

**EVALUATION OF PHARMACOGNOSTIC AND PRELIMINARY PHYTOCHEMICAL PROPERTIES IN THE WHOLE PLANT SAMPLES OF *POLYGONUM GLABRUM* (WILLD)****B. Ezhilan and R. Neelamegam***

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ABSTRACT

Evaluation of pharmacognostic properties and preliminary phytochemical constituents in the whole plant sample of *Polygonum glabrum* (Willd) was carried out to record the macro- and micro -scopic characters, physic-chemical parameters, extractive and successive extractive values, fluorescence behaviour and preliminary phytochemical constituents in several solvent extracts. The studies of morphological and anatomical characteristics in *Polygonum glabrum* mainly used to identify the plant. Maximum extractive values (4% in aqueous and ethanol extracts) and the successive extractive values (3.5% in the ethanol and chloroform extracts) of *P. glabrum* whole plant samples were noted. The phytochemical screening of aqueous extract shows the presence of all compounds (alkaloids, flavonoids, phenol, proteins, steroids and tannins) as compared to other solvents tested. In the ethanol extract except flavonoids all other compounds are present. The physic-chemical, fluorescence and preliminary phytochemical compounds along with macro- and micro- scopic characters recorded in this study would helpful for botanical identification of their crud drug forms.

KEYWORDS: *Polygonum glabrum*, Polygonaceae, Morphology, Anatomy, Physico-chemical properties, extractive values, Fluorescence.

1. INTRODUCTION

Polygonaceae family has several important medicinal plants with wide range of biological activities and phytochemical constituents. *Polygonum glabrum* Willd belongs to Polygonaceae, mostly found in riverbanks, stream sides and marshy places and growing up to 2.5 meters. The root stocks of *P. glabrum* are reported to be used in piles, jaundice, debility and consumption. The herb possesses antibacterial activity (against *Micrococcus pyrogens* and *Diplococcus pyrogens*), antifungal activity and antihelmenthic activity. It is also claimed to have medicinal uses such as astringent, diuretic, rubifacient, vermifuge, rheumatism, relieves pain and in some areas used as remedy for fever.^[1] The epidermal structure and ontogeny of stomata in two of Polygonales are described by Inamdar.^[2] Prakash *et al.*^[3] studied the macroscopic, microscopic and fluorescence characters of cell contents and ash values of *Polygonum glabrum*. Sinha *et al.*^[4] carried out pharmacognostic studies on leaf of *P. glabrum* along with fluorescence characteristics, ash and extractive values. Since, the medicinal potential of plants depends on biotic and abiotic elements of the plant growing area, the present study carried out to evaluate the macroscopic and

microscopic characteristics, physico-chemical characters and fluorescence analysis of the *P. glabrum* plants.

2. MATERIALS AND METHODS**2.1. Collection and Preparation of Plant Samples**

Polygonum glabrum was collected from *Srivikundam*, Thoothukudi District, Tamil Nadu, India (Figure 1a-1c). Mature and healthy plants of *P. glabrum* were collected and recorded the morphological characters of the plant by examined using hand lens in the field and by dissection microscope in the laboratory. *P. glabrum* plant parts (Figure 1c) were cut, removed from the plant and fixed in FAEA (5ml of Formalin + 5ml of Acetic acid + 90ml of 70% Ethyl Alcohol) mixture. After 24h of fixing, the specimens were dehydrated with graded series of tertiary-butyl alcohol.^[5] Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60°C) until TBA solution attained super saturation. The specimens were cast into paraffin blocks.

2.2. Sectioning

The paraffin embedded specimens were sectioned with 10-12µm thickness using *Rotary Microtomes*. Dewaxing of the sections was done by customary procedure (Johansen, 1940). The sections were stained with

Toluidine Blue.^[6] The dye rendered 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 and Fast-green and IKI (Iodine Potassium Iodide) for starch.



Figure 1: Maps showing the location of *Polygonum glabrum* Willd (Polygonaceae) collected from Srivaikundam, Thoothukudi District.



Plate I: Diagrammatic illustrations of *Polygonum glabrum* Willd.



Plate II: *Polygonum glabrum* -Habit (A, B, C & D); Plant parts used for whole plant extract (E).

The stomatal morphology, venation pattern and trichome distribution, paradermal sections (sections taken parallel to the surface of leaf) as well as clearing of leaf with 5% sodium hydroxide or epidermal peeling were analysed by partial maceration employing Jeffrey's Maceration Fluid.^[5] Glycerine mounted temporary preparations were made for macerated/ cleared materials. Powdered materials of different parts of the plants were cleared with NaOH and mounted in glycerine medium after staining. Different cell component were observed and measured under microscope.

2.3. Photomicrographs

Wherever necessary, microscopic descriptions of tissues are supplemented with photo micrographs with different magnifications taken by Nikon Labphoto-2 microscopic unit. Normal observations were made with bright field microscope whereas the study of crystals, starch grains and lignified cells were carried out with polarized light microscope. Magnifications of the figures are indicated by the scale-bars. Descriptive terms of the anatomical features are as given in the standard anatomy books.^[7]

2.4. Physico-chemical characters

Various physical and chemical properties such as loss of weight on drying, total ash, acid soluble/insoluble ash, water soluble/insoluble ash, sulphated ash, residue on ignition, extractive value and successive extractive yield were determined from *P. glabrum* whole plant samples by using standard methods.^[8-10]

2.5. Fluorescence Analysis

The powdered whole plant sample of *P. glabrum* and their extracts in various solvents and chemical reagents (Table 3) were examined under day light, fluorescent light and ultra violet light (365nm-254nm) and the change in colour was recorded.

2.6. Preliminary Phytochemical Analysis

The whole plant dry powder sample of *P. glabrum* was extracted separately with different solvents (water, ethanol, ethyl acetate, methanol, hexane, chloroform) at 20% level in a Soxhlet apparatus. These extracts were concentrated and used separately for qualitative preliminary phytochemical analysis following standard methods.^[11-15]

3. RESULTS AND DISCUSSION

3.1. Morphological properties

Polygonum glabrum (Figure 1b & 1c; Photos A to E) is an erect glabrous annual herb, reaching even 5.0m height and rooting from lower nodes. Stem is 0.6-1.5m tall, stout, simple or slightly branched, procumbent then erect and usually reddish below and the young stem usually green, polished with a dark-reddish ring at each node with tubular ocreae. Leaves are 7.5-23cm long and 1.6-3.0cm wide, lanceolate, finely acuminate, glabrous, closely gland-dotted, tapering at the base and lateral nerves numerous close slender. Petioles are 3.0-13cm long and the young leaves are usually red. Stipules are 2.2-3.2cm long and those on the old stems torn and ragged when young completely and closed sheathing the stem conspicuously veined, the mouth truncate not

ciliate. Flowers are pink in terminal long paniculate slender racemes, peduncles usually glabrous and pedicels short. Bracts are 4.0mm long, ovate obtuse, with membranous and non-ciliate margins. Perianth is 3.0-5.0mm long and the segments oblong obtuse. Stamens are 6-8 and 2 styles, connate at the base and the free portions are 1.5cm long filiform. Nutlets are 3.0mm in diameter, broadly ovoid or suborbicular, compressed, biconvex, black and shining.

3.2. Microscopic (Anatomical) Features

Leaf anatomy of *P. glabrum* shows broad thick (1.0mm) midrib (Figure 2; Photos A & B). The blunt adaxial part is 700 μ m wide and the broad semicircular abaxial part 1.0mm wide (Figure 2; Photo A). The midrib consists of narrow epidermal layer of thick walled rectangular cells followed by a narrow zone of small

thick walled collenchymatous cells. The palisade cells of the leaf extend up to the lateral shoulders of the adaxial hump. The central ground tissue is parenchymatous, thin walled and compact. Some of the cells of the ground tissues possess calcium oxalate druses (Figure 2; Photo B). Vascular system of *P. glabrum* leaf is multi-stranded with eight discrete vascular bundles arranged in a ring. Of the eight bundles, the adaxial median bundle is the larger than other bundles which are smaller and vary in size. The bundles are collateral with outer phloem and inner xylem. The xylem elements are circular, thick walled and diffuse in distribution. Phloem elements are in separate circular masses located on the outer part of the xylem elements. The vascular bundles have sclerenchymatous sheath which is thicker on the outer part than in the inner part (Figure 2; Photo B).

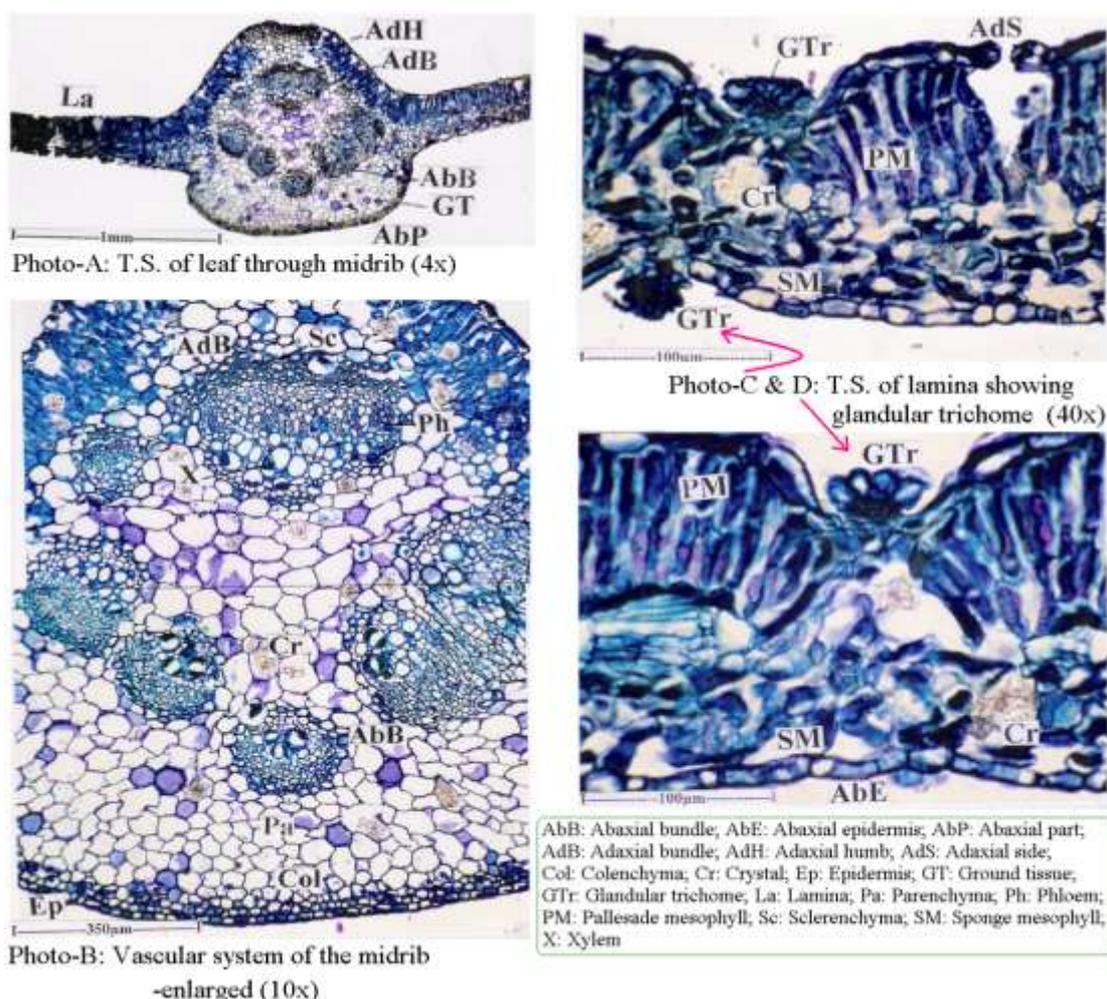


Figure 2: Photos showing anatomical features of *Polygonum glabrum* leaf (A & B) and lamina (C & D).

Lamina internal structure: (Figure 2; Photos C to D) of *P. glabrum* leaf is thin, dorsiventral, amphistomatic and gland bearing with 150 μ m thick. The adaxial stomata are developed with the guard cells situated at the level of the epidermis (Figure 2; Photo C). The epidermal layers possess rectangular thick (10 μ m) walled cells. There are shallow, wide cavities on the epidermal layer where prominent peltate type glandular trichomes are located.

The trichome is sessile and consists of a short one celled stalk. At the apex of the stalk occurs a circular disc of 4-8 radiating glandular cells which are 20 μ m in height and 40-50 μ m in diameter (Figure 2; Photos B to D). The mesophyll tissue includes thick palisade zone comprising two layers of wide cylindrical cells (Figure 2; Photos C & D). The spongy parenchyma cells are 4 or 5 layered and the cells are lobed and loosely arranged.

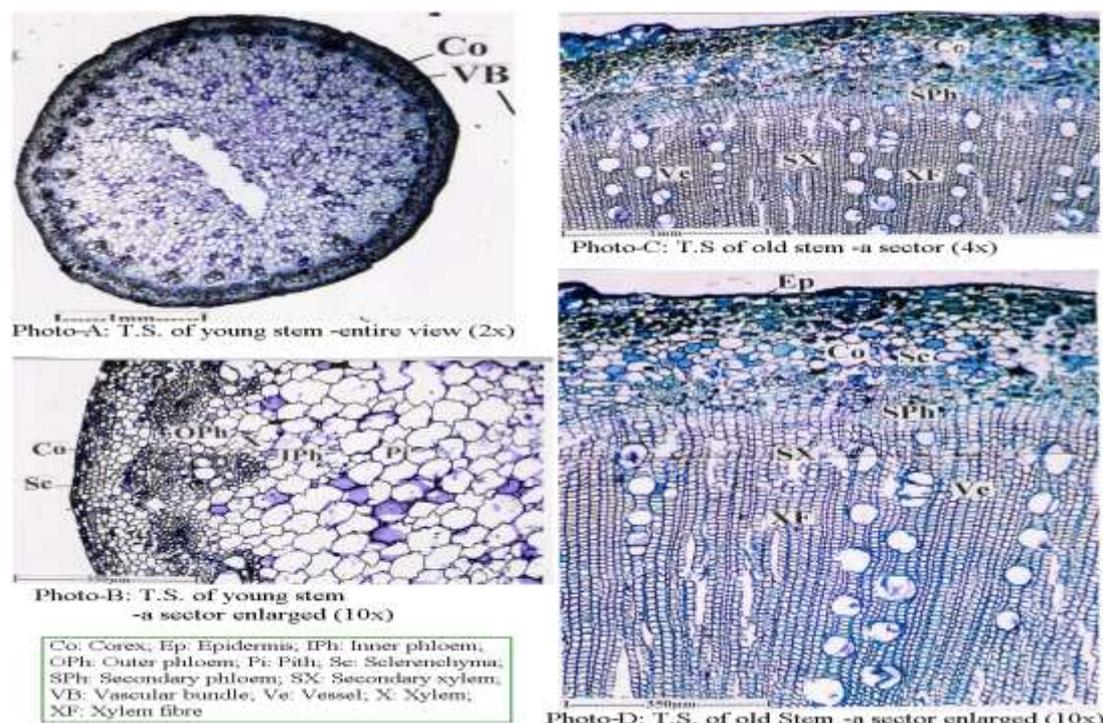


Figure 3: Photos showing anatomical features of *Polygonum glabrum* stem –young stem (A & B) and old stem (C & D).

Stem anatomy of both young and old stems of *P. glabrum* are observed (Figure 3; Photos A to D). The young stem is circular in sectional outline and the surface is smooth and even, nearly 2.5mm thick (Figure 3; Photo A). The young stem consists of a thin continuous epidermal layer of rectangular thick walled cells. The cortex is narrow comprising four outer layers of small rectangular cells and about three layers of larger polygonal compact cells. The pith is quite wide, homogenous and the parenchymatous cells are large, thin walled and wavy (Figure 3; Photo B). The vascular system is of eustele type, comprising numerous independent primary vascular bundles, arranged in a wide ring (Figure 3; Photo A). The vascular bundles are bicollateral with outer and inner phloem and xylem in between. The xylem elements are few in each bundle and

are wide, circular and thin walled. The medullary rays are narrow. A thin arc of fibres occurs on the outer part of each vascular bundle. The internal structure of *old stem* (Figure 3; Photos C & D) shows secondary growth and the epidermis is intact with broken fissures at certain places. The cortex is wide (300µm) and parenchymatous. There is thin layer of less prominent fibres along the inner boundary of the cortex. Secondary phloem is a wide zone of radial files of thin walled elements. Secondary xylem consists of thick solid and dense cylinder of fibres and vessels. The fibres are fairly thick walled, wide and lignified, and occur in strict parallel compact rows. The vessels are up to 70µm wide, diffuse in distribution, solitary, circular and thin walled (Figure 3; Photo D).

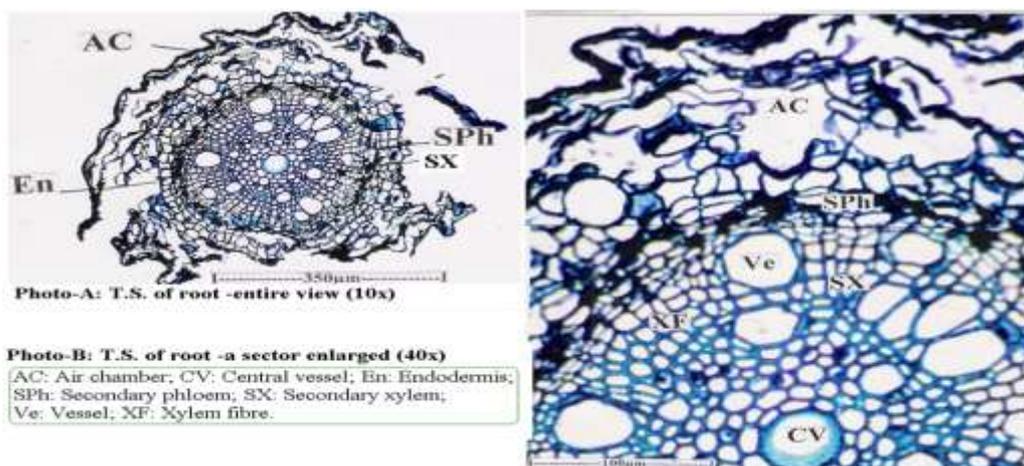


Figure 4: Photos showing anatomical features of the root (A & B) of *Polygonum glabrum*.

Root anatomy (Figure 4; Photos A & B) shows some hydromorphic features. The epidermis and outer cortex are thin and are broken in most of the roots. The cortex consists of wide air chambers separated laterally by thin partitions and is irregular in outline. The vascular cylinder is prominent solid cylinder with 550 μ m in diameter. Secondary phloem occurs around the xylem cylinder as thin layer. The outer phloem elements are

crushed into dark amorphous patches. The xylem cylinder consists of a wide circular central metaxylem element and outer sparse wide and angular vessels. The vessels are solitary or in small clusters with up to 40 μ m in diameter (Figure 4; Photo B). The ground tissue in the xylem cylinder includes libriform fibres which are thick walled and lignified.

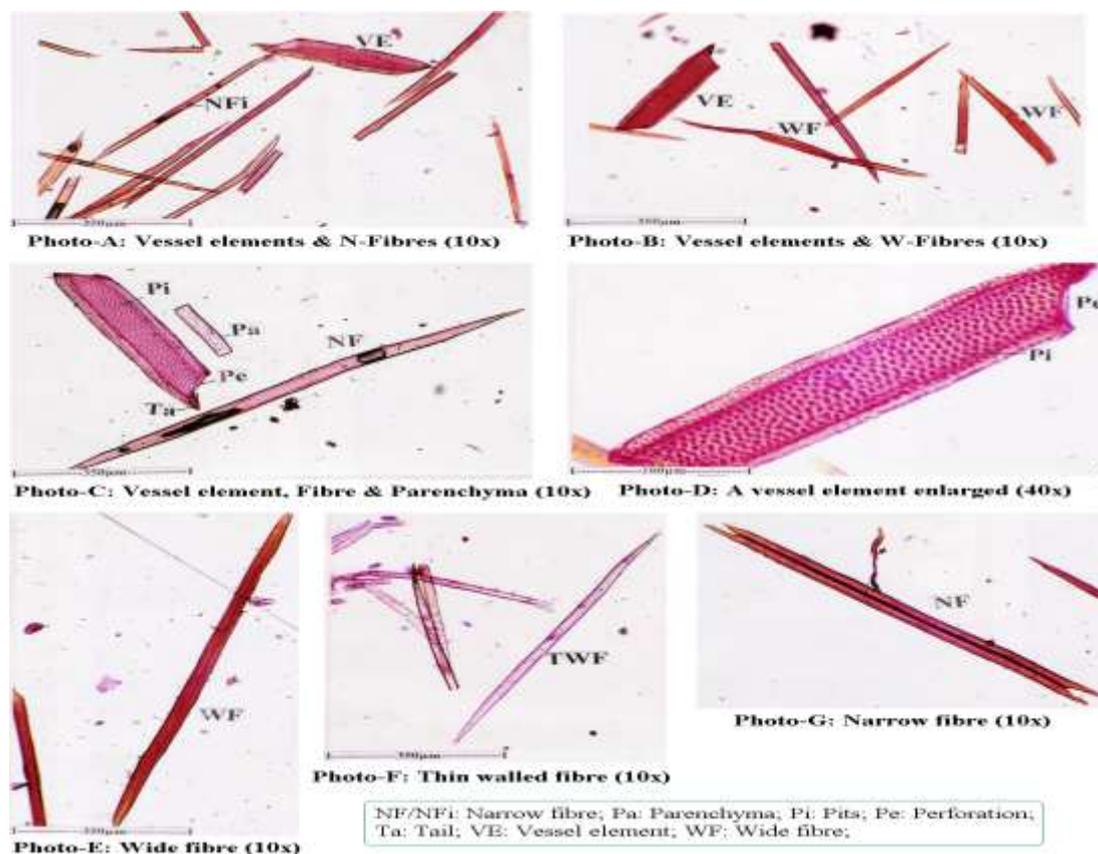


Figure 5: Photos –A to G showing powder microscopic features of *Polygonum glabrum*.

3.3. Powder microscopic observation

Macerated preparations and powder samples of *P. glabrum* show elements like parenchyma cells, vessel elements, and xylem fibres.

Parenchyma cells (Figure 5; Photo C) are long, rectangular, 40 x 70 μ m in size and thin walled with wide lumen parenchyma cells seen occasionally in the powder samples. Some of the parenchyma cells have simple pits and no specific inclusions are seen in the cells. The cells are.

Vessel elements (Figure 5; Photos A to D) are long (320-360 μ m) cylindrical cells with thick lignified walls and have short, thick conical tails. The lateral walls have dense, multiseriate, alternate, circular bordered pits (Figure 5; Photo D). The end walls have single, wide, oblique perforations.

Xylem fibres (Figure 5; Photos F & G) are varying in size and abundant in the powder samples. Thick walled wide fibres (Figure 5; Photos B & E) are 20 μ m thick and 350 μ m long. Some of fibres have thin walls and wide lumen in the middle part with thin tapering ends (Figure 5; Photo B). Thick walled narrow fibres (Figure 5; Photos A, C & G) are 500-600 μ m long and 10 μ m thick and have thick walls, narrow lumen with pointed ends. Thin walled fibres (Figure 5; Photo F) are thin walled, narrow long fibres of up to 700 μ m long and 20 μ m wide and are occasionally seen in the powder samples. The lumen is wide and the ends are tapering.

3.4. Physical properties: Various pharmacognostic physical properties of *P. glabrum* were recorded and the data are presented in Table 1. The ash value furnishes a basis for judging the identity and cleanliness of a drug in powder form. They were determined with a purpose to find out the total amount of inorganic solutes present in the plant samples.

Table 1: Pharmacognostic physical characters of whole plant dry samples of *Polygonum glabrum*.

Parameters Test in <i>Polygonum glabrum</i>	
1. Dry weight (gm)	55.20
2. Moisture content (%) (or) Weight loss (%)	44.80
3. Total ash (%)	8.33
4. Water soluble ash (%)	10.00
5. Water insoluble ash (%)	90.00
6. Acid soluble ash (%)	90.00
7. Acid insoluble ash (%)	10.00
8. Sulphated ash (%)	69.33
9. Residue on ignition (%)	93.00

3.5. Extractive values

Extractive values of crude drug are useful for their evaluation especially when the constituents of drug cannot be readily estimated by any other means.^[14] The extractive values of *P. glabrum* whole plant samples in different solvent extracts varied from 1.5 to 4% in *P. glabrum*. Among all the tested solvent extracts, the

highest extractive value of 4% was noted in water and ethanol extracts. The extractive value of different whole plant solvent extracts is arranged in the following order: water = ethanol > chloroform > ethyl acetate > hexane > methanol. The difference in extractive value depends on the solubility of major components of the plant parts in solvents.^[15]

Table 2: Determination of extractive values and successive extractive yield in whole plant solvent extracts of *Polygonum glabrum*.

Solvent extracts analyzed	Extractive values (%)	Successive extractive yield (%)
Chloroform	3.0	3.5
Ethanol	4.0	3.5
Ethyl acetate	2.5	1.0
Hexane	2.0	1.0
Methanol	1.5	0.5
Water	4.0	1.0

3.6. Successive extractive yield

Successive extractive values of *Polygonum glabrum* whole plant samples in different solvents were ranged from 0.5% to 3.5% (Table 2). Among the solvent extracts tested, the maximum yield (3.5%) was noted in the ethanol and chloroform extracts. The yield of different successive whole plant sample extracts of *P. glabrum* shows in the following order: chloroform = ethanol > hexane = water = ethyl acetate > methanol. The plant extracts were good sources of different classes of bioactive compounds. The solvent extracts will be effective in isolating active biological compounds due to their high non-polarity. That is the non-polar solvents are more effective than the polar solvents in relation to extractive values.^[16] Extractive values and successive extractive yield of different solvent extracts help 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.

3.7. Fluorescence analysis

The behaviour of the whole plant dry powder samples of *P. glabrum* in different solvents and their extracts towards ordinary day light, fluorescence tube light and UV light (at 254nm and 365nm) was observed and compared and the results presented in Tables 4. 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 *Polygonum* species 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.

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

Whole plant dry powder (WDP) + Solvents used	Fluorescence characters of <i>Polygonum glabrum</i>			
	Day light	Fluorescent light	UV-254	UV -365
1. Whole plant Dry Powder (WDP)	Brown	Brown	Black	Black
2. WDP + H ₂ SO ₄ (1N)	Pink	Brown	Green	Black
3. WDP + CH ₃ COOH (1N)	Pink	Brown	Green	Black
4. WDP + HNO ₃ (1N)	Brown	Brown	Green	Black brown
5. WDP + KOH (1N)	Brown	Black green	Dark green	Black

6. WDP + Na OH (1N)	Brown	Brown	Dark green	Black
7. WDP +Petroleum ether	Brown	Brown	Green	Black
8. WDP +Acetone	Brown	Brown	Green	Black brown
9. WDP +Chloroform	Brown	Brown	Green	Black brown
10. WDP +Ethyl alcohol	Red brown	Brown	Black	Black
11. WDP +Ethyl acetate	Brown	Brown	Black	Black
12. WDP +Water	Brown	Brown	Green	Black
Solvent extracts				
1. H ₂ SO ₄ (1N)	Colourless	Colourless	Pale green	Black
2. CH ₃ COOH (1N)	Pale brown	Pale brown	Green	Yellow
3. HNO ₃ (1N)	Pale yellow	Pale yellow	Pale green	Yellow brown
4. KOH (1N)	Brown	Brown	Green	Black
5. Na OH (1N)	Pale brown	Pale brown	Dark green	Black
6. Petroleum ether	Colourless	Colourless	Green	Brown
7. Acetone	Brown	Brown	Green	Dark brown
8. Chloroform	Green	Green	Green	Dark brown
9. Ethyl alcohol	Brown	Brown	Dark green	Brown
10. Ethyl acetate	Green	Green	Green	Brown
11. 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: Preliminary phytochemical screening in the whole plant extracts of *Polygonum species*.

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

3.8. Preliminary Phytochemical Screening

The phytochemical screening of different extracts of *P. glabrum* whole plant dry sample shows the presence of chemical compounds like alkaloids, flavonoids, phenols, proteins, steroids, and tannins. The variations noted in the presence or absence of chemical compounds is the solvent dependent on their solubility in the polar and non-polar solvents. In this study, the aqueous (water) extract of whole plant sample of *P. glabrum* shows all phytochemicals tested as compared to other solvents. Among the chemical compounds, phenols and tannins detected in all solvent extract (Table 3). The chloroform extract of *P. glabrum* shows the presence of flavonoids, phenols and tannins while Sivakumar *et al.*^[1] reported the presence of alkaloids, carbohydrates and flavonoids in the chloroform extracts of *P. glabrum*. This indicates the influence of environmental factors on phytochemicals of plants as reported by Xin *et al.*^[17] and these factors can be applied to improve and enhance the phytochemical content in medicinal plants.

4. CONCLUSION

Plants are known to contain numerous biologically active compounds which possess curative properties. Physico-chemical methods employed for determining the quality and purity of drugs. A combination of anatomical

characters such as vessels, fibres and parenchyma cells of leaf, stem and root of *P. glabrum* are very significant macro and microscopic characters, physico-chemical properties and fluorescence behaviour observed in this study may be used in the identification of crude drugs prepared from the *Polygonum glabrum* species.

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