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Pharmacognostical Studies on the Roots of *Clerodendrum serratum*

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Abstract

Sirutekku is a plant drug used in Indian medicine. *Clerodendrum serratum* is deemed to be the source of authentic *Sirutekku*. Many taxa belonging to the Verbenaceae family are used to adulterate the genuine drug, posing problems in identification. The root of *C. serratum* is considered for this study with the aim of producing an anatomical description for diagnosis and for distinguishing it from its adulterants. The studies include gross anatomical features, cellular composition, tissue organization and cellular inclusions of the root.

Keywords: *Sirutekku*, *Clerodendrum serratum*, *Premna herbacea*, *Ayurveda*, *Siddha*.

Introduction

Sirutekku is a plant drug which enters into more than 10 polyherbal formulations in the Indian systems of medicine, such as Bharangirasayanam, Nochithailam, Kaphasurakkudineer, Vathasurakkudineer, Rasaganthimezhugu, Sarabungavilvathiezhagam, Sirutekkukudineer, Kantankatthirikudineer, Thonthasurakiyazham, Thalisapatthirichoornam etc. (Anonymous, 1984; Murugesa Mudaliar, 1969). While the efficacy of the drug is widely recognised by *Ayurveda* and *Siddha* physicians, the botanical identification of the drug continues to cause problems. While *Clerodendrum serratum* (L.) Moon (Verbenaceae) (synonyms: *Volkameria serrata* L., *Rotheca serrata* (L.) Steane & Mabb.) is deemed to be the authentic *Sirutekku*, the genuineness of the drug has been disputed and pharmacists are skeptical over its identity. This situation has arisen due to the fact that drugs from many taxa belonging to the Verbenaceae, and few others of different plant families, have been sold in the market under the name *Sirutekku*. Some such adulterants are *Clerodendrum indicum* (L.) Kuntze, *Premna obtusifolia* R. Br., and *P. herbacea* Roxb. of the Verbenaceae, *Gardenia latifolia* Aiton, *G. resinifera* Roth, and *G. turgida* Roxb. of the Rubiaceae and *Picrasma quassioides* (D.Don) Benn. of the Simaroubaceae. All these taxa can be distinguished from *C. serratum* with ease when the specimens are available with flowers. However, when the samples are available only in the vegetative condition or in fragmentary form, as often happens in dealing with the crude drugs, identification of the specimen poses considerable difficulties.

In a situation when incomplete non-flowering samples are to be identified, one has to resort to the histological analysis of the samples, employing certain special botanical microtechniques. This work is devoted to an anatomical investigation of the roots of *C. serratum*, the true *Sirutekku*, in order to provide an account of the anatomical features, which can be used for diagnosing and distinguishing the drug from its adulterants.

A perusal of the literature showed that Nayar et al. (1976) have studied the anatomical features of *Premna herbacea* and also described certain diagnostic features that separate *C. serratum* from *P. herbacea*. Considering the importance of *Sirutekku* and its various applications in Indian Medicine, it is felt that a more thorough examination of the root of *C. serratum* would be worthwhile, since such a study may enable one to identify the taxon in any fragmentary form.

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Materials and methods

The specimens for the present studies were collected from Kolli Hills, (altitude 1000 m) in the Namakkal district of Tamilnadu State. Young and old roots were collected, trimmed and fixed in the field. Formalin: acetic acid: alcohol (FAA) was used as the fixative for all soft specimens. In the case of root-wood, the samples were cut and sliced into small pieces and soaked in glycerine-alcohol, which served both as a softening and preserving fluid of the tissues, which were also, when necessary, softened with the help of ethylenediamine (ETD) (Carlquist, 1982). Root bark and thin lateral roots were sectioned with a rotary microtome after paraffin embedding. Sections were cut 8–12 µm thick, stained with tannic acid and ferric chloride plus safranin (Foster, 1934) and with toludine blue O (O'Brien et al., 1964). Using the Johansen (1940) method, xylem elements were isolated and the dimensional values of the elements were obtained by taking at least 25 measurements for each element. Vessel (pore) diameter was measured from transverse sections (TS) of wood; ray height and breadth were measured from tangential longitudinal sections (TLS) of the wood; other measurements were taken from macerated preparations. Descriptive terms of the wood anatomy are based upon the terminology recommended by the IAWA Bulletin (1989). Photomicrographs were taken with a NIKON-Lab phot-2.

Results

Anatomy of the root

Young root (Fig. 1.1–3)

Specimen examined: lateral root measuring about 3.8 mm thick. Root roughly circular in cross sectional outline with shallow fissures and thin membranous peelings of phellem tissue (Fig. 1.1).

Periderm (Fig. 1.2)

This consists of old phellem, which scales off in membranous fragments along the outermost boundary of the newly formed periderm. In regions where old phellem has peeled off, the surface of the new periderm appears smooth and even. Newly formed periderm ranges from 150–250 µm wide in the radial direction. It consists of a phellogen layer, one or two phelloderm layers toward the inner side and 6–10 phellem layers towards the outer side. The entire periderm is 300 µm wide. The cells of the phellem are transversely and narrowly oblong, thin walled and suberised, uniform in size and shape. The outermost layer of the newly formed phellem consists of comparatively thick-walled, transversely linear cells with granular contents and this layer marks the line of peeling-off of the old phellem tissue. The phelloderm cells become indistinct.

The cortical zone measures 450–500 µm wide in the radial plane, the inner boundary being marked by isolated small groups of fibres. The cortical cells are elliptical or oblong in the tangential view, thin-walled, compact, with small intercellular spaces (Fig. 1.2). The cortical cells contain abundant starch grains that are mostly concentric; very few grains are eccentric. The size is 17–22 µm, mostly present in ground parenchyma of secondary xylem and secondary phloem. Some of the inner cortical cells, lying just outer to the groups of fibres, have differentiated into sclereids, which occur in a thin tangential, discontinuous band of one or two cells thick. The cortical sclereids can be distinguished from the fibres by their larger size, wider lumen, lamellate wall and canal like prominent pits (Fig. 1.3). In vertical plane, the sclereids are elliptic, oblong or much elongated and linear.

The vascular cylinder is solid and compact with an even outer margin and without pith in the centre. The central core of the xylem cylinder is occupied by a large metaxylem element with three to five exarch primary xylem strands. The secondary xylem is fairly wide and consists of fibrous ground tissue and angular vessels (Fig. 1.2). The vessels are either solitary or in small clusters (Fig. 1.3). The diameter of the vessels ranges from 60–100 (67) µm and their wall thickness is about 5 µm. The vessel elements have simple perforations and a slightly oblique perforation plate. The lateral wall thickenings are scalariform and reticulate in the primary xylem vessels and show alternate elliptic pits in the case of secondary xylem vessels.

The fibres are thin walled and wide lumened. Almost all fibres contain abundant starch grains. Septate libriform fibres are also frequently seen. No distinct pits are evident in the fibre walls. Axial parenchyma cells occur in vertical strands.

Phloem (Fig. 1.3)

The primary phloem has been crushed and obliterated. The intact secondary phloem occurs as a continuous cylinder outside the xylem. It consists of sieve tubes, companion cells and axial parenchyma. The sieve tubes occur in groups; they are polyhedral in cross section with companion cells located along the corners. Sclerenchyma (i.e., sclerieds) is not present in young roots. No specific cell inclusions are seen in the phloem parenchyma.

Old root

Wood

Old roots, ranging in thickness from 3–7 cm were examined.

Physical properties

Wood hard, heavy, light brown, fine-grained smooth textured; no taste, no odour. Pores minute, not visible to the naked eye; growth rings fairly distinct and visible to the naked eye.

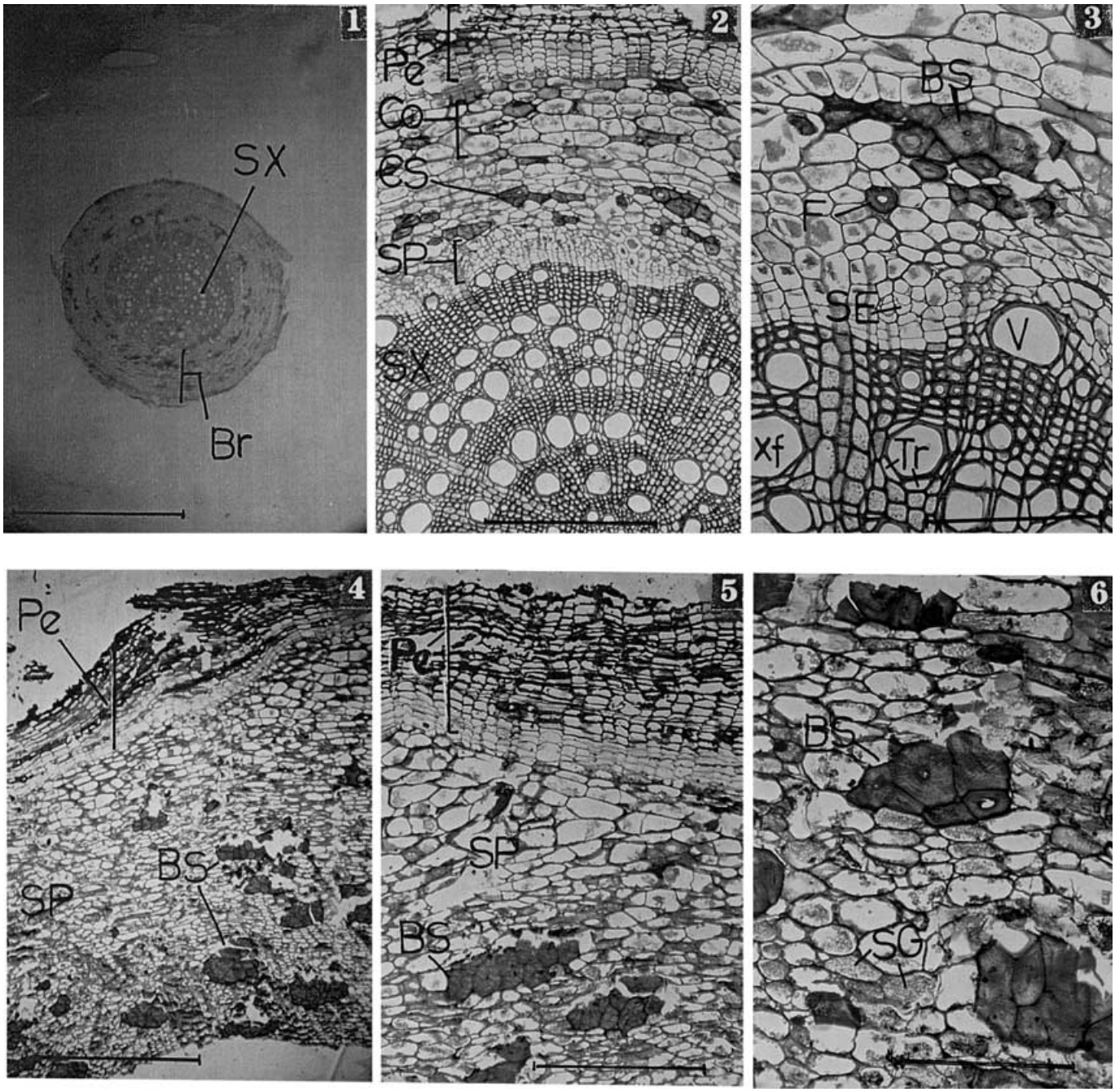

Figure 1. Young root and bark of mature root.

1. T S of young root in ground plan;
2. T S of young root, a sector showing periderm, cortex, secondary xylem.
3. Secondary xylem and secondary phloem of the previous section enlarged
4. T S of bark of old root showing the periderm and granular secondary phloem.
5. Periderm and old phloem tissue enlarged
6. Sclereids and starch bearing phloem parenchyma.

(Br: Bark tissues; BS: Brachysclereids; Co: Cortex; CS: Cortical sclerenchyma; Pe: Periderm (Phellem); SE: Sieve elements; SG: Starch grains; SP: Secondary phloem; SX: Secondary xylem; V: Vessel; Tr: Tracheids; XF: Xylem fibres)

Scale bars in Fig. 1 = 4 mm; 2 and 5 = 430 µm; 3 and 6 = 200 µm; 4 = 700 µm.

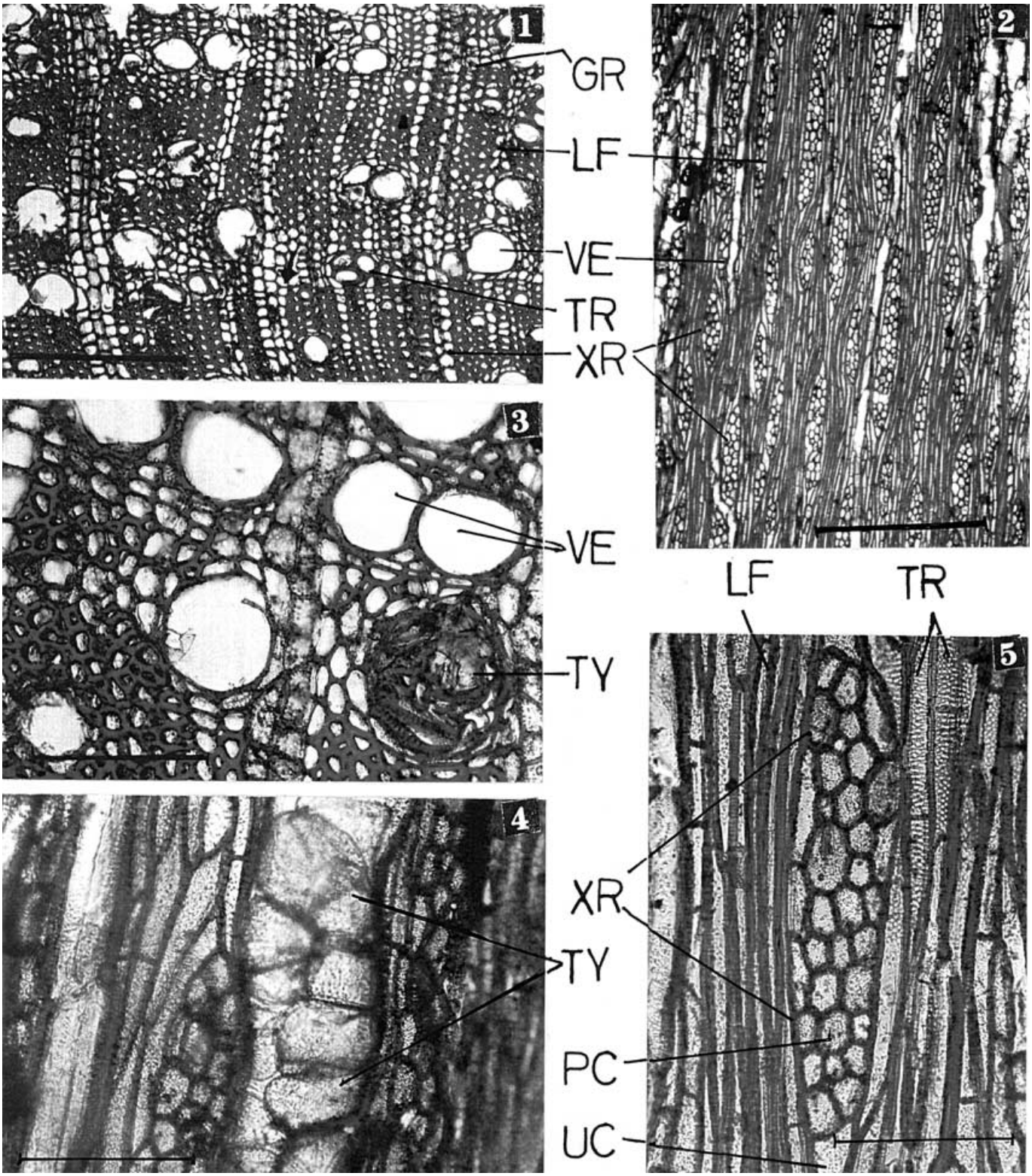

Figure 2. Anatomy of old-root.

1. TS of root wood showing growth rings.
2. TLS of root wood
3. TS of root wood, enlarged
4. TLS of wood showing abundant tyloses in a vessel.
5. TLS of wood showing a heterocellular ray, tracheids and libriform fibres.

(GR: Growth ring; LF: Libriform Fibres; PC: Procumbent cells; TR: Tracheids; TY: Tyloses: sclerosed and abundant; UC: upright cells; VE: Vessel element; XR: Xylem ray)

Scale bars in Figure 1 = 430 µm; 2 = 700 µm; 3–5 = 200 µm.

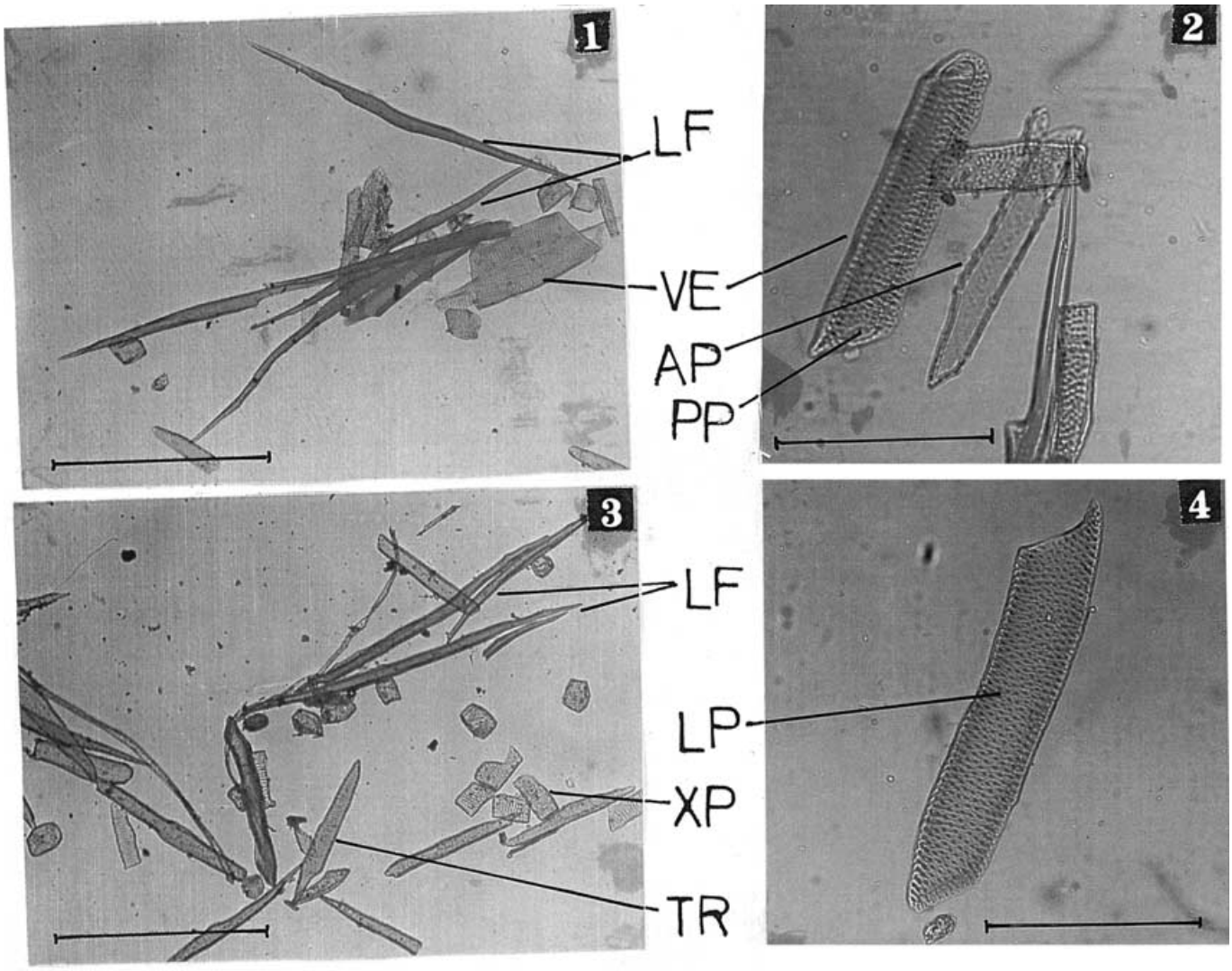

Figure 3. Root-wood elements.
1. Many libriform fibres and one wide vessel element
2. A narrow vessel element and an axial parenchyma
3. Libriform Fibres, parenchyma and tracheid;
4. Single vessel element showing lateral wall pittings

(AP: Axial parenchyma; LF: Libriform fibres; LP: lateral wall pittings; PP: perforation plate; TR Tracheid; XP: Xylem parenchyma; VE: Vessel elements)

Scale bars in Fig. 1 and 3 = 430 µm; 2 and 4, = 5 = 200 µm.

Microscopical features

Growth rings distinct, marked by a broad zone of boundary parenchyma and isolated groups of vascular tracheids (Fig. 2.1,3). Tracheids diffusely-porous, the pores circular or elliptic, solitary or less frequently in multiples of 2 or 3; tangential diameter 120 (50–170) µm; pore frequency 17/mm^2; wall thickness 10 µm; length of the vessel elements 328 (250–430) µm; cylindrical, more frequently short and barrel shaped; perforation plate simple, horizontal short elements or slightly oblique in longer elements and tailed at one or both ends, or tailless; lateral wall pits tangentially elongated and linear (Fig. 3.1–4); tyloses abundant and due to mutual compression, form pseudoparenchymatous structure within the vessel lumen (Fig. 2.3,4). Pore lines wavy, as seen in longitudinal section (LS) views.

Axial parenchyma paratracheal and scanty, and apotracheal and boundary; cells vertically oblong, thin walled, simple pitted, occurring in strands. Fibres libriform type, thick walled, non-septate (Fig. 3.1,3), length 486 (320–840) µm; walls 5 µm thick; pits not evident.

Tracheids-vascular tracheids occurring at the growth ring boundary and vasicentric tracheids abutting the vessels; cells shorter, walls thinner and lumen wider when compared to the

length, wall thickness and cell lumen of the fibres; lateral wall pits circular, bordered and abundant.

Xylem rays-multiseriate, occasionally uni- or biseriate; heterocellular, with marginal upright cells and rectangular body cells (Fig. 2.2 and 5); uni- or biseriate rays are exclusively homocellular with upright cells only; rays 559 (170–950) µm high, 94 (50–130) µm broad. Ray frequency 3–5/mm.

Root bark

Bark, chiselled out from old roots measuring about 6 cm in diameter was studied. In surface view, the bark appears light brown, shallow fissured, flaky, flakes firm, scaling off in powder.

Slash features

Bark samples ranging in thickness from 3–4.5 mm were slashed. The inner surface of the bark is pearly white; inner boundary of the bark even and smooth. In sectioned view, the inner bark (secondary phloem) appears distinct in colour and texture from the outer bark (periderm). The inner bark is dull white and granular in texture; medullary rays are not evident; outer bark dark and homogeneous.

Microscopical features

The inner bark consists of a narrow zone of non-collapsed conducting phloem and a broad zone of outer non-conducting phloem. The inner phloem has small sieve tubes, companion cells and axial parenchyma. The outer zone has brachysclereids in scattered groups (Fig. 1.4–6). The ground tissue consists of enlarged parenchymatous cells. The cells of the ray parenchyma are tangentially oblong, less compact, so that minute intercellular spaces occur. The axial parenchyma cells are polygonal in shape and are smaller than the ray cells. Both the axial and ray parenchyma cells contain densely-packed granular starch grains (Fig. 1.6).

The inner bark is delimited externally by a distinct zone of cortex, where the homogeneous cortical parenchyma cells are tangentially elongated, some of them radially divided. The cortex seems to persist, not being used up in the periderm development.

The outer bark is represented by a narrow zone of periderm. A single persistent phellogen functions to produce the simple periderm (Fig. 1.4 and 5). The periderm consists of homogeneous phellem and one or two layers of phelloderm. The latter is not distinct from the cortical cells. The phellem cells are thin walled, tangentially oblong and suberised. Their tangential walls are raised outward. The phellem breaks in the form of small flakes along larger, non-suberised layers of cells formed at frequent intervals in the periderm.

Discussion

The comprehensive analysis of the anatomical features of roots of *Clerodendrun serratum* here presented may help to evaluate the usefulness of these characters in establishing the botanical identity of the taxon. While discussing the purpose of systematic anatomy, Metcalfe (1979) has correctly pointed out that any exercise that involves the identification of vegetable material when it is in a fragmentary or partly decomposed condition, can be achieved only by the methods of comparative histology. This applies in establishing the identification of economic plant products ranging from timbers to foodstuffs as well as crude drugs of vegetable origin. Adulterants and substitutes can also be detected. Concerning to the subterranean part of the plant, it may be noted that the correct identification of roots can often be achieved only by microscopical investigation. In the case of *C. serratum*, the dried root is used as a crude drug and when a question arises regarding the purity of the drug, microscopic features help us to solve the problem. It has been reported that substitution and adulteration happen quite frequently in samples of *C. serratum.*

The origin of the first phellogen in the young root of *C. serratum* is evidently from the sub-epidermal cell layers. This observation is in accordance with that of Prasad et al. (1967). In the root of *Clerodendrum indicum*, the phellogen is reported to be pericyclic in origin, so that the cortex is lost during the process of periderm development. This feature is an important character identifying the root of *C. serratum* and distinguishing it from *C. indicum*. The secondary xylem of the root (wood) of *C. serratum*, exhibits certain features not shared by those of the adulterants. In the secondary xylem of old root of *C. serratum*, fairly distinct growth rings are evident, which are marked by the occurrence of boundary parenchyma and isolated nests of vascular tracheids. In the roots of *Premna* and *Gardenia*, this feature seems to be lacking. Further, in *C. serratum*, the bark is characterised by the presence of groups of scattered brachysclereids in the secondary phloem, which constitute the sclerenchymatous components. In *Premna* and *Gardenia* species, the sclerenchymatous elements of the bark are true, libriform fibres. Thus, the type of sclerenchyma in the root barks of *Clerodendrum*, *Premna* and *Gardenia* offer an easily accessible clue to their identity.

Nayar et al. (1976) procured a root drug from Bangalore crude drug market, which was sold under the name of Bharangi. In their attempt to identify the drug, they found that its source was *Premna herbacea* of the Verbenaceae. They were also able to detect some differences between the roots of *P. herbacea* and *C. serratum* in the microscopical features. According to them, *P. herbacea* has nodulated roots, "inconspicuous" phellogen, compound starch grains in the "secondary" cortex, secondary phloem and xylem parenchyma, pericycle without "hard bast," parenchymatous pith and "fibres" with "scalariform" or simple pits. Presumably, their observation might have been based on a sample of

root-bark in which the phellogen was inactive. They seem to refer to the vessel elements with scalariform lateral wall thickenings, that were mistaken for the fibres. The presence of a parenchymatous pith in the root claimed by them also seems to be subject to verification. However, some of their observations on *P. herbacea* provide valuable clues that help to differentiate *P. herbacea* from *C. serratum.* Nodulated roots, the presence of true fibres in the bark and compound starch grains in the bark tissue and absence of stone cells in the cortex are some of the characters of *P. herbacea*, which demarcate *P. herbacea* from *C. serratum.*

From this investigation on roots of *C. serratum*, the plant considered to be the source of *Sirutekku*, the following diagnostic characters are presented:

1. Bark with a simple, homogeneous periderm and a single persistent phellogen.
2. Secondary xylem with fairly distinct growth rings, boundary parenchyma, vascular tracheids and vessels fibres.
3. Secondary phloem with scattered masses of brachysclereids.
4. Xylem rays mostly multiseriate and heterocellular.
5. Vessel elements short and cylindrical with simple, horizontal perforation plate circular or tangentially elongated lateral wall pits and abundant tyloses.

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