WORMWOOD

Absinthii herba

DEFINITION

Wormwood consists of the basal leaves or slightly leafy, flowering tops, or of a mixture of these dried, whole or cut organs of *Artemisia absinthium* L. It contains not less than 2 ml/kg of essential oil, calculated with reference to the dried drug.

CHARACTERS

It has the macroscopic and microscopic characters described under identification tests A and B.

IDENTIFICATION

A. The leaves are greyish to greenish, densely tomentose on both surfaces. The basal leaves, with long petioles, have triangular to oval bipinnatisect to tripinnatisect lamina, with rounded to lanceolate segments. The cauline leaves are less segmented and the apical leaves are lanceolate. The stem of the flower-bearing region is greenish-grey, tomentose, up to 2.5 mm in diameter and usually with five flattened longitudinal grooves. The capitula are arranged as loose, axillary panicles, inserted at the level of the lanceolate to slightly pinnatisect leaves; they are spherical to flattened hemispherical, 2 mm to 4 mm in diameter and consist of a grey, tomentose involucre, the outer bracts linear, inner layer ovate, blunt at the apices with scarious margins, a receptacle with very long paleae up to 1 mm or more long, numerous yellow, tubular, hermaphroditic florets about 2 mm long and few yellow, ray florets.

B. Reduce to a powder (355). The powder is greenish-grey. Examine under a microscope using chloral hydrate solution R. The powder shows many T-shaped trichomes with a short uniseriate stalk consisting of one to five small cells, perpendicularly capped by a very long, undulating terminal cell tapering at the ends; fragments of epidermises with sinuous to wavy walls, anomocytic stomata (2.8.3) and secretory trichomes each with a short, biseriate, two celled stalk and a biseriate head with two or four cells ; fragments of the tubular and ray florets, some containing small cluster crystals of calcium oxalate; numerous paleae each composed of a small cell forming a stalk and a very long, cylindrical and thin-walled terminal cell about 1 mm to 1.5 mm long; spheroidal pollen grains, about 30 µm in diameter, with three pores and a finely warty exine; groups of fibres, small vessels with spiral and annular thickening, larger vessels with bordered pits and parenchyma with moderately thickened and pitted walls, from the stem.

C. Examine by thin-layer chromatography (2.2.27), using a suitable silica gel as the coating substance. Test solution. Place 2 g of the powdered drug (355) in 50 ml of boiling water R and allow to stand for 5 min, shaking the flask several times. After cooling, add 5 ml of a 100 g/l solution of lead acetate R. Mix and filter. Rinse the flask and the residue on the filter with 20 ml of water R. Shake the filter with 50 ml of methylene chloride R. Separate the organic layer, dry over anhydrous sodium sulphate R, filter and evaporate the filtrate to dryness on a water-bath. Apply to the plate as bands 10 µl of each solution. Develop over a path of 15 cm using a mixture of 10 volumes of acetone R, 10 volumes of glacial acetic acid R, 30 volumes of toluene R and 50 volumes of methylene chloride R. Allow the plate to dry in air. Spray the plate with acetic anhydride-sulphuric acid solution R and examine in daylight. The chromatogram obtained with the test solution shows the blue zone of artabsin shortly above the red zone of methyl red in the chromatogram obtained with the reference solution. Examine in daylight while heating at 100 °C to 105 °C for 5 min. The chromatogram obtained with the reference solution shows in the middle third the red zone of methyl red and below it the light pink zone of resorcinol. The chromatogram obtained with the test solution shows an intense red to brownish-red zone of absinthin with a similar Rf value to that of the zone due to resorcinol in the chromatogram obtained with the reference solution. Other zones are visible, but less intense than that of absinthinDissolve the residue in 0.5 ml of alcohol R. Reference solution. Dissolve 2 mg of methyl red R and 2 mg of resorcinol R in 10.0 ml of methanol R.

DANDELION HERB WITH ROOT

Taraxaci officinalis herba cum radice

DEFINITION

Mixture of whole or fragmented, dried aerial and underground parts of *Taraxacum officinale* F.H. Wigg.

CHARACTERS

Bitter taste.

IDENTIFICATION

A. The underground parts consist of dark brown or blackish fragments 2-3 cm long, deeply wrinkled longitudinally on the outer surface. The thickened crown shows many scars left by the rosette of leaves. The fracture is short. A transverse section shows a greyish-white or brownish cortex containing concentric layers of brownish laticiferous vessels and a porous, pale yellow, non-radiate wood. Leaf fragments are green, glabrous or densely pilose. They are crumpled and usually show a clearly visible midrib on the inner surface. The lamina, with deeply dentate margins, is crumpled. The solitary flower heads, on hollow stems, consist of an involucre of green, foliaceous bracts surrounding the yellow florets, all of which are ligulate; a few achenes bearing a white, silky, outspread pappus may be present.

B. Microscopic examination (2.8.23). The powder is yellowish-brown. Examine under a microscope using chloral hydrate solution R. The powder shows the following diagnostic characters (Figure 1851.-1): fragments of cork [G] with flattened, thin-walled cells; reticulate lignified vessels [H] from the roots; fragments of parenchyma containing branched laticiferous vessels [F]; fragments of leaves, in surface view, showing upper [E] and lower [C] epidermises consisting of interlocking lobed cells and anomocytic stomata (2.8.3) [Ca, Ea]; elongated, multicellular covering trichomes with constrictions, which are more or less abundant depending on the variety or sub-variety [B, D]; fragments of the upper [E] epidermis usually accompanied by underlying palisade parenchyma [Eb] and fragments of the lower [C] epidermis accompanied by underlying spongy parenchyma [Cb]; lignified, spirally or annularly thickened vessels; fragments of flower-stem epidermis with stomata and rigid-walled, elongated cells [A]; pollen grains with a pitted exine [J]. Examine under a microscope using glycerol R. The powder shows angular, irregular inulin fragments, free or included in the parenchyma cells.

C. Thin-layer chromatography (2.2.27).

Test solution. To 2.0 g of the powdered herbal drug (355) (2.9.12) add 10 mL of methanol R. Heat in a water-bath at 60 °C or sonicate for 10 min. Cool and filter.

Reference solution. Dissolve 2 mg of chlorogenic acid R and 2 mg of rutin R in methanol R and dilute to 20 mL with the same solvent.

Plate: TLC silica gel plate R (5-40 µm) [or TLC silica gel plate R (2-10 µm)].

Mobile phase: anhydrous formic acid R, water R, ethyl acetate R (10:10:80 *V/V/V*).

Application: 20 µL [or 5 µL] as bands of 10 mm [or 8 mm].

Development: over a path of 12 cm [or 7 cm].

Drying: in air.

Detection: heat at 100 °C for 5 min; spray with or dip briefly into a 10 g/L solution of diphenylboric acid aminoethyl ester R in methanol R and dry at 100 °C for 5 min; spray with or dip briefly into a 50 g/L solution of macrogol 400 R in methanol R; heat at 100 °C for 5 min and examine in ultraviolet light at 365 nm.
Results: see below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other faint zones may be present in the chromatogram obtained with the test solution.

Top of the plate	
	A faint red zone
	A faint yellow zone
_____	_____
Chlorogenic acid: a blue zone	2 light blue zones
_____	_____
Rutin: a yellowish-brown zone	
	A light blue zone
Reference solution	**Test solution**

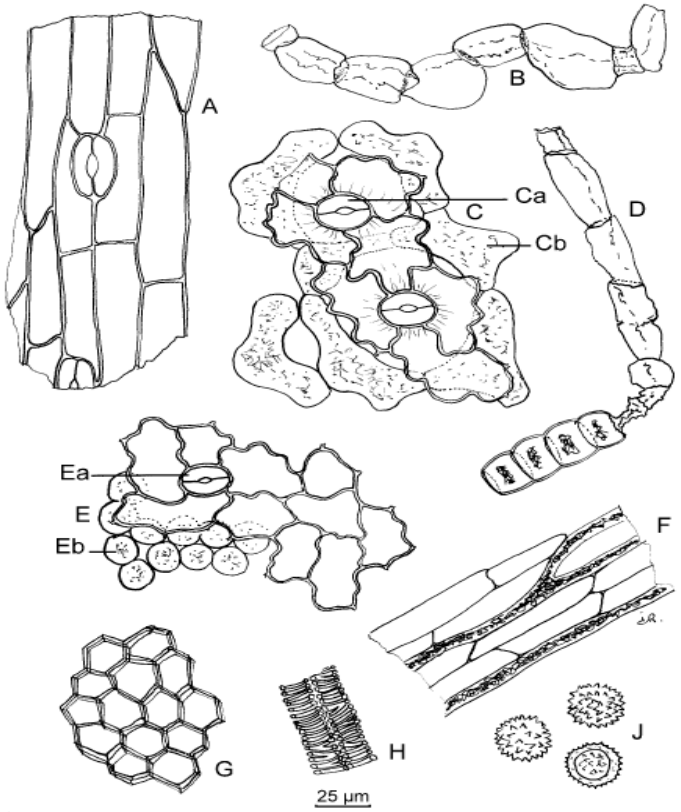

Figure 1851.-1. – *Illustration for identification test B of powdered herbal drug of dandelion herb with root*

OREGANO

Origani herba

DEFINITION

Dried leaves and flowers separated from the stems of *Origanum onites* L. or *Origanum vulgare* L. subsp. *hirtum* (Link) Ietsw., or a mixture of both species.

Content:

– *essential oil*: minimum 25 mL/kg (anhydrous drug);
– *sum of the contents of carvacrol and thymol* (both $C_{10}H_{14}O$; M_r 150.2): minimum 60 per cent in the essential oil.

IDENTIFICATION

A. *O. onites*. The leaf is yellowish-green, usually 4-22 mm long and 3-14 mm wide. It has a long or short petiole or is sessile. The lamina is ovate, elliptic or ovate-lanceolate. Margins are entire or serrate, the apex is acute or obtuse. The veins are yellowish and conspicuous on the adaxial surface. Flowers are solitary or seen as broken parts of the corymb. The calyx is bract-like and inconspicuous. The corolla is white, on top of inflorescences or single flowers, or inconspicuous. The bracts are imbricate and green like the leaves. The drug contains yellowish or yellowish-brown stem parts.

O. vulgare (subsp. *hirtum*). The leaf is green and usually 3-28 mm long and 2.5-19 mm wide. It is petiolate or sessile. The lamina is ovate or ovate-eliptic. The margins are entire or serrate, the apex is acute or obtuse. Flowers are rare, found as broken parts of the corymbs. Bracts are greenish-yellow and imbricate. The calyx is corolla-like and inconspicuous. The corolla is white, on top of inflorescences, slightly conspicuous or inconspicuous.

B. Reduce to a powder (710) (2.9.12). The powder is green (*O. vulgare*) or yellowish-green (*O. onites*). Examine under a microscope using chloral hydrate solution R (Figure 1880.-1).

O. onites powder shows fragments of leaf epidermis [A, D, G] composed of cells with sinuous walls, diacytic stomata (2.8.3) [Ga], covering trichomes and glandular trichomes; there are 2 types of glandular trichomes: some of lamiaceous type with 8-16 cells, in surface view [Da], and a very common type with a unicellular head and uni- [Gc], bi- [H] or tricellular stalk; the covering trichomes have smooth, thick walls; some are multicellular [B, Gb], often broken [Aa], and contain prisms of calcium oxalate, while others, which are rare, are unicellular and conical [C]; scars from covering and glandular trichomes are visible on the epidermises [Gd, Ge]; pollen grains, with smooth exine, are frequent [E, F].

O. vulgare subsp. *hirtum* powder shows fragments of the upper epidermis with cells with sinuous, beaded walls, accompanied by palisade parenchyma [J]; fragments of the lower epidermis [N] composed of cells with finely and irregularly thickened walls, diacytic stomata (2.8.3) [Na], covering trichomes and glandular trichomes; there are 2 types of glandular trichomes: some of lamiaceous type with 12 cells, in surface view [Nb], and a rare type with a unicellular head [Nc] and bi- or tricellular stalk; the covering trichomes have thick, warty walls and contain fine needles of calcium oxalate; some are conical, multicellular and serrate [L, M], while others, which are rare, are unicellular [K]; there are occasional pollen grains, with smooth exine [E, F].

C. Thin-layer chromatography (2.2.27).

Test solution. To 1.0 g of the powdered herbal drug (355) (2.9.12) add 5 mL of methylene chloride R and shake for 3 min, then filter through about 2 g of anhydrous sodium sulfate R.
Reference solution. Dissolve 1 mg of thymol R and 10 µL of carvacrol R in 10 mL of methylene chloride R.
Plate: TLC silica gel plate R.
Mobile phase: methylene chloride R.
Application: 20 µL as bands.
Development: over a path of 15 cm.
Drying: in air.
Detection: spray with anisaldehyde solution R using 10 mL for a plate 200 mm square and heat at 100-105 °C for 10 min.
Results: see below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other zones are present in the lower third and upper part of the chromatogram obtained with the test solution.

Top of the plate	
	A bluish-purple zone
_____	_____
	A pale green zone
Thymol: a pink zone	A pink zone (thymol)
Carvacrol: a pale violet zone	A pale violet zone (carvacrol)
_____	_____
	A pale purple zone
	A grey zone
	A pale green zone
	A bluish-purple zone
	An intense brown zone

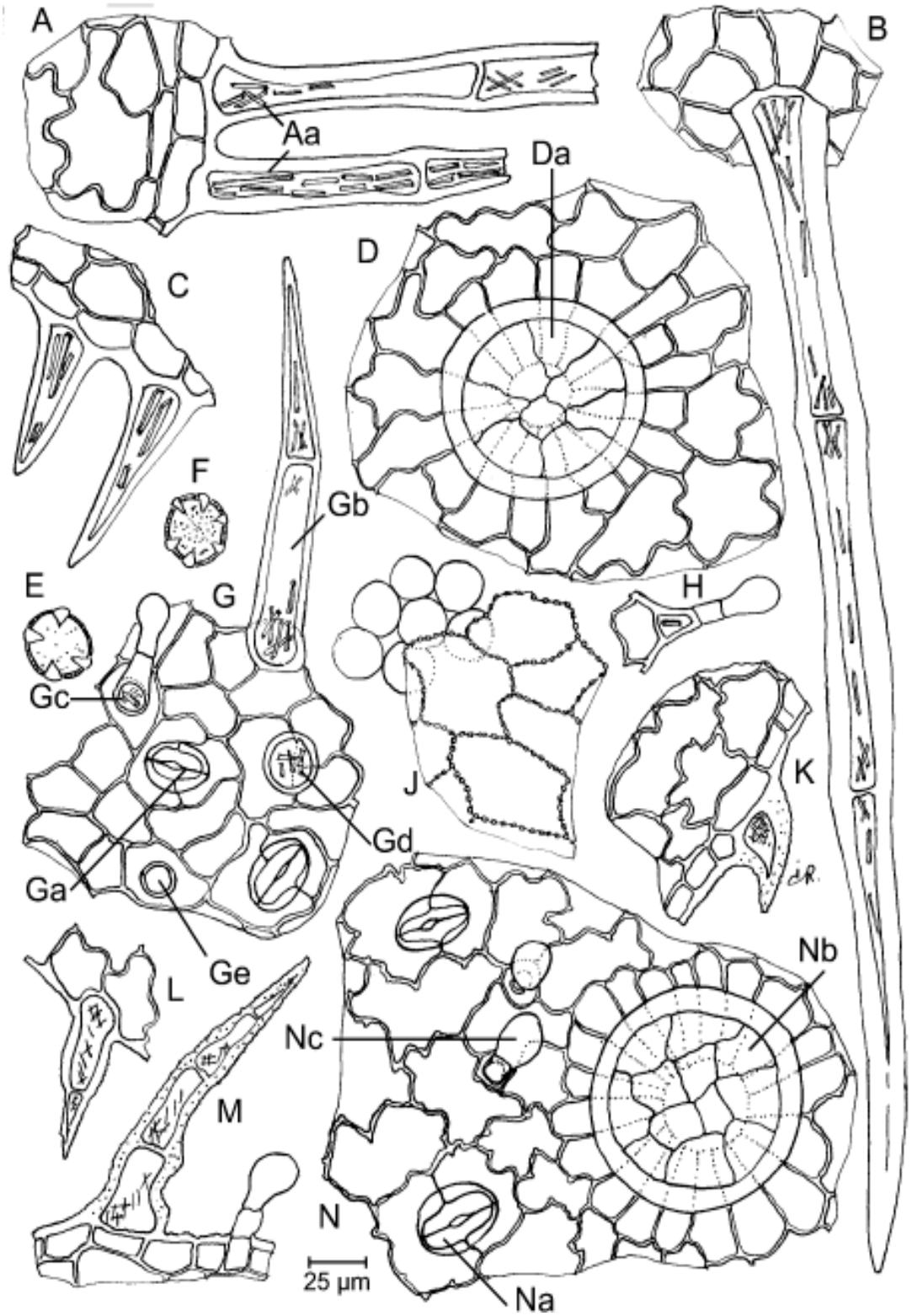

Figure 1880.-1. – *Illustration for identification test B of powdered herbal drug of oregano*

THYME

Thymi herba

DEFINITION

Whole leaves and flowers separated from the previously dried stems of *Thymus vulgaris* L. or *Thymus zygis* L. or a mixture of both species.

Content:

– *essential oil*: minimum 12 mL/kg (anhydrous drug);
– *sum of the contents of thymol and carvacrol* (both $C_{10}H_{14}O$; M_r 150.2): minimum 40 per cent in the essential oil.

CHARACTERS

▶Strong odour◀ reminiscent of thymol.

#IDENTIFICATION

A. The leaf of *Thymus vulgaris* is usually 4-12 mm long and up to 3 mm wide, sessile or with a very short petiole. The lamina is tough, entire, lanceolate or ovate, covered on both surfaces by a grey or greenish-grey indumentum; the edges are markedly rolled up towards the abaxial surface. The midrib is depressed on the adaxial surface and is very prominent on the abaxial surface. The calyx is green, often with violet spots and is tubular; at the end are 2 lips of which the upper one is bent back and at the end has 3 lobes, the lower is longer and has 2 hairy teeth. After flowering, the calyx tube is closed by a crown of long, stiff hairs. The corolla, about twice as long as the calyx, is usually brownish in the dry state and is slightly bilabiate.

The leaf of *Thymus zygis* is usually 1.7-6.5 mm long and 0.4-1.2 mm wide; it is acicular or linear-lanceolate and the edges are markedly rolled towards the abaxial surface. Both surfaces of the lamina are green or greenish-grey and the midrib is sometimes violet; the edges, in particular at the base, have long, white hairs. The dried flowers are very similar to those of *T. vulgaris*.

C. Microscopic examination (2.8.23). The powder of both species is greyish-green or greenish-brown. Examine under a microscope using chloral hydrate solution R. The powder shows the following diagnostic characters (Figure 0865.-1 and Figure 0865.-2): fragments of the outer epidermis of the corolla (surface view [A, C, F]), consisting of cells with wavy and slightly thickened [Fc] or unthickened [Ac] walls, numerous uniseriate, multicellular, covering trichomes, often with 1 cell collapsed [Aa], glandular trichomes with a unicellular head and a unicellular [Ca, Fb] or multicellular [Ab] stalk, diacytic stomata (2.8.3) [Fa] and glandular trichomes generally with 12 cells [D]; cells of the epidermis from the base of the corolla, isodiametric with slightly thickened walls [C]; pollen grains, relatively rare, spherical and smooth, with 6 germinal slit-like pores, measuring about 35 µm in diameter [B]; the powder of *T. zygis* also contains numerous thick bundles of fibres from the main veins and from fragments of stems; the epidermises of the leaves (surface view [G, K]) have cells with anticlinal walls that are sinuous and beaded [Ga, Ka], and diacytic stomata (2.8.3) [Gb]; numerous glandular trichomes made up of 12 secretory cells, the cuticle of which is generally raised by the secretion to form a globular or ovoid, bladder-like covering [Kb]; glandular trichomes with a unicellular stalk and a globular or ovoid head [Kc]; in both species, the adaxial epidermis bears covering trichomes with warty walls that are shaped as pointed teeth [Gc], and is usually associated with underlying palisade parenchyma [Gd, Kd]; the abaxial epidermis (transverse section [H, L]) bears covering trichomes of different types: unicellular, straight or slightly curved [Ha, La];

bicellular or tricellular, articulated and most often elbow-shaped [Hb, J] (*T. vulgaris*); bicellular or tricellular, more or less straight [N], or very large, multicellular [M], at the base of the lamina (*T. zygis*); fragments of calyx covered by numerous, uniseriate trichomes with 5-6 cells and a weakly striated cuticle (surface view [E]). ◄

C. Thin-layer chromatography (2.2.27).

▶*Test solution*. To 0.5 g of the powdered herbal drug (355) (2.9.12) add 5 mL of methanol R. Sonicate for 10 min. Centrifuge or filter; use the supernatant or the filtrate.

Reference solution. Dissolve 1 mg of rutin R and 1 mg of rosmarinic acid R in 5 mL of methanol R.

Plate: TLC silica gel F_{254} plate R (5-40 µm) [or TLC silica gel F_{254} plate R (2-10 µm)].

Mobile phase: anhydrous formic acid R, water R, ethyl acetate R (1:1:15 *V/V/V*).

Application: 20 µL [or 5 µL] as bands of 20 mm [or 8 mm].

Development: over a path of 15 cm [or 6 cm].

Drying: in air.

Detection: heat at 100 °C for 3 min, treat the still-hot plate with a 5 g/L solution of diphenylboric acid aminoethyl ester R in ethyl acetate R, then treat with a 50 g/L solution of macrogol 400 R in methylene chloride R; examine in ultraviolet light at 365 nm.

Results: see below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other faint fluorescent zones may be present in the chromatogram obtained with the test solution.

D. Examine the chromatograms obtained in the assay for thymol and carvacrol.

Results: the characteristic peaks in the chromatogram obtained with the test solution are similar in retention time to those in the chromatogram obtained with ▶reference solution (a)◀.

Top of the plate	
	2 red fluorescent zones
Rosmarinic acid: a blue fluorescent zone	A blue fluorescent zone (rosmarinic acid)
_____	_____
	1 or 2 blue fluorescent zones
_____	_____
	2 yellow or orange fluorescent zones
	A green fluorescent zone may be present
Rutin: an orange-yellow fluorescent zone	
Reference solution	**Test solution**

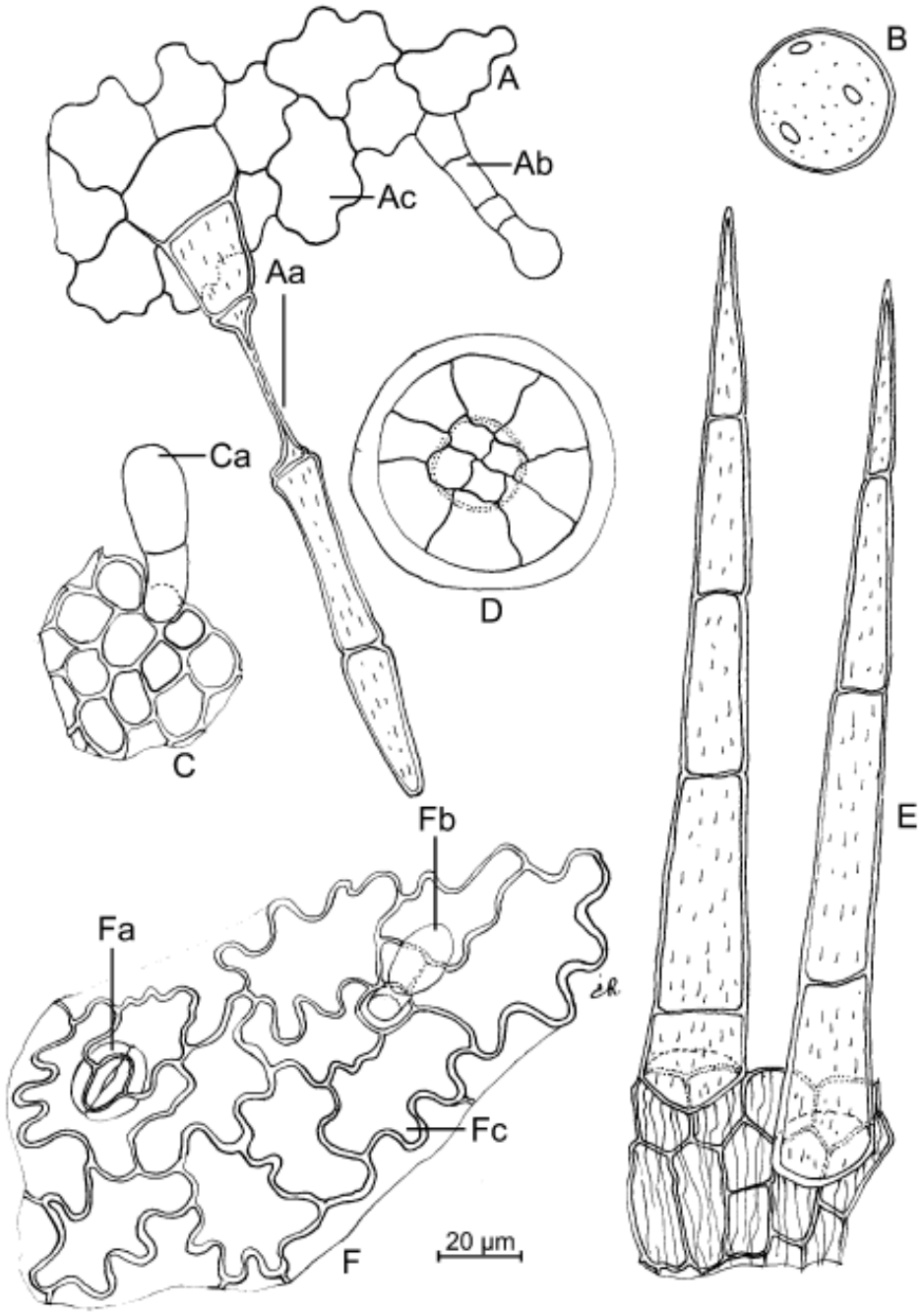

Figure 0865.-1. – *Illustration for identification test B of powdered herbal drug of thyme* ◀

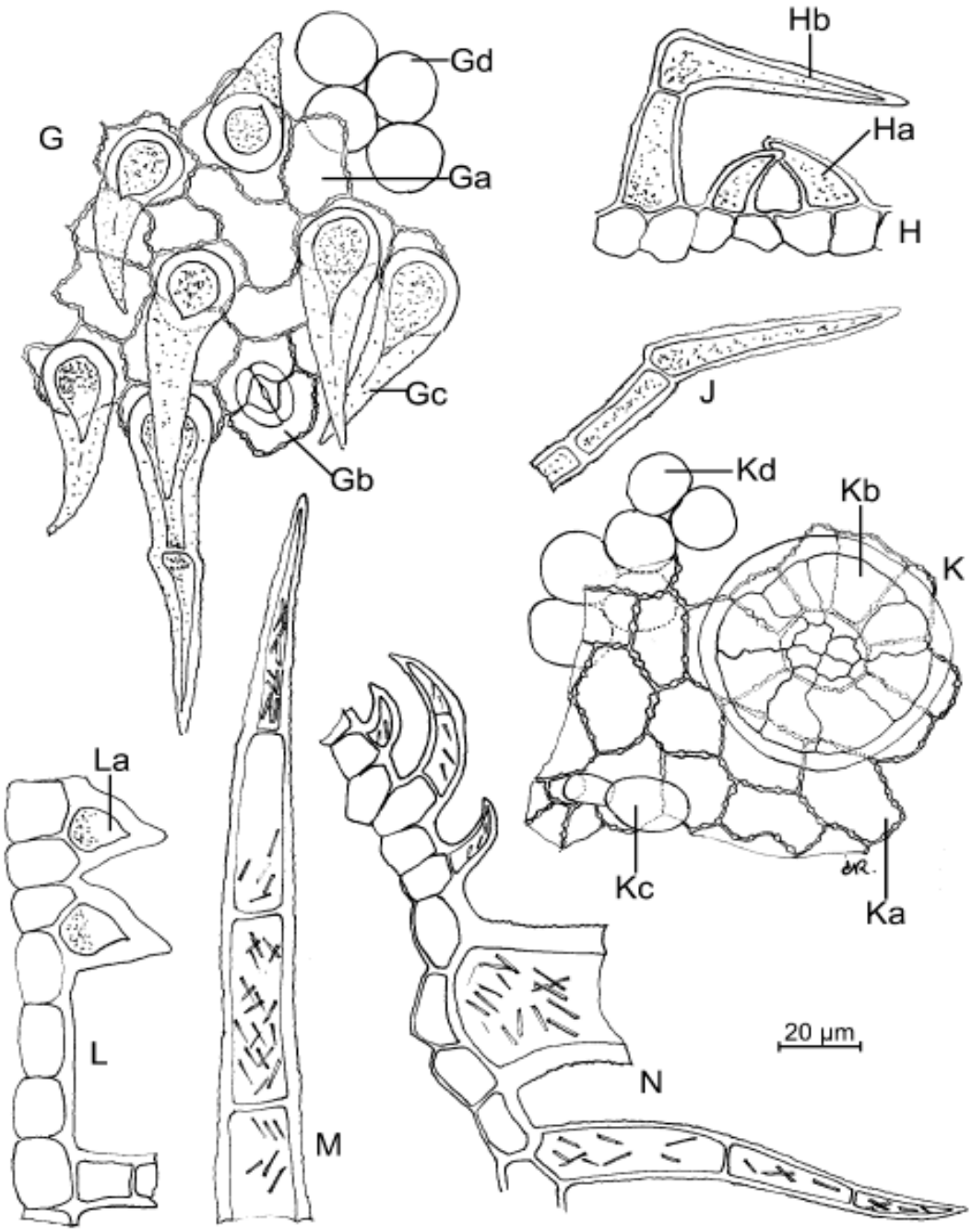

Figure 0865.-2. – *Illustration for identification test B of powdered herbal drug of thyme* ◀

YARROW

Millefolii herba

DEFINITION

Whole or cut, dried flowering tops of *Achillea millefolium* L.

Content:

– *essential oil*: minimum 2 mL/kg (dried drug);
– *proazulenes, expressed as chamazulene* ($C_{14}H_{16}$; M_r 184.3): minimum 0.02 per cent (dried drug).

IDENTIFICATION

A. The leaves are green or greyish-green, faintly pubescent on the upper surface and more pubescent on the lower surface, 2-3 pinnately divided with linear lobes and a finely pointed whitish tip. The capitula are arranged in a corymb at the end of the stem. Each capitulum, 3-5 mm in diameter, consists of the receptacle, usually 4-5 ligulate ray-florets and 3-20 tubular disk-florets. The involucre consists of 3 rows of imbricate lanceolate, pubescent green bracts arranged with a brownish or whitish, membranous margin. The receptacle is slightly convex and, in the axillae of paleae, bears ligulate ray-florets with a three-lobed, whitish or reddish ligule and tubular disk-florets with a radial, five-lobed, yellowish or light brownish corolla. The pubescent green, partly brown or violet stems are longitudinally furrowed, up to 3 mm thick with a light-coloured medulla.

B. Microscopic examination (2.8.23). The powder is green or greyish-green. Examine under a microscope using chloral hydrate solution R. The powder shows the following diagnostic characters (Figure 1382.-1): fragments of the stem epidermis (surface view [K]), with cells having a smooth cuticle and anomocytic stomata (2.8.3); fragments of leaf and bract epidermises (surface view [B]), with cells having wavy and irregularly thickened walls, a finely striated cuticle and anomocytic stomata (2.8.3); very rare glandular trichomes with a short stalk and a head formed of 2 rows of 3-5 cells enclosed in a bladder-like membrane [H]; uniseriate, whole or fragmented covering trichomes [A] consisting of 4-6 small, more or less isodiametric cells at the base and a thick-walled, often somewhat tortuous terminal cell, about 400 µm to greater than 1000 µm long; fragments of the ligulate corolla with papillary epidermal cells [D]; fragments of the corolla tubes, with sinuous epidermal cells, covered by a thin striated cuticle (surface view [F]); small-celled parenchyma from the corolla tubes containing cluster crystals of calcium oxalate [E]; groups of lignified and pitted cells from the bracts [G]; spherical pollen grains, about 30 µm in diameter, with 3 germinal pores and a spiny exine [C]; groups of sclerenchymatous fibres and small vessels with spiral or annular thickening, from the stem [J].

c.To 2.0 g of the powdered herbal drug (710) (2.9.12) add 25 mL of ethyl acetate R, shake for 5 min and filter. Evaporate to dryness on a water-bath and dissolve the residue in 0.5 mL of toluene R (solution A). To 0.1 mL of this solution add 2.5 mL of dimethylaminobenzaldehyde solution R8 and heat on a water-bath for 2 min. Allow to cool. Add 5 mL of light petroleum R and shake the mixture vigorously. The aqueous layer shows a blue or greenish-blue colour.

D. Thin-layer chromatography (2.2.27).

Test solution. Use solution A prepared in identification test C.

Reference solution. Dissolve 10 mg of cineole R and 10 mg of guaiazulene R in 20 mL of toluene R.
Plate: TLC silica gel plate R.
Mobile phase: ethyl acetate R, toluene R (5:95 *V/V*).
Application: 20 µL as bands.
Development: over a path of 10 cm.
Drying: in air.
Detection: ▶treat◀ with anisaldehyde solution R, heat at 100-105 °C for 5-10 min and examine in daylight.
Results: the chromatogram obtained with the reference solution shows in the upper part a red zone (guaiazulene) and in the middle part a blue or greyish-blue zone (cineole). The chromatogram obtained with the test solution shows a violet zone a little above the zone due to guaiazulene in the chromatogram obtained with the reference solution; below this zone a reddish-violet zone; below which, 1-2 not clearly separated greyish-violet or greyish zones (which changes to greenish-grey after a few hours) and a reddish-violet zone a little above the zone due to cineole in the chromatogram obtained with the reference solution. Furthermore, other faint zones may be present.

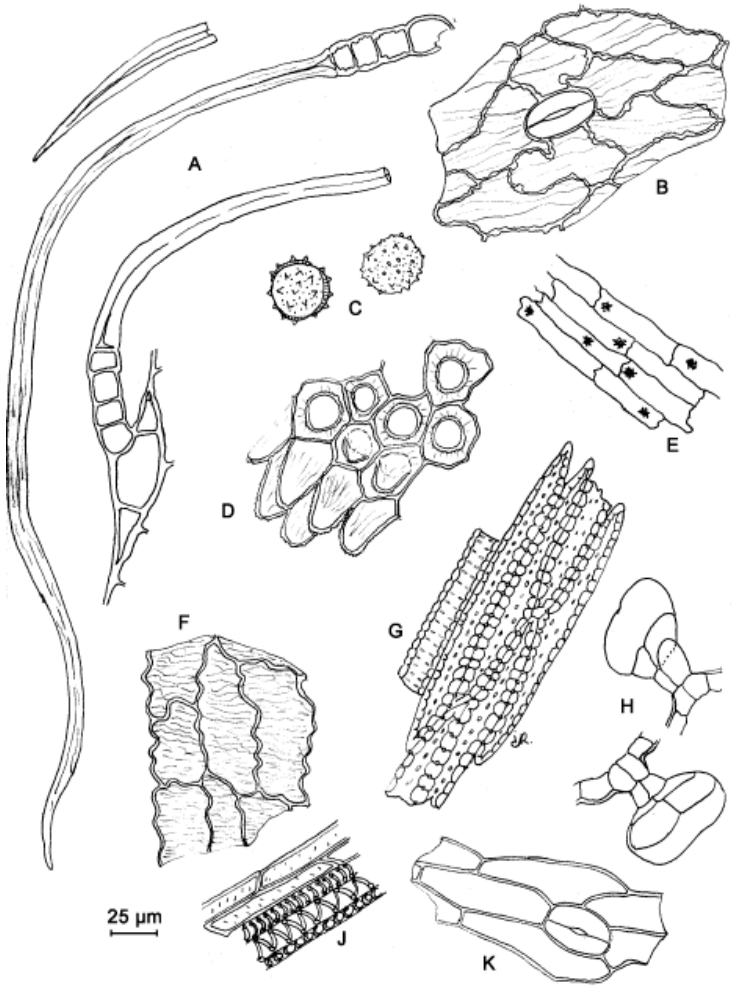

Figure 1382.-1. – *Illustration for identification test B of powdered herbal drug of yarrow*

AGRIMONY

Agrimoniae herba

DEFINITION

Dried flowering tops of *Agrimonia eupatoria* L.

Content: minimum 2.0 per cent of tannins, expressed as pyrogallol ($C_6H_6O_3$; M_r 126.1) (dried drug).

IDENTIFICATION

A. The stem is green or, more usually, reddish, cylindrical and infrequently branched. It is covered with long, erect or tangled hairs. The leaves are compound imparipennate with 3 or 6 opposite pairs of leaflets, with 2 or 3 smaller leaflets between. The leaflets are deeply dentate to serrate, dark green on the upper surface, greyish and densely tomentose on the lower face. The flowers are small and form a terminal spike. They are pentamerous and borne in the axils of hairy bracts, the calyces closely surrounded by numerous terminal hooked spires, which occur on the rim of the hairy receptacle. The petals are free, yellow and deciduous. Fruit-bearing obconical receptacles, with deep furrows and hooked bristles, are usually present at the base of the inflorescence.

B. Reduce to a powder (355) (2.9.12). The powder is yellowish-green or grey. Examine under a microscope using chloral hydrate solution R. The powder shows the following diagnostic characters (Figure 1587.-1): numerous straight or bent, unicellular, long, thick-walled (about 500 µm) covering trichomes [Ab, Ca, F], finely warty, and sometimes spirally marked, often fragmented (F); fragments of the epidermis of the stems [A] with stomata [Aa], covering trichomes (Ab) and glandular trichomes [Ac]; fragments of upper leaf epidermis in surface view [C] with straight walls bearing covering trichomes (Ca), accompanied by palisade parenchyma [Cb], with some of the cells containing calcium oxalate prisms [Cc]; fragments of lower leaf epidermis in surface view [J] with sinuous walls and abundant stomata [Ja], mostly anomocytic (2.8.3) but occasionally anisocytic, and glandular trichomes [Jb]; ovoid to subspherical pollen grains, with 3 pores and a smooth exine [D]; glandular trichomes with a multicellular, uniseriate stalk and a unicellular to quadricellular head [B, Jb]; fragments of the stems [H] with groups of fibres [Ha] and parenchymatous cells, some of which contain cluster crystals of calcium oxalate [Hb]; small spiral vessels from the leaflets [G]; fragments of large, spiral or bordered-pitted vessels from the stem [E]

C. Thin-layer chromatography (2.2.27).

Test solution. To 2.0 g of the powdered herbal drug (355) (2.9.12) add 20 mL of methanol R. Heat with shaking at 40 °C for 10 min. Filter.

Reference solution. Dissolve 1.0 mg of isoquercitroside R and 1.0 mg of rutin R in 2 mL of methanol R.

Plate: TLC silica gel plate R.

Mobile phase: anhydrous formic acid R, water R, ethyl acetate R (10:10:80 *V/V/V*).

Application: 10 µL as bands.

Development: over a path of 12 cm.

Drying: at 100-105 °C.

Detection: spray the still-warm plate with a 10 g/L solution of diphenylboric acid aminoethyl ester R in methanol R and then with a 50 g/L solution of macrogol 400 R in methanol R; allow the plate to dry in air for 30 min and examine in ultraviolet light at 365 nm.
Results: see below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution.

Top of the plate	
	An orange fluorescent zone may be present (quercitroside)
Isoquercitroside: an orange fluorescent zone	An orange fluorescent zone (isoquercitroside)
	An orange fluorescent zone (hyperoside)
Rutin: an orange fluorescent zone	An orange fluorescent zone (rutin)
Reference solution	**Test solution**

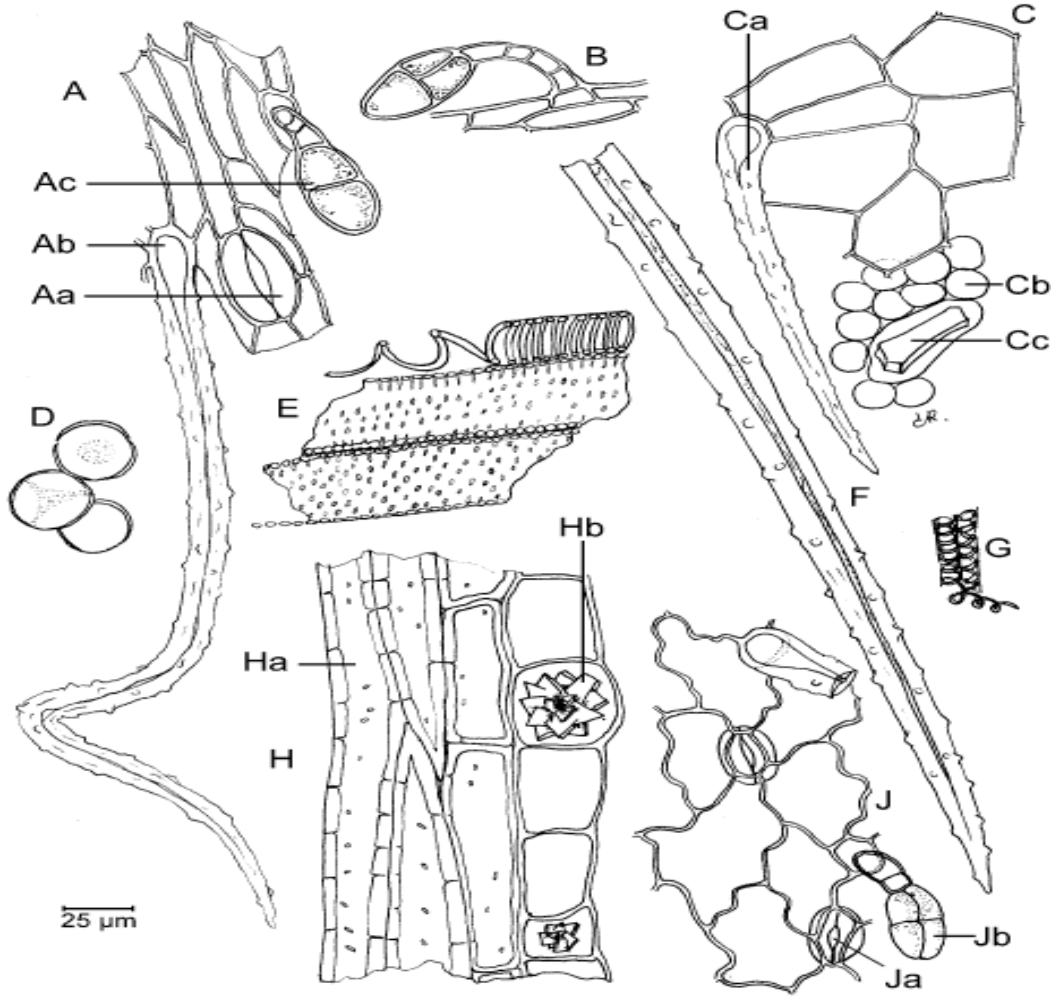

Figure 1587.-1. – *Illustration for identification test B of powdered herbal drug of agrimony*

CENTAURY

Centaurii herba

DEFINITION

Whole or fragmented dried flowering aerial parts of *Centaurium erythraea* Rafn s. l. including *C. majus* (H. et L.) Zeltner and *C. suffruticosum* (Griseb.) Ronn. (syn.: *Erythraea centaurium* Persoon; *C. umbellatum* Gilibert; *C. minus* Gars.).

CHARACTERS

Bitter taste.

IDENTIFICATION

A. The hollow cylindrical, light green to dark brown stem has longitudinal ridges, and is branched only in its upper part. The sessile leaves are entire, decussately arranged, and have an ovate to lanceolate lamina, up to about 3 cm long. Both surfaces are glabrous and green to brownish-green. The inflorescence is diaxially branched. The tubular calyx is green and has 5 lanceolate, acuminate teeth. The corolla consists of a whitish tube divided into 5 elongated lanceolate pink to reddish lobes, about 5-8 mm long. 5 stamens are present attached to the top of the corolla tube. The ovary is superior and has a short style, a broad bifid stigma and numerous ovules. Cylindrical capsules, about 7-10 mm long, with small brown markedly rough seeds are frequently present.

B. Reduce to a powder (355) (2.9.12). The powder is greenish-yellow or brownish. Examine under a microscope, using chloral hydrate solution R. The powder shows the following diagnostic characters: fragments from the stem with lignified groups of fibres associated with narrow vessels, tracheidal vessels occasional vessels with spiral thickening; pitted parenchyma of the pith and medullary rays; fragments of leaf lamina with sinuous epidermal cells and striated cuticle, especially over the margins and surrounding the stomata; numerous stomata, mainly anisocytic (2.8.3); fragments of the palisade mesophyll, each cell containing a single prism crystal or, less frequently, a cluster crystal of calcium oxalate; fragments of calyx and corolla, those of the calyx with straight-walled epidermal cells, those of the inner epidermis of the corolla with obtuse papillae and radially striated cuticle; parts of the endothecium with reticulate or ridge-shaped wall thickenings; triangularly rounded or elliptical, yellow pollen grains, about 30 µm in diameter, with a distinctly pitted exine and 3 germinal pores; fragments of the wall of the fruit capsule composed of crossed layers of fusiform cells; oil droplets from the seeds, fragments of the epidermis of the testa showing large, brown reticulations and a pitted surface.

C. Thin-layer chromatography (2.2.27).

Test solution. To 1.0 g of the powdered herbal drug (355) (2.9.12) add 25 mL of methanol R, shake for 15 min and filter. Evaporate the filtrate to dryness under reduced pressure and at a temperature not exceeding 50 °C. Take up the residue with small quantities of methanol R so as to obtain 5 mL of solution, which may contain a sediment.

Reference solution. Dissolve 1 mg of rutin R and 1 mg of swertiamarin R in methanol R and dilute to 1 mL with the same solvent.

Plate: TLC silica gel F_{254} plate R (5-40 µm) [or TLC silica gel F_{254} plate R (2-10 µm)].

Mobile phase: water R, anhydrous formic acid R, ethyl formate R (4:8:88 *V/V/V*).
Application: 10 µL [or 5 µL] as bands.
Development: in an unsaturated tank over a path of 12 cm [or 6 cm].
Drying: in air.
Detection A: examine in ultraviolet light at 254 nm.
Results A: see below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other less intense quenching zones may be present in the chromatogram obtained with the test solution.

Top of the plate	
_____	_____
_____	_____
Swertiamarin: a quenching zone	A prominent quenching zone (swertiamarin)
Rutin: a quenching zone	
Reference solution	**Test solution**

Detection B: spray with anisaldehyde solution R and heat at 100-105 °C for 5-10 min. Examine in daylight.
Results B: see below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other less intense coloured zones may be present in the chromatogram obtained with the test solution.

Top of the plate	
_____	_____
_____	_____
Swertiamarin: a brown zone	A brown zone (swertiamarin)
Rutin: a yellow zone	
	A brownish-grey zone
	A yellow zone
	A grey zone
Reference solution	**Test solution**

GREATER CELANDINE

Chelidonii herba

DEFINITION

Dried, whole or cut aerial parts of *Chelidonium majus* L. collected during flowering.

Content: minimum 0.6 per cent of total alkaloids, expressed as chelidonine ($C_{20}H_{19}NO_5$; M_r 353.4) (dried drug).

IDENTIFICATION

A. The stems are rounded, ribbed, yellowish or greenish-brown, somewhat pubescent, about 3-7 mm in diameter, hollow and mostly collapsed. The leaves are thin, irregularly pinnate, the leaflets ovate to oblong with coarsely dentate margins, the terminal leaflet often 3-lobed; the adaxial surface is bluish-green and glabrous, the abaxial surface paler and pubescent, especially on the veins. The flowers have 2 deeply concavo-convex sepals, readily removed, and 4 yellow, broadly ovate, spreading petals about 8-10 mm long; the stamens are numerous, yellow, and a short style arises from a superior ovary; long, capsular, immature fruits are rarely present.

B. Microscopic examination (2.8.23). The powder is dark greyish-green or brownish-green. Examine under a microscope using chloral hydrate solution R. The powder shows the following diagnostic characters (Figure 1861.-1): numerous fragments of upper epidermis, composed of cells with sinuous walls in surface view [B], accompanied by underlying palisade parenchyma [Ba]; numerous fragments of lower epidermis in surface view [A, E] bearing anomocytic stomata (2.8.3) [Aa] and bases of covering trichomes [Ab], sometimes accompanied by underlying spongy parenchyma [Ea]; long, uniseriate, multicellular covering trichomes, usually fragmented, with thin-walled cells, sometimes collapsed [G]; vascular tissue from the leaves and stems consisting of pitted and spirally thickened vessels [D]; groups of fibres [C]; articulated latex tubes with yellowish-brown contents [F]; occasional fragments of the corolla [H] consisting of thin-walled cells containing numerous pale yellow droplets of oil [Ha]; spherical pollen grains about 30-40 µm in diameter with 3 pores and a finely pitted exine [J].

C. Thin-layer chromatography (2.2.27).

Test solution. To 0.4 g of the powdered herbal drug (710) (2.9.12) add 50 mL of dilute acetic acid R. Boil in a water-bath under a reflux condenser for 30 min. Cool and filter. To the filtrate add concentrated ammonia R until a strong alkaline reaction is produced. Shake with 30 mL of methylene chloride R. Dry the organic layer over anhydrous sodium sulfate R, filter and evaporate *in vacuo* to dryness. Dissolve the residue in 1.0 mL of methanol R.

Reference solution. Dissolve 2 mg of methyl red R and 2 mg of papaverine hydrochloride R in 10 mL of ethanol (96 per cent) R.

Plate: TLC silica gel plate R.

Mobile phase: anhydrous formic acid R, water R, propanol R (1:9:90 *V/V/V*).

Application: 10 µL as bands.

Development: over a path of 10 cm.

Drying: in air.

Detection: spray with potassium iodobismuthate solution R and dry in air; spray with sodium nitrite solution R and allow to dry in air; examine in daylight.

Results: see below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other weaker zones may be present in the chromatogram obtained with the test solution.

Top of the plate	
_____	_____
Methyl red: a red zone	A brown zone
	A brown zone
Papaverine: a greyish-brown zone	A greyish-brown zone
_____	_____
	2 brown zones
Reference solution	**Test solution**

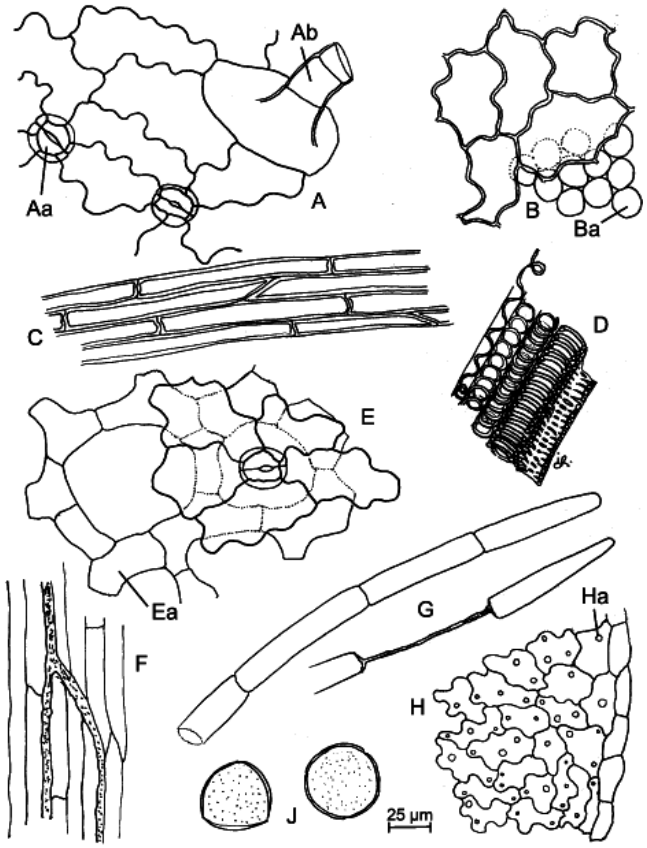

Figure 1861.-1. – *Illustration for identification test B of powdered herbal drug of greater celandin*

MEADOWSWEET

Filipendulae ulmariae herba

DEFINITION

Whole or cut, dried flowering tops of *Filipendula ulmaria* (L.) Maxim. (syn. *Spiraea ulmaria* L.).
Content: minimum 1 mL/kg of essential oil (dried drug).

CHARACTERS

Aromatic odour of methyl salicylate, after crushing.

IDENTIFICATION

A. The stem, up to 5 mm in diameter, is greenish-brown, stiff, angular, hollow except at the apex, and has regular, straight, longitudinal furrows. The petiolate leaf, compound imparipinnate, has 2 reddish-brown angular stipules. It consists of 3-9 pairs of leaflets, unevenly dentate, some of which are small and fan-shaped. The leaflets are dark green and glabrous on the upper surface, tomentose and lighter, sometimes silvery on the lower surface. The terminal leaflet, the largest, is divided into 3 segments. The veins are prominent and brown on the lower surface. The inflorescence is complex and composed of very numerous flowers arranged in irregular cymose panicles. The flowers are creamish-white and about 3-6 mm in diameter; the calyx consists of 5 dark green, reflexed and hairy sepals fused at the base to a concave receptacle; the 5 free petals, which are readily detached, are pale yellow, obovate and distinctly narrowed at the base; the stamens are numerous with rounded anthers and they extend beyond the petals; the gynoecium consists of about 4-6 carpels, each with a short style and a globular stigma; the carpels become twisted together spirally to form yellowish-brown fruits with a helicoidal twist. Unopened flower buds are frequently present. If the fruit is present, it has a helicoidal twist and contains brownish seeds.

B. Microscopic examination (2.8.23). The powder is green or yellowish-green. Examine under a microscope using chloral hydrate solution R. The powder shows the following diagnostic characters (Figure 1868.-1): fragments of the epidermises of the leaves and sepals [C, E, F] with sinuous or wavy cells [Ca, Ea, Fa], short, thick-walled, conical covering trichomes thickened at the base, in surface view [Eb] and in side view [J], unicellular covering trichomes, thin-walled, very long and flexuous, with pointed ends in surface view [Fc] and in side view [A], or their scars (flexuous trichome [Fd], conical trichome [Fe]) and occasional clavate glandular trichomes with a 1- to 3-celled ([Ed] and [G], respectively), uniseriate stalk, a multicellular head and dense brown contents; fragments of the upper epidermis often accompanied by palisade parenchyma [Cb] including some hypertrophied cells containing a cluster crystal of calcium oxalate [Cc]; fragments of the lower epidermis with anomocytic stomata (2.8.3) [Ec, Fb], sometimes accompanied by spongy parenchyma [Ff] with some cells containing cluster crystals of calcium oxalate [Fg]; fragments of the petals [H] with thin-walled epidermal cells, some showing rounded papillae [Ha]; numerous spherical pollen grains with 3 pores and a faintly pitted exine [Bb]; fragments of the anther [B, D] whose fibrous layer shows specific thickenings, in surface view [D] and in side view [Ba]; fragments of the ovary [K] with an epidermis bearing stomata [Ka] and with parenchyma containing prism crystals of calcium

oxalate [Kb]; fragments of vascular tissue [L] with annular, spiral or pitted vessels from the leaves and stems.

C. Thin-layer chromatography (2.2.27).

Test solution. Xylene solution obtained in the assay.

Reference solution. Dissolve 0.1 mL of methyl salicylate R and 0.1 mL of salicylaldehyde R in xylene R and dilute to 5 mL with the same solvent.

Plate: TLC silica gel plate R.

Mobile phase: hexane R, toluene R (50:50 *V/V*).

Application: 10 µL as bands.

Development: over a path of 10 cm.

Drying: in air.

Detection: treat with 3 mL of ferric chloride solution R3 and examine in daylight.

Results: see below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other zones are present in the chromatogram obtained with the test solution.

Top of the plate	
_____	_____
Methyl salicylate: a violet-brown zone	A violet-brown zone (methyl salicylate)
Salicylaldehyde: a violet-brown zone	A violet-brown zone (salicylaldehyde)
_____	_____
Reference solution	**Test solution**

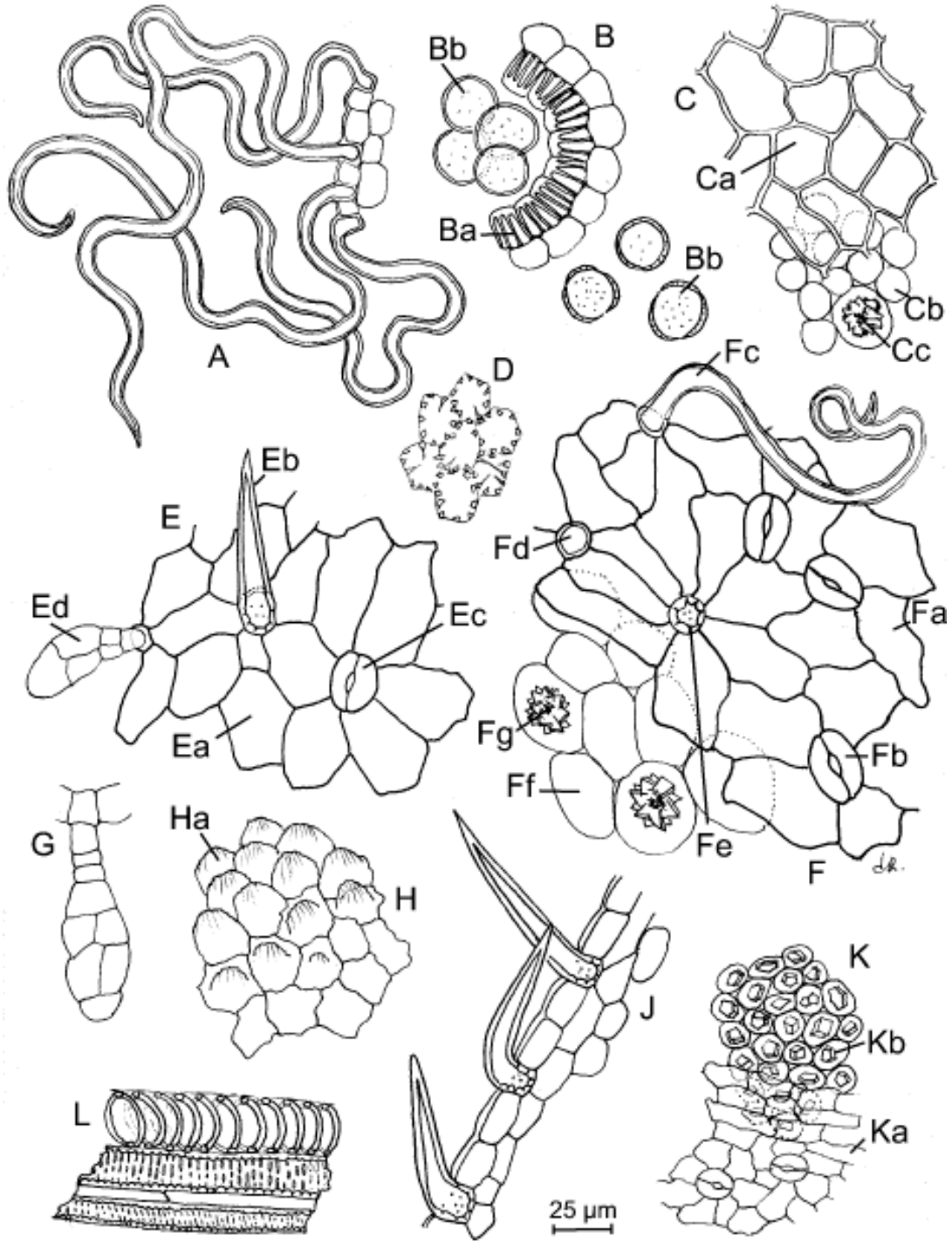

Figure 1868.-1. – *Illustration for identification test B of powdered herbal drug of meadowsweet*

MELILOT

Meliloti herba

DEFINITION

Whole or cut, dried aerial parts of *Melilotus officinalis* (L.) Lam.

Content: minimum 0.3 per cent of coumarin ($C_9H_6O_2$; M_r 146.1) (dried drug).

IDENTIFICATION

A. The stem is green, cylindrical, glabrous and finely ridged. The leaves are alternate, petiolate and trifoliate with 2 lanceolate stipules; the leaflets are up to about 3 cm long and 20 mm wide, elongated or ovate with a finely dentate margin, acute at the apex and base; the upper surface is dark green and glabrous, the lower surface paler green with short, fine hairs, especially at the base. The inflorescence is racemose with numerous pale yellow flowers, about 7 mm long, each having a hairy calyx with 5 deeply-divided, unequal teeth, and a papilionate corolla. The fruit is an indehiscent pod, often persistent within the calyx, yellowish-brown, short and tapering at the apex; the surface is glabrous and transversely wrinkled.

B. Microscopic examination (2.8.23). The powder is yellowish-green. Examine under a microscope using chloral hydrate solution R. The powder shows the following diagnostic characters (Figure 2120.-1): fragments of the leaf lamina in surface view [D] showing unevenly thickened, slightly sinuous epidermal cells; numerous stomata [Db], mostly anomocytic (2.8.3) with 3-6 subsidiary cells [Da] and frequently, underlying palisade parenchyma [Dc]; uniseriate covering trichomes with 2 short, smooth-walled basal cells and a long terminal cell, bent at right angles, with a thick wall and a warty cuticle [A, B]; occasional glandular trichomes with a short, 2- or 3-celled stalk and ovoid, biseriate head with 4 indistinct cells [H]; fragments of the petals composed of cells with wavy walls [M]; fragments of vascular tissue from the stem [F, G], including large vessels [G], sometimes associated with unlignified septate fibres [Fa] and a sheath of parenchymatous cells containing prisms of calcium oxalate [Fb]; fragments of mesophyll [J] including some cells which may occasionally contain cluster crystals of calcium oxalate [Ja]; fragments of the stem epidermis with elongated, straight-walled cells and anomocytic (2.8.3) stomata [L]; fragments of the fibrous layer of the anthers in surface view [E] and in transverse section [K]; spherical or ovoid pollen grains about 25 µm long with 3 germinal pores and a smooth exine [C].

C. Thin-layer chromatography (2.2.27).

Test solution. To 0.3 g of the powdered herbal drug (355) (2.9.12) add 3 mL of methanol R. Heat on a water-bath at 100 °C for 1 min and filter.

Reference solution. Dissolve 50 mg of coumarin CRS and 20 mg of *o*-coumaric acid R in 50 mL of methanol R.

Plate: TLC silica gel plate R (5-40 µm) [or TLC silica gel plate R (2-10 µm)].

Mobile phase: dilute acetic acid R, ether R, toluene R (10:50:50 *V/V/V*); use the upper layer.

Application: 25 µL [or 3 µL] as bands of 10 mm [or 8 mm].

Development: over a path of 12 cm [or 6 cm].

Drying: in air.

Detection: spray with 2 M alcoholic potassium hydroxide R and examine in ultraviolet light at 365 nm.
Results: see below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other faint zones of various colours may be present in the chromatogram obtained with the test solution.

Top of the plate	
Coumarin: a greenish-yellow fluorescent zone	A greenish-yellow fluorescent zone (coumarin)
_____	_____
	A blue fluorescent zone
o-Coumaric acid: a greenish-yellow fluorescent zone	A greenish-yellow fluorescent zone (*o*-coumaric acid) may be present
_____	_____
Reference solution	**Test solution**

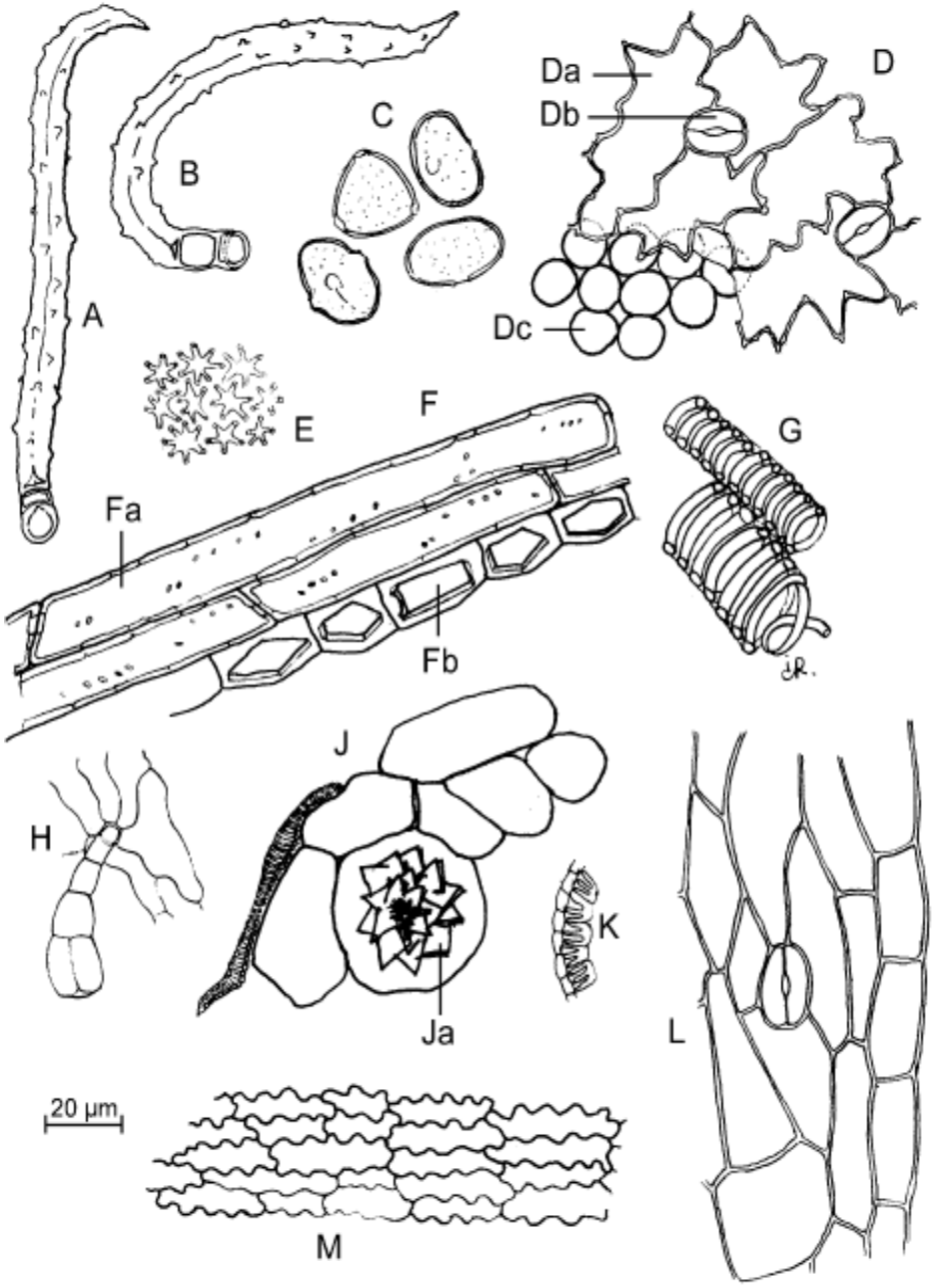

Figure 2120.-1. – *Illustration for identification test B of powdered herbal drug of melilot*

ST. JOHN'S WORT

Hyperici herba

DEFINITION

Whole or fragmented, dried flowering tops of *Hypericum perforatum* L., harvested during flowering time.

Content: minimum 0.08 per cent of total hypericins, expressed as hypericin ($C_{30}H_{16}O_8$; M_r 504.4) (dried drug).

IDENTIFICATION

A. The branched and bare stem shows 2 more or less prominent longitudinal ridges. The leaves are opposite, sessile, exstipulate, oblong-oval and 15-30 mm long; present on the leaf margins are glands which appear as black dots and over all the surface of the leaves many small, strongly translucent excretory glands which are visible in transmitted light. The flowers are regular and form corymbose clusters at the apex of the stem. They have 5 green, acute sepals, with black secretory glands on the margins; 5 orange-yellow petals, also with black secretory glands on the margins; 3 staminal blades, each divided into many orange-yellow stamens and 3 carpels surmounted by red styles.

The drug may also show the following: immature and ripe fruits and seeds. Immature fruits are green or yellowish, seeds are whitish. Occasional ripe fruits may be present; these are dry trilocular capsules containing numerous seeds, brown, broad or small-ovate, 5-10 mm long, with broad linear or punctiform glands, irregularly striated ducts, conducting secretions. Ripe seeds are 1-1.3 mm long, cylindrical or trigonous, shortly pointed at both ends, brown or almost black, minutely pitted longitudinally.

B. Microscopic examination (2.8.23). The powder is greenish-yellow. Examine under a microscope using chloral hydrate solution R. The powder shows the following diagnostic characters (Figure 1438.-1): fragments of the leaf epidermis [A, B] or stems [H] with paracytic [Ab, Ha], anisocytic [Ac, Bb, Hb] or anomocytic [Ae] stomata (2.8.3); fragments of the leaf epidermis often accompanied by palisade parenchyma [Ad, Bc]; polygonal cells of the upper epidermis with thickened and beaded walls [Ba]; more or less sinuous, thin-walled cells of the lower epidermis [Aa]; fragments of the leaf and sepal [E] with large, red-pigmented oil glands [Ea] associated with palisade parenchyma [Eb] and small vessels [Ec]; elongated cells of fragments of the petal epidermis with straight or wavy anticlinal walls [J]; vessels [D] with reticulate or pitted walls [Da] and groups of thick-walled fibres [Db]; fragments of the central parenchyma of the stems [K] with lignified and pitted rectangular cells [Ka] sometimes associated with vessels [Kb]; fragments of the anthers [F] showing the central part consisting of small cells containing cluster crystals of calcium oxalate [Fb] and cells from the fibrous layer [Fa]; fragments of the staminal filament with elongated, thin-walled cells with a striated cuticle [C]; numerous pollen grains with 3 germinal pores and a smooth exine, occurring singly [G] or in dense groups.

C. Thin-layer chromatography (2.2.27).

Test solution. Stir 0.5 g of the powdered herbal drug (500) (2.9.12) in 10 mL of methanol R in a water-bath at 60 °C for 10 min and filter.

Reference solution. Dissolve 5 mg of hyperoside R and 5 mg of rutin R in methanol R, then dilute to 5 mL with the same solvent.
Plate: TLC silica gel plate R.
Mobile phase: anhydrous formic acid R, water R, ethyl acetate R (6:9:90 *V/V/V*).
Application: 10 µL of the test solution and 5 µL of the reference solution, as bands of 10 mm.
Development: over a path of 10 cm.
Drying: at 100-105 °C for 10 min.
Detection: treat with a 10 g/L solution of diphenylboric acid aminoethyl ester R in methanol R and then with a 50 g/L solution of macrogol 400 R in methanol R. After about 30 min, examine in ultraviolet light at 365 nm.
Results: the chromatogram obtained with the reference solution shows in the lower third a zone due to rutin and above it a zone due to hyperoside, both with yellow-orange fluorescence. The chromatogram obtained with the test solution shows in the lower third 2 reddish-orange fluorescent zones due to rutin and hyperoside, and in the lower part of the upper third a zone due to pseudohypericin and above it a zone due to hypericin, both with red fluorescence. Other yellow or blue fluorescent zones are visible.

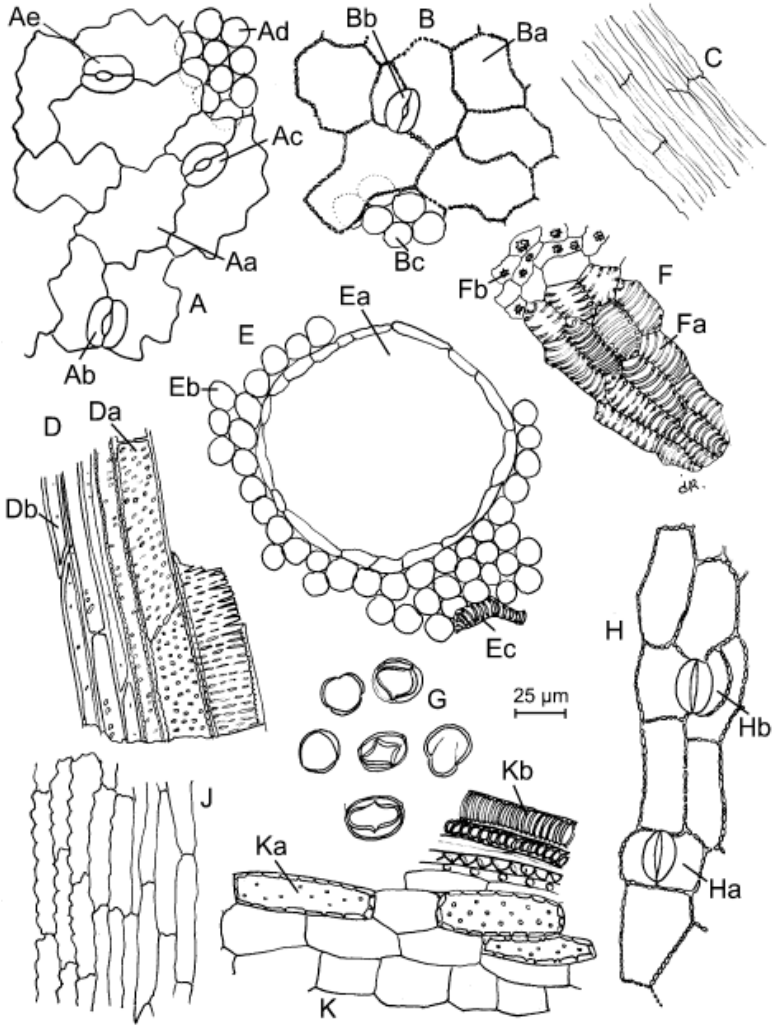

Figure 1438.-1. – *Illustration for identification test B of powdered herbal drug of St. John's wort*

EQUISETUM STEM

Equiseti herba

DEFINITION

Whole or cut, dried sterile aerial parts of *Equisetum arvense* L.
Content: minimum 0.3 per cent of total flavonoids, expressed as isoquercitroside ($C_{21}H_{20}O_{12}$; M_r 464.4) (dried drug).

IDENTIFICATION

A. It consists of fragments of grooved main stems, branches with longitudinal sharp ridges and leaves in whorls, united at the base into a sheath, light green or greenish-grey. The fragments are rough to the touch, brittle and crunchy when crushed. The main stems are about 1-4.5 mm in diameter, hollow, jointed at the nodes, which occur at intervals of about 1.5-4.5 cm; distinct vertical grooves are present on the internodes, ranging in number from 4 to 14 or more. The central hollow is less than 50 per cent but more than 25 per cent of the diameter of the main stem. Verticils of widely spaced and erect branches, usually simple, each about 1 mm thick with 3-5 longitudinal, sharp ridges, occur at the nodes; at the end of each ridge is a protruding, distinct collenchymatic bundle under the epidermis. The branches are not hollow. The leaves are small, linear, verticillate at each node, concrescent at the base; they form a toothed sheath around the stem with the number of teeth corresponding to the number of grooves on the stem. Each tooth, often brown, is lanceolate-triangular. The lowest internode of each branch is longer than the sheath of the stem to which it belongs.

B. Microscopic examination (2.8.23). The powder is greenish-grey. Examine under a microscope using chloral hydrate solution R. The powder shows the following diagnostic characters (Figure 1825.-1): fragments of the epidermis in surface view [B, C] composed of rectangular cells with wavy walls and paracytic stomata (2.8.3) in 2-4 rows, the 2 subsidiary cells are in the same plane as the epidermis, cover the guard cells and show radial ridges; small silica pilulae are scattered on the surface of the subsidiary cells and appear more frequent at the margin forming a distinct ring surrounding the subsidiary cells (C); 2-celled papillae on the ridges, less distinct on the main stem [A] but large and rectangular on the branches, oriented longitudinally [F]; in surface view, the epidermis of the main stems consists of elongated cells [G], the epidermis of the secondary branches shows the 2-celled papillae which resemble pairs of small cells separated by a larger cell [D]; fragments of large-celled parenchyma [H] and groups of long unlignified fibres with narrow lumens; small vessels with spiral or annular thickening [E].

C. Examine the chromatograms obtained in the test for *Equisetum palustre*.
Results: see below the sequence of zones present in the chromatograms obtained with reference solution (b) and the test solution. Furthermore, other weak fluorescent zones may be present in the chromatogram obtained with the test solution.

Top of the plate	
	2 red fluorescent zones
Caffeic acid: a greenish-blue fluorescent zone	
	2 greenish-blue fluorescent zones
_____	_____
	An orange fluorescent zone
Hyperoside: an orange fluorescent zone	
	2 greenish-blue fluorescent zones
_____	_____
Rutin: an orange fluorescent zone	
Reference solution (b)	**Test solution**

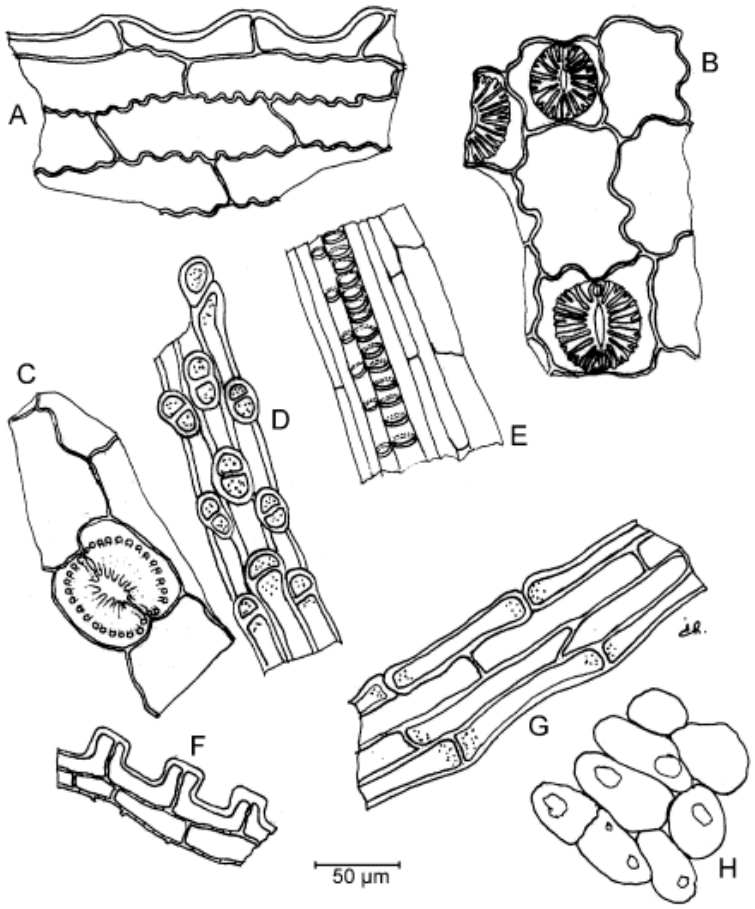

Figure 1825.-1. – *Illustration for identification test B of powdered herbal drug of equisetum stem*

PASSION FLOWER

Passiflorae herba

DEFINITION

Fragmented or cut, dried aerial parts of *Passiflora incarnata* L. It may also contain flowers and/or fruits.

Content: minimum 1.5 per cent of total flavonoids, expressed as vitexin ($C_{21}H_{20}O_{10}$; M_r 432.4) (dried drug).

IDENTIFICATION

A. The green or greenish-grey or brownish stem is ligneous, hollow, longitudinally striated, glabrous or very slightly pubescent, with a diameter that is generally less than 8 mm. The green or greenish-brown leaves are alternate, finely dentate and pubescent, deeply divided into 3 acute lobes of which the central lobe is the largest. The midrib is much more prominent on the lower surface. The petiole is pubescent and bears 2 dark nectaries near the lamina. The tendrils are very numerous and grow from the axils of the leaves; they are fine, smooth, round and terminated in cylindrical spirals. The radiate flowers, if present, have 3 small bracts and a corolla consisting of 5 white, elongated petals with several rows of filiform, petaloid appendices. If present, the greenish or brownish fruit is flattened and oval; it contains several flattened, brownish-yellow, pitted seeds.

B. Reduce to a powder (355) (2.9.12). The powder is light green. Examine under a microscope using chloral hydrate solution R. The powder shows the following diagnostic characters: fragments of the leaf epidermis with sinuous walls and anomocytic stomata (2.8.3); numerous cluster crystals of calcium oxalate isolated or aligned along the veins; many isolated or grouped fibres from the stems associated with pitted vessels and tracheids; uniseriate trichomes with 1-3 thin-walled cells, straight or slightly curved, ending in a point or sometimes a hook. In addition, the powder shows, if flowers are present, papillose epidermises of the petals and appendages and pollen grains with a reticulate exine; and if mature fruits are present, scattered brown tannin cells and brownish-yellow, pitted fragments of the testa.

C. Examine the chromatograms obtained in the test for other species of *Passiflora*.

Results: the chromatogram obtained with the test solution shows below the zone due to rutin in the chromatogram obtained with the reference solution a zone of intense yellow fluorescence, above it a zone of green fluorescence (diglycosylflavone), below the zone due to hyperoside in the chromatogram obtained with the reference solution a zone of yellow fluorescence (iso-orientin) and above a zone of green fluorescence (isovitexin), above the zone due to hyperoside in the chromatogram obtained with the reference solution a zone of brownish-yellow fluorescence (orientin) and above it a zone of green fluorescence (vitexin). These latter 2 zones may be absent. Further zones may be present.