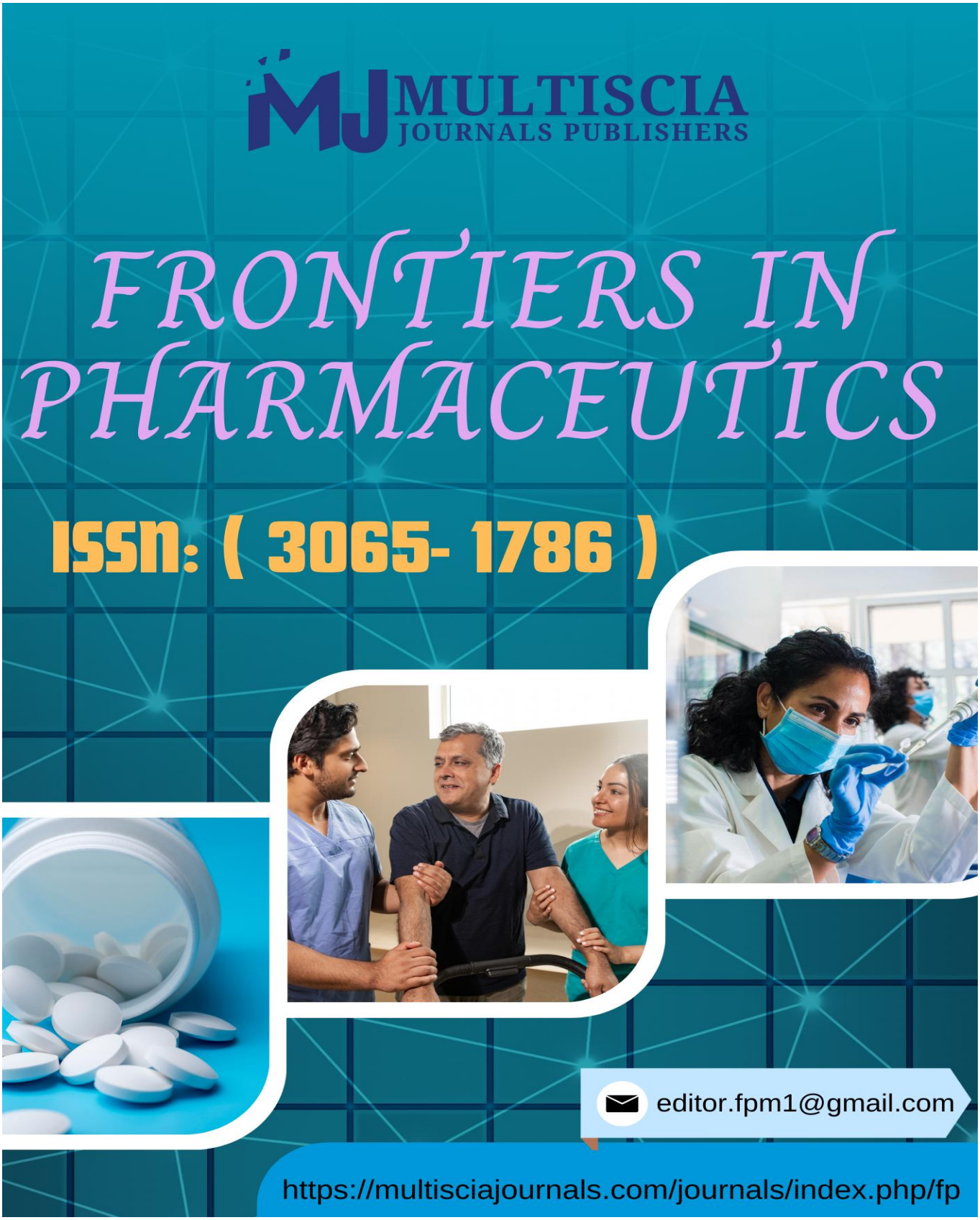
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Methanol Extract of Bryophyllum pinnatum Leaf Harvested from Southeast Nigeria Has Antidiarrheal and Antioxidant Properties in Mice

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1. Introduction

In the majority of developing nations, diarrhea is a leading cause of hospitalization for children and even older adults, and it is responsible for approximately 5 million fatalities per year [1]. Increased frequency, fluidity, and three or more daily bowel movements, together with oxidative stress and an inflammatory response, are its defining characteristics [2, 3]. It arises from an imbalance in the gut's secretory and absorptive physiologies of water and electrolytes, which can be brought on by helminths, toxins, food, allergies, and microorganisms (bacteria, viruses, protozoa, and fungus) [4]. Antispasmodic, antisecretory, and prostaglandin secretion inhibitors including loperamide, diaretyl, and atropine are frequently used to treat diarrhea [3, 5]. Constipation, sleepiness, and colorectal cancer are among the adverse effects of these drugs that restrict their use [5]. Diarrhea and other gastrointestinal diseases are treated in the tropics with a variety of naturally occurring substances [1]. Bryophyllum pinnatum is one of the medicinal plants used in herbal remedies for diarrhea in South-Eastern Nigeria.
Common names for Bryophyllum pinnatum include miracle leaf, life plant, love plant, and Canterbury bells. It is a member of the Crassulaceae family. Tropical Africa, America, Hawaii, India, China, Australia, and Madagascar are among its many locations [6]. As an astringent, emollient, hemostatic, carmi-native, disinfectant, and tonic, the leaf is widely employed in traditional medicine. Hematemesis, hemorrhoids, menstrual discomfort, wounds, boils, sloughing ulcers, ophthalmia, burns, scalds, diarrhea, and dysentery are among the conditions for which it is also used [7]. Alkaloids, triterpenes, lipids, flavonoids, and other biologically active compounds

The plant has been shown to include glycosides, kaempferol rhamnoside, bufadienolides, phenols, and organic acids [6–8]. Hepatoprotective and antineoplastic properties [6, 9], anti-asthmatic and antitussive properties [9, 10], antidiabetic properties [10], antihypertensive properties [11], antimicrobial properties [12], anti-inflammatory and analgesic properties [13, 14], and antiulcer properties [15] have all been documented for B. pinnatum leaves. It has been observed that B. Pinnatum, which is gathered from the Southwest ecological zone, possesses antidiarrheal properties [16]. The phytochemical composition and pharmacological actions of medicinal plants are influenced by ecological conditions, including rainfall, soil type, light intensity, and humidity [17]. Few studies have been conducted on the antidiarrheal properties of B. pinnatum leaves cultivated in the ecological zone of southeast Nigeria. The methanol extract of B. Pinnatum leaves collected from southeast Nigeria was tested for its antidiarrheal properties in mice.

1. Supplies and Procedure

1.1. Plant Identification and Collection. In June 2016 (rainy season), fresh Bryophyllum pinnatum leaves were gathered from Ugba Junction in Isiala Ngwa South Local Government Area of Abia State, South-Eastern Nigeria. Plant taxonomist Dr. M. C. Dike of the College of Natural Resources and Environmental Management, Michael Okpara University of Agriculture, Umudike, verified that the leaves were indeed Bryophyllum pinnatum. For reference, a voucher specimen bearing the catalogue number MOUAU/CVM/VPP/2016/44 was placed in the herbarium of the Department of Veterinary Physiology and Pharmacology.

1.2. Drugs and Chemicals. Methanol, sodium acetate trihydrate, glacial acetic acid, 2,4,6-tripyridyl-s-triazine (TPTZ), hydrochloric acid, charcoal, castor oil, gum acacia (Merck, Germany), ascorbic acid (Hopkin and Williams, England), loperamide hydrochloride (Xian-Janssen Pharmaceutical Ltd., China), and 2,2-diphenyl-2-picrylhydrazyl (DPPH).

2.4. Animals. In this study, 90 (90) mice weighing between 28 and 34 g and between 8 and 10 weeks of age were obtained from the laboratory animal unit of the Department of Veterinary Physiology and Pharmacology at Michael Okpara University of Agriculture Umudike, Abia State. The animals were kept in aluminum cages with natural light/dark cycles and at ambient temperature. The mice were fed regular commercial pelleted grower feed (Vital feed® Nigeria) at will and were given access to clean drinking water. Before the investigation, the mice were acclimated for two weeks. The institution's ethics committee accepted the experimental protocol, and they were kept in compliance with the guidelines provided in the Guide for the Care and Use of Laboratory Animals [18].

2.5. Research on Antidiarrhea

2.5.1. Diarrhea Caused by Castor Oil. We adhered to Onoja and Udeh's [19] methodology. To put it briefly, thirty (30) mice were split up into five groups (A–E), each consisting of six mice. They had free access to water during their roughly ten-hour fast, but two hours before the test, the water was taken away. Groups C, D, and E received 50, 100, and 200 mg/kg of BPE, respectively, while Group A received 10 mL/kg of distilled water and Group B received loperamide (5 mg/kg). Oral dose of 0.3 ml of castor oil caused diarrhea one hour after the medicines were administered. Each animal was put in a cage with white paper lining it. Over the course of four hours, the quantity of both moist and dry stool droppings was counted at one-hour intervals. The treated groups' mean percentage of wet stool passed was compared to the control group's [20].

On a laboratory bench, pinnatum were allowed to dry at ambient temperature before being manually milled into a coarse powder (Corona, China). The electronic balance (PI 303 model, China) was used to weigh the powdered material. For 48 hours, the 150 g of plant material was macerated with 80% methanol while being shaken intermittently every three hours. Whatman No. 1 filter sheets were used to filter the resultant slurry, which was then dried at 40°C in a hot air oven. Before being used, the extract was kept in a refrigerator at 4°C. The following formula was used to determine the Bryophyllum pinnatum extracts' (BPE) percentage yield (w/w): Weight of extract ÷ weight of initial plant material equals percentage yield (w/w).

Small Intestinal Transit (2.5.2). In this experiment, the modified Chime [21] approach as outlined by Onoja and Udeh [19] was used. In summary, thirty mice were randomly assigned to five groups (A–E), each consisting of six mice, and they were given a 10-hour fast before to the experiment. Groups C, D, and E received 50, 100, and 200 mg/kg of BPE, respectively, while Group A received 10 mL/kg of distilled water and Group B received loperamide (5 mg/kg). One hour following treatment, all of the animals received the normal charcoal meal, which consists of 5% activated charcoal suspended in 5% gum acacia. The animals were killed by cervical dislocation 30 minutes after the charcoal meal was administered, and the intestines were promptly separated and tied off at the ileocecal junction and pyloric sphincter. The small intestine One hundred

weight of the initial plant material

(1)

The percentage of the small intestine's length from the pyloric sphincter to the ileocecal junction that the charcoal meal covered was known as transit.

TABLE 1: Effect of BPE and loperamide on castor oil induced diarrhea on mice.

Percentage (%) wet stool				
Treatment	1 h	2 h	3 h	4 h
Distilled water 10 ml/kg	76.66 ± 2.35	81.38 ± 2.00	82.57 ± 2.27	84.62 ± 2.23
Loperamide 5 mg/kg	0.00 ± 0.00*	40.00 ± 16.32*	88.75 ± 3.39	90.00 ± 2.98
BPE 50 mg/kg	48.33 ± 9.06*	63.54 ± 11.01	83.06 ± 2.01	83.61 ± 2.18
BPE 100 mg/kg	28.33 ± 9.77*	50.00 ± 11.57	61.66 ± 8.48*	72.14 ± 5.13*
BPE 200 mg/kg	20.83 ± 10.75*	61.11 ± 2.02	63.49 ± 2.89*	62.50 ± 4.56*

*$p < 0.05$ when compared with distilled water treated group. BPE = *Bryophyllum pinnatum* Extract.

$$\% \text{ distance travelled} = \frac{\text{distance travelled by the charcoal meal}}{\text{Full length of the small intestine}} \times \frac{100}{1} \quad (3)$$

$$\% \text{ inhibition} = \frac{\% \text{ distance travelled of control group} - \% \text{ distance travelled of treated group}}{\% \text{ distance travelled of control group}} \times \frac{100}{1}$$

2.4.1. Enteropooling. The method of Hassan et al. [22] as described in Onoja and Udeh. [19] was followed in this study. Briefly, thirty mice were randomly divided into five groups of six mice each and were fasted for 10 h before the experiment. Group A received 10 mL/kg of distilled water and Group B received loperamide (5 mg/kg) while Groups C, D, and E received 50, 100, and 200 mg/kg of BPE, respectively. One h after the treatment, the animals were sacrificed by cervical dislocation and laparotomized and the intestines were immediately isolated and ligated at the pyloric sphincter and ileocecal junction. The small intestines were weighed, the content of each intestine was milked out, and the empty intestines were reweighed. The difference in weight between the full and empty intestines was recorded as the weight of the intestinal content.eight of intestinal content = wt of intestine with content − wt of empty intestine

$$\% \text{ inhibition} = \frac{\text{wt of intestinal content of control} - \text{wt of intestinal content of treated group}}{\text{wt of intestinal content of control}} \times 100, \quad (4)$$

where wt = weight.

2.5. Antioxidant Study

2.5.1. 2,2-diphenyl-1-picrylhydrazyl (DPPH) Photometric Assay. The DPPH radical scavenging activity of the extract was analyzed as reported by Onoja et al., [23] using spectropho- tometer. The extract at various concentrations (25, 50, 100, 200, and 400 μg/ml) was assayed in triplicate and ascorbic acid was also assayed as a reference standard.*Ferric Reducing Antioxidant Power.* The ferric reducing antioxidant power of BPE was evaluated as described by Onoja et al. [24]. The concentrations of 25, 50, 100, 200, and 400 μg/ml of BPE in triplicate were used in the study. It was compared with ascorbic acid at 125 μg/ml concentration.

2.6. Data Analysis. The obtained data were statistically eval- uated using one-way ANOVA, followed by least significant difference test with SPSS software. The mean values were considered significant at $p < 0.05$.

2. Results

2.1. Effect of BPE and Loperamide on Castor Oil-Induced Diarrhea on Mice. The extract caused a significant ($p < 0.05$) dose-dependent decrease in the percentage of wet stools (Table 1) in the treated groups throughout the period of observation when compared with distilled water treated groups. At 4 h after diarrhea induction, the percentages of wet stool of the distilled water, loperamide, and BPE 50, 100, and 200 mg/kg were 84.62, 90.00, 83.61, 72.14, and 62.50%, respectively. The optimum antidiarrheal effect of BPE was produced at 200 mg/kg dose.

2.2. Effect of BPE and Loperamide on Small Intestinal Transit in Mice. The loperamide and BPE (50, 100, and 200 mg/kg) significantly ($p < 0.05$) reduced the small intestinal transit of charcoal meal in the treated mice (Table 2) when compared with distilled water treated mice. The extract did not produce dose-dependent effect. The maximal effect of the extract was observed at 50 mg/kg.

2.3. Effect of BPE and Loperamide on Enteropooling in Mice.
The loperamide and BPE (50, 100, and 200 mg/kg) caused a

TABLE 2: Effects of BPE and loperamide on small intestinal transit in mice.

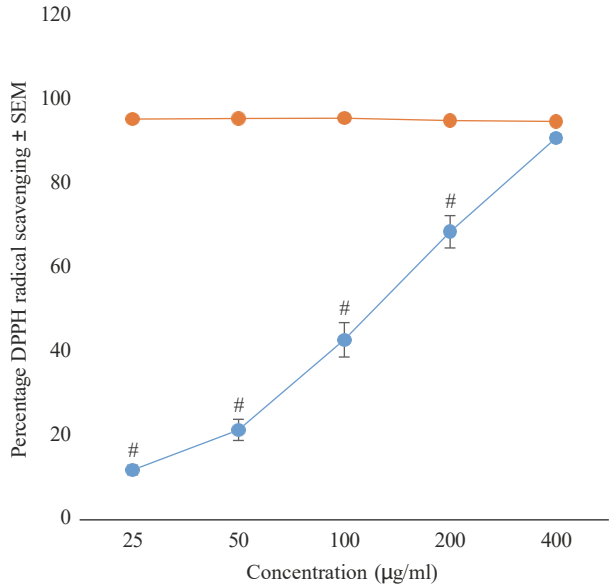
Treatment	% Distance travelled	% Inhibition
Distilled water 10 ml/kg	60.65 ± 0.46	0
Loperamide 5 mg/kg	45.10 ± 2.20*	25.63
BPE 50 mg/kg	43.78 ± 2.96*	27.81
BPE 100 mg/kg	58.93 ± 2.76	2.83
BPE 200 mg/kg	54.60 ± 0.49	9.97

*$p < 0.05$ when compared with distilled water treated group. BPE = *Bryophyllum pinnatum* extract.

TABLE 3: Effects of BPE and loperamide on the enteropooling in mice.

Treatment	WT of intestinal content (g)	% inhibition
Distilled water 10 ml/kg	0.91 ± 0.01	-
Loperamide 5 mg/kg	0.45 ± 0.07*	50.55
BPE 50 mg/kg	0.60 ± 0.01*	34.07
BPE 100 mg/kg	0.67 ± 0.01*	26.37
BPE 200 mg/kg	0.66 ± 0.01*	27.47

*$p < 0.05$ when compared with distilled water treated group. BPE = *Bryophyllum pinnatum* extract, WT = weight.



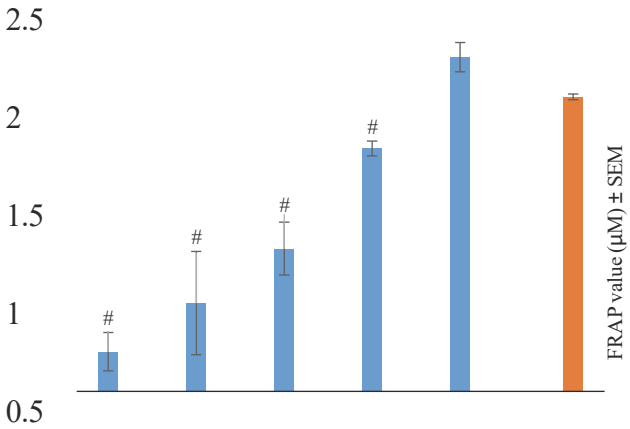
significant ($p < 0.05$) reduction in the weight of the intestinal content in the treated mice when compared to the distilled water treated mice (Table 3). The BPE did not produce a dose-dependent effect. The weights of the intestinal content of the distilled water, loperamide, and BPE 50, 100, and 200 mg/kg were 0.91, 0.45, 0.60, 0.67, and 0.66 g, respectively.

2.4. DPPH Radical Scavenging Activities of BPE. The extract produced a concentration dependent increase in percentage antioxidant activity. The optimum antioxidant activity of BPE was produced at 400 μg/ml concentration (Figure 1).

2.5. Ferric Reducing Antioxidant Power of BPE. The extract produced a concentration dependent increase in antioxidant power. At 400 μg/ml the assay gave 2.27 (μM), showing that

BPE
Ascorbic acid

FIGURE 1: DPPH radical scavenging activities of BPE. #$p < 0.05$ when compared with ascorbic acid, BPE = *Bryophyllum pinnatum* extract.



BPE has a high antioxidant power (Figure 2).

3. Discussion

The antidiarrheal property of methanol extract of *Bryophyl- lum pinnatum* was evaluated using castor oil-induced diar- rhea, intestinal transit, and enteropooling models in mice. The extract significantly ($p < 0.05$) inhibited castor oil- induced diarrhea, reduced intestinal transit, reduced intesti-

nal fluid accumulation, and elicited potent antioxidant activ- ities. These pharmacological activities could be mediated by the phytochemical constituents of *Bryophyllum pinnatum* [25]. The presence of phytoconstituents like alkaloids, ter- penes, glycosides, and flavonoids has been reported on *B. pinnatum* and the antidiarrheal and antioxidant activities of these phytoconstituents have been reported as well [26].

The inhibition of castor oil-induced diarrhea might be linked to protection against gastric irritation and inflamma- tion as well as reduction in prostaglandin release [27, 28].0

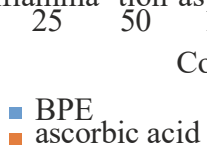


FIGURE 2: Ferric reducing antioxidant power (FRAP) of BPE. $^{\#}p < 0.05$ when compared with ascorbic acid, BPE = *Bryophyllum pinnatum* extract.

Ricinoleic acid, the active metabolite of castor oil, causes irritation and inflammation of the intestinal mucosa which leads to increased prostaglandin release, enhanced peristalsis, and reduced reabsorption of sodium ion, chloride ion, and water from the gut which give rise to diarrhea [29]. The inhi- bition of prostaglandin synthesis has been incriminated in the anti-inflammatory and analgesic activities of *B. pinnatum* [13, 14].

The reduction in intestinal transit may be due to reduced peristalsis which can be attributed to relaxation of the intesti- nal smooth muscle [30, 31]. Drugs that relax the intestinal smooth muscles are often used as antidiarrheal agent because they inhibit intestinal hypermotility (increase peristalsis) which usually accompanies diarrhea [26]. Antidiarrheal activity of loperamide is linked to inhibition of peristalsis [32, 33]. The reduction in intestinal fluid accumulation is linked to antisecretory activity. Tannins and flavonoids present in the plant extract are reported to inhibit release of prostaglandins, thereby inhibiting motility and secretion induced by castor oil. The antidiarrheal activity of the extract may also be due to denature of proteins by tannates that make intestinal mucosa more resistant and reduce secretion [25]. The reported antimicrobial activities of *B. pinnatum* suggest that the extract could be effective in the management of susceptible microbial induced diarrhea [34, 35]. The findings of this study corroborate the antidiarrheal activities of *Combretum dolichopetalum* [19] as well as the report of Adeyemi et al. [16] on the antidiarrheal effects of *B. pinnatum* harvested from South-Western Nigerian.

The antidiarrheal effects of *B. Pinnatum* may also be associated with its potent antioxidant potential as observed in this study. Umukoro and Ashorobi reported that ascorbic acid and α-tocopherol reduce prostaglandin level through the inhibition of perioxidation of phospholipids and thus ameliorate castor oil-induced diarrhea [27]. The antidiarrheal and antioxidant activities of *Bryophyllum pinnatum* could be mediated by the phytochemical composition. Phytochemical investigations have shown the presence of alkaloids, triter- penes, lipids, flavonoids, glycosides, kaempferol rhamnoside, bufadienolides, phenols, and organic acids [6–8].

4. Conclusion

The findings of this study demonstrated the pharmacological basis for ethnomedical use of *Bryophyllum pinnatum* in diar- rhea treatment. However further studies are desired toward the isolation and characterization of the active compound.

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