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PHARMACOGNOSTICAL STUDIES ON LEAF OF POLYGONUM GLABRUM WILLD.

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ABSTRACT: *Pharmacognostic studies on leaf of Polygonum glabrum Willd. has been carried out along with its numerical values, fluorescence characteristics and ash and extractive values.*

INTRODUCTION

Rasna a herbal drug of indigenous system of medicine is well known for its medicinal uses (Anonymous, 1969; Chopra et al, 1956). The correct botanical source of the drug is highly controversial (Bapalal, 1927, 1968, 1970; Chopra et al. 1956, 1958; Dey, 1973; Dutt, 1922; Dymock, et al, 1891; Kirtikar and Basu, 1933; Nadkarni, 1976 and Sharma, 1979, 1981). In the recent study it was observed that the stem and leaves of quite a new plant, botanically identified as *Polygonum glabrum* willd. Belonging to family Polygonaceae (Mishra, 1982) are sold as Rasna.

The drug has been the subject of some chemical (Adinarayana, 1980; Tiwari et al, 1979) and pharmacological (Singh et al, 1985) investigations also. In the pharmacognostic field Prakash et al, (in Press) have worked on stem of *Polygonum glabrum* willd. The present investigation was therefore undertaken with a view to bring out all the diagnostic characters of leaf, along with its numerical values, fluorescence characteristics and ash and extractive values.

MATERIALS AND METHODS

Fresh leaves of botanically identified plants of *Polygonum glabrum* Willd. were collected from Varanasi District. Free hand sections were taken, stained and mounted in the usual way. Representative diagrams were sketched with the help of cameralucida. Micro – chemical tests for cell content and cell – wall structures were performed according to the methods described by Johansen (1940), Kay (1938) and Trease and Evans (1972). Quantitative values of leaf were determined according to Wallis (1946) while fluorescence analysis under ultra – violet light was performed according to Kokoski et al (1958). The extractive values were determined according to the procedure of Indian Pharmacopoeia (1966).

Macroscopical Characters (Plate I)

The leaves are simple, petiolate, stipulate, narrow lanceolate in shape, very variable in size, from 7.5 – 25 cm in length and 1.6 – 3.0 cm at the widest part. The petiole is short, 0.3 – 1.3 cm long and more or less

flattened on the ventral side; those on the young leaves are usually red. The stipule in the form of a very characteristic membranous sheath – ochrea is present at the base. On the old stems the stipules are torn and ragged but they make complete and close sheathing on younger ones. They are 2.2 – 3.2 cm long, conspicuously veined with truncate mouth and have very small and few cilia like projections originating from the veins. The lamina is thick with finely acuminate apex, tapering base and entire margin which bears cilia like projections. The surface is closely dotted and somewhat glabrous. The midrib is prominent on either side. Many slender, lateral veins leave the midrib at an angle of 45^{o}. Both odour and taste are indistinct.

Microscopical Characters (Plate II)

Petiole : The cross – section of petiole (Fig. 1) is plano – convex in outline throughout its length. It shows a single layered epidermis, a well developed collenchymatous tissue and a broad zone of parenchymatous ground tissue in which a number of vascular bundles associated with sclerenchymatous fibres are present. The surface is characterized by the present of a number of sclerenchymatous elements. In anatomical detail it is similar to that of midrib as described below.

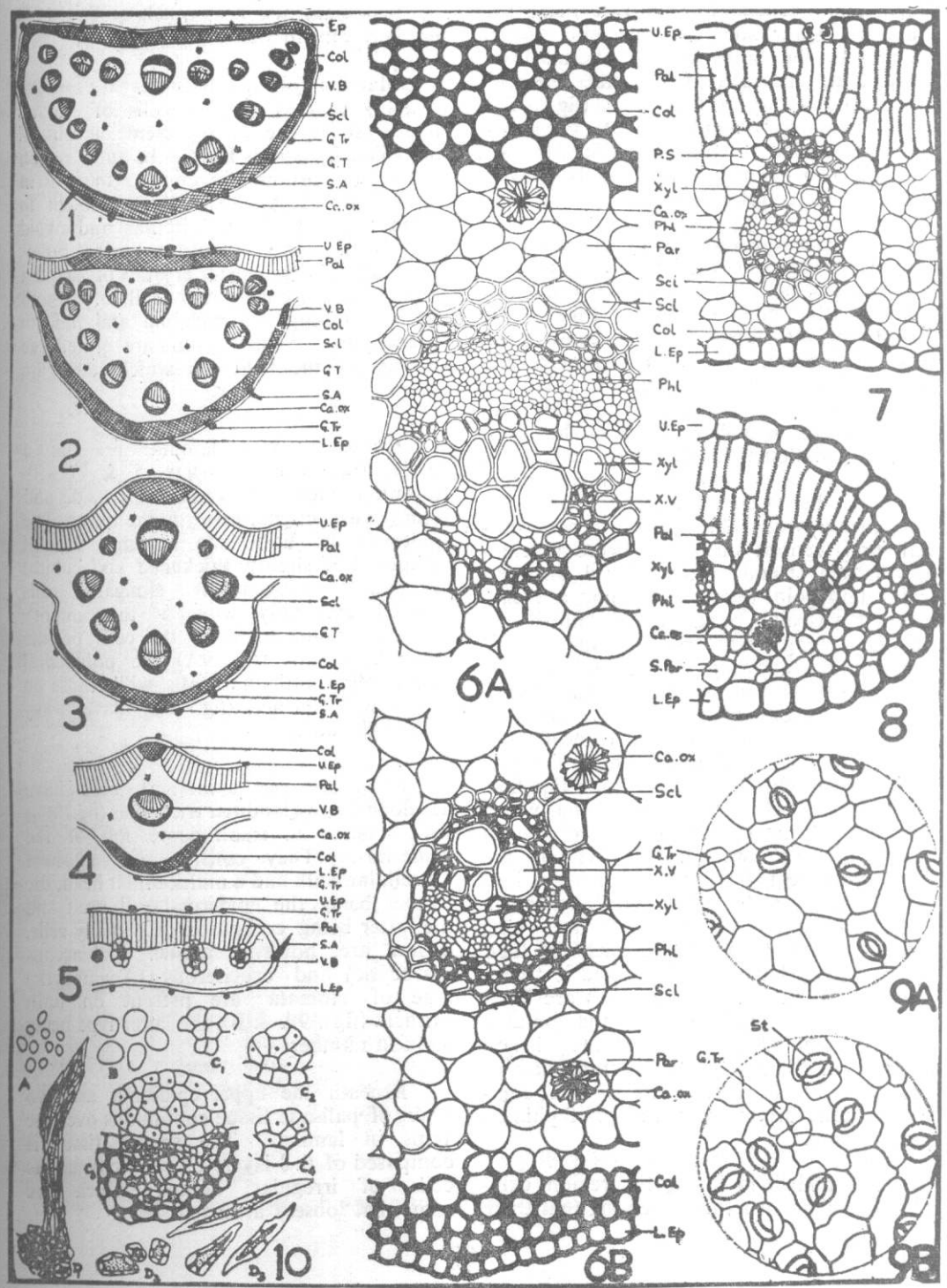
FIG No. 1.

Midrib: The midrib in its cross – section exhibits a plano – convex outline in basal region (fig. 2). In the middle and the apical region the outline becomes somewhat biconvex due to the bulging out of the ventral surface (Fig. 3 & 4). The epidermis of either side in single layered consisting of cubical to rectangular cells which are slightly arched on their outer side and are covered externally by a thick cuticle. The epidermal cells of the dorsal side are smaller in dimensions and more thickened as compared to those of the ventral side which are distinctly tangentially elongated (figs.

6A & B). A number of glands which are very variable in size and mostly sessile consisting of multicellular head and containing conspicuous circular bodies are present on the epidermis (figs. $10C_2$ & C_3). Immediately beneath some of these sessile glands 2 – 3 layers of sub – epidermal cells including the cells of the epidermis form a small sclerenchymatous strand which consists of strongly lignified cells having numerous slit like pits (fig. $10C_3$). A few small glands consisting of comparatively smaller number of cells, the smallest gland being quadricellular, however, show a short stalk of one cell only, the major portions of which remains embedded in the epidermis (Fig. $10C_1$). Near trichomes the epidermal cells are short and divided tangentially. Following the epidermis on either side is collenchyma which is 3 – 5 cells thick on the lower side and 4 – 6 cells thick on the upper and show characteristic angular thickenning on their walls. Opposite many glandular trichomes in the region of collenchyma, a few sclerotic cells are present. These gradually increase in number and form several small strands in the collenchymatous ring. Some of the collenchymatous cells immediately beneath the epidermis become sclerosed and show numerous pits on their walls. The epidermal cells present immediately above the sclerotic cells also become thick walled, pitted and strongly lignified. These cells gradually elongate to form small stiff conical bodies which on further elongation form a cluster of long teeth like structure. These sub – epidermal appendages finally form long peg like structures by the addition of more of sclerenchymatous elements (fig. $10D_1$). The foot of the appendage is composed of mostly small rectangular cubical to slightly conical cells (fig. $10D_2$) while the elements which constitute the body consist of small but elongated, fusiform, fibre like elements with tubular pits on their walls (fig.$10D_3$). The remaining ground tissue is composed of thin walled circular to more or less isodiametric parenchymatous cells showing distinct intercellular spaces.

The midrib receives 15 – 17 vascular bundles from the petiole. The number of vascular bundle becomes 6 – 8 proceeding towards the middle region. Finally the apical regions show only one conspicuous vascular strand. The widely spaced collateral vascular bundle some of which are still in the process of their development are arranged in a ring which is however, adaxially flattened in the basal region of the midrib. Each vascular bundle is accompanied by well developed strand of sclerenchymatous fibres towards both dorsal and ventral surfaces. These sclerenchymatous strands often extend on their sides and join together forming a sheath around vascular bundles. The fibres are lignified, long and tapering with pointed extremities, showing lumen of varying thickness. Numerous simple and oblique pits are present on their walls. Some of the fibres show chisel like, truncated or bifurcated ends. The elements of xylem are moderately thickened and lignified, however, a few xylem parenchyma which are present mostly on the inner side of xylem are thin walled. The vessels are long cylindrical, having simple perforations and show mostly spiral thickenning on their walls while tracheids are long and tapering with simple pits on their walls. They have short pointed extremities or blunt ends. Xylem fibres are long, narrow with tapering and the pointed ends showing simple and oblique pits on their wall. The phloem is composed of several strands of sieve tubes and companian cells dispersed in a mass of phloem parenchyma.

FIG No. 2


Ep
Col
V.B
Scl
G.Tr
G.T
S.A
Cc.ox
1
U.Ep
Pal
V.B
Col
Scl
G.T
S.A
Ca.ox
G.Tr
L.Ep
2
U.Ep
Pal
Ca.ox
Scl
G.T
Col
L.Ep
G.Tr
S.A
3
Col
U.Ep
Pal
V.B
Ca.ox
Col
L.Ep
G.Tr
4
U.Ep
G.Tr
Pal
S.A
V.B
L.Ep
5
A
B
C1
C2
C3
D1
D2
D3
10
U.Ep
Pal
Col
P.S
Xyl
Ca.ox
Phl
Par
Sci
Scl
Col
L.Ep
7
U.Ep
Xyl
X.V
Pal
Xyl
Phl
Ca.ox
S.Par
L.Ep
8
6A
Ca.ox
Scl
G.Tr
X.V
Xyl
Phl
Scl
Par
Ca.ox
Col
L.Ep
6B
9A
St
G.Tr
9B

The midrib in its apical region exhibit a similar structure excepting that there is only one small vascular strand enclosed in a sclerenchymatous sheath. However, all the elements are much reduced in number, midrib being small (Fig. 4).

Many simple starch grains are present in many parenchymatous cells of ground tissue. A few are also present in cells of the collenchymatous layer. In outer region these grains are mostly circular to oval in outline (fig. 10A) while those present in ground tissue are large, circular and ovoid (fig. 10B). The striations are rather indistinct. Large cluster crystals of calcium oxalate are present in many cells of ground tissue including collenchyma and phloem parenchyma. Some crystals are quite large and are of the cells in which they are present.

Lamina : The lamina presents a dorsiventral structure (Fig. 5 & 7) with palisade tissue towards the upper side and spongy parenchyma towards the lower. The epidermis of both side is single layered composed of slightly thickened and cubical to greatly tangentically elongated cells covered externally with a thin smooth cuticle. In surface view, the cells of the upper epidermis (fig. 9A) are polyhedral with straight anticlincal walls while those on the lower surface (fig. 9B) have wavy anticlinical walls.

Some of the epidermal cells have developed into glandular trichomes (Fig. $10C_4$) which occur in small depression of the epidermis. They consist of a short unicellular stalk and a multicellular head, the former being composed of 4 – many cells; a few are, however, sessile. Rubiaceous (paracytic) and cruciferous (Anamocytic) type of stomata are present on both surfaces (figs. 9A & B), the latter type being more in number.

Beneath the upper epidermis are two layers of palisade tissue continuous over the veins in lamina. The spongy tissue is composed of 6 – 8 layers of parenchymatous cells of irregular outline which are somewhat loosely arranged.

The mesophyll is traversed by numerous veins. In the bigger veins the palisade occurs beneath the upper epidermis while immediately above the lower a few collenchymatous cells are present. The vascular bundle present in between the two tissues is sheathed all round by a parenchymatous sheath. In small veins, both palisade and spongy parenchyma are present beneath upper and lower epidermis respectively, the vascular strand remains enclosed by a parenchymatous sheath. The sclerenchymatous fibres do not form a covering round the vascular bundles but are present on either side, the number of fibres being small.

At the extreme margin (Fig. 5 & 8) the palisade cells are absent and the spongy parenchyma which are circular to oval are arranged in a compact manner. The vascular strands present near the margin are much reduced in size and composed of only a few phloem and xylem elements, the sclerenchymatous fibres being absent on either side.

Stiff peg like projections originating from the sub epidermal layer similar to those as described above are present on the margin at regular intervals (Fig. 5).

Large crystals of calcium oxalate and simple starch grains are also present in many parenchymatous cells of lamina.

Measurements of microns of different elements in t.s. of leaf are given below

	Petiole	*Midrib*	*Lamina*
Upper epidermal cells	12 – 15 – 21 – 30 x 9 – 12 – 15 – 18	9 - 15 – 18 -30 x 9 – 12 – 15 – 18	9 - 15 -25 -40 x 10 – 14 - 17 – 20
Lower epidermal cells (Petiole)	10 – 15 – 20 – 30 x 8 – 10 – 12 – 15	12 – 21 – 27 – 33 x 6 – 15 – 18 – 24	10 – 15 – 20 – 40 x 10 – 12 – 15 – 20
Collenchymatous cells	9 – 24 – 45 – 96 in diam	6 – 9 – 15 – 21 in diam	-
Spongy parenchyma	27 – 45 – 75 – 96 in diam	21 – 36 – 45 – 70 in diam	18 – 33 – 43 – 60 in diam
Palisade cells	-	-	24 – 45 – 57 – 72 x 9 - 12 – 15 – 18
Glandular trichomes (head)	50 – 60 – 75 – 90 in diam	45 – 55 – 60 – 75 in diam	30 – 40 – 50 – 55 in diam
Sub-epidermal appendages	150 – 240 – 300 – 690	150 – 240 – 300 – 700	-
Cluster crystals of calcium oxalate	18 – 25 – 40 – 60 in diam	27 – 45 – 50 – 55 in diam	15 – 30 – 40 – 55 in diam
Starch grains (big)	10 – 15 – 25 – 30 in diam	12 – 18 – 27 – 33 in diam	10 – 15 – 25 – 30 in diam
(small)	5 – 10 – 12 – 15 in diam	6 – 8 – 10 – 12 in diam	5 – 8 – 9 – 11 in diam

Quantitative values

Stomatal number of upper surface; lower surface; stomatal index, upper surface; lower surface; palisade ration, vein islet and vein termination number of leaf are 113. 3, 175. 7, 12.1, 19.1, 5.41, 9.72 and 8.9 respectively.

Ash and extractive values

Total ash, acid insoluble ash and water soluble ash are 12. 52, 3.65 and 1.09% (w/w) respectively. Water and alcohol soluble extractive values are 13.05 and 12.66 % (w/w) respectively

Fluorescence analysis

Fluorescence characteristics of leaf under ultra – violet light are recorded in Table I.

TABLE – I

Sl .No	Treatment	Fluorescence
1	Powder as such	Dark green
2	Powder mounted in nitrocellulose	Black
3	Powder treated in IN – NaOH in methanol	Purple
4	Powder treated with IN – NaOH in methanol, dried and mounted in nitrocellulose	Greenish – black
5	Powder treated in 1N – HCl	Yellowish – green
6	Powder treated with 1N – HCl dried and mounted in nitrocellulose	Black
7	Powder treated with HNO_3 (1:1)	Dull green
8	Powder treated with H_2SO_4 (1: 1)	Blackish - green

LEGEND TO FIGURES

PLATE II

Microscopical characters of the leaf of *Polygonum glabrum* Willd.

Fig. 1 : A. T. S. of petiole from its middle region (diagrammatic) x 30

Fig. 2 : A. T. .S. of midrib from its basal region (diagrammatic) x 30

Fig. 3 : A. T. S. of midrib from its middle region (diagrammatic) x 40

Fig. 4 : A. T. S. of midrib from its apical region (diagrammatic) x 60

Fig. 5 : T. S. of a portion of lamina showing margin (diagrammatic) x 90

Fig. 6 : Cellular details in t.s. of a portion (A – B) of midrib all x 300

A: Ventral side

B: Dorsal side

Fig. 7 : Cellular details in t.s. of a portion of lamina		x	300
Fig. 8 : Cellular details in t.s. margin		x	300
Fig. 9 : Surface view of epidermis (A – B)	all	x	300
A: Ventral surface			
B: Dorsal surface			
Fig. 10. (A – B) : Starch grains	all	x	300
$C_1 – C_3$: Glandular trichomes of petiole and midrib	all	x	300
C_4: Glandular trichome of lamina		x	300
D_1: Sub – epidermal appendage		x	140
$D_1 – D_3$: Elements of sub – epidermal appendage		x	260

Ca. ox, Cluster crystals of calcium oxalate; Col. Collenchyma; Ep. Epidermis; G. T., ground tissue; G. Tr, glandular trichome; L. Ep., lower epidermis; Pal, palisade; Phl. Phloem; P. S., parenchymatous sheath; S. A., sub-epidermal appendage; Scl. Sclerenchyma; S. Par, spongy parenchyma; St., Stomata; U. Ep., Upper epidermis; V. B., Vascular bundle; X. v., xylem vessel; Xyl, xylem

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