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Characterization of the *Bryophyllum pinnatum* leaf's Active Component and It's Antidiarrheal Potential

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ABSTRACT

Diarrhea is a symptom experienced by nearly everyone, generally considered an increase in the volume, fluidity, and/or frequency of stools. Chronic diarrhea may affect ~5% of people in industrialized countries, and acute or chronic infectious diarrhea remains an important cause of morbidity in developing countries. The Crassulaceae family of plants includes the *Bryophyllum pinnatum* (Lam.) Oken plant, which is well renowned for its therapeutic properties. The main aim of the research is to assess the antidiarrheal property using Mgso4 induced diarrheal model and isolate an active compound. Both EAE and EE exhibited consistent effects. The Percentage of Inhibition of T1 (EE 200 mg/kg), T2 (EE 400 mg/kg), T3 (EAE 200 mg/kg) and T4 (EAE 400mg/kg) groups was found 41.51%, 61.32%, 32.56% and 52.78% respectively with standard Loperamide drug (at 3 mg/kg dose, p.o.). T2 (EE 400mg/kg) showed better outcomes because of isolated flavonoid substance, 4, 5, 7-trihydroxy flavones as a yellow pigment from ethanol-DCM, with the aid of analysis using UV, IR, NMR and mass spectroscopy, the substance, was characterized and identified as 4, 5, 7-trihydroxy flavones, with the chemical formula $C_{15}H_{10}O_5$ from EE extract of *Bryophyllum pinnatum* (Lam.) Oken plant leaves. Finally, it can be concluded that flavonoids are responsible for the antidiarrheal activity and this may be used to determine the precise function of herbal medicine in contemporary society.

Keywords: Antidiarrheal activity, *Bryophyllum pinnatum* Lam. (Oken), Ethanol extract, Ethyl acetate extract, Flavonoid.

INTRODUCTION

In, 2017 the mortality from diarrheal diseases is 1.6 million and is one of the leading causes of death around the globe. This number exceeds the sum of all "intentional injury" fatalities in the same year. One-third of fatal diarrheal disease cases included youngsters under the age of five, according to the graphic. Children under the age of five have been the group most frequently afflicted by diarrheal diseases for the majority of the last three decades; in 1990, 1.7 million children died as a result. The tenth biggest cause of child death in 2017 was diarrheal disease, which claimed the lives of more than 500,000 of the 5.4 million children who died. After preterm birth complications and pneumonia,

diarrheal infections are the 3rd main cause of child death worldwide[1-2].

An effective treatment must concentrate on fluid loss since diarrhea might result in potentially deadly dehydration. Oral rehydration therapy is one of the most well-liked strategies for reducing diarrhea-related dehydration (ORT, commonly known as ORS). ORT is a very simple therapy that makes use of a salt and sugar in water and it is not required any specialty for administration orally. To prevent mortality brought on by various diarrheal illnesses, we need further interventions and treatments.

Most population in developing countries rely on Herbal medicines for health safety. Todays, many traditional medicines prepared from herbal plants are used for treating the diarrhoeal problem like as *Coffea arabica, Plumbago zeylanica, Mentha longifolia, Cissampelos pareira*, and *Solanum hastifolium* etc.

Medicinal plants are favorable source of novel antidiarrheals. Hence, the WHO has uplifted studies concern to the treatment and therapy of diarrheal diseases by following traditional medical practices. Presently accessible drugs related with adverse effects and complications. Drug defiance is one more challenge to ponder about antibiotics used in the therapy or treatment of diarrhea. The frequency of diarrhea in developing countries are high which integrate with restrictions of obtainable antidiarrheal drugs and bad health care may construct traditional medicines good substitute for the administration of diarrhea[3-6].

The literature review demonstrated that plant *Bryophyllum pinnatum*, a member of the family Crassulaceae. The pharmacological properties of this amazing plant are caused by a variety of its active components. The stigmasterols and bufadienolides, which are steroids were found in or filtered out of the dried shoots/leaves of *Bryophyllum pinnatum*. The extracts of the all-plant's parts are rich in alkaloids and anthocyanins, saponins and tannins, phenols, quinines, phenylpropanoids, bufadienolides, phenylpropanoids, flavonoids, fatty acids, and glycosides. The aerial parts of this plant are rich in macro elements including calcium, potassium, magnesium, sodium, phosphorous and microelements including zinc, vitamins are tocopherol, ascorbic acid, thiamine, glycine, riboflavin, niacin, pyridoxine, cysteine[7-10].

It is a plant that is grown all over the world and is used as a folk remedy in many nations, including Australia, tropical Africa, India, tropical America, and China. It is astringent and has a sour flavor, but it turns sweet after digestion. The plants have a wide range of therapeutic qualities. Both hemostatic and wound-healing, antidiabetic effects are present[13-14]. The active ingredients extracted from *Bryophyllum pinnatum* have been broadly applied as folk medicines to treat a variety of illnesses, including kidney stones, lung infections, rheumatoid arthritis, and hypertension. The leaves of *Bryophyllum pinnatum* are utilised as an antifungal and an antiallergen Emollient, refrigerant, hemostatic, mucilaginous, depurative, anodyne, disinfectant, constipating, and antitonic are some of the properties of *Bryophyllum pinnatum*. The herb was effective in treating vitiated diseases (pitta and vat), epilepsy, piles, hematemesis, menorrhagia, wound healing, hemorrhoids, skin discolorations, boils, and ophthalmia[9-12].

Here, our main objects are to investigate the better anti-diarrheal properties of *Bryophyllum pinnatum* leaf extracts and to identify, describe a novel active constituent which is responsible for reproducible antidiarrheal activity. This study constructs a benefit in pharmaceutical industries for newer antidiarrheal formulations with high safety and lessen inconsistencies.

MATERIAL AND METHODS

Preparation of plant extracts

The study's essential leaves obtained from Lucknow. The plant was verified and validated at the NBRI-CSIR, Lucknow. The first step after collecting the leaves was to clean them of the dirt. Dusting was done first, and then the area was cleaned using a cloth made entirely of cotton. The leaflets were dried in the shade once the leaves had been thoroughly cleaned. The drying procedure was followed by size reduction. The use of a grinder was a sufficient method for size reduction because it reduced time and provided leaf powder material in an adequate size shown in Fig. 1. The crude medicine was extracted in the Soxhlet assembly with petroleum ether, ethyl acetate, and 70% ethanol to reduce any potential mistakes in the elution of phytoconstituents. To reduce the margin of error and quicken the extraction process, the Soxhlet extraction method

was applied consecutively. We would get a decisive strategy and analytical procedure by discussing the step-by-step elution process. This would guarantee the chemical contents eluted in the process. The *Bryophyllum pinnatum* had a few active components that could be isolated through a series of extractions. It is initially extracted using petroleum ether with the influence of extraction parameters (temperature, time/pressure 60-70°C, 4-6 h/no pressure), which increases the amount of non-polar elements in the extract at suitable temperature 60-70°C, and then it is extracted once more using ethyl acetate (temperature, time/pressure 65-80°C, 5-6 h/no pressure), and ethanol (temperature, time/pressure 65-80°C, 5-7 h/no pressure), that are polar solvents.

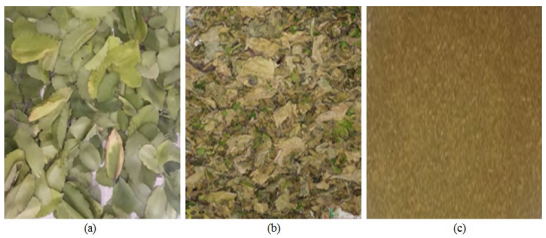

Fig. 1. Preparation of Fine powder A: Leaves of *Bryophyllum pinnatum* B: Dried leaves C: Grind fine powder.

Approximately to 120 g of dried and fresh Patharchatta *(Bryophyllum pinnatum)* leaves were loaded for extraction over the development of 8 to 10 hours. The dry substance was extracted using a variety of organic solvents, such as petroleum ether (100%), ethyl acetate (100%), and ethanol (70%) with water. Following that, extracts were distilled, dried, and weighed displaying below in Figure 2.

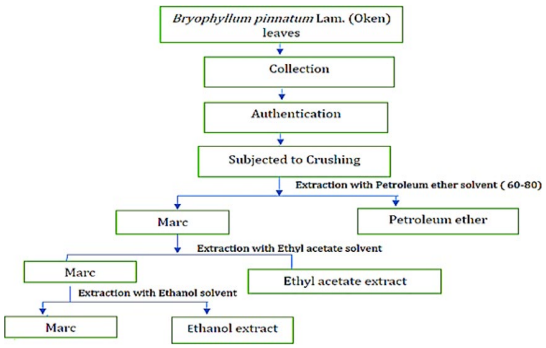

Fig. 2. Successive extraction of leaves of *Bryophyllum pinnatum*

Quantification of Secondary metabolites

Determination of Alkaloids

Sample (2.50 g) was added to a beaker with a volume of 250 cm^3 and 10% glacial acetic acid in ethanol. The beaker was then sealed with a lid and let to stand for 5 hours. 15 drops of conc. NH_4OH were added to the solution dropwise up to precipitation, followed by filtering the solution and was concentrated to quarter volume on a water bath. After a 3 h sedimentation period, the mixture's top layer was removed. The precipitates were then rinsed with 0.1 M NH_4OH before being filtered and oven-dried[26]. The formula used to compute the percentage (%) of alkaloid is equals to the weight of Alkaloid/Weight of Sample X 100 and is shown mathematically in Table 1.

Table 1: Quantitative estimation of Flavonoids and Alkaloids

S. No	Extracts	Flavonoids(%)	Alkaloids(%)
1	EAE	7.04%	9.36%
2	EE	21.6%	16%

Determination of Flavonoids

50 mL of 80% aqueous CH_3OH was added to the sample after it had been weighed to 2.50 g. The sample was then placed in a 250 cm^3 glass container covered with lid for one day at room temperature. The supernatant was evacuated and the sediment was recovered again using the identical quantity of ethanol. Each plant extract was filtered through a full solution using Whatman filter paper number 42. The filtrate from each extract sample was then put into a crucible and dried over a water bath. After cooling in a desiccator, the material inside the crucible was weighed to ensure a steady weight[28]. The formula used to determine the flavonoid percentage was flavonoid %=weight of flavonoid/weight of sample X 100.

Acute toxicity test

The acute toxicity study was conducted according to maximal test dose of 4000 mg/kg recommended by the OECD 423 guideline[27]. Three albino female rats were ordered to starve for 24 h while being given free access to water. After receiving maximal dose of 4000 mg/kg of *Bryophyllum pinnatum* extract to each animal and was examined separately for behavioral profile, autonomic profile, neurologic profile, physical states, and morbidity or mortality. This observation was made following dosing continuously for 4 h, intermittently for the first 72 h (with special focus on the first 4 h), and then daily for the following 15 days.

Albino Wistar rats of either sex, weighing 120–220 g, were employed in the investigation. This research complied with the Institutional Animal Ethics Committee's regulation (Reg. No. 1088/PO/Re/S/07) regarding the experimental setup and protocol, which was issued in accordance with Rule 5(a) of the "Breeding of and Experiments on Animal Control and Supervision Rules 1998."

Five rats were randomly selected from each set of animals. All treatments were given orally using oral gavage number 14 one hour before to the experiment. The doses were established based on acute toxicity studies in accordance with OECD standards.

Antidiarrheal potential in MgSO4 induced diarrhea model

One hour after receiving the various therapies, the animals will receive an oral dose of $MgSO_4$ (2 g/kg body weight) for the development of diarrhea model. 18 h fasted rats of both sexes were grouped into seven as Group 1: Positive Control symbolized as PC (Saline); Group 2: Negative Control symbolized as NC ($MgSO_4$); Group 3: Standard group symbolized as ST (Loperamide); Group 4: Test-1 symbolized as T1 (with low dose at 200mg/kg of Ethanol extract); Group 5: Test-2 designated as T2 (with high dose at 400mg/kg of Ethanol extract); Group 6: Test-3 denoted as T3 (with low dose at 200mg/kg of Ethyl acetate extract); Group 7: Test-4 designated as T4 (with high dose at 400mg/kg of Ethyl acetate extract). Each rat will receive 2g/kg of magnesium sulphate via oral delivery an hour after treatment, which will cause diarrhea. The animals will subsequently be kept in an individual, clear cage with a floor of white paper. For a total of 4 h, the paper will be replaced in 1 hour. The beginning of diarrhea, the quantity and weight of wet stools, and the overall volume and weight of faeces will all be noted during the observation time. Finally, the percentage of faecal output (% FOP) and diarrheal inhibition (% inhibition of defecation) will be calculated as percentage of inhibition of defecation equals to negative control means minus treated mean divided by negative control means and multiplied by hundred.

Column chromatography

It is one of the widely used methods for removing molecules from substances. This is based on a method where a group of molecules are combined and placed on top of or inside a solid. Some of the mixture's components remain in the solid phase or move slowly in the chromatographic solid system, while others move quickly alongside the mobile solvent phase and quickly leave the system. Based on polarity and absorption differences, many substances separated. Therefore, any molecule can be purified or separated as a result of these variations and causes. Various mesh sizes of Silica gel, ranging from 250 to 300 mesh size, are employed in this procedure. The column is 130 cmx 6 cm. Weigh the ethanol extract 8.0 g and then it was diluted in the appropriate solvent and combined with 200 gm of silica gel in this column by wet loading method, which has a 100 cm height. Both were properly combined and dried until the extract had adhered to the silica gel's surface. To prevent reverse flotation of the extraction during the solvent pour, this dried silica with plant extract was loaded on top of a packed column and capped with a cotton plug.

Isolation of active compounds from EE of *Bryophyllum pinnatum* leaves

The solvent was flushed in a gradient. 100% Dichloromethane was poured in series. After 100% dichloromethane, column was run by the ratio of DCM-ethanol which is tabulated in Table 5. Total 130 fractions were gathered and condensed during distillation. Every fraction was placed onto a TLC plate, developed using several solvent systems, and then examined under a UV lamp. 90 to 110 fractions were pooled and concentrated to produce (M2-yellow) 42 mg, and both separated chemicals were collected in flasks. Rf value of all 90 to 110 fractions were shown by running in the same 35% Ethanol: DCM as mobile phase for TLC at 0.6 Rf.

Structure elucidation of active isolated compound

It takes a lot of time and effort to use standard methods to identify the structures of unknown substances in complex metabolomics mixtures. The MS/NMR strategy substantially speeds up this job by minimizing sample separation and utilizing an integrated platform that exploits the high complementarity of high-resolution NMR and MS studies in conjunction with cheminformatics. The overall structure elucidation of challenging natural substances has gotten easier during the last few decades. Unambiguous structure elucidation is made possible by new MS technologies by the operation with NIST MS search 2.4 like tandem MS (MS/MS) and

ultra-high-resolution MS (HRMS), as well as further developments in recently discovered NMR probes. As previously noted, repeated column chromatography employing silica gel was used to purify the 70% ethanolic extract (EE) of dried *Bryophyllum pinnatum* plant leaves. To obtain the active chemical, the eluents were subjected to crystallization using acetone (M2). Based on their examination of the chemical using UV, IR, and mass spectroscopy.

RESULT

The leaves were roughly 2.7 kg in weight during sample processing, had a dark green colour, and had a rough texture (Fig. 1). Petroleum ether, ethyl acetate, and (70%) ethanol was utilized as the extraction solvents in this work to get the extracts depicted in Fig. 2. The percentage of flavonoid was calculated and showed in Table 1 and was calculated as Percentage of Flavonoid=Weight of flavonoid/ Weight of sample×100.

Acute toxicity investigations showed that the *Bryophyllum pinnatum* ethanolic and ethyl acetate extract is not harmful (EEBP, EAEBP). Until the completion of the trial period, no mortality or adverse effects were seen at any of the chosen doses. Table 2 lists the observational parameters.

Antidiarrheal potential

$MgSO_4$ induced diarrhea model

Table 3 displays how the animals have been grouped. The percentage of inhibition of the standard group was determined to be 73.81%, whereas the mean of the negative control group's defecation was 1.474 and the mean of the standard group's defecation was 0.386. However, mean of T1 (EE 200mg/kg), T2 (EE 400mg/kg), T3 (EAE 200mg/kg) and T4 (EAE 400mg/kg) groups of defecation were 0.862, 0.570, 0.994 and 0.696 respectively. The Percentage of Inhibition of T1 (EE 200mg/kg), T2 (EE 400mg/kg), T3 (EAE 200mg/kg) and T4 (EAAE 400mg/kg) groups was found 41.51%, 61.32%, 32.56% and 52.78% respectively.

Both ethanol and ethyl acetate extracts demonstrated notable antidiarrheal benefits in mice when administered to them in a model of diarrhea caused by magnesium sulphate. Following the administration of $MgSO_4$, the faecal production, total number of wet faeces, and average weight of faeces produced by the ethanol extract and ethyl acetate extract reduced as compared to the negative control group, as demonstrated in the observation above. But as compared to other groups, ethanol extract at a high dose shown remarkable antidiarrheal results. We have conclusively established a link between diarrhea, quantity of faeces, total number of wet faeces, and average weight of wet faeces. It was noticed normal during the acute toxicity assessments of ethanol and ethyl acetate extract for the symptomatic examinations for the extract's detrimental effect, as given in Table 2.

Table 2: Observations for acute toxicity studies of ethyl acetate and ethanol extract

Parameters		Observations									
		Ethyl acetate					Ethanol				
		01	04	24	72	14 days Hours	01	04	24	72	14 days Hours
Behavioural profile	Alertness	N	N	N	N	N	N	N	N	N	N
	Restlessness	N	N	N	N	N	N	N	N	N	N
	Irritability	N	N	N	N	N	N	N	N	N	N
	Fearfulness	N	N	N	N	N	N	N	N	N	N
Autonomic profile	Defecation	N	N	N	N	N	N	N	N	N	N
	Urination	N	N	N	N	N	N	N	N	N	N
Neurologic profile	Locomotion	N	N	N	N	N	N	N	N	N	N
	Reactivity	N	N	N	N	N	N	N	N	N	N
	Touch response	N	N	N	N	N	N	N	N	N	N
	Pain response	N	N	N	N	N	N	N	N	N	N
Physical profile	Texture of fur	N	N	N	N	N	N	N	N	N	N
	Nasal secretions	N	N	N	N	N	N	N	N	N	N
	Faeces color/texture	N	N	N	N	N	N	N	N	N	N
Lethality	A	A	A	A	A	A	A	A	A	A	

*N=Normal; A=Absent

Table 3: Grouping and dosing of animals

S. No	Drug	Dose	Route ofAdministration	No ofAnimals
1	PC	0.5mL	Oral	05
2	NC	2g/kg	Oral	05
3	ST	3mg/kg	Oral	05
4	T1	200mg/kg	Oral	05
5	T2	400mg/kg	Oral	05
6	T3	200mg/kg	Oral	05
7	T4	400mg/kg	Oral	05

Table 4: Mean±S.D. of Onset of Diarrhea, Number of faeces, Total wet faeces and Average weight of wet faeces

Animals	NC	ST	T1	T2	T3	T4
Onset of Diarrhea	46.40±3.736***	129.80±4.641***	54.40±4.89***	96.20±5.034***	51.80±5.687***	81.80±4.188***
Number of faeces	19.00±2.608***	8.60±1.03***	15.00±1.304**	11.40±1.631***	16.20±1.855***	17.20±1.744***
Total wet feaces	15.80±1.924***	3.80±1.304***	11.40±2.702***	7.20±1.789***	14.20±0.837***	12.80±2.168***
Average weight of faeces	1.47±0.437***	0.39±0.147***	0.86±0.213***	0.57±0.189***	0.99±0.230***	0.70±0.191***
Average weight of faeces	1.62±0.225***	0.84±0.096***	1.04±0.145***	0.64±0.0.182***	1.26±0.378***	0.89±0.152***

Values are mean ± SD of 05 rats in each group p value: <0.001 compare the respective group with the Negative control group ****<0.001, ***<0.01, ns= non-significant

Isolation of active compounds from EE of Bryophyllum pinnatum leaves.

130 fractions totaling 6.5 litres of the extracted material were collected and concentrated by distillation. The 90 to 110 fractions were pooled and concentrated to produce (M2-yellow) 42 mg, and both separated compounds were collected in flasks. The Rf value in TLC of all 90 to 110 fractions was shown to be the same TLC at 0.6 Rf by running in the same 35% Ethanol: DCM as mobile phase for TLC.

Table 5: Nature of fractions and polarity of the solvent used in EE column

S. No.	Volume of Ethanol(ml)	Volume of n-DCM (ml)	Fraction combined	% Used in column	Total volume (mL)	Remarks
1	25	475	10-Jan	5%	500	No spot
2	50	450	20-Oct	10%	500	Impurity
3	50	450	20-30	10%	500	Tailing
4	75	425	30-40	15%	500	Greenish Yellow spot
5	75	425	40-50	15%	500	light greenish Yellow spot
6	75	425	50-60	15%	500	Green yellow
7	75	425	60-70	15%	500	light spot
8	75	425	70-80	15%	500	very light spot
9	125	375	80-90	25%	500	No spot
10	175	325	90-100	35%	500	M2-yellow spot
11	200	300	100-110	40%	500	Light yellow spot
12	250	250	110-120	50%	500	Pooled
13	500	-	120-130	100%	500	Finished

Table 5 shows the polarity of the elution-related solvents. Yellow pigment M2, designated as 4, 5, and 7-trihydroxy flavones, was extracted from ethanol-Dichloromethane. As shown in Fig. 3, the UV absorption bands that occurred at 253.2 and 349.0nm were identified as flavones. Its IR spectrum showed characteristics absorption bands for hydroxyl (3307 cm^{-1}), carbonyl stretching (1246 cm^{-1}), aromatic vibrations (1412 cm^{-1}) and ether (1106 cm^{-1}), carbon-carbon stretching (1508 cm^{-1}) functional group as displayed in Fig. 4. The ^{1}HNMR spectrum of compound M2 displayed protons at 12.95 (H-5, s, OH attached), δ7.90 (H-2, 6, d, 2H), δ6.91 (H-3, 5, d, 2H), δ6.75 (H-3, s, 1H), δ6.46 (H-6, s, 1H), δ6.17 (H-8, s, 1H) as shown in Fig. 5. The electron impact mass spectrum of M2 displayed a molecular peak atm/z270.1428(M^{+}) corresponding to a flavone's nucleus having

hydroxyl groups with molecular formula $C_{15}H_{10}O_5$ (m/z270.1428 calculated) as observed in Figure 6.

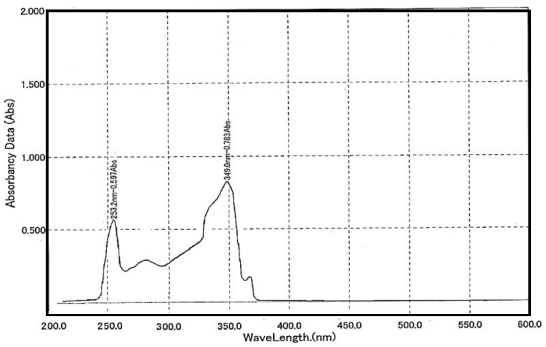

Fig. 3. UV absorption spectra of isolated compound M2

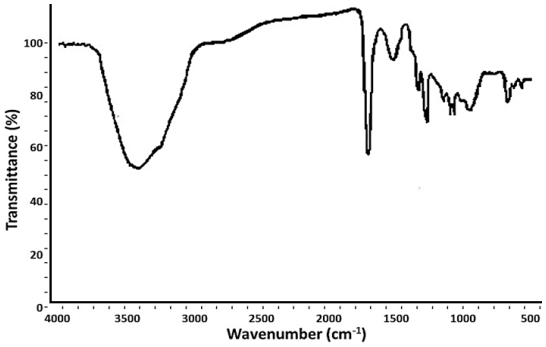

Fig. 4. IR spectrum of isolated compound M2

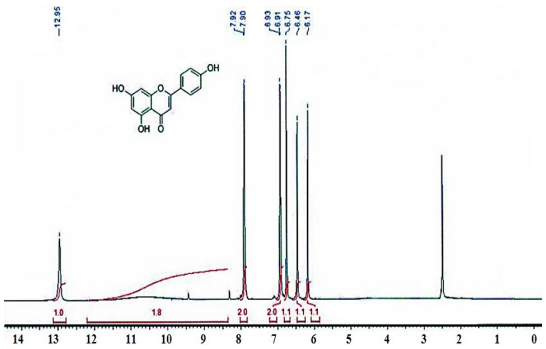

Fig. 5. NMR spectra of isolated compound M2

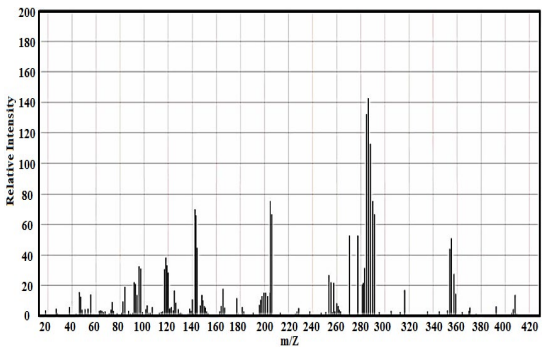

Fig. 6. Mass spectra of isolated compound M2.

The above spectroscopic data suggest that compound M2 was a flavonoid and was identified as 4',5,7-trihydroxy flavone (Figure 7).

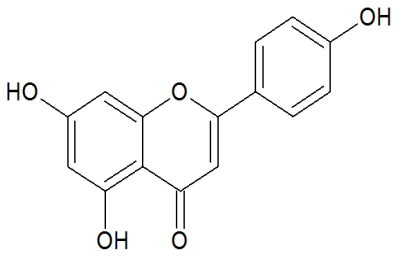

Fig. 7. Structure of isolated M2 compound

DISCUSSION

Different types of *Bryophyllum pinnatum* are medicinally utilized in Islands of Indo China/ Philippines. The hot and humid parts of India have seen its naturalization. The leaves and bark have digestive astringent, analgesic, carminative, and bitter tonic properties that are helpful for diarrhea and vomiting.[18] Leishmaniasis, various bacterial/viral/ fungal diseases and are among the conditions it is used to treat both topically and internally.[19] The leaf of this plant have been used in traditional medicine for their effective anti-histamine and anti-allergic activity, antimicrobial[20-21], antifungal[22], antiulcer[23], anti-inflammatory and analgesic[24] properties.[25] It is vital to quantify secondary metabolites because they are responsible for the biological features of medicinal plants. When administered as drugs, these secondary metabolites may benefit health by preventing chronic diseases like cancer, cardiovascular, and neurological conditions.

Quantitative analysis is a key technique for determining the amount of phytoconstituents present in plant extracts. For this, total flavonoid contents (TFC) have been established[26,28] from the both EAE and EE extracts made from leaves of the *Bryophyllum pinnatum* plant that were tested for the presence of TFC using traditional methods. When the flavonoids in the plant were counted, it was discovered that the EE has high 21.6% in flavonoid content than the EAE. Magnesium sulphate-induced diarrhea model has been approximately used for initiation for assessment of the antidiarrheal movement. $MgSO_4$ increases the volume of intestinal content through prevention of reabsorption of water. It is in a class of osmotic laxatives, producing watery diarrhea by which stool can be deflated from the

colon by means of irritation of the gastrointestinal mucosa, stimulating gastrointestinal motility and fascinates water from the surrounding tissues of colon which results reduction in the absorption of electrolytes from the stomach and these are comparable to the pathophysiological processes that lead to diarrhea and to lessen such changes antidiarrheal agents required So, the research team evaluated the traditional properties of *Bryophyllum pinnatum* plant extracts by Magnesium sulphate-induced diarrhea model, produced anticholinergic effect, decreasing secretion and intestinal motility significantly with P value<0.001. Additionally, several parameters relating to EE that showed antidiarrheal actions were analyzed and compared with the negative controls ($MgSO_4$) showed lessen number of diarrhea, average weight of stool and showed high percentage of inhibition by EE at high dose 400mg/kg in comparison to EAE extract doses. The results of Magnesium Sulphate induced model proved the antidiarrheal potency of the plant due to the presence of phytoconstituents.

Studies on phytochemistry have revealed the presence of many secondary metabolites. According to phytochemical studies, two flavonoids isolated that stop the growth of bacteria are trihydroxyl flavones and 4,3,5,7 tetrahydroxy 5 methyl 5 propenamine anthocyanidins. Bacterial growth is inhibited by a 60% methanolic concentrate of *Bryophyllum pinnatum*.[30] Based on certain studies, *Bryophyllum pinnatum's* active ingredients, such as the bufadienolides bryophyllin A and bryophyllin C, have strong insecticidal properties.[31-32] Certain kinds of acute ulcers cannot spread when methanol and leaf extract from *Bryophyllum pinnatum* are combined.[32] For the cell culture study of the anthelmintic effect. The findings demonstrate that *Bryophyllum pinnatum* root extract displays significant antihelminth activity. But the extract of *Bryophyllum pinnatum* roots in methanol was most effective against helmentic activity. Because of its high efficacy, root extract paralyses and kills worms more quickly than medications like piperazine citrate (100 mg/mL). Phyto-chemical investigation of crude extracts of *Bryophyllum pinnatum* revealed the presence of tannins with anthelmintic action.[33] According to a study, sedated rabbits and cats' liver, kidney, blood pressure, and general health were affected by *Bryophyllum pinnatum* leaf extract in aqueous form. This investigation found that the concentrate lowered the circulatory strain slightly and further decreased the high adrenaline that was causing the sedated cat's hypertension. It was considered that *Bryophyllum pinnatum's* ability to reduce pulse had a solid pharmacological foundation. However, due to its organotoxic nature and negligible effect on decreasing blood pressure, the *Bryophyllum pinnatum* leaf extract is ineffective as an antihypertensive medication.[34] Rat wound models were utilised to evaluate pinnatam's efficacy in healing wounds. According to the histological analysis, it has shown good potential against wound.[35-36] *Bryophyllum pinnatum* has been shown to have hepatoprotective effects in numerous investigations. It has been found to be a very effective hepatoprotective.[37] The ability of its leaf juice and ethanolic extract to shield rats' livers from CCl_4-induced hepatotoxicity was examined.[38]

The aqueous extract of *Bryophyllum pinnatum* also showed a positive association with the glucose lowering activity in diabetic model rats.[36] The extract exhibits nephroprotective activity, the researchers demonstrated.[39] Additionally, it has been observed that hydroalcoholic extract of *Bryophyllum pinnatum* leaves showed a significant diuretic and antiurolithitic effect when given intravenously to female rats and orally to male rats. Uneven homeostatic phenomena between oxidants-antioxidants are mainly by the physiological overload of free radicals in the body. Since oxidative stress is the outcome of this randomness, it is believed to be the primary cause of human cancer, diabetes, Alzheimer's disease, arteriosclerosis, and other neurological ailments. According to Morales and colleagues, quercetin may reduce the nephrotoxicity brought on by cadmium by increasing eNOS, which suppresses the COX-2/iNOS, as well as a small cysteine-rich protein known as metallothionein.[40] *Bryophyllum pinnatum* leaf material contains five isolated bufadienolides. Bufadienolides stop Raji cell activation by the tumor promoter 12-Otetradecanoylphorbol-13-acetate. According to these investigations, bufadienolides derived from Bryophyllum pinnatum may be useful as cancer chemopreventives.[40] MTT assays were performed on HT-1080 cell line, to assess the antiproliferative effectiveness of the methanolic aqueous extract. Once this is accomplished, the new curative methodology will surely advance and

influence the growth of further therapeutic modalities. But a lot of plant lectins have a strong anti-diarrheal effect that is harmful and has a lot of undesirable effects in clinical patients. In order to ensure patient protection, the stiffness of pre-clinical research is considered. Plant lectins also negatively affect the functionality and health of the clinical patients who are the focus of the inquiry since they are derived from plants. To put it another way, a select group of well-studied plant lectins are supporting a few critical processes, making them useful therapeutic agents.[41]

Regarding the quantitative assessment for phytochemicals of EAE and EE of *Bryophyllum pinnatum* leaves. The calculation was found constructive for the presence of alkaloids and flavonoids and their percentage of concentration is represented in Table 1. Intelligences in the works showed that, flavonoids are stated as antioxidants which are accountable for overturning the initiation of prostaglandin synthesis enzymes and detected to prevent intestinal motility and fluid/water and electrolyte discharge too[42,43]. The *Bryophyllum pinnatum* has a high antioxidant capacity, which may enlighten on antidiarrheal properties according to Umukoro and Ashorobi by the mechanism of preventing phospholipid perioxidation, ascorbic acid and tocopherol and decrease Magnesium sulphate induced diarrhea. So, this study reveals antidiarrheal property of this plant may depend on its phytochemical composition which was proved by quantitative analysis and isolation process.

Similarly, the EE contains flavonoids which has played a significant role in showing positive antidiarrheal actions as reported previously[42]. The outcome of the work proposed that EE produces a remarkable result and highest inhibition in diarrhea droppings when compare with EAE. So, the active phytoconstituents flavonoids, that may be responsible for antidiarrheal actions was isolated from EE by column chromatography.

In conclusion, the 70% ethanolic extract of dried leaves of *Bryophyllum pinnatum* plant was purified by repeated column chromatography using silica gel. The eluents were submitted to crystallization using acetone to afford compounds M2. This compound was also elucidated because of their UV, IR, and Mass spectroscopy analysis and identified as 4,5,7-trihydroxy flavones which was isolated as a yellow pigment from ethanol-DCM. The UV absorption bands, IR spectrum, ^{1}HNMR spectrum and electron impact mass spectrum confirmed the structure having the molecular formula ($C_{15}H_{10}O_5$). The above spectroscopic data suggest that compound M2 was a flavonoid and was characterized as 4,5,7-trihydroxy flavone.

The unique isolated compounds 4,5,7-trihydroxy flavone is new and has been first time reported from India. More work is required to find the exact role in the modern word of herbal medication is required to explore.

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Conflict of interests

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