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PHARMACOGNOSTICAL STUDIES ON 'JIVANTI' PART-III—*SARCOSTEMMA BREVISTIGMA* W. & A.

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ABSTRACT

The present paper deals with the macro- and microscopical studies of the vegetative parts of *Sarcostemma brevistigma* W. & A. The cell-contents, percentage extractives and ash values, fluorescence characters and behaviour of drug powder on treatment with different chemical reagents is also described.

The present paper deals with the Pharmacognosy of *Sarcostemma brevistigma* W. & A. (Asclepiadaceae) which is a latex bearing, leafless, jointed and trailing shrub, a meter or more in length, with green succulent branches ; and usually distributed in tropical and sub-tropical areas. The plant is reputed for its medicinal properties and is denominated differently in different parts of the country (Kirtikar and Basu, 1933 ; Bhandari, 1945 ; Chopra *et al.*, 1956 ; Anonymous, 1972 ; and Watt, 1972).

Phytochemical investigations of the plant have revealed presence of octacosane, lupeol, α-amyrin, β-amyrin, β-sitosterol, a phytosterol (m.p. 142°), malic acid, succinic acid, reducing sugars, sucrose, tannins and an alkaloid (Van, 1937 ; Beri and Sharma, 1963 ; Hajarnavis, 1964 ; Sharma and Misra, 1975). No previous work on pharmacognosy of the species, however, seems to have been reported so far. The present communication, accordingly, deals with detailed diagnostic characters of the root and the stem of *Sarcostemma brevistigma* with a view to help in checking adulteration and differentiating it from its known substitutes (Rao, 1914 ; Datta and Mukerji, 1950 ; Chunekar, 1969 ; Gupta *et al.*, 1970 ; Gupta and Kapoor, 1971 ; Singh and Chunekar, 1972 ; Bapalal, 1975).

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MATERIAL AND METHOD

Fresh specimens of stem and root, at various stages of their development, were collected from plants growing in the National Botanical Research Institute Campus, Lucknow and were preserved in formalin-aceto-alcohol (F.A.A.) mixture for microscopic studies.

The methods adopted for investigation were the same as in case of *Leptadenia reticulate* W. & A. (Gupta and Kapoor, *loc. cit.*), excepting for fluorescence analysis of drug-powder which was performed according to the methods of Kokosky *et al.* (1958).

OBSERVATIONS

Macroscopical Characters (Plate I):

Root: The root system consists of a few long (35 mm) and fairly stout (1-4 mm) adventitous roots. The cylindrical roots are bent irregularly or slightly nodular at places, and externally rough, brownish to pale brown in colour. The transversely cut surface shows a narrow bark and a wide wood which is creamish white in fresh root

but turns brown on drying. Its taste is slightly bitter with no perceptible odour.

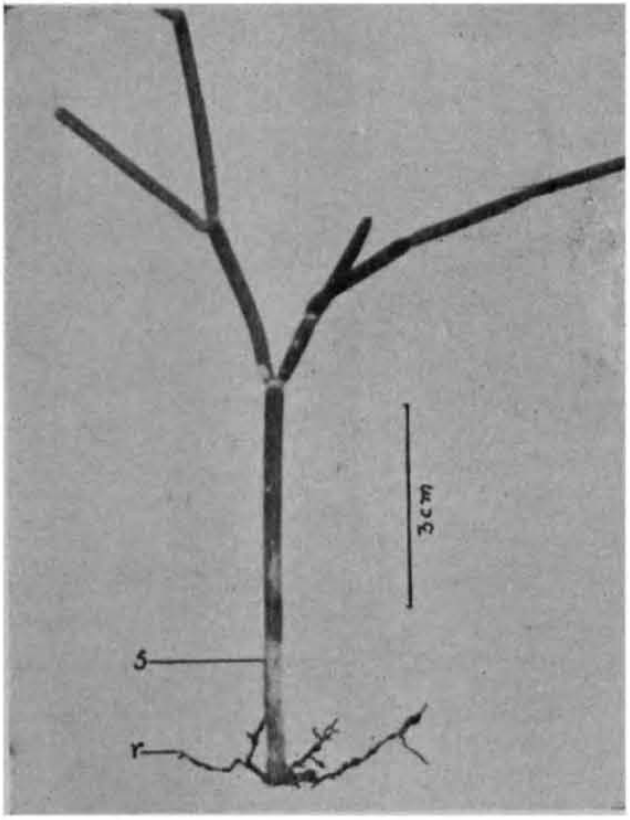


Plate I : Macroscopical characters of a whole plant of *Sarcostemma brevistigma* W. & A.

Stem: The stem is succulent, 2-7 cm in diameter, divaricately branched with smooth surface when fresh, but yellowish brown and wrinkled with distinct longitudinal ridges in thicker parts especially near the nodes in dried samples. The internodes are 6-12 cm long. Fracture is short. The stem in transverse section shows a large hollow in the centre, surrounded by a cylinder of wood and narrow bark. It tastes bitter and has no distinct odour.

Microscopical Characters (Figs. 1-16):

Young root: T. S. of young root (Fig. 1 and 3) shows a single layer of epiblema composed of thin-walled, cubical cells measuring 12-20-30 × 6-12-18 μ with slightly wavy radial walls. The epiblema is followed by cortex composed of 6-8 layers of isodiametric, parenchymatous cells measuring 30-40-55 × 24-34-45μ. Some of the cells contain cluster crystals of calcium oxalate. The endodermis consists of tangentially elongated, barrel shaped cells showing distinct Casparian-thickenings on the radial walls. Beneath the endodermis is a layer of parenchymatous pericycle. The xylem and phloem are radially arranged with primary xylem exhibiting a tri- to pentarch condition. The pith is small consisting of 4-6 polyhedral cells measurng 25-30-40 μ in diameter.

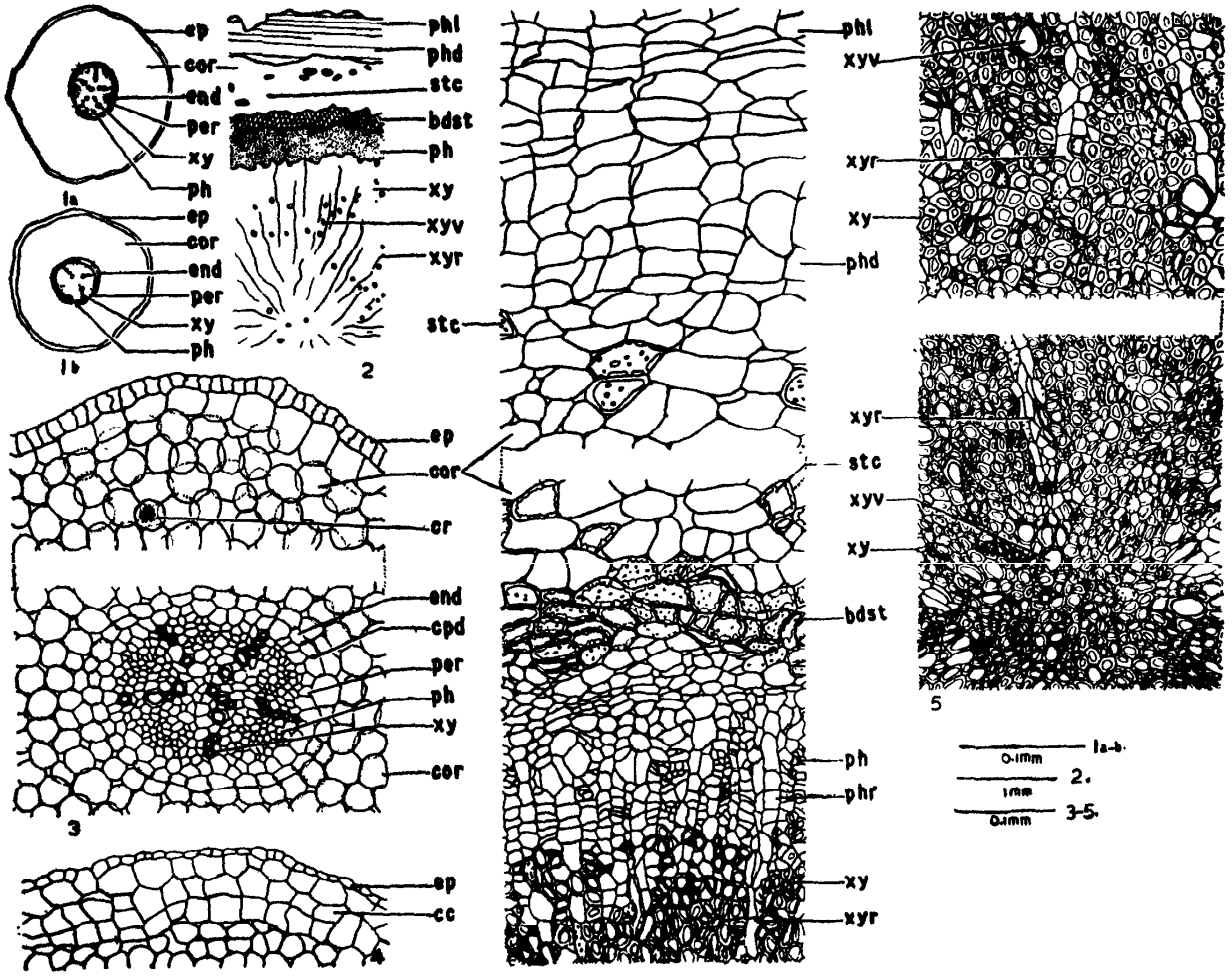
The secondary growth starts quite early. The activity of the cambium is much more vigorous towards the inner side, thus leading to the formation of a wide xylem and a comparatively narrow phloem. The phellogen arises in the second layer of the cortex (Fig. 4). It produces more cork cells on its outer side and only a few layers of phelloderm cells on the inner side. With the formation of phellogen, some of the cortical cells become thick-walled and lignified, forming stone-cells which are either solitary or are distributed in groups of 2-4. The number of stone cells increases as growth proceeds, ultimately leading to a continuous band of stone cells in the innermost region (Figs. 2 and 5).

Mature root: In a 3-4 mm thick root the cork is represented by 13 to 30 layers of suberised cells measuring 22-50-75-115 × 10-20-25-35μ. The phelloderm is a narrow zone of 4-6 layers of parenchymatous cells, measuring 18-40-70-95 × 12-25-50 μ (Fig. 5). The primary cortex is composed of parenchyma and the stone cells, mostly isolated but some times in small groups of 2-4. The innermost region is represented by a continuous ring of stone cells, 2-6 cells thick. These stone cells vary in shape and size, measuring 30-50-65-85 × 12-18-25-40 μ ; and having a wide lumen and simple pits (Figs. 6-8 f1-f8).

The phloem consists of sieve elements and phloem parenchyma, transversed by phloem rays (Figs. 5 and 6). Non-articulated but

branched laticifers are present in this region and have wavy walls, with notches at places (Fig. 8h). In a tangential section uni- and bi-seriate rays are 1-7-9 cells high and tri-seriate ones 3-6-13 cells high (Fig. 6). The ray cells measure 24-49-61-78 × 12-16-20-25 μ. A thin continuous strip of 4-6 cambium cells separates phloem from the xylem (Fig. 5).

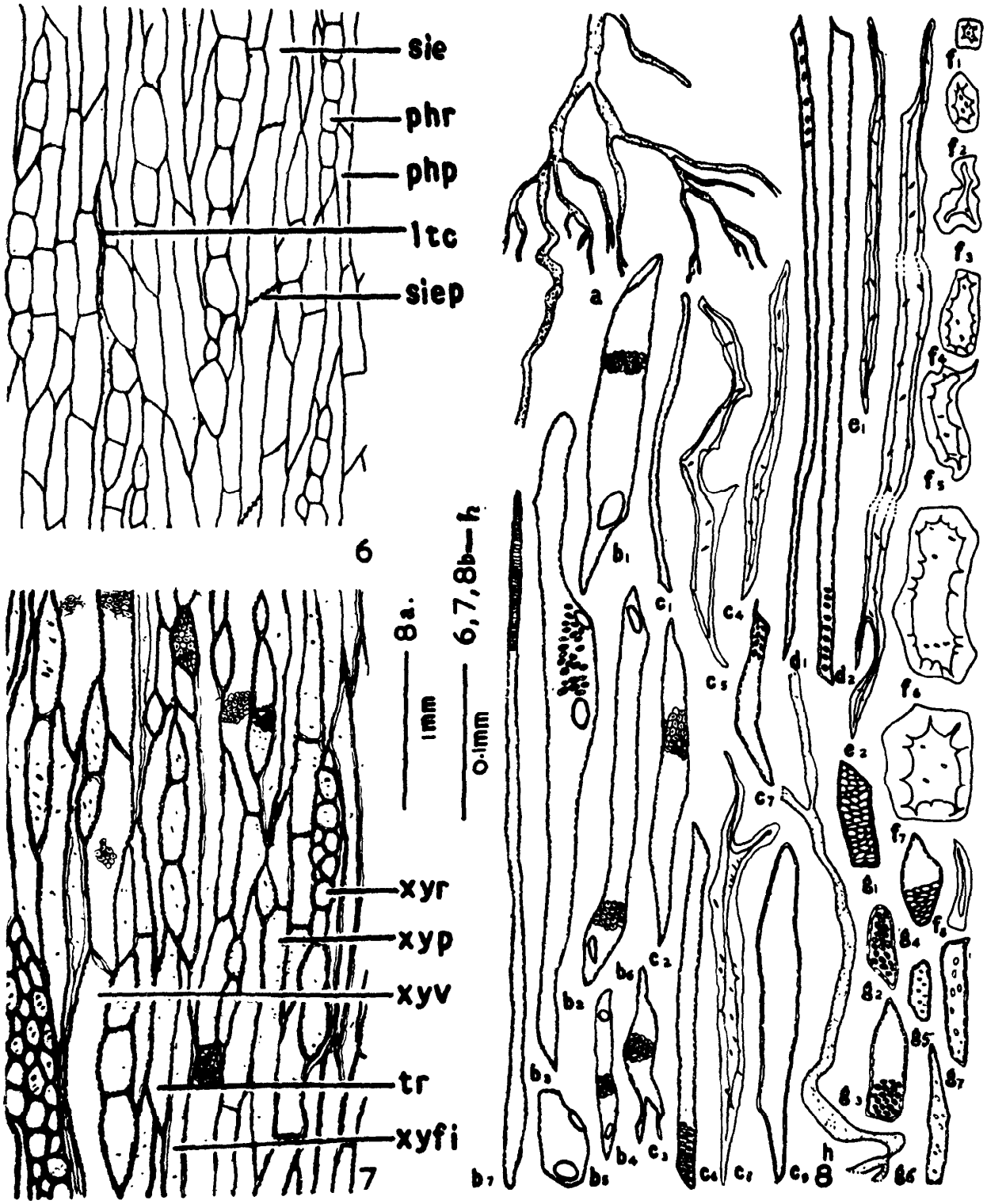
The wood is diffused, porous and com-



Microscopic characters of root of *Sarcostemma brevistigma* W. & A.

Figs. 1-5 : 1. T. S. of young root (diagrammatic). *a*. showing pentarch condition of xylem. *b*. showing triarch condition of xylem. 2. T. S. of a portion of mature root (diagrammatic). 3. Details in t. s. of a portion of young root showing pentarch conditions. 4. Details in t. s. of a slightly mature root showing formation of phellogen in outermost cortical layer. 5. Details in t.s. of a portion of fig. 2.

posed of vessels, tracheids, fibres and parenchyma traversed by xylem rays (Fig. 5). All the elements of xylem are thick-walled and highly lignified. In a tangential section the uni- and bi-seriate rays are 1-4-9-12 cells high and the multi-seriate (tri-seriate) ones 12-14-16 cells high (Fig. 7). The rays cells are slightly thick walled, lignified with simple pits, and measure 16-33-61-82 × 12-16-20-29 μ. Isolated vessels vary in shape and size measuring 65-155-210-320 × 20-40-60 μ (Fig. 8 b1-b6). The bordered pits may be alternate or opposite types. The few spiral vessels encountered are very long (Fig. 8 h7). The tracheids, measuring 25-75-110-170 × 6-12-18 μ, have tapering, truncated and bifurcated ends and bordered pits arranged in opposite or alternate manner (Fig. 8 c1-c9). The tracheid fibres, measuring 120-346-480 × 9-12-18 μ, have similar extremeties and a few



Microscopic characters of root of *Sarcostemma brevistigma* W. & A. and a latifer from the stem bark.

Figs. 6-8 : 6. L. S. of mature root through a portion of phloem region. 7. L. S. of mature root through a portion of xylem region. 8. Isolated elements of root and a laticifer isolated from stem bark. [a-a portion of laticifer isolated from stem bark. b1-b7-xylem vessels. c1-c9-tracheids. d1 & d2-tracheid fibres, e1 & e2-libriform xylem fibres. f1-f8-stone cells. g1-g7-xylem parenchyma. h-a portion of laticifer isolated from phloem region of root bark.]

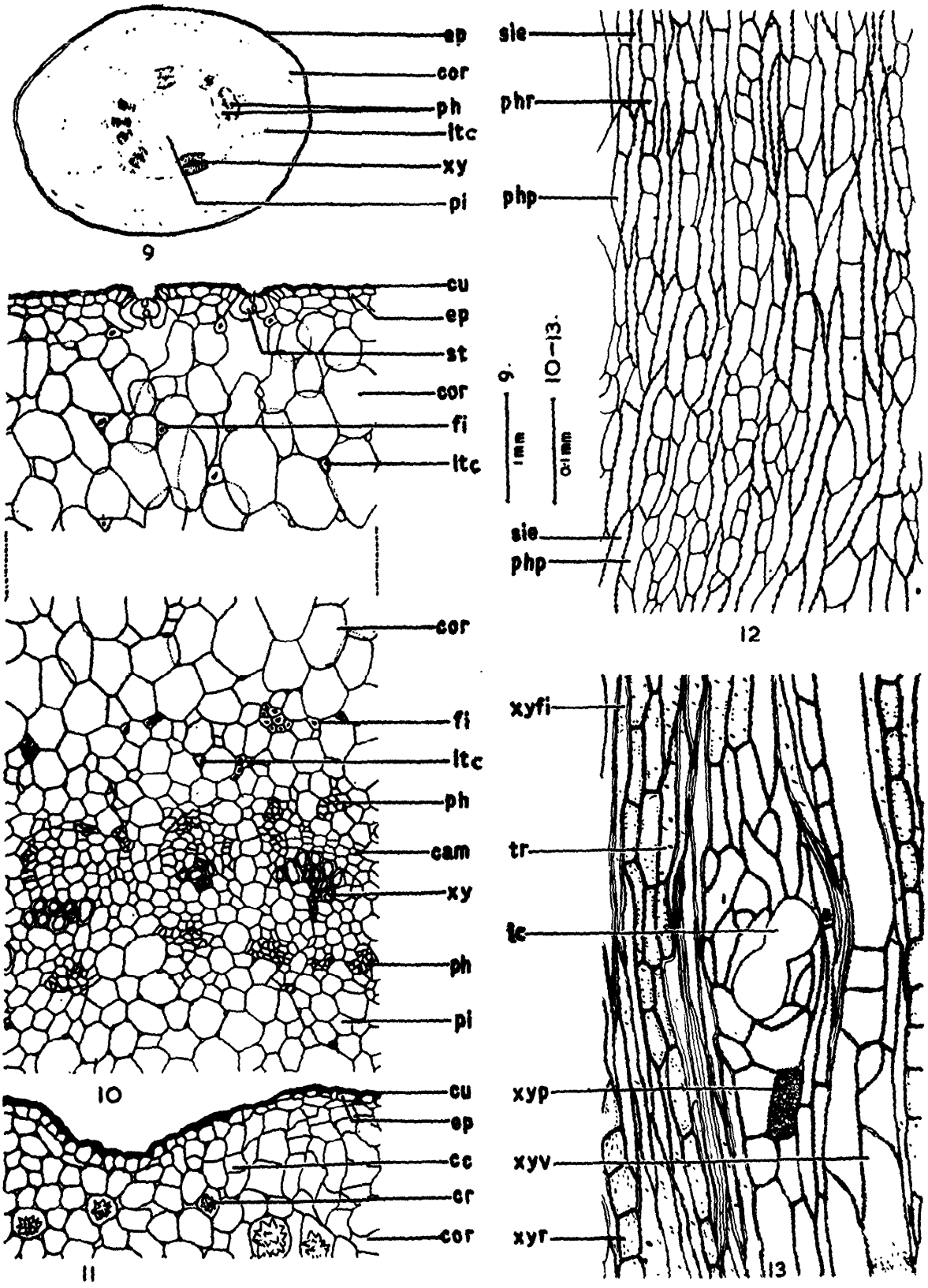
bordered pits on walls arranged in a scalariform manner (Fig. 8 d1-d2). The libriform xylem fibres, however, measure 255-485-740 × 9-15-20-22 μ and have comparatively wider lumen and bordered pits so reduced as to be essentially simple (Fig. 8 e1-e2). The xylem parenchyma are small and have bordered pits arranged similar to those in vessels. Some of the xylem parenchyma are unlignified with simple pits, or partly lignified with reticulate type of secondary thickening (Fig. 8 g1-g7).

Young Stem: T. S. of the young stem shows a single layered epidermis composed of more or less cubical cells measuring 15-18-21 × 12-15-18 μ, covered externally with a thin cuticle. Dispersed in the epidermal layer are some sunken stomata (Fig. 10). In surface view the epidermal cells are mostly polyhedral in outline and show uniform thickenings on their walls. 8 to 10 subsidiary cells surrounding the stomata are, however, thin-walled and much larger than the adjoining epidermal cells. Beneath the epidermis is a wide cortex consisting of 12-16 layers of thin-walled, isodiametric, elliptic to polyhedral, parenchymatous cells, measuring 57-76-110 × 36-40-67 μ The cells of upper few layers are comparatively smaller and are closely packed. Some cellulosic fibres, isolated or in groups of 2-3, and laticifers are also distributed in the cortical region (Fig. 9 and 10). The endodermis is not discernible at an early stage but become distinct later on. The pericycle is represented by small groups of fibres arranged in two discontinuous bands alternated by parenchyma. Followng the pericycle is an amphiphloic siphonostele consisting of a ring of small, open, bicollateral bundles. The pith cells are thin-walled, measuring 27-50-100 μ in diam. The presence of laticifers is also noticeable in the phloem and pith regions.

As growth proceeds more fibres are formed in outer ground tissue and pericycle. Besides, some parenchymatous cells in the cortex and just below the inner ring of the pericyclic fibre strands gradually get converted into stone cells. The phellogen formation occurs in the outermost cortical layer (Fig. 11). Cluster crystals of calcium oxalate are deposited in the parenchymatous cells of the cortex and pith region. The epidermis persists for quite some time before it is ruptured, alongwith a few cork cells. Inner phloem tissues in the outer pith region gradually collapse.

Mature stem: In a 4 to 5 mm thick stem the periderm is composed of about 20 layers of suberised cork cells measuring 25-50-65 × 15-25-50 μ, and 5-6 layers of phelloderm cells measuring 35-60-75 × 25-35-65 μ. It is followed by 10-14 layers of primary cortical cells (Fig. 14 and 16). The latter consists of isolated cellulosic fibres, stone cells, both isolated and in groups of 2-3, and thin-walled parenchyma measuring 28-85-110 × 20-55-85 μ The pericycle is represented by two discontinuous rings of fibre strands alternating with parenchyma. Each ring has of 35-43 strands and each strand consists of 3-14-20-33 fibres (Figs. 14 and 16). The cortical and pericycle fibres are very long, have tapering pointed and bifurcated ends; their walls are wavy and show notches at places. These walls are cellulosic in nature and show distinct anastomosing system of thread like microfibrils (Fig. 15 a1-a2). Unlike the root, stone cells form a 1-4 cells thick continuous ring just outside the phloem (Fig. 16). These cells are similar to those in the root in structure excepting in a few cases where ramifying canals are formed due to fusion of pits (Fig. 15 C1-C8). In this case also, the phloem is composed of seive elements and phloem parenchyma traversed by phloem rays (Figs. 12 and 16). In a tangential section uni- and bi-seriate rays are 1-5-11-20 cells and multiseriate (triseriate) ones 6-9-12-19 cells high (Fig. 12). The ray cells measure 24-41-61-86 × 12-16-20-25 μ

The xylem is composed of tracheae, tracheids, fibres and xylem parenchyma traversed by xylem rays consisting of squarish and



Microscopic characters of stem of *Sarcostemma brevistigma* W. & A. showing t. s. of young stem and t. l. s. of mature stem.

Figs. 9-13 : 9. T. S. of a young stem (diagrammatic). 10. Details in t. s. of a portion of Fig. 9. 11. Details in t. s. of a portion of a slightly mature stem showing formation of phellogen in outermost cortical layer. 12. L. S. of mature stem through a portion of phloem region. 13. L.S. of mature stem through a portion of xylem region.

radially elongated parenchyma with simple pits (Fig. 13 & 16). The uni- and bi-seriate rays are 1-16-22-37 cells and tri-seriate ones 22-27-33-43 cells high (Fig. 13). The ray cells measure 25-49-78-103 × 12-33-49-70μ Dark brown contents are also found in intercellular canals having no walls of their own. In case where the ray is narrow, the presence of a such canal causes it to widen locally but the shape of a wider ray is not altered (Fig. 13). Intraxylary phloem usually gets crushed. Pith cells are thin-walled, isodiametric to oval in shape, measuring 35-70-120 μ in diameter (Fig. 16). Non-articulated, much branched laticifers measuring 1350-2720-3270-7175 × 13-25-40 μ are distributed in the cortex, phloem and the pith (Fig. 8a). On maceration isolated elements are found to be structurally similar to those of the root (Fig.15). However, their measurements are as follows:

Stone cells: 35-80-100-110 × 20-30-55-80 μ

Cellulosic fibres: 2850-6850-7884 × 13-16 μ.

Xylem vessels: 120-266-530 × 13-40-80 μ.

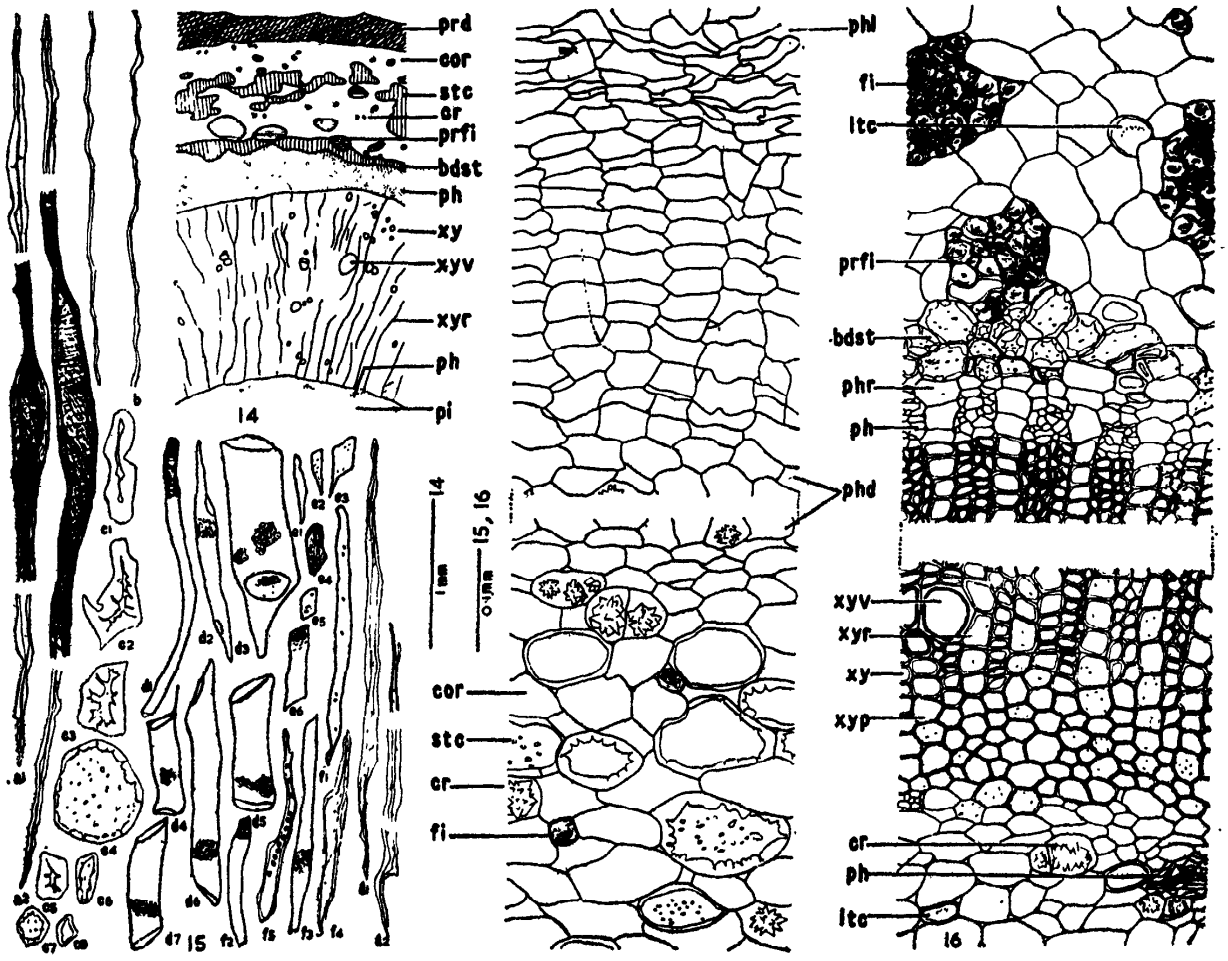
Tracheids: 250-385-465 × 13-20 μ.

Tracheid fibres: 255-346-480 × 9-12-18μ .

Libriform xylem fibres: 545-665-870 × 13-20 μ.

Xylem parenchyma: 65-95-160 × 13-25-40μ.

Cell contents: Besides pale-yellow latex in laticifers and a dark brown content in intercellular canals mentioned earlier, cluster crystals of calcium oxalate measuring 20-25-30 μ in diameter are present in some



Microscopic characters of mature stem of *Sarcostemma brevistigma* W. & A.

Figs. 14-16 : 14. T. S. of a portion of mature stem (diagrammatic). 15. Isolated elements. [a1 & a2-cellulosic fibres. b-a portion of laticifer. c1-c8-stone cells. d1-d7-vessels. e1-e6-xylem parenchyma. f1-f5-tracheids. g1 & g2-libriform xylem fibres] 16. Details in t. s. of portion of fig. 14.

of the parenchymatous cortical cells of root and stem bark and also in pith cells of the latter. Some small simple starch grains measuring 10-15-20-30 μ in diam. are also present in the cortex, phloem and xylem parenchyma and ray cells of root and stem.

Preliminary phytochemical examination showed the presence of sterols, triterpenes, reducing sugars, polysaccharides/glycoside, tannin and alkaloids. Extracts of the drug, obtained successively in petroleum-ether (60-80°), benzene and alcohol by sulphation, were subjected to chromatographic resolution over Brockmann alumina. From petroleum ether extract three compounds *viz.* hentriacontane, m.p. 67-68°, α-amyrin, m.p. 184-86°(Acetate 220-21°, benzoate 198-200°); β-sitosterol, m.p. 134-36° (Acetate 126-27°; benzoate 138-40°), and from the benzene extract four compounds *viz.* β-amyrin, m.p. 194°; lupeol, m.p. 201°; β-sitosterol-β-D-glucoside, m.p. 282-84° (triacetate 163-65° benzoate 201°); and β-sitosterol, m.p. 132-34° were isolated. However, from alcoholic extract only β-sitosterol β-D glucoside m.p. 282-84° was isolated.

Examination of drug powder: The powder of the drug is brownish pale-green having bitter taste with no characteristic odour.

Under the microscope the powder is observed to have small group of cork-cells, parenchyma, cluster crystals of calcium oxalate, isolated and small groups of broken fibres, tracheids and vessels and starch grains. Pieces of cellulosic fibres show distinct anastomosing system of microfibrils. Parts of laticifers fiilled with palebrown contents are also noticeable. On treatment with different chemical reagents the powder behaves as shown in Table I. The fluoresence emited by the drug-powder mounted in different media under ultraviolet light is recorded in Table-II. The percentage extractives and ash values (Table-III) are also helpful in characterising the drug powder.

Table I : Effect of different chemical reagents on the root and the stem powder of *Sarcostemma brevistigma* W. & A.

TREATMENT	COLOUR	
	Root	Stem
1. Powder treated with 1N. NaOH in methanol	Light brown	Light yellow
2. Powder treated with 1N. Hydrochloric acid	Creamish-yellow	Greyish yellow
3. Powder treated with 1N. NaOH. aq.	Creamish brown	Brownish yellow
4. Powder treated with 1N. Nitric acid	Orange	Yellowish light brown
5. Powder treated with sulphuric acid (1:1)	Light brownish dirty green.	Dirty greenish yellow
6. Powder treated with N/10 Iodine solution	Reddish brown	Chocolate brown
7. Powder treated with 50% potassium hydroxide soln.	Yellowish brown	Yellowish brown
8. Powder treated with 10% ferric chloride soln.	Creamish yellow	Greenish yellow

Table II : Fluorescence characteristic of *Sarcostemma brevistigma* W. & A.

TREATMENT	FLUORESCENCE	
	Root	Stem
1. Powder as such	Greyish yellow	Creamish yellow
2. Powder mounted in nitrocellulose	Greyish yellow	Creamish yellow
3. Powder treated with 1N. NaOH in methanol	Dark brown	Greenish yellow
4. Powder treated with 1N. NaOH in methanol dried and mounted in nitrocellulose	Dark brown	Brown with greenish yellow tinge
5. Powder treated with 1N. Hydrochloric acid	Chocolate brown with yellowish tinge	Creamish violet
6. Powder treated with 1N. Hydrochloric acid dried and mounted in nitrocellulose	Dark brown	Creamish purple
7. Powder treated with 1N. NaOH aq.	Dark brown	Greenish brown
8. Powder treated with nitric acid (1:1)	Dark brown	Dark reddish brown
9. Powder treated with sulphuric acid (1:1)	Dirty green with yellowish tinge	Greenish dark brown

Table III : Determination of Ash values and percentage extractives of root and stem of *Sarcostemma brevistigma* W. & A.

Plant parts	Total ash	Acid insol. ash	Alcohol sol. extractive	Water sol. extractive
Root	6.087%	0.998%	9.597%	7.700%
Stem	7.613%	0.358%	14.81%	26.602%

DISCUSSION

'Jivanti' is one of the several drugs of the traditional system of medicine, the identity of which is still unsettled. Besides *Sarcostemma brevistigma* W. & A., several other plants *viz. Desmotrichum fimbriatum* Blume and *Coelogyne ovalis* Bl. (Orchidaceae); *Trema orientalis* Bl. (Urticaceae); *Cimicifuga foetida* Linn. (Ranunculaceae); *Wattakaka volubilis* (Linn.) Stapf, *Holostemma annulare* K. Schum and *Leptadenia reticulata* W. & A. (Asclepiadaceae), are also associated with this drug. Only a detailed study of various characters including pharmacognosy of these taxa can clear up the prevailing coufusion. Earlier works on *Desmotrichum fimbriatum* (Gupta *et al., loc. cit.*) and *Leptadenia reticulata* (Gupta and Kapoor, *loc. cit.*) have elucidated the diagnostic characters of these two species. Similar study on root of *Wattakaka volubilis* and bark of *Trema orientalis* was done by Datta and Mukerji (1950) and Datta (1948) respectively.

From foregoing observations it is evident that certain characters as thin-walled subsidiary cells surrounding the stomata and cellulosic fibres in the cortex and pericyclic zone of the stem, occurrence of a band of stone cells round the phloem tissue both in root and stem, ditribution and structures of laticifers, alongwith certain phytochemical constants and fluorescence tests can provide useful clues for differentiating *Sarcostemma brevistigma* from its known substitutes.

List of abbreviations : bdst, band of stone cells; cam, cambium; cc, cork cambium; cor, cortex; cpd, casparian thickening; cr, calcium oxalate crystal; cu, cuticle; end, endodermis; ep, epidermis; epi, epiblema; fi, cellulosic fibre; ic, intercellular canal; ltc, laticifer; per, pericycle; ph, phloem tissue; phd, phelloderm; phl, phellem; php, phloem parenchyma; phr, phloem ray; pi, pith region; prd, periderm; prfi, pericyclic fibre; r, root; s, stem; sie, sievetubes; siep, sieveplate; st, stomata; stc, stone cell; tr, tracheid; xy, xylem tissue; xyfi, xylem fibre; xyp, xylem parenchyma; xyr, xylem ray; xyv, xylem vessel.

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