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Original Research Article

PHYTOCHEMICAL CHARACTERIZATION AND SUB-ACUTE TOXICITY EVALUATION OF RAW *Bryophyllum pinnatum* (LAM.) OKEN EFFECTS ON THE METABOLIC PROFILE IN WISTAR RATS

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ABSTRACT

In many Nigerian households, the succulent leaves of *Bryophyllum pinnatum* (*B. pinnatum*), also called *Ewe Abamoda, Oda opue, Mgbitingbi* are commonly squeezed without using extraction solvents and taken orally. Despite the common use of this traditional preparation, scientific information regarding its safety remains limited. This research investigated the phytochemical constituents and sub-acute toxicity of raw *B.pinnatum* leaves preparation (mimicking traditional mode of administration) in male Wistar rats for 16 days. Fifteen rats were grouped into normal control, low-dose (702.5 mg/kg body weight, equal to 1-teaspoon) and high-dose (1405 mg/ kg body weight, equal to 2-teaspoons) treatments. Phytochemical screening revealed flavonoids, coumarins, steroids, cardiac glycosides, tannins and phlobatannins. Using standard methods, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline-phosphatase (ALP), bilirubin, total protein, albumin, globulin, cholesterol, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), creatinine and uric acid, were evaluated using serum. No significant differences ($p > 0.05$) were observed in ALT, total bilirubin, conjugated bilirubin, total protein, albumin, globulin, cholesterol, HDL, LDL, or uric acid among the groups. However, AST, ALP and creatinine remained comparable between control and low-dose groups but were significantly increased ($p < 0.05$) in high-dose group. Triglycerides were significantly elevated in high-dose group, whereas low-dose group did not differ significantly from control. These findings indicate that phytochemicals present may contribute to therapeutic properties of *B. pinnatum* and traditionally consumed quantity equivalent to approximately 1-teaspoon appears relatively safe. However, doubling the intake triggers localized hepatic-renal stress and metabolic disruption, emphasizing importance of dose standardization in traditional herbal medicine.

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INTRODUCTION

Plants remain an important therapeutic agent's source and has continue to play a significant part in primary healthcare worldwide. The World Health Organization reports that approximately 80 % of the population in developing countries depends on the traditional usage of herbal medicine for the treatment of various diseases[1]. The therapeutic efficacy of medicinal herbal plants is predominantly attributed to the phytochemical constituents, which include alkaloids, flavonoids, tannins, glycosides, steroids, saponins, terpenoids, and coumarins. These secondary metabolites possess different biological activities like anti-inflammatory, antioxidant, antimicrobial, antidiabetic, cardioprotective,

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and hepatoprotective activities [2,3]. *Bryophyllum pinnatum* (*B. pinnatum*) (figure 1) is from a family of Crassulaceae, an evergreen plant that lives for many years. Its leaves are succulent and it can grow tall to about 1.4 m. The plant can easily be cultivated via stems or leaf cutting. *B. pinnatum* is employed in ethnomedicine for the management of whitlow, insect bites, diarrhea, earache, ulcers, abscesses and burns [4]. *B. pinnatum*, can also be called "life plant" or "miracle leaf" or "Never Die" plant [5].



Figure 1: *Bryophyllum pinnatum*

B. pinnatum have been used to treat diseases related to rheumatism, infection and inflammation in traditional medicinal practices. It has been reported that *B. pinnatum* have immunosuppressive, hepatoprotective, nephroprotective, antimicrobial and antimutagenic effect [4,6].

B. pinnatum has been widely studied for its medicinal value, particularly for its antioxidant, anti-inflammatory and hormone-regulating potentials. The plant's leaves comprise different phytochemicals like flavonoids, alkaloids and polyphenols, which are predominantly responsible for its health-promoting properties [7,8]. Quercetin is reported for its antioxidant capacity and has shown to have anti-inflammatory, anticancer, and cardioprotective benefits by attenuating oxidative stress [9]. Kaempferol has also been reported to have anti-inflammatory, anticancer, and neuroprotective properties[10]. Because of its high-water content and nutritional content, *B. pinnatum* leaves are used in some areas as a supplement in feed for poultry, cattle and goats [10]. While *B. pinnatum* has indeed been reported for its pharmacological properties like antioxidant, anti-inflammatory, and hormonal effects, most of these studies have focused on its medicinal applications in humans or used for *in vitro* or *in vivo* models with the use of rodents[8,11]. Also, previous literature has sporadically explored the extracts of *B. pinnatum* using aqueous or organic solvents but the toxicological profiles of raw, un-extracted preparations remain scarce. This research was therefore structured to bridge this information gap by investigating qualitative phytochemical screening raw *B. pinnatum* and establishing its metabolic sub-acute safety margins in a Wistar rat model at single and double traditional teaspoon dose equivalents (702.5 mg/ Kg and 1405 mg/Kg body weight) for 16-day period.

MATERIALS AND METHODS

Plant material: The plant materials were collected at a garden in Benin-City and authenticated by a botanist in the department of Plant Biology and Biotechnology, University of Benin where a voucher number UBH-B593 was allocated. *Bryophyllum pinnatum* leaves juice or crude extract preparations was produced by grinding the leaves and squeezed and thereafter sieved to remove any shaft to get the crude juice/extract without any extracting solvent. The extract/juice was separated, prepared freshly daily and used for this experiment.

Ethical approval: Ethical permission was obtained with the reference number WSU/26/005 from Wellspring University, Research Ethics Committee.

Reagent: Reagents and chemicals used are of analytical grade. All assays employed ready-to-use kits from Randox (United Kingdom) for cholesterol, high density lipoprotein (HDL), triglycerides, aspartate transaminase (AST), alkaline phosphatase (ALP), alanine transaminase (ALT), bilirubin, total protein, albumin, globulin, uric acid and creatinine.

Phytochemical Screening

The phytochemical screening were done according to the methods as described by Sofowora [12], Trease and Evans[3], and Harborne [13].

Test for Alkaloids: A solution of the extract (2 mL) was added to a small portion of dilute hydrochloric acid (HCL) and Mayer's reagent. The generation of a white/cream precipitate indicated that alkaloids were present.

Test for Flavonoid (Shindo's test): Two milliliters (2 mL) of the extract was added to 2mL 5% dilute

NaOH then mixed well. Thereafter, 2mL of dilute HCl was added into the test tubes and observed for a colour change (a yellow colour was observed).

Test for Phenols (Ferric chloride test): An aliquot of the extract (2mL) was treated with aqueous 5% Ferric chloride and observed for the formation of a deep blue or black color.

Test for Saponins (Foam test): To 2mL of the extract was added 6mL of water, mixed in a test tube, then shaken vigorously and observed for the formation of persistent foam, confirming the presence of saponins.

Test for Steroids: This used Libermann- Burchard test. 5 drops of chloroform, 4 drops of acetic anhydride, and one drop of concentrated sulfuric acid were added to 2 mL of the test solution. The presence of a purple color, which changes to blue or green, showed that steroids is present.

Test for Tannins: Three drops of 0.1 % ferric chloride were added to the extract and observed for the formation of a brownish-green precipitate.

Test for Cardiac glycosides (Killaini's test): Aliquot of the extract (1 mL) in a test tube was added to 2 mL of glacial acetic acid, then one drop of 15 % Ferric chloride and 1 mL of concentrated H_2SO_4 were added to the mixture. Brown colouration formed at the interface indicated that cardiac glycosides was present.

Anthraquinones Test (Borntrager's test): Boil 0.5 mL extract with 2 mL of 10 % HCl, cool and filter. Equal volume of chloroform(2.5ml) was added, then a few drops of 10% NH_3. Red colouration in the ammoniacal layer indicates the presence of anthraquinones.

Terpenoids Test (Salkowski test): Mixing of 0.5 mL extract with 2 mL chloroform was done while 3 mL concentrated H_2SO_4 was carefully added down test tube to form a layer. Reddish-brown colouration at the interface indicates terpenoids.

Phlobatannins Test (Hydrochloric acid test): Boil 2 mL extract with 2 mL of 1% aqueous HCl. The formation of a red precipitate indicates phlobatannins.

Coumarins Test: Alkaline reagent test was employed. 1 mL of 10% NaOH was mixed with 2 mL of extract. The development of an intense yellow colour, indicates coumarins.

Animals: A 16-day sub-acute toxicity study was conducted with the use of fifteen healthy adult Wistar rats of both sexes weighing approximately 170-200g bought in Anatomy department of the University of Benin and housed in the animal house where they acclimatized for two weeks (14days) before the start of the experiment and were fed without restriction with chow and clean water. The study was designed with reference to relevant principles of OECD Test Guidelines 407 for repeated-dose oral toxicity studies in rodents, even though a shorter period of 16 days was used. Group one was the control (5rats) and were given 1 mL of distilled H_2O, group two (5rats) were given 702.5 mg/Kg body weight (1-tea spoon) of the crude extract/juice of *Bryophyllum pinnatum* while group three (5rats) were given 1405 mg/Kg body weight (2-tea spoons) of the crude extract of *Bryophyllum pinnatum* for 16 days using a gastric gavage. Clinical observations were made daily for: mortality, behavioral change, skin and fur appearance, eyes and mucour membranes, tremors, convulsions, salivation, diarrhea, lethargy, sleep or coma. After 16 days, blood samples were collected under appropriate anaesthesia by cardiac puncture into plain tubes and serum separated by centrifugation and stored for biochemical analysis. All animals were humanely euthanized subsequently.

Biochemical assays on the serum

Analytical-grade reagents and chemicals were used. All assays employed ready-to-use kits from Randox (Randox United Kingdom) for cholesterol, HDL, Triglycerides, AST, ALP, ALT, Bilirubin, Total protein, Albumin, Globulin, Uric acid and Creatinine.

Lipid profile

Estimation of Total Cholesterol

The concentration of cholesterol was estimated with the method of Allain *et al*.[14]. Cholesterol present in the serum as cholesterol esters are broken down by cholesterol esterase (C.E) to free cholesterol, which is oxidized by cholesterol oxidase (C.O) to generate H_2O_2, that reacts with phenol and 4-aminoantipyrine to produce red quinoneimine dye, whose intensity correlates to the level of cholesterol in the sample.

HDL-Cholesterol Estimation

HDL-cholesterol was determined employing cholesterol CHOD-PAP assay[15]. Low density lipoproteins (LDL) and fractions of chylomicron are precipitated quantitatively by adding phosphotungstic acid in the presence of magnesium ions. The cholesterol concentration in the HDL fraction remaining in the supernatant is determined after centrifugation.

LDL- cholesterol Determination
The level of Low-Density Lipoprotein (LDL)-cholesterol in the serum sample was calculated by modification of Friedewald's formular [16].

Triacylglycerol Estimation
Triacylglycerol level in serum was assayed using the method described in Jiao et al, 2024 [17]. This is based on the breakdown of triacylglycerol with water, to glycerol and free fatty acids by triacylglycerol lipase. The phosphorylation of glycerol to glycerol-3-phosphate by adenosine triphosphate is catalyzed by glycerokinase. The oxidation of glycerol-3-phosphate to dihydroxyacetone phosphate and hydrogen peroxide is catalyzed by glycerol phosphate oxidase. A red-coloured compound was generated by the peroxidase-catalyzed coupling of 4-aminoantipyrine and phenol with hydrogen peroxide. The colour correlates to the triacylglycerol concentration in the serum.

Liver Function Assays
Aspartate aminotransferase (AST) Activities Determination
In the analysis of aspartate aminotransferase (AST), the method described by Reitman and Frankel[18]. was used. The unstable oxaloacetate undergoes spontaneous decarboxylation to generate pyruvate. The absorbance of the resulting red-coloured complex, formed from the reaction of pyruvate with p-nitrophenyl hydrazine, is measured at 546 nm using a spectrophotometer.

Alkaline phosphatase (ALP) Activities Estimation
Serum Alkaline Phosphatase (ALP) was estimated following Henry's method,1974 [19]. The procedure involves the catalytic hydrolysis of the colourless organic phosphate ester substrate, P-nitrophenylphosphate, which leads to the formation of the yellow-coloured product P-nitrophenol and phosphate. This reaction takes place in an alkaline pH of 10.3. The assay monitors the rate of change in absorbance at 405 nm over a fixed-time interval. The observed rate of absorbance change is directly proportional to the ALP activity present in the serum. This method provides a quantitative measure of alkaline phosphatase activity.

Estimation of Alanine aminotransferase (ALT) Activities
The enzymatic activity of alanine aminotransferase (ALT) involves the reversible transamination of alanine and α-ketoglutarate to yield pyruvate and L-glutamate. This reaction, outlined by Reitman and Frankel, 1957 [18], is quantified by monitoring the concentration of the formed pyruvate hydrazone, which reacts with 2,4-dinitrophenylhydrazine. Measuring this hydrazone allows for the accurate assessment of ALT activity in the sample. The absorbance was measured at 546 nm.

Serum Bilirubin Estimation
Jendrassik and Grof, 1938 [20] method was employed for bilirubin estimation. Direct bilirubin reacted with diazotized sulphanilic acid in an alkaline medium to create a blue-coloured complex. Total bilirubin was determined in the presence of caffeine, which liberated albumin-bound bilirubin, through the reaction with diazotized sulphanilic acid.

Serum Total Protein Estimation
The Biuret method, as described by Tietz, 1995[20] was followed. Cupric ions react with protein peptide bonds in an alkaline environment, which lead to the development of a coloured complex.

Estimation of serum Albumin
The colorimetric method described by Doumas *et al*,1991[22] was employed to quantify albumin. Serum albumin quantitatively binds to the indicator 3,3',5,5'-tetrabromo-m cresol sulphonephthalein (bromocresol green, BCG). The albumin-BCG complex exhibits maximum absorption at 578 nm, with the absorbance directly proportional to the albumin concentration in the serum.
Estimation of serum Globulin
This is done by subtracting albumin level from the total protein level [23].

Kidney Function Test
Estimation of serum uric acid
Uric acid quantification was done according to the method described by Tietz, 1999[23]. This involved the action of uricase on uric acid to produce allantoin, carbon(iv)oxide and H_2O_2. Hydrogen peroxide in the presence of peroxidase (and enzyme) reacts with a chromogen to yield quinoneimine (red coloured complex). The absorbance was measured at 505 nm. The colour intensity is equivalent to the amount of uric acid present in the serum sample.

Estimation of serum Creatinine
The quantification of creatinine in serum followed the procedure described by Bartels and Bohmer, 1972 [24]. Creatinine, when in an alkaline solution, interacts with picric acid, resulting in the formation of a coloured complex. The intensity of the complex's colour is proportional to the amount of creatinine in the serum.

Statistical analysis

Data obtained were expressed as mean ± standard error of mean (SEM). Values were subjected to one-way analysis of variance (ANOVA) and differences between samples were analysed by Tukey's multiple comparison using GraphPad Prism 10 Statistical package. P values < 0.05 were considered as significant.

RESULTS

Phytochemical screening of *B. pinnatum*: The phytochemical screening (Table 1) showed the presence of flavonoids, tannins, cardiac glycosides, steroids, phlobatannins, and coumarins, whereas alkaloids, phenols, terpenoids, saponins, and anthraquinones were absent.

The aspartate aminotransferase (AST) activity (Table 2 and Figure 2) showed that there was no statistically significant differences ($p>0.05$) observed in the activity of the control group and the group administered 702.5 m/kg body weight of *B. pinnnatum* juice, while there was a statistically significant ($p<0.05$) increase in AST activity in the group administered 1405 mg/kg body weight of *B. pinnnatum* compared to the control group.

In this study, the result of alanine aminotransferase (ALT) (Table 2 and Figure 2) indicates that there was no statistically significant differences ($p>0.05$) observed in the activity of ALT of the control group and the groups administered 702.5 and 1405 mg/kg body weight of *B. pinnnatum* juice.

The alkaline phosphatase (ALP) activity (Table 2 and Figure 2) showed that there was no statistically significant differences ($p>0.05$) observed in the activity of the control group and the group administered 702.5 m/kg body weight of *B. pinnnatum* juice. While there was a statistical significant ($p<0.05$) increase in ALP activity between the control group and the group administered 1405 mg/kg body weight of *B. pinnnatum*.

There was no statistically significant differences ($p>0.05$) observed in the total bilirubin (Table 3 and Figure 3) in all the animals used in this experiment across the groups (both the control group and the group administered *Bryophyllum pinnatum* crude leave juice at different doses). This same trend are observed for the conjugated bilirubin level (Table 3 and Figure 3), total protein, albumin level and the globulin level (Table 4 and Figure 4).

The concentrations of cholesterol, HDL and LDL (Table 5 and Figure 5) showed no significant difference ($p>0.05$) between the control group and the group administered with *Bryophyllum pinnatum* crude leave juice at different doses for sixteen days. This suggests that at various tested doses and duration, the juice did not exert any measurable influence on the overall cholesterol, HDL and LDL levels in the experimental animals used.

There was no statistically significant differences ($p>0.05$) observed in the triglycerides level (Table 5 and Figure 5) of the control group and the group administered 702.5 m/kg body weight of *B. pinnnatum* juice. While there was a statistically significant ($p<0.05$) decrease in triglycerides level between the control group and the group administered 1405 mg/kg body weight of *B. pinnnatum*.

Regardless of the dosage administered, *Bryophyllum pinnatum* leaves juice had no statistically significant ($p>0.05$) impact on uric acid level (Table 5 and Figure 5).

The creatinine level (Table 5 and Figure 5) showed that there was no statistically significant differences ($p>0.05$) observed in the concentration of the control group and the group administered 702.5 mg/kg body weight of *B. pinnnatum* juice but there was a statistically significant ($p<0.05$) increase in creatinine level observed between the control group and the group administered 1405 mg/kg body weight of *B. pinnnatum*.

Table 1: Phytochemical screening of *Bryophyllum pinnatum* leaf juice

PHYTOCHEMICALS	*Bryophyllum pinnatum*
Flavonoids	+
Tannins	+
Cardiac Glycosides	+
Saponins	-
Steroids	+
Terpenoids	-
Phenols	-
Phlobatannins	+
Coumarin	+
Alkaloids	-
Anthraquinone	-

Key: + = present; - = absent

Table 2: Effect of *Bryophyllum pinnatum* crude extract on AST, ALT and ALP activities (U/L)

Dose (mg/Kg body weight)	Mean	SEM	P values	Significant difference
Aspartate aminotransferase (AST) activity (U/L)				
Control (1 mL distilled water)	289.80	17.92[a]	-	-
702.5	337.60	38.80[a]	0.6448	No
1405	436.20	48.13[b]	0.0406	Yes
Alanine aminotransferase (ALT) activity (U/L)				
Control (1 mL distilled water)	119.80	8.22[a]	-	-
702.5	129.00	1.15[a]	0.8630	No
1405	142.00	19.92[a]	0.4452	No
Alkaline phosphatase (ALP) activity (U/L)				
Control (1 mL distilled water)	88.40	3.27[a]	-	-
702.5	94.60	1.89[a]	0.6501	No
1405	111.80	7.54[b]	0.0135	Yes

Values are presented as mean ± SEM, n=5. Different letters represent significant difference at $p < 0.05$ in comparison to the control.

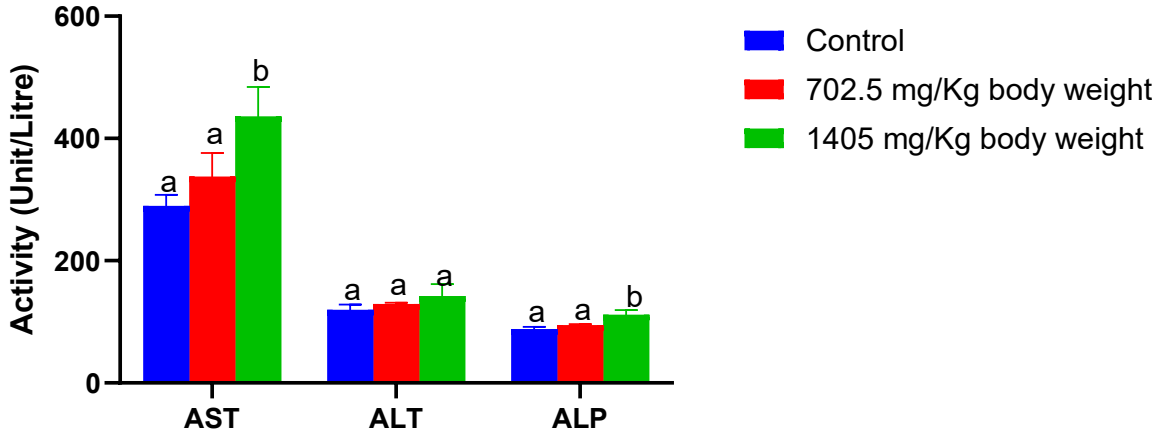


Figure 2: Effect of *Bryophyllum pinnatum* crude extract on AST, ALT and alkaline phosphatase ALP activities (U/L) Values are presented as mean ± SEM, n=5. Different letters represent significant difference at $p < 0.05$ in comparison to the control.

Table 3: Effect of *Bryophyllum pinnatum* crude extract on Total bilirubin and Conjugated bilirubin (mg/dL)

Dose (mg/Kg body weight)	Mean	SEM	P values	Significant difference
Total bilirubin (mg/dL)				
Control (1 mL distilled water)	0.24	0.02	-	-
702.5	0.30	0.00	0.0906	No
1405	0.28	0.02	0.3040	No
Conjugated bilirubin (mg/dL)				
Control (1 mL distilled water)	0.10	0.00	-	-
702.5	0.10	0.00	-	No
1405	0.10	0.00	-	No

Values are presented as mean ± SEM, n=5. No significant difference at $p < 0.05$ in comparison to the control.

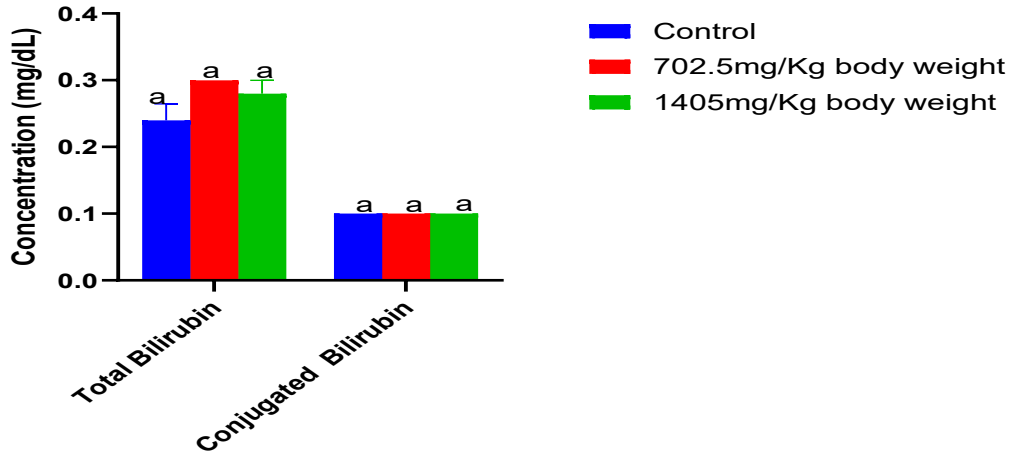


Figure 3: Effect of *Bryophyllum pinnatum* crude extract on Total bilirubin and Conjugated bilirubin (mg/dL). Values are presented as mean ± SEM, n=5. No significant difference at $p < 0.05$ in comparison to the control.

Table 4: Effect of *Bryophyllum pinnatum* crude extract on the concentrations of Total protein, Albumin and Globulin (g/dL)

Dose (mg/Kg body weight)	Mean	SEM	P values	Significant difference
Total protein concentration (g/dL)				
Control (1 mL distilled water)	6.080	0.29	-	-
702.5	5.82	0.14	0.6872	No
1405	6.02	0.21	0.9796	No
Albumin concentration (g/dL)				
Control (1 mL distilled water)	3.1405	0.18	-	-
702.5	3.08	0.16	0.4941	No
1405	3.06	0.27	0.4562	No
Globulin concentration (g/dL)				
Control (1 mL distilled water)	2.62	0.17	-	-
702.5	2.75	0.06	0.9171	No
1405	2.90	0.39	0.7129	No

Values are presented as mean ± SEM, n=5. No significant difference at $p < 0.05$ in comparison to the control.

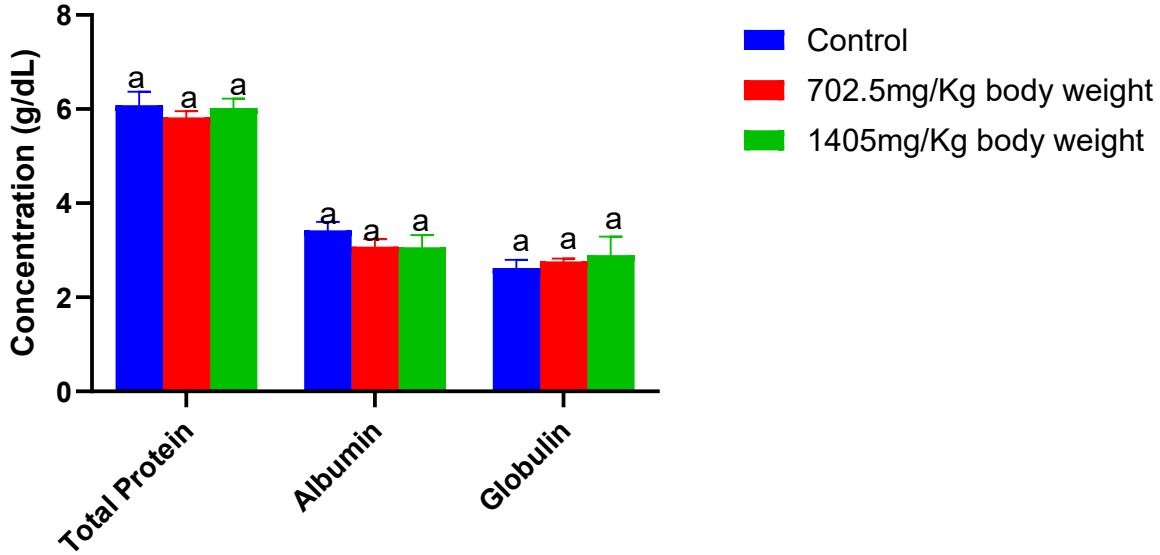


Figure 4: Effect of *Bryophyllum pinnatum* crude extract on the concentrations of Total protein, Albumin and Globulin (g/dL). Values are presented as mean ± SEM, n=5. No significant difference at $p < 0.05$ in comparison to the control.

Table 5: Effect of *Bryophyllum pinnatum* crude extract on the concentrations of Cholesterol, HDL, LDL and triglycerides (mg/dL)

Dose (mg/Kg body weight)	Mean	SEM	P values	Significant difference
Cholesterol concentration (mg/dL)				
Control (1 mL distilled water)	101.60	7.36[a]	-	-
702.5	92.80	6.98[a]	0.8399	No
1405	112.00	16.08[a]	0.7849	No
High density lipoprotein (HDL) concentration (mg/dL)				
Control (1 mL distilled water)	30.00	1.643[a]	-	-
702.5	27.20	2.04[a]	0.7987	No
1405	34.20	4.63[a]	0.6103	No
Low density lipoprotein (LDL) concentration (mg/dL)				
Control (1 mL distilled water)	51.00	3.834[a]	-	-
702.5	43.00	4.35[a]	0.8429	No
1405	61.00	11.75[a]	0.6296	No
Triglycerides concentration (mg/dL)				
Control (1 mL distilled water)	109.80	6.288[a]	-	-
702.5	109.00	4.85[a]	0.9944	No
1405	84.00	5.57[b]	0.0174	Yes

Values are presented as mean ± SEM, n=5. Different letters represent significant difference at $p < 0.05$ in comparison to the control.

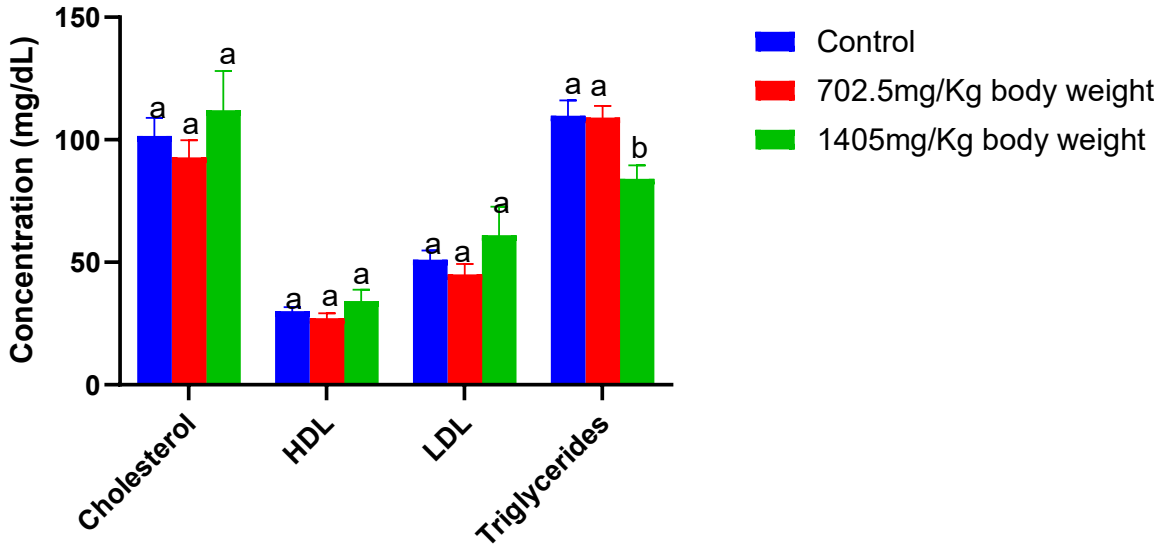


Figure 5: Effect of *Bryophyllum pinnatum* crude extract on the concentrations of Cholesterol, HDL, LDL and triglycerides. Values are presented as mean ± SEM, n=5. Different letters represent significant difference at $p < 0.05$ in comparison to the control.

Table 6: Effect of *Bryophyllum pinnatum* crude extract on the concentrations of Uric acid and Creatinine (mg/dL)

Dose (mg/Kg body weight)	Mean	SEM	P values	Significant difference
Uric acid concentration (mg/dL)				
Control (1 mL distilled water)	1.00	0.05[a]	-	-
702.5	0.98	0.04[a]	0.9812	No
1405	1.20	0.11[a]	0.1935	No
Uric acid and Creatinine concentration (mg/dL)				

Control (1 mL distilled water)	4.1405	0.32[a]	-	-
702.5	5.08	0.22[a]	0.3955	No
1405	5.80	0.46[b]	0.0378	Yes

Values are presented as mean ± SEM, n = 5. Different letters represent significant difference at $p < 0.05$ in comparison to the control.

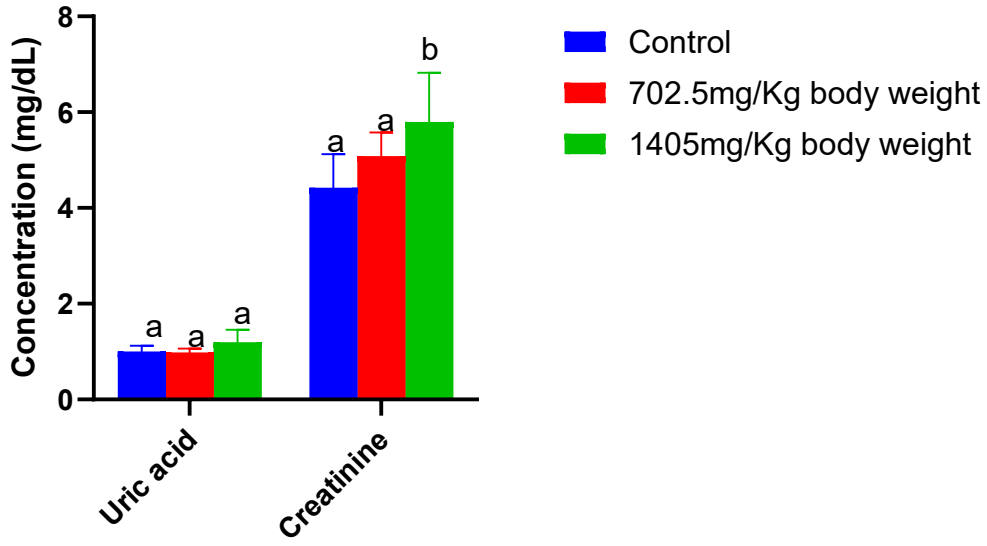


Figure 6: Effect of *Bryophyllum pinnatum* crude extract on the concentrations of Uric acid and Creatinine (mg/dL). Values are presented as mean ± SEM, n=5. Different letters represent significant difference at $p < 0.05$ in comparison to control.

DISCUSSION

The phytochemical screening of raw *B. pinnatum* sample provides crucial insights into its expected pharmacological activity and baseline toxicity. The co-existence of flavonoids, tannins, steroids, phlobatannins, and coumarins accounts for the plant's documented wound-healing, anti-inflammatory, and antioxidant potentials [25,26]. The structural preservation of critical baseline biomarkers seen in this study can be directly attributed to the reactive oxygen species (ROS) scavenging capacity of these polyphenols.

Furthermore, the result showed the absence of alkaloids, saponins, and anthraquinones (Table 1). Saponins are well-known to cause significant gastrointestinal irritation and red blood cell hemolysis, while localized plant alkaloids frequently present severe parenchymal or neurotoxic profiles. Anthraquinones, when present, can induce unwanted gut motility and severe electrolyte imbalances through hyper-purgative pathways. Meanwhile, the absence of total phenols and terpenoids in this screening contrasts with some established literature profiles [25]. This variation underscores the significant role that environmental stressors, geographic variations, or local soil composition play in altering a plant's secondary metabolic pathways [27, 28].

The stability of ALT, total bilirubin, conjugated bilirubin, total protein, albumin, and globulin across all groups indicates that *B. pinnatum* does not induce direct hepatocellular necrosis or impair the liver's capacity for protein synthesis, even at the high dose of 1405 mg/kg. Because ALT is highly specific to hepatocyte cytoplasm, its stable baseline across groups establishes that the raw sample avoids systemic parenchymal destruction. This matches previous observations by Ozolua *et al,* 2010 [27], who found that baseline sub-acute administration of aqueous extract of B. pinnatum leaves left overall serum bilirubin and functional liver proteins intact.

Furthermore, the results showed a clear toxicity threshold when examining AST, ALP, and creatinine. The lack of any significant difference between the control and the 702.5 mg/kg group highlights a clean safety profile at a one-teaspoon equivalent dose. Yet, the high significant variance between the control and the 1405 mg/kg group indicates the activation of cellular stress pathways. Because ALT remained stable, the isolated increase in AST and ALP at 1405 mg/kg suggests sub-clinical biliary irritation or extrahepatic muscular stress. This specific elevation may be driven by the accumulated cardiac glycosides (bufadienolides) identified in the qualitative screening, which are known to exert dose-dependent tissue strain [26]. Simultaneously, the significant rise in serum creatinine at 1405 mg/kg

indicates a distinct reduction in glomerular filtration efficiency, proving that doubling the traditional intake over a sub-acute 16-day window induces mild renal impairment.

The lipid architecture observed in this study provides additional context regarding metabolic processing. While structural cholesterol fractions (Cholesterol, HDL, and LDL) remained entirely unaffected, the high elevation of triglycerides exclusively at 1405 mg/kg reveals a rate targeted metabolic saturation point. High doses of crude phytochemical mixtures can inhibit hepatic triglyceride lipase or transiently downregulate the clearance of very-low-density lipoproteins (VLDL) within systemic circulation, giving rise to localized hypertriglyceridemia [29].

The absence of significant changes in the serum total protein, albumin, and globulin further shows that the synthetic capacity of the liver was not adversely affected. Albumin synthesis is one of the main metabolic functions of the liver, and reductions are commonly associated with chronic liver dysfunction or impaired protein synthesis [30]. Therefore, the maintenance of normal protein concentrations suggests that prolonged administration of the extract did not compromise hepatic protein synthesis.

CONCLUSION

The study indicated that *B. pinnatum* possesses several beneficial phytochemicals that may account for its traditional medicinal uses. Moreover, repeated administration at 702.5 mg/kg body weight appeared relatively safe. However, prolonged administration at 1405 mg/kg body weight produced mild dose-related alterations in AST, ALP, creatinine, and triglycerides without evidence of severe hepatotoxicity or nephrotoxicity. These findings support the traditional usage of the plant at lower doses while highlighting the importance of dose optimization during prolonged use and validating the traditional need for standardized dosing regimens.

Limitations: The study focused on the phytochemical characterization of the specie of *B. Pinnatum* (Lam.) Okem found in Benin-city, Nigeria and the sub-acute toxicity on the metabolic profiles but did not assess the histopathology of the main organs.

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AUTHORS' CONTRIBUTION

The conceptualization and design of this study was done equally by the two authors.

CONFLICT OF INTEREST

None declared by the authors.

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