C H A P T E R - VII

Chemistry of the roots of GLINUS LOTOIDES Linn.

A - General :

Saponins are often classified as acidic saponins and neutral saponins. Triterpenoid saponins, acidic in reaction, contain free carboxylic groups. They are insoluble in water but form salts with alkali which are soluble in water. Saponins on hydrolysis yield sugars and sapogenins. The sugars are generally hexoses like glucose or galactose, but sometimes a pentose is obtained. In a few cases, uronic acid is also found (Trese, 1961). The sapogenins of acid saponins on degradation, especially with zinc dust, always yield the same substance, sapotalene or 1:2:7-trimethylnaphthalene (McIlroy, 1951). By selenium dehydrogenation 1 : 8-dimethylpicene, belonging to polyterpenoid series was obtained. Many of the saponins which are used in medicine as expectorant or diuretic, usually as local irritant, like quillaic acid, glycyrrhizinic acid, senegin, primulic acid, etc. belong to the above group.

The aglycone of neutral saponins, on selenium dehydrogenation, yield Diel's hydrocarbon, methyl

cyclopentanophenanthrene, and are called steroidal sapogenins (McIlroy, 1951). The steroidal sapogenins thus belong to the important group of natural substances like sterols, bile acids, vitamin D, sex hormones, adrenal cortical hormones, steroid alkaloids, etc. Sarsasapogenin from sarsaparilla, digitonin from Digitalis species, and sapogenins of Dioscorea and Agave species belong to this group. The steroidal sapogenins, especially those from Dioscorea and Agave species, on oxidation yield products which are used in the preparation of synthetic hormones like cortisone. Systematic search for plants with steroidal sapogenins is carried out; the steroidal sapogenins being identified by characteristic infra-red spectra. There is no specific colour reaction for identifying saponins. With concentrated sulphuric acid or better with concentrated sulphuric acid and alcohol in equal parts, a yellow colour changing to red or red-violet is obtained.

The property of frothing is due to change in surface tension and viscosity. Saponins are amorphous, sometimes hygroscopic, soluble in water forming viscous solutions, sparingly soluble in alcohol and insoluble in many organic solvents like ether, light petroleum and acetone. Saponin kill mercury and their aqueous solutions form emulsion of fixed oils. Their odour is usually sternutatory. Most of them possess a pungent, metallic taste except Glycerrhiza which is 150 times sweeter than

sucrose and has an agreeable taste (Ramsted, 1959).

The positive froth number and haemolytic index in almost all the plant parts of Glinus lotoides Linn. and G.oppositifolius Linn. suggest that saponin is present in entire plant, but the nature of saponin is not the same in different parts, as from root to stem to leaf froth number increases and haemolytic index decreases. Thus, the different parts of both the plants may contain different saponins or mixture of saponins. It is also likely that the presence of other constituents in the drug might have affected haemolytic index and froth number (Wasicky, 1932).

Thus, it was found necessary to carry out phytochemical investigations of roots of Glinus lotoides Linn. The roots show haemolytic value that is higher than that shown by other parts of the same plant as well as different parts of G.oppositifolius Linn.

T A B L E - 23

Glinus lotoides Linn.			Glinus oppositifolius Linn.		
Root*	500	(unco-rrected)	Root	Method 1	145 (Ridgway & Rowson 1956)
				Method 2	
				Method 3	
				Method 4	
Stem*	200				
Leaf*	-		Stem and Leaf*		143
Fruit with Seeds*	-				
Entire plant*	285				

B - Phytochemistry of the roots :-

E X P E R I M E N T A L

Petroleum Ether Extract :- Isolation of Substance A.

The powdered drug (750g.) was extracted with petroleum ether (b.p. 60-80°) to exhaustion. Most

* See page No. 108

of the solvent from the yellow coloured extract was removed by distillation and the remaining by spontaneous evaporation, when a light brownish semisolid mass was obtained.

The brownish mass was found to be completely soluble in ether, benzene, toluene, etc. and partly soluble in alcohol (90%), acetone and ethyl acetate. As nothing was obtained from the semisolid mass by solvent treatment, it was saponified with N/2 alcoholic potassium hydroxide for two hours. Most of the alcohol was then removed by distillation under reduced pressure, the aqueous solution remaining diluted with water, and unsaponifiable matter extracted repeatedly with ether. The other solutions were washed with water. The combined aqueous washings were shaken with ether; the ether solution washed with water and combined with the main ether solution. The aqueous washings were discarded.

On removing ether by distillation, an orange-coloured residue was obtained. This unsaponifiable matter was treated with boiling absolute alcohol when alcohol-soluble and alcohol-insoluble portions were obtained. The alcohol insoluble portion was negligible and, therefore, not further worked up.

The alcohol-soluble portion was concentrated under reduced pressure and then kept in a refrigerator

for 24 hours, when a pale yellow flocculent precipitate was obtained. This was separated by filtration. On repeating the above treatment, the filtrate yielded further smaller quantities of the pale yellow precipitate. All these fractions were found to melt in the range 127-33°. They were, therefore, combined. After several crystallisations from alcohol (95%), white plates, m.p. 139-40°, were obtained (Substance A). The melting point of substance A did not rise on further crystallisation.

Substance A was found to be soluble in ether, benzene, alcohol (90%), methanol, acetone, chloroform, etc. It dissolved in cold, concentrated sulphuric acid giving an orange-red colour with a green fluorescence. A solution of the substance in alcohol (90%) formed a white precipitate with a saturated solution of digitonin in alcohol (90%). The substance was found to answer the following tests characteristic of sterols.

Liebermann (1885) reaction :

A small crystal of the substance was dissolved in acetic anhydride and a few drops of concentrated sulphuric acid added cautiously. A greenish blue colour developed in the layer of acetic anhydride.

Libermann-Burchard (1890) reaction :

Chemistry

A small crystal of the substance was dissolved in chloroform and treated with a few drops of acetic anhydride and concentrated sulphuric acid. The pink colour which developed, slowly changed to green and then to brown after standing for a long time.

Hesse's test (Rosenthaler, 1930):

A solution of a small amount of the substance in chloroform was layered on concentrated sulphuric acid in a test-tube. A violet ring developed at the junction.

Moleschott's test (Rosenthaler, 1930) :

A drop of dilute sulphuric acid was added on a small crystal of the substance on a slide, when a violet tinge was observed.

Hirschsohn's test (Rosenthaler, 1930):

A small crystal of the substance was mixed with two fragments of trichlor-acetic acid and heated to boiling. A red colour developed which changed to violet.

	Found	C	83.7%	:	H	12.4%
$C_{29}H_{50}O$	Requires	C	84.1%	:	H	12.1%

Preparation of the Acetyl Derivative of the Substance A :

The substance A, (0.25 g.), was refluxed on

a sand-bath with acetic anhydride, (7 ml.) and fused sodium acetate (2.5 g.). The resulting mass was treated with cold water and the aqueous solution neutralised with sodium bicarbonate. The precipitate obtained was washed thoroughly with water and crystallised twice from methanol, when it melted at 126-7°. The melting point of the substance did not rise further on recrystallisation.

	Found	C	81.2 %	:	H	11.6 %
$C_{31}H_{52}O_2$	Required	C	81.5 %	:	H	11.4 %

The properties of the Substance A and of its acetate derivative agree closely with those described for β-sitosterol and its acetate in the literature. The identity of the Substance A was finally established by the mixed melting point determination with an authentic specimen of β-sitosterol.

Alcohol Extract : Isolation of Substances B and C :

The petroleum ether exhausted drug was dried in air and extracted with 70% alcohol to exhaustion. The combined extracts were distilled under low pressure to reduce the percentage of alcohol to about 50%. On allowing to stand, a considerable amount of colloidal matter precipitated. The extract was heated and filtered through Buchner funnel with kieselguhr as a filter aid. The

resultant filter cake was stirred with a sufficient quantity of 50% alcohol and filtered. The filter cake was then washed with more 50% alcohol and the filtrate and washings combined.

Alcohol from the combined filtrate and washings was removed by distillation under reduced pressure, and the resulting aqueous solution concentrated on a steam-bath to a syrupy, yet free-flowing, consistency. To this turbid, aqueous solution, sodium chloride (5 g./100 ml.) was added, followed by sufficient hydrochloric acid to bring the pH of the solution to about 4.5. The extract thus obtained was shaken repeatedly in a separating funnel with n-butanol saturated with water. The butanol extracts were combined and washed once with 5% aqueous sodium chloride solution, the washings being extracted with n-butanol saturated with water. The aqueous layer was discarded.

The combined butanol extracts were concentrated by distillation under reduced pressure to a syrupy mass and triturated with a sufficient volume of ether, when a precipitate was obtained. The precipitate was allowed to settle and the clear, supernatant liquid decanted off. The precipitate was washed by decantation with ether. It was then taken up in a minimum volume of absolute alcohol and dry ether added. The buff coloured precipitate obtained was allowed to settle, the clear supernatant liquid decanted off, the precipitate washed with a little dry ether, transferred to a vacuum desiccator and dried

over night over anhydrous calcium chloride. On several recrystallisations from isobutanol a light buff coloured substance B (3 g.), m.p. 248° (decomp.) was obtained.

The substance B was soluble in water, alcohol, n-butanol, isobutanol etc. but was insoluble in petroleum ether, ether, acetone and chloroform. The dry powder had a marked sternutatory property. On shaking a small amount of the substance with water, a large amount of frothing was produced. The substance gave a violet colour with concentrated sulphuric acid. Even in very dilute solutions, it showed haemolytic activity. It gave a violet colour with 1% solution of vanillin in hydrochloric acid.

Hydrolysis of the saponin :

The saponin (1 g.) was dissolved in water (200 ml.) and concentrated sulphuric acid (4 ml.) added. The mixture was refluxed on a steam-bath for two hours. The dark, flocculent precipitate obtained was filtered and washed with water. It was then taken up in absolute alcohol (10 ml.), activated charcoal (0.2 g.) added and the mixture refluxed for half an hour and filtered. The pale yellow coloured filtrate obtained was concentrated and set aside to crystallise. A white amorphous-looking substance was obtained. After several crystallisations from absolute alcohol, needle-shaped crystals melting at 305-7° C were obtained (Substance C).

Chemistry

The substance C was found to be soluble in benzene, ether, chloroform, alcohol, methanol, etc. It was insoluble in water and dilute hydrochloric acid.

A small quantity of the sapogenin was dissolved in commercial thionyl chloride. The solution gave the following colour sequence:

colourless ---→ light blue (5 minutes ---→ purple (10 minutes).

A few mgs. of the substance was dissolved in a few mls. of chloroform and 2-3 drops of concentrated sulphuric acid, followed by addition of 2-3 drops of a solution of acetic anhydride in chloroform. The following colour sequence was observed :

red-violet -----→ blue ----------→ green.

A few mgs. of the substance was treated with a few drops of a 0.01% solution of antimony trichloride in chloroform. A violet colour developed which changed to red.

Substance C did not react with 2 : 4- dinitrophenylhydrazine, thus showing absence of carbonyl group.

	Found	C 79.1%	:	H 10.1%
$C_{30}H_{48}O_3$	required	C 78.9%	:	H 10.5%

Preparation of Acetyl Derivative of the Substance C :

The sapogenin (0.1 g.) was heated for

15 minutes at 130° C with anhydrous sodium acetate (0.1 g.) and acetic anhydride (1 ml.). The product of the reaction was dissolved in hot alcohol (80% ; 5 ml.), the solution cooled and the crystals filtered and washed with a small quantity of the same alcohol. On two recrystallisations from methyl alcohol and water, colourless needles m.p. 264-5° were obtained. The melting point of the derivative did not rise on further crystallisations.

	Found	C	77.3%	:	H	9.8%
$C_{32}H_{50}O_4$	required	C	77.1%	:	H	10.0%

The infra-red absorption spectrum of the acetyl derivative (Plate I, 3) showed an intense band at 1252 cm^{-1} which is indicative of the presence of acetyl group. The band at 1728 cm^{-1} is also due to the presence of the acetyl group. The band at 1678 cm^{-1} is due to the ketone of the acid group present in the compound. There is evidence of absorption between 850 and 800 cm^{-1} which ~~can~~ may be assigned to the presence of trisubstituted double bond.

Infra-red spectrum resembles remarkably well with spectra of the acetate of the triterpenic acids and do not resemble the acetate of steroidal sapogenins in the 'finger-print' region. The presence of the acid group and the resemblance to the triterpenic acids led to the conclusion on the spectrographic basis that the substance is an acetate of a triterpenic acid sapogenin.

Chemistry

The properties of Substance C and its acetyl derivative agree closely with those of oleanolic acid and its acetyl derivative described in the literature. Mixed melting point determination and comparison of infra-red spectrum with spectra of various triterpene sapogenins and their derivatives led to the conclusion that Substance C is identical with and should have the structure of oleanolic acid.

S U M M A R Y

The roots of Glinus lotoides Linn. have been ~~been~~ investigated. From the petroleum ether extract was isolated Substance A, $C_{29}H_{50}O$, m.p. 139-40°, giving tests characteristic of sterols. It gave an acetyl derivative, $C_{31}H_{52}O_2$, m.p. 126-27°. These data agree closely with those of β-sitosterol. Its identity was confirmed by determination of mixed melting point.

From the alcoholic extract was obtained Substance B, m.p. 248° (decomp.) showing properties characteristic of saponins. On hydrolysis it gave Substance C.

Substance C, $C_{30}H_{48}O_3$, m.p. 305-7°, giving a variety of colour reactions with thionyl chloride, concentrated sulphuric acid and acetic anhydride, and antimony trichloride. Its acetyl derivative, $C_{32}H_{50}O_4$, m.p. 264-5°,

was also prepared. The Substance C was unaffected by treatment with 2 : 4- dinitrophenylhydrazine. The infra-red spectrum of the Substance C showed bands characteristic of acetyl group and carbonyl group, and it resembled closely to spectra of triterpenic acid sapogenins. The properties of the Substance C and its acetyl derivative agree closely with those described for oleanolic acid and its acetyl derivative.

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PHARMACOGNOSTIC STUDY OF *MOLLUGO HIRTA* THUNB.

(*GLINUS LOTOIDES* LINNE)

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The morphological and microscopical characters of *Mollugo hirta* leaf, stem and root are described. The froth number and haemolytic index of leaf, stem, root, fruit with seeds and entire plant have been determined.

In different parts of India, the entire plant or the leaf of *Mollugo hirta* Thunb. (*Glinus lotoides* Linne.)[1] belonging to the family Molluginaceae (Ficoideae), is used as a diuretic, purgative or as an antiseptic. Decoction of the leaves is used in dropsy. Entire plant or its ash mixed with oil is applied to boils and ulcers.[2] The dried plant is used for diarrhoea in Sind. In the Punjab, it is given as a purgative for abdominal diseases. In Las Bela, it is prescribed for the cure of boils and bilious attacks. In Puducotta, the juice is administered internally to weak children.[3]

Ridgway and Rowson[4] have studied an Indian drug *Mollugo oppositifolia* Linn. (*Glinus oppositifolius* L.). Shah and Aghara[5] investigated Tuticorin Yellow and found that it contains saponin and consists of the roots of *M. oppositifolia* or its closely allied species. They also men-

tioned that other species of Mollugo would be worth studying for their saponin content and probably as substitutes for senega. Up till now no pharmacognosy of *M. hirta* seems to have been carried out and therefore, the present work was undertaken. According to this investigation all the parts of the plant contain saponin.

The plant is found throughout India in November-December, just after the rains are over. It is usually found on roadsides and in the dry pits on river banks and around ponds. The plant grows widely and abundantly in Gujarat and Saurashtra.

MATERIAL AND METHODS

Young and old entire plants were obtained from the local areas. The plant was identified by us according to Cooke's[6] and Hooker's[7] flora. The plants were dried in the shade. Entire plants, roots, stems, leaves and fruits with seeds were separated and forty-mesh powder of each was prepared.

Free-hand and microtome sections were taken for leaf, stem and root and, after staining with safranin, were made permanent. Stem and root were macerated according to Schultze's[8] maceration process.

Description of the plant:

The plant is an annual herb and is covered more or less by white, stellate hairs which impart it a white appearance. Stems are many, prostrate and spreading from a centre, sometimes ascending. Leaves are usually opposite and several at a node and pubescent (Fig. 1). Flowers are pinkish white or green in axillary fascicles of one to six.

Morphology of leaf, stem and root:

Leaf: Leaves are opposite or several at a node. Those at the base are narrow while those at the apex are broad and having small petioles. They are thick and measure 1-2 cm. by 0.8-1.3 cm. and are densely stellately villous on both sides. Shape is broadly obovate or suborbicular. Apex is obtuse and base is cuneate. Margin is entire. Venation is reticulate. Petioles are slender, stellately hairy and measure 6-10 mm.

Stem: Stems are about 13-15 in number arising from the top of the root and forming a crown. They are prostrate 15-45 cm. in length and directed at right angles to the tap root. They show distinct nodes and internodes. Internodes are about 1-3 cm. in length. Nodes are slightly swollen. They are cylindrical, green and covered with stellate hairs. Fracture is smooth and green.

Root: Roots are straight, unbranched and tapering with a crown at the top (Fig. 2). Direction of growth is vertical. They are about 6-16 cm. in length and about 3-9 mm. in breadth just below the crown. Outer surface is pale yellow in colour and wrinkles are longitudinal and irregular. They show slightly spiral appearance just below the crown. Their surface bears a few scattered pits in which the scars of the detached rootlets are seen as slight protrusions. Crown is

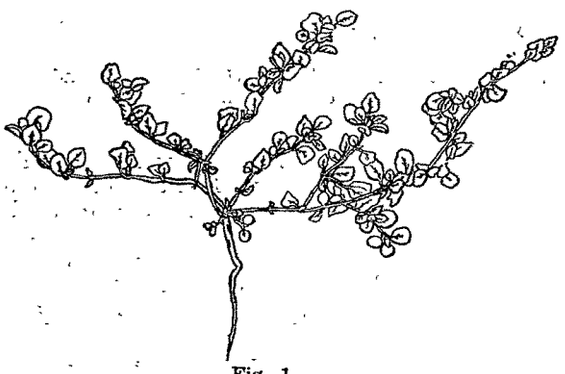

Fig. 1
Entire plant -x 1/3
Mollugo hirta Thunb (*Ficoidae*)

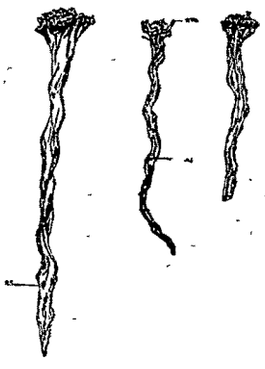

Fig. 2
Entire root
x-1/3

rs—rootlet scar
sm—stem

small and consists of about fifteen remains of the stem bases. Fracture of the stem bases shows greenish colour. Roots break with short and uneven fracture. Smoothened fractured surface shows alternate rings of vascular bundles and parenchyma forming an anomalous structure. Centermost cylinder is solid. Vessels of the xylem are seen distinctly as pores. Vascular rings vary from two to six according to the thickness of the root (Fig. 3). Taste is slightly acrid and odour is none.

Fig. 3
T.S. root from near the crown x 20

xy2—secondary xylem
v—vessel

Microscopy

Leaf: The lamina is dorsiventral, having palisade beneath the upper epidermis only. The epidermis consists of tabular cells having straight anticlinical walls. Some of the epidermal cells bear stellate hairs (Fig. 4). Stomata are present on both the upper and lower epidermis. Their number is more on the lower epidermis than on the upper one. They are both of ranunculaceous and cruciferous types. Palisade consists of three layers of long columnar cells. Spongy tissue consists of three to four layers of rounded cells. The cells of mesophyll tissue contain calcium oxalate crystals of various shapes (Fig. 5). Their shape may

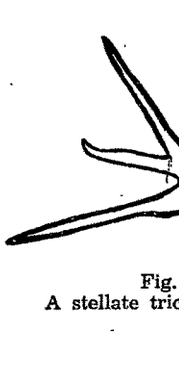

Fig. 4
A stellate trichome x 117

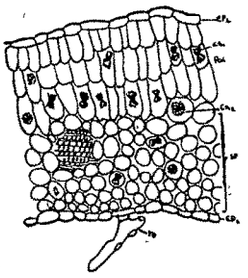

Fig. 5
T.S. of lamina x 57

cr_1—boe-shaped crystal
cr_2—rosette crystal
ep_1—upper epidermis
ep_2—lower epidermis
pal—palisade
sp—spongy tissue
tb—trichome broken

be squarish, rectangular, boe-shaped, aggregate or rosettes. Obliquely and longitudinally cut veinlets are present in this tissue.

Section through the midrib shows the palisade continuous over the meristele but only two layers of this tissue are observed. The midrib slightly projects below and has a shallow groove above. The meristele consists of a radiate xylem and phloem. The cortical tissue of the midrib is parenchymatous (Fig. 6). The cells of

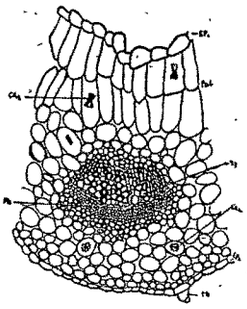

Fig. 6
T.S. of lamina passing through x 57

cr_1—boe-shaped crystal
cr_2—rosette crystal
ep_1—upper epidermis
ep_2—lower epidermis
pal—palisade
ph—phloem
tb—trichome broken
xy—xylem

the palisade and parenchyma also contain calcium oxalate crystals of various shapes.

Stem: Epidermis is the outermost layer covered by cuticle. Some of the cells bear stellate hairs (Fig. 4). Cortex consists of three to five layers of parenchymatous cells. They contain chloroplasts. Stem base develops two to three layers of thin-walled and lignified cork cells.

termination of froth number, the following results were obtained at pH 7.38.

Root — 200; stem — 555; leaf — 2500; fruit with seeds — 1000; entire plant — 1428.

Haemolytic Index

Adopting Jaretzky's[10] method for haemolytic index using buffer solution at pH 7.38, the following results were obtained, 'a' represents the uncorrected haemolytic index and 'b' represents the haemolytic index corrected to digitonin:—

Root — (a) 500, (b) 780; stem — (a) 200, (b) 312; leaf — (a) nil; fruit with seeds — (a) partial; entire plant — (a) 285, (b) 444.6.

Distinguishing characters between the roots of *Mollugo hirta* and Tuticorin yellow (*M. oppositifolia*)

It may be noted that *Mollugo hirta* roots contain stone cells and calcium oxalate crystals but do not contain starch. Tuticorin yellow roots on the other hand do not contain stone cells and calcium oxalate crystals but does contain starch. These differences afford a method of distinguishing the two drugs.

DISCUSSION

The positive froth number and haemolytic index in almost all parts of the plant suggest that saponin is present in the entire plant, but the nature of saponin is not the same in different parts, as from root to stem to leaf the froth number increases and haemolytic index decreases. Thus, the different parts of the plant may contain different saponins or mixtures of saponins. It is also likely that the presence of other constituents in the drug might have affected haemolytic index and froth number.[11] Naturally, the entire plant shows intermediate results as regards both the haemolytic index and froth number.

Some of the medicinal activities[12] mentioned earlier may be attributed to the presence of saponins. The plant is a closely allied species of Tuticorin Yellow or of *Glinus oppositifolius*, which is used widely as a substitute for senega and it would be worth-while to study the expectorant action of the entire plant and compare it with that of senega. Further work is in progress.

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Pharmacognostic Study of the Roots of *Primula denticulata* Smith

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The morphological description of the entire plant and detailed macroscopical and microscopical characters of the root of *Primula denticulata* Smith are presented. From the froth number and haemolytic index of the root, which are 5000 and 66,000 respectively, it appears that the roots of *P. denticulata* can be a suitable substitute for *Polygala senega* roots.

ROOTS of several species of the family *Primulaceae* are used as a substitute for senega. Thus, in German Pharmacopoeia Supplement[1], the roots of *Primula elatior* and *P. veris* (syn. *P. officinalis*) are official. At present, in the *Indian Pharmacopoeia*, there is no suitable substitute for senega. It has been shown[2] that the botanical identity of Indian senega or *Chinensis* I.P. is not *Polygala chinensis* but *Andrachne aspera* and it does not contain saponin. The roots of several species of Polygalaceae such as those of *P. chinensis*[3], *P. abyssinica*[4] and *P. erioptera* (Shah, C. S. and Pandya, K. H., private communication) are not considered suitable because of their small size or low haemolytic index. As *Primula denticulata* is found abundantly in Kashmir and can be a suitable substitute for senega, the present investigation was undertaken.

Materials and methods

Entire plants were collected by the authors at Gulmarg (altitude 9000 to 10,000 ft), Kashmir, in May 1959 and later on identified[5] as *Primula denticulata* Smith. This plant is reported[5] to occur from Kashmir to Bhutan (altitude 7000 to 13,000 ft), Khasia hills (5000 ft) and Afghanistan.

Microtome sections of the root from different levels were made permanent after staining with safranin and light green. The root pieces were macerated as per Schultz's maceration process.

Description of the plant

The plant is a perennial herb, 15-25 cm. in height above soil. Leaves are simple, entire and radical, and develop fully during the flowering season. They are obovate-spathulate in shape with a sheathing base and obtuse apex (Fig. 1C). Margin is dentate with sharp minute teeth. Normal leaves are completely surrounded by a number of thick, fleshy and ovate scale leaves, which are about 3 to 4 cm. long and are equally wide (Fig. 1A). Flowers are crowded to form heads on a short 18-23 cm. long scape (Fig. 1A). Bracts are short, unequal and sometimes connate. Corolla is pale purple and salver-shaped.

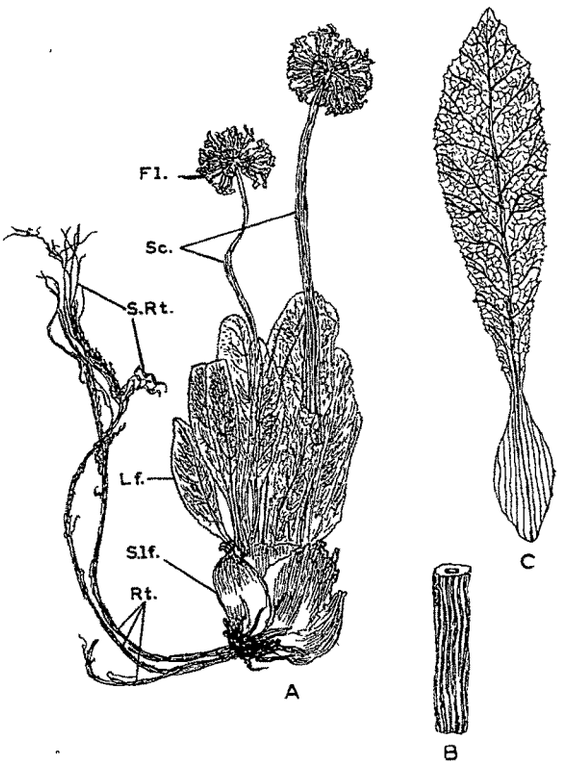

Fig. 1 — Morphological characters of *P. denticulata* [(A) Entire plant × 0·5, (B) a piece of root × 3; (C) leaf × 1·25. *R*, root; *Fl*, flower; *Sc*, scape; *S.Rt*, secondary root; *Lf*, normal leaf; *S.lf*, scaly leaf]

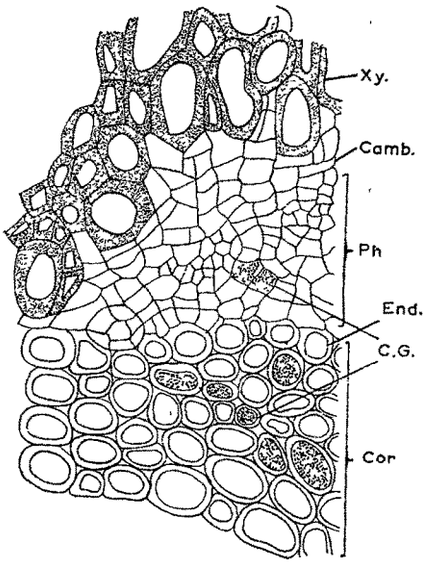

Fig. 4 — Part of phloem and inner cortex showing distribution of granular cell contents × 375 [*Xy*, xylem; *Camb*, cambium; *Ph*, phloem; *End*, endodermis; *C.G*, cell with granular contents; *Cor*, cortex]

The central stele is pentarch. Five groups of radiating xylem alternate with equal groups of phloem. Xylem is exarch (Fig. 3). In the centre, a small parenchymatous pith is present in young roots. In old roots, the parenchymatous pith is gradually reduced and replaced by xylem. Xylem consists of only vessels which show elongate bordered pits as well as reticulate types of wall thickenings (Fig. 5). Vessels measure 97-135-**176**-206-255 μ in length and 21-**37**-52 μ in width. Cambium is distinct. Phloem consists of sieve tubes, companion cells and phloem parenchyma (Fig. 4).

Cell contents

Granular contents — The granular contents in the outer cortical cells are stained orange red with 10 per cent iodine solution. The granular contents of the inner cortical cells and of the phloem cells are stained dark brown with ferric chloride and iodine solutions; with Sudan III, they are stained orange red.

Starch — A few simple or compound grains of three, four or many components occur in some of the parenchymatous cells. Simple grains are mostly elliptical. Some show a central fissured hilum. Simple grains measure 9-23 μ in diameter.

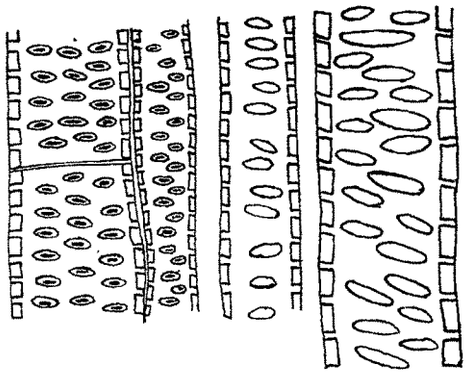

Fig 5 — Xylem with bordered pits and reticulate wall thickening × 750

Froth number

The froth number of the root of *P. dendiculata* determined at *p*H 7·8 according to Kofler's method[6] was 5000; the froth number of *Polygala senega* root[7] and Quilalia bark is 3000.

Haemolytic index

Haemolytic index[8] of *P. denticulata* root, determined using ox blood with isotonic buffer solution at *p*H 7·38 was 66,000; the haemolytic indices[9] for *Polygala senega* and Quilalia bark are 2500-6000 and 4000-6000 respectively.

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