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PHARMACOGNOSTICAL, PHYTOCHEMICAL AND ANTHELMINTIC ACTIVITY OF POLYGONUM MURICATUM

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ABSTRACT
Polygonum muricatum is widely used by the local people of Meghalaya for anthelmintics. An attempt has been made to evaluate the pharmacognostical, preliminary phytochemical and pharmacological parameters of leaves of *Polygonum muricatum*. The physicochemical constants like moisture content, 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. The extract obtained by successive solvent extraction was subjected to preliminary phytochemical analysis to find out the presence of compounds. The plant *Polygonum muricatum* leaves were extracted with the solvent benzene, acetone, ethanol and water by soxhlet apparatus method. The extract was evaluated for anthelmintic activity with Indian earthworm. Ethanol extract of the plant *Polygonum muricatum* has shown the significant activity.

INTRODUCTION

Traditional system of medicine is the most widely practiced system of medicine. Plants have been an exemplary origin for medication since ancient age. As per the report of the World Health Organization around 80 percent of people still rely on traditional/ ethnic medicines [1].

Most trusted Indian literature like Ayurveda sushruta Samhita, and charak Samhita provide classified information about the use of plants in the treatment of various human ailments. India has about 45000 plant species and among them, several thousand have been claimed to possess medicinal properties [2].

The plant *Polygonum muricatum* belongs to the family Polygonaceae, grown up in preferably between 800 and 1800 m altitude [3]. In India, this plant widely available in the North-eastern part of Meghalaya elevation of 850 m-1700 m. Traditional healers from Meghalaya most effectively used the juice of the plant leaves for the treatment anthelmintic and antidiarrhoeal. Taking under consideration these facts the

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present research has been planned to determine the medicinal values of the plant as well as to investigate the pharmacognostic, phytochemical and anthelmintic activity of leaves of *Polygonum muricatum*.

MATERIALS & METHODS

Collection of Plant: The entire plant was brought from Meghalaya during the month of August-September and washed in continuous water, set apart from the grass and other additional material and the field data of the plant like its height, soil condition and flower colour was noted in the note book.

Authentication of Plant: The selected plant was collected in flowering condition and submitted for authentication to the authorized person at Department of Botany, Guwahati University Assam, India. The reference of authentication is mentioned herewith. (Herb./Bot/GU/2016/183)

Macroscopical Observation: The macroscopical observation was carried out to determine the shape, size, taste, colour, odour etc. of the powdered drug [4]

Microscopical Examination: Transverse section of leaves- The leaves of the plant *Polygonum muricatum* was sectioned by using a new blade, a clean glass slide taken and placed a drop of glycerine water in the center of a slide into this the section was placed then into this one drop of phloroglucinol and HCl (1:1) given. Placed the coverslip by using the finger and thumb of the left hand and let the edge of the coverslip rest on the slide at the left-hand edge of the drop. Insert a dissecting needle under the right-hand edge of the coverslip and let the latter rest on the needle. Lower the coverslip slowly on to the drop of the liquid exactly fills the space between the slide and the coverslip without any air bubbles being trapped inside. Then placed the slide in position on the stage of the microscope and observed the T.S of the leaf by using 10X and 45X lens [4]

Powder microscopy: For powder analysis, the whole plant was collected and washed thoroughly with water to eliminate any unwanted matter. This was further dried in the shade. After complete drying, it was powdered and this was further subjected to 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 [4]

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. Different parameters like Total ash, Acid insoluble ash, Water soluble ash was determined as per standard protocol [5]

(ii) Determination of Extractive Values:The Alcohol-soluble extractive and Water-soluble extractive value was determined. Extractive values of a crude drug determine the number of active constituents extracted with solvents from a given amount of medicinal plant material [5]

(iii) Determination of moisture content: Moisture will lead to the activation of enzymes and gives the suitable condition, to the proliferation of living micro-organisms. Drying plays a very important role in the quality as well as the purity of the material [6]

(iv)Fluorescence analysis: Fluorescence analysis is considered as an important parameter as per as drug crud drug is concern. The powdered drug was examined under long wavelength and short wavelength and at an 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 [7]

Preliminary Phytochemical Screening

Preparation of Plant Extract- The leaves of the plant was collected and washed thoroughly with water to remove any unwanted matter. This was further dried in shade. After complete drying, it was powdered and stored in an airtight container. Then using this air-dried powder Successive Solvent Extraction was done using a 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 [8 – 10]

Pharmacological activity: Earthworms, each of average length of 6 cm, were placed in petridishes containing 2 ml of various drug concentrations, 25 mg/ml, 50 mg/ml, and 100 mg/ml of solution. Albendazole solution was used as reference standard drug and normal saline as control. By tapping the end of each

worm with the index finger and applying a bit of pressure, the worms that were a live showed motility and those dead were non-motile. The motile worms were returned to the respective petridishes [11–12]

RESULTS& DISCUSSIONS

Table No. 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 leaves

Microscopical Examination

T.S. of leaf

The fresh leave of *Polygonum muricatum was* taken for the transverse section (T.S.), When T.S. of the leave was initiated with chloral hydrate, phloroglucinol, and dilute HCl and stained with safranin, iodine solution following component were observed-Upper epidermis, Vascular bundle, Lower epidermis, Starch grains, Fragment of vessels, Fibres, Calcium oxalate crystal, Xylem, Phloem, Stomata.

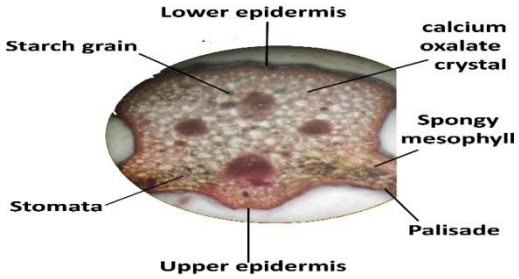

Fig. No.1

Powder microscopy of leaves

The powdered of leaves of *Polygonum muricatum*is light brown in color and has a characteristic odour. When the powder was mounted with chloral hydrate, phloroglucinol, and dil. HCl and stained with safranin, iodine solution following component were observed – FlattedStarch grains, Fragment of vessels, Fibres, Calcium oxalate crystal, Xylem, Phloem, Stomata.

Ash Value, Extractive value & Moisture content- Ash values are helpful in determining the purity and quality of a crude drug, especially in a powdered form. On incineration, a crude drug normally leaves an ash consisting of carbonates, phosphates, and silicates of sodium, potassium, calcium and magnesium. Extractive values of a crude drug determine the number 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. 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 the purity of the material.

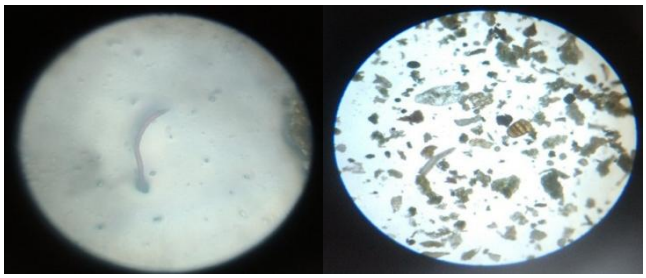

Fig. No.: 3 Fig. No.: 4

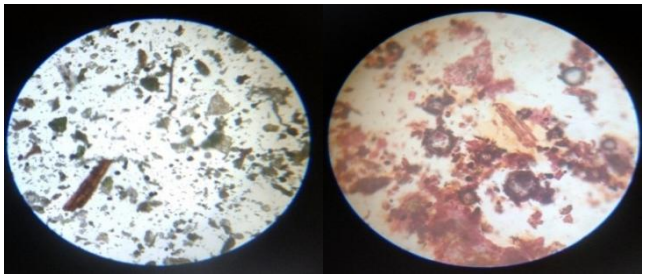

Fig. No.: 5 Fig. No.: 6

Table No.2: Ash Value, Extractive Value, Moisture Content

Particulars		
Ash Value	Total ash	10% (w/w)
	Acid insoluble ash	1% (w/w)
	Water soluble ash	2% (w/w)
Extractive Value	Extracts	Extractive value (% w/w)
	Alcohol soluble extracts	6.66 %
	Water soluble extracts	8.11 %
Moisture content	12.22% (w/w)	

Fluorescence Analysis- These were observed under different wavelengths i.e., visible rays, short wavelength (254 nm) and long wavelength (365 nm). Various colour radiations emitted were observed and noted.

Table No. 3. Fluorescence Analysis

Treatment of powder of *Polygonum muricatum leave*s	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 MethanolicNaOH	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

Preparation of Extracts: The shade-dried leaves were powdered and about 500 grams of dried powder was extracted first with Benzene at 60 to 65°C by continuous hot percolation, using Soxhlet apparatus. Solvents for extraction were selected based on the increasing polarity starting from Benzene, Acetone, Ethanol, and water respectively. The extraction was continued for 72 hours. After completion of the extraction process, all the extracts were concentrated to dry mass by using vacuum distillation. The final prepared extracts were stored in a sealed airtight container and kept it inside the refrigerator until further use.

Phytochemical Screening: 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 No. 4: Phytochemical Evaluation of *Polygonum muricatum* extracts

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

B = Benzene, H= Hexane, A = Acetone, E = Ethanol, W = Water

Pharmacological Screening

The anthelmintic activity was carried out on adult Indian earthworm *Pheretima posthuma* in view of anatomical and physiological resemblance with the intestinal roundworm parasite of a human being. Ethanolic extract of *Polygonum muricatum* leaves exhibits more potent activity at higher concentration (100 mg/ml) against *Pheretima posthuma* (earthworm). Evaluation of anthelmintic activity was compared with reference standard albendazole is shown below.

Table No. 5. Evaluation of Anthelmintic activity

Group	Treatment	Conc. (mg/ml)	Paralysis time (min)	Death Time (min)
1	Control (NS)	-	-	-
2	Albendazole	25	20 ± 0.00	30 ± 0.00
3	Leaves ethanol extract	25	90 ± 2	-
		50	60 ± 1.2	120 ±.9
		100	35 ± 0.47	46 ± 0.47

P value is ≤ 1, F value = 0.170001

Leaf extract of *Polygonum muricatum* showed the significant against Indian earthworm (*Pheretima posthuma)*. Earthworm in normal saline solution has not showed paralysis or death which ideal about selection of solvent is suitable.

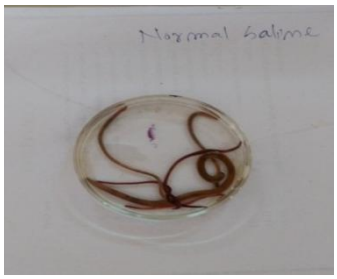

Fig. No. 7: Indian earthworm treatedwith normal saline

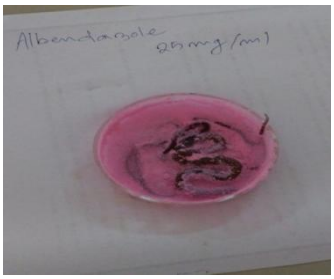

Fig. No. 8: Indian earthworm treatedwith albendazole

Fig. No.: 9. Indian earthworm treatedwith ethanol extract (25 mg/ml)

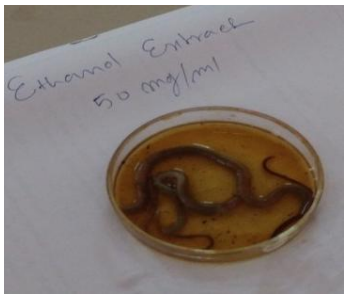

Fig. No.: 10. Indian earthworm treatedwith ethanol extract (50mg/ml)

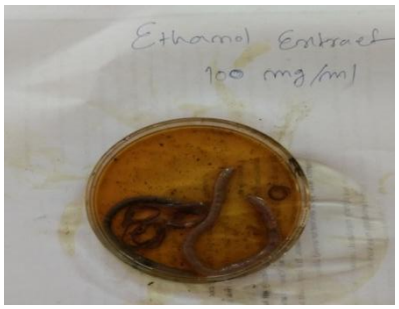

Fig. No.: 11. Indian earthworm treated with ethanol extract (100 mg/ml)

An effort has been made to determine the pharmacognostical, phytochemical and pharmacological properties of leaves of *Polygonum muricatum*. The macroscopical evaluation of the leaf of *Polygonum muricatum* shows that the leaf was green in color with a characteristic odour, the taste was found to be acrid and size of the leaf was around 6 cm – 8 cm in length and 2 cm – 4 cm in diameter. Further, the microscopical studies of the fresh leaf showed the presence of Fibres, Calcium oxalate crystal, Xylem, Phloem, Stomata, Strauch Grains, Upper epidermis, Lower epidermis (shown in Fig no. 1). The powdered leaf microscopy of *Polygonum muricatum* has also been carried out the finding are shown in fig No- 2- 6. The powdered leaf was subjected for physiochemical evaluations like moisture content, ash value, extractive value such as alcohol soluble extractive and water-soluble extractive value were determined. The results state that the percent water-soluble extractive values were higher than the alcohol; which indicates the presence of more amounts of water-soluble contents in the plants (Table no. 02). Fluorescence analysis powder drug was performed using different solvent under different wave length.

The result of the study shows brown, yellow colour as well as black colour under short wave length and green colour appear under long wave (Table no- 03) . The powder plant was extracted by solvent extraction method using ethanol as solvent. The different concentration of ethanolic extract were prepared using the concentrations 25 mg/ml, 50 mg/ml and 100 mg/ml respectively and subjected for phytochemical analysis study and anthelmintic activity against indian earthworm (*Pheretima posthuma).* The result of the study indicates that more of less all the concentration shows anthelmintic activity against indian earthworm. However, the activity increases with the increase in the concentration of extract. The phytochemical analysis studies indicates the presence of alkaloid, carbohydrate, glycoside, protein and amino acid, phytosterol, tannin etc.

CONCLUSION

The aim of the present work was to determine the presence of phytochemicals in the leaves of *Polygonum muricatum* and to establish the medicinal importance of the plant. From the above mentioned study and obtained results, it may be concluded that the ethanolic extract of the leave of *Polygonum muricatum* shows potent anthelmintic activity. However, further research activities are required to carried out to determine the exact phytochemical and mechanism responsible for the anthelmintic activities of the leave of *Polygonum muricatum.*

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FINANCIAL ASSISTANCE

NIL

CONFLICT OF INTEREST

The authors declare no conflict of interest

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