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Journal of Advanced Scientific Research

J.Adv.Sci.Res, 2011, 2(1); 42-49
Published on 10 Feb 2011
Short Communication

ISSN: 0976-9595

Pharmacognostic & Phytochemical Evaluation of *Bryophyllum Pinnatum* Leaves

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ABSTRACT

In the present study leaves of *Bryophyllum Pinnatum* (life plant) were subjected to pharmacognostical studies such as determination of epidermal cell, parenchyma cell, cambium, xylem, phloem, stomata. Quantitative microscopy including stomatal number, stomatal index, vein islet and vein terminations were determined by using fresh leaves of the plant and results were observed.

Physicochemical studies such as ash values, extractive values of plant part were carried out to confirm the identity of plant and to ascertain the quality and purity of the drug. Ash values such as total ash, acid insoluble ash, water soluble ash and sulphated ash of the plant were determined and recorded. Extractive values such as alcohol soluble extractives and water soluble extractive values were determined. These parameters may be useful for the identification and authentification of the plant for the future investigators.

Keywords: Life plant, Phytochemical Evaluation, Physicochemical Parameters

INTRODUCTION

Traditional medicine involves the use of herbal medicine, animal parts and minerals. However, herbal medicines are the most widely used of the three. Herbal medicines contain an active ingredient, aerial or underground parts of plants as their petal or seeds materials or combinations thereof, whether in the crude state or as plant preparations[1]. Despite the immense technological advancement in modern medicine, many people in world (approximately 75% of the population) still rely on traditional healing practices and medicinal plants for their daily healthcare needs[2]. In most of the developing countries of the world, rural and urban dwellers, literate or illiterate rely heavily on herbal preparations for the treatment of various diseases despite the availability of orthodox medicine[3]. One of such frequently used medical plants is *Bryophyllum pinnatum*.

Bryophyllum pinnatum (Lam.) Oken (family: Crassulaceae) (synonym: Kalanchoe pinnata, Lam.; common names: Life plant, air plant (Mexican), love plant, Canterbury bells, Cathedral bells, etc) is a perennial herb growing widely and used in folkforic medicine in tropical Africa, India, China, Australia and tropical America[4-5]. It is a succulent glabrous herb 0.3-1.2 m high, stems obtusely 4-angled, the older light colored, the younger reddish speckled white, leaves variable, decussate, the lower usually simple or occasionally compound, the upper usually 3-5 or 7-folliolate, long petiole, petiole united by a ridge round the stem[6]. Extracts of Bryophyllum pinnatum, have been used by modern physicians mainly as a psychiatric sedative. Identified active ingredients include bufadienolides, flavonoids, glycosides, steroids and organic acids[7-11].

In traditional medicine, the leaves of this plant have been reported to possess antimicrobial[12-14], antifungal[15], anti ulcer[16], anti-inflammatory& analgesic[17-18] and antihypertensive[19] activities. The methanol extract of the leaves of

the plant has also been reported to have histamine receptor (H1) antagonism in the ileum, peripheral vasculature and bronchial muscle[20].

In order to scientifically apprise some of the ethnomedical uses of the plant, the present study was undertaken to determine various physicochemical parameters and to interpretate the main phytoconstituents present in *Bryophyllum pinnatum* leaves.

MATERIAL AND METHODS

Collection of plant material

The leaves part of plant were collected from the field of Allahabad and Meerut (U.P), authenticated as *Bryophyllum pinnatum* (Lam.) Oken (family: Crassulaceae) by Prof. Virendra Kumar Singh (C.C.S university, Meerut, U.P., India). A voucher specimen has been kept in our research laboratory for further reference. After authentication, fresh leaves were collected in bulk, washed, shade dried and pulverized in a mechanical grinder to obtain coarse powder.

Anatomical studies

Transverse section taken from the middle part of the leaves was observed. Microscopic studies were done by preparing a thin section of leaves of *Bryophyllum pinnatum*. The section was cleared with chloral hydrate solution and then stained with phloroglucinol and hydrochloric acid, mounted in glycerin. Transverse section of leaves was observed to have epidermal cell, parenchyma cell, cambium xylem, phloem, stomata. The results of microscopic studies are expressed in table 1 and figure 1.

Table 1: Quantitative Microscopy of *Bryophyllum pinnatum* Leaves

Particulars	***Bryophyllum pinnatum***
Vein islet number	10-15
Vein termination number	4-6
Stomatal number	
• Upper surface	7-10
• Lower surface	10-13
Stomatal index	
• Upper surface	8-11
• Lower surface	12-14

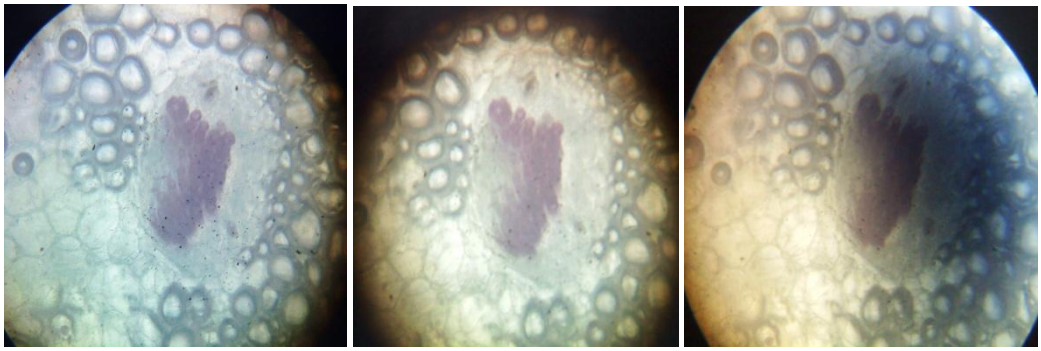

Fig. 1: Microscopic section of *Bryophyllum pinnatum* Leaves

Determination of Physicochemical Parameters

Moisture content

The percentage of active chemical constituents in crude drugs is given in terms of air dried drugs. Hence the moisture content of a drug should be determined. 2gm of powdered drug was transferred into a china dish and the contents were distributed evenly to a depth not exceeding 10mm. The loaded plate was heated at 105ºC in hot air oven and weighed at different time intervals until a constant weight was obtained. The difference in weight after drying and initial weight is the moisture content. Same experiment was repeated six times for precision and percent moisture for the sample was calculated.

Total ash value

About 2gm of powdered drug was weighted accurately into a tarred silica crucible and incinerated at 450ºC in muffle furnace until free from carbon. The crucible was cooled to room temperature and weighted. Percentage of ash was calculated with reference to air dried substance.

Acid soluble ash

Ash obtained from total ash was boiled with 25ml of 2N HCl for few minutes and filtered through an ashless filter Paper. The filter paper was transferred into a tarred silica crucible and incinerated at 650º C in muffle furnace until free from carbon. The crucible was cooled and weighted. Percentage of acid insoluble ash was calculated with reference to air dried substance.

Water soluble ash

Ash obtained from total ash was boiled with 25 ml of distilled water for few minutes and filtered through an ashless filter paper. The filter paper was transferred into a tarred silica crucible and incinerated at 450ºC in muffle furnace until free from carbon. The crucible was cooled and weighted. Percentage of water soluble ash was calculated with reference to air dried substance.

All the experiment was repeated six time for precision and result were expressed as mean ± SD.

Table 2: Physiochemical parameters of *Bryophyllum pinnatum.*

Parameter	*Bryophyllum pinnatum*
LOD	12%
Crude fibre	3%
Ash values	
• Total ash values	8%
• Acid insoluble	3%
• Water soluble	5%
Extractive values	
• Pet. Ether	1.5%
• Alcoholic	3.2%
• Aqueous/ water	5.8%

Ether soluble extractive values

5gm drug was refluxed with 100 ml of petroleum ether for 2hrs and filtered through whattman filter paper. 10 ml of the filtrate was evaporated in a tarred dish at 105ºC and weighed. Ether soluble extractive values were calculated as mean of six specimen ± SD.

Alcohol soluble extractive values

5gm of powdered drug was refluxed with 100ml of alcohol for 2hrs and filtered through whattman filter paper. 10ml of filtrate was evaporated in a tarred dish at 105ºC and weighed. Alcohol soluble extractive values were calculated as mean of six specimen ± SD.

Water soluble extractive values

5gm of powdered drug was treated with 100 ml water at in a stoppered flask with frequent shaking during first 6 hrs using electrical shaker and allowed to stand for 24 hrs. Temperature was maintained at 45°c during entire process. Extract was filtered and 10ml of filtrate was evaporated in a tarred dish at 105ºc and weighed. Water soluble extractive values were calculated.

EXTRACTION OF PLANT MATERIAL

The powdered drug (500 g) after defatting with petroleum ether (60-80°) for 48 h was successively extracted with chloroform, methanol and water for 48 h in a soxhlet extractor. Following extraction, the liquid extracts were concentrated under vacuum to yield dry extracts. Standard methods were used for preliminary phytochemical screening of the different extracts to know the nature of phytoconstituents present within them.

Table 3: Extraction of *Bryophyllum pinnatum*

Extracts	Colors	Consistency	% yield
Pet. ether	Pale greenish yellow	Semisolid	2.85%
Chloroform	Dark green	Semisolid	5.5%
Ethanolic	Pale green	Semisolid	20.5%
Aqueous	Light brown	Semisolid	36.5%

PHYTOCHEMICAL SCREENING

Detection of Carbohydrates

Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrated were used to test for the presence of carbohydrates.

Molisch's test

Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube and 2 ml of conc. sulphuric acid was added carefully along the sides of the test tube. Formation of violet ring at junction indicates the presence of carbohydrates.

Benedict's test

Filtrates were treated with Benedict's reagent and heated on water bath. Formation of orange red precipitate indicates the presence of reducing sugar.

Fehling's test

Filtrates were hydrolyzed with dil. HCl, neutralized with alkali and heated with Fehling A & B solution. Formation of red precipitate indicates the presence of reducing sugars.

Detection of Alkaloids

The small portions of solvent free chloroform, alcoholic and water extracts are stirred separately with a few drops of dil. HCl and filtered and then subjected to test for alkaloids.

Dragendorff's test

Extracts were treated with dragendorff's reagent. Formation of orange brown precipitate indicates the presence of alkaloids.

Mayer's test

Extracts were treated with Mayer's reagent. Formation of cream precipitate indicates the presence of alkaloids.

Wagner's test

Extracts were treated with Wagner's reagent. Formation of reddish brown precipitate indicates the presence of alkaloids.

Detection of Saponins

Foam test

Extracts were diluted with distilled water to 20 ml and this was shaken in a graduated cylinder for 15 min. Formation of 1 cm layer of foam indicates the presence of saponins.

Detection of Phytosterols

Salkowski's test

Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of conc. sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of trierpenes.

Libermann Burchard's test

Extracts were treated with chloroform and filtered. The filtrated were treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric scid was added carefully along the sides of the test tube. Formation of brown ring at the junction indicates the presence of phytosterols.

Detection of Phenolics

Ferric chloride test

Extracts were treated with few drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Table 4: Phytochemical Screening of *Bryophyllum pinnatum*

Chemical Constituent	Tests	Pet. ether. Ext.	Chloroform. Ext.	EtOH. Ext.	Aq. Ext.
Alkaloids	• Dragendroff'stest	-	++	++	-
	• Mayer's test	-	++	++	-
	• Wagner's test	-	++	++	-
Carbohydrates	• Benedicts test	-	-	-	-
	• Fehling's test	-	-	-	-
	• Iodine test	-	-	-	-
	• Glucose test	-	-	-	-
	• Molish's test	-	-	-	-
Flavonoids	• Lead acetate test	++	++	++	++
	• Sodium hydroxide test	++	++	++	++
	• Shinoda test	++	++	++	++
Carotenoids	• Ext. + conc. HCl+ PhOH.	++	-	-	-
	• Ext. + 85% sulphuric acid	++	-	-	-
Saponin	• Foam test	++	-	++	++
Steroid	• Libermann burchard's test	-	-	-	-

Detection of Tannins

Gelatin test

To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

Detection of Flavonoids

Alkaline reagent test

Extracts were treated with few drops of sodium hydroxide solution. Formation of yellow colour, which becomes colourless on addition of dilutes acid, indicates presence of flavonoids.

Lead acetate test

Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

Shinoda test

To the alcoholic solution of extracts, a few fragments of magnesium ribbon and conc. HCl were added. Appearance of magenta colour after few minutes indicates presence of flavonoids.

Optimization of TLC solvent system

Different solvent systems were tried for developing a TLC system for study of *Bryophyllum Pinnatum*. Solvent systems were tried identification of constituents in the extract based on the literature survey and the one showing maximum separation was selected as mobile phase for study.

Solvent System for *Bryophyllum pinnatum*

For Pet. Ether extract- Toluene: ethyl acetate: glacial acetic acid (5:7:1)
For chloroform extract- Toluene: ethyl acetate: glacial acetic acid (6:4:1)
For ethanolic extract- Chloroform: methanol (8:2)

In the calculated Rf values 3 spots in pet. ether extract, 3 spots in chloroform extract and 1 spot in ethanolic extract were found and showed good separation. The results are compiled in table 5.

Table 5: TLC analysis of *Bryophyllum pinnatum*

Extracts	Spots	Colors	Rf values
Pet. ether	1	Yellow	0.55
	2	Greenish yellow	0.65
	3	Dark yellow	0.75
Chloroform	1	Bluish green	0.4
	2	Yellow	0.5
	3	Pale green	0.8
Ethanolic	1	Light pale green	0.6

CONCLUSION

The plant *Bryophyllum pinnatum* is a perennial herb belongs to family Crassulaceae, which is used in ayuredva. In study of plant *Bryophyllum pinnatum* pharmacognostic, preliminary phytochemical screening, physicochemical properties have been evaluated.

For the proper identification of plant, physicochemical parameters (ash value, crude fibre, extractive values, and moisture content) provide useful information. In morpho-anatomical studies the transverse section has been examined. Form the present investigation it is evident that certain characters such as color of leaves, stoma in epidermal layer, xylem, phloem, cambium can provide useful parameter. Preliminary phytochemical investigation of different extracts of leaves *Bryophyllum pinnatum* shows the presence of alkaloids, carotenoids, flavonoids and saponin. TLC profile analysis was found to be a useful tool to provide the in plant *Bryophyllum pinnatum.*

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