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Evaluation of pharmacognostic, physico-chemical and fluoresence properties of *Polygonum barbatum* Linn.

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Abstract

Polygonum barbatum, belongs to family Polygonaceae, is used in traditional system of medicine for healing various diseases. This study includes morphological, microscopic and physic-chemical investigations of the Polygonum barbatum. The results of present study used to ascertain the originality of the drugs prepared from the whole plant samples of P. barbatum.

Keywords: *Polygonum barbatum,* Polygonaceae, Anatomy, Fluorescence, Physical and chemical properties.

Introduction

Polygonum barbatum Linn (Family: Polygonaceae) distributed throughout India, particularly in wetlands. Many reports indicate the various medicinal properties of *P. barbatum* and its potential use in the traditional system of Indian medicine to cure various human diseases. Several reports indicate the uses of different parts of *Polygonum* species in the treatment of gripping colic pain[1,2] with seeds; ulcers and scarred tissues with leaf[3] and shoot/stalk[4] decoctions, respectively; and scabies with root past[5]. Dichloromethane extract of *P. barbatum* reported to have brain shrimp toxicity and sapsmolytic activity and the methanol extract have chlorogenic activity[6]. Aerial parts of *P. barbatum* have anticeptive, antiinflamatory and diuretic properties[7,8] and roots are used as astringent and cooling remedy[9,10]. Choudhary *et al*.[11] reported the uses of *P. barbatum* as astringent, colic and others. Sheela *et al*.[10] reported on pharmacognostic standardization and phytochemical screening of *P. barbatum* leaves. In this study, various pharmacognostic, physic-chemical and fluorescence characteristics of *Polygonum barbatum* Linn whole plant samples were evaluated.

Materials and methods

Study Plant: Normal, healthy, *Polygonum barbatum* plants were collected from *Nallur*, Thoothukudi District, Tamil Nadu, India (Figure-1a) and used to study their morphological and anatomical characteristics. Plant characters were examined using hand lens in the field and dissection microscope in the laboratory. Photographs of the study plant and its parts were also taken for future utilization (Figure-1b).

Plant sample preparation: *Polygonum barbatum* plant parts (root + shoot + leaf) were prepared and fixed for 24 hours in FAEA (5ml of Formalin + 5ml of Acetic acid + 90ml of 70% Ethyl Alcohol) mixture. Then the plant parts were dehydrated with graded series of tertiary-butyl alcohol[12]. Infiltration of the plant parts was carried by gradual addition of paraffin wax (melting point 58-60°C) until toluene butyric acid (TBA) solution attained super saturation. The plant parts were cast into paraffin blocks.

Sectioning: The paraffin embedded *P. barbatum* plant parts (leaf, stem and roots) were sectioned using *Rotary Microtomes* with a thickness of 10-12µm. The plant sections were dewaxed[13]and stained with Toluidine Blue[14]. The staining produce pink colour to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies, etc. The plant sections were also stained with safranin, Fast-green and IKI (Iodine Potassium Iodide) for starch.

Paradermal sections (taken parallel to the surface) of *P. barbatum* leaves were taken to study the morphology of stomata, venation pattern and trichome distribution in *P. barbatum* leaves. Sodium hydroxide (5%) was used for leaf clearing and Jeffrey's Maceration Fluid[12] was used for epidermal peeling by partial maceration method. Temporary slides were prepared for macerated/cleared plant materials. *Polygonum barbatum* powder samples were cleared with NaOH and stained. Different cell components of *P. barbatum* plant powder samples were observed and measured.

Photographs of anatomical features of leaves, shoots and roots of *P. barbatum* were taken with different magnifications and the microscopic descriptions of plant tissues are added with micrographs. Bright field microscope was used for normal observations. Crystals, starch grains and lignified cells were studied using polarized light microscope. Magnifications of the figures are indicated by the scale-bars and the descriptive terms of the anatomical features used are as given in the standard anatomy books[15].

Physical characters such as weight loss on drying, total ash, acid soluble and insoluble ash, water soluble and insoluble ash, sulphated ash and residue on ignition were recorded in the whole plant sample of *P. barbatum* by standard methods[16].

Weight loss on drying: Known quantity of *P. barbatum* powder samples was weighed separately and allowed to dry until a constant weight was obtained. The loss of weight on drying was determined from the initial and final weight.

Total ash: *Polygonum barbatum* whole plant dry powder sample (3g) was ignited to dull red heat in a silica crucible until the ash without carbon. The amount of ash with reference to the amount of dry sample taken was determined by weighing the crucible at room temperature.

Water soluble and insoluble ash: Known amount of *P. barbatum* ash sample (0.15g) was mixed in distilled water (25ml) and boiled. Insoluble ash content was collected and weighed. The insoluble ash content was subtracted from the amount of ash taken to determine the soluble ash content in water.

Acid soluble and insoluble ash: *Polygonum barbatum* ash samples (0.15g) was mixed and boiled with 25ml 2N HCl. The insoluble ash content was collected in sintered crucible and weighed. The amount of acid-insoluble ash and acid soluble ash was determined separately.

Residue on ignition: *Polygonum barbatum* powdered sample (3g) was put in a previously weighed crucible. Then the plant sample containing crucible was ignited, cooled to room temperature and weighed to determine the amount of residue on ignition.

Sulphated ash: *Polygonum barbatum* powder sample (3g) was taken in weighed silica-crucible and mixed with conc. H_2SO_4. Then, the plant sample containing crucible was ignited, cooled to room temperature and weighed to determine the amount of sulphated ash.

Extractive value of *P. barbatum* whole plant samples dissolved in various solvents was recorded separately to study the distribution of various constituents. Whole plant dry samples of *P. barbatum* (4.0g) were macerated with 100ml of different solvents (hexane, chloroform, ethyl acetate, ethanol and water) for 18 hours with frequent shaking. Then it was filtered and the filtrate (25ml) was transferred to a Teflon coated-dish and evaporated in a water bath. The resulting residue was dried at 105°C for 6h, cooled in desiccators for 30 min and weighed to determine extractive values by standard method[17].

Successive extractive yield is a measure of the solvents effectiveness to extract components from the plant samples. The successive extractive yield of *P. barbatum* whole plant samples in different solvents (hexane, chloroform, ethyl acetate, ethanol and water) was determined by standard method[17].

Fluorescence analysis: The fluorescence behaviour of *P. barbatum* samples was assessed by treating with various chemical agents and solvents under day light, fluorescent light and UV light (254nm and 365nm) and the colour change was recorded.

Preliminary phytochemical analysis: Different solvent extracts of *P. barbatum* samples was used to assess the presence of various phytochemicals by standard methods[18-20].

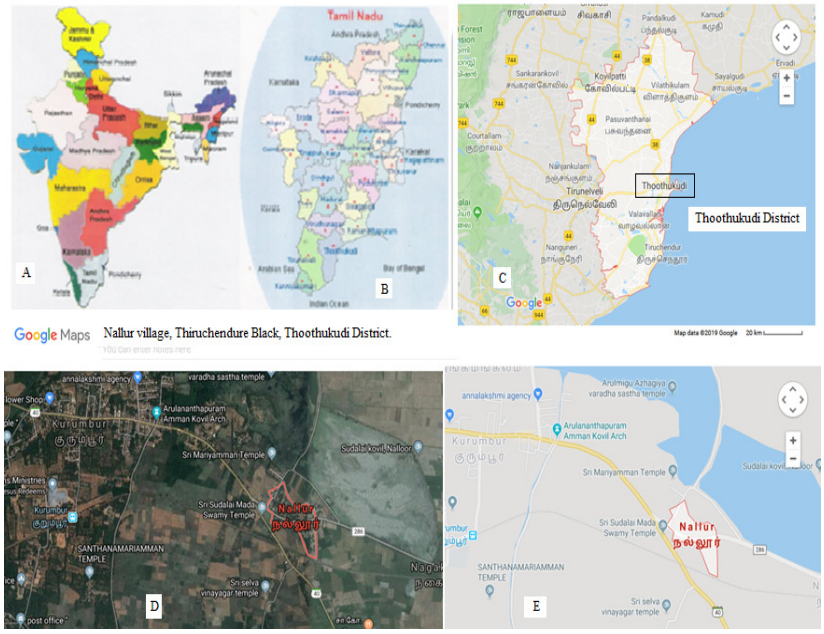

Figure-1a: Maps showing the location (Nallur in Thoothukudi District, Tamil Nadu) of *Polygonum barbatum* collected.

Figure-1b: Diagrammatic illustrations of *Polygonum barbatum* (A. One twig, B. Leaf, C. Inflorescence, D. Portion of stem with tubular strigse ocreae, E. Root.), and Habit of *Polygonum barbatum* (F, G, H) and Plant Parts used for whole plant samples (I).

Results and discussion

Pharmacognosy Studies: Morphological features: *Polygonum barbatum* (Figure-1b and 1c), is an erect annual herb with tubular strigose ocreae, conspecuous for the long fimbriate ciliae of the ocreae; Leaves are lanceolate or linear lanceolate; accumulate both ends glabrous, or more or less strigose, 7.15-12.5cm long, leaf sheath much shorter than the length (7.5cm); Internodes, much truncate stiff, ciliate, spaciform racemes rather thick (3.8-5.0cm) long peduncles (2.5cm long or more); Flowers are white; Pedicellate; Pedicel short, jointed under perianth; Perianth white, short, broad and rounded; Stamens-8, arranged in two whorls, 5-in outer whorl and 3-in inner whorl; Gynoecium-3, syncarpous, ovary superior, unilocular, ovule basal placentation, 3-angled nectar secreting disc present below ovary; Nutlet trigonous.

Microscopic (Anatomical) Features: ***Leaf*** (Figure-2; A to G): The lamina of *Polygonum barbatum* leaf is thin and smooth and have a thick midrib (Figure-2; A). The midrib of leaves is planoconvex. The abaxial side of the leaf is wide (1.11mm), thick (1.6mm) and semicircular while the adaxial side is more or less flat (Figure-2; A, B). The leaf midrib is multi-stranded with parenchyma ground tissue. Calcium oxalate crystals are druses and distributed palisade region of the midrib. Similar observations also reported by Sheela *et al.*[10]. Thin layered epidermis consists of small, squared and thick walled cells. Sub-epidermal cells are 2 or 3 layers of collenchymas. The ground tissue is homogenous, comprising large, thin walled, compact parenchyma cells with abundant large calcium oxalate druses (Figure-2; C, E, F). The multi-stranded vascular system includes about 10 discrete vascular bundles arranged in a planoconvex ring. Of the 10 bundles, the adaxial median bundle is the largest and others are of equal size (Figure-2; B). Collateral vascular bundles posses cluster of wide, thick walled circular xylem elements and thick band of phloem elements. The bundles have an arc of sclerenchyma caps abutting the phloem (Figure-2; D, E).

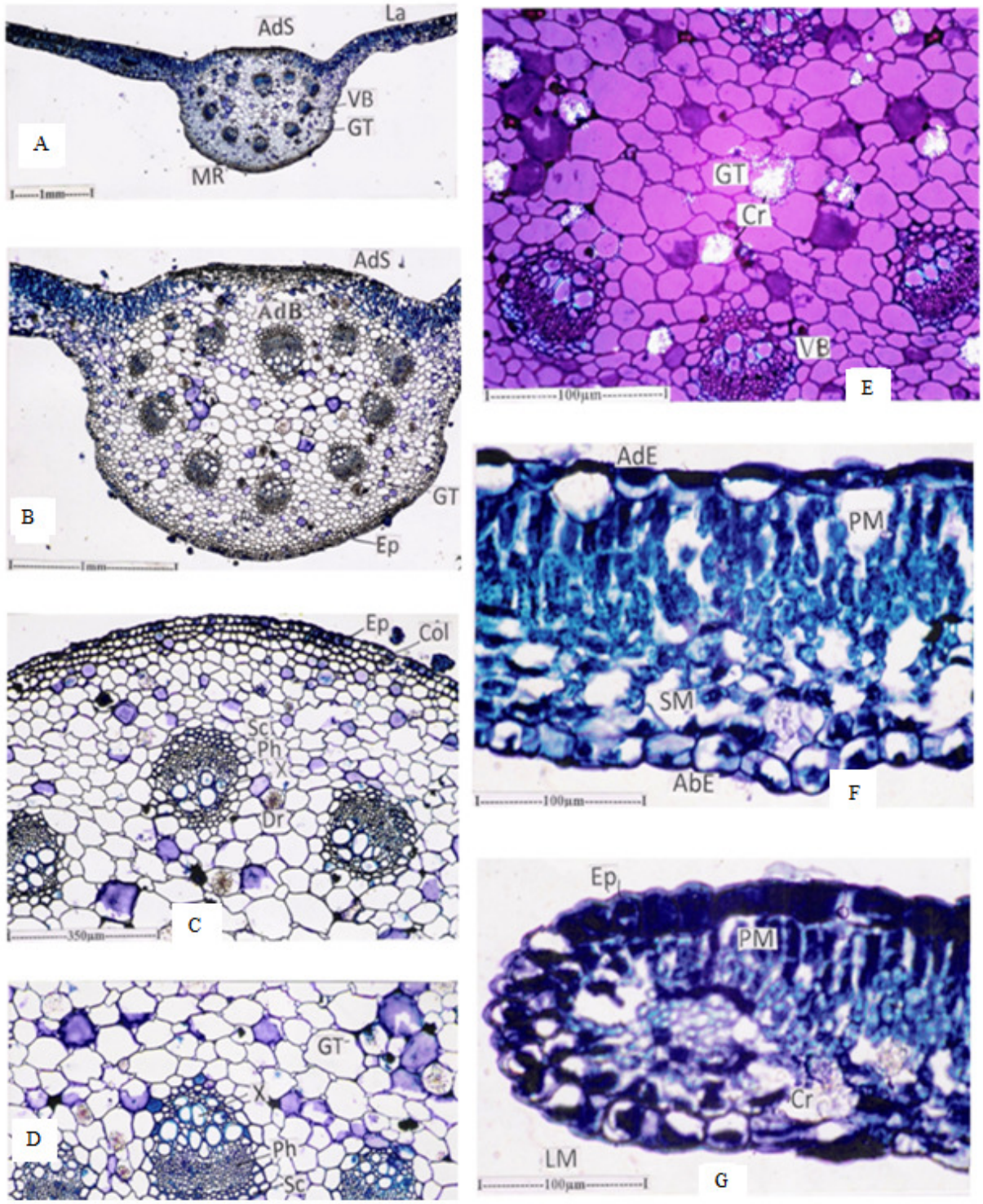

Figure-2: Anatomical features of *Polygonum barbatum* leaf [A - T.S. of leaf through midrib (2x), B - T.S. of midrib enlarged (4x), C - T.S. of midrib –a section enlarged (10x), D - Vascular system of the midrib –enlarged (10x), E - Crystals in the midrib (40X) and lamina, F - T.S. of lamina (40x), G - T.S. of lamina showing leaf margin-(40x)]. (AdB – Adaxial bundle; AbE – Abaxial epidermis; AdE – Adaxial epidermis; AdS – Adaxial side; Col – Colenchyma; Cr – Crystal; Dr – Druses; Ep – Epidermis; GT – Ground tissue; La – Lamina; LM – Leaf margin; MR – Midrib; Ph – Phloem; PM – Pallesade mesophyll; Sc – Sclerenchyma; SM – Spongy mesophyll; VB – Vascular bundle; X – Xylem).

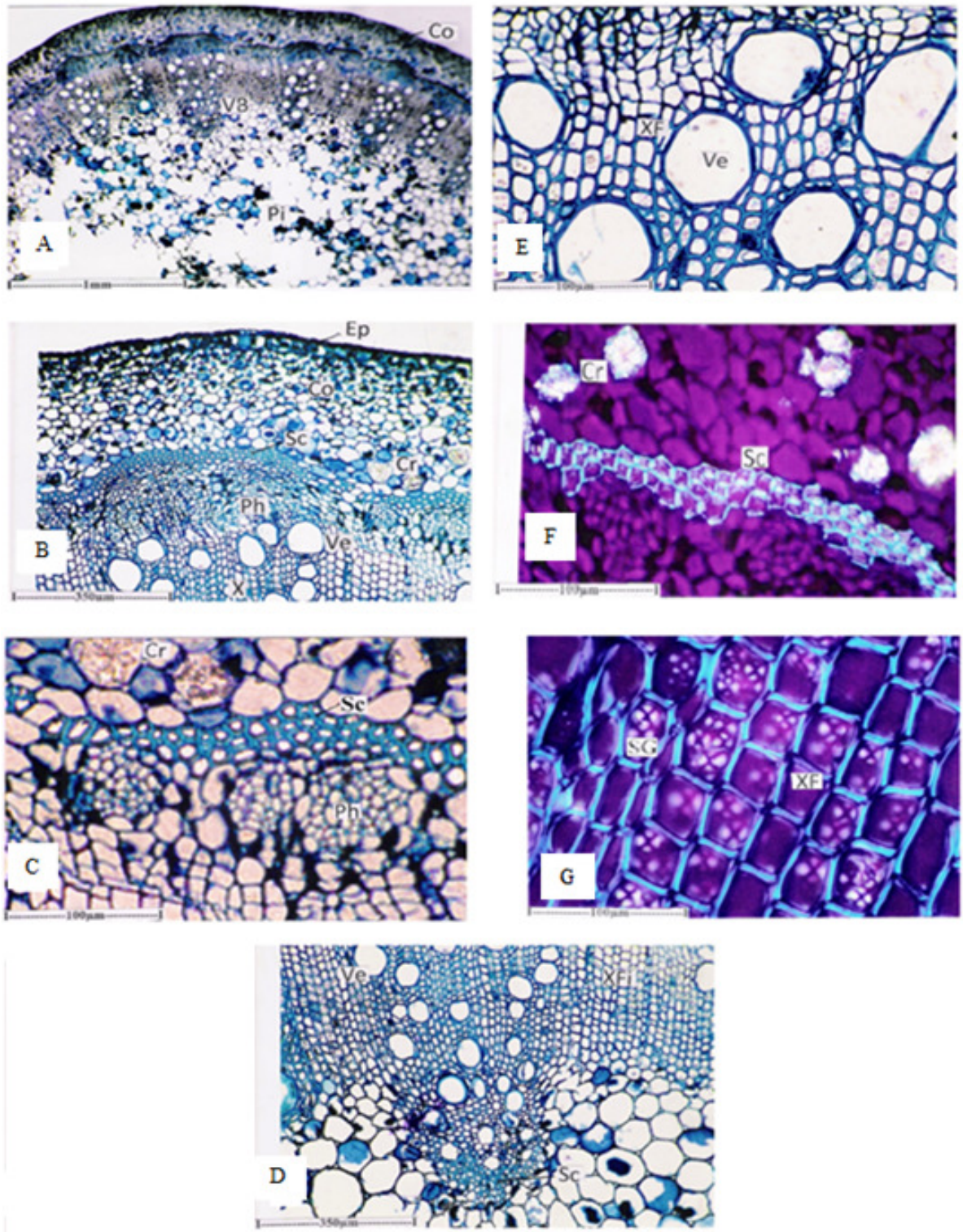

Figure-3: Anatomical features of *Polygonum barbatum* stem [A - T.S. of stem (4x), B - T.S. of stem enlarged (10x), C - T.S. of stem – phloem and sclerenchyma sheath (40x), D - T.S. of stem – xylem cylinder enlarged (10x), E - T.S. of stem –vessels and fibres enlarged (40x), F - T.S. of stem –crystals in the cortical zone (40x), G - T.S. of stem-starch grains in the xylem fibres – enlarged (40x)]. (Co – Cortex; Cr – Crystal; Ep – Epidermis; Ph – Phloem; Pi – Pith; Sc – Sclerenchyma; SG – Starch grains; Ve – Vessel; X – Xylem; XF/XFi – Xylem fibres).

Lamina (Figure-2; F, G) is dorsiventral and the adaxial epidermal cells are semi-circular, spindle shaped with 20µm in thickness. The aboxial epidermal layer is also thick with circular or barrel shaped cells. Palisade and spongy parenchyma cells are more or less similar. The palisade region has 2 to 3 layers of cylindrical cells. Spongy parenchyma tissue has 5 to 6 layers of lobed cells with large air spaces. Leaf margin is semi-circular and 130µg in thickness. Along the leaf margin, the epidermal cells are thick walled and conical in shape. The lamina median contains druses of calcium oxalate crystals. Both upper and lower epidermises are not interrupted by the glandular trichomes as reported by Sheela *et al*.[10] but it is noted in the epidermis of *P. glabrum*[21].

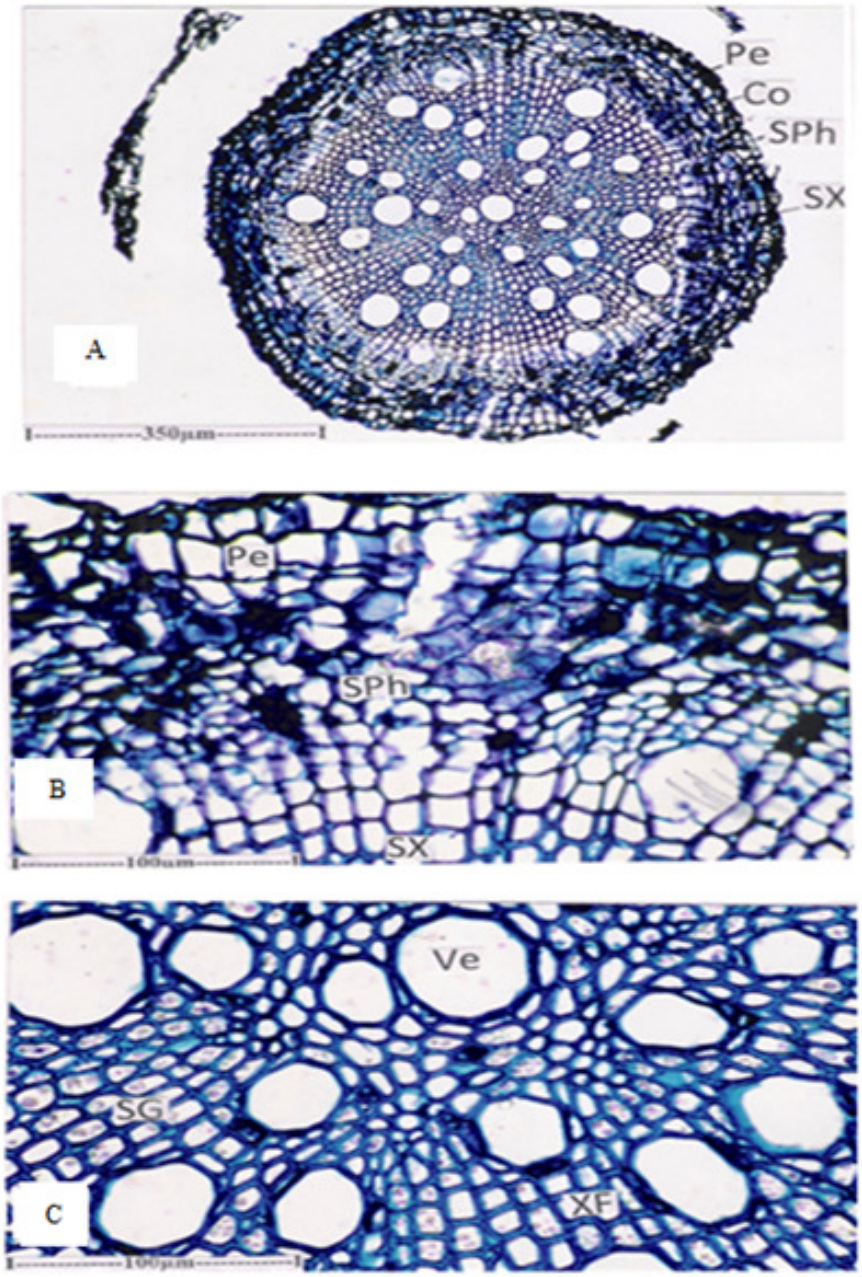

Figure-4: Anatomical features of *Polygonum barbatum* root samples [A –T.S. of root entire (10x), B –T.S. of root showing secondary phloem (40x), C –T.S. of root showing secondary xylem (40x)]. (Co – Cortex; Pe – Periderm; SG – Starch grain; SPh – Secondary phloem; SX – Secondary xylem; Ve –Vessel; XF – Xylem fibre).

T.S of *stem* (Figure-3; A to G) is circular with a thin intact epidermal layer followed by 250µm wide cortical region of parenchyma cells. Along the inner boundary of epidermis, the cortex is a thick cylinder of sclerenchyma cells (Figure-3; A to C). The cortical parenchyma cells contain calcium oxalate druses with 20-40µm in thick (Figure-3; C, F). The cortex is followed by the phloem elements in thick circular masses (Figure-3; C). Xylem cylinder consists of several vessels with radial rows of fibres (Figure-3; D). The vessels in the radial segments are wide (70µm), circular to elliptical, solitary and thick walled (Figure-3; D, E). The xylem fibres have dense accumulation of starch grains (Figure-3; G) which are simple, spherical with central hilum.

T.S of root (Figure-4; A to C): is circular with somewhat smooth surface and measuring 700µm in diameter. The root consists of a distinct superficial periderm which includes 4 or 5 layers of radial and oblong cells (Figure-4; B). Periderm is followed by secondary phloem. Phloem zone is narrow and it includes discrete mass of sieve elements with parenchymatous gaps in between (Figure-4; B). Secondary xylem cylinder is circular, solid and dense (Figure-4; C) with vessels and fibres. The vessels are angular, wide, thick walled, solitary and diffuse in distribution. The vessels increase in size from centre towards the periphery (Figure-4; C). The widest vessel is 70µm in diameter. The xylem-fibres are rectangular with thick lignified walls, occur in compact radial rows. Most of the fibres contain starch grains.

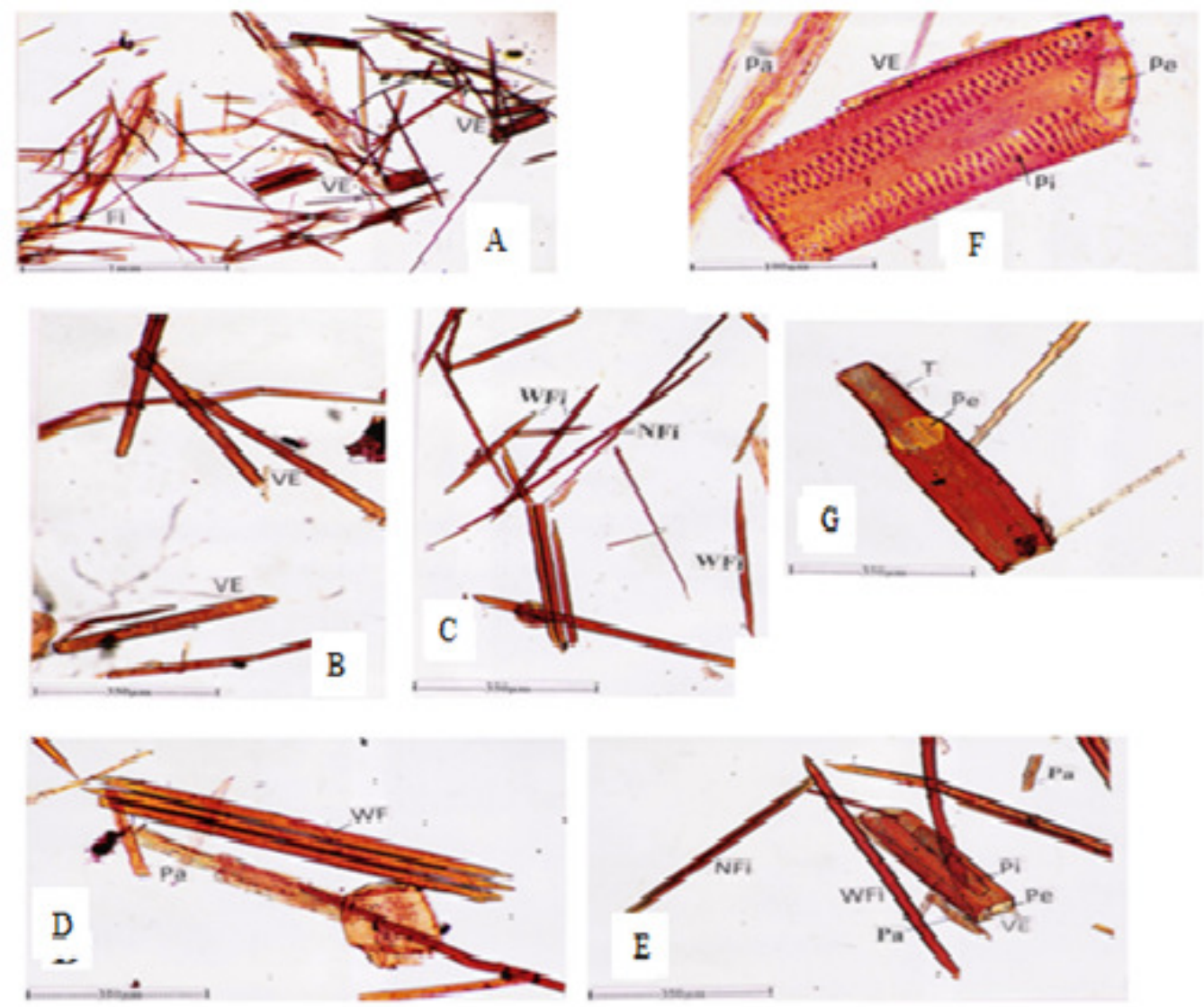

Figure-5: Microscopic features of *Polygonum barbatum* whole plant powder samples [A –Vessel elements and fibres (4x), B – Vessel elements (10x), C –Fibres (10x), D –Wide fibres (10x), E –Narrow fibre and vessel elements (10x), F –Vessel elements showing pits and perforations (40x), G –One vessel element with sub-terminal perforation (10x)]. (Fi – Fibre; NFi – Narrow fibres; Pa – Parenchyma; Pe – Perforation; Pi – Pits; T – Tail; VE – Vessel element; WFi – Wide fibres).

Microscopic observation of *P. barbatum* powder samples: The powder of *P. barbatum* includes vessel elements, fibres and parenchyma cells (Figure 5; A to G).

Vessel elements (Figure-5; A, B): Vessel elements are prominent inclusion in the powder samples. The vessel elements are long (200-400µm), narrow and cylindrical. The pits on the walls are elliptical and multi-seriate (Figure-5; F, G). The perforation is circular, wide and horizontal (Figure-5; G). Some of the vessel elements have long, thick and blunt, tail-like extensions beyond the perforation (Figure-5; F).

Parenchyma cells: Long narrow, scale like parenchyma cells are commonly found in the powder samples (Figure-5; D). The xylem parenchyma cells occur in small bundles and are short, narrow elongated with dense simple pits (Figure-5; E).

Fibres: There are two types of fibres (wide and narrow fibres) in the whole powder samples of *P. barbatum* (Figure-5; C).

Wide fibres (Figure-5; C to E) have thin walls and wide lumen. They are generally shorter and thicker than other fibres. The wide fibres are 370-600µm long and 25µm wide.

Narrow fibres (Figure-5; C to E) are more common in the whole plant powder samples of *P. barbatum.* The fibres have thick walls and narrow lumens and are longer than the wide fibres measuring 600-750µm long and 10µm wide.

Physico-chemical properties: Various pharmacognostic physical properties of *P. barbatum* were determined and the data are presented in Table-1. Air dried powdered whole plant sample of *P. barbatum* was used to analyze different physical properties. The results indicate that the *P. barbatum* whole plant sample shows 54.87% dry weight, 45.13% moisture content, 11.96% total ash, 20% water soluble ash, 80% water insoluble ash, 70% acid soluble ash, 30% acid insoluble ash, 75.67% sulphated ash and 93.67% residue on ignition (Table-1).

The ash values give a basis for judging the identity and cleanliness of a drug in powder form and also used to find out the total amount of inorganic elements present in the plants.

Extractive values and Successive extractive values: Extractive values and successive extractive yield help to determine the active polar, medium polar and non-polar components in solvent extracts of plant materials.

Extractive values of different solvents are determined in the whole plant dry powder samples of *P. barbatum* and the results (Table-2) shows variations in the extractive values ranged from 1.5 to 4%. Among the extracts tested, the maximum extractive value of 4% was noted in the water extract. The extractive value of different whole plant solvent extracts of *P. barbatum* is found in the following order: water > ethanol > chloroform = ethyl acetate = methanol > hexane.

Table-1: Pharmacognostic physico-chemical characters of *Polygonum barbatum* whole plant dry samples.

Parameters Tested	Physical Properties
Dry weight (gm)	54.87
Moisture content (%) (or) Weight loss (%)	45.13
Total ash (%)	11.96
Water soluble ash (%)	20.00
Water insoluble ash (%)	80.00
Acid soluble ash (%)	70.00
Acid insoluble ash (%)	30.00
Sulphated ash (%)	75.67
Residue on ignition (%)	93.67

Table-2: Extractive values and successive extractive yield of *Polygonum barbatum* whole plant samples.

Solvent extracts analyzed	Extractive values (%)	Yield (%) of successive extracts
Hexane	1.5	1.0
Chloroform	2.0	1.0
Ethyl acetate	2.0	0.5
Ethanol	3.0	3.5
Methanol	2.0	0.5
Water	4.0	1.5

Successive extractive yield of *P. barbatum* whole plant dry powder successive extracts, ranged from 0.5% to 3.5% (Table-2). Among the extracts tested, the maximum percentage of 3.5% extract yield was noted in the ethanol extract of *P. barbatum.* The percentage yield of different successive whole plant extract of *P. barbatum* is noted in the following order: ethanol > water > hexane = chloroform > ethyl acetate = methanol. The low extractive yield of successive solvent extracts may be due to the low solubility of the major components of the plant parts in solvents as suggested by Pattanayak *et al.*[22]

Fluorescence analysis: The whole plant dry powder samples of *P. barbatum* was treated in different solvents and their extracts (Table-3) and subjected to fluorescence analysis under day light, fluorescence light and UV light (at 254nm and 365nm). The fluorescence analysis utilizes the fluorescence produced by the compounds in the ultraviolet light for analytical evaluation. The

behaviour of the powdered plant material of *P. barbatum* observed in different solutions and their extracts towards ordinary light, fluorescent light and UV light can be used as analytical device for testing adulteration if any.

Table-3: Fluorescence characters of the whole plant dry powder* and extracts** of *Polygonum barbatum*.

Whole plant dry powder (WDP) + Solvents used	Fluorescence characters of *Polygonum barbatum*			
	Day light	Fluorescent light	UV-254	UV -365
Whole plant Dry Powder (WDP)	Dark green	Dark green	Dark green	Black brown
WDP + H_2SO_4 (1N)	Brown	Dark green	Black	Black
WDP + CH_3COOH (1N)	Brown	Dark green	Green	Black
WDP + HNO_3 (1N)	Brown	Brown	Green	Black brown
WDP + KOH (1N)	Brown	Black green	Dark green	Black
WDP + Na OH (1N)	Brown	Green	Pale green	Black
WDP +Petroleum ether	Brown	Brown	Black	Black
WDP +Acetone	Brown	Brown	Black	Black
WDP +Chloroform	Brown	Brown	Black	Black
WDP +Ethyl alcohol	Brown	Brown	Black	Black
WDP +Ethyl acetate	Brown	Brown	Black	Black
WDP +Water	Green	Green	Green	Black
Solvent extracts used				
H_2SO_4 (1N)	Colourless	Colourless	Pale green	Green
CH_3COOH (1N)	Colourless	Colourless	Green	Yellow
HNO_3 (1N)	Pale yellow	Pale yellow	Pale green	Green
KOH (1N)	Brown	Brown	Green	Yellow green
Na OH (1N)	Colourless	Colourless	Green	Green
Petroleum ether	Colourless	Colourless	Colourless	Brown
Acetone	Green	Green	Green	Dark brown
Chloroform	Green	Green	Green	Brown
Ethyl alcohol	Green	Green	Pale green	Brown
Ethyl acetate	Green	Green	Green	Brown
Water	Brown	Brown	Green	Black

*Fluorescent characters of whole plant dry powder were observed immediately in different solvents. **Fluorescent characters of whole plant dry powder solvent extracts were observed after 1h of incubation.

Table-4: Evaluation Preliminary phytochemicals in the whole plant extracts of *Polygonum barbatum*.

Solvent extracts	Phytocompounds tested					
	Alkaloids	Flavonoids	Phenols	Proteins	Steroids	Tannins
Hexane	+	+	+	-	-	+
Chloroform	-	+	+	-	-	+
Ethyl acetate	-	+	+	-	-	+
Ethanol	+	-	+	+	+	+
Methanol	+	-	+	-	+	+
Water	+	+	+	+	+	+

Preliminary phytochemical screening: Various extracts of *P. barbatum* whole plant dry samples were subjected to different qualitative chemical tests to assess (Table-4) the presence of alkaloids, flavonoids, phenols, proteins, steroids, and tannins. However, there are variations in the presence and absence of these phytochemical compounds in the solvent extracts of the *P. barbatum* tested.

Knowledge of pharmacognosy is useful in identification and finding the quality of the drug. Based on the Pharmacognostic characters, not only the drug can be identified. But also, the adulteration of drug can be checked. It is imperative that correct identification and nomenclature of the plant concerned are determined for standardization of plant products and for effective utilization of economic plants. The standardization prevents misidentification and adulteration.

The medicinal value of several plant species have yet to be screened for their biologically active compounds which are the source of important therapeutic agents as well as for their possible curative properties[23]. The indigenous population has developed vast knowledge on themselves and their crops. The evaluation or standardization of a crude drug involves pharmacognostic methods[24]. Scientific methods of standardization are needed to confirm the authenticity of medicinal plants used in the preparation of medicines. The search for biologically active compounds from natural sources has always been of great interest to researchers looking for new sources of drugs useful in infectious diseases. The study of pharmacognosy related to the structural, physical, chemical and sensory characters of crude drugs of plants origin. Various physicochemical methods used to determine the quality as well as purity of drugs.

Conclusion

In *P. barbatum,* the morphological and anatomical attributes, of vessels, fibers and parenchyma cells of leaf, stem and root, observed in this study are very significant and can be used in the identification of crude drugs prepared from whole plant samples of *P. barbatum*.

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