Evaluation of ulcer-protective effect of the ethanolic extract of *Bryophyllum pinnatum* leaves on ethanol-induced ulcer

Research article

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Abstract

Bryophyllum pinnatum extract has been employed for different pharmacological uses ranging from antimicrobial activity, anti-inflammatory, antidiabetic, anticonvulsant, antihypertensive and anticancer activity, antitumuour, anthelmentic, hepatoprotective, antinociceptive, nephroprotective, antioxidant activity, analgesic, anticonvulsant, neuropharmacological, antipyretic, haemostatic and wound healing properties. However, the use of ethanolic extract of *B. pinnatum* leaves (EEBPL) in the management of ulcer not known. The phytochemical analyses, acute toxicity studies and ulcer studies of ethanolic extract of *B. pinnatum* leaves was carried out using standard methods. *Bryophyllum pinnatum* leaves extract contains flavonoids, tannins, terpenoids, alkaloids, reducing sugars, phenols and carbohydrates. Acute toxicity study revealed that EEBPL was not toxic at all doses studied. Ulcer protective studies demonstrated ulcer-protective activity of EEBPL which compared favorably with the standard drug (omeprazole). We hereby submit that EEBPL is safe regarding toxicity in ulcer protection and could be used in anti-ulcer formulations.

Public Interest Statement

Apart from the local use in traditional medicine, *Bryophyllium pinnatum* has demonstrated promising results in the treatment of several diseases under scientific experimental conditions as documented in several scientific literatures. However, the ulcer-protective property remains unexploited. We therefore seek to evaluate the possible use of *B. pinnatum* in the management of ulcer and ulcer-related pathologies.

1.0 Introduction

It is no news that medicinal plants possess various compounds that has accounted for their vast application in the management of human pathologies. Generally, the biological friendly nature, availability and demonstrated potentials of these medicinal plants in the face of emerging human diseases with multiple etiologies has necessitated novel research on known and novel plants for the treatment of several diseases. Recently, various drug discovery enquiries have depended on preliminary studies and isolation of substances of pharmacological potentials from plant extracts. Some of these research projects have graduated from mere studies designed to give scientific explanation to local claims by traditional users, to novel drug discovery roadmaps.

With the increasing incidence of ulcer in individuals, especially human populations exposed to various levels of stress, alcoholism and unlearned use of non-steroidal anti-inflammatory drugs, investigations into medicinal plants with ulcer protective properties becomes very necessary. Various documented research abound, that has reported the use of plant extracts in the management of ulcer (Madueke and Anosike, 2017), among others. The different potentials of different medicinal plants in the management of a single disease make it necessary for the advancement of such studies, using as many plants as could be achieved.

Apart from the local use in traditional medicine, *Bryophyllium pinnatum* has demonstrated promising results in the treatment of several diseases under scientific experimental conditions. The antimicrobial property was reported by Okwu and Nnamdi (2010), while Ojewole (2005) reported the hypoglycaemic and hypolipidemic properties of the plant extract on STZ-induced diabetic rats. Flavanoids obtained from the plant like Quacertin, leuteolin, Proanthocyanidins, kaempferol di-glycoside, flavonol and flavone glycosides were found to show potent anti-leishmanial activity (Afzal *et al*., 2012). All these pharmacological activities of *Bryophyllum pinnatum* have shown that it could be an indispensable target for the synthesis of novel drugs. We therefore report the ulcer-protective effect of the ethanol leaf extract.

2. Materials and methods

2.1 Chemicals and reagents.

The Ethanol used for the extraction of the crude-extract was obtained from Chemistry Department, University of Nigeria, Nsukka manufactured by Guangdong Guanghua chemical Ltd, Shanghai-China. Other chemicals to be used were gotten from commercial vendors in the environs of Nsukka: Tween 80(2%) (Sigma-Aldrich, Germany), chloroform (Sigma-Aldrich, Germany), distilled water (UNN, Nigeria), normal saline, omeprazole tablets.

2.2 Collection of Plant Sample and Extraction

The fresh leaves of the plant were collected and authenticated by a taxonomist at the International Centre of Ethnomedicine and Drug Discovery, (InterCEED) Nsukka with voucher specimen number #Interceed/803. The plant material was air-dried, powdered, macerated with ethanol for 72hrs and filtered using Whatman No. 1 filter paper. The filtrate obtained was dried in a rotary evaporator (IKA, Germany) at an optimum temperature of 40°-50°C. The dried crude extract obtained was stored in a sterile container for use.

2.3 Experimental animals

Eighteen (18) mice and Twenty-five (25) adult male albino rats (60- 140g) were used for the study. Animals were collected from the Animal house of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. Ethical clearance for experimental animals was obtained from the Ethical Clearance Committee for Biological Sciences, University of Nigeria, Nsukka.

2.4 Acute toxicity studies

Acute toxicity studies of EEBPL was carried out following the method of Lorke (1983).

2.5 Experimental Design

Animals were allowed to acclimatize for 7 days prior to experiment and maintained in cages with food and water under a 12 hours light/dark cycle. The rats were randomly divided into five groups of five animals each and administered thus;

Group 1: 2 ml/kg bwt 2% Tween 80 + Induction of ulcer (positive control).
Group 2: 100 mg/kg bwt Omeprazole + Induction of ulcer (standard control).
Group 3: 100 mg/kg bwt EEBPL+ Induction of ulcer.
Group 4: 200 mg/kg bwt EEBPL+ Induction of ulcer.
Group 5: 400 mg/kg bwt EEBPL + Induction of ulcer.

2.6 Phytochemical and Macronutrients Analysis

The qualitative phytochemical and macronutrient analyses were done using the methods of Harborne, (1998); Trease and Evans, (2002).

2.7 Ulceration index

Ulcer induction followed the method of Sayanti *et al*. (2007). Animals were fasted for 24hrs with access to drinking water. Tween 80, graded doses of EEBPL and omeprazole as stated in the experimental design orally. Thirty minutes after treatment, absolute ethanol was used to induce ulcer in all groups. After eight (8) hours, animals were sacrificed and stomach removed and opened along the greater curvature, rinsed with normal saline and pinned to flat board. The stomach was examined using magnifying lens (10x) for lesions, counted and scored as

0 = no ulcer;
1 = superficial ulcer;
2 = deep ulcer;
3 = perforations. The ulcer index and % ulcer inhibition was calculated using the formula prescribed by Onwukwe *et al.* (2016):

$$\text{Ulcer index} = \frac{\text{Total ulcer score}}{10}$$

$$\text{Ulcer inhibition (\%)} = \frac{\text{Ulcer index of untreated} - \text{Ulcer index of treated}}{\text{Ulcer index of untreated}} \times 100$$

2.9 Statistical analysis

Statistical analysis of One-way ANOVA was carried out on the result using the statistical package, IBM SPSS Statistics version 20 at the level of significance, $p < 0.05$

3. Results

3.1 phytochemical analyses

Phytochemistry of EEBPL were determined using prescribed standard procedures and the result shows that the ethanol extract of *Bryophyllum pinnatum* contain alkaloids, glycosides, terpenoids, saponins, tannins, carbohydrates, reducing sugar, flavonoids and total phenol. The test conducted for glycosides and saponins were found to be negative for the two (Table 1).

Table 1: Phytochemical screening of dried powdered of *Bryophyllum pinnatum*

Phytochemical	Inference	Concentration (mg/ml)
Alkaloids	+++	171.58 ± 6.21
Total phenol	++	66.67 ± 12.73
Reducing sugar	+	7.82± 0.58
Terpenoids	+++	476.89 ± 76.59
Carbohydrates	++	30.87 ± 2.56
Flavonoid	+++	269.64 ± 50.38
Tannins	+	2.36 ± 0.37
Steroids	+	11.31± 1.41

Key: +Low concentration, ++Moderate concentration, +++High concentration

3.2 Acute toxicity studies

Acute toxicity studies of EEBPL showed safety of the extract for oral use at all studied doses and thus, no death was recorded nor changes in behavior.

Table 2: Acute toxicity studies of EEBPL on albino mice.

Phase I

Dose (mg/kg)	Mortality
10	0/3
100	0/3
1000	0/3

Phase II

Dose (mg/kg)	Mortality
1600	0/3
2900	0/3
5000	0/3

3.3 Mean Ulcer index and percentage ulcer inhibition

Table 4 shows the result of ulcer index and % ulcer inhibition. Mean ulcer index of the treated groups were significantly reduced relative to the untreated control (group 1). There was a considerable decrease in ulcer indices of groups 3, 4 and 5 in a dose dependent manner showing

increased ulcer protection conferred by EEBPL as dosage increases. Our data also revealed that EEBPL demonstrated a higher ulcer-protective effect relative to the standard drug at 400 mg/kg body weight.

Table 4: Mean Ulcer Index and % ulcer inhibition of Experimental Groups

Groups	Mean ulcer index	% ulcer inhibition
1	2.40 ± 0.79^{c}	-
2	$1.08\pm0.63^{a, b}$	55.00
3	$2.10\pm1.16^{b, c}$	12.50
4	$1.84\pm0.40^{a, b,c}$	23.30
5	0.95 ± 0.30^{a}	60.40

Values are written as Mean ± SD, (n=5).

*Values in the same column having different superscripts differ significantly ($p<0.05$).

4. Discussion

Our study investigated the ulcer-protective effect of EEBPL on ethanol-induced ulcer in rats. The phytochemical screening demonstrated the presence of various phytochemicals which are known to be responsible for the healing properties of medicinal plants. Flavonoids and tannins have been reported to possess anti-ulcer properties through free radical scavenging mechanisms and protection of the gastric mucosa (Francesca and Angelo, 2000). Although our study is a preliminary level study, however, we submit, on the basis of observed phytochemicals, that EEBPL is a promising raw material for novel drug discovery and thus provide a scientific explanation for the reported pharmacological potentials of *B. pinnatum*.

Acute toxicity studies suggest that EEBPL is safe for oral administration at all doses studies. This follows from our observation that even at a dose of 5000 mg/kg body, no death was recorded in the experimental mice and no negative behavioral changes were observed for the period of the study. Drug side toxicity has become a burden to researchers and drug discovery scientists. Our data reveal that the use of EEBPL in drug development might pose little or no side effect with respect to oral administration for ulcer management.

Our collated data on ulcer index and ulcer inhibition demonstrated a promising solution for ulcer management as our extract possess a higher ulcer-protective effect at 400 mg/kg relative to the standard drug omeprazole. Ulcer induction by alcohol has been explained to occur through many mechanisms, one of which is through irritation of the gastric mucosa and disruption of vascular endothelium leading to elevated vascular permeability and lifting of the epithelium (Anosike and Ofoegbu, 2013). These events can stimulate the proton pump resulting

in elevated secretion of gastric acid. Omeprazole on the other hand, mediates ulcer inhibition through blocking the proton pump of the parietal cells. We therefore propose that EEBPL ulcer-protective effect could be through a similar mechanism. It is also possible that EEBPL confers a favorable effect on the enzymes of prostaglandin synthesis and thus could have a hand in mucosa regeneration due to positive adjustment of prostaglandin levels and function.

5. Conclusion

Despite the growing interest into the pharmacological importance of *Bryophyllum pinnatum* plant parts in terms of its anti-ulcerogenic effects, no research work has been carried out on its ulcer-protective effects. Ethanolic extract of *Bryophyllum pinnatum* had significant protective effects against ethanol-induced ulcer possibly by its free radical scavenging mechanism, anti-secretory effect, mucous secretion activation and gastric mucosa stabilization property which could be attributed to the antioxidant property of the identified phytoconstituents and other bioactive components of its extract. *Bryophyllum pinnatum* was also found to be safe for pharmacological usage. The present study has provided an indispensable piece of pharmacological information on *Bryophyllum pinnatum* for pharmacists in the formulation of novel drugs. We therefore conclude that *B. pinnatum* is a promising safe anti-ulcer formulation raw material.

Recommendation

We recommend that the study be advanced to isolate and purify these bioactive components of the extract for future drug discovery which could be applied in the treatment of ulcer and ulcer-related diseases.

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Competing Interest

The last co-author is a member of the editorial board. There could be potential conflict of interest.

Biographies

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Madueke Augustine Chidi is a research and teaching assistant in the department of biochemistry, university of Nigeria Nsukka. He holds an MSc in pharmacological biochemistry with research interest in the areas of drug delivery, medicinal chemistry, nutritional biochemistry and molecular biology. He is passionate about unraveling the molecular basis of diseases and drug discovery. He has published papers in local and international journals.

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