International Journal of Research in Pharmacy and Pharmaceutical Sciences
ISSN: 2455-698X; Impact Factor: RJIF 5.22
www.pharmacyjournal.in
Volume 2; Issue 3; May 2017; Page No. 06-10

Pharmacognostical and preliminary phytochemical screening of leave of *Polygonum muricatum*

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Abstract

Traditional system of Medicine is the most widely practiced system of medicine, which incorporates natural sources like entire plants or their parts. The therapeutic of drugs used in various systems greatly depends on the use of proper and genuine raw materials. Because of these, assurance of safety, quality, and of medicinal plants and herbal products has now become a key issue. Pharmacognostic studies play a vital role in assessing the quality, purity and identification of a plant drug. The misuse of herbal medicine or natural product starts with wrong identification which can be solved by pharmacognostic studies of medicinal plants. According to WHO, the pharmacognostic evaluation of a medicinal plant is the first step to be carried out to establish its identity and purity? The Physicochemical screening are also important to cheek if the drug is adulterant or not. Phytochemical screening is also important to find out the active chemical constituents like Alkaloid, glycoside, tannin, protein etc present or not in the extracts. Hence the present study deals with the details pharmacognostical and preliminary phytochemical studies of leaves of *Polygonum muricatum* commonly known as Jabuit from Polygonaceae family.

Keywords: pharmacognostical, physiochemical, extractive value, moisture content, *polygonum muricatum*

1. Introduction

Natural product are known to play an important role in pharmaceutical biology. Plants have been an exemplary source of medicine since ancient age. Even today, the World Health Organization estimates that up to 80 percent of people still rely mainly on traditional medicines. Ayurveda and other Indian literature mention the use of plants in treatment of various human ailments. India has about 45000 plant species and among them, several thousands have been claimed to possess medicinal properties. The selected plant *Polygonum muricatum* belonging to family polygonaceae, grown up in many regions of the world preferably between 800 and 1800 m altitude. Though the plant is native to china but, in India, it is widely found in the Meghalaya elevation of 850m-1700m. Traditionally it is reported that juice from the leaves of the plant from *Polygonum muricatum* from Polygonaceae family used for the treatment anthelmintic and in diarrhoea. So in the present study been made to evaluate the pharmacognostical and phytochemical parameters of leaves of *Polygonum muricatum.* The identification and authentication of the plant material taxonomically and pharmacognostically is important to standardized and also to avoid spurious or adulterated drugs and phytochemical analysis is also important to find out the presence of active constituents present in the leaves of *Polygonum muricatum*.

2. Materials and Methods

- **Collection of Plant -** The entire plant and the plant parts were collected from Meghalaya during the month of August-September and washed in running water, segregated from the grass and other extraneous material and the field data of the plant like its height, flower colour and soil condition were noted in the note book.
- **Authentication of Plant**- The selected plant was collected in flowering condition and deposited in the form of herbarium and submitted to the Botany Department of Guwahati University and authentication of submitted plant was carried out by the authorised person of Department of Botany, University of Guwahati, Assam, India.
- **Macroscopical Observation**-The macroscopical observation were carried out as per performed by the standard methods to determine the shape, size, taste, colour, odour of powdered drug etc.

Microscopical Examination

- **Transverse section of leaves-** The leaves of the plant was sectioned by using a new blade, a clean glass slide taken and placed a drop of glycerine water in the centre of slide into this the section was placed then into this one drop of phloroglucinol and HCL (1:1) given. Placed the cover slip by using the finger and thumb of the left hand and let the edge of the cover slip rest on the slide at the left hand edge of the drop. Insert a dissecting needle under the right hand edge of the cover slip and let the latter rest on the needle. Lower the cover slip slowly on to the drop of the liquid exactly fills the space between the slide and the cover slip without any air bubbles being trapped inside. Then placed the slide in position on the stage of microscope and observed the T.S of the leaf by using 10X and 45X lens.
- **Powder microscopy-** For powder analysis the whole plant was collected and washed thoroughly with water to remove any unwanted matter. This was further dried in the shade. After complete drying, it was powdered and passed through sieve no. 40. This was further subjected with different reagents like chloral hydrate, phloroglucinol and conc. HCl (1:1), iodine solution for the presence of the constituents like lignin, starch and calcium oxalate crystals.

Physicochemical constants

i) **Determination of Ash Value-** Ash values are helpful in determining the quality and purity of a crude drug especially in a powdered form. The following parameters were performed like Total ash, Acid insoluble ash, Water soluble ash as per standard procedure.

ii) **Determination of Extractive Values-** Extractive values of a crude drug determine the amount of active constituents extracted with solvents from a given amount of medicinal plant material. It is employed for materials for which no suitable chemical or biological assay exists. Under this parameter Alcohol soluble extractive and Water soluble extractive value were performed as per standard procedure.

iii) **Determination of moisture content-** Moisture is an inevitable component of crude drugs, which must be eliminated as far as practicable. Drying plays a very important role in the quality as well as purity of the material. Moisture will lead to the activation of enzymes and gives suitable condition, to the proliferation of living micro-organisms.

iv) **Fluorescence analysis-**The organic molecules absorbs light usually over a specific range of wavelength, get excited to a high energy level and many of them emit such radiations while coming back to the original state. Such a phenomenon of re-emission of absorbed light that occurs only when the substance is receiving the exciting rays is known as "Fluorescence". The powdered drug was examined under U.V. and ordinary light with different reagents. The powdered drug was taken in a petridish and treated with different reagents. These were observed under different wavelengths i.e., visible rays and ultraviolet rays (254 nm and 365 nm). Various colour radiations emitted were observed and noted.

v) **Behaviour of powder of leaves of *Polygonum muricatum* with different reagents / solvents-**Powdered drug was examined by mixing with different solvents or reagents as per the procedure and the colour changes was observed in naked eyes under sufficient light.

Preliminary Phytochemical Screening

- **Preparation of Plant Extract-** The leaves of the plant were collected and washed thoroughly with water to remove any unwanted matter. This was further dried in shade. After complete drying it was powdered and passed through sieve no 40 and stored in an air tight container. Then using this air dried powder Successive Solvent Extraction was done using Soxhlet apparatus. The extraction was carried out, by using solvents of increasing polarity starting from Benzene, Acetone, Ethanol and water respectively.The concentrated extracts were re-dissolved in respective solvents& subjected to various chemical tests as per the standard methods for the identification of the various constituents.

3. Result and Discussion

Table 1: Macroscopical Evaluation-

Colour	Green
Odour	Characteristic
Taste	Acrid
Size	Length of leaves is 6 cm to 8 cm, diameter of leaves is 2 cm to 4 cm
Shape	Lanceolate
Extra features	White 'V' shape shiny patches on leave

Microscopical Examination

T.S. of leaf

The fresh leave of *Polygonum muricatum was* taken for transverse section (T.S.),When T.S. of the leave was mounted with chloral hydrate, phloroglucinol and dil. HCl and stained with safranin, iodine solution following elements were observed-

Upper epidermis, Vascular bundle, Lower epidermis, Starch grains, Fragment of vessels, Fibres, Calcium oxalate crystal, Xylem, Phloem, Stomata.

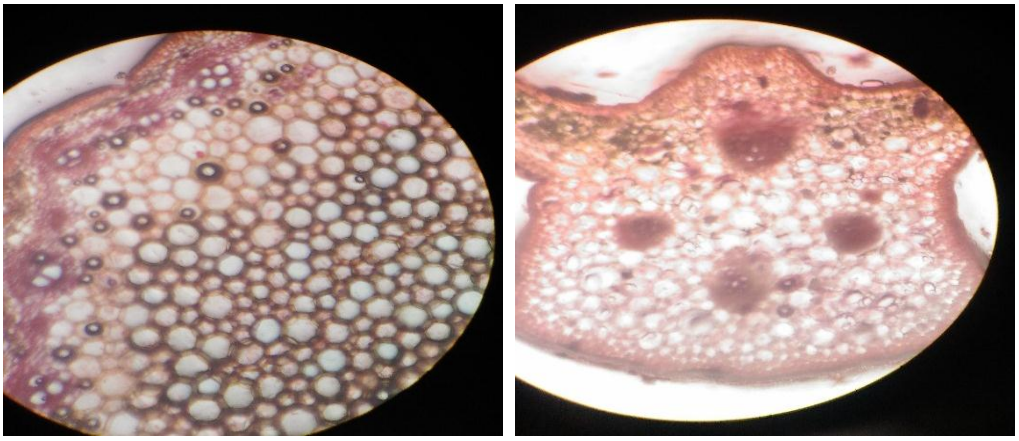

Fig 1

Powder microscopy of leaves

The powdered of leaves of *Polygonum muricatum* is light brown in color and has characteristic odour. When powder was mounted with chloral hydrate, phloroglucinol and dil.

HCl and stained with saffranin, iodine solution following elements were observed- Flatted Starch grains, Fragment of vessels, Fibres, Calcium oxalate crystal, Xylem, Phloem, Stomata.

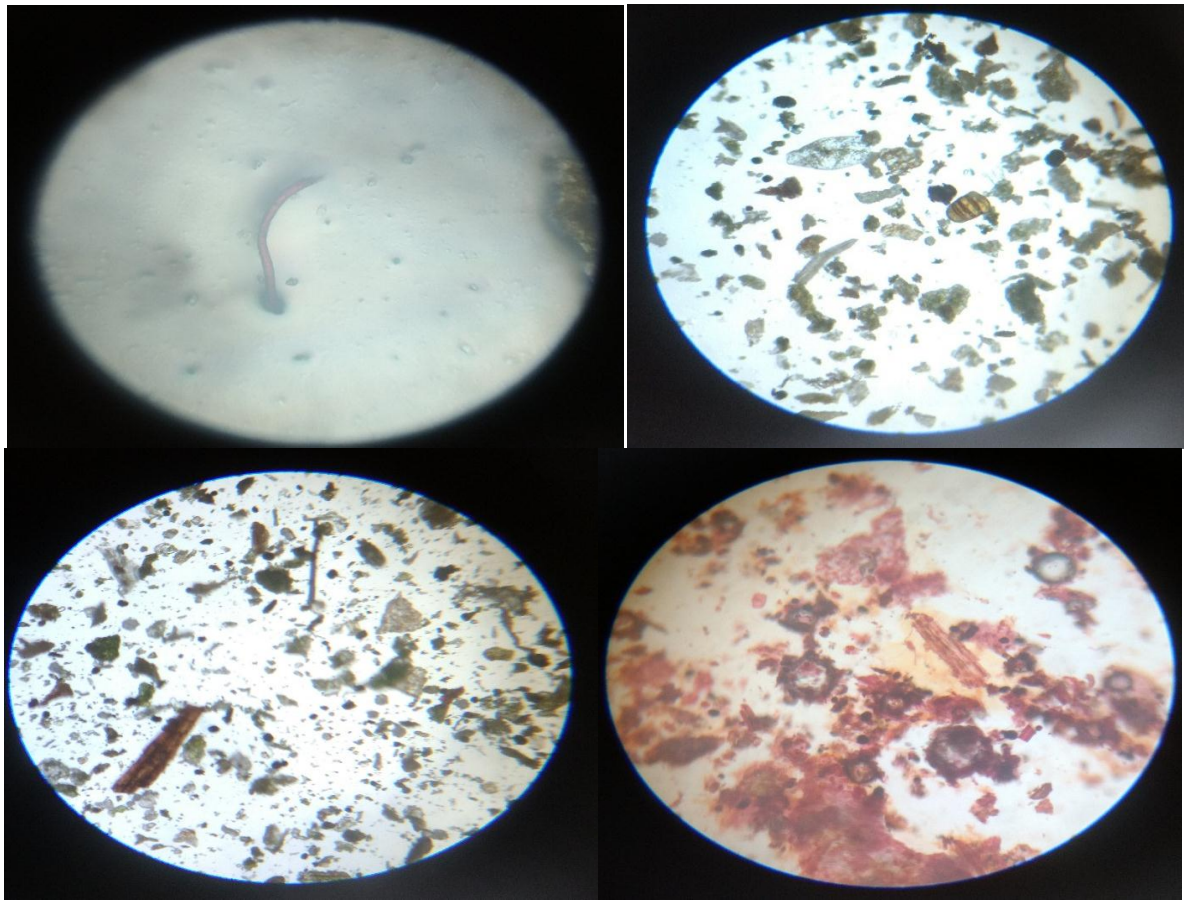

Fig 2

Ash Value- Ash values are helpful in determining the quality and purity of a crude drug especially in a powdered form. The object of ashing the vegetable drug is to remove all traces of organic matter that may otherwise interfere in an analytical determination. On incineration, a crude drug normally leaves an ash consisting of carbonates, phosphates and silicates of sodium, potassium, calcium and magnesium.

Table 2: Ash Value-

Sl. No.		
1.	Total ash	10% (w/w)
2	Acid insoluble ash	1% (w/w)
3	Water soluble ash	2% (w/w)

Extractive Values- Extractive values of a crude drug determine the amount of active constituents extracted with solvents from a given amount of medicinal plant material. It is employed for materials for which no suitable chemical or biological assay exists.

Table 3: Extractive Value

Sl. No.	Extracts	Extractive value (% w/w)
1	Alcohol soluble extracts	6.66 %
2	Water soluble extracts	8.11 %

Moisture content

Moisture is an inevitable component of crude drugs, which must be eliminated as far as practicable. Drying plays a very important role in the quality as well as purity of the material. Moisture will lead to the activation of enzymes and gives suitable condition, to the proliferation of living micro-organisms.

Table 4: Moisture Content

1st observation	2nd observation	3rd observation	Avg. Value
10%(w/w)	13.33% (w/w)	13.33% (w/w)	12.22% (w/w)

Fluorescence Analysis- The organic molecules absorbs light usually over a specific range of wavelength, get excited to a high energy level and many of them emit such radiations while coming back to the original state. Such a phenomenon of re-emission of absorbed light that occurs only when the substance is receiving the exciting rays is known as "Fluorescence". These were observed under different wavelengths i.e., visible rays and ultraviolet rays (254 nm and 365 nm). Various colour radiations emitted were observed and noted.

Table 5: Fluorescence Analysis

Treatment of powder of *Polygonum muricatum leaves*	Visible rays	Ultra -violet light	
		short wave (254 nm)	long wave (365 nm)
Powder as such	Brown	Black	Green
Powder+50% H_2SO_4	Light brown	Black	Brown
Powder+50% HNO_3	Brown	Black	Green
Powder+5% KOH	Yellow	Brown	Brown
Powder+Methanol	Brown	Black	Brown
Powder+1N HCl	Yellow	Brown	Green
Powder+1N Methanolic NaOH	Yellow	Black	Green
Powder+Cold water	Light brown	Black	Green
Powder+Hot water	Brown	Black	Dark green
Powder+Picric acid	Light brown	Black	Dark brown
Powder+Ammonia solution	Brown	Black	Green
Powder+Chloroform	Deep brown	Dark brown	Black
Powder+Glacial acetic acid	Deep brown	Dark brown	Black
Powder+5% Iodine solution	Deep brown	Dark brown	Black
Powder+$FeCl_3$	Yellow	Black	Green

Behavior of powder of *Polygonum muricatum* leaves with different reagents / solvents- Powdered drug was examined by mixing with different solvents or reagents as per the procedure and the colour changes was observed in naked eyes under sufficient light.

Table 6: Behavior of powder of *Polygonum muricatum leaves* with different reagents / solvents

Treatment of powder of *Polygonum muricatum leaves*	Visible rays
Powder as such	Light brown
Powder+50% H_2SO_4	Brown
Powder+50% HNO_3	Brown
Powder+5% KOH	Brown
Powder+Methanol	Brown
Powder+1N HCl	Light brown
Powder+1N MethanolicNaOH	Deep brown
Powder+Cold water	Light brown
Powder+Hot water	Light brown
Powder+Picric acid	Deep brown
Powder+Ammonia solution	Brown
Powder+Chloroform	Deep brown
Powder+Glacial acetic acid	Deep brown
Powder+5% Iodine solution	Deep brown
Powder+$FeCl_3$	Brown

Preparation of Extracts- The shade-dried leaves were powdered to get a coarse granule. About 500 grams of dried powder was extracted first with Benzene at 60 to 65ºC by continuous hot percolation, using Soxhlet apparatus. The extraction was carried out, by using solvents of increasing polarity starting from Benzene, Acetone, Ethanol and water respectively. The extraction was continued for 72 hours. The Benzene extract was filtered and concentrated to dry mass by using vacuum distillation. The marc left after benzene extraction was taken and then subsequently extracted with acetone, ethanol & water for 72 hrs. These extracts were filtered & concentrated to a dry mass.

Table 7: Successive solvent extraction & Nature of extracts

Sl. No.	Solvent	Color	Consistency	% of Yield (%w/w)
1.	Benzene	Dark green	Sticky	1.73
2.	Acetone	Black	Sticky	1.27
3.	Ethanol	Dark green	Sticky	3.84
4.	Water	Dark brown	Sticky	1.52

Phytochemical Screeing- The concentrated extracts were re-dissolved in respective solvents & subjected to various chemical tests as per the standard methods for the identification of the various constituents.

Table 8: Phytochemical Evaluation of *Polygonum muricatum* extracts

Test	B	A	E	W
Alkaloids	_	+	+	_
Carbohydrates	_	+	+	+
Glycosides	_	_	+	_
Phytosterols	_	_	+	_
Fixed oil and fats	+	+	_	_
Phenolic compound and Tannins	_	_	+	+
Saponins	_	+	+	+
Proteins&Aminoacids	+	_	+	_
Gums and Mucilage	_	_	_	_

B = Benzene**, A** = Acetone**, E** = Ethanol**, W** = Water

4. Conclusion

An attempt has been made to evaluate the pharmacognostical and preliminary phytochemical parameters of leaves of *Polygonum muricatum.* The identification of the plant material taxonomically and pharmacognostically is important to provide pharmacognostical standards and also to avoid spurious or adulterated drugs. The physicochemical constants like moisture constant, ash values such as total ash, acid insoluble ash, and water soluble ash, extractive values such as water soluble extractive value and alcohol soluble extractive value were determined. These help in formulating pharmacopoeial standards for the drug. The extracts obtained by successive solvent extraction were subjected to preliminary phytochemical analysis to find out the presence of compounds.

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