AN ANATOMICAL STUDY OF ETHIOPIAN KHAT (LEAF OF *CATHA EDULIS* FORSK)

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The morphology and anatomy of the leaves of *Catha edulis* Forsk. obtained from Ethiopia is described and illustrated. A list of the characters of powdered Khat is also given.

KHAT is the arabic name for the shrub, *Catha edulis* Forsk. (Family Celastraceae), the leaves of which are used as a traditional medicine in various regions of East Africa and Arabia. The leaves are, however, more commonly used for other reasons and the habitual consumption of khat has created deep social problems in many of these countries.

The United Nations Bulletin on Narcotics (1956) includes a comprehensive review on khat which indicates that among the poorer sections of the people of East Africa and Arabian countries, where the leaf is chewed to alleviate the sensations of hunger and fatigue, both its occasional and habitual use may lead to serious illness and even madness or death. In 1957 the Bulletin contained an article which deals specifically with the social and medical problems created by its use in Djibouti. However, the 7th Report of the Expert Committee on Addiction Producing Drugs (1957) did not consider that khat could be classified within the accepted definitions of drug addiction or drug habituation. Nevertheless, the French Government decided to classify Khat as a narcotic and for this purpose defined khat as the leaves only of this plant.

According to Paris (1958) there are several varieties of the plant obtained from different geographical sources but the most esteemed, and, according to some authorities, the only acceptable material is that obtained from Ethiopia where it is specially cultivated in the Harar district. There is certainly a regular air transport of freshly collected khat between Addis Ababa and cities and towns in neighbouring African and Arab countries where it is in great demand in the bazaars.

The identification of the leaves of *Catha edulis* Forsk in the broken or powdered condition as well as when whole, may frequently be necessary for forensic purposes and as previous descriptions appear to be inadequate, poorly illustrated and sometimes inaccurate, it was decided to make a detailed examination of the leaf.

Previous Investigations

The first anatomical description of the leaf was given by Collin (1893) and apart from the inadequate description of the fibres it is substantially

In recording the measurements, the system adopted by Moll and Janssonius (i.e. the dimensions in the radial (R), tangential (T) and longitudinal (L) directions of growth) is used in most instances for the sizes of cells. Where, however, this system might be confusing or ambiguous, the ordinary method of recording the longest and shortest axes irrespective of direction of growth, is given.

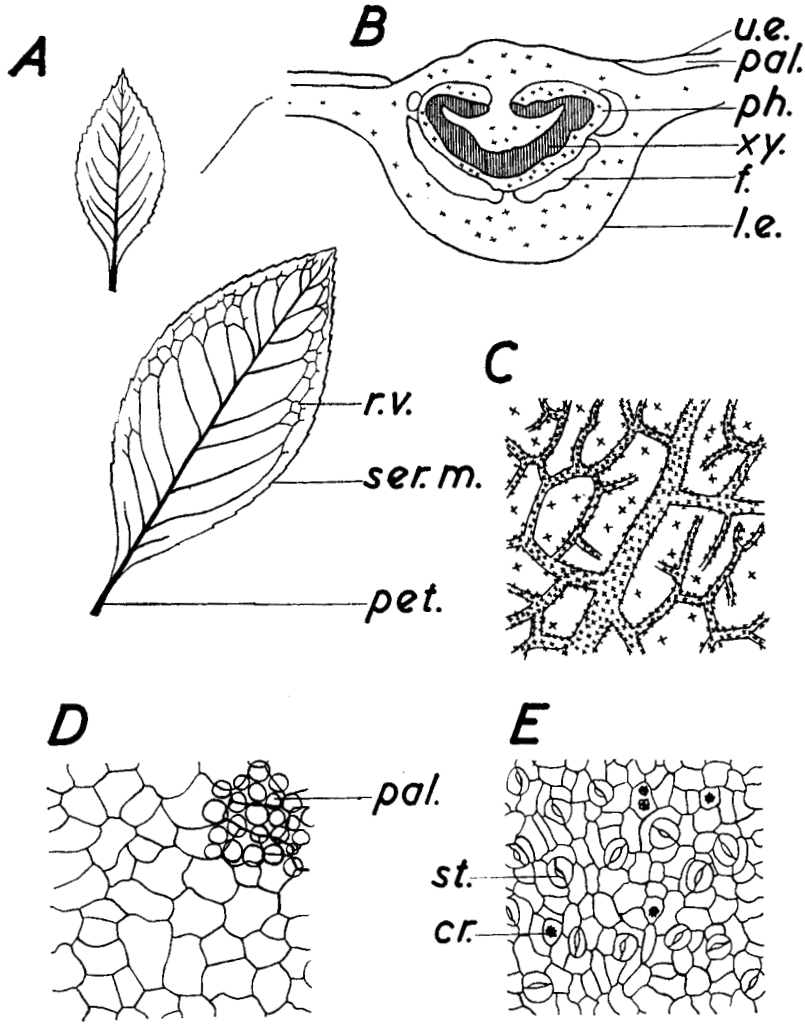

FIG. 1. A, Leaf, natural size. B, Transverse section through midrib × 15. C, Lamina showing venation and distribution of calcium oxalate × 240. The x represents cluster crystals. D, Upper epidermis (surface view) × 240. E, Lower epidermis (surface view) × 240. cr., cluster crystals of calcium oxalate; f., fibres; l.e., lower epidermis; pal., palisade; pet., petiole; ph., phloem; r.v., reticulate venation; ser.m., serrate margin; st., stomata; u.e., upper epidermis; xy., xylem.

correct. The drawings, however, are not good in comparison with present day standards. Perrot (1943–44) gives only a brief and incomplete description in which he refers to the crystals of calcium oxalate as twin crystals. The British Pharmaceutical Codex (1949) includes a short account of the leaf (as an adulterant or substitute for Tea). The crystals of calcium oxalate are correctly described though no sizes are given and reference is also made to tanniferous cells. Paris (1958) gives a very

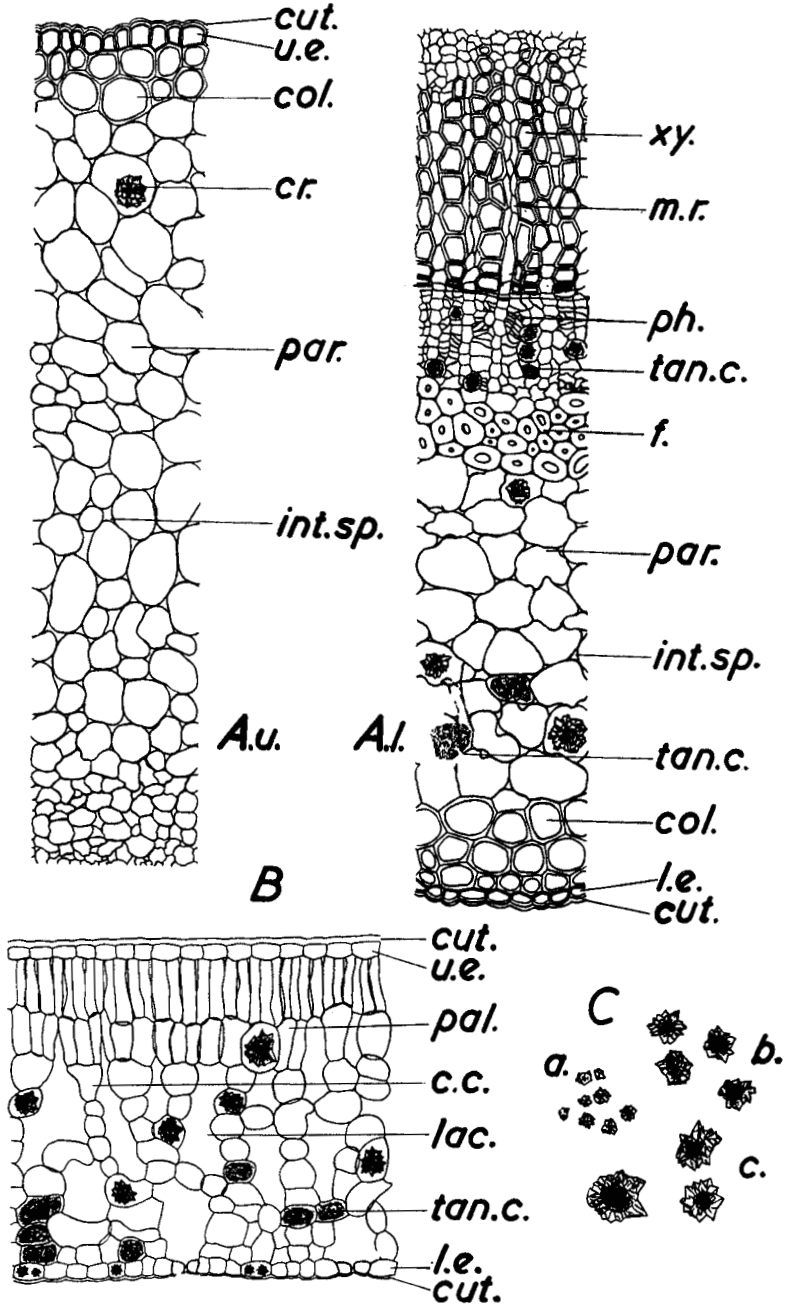

FIG. 2. A, Transverse section through midrib × 180. A u., upper portion, A l., lower portion. B, Transverse section through lamina × 180. C, Cluster crystals of calcium oxalate × 275: a, from the lower epidermis; b, from the phloem; c, from the parenchyma and mesophyll. c.c., collecting cell; cr., cluster crystals of calcium oxalate; col., collenchyma; cut., cuticle; f., fibres; int.sp., intercellular space; lac., lacunae; l.e., lower epidermis; m.r., medullary ray; par., parenchyma; ph., phloem; tan.c., tanniferous cell; u.e., upper epidermis; xy., xylem.

brief description of the features of the powdered leaf with some drawings but totally inadequate to be of great help in identifying the powdered leaf with any certainty.

Materials

The samples of khat used in this investigation were obtained as follows:

(*a*) a sample purchased in a bazaar in Aden in October 1959 by Mrs. V. Stewart and flown to England the same day.

(*b*) a sample collected in January, 1960, from cultivated plants of *Catha edulis* Forsk. in Ethiopia by J. Donner, Esq.

Both samples were presented to the Pharmacognosy Museum of the Chelsea School of Pharmacy.

Macroscopical Features

The slender twigs have from 10 to 16 leaves arranged in opposite pairs with occasional displacement at the base of the stem so that the lower leaves may appear to be arranged alternately. The leaves are simple and measure 4 to 11 cm. long and 1·8 to 5 cm. wide at the widest part. They have a short, round petiole, 3 to 7 mm. long.

In the fresh condition the leaves are bright green and the surfaces are glossy. When dry the leaves are paler, the colour of both surfaces being similar. The lamina is glabrous and slightly coriaceous. It is oval-lanceolate in shape with an acute or sometimes slightly acuminate apex and an acute, symmetrical base. With the exception of a small portion near the base which is entire the margin is serrate.

The upper surface shows distinct pinnate venation but this is more marked on the lower surface, the midrib being prominent, round and yellow to reddish brown in colour. A few of the lateral veins leave the midrib at a fairly wide angle (45°) but the majority leave at a narrow angle (25 to 30°) and immediately curve upwards. Near the margin the lateral veins anastomose to form a reticulate venation (Fig. 1,A).

The leaves are odourless and have a slightly astringent taste.

Microscopical Features

Lamina. The upper epidermis consists of a single layer of polygonal cells covered with a fairly thick, smooth cuticle. The outer walls of the cells are thickened with cellulose but the anticlinal walls which are somewhat sinuous are not thickened to any great extent. No trichomes or stomata are present (Figs. 1,D and 2,B).

The cells measure about 12 to 55 μ long, 10 to 36 μ wide and 10 to 16 μ high.

The appearance of the epidermal cells over the veins is similar to the remainder of the upper epidermal cells.

The mesophyll is clearly differentiated into palisade and spongy mesophyll. The palisade consists of two rows of thin walled cylindrical cells, those of the inner row being usually shorter and wider than those of the upper row.

The Palisade Ratio is 2·75–**3·18**–4·0.

Apart from the chloroplasts there are no distinctive cell inclusions in the upper row but occasionally the cells of the lower row are enlarged and contain a cluster crystal of calcium oxalate. Frequently the cells of the lower row are in groups of 2 or 3 with spaces between them. Sometimes a cell of the inner row is "Y" or funnel shaped and probably functions as a collecting cell from the adjacent cells of the upper row.

The spongy mesophyll which consists chiefly of thin walled rounded to elongated cells show three interesting features: (i) the cells adjacent to the palisade cells are often larger and "Y" shaped, each being in contact with 2 or 3 palisade cells and probably function as collecting cells ("sammezellen" Haberlandt, 1909). (ii) the cells are arranged to show a pattern of regularly spaced large air spaces (lacunae). (iii) the cells adjacent to the lower epidermis are more closely arranged in 2 or 3 rows and except where there are stomata in the epidermis show no large air spaces.

Some of the cells of the mesophyll contain cluster crystals of calcium oxalate measuring 12 to 32 μ in diameter. Other cells, particularly those near to the lower epidermis contain slightly granular contents which stain brown with dilute solution of iodine, greenish black with solution of ferric chloride and orange red with Sudan III. Sometimes the contents are coloured pale brown and in such cases the colour reaction with Sudan III is not obvious. These are the cells referred to in the British Pharmaceutical Codex as "tanniferous cells" but it is probable that the contents are resinous since they are not readily soluble in water but are soluble in ethanol (90 per cent).

The lower epidermis consists of a single layer of polygonal cells, the walls of which are thickened in a similar manner to those of the upper epidermis. The anticlinal walls are only slightly sinuous. The epidermis is covered with a fairly thick, smooth cuticle. Some of the cells contain one or two small crystals of calcium oxalate which are either clusters or conglomerates of few components; these crystals are smaller than those in the mesophyll and measure 4 to 8 μ in diameter. No trichomes are present but there are a large number of anomocytic stomata surrounded by 3 or 4 (occasionally 5) cells. The epidermal cells measure about 10 to 40 μ long, 8 to 20 μ wide and 8 to 12 μ high. (Figs. 1,E and 2,B.)

The Stomatal Index is 10·4–**14·2**–18·8.

The appearance of the lower epidermal cells over the veins differs from the remainder of the epidermis. There are no stomata, no cells containing crystals of calcium oxalate and seen in surface view the cells are irregularly rectangular in shape.

When a cleared piece of lamina is examined microscopically cluster crystals of calcium oxalate are seen to accompany all the veins (Fig. 1,C). The crystals measure 7 to 25 μ in diameter. Examination of the transverse sections of the lamina through the lateral veins show them to have similar features to the midrib, the crystals of calcium oxalate being in the phloem parenchyma.

The Vein Islet Number is 4·0–**5·1**–7·0.

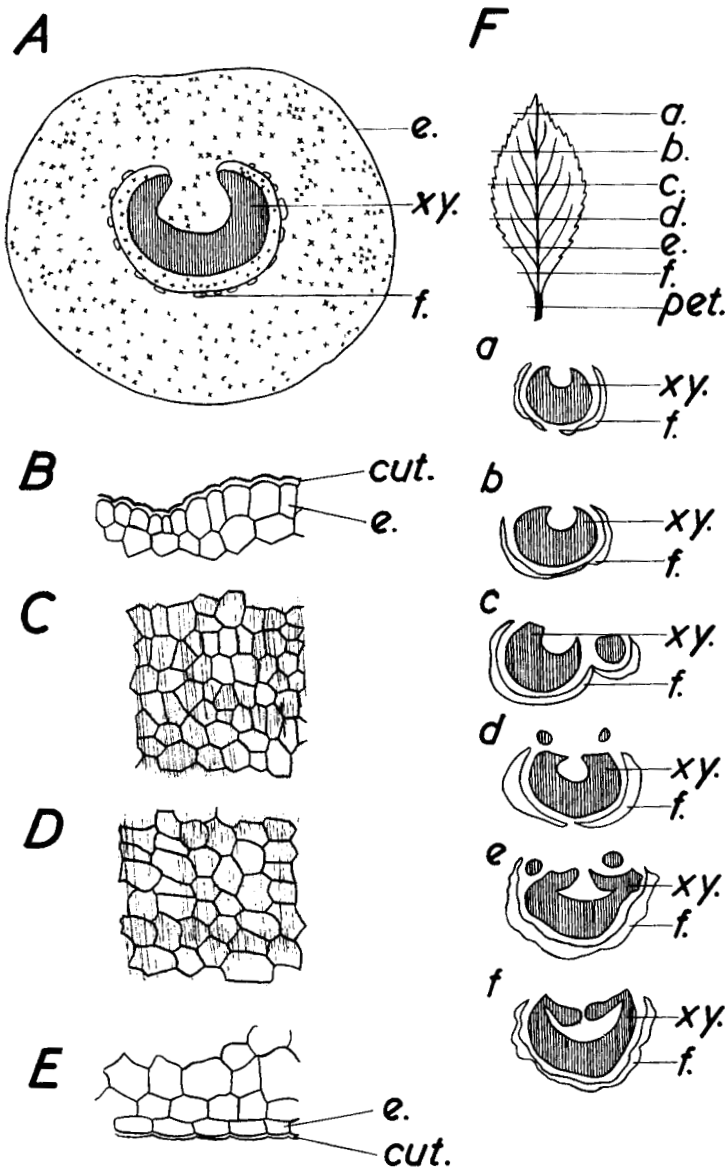

FIG. 3. A, Transverse section through petiole × 25. B, Transverse section through upper epidermis of petiole × 180. C, Surface view of upper epidermis of petiole × 180. D, Surface view of lower epidermis of petiole × 180. E, Transverse section through lower epidermis of petiole × 180. F, Transverse sections through midrib of leaf in position as shown (a to f) × 10. cut., cuticle; e., epidermis; f., fibres; xy., xylem. The x represents cluster crystals of calcium oxalate.

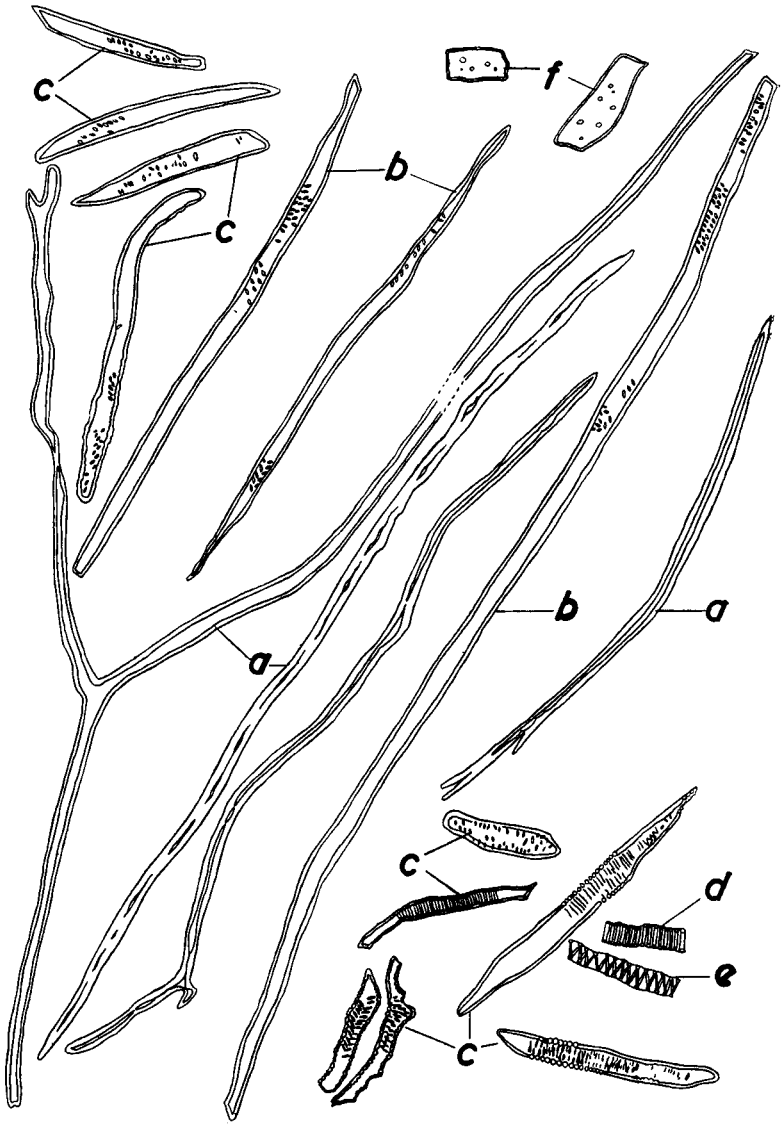

FIG. 4. Isolated elements from the veins × 180: a, fibres from pericyle; b, fibre tracheids; c, tracheids; d, annular vessels; e, spiral vessels; f, parenchyma from medullary rays.

Midrib. Both upper and lower epidermises are similar to those already described as covering the veins of the lamina except that the cell walls are thicker and that over the lower portion of the midrib they gradually change in appearance to resemble the epidermises of the petiole.

The palisade of the lamina is discontinuous over the midrib; collenchyma occurs beneath the epidermis and in older leaves may be quite extensive though in younger leaves may only constitute a few rows of cells. The remainder of the midrib cortex consists of large, rounded parenchymatous cells with small intercellular spaces. In the younger leaves the cell walls are thin but in the older leaves they may be thickened with cellulose. Tanniferous cells are present, particularly near to the lower epidermis.

No starch sheath (endodermis) appears to be present.

The meristele is arcuate in shape but the actual shape of the xylem and the presence or absence of additional meristeles depends upon the position along the midrib at which the section is cut (Fig. 3,F). The phloem consists of sieve tubes, companion cells, phloem parenchyma and some small tanniferous cells. No fibres are present in the phloem which is subtended by a crescent of pericyclic fibres. The extent of the development of this fibrous tissue depends upon the age of the leaf—the older leaves containing many fibres and the younger leaves having fewer fibres occurring in groups of 4 to 10 fibres only. The individual fibres have thick, highly refractive walls up to 5 μ thick. The walls give intense colours with cellulose stains but they are also slightly lignified. The lumen varies in diameter and in places may be entirely obliterated. Many of the fibres are branched tripartly and the ends of the fibres are frequently bifurcated (Fig. 4,a). The fibres have the following dimensions: R and T = 8 to 18 μ, L = frequently greater than 1,000 μ.

The xylem consists of radial rows of vessels, tracheids and fibre tracheids with well defined medullary rays (Fig. 2,A). The cell walls of all the xylem elements are lignified. No crystals of calcium oxalate occur in the cells of the medullary rays. The vessels show spiral and annular thickening and may be up to 20 μ in diameter (Fig. 4,d and e). Most tracheids have elongated oval bordered pits though some tracheids show reticulate and scalariform thickening. These are more common in the xylem of the capillary veins. The tracheids measure R and T = 15 to 20 μ, L = 100 to 200 μ (Fig. 4,c). The fibre tracheids also have elongated bordered pits and measure R and T = 10 to 18 μ, L = 270 to 850 μ (Fig. 4,b).

Petiole. The upper epidermis consists of a single layer of cells covered with a fairly thick, rough cuticle which gives to the epidermis in surface view, a striated appearance. The outer walls are convex and the anti-clinal walls are straight. No trichomes or stomata are present. The cells measure about 8 to 24 μ long, 6 to 18 μ wide and 8 to 24 μ high. The lower epidermis is similar in appearance to the upper epidermis except that the outer walls are not so convex and, seen in surface view the cells are larger. The cuticle is striated; and trichomes, stomata and crystals of calcium oxalate are absent. The cells measure about 12 to 48 μ long, 8 to 24 μ wide, and 4 to 8 μ high (Figs. 3,D and E).

The cortical region consists of parenchymatous cells, some of which are tanniferous cells. A large number contain cluster crystals of calcium oxalate the maximum size of which is 40 μ in diameter.

The stelar region is similar to that in the midrib, the only difference being in the smaller number of pericyclic fibres which occur in groups of 2 to 8 fibres arranged as a crescent shaped tissue. All the cells of the meristele are similar to those already described for the midrib (Fig. 3,A).

Characters of Powdered Khat

1. Fragments of lamina showing veins accompanied by cluster crystals of calcium oxalate measuring 7 to 25 μ in diameter.
2. Fragments of lamina showing the upper epidermal cells with sinuous anticlinal walls. Stomata are absent and cells of the palisade may be present.
3. Fragments of lamina showing the lower epidermal cells with slightly sinuous anticlinal walls. Numerous anomocytic stomata surrounded by 3 or 4 (occasionally 5) cells; some cells may contain one or two cluster crystals of calcium oxalate 4 to 8 μ in diameter.
4. Parenchymatous cells containing cluster crystals of calcium oxalate measuring chiefly 12 to 32 μ in diameter with some up to 40 μ.
5. Cluster crystals of calcium oxalate 4 to 40 μ in diameter.
6. Fragments of thick walled, slightly lignified pericyclic fibres with lumen of variable width, often obliterated. The cell walls also show intense colouration with cellulose stains. The fibres may show tripartite branching and the ends may be bifurcated.
7. Tanniferous cells, the contents of which stain brown with dilute solution of iodine, greenish black with solution of ferric chloride and orange red with Sudan III.
8. Fragments of striated, straight walled epidermal cells from the petiole.
9. Fibre tracheids with lignified walls and having elongated oval bordered pits.
10. Tracheids, some of which show scalariform and reticulate thickening and others with elongated bordered pits.

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