THE MORPHOLOGY AND ANATOMY OF THE LEAF OF *PODOPHYLLUM PELTATUM* L.*

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A brief review of the history of *Podophyllum peltatum* L. is given, together with an illustrated account of the macroscopical and anatomical structure of the leaf. The diagnostic characters of the powdered leaves are recorded and compared with those previously reported for *Podophyllum hexandrum* Royle. To distinguish the leaves of the two species in powder form, recourse must be made to palisade ratios: for *P. peltatum*, the values are 7·0–**8·5–12·16**–17, and for *P. hexandrum*, 4·0–**4·96–7·68**–11·25.

Podophyllum peltatum L. was first introduced into Britain as a horticultural plant and was listed in Evelyn's Kalendarium (1699) under the name *Anapodophyllum* Tournefort. This name, later shortened by Linnaeus (1737) to *Podophyllum*, was derived from πούς, a foot and φύλλον, a leaf. Other common names were listed by Britten and Brown (1897) and Holm (1907) and include May Apple, Wild Lemon, Raccoon Berry, Duck's Foot and Wild or American Mandrake. Holm (1907) reported that the root and rhizome were used by the Cherokees as an anthelmintic and by the Osage Indians as a cure, by purgation, for poisoning; Lloyd (1910) noted that the Wyandottes regarded them as cathartics and emetics. The leaves appear to have been little used and Bentley (1861) reported that they contained a poisonous principle which was lost on drying. Duffield (1868) isolated only 0·3 per cent w/w of resin from the leaves whilst Husband (1869) reported resin absent but Carter (1886) obtained 6 per cent w/w of resin from leaves collected soon after flowering. Recent investigations by Hussain, Chaudhri, Muhammed and Wahhab (1954) of the resin content of *P. hexandrum* Royle indicated that further work was necessary and our preliminary estimations by the method previously described (Ellis and Fell, 1962) have established an average resin content of 4·2 per cent w/w in leaves of *P. peltatum*.

Holm (1907) described the anatomy of the leaf briefly together with that of the stem and underground organs and the present work was undertaken to expand this description and to note the diagnostic characters necessary for the identification of whole or powdered leaves and to distinguish them from those of *P. hexandrum* Royle. As it proved impossible to raise *P. peltatum* L. from seed under the conditions available and as the germination of the seeds and the variations in the morphology of the flowering plants have already been described by Holm (1899) and Porter (1877) no details of development are given.

* The subject-matter of this text forms part of a thesis submitted by one of us (S. E.) for the external degree of M.Pharm., University of London.

Material and Methods

The leaves were supplied (i) by Philadelphia College of Pharmacy and Science, Philadelphia (ii) Lafayette College, Easton, Pennsylvania, (iii) by Dr. T. E. Wallis, from plants grown in Kew Gardens, Surrey or (iv) were collected from plants supplied by various nurserymen and transplanted to a light peaty soil in West Yorkshire.

The techniques used in the examination of these leaves were similar to those described for *P. hexandrum* leaves (Ellis and Fell, 1962) excepting that the length of the maceration process with 5 per cent w/v potassium hydroxide solution was reduced to 10 to 15 min. in order to isolate the secretion cells in the whole condition as well as the lignified material.

Macroscopy

A high degree of variation occurs in the macroscopical appearance of radical and cauline leaves, but no significant differences were observed between the two types. The leaves (Fig. 1, *A–C*) measure from 10 to 25 cm. across and are peltate with a centric, or slightly eccentric, erect, cylindrical petiole. They are polygonal in outline, the lamina being palmatisect with 4 to 7 obovate, or ob-lanceolate, bifid lobes. The margin of the leaf is entire in the basal region and serrate near the tip. The leaf is curled around the petiole on emergence and the lobes are revolute. The acute apices of the lobes may retain this revolute margin and be permanently reflexed. The venation is palmate with one main vein per lobe arising from the apex of the petiole and running straight to the point of conjunction of the segments where it divides, each branch running to the apex of a segment. In each marginal tooth three ultimate veinlets unite to form the terminal network (Fig. 2, *A*). The veins, which are prominent on the under surface, are lighter in colour than the interneural tissue and are covered with long, silky, covering trichomes. Trichomes are also present along the margin and sparsely scattered over the interneural tissue. The lamina shows little or no mottling, but the petiole is mottled red and green and in some cases bears numerous covering trichomes at its upper end (Fig. 2, *A*, *B*, *D* and *G*; Fig. 6, *B*).

Anatomy

Lamina

Interneural Tissue (Fig. 2, *A–E* and Fig. 3, *B*).

The upper epidermis is covered by a thin, smooth cuticle. The epidermal cells are irregular in shape and measure about Lev L and B 55 to **84** to 119 μ and H 19 to **33** to 46 μ, with very wavy anticlinal walls. They contain prominent nuclei situated on the base of the cell. *Trichomes* are absent from this surface except along the margin and *stomata* are very rare. Within each marginal tooth 1 to 4 hydathodes, measuring about Lev L 37 to **40** to 44 μ and Lev B 25 to **32** to 35 μ, occur on a small proportion of the leaves examined (Fig. 2, *A* and *C*).

The mesophyll is not well differentiated and all the cells contain chloroplasts, which are spherical or subspherical. The *palisade* consists

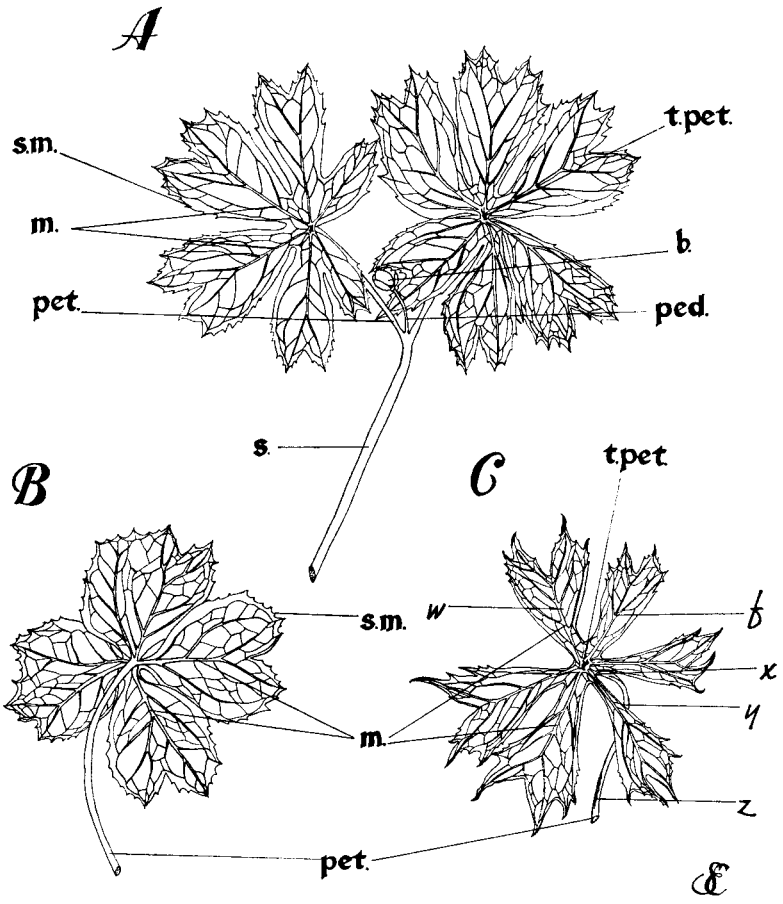

FIG. 1. Leaf of *Podophyllum peltatum* L. *A*, Flowering shoot; *B*, under surface of whole mature leaf; *C*, whole mature leaf of non-flowering plant. All × 1/5. "w," position of sections in Fig. 3, *A*; Fig. 4; Fig. 5, *A*; "x"–"z," position of sections in Fig. 5, *B–D*; "y" position of sections in Fig. 6; *b.*, flower bud; *m.*, main vein; *ped.*, pedicel; *pet.*, petiole; *s.*, stem; *s.m.*, serrate margin; *t.pet.*, top of petiole.

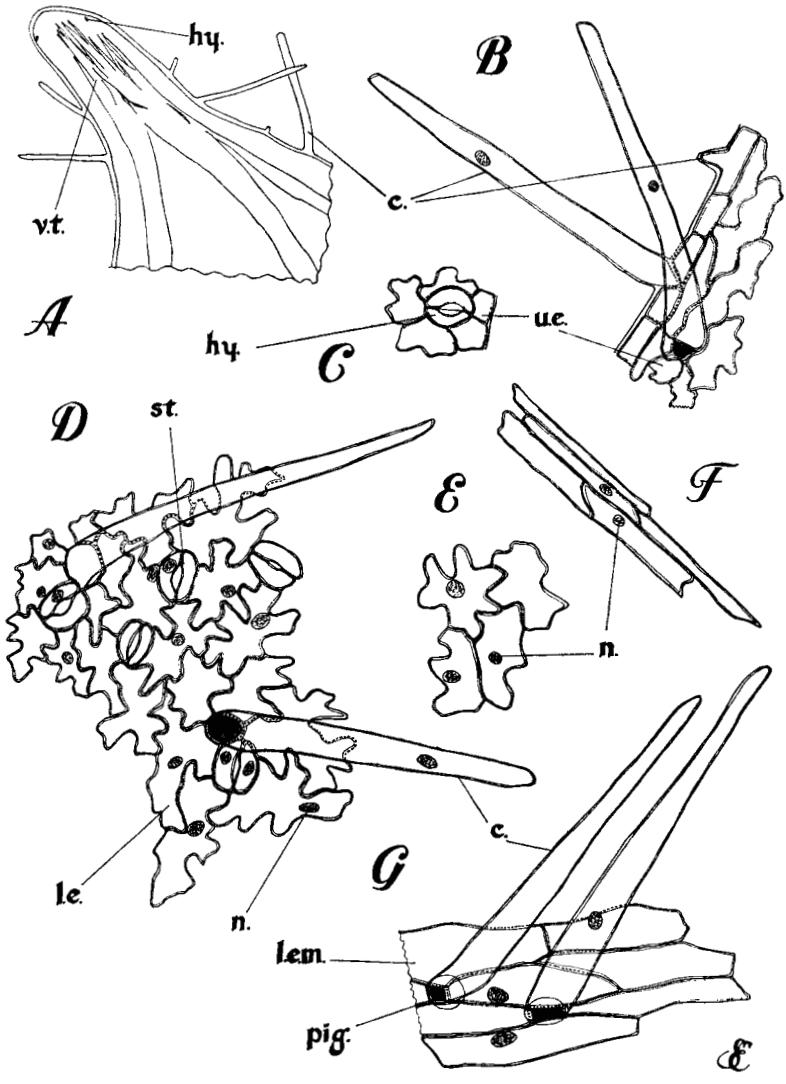

FIG. 2. Leaf of *P. peltatum* L. *A*, marginal tooth of leaf; *B*, upper epidermis of lamina; marginal region; *C*, upper epidermis of lamina with hydathode; *D*, lower epidermis of lamina, interneural region; *E*, upper epidermis of lamina; *F*, upper epidermis of main vein; *G*, lower epidermis of main vein. *A* × 30; *B–G* × 150. *c.*, covering trichome; *hy.*, hydathode; *l.e.* lower epidermis; *l.e.m.*, lower epidermis of main vein; *n.*, nucleus; *pig.*, pigment; *st.*, stoma; *u.e.*, upper epidermis; *v.t.*, veinlet termination.

of an ill-differentiated single layer of irregularly cylindrical cells measuring about Lev L and B 15 to **27** to 46 μ and H 27 to **43** to 78 μ. The *spongy mesophyll* is aereolate and consists of five or six layers of trabeculate parenchyma cells about 11 to **32·5** to 78 μ in diameter, and occasional rounded cells of the same diameter, with brown amorphous contents staining red with 50 per cent v/v nitric acid, with large intercellular spaces. *Calcium oxalate* occurs only rarely in the mesophyll but in some leaves rosette crystals are found near the veins (Fig. 3, *B* and *C*).

The LOWER EPIDERMIS is covered by a thin, smooth cuticle. The epidermal cells are irregular in shape, measuring about Lev L and B 55 to **91** to 137 μ and H 15 to **25** to 35 μ, with very wavy anticlinal walls. Anomocytic *stomata* are numerous, raised slightly above the level of the epidermis, ellipitical, or circular, in outline and measure about 36 to **45·5** to 61 μ in length and 25 to **34** to 40 μ in breadth, or 32 to **40** to 46 μ in diameter (Fig. 2, *D*; Fig. 3, *B*). *Covering trichomes* occur over the whole surface of the lamina but are most numerous near the larger veins and in the marginal region. Those of the interneural tissue measure about 200 to **310** to 470 μ in length and 29 to **37** to 47 μ in diameter at the base whereas those of the marginal region show a much wider variation in size measuring about 14 to **330** to 865 μ in length and about 14 to **32·5** to 61 μ in diameter at the base. They are thin walled, cellulosic and unicellular with a blunt, rounded apex (Fig. 2, *B* and *D*).

The lamina has a smooth margin with a few scattered acutely pointed teeth near the apices of the lobes. The ultimate veinlets extend to within about 0·1 mm. of the teeth apices and terminate in several small spiral elements. Three ultimate veinlets unite to form this terminal group about 0·6 mm. from the tip (Fig. 2, *A*).

Main Vein (Fig. 2, *F* and *G*; Fig. 3, *A* and *D*; Fig. 4; Fig. 5, *A*).

The central veins of each lobe and its segments are similar in construction. The transverse section, in the basal half of the lobe, shows three unequal bundles embedded in a central, cordate shaped mass of collenchyma (Fig. 5, *A*). The extreme tip of the lobe shows three, small, equal bundles which unite and subsequently redivide to give three unequal bundles after the point of conjunction of the two segments has been reached. The size of the central bundle increases gradually as small secondary veins enter and the amount of collenchyma increases proportionately. The entry of larger secondary veins alters the arrangement of the bundles temporarily, but it always returns to three. No unification of the main veins occurs before they enter the petiole.

The UPPER EPIDERMIS is composed of polygonal straight, or slightly wavy walled cells elongated along the axis of the lobe (Fig. 2, *F*; Fig. 3, *A*). They measure about Lev L 68 to **127** to 200 μ, Lev B 10 to **18** to 25 μ and H 23 to **31** to 39 μ. The cuticle is thicker than on the lamina but the nuclei are similar to those already described. *Trichomes* and *stomata* are absent.

The CORTEX is divided into two main regions. The upper hypodermal region is composed of thick-walled, collenchymatous cells measuring

about L 82 to **160** to 277 μ and R and T 19 to **38** to 74 μ throughout, but the lower hypodermal region becomes collenchymatous only in the basal half of the lobe, where it is large-celled, measuring about L 82 to **160** to 277 μ and R and T 21 to **39** to 58 μ. Both hypodermal regions contain scattered spherical, or sub-spherical chloroplasts. The remaining cortex is parenchymatous, the cells measuring about L 93 to **135** to 190 μ and R and T 20 to **38** to 75 μ above the stele and L 62 to **109** to 292 μ, R and T 45 to **76** to 117 μ below the stele. Rosette crystals of *calcium oxalate* occur scattered throughout the cortex in the larger cells, measuring about 15 to **28** to 43 μ in diameter.

The ENDODERMIS is not well differentiated but a continuous band of cells, which contain starch grains, measuring about 4 to **6** to 10 μ in diameter, can be traced around the central collenchymatous tissue surrounding the stele. As the parenchyma of the cortex does not contain starch this layer may be regarded as a starch sheath (Fig. 3, *A*).

The MERISTELE consists of three well defined bundles embedded in a mass of relatively thin walled collenchyma (Fig. 3, *A*; Fig. 5, *A*), measuring about L 117 to **195** to 320 μ and R and T 9 to **25** to 55 μ. The cells frequently contain nuclei and occasionally brown amorphous material, which stains red with 50 per cent v/v nitric acid.

The PHLOEM consists of sieve tissue with well defined companion cells and patches of small celled phloem parenchyma. The sieve tubes measure about R and T 11 to **17** to 31 μ, the individual segments being about 177 μ in length, with transverse, or oblique sieve plates. The companion cells are narrow being only 2 to **6·3** to 12 μ in diameter and 66 to **96** to 140 μ in length. There are distinct medullary rays between the three bundles, composed of cellulosic parenchyma, slightly elongated radially, measuring about R 19 to **31** to 43 μ and T 11 to **16** to 23 μ. There is no radial arrangement within the bundles, but phloem parenchyma cells, measuring about R and T 17 to **26** to 39 μ and L 78 to **130** to 200 μ and occasionally containing brown amorphous contents similar to those of the collenchyma, occur in small groups. Large secretory structures, which are unicellular with brown, thin, cellulosic cell walls and brown cell contents, staining red with 50 per cent v/v nitric acid, grey with ferric chloride solution and a faint pink with Sudan III, and measuring about R and T 19 to **33** to 51 μ and L 225 to **452** to 875 μ occur in small numbers in the phloem. In addition, in the main veins of larger leaves, in the region of the petiole apex, thick walled, non-lignified fibre-like cells occur in the pericyclic region. They measure about R and T 15 to **26** to 35 μ and L 70 to **84** to 175 μ and occur scattered in the outer region of the vascular collenchyma (Fig. 3, *A*; Fig. 4).

The *cambium* consists of an ill defined layer of thin walled, tangentially elongated cells.

The XYLEM consists of irregularly arranged vessels and tracheids with patches of xylem parenchyma. The vessels are lignified with spiral, annular or rarely reticulate thickening and measure about 11 to **25** to 39 μ in diameter. The tracheids and tracheidal vessels are of similar diameter and measure about 245 to **391** to 665 μ in length and show spiral, annular,

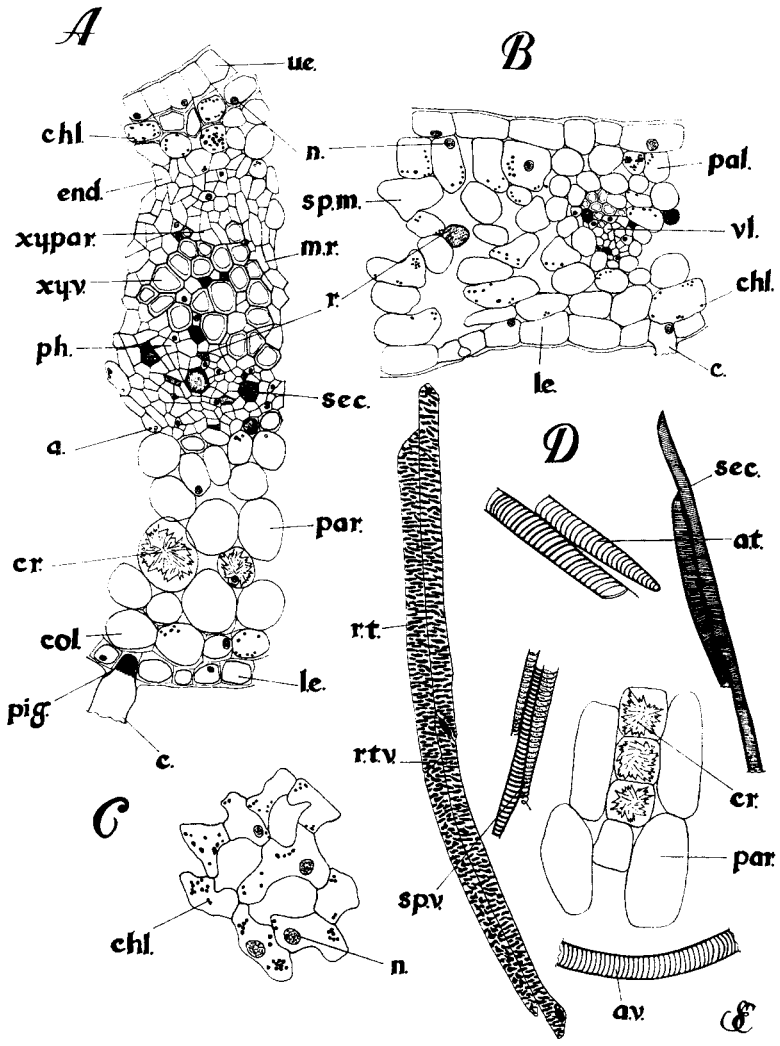

FIG. 3. Leaf of *P. peltatum* L. *A*, transverse section of the main vein cut at position "w" (see Fig. 1, *C*); *B*, transverse section of the lamina; *C*, spongy mesophyll; *D*, isolated elements of the main vein obtained by maceration. *A*, *B* and *C* × 150; *D* × 75. *a.*, starch; *a.t.*, annular tracheid; *a.v.*, annular vessel; *chl.*, chloroplast; *col.*, collenchyma; *cr.*, crystal of calcium oxalate; *end.*, endodermis; *l.e.*, lower epidermis; *m.r.*, medullary ray; *n.*, nucleus; *pal.*, palisade; *par.*, parenchyma; *ph.*, phloem; *pig.*, pigment; *r.*, resinous material; *r.t.*, reticulate tracheid; *r.t.v.*, reticulate tracheidal vessel; *sec.*, secretion cell; *sp.m.*, spongy mesophyll; *sp.v.*, spiral vessel; *st.g.c.*, stoma guard cell; *u.e.*, upper epidermis; *v.col.*, vascular collenchyma; *vl.*, veinlet; *xy.par.*, xylem parenchyma; *xy.v.*, xylem vessel.

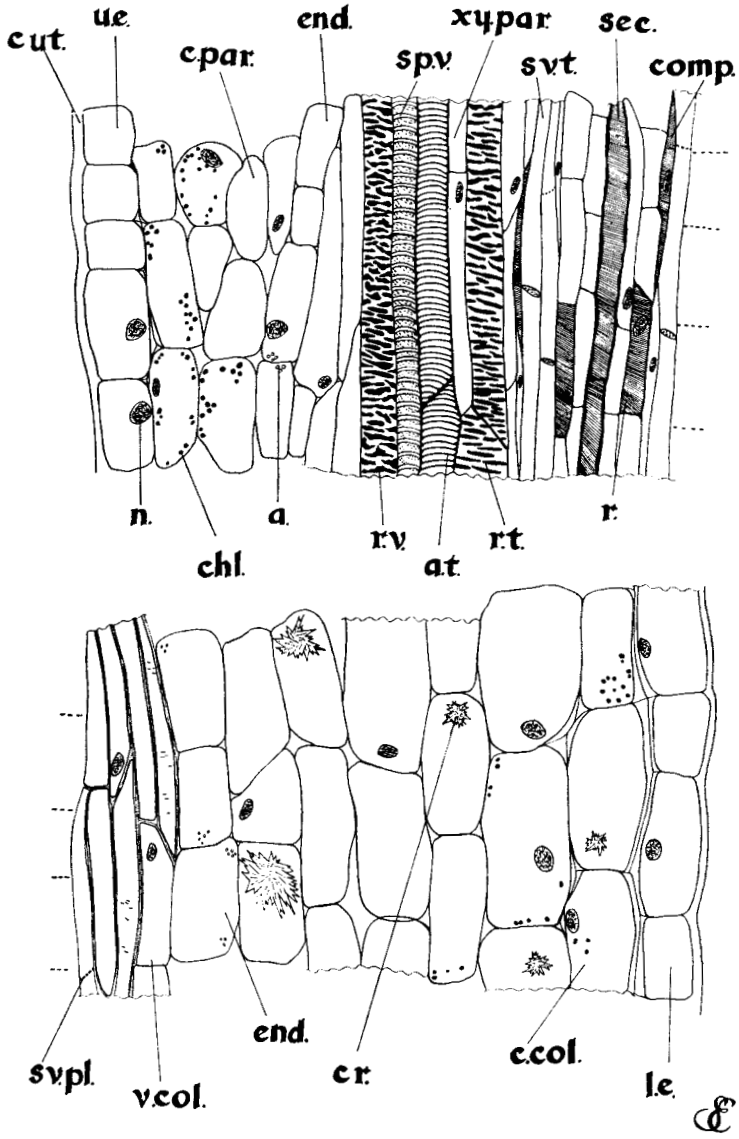

FIG. 4. Leaf of *P. peltatum* L. Longitudinal section of the main vein cut approximately at position "w" (see Fig. 1, *C*). × 150. *a.*, starch; *a.t.*, annular tracheid; *chl.*, chloroplast; *c.col.*, cortical collenchyma; *comp.*, companion cell; *c.par.*, cortical parenchyma; *cr.*, crystal of calcium oxalate; *cut.*, cuticle; *end.*, endodermis; *l.e.*, lower epidermis; *n.*, nucleus; *r.*, resinous material; *r.t.*, reticulate trachieid; *r.v.* reticulate vessel; *sec.*, secretion cell; *sp.v.*, spiral vessel; *sv.pl.*, sieve plate; *sv.t.*, sieve tube; *u.e.*, upper epidermis; *v.col.*, vascular collenchyma; *xy.par.*, xylem parenchyma.

reticulate or pitted thickening. The number of tracheids increases towards the tip of the lobe. The xylem parenchyma is thin walled, cellulosic; its cells have similar cell contents to those of the phloem parenchyma.

The LOWER EPIDERMIS (Fig. 2, *G*; Fig. 3, *A*; Fig. 4) is composed of large polygonal cells elongated along the axis of the lobe, measuring about Lev L 29 to **44** to 60 μ, Lev B 21 to **31** to 43 μ and H 27 to **40** to 63 μ with straight anticlinal walls. *Stomata* are absent but numerous unicellular *covering trichomes* occur. They measure about 288 to **550** to 820 μ in length and 25 to **38** to 54 μ in diameter at the base. The walls are thin and cellulosic and the trichomes have a blunt apex (Fig. 2, *G*).

PETIOLE (Fig. 5, *B—D*; Fig. 6).

The petiole is smoothly cylindrical, from 5 to 10 cm. long in cauline leaves and from 8 to 20 cm. long in radical leaves and from 3 to 6 mm. in diameter. The upper region, for a length of about 1 to 2 cm., bears long, silky covering trichomes similar to those of the main vein and the lower half of the petiole is frequently mottled red and green.

The vascular tissue occurs in two regions: (1) the outer ring of 15 to 25 bundles situated near the periphery and containing pericyclic fibres, phloem, cambium and xylem embedded in a mass of collenchyma and surrounded by a starch sheath. At the distal end of the petiole the pericyclic fibres are few, or absent, but the amount of lignified tissue gradually increases towards the base (Fig. 5, *B–D*). About 4 cm. below the leaf, the pericyclic fibres form an arc extending from cambium to cambium around the phloem and, 4 cm. further down, the whole of the vascular collenchyma within the starch sheath has been replaced by lignified fibres. Near the junction of the petiole with the stem, or rhizome, lignification of the interfascicular parenchyma in the pericyclic region occurs in a small proportion of leaves. In none of the leaves examined had this lignification become a complete ring (Fig. 5, *D*). (2) The central group of 6 bundles are asymmetrically placed in the pith parenchyma. These bundles contain a smaller number of pericyclic fibres, phloem, cambium and xylem embedded in collenchyma and surrounded by a starch sheath, one or two cells in breadth. The amount of lignified material increases towards the base, but in none of the leaves examined did the arc of pericyclic fibres extend to the cambium and the interfascicular parenchyma does not become lignified. The medullary bundles are irregularly arranged in the upper half of the petiole but tend to form a second, eccentric circle in the basal half (Fig. 5, *B–D*).

Tracing the entry of the primary leaf veins into the petiole shows that each vein divides on entry, part of the vascular tissue forming bundles in the outer ring and part entering the central bundles.

The EPIDERMIS consists of straight walled cells elongated longitudinally and measuring about Lev L 165 to **310** to 440 μ, Lev B 18 to **29** to 41 μ and H 19 to **28·5** to 39 μ. The cell walls are thin and cellulosic and the outer surface is covered by a thin cuticle. Anomocytic *stomata* measuring about 63 μ in length and 48 μ in breadth occur infrequently over the whole

of the petiole surface and unicellular, *covering trichomes*, closely resembling those of the main vein and measuring about 170 to **584** to 957 μ in length and 24 to **36** to 50 μ in diameter at the base, occur near the apex. Many cells contain red-brown pigment (Fig. 6; Fig. 7, B).

The CORTEX, like that of the main vein, consists of two layers of tissue. The outermost layer is a band of collenchyma several cells wide with thick cellulosic walls, measuring about R and T 15 to **29** to 39 μ and L 66 to **114** to 195 μ. The cells contain lenticular chloroplasts and in the outermost layers red-brown pigment. The remaining cortex is parenchymatous, occasionally becoming lignified and pitted in the pericyclic region of the basal half of the petiole. The cells are similar in size to the collenchyma being about R and T 15 to **39** to 63 μ and L 70 to **106** to 148 μ and frequently contain rosette crystals of calcium oxalate measuring about 27 to **36** to 59 μ in diameter (Fig. 6).

The ENDODERMIS takes the form of a starch sheath of one, or rarely two, layers of cells surrounding each bundle. The cells are similar in size and shape to the surrounding parenchyma but they contain numerous starch grains about 2 to **5** to 8 μ in diameter.

The PERICYCLIC FIBRES increase in number from the apex to the base of the petiole and eventually replace the whole of the vascular collenchyma in the outer bundles. The fibres are extremely long, measuring about L 580 to **1050** to 1,450 μ and R and T 5 to **16** to 31 μ, with thick, lignified, pitted walls and acute apices (Fig. 5, *B–D*; Fig. 7, *A*).

The *vascular bundles* of the inner and outer rings differ only slightly, the main difference being in the relative proportions of lignified fibres and collenchyma to conducting tissue; the inner ones have more conducting tissue and collenchyma and fewer fibres. In both cases at the apex of the petiole the bundles are embedded in collenchyma, the cells of which measure about L 97 to **215** to 390 μ and R and T 11 to **24** to 43 μ and have thin cellulosic walls.

The PHLOEM consists of groups of sieve tubes about 11 to **17** to 27 μ in diameter, the individual segments being about 78 to **162** to 280 μ in length; irregular groups of phloem parenchyma occur, individual cells measuring about L 46 to **107** to 226 μ and R and T 11 to **19·5** to 31 μ, and frequently containing brown cell contents similar to those of the main vein.

The XYLEM is well defined, the elements being irregularly arranged. The conducting elements resemble those of the main vein with a higher proportion of vessels to tracheids and a larger amount of reticulate and pitted thickening (Fig. 7, *A*). A little xylem parenchyma, measuring about 7 to **15** to 28 μ in diameter and 58 to **107** to 195 μ in length occurs close to the indistinct cambium and associated with the groups of vessels. The parenchyma frequently contains nuclei and, less often, brown cell contents similar to those of the phloem. The vessels are larger than those of the main vein being about 11 to **30** to 47 μ in diameter. The tracheids and tracheidal vessels have diameters within this range and measure about L 400 to **655** to 1260 μ (Fig. 7, *A*).

The PITH is composed of large celled parenchyma in which little or no lignification occurs. The cells are thin walled and measure about L 39 to

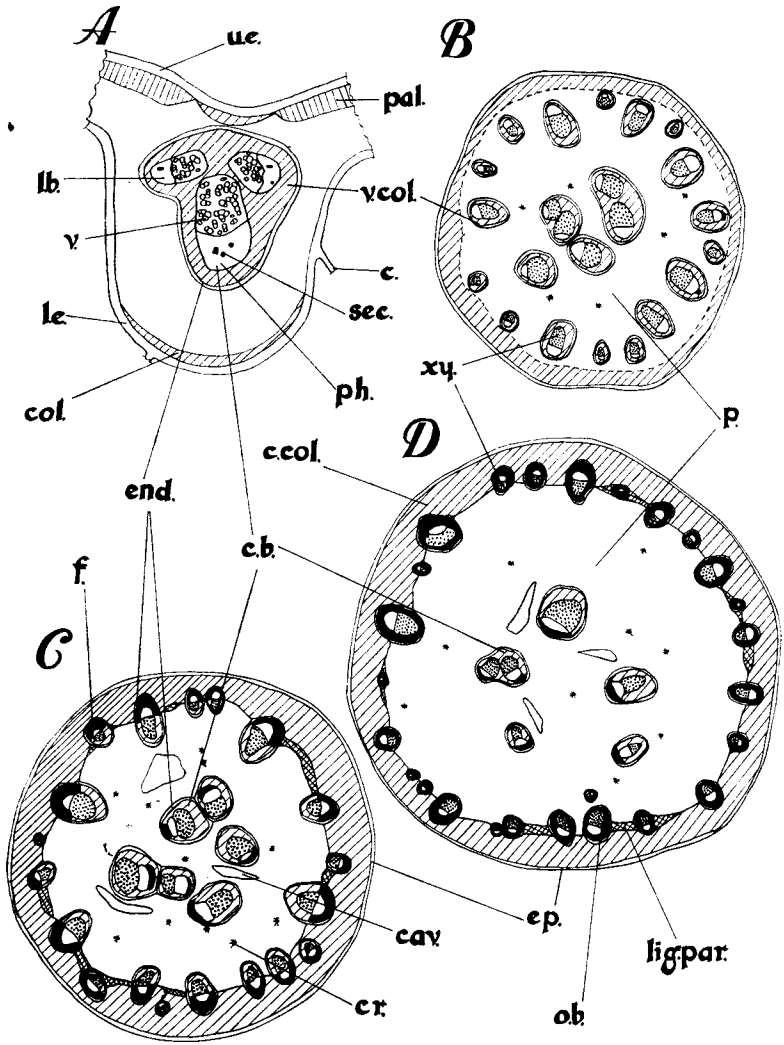

FIG. 5. Leaf and Petiole of *P. peltatum* L. *A*, transverse section of the main vein cut at position "w"; *B–D*, transverse sections of the petiole cut at positions "x," "y" and "z" (see Fig. 1, *C*). *A* × 40; *B–D* × 10. *c.*, covering trichome; *cav.*, cavity; *c.b.*, central bundle; *c.col.*, cortical collenchyma; *col.*, collenchyma; *cr.*, crystal of calcium oxalate; *end.*, endodermis; *ep.*, epidermis; *f.*, fibre; *l.b.*, lateral bundle; *l.e.*, lower epidermis; *lig.par.*, lignified parenchyma; *o.b.*, outer bundle; *p.* pith; *pal.*, palisade; *ph.*, phloem; *sec.*, secretion cell; *v.*, vessel; *v. col.*, vascular collenchyma. *xv.*, xylem.

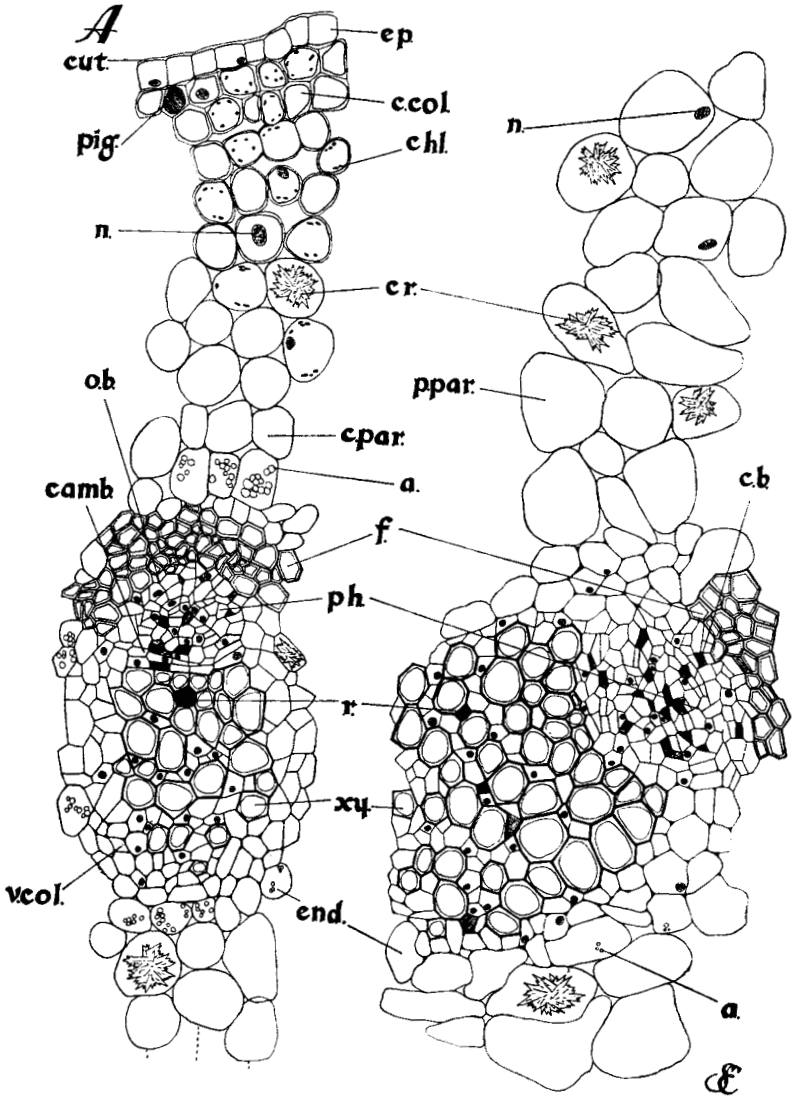

FIG. 6. Petiole of *P. peltatum* L. Transverse section of petiole cut at position "y" (see Fig. 1, *C*). × 150. *a.*, starch; *camb.*, cambium; *c.b.*, central bundle; *c.col.*, cortical collenchyma; *chl.*, chloroplast; *c.par.*, cortical parenchyma; *cr.*, crystal of calcium oxalate; *cut.*, cuticle; *end.*, endodermis; *ep.*, epidermis; *f.*, fibre; *n.*, nucleus; *o.b.*, outer bundle; *ph.*, phloem; *pig.*, pigment; *p. par.*, pith parenchyma; *r.*, resinous material; *v.col.*, vascular collenchyma; *xy.*, xylem.

85 to 183 μ and R and T 23 to 77 to 175 μ. Large rosette crystals of *calcium oxalate* measuring about 27 to 36 to 59 μ in diameter occur in vertical files of more or less cylindrical cells scattered throughout the pith but more common near the vascular bundles (Fig. 6; Fig. 7, *A*).

Powder

The colour of a No. 40 powder varied from light green to brown, according to the time of collection of the leaves; it has a slightly acrid odour and a bitter taste. When a small amount of powder is mixed with (a) 5 per cent w/v copper acetate solution a bright green colour develops and (b) with 5 per cent w/v ferric chloride solution, a gradual darkening of the fragments of tissue is observed.

To examine the structural features of the powder mounts were prepared using 50 per cent v/v glycerol solution, solution of chloral hydrate or phloroglucinol and hydrochloric acid. The characters of the powder (Fig. 8) are:

1. Very numerous whole, or fragmented unicellular *covering trichomes.*
2. Fragments showing, in surface view, the wavy walled cells of the *upper epidermus of the interneural lamina* and usually the underlying palisade and, in the marginal region, covering trichomes of varying lengths or an occasional cicatrix.
3. Fragments of the *lower epidermis* showing, in surface view, the very wavy walled epidermal cells, anomocytic *stomata* and the bases or cicatrices of covering trichomes.
4. Less frequent particles showing the straight walled cells of the *upper epidermis of the main vein* or the elongated straight walled cells of the *lower epidermis of the main vein,* with numerous trichomes or cicatrices.
5. Fragments of lamina in transverse sectional view, about 160 μ wide with a single ill-differentiated palisade.
6. Small spiral and annular vessels from the veins; large annular tracheids and vessels associated with secretory cells, which are thick walled and have orange-brown resinous contents, from the main veins; reticulate vessels with associated collenchyma and large sieve tubes from the petiole.
7. Lignified acutely pointed *fibres* with pitted walls from the petiole.
8. Large thin walled parenchyma cells, frequently containing rosette crystals of *calcium oxalate* from the pith of the petiole, the cortex of the main vein or more rarely the mesophyll of the leaf.
9. Scattered rosette crystals of *calcium oxalate.*

Proportion of Petiole. Investigations on samples available showed that the percentage of petiole present was about 18 to 24 per cent by weight of the dry leaf.

Discussion

Although it is comparatively easy to distinguish the whole leaves of *P. peltatum* L. and *P. hexandrum* Royle by the degree of incision, the number and shape of the lobes and the eccentricity of the petiole in relation to the lamina, they show a close similarity in broken or powdered condition.

These two species of the genus *Podophyllum* are characterised by the presence of long, unicellular, *covering trichomes* with cellulosic walls and blunt, rounded apices; long, lignified, pitted *fibres* with acute apices, frequently associated with sieve tissue or collenchyma; epidermal tissue from the *lower epidermis* of the *interneural lamina* composed of very wavy walled cells and having anomocytic *stomata*; rosette crystals of *calcium oxalate*; brown amorphous material staining orange-red with 50 per cent v/v nitric acid and the development of a green colour on the addition of 5 per cent w/v copper acetate solution. Only three structures can be considered of diagnostic value in distinguishing the two species *P. peltatum* and *P. hexandrum* by microscopical examination alone.

1. The presence of long, cellulosic, *secretory cells* with brown cell walls and orange-brown cell contents from the phloem parenchyma of *P. peltatum*.

2. The character of the *upper epidermis* of the *interneural lamina*, which is wavy walled in *P. peltatum* and almost straight walled in *P. hexandrum*; stomata are absent from this surface in both species.

3. The presence in *P. hexandrum* of fragments of lamina in transverse sectional view which show a *double palisade*. The palisade of *P. peltatum* is single and ill-differentiated.

Other differences which occur are merely a matter of degree. *P. peltatum* contains a large number of lignified *fibres*, but *lignified parenchyma* is rare, whilst in *P. hexandrum* fibres are fewer and frequent fragments of lignified parenchyma occur. The amount of lignified parenchyma will depend upon the number of long petioles included in the sample and hence may vary widely.

P. peltatum contains a larger number of *calcium oxalate* crystals than *P. hexandrum* but the amount of calcium oxalate in either species varies according to the location of the plant and may not be constant.

TABLE I

DIAMETER OF THE CALCIUM OXALATE CRYSTALS

Leaf	Range of diameters in μ from 50 readings	Mean diameter	Standard deviation	t	t (from tables) P=0·01	t (from tables) P=0·05	Minimum number of readings
P. peltatum	17·5–26·98–38·51–50·04–63	38·51	11·53	4·205	2·571	1·970	21·3 adjusted to 22
P. hexandrum	11·5–20·91–29·84–38·77–52·5	29·84	8·95				

Note. $t = \dfrac{m_p - m_h}{\sqrt{\dfrac{s_p^2 + s_h^2}{N}}}$ Minimum number of readings $= \dfrac{\left(\dfrac{s_p + s_h}{2}\right)^{t^2}}{\left(\dfrac{m_p - m_h}{2}\right)^2}$

There is a variation in size between the crystals in the leaves of the two species and the examination of powders, prepared from leaves gathered in two different locations for each species, gave an average diameter of crystal of 38·5 μ for *P. peltatum* and 29·8 μ for *P. hexandrum* (see Table I).

The ranges of diameters overlap considerably but the variation in the means can be shown to be highly significant at the P = 0·05 and P = 0·01 probability levels. Further statistical calculations indicate that the size of the calcium oxalate crystals can be used as a diagnostic character provided 22 or more measurements are made.

P. peltatum also contains more trichomes, or trichome fragments, than *P. hexandrum* although they are of similar size, the lengths lying between 150 and 960 μ in the case of *P. peltatum* and 150 and 825 μ in *P. hexandrum*. The number of trichomes present on the whole leaf of *P. hexandrum*,

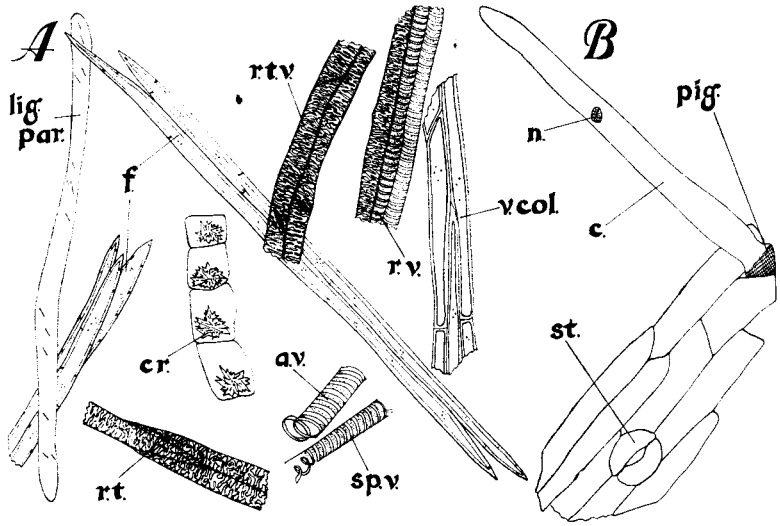

FIG. 7. Petiole of *P. peltatum* L. *A*, isolated elements from the petiole obtained by maceration; *B*, epidermis of petiole. *A* × 75; *B* × 150. *a.v.*, annular vessel; *c.*, covering trichome; *cr.*, crystal of calcium oxalate; *f.*, fibre; *lig.par.*, lignified parenchyma; *n.*, nucleus; *pig.*, pigment; *r.t.*, reticulate tracheid; *r.t.v.*, reticulate tracheidal vessel; *r.v.*, reticulate vessel; *sp.v.*, spiral vessel; *st.*, stoma; *v.col.*, vascular collenchyma.

especially in the marginal regions, has been observed to vary with the age of the leaf. Hence the proportion of trichomes present in a powder will vary according to the time of collection and any numerical differences between the two species based on this character can not be diagnostic.

The identification of these powders as belonging to the genus *Podophyllum* is straightforward, but there is insufficient differential evidence to assign them to their specific rank. The significance of palisade ratio and stomatal index of these species was examined in an attempt to effect a clearer distinction. The values were determined using the methods of Wallis and Dewar (1933) and Salisbury (1927) respectively and the figures from 20 positions on each of 20 leaves were subjected to statistical analysis.

The *Palisade Ratios* show a highly significant variation and this value could be used to distinguish the leaves of the two species in both broken

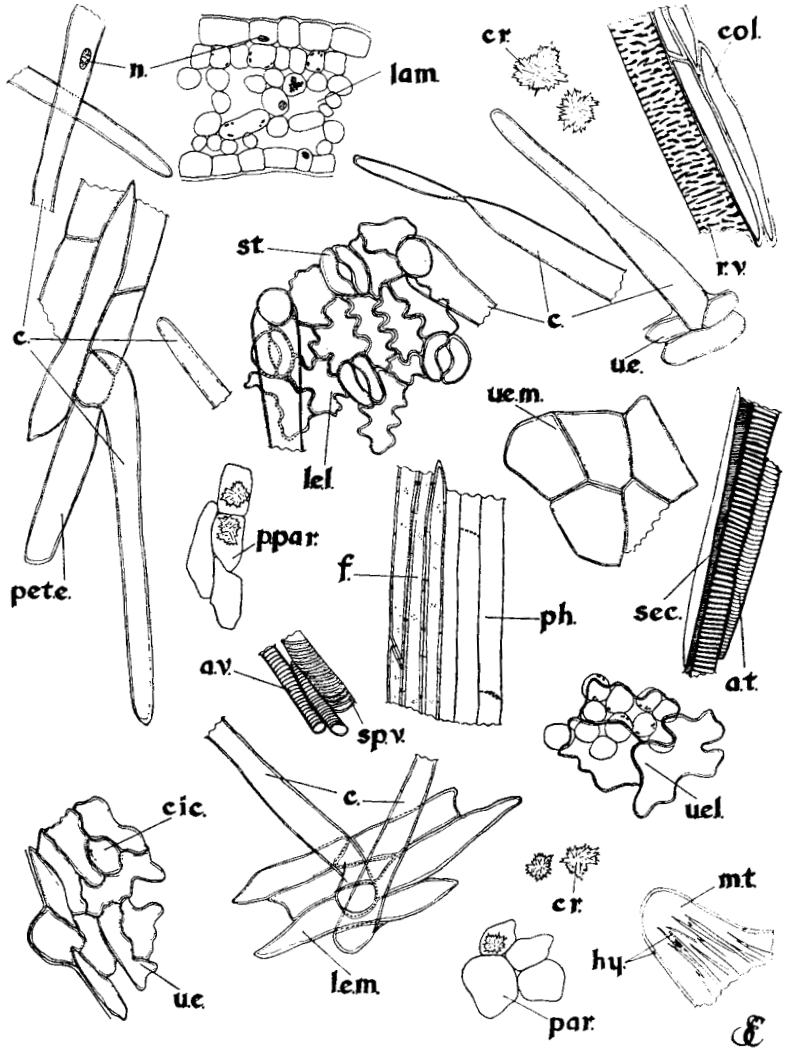

FIG. 8. Powder of the Leaf and Petiole of *P. peltatum* L. Marginal tooth × 30; other fragments × 150. *a.t.*, annular tracheid; *a.v.*, annular vessel; *c.*, covering trichome; *cic.*, cicatrix; *col.*, collenchyma; *cr.*, crystal of calcium oxalate; *f.*, fibre; *hy.*, hydathode; *lam.*, lamina; *l.e.l.*, lower epidermis of lamina; *l.e.m.*, lower epidermis of main vein; *m.t.*, marginal tooth; *n.*, nucleus; *par.*, parenchyma; *pet.e.*, petiole epidermis; *ph.*, phloem; *p.par.*, pith parenchyma; *r.v.*, reticulate vessel; *sec.*, secretion cell; *sp.v.*, spiral vessel; *st.*, stoma; *u.e.*, upper epidermis; *u.e.l.*, upper epidermis of lamina; *u.e.m.*, upper epidermis of main vein.

or powdered condition (see Table II). The *Stomatal Indices* show a statistically significant variation of the means but the high standard deviations cause the ranges to overlap to such an extent that 350 readings would be necessary. To attempt a distinction of the two species *P. peltatum* and *P. hexandrum* by this method would be impracticable.

TABLE II

RESULTS FROM PALISADE RATIOS AND STOMATAL INDICES OF THE TWO SPECIES

Leaf	Range of values from 400 readings	Mean ratio	Standard deviation	*t*	*t* (from tables) P=0·01	*t* (from tables) P=0·05	Minimum number of readings
Palisade Ratios *P. peltatum*	7·0–8·5–12·16–17·0	10·25	1·93	33·3	2·571	1·97	4·6 adjusted to 5
P. hexandrum	4·0–4·96–7·68–11·25	6·32	1·36				
Stomatal Indices *P. peltatum*	9·09–15·57–23·65–33·33	19·61	4·04	4·03	2·571	1·97	335
P. hexandrum	4·0–14·76–22·24–30·78	18·5	3·74				

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