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Original Research Article

Ulcer-Protective Property of *Bryophyllum pinnatum* Leaf Extract and their Phytosomal Formulations

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ABSTRACT

Active phytoconstituents usually exhibit robust in vitro pharmacological properties that do not correspond proportionately to the in vivo clinical benefits owing to their low gastrointestinal absorption. To improve on the bioavailability of the plant natural products, a strategy to enhance their in vivo absorption is developed via novel formulation of phytosome – a phospholipid-phytoconstituent complex. This study formulated *Bryophyllum pinnatum* extract into phytosome, evaluated and compared the ulcer-protective properties of the phytosome and the *Bryophyllum pinnatum* free extract in indomethacin-induced ulcerative animal ulcer model. Phytosomal formulations of *Bryophyllum pinnatum* leaves were prepared by combination of ethanol extract of *Bryophyllum pinnatum* and phosphatidylcholine (phospholipon® 90H) in different ratios of 1:1, 1:3, and 1:5, respectively. The phyto-phospholipid complexes were evaluated for some physical and chemical properties, which include entrapment efficiency, Fourier transform infrared (FTIR) spectroscopy and *in vitro* drug release. Standard laboratory methods were employed in the phytosome formulation, evaluation and ulcer-protective studies. Ulcer protective studies were carried out using 400 mg/kg of phytosome and crude extract. All the phytosomal formulations showed increased drug release profile in phosphate buffer (pH 6.8) when compared to the free extract. At 400 mg/kg, the complexes significantly ($p < 0.05$) improved ulcer-protective property and oxidative stress parameters when compared to the free extract, and hence, could be a better form for ulcer management.

***Key words**: Bryophyllum pinnatum*, Phytosome, *In vitro* drug release, Ulcer inhibition.

Introduction

Traditional medicine has developed in various communities in Nigeria due to the health needs of the people.[1] Medicinal plants constitute the major sources of active drug in both traditional and modern medicine.[1] Even with the discovery of modern drugs, it has been reported by researchers that many of such drugs are always associated with adverse effects.[2] There is a shift in view of researchers into finding drugs of high efficacy with lower or no associated adverse reaction. Several studies have been performed on different plants for chemical compositions, and subsequently, their therapeutic benefits have been well ascertained and documented.[1] *Bryophyllum pinnatum* (Lam.) (*BP*) (fam: Crassulaceae) is a crassulescent perennial herb of about a metre in height, with smooth and fleshy leaves,[3] distributed all over the world but grows primarily in the rain forest and in many parts of Nigeria.[4] The plant is well known as an agent for wound healing.[5] It is used as a herbal remedy in almost all parts of the world.[6,7] The plant is widely used in folklore medicine for the management of a number of ailments which include diarrhoea and vomiting, gastric ulcers, abscesses, earache, burns, lithiasis and insect bites.[8] It is also used to hasten the dropping of the placenta of newly born baby, in southern Nigeria.[9] Most of the chemical constituents in *BP* that are responsible for these therapeutic functions are water-soluble molecules, which include phenols, glycosides, flavonoids etc.[10] However, these water-soluble plant molecules are limited in the therapeutic efficacy because they are poorly absorbed in both oral and topical administration which subsequently lead to poor bioavailability of these drug molecules.[11] The effectiveness of a herbal product or drug is dependent on the amount of the active compound that is actually delivered to the site of action. In order to overcome this limitation, these water-soluble phyto-constituents or the crude extract are converted into a lipid-compatible molecule called phytosome. Phytosome technology generally improve the pharmacokinetic and pharmacodynamic properties of metabolic principles in plant extract because it enhances ability of crossing cellular membrane and access into blood vessels easily.[12] Phytosome therefore, helps to increase the bioavailability of drugs and eventually leads to high efficacy. There are scanty or absence of information on the phytosome dosage forms of many traditional herbs that have been found to be active in their crude forms. Therefore, there is need to document the effect of these conventional herbal drugs in line with their phytosome dosage forms in the treatment of diseases. The study investigated the ulcer protective property of ethanol extract of *BP* leaves to validate the claim of traditional practitioners and as well document the enhanced ulcer protective effect of phytosome dosage forms of *BP* in indomethacin-induced ulceration in rats.

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Materials and Methods

Chemicals and reagents

The chemicals and reagents used in the study include: commercial Cimetidine (Yangzhou Pharmaceutical co Ltd Jiangdu, China; marketed by Greenlife Pharmaceuticals Nigeria), Phospholipid (Phospholipon® 90H; P90H) (Lipoid GMBH-FRIGENSTRASSE-4-D 67065 LUDWIGSHAFEN Germany), distilled water (Department of Industrial Chemistry, UNN), dichloromethane (Lobal Chemie Laboratory Reagents, India), ethanol (BDH, England), Fehling's solutions A and B (BDH, England), ferric chloride (Merck, Germany), hydrochloric acid (BDH, England), indomethacin (Greenlife Pharmaceuticals, Nigeria), monobasic potassium phosphate, sodium chloride (BDH, England), sulphuric acid (BDH, England). Analytical grade chemicals were employed in this study.

Collection and preparation of plant material

The plant leaves were collected from Amaezi ugwunani Aku in Igbo-etiti Local Government Area, Enugu State, Nigeria in August 2019, and was identified by a Plant Taxonomist, Mr. Alfred Ozioko of International Centre for Ethno-medicine and Drug Development (INTERCEDD) Nsukka, Enugu State, Nigeria. Voucher specimen number (INTERCEDD/051).

Extraction was by maceration of 115.23 g of *Bryophyllum pinnatum* dry leaves (powder) with 2.5 L of absolute ethanol. The mixture was left for 48 h. The solution was filtered using Whatman No1 filter paper.and the filtrate collected and concentrated with a rotary evaporator at 40°C. The concentrated extract (17.63 g) with percentage yield of 15.3% was stored in the refrigerator for future use.

Experimental animal

Male Wistar rats (100 – 160 g) were obtained from the Department of Zoology and Environmental Biology, University of Nigeria Nsukka. Ethical clearance was approved by the Faculty of Biological Science Ethics and Biosafety Committee, University of Nigeria Nsukka, Enugu State Nigeria (Approval N0: UNN/FBS/EC/1022). The study was carried out following the guidelines for the care and use of animals provided by the ethics committee.

Qualitative and quantitative phytochemical analysis

The qualitative and quantitative phytochemical constituents of ethanol extract of *BP* leaves were determined using standard methods.[13-15]

Preparation of phytosome complex

Different phytosome complexes were formulated by a stoichiometric combination of ethanol extract of *BP* leaves and P90H solutions in a suitable solvent using a standard method.[16] Phytosome preparations of *Bryophyllum pinnatum* ethanol extract and P90H in ratios of 1:1, 1:3 and 1:5 were formulated by dissolving a given quantity of the extract (1g of extract and 1 g of P90H for 1:1 phytosome formulation) in ethanol and mixed with appropriate solution containing (1g of P90H for 1:1 phytosome formulation) P90H in dichloromethane under constant reflux, at temperature not above 40°C. The formulated phytosomes were dried and stored in amber bottle for use. Commercial Cimetidine and P90H complex of 1:1 ratio was also formulated in the same manner to serve as controls.

Selection of wavelength

Bryophyllum pinnatum leaf extract (10 mg) and commercial cimetidine (10 mg) were each dissolved in 30 mL alcoholic buffer (pH 1.5) to form solutions. These solutions were separately scanned spectrophotometrically (E312 model Jenway, England) from 300 nm to 700 nm and the maximum wavelength (λ max) of absorption observed for *BP* was 420 nm and 340 nm for commercial Cimetidine. These wavelengths were used for the analysis of samples.

Entrapment efficiency

Entrapment efficiency was carried out according to previously described method.[17] A given quantity of each of the formulated phytosome complexes of *BP* (1:1, 1:3 and 1:5) was dissolved in freshly prepared alcoholic buffer (pH 1.5) and placed in a mechanical shaker at room temperature. The content was filtered with a Whatman N0 1 filter paper and the concentrations were determined using a spectrophotometer (E312 model Jenway, England) at wavelengths of 420 nm for *BP* and 340 nm for the commercial Cimetidine. The encapsulation efficiency is expressed as follows:

$$\text{Entrapment Efficiency (\%)} = \frac{Actual\ content}{Theoretical} \times 100 \quad \text{---------- ---- (1)}$$

Fourier transform infrared spectroscopy (FTIR)

Spectra were obtained by transmittance method using an FT-IR spectrometer (Shimadzu 4300, Japan). Spectra of the crude ethanol extract of *BP*, P90H and the different phytosome complexes (1:1, 1:3 and 1:5) were recorded separately in the FTIR spectra. This was carried out by mixing each of the samples with potassium bromide. The potassium bromide discs were prepared by compressing the powders at pressure of 15 tons for 10 min in hydraulic press. Scans / spectrum were obtained at a resolution of 8 cm^{-1}, from 4000 to 650 cm^{-1}.[18]

Preparation of alcoholic buffer (pH 1.5) and phosphate buffer (pH 6.8) mimicking intestinal environment

These were prepared using standard methods.[19] Phosphate buffer (pH 6.8) was prepared in order to mimic the intestinal environment of very weak acidity. This was formulated without the addition of pancreatin. A given quantity (2 g) of monobasic potassium phosphate was dissolved in 190 mL of 0.2 N sodium hydroxide solution, the pH was adjusted and the volume of the content was finally made up to 1000 mL with distilled water.

In vitro drug release studies

This was carried out using a magnetic stirrer. Freshly prepared phosphate buffer (pH 6.8) was used. A volume of the phosphate buffer (pH 6.8), 200 mL maintained at 37°C was used as the acceptor phase. Quantity of 300 mg each of different samples (1:1 cimetidine in phospholipid, 1:1, 1:3 and 1:5 phytosome complexes) and 150 mg of plant extract were analysed separately by dissolving each of them in 5 mL of phosphate buffer (as donor phase) and then placed in a dialysis membrane and tied securely with thread. The dialysis membrane and its content (the donor phase) were suspended in the 200 mL of the phosphate buffer (the acceptor phase) and the whole set up were maintained at 37°C. The stirrer was set at 50 rpm and a 5 mL aliquot was removed and replaced with an equal volume of fresh phosphate buffer at different sampling time intervals (30, 60, 90, 120, 150 and 180 min). The drug content in the samples were analysed spectrophotometrically (Spectrophotometer, E312 model, Jenway, England) at 420 nm for *BP* and at 340 nm for Cimetidine. The above procedure was repeated three times for each of the sample.

In vivo studies

Determination of Median Lethal Dose (LD_{50})

The acute toxicity test of ethanol extract of *BP* leaves was conducted in accordance with the method of Lorke.[20] It was found that the median lethal dose (LD_{50}) of extract is more than 5000 mg/kg.

Experimental design

Forty (40) Wistar rats were randomly divided into ten groups of four rats each. Five (5) mL/kg of normal saline was given to the animals in control group (Group I). Groups II and III animals were ulcer induced and were given normal saline (5 mL/kg) and 2% Tween 80, respectively. Groups IV, V and VI animals were ulcer induced and received 100 mg/kg of P90H, Cimetidine, and cimetidine-P90H complex, respectively. While Group VII animals were ulcer induced and received 400 mg/kg b.w of crude ethanol extract of *BP*, groups VII, IX and X were ulcer induced and treated with 400 mg/kg of 1:1, 1:3, and 1:5 phytosome formulations, respectively.

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Induction of ulcer with indomethacin

Wistar rats (100 – 160 g) were pre-treated with the extract and the formulated phytosomes for three (3) weeks before ulcer induction. Ulcer was induced using the method of Urushidani[21] with slight modifications. Rats were fasted for 24 h having access to water *ad libitum.* Water was removed 1 h before treatment of animals as stated above. All drugs were administered by oral route. Thirty minutes after treatment on the last day, ulcer was induced in the animals by oral administration of 50 mg/kg of commercial indomethacin in all the groups except group 1 (normal rats). After 8 h of induction, the animals were sacrificed, followed by the removal of their stomachs. After opening along the greater curvature, the stomachs were washed and rinsed under a stream of water and the erosions formed on their glandular areas observed and placed on a scale (0 – 3 mm) according to the severity of the ulcer; 0 = normal; 1 = < 1 mm; 2 = 1- 2 mm; 3 = > 2 mm.[22]

The extent of ulcer protection was estimated thus:

$$\text{Ulcer protection (\%)} = \frac{U_c - U_t}{U_c} \times 100 \qquad \text{---------- ---- - -----} \quad (2)$$

Where U_t = ulcer index of the test group; U_c = ulcer index of control group.,

Test for gastric volume and total acidity

This was done by opening the stomach along the greater curvature and draining the content into a calibrated tube. This was centrifuged for 10 min at 1000 rpm. The supernatant was measured and the volume was recorded. Total acidity was determined by the method of Kulkarni.[23] The gastric supernatant (1 mL) was diluted to 10 mL with distilled water, and titrated against 0.01N NaOH using thymol blue as indicator until reddish orange colour changed to blue colour. The level of acidity was measured by the amount of base (sodium hydroxide) used to neutralize 1 ml of the gastric content during the titration. Acidity was stated as follows:

$$\text{Acidity (mmol/L)} = \frac{\text{Volume of NaOH used x Normality of NaOH (0.01) x 1000}}{\text{Volume of gastric content used}} \quad \text{----- (3)}$$

Determination of effects of Bryophyllum pinnatum extract and phytosomes on oxidative status of ulcerated rats

The stomach tissues of rats were homogenized in ice cold 0.1 M phosphate saline buffer (pH 7.4) followed by centrifugation of the homogenate for 10 min at 2500 rpm. The supernatant gotten was used for the assessment of oxidative status. The concentration of malondialdehyde (MDA), and the activities of catalase (CAT), glutathione peroxidase (GPX) and superoxide dismutase (SOD) were estimated by standard methods.[24-27]

Statistical Analysis

The data generated were analysed using statistical product for service solution (SPSS) version 22. The expression of results were as mean ± SD and one-way analysis of variance (ANOVA) was employed for the test of statistical significance where $p < 0.05$ was considered significant.

Results and Discussion

Phytochemical compositions of ethanol extract of Bryophyllum pinnatum leaves

Table 1 shows the presence of reducing sugars, tannins, alkaloids, flavonoids, steroids, phenols, carbohydrates and terpenoid at varying concentrations. *Bryophyllum pinnatum* has previously been reported to be rich in these phytochemicals. The result collaborates the findings of other researchers [28,29] and they have been found to be effective in the treatment of various diseases, which include bacterial infections and ulcer diseases.[8]

Entrapment efficiency of phytosomes

Different phytosome formulation ratios (1:1, 1:3 and 1:5) showed different entrapment efficiencies (Table 2). Among the formulated phytosome complexes of *BP*, 1:5 showed the highest encapsulation yield when compared to the encapsulation efficiency of the other phytosome complexes. The entrapment efficiency represents the amount of the active ingredient that actually binds or is entrapped in the phospholipid (P90H). Therefore, perfect loading of extracts on the phytosomes and minimal loss of it throughout the preparation steps are reflected in the entrapment efficiency.[30]

Fourier transform infrared spectroscopy

The interaction of infrared radiation with an organic compound leads to absorption or transmittance of certain frequencies by the functional groups present in the compound. Such absorption or transmittance of frequencies in infrared spectrum is a direct measure of the degree of molecular bond rotations or vibrations of the bonds in the compound's functional groups.[31] As shown in Figure 1, there were shifts in the transmittance spectra of O-H and C-H groups in the various phytosomal formulations to lower frequency when compared to the O-H and C-H groups of the individual components (*Bryophyllum pinnatum* extracts and the P90H). These downward shifts observed in the phytosomal formulations indicate the formation of ionic bonds[32] leading to decrease in asymmetric stretches of the O-H and C-H bonds in the individual compounds (*BP* extracts and the P90H, both are present in phytosomal formulations). The peaks at 2922.2 cm^{-1} and 2914.8 cm^{-1} in *BP* extract and phosphatidylcholine, respectively, and the various complexes at 2918.5 cm^{-1} indicate symmetric stretching mode of C-H alkane group which are seen as shoulders in the FTIR transmittance spectra in all the samples. The result shows that in all the samples, there is presence of anti-symmetric stretching of C=O of ketone, symmetric stretching mode of O-H of alcohols and phenols and symmetric stretching mode of C-H of aliphatic chains of molecules present in the complex. These findings suggest that a net change occurred in the conformations of the bioactive molecules in the crude extract upon phospholipid complexation, which results to a new set of molecular vibrations in the phytosomes. The results are also in support of the findings of Esther and co-workers,[33] who analysed aqueous, methanol, and dichloromethane leaf extracts of *BP* using FTIR Spectroscopy and also reported the presence of O-H stretching bond and carboxylic acid stretching bond (C-H) at 2922 cm^{-1}.

In vitro drug release study

In vitro dissolution was carried out in this study to predict efficacy, bioavailability and the potential *in vivo* behaviour of the phytosomal formulations of *BP* in comparison with the free leaf extract. *Bryophyllum pinnatum* extract-phospholipid complexes (1:1, 1:3 and 1:5) had cumulative percentage release of 13.47%, 7.73% and 8.00%, respectively when compared to the free leaf extract (6.4%) (Figure 2). This shows that the drug release profile of *BP* extract is improved when administered in the phytosome dosage form. However, no considerable ($p < 0.05$) difference was observed in the release between 1:3 and 1:5 phytosome complexes. Also, it was observed that 1:1 commercial cimetidine enclosed in P90H showed a lower drug release when compared to the cimetidine that was not enclosed in P90H (Figure 2). The small release observed in phytosome complexes and *BP* extract could be due to sticky nature of ethanol extract of *BP*, which may affect the release, and permeability of the active component through the dialysis membrane. Apart from temperature and pH of the medium, the release may be affected due to the fact that phyto-phospholipid complex powders have poor rheology, less dense and sticky tendency.[34] *In vitro* drug release may be non-discriminate or over-discriminate when compared to the *in vivo* performance.[35] This implies that result of the *in vitro* study does not always correspond with the *in vivo* effect and so may differ when compared to the results produced when administered in the biological system.

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Ulcer-protective study

Table 3 shows the index of ulcer and degree of ulcer protection of *BP* and the doses of the different phytosomal formulations. The highest severity (ulcer index, 2.85 ± 1.03) among the treated groups was found in the group treated with 400 mg/kg body weight (b.w) of *BP* extract. The group treated with 400 mg/kg b.w of 1:3 phytosome showed the least ulcer index of 1.55 ± 0.44 which is comparable to the index produced by 100 mg/kg of commercial cimetidine-loaded P90H. Treatment of animals with 400 mg/kg of 1:1, 1:3 and 1:5 phytosomes markedly increased ulcer protective property of ethanol extract of *BP* from 59.29% to 73.57%, 77.56%, and 71.71%, respectively (Table 3). This effect was due to improved absorption and bioavailability of plant extract in the phytosomal formulations. There was no considerable ($p < 0.05$) difference in percentage ulcer protection in groups pre-treated with phytosomes when compared to the percentage ulcer protection of groups pre-treated with cimetidine. However, the increase in potency of the phytosome dosage forms did not increase with the increase in P90H concentration, because 1:5 phytosome complex produced the least effect among all the phytosomes. The higher ulcer-protective effect of 1:3 phytosome compared to the 1:5 phytosome could possibly be due to formation of aggregate of excess phospholipid molecules, which sterically limit the accessibility of the phytosomes to the cell membrane surface, leading to overall lower absorption of the extract by the cells. Hence, performance of every drug has to be determined by experiment because each active drug has different nature and formulation ratio at which it will produce the best efficacy. The high level of ulceration observed among the untreated controls were in agreement with findings in literiature[36,37] where it was shown that indomethacin caused alterations in gastric secretions of rats.

Table 1: Qualitative and quantitative phytochemical compositions of Ethanol Extract of *Bryophyllum pinnatum* Leaves

Phytochemicals	Presence of component	Conc. (mg/100g)
Reducing sugar	+	7.82 ± 0.58
Tannins	+	2.36 ± 0.37
Alkaloids	+	171.58 ± 6.21
Flavonoids	+	269.64 ± 50.38
Steroids	+	11.31 ± 1.41
Phenols	+	66.67 ± 12.73
Carbohydrates	+	30.87 ± 2.56
Terpenoid	+	476.89 ± 76.59

+ indicates presence of component**.** Results are expressed as mean ± SD (n = 3)

Table 2: Entrapment Efficiency of phytosome complexes of *Bryophyllum pinnatum*

Phytosome formulation	Entrapment Efficiency (%)
1:1 *B. pinnatum* extract-P90H complex	97.13 ± 1.33
1:1 Commercial cimetidine-P90H complex	99.71 ± 0.02
1:3 *B. pinnatum* extract-P90H complex	94.93 ± 2.38
1:5 *B. pinnatum* extract-P90H complex	97.42 ± 0.58

Results are expressed as mean ± SD. (n = 3).

Table 3: Ulcer index and percentage ulcer protection of *Bryophyllum pinnatum* and the phytosomal formulations

Treatment Groups	Dose (mg/kg)	Ulcer index	% ulcer protection
Normal	5 mL/kg	0.00^{a}	-
Ulcer untreated	5 mL/kg	7.00 ± 3.69^{c}	0.00
2% tween 80	5 mL/kg	6.98 ± 1.11^{c}	0.29
P90H	100	6.95 ± 2.09^{c}	0.71
Commercial Cimetidine	100	1.75 ± 1.30^{ab}	75.00
Commercial Cimetidine in P90H	100	1.63 ± 1.08^{ab}	76.71
B. pinnatum extract	400	2.85 ± 1.03^{b}	59.29
1:1 phytosome	400	1.85 ± 0.85^{ab}	73.57
1:3 phytosome	400	1.55 ± 0.44^{ab}	77.86
1:5 phytosome	400	1.98 ± 1.51^{ab}	71.71

Values down the column with different superscripts (^{a,b,c,}) are statistically different. ($p < 0.05$) while those with the same superscript down a column are not statistically different ($p > 0.05$). Results are expressed as mean ± SD (n = 4).

Gastric volume and total gastric acidity

Among the pre-treated animals, least volume and acidity was detected among groups pre-treated with 400 mg/kg of phytosomal formulations when compared to the group pre-treated with 400 mg/kg b.w of leaf extract. The decrease in the gastric volume of the pre-treated ulcerated rats indicates the inhibitory effect of *BP* extract on gastric acid secretion when compared to the animals in the untreated group (Table 4). This report agrees with the findings in literature[38]. Gastric secretion and total gastric acidity significantly ($p < 0.05$) improved among the groups pre-treated with phytosomal formulations when compared to the volume and total acidity of group pre-treated with leaf extract. Generally, formulation and treatment with the phytosome dosage forms significantly ($p < 0.05$) reduced the total gastric acidity of the animals from 10.25 ± 0.5 to 6.74 ± 2.88 mmol/L (Table 4). The results showed that 1:3 phytosome complex exerted the most ulcer protective activity among the phytosome formulations followed by 1:1 and 1:5 phytosomes, respectively which is in agreement with the report[32] of the bioequivalence of fast, medium and slow dissolution drugs. It was found that all the drugs (fast, medium and slow dissolution) produced the same potency *in vivo* with their corresponding formulations and reported that *in vitro* drug release may over-discriminate drug performance. This effect is observed in Table 4 and Figure 2. The ulcer protective property produced by phytosomes were comparable and equipotent as that of commercial cimetidine. Ethanol extract of *BP* leaves and their various complexes with P90H may exert their ulcer protective effect by enhancing the production and release of endogenous prostaglandins by trophic effect in which they bind to the epithelial growth factor (EGF). This in turn promotes regulation of gastric secretion and enhances the protection of mucosal barrier from the damaging effect of indomethacin on the gastric mucosa.

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Table 4: The gastric volume and the total gastric acidity of indomethacin-induced ulcer rats

Treatment Groups	Dose (mg/kg)	Gastric volume (mL)	Total gastric acidity (mmol/l)
Normal	5 mL/kg	2.03 ± 0.56[a]	6.50 ± 2.74[a]
Ulcer untreated	5 mL/kg	4.03 ± 0.60[c]	18.25 ±1.71[b]
2% tween 80	5 mL/kg	3.98 ± 0.46[c]	18.07 ± 5.83[b]
P90H	100	3.75 ± 0.83[bc]	17.34 ± 5.67[b]
Commercial	100	2.37 ±1.08[a]	7.50 ± 3.11[a]
Cimetidine			
Commercial	100	2.25 ± 0.06[a]	7.52 ± 3.82[a]
Cimetidine in p90H			
B. pinnatum extract	400	3.43 ± 0.42[bc]	10.25 ± 0.5[a]
1:1 phytosome	400	2.85 ± 0.72[ab]	8.00 ± 0.82[a]
1:3 phytosome	400	3.03 ± 0.54[abc]	6.74 ± 2.88[a]
1:5 phytosome	400	3.00 ± 0.71[abc]	7.72 ± 1.31[a]

Values down the column with different superscripts ([a,b,c]) are statistically different. ($p < 0.05$) while those with the same superscript down a column are not statistically different ($p > 0.05$). Results are expressed as mean ± SD (n = 4).

Effects of Bryophyllum pinnatum extract and phytosome on the oxidative status

The statuses of biomarkers for oxidative stress were evaluated to ascertain the levels of antioxidant defences on the ulceration process of the gastric tissues of the animals (Table 5). Persistent use of non-steroidal anti-inflammatory drugs (NSAIDs) of which indomethacin is an example leads to a decrease in the prostaglandin (PG) level by inhibition of cyclooxygenase-1 (COX-1), resulting in damage to gastric mucosa by production of free radicals.[39] Oxidative stress induces generation of reactive oxygen species (ROS) and their overproduction is one of the prime etiologic factors of gastric ulcer. In excess of overproduction of ROS, an imbalance of antioxidant system occurs and some important biochemical marker conditions are affected such as peroxidation of lipids, nucleic acids and proteins, which may lead to cell destruction and necrosis.[40] The decreased activities of GPX, CAT and SOD, and increased concentration of MDA found among the untreated animals is an indication of oxidative stress among the untreated ulcer control groups which led to higher tissue damage.[41,42] This implicated the antioxidant property of ethanol extract of *BP*. Phenolic compounds (phenol, flavonoids, tannin, and terpenoids) are known as powerful chain breaking antioxidants and have free-radical scavenging activity. They were found to be in high concentrations in the phytochemical composition of *BP* (Table 1). *Bryophyllum pinnatum* leaves contain antioxidant constituents which has the ability of donating hydrogen to a free radical and thereby preventing the potential damage of biological molecules caused by unstable radicals.[43] The highest antioxidant enzyme activities were found among the groups pre-treated with 400 mg/kg of phytosomes which implies that phytosome dosage forms improved the oxidative status better than the free plant extract.

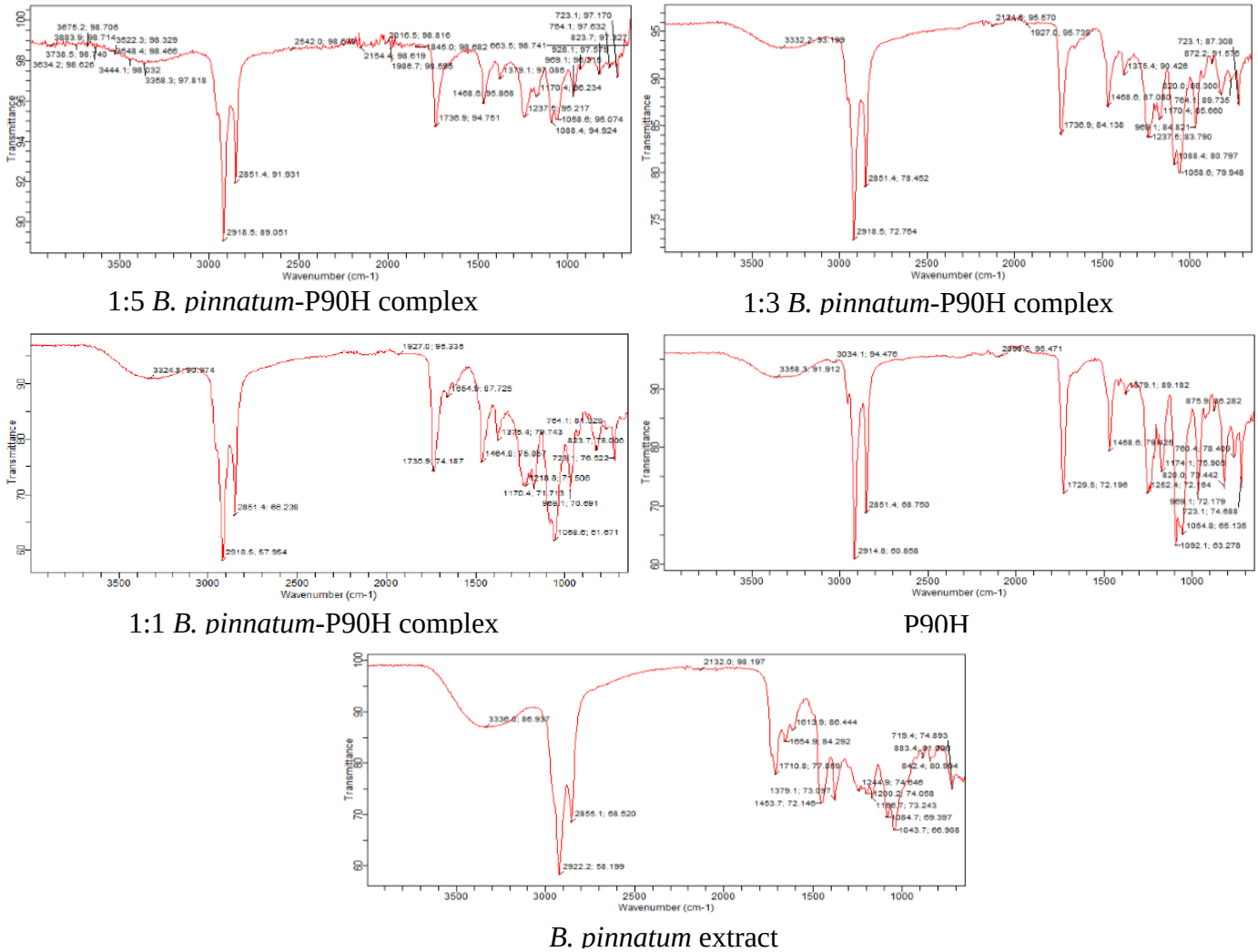

Figure 1: FTIR Spectra of *B. pinnatum* extract, P90H and phytosomal formulations (1:1, 1:3 and 1:5)

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Table 5: Effect of *B. pinnatum* extract and phytosomes on the oxidative status of indomethacin-induced ulcer in rats

Groups	SOD (iu/L)	CAT(iu/L)	GPX (iu/L)	MDA (mg/dL)
Normal	12.01 ± 0.41^{c}	1.17 ± 0.22^{c}	11.00 ± 1.90^{b}	1.19 ± 0.23^{a}
Ulcer untreated	8.17 ± 1.64^{a}	0.86 ± 0.15^{ab}	8.03 ± 1.77^{a}	3.45 ± 1.10^{d}
2% Tween 80	8.20 ± 2.76^{a}	0.84 ± 0.26^{a}	8.07 ± 1.60^{a}	2.44 ± 0.31^{bc}
P90H	8.43 ± 1.56^{a}	0.90 ± 0.29^{abc}	8.10 ± 0.72^{a}	2.33 ± 0.57^{bc}
Commercial Cimetidine	11.85 ± 0.64^{e}	1.07 ± 0.05^{abc}	10.94 ± 1.93^{b}	1.26 ± 0.20^{ab}
Commercial Cimetidine in p90H	11.87 ± 0.65^{e}	1.11 ± 0.02^{bc}	11.00 ± 1.90^{b}	1.20 ± 0.25^{abc}
400 mg extract	8.26 ± 2.91^{ab}	1.14 ± 0.04^{c}	9.22 ± 2.09^{ab}	2.31 ± 1.24^{bc}
400 mg 1:1 phytosome	9.48 ± 2.19^{abc}	1.13 ± 0.06^{c}	10.84 ± 1.19^{b}	1.25 ± 0.06^{ab}
400 mg 1:3 phytosome	11.87 ± 0.91^{c}	1.17 ± 0.19^{c}	10.57 ± 0.84^{b}	1.21 ± 0.07^{a}
400 mg 1:5 phytosome	11.25 ± 0.14^{bc}	1.10 ± 0.06^{bc}	10.37 ± 0.71^{ab}	1.25 ± 0.08^{ab}

Values down the column with different superscripts (^{a,b,c,}) are statistically different. ($p < 0.05$) while those with the same superscript down a column are not statistically different ($p > 0.05$). Results are expressed as mean ± SD ($n = 4$).

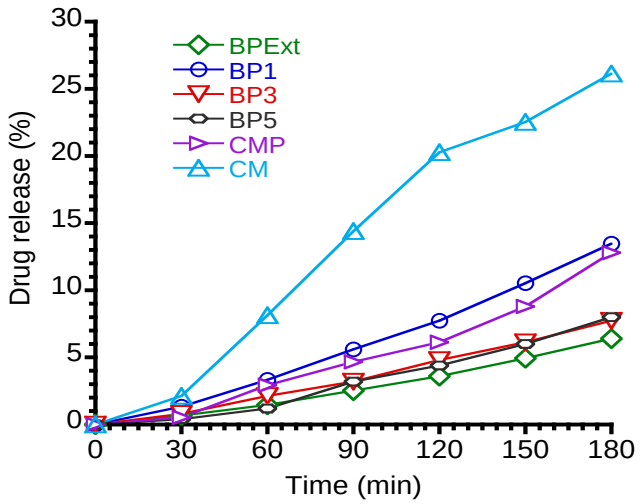

Figure 2: *In vitro* drug release profile of *Bryophyllum pinnatum* phytosome complexes in a phosphate buffer (pH 6.8).
BP Extract = *Bryophyllum pinnatum* crude extract, BP1= 1:1 *Bryophyllum pinnatum* phytosome complex, BP3= 1:3 *Bryophyllum pinnatum* phytosome complex, BP5= 1:5 *Bryophyllum pinnatum* phytosome complex, CM = Commercial Cimetidine, CMP= Cimetidine complexed with phospholipid.

Conclusion

Ethanol extract of *BP* leaves is rich in phytochemical compositions and possess ulcer protective property. Formulation of the extract into phytosomes improved the ulcer protective property of *B. pinnatum* extract. However, increase in P90H in the formulation of phytosome did not guarantee increase in efficacy.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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