

Saponins-containing medicinal plants and herbal drugs

MORPHOLOGICAL, ANATOMICAL AND CHEMICAL ANALYSIS OF SAPONINS-CONTAINING MEDICINAL PLANTS AND HERBAL DRUGS

Saponins are a group of natural organic compounds, which due to their chemical structure appear to be glycosides, possessing **high surface activity**. They exert **hemolytic** and **toxic** action, especially on cold-blooded animals. Many saponins are used as fish poisons.

Saponin glycosides are widely distributed in higher plants and possess the following properties:

- a. They form colloidal solutions in water which *foam on shaking*.
- b. There are usually *sternutatory and irritating to mucous membranes* and possess a bitter taste.
- c. They *destroy erythrocytes via hemolysis* and are especially toxic to cold-blooded animals (fish poison).
- d. Hydrolysis affords *aglycone (genin)* known as a sapogenin.
- e. The more poisonous saponins are called sapotoxins.
- f. The saponins themselves are frequently amorphous and difficult to separate and purify but do form nicely crystalline acetylation products.

Questions on topic "Saponins"

1. Which compounds are called saponins?
2. What physical and chemical properties saponins do have?
3. What do underlie the classifications of the saponins?
4. What does underlie the chemical classification of the saponins?
5. Which methods of qualitative analysis of saponins are used?
6. Which methods of quantitative analysis of saponins are used? What is advantage of these methods?
7. What kinds of pharmacological activities do saponins have? (What purposes are saponins employed for?)
8. How should herbal drugs contained saponins be picked up?
9. How should herbal drugs contained saponins be stored?
10. List the Latin name of plant sources of steroidal hormones.

Quantitative analysis of saponins in herbal drugs

Definition of haemolytic index by Koffler method. Haemolytic index is minimal concentration of the substance (mkg/ml) that exerts complete haemolysis of 2 % suspension of defibrinated blood.

Place 2 g of a powdered plant drug (weigh with precision $\pm 0,01$ g), into a flask and add 100 ml of hot physiological solution with phosphate buffer pH 7,4. Weigh the flask with the solution (with precision $\pm 0,01$ g) and make an incision in a boiling water bath for 15 min. Add water into flask to maintain the previous weight of the flask, and filtrate. Carry out the experiment using 10 test tubes. Add 1,0, 0,9, 0,8; 0,7; 0,6; 0,5; 0,4; 0,3; 0,2; 0,1 ml of the obtained extract in each of 10 test tubes. Add the necessary quantity of physiological solution to maintain 1 ml, then add 1 ml erythrocyte suspension and shake. Find the last test-tube, where haemolysis occurred after 15 min. If haemolysis occurs in all test tubes, prepare a new portion of solutions. The results write in

Calculate haemolytic index from the expression:

$$X = \frac{2 \cdot 100}{a \cdot b} =$$

a - primary concentration of extraction in %,

b - quantitative of primary solution in final test-tube, where haemolysis occurred, ml.

Determination of foaming index

Many medicinal plant materials contain saponins that can cause persistent foam when an aqueous decoction is shaken. The foaming ability of an aqueous decoction of plant materials and their extracts is measured in terms of a foaming index.

Recommended procedure

Reduce about 1 g of the plant material to a coarse powder (sieve size no. 1250), weigh accurately and transfer to a 500-ml conical flask containing 100 ml of boiling water. Maintain at moderate boiling for 30 minutes. Cool and filter into a 100 ml volumetric flask and add sufficient water through the filter to dilute to volume.

Pour the decoction into 10 stoppered test-tubes (height 16 cm, diameter 16 mm) in successive portions of 1 ml, 2 ml, 3 ml, etc. up to 10 ml, and adjust the volume of the liquid in each tube with water to 10 ml. Stopper the tubes and shake them in a lengthwise motion for 15 seconds, two shakes per second. Allow to stand for 15 minutes and measure the height of the foam. The results are assessed as follows.

If the height of the foam in every tube is less than 1 cm, the foaming index is less than 100.

• If a height of foam of 1 cm is measured in any tube, the volume of the plant material decoction in this tube (a) is used to determine the index. If this tube is the first or second tube in a series, prepare an intermediate dilution in a similar manner to obtain a more precise result.

If the height of the foam is more than 1 cm in every tube the foaming index is over 1000. In this case repeat the determination using a new series of the decoction in the order to obtain a result.

Calculate the foaming index using the following formula

$$X = \frac{1000}{a}$$

a - the volume in ml of the decoction used for preparing the dilution in the tube foaming to a height of 1 cm is observed

Tests for saponins

1. Identification of saponin according to the physical properties.

a) Shake vigorously 3 ml of the extract for several seconds. Foam appears if saponin are present.

b) Carry out of Fontan-Kandell reaction for determination of a chemical group of saponins.

Take two test-tubes of the same colour and diameter, containing 2 ml of the extract; add 2 ml of 0,5 N solution of HCl solution (pH=1,0) to the first test-tube; and 2 ml 0,5 N NaOH solution (pH= 13) to the second one and shake both test-tubes. Determine the height of the foam and its stability. Indicate conditions of letter forming of foam (pH of medium) and make the conclusion about the chemical group of saponins.

If foam is stable in an alkaline medium, the sample contains steroid saponins. The foam of triterpenoid saponins is stable both in alkaline and acid mediums

2. Identification of saponins by their chemical properties.

a) **Precipitation reactions.**

add several drops of Pb (CH₃COOH)₂ to 2 ml of the extract; white precipitation is foamed

add several drops of Nessler reagent to 2 ml of the extract

add several drops of saturated solution of Ba(OH)₂ to 2 ml of the extract, white precipitation is foamed

b) **reactions of forming of colour substances:**

Libermann-Burchard reaction. Evaporate 2 ml of the extract in the porcelain cup, dissolve the residue in the 0,5 ml of acetate anhydride and place it into the test tube. **Carefully** (!) add the equal volume of concentrate sulphuric acid.

3. Identification of saponins by biological properties.

Add 2 ml of erythrocyte suspension in a physiological solution to 2 ml of the extract. Note the result of the reaction after 15 min. Describe changes you watch and explain them.

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Yam rhizome with roots - *Rhizoma cum radicibus Dioscoreae*

Plant Yam - *Dioscorea nipponica* Makino, *D.villosa*

Family – *Dioscoreaceae*



Fig. 12.1. Yam rhizome

Description. The raw material of yam is represented by pieces of rhizomes up to 30 cm long and to 2 cm in diameter. The rhizomes are cylindrical, slightly curved, unbranched, longitudinally wrinkled. They are covered by thin layer of cork, which usually easily breaks off. The upper surface of rhizomes bears leaf-scars. Thin unbranched adventitious roots stem from rhizomes; they are up to 40 cm long and about 1 mm in diameter. The outer surface of rhizome is light-brown or yellowish. The fracture is even, white. The odour is weak, specific. The taste is bitter, slightly burning.

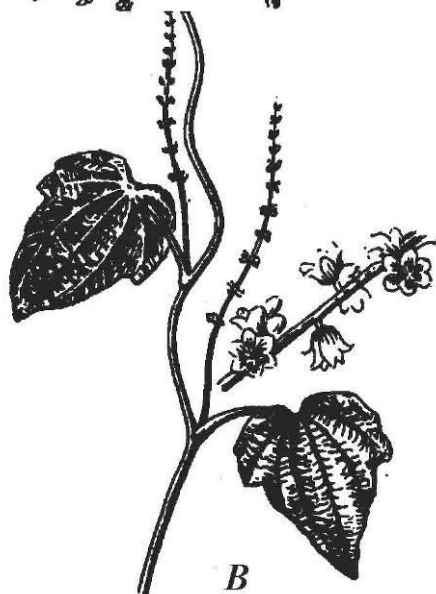


Fig. 12.2. Yam, Dioscorea nipponica (A) and D.caucasica (B)

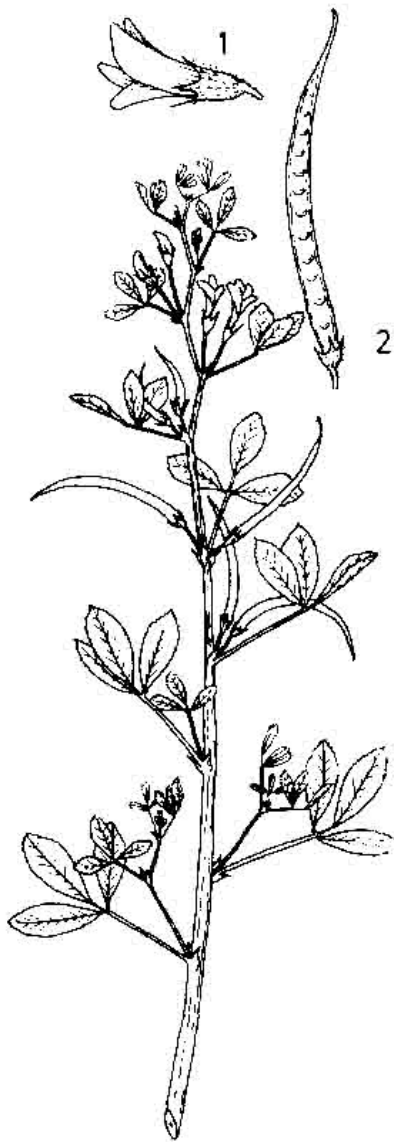
Fenugreek seeds - *Semen Foeni-graeci*

Fenugreek - *Trigonella foenum graecum* L.

Family *Fabaceae*

Definition. Fenugreek consists of the dried, ripe seeds of *Trigonella foenum-graecum* L.

Description. The seed is hard, flattened, brown to reddish-brown and more or less rhomboidal with rounded edges. It is 3 mm to 5 mm long, 2 mm to 3 mm wide and 1.5 mm to 2 mm thick. The widest surfaces are marked by a groove that divides the seed into two unequal parts. The smaller part contains the radicle; the larger part contains the cotyledons. Fenugreek has a strong characteristic aromatic odour.



Fenugreek seeds. A spice cultivated in many parts of the world and derived from *Trigonella foenum-graecum* (Fam. Leguminosae). Different commercial samples vary according to their geographic origin (Fig. 12.3). The seeds may be irregularly rhomboidal, oblong or square in outline, and yellow, olive-green or yellowish-brown to dark brown in colour. The Indian and Moroccan varieties are quoted on the London Drug Market.

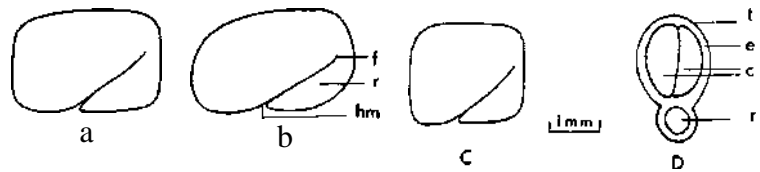


Fig. 12.3. Commercial fenugreek seed. *a*, Morocco, Israel; *b*, Ethiopia; *c*, India, Pakistan; *d*, transverse section of a seed: *c*, Cotyledons; *e*, endosperm, *f*, furrow; *hm*, hilum and micropyle region; *r*, radicle; *t*, testa.

Fig. 12.4. Fenugreek (1 - flower; 2 - legume)

Anatomical characteristics. The powder shows fragments of the testa in sectional view with thick cuticle covering lageniform epidermal cells, with an underlying hypodermis of large cells, narrower at the upper end and constricted in the middle, with bar-like thickenings of the radial walls; yellowish-brown fragments of the epidermis in surface view, composed of small, polygonal cells with thickened and pitted walls, frequently associated with the hypodermal cells, circular in outline with thickened and closely beaded walls; fragments of the hypodermis viewed from below, composed of polygonal cells whose bar-like thickenings extend to the upper and lower walls; parenchyma of the testa with elongated, rectangular cells with slightly thickened and beaded walls; fragments of endosperm with irregularly thickened, sometimes elongated cells, containing mucilage.

False aloe root – *Radix Agavae*

False aloe, Rattlesnake's master - *Agava virginica* L.

Family *Amaryllidaceae*

Plant. Leaves linear-lanceolate, fleshy, glabrous, and radical, with cartilaginous, marginal serratures.

Soap Bark Quillaia Bark – *Cortex Quillajae*

Quillaja, Soap tree – *Quillaja saponaria* Mol.

Family *Rosaceae*

Definition Quillaia is the dried inner part of the bark of *Quillaja saponaria* Molina and of other

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species of *Quillaja*. Extractive soluble in ethanol (45%). Not less than 22.0%.



Description Pieces flat, up to about 1 metre long, 10 to 20 cm broad and 3 to 10 mm, usually 6 mm, thick. Outer surface brownish white or pale reddish brown, longitudinally striated or coarsely reticulated, with occasional blackish brown patches of adherent outer bark; inner surface yellowish white, smooth and very hard; fracture splintery and laminated, the broken surface showing numerous large prisms of calcium oxalate as glistening points. Smoothed transversely cut surface appearing chequered, with delicate radial lines representing medullary rays and tangential lines formed by alternating tangential bands of fibrous and non-fibrous phloem. Odourless or almost odourless; dust strongly sternutatory.

Fig. 12.5. Flowering Soap tree

Anatomical characteristics Outer bark, when present, consisting of reddish brown cork cells alternating with bands of brown parenchyma containing numerous groups of phloem fibres and large prisms of calcium oxalate. Inner bark consisting of alternating bands of tortuous fibres, irregularly enlarged at intervals, about 500 to 1000 μm long and 20 to 50 μm wide and of sieve tissue mixed with parenchyma. Medullary rays mostly three to four, but sometimes up to six cells wide, with occasional pitted, subrectangular sclereids adjacent to the bundles of phloem fibres. Starch granules 5 to 20 μm , usually about 10 μm , in diameter and prisms of calcium oxalate usually 50 to 170 μm long and up to 30 μm wide present in the parenchymatous cells.

Sarsaparilla root - *Radix Sarsaparillae*

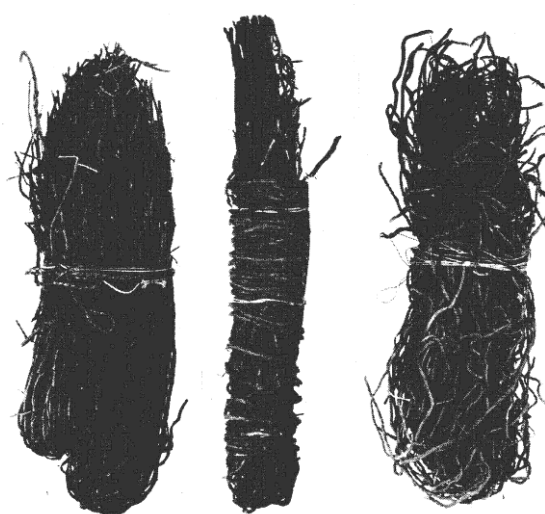
Smilax - *Smilax china* L.

***Smilax officinale* - *Smilax officinalis* H.B.K.**

Family *Liliaceae*



A



B

Fig. 12.6. A - Sarsaparilla or smilax, B - Sarsaparilla roots

Description. Mexican Sarsaparilla. In long roots up to 6 mm. in diameter, frequently attached to a tough, woody crown possessing one or more stem bases; externally light grayish-brown or weak

reddish-brown to yellowish-brown, longitudinally ridged and broadly furrowed, the furrows sometimes containing blackish earth; with relatively few fibrous rootlets; fracture of cortex brittle, central cylinder tough and fibrous; the cut or fractured surface exhibiting *It* mealy and pale orange, or light yellowish brown and horny cortex, a yellow band porous woody zone and a lighter colored central pith.

Liquorice root - *Radix Glycyrrhizae*

Liquorice - *Glycyrrhiza glabra* L., *Liquirita glabra*

Family *Fabaceae* (*Leguminosae*)

Definition. Liquorice root consists of the dried unpeeled or peeled, whole or cut root and stolons of *Glycyrrhiza glabra* L. It contains not less than 4.0 per cent glycyrrhizic acid ($C_{42}H_{62}O_{16}$, M_r 823), calculated with reference to the dried drug.

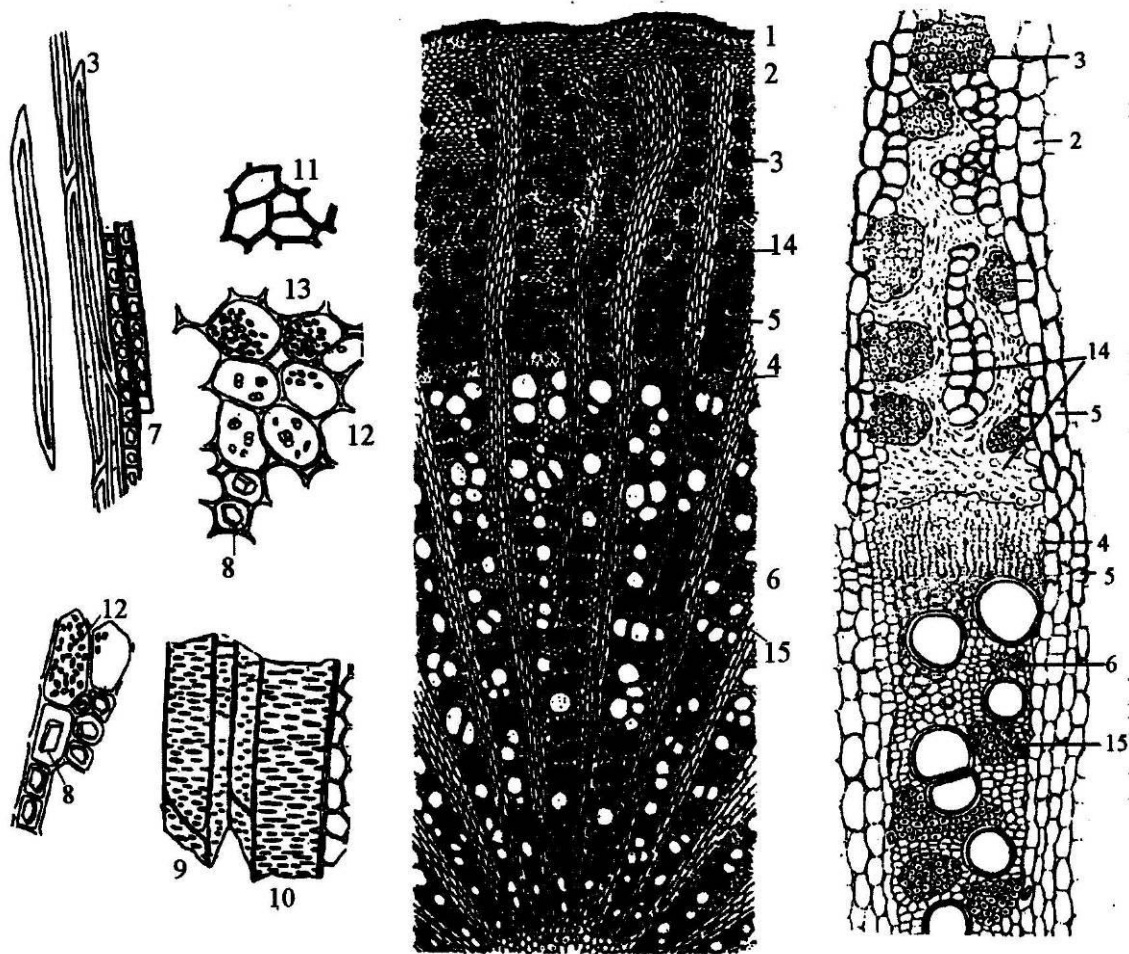


Fig. 12.7. A – Liquorice, B - Liquorice root

Description. The commercial variety, *G. glabra* var. *typica* Regel St Herd, known as Spanish liquorice, consists generally of roots and rhizomes in nearly cylindrical pieces, up to 1 m long and 5-20 mm in diameter; externally, the bark is brownish grey to dark brown, longitudinally wrinkled, occasionally bearing small dark buds in rhizomes or small circular or transverse rootlet-scars in roots. The peeled root is yellow, smooth, fibrous, finely striated; fracture, fibrous, in the bark and splintery in the wood; internally, bright yellow. A distinct cambium ring separates the yellowish grey bark from the finely radiate yellow wood; central pith, only in rhizomes.

The commercial variety, *G. glabra* var. *glandulifera* (Wald et Kit) Regel & Herd, known as Russian liquorice, consists mainly of roots, in cylindrical pieces somewhat tapering and sometimes longitudinally split; 15-40 cm long, 1-5 cm in diameter. The enlarged crown of the root may attain up to 10 cm in diameter; externally, the unpeeled root purplish brown, somewhat scaly, with stem scars at the top; the peeled root yellowish, coarsely striated; fracture as for Spanish type; internally, yellow, radiating.

Anatomical characteristics



The raw material is represented by segments of roots and rhizomes of various length and thickness. The surface of roots is longitudinally-wrinkled, dark or grey-brown in colour. The fracture of roots is fibrous, yellow in colour. The odour is absent, the taste is sickly-sweet.

Powdered liquorice: 1. numerous fragments of fibers accompanied by crystal sheath (8, 12). 2. Dark-yellow fragments of lignified and usually bordered pitted vessels (9, 10). 3. Prismatic crystals of calcium oxalate, free or in cells. 4. Numerous starch grains free or in parenchyma cells, mostly simple, oval, rounded or fusiform. Fragment of cork may be present.

Transersial Section: 1- cork composed of thin-walled cells; 2 –parenchyma’s cell with abundant starch grains, 3- group of fibres, 4- medullary ray, 5 – cambium, 6- sieve, 7 – vessel, 8- Lignified xylem parenchyma with part of an underlying bordered pitted vessel and adjacent thin-walled parenchyma, collenchyma

Transverse sections show a periderm of numerous layers of yellowish-brown cork cells, a phellogen and one or more rows of cells of the phelloderm, the cells showing a tendency to collenchymatous thickenings and with occasional monoclinic prisms of calcium oxalate; a middle bark of starch-bearing parenchyma and whitish groups of bast-fibers surrounded with crystal-fibers; inner bark with a very characteristic radial arrangement of phloem and medullary rays, the phloem consisting of wedges of small groups of bast-fibers and parenchyma, separated by an almost continuous, obliterated sieve tissue, the cells of the latter being very irregular in outline and with thick, highly refracting walls; medullary rays from 1 to 8 cells wide; wood characterized by broad wedges consisting of large tracheae with yellowish walls, small compact groups of wood-fibers and starch-bearing parenchyma alternating with the broad

medullary rays; pith composed of parenchyma, the cells being large, more or less polygonal in outline and containing numerous starch grains, or prisms of calcium oxalate. In sections of roots the pith is absent.

Java tea leaves (dried leaves and tops of stems) = *Folium Orthosiphonis*

Java tea = *Orthosiphon stamineus* Benth. (*O. aristatus* Miq.; *O. spicatus* Bak.).

Family *Lamiaceae*

Definition. Java tea consists of the fragmented, dried leaves and tops of stems of *Orthosiphon stamineus* Benth. (*O. aristatus* Miq.; *O. spicatus* Bak.). Content: minimum 0.05 per cent of sinensetin ($C_{20}H_{20}O_7$; M_r 372.4) (dried drug).

Description. The leaves are friable and may reach 7.5 cm in length and 2.5 cm in width. The petiole is short. The lamina is oval to lanceolate, the apex acuminate and the base cuneate. The abaxial surface of the leaves is light greyish-green and the adaxial surface is dark green to brownish-green. The venation is pinnate with few secondary veins. Examined under a lens (10x), the secondary veins, after running parallel to the midrib, diverge at an acute angle. The margin is irregularly and roughly dentate, sometimes crenate and the abaxial surface is slightly curved. The petioles are thin, quadrangular, 4 mm to 8 mm long and, like the primary venation, usually violet-coloured. Occasionally, inflorescences in clusters of bluish-white to violet flowers, not yet opened, are found.

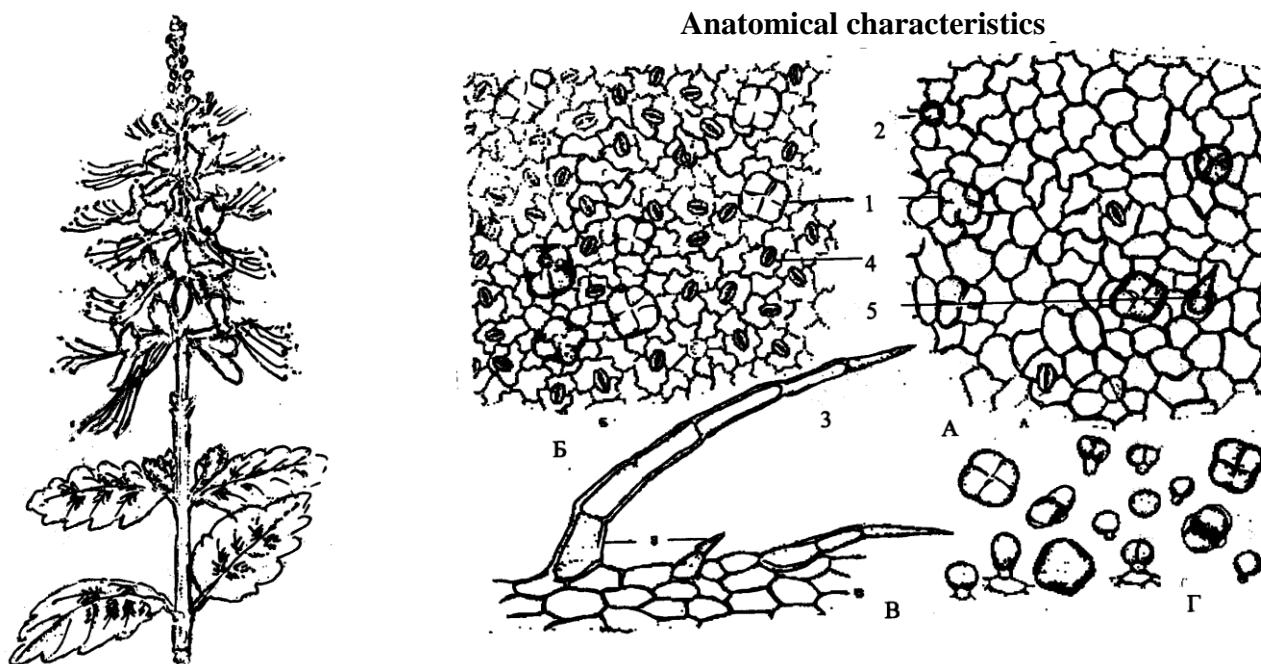


Fig. 12.8. Flowering Java tea plant

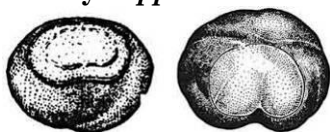
The edipermis and powder shows fragments of epidermis, with cells with sinuous outlines bearing unicellular or bicellular conical covering trichomes (3) and articulated uniseriate trichomes up to 450 μ long, consisting of three to eight cells with thick pitted walls - capitate trichomes (1) with unicellular or bicellular heads; secretory trichomes (5) with unicellular stalks and usually tetracellular heads; diacytic stomata (4), which are more numerous on the lower epidermis.

Tests. Foreign matter. Not more than 5 per cent of stems with a diameter greater than 1 mm and not more than 2 per cent of other foreign matter; Loss on drying. Not more than 11.0 per cent, determined on 1.000 g of the powdered drug by drying in an oven at 100 $^{\circ}C$ to 105 $^{\circ}C$ for 2 h. Total ash. Not more than 12.5 percent.

Horse chestnut seeds = *Semen Hippocastani*

Horse chestnut = *Aesculum hippocastanum* L.

Family *Hippocastanaceae*



Definition. Semen Hippocastani consists of the dried ripe seeds of *Aesculum hippocastanum* L. It contains not less than 3, 0% triterpen saponins calculated as aescin (escin) determined by spectrophotometry at 540 nm.

Description. Globulous or ovoid, 2-4 cm in diameter. The 2 large cotyledons, oily and starchy, often connate with a line of suture more or less visible; covered by a shiny dark-brown tegument with a large whitish spot corresponding to the hilum; tegument creamy white in the immature seed, takes on a brown tinge during maturation, becoming dark brown when mature. Curved radicle occupies a depression either on the commissure of the cotyledons or the dorsal side of the cotyledons. Odour slight; taste bitter, acrid.

Ginseng root - *Radix Ginseng*

Ginseng - *Panax Ginseng* C.A. Mey

Family *Araliaceae*



Definition. Radix Ginseng consists of dried roots of Ginseng, it contains not less 1,5% ginsekosides calculated as ginsecoside.

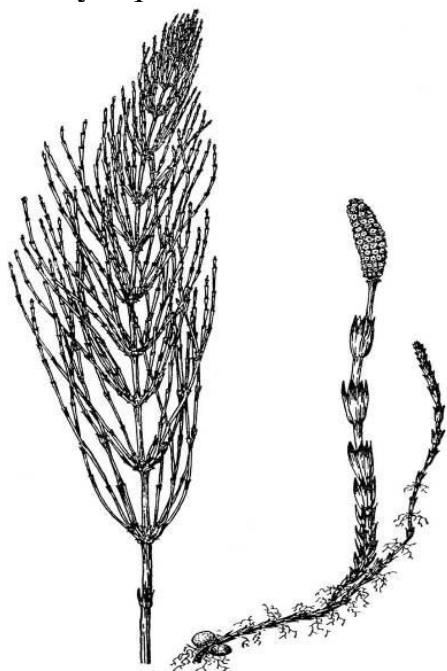
Description. Ginseng root occurs in rachis-like root, to 25 cm long with 2-5 large branchings, rarer without them. The body of the root is thickened, nearly cylindrical with clearly expressed ringed thickenings in the upper part. The outer surface is longitudinally, rarer spirally wrinkled. Their colour is yellowish-white. The fracture of the root is even. The upper part of the root narrows into transverse wrinkled rhizome-root-collar. The rhizome is short with several scars of fallen-off stems. The rhizome is represented by extended traces of the stem and topical bud. Sometimes from the root-collar one or several adventitious roots go out. The odour is specific; the taste is sweet, burning, and then bitterish.

Fig. 12.9. *Panax ginseng*

Horse-tails herb - *Herba Equiseti*

Field Horse-tail - *Equisetum arvense* L.

Family Equisetaceae



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).

Collection. The herbs (green vegetative runners) are collected in summer, cut at about 5 cm from surface of soil; then dried at temperature about 40-50°C. **Content:** minimum 0.3 per cent of total flavonoids expressed as isoquercitroside ($C_{21}H_{20}O_{12}$; M_r 464.4) (dried drug).

Description. The herb consists of dried overground vegetative parts of Field Horse-tail. The stems are rigid, articulate, branched, hollow, grooved with 6-18 longitudinal ribs of 30 cm length. The branches are joined into whorls by 6-18 in each, directed obliquely to the top, usually they are unbranched, articulate, 4-5 ribbed without cavity. The leaves are abortive and united in tubular dentate sheaths. The cryptogamous spikelets on the apex of stems and branches are absent.

Adulterants: *Equisetum sylvaticum*, *E.pratense*, *E.fluviatile*, *E.palustre*

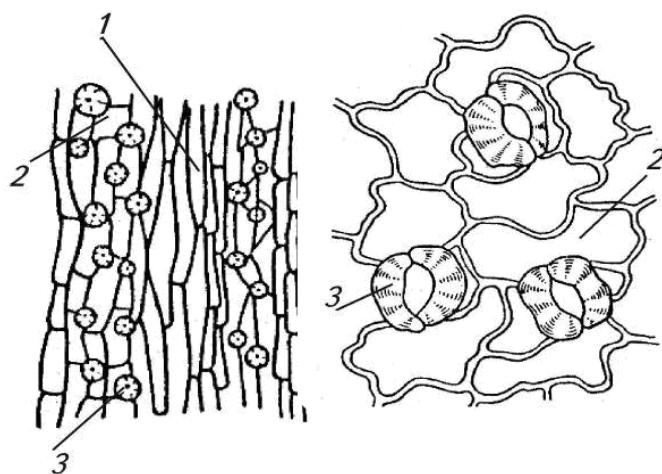
Fig. 12.10. Shoot of Horse-tails

Identification. Macroscopic and microscopic characters described under identification tests A and B. (EuPh)

A. It consists of fragments of grooved stems and linear leaves, light green to greenish-grey. They are rough to the touch, brittle and crunchy when crushed. The main stems are about 0.8 mm to 4.5 mm in diameter, hollow, jointed at the nodes which occur at intervals of about 1.5 cm to 4.5 cm; distinct vertical grooves are present on the internodes, ranging in number from 4 to 14 or more. Verticils of widely spaced and erect branches, usually simple, each about 1 mm thick with 2 to 4 longitudinal grooves, occur at the nodes. 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 it belongs to.

B. Reduce to a powder. The powder is greenish-grey.

Anatomical analysis.



Examine under a microscope using chloral hydrate solution R. The powder shows the following diagnostic characters: fragments of the epidermis in surface view, composed of rectangular cells (1) with wavy walls and paracytic stomata (3) with the 2 subsidiary cells covering the guard cells and having conspicuous radial ridges; in transverse sectional view the epidermis is crenate, with the protuberances formed from the contiguous walls of 2 adjacent, U-shaped cells. Fragments of large-celled parenchyma and groups of long, non-lignified fibres with narrow lumens; scattered small, lignified vessels with spiral or annular thickening.

Ivy leaf - *Folium Hederae helicis*
Ivy - *Hedera helix* L.

Family Araliaceae



Fig. 12.11. Ivy, A- ivy shoot, B- leaves, C- inflorescence

Description. The 3-5-lobed leaves, with more or less triangular lobes, collected between spring and early summer from the non-flowering shoots in the lower reaches of evergreen woody plant are the part used as a drug. The 4-10cm long and equally wide, dark green, shiny leaves are cordate at the base; they are firm and leathery, with a white, fan-like nervature which is easily recognized in the cut drug on the lower surface. Young leaves are pubescent, older ones glabrous. Occasionally, the ovate-rhombic to lanceolate leaves with a long acumen and an entire margin, which are found on the flowering shoots from the upper part of the plant are collected along the rest; the dark green petiole is mostly round and longitudinally grooved. Odour is faint, characteristic, and somewhat musty. Taste is bland, mucilaginous, somewhat bitter and slightly irritating.

The root of Soapwort - *Radix Saponaria*
Soapwort - *Saponaria officinalis*
Family *Caryophyllaceae*



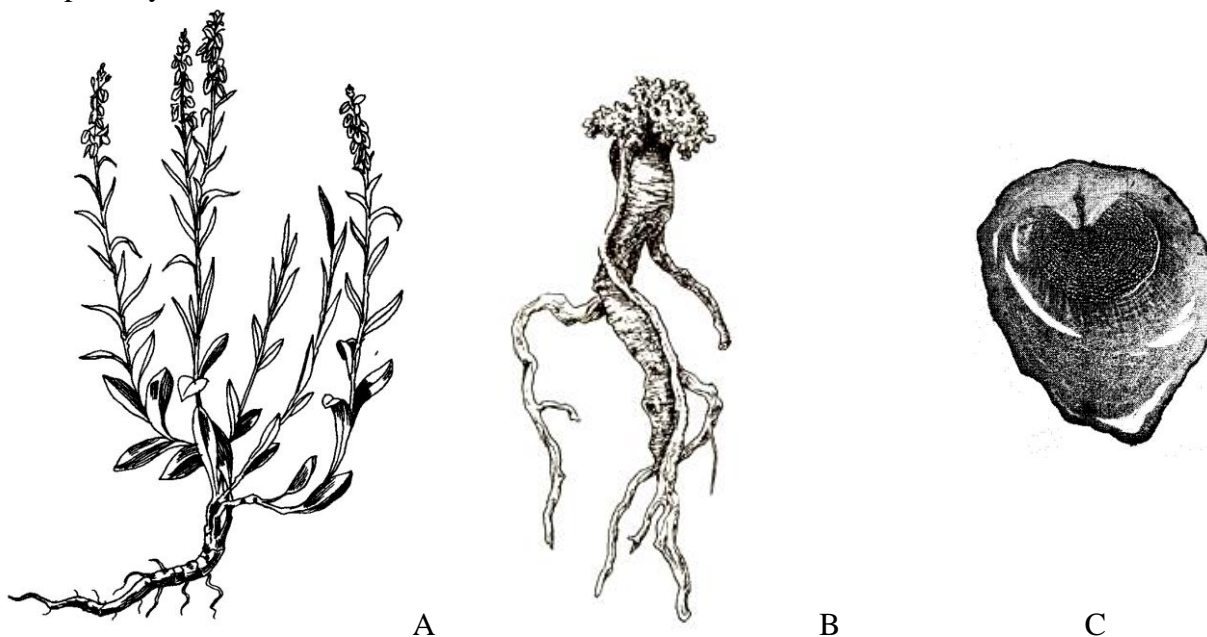
Description. It occurs in cylindrical, long, hard, thin pieces. The outer surface is red-brown in colour, the fracture is yellowish. The odour is absent, the taste is slightly burning, irritating remaining for a long time.

Fig. 12.12. Flowering shoot of soapwort

Senega root = *Radix Senegae*
Senega = *Polygala senega*
Family *Polygalaceae*

Definition. Senega root consists of the dried and usually fragmented root and root-crown of *Polygala senega* L. or of certain other closely related species or of a mixture of these *Polygala* species.

Description. Senega occurs in pieces 5-10 cm long and 2-12 mm in diameter. The lower part is yellowish in colour, but the crown is somewhat darker. The latter is knotty and bears numerous, often purplish buds and the remains of aerial stems. The tapering and often curved root frequently divides into two or more branches. It is longitudinally and sometimes especially near the crown, transversely wrinkled. Very frequently but not always it exhibits a keel or ridge in the form of rapidly descending spiral, this keel is prominent on the concaves surface of the curves of the tap-root and often extends a considerable distance. The fracture is short, which is smooth in the bark and splintery in the wood.



*Fig.12.13. A - *Polygala senega*, B - roots (Canadian drug); C - transverse section of root, showing keel and wedges of parenchyma.*