

# Effect of hydro-ethanol leaf extract of miracle plant (*Bryophyllum pinnatum*) on the kidneys of streptozotocin-induced diabetic Albino rats

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## ABSTRACT

**Background:** Diabetes is a global health concern with increasing prevalence, often leading to kidney complications. This study evaluates the effects of hydro-ethanol leaf extract of *Bryophyllum pinnatum* on the kidneys of streptozotocin-induced diabetic albino rats.

**Method:** The extract was analysed for its phytochemical constituents. Twenty-five albino rats were divided into five groups (n=5). Twenty (20) of these rats (groups 2-5) were induced intraperitoneally with a single dose of streptozotocin (35 mg/Kg) to cause diabetes. On the third day, diabetes was confirmed. Groups 1 and 2 (basal control and negative control) received 1 ml of distilled water only. Groups 3 and 4 were treated with 25 and 50 mg/Kg of the extract, respectively. Group 5 received 200 mg/Kg Metformin. Treatments lasted for 14 days, after which the animals were euthanised 24 hours after the last treatment. Kidneys were collected and weighed. Serum was prepared to assess kidney biomarkers.

**Results:** Phytochemical screening showed terpenoids, phenols, tannins, saponins, glycosides, triterpenes, cardiac glycosides, gums, and mucilage, while steroids, alkaloids, anthraquinones, flavonoids, phlobatannins, and fixed oils/fats were absent. The extract reduced renal damage by lowering creatinine and bilirubin levels. At 50 mg/kg, it significantly decreased urea and uric acid. However, it was less effective in correcting electrolyte imbalance in streptozotocin-induced diabetic albino rats

**Conclusion:** The hydro-ethanol leaf extract of *Bryophyllum pinnatum* improved glycemia, kidney markers balance in streptozotocin-induced diabetic rats in a dose-dependent manner, suggesting its potential in managing diabetic kidney complications.

**Keywords:** *Bryophyllum pinnatum*, Diabetes, Hydro-ethanol leaf extract, Kidney, Streptozotocin.

## 1. INTRODUCTION

Diabetes is a group of metabolic disorders marked by high blood glucose levels (hyperglycemia) due to inadequate insulin action, secretion, or both. This condition affects the metabolism of carbohydrates, proteins, and fats [1]. Beyond merely affecting blood sugar, diabetes is a systemic condition that affects various organ systems, including the kidneys, resulting in complications that elevate morbidity and mortality rates [2]. It undeniably leads to severe and persistent complications in both microvascular (such as nephropathy, neuropathy, and retinopathy) and macrovascular (like coronary and peripheral arterial conditions and stroke) systems. These complications manifest across all types of diabetes mellitus. The consequences of these issues are profound, causing harm to multiple organs, especially when the diagnosis is delayed [3]. Diabetic nephropathy is a microvascular complication that gradually

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undermines kidney function, resulting in end-stage renal disease. It significantly affects the peripheral nerves, causing pain, sensory deficits, and potential limb amputations. Since microvascular complications frequently evolve without noticeable symptoms, they severely influence and affect the quality of life and healthcare expenditures [4]. Historically, medicinal plants have been utilised to treat and manage diabetes mellitus in traditional medicine across the globe. They play a crucial role in diabetes management, particularly in developing countries where access to conventional diabetes treatments is limited. In developed countries, the use of herbal treatments for diabetes has met a decline with the advent of insulin and synthetic oral diabetes medications in the early 20<sup>th</sup> century. However, there is a resurgence of interest in medicinal plants that can help reduce blood sugar levels. More people in developed nations are turning to herbal remedies for diabetes [5]. This shift is likely influenced by several factors, such as adverse effects of standard diabetes treatments, high rates of treatment failure, and the cost associated with these synthetic drugs [5]. Moreover, medicinal plants serve as valuable sources for developing new drugs, and traditional medicinal knowledge has proven effective in producing new therapeutics [6]. Various medicinal plants contain different phytochemicals capable of lowering blood glucose levels and exhibiting renal protective potentials [7]. Due to its healing attributes, *Bryophyllum pinnatum* has been used in traditional medicines [8]. *Bryophyllum pinnatum* is a succulent plant in the Crassulaceae family. It is also referred to as the Air plant, Cathedral bell, Life plant, and Miracle plant. The plant is recognised for its thick, fleshy leaves arranged alternatively along its stems [9]. It can propagate through seeds and vegetative methods, aiding its widespread growth. In various regions, it holds cultural importance within traditional medicine practices. Different parts of the plant are credited with a range of medicinal benefits [8]. Additionally, *Bryophyllum pinnatum* has been traditionally employed to treat various diseases, including diabetes and kidney-related diseases [7]. This current study, however, focused on examining the effects of the hydro-ethanol leaf extract of *Bryophyllum pinnatum* on the kidneys of streptozotocin-induced diabetic albino rats.

## **2. MATERIALS AND METHODS**

### **2.1 Materials**

#### *2.1.1 Biological Materials*

Plant materials - The leaves of Miracle plant (*Bryophyllum pinnatum*) were collected from a local farm in Aiyegbaju Ekiti, Ekiti State, Nigeria. It was authenticated at the Department of Botany, Ekiti State University Ado Ekiti, Ekiti State, with voucher herbarium number: UHAE 2023076. Experimental Animals - Twenty-five (25) female albino rats weighing  $120 \pm 20$  g were obtained from the Animal Holding Unit of Ekiti State University, Ekiti State, Nigeria. The rats were kept in a clean plastic cage and allowed to acclimatise for two weeks before the commencement of experiments. They were fed a standard pellet diet and water *ad libitum*.

#### *2.1.2 Chemicals and Reagents*

Streptozotocin used in this study was acquired from Elabscience Biotechnology Inc. (USA). Other chemicals and reagents used were of analytical grade.

### **2.2 Methods**

#### *2.2.1 Extraction of hydro-ethanol leaf of Miracle plant (*Bryophyllum pinnatum*)*

One thousand and five hundred (1500) grams of the fresh leaves were weighed and blended using an electric blender. The blended leaf was extracted using 1.5 litres each of distilled water and 95% absolute ethanol (1:1) for 24 hours at room temperature. The hydro-ethanol extract of the leaf was filtered with a Muslin cloth, and the filtrate was evaporated using a rotary evaporator. The leftovers were placed undisturbed in the desiccator for two (2) days to dry completely. The obtained powder was kept for biochemical analysis.

#### *2.2.2 Experimental design*

Twenty-five (25) female Albino rats weighing  $120 \pm 20$ g were divided into five groups of 5 animals each. Groups 2-5 were induced intraperitoneally with a single dose of 35 mg/Kg body weight of streptozotocin dissolved in 0.1M citrate buffer at pH 4.5 [10]. On the third day of the induction, the blood glucose levels of the induced animals were measured with a Fine test glucometer using blood from a tail prick to confirm the induction of diabetes. Animals with blood glucose  $>150$  mg/dl were considered diabetic and used for this experiment. The experiment lasted for 14 consecutive days. The details of animal grouping and treatment are as shown below:

Group 1: Basal control treated with 1 ml of distilled water only.



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Group 2: Negative control treated with 1ml of distilled water only.

Group 3: Treated with 25 mg/Kg body weight of hydro-ethanol leaf extract of *Bryophyllum pinnatum*.

Group 4: Treated with 50 mg/Kg body weight of hydro-ethanol leaf extract of *Bryophyllum pinnatum*.

Group 5: Standard control treated with 200 mg/Kg body weight of standard drug, Metformin.

### 2.2.3 Oral glucose tolerance test

On the last day of the 2 weeks experiment, an oral glucose tolerance test was carried out: The rats were made to undergo a 12-hour overnight fast, after which each group of rats was given the appropriate treatment (distilled water, extract, and Metformin). After 30 minutes of this pre-treatment interval, all rats received 2 g/Kg glucose solution orally. Then, at baseline, 0 (prior to the glucose load), and 30, 60, and 120 minutes post-glucose administration, blood samples were collected from the retro-orbital plexus of all rats to evaluate the extract's impact on their serum glucose levels [11].

### 2.2.4 Serum preparation

Twenty-four hours after the final treatments, the animals were euthanised after being anaesthetised with chloroform and blood was carefully drawn through cardiac puncture. The collected blood was then allowed to clot before being centrifuged for 10 min at 300 g to prepare serum [11]. The resulting supernatant was used to assess serum biochemical markers.

### 2.2.5 Removal of kidney

Prior to anaesthesia, each rat was weighed. The rats were anaesthetised and the kidneys were then extracted, rinsed with ice-cold phosphate buffer saline and also weighed.

### 2.2.6 Qualitative phytochemical analysis of hydro-ethanol leaf extract of Miracle plant (*Bryophyllum pinnatum*):

This was carried out according to the methods of [12] and [13].

### 2.2.7 Biochemical analyses

The urea concentration was measured using the urease method as described by [14].

Creatinine concentration was determined by Jaffe's reaction through the reaction of creatinine with sodium picrate [15]. Uric acid concentration was assessed using the Uricase method outlined by [16]. The bilirubin concentration was measured according to the method developed by [17]. The concentrations of electrolytes were determined using the ion-selective electrode (ISE) method, as described by [18].

## 2.3 Statistical Analysis

The results are presented as mean  $\pm$  SD for each group. Differences among groups were analysed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Data were analysed using GraphPad Prism Version 5.0, and values were considered significant at  $P < 0.05$ .

## 3. RESULTS

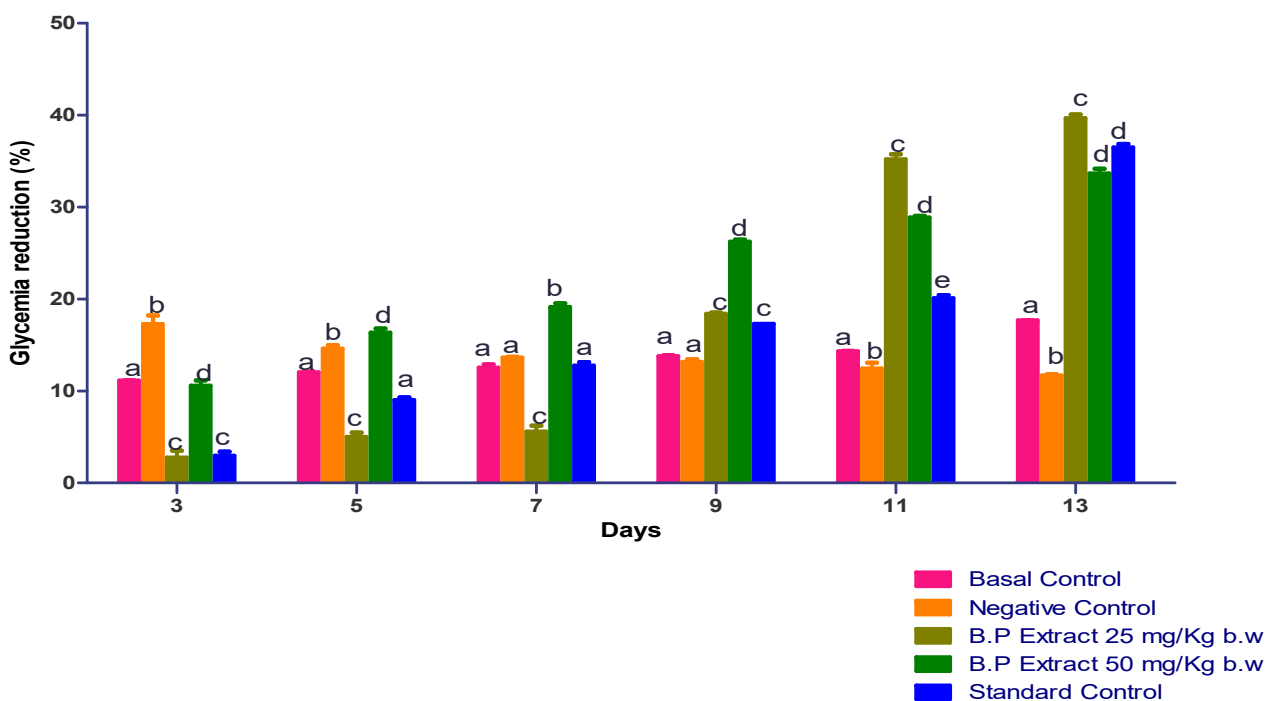
Phytochemical analysis of the hydro-ethanol leaf extract of *Bryophyllum pinnatum* revealed the presence of terpenoids, phenol/phenolic acids, tannins, saponins, glycosides, triterpenes, cardiac glycosides, gums, and mucilage. However, steroids, alkaloids, anthraquinones, flavonoids, phlobatannins, fixed oils and fats were not detected in the hydro-ethanol extract of *Bryophyllum pinnatum* (Table 1). Figure 1 showed that the highest percentage reduction in glycemia occurred on day 13. The percentage reduction in glycemia of the negative control significantly ( $P < 0.05$ ) decreased compared with the basal control, extract-treated group (groups 3 and 4) and standard control. It was evident in Figure 2 that negative control (diabetic control) showed a significant ( $P < 0.05$ ) increase in the percentage kidney-to-body weight ratio compared with basal control. Whereas a 14-day administration of 25 and 50 mg/Kg body weight of hydro-ethanol leaf extract of *Bryophyllum pinnatum* caused a significant ( $P < 0.05$ ) decrease in the percentage kidney to body weight ratio of the treated diabetic groups compared with negative control. Furthermore, the group treated with the least dose of the extract (25 mg/Kg) showed no significant ( $P > 0.05$ ) difference when compared with basal control. The group treated with the highest dose of the extract (50 mg/Kg) showed no significant difference compared with the Metformin-treated group.



Table 1: Qualitative phytochemical constituents of hydro-ethanol leaf extract of Miracle plant (*Bryophyllum pinnatum*)

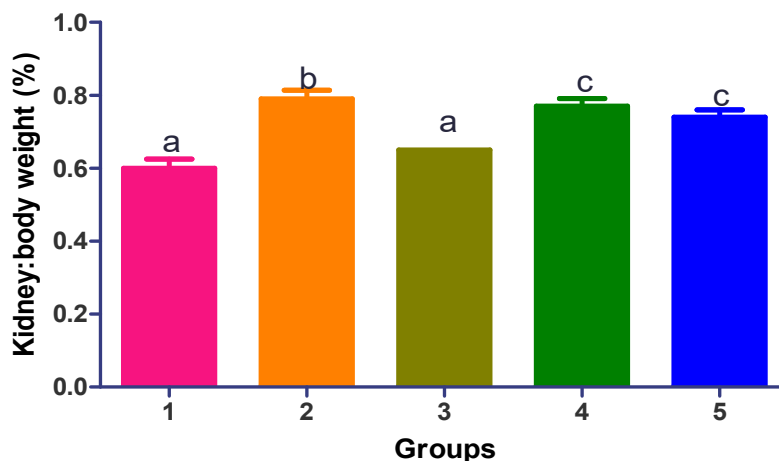
Phytochemicals	Status
Terpenoids	+
Phenol/phenolic acid	+
Tannins	+
Saponins	+
Glycosides	+
Triterpenes	+
Cardiac glycosides	+
Gums and mucilage	+
Steroids	+
Alkaloids	-
Anthraquinones	-
Flavonoids	-
Phlobatannins	-
Fixed oils and fats	-

Note: Positive (+) sign represents the presence, while negative (-) sign represents the absence of the phytochemicals.



**Figure 1:** Percentage reduction of glycemia in streptozotocin-induced diabetic albino rats treated with hydro-ethanol leaf extract of *Bryophyllum pinnatum*

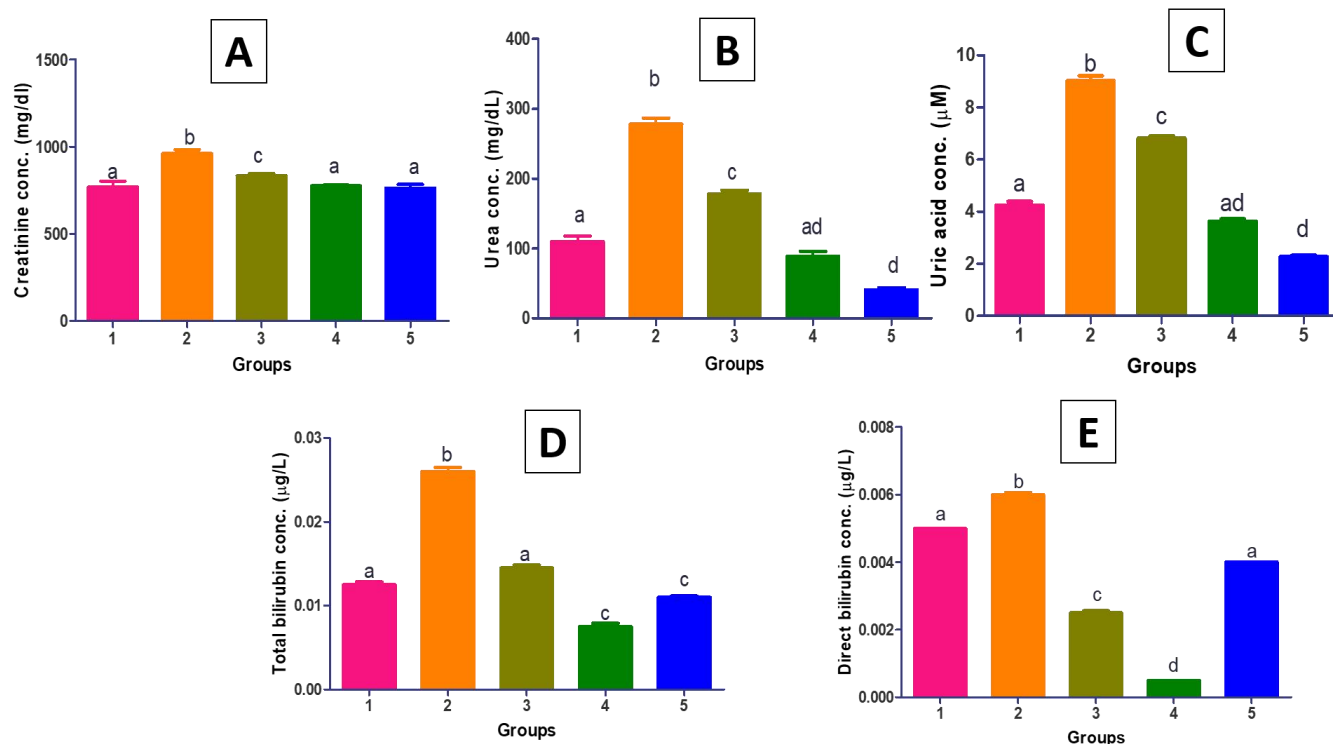
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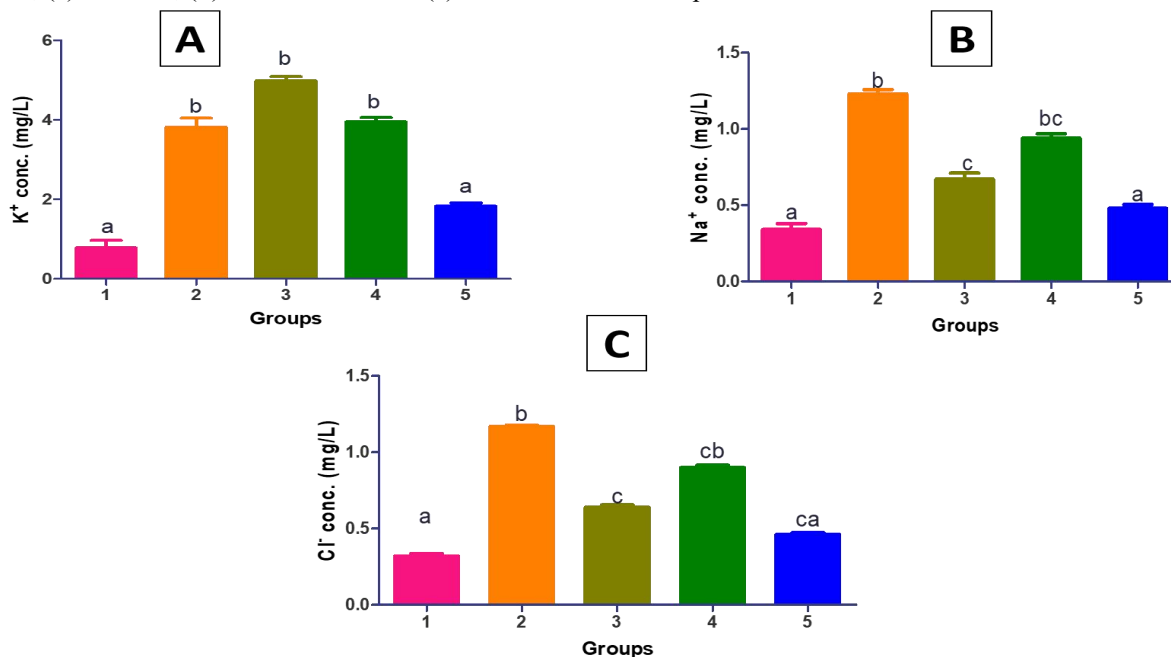
Key: B.P – *Bryophyllum pinnatum*

Figure 2: Percentage kidney to body weight ratio of streptozotocin-induced diabetic albino rats treated with hydro-ethanol leaf extract of *Bryophyllum pinnatum*.

Generally, the biochemical analysis results indicated that inducing albino rats with streptozotocin significantly ( $P < 0.05$ ) increased the concentrations of creatinine, urea, uric acid, total and direct bilirubin (Figure 3) as well as sodium ion ( $\text{Na}^+$ ), potassium ion ( $\text{K}^+$ ), and chloride ion ( $\text{Cl}^-$ ) (Figure 4). Treatment with the extract at a dosage of 25 mg/Kg body weight and Metformin reduced the creatinine concentration to a level that was not significantly ( $P > 0.05$ ) different from the basal group. Additionally, the group treated with the highest dose of the extract (50 mg/Kg body weight) also showed no significant ( $P > 0.05$ ) difference in urea concentration compared with the basal group and the Metformin-treated group (Figure 3a). Administering 25 mg/Kg and 50 mg/Kg of *Bryophyllum pinnatum* leaf extract significantly ( $P < 0.05$ ) reduced urea concentration in a dose-dependent manner compared with the negative control. The lowest dose (25 mg/Kg b.w) of hydro-ethanol leaf extract of *Bryophyllum pinnatum* showed a significant ( $P < 0.05$ ) increase compared to the basal control. No significant ( $P > 0.05$ ) difference in urea concentration was found for the highest dose (50 mg/Kg b.w) compared to the basal and standard controls (Figure 3b). Similarly, the uric acid levels in the group that received the highest dose of the extract (50 mg/Kg body weight) demonstrated no significant ( $P > 0.05$ ) difference when compared with the basal group and the Metformin group (Figure 3c). The total bilirubin concentration for the group treated with 25 mg/Kg body weight of the extract was not significantly ( $P > 0.05$ ) different from the basal control. However, the group treated with 50 mg/Kg body weight of the extract showed no significant ( $P > 0.05$ ) difference compared with the standard Metformin group (Figure 3d). Regarding direct bilirubin, the concentration in the Metformin-treated group was significantly ( $P < 0.05$ ) higher than in the extract-treated group, but it was not different from the basal group (Figure 3e). For potassium ion ( $\text{K}^+$ ) concentration, the extract-treated group had significantly ( $P < 0.05$ ) higher levels than both the basal and standard control groups (Figure 4a). Likewise, the sodium ion ( $\text{Na}^+$ ) concentration in the extract-treated group was significantly ( $P < 0.05$ ) higher than in both the basal and standard control groups (Figure 4b). In contrast, the chloride ion ( $\text{Cl}^-$ ) concentration in the group treated with 25 mg/Kg body weight of the extract was significantly ( $P < 0.05$ ) higher than the basal control but not significantly ( $P > 0.05$ ) different from the standard control, which itself showed no significant ( $P > 0.05$ ) difference from the basal control (Figure 4c).



**Figure 3:** Effects of hydro-ethanol leaf extract of *Bryophyllum pinnatum* on the concentrations of (a) creatinine, (b) urea, (c) uric acid, (d) total bilirubin and (e) direct bilirubin in streptozotocin-induced diabetic albino rats.



**Figure 4:** Effects of hydro-ethanol leaf extract of *Bryophyllum pinnatum* on the concentrations of (a)  $\text{K}^+$ , (b)  $\text{Na}^+$  and (c)  $\text{Cl}^-$  ions in streptozotocin-induced diabetic albino rats.

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### 4. DISCUSSION

Diabetes mellitus is a global health issue in which insulin function is impaired. It is related to kidney diseases because they are intricate and significantly impact public health, medical practice, and research [19, 20]. Even though there are various conventional anti-diabetic drugs for sale [21], the search for new antidiabetic sources from natural plants remains a significant area of interest [11] as they are reported to possess considerable anti-diabetic benefits and fewer side effects [22]. Therefore, this study aims to evaluate the effects of hydro-ethanol leaf extract of *Bryophyllum pinnatum* on the kidneys of streptozotocin-induced diabetic albino rats. The bioactive compounds, including terpenoids, phenol/phenolic acids, tannins, saponins, glycosides, triterpenes, cardiac glycosides, gums, and mucilages that are present in this extract are attributed to function in kidney health and other medicinal benefits [9]. This result corroborates that of [23], who revealed the presence of carbohydrates, proteins, phenols, flavonoids, saponins, glycosides, alkaloids, terpenoids and steroids in alcohol extracts of *Bryophyllum pinnatum* during their study on the application of *Bryophyllum pinnatum* leaf extracts in lithiasis rats against the formation of renal calculi. Type 2 diabetes is marked by insulin resistance and dysfunction of beta cells. Initially, the body tries to compensate for this by increasing insulin production to maintain normal blood sugar levels [24]. The increase in the percentage reduction in glycemia in the treated group revealed that the extract may be responsible for the reduction in the glucose level in the extract-treated rats. The phenomenon of diabetic renal hypertrophy is widely recognised. Soon after experimental diabetes is induced in rats, there is a notable rise in kidney weight within a brief period of hyperglycemia and glycosuria. This swift kidney enlargement phase encompasses cellular hypertrophy and hyperplasia [25]. However, the ability of the hydro-ethanol leaf extract of *Bryophyllum pinnatum* to reverse the increased kidney-body weight ratio of the diabetic experimental animals corroborates the positive influence on weight loss generally associated with diabetes [26]. The observed significant increase in the negative control compared with the basal control may indicate kidney impairment due to diabetes. This finding is similar to the research carried out by [27], where the increase in the concentration of creatinine in the negative group indicated kidney dysfunction. The observed high creatinine concentration in the negative control may be caused by high levels of creatine breakdown because glucose was not available for muscle metabolism. The observed reductions in creatinine levels in groups treated with the hydro-ethanol leaf extract of the plant compared with the negative control may suggest that the extract may help protect kidney function. This might be as a result of the renal protective effects of the hydro-ethanol leaf extract of *Bryophyllum pinnatum* [28]. The observed high urea and uric acid concentrations in the negative group may suggest that the diabetes in this group negatively impacted kidney function by reducing its ability to excrete waste [29]. The decline in the concentration of urea and uric acid in the extract-treated group may therefore imply its potential nephroprotective effects. Similarly, the observed elevated concentrations in total and direct bilirubin in the negative control group may suggest possible liver-kidney complications in streptozotocin-induced diabetic albino rats [30]. However, this extract appeared to counteract this effect in the extract-treated groups, thereby indicating potential improved kidney function and its complex effects on metabolic parameters in diabetic conditions. Elevated electrolytes (potassium  $K^+$ , sodium  $Na^+$  and chloride  $Cl^-$ ) concentrations observed in the negative control may suggest a decline in kidney function, as the kidney struggles to excrete excess electrolytes. Imbalance in the concentrations of electrolytes disrupts normal bodily functions and can lead to various critical complications [31]. The reduction in the concentrations of these electrolytes in the extract- and metformin-treated groups may be due to the bioactive present in the extract, most especially at the higher dose (50 mg/Kg body weight) which appeared to be more effective in mitigating electrolyte imbalances, thereby reinforcing the nephroprotective potential of the hydro-ethanol leaf extract of Miracle plant in streptozotocin-induced diabetic albino rats.

### 5. CONCLUSION

This study revealed that the hydro-ethanol leaf extract of *Bryophyllum pinnatum* consists of various bioactive compounds, including terpenoids, phenol/phenolic acids, tannins, saponins, glycosides, triterpenes, cardiac glycosides, gums, and mucilage, which may be responsible for its various therapeutic effects. This extract demonstrated significant renal protective effects in streptozotocin-induced diabetic albino rats as observed in the improvements in glycemia as well as kidney function markers (creatinine, urea, uric acid, total and direct bilirubin) and electrolyte balance ( $K^+$ ,  $Na^+$  and  $Cl^-$ ) in the extract-treated groups. In addition, the activity of the extract is concentration-dependent, with the highest dose (50 mg/Kg b.w) which appeared more effective. These findings highlight the potential of the hydro-ethanol leaf extract of *Bryophyllum pinnatum* in managing diabetic kidney complications.



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## DECLARATIONS

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### Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

### Conflict of interest

The authors declare no conflicts of interest concerning the authorship, research work and/or publication of this work.

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### Authors Contribution

- Idowu O. Aluko: Methodology, Data Collection, Data Analysis, Visualization, Writing- Original Draft
- Fisayo A. Bamisaye: Methodology, Laboratory Analysis, Supervision, Writing – Review and Editing

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