

Standardisation and Quality Evaluation of *Centella asiatica* Linn.

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ABSTRACT: *Centella asiatica* Linn. Is a well-known medicinal herb used in various types of diseases, it was noticed that the herb is being heavily adulterated with the cheaper substances. A critical study of the authentic and market samples (available in powder form) was carried out to study the current status of the drug in the local market. Powder analysis of the market samples shows fragments of sclerenchymatous net, which is a characteristic feature of some umbelliferous fruits. Occurrence of prismatic crystals of calcium oxalate and large number of starch grains shows that the powdered materials are heavily adulterated with some cheaper substances. Fluorescence analysis of authentic and market samples exhibits 23.28% and 12.34% -18.13% respectively and there is a difference in crude fibre content also. Moreover there is remarkable difference in the quantitative value of Asiatic acid (3.25% - 0.12%) which is one of the chief constituents of *C. asiatica*.

INTRODUCTION

Centella asiatica Linn. is a well-known medicinal herb used in various types of diseases. Especially, the plant is known for its spectacular effect on the most important organ of human body, the brain. Botanical aspects of this plant have been worked out^{1,2}. Various workers have isolated some of the active constituents viz., Brahmoside, Brahminoside, Brahmic acid, Isobrahmic acid, Betulic acid, stigma sterol, thalictoside, thalictolic acid Asiatic acid etc^{3,4,5,6,7}. Diallo⁸ have separated Asiatic acid and medecassoside by using gas-speed counter-current chromatography to thin layer chromatography. Variation in the chemical composition of Indian samples of *C. asiatica* was worked out⁹. CNS effects, anti-anxiety profile, antibacterial activity of the plant was carried out by various workers^{10,11,12}. Since the plant is being used widely, substitutes or adulterants with a similar appearance have entered the market, but these materials do

not possess the desired medicinal properties. Also at times different herbal drugs have same commercial names and same drug has different names in different localities and regions of the country. One of the best examples of such a situation is *C. asiatica* Linn, it is commonly known as Bramhi. In Bengal *Bacopa monnieri* Pennel Syn *Herpestis monniera* (Linn) Pennel is known as nrami¹³. In present investigation efforts were made to study the different commercial samples from the local market. Critical studies of the authentic and market samples (available in powder form) were carried out to study the current status of the drug in the market.

MATERIALS AND METHODS

Authentic sample was collected from the regional Research Institute (Ayu) Kothrud, Pune. Market samples (tree in number)

were obtained from the local wholesale dealers viz.

1. Ambadas Ayurvedic Medicines, Pune.
2. Sahyadra Aushadhi Bhandar, Pune.
3. Abhital Ayurvedic Aushadhalaya, Pune.

These samples were labeled as MS1, MS2 and MS3 respectively. Semi permanent sections were made and stained either with toluidine blue or methylene blue Epidermal peelings were taken from both sides of the lamina and stained with Delafield haematoxylin¹⁴. Standard procedure was followed for histochemical tests¹⁵ (calcium oxalate crystals, tannin, starch, lignin), for counting vein islet number¹⁶, crude fibre content¹⁷ and fluorescence analysis¹⁸. Ash and extractive values of the leaves were determined by pharmacopoeial standards¹⁹.

TLC identity test: for TLC identity test 5 gm of the powdered drug was refluxed in 50ml of methanol for 15min filter and evaporate the filtrate to dryness, Dissolve the residue in methanol (2ml). Umbelliferone (1mg dissolved in 20l methanol) is used as reference solution Apply 20 µl each of reference and test solution on two different tracks on a precoated silica gel plate, develop the plate with the equal volume of toluene: methanol (saturated with 10% acetic acid) runs to a distance of 18 cm . Observations were made under UVlight (360) and the Rf values were estimates.²⁰

RESULTS MACROSCOPY

Leaves are 1-6 cm in diameter. Several leaves spring from the rootstock and have elongated petioles. One to tree leaves arise from each node of the stems and rare orbicular, reniform, rather broader than long, more or less cupped, entire or shallowly

crenate, glabrous on both sides and with numerous slender nerves from a deeply cordate base; petioles are 7-15 cm or more in length channeled, glabrous or nearly so stipules short adnate to the petioles, forming as sheathing base venation pattern is of palenactiondromous type development confirms it to be perfect marginal.

MICROSCOPY

Petiole (Fig 1&3): in transaction (TS) it shows single layered epidermis covered by a cuticle Inner cell walls of the epidermis adjoining cortex are much thickened. 2-3 layers of collenchyma tissue follow epidermis and following this is a broad zone of parenchyma five to seven vascular bundles are located in the projecting arms of the petiole. Chlorophyll pigment is seen in the outer peripheral layers of the parenchyma Crystals of calcium oxalate are also noticed in the parenchymatous zone.

Midrib (Fig 2&7): TS of the midrib shows single layered Epidermis. Epidermis Epidermal structure is similar to that of petiole two to three layers of collenchyma cells are present just beneath the Epidermis. Collenchyma region is followed by 4-5 layers of parenchyma cells which do not contain chloroplast. The vascular bundle is present in central region and is differentiated into xylem towards the dorsal side and phloem towards ventral side. The xylem consists of radial rows of xylem vessels with xylem parenchyma in between.

Lamina (Fig 4): in TS the lamina shows dorsiventral structure. In surface view (Fig. 5&6) the amphistomatic leaf lamina shows paracytic and anomocytic types of stomata. Upper epidermis is followed by palisade tissue, which is differentiated, into two layers, Spongy parenchyma cells with large

intercellular spaces, some cells of which contain crystal of calcium oxalate.

Quantitative values such as palisade ratio, vein islet number, and stomatal index are tabulated in table number 1.

POWDER ANALYSIS

Authentic sample: Powder is greenish-to-greenish brown in colour with bitter taste. Under the microscope the powder shows the fragments of epidermal cell (Fig 8&9), collenchyma tissues (Fig 10) fibres (Fig 11), few trichomes (fig 12) treachery elements with spiral thickening (Fig14i) and pits on their secondary wall (Fig 14ii) rosettes crystals of calcium oxalate (Fig 13etc).

Marker samples: Powder is pale brown in colour with slight bitter taste. More or less all the three market samples under the microscope have shown the following characters.

Sclereids (Fig 20) are fairly small, square to rectangular or oval in outline and are comparatively thin walled with numerous pits. Fibres (Fig 23) are thick walled lignified with an irregular lumen and few pits. Treachery elements (Fig 22) usually occur in small groups; they are lignified and usually spirally thickened but a few have small pits on their secondary wall. The prisms and cluster crystals of calcium oxalate which are found scattered as well as in the parenchymatous tissue; some of the cluster crystals are rather irregular and appear fragmented (Fig 18). The very occasional covering trichomes (Fig 17), which are unicellular, conical and moderately thick walled and may be slightly swollen at the base. Occasional fragments of the lower epidermis in surface view show the presence of cicatrices where the trichomes have been attached (Fig 15). Fragments of parenchyma

cells in longitudinal view show that the tangential walls have a conspicuously sinus outline (Fig21) The starch granules, mainly simple rather small, spherical to slightly polyhedral; compound granules also occur a small point or cleft hilum is some times visible (Fig 19), Sclerenchymatous layer (Fig 16) groups of fusiform fibres of sclerenchyma running wavy and at times crossing with each other or with thin walled lignified cells. These fragments are more in number in market sample no 1. Quantitative values such as ash values, extractive values and crude fibre content are tabulated in tables 2,3 and 4 respectively. Chemical tests are mentioned in table no. 5. Fluorescence analysis under the U.V. light at long and short wave length are given in table no 6. Alcoholic extracts of the sample when they are subjected for Asiatic acid estimation shows 3.25% in the authentic samples and 0.12-1.73% for market samples.

DISCUSSION

Anatomical studies described by some earlier workers¹² are more or less matching with our observations. However in the present investigation nonglandular uniseriate trichomes are observed in *C. asiatica* Linn.

Powder analysis of the market samples shows group of fusiform sclereids, which is a characteristic feature of prismatic crystals of calcium oxalate and large number of starch grains show that the powdered materials are heavily adulterated with some cheaper substances. Fluorescence analysis of authentic and market samples show remarkable difference in the colour. Water extractive values of authentic and market samples exhibit 23.28% and 12.34% - 18.13% respectively and there is a difference in crude fibre content also. Moreover there is a remarkable difference in the quantitative value of Asiatic acid (3.25-

0.12%), which is one of the chief constituents of *C. asiatica*. From the above observations it is quite clear that all the above observations it is quite clear that all the market samples of *Centella asiatica*

Linn. Have been heavily adulterated and it is anticipated that the present investigation will certainly help in standardization of market samples.

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Table 1
Microscopical observation of C. asiatica:

1	Palisade ratio	2-3
2	Vein islet number	8-11
3	Stomatal index:	
4	Lower.Epi	12-14
5	UpperEpi	8-10

Table 2
Ash values

Sr.No	Plant material market sample	Total ash in %	Acid insoluble as in %	Sulphated ash. In %
1	Centella asiatica	11.16	0.318	13.84
2	Market sample 1	25.85	15.87	28.86
3	Market sample 2	28.31	10.91	20.17
4	Market sample 3	20.71	17.19	24.84

Table 3
Extractive values

Sr.No	Plant material market sample	Alcohol extract in %	Water extract in %
1	Centella asiatica	9.52	23.28
2	Market sample 1	9.68	15.04
3	Market sample 2	10.79	12.34
4	Market sample 3	8.34	18.13

Table 4
Crude fibre content

Sr.No	Plant material market sample	Crude fibre content (in%)
1	Centella asiatica	20.17
2	Market sample 1	15.95
3	Market sample 2	10.17
4	Market sample 3	16.13

Table 5
Chemical tests

Sr.No	Tests	C. asiatica	Market sample1	Market sample2	Market sample3
1	Coumarin	Present	Present	Present	Present
2	Strach	Present	Present	Present	Present
3	Cholcone	Absent	Absent	Absent	Absent
4	Tannin	Present	Present	Present	Present
5	Saponins	Absent	Absent	Absent	Absent
6	Alkaloids	Present	Present	Present	Present

Table 6
Fluorescence analysis
A)C. asiatica, Linn.

Sr.no	Solvent	Ordinary light	U.V. (sort wave)	U.V. (long wave)
1	Pet ether	Amber	Light green	Amber
2	Carbon tetrachloride	Amber	Greenish yellow	Red
3	Chloroform	Olivaceous	Slate purple	Scarlet
4	Acetone	Dark green	Dark green	Scarlet
5	Ethyl acetate	Dark herbage green	Dark herbage green	Scarlet
6	Ethyl alcohol	Dark herbage green	Dark herbage green	Scarlet
7	Methyl alcohol	Dull green	Dull green	Red
8	Distilled water	Primorse	Leek green	Primorse

B) Market samples

Sr	Solvent	Ordinary Light			U.V	Short	Wave	U.V	Long	Wave
		MS -1	MS-2	MS-3	MS -1	MS-2	MS-3	MS -1 Peach	MS-2	MS-3
1	Pet ether	Amber	Luteous	Amber	Light green	Light green	Greenish Yellow		Rose	Peach
2	Ccl4	Olive	Olive	Grey	Olive	Olive	Grey	Brick	Red	Red
3	Chloroform	Umber	Umber	Umber	Dull green	Dull green	Dull green	Scarlet	Scarlet	Scarlet
4	Acetone	Olive	Amber	Amber	Grass green	Grey	Grass green	Red	Rose	Red
5	Ethyl	Umber	Olive	Olive	Olive	Olive	Grey	Red	Red	Scarlet

	acetate									
6	Ethyl alc	Amber	Olive	Olive	Light green	Light green	Light green	Scarlet	Red	Red
7	Methyl alc	Olive	Greyish	Olive	Dull green	Dark green	Dark green	Red	Red	Citrine blue
8	Distilled water	Primrose	Flesh	Primrose	Leek green	Leek green	Citrine blue	Citrine blue	Bluish green	Scarlet

ILLUSTRATION TO THE FIGURES:

Fig. 1 : Schematic diagram of the petiole through transaction (TS)

Fig. 2 : Schematic diagram of the midrib through TS

Fig. 3 : A portion of the petiole through TS

Fig. 4 : TS of the leaf lamina

Fig. 5 : Upper epidermis in surface view

Fig. 6 : Lower epidermis in surface view

Fig. 7 : TS of midrib.

Fig. 8 : Epidermis in surface view showing anomocytic type of stomata.

Fig. 9 : Epidermis in surface view at midrib region

Fig. 10 : Fragment of collenchyma tissue associated with epidermis

Fig. 11 : Fibers

Fig. 12 : Nonglandular trichomes

Fig. 13 : Rosettes of calcium oxalate crystals

Fig. 14 : Tracheary elements showing (i) Spiral wall thickenings (ii) Pits.

Fig. 15 : Epidermis in surface view showing cicatrix surrounded by cuticular striations.

Fig. 16 : Groups of fusiform sclerids

Fig. 17 : Non glandular trichomes

Fig. 18 : Crystals of calcium oxalate.

Fig. 19 : Starch grains

Fig. 20 : Sclereids

Fig. 21 : Parenchyma cells showing sinus walls.

Fig. 22 : Tracheary elements showing (i) Spiral wall thickenings (ii) Pits.

Fig. 23 : Part of group of fibres

AC: Anomocytic type of stomata

CR: Crystals

CO: Colleenchyma

PA: Palisade tissue

LE: Lower epidermis

SP: Spongy Parenchyma

PC: Paracytic type of stomata

UE: Upper epidermis

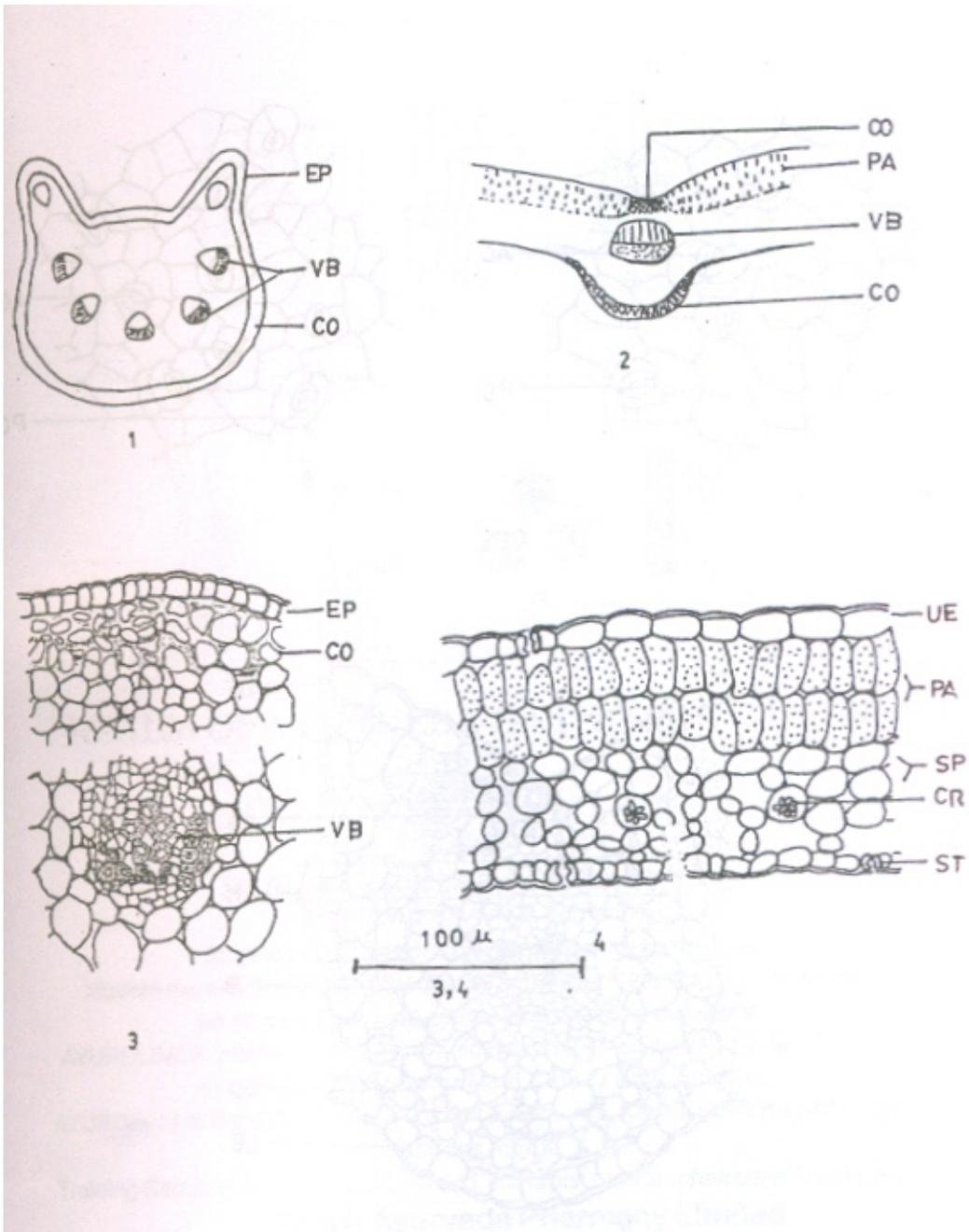
ST: Stomata

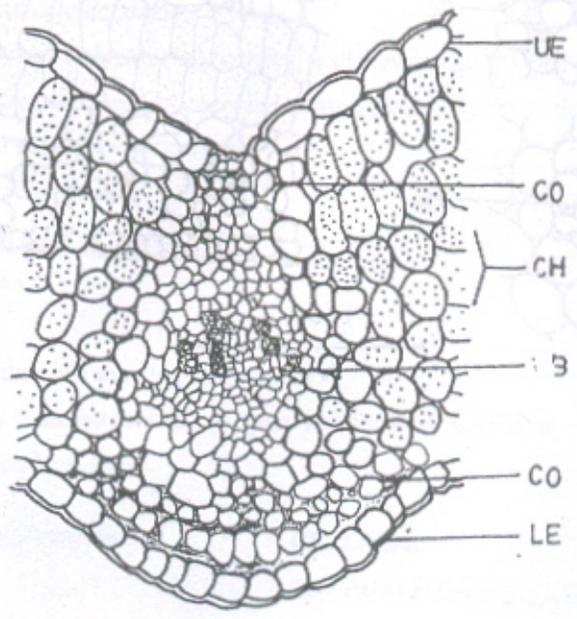
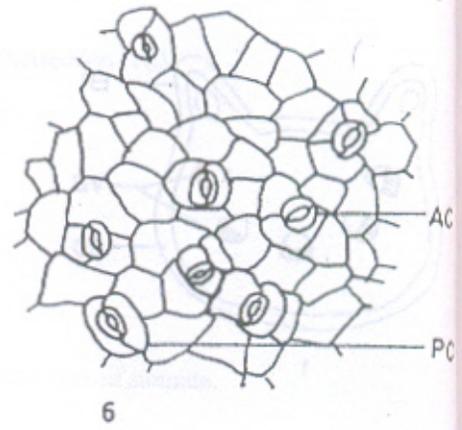
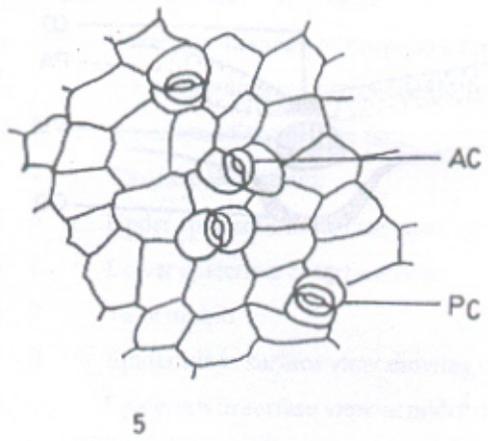
CI: Cicatrix

VB: Vascular bundle

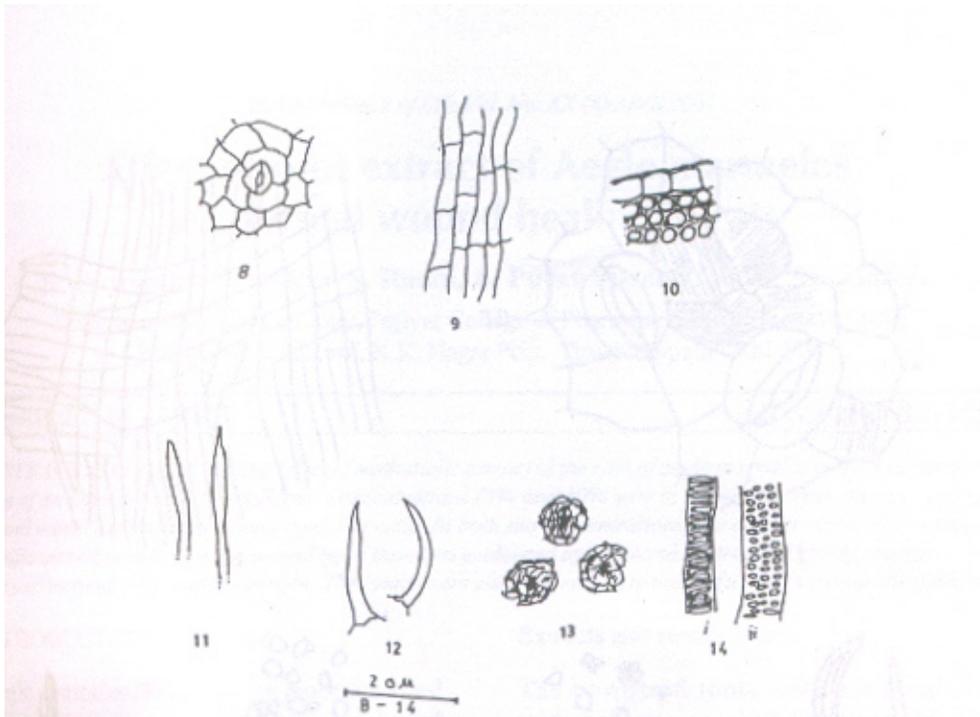
EP: Epidermis

CH: Cholrencyama





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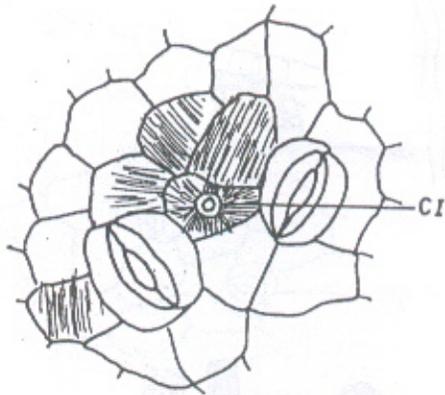
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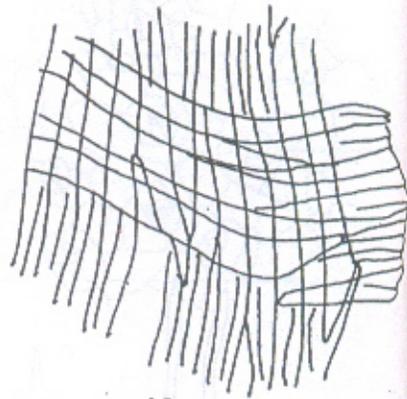
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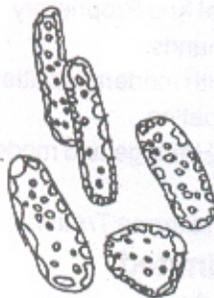
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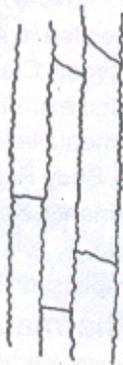
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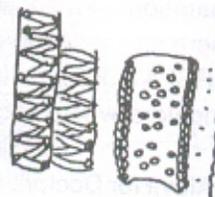
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