groups in which diabetes was not induced. The extract was administered orally to groups B and D at a dose of 200 mg/kg body weight for 7 days after the confirmation of the effect of alloxan in the induced groups. The *Buchholzia coriacea* aqueous seed extract was screened for phytochemicals. At the end of the experimental period, the rats were sacrificed by decapitation, blood was collected and

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used for the biochemical analysis.
**Place and Duration of Study:** Department of Biochemistry, College of Medical Sciences, University of Jos, Nigeria. Between August and December 2014
**Results:** The administration *of Buchholzia coriacea* seed extract caused significant (p = 0.05) reduction in the levels of glucose, total cholesterol, and triglyceride, increased protein levels in both treated and normal groups, but had no significant ameliorative nor adverse effect on liver and kidney parameters (liver enzymes, urea, creatinine and electrolytes levels) of both normal and diabetic rats administered the extract.
**Conclusion:** In conclusion, the aqueous extract of *Buchholzia coriacea* seed possess hypolipidemic and hypoglycemic properties and may therefore be beneficial in the management of diabetes mellitus at the above dosage and treatment period with no observed adverse effect on the liver and kidney.

*Keywords: Alloxan; Buchholzia coriacea; diabetes mellitus; hypoglycemic; hypolipidemic.*

## 1. INTRODUCTION

*Buchholzia coriacea* belongs to the family capparidacea and is widely distributed in several tropical countries [1]. The plant was named after R.W Buchholz who collected them in Cameroon in the late 19$^{th}$ century [2]. *B. coriacea* is a forest tree with large, glossy leaves and conspicuous cream white flowers in racemes at the end of the branches [3]. The seeds of *B. Coriacea* have medicinal value and this gave the plant a common name 'wonderful kolanut' because of its usage in traditional medicine to treat a variety of illnesses. The seeds or kernels of the plant *B. coriacea* are edible (can be eaten raw or cooked) and they have a spicy taste [4,5].

Scientific research on different parts of the plant has revealed various medicinal properties such as antihelmintic [6], antibacterial [7], antimicrobial [8], hypoglycemic [9-11], antiplasmodial [12], antidiarhoeal and antispasmodic [4], analgesic [13] effects.

Diabetes mellitus is a disorder in which the body is not able to properly metabolize carbohydrates [14]. It is a metabolic disorder of multiple etiologies, characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both with consequence in derangement of carbohydrate, fat, and protein metabolism [15]. Thirst, hunger and loss of weight are also characteristic of diabetes [14] and this may result in chronic complications including microvascular, macrovascular, and neuropathic disorders [16]. Diabetes eventually leads to diseases of the coronary arteries and the cerebrovascular system, renal failure, retinopathy, neuropathy and premature death [17,18].

Diabetes Mellitus has an estimated world prevalence of 285 million adults (aged 20-79 years) in 2010, it is postulated to rise to 439 million adults worldwide in 2030 [19]. This indicates the growing scourge of Diabetes Mellitus with high morbidity and mortality rate especially in the developing countries. A 69% and 20% increase in numbers of adults with diabetes in developing countries and developed countries respectively is projected to occur between 2010 and 2030 [19].

Type 2 Diabetes mellitus is by far the commonest form of the disease globally, with rapidly developing countries being at the forefront as far as this epidemic is concerned. Despite the availability of several oral hypoglycemic agents and insulin which are used for the treatment of diabetes, there is an increasing demand by patients to use herbal drugs even when their biologically active compounds are unknown, because of their effectiveness, fewer side effects and relative low cost [11]. This has prompted active research efforts to find more effective, safer and cheaper alternative agents of plant origin that possess hypoglycemic properties. Although the hypoglycemic activity of various organic solvent extracts of *B. coriacea* seed have been researched [9-11], this study was undertaken to evaluate the effect of the aqueous extract of the seeds in alloxan-induced diabetic rats and in normal rats.

## 2. MATERIALS AND METHODS

### 2.1 Collection and Identification of Plant Material

*B. coriacea* seeds (wonderful kolanut) were purchased from Terminus market in Jos, Plateau

state in the month of September 2014. The plant was identified and authenticated by Mr. Agyeno Otuse at the Department of Plant Science and Technology, University of Jos, Jos. Nigeria.

## 2.2 Chemicals and Reagents

Alloxan monohydrate was obtained from Sigma-Aldrich Chemical Company, St. Louis, U.S.A. All other chemicals and reagents used were of analytical grade and were obtained from reputable scientific and chemical companies.

## 2.3 Experimental Animals

White albino rats (Wister strain) were purchased from the animal house of the University of Jos, Plateau State, Nigeria. All the rats were young adult males. The animals were housed in cages under standard laboratory conditions, allowed free access to standard rat pellet (Grand Cereal and Oil Mills Ltd, Jos.) and water *ad libitum*. The animals were acclimatized for 10 days prior to commencement of the experiment. All experiments on animals were in accordance with the guidelines of both the University's ethical committee and the International Guidelines for Handling of Laboratory Animals [20].

## 2.4 Preparation of *Buchholzia coriacea* Seed Extracts

The fresh seeds of *B. coriacea* were cleaned, cut in pieces and air dried at room temperature under the shade until a constant weight was obtained. The dried seeds were pulverized; 100 g of the powdered seeds were dissolved in 500 mL of distilled water and allowed for 72 hours with intermittent shaking. Afterwards, the mixture was then stirred vigorously and filtered with Whatman No. 1 paper and then concentrated on a steam bath. Extract to be administered is freshly reconstituted in distilled water daily to give the required dose (200 mg/kg body weight) used in this study. The reconstituted extract was administered orally to the rats using cannula.

## 2.5 Induction of Diabetes

Five percent solution of freshly prepared Alloxan monohydrate (2, 4, 5, 6 tetraoxypyridine 5, 6-dioxyuracil) was used to induce diabetes in overnight fasted rats by intraperitoneal injection at a dose of 150 mg/kg body weight. This drug has been reported to act by selectively destroying the beta cells of the pancreas thereby, reducing insulin secretion [21]. Induction of diabetes was confirmed through intragastric tube touch Glucometer when fasting blood glucose levels reach 126 mg/dl and accompanied with positive hyperglucosuric test [22].

## 2.6 Animal Grouping and Treatment

After animals were acclimatized to laboratory conditions for 10 days, 20 rats weighing between 150-180 g were randomly selected and divided into four groups of 5 animals each as follows:

GROUP A: Diabetic control group
GROUP B: Diabetic + extract treated group
GROUP C: Normal control group
GROUP D: Normal + extract treated group

Animals in groups B and D were given 200 mg/kg body weight of the *B. coriacea* seed extract per day by oral administration for 7 days.

## 2.7 Collection of Blood Sample

At the end of the experiment, the rats were fasted for 24 hours before they were sacrificed by decapitation. The blood was collected in clean dry centrifuge tubes and was allowed to clot for 40 minutes and spinned at 5,000 rpm for 10 minutes. The serum was collected and transferred into bijou bottles and kept for analysis.

## 2.8 Phytochemical Screening of *Buccholzia coriacea* Seed Extract

The phytochemical screening of the aqueous extract was carried out using standard qualitative procedures [23,24]. This is with a view to assess the secondary metabolites therein.

## 2.9 Determination of Weight Change

Total body weight of both the diabetic and non-diabetic rats was measured using digital chemical balance (M/s Contech Instruments Limited, Mumbai, India) and recorded on days 1, 3, 5 and 7 and the mean body weights for each day was calculated at the end of the experimental period.

## 2.10 Biochemical Assays

### 2.10.1 Serum glucose, total protein and albumin determination

Serum concentrations of glucose were determined using the digital glucometer (Accu-

Chek Advantage, Roche Diagnostic,Germany) while total protein, and albumin were estimated using Randox kits (Randox Laboratories Ltd, United Kingdom).

#### 2.10.2 Lipid profile determination

Serum concentrations of Triglyceride (TG), Total Cholesterol (TC), High-Density Lipoprotein (HDL), and Low-Density Lipoprotein (LDL) were determined using commercially available kits (BIOSINO Biotechnology and Science INC, China).

#### 2.10.3 Liver enzymes (ALP, AST and ALT) determination

Alkaline Phosphatase-ALP (EC. 3.1.3.1) activity was determined by the Para- Nitrophenyl Phosphate (PNPP) method of Wright et al. [25]. Alanine aminotransferase- ALT (EC. 2.6.1.2), and aspartate aminotransferase-AST (EC. 2.6.1.1) were assayed according to the method described by Mohun and Cook [26].

#### 2.10.4 Serum urea and creatinine determination

Determination of serum creatinine was carried out using Jaffe's method described by Bowers and Wong [27]. Urea was estimated using urease-Berthelot's method described by Richterich and Kuffer [28].

#### 2.10.5 Serum electrolytes ($Na^+$, $K^+$, $Cl^-$ and $HCO_3^-$) determination

Serum sodium and potassium ions were measured by the flame photometry method of Vogel [29] and bicarbonate ion was determined using the titration method of Segal [30], Chloride ion was analyzed using the method of Schales and Schales [31].

### 2.11 Statistical Analysis

Results were expressed as mean SD and statistical analysis was carried out using ANOVA and were considered statistically significant at P=0.05.

## 3. RESULTS

### 3.1 Phytochemical Screening

Phytochemical screening of the aqueous extract of *B. coriacea* shows the presence of Alkaloids, flavonoids, Tannins, Saponins, Terpenes, Steroids, Cardiac Glycosides, Carbohydrate, Phenols and Resins as shown on Table 1.

**Table 1. Phytochemical screening of aqueous extract of *Buchholzia coriacea***

| Phytochemical parameters | Inference |
|---|---|
| Alkaloids | + |
| Flavanoid | + |
| Tanins | + |
| Saponins | + |
| Terpenes/Steriod | + |
| Cardiac Glycoside | + |
| Balsam | - |
| Carbohydrate | + |
| Phenols | + |
| Resins | + |

*Key: Positive (+) = Present, Negative (-) = Absent*

### 3.2 Changes in Body Weight

There was a progressive decrease in weight of the rats in the diabetic control group as the days increased while weights increased in both the normal control and extract treated groups.

### 3.3 Glucose, Total Protein and Albumin

Serum glucose level was significantly raised while protein and albumin decreased significantly in group A animals compared to the normal. Administration of the aqueous extract of *B. coriacea* for 7 days to the group B animals resulted in lowering of the glucose level, and increase in protein and albumin to near normal levels. No significant effect was observed in glucose and albumin levels for the normal treated group (Group D) compared to normal control but the total protein increased significantly.

### 3.4 Lipid Profile

The result of lipid profile analysis revealed significant increase in triglyceride and total cholesterol and a decrease in HDL levels in the diabetic control group compared to the normal. Extract administration resulted in lowering of these values significantly as seen in the diabetic treated group to near normal levels. There was no significant change in the normal + extract treated group compared to the normal for TG and TC. HDL level decreased significantly in group A compared to normal, extract administration did not result in any significant change in both the diabetic and normal groups compared to the respective controls. The LDL levels did not have

any difference across all groups, that is, both alloxan induction of diabetes and extract administration did not have any effect on LDL levels compared to the normal.

### 3.5 Liver Enzymes Determination

Serum levels of all liver marker enzymes assayed for increased significantly in the diabetic control group compared to normal but administration of the extract had no significant effect on both the diabetic and normal group of rats.

### 3.6 Urea and Creatinine Determination

No significant difference was observed in the levels of Urea and creatinine in both the alloxan-induced diabetic groups nor in the extract treated groups compared to the normal control group.

### 3.7 Electrolytes Determination

No significant change was observed for all electrolytes in all groups compared to the normal control group. Neither the induction of diabetes nor extract administration resulted in any significant change.

## 4. DISCUSSION

Alloxan is cytotoxic to the β-cells of pancreatic islets of Langerhans, this cytotoxic effect results in a decrease in endogenous insulin secretion and causes decreased glucose utilization by body tissues [32]. Alloxan is a known diabetogenic agent used to induce Type 2 diabetes in animals, it functions by causing necrosis of the pancreatic beta cells via the generation of free radicals [33] resulting in metabolic derangements such as increase in blood glucose level, decreased protein content, increased levels of cholesterol and triglycerides [34]. Alloxan-induced diabetes model seem to be the best known drug induced diabetes as it appears to be the easiest, most reliable and most practicable method of inducing diabetes, although chemical induction of diabetes with streptozotocin is most widely used [33,35].

Our preliminary phytochemical screening of the aqueous seed extract of *Buchholzia coriacea* revealed the presence of Alkaloids, Flavonoids, Tannins, Saponins, Terpenes, Steroids, cardiac Glycosides, Carbohydrate, Phenols and Resins (Table 1). This result is in consonance with those of [36] and [7]. The hypoglycaemic effects of alkaloids and flavonoids have been reported [37], thus it is not unreasonable to speculate that some of these phyto constituents among others are presumably responsible for the glucose lowering ability of the aqueous seed extract of *Buchholzia coriacea* seen in this study.

The mean body weights of all rats in all experimental groups measured on days 1, 3, 5 and 7 of the experiment is shown in Table 2. Diabetes is associated with the characteristic loss of body weight which is due to increased muscle wasting and due to loss of tissue proteins [38]. As expected in the diabetic control group, the body weight of rats was progressively reduced; this alloxan caused body weight loss was regained to its above-initial values by *Buccholzia cariacea* seed extract treatment, which indicates the prevention of muscle tissue damage due to hyperglycemic condition reflecting an improved health of treated animals. Worthy of note is the increase in body weight recorded in normal rats administered with the extract (group D). This observation gains support from the previous study by Ibrahim and Fagbohun [39], where they showed that *Buchholzia coriacea* seeds contain high percentage of carbohydrate, protein and fat and therefore the seeds could be used when considering natural food and feed additives to improve human and animal health.

From Table 3, Treatment of the alloxan-induced diabetic rats with 200 mg/kg body weight/day of the aqueous extract of *Buchholzia coriacea* for 7 days showed a significant hypoglycemic activity but glucose levels remained within normal range for normal rats treated with the extract indicating that the extract did not cause any alteration in glucose levels of normal rats. Hypoglycemic activity of ethanol and butanol fractions [9], and methanol extracts [10,40] have also been reported. The probable mechanisms of action of the plant extract could be via several mechanisms which are linked to either potentiation of insulin from beta cells, increasing peripheral glucose uptake, slowing down the absorption of sugar from the intestinal gut or by decreasing the release of glucose from the liver [41,42]. Studies by Pereira et al. [43] also indicated that the clinical entity of type 2 diabetes involves defective protein metabolism leading to decreased protein content. This is as a result of increased muscle wasting due to loss of tissue proteins [38]. Also, impaired insulin action as in type 2 diabetes may result in reduced average

synthesis rates of whole body protein. The above findings are evident in the drastic reduction in total protein level observed in the diabetic control group when compared to the normal control group. Again, administration of the aqueous *Buchholzia coriacea* extract ameliorated the loss in protein by increasing the protein content significantly almost to the normal levels in the diabetic treated group and also in the normal treated group. Albumin is the most extensively studied protein as it is by far the most abundant protein in nephrotic urine [44]. The association of low serum albumin with faster rate of Glomerular Filtration Rate (GFR) decline was consistent in studies of diabetic patients carried out by the National Kidney Foundation [45]. In our study, serum albumin concentration decreased but not significantly (P=0.05) in the diabetic control group. Also, no significant difference (P=0.05) was observed in the extract treated groups compared to control indicating that there was no effect of extract administration on kidney function as albuminuria is a marker of kidney dysfunction.

Hyperglycemia is accompanied with dyslipidemia in diabetes [46]. Most diabetics have a high blood triglyceride level, a high low-density lipoprotein level and a low high-density lipoprotein level. Under normal circumstances insulin activates the enzyme lipoprotein lipase which hydrolyses triglycerides thus, increasing uptake of fatty acids into adipose tissue and triglyceride synthesis. It also inhibits lipolysis [47,48]. Deficiency of insulin results in failure to activate the enzyme lipoprotein lipase, thereby causing hypertriglyceridemia [49]. The abnormal high concentration of serum lipids in the diabetic untreated rats may be due, mainly to the increase in the mobilization of free fatty acids from the peripheral fat depots, since insulin inhibits the hormone sensitive lipase [50]. Therefore, the normalization of serum total cholesterol and triacylglycerol by aqueous seed extract of *Buchholzia coriacea* suggest that the extract is effective in reversing the abnormalities associated with lipid metabolism in diabetes. The extract had no significant effect on the HDL and LDL.

**Table 2. Effect of aqueous extract of *Buchholzia coriacea* on body weight changes in diabetic and normal rats**

| Groups | Day 1 | Day 3 | Day 5 | Day 7 |
|---|---|---|---|---|
| A – Diabetic control | 166±5.6 | 160±5.3 | 158±5.1 | 154±5. 2 |
| B – Diabetic treated | 162±9.6 | 164±9.5 | 164±6.2 | 165±9. 3 |
| C – Normal control | 160±6.3 | 165±5.7 | 168±5.1 | 170±8.4 |
| D – Normal treated | 162±8.5 | 162±9.3 | 167±5.5 | 169±10.2 |

*The mean weights ± standard deviation for the five rats in each group.*

**Table 3. Effect of aqueous extract of *Buchholzia coriacea* on blood glucose, total protein and albumin levels of diabetic and normal rats**

| Groups | Glucose (mmol/L) | Protein (g/L) | Albumin (g/L) |
|---|---|---|---|
| A- Diabetic control | 13.13±0.24[a] | 45.25±3.78[a] | 33.50±3.00[a] |
| B- Diabetic treated | 7.20±0.38[b] | 66.50±2.52[b] | 35.00±3.37 |
| C- Normal control | 5.67±0.57[b] | 69.33±1.56[b] | 37.33±2.08[b] |
| D- Normal treated | 5.93±0.10[b] | 78.00±4.90[ab] | 36.00±2.00 |

*Values are expressed as mean ± SD, n= 5 for each group*
*[a]values are significantly different from normal control group at (P= 0.05)*
*[b]values are significantly different from the diabetic control group at (P= 0.05)*

**Table 4. Effect of aqueous extract of *Buchholzia coriacea* on serum lipid profile parameters of diabetic and normal rats**

| Groups | Lipid profile parameter (mmol/L) | | | |
|---|---|---|---|---|
| | TG | TC | HDL | LDL |
| A- Diabetic control | 1.18±0.09[a] | 1.98±0.15[a] | 0.73±0.05[a] | 0.43±0.15 |
| B- Diabetic treated | 0.55±0.06[b] | 1.13±0.05[b] | 0.83±0.05[a] | 0.43±0.05 |
| C- Normal control | 0.77±0.05[b] | 1.03±0.05[b] | 1.10±0.08[b] | 0.48±0.10 |
| D- Normal treated | 0.62±0.15[b] | 1.20±0.21[b] | 1.18±0.10[b] | 0.43±0.05 |

*Values are expressed as mean ± SD, n= 5 for each group*
*[a] values are significantly different from normal control (P=0.05)*
*[b] values are significantly different from the diabetic control group (P=0.05)*

**Table 5. Effect of aqueous extract of *Buchholzia coriacea* on selected liver enzymes in diabetic and normal rats**

| Groups | Liver enzymes (IU/L) | | |
|---|---|---|---|
| | ALT | AST | ALP |
| A- Diabetic control | 117.75±0.50[a] | 225.25±0.96[a] | 165.50±1.92[a] |
| B- Diabetic treated | 112.25±0.50[a] | 217.25±0.96[a] | 162.50±1.73[a] |
| C- Normal Control | 74.50±3.00[b] | 117.75±0.96[b] | 63.250±1.50[b] |
| D- Normal treated | 75.25±1.50[b] | 117.50±0.58[b] | 62.00±0.82[b] |

*Values are expressed as mean ± SD, n= 5 for each group*
*[a] values are significantly different from the normal control (P= 0.05)*
*[b] values are significantly different from the diabetic control group (P= 0.05)*

**Table 6. Effect of aqueous extract of *Buchholzia coriacea* on urea and creatinine in diabetic and normal rats**

| Groups | Urea (mmol/L) | Creatinine (mmol/L) |
|---|---|---|
| A- Diabetic control | 10.05±0.38 | 68.75±0.96 |
| B- Diabetic treated | 9.50±0.08 | 65.75±2.87 |
| C- Normal control | 9.13±0.15 | 64.90±1.16 |
| D- Normal treated | 9.75±0.06 | 64.75±2.06 |

*Values are expressed as mean ± SD, n= 5 for each group*

**Table 7. Effect of *aqueous* extract *of Buccholzia coriacea* on sodium, potassium, chloride and bicarbionate ions in serum of diabetic and normal rat**

| Groups | Electrolytes (mmol/L) | | | |
|---|---|---|---|---|
| | $Na^+$ | $K^+$ | $Cl^-$ | $HCO_3^-$ |
| A- Diabetic control | 143.75±1.26 | 6.18±0.15 | 103.75±0.96 | 28.50±1.73 |
| B- Diabetic treated | 141.00±1.41 | 6.13±0.13 | 103.75±0.15 | 26.75±0.96 |
| C- Normal control | 142.75±1.96 | 5.78±0.22 | 105.75±0.05 | 26.25±0.15 |
| D- Normal treated | 141.75±1.26 | 5.03±0.38 | 105.75±0.05 | 24.75±2.06 |

*Values are expressed as mean ± SD, n= 5 for each group*

Chronic mild elevations of transaminases are frequently found in type 2 diabetic patients and often reflect underlying insulin resistance [51]. ALP is a membrane bound enzyme while ALT and AST are cytosolic enzymes. These enzymes are highly concentrated in the liver and kidney and are only found in the serum in significant quantities when the cell membrane becomes leaky and even completely ruptured [52,53]. The fact that no significant effect (P=0.05) on liver enzymes was observed after extract administration for 7 days in both the diabetic and normal groups shows that the extract did not have toxic effect on the liver of normal rats neither did it ameliorate the high levels of serum liver enzymes seen as a consequence of induction of diabetes. The lack of associated lethality with a high dose (LD50 greater than 5000 mg/kg body weight) as observed by Theophine et al. [10] indicates a high safety profile of the extract of *Buchholzia coriacea* seeds.

Plasma creatinine and urea are established markers of Glomerular Filtration Rate (GFR) [54]. Judykay [55] in his submission suggested that high creatinine levels observed in diabetic patients may be due to impaired function of the nephrons. The researcher also posited that high urea levels in diabetes mellitus patients could be attributed to a fall in the filtering capacity of the kidney thus leading to accumulation of waste products within the system. Also, alteration in serum levels of $Na^+$ and $K^+$ has been associated with renal function impairment [56,57]. Previous report by Uladimir [58] state that hyperglycemia is associated with long-term damage, dysfunction and failure of various organs, like the kidneys [59]. The observed non alteration in serum urea and creatinine as well as electrolytes in animals in all the groups compared with the normal control is an indication that both the induction of diabetes and the administration of the extract might not have altered renal function in the rats.

## 5. CONCLUSION

In conclusion, the results from this study indicated that administration of aqueous seed extract of *Buchholzia coriacea* to alloxanized

diabetic rats at the doses considered and the duration of administration showed that the extract had hypoglycemic and hypolipidemic effects but did not have any adverse effects on liver and kidney functions in rats and thus there could be scientific merit in the folkloric use of the extract in the management of diabetes.

Present work was a preliminary effort, therefore, further studies are needed for characterization of active compounds, and to investigate and elucidate the possible mechanism of action of the active ingredients, establish complete safety profiles, pre-formulation studies for development of a potential dosage form and evaluate the potential value of *Buccholzia coriacea* aqueous seed extract for the management of diabetes mellitus.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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