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Protective Effect of *Bryophyllum pinnatum* Extracts Against Carbon Tetrachloride (CCl_4) Induced Toxicity in Male Wistar Rats

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ABSTRACT

Background and Objective: *Bryophyllum pinnatum* is used for remedy of jaundice, hypertension, renal stones, and diabetes. This study estimated the protective potential of ethanol leaf extract of *Bryophyllum pinnatum* against CCl_4-induced biochemical injury in Wistar albino rats using serum biochemical parameters. **Materials and Methods:** The study evaluated the antioxidant and hepatoprotective effects of *Bryophyllum pinnatum* leaves. Ethanol and aqueous extracts were prepared and screened for phytochemicals. Antioxidant activities were assessed using DPPH, FRAP, and phosphomolybdenum assays. Thirty male Wistar rats were grouped, induced with carbon tetrachloride (CCl_4), and treated with extracts. Histopathological analysis and statistical evaluations demonstrated significant antioxidant and hepatoprotective properties of the extracts ($p<0.05$). **Results:** It was observed that there was a significant ($p<0.05$) increase in the AST, ALT, ALP, conjugated bilirubin, and total bilirubin in serum levels of the rats administered with CCl_4 group compared to the negative control group. However, pre-treatment of rats with ethanol extract of *B. pinnatum* significantly ($p<0.05$) decreased these biochemical parameters compared to the positive control group. In addition, *B. pinnatum* extract significantly modulated hematological parameters in the CCl_4-induced toxicity in the rats administered with the extract though the 500 mg/kg body weight group was the most potent. The liver histopathological screening showed severe diffuse vacuolar degeneration and necrosis of hepatocytes in the induced group when compared to the negative control but was reversed after treatment with the extract. **Conclusion:** The results obtained from this study showed that the ethanol leaf extract of *B. pinnatum* has a protective effect against CCl_4-induced hepatic damage which might be due to the presence of phytochemical and antioxidant compounds.

KEYWORDS

Bryophyllum pinnatum, hematological indices, hepatoprotective, carbon tetrachloride, liver enzymes

INTRODUCTION

Most developing countries of the world including Nigeria rely heavily on herbal preparations for the treatment of various diseases despite the availability of orthodox medicine[1]. Conventional drugs used in the treatment of hepatic diseases are costly, limited, and can have serious adverse effects. Hence the search for relatively effective regimens and inexpensive drugs with little or no side effects is recently being sought from medicinal plants especially those with antioxidant properties since hepatic diseases are associated with oxidative stress. *Bryophyllum pinnatum* (family: Crassulaceae) is also known as *Kalanchoe*

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pinnatum or *Bryophyllum calycinum*[2]. *Bryophyllum pinnatum* is a perennial herb with a height of 3 to 5 m high with opposed glabrous leaves[3-5]. The herb contains a wide range of valuable chemicals that could be responsible for its various pharmacological effects[6]. The plant parts are frequently applied for the cure of burns, rheumatoid arthritis, antiseptic, blisters, cough suppression, insect bites, psychiatric disorders, and abdominal discomforts[2]. *Bryophyllum pinnatum* is used for the treatment and management of various pathologies such as conjunctivitis, edema, piles, cuts, eczema, constipation, epilepsy, cholera, asthma, chest colds, menstrual disorders, chicken pox, and fever[7]. It is well-known for its anti-inflammatory, wound healing, analgesic, and hemostatic qualities[8]. Also, slightly heated leaves act as a tocolytic agent to prevent premature labor are applied on superficial skin infections, and are also used for the dropping of placenta in Southeast Nigeria, hence it is used[9]. Furthermore, various plant parts like root extract is being used for their laxative, diuretic, and anti-psychotic effects[10,11] and the paste from crushed leaves can be applied on the skin for the treatment of boils and abscesses[12]. In Germany, anthroposophical physicians prescribed *Bryophyllum pinnatum* preparations for tocolysis and behavioral disorders[13]. Environmental contaminants including carbon tetrachloride (CCl_4) have added to the global burden of hepatotoxicities. Human exposure to CCl_4 occurs at the workplace in polluted air, soil, and water through dermal inhalation, oral, and dermal routes. Carbon tetrachloride (CCl_4) is a colorless, volatile, non-inflammable liquid that is produced by the mixture of chlorine with chloroform in the presence of light. The CCl_4 toxicity develops through the generation of free radical CCl_3 and other metabolites produced by cytochrome P450. Severe conditions may develop through multiple organ dysfunction by these free radicals[14]. Upon metabolism, they lead to cellular damage by alteration of cellular structure through lipid peroxidation. The CCl_4 is a known hepatotoxic agent that induces the disorders through the formation of a trichloromethyl radical (CCl_3^-) and its peroxyl radical derivative, CCl_3OO^-, formed during the metabolism by the phase 1 enzymes present in the endoplasmic reticulum of hepatocytes. The acute toxicity of CCl_4 in the liver has been investigated by oral administration of CCl_4 with the effect which could be felt in the hematological indices[15]. The liver is an important organ responsible for various metabolic processes of detoxification of the organism through a complex enzyme system that regulates Red Blood Cells (RBCs), glucose synthesis, and storage[16]. A large percentage of liver disorders occur as a result of exposure to chemical compounds capable of inducing oxidative stress, which leads to an increase in the production of radical compounds thereby leading to liver diseases with extremely poor prognosis and high mortality which is due to lack of effective therapy[17]. Carbon tetrachloride (CCl_4) is one of the xenobiotics that has been used extensively to study hepatotoxicity in animal models by initiating lipid peroxidation, thereby causing injuries to the kidney, heart, testis, and brain in addition to liver pathogenesis[18,19]. The liver is particularly susceptible to oxidative stress due to the direct release of CCl_4 metabolites and cytokines, which propagate inflammatory responses[20]. Elevations in ALT and AST in out of proportion to ALP and bilirubin denotes a hepatocellular disease. This study, assessed the protective effect of *Bryophyllum pinnatum* extract against carbon tetrachloride (CCl_4) induced toxicity in rats.

MATERIALS AND METHODS

Study area: The study was carried out at the Department of Chemistry, The Polytechnic, Ibadan Nigeria from October, 2023 to April, 2024.

Identification and extraction of plant material: *Bryophyllum pinnatum* leaves were obtained at the Botanical Garden, University of Ibadan. The leaves were identified and authenticated at the Herbarium Unit, Botany Department, University of Ibadan, Nigeria. The leaves were air dried under shade for four weeks and it was pulverized to a fine powder using the blender.

Ethanol extraction: As 450 g of the pulverized leaves of *Bryophyllum pinnatum* was macerated in 4 L of ethanol for 72 hrs and it was agitated frequently. It was then filtered with muslin cloth and filter paper and the filtrate was kept in a refrigerator. The marc was re-soaked in 4 L of ethanol for 24 hrs and then filtered and concentrated using a rotary evaporator.

Aqueous extraction: As 165 g of the pulverized leaves of *Bryophyllum pinnatum* was macerated in 5 L of distilled water for 72 hrs and it was agitated frequently. It was then filtered with muslin cloth and filter paper. The filtrate was concentrated using a rotary evaporator.

Qualitative phytochemical screening: The phytochemicals of the crude extract and its fractions with a slight modification were determined[21]. Terpenoids, saponins, tannins, alkaloids, flavonoids, glycosides, steroids, phenol, and anthraquinones were present in the extracts.

Quantitative phytochemical screening: Total alkaloids, flavonoids, phenol, tannins, terpenoids, and saponins were quantitatively determined.

DPPH scavenging activity: The DPPH scavenging activity based on the scavenging activity of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical determined[22]. The fractions were prepared with methanol into five different concentrations ranging from 200-1000 µg/mL 1 mL of the different concentrations were transferred into the test tube and 1 mL of DPPH solution (0.3 mM) was added. After 30 min, the absorbance was measured at 517 nm. The percentage of the DPPH radical scavenging was calculated using the equation given below:

$$\text{Inhibition of DPPH radical (\%)} = \frac{\text{Abr - Aar}}{\text{Abr}} \times 100$$

where, Abr is the absorbance before the reaction (blank) and Aar is the absorbance after the reaction has taken place.

Ferric Ion-Reducing Antioxidant Power Assay (FRAP): Ferric Ion-Reducing Antioxidant Power Assay was determined[23]. The fractions in different concentrations were mixed with 2.5 mL of 20 mM phosphate buffer and 2.5 mL 1%, w/v potassium ferricyanide, and then the mixture was incubated at 50°C for 30 min. Afterward, 2.5 mL of 10%, w/v trichloroacetic acid, and 0.5 mL 0.1%, w/v ferric chloride was added to the mixture, which was kept aside for 10 min. Finally, the absorbance was measured at 700 nm. Ascorbic acid was used as the positive reference standard. All assays were run in triplicate way and averaged.

Total antioxidant determination: Total antioxidant activity was estimated by phosphomolybdenum assay preparation of Molybdate reagent solution[24].

One milliliter each of 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate were added to 20 mL of distilled water and made up volume to 50 mL by adding distilled water.

Sample in different concentrations ranging from 200-1000 µg/mL was added to each test tube individually containing 3 mL of distilled water and 1 mL of Molybdate reagent solution. These tubes were kept incubated at 95°C for 90 min. After incubation, these tubes were normalized to room temperature for 30 min and the absorbance of the reaction mixture was measured at 695 nm. Mean values from three independent samples were calculated for each extract.

Ascorbic acid was used as the positive reference standard.

Experimental animals: Thirty healthy male Wistar rats with weights ranging from 90-135 g were obtained from Lifelong farm, Lagelu Road, Oremeji Bus stop, Ibadan, Oyo State, Nigeria. They were kept in clean cages (wooden with wire mesh), maintained under standard laboratory conditions and were allowed free access to standard grower's mash with clean water. The experimental animals were acclimatized for 14 days in the Animal House of The Polytechnic, Ibadan, Nigeria. All animal procedures were performed by the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH publications No. 8023, revised 1978).

Experimental design: The rats were selectively distributed into six groups of five rats per group. The procedure for administering plant extracts includes:

Group 1 (Control): Fed with normal feed and water daily for the period of the experiment
Group 2: It was induced with 3 mL/kg body weight of carbon tetrachloride (CCl_4) once
Group 3: It was administered 250 mg/kg body weight of ethanol extract of *Bryophyllum pinnatum* daily for 1 week
Group 4: It was administered 500 mg/kg body weight of ethanol extract of *Bryophyllum pinnatum* daily for 1 week
Group 5: It was induced with 3 mL/kg b.wt., once and administered 250 mg/kg body weight of ethanol extract of *Bryophyllum pinnatum* daily for 1 week
Group 6: It was induced with 3 mL/kg b.wt., once and administered 500 mg/kg body weight of ethanol extract of *Bryophyllum pinnatum* daily for 1 week

Experimental protocol: The toxicant (CCl_4) was administered intraperitoneally and the extract was administered orally to the animals for 1 week (7 days). After 7 days of administration, the animals were sacrificed, having been placed on 12 hrs overnight fast, and weighed to obtain the final body weights. Blood samples were collected and liver samples of experimental rats were harvested for histopathology examination.

Histopathological analysis: The harvested liver was fixed in 10% buffered formalin saline and dehydrated in ascending grades of ethanol, after which the liver tissues were cleared in chloroform overnight, infiltrated, and embedded in molten paraffin wax. The blocks were then trimmed and sectioned at 5 µm. The sections were deparaffinized in xylene, mounted on clean slides, stained with Haematoxylin and Eosin (H&E), and examined under an Olympus/3H light microscope. Photomicrographs of the liver were captured using a Moticam Images Plus 2.0 digital fitted to the light microscope.

Statistical analysis: Data generated were presented as Mean±Standard Deviation of triplicate measurements; analysis of variance was used to assess significant differences in the means of the measured parameters in the group treatments, while Tukey's *post hoc* test was used to identify specific differences between groups. Probability level at $p<0.05$ was considered significant. Statistical analysis was performed using SPSS version 20.

RESULTS

The result as presented in Table 1 revealed the presence of saponins, tannins, flavonoids, cardiac glycosides, anthraquinones, steroids, terpenoids, alkaloids, and phenol in extracts of the plant though anthraquinone and steroid were absent in the aqueous extract.

In the quantitative result presented in Table 2, the ethanol extract had a higher percentage of flavonoids, saponin, terpenoids, and phenols while the aqueous extract had a higher percentage of alkaloid and tannin. This indicated that the ethanol extract possesses a higher phytochemical constituent than the aqueous extract.

The result of the DPPH scavenging activity of the ethanolic and aqueous extract of the plant is shown in Table 3. This result shows that the ethanolic extract possess more potent scavenging activity than the corresponding aqueous extract.

Table 4 shows the comparative ferric-reducing antioxidant power (FRAP) of the ethanol extract, aqueous extract, and standard ascorbic acid. It was observed that the ethanol extract had better antioxidant activity compared with the aqueous extract.

Table 5 presents the result of the total antioxidant capacity of ethanol and aqueous leaf extract of *B. pinnatum*. The antioxidant activities of the plant extracts showed a concentration-dependent increase with the ethanolic extract showing more activity than the aqueous extract.

Table 1: Qualitative phytochemical screening of ethanol and aqueous extract of *Bryophyllum pinnatum* leaves

Parameter	Ethanol	Aqueous
Saponins	++	+
Tannins	++	++
Flavonoids	++	++
Cardiac glycosides	+	++
Anthraquinones	++	-
Steroids	+	-
Terpenoids	+	+
Alkaloids	+	+
Phenol	++	++

+: Present, ++: Abundantly present and -: Absent

Table 2: Quantitative phytochemical screening of ethanol and aqueous extract of *Bryophyllum pinnatum* leaves

Sample	Alkaloids content (% w/w)	Flavonoids content (% w/w)	Saponins content (% w/w)	Tannins content (% w/w)	Terpenoids content (% w/w)	Total phenol (% w/w)
Ethanol extract	9.75±0.98	1.69±0.13	1.7±0.20	0.95±0.03	1.0±0.00	0.69±0.03
Aqueous extract	10.75±1.25	0.97±0.09	0.65±0.07	0.99±0.02	0.4±0.01	0.63±0.02

Values are Mean±SD of triplicate determinations

Table 3: DPPH scavenging activity of ethanol and aqueous leaves extracts of *Bryophyllum pinnatum*

Concentration (µg/mL)	Ethanol extract	Aqueous extract
200	0.722±0.06	0.802±0.06
400	0.712±0.15	0.8±0.10
600	0.651±0.06	0.756±0.06
800	0.639±0.10	0.703±0.06
1000	0.618±0.06	0.685±0.06

Values are Mean±SD of triplicate determinations

Table 4: FRAP activity of ethanol and aqueous leave extracts of *Bryophyllum pinnatum*

Concentration (µg/mL)	Ethanol extract	Aqueous extract
20	1.606±0.004	1.412±0.001
40	1.615±0.002	1.417±0.001
60	1.621±0.002	1.435±0.004
80	1.655±0.000	1.476±0.004
100	1.676±0.001	1.544±0.00.5

Values are Mean±SD of triplicate determinations

Table 5: Total antioxidant capacity of ethanol and aqueous leave extracts of *Bryophyllum pinnatum*

Concentration (µg/mL)	Ethanol extract	Aqueous extract
200	0.166±0.003	0.134±0.002
400	0.213±0.001	0.140±0.002
600	0.285±0.001	0.149±0.000
800	0.304±0.000	0.165±0.001
1000	0.479±0.002	0.177±0.002

Values are Mean±SD of triplicate determinations

Table 6: Effect of ethanolic extract of *B. pinnatum* on red blood cell indices in CCl_4-induced toxicity in Wistar rats

Treatment	HCT (%)	RBC (10^{12}/L)	HGB (g/dL)	MCV (fl)	MCH (pg)	MCHC (g/dL)
Control	$36±2^{b}$	$5.85±0.1^{d}$	$13.4±0.2^{c}$	$75±4^{a}$	$15.2±0.6^{a}$	$29.9±1.2^{ab}$
CCL_4 treatment	$20±2^{a}$	$2.44±0.1^{a}$	$8.6±0.3^{a}$	$92±3^{b}$	$18.0±0.8^{b}$	$40.8±1.0^{b}$
250 mg/kg BPE	$36±3^{b}$	$5.57±0.0^{cd}$	$13.5±0.7^{c}$	$76±4^{a}$	$15.3±1.0^{a}$	$29.9±1.2^{ab}$
500 mg/kg BPE	$34±4^{b}$	$5.65±0.1^{cd}$	$13.1±0.8^{c}$	$79±3^{a}$	$15.4±0.9^{a}$	$27.5±2.0^{a}$
CCL_4+250 mg/kg BPE	$29±3^{ab}$	$4.19±0.2^{b}$	$10.9±0.5^{b}$	$86±7^{ab}$	$16.8±0.7^{ab}$	$30.5±0.1^{ab}$
CCL_4+500 mg/kg BPE	$35±3^{b}$	$5.44±0.2^{c}$	$12.7±0.5^{c}$	$77±3^{a}$	$15.4±1.0^{a}$	$30.0±0.8^{ab}$

Means with different alphabets in any pair in the same column are significantly different ($p<0.05$), HCT (%): Hematocrit, RBC (1012L): Red blood cell count, HB (g/dL): Haemoglobin concentration, MCV (fl): Mean Corpuscular Volume, MCH (pg): Mean corpuscular haemoglobin, MCHC (g/dL): Mean corpuscular haemoglobin concentration and BPE is *Bryophyllum pinnatum* extract

The effect of a graded dose of the ethanol extract of *B. pinnatum* on liver function indices of carbon tetrachloride-induced toxicity in the Wistar rats is shown in Table 6. Results showed that no significant differences were observed in the mean levels of the total protein and albumin among the various group treatments. However, there are significant differences ($p<0.05$) in the mean values between the control group and the CCl_4 treated groups as well as the ethanol extract treated groups in the total bilirubin levels, conjugate bilirubin, and the activities of the enzymes: AST, ALT, and ALP, where the CCl4 treated groups showed significantly elevated levels of the measured indices.

The hematological parameters indicate that CCL_4 treatment significantly reduced HCT, RBC count, and HGB levels while increasing MCV, MCH, and MCHC compared to the control group. However, co-treatment with BPE at 250 or 500 mg/kg ameliorated these effects, with the 500 mg/kg dose showing near-restoration of values to control levels. This suggests that BPE effectively mitigates CCL_4-induced hematotoxicity, with higher doses providing better protection (Table 6).

The data reveals that CCL_4 treatment significantly reduced WBC, PLT, and MID levels compared to the control, with WBC decreasing from 7.65±0.19 to 5.40±0.14 (10 L^{-1}) and PLT dropping from 354±38 to 160±8 (10 L^{-1}). Conversely, CCL_4 treatment elevated LYM levels to 47.4±1.0% from 35.7±1.9% in the control group. Treatment with BPE at 250 and 500 mg/kg alone maintained WBC, PLT, and MID levels similar to the control. Notably, co-administration of BPE with CCL_4 partially mitigated the adverse effects, particularly at 500 mg/kg, where WBC and PLT levels improved to 7.03±1.39 (10 L^{-1}) and 339±30 (10 L^{-1}), respectively (Table 7).

The effect of a graded dose of the ethanol extract on hematological parameters of carbon tetrachloride-induced toxicity in Wistar rats is shown in Table 7 and 8. In the red blood cell indices (Table 7), there are significant differences between the control group, CCl_4 treated group, and the *B. pinnatum* ethanolic leaf extract treated groups in the hematocrit level, red blood cell count, hemoglobin levels, mean corpuscular volume, mean corpuscular hemoglobin, while no significant differences exist between the control group and the CCl_4 treated group as well as the extract treatment group. In the white blood cell indices (Table 8), a significant difference exists in the mean of all the white blood cell parameters between the control and CCl_4 treated group, and also in the extract treatment.

The findings indicate that CCl_4 treatment significantly elevated serum TB, CB, AST, ALT, and ALP levels compared to the control group, suggesting hepatic damage. Treatment with BPE alone at both 250 and 500 mg/kg doses maintained parameters close to control values, indicating no hepatotoxic effects. The Co-treatment with BPE at 250 and 500 mg/kg in CCl_4-exposed groups mitigated the elevated levels of these biomarkers, with the 500 mg/kg dose showing greater efficacy. TP and ALB levels remained relatively consistent across all groups, reflecting no significant impact on protein metabolism (Table 8).

The pathological changes caused by CCl_4 intoxication in the liver and the protective effects of the ethanol extract of *Bryophyllum pinnatum* are depicted in Fig. 1. In Fig. 1a, the negative control rats show no visible lesions, indicating normal hepatic architecture. In contrast, Fig. 1b illustrates the effect of 3 mL/kg body weight (b.wt.) of CCl_4, revealing severe diffuse vacuolar degeneration and necrosis of hepatocytes. Administration of 250 mg/kg b.wt., of the ethanol extract of *Bryophyllum pinnatum* is shown in Fig. 1c, where moderate portal fibrous hyperplasia is observed. Figure 1d demonstrates the effects of

Table 7: Effect of ethanolic extract of *B. pinnatum* on white blood cell indices in CCl_4-induced toxicity in Wistar rats

Treatment	WBC (10^9/L)	GRA (%)	LYM (%)	MID (%)	PLT (10^9/L)
Control	7.65±0.19^{b}	56.4±1.5^{ab}	35.7±1.9^{ab}	7.9±0.5^{c}	354±38^{b}
CCL_4 treatment	5.40±0.14^{a}	52.3±0.9^{a}	47.4±1.0^{c}	0.3±0.2^{a}	160±8^{a}
250 mg/kg BPE	7.62±0.34^{b}	56.7±2.1^{ab}	33.5±2.1^{a}	9.8±1.7^{c}	337±54^{b}
500 mg/kg BPE	7.74±0.11^{b}	56.3±2.5^{ab}	34.7±1.3^{a}	9.0±1.2^{c}	361±51^{b}
CCL_4+250 mg/kg BPE	5.97±0.20^{a}	52.0±2.0^{a}	40.8±2.3^{b}	7.2±1.8^{c}	279±23^{b}
CCL_4+500 mg/kg BPE	7.03±1.39^{ab}	60.5±3.2^{b}	35.8±3.1^{ab}	3.7±1.2^{b}	339±30^{b}

Means with different alphabets in any pair in the same column are significantly different ($p<0.05$), WBC (10^9/L): White blood cell count, GRA (%): Granulocyte, LYM (%): Lymphocytes and PLT (10^9/L): Platelets

Table 8: Effect of ethanol extract of *B. pinnatum* on liver function indices of CCl_4 induced toxicity in Wistar rats

Treatments	TB (mg/dL)	CB (mg/dL)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	TP* (mg/dL)	ALB* (mg/dL)
Control	0.3±0.2^{a}	0.1±0.1^{a}	13±1^{a}	12±2^{ab}	41±2^{a}	6.7±0.3^{a}	3.8±0.2^{a}
CCL_4 treatment	3.1±0.1^{c}	1.1±0.2^{c}	28±2^{c}	27±2^{d}	76±10^{c}	6.8±0.8a	3.8±0.6^{a}
250 mg/kg BPE	0.7±0.2^{ab}	0.2±0.1^{ab}	14±2^{a}	11±2^{ab}	49±3^{ab}	7.0±0.2^{a}	3.8±0.2^{a}
500 mg/kg BPE	0.7±0.2^{ab}	0.2±0.1^{ab}	16±1^{ab}	10±2^{a}	51±3^{ab}	6.7±0.4^{a}	3.7±0.3^{a}
CCL_4+250 mg/kg BPE 1	0.0±0.2^{b}	0.5±0.1^{b}	19±2^{b}	18±2^{c}	60±3^{b}	6.5±0.3^{a}	3.5±0.2^{a}
CCL_4+500 mg/kg BPE	0.6±0.1^{ab}	0.2±0.1^{ab}	14±2^{a}	15±2^{bc}	54±5^{ab}	7.0±0.2^{a}	4.0±0.1^{a}

*Not significant ($p>0.05$), means differences between any two groups carrying the same subscript alphabet are not significantly different ($p>0.05$), TB: Total bilirubin CB: Conjugate bilirubin AST: Aspartate aminotransferase ALT: Alanine transaminase, ALP: Alkaline phosphatase, TP: Total protein and ALB: Albumin

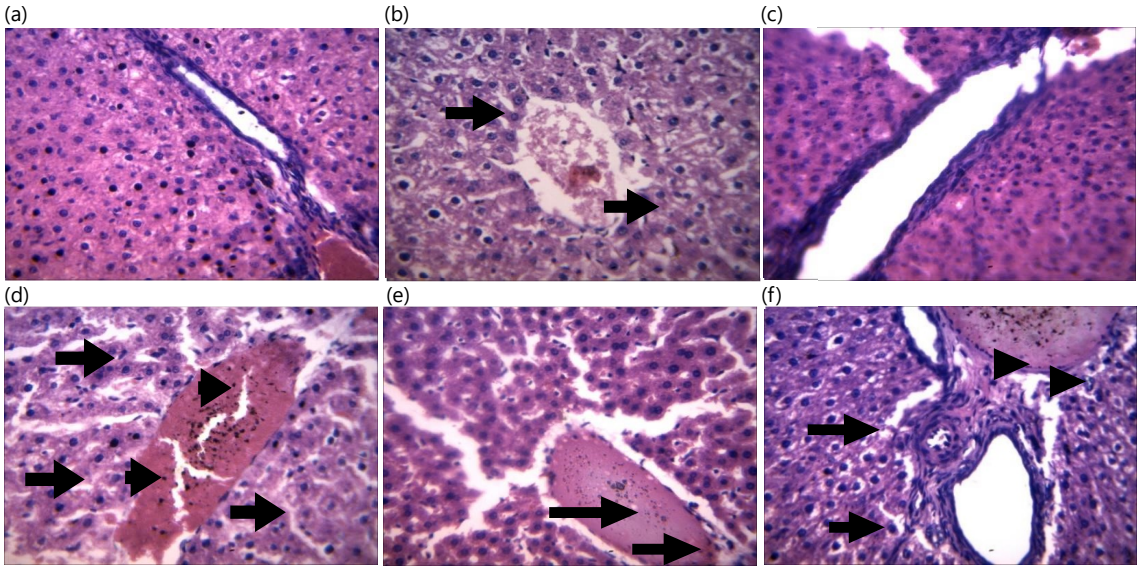

Fig. 1(a-f): Photomicrograph showing liver sections of experimental rats, (a) Negative control rats, (b) 3 mL/kg b.wt., of CCl_4 showing severe diffuse vacuolar degeneration and necrosis of hepatocytes (arrows), (c) 250 mg/kg b.wt., of ethanol extract of *Bryophyllum pinnatum* showing no visible lesion, (d) 500 mg/kg b.wt., of ethanol extract of *Bryophyllum pinnatum* showing moderate portal congestion (arrowheads), with mild hydropic degeneration of hepatocytes (arrows) (e) 3 mL/kg b.wt., of CCl_4 with 250 mg/kg b.wt., of ethanol extract of *Bryophyllum pinnatum* showing mild central venous congestion (arrows) and (f) 3 mL/kg b.wt., of CCl_4 with 500 mg/kg b.wt., of ethanol extract of *Bryophyllum pinnatum* showing mild portal congestion (arrowheads), with mild vacuolar degeneration of hepatocytes (arrows)

500 mg/kg b.wt., of the ethanol extract, showing moderate portal congestion accompanied by mild hydropic degeneration of hepatocytes. The combined treatment of 3 mL/kg b.wt., of CCl_4 and 250 mg/kg b.wt., of the ethanol extract is shown in Fig. 1e, indicating mild central venous congestion. Lastly, Fig. 1f presents the combined treatment of 3 mL/kg b.wt., of CCl_4 with 500 mg/kg b.wt., of the ethanol extract, showing mild portal congestion and mild vacuolar degeneration of hepatocytes.

DISCUSSION

Medicinal plants are a potential source of biomolecules that have been used as phytomedicine for the treatment of various diseases and disorders for centuries[25]. The chemical constituents in medicinal plants possess biological activities that can scavenge free radicals and improve human health via the pharmaceutical and food industries though they also represent important value in perfume, agrochemical, and cosmetic industries[26].

Preliminary phytochemical investigation of the plant extracts of *B. Pinnatum* confirmed the presence of a wide range of secondary metabolites in the plant such as alkaloids, flavonoids, tannins, and saponins. This observation is in agreement with that reported in the ethanol extract of *B. pinnatum* leaves[27]. The result of the study showed that the ethanol and aqueous extracts of *B. pinnatum* contain high levels of total phenols which is quite higher than those levels reported in other studies[28,29].

The *in vitro* antioxidant activity of the extracts as measured via the DPPH assay showed that the percentage inhibition of the DPPH radicals presented in Table 3 shows that the radical scavenging activity of both extracts increased with an increase in concentration, however, the ethanol extract had better scavenging ability than the aqueous extract. Also, results from the IC_{50} which estimates the concentration at which 50% of the radicals would have been scavenged revealed that the ethanol extract had better radical scavenging ability than the aqueous extracts, though, the ascorbic acid standard had better activity (the lower the IC_{50} the better the antioxidant activity) than both extracts. The IC_{50} obtained in this study

for both ethanol (1.54 mg/mL) and aqueous extract (1.89 mg/mL) is comparable with the range (2.35 mg/mL) reported[30] in ethanol root extract of *B. pinnatum*. However, the concentration in both extracts was well above the levels reported in various extract fractions of *B. pinnatum*[29]. The reduction of Fe^{3+} is usually used as an indicator of electron-donating ability in the FRAP assay, therefore, the presence of antioxidants in the extracts would result in the reduction of Fe^{3+} to Fe^{2+} through the donation of an electron in the FRAP assay[31]. A dose-dependent increase in the ferric-reducing ability in the ethanol and aqueous extract of *B. pinnatum* and standard, with the standard having better ferric-reducing ability followed by the ethanol extract and then the aqueous extract was observed in the FRAP assay (Table 4); this trend is similarly observed in different extract fractions of *B. pinnatum*[29]. The total antioxidant capacities of the ethanol and aqueous extract show an increase with an increase in concentration as presented in Table 5 though the ethanol extract showed a higher TAC at all the measured concentration ranges compared with the aqueous extracts of *B. pinnatum*. This trend is comparable with those reported in previous studies for *B. pinnatum*[32]. The current study shows that the *in vitro* antioxidant activities of both extracts via the DPPH, FRAP, and TAC indicate that the ethanol extract possesses better DPPH radical scavenging activity though, not comparable with the ascorbic acid standard; a concentration-dependent reducing power in the FRAP assay with the trend: Ascorbic acid>ethanol extract>aqueous extract; while the ethanol extract had the better total antioxidant capacity.

Furthermore, the effect of graded doses of the ethanolic extract of the plant on the hematological indices against carbon tetrachloride-induced toxicity in animal models was assessed. From the result, as presented in Table 6 and 7, the red and white blood cell indices were found to be significantly altered ($p<0.05$) by the CCl_4 treatment except in the Mean Corpuscular Hemoglobin Concentration (MCHC) in the red blood cell indices and granulocyte levels in the white blood cell count. This observation had been similarly observed in other studies[33-35]. The extract-only treated groups (250 and 500 mg/kg b.wt.) had no significant effect on the hematological parameters as levels are comparable with the control group ($p>0.05$). The treatment of the CCl_4-induced rats with the ethanolic extracts of *B. pinnatum* shows that the higher dosage (500 mg/kg) of BPE significantly modulated the hematocrit, hemoglobin, mean corpuscular volume, and the mean corpuscular hemoglobin levels in the red blood cell parameters, the red blood cell count was equally modulated by 500 mg/kg b.wt., of *B. pinnatum* ethanolic extract though below the control level. In the white blood cell indices, a similar trend is observed; the extract-alone treated groups had comparable levels with the control which suggests the extract may have a protective effect in the experimental rats. Similarly, the higher dosage of the extract (500 mg/kg) significantly ($p<0.05$) elevated the granulocytes, lymphocytes, and platelet concentration. The mid cells were also modulated but not to the level of the control. These suggest a dose-dependent effect of the extract on the hematological parameters in the rats where the ethanolic extract at a level of 500 mg/kg b.wt., had a significant positive effect on the hematological parameters. These findings agree with those observed with other plants that ameliorated the negative effect of CCl_4 toxicity in animal models. The effect of ethanol extract of *Bryophyllum pinnatum* on the pathological changes caused by CCl_4 intoxication in the liver is displayed in Fig. 1. The liver photomicrographs of the experimental rats in groups 1 and 2 fed with distilled water and 250 mg/kg extract only showed intact and normal liver architecture as presented in plates A and C. However, the CCl_4-treated group revealed severe diffuse vacuolar degeneration and necrosis of hepatocytes as presented in plate B which is in line with what has been reported by previous findings that CCl_4 causes necrosis[36]. Also, the photomicrograph of the liver of rats treated with 500 mg/kg body weight of ethanol extract of *Bryophyllum pinnatum* as presented in plate D showed moderate portal congestion with mild hydropic degeneration of hepatocytes. The liver section of pretreated rats that received 3 mL/kg b.wt., of CCl_4 and 250 and 500 mg/kg body weight of ethanol extract of *Bryophyllum pinnatum* showed mild portal venous congestion. This indicated that the extract may have exhibited hepatoprotective activity due to its antioxidant properties attributable to these powerful antioxidants. Folashade *et al.*[37], reported that the terpenoid fraction of *V. amygdalina* leaf extract ameliorates carbon tetrachloride-induced hepatotoxicity in rats. Pretreatment with *Bryophyllum pinnatum* ethanol extract significantly improved the structure of hepatic cells. Also, rat treatments with the *Bryophyllum pinnatum* markedly attenuated the hepatotoxicity caused by CCl_4.

Medicinal plants are a potential source of biomolecules that have been used as phytomedicine for the treatment of various diseases and disorders for centuries. The plant contains numerous bioactive components that can scavenge free radicals. It may be essential to consume natural oxidants to enhance a weakened immune system. The antioxidants protect the body from free radical-induced oxidative stress and help to prevent cancer and heart disease.

CONCLUSION

Despite the development of various major therapies, the tilt toward herbal medicine is gaining momentum due to the rising concerns about the increasing toxicities associated with main-line therapies. Plants contain numerous antioxidants which help to confer protection against free radicals associated diseases like cancer. The administration of *Bryophyllum pinnatum* extract at the doses used (250 and 500 mg/kg) attenuated the increased liver enzymes and subsequent normalization of the liver structure compared to the animals in the groups treated with CCl_4 alone thereby confirming the protective effect of *Bryophyllum pinnatum*. Current study findings suggest that ethanol extract of *Bryophyllum pinnatum* protects against oxidative stress and hepato-renal injury caused by CCl_4 intoxication. *Bryophyllum pinnatum* could therefore be a potential therapeutic agent for managing hepatic injury. However, further studies are required to isolate the active principles in the plant.

SIGNIFICANCE STATEMENT

The study highlights the protective potential of *Bryophyllum pinnatum* ethanol extract against oxidative stress and hepato-renal injuries caused by CCl_4 intoxication. The extract significantly normalized liver enzymes improved hematological parameters, and reversed histopathological damage, with 500 mg/kg showing the most potent effect. These findings suggest *B. pinnatum* is a promising therapeutic agent for managing hepatic injury, attributed to its phytochemical and antioxidant properties.

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