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PHARMACOGNOSTICAL AND PRELIMINARY PHYTOCHEMICAL ANALYSIS OF *POLYGONUM CHINENSE* L.

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ABSTRACT

Evaluation of pharmacognostic properties, fluorescence behaviour and preliminary phytochemical analysis was done in whole plant sample of *Polygonum chinense*, collected from Nallur village, Thoothukudi District, Tamil Nadu, India. Anatomical structure of *P. chinense* leaf, lamina, young and old stem, root samples and powder samples were studied. Pharmacognostic physical properties, –total ash (19.13%), water soluble ash (15%), acid soluble ash (70%), sulphated ash (96%) and residue on ignition (93.67%), were estimated. Maximum extractive value was noted in water extract (8%) while the maximum successive extractive value was noted in ethanol extract (2.5%). The fluorescence behaviour of *P. chinense* whole plant sample was observed in various solvents and their extracts under day light, fluorescence light and UV light (at 254nm and 365nm). Preliminary phytochemical analysis in the whole plant sample of *P. chinense* indicates the presence of phenols and tannins in all solvents, tested. The evaluation of anatomical and physico-chemical properties of *P. chinense* is useful for determining the quality and purity of the crude drug prepared from this plant.

KEYWORDS: *Polygonum chinense*, anatomy, pharmacognosy properties, fluorescence properties, preliminary phytochemicals.

I-INTRODUCTION

Traditional medicines and medicinal plants used in most of the developing countries have been noted as a basis for the maintenance of good health.[1] Traditional medicines are prepared from a single plant or combination of more than one plant part or one plant. The potential of these plants depend upon the current knowledge about taxonomic features of plant species, plant parts and biological properties of medicinal plants which in turn depends upon the occurrence of primary and secondary metabolites.[2] There are various factors influencing the variability of phytochemical in plants comprising genotype, size and maturity, soil conditions, irrigation, pesticide utilization, disease and pests, location and climate condition, etc.[3] Various forms of raw plant materials, processed plant materials and medicinal herbal products are used as finished labeled herbal drugs. Due to increased awareness of herbal medicines, it is essential to assess constantly for their possible pharmacognostic values that useful in identification, quality verification, check adulteration and effective utilization.

Polygonum is a genus of about 220 species of flowering plants in the buckwheat and knotweed family, Polygonaceae (Tropicos). *Polygonum chinense*, commonly known as creeping smart weed[4] or Chinese knotweed. It is wide spread across China, Jappan, the Indian subcontinent, Indonesia, Malaysia and Vietnam.[5] Tanaka *et al*.[6] reported that *Polygonum* is used in the treatment of dysentery, enteritis and sore throat in Malaysia and Vietnam. In Arunachal Pradesh, the leaf infusion of *P. chinense* used in colic pain; leaf paste used as essential application in boils; and stem juice is taken internally as a fever herb tonic and vulnerary.[7] It is also reported to use as a tonic and in colic and other uses.[8] Earlier reports indicate the presence of enormous amount of tannins and phenol compounds in the roots of *P. chinense*.[9,10,11] Hence, the present study carried out to record the nature of pharmacognostic and preliminary phytochemical properties in the whole plant sample of *Polygonum chinense*.

II-MATERIALS AND METHODS

Collection and preparation of plant specimens

The plant *P. chinense* was collected from *Mekkarai*, Shenkottai taulk (Figure 1a), Tirunelveli district, and elevation about 300meters MSL (Mean Sea Level). Care was taken to select healthy plants of normal growth.

Macroscopic studies

Mature and healthy plants of *P. chinense* were collected to study the morphological characters. Plant was examined using hand lens in the field and dissection microscope in the laboratory and the characters were recorded. Photographs of the *P. chinense* and its parts were also taken for future utilization.

Microscopic (Anatomy) studies

The samples of *P. chinense* plant parts (root, shoot and leaf) were cut and removed from the plant and a mixture of them (1:1:1, respectively) fixed in *Formalin* (*5ml), Acetic acid (5ml) and 70% Ethyl Alcohol (90ml*) (FAEA) mixture for 24 hours. The specimens were then dehydrated with graded series of tertiary-butyl alcohol.[12] Infiltration of the specimens was carried by gradual addition of paraffin wax (at 58-60°C) until TBA solution attained super saturation and the specimens were cast into paraffin blocks.

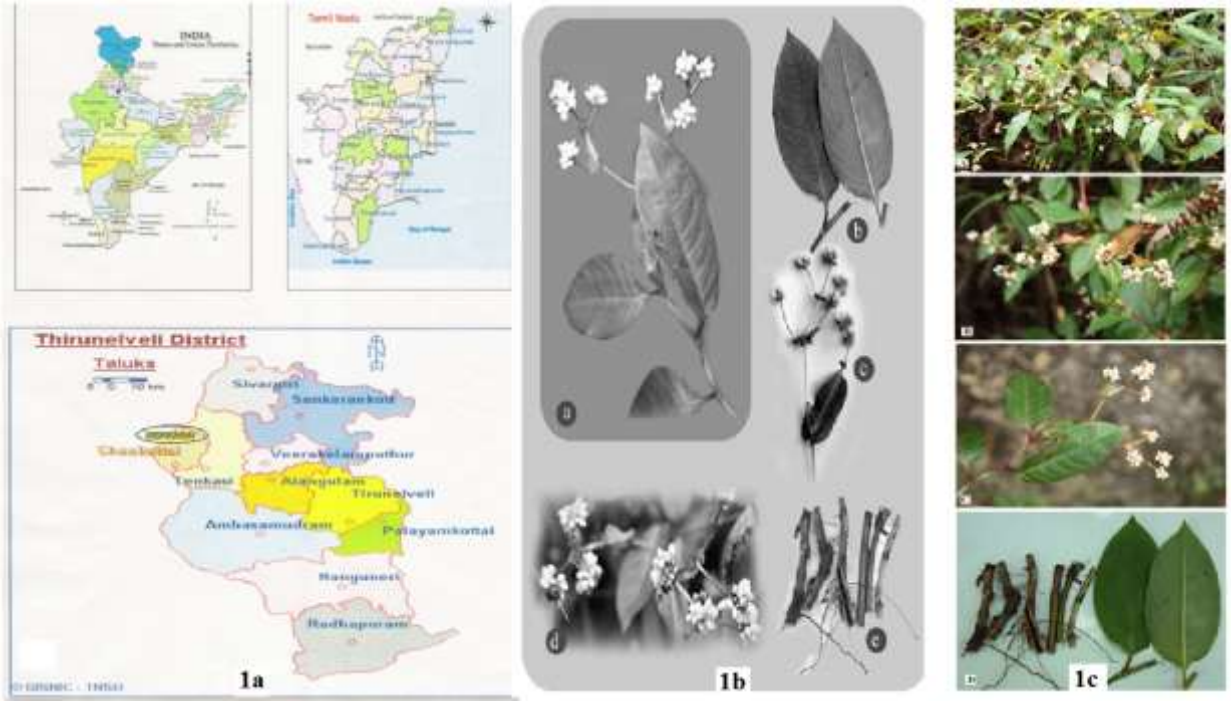

Figure 1: 1a. Maps showing the location of *Polygonum chinense* Linn. collected from Mekkarai village, Tirunelveli District, Tamil Nadu, India. 1b. A diagrammatic illustrations of *Polygonum chinense* Linn. 1c. Habit (A, B & C) and parts (D) of *Polygonum chinense* Linn used for whole plant extracts.

Sectioning

The paraffin embedded *P. chinense* (leaf, stem and root) specimens were sectioned using Rotary Microtomes, with 10-12µm thickness. The sections were dewaxed[13] and stained with *Toluidine Blue*.[14] The stain gave 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. Wherever necessary, sections were also stained with *Safranin*, *Fast-green* and *Iodine Potassium Iodide* for starch.

The stomatal morphology, venation pattern, trichome distribution and paradermal sections (sections taken parallel to the surface of leaf) of *P. chinense* as well as clearing of leaf with 5% sodium hydroxide or epidermal peeling were studied by partial maceration method employing with Jeffrey's Maceration Fluid[12] and for macerated/ cleared materials glycerine mounted temporary preparations were made. Different cell component were observed and measured in powdered materials of *P. chinense* plant parts after cleared with NaOH, stained and mounted in glycerine.

Photomicrographs

Microscopic descriptions of *P. chinense* plant tissues are supplemented with micrographs of different magnifications taken with Nikon Labphoto-2 microscopic unit, wherever necessary. Normal observations were made with bright field microscope. Crystals, starch grains and lignified cells were observed under polarized light microscope. Magnifications of the figures are indicated by the scale-bars. Standard descriptive terms used for anatomical features as given in the standard anatomy books.[15]

Physico-chemical characters

The percentage of loss of weight on drying, total ash, acid soluble/insoluble ash, water soluble/insoluble ash, sulphated ash and residue on ignition, and extractive values and successive extractive yield of different solvent extracts[16,17,18] were obtained from *P. chinense*

by employing standard methods of analysis as described in Pharmacopoeia of India.[19]

Fluorescence analysis

The *P. chinense* powdered sample and the extract of the powder in various solvents (Table 4) were examined under day light, fluorescent light and ultra violet light (365nm-254nm) and the fluorescence characters were determined by observing and recording the colour change.

Preliminary phytochemical analysis

Several chemical compounds were analyzed qualitatively in different *P. chinense* solvent extracts separately using standard methods.[20,21,22]

III-RESULTS AND DISCUSSION

Pharmacognosy Studies

Morphological descriptions

Figure 1b and 1c shows the morphological features of *P. chinense*. *P. chinense* is a rambling under-shrub, semi-scandent over bushes with ocreae cleft to base and grows about 2m long. The leaves are stiff, ovate acute, base truncate or round, sometimes cordate, glabrous nerves conspicuous, and with 12.5cm long and 7.5cm wide. The petioles are 1cm long with round auricles at base and the leaf sheath is about 1.3cm long. The inflorescence is a panicled cymes, 2.5-7.5cm long with leafy bracts and the flowers are white in colour, terminal corymbosely panicled heads. There are 7-8 stamens in 2 whorls. Overy is trigonous and the style is trified above. The nutlet is trigonous, pulby and black. Tanaka *et al*.[6] reported that *P. chinense* is a perennial climber grows to 2 to 3m height and the stems are glabrous and red brown with longitudinal strips; leaves have oval blades with 4-8cm long and 3-5cm wide, with pointed apex and round or nearly cordate base, the cyms emerge at terminal and are 5-7cm long with small white or pink flowers.

Microscopic/Anatomical Features

Leaf of *P. chinense* consists of a prominent shield shaped midrib and thin lamina (Figure 2; Photo-A). The adaxial part of the midrib is semicircular and wide. The adaxial dome is 500µm wide and the abaxial part is 900µm wide. The abaxial part is squarish with short peg-like out growth at the median part (Figure 2; Photo-B). The midrib is 1.4mm thick, consists of a thin well defied epidermis with small and circular epidermal cells (Figure 2; Photo-B). The ground tissue is homogenous and aerenchymatous with small air chambers of irregular shape, separated by thick partitions. There are two thick (200µm) and wide (450µm) vascular bundles placed at the middle part of the midrib. The bundles are broadly semi-circular with flat inner side and curved outer side. The xylem part of the vascular bundles is just opposed to each other. The vessels of the vascular bundles are wide, circular, fairly thick walled and diffuse in distribution. Phloem occurs in thick continuous band on the outer part of the xylem strand. There is a thick layer of sclerenchyma cells abutting the phloem. The sclerenchyma cells are thin walled, poorly lignified and have wide lumen (Figure 2; Photo-B).

Lamina of *P. chinense* (Figure 2; Photos C & D) have smooth and even surface with 150µm in thickness. The adaxial epidermis is apostomatic consists of thick (25µm) and wide cells. The abaxial epidermis is stomatiferous consists of thin cylindrical cells. The mesophyll tissue consists of a single row of short, thick palisade cells which are arranged with wide gaps. The spongy mesophyll is 5-layered with lobed and loosely arranged cells (Figure 2; Photo-D). The lateral view of the vascular bundles consists of fibres enclosing small xylem and phloem elements (Figure 2; Photo-C).

Young Stem of *P. chinense* (Figure 3; Photos-A & B) is circular in outline with shallow ridges and 1.7mm in thickness (Figure 3; Photo-A). It has a thin epidermal layer of small circular cells and the cortex is 200µm wide with circular compact thin walled parenchyma cells. The vascular system is a eustele and consists of several independent collateral vascular bundles arranged in a ring and possesses a few wide circular vessels or single vessel and thick mass of phloem elements. There is a thick continuous sclerenchyma cylinder ensheathing the vascular bundles along the phloem end. The sclerenchyma cells are thin walled, poorly lignified and have wide lumen. In between the vascular bundles are narrow medullary rays (Figure 3; Photo-B).

Old Stem of *P. chinense* (Figure 3; Photos-C & D) has initial stage of secondary growth. The epidermis is intact; cortex consists of 6-8 layers of compact parenchyma cells. A wavy thick sclerenchyma cylinder of 40-60µm thickness runs all around the vascular cylinder. Phloem is continuous and includes sieve-elements and parenchyma cells (Figure 3; Photos-E & F). Xylem cylinder is continuous possessing thin porous xylem fibres arranged in radial parallel rows which alternate with radially elongated wedges possessing wide (50µm), circular and solitary vessels. These vessel clusters are primary xylem and fibre based. The secondary xylem produced by inter-fascicular cambium.

Root anatomy of *P. chinense* (Figure 4; Photos-A to C) shows high degree of secondary growth with 800µm in thickness. The periderm is thin and exfoliates from the root (Figure 4; Photo-B). The cortex is narrow with parenchyma cells and isolated fibres. Secondary phloem is thin and uneven with sieve-elements and tannin containing parenchyma cells. No growth rings are evident in the secondary xylem. The vessels in the xylem are diffuse, mostly solitary, occasionally in multiples of two, thin walled and up to 50µm wide. The xylem fibres are thick walled and lignified and the lumen is wide and rectangular (Figure 4; Photo-C).

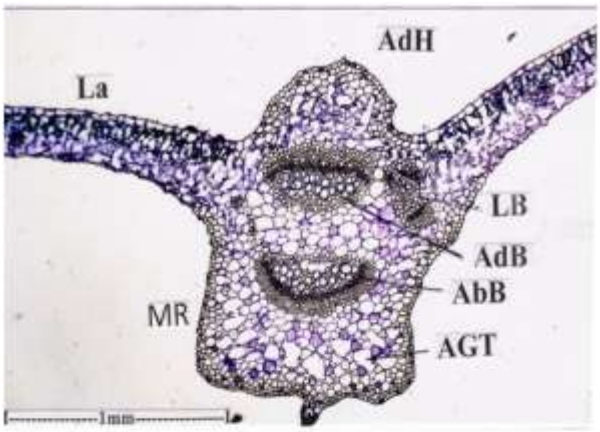

Photo-A: T.S. of leaf through midrib (4x)

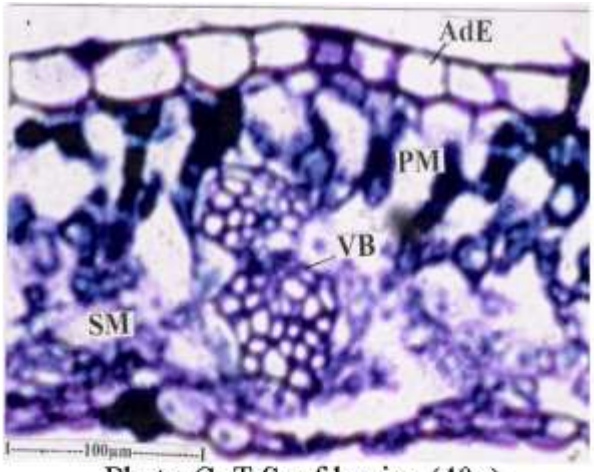

Photo-C: T.S. of lamina (40x)

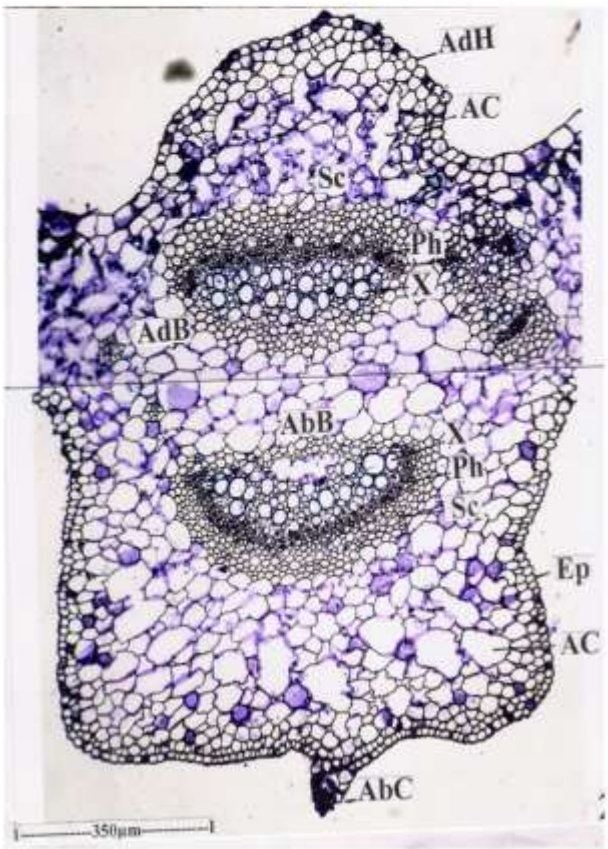

Photo-B: T.S. of midrib enlarged (10x)

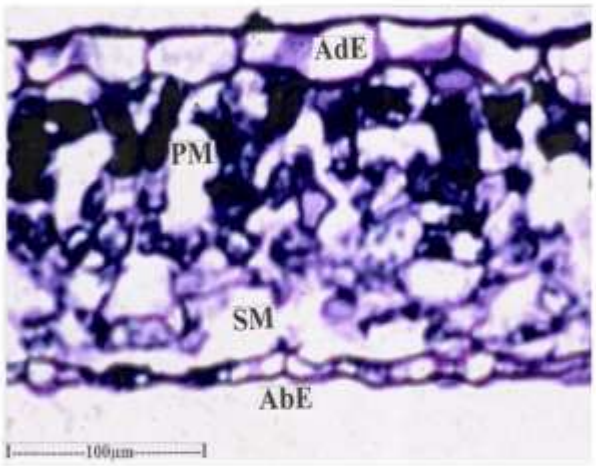

Photo-D: T.S. of lamina (40x)

AdH: Adaxial hump; AC: Air chamber;
AdB: Adaxial bundle; AbB: Abaxial bundle;
AGT: Abaxial ground tissue; AbC: Abaxial cone;
Ep: Epidermis; La: Lamina; LB: Lateral bundle;
MR: Midrib; Ph: Phloem; Sc: Sclerenchyma;
X: Xylem; AdE: Adaxial epidermis;
AbE: Abaxial epidermis; SM: Spongy mesophyll;
PM: Palisade mesophyll; VB: Vacular bundle

Figure 2: Photos showing anatomical features of the leaf (A & B) and lamina (C & D) of *Polygonum chinense*.

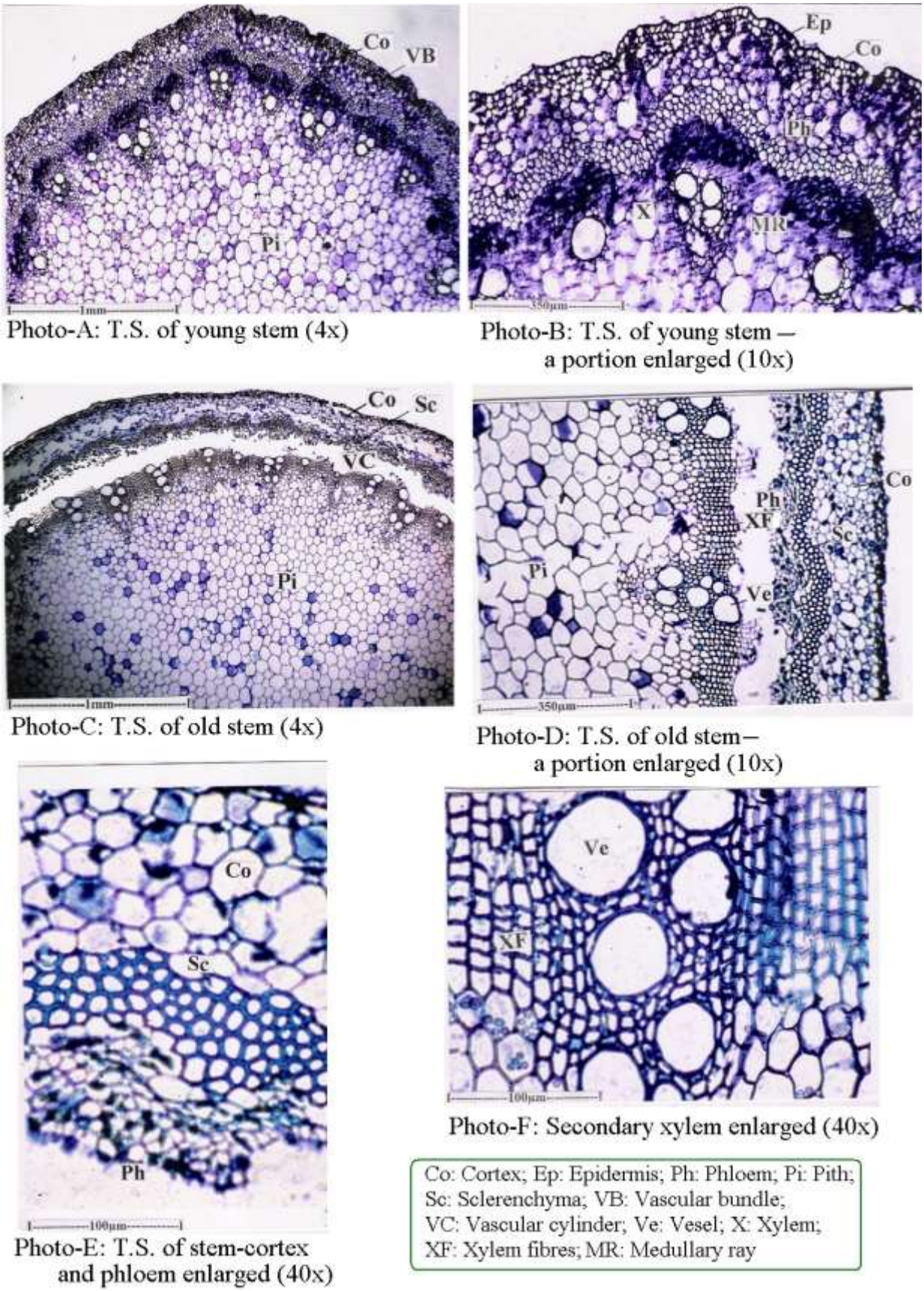

Figure 3: Photos showing anatomical features of the stem –young stem (A & B), old stem (C, D, E & F) of *Polygonum chinense*.

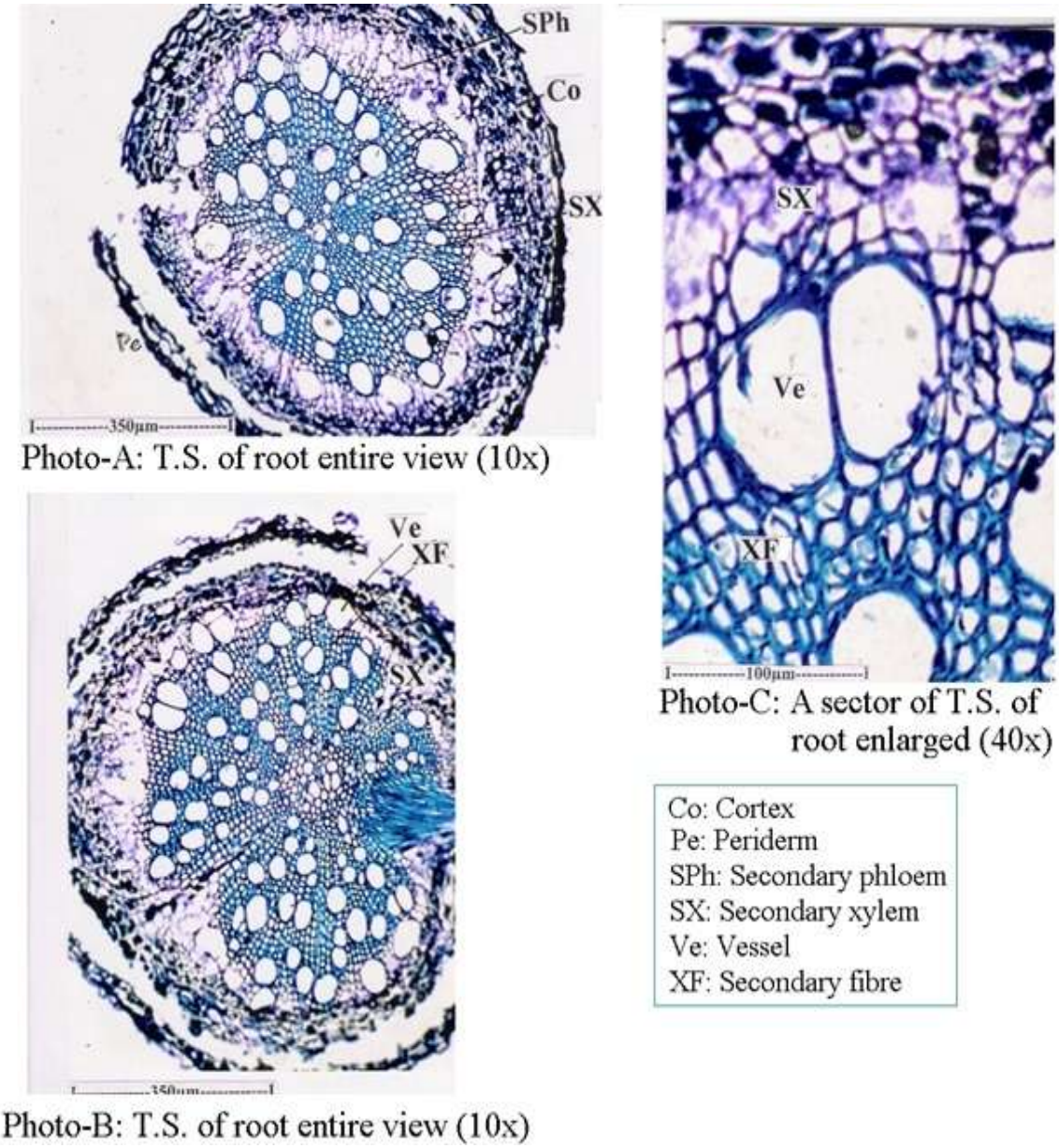

Figure 4: Photos showing anatomical features of the root (A, B & C) of *Polygonum chinense*.

Microscopic analysis of powder samples

Macerated preparations and powder of *P. chinense* shows various elements like parenchyma cells, vessels, fibres, etc. Rectangular thin/thick walled, wide/narrow parenchyma cells possess some amorphous inclusions are common in the powder samples (Figure 5; Photos-E & F).

Vessels (Figure 5; Photos-A & B) are narrow, cylindrical and are common in the powder samples of *P. chinense*. The lateral walls have multiseriate bordered pits (Figure 5; Photos-A to C). The primary xylem vessels elements have spiral lateral wall thickenings (Figure 5; Photo-D). The end wall perforation is wide, circular and horizontally oriented (Figure 5; Photo-C).

Fibres of different types such as thick walled narrow fibres, thin walled narrow fibres and wide fibres are common in the powder samples of *P. chinense* (Figure 5; Photo -E). Thick walled narrow fibres (Figure 5; Photo-F) are narrow, thick walled, long (1.3mm) and have narrow (10µm) lumen and have no pits on the lateral walls. Thin walled narrow fibres (Figure 5; Photos-E & F) are as long as the thick walled fibres and are also narrow with reduced lumen. Their walls are thin and poorly lignified. Wide fibres (Figure 5; Photos-E & F) are shorter than the narrow fibres and have comparatively thin walls and wide lumen. Further the wide fibres have distinct slit-like simple multiseriate pits (Figure 5; Photos-G) with less than 1mm long and 20µm wide.

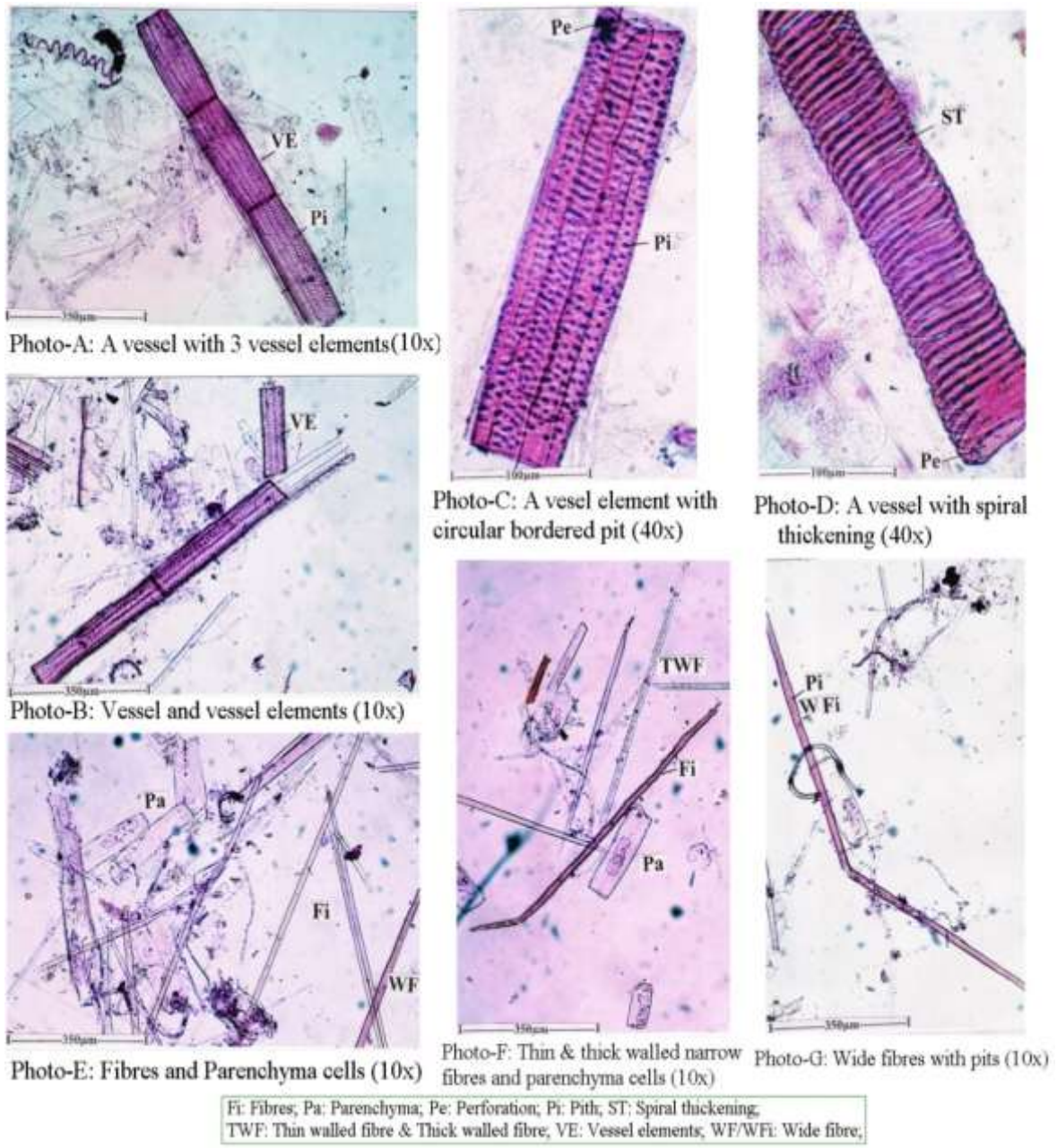

Figure 5: Photos –A to G showing powder microscopic features of *Polygonum chinense*.

Physico-Chemical Properties

Various pharmacognostic physical properties of *P. chinense* were determined and the data are presented in Table 1. Ash properties of the whole plant dry powder of *P. chinense* showed 19.13% total ash 15% water soluble ash, 85% water insoluble ash, 70% acid soluble ash, 30% acid insoluble ash and sulphated ash (96%) was noted in the *P. chinense*. The acid insoluble content was 30% in *P. chinense*.

Extractive values and Successive extractive yield

Extractive values of *P. chinense* whole plant extracts are presented in Table 2. The extractive values of different solvent extracts varied from 1% (methanol extract) to 8% (water extract) in *P. chinense* and are shown in the following order: water > chloroform = ethyl acetate = ethanol > hexane > methanol.

Successive extractive yield of *P. chinense* whole plant sample successive extracts are presented in Table 2. It was ranged from 0.5% to 2.5%. Among the extracts tested, the maximum extract yield was noted in the ethanol extract (2.5%) of *P. chinense*. The yield of different successive whole plant extracts *P. chinense* is recorded in the following order: ethanol > water > ethyl acetate > hexane > chloroform = methanol.

Fluorescence analysis

The behaviour of the whole plant dry powder of *P. chinense* in different solvents and their extracts towards ordinary day light, fluorescence tube light and UV light (at 254nm and 365nm) was observed and the results presented in table 3.

Table 1: Pharmacognostic physical properties of whole plant dry samples of three *Polygonum chinense*.

Sl. No.	Parameters Tested		Sl. No.	Parameters Tested	
1.	Dry weight (gm)	15.40	6.	Acid soluble ash (%)	70.00
2.	Moisture content (%) (or) Weight loss (%)	84.60	7.	Acid insoluble ash (%)	30.00
3.	Total ash (%)	19.13	8.	Sulphated ash (%)	96.00
4.	Water soluble ash (%)	15.00	9.	Residue on ignition (%)	93.67
5.	Water insoluble ash (%)	85.00			

Table 2: Determination of extractive values and successive extractive yield of *Polygonum chinense*.

Solvent extracts analyzed	Extractive values (%)	Successive extractive yield (%)
1. Chloroform	2.0	0.5
2. Ethanol	2.0	2.5
3. Ethyl acetate	2.0	1.5
4. Hexane	1.5	1.0
5. Methanol	1.0	0.5
6. Water	8.0	2.0

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

Whole plant dry powder (WDP) + Solvents used	Fluorescence characters of *Polygonum chinense*			
	Day light	Fluorescent light	UV-254	UV -365
1. Whole plant Dry Powder (WDP)	Green	Green	Green	Black
2. WDP + H_2SO_4 (1N)	Green-brown	Pale green	Green	Black
3. WDP + CH_3COOH (1N)	Brown	Green	Green	Black
4. WDP + HNO_3 (1N)	Brown	Brown	Green	Brown black
5. WDP + KOH (1N)	Brown	Brown	Green	Black
6. WDP + NaOH (1N)	Brown	Brown	Dark green	Black
7. WDP +Petroleum ether	Green	Green	Green	Black
8. WDP +Acetone	Green	Green	Green	Black
9. WDP +Chloroform	Dark green	Green	Black	Black
10. WDP +Ethyl alcohol	Brown	Brown	Black	Black
11. WDP +Ethyl acetate	Green	Green	Green	Black
12. WDP +Water	Green	Green	Green	Black
Solvent extracts				
1. H_2SO_4 (1N)	Colourless	Colourless	Pale green	Black
2. CH_3COOH (1N)	Colourless	Colourless	Green	Black
3. HNO_3 (1N)	Pale yellow	Pale yellow	Pale green	Black
4. KOH (1N)	Brown	Brown	Black	Black
5. Na OH (1N)	Dark brown	Dark brown	Dark green	Black
6. Petroleum ether	Colourless	Colourless	Pale green	Brown
7. Acetone	Yellow green	Yellow green	Green	Dark brown
8. Chloroform	Green	Green	Green	Brown
9. Ethyl alcohol	Yellow green	Yellow green	Yellow green	Brown
10. Ethyl acetate	Yellow green	Yellow green	Green	Brown
11. Water	Pale brown	Pale 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.

Preliminary phytochemicals

Chemical compounds such as carbohydrates, reducing sugar, starch, protein and lipid were estimated qualitatively in the whole plant aqueous extracts of *P. chinense* and the data are presented in Table 4. The results indicate the presence of alkaloids, flavonoids, phenols, proteins, steroids, tannins and the absence of saponins. Earlier studies on *P. chinense* shows.[11] reported the presence of glycosides, tannins, flavonoids, terpenoids, proteins and absence of saponins, steroids and carbohydrates in the whole plant samples of methanol extract, whereas the aqueous leaf extract *P. chinense* contains tannins, steroids, cardiac glycosides and the absence of quinons and the methanolic leaf extract shows the presence of tannins, saponins, cardiac glycosides and the absence of quinons and steroids.[23]

The structural, physical, chemical and sensory characters of crude drugs of plants origin are analytical in values. 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. Higher plants are the source of important therapeutic agents.[24] Standardization of pharmacognostic methods are needed to confirm the authenticity of medicinal plants used in the preparation of a drug.[25]

Table 4: Preliminary phytochemical screening in the whole plant extracts of *Polygonum chinense*.

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

V-CONCLUSION

The results of this study reveal the importance of *P. chinense* in various aspects. A combination of anatomical characters such as vessels, fibres and parenchyma cells of leaf, stem and root of *P. chinense* are very significant macro/microscopic characters, observed in this study, may be used in the identification of crude drugs prepared from the *P. chinense*. The ash value gives a basis for judging the identity and cleanliness of a drug in powder form. Ash values were determined with a purpose to find out the total amount of inorganic solutes present in the medicinal plant materials. It is known that ash of any plant does not contain any organic material and therefore inorganic salts are used medicinally. Only very few herbal therapies make use of ash. Extractive values and successive extractive yield of different solvent extracts helps to determine the amount of active constituents in a given amount of medicinal plant material when extracted with solvents. These values provide an indication of the extent of polar, medium polar and non-polar components present in the plant material. It is employed for those plant materials for which no suitable or biological assay method exists. 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*.[26] 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. chinense* observed in this study in different solution and their extracts towards ordinary light, fluorescent light and UV light can be used as diagnostic tool for testing adulteration if any. Hence, the macroscopic (morphological), and microscopic (anatomical) studies, physic-chemical parameters and fluorescence behaviour are tools for the standardization of medicinal plant materials and their crude form drugs.

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