GLINUS OPPOSITIFOLIUS L. ROOT—A SUBSTITUTE FOR SENEGA

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In recent years, considerable quantities of materials named as "Senegas" derived from the Indian sub-continent have been offered on European markets, the extensive establishment of these materials probably having connection with the prevailing high price of *Polygala senega* L. The materials appear to be of varied origins and some have been investigated by a number of workers. Datta and Mukerjii[1] have described *Polygala chinensis* L. under the names of Chinensis, or Indian Senega, the material now being the subject of a monograph in the Indian Pharmaceutical Codex. Their description includes a somewhat sparsely illustrated account of the anatomy of the drug, which is said to have a wide geographical distribution throughout India, from the Punjab to Burma, S. India and Ceylon, but to be obtained in large quantities from the N.W. Provinces. Qazilbash[2] has ascertained that so-called Pakistan Senega, previously supposed to be derived from *P. chinensis*, is in fact derived from *Andrachne aspera* L. (Fam. Euphorbiaceæ) and is collected mainly from the Khattak hills, in the Peshawar district, N.W. Frontier Province. Dequeker[3] has reported the presence on the Belgian market of so-called Indian Polygala and has pointed out characters by which this material may be distinguished from *P. senega* and from Pakistan Senega. Dequeker has carried out a comparative evaluation of *P. senega* and two samples of Indian Polygala; the investigation was a preliminary one and the origin of the Indian Polygala was to be established at a later date. A further material, not of Indian origin, has been described by Paris and Lys[4] under the name of Syrian Polygala. This material, *Spergularia marginata* Kittel (Fam. Caryophyllaceæ) was investigated chemically and pharmacologically.

Samples of a material labelled Indian Senega Root have been offered on the English drug market, and also on the European continent, being claimed to possess the medicinal properties of *P. senega.* They are stated to be obtained from South Kanara, Malabar and the districts about Madura and Ramnad in the province of Madras. Samples of this material examined in the Museum of the Pharmaceutical Society did not appear to conform in structural characters with any of the other materials mentioned above, and it was thought worthwhile to investigate the macroscopical and microscopical characters of the material, in order that it might be adequately compared with the commercially related material. In order to determine the botanical origin, a specimen of an entire plant, including flowers, stem and root, was acquired; the root of this specimen possessed the macroscopical and microscopical

characters of the commercial Indian Senega Root. The material was identified in the Herbarium of the Royal Botanic Gardens, Kew, as *Glinus oppositifolius* L. A.DC. (= *Mollugo spergula* L. and *Mollugo oppositifolia* L.) Family Molluginaceæ (or Ficoidaceæ). Herbarium specimens seen in the Herbaria at Kew and at the National Science Museum, South Kensington, indicate that this plant is of a wide geographical distribution. References are made to its having grown in many different regions of India from the north to the south, it appearing to favour sandy soil and river banks. It grows also in China, Indo-China, the Malay Archipelago, the Phillipines, Australia, Africa (Sudan, Uganda, Nigeria, Tanganyika, Sierra Leone, Natal and the island of Madagascar) and South America.

Materials

The investigation was carried out on two samples of commercial material and a further sample sent from the University of Louvain through the kindness of Professor R. Dequeker. These samples, and the entire plant specimen identified at Kew, all showed the same morphological characters.

Macroscopical and Sensory Characters

The drug appears in commerce as pieces of root, largely unbroken, of a vertical direction of growth, and cylindrical in shape, with a slight taper from crown to tip (Fig. 1, *A*). The roots are unbranched, usually straight and only infrequently slightly contorted. The pieces vary in size from 6 to 16 cm. in length and from 2 to 6 mm. in diameter, and are of a pale brown colour. Rootlets are only occasionally attached as threadlike appendages up to 3 cm. in length and about 0·5 mm. in diameter, but the roots bear rather indistinct scars of detached rootlets, often as minute depressions in the surface. The root surface is longitudinally wrinkled, the wrinkles running spirally and being deeper in the more mature pieces. The crown of the root is knotty and bears the vestigial remains of from two to twelve aerial stems, the bases of which are surrounded by membranous scaly leaves which are generally green but sometimes pale brown in colour. The crown may occasionally bear buds as well as aerial stem remains. The stem portions, where not merely represented by scars, are the same colour as the roots; they are from 1 to 5 mm. in length and about 1 mm. in diameter, cylindrical, hollow, with a longitudinally striated surface and white interior parts. Examination of the transverse sections of the stems (Fig. 3, *A*) show a wide continuous ring of secondary xylem about a central parenchymatous pith, which latter commonly borders a wide lacuna. An anomalous structure is found due to a tertiary cambium having arisen in the phloem, producing an arc or ring of collateral bundles. These are separated, at least initially, by parenchymatous medullary rays which are wider towards the edge of the arc, where the youngest bundles are developing.

The smoothed transverse section of the root shows a number of rings of vascular tissue surrounding an eccentric vascular core, these appearing

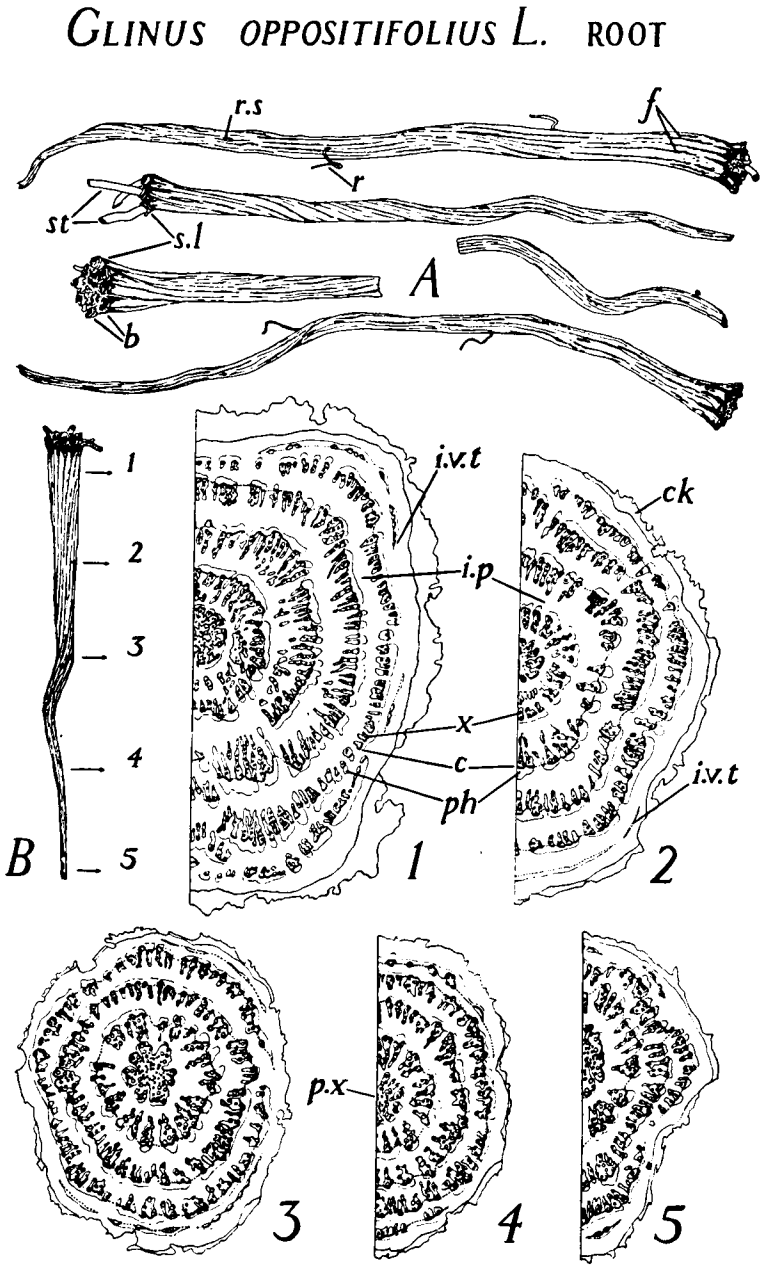

FIG. 1. Root of *Glinus oppositifolius* L. *A*, pieces of commercial root × 1. *B*, root (× 1) showing levels at which the corresponding smoothed transverse sections 1 to 5 were made. All sections × 20. *b*, stem buds; *c*, cambium; *ck*, cork; *f*, furrows; *i.p.*, intervascular parenchyma; *i.v.t.*, initiating vascular tissue; *ph*, phloem; *p.x.*, primary xylem; *r*, rootlet; *r.s.*, rootlet scar; *s.l.*, scale leaves; *st*, stems; *x*, xylem.

as white rings on a brown background (Fig. 1, *B*: 1 to 5). This appearance is due to the anomalous formation of successive rings or arcs of meristematic tissue. These successive meristems produce a number of annular zones or rings of growth which are more or less equidistant from one another and consist of xylem internally and phloem externally. The number of rings seen about the vascular core varies from one or more at the root tip to up to six at the crown. In the growth of these rings, an arc of vascular bundles appears intermittently on that side of the root having the greatest radius from the eccentral vascular core, developing in time to form a complete ring (Fig. 1, *B*; 2, *i.v.t.*). The outermost ring seen in a smoothed transverse section is consequently often discontinuous, the missing or least developed part of the ring usually occurring in that part of the section through which runs the shortest possible radius from the innermost xylem tissue to the cork. The rings of vascular tissue are separated from one another by intervening brown bands of about the same radial thickness as the rings. The central vascular core occupies from about one-tenth to a half of the total root diameter, the proportion decreasing with an increasing number of vascular rings in the section. Medullary rays between the individual vascular bundles of the rings may be made out with difficulty on viewing with a hand-lens magnifying 10 diameters; they appear as fine brown threadlines. The fracture is short and the roots have a springy resilience to bending. The odour is faint and the taste is not marked, being reminiscent of wheat grain. Chewing produces a very fibrous residue.

Anatomy of Root

In typical roots, the external cork tissue is of variable thickness, being made up of from 2 to 8 layers and frequently broken (Fig. 2, *A*). Individual cells are tabular and irregularly polygonal in anticlinal outline (Fig. 2, *E*); they measure about R = 7 to **15** to 26 μ, T = 12 to **33** to 53 μ and L = 17 to **48** to 91 μ*. The cells of the cork are suberised and slightly lignified. They stain brown with iodine and occasional cell contents stain black with ferric chloride. The phellogen is not distinct. Within the cork, the phelloderm is seen as a parenchymatous tissue up to about 8 cells in radial depth (Fig. 2, *F*), the individual cells being perceptibly larger than those of the parenchyma of the internal bands which separate the vascular rings and which are made up of from 10 to 18 layers of cells. In each of these bands of parenchyma, the cells are larger towards the outer part, and smaller towards the inner part, of the band. In the inner bands, the parenchyma cells tend to be tangentially elongated in a manner suggesting partial distortion by pressure. In roots showing five or more rings of vascular tissue, such elongated cells are commonly seen in the central and next outer band of conjunctive parenchyma tissue. The cells of the parenchyma measure about R = 10 to **26** to 51 μ, T = 20 to **43** to 89 μ and L = 24 to **46** to 71 μ.

* The letters R, T and L have the same connotations as devised by Moll and Janssonius[5]. That is to say, they symbolise the measurements of the cells in radial, transverse and longitudinal directions, respectively.

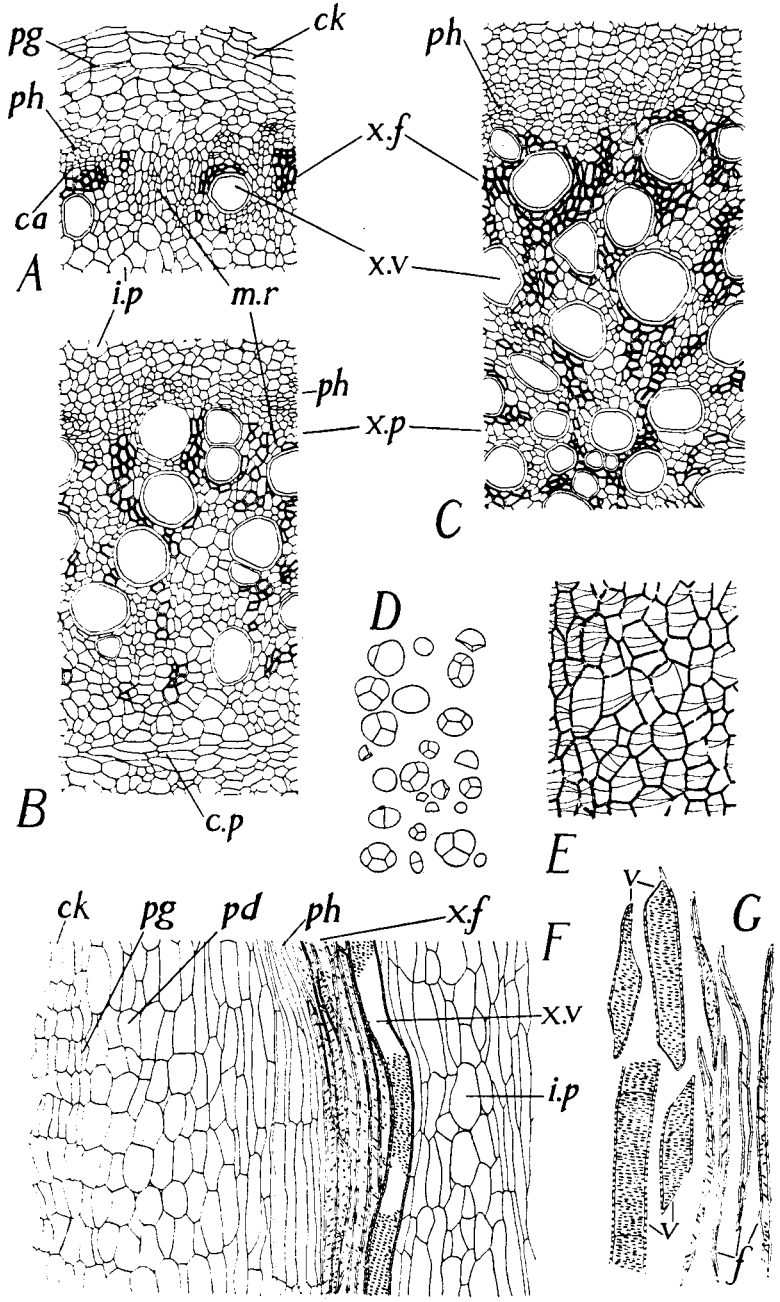

FIG. 2. Root of *Glinus oppositifolius* L. *A*, transverse section at level *B*1 (see Fig. 1), showing part of outermost ring of vascular tissue. *B*, transverse section at level *B*1, showing part of ring immediately outside the central vascular core. *C*, transverse section at level *B*1, showing part of central vascular core. *D*, starch grains. *E*, cork cells in surface view. *F*, radial longitudinal section of outermost ring of vascular tissue. *G*, isolated elements from the disintegrated xylem. *A–C*, *E–G*, × 140; *D* × 750. *ca*, cambium; *ck*, cork; *c.p.* collapsed parenchyma; *f*, fibres; *i.p.*, intervascular parenchyma; *m.r.*, medullary ray; *pd*, phelloderm; *pg*, phellogen; *ph*, phloem; *v*, vessels; *x.f.*, xylem fibres; *x.p.*, xylem parenchyma; *x.v.*, xylem vessels.

They contain numerous small starch grains, both simple and compound (Fig. 2, *D*); the latter are the commoner form, being made up of from 2 to 6 components and the most frequently occurring forms are those with 3 or 4 components. Hila were not made out, but the grains exhibit distinct crosses in polarised light. If compound grains be broken apart, as is commonly effected in powdering or scraping the root, the individual components are seen to have one larger curved side and 1 to 4 smaller flat surfaces depending on the number of components in the compound grain. Simple grains are subspherical or ovoid, two-component grains are ovoid and the more compound grains are spherical in outline. The complete grains vary in diameter from 1·5 to **4·5** to 13 μ.

The numerous collateral bundles which make up each ring are separated from one another by parenchymatous medullary rays of varying widths (Fig. 2, *A* and *B*); the cells tend to be smaller and more radially elongate than those of the parenchyma of the bands. The wider rays contain starch grains. There are more bundles in the rings as they occur nearer to the surface of the root; otherwise each ring of vascular tissue is of similar nature except for the peripheral incomplete ring. This peripheral ring shows immature bundles with a marked lack of vessels and, particularly at its edges, may be represented by undifferentiated cambium, elsewhere difficult to make out (Fig. 1, *B*:2, *i.v.t.*). The eccentral core contains both primary and secondary tissues and is of greater radial width than any of the rings.

Within the bands, the secondary phloem occurs in groups collateral with the xylem. These groups are up to 12 layers of cells in radial width and are made up of axially elongated parenchyma cells which are polygonal in transverse section (Fig. 2, *A*, *B* and *C*); the end walls may be tapering or, less frequently, at right angles. They measure about R and T = 3·5 to **7** to 15 μ and L = 17 to **100** to 160 μ. The sieve tubes are narrow and tend to be collapsed; the sieve areas are with difficulty distinguished. The cambia of the inner cylinders are not seen in a state of active division, but, where the outermost ring is initiating, an active meristem is found, consisting of from 2 to 6 layers of rectangular prisms measuring R = 2 to 6 μ, T = 3·5 to **8** to 15 μ and with longitudinally directed axes (Fig. 2, *A*, *ca*). Such adventitiously differentiated cambium arising in the external part of the phloem produces, firstly, new phloem externally; subsequently, xylem elements are produced internally. The xylem groups in the vascular rings, as seen in transverse section, are arranged in radially directed rectangular or wedge-shaped masses from 2 to 12 cells tangentially and up to about 30 cells radially (Fig. 2, *B*). The groups are made up of vessels, fibrous tracheids, sclerenchyma fibres and parenchyma, all but the latter having yellow lignified walls. The vessels appear singly or only infrequently paired. In transverse section, single vessels are more or less spherical in outline. They measure about R and T = 10 to **45** to 89 μ and the individual articulations measure L = 90 to **180** to 260 μ (Fig. 2, *F* and *G*). They are thick-walled with numerous small elongated bordered pits; the end-walls are either slightly tapering or at right angles to the longitudinal

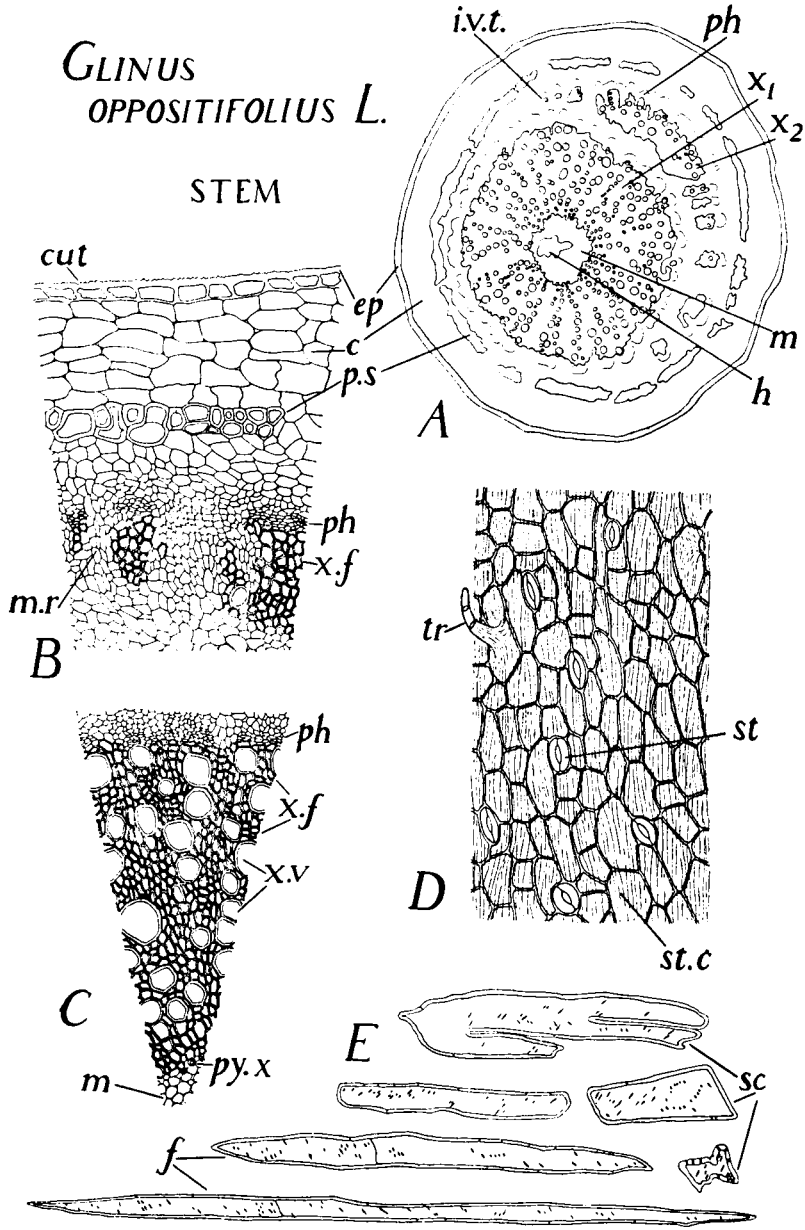

FIG. 3. Stem of *Glinus oppositifolius* L. *A*, transverse section of stem. *B*, transverse section of outer part of stem in region of initiating vascular ring. *C*, transverse section of inner part of stem. *D*, surface view of epidermis. *E*, isolated elements of the pericyclic sclerenchyma. *A* × 40; *B–D*, × 140; *E* × 160. *c*, cortex; *cut*, cuticle; *ep*, epidermis; *f*, fibres; *h*, lacuna; *i.v.t.*, initiating vascular tissue; *m*, medulla; *m.r.*, medullary ray; *ph*, phloem; *p.s.*, pericyclic sclerenchyma; *py.x.*, primary xylem; *sc*, sclereids; *st*, stoma; *st.c.*, striated cuticle; *tr*, trichome; x_1, secondary xylem; x_2, tertiary xylem; *x.f.*, xylem fibres; *x.v.*, xylem vessels.

axis, with, frequently, a small upper or lower tapering projection at the extremity. They have a large circular perforation. Sclerenchymatous xylem fibres are long and narrow with tapering ends, thickened walls and an irregularly penta- or hexagonal shape as seen in transverse section. They measure 7 to **12** to 17 μ in diameter, L = 130 to **285** to 450 μ and have a restricted slit-like pitting. The fibrous tracheids are comparatively broader, with extensive oval bordered pitting; they fall within the following measurements: R and T = 10 to **15** to 24 μ, L = 180 to **300** to 510 μ. Some fibres of intermediate characters also occur. Xylem parenchyma consists of unlignified axially elongated elements. The vascular tissue commonly does not run in any constant vertical plane, but tends to waver in its longitudinal direction.

Anatomy of Stem

The epidermal cells of the stem are tetra- to hexagonal prisms, measuring about R = 11 to **14** to 16 μ, T = 18 to **26** to 36 μ, L = 20 to **38** to 70 μ. The outer and inner walls are thickened, the outer walls having marked longitudinal cuticular striations (Fig. 3, *B* and *D*). There is infrequent beading of the anticlinal walls. Anomocytic stomata are common; they measure about 21 to 30 μ in length and have from two to four subsidiary cells. The axes of the stomata run more or less parallel to one another in the direction of the longitudinal axis of the stem. Trichome cicatrices are common. Trichomes are infrequent, uniseriate, up to 3 cells long. The basal part has a longitudinally striated cuticle, the cuticular striations of neighbouring cells of the epidermis converging upon this region. It has the shape of a truncated cone, and the remaining cells of the trichome are slightly warty, conical, often wider than the basal cell and the terminal cells is bluntly pointed. The cortex consists of parenchyma, about 5 or 6 cells in radial width (Fig. 3, *B*, *C*). The cells measure R = 8 to **21** to 23 μ, T = 30 to **52** to 83 μ and L = 20 to **52** to 95 μ. They show intercellular spaces and contain starch grains having the same characters as those of the root. The pericycle shows an interrupted ring of yellow lignified sclerenchymatous cells, one or two layers in radial width (Fig. 3, *B*, *p.s.*). The cells measure R = 8 to **23** to 46 μ, T = 9 to **22** to 44 μ and L = 150 to **310** to 710 μ; they comprise fibres and sclereids, the former being less frequent (Fig. 3, *F*). The fibres have thin walls and may be of variable width at different levels; they have a notched outline. Transverse partitions occur in some of the fibres. The sclereids are few and vary in shape from polygonal prisms with a longitudinally directed axis, or short cylinders, to very irregularly shaped cells, the lumen on occasion being U-shaped. They have thin walls and wide lumens. The pericyclic sclerenchyma cells exhibit simple pitting.

The conjunctive parenchyma lying within the pericycle consists of longitudinally directed thin-walled cells measuring about R = 5 to **11** to 26 μ, T = 6 to **18** to 38 μ and L = 33 to **70** to 85 μ, the R and T dimensions becoming progressively smaller from the pericycle inwards. The cells are densely filled with starch grains possessing the same characters as those of the root. Collateral with each ray of xylem, either secondary

or tertiary, is a cap of tangentially flattened phloem cells up to about six layers in radial width. The cells measure about R = 4 μ, T = 8 μ and L = 120 μ. The walls are thickened, especially in the angles of the cells and, in transverse section, the tissue appears somewhat whiter and more refractive than the cells of the conjunctive parenchyma (Fig. 3, *B* and *C*, *ph*).

The tertiary xylem resembles the secondary xylem except that there is an absence of vessels from its younger parts and that these are separated by parenchymatous medullary rays (Fig. 3, *A*, x_2 and *B*). The secondary xylem forms a continuous ring 35 to 40 cells in radial thickness and is made up of yellow lignified elements consisting of vessels in a ground tissue of fibrous tracheidal elements (Fig. 3, *A*, x_1 and *C*). Medullary rays, of which there are about 40, are up to five cells wide. The xylem vessels are more or less spherical in transverse section and occur singly. They measure about R and T = 10 to **24** to 48 μ and the individual articulations measure L = 100 to **186** to 290 μ; the end-walls are at right angles or slightly oblique, with a large circular perforation. They are covered with numerous small oval bordered pits. The tracheidal elements measure about R = 6 to **11** to 20 μ, T = 6 to **9** to 18 μ and L = 110 to **215** to 315 μ; they comprise slender fibrous cells with tapering ends and shorter elements with oblique or bluntly pointed ends. All have oval or elongated bordered pits. Primary xylem elements occur on the inside of the xylem rays as groups of lignified spiral and annular vessels. The pith consists of parenchyma cells measuring R and T = 10 to **21** to 34 μ, L = 66 to **110** to 150 μ. The cells are smaller towards the outside.

Differential Characters

Features by which *Glinus oppositifolius* root may be distinguished from that of senega are numerous and marked. In the entire condition, the roots of the former are longer and more slender; they are very much straighter and less branched. The colour is a uniform light brown, without any of the purple tints seen in senega; the crown is less bulbous and knotty, with fewer buds and aerial stem bases. The external surface shows uniform spiral furrowing and has neither the keel nor the transverse wrinkles of senega root. The transverse section of the root of *G. oppositifolius* shows up to about six characteristic concentric rings of anomalous xylem and phloem tissues arranged round an eccentric vascular core of secondary xylem and phloem. This differs markedly from the distribution of xylem and phloem tissues characteristic of senega. The taste lacks marked acridity and there is no distinct odour. When in the powdered condition, *G. oppositifolius* is a pale buff colour and has a dry starchy texture as against the grey colour and oily-damp texture of senega. Microscopically, the main distinguishing feature between the two powders lies in the contents of the cells of the parenchymatous tissues. In the case of *G. oppositifolius*, small starch grains, simple or 2 to 6 compound, are present in the cells and oily droplets are absent, whilst the converse condition is true of senega. There is no collenchymatous

parenchyma in *G. oppositifolius*. The xylem elements are rather similar in both roots, but the vessels in *G. oppositifolius* are up to about 90 μ in diameter and those of senega are up to 50 μ; the former has a few sclerenchymatous fibres. There are salient histological differences between the stems of the two materials. The epidermal cells of the stems of *G. oppositifolius*, described elsewhere in the paper, are readily distinguished from those of senega, which have sinuous anticlinal walls; the pericyclic sclerenchyma of *G. oppositifolius* is lignified whilst that of senega is unlignified. In powder, the very small amounts of stem present preclude their use as very significant diagnostic characters.

Evaluation by Haemolytic Indices

The root of *G. oppositifolius* is offered as a "Senega", and the powder, shaken with water, gives a copious persistent froth. Some preliminary investigations to assess the saponin content by determining the haemolytic index of the root have been made, using a moderately fine powder in an air-dry condition. After examining a number of methods for the determination of haemolytic index, that due to Runge[6], employing a geometric progression of dilutions, was adopted. The standard used was desoxycholic acid, maintained in a vacuum desiccator and prepared as a solution according to the method of Runge, immediately prior to its use. The use of a standard of purified white saponin (Saponinum purum album, Merck) was attempted, but rejected due to difficulties in obtaining consistent haemolytic responses with it under controlled conditions. Four extraction methods were employed, as follows:

1. 10 g. of the powdered drug was macerated for 24 hours with a little 60 per cent. ethanol and packed into a percolator. More of the solvent was added and the first 8 ml. of percolate collected and reserved. The drug was further percolated with solvent so as to yield 600 ml. of percolate, which was then evaporated in a stream of warm air, added to the reserved percolate and the whole made up to 10 ml. After 48 hours, the extract was centrifuged to obtain a clear liquid, of which one part was diluted for use with 49 parts of Runge's phosphate buffer pH 7·4 to give a 2 per cent. drug-to-solvent extractive.

2. 10 g. of the powdered drug was macerated as previously and percolated with 60 per cent. ethanol to yield 600 ml. of percolate. 60 ml. of this was evaporated to dryness in a steam of warm air and the residue dissolved in 60 ml. of methanol and filtered. The solvent was again evaporated and the residue taken up in 50 ml. of phosphate buffer pH 7·4, centrifuging if necessary to obtain a clear 2 per cent. drug-extractive.

3. One g. of the powdered drug was extracted with chloroform in a soxhlet apparatus for 6 hours and the solvent removed. The powder was then extracted for 10 hours with methanol in a soxhlet apparatus and the solvent removed and evaporated under reduced pressure. The residue was taken up in 50 ml. of phosphate buffer pH 7·4 to obtain a 2 per cent. drug-extractive.

4. A method due to Ruyssen (private communication) was employed, involving preliminary extractions with light petroleum and acetone and,

after drying, a form of controlled decoctive extraction of the powder with a phosphate buffer to yield a 1 per cent. drug-extractive.

Each of these extractions were applied at the same time to portions of a bulked sample of powdered senega root and the haemolytic indices determined.

The results were:—

	H.I. *P. senega* L.	H.I. *G. oppositifolius* L.
Method 1	2240	145
Method 2	1620	120
Method 3	1860	145
Method 4	1500	410

The first three of these methods give results which for *G. oppositifolius* are more or less consistent; for senega, there is a proportionately somewhat wider variation in values of haemolytic indices. Using these methods, the roots of *G. oppositifolius* show haemolytic indices which, by Runge's procedure, are from about 13 to 15 times lower than those of senega root. Method 4, which gives a lower H.I. value for senega, gives an appreciably higher H.I. value for *G. oppositifolius* than do the other three methods of extraction; in this case the H.I. value for *G. oppositifolius* is 3 to 4 times lower than that for senega. By this method of extraction, the total saponins were not taken up, as indicated by the capacity of the "exhausted" powders to yield a persistent froth on shaking with water. This may account for the lower H.I. value in the case of senega and indicates that the value for *G. oppositifolius*, though higher than by the other three methods, is none the less a minimal and provisional one. Work is being continued to elucidate the optimum extractive conditions for the material.

Summary and Conclusions

The diagnostic characters of *G. oppositifolius* are:

1. Macroscopical. The roots, pale brown in colour, are straight and unbranched, with small knotty crowns, longitudinally wrinkled surfaces and very few rootlets. The transverse section shows a series of concentric rings of vascular tissue arranged about an eccentric vascular core, the appearance being due to the formation of successive anomalous meristems external to a normal secondary cambium. Cylindrical, hollow stems, about 1 mm. in diameter, or their scars, or stem buds, occur on the crown.

2. Microscopical. Diagnostic characters include the thin-walled parenchymatous tissue containing small starch grains, single and compound, averaging about 4 or 5 μ and with up to six components. The xylem comprises pitted vessels up to about 90 μ in diameter, pitted fibrous tracheids and occasional sclerenchymatous fibres.

Preliminary determinations of the haemolytic index of *G. oppositifolius* show values ranging from about one-fifteenth to one-quarter of those of senega roots, depending on the methods of extracting the roots.

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DISCUSSION

The paper was presented by MR. R. M. RIDGWAY.

DR. J. W. FAIRBAIRN (London) said it was remarkable that a substitute for *Polygala senega* had such an increased haemolytic index with the fourth method used by the author, and yet the value for *Polygala senega* by that method had been reduced. The authors described the stomata as anomocytic. The term anomocytic indicated a group in which no definite arrangement was discernible: he had always hoped that this group would itself yield further categories. He noticed that the stomata tended to form a pattern of one large and three small subsidiary cells; it would be interesting to know whether the authors had examined a larger number than was shown in order to see whether the irregularity persists. He suggested that the word "sclerenchyma" used to describe xylem fibres was redundant.

DR. T. E. WALLIS (London) said that the description of the morphology and anatomy of root and stem provided a needed means of determining the origin of the material should it occur again. The authors did not state how many primary xylem groups were in the root.

MR. A. R. G. CHAMINGS (Horsham) commented on the use of the haemolytic index and enquired whether it had any relation to the toxicity of the drug.

MR. T. C. DENSTON (London) said that the new Indian Pharmacopœia of 1955 contained a monograph on Indian senega. That monograph was not very different from that which appeared in the precursor of the Indian Pharmacopœia. The paper referred to two samples of commercial material: were these available in commerce in Britain, or offered in India as complying with the Indian Pharmacopœial list?

MR. R. M. RIDGWAY, in reply, agreed it was remarkable that there was so great a difference in the haemolytic index of the two drugs by various methods of extraction. The particular pattern of stomata probably did not occur over a large number of stomata but was peculiar to the one drawing shown. He accepted Dr. Fairbairn's comment on the use of the word sclerenchyma. It had not been possible to determine the archy of *G. oppositifolius* as the material developed at too great a rate. The haemolytic index was not necessarily a measure of toxicity, and the pharmacological action of the drugs was not proportional to their haemolytic indices. The haemolytic index was merely a way of evaluating the drugs. The samples came from an English source.