Pharmacognosy practice II.
for pharmacy students

Prescriptions for examinations of plant drugs
1. practice

Alkaloid-containing drugs I.

Belladonna leaf
Stramonium leaf

Belladonna Leaf (Ph Eur monograph 0221)
Belladonna Herb

Preparations
- Prepared Belladonna
- Standardised Belladonna Leaf Dry Extract
- Belladonna Tincture

(When Belladonna Herb, Belladonna Leaf or Powdered Belladonna Herb is prescribed, Prepared Belladonna shall be supplied.)

DEFINITION
Dried leaf or dried leaf and flowering, and occasionally fruit-bearing tops of *Atropa belladonna* L.

Content
Minimum 0.30 per cent of total alkaloids, expressed as hyoscyamine (C₁₇H₂₃NO₃; M₂89.4) (dried drug). The alkaloids consist mainly of hyoscyamine together with small quantities of hyoscine (scopolamine).

CHARACTERS
Slightly nauseous odour.

IDENTIFICATION
A. The leaves are green or brownish-green, slightly darker on the upper surface, often crumpled and rolled and partly matted together in the drug. The leaf is petiolate and the base of the lamina is acute and decurrent and the margin entire. The flowering stems are flattened and bear at each node a pair of leaves unequal in size, in the axils of which occur singly the flowers or occasionally fruits. The flowers have a gamosepalous calyx and campanulate corolla. The fruits are globose berries, green or brownish-black and surrounded by the persistent calyx with widely spread lobes.

B. Reduce to a powder (355) (2.9.12). The powder is dark green. Examine under a microscope, using chloral hydrate solution R. The powder shows the following diagnostic characters: fragments of leaf lamina showing sinuous-walled epidermal cells, a striated cuticle; stomata more frequent on the lower epidermis (anisocytic and also some anomocytic) (2.8.3); multicellular uniseriate covering trichomes with smooth cuticle, glandular trichomes with unicellular heads and multicellular, uniseriate stalks or with multicellular heads and unicellular stalks; parenchyma cells including rounded cells containing microsphenoidal crystals of calcium oxalate; annular and spirally thickened vessels. The powdered drug may also show: fibres and reticulately thickened vessels from the stems; subspherical pollen grains, 40-50 µm in diameter, with 3 germinal pores, 3 furrows and an extensively pitted exine; fragments of the corolla, with a papillose epidermis or bearing numerous covering or glandular trichomes of the types previously described; brownish-yellow seed fragments containing irregularly sclerified and pitted cells of the testa.
**Stramonium Leaf (Ph Eur monograph 0246)**

**Preparation**
- Prepared Stramonium

When Stramonium Leaf or Powdered Stramonium Leaf is prescribed, Prepared Stramonium shall be dispensed.

**DEFINITION**
Dried leaf or dried leaf and flowering, and occasionally fruit-bearing, tops of *Datura stramonium* L. and its varieties.

**Content**
Minimum 0.25 per cent of total alkaloids, expressed as hyoscyamine (C_{17}H_{23}NO_{3}; M, 289.4) (dried drug). The alkaloids consist mainly of hyoscyamine with varying proportions of hyoscine (scopolamine).

**CHARACTERS**
Unpleasant odour.

**IDENTIFICATION**

A. The leaves are dark brownish-green or dark greyish-green, often much twisted and shrunken during drying, thin and brittle, ovate or triangular-ovate, dentately lobed with an acuminate apex and often unequal at the base. Young leaves are pubescent on the veins, older leaves are nearly glabrous. Stems are green or purplish-green, slender, curved and twisted, wrinkled longitudinally and sometimes wrinkled transversely, branched dichasially, with a single flower or an immature fruit in the fork. Flowers, on short pedicels, have a gamosepalous calyx with 5 lobes and trumpet-shaped brownish-white or purplish corolla. The fruit is a capsule, usually covered with numerous short, stiff emergences; seeds are brown or black with a minutely pitted testa.

B. Reduce to a powder (355) (2.9.12). The powder is greyish-green. Examine under a microscope using chloral hydrate solution R. The powder shows the following diagnostic characters: fragments of leaf lamina showing epidermal cells with slightly wavy anticlinal walls and smooth cuticle; stomata are more frequent on the lower epidermis (anisocytic and anomocytic) (2.8.3); covering trichomes are conical, uniseriate with 3-5 cells and warty walls; glandular trichomes are short and clavate with heads formed by 2-7 cells; dorsiventral mesophyll, with a single layer of palisade cells and a spongy parenchyma containing cluster crystals of calcium oxalate; annularly and spirally thickened vessels. The powdered drug may also show: fibres and reticulately thickened vessels from the stems; subspherical pollen grains about 60-80 µm in diameter with 3 germinal pores and nearly smooth exine; fragments of the corolla with a papillose epidermis; seed fragments containing yellowish-brown, sinuous, thick-walled sclereids of testa; occasional prisms and microsphenoidal crystals of calcium oxalate.
1., TLC (Belladonna leaf, Stramonium leaf, Dry belladonna extract)

Extraction:

- 0.6 g drug
- extraction with 15 mL 0.05 M H$_2$SO$_4$, with heating
- filter
- + 1 mL cc NH$_3$
- extraction with 10 mL of Et$_2$O
- filter Et2O fraction through dry Na$_2$SO$_4$
- evaporate
- re-uptake in MeOH (200 μL)
- apply on TLC (20-40 μL)

Standard: atropine sulphate solution (1mg/mL) in MeOH (10 μL = 10 μg).
Standardized dry belladonna extract (100 mg / 1 mL) in MeOH (10 μL)

Stationary phase: Silicagel G

Eluent: cc NH$_3$ + H$_2$O + acetone (3+7+90)

Dry the plate until it is solvent-free.

Visualization, examination: Spray with Dragendorff reagent, followed by heating at 100-105 ºC; examine in VIS.

2., Microscope preparation (Belladonna leaf, Stramonium leaf)

Observe the shape, density and distribution of Ca-oxalate rosettes.

3., „Vitali” reaction (detection of tropane alkaloids) (Belladonna leaf, Stramonium leaf)

- on the evaporated sample prepared for the TLC
- drop 4-5 drops of cc. HNO$_3$
- evaporate to dryness
- add one pastille of dry KOH
- add 100-200 μL EtOH

- positive reaction: purple color
2. Practice

Alkaloid-containing II.

Poppy fruit without seeds
Cinchona bark
Xantin-alkaloid containing drugs
(Tea leaves, Coffee seed, Cacao seed)

Cinchona Bark (Ph Eur monograph 0174)

Cinchona; Red Cinchona Bark

When Powdered Cinchona is prescribed or demanded, material complying with the requirements below with the exception of Identification test A and the test for Foreign matter shall be dispensed or supplied.

DEFINITION

Whole or cut, dried bark of Cinchona pubescens Vahl (Cinchona succirubra Pav.), of Cinchona calisaya Wedd., of Cinchona ledgeriana Moens ex Trimen or of their varieties or hybrids.

Content
Minimum 6.5 per cent of total alkaloids, of which 30 per cent to 60 per cent consists of quinine-type alkaloids (dried drug).

CHARACTERS

Intense bitter, somewhat astringent taste.

IDENTIFICATION

A. The stem and branch bark is supplied in quilled or curved pieces 2-6 mm thick. The outer surface is dull brownish-grey or grey and frequently bears lichens; it is usually rough, marked with transverse fissures and longitudinally furrowed or wrinkled; exfoliation of the outer surface occurs in some varieties. The inner surface is striated and deep reddish-brown; the fracture is short in the outer part and fibrous in the inner part.

B. Reduce to a powder (355) (2.9.12). The powder is reddish-brown. Examine under a microscope using chloral hydrate solution R. The powder shows the following diagnostic characters: thin-walled cork cells filled with reddish-brown contents; yellow, spindle-shaped striated phloem fibres up to 90 µm in diameter and up to 1300 µm in length, very thick-walled with an uneven lumen and with conspicuous, funnel-shaped pits; parenchymatous idioblasts filled with microprisms of calcium oxalate; clusters of thin-walled phloem parenchyma cells. Examine under a microscope using a 50 per cent V/V solution of glycerol R. The powder shows a few starch granules 6-10 µm in diameter; mostly simple but occasionally with 2 or 3 components.
1., TLC #1 (Poppy fruit without seeds)

Extraction:

- 1 g drug
- mix with few drops of 10% NH₃
- extraction with 10 mL CHCl₃
- filter
- evaporate to dryness
- dissolve (200 μL MeOH)
- apply to TLC (20 and 40 μL)

Standard: morphine (10 μL), codeine (10 μL), papaverine (10 μL)

Eluent: cc. NH₃ + EtOH + aceton + toluol (6+6+40+40)

Dry the plate until it is solvent-free.

Visualisation, examination:

1., Spray with Dragendorff-reagent, heating, examine in VIS.
2., Spray with Marquis-reagent, heating, examine in VIS.

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2. TLC #2 (Cinchona bark)

Extraction:

- 0.1 g drug
- extraction with 10 mL 1 M HCl, heating
- filter
- + 6 M NH₃ (until color changes)
- extraction with 10 mL CHCl₃
- filter through Na₂SO₄
- evaporate to dryness
- dissolve (200 μL MeOH)
- apply to TLC (20 and 40 μL)

Standard: quinine (10 μL), quinidine (10 μL)

Stationary phase: Silicagel G, impregnated with 3% K-Na-tartarate

Eluent: MEK + MeOH + water (30+10+5)

Dry the plate until it is solvent-free.

Visualization, examination:

1., Spray with Dragendorff-reagent, heating; examine in VIS.

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3., Detection of rubanol-skeleton alkaloids (Cinchona bark)

- heat 0.1 g dry drug in a test tube
- purple steam develops, and condenses as tiny, red droplets on the cooler parts of the test tube
- dissolve the droplets in MeOH
- add 1 drop from the solution to 20 mL water
- examine fluorescence under UV (356 nm)

- positive: intense light fluorescence
4., Microsublimation (caffeine) (tea, coffee)

- put about 0.5 g drug in the microsublimator, put a slide on it
- gently heat
- examine the caffeine crystals on the slide with microscope

5., Murexid reaction (detection of xanthine derivates, here: caffeine) (tea, coffee)

- use the results of microsublimation (crystals of caffeine), from point 4.
- add 20 μL cc. H₂O₂
- add 60 μL 0,05 M HCl
- evaporate to dryness
- the residue is *orange*
- add 20 μL 3% NH₃
- positive reaction: violet color
3. Practice

Alkaloid-containing drugs III.

Capsicum
Ipecacuanha root

Capsicum (Ph Eur monograph 1859)

DEFINITION

Dried ripe fruits of Capsicum annuum L. var. minimum (Miller) Heiser and small-fruited varieties of Capsicum frutescens L.

Content
Minimum 0.4 per cent of total capsaicinoids, expressed as capsaicin (C18H27NO3; M r 305.4) (dried drug).

CHARACTERS

Extremely pungent taste.

IDENTIFICATION

A. The fruit is yellowish-orange or reddish-brown, oblong conical with an obtuse apex, about 1-3 cm long and up to 1 cm in diameter at the widest part, occasionally attached to a 5-toothed inferior calyx and a straight pedicel. Pericarp somewhat shrivelled, glabrous, enclosing about 10-20 flat, reniform seeds 3-4 mm long, either loose or attached to a reddish dissepiment.

B. Reduce to a powder (355) (2.9.12). The powder is orange. Examine under a microscope using chloral hydrate solution R. The powder shows the following diagnostic characters: fragments of the pericarp having an outer epicarp with cells often arranged in rows of 5 to 7, cuticle uniformly striated, parenchymatous cells frequently containing droplets of red oil, occasionally containing microsphenoidal crystals of calcium oxalate, endocarp with characteristic island groups of sclerenchymatous cells, the groups being separated by thin-walled parenchymatous cells; fragments of the seeds having an episperm composed of large, greenish-yellow, sinuous-walled sclereids with thin outer walls and strongly and unevenly thickened radial and inner walls which are conspicuously pitted, endosperm parenchymatous cells with drops of fixed oil and aleurone grains 3-6 µm in diameter; occasional fragments from the calyx having an outer epidermis with anisocytic stomata (2.8.3), inner epidermis with many trichomes but no stomata, trichomes glandular, with uniseriate stalks and multicellular heads, mesophyll with many idioblasts containing microsphenoidal crystals of calcium oxalate.
**Ipecacuanha (Ipecacuanha Root, Ph Eur monograph 0094)**

**Preparations**
- Prepared Ipecacuanha
- Ipecacuanha Liquid Extract

**DEFINITION**

Fragmented and dried underground organs of *Cephælis ipecacuanha* (Brot.) A. Rich., known as Matto Grosso ipecacuanha, or of *Cephælis acuminata* Karsten, known as Costa Rica ipecacuanha, or of a mixture of both species. The principal alkaloids are emetine and cephaeline.

**Content**
Minimum 2.0 per cent of total alkaloids, expressed as emetine (C29H40N2O4; Mr 480.7) (dried drug).

**CHARACTERS**
Slight odour.

**IDENTIFICATION**

A. *C. ipecacuanha*. The root occurs as somewhat tortuous pieces, dark reddish-brown or very dark brown, seldom more than 15 cm long or 6 mm thick, closely annulated externally, having rounded ridges completely encircling the root; the fracture is short in the bark and splintery in the wood. The transversely cut surface shows a wide greyish bark and a small uniformly dense wood. The rhizome occurs as short lengths usually attached to roots, cylindrical, up to 2 mm in diameter, finely wrinkled longitudinally and with pith occupying approximately one-sixth of the whole diameter. *C. acuminata*. The root in general resembles the root of *C. ipecacuanha*, but differs in the following particulars: it is often up to 9 mm thick; the external surface is greyish-brown or reddish-brown with transverse ridges at intervals of usually 1-3 mm, the ridges being about 0.5-1 mm wide, extending about half-way round the circumference and fading at the extremities into the general surface level.

B. Reduce to a powder (355) (2.9.12). The powder is light grey or yellowish-brown. Examine under a microscope, using chloral hydrate solution R. The powder shows the following diagnostic characters: parenchymatous cells, raphides of calcium oxalate up to 80 µm in length either in bundles or scattered throughout the powder; fragments of tracheids and vessels usually 10-20 µm in diameter, with bordered pits; larger vessels and sclereids from the rhizome. Examine under a microscope using a 50 per cent V/V solution of glycerol R. The powder shows simple or two- to eight-compound starch granules contained in parenchymatous cells, the simple granules being up to 15 µm in diameter in *C. ipecacuanha* and up to 22 µm in diameter in *C. acuminata*. 
1., TLC #1 (Capsicum, „spicy” and „sweet” variants)

Extraction:
- 0.2 g drug
- extraction with 2 mL acetone, short warming
- filter
- apply to TLC (30 μL)

Standard: none
Stationary phase: Silicagel G
Eluent: CHCl₃ + MeOH (95+5)
Dry the plate until it is solvent-free.

Visualization, examination:
1., examine in VIS, without spraying (for carotenoids)

2., Spray with the 1:1 mixture of 1% FeCl₃ solution and 1% K₃[Fe(CN)₆] solutions. Prepare right before use, spray immediately. Examine in VIS, (for capsaicin).

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2., Detection of Capsicum carotenoids (Capsicum, „spicy” and „sweet” variants)
- To the dry acetone extract (from TLC)
- add few drops of cc. H₂SO₄
- Positive: Blue-purple color reaction
3., TLC #2 (Ipecacuanha root)

Tincture is applied to TLC directly (20 and 40 μL).

Standard: none

Stationary phase: Silicagel G

Eluent: cc NH₃ + MeOH + EtOAc + toluene (2+15+18+65)

Dry the plate until it is solvent-free.

Visualization, examination: Spray with 0,5% I₂ (in EtOH), heat gently. Examine in A., UV 365 nm, B., VIS.

Reference data:

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<td>emetine</td>
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In extracts from roots of *Cephaelis acuminata*, zones of emetine and cephaeline are of similar intensity, while in extracts from roots of *Cephaelis ipecacuanha*, the zone of emetine is much more intensive, than that of the cephaeline.
4. Practice

Drugs containing antrakinon-glycosides

Frangula bark
Rhubarb
Senna leaf
Senna fruit (Alexandrian and Tinnevelly)

Frangula Bark (Ph Eur monograph 0025)

Preparation
  - Standardised Frangula Bark Dry Extract

When Powdered Frangula Bark is prescribed or demanded, material complying with the requirements below, with the exception of Identification test A and the test for Foreign matter, shall be dispensed or supplied.

DEFINITION

Dried, whole or fragmented bark of the stems and branches of Rhamnus frangula L. (Frangula alnus Miller).

Content

Minimum 7.0 per cent of glucofrangulins, expressed as glucofrangulin A (C_{27}H_{30}O_{14}; M, 578.5) (dried drug).

IDENTIFICATION

A. The bark occurs in curved, almost flat or rolled fragments or in single or double quilled pieces usually 0.5-2 mm thick and variable in length and width. The greyish-brown or dark brown outer surface is wrinkled longitudinally and covered with numerous greyish, transversely elongated lenticels; when the outer layers are removed, a dark red layer is exposed. The orange-brown or reddish-brown inner surface is smooth and bears fine longitudinal striations; it becomes red when treated with alkali. The fracture is short, fibrous in the inner part.

B. Reduce to a powder (355) (2.9.12). The powder is yellowish or reddish-brown. Examine under a microscope using chloral hydrate solution R. The powder shows the following diagnostic characters: numerous phloem fibres, partially lignified, in groups with crystal sheaths containing calcium oxalate prisms; reddish-brown fragments of cork; fragments of parenchyma containing calcium oxalate cluster crystals. Sclereids are absent.
**Rhubarb (Ph Eur monograph 0291)**

**Preparation**
- Compound Rhubarb Tincture

When Powdered Rhubarb is prescribed or demanded, material complying with the requirements below with the exception of Identification test A and the test for Foreign matter shall be dispensed or supplied.

**DEFINITION**

Rhubarb consists of the whole or cut, dried underground parts of *Rheum palmatum* L. or of *Rheum officinale* Baillon or of hybrids of these two species or of a mixture. The underground parts are often divided; the stem and most of the bark with the rootlets are removed. It contains not less than 2.2 per cent of hydroxyanthracene derivatives, expressed as rhein (C₁₅H₁₈O₆, M, 284.2), calculated with reference to the dried drug.

**CHARACTERS**

Characteristic, aromatic odour.

**IDENTIFICATION**

A. The appearance is variable: disc-shaped pieces up to 10 cm in diameter and 1 cm to 5 cm in thickness; cylindrical pieces; oval or planoconvex pieces. The surface has a pinkish tinge and is usually covered with a layer of brownish-yellow powder. It shows, especially after moistening, a reticulum of darker lines. This structure causes the marbled appearance of the drug. The fracture is granular. The transverse section of the rhizome shows a narrow outer zone of radiating brownish-red lines. These medullary rays are crossed perpendicularly by a dark cambial ring. Inside this zone is a ring of small star-spot formations of anomalous vascular bundles. The root shows a more radiate structure.

B. Reduce to a powder (355) (2.9.12). The powder is orange to brownish-yellow. Examine under a microscope using chloral hydrate solution R. The powder shows the following diagnostic characters: large calcium oxalate cluster crystals, which may measure more than 100 µm, and their fragments; reticulately thickened non-lignified vessels measuring up to 175 µm. Numerous groups of rounded or polygonal, thin-walled parenchyma cells. Sclereids and fibres are absent. Examine under a microscope using a 50 per cent V/V solution of glycerol R. The powder shows simple, rounded or compound (2 to 4) starch granules with a star-shaped hilum.
**Senna Leaf (Ph Eur monograph 0206)**

**Preparation**
- Standardised Senna Leaf Dry Extract

When Powdered Senna Leaf is prescribed or demanded, material complying with the requirements below with the exception of Identification test A and the test for Foreign matter shall be dispensed or supplied.

**DEFINITION**

Dried leaflets of Cassia senna L. (C. acutifolia Delile), known as Alexandrian or Khartoum senna, or Cassia angustifolia Vahl, known as Tinnevelly senna, or a mixture of the 2 species.

**Content**

Minimum 2.5 per cent of hydroxyanthracene glycosides, expressed as sennoside B (C42H38O20; Mr 863) (dried drug).

**CHARACTERS**

Slight characteristic odour.

**IDENTIFICATION**

A. *C. senna* occurs as greyish-green or brownish-green, thin, fragile leaflets, lanceolate, mucronate, asymmetrical at the base, usually 15-40 mm long and 5-15 mm wide, the maximum width being at a point slightly below the centre; the lamina is slightly undulant with both surfaces covered with fine, short trichomes. Pinnate venation is visible mainly on the lower surface, with lateral veins leaving the midrib at an angle of about 60° and anastomosing to form a ridge near the margin. Stomatal index (2.8.3) 10-12.5-15.

*C. angustifolia* occurs as yellowish-green or brownish-green leaflets, elongated and lanceolate, slightly asymmetrical at the base, usually 20-50 mm long and 7-20 mm wide at the centre. Both surfaces are smooth with a very small number of short trichomes and are frequently marked with transverse or oblique lines. Stomatal index (2.8.3) 14-17.5-20.

B. Reduce to a powder (355) (2.9.12). The powder is light green or greenish-yellow. Examine under a microscope using chloral hydrate solution R. The powder shows the following diagnostic characters: polygonal epidermal cells showing paracytic stomata (2.8.3); unicellular trichomes, conical in shape, with warty walls, isolated or attached to fragments of epidermis; fibres with a crystal sheath of prismatic crystals of calcium oxalate; cluster crystals isolated or in fragments of parenchyma.
**Alexandrian Senna Fruit (Ph Eur monograph 0207)**

Alexandrian Senna Pods

Preparations
- Senna Liquid Extract
- Standardised Senna Granules
- Senna Tablets

When Powdered Alexandrian Senna Fruit is prescribed or demanded, material complying with the requirements below with the exception of Identification test A and the test for Foreign matter shall be dispensed or supplied.

**DEFINITION**

Dried fruit of *Cassia senna* L. (*C. acutifolia* Delile).

Content
Minimum 3.4 per cent of hydroxyanthracene glycosides, expressed as sennoside B \((C_{42}H_{38}O_{20}; M, 863)\) (dried drug).

**CHARACTERS**

Slight odour.

**IDENTIFICATION**

A. Flattened reniform pods, green or greenish-brown with brown patches at the positions corresponding to the seeds, usually 40-50 mm long and at least 20 mm wide. At one end is a stylar point and at the other a short stalk. The pods contain 6-7 flattened and obovate seeds, green or pale brown, with a continuous network of prominent ridges on the testa.

B. Reduce to a powder (355) (2.9.12). The powder is brown. Examine under a microscope using chloral hydrate solution R. The powder shows the following diagnostic characters: epicarp with polygonal cells and a small number of conical warty trichomes and occasional anomocytic or paracytic stomata (2.8.3); fibres in 2 crossed layers accompanied by a crystal sheath of calcium oxalate prisms; characteristic palisade cells in the seed and stratified cells in the endosperm; clusters and prisms of calcium oxalate.

**Tinnevelly Senna Fruit (Ph Eur monograph 0208)**

Tinnevelly Senna Pods

Preparations
- Senna Liquid Extract
- Senna Tablets

When Powdered Tinnevelly Senna Fruit is prescribed or demanded, material complying with the requirements below with the exception of Identification test A and the test for Foreign matter shall be dispensed or supplied.

**DEFINITION**

Dried fruit of *Cassia angustifolia* Vahl.

Content
Minimum 2.2 per cent of hydroxyanthracene glycosides, expressed as sennoside B \((C_{42}H_{38}O_{20}; M, 863)\) (dried drug).

**CHARACTERS**

Slight odour.

**IDENTIFICATION**

A. Flattened, slightly reniform pods, yellowish-brown or brown with dark brown patches at the positions corresponding to the seeds, usually 35-60 mm long and 14-18 mm wide. At one end is a stylar point and at the other a short stalk. The pods contain 5-8 flattened and obovate seeds, green or pale brown, with incomplete, wavy, transverse ridges on the testa.

B. Reduce to a powder (355) (2.9.12). The powder is brown. Examine under a microscope using chloral hydrate solution R. The powder shows the following diagnostic characters: epicarp with polygonal cells and a small number of conical warty trichomes and occasional anomocytic or paracytic stomata (2.8.3); fibres in 2 crossed layers accompanied by a crystal sheath of calcium oxalate prisms; characteristic palisade cells in the seed and stratified cells in the endosperm; clusters and prisms of calcium oxalate.
1., TLC #1 (Frangula bark, Rhubarb)

Extraction:
- 0.5 g drug
- extraction with 5 mL EtOH (heat until boiling)
- filter
- apply to TLC (20 μL)

Standard: -

Stationary phase: Silicagel G

Eluent: water + MeOH + EtOAc (10+13.5+100)

Dry the plate until it is solvent-free.

Visualisation, examination:
1. Without spraying, examine in A., UV 365 nm, B., VIS.
2. Spray with 0.5% KOH (in 50% EtOH), heat. Examine in VIS.

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2., TLC #2 (Sennae folium)

Extraction:
- 1 g drug
- 10 mL EtOH, 5 min. mild boiling
- filter
- apply to TLC (20 μL)

Standard: sennoside A+B (in MeOH) (20 μL)

Stationary phase: Silicagel G

Eluent: EtOAc+MeOH+water+Et₂N (40+20+10+5)

Dry the plate until it is solvent-free.

Visualization, examination: Spray with 0,5% KOH (in 50% EtOH), heat, examine in A., VIS., B., UV 365 nm

Reference data:

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<td>Sennoside A</td>
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</tbody>
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3., Detection of Senna anthraquinones (Sennae folium)

- 0,5 g drug
- extraction with 10 mL 5% KOH (in EtOH-al) (gentle heating)
- add 10 mL water
- filter
- + 2 M HCl (until color changes)
- extraction with 10 mL Et₂O
- to the Et₂O phase add 5 mL 2M NH₃ in a test tube
- positive reaction: water phase orange-red
4., Detection of anthraquinone glycosides (Frangula bark)

- To the inner side of the bark add a drop of 2M NaOH
- Positive reaction: red color

5., Detection of anthraquinone glycosides (Frangula bark and Rhubarb)

- 0.2 g drug
- Extraction with 10 mL water + 5 drops of 2M NaOH (heat until boiling)
- Filter
- + 2 M HCl (until color changes)
- Extraction with 10 mL Et₂O
- Shake Et₂O phase with 5 mL 6M NH₃ in a test tube
- Positive reaction: water phase intensive red, Et₂O phase yellow

6., Contamination with Rheum raponticum (Rhubarb)

- Examine the TLC plate
- No raponticin should be present (see Table).
5. Practice

St John's Wort
Milk-Thistle Fruits

St John's Wort (Ph Eur monograph 1438)
Hypericum

DEFINITION
Whole or cut, dried flowering tops of Hypericum perforatum L., harvested during flowering time.

Content
Minimum 0.08 per cent of total hypericins, expressed as hypericin (C30H16O8; Mr 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. Reduce to a powder (355) (2.9.12). The powder is greenish-yellow. Examine under a microscope using chloral hydrate solution R. The powder shows the following diagnostic characters: fragments of polygonal cells of the epidermis with thickened and beaded walls and paracytic or anomocytic stomata (2.8.3); fragments of the leaf and sepal with large oil glands and red pigment cells; thin-walled, elongated cells of the petal epidermis with straight or wavy anticinal walls; tracheids and tracheidal vessels with pitted walls and groups of thick-walled fibres; fragments of rectangular, lignified and pitted parenchyma; fibrous layer of the anther and elongated, thin-walled cells of the filament with a striated cuticle; numerous pollen grains with 3 pores and a smooth exine, occur singly or in dense groups, and calcium oxalate cluster crystals. The powder may also show the following diagnostic characters: fragments of the fruit, exocarp solid with rounded polygonal-cells, endocarp with blunt, thick-walled fibres; fragments of the seed testa, whitish or brown with thick-walled hexagonal or rounded polygonal cells; fragments of the nutritive tissue and embryo, abundant oil droplets.
Milk-thistle Fruit (Ph Eur monograph 1860)

DEFINITION

Mature fruit, devoid of the pappus, of *Silybum marianum* L. Gaertner.

Content

Minimum 1.5 per cent of silymarin, expressed as silibinin (C25H22O10; Mr 482.4) (dried drug).

CHARACTERS

No rancid odour.

IDENTIFICATION

A. The achene is strongly compressed, elongate-ovate, about 6-8 mm long, 3 mm broad and 1.5 mm thick; the outer surface is smooth and shiny with a grey or pale brown ground colour variably streaked dark brown longitudinally to give an overall pale greyish or brown colour; the fruit is tapering at the base and crowned at the apex with a glistening, pale yellow extension forming a collar about 1 mm high surrounding the remains of the style. Cut transversely, the fruit shows a narrow, brown outer area and 2 large, dense, white oily cotyledons.

B. Reduce to a powder (355) (2.9.12). The powder is brownish-yellow with darker specks. Examine under a microscope using chloral hydrate solution R. The powder shows the following diagnostic characters: fragments of the epicarp composed of colourless cells, polygonal in surface view, the lumen appearing fairly large or as a small slit, depending on the orientation; groups of parenchymatous cells from the pigment layer, some of them containing colouring matter which appears bright red; very abundant groups of large sclereids from the testa with bright yellow pitted walls and a narrow lumen; occasionally fragments of small-celled parenchyma with pitted and beaded walls; abundant thin-walled parenchymatous cells from the cotyledons containing oil globules and scattered cluster crystals of calcium oxalate; a few larger, prismatic crystals of calcium oxalate.
1., TLC #1 (St John's wort)

Extraction:
- 0,5 g drug
- extraction with 5 mL MeOH
- filter
- apply to TLC (20 µL)

Standard: -

Stationary phase: Silicagel G

Eluent: EtOAc + HCOOH + AcOH + water (100+11+11+27)

Dry the plate until it is solvent-free.

Visualization, examination: Examine in UV 365 nm without spraying.

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<td>hypericin</td>
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2. TLC #2 (Milk-thistle Fruit)

Extraction:

- 0,5 g drug
- shake with 2x10 mL Et₂O-el for 5-5 min.
- discard Et₂O extracts
- dry the drug
- extract with 5 mL MeOH (gentle boiling for a few minutes)
- evaporate to dryness
- dissolve in MeOH (200 μL)
- apply to TLC (20 μL)

Standard: Legalon drg. MeOH extract (20 μL)

Stationary phase: Silicagel G

Eluent: CHCl₃ + acetone + HCOOH (75+16,5+8,5)

Dry the plate until it is solvent-free.

Visualization, examination: Spray with alkalic Pb(OAc)₂, heat; examine in UV 365 nm

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6. Practice

**Flavonoid-containing plant drugs I.**

Comparative analysis of flavonoid patterns of *Tilia* species (lime flowers and bracts)

**Lime Flower (Ph Eur monograph 0957)**

**DEFINITION**

Whole, dried inflorescence of *Tilia cordata* Miller, of *Tilia platyphylos* Scop., of *Tilia × vulgaris* Heyne or a mixture of these.

**CHARACTERS**

Faint aromatic odour. Faint, sweet and mucilaginous taste.

**IDENTIFICATION**

A. The inflorescence is yellowish-green. The main axis of the inflorescence bears a linguiform bract, membranous, yellowish-green, practically glabrous, the central vein of which is joined for up to about half of its length with the peduncle. The inflorescence usually consists of 2-7 flowers, occasionally up to 16. The sepals are detached easily from the perianth; they are up to 6 mm long, their abaxial surface is usually glabrous, their adaxial surface and their borders are strongly pubescent. The 5 spatulate, thin petals are yellowish-white, up to 8 mm long. They show fine venation and their borders only are sometimes covered with isolated trichomes. The numerous stamens are free and usually constitute 5 groups. The superior ovary has a pistil with a somewhat 5-lobate stigma.

B. Separate the inflorescence into its different parts. Examine under a microscope using chloral hydrate solution R. The adaxial epidermis of the bract shows cells with straight or slightly sinuous anticlinal walls; the abaxial epidermis shows cells with wavy-sinuous anticlinal walls and anomocytic stomata (2.8.3). Isolated cells in the mesophyll contain small calcium oxalate cluster crystals. The parenchyma of the sepals shows, particularly near the veins, numerous mucilaginous cells and cells containing small calcium oxalate clusters. The adaxial epidermis of sepals bears bent, thick-walled covering trichomes, unicellular or stellate with up to 5 cells. The epidermal cells of the petals show straight anticlinal walls with a striated cuticle without stomata. The parenchyma of the petals shows small calcium oxalate clusters and especially in its acuminate part mucilaginous cells. The pollen grains have a diameter of about 30-40 µm and are oval or slightly triangular with 3 germinal pores and a finely granulated exine. The ovary is glabrous or densely covered with trichomes, often very twisted, unicellular or stellate with 2-4 branches.
1., TLC (Lime flower)

Extraction:

- 0.5 g drug
- extraction with 5 mL MeOH
- filter
- apply to TLC (30 μL)

Standard: -, identification by reference Rf values (see Table)

Eluent: EtOAc + HCOOH + AcOH + H₂O (100+11+11+27)

Dry the plate until it is solvent-free.

Visualization, examination:
1., Examine in UV 365 nm, without spraying
2., Spray with AlCl₃, examine in UV 365 nm

Rf values for different flavonoids in the used system:
- Apigenin-6-C-glu-7-0-glu (Saponarin) 0.20
- Quercetin-3-O-rut (Rutin) 0.30
- Diosmetin-7-O-rhaglu (Diosmin) 0.31
- Tamarixetin-7-O-rut 0.34
- Isorhamnetin-3-O-rut (Narcissin) 0.36
- Apigenin-7-O-apiosylglu (Apiin) 0.39
- Myricetin-3-O-gal 0.45
- Myricetin-3-O-glu 0.46
- Quercetin-3-O-gal (Hyperoside) 0.51
- Quercetin-3-O-glu (Isoquercitrin) 0.53
- Luteolin-7-O-glu 0.54
- Apigenin-8-C-glu (Vitexin) 0.56
- Apigenin-7-O-glu 0.57
- Isorhamnetin-3-O-glu 0.58
- Myricetin-3-O-rha 0.58
- Kaempferol-3-O-gal 0.59
- Quercetin-3-O-ara 0.61
- Kaempferol-3-O-glu (Astragalin) 0.65
- Quercetin-3-O-rha (Quercetin) 0.69
- Kaempferol-3-O-rha 0.72
- Quercetin-3-O-ara 0.72
7. Practice

Flavonoid-containing plant drugs II.

Elder flower
Hawthorn leaf
Hawthorn fruit
Mullein flower
Ginkgo leaf
**Elder Flower (Ph Eur monograph 1217)**

**DEFINITION**
Dried flowers of *Sambucus nigra* L.

**Content**
Minimum 0.80 per cent of flavonoids, expressed as isoquercitrinose (C_{21}H_{20}O_{12}; M, 464.4) (dried drug).

**IDENTIFICATION**
A. The flower, about 5 mm in diameter, has 3 small bracts (hand lens) and may have a peduncle. The 5-toothed calyx is small; the corolla is light yellow, with 5 broadly oval petals fused at their bases into a tube. The filaments of the 5 yellow stamens alternate with the petals. The corolla is often isolated or attached to the stamens, to which it is fused at the base. The ovary is inferior with 3 locules and it bears a short style with 3 obtuse stigmata.

B. Reduce to a powder (355) (2.9.12). The powder is greenish-yellow. Examine under a microscope using chloral hydrate solution R. The powder shows the following diagnostic characters: numerous spherical, sometimes ellipsoidal, pollen grains about 30 µm in diameter, with 3 germinal pores and very finely pitted exine; calyx epidermal cells with a striated cuticle and occasional unicellular marginal teeth from the basal region; corolla fragments with numerous small globules of volatile oil, those of the upper epidermis with slightly thickened and beaded walls and a striated cuticle; mesophyll cells of petals and sepals with idioblasts containing numerous sandy crystals of calcium oxalate.

**Hawthorn Leaf and Flower (Ph Eur monograph 1432)**

**Preparation**
- Hawthorn Leaf and Flower Dry Extract

**DEFINITION**
Whole or cut, dried flower-bearing branches of *Crataegus monogyna* Jacq. (Lindm.), *C. laevigata* (Poiret) D.C. (*C. oxyacanthoides* Thuill.) or their hybrids or, more rarely, other European *Crataegus* species including *C. pentagyna* Waldst. et Kt. ex Willd., *C. nigra* Waldst. et Kt., *C. azarolus* L.

**Content**
Minimum 1.5 per cent of flavonoids, expressed as hyperoside (C_{21}H_{20}O_{12}; M, 464.4) (dried drug).

**IDENTIFICATION**
A. The stems are dark brown, woody, 1-2.5 mm in diameter, bearing alternate, petiolate leaves with small, often deciduous stipules and coryombs of numerous small white flowers. The leaves are more or less deeply lobed with slightly serrate or almost entire margins; those of *C. laevigata* are pinnately lobed or pinnatifid with 3, 5 or 7 obtuse lobes, those of *C. monogyna* pinnatifid with 3 or 5 acute lobes; the adaxial surface is dark green or brownish-green, the abaxial surface is lighter greenish-brown and shows a prominent, dense, reticulate venation. The leaves of *C. laevigata*, *C. monogyna* and *C. pentagyna* are glabrous or bear only isolated trichomes, those of *C. azarolus* and *C. nigra* are densely pubescent. The flowers have a brownish-green tubular calyx composed of 5 free, reflexed sepals, a corolla composed of 5 free, yellowish-white or brownish, rounded or broadly ovate and shortly unguiculate petals and numerous stamens. The ovary is fused to the calyx and consists of 1-5 carpels, each with a long style and containing a single ovule; in *C. monogyna* there is 1 carpel, in *C. laevigata* 2 or 3, in *C. azarolus* 2 or 3, or sometimes only 1, in *C. pentagyna* 5 or, rarely, 4.

B. Reduce to a powder (355) (2.9.12). The powder is yellowish-green. Examine under a microscope using chloral hydrate solution R. The powder shows the following diagnostic characters: unicellular covering trichomes, usually with a thick wall and wide lumen, almost straight or slightly curved, pitted at the base; fragments of leaf epidermis with cells which have sinuous or polygonal anticlinal walls and with large anomocytic stomata (2.8.3) surrounded by 4-7 subsidiary cells; parenchymatous cells of the mesophyll containing calcium oxalate clusters, usually measuring 10-20 µm, those associated with the veins containing groups of small prism crystals; fragments of petals showing rounded polygonal epidermal cells, strongly papillose, with thick walls, the cuticle of which clearly shows wavy striations; fragments of anthers showing endothecium with an arched and regularly thickened margin; fragments of stems containing collenchymatous cells, bordered pitted vessels and groups of lignified sclerenchymatous fibres with narrow lumina; numerous spherical to elliptical or triangular pollen grains up to 45 µm in diameter, with 3 germinal pores and a faintly granular exine.
Hawthorn Berries (Ph Eur monograph 1220)

DEFINITION

Dried false fruits of *Crataegus monogyna* Jacq. (Lindm.), or *Crataegus laevigata* (Poir.) D.C. (synonym: *Crataegus oxyacantha* L.) or their hybrids or a mixture of these false fruits.

Content
Minimum 1.0 per cent of procyanidins, expressed as cyanidin chloride (C₁₅H₁₁ClO₆; M, 322.7) (dried drug).

CHARACTERS
Sweet mucilaginous taste.

IDENTIFICATION

A. The false fruit of *Crataegus monogyna* is obovate or globular, generally 6-10 mm long and 4-8 mm wide, reddish-brown or dark red. The surface is pitted or, more rarely, reticulated. The upper end of the fruit is crowned by the remains of 5 reflexed sepals surrounding a small sunken disc with a shallow, raised rim. The remains of the style occur in the centre of the disc with tufts of stiff, colourless hairs at the base. At the lower end of the fruit is a short length of pedicel or, more frequently, a small pale circular scar where the pedicel was attached. The receptacle is fleshy and encloses a yellowish-brown, ovoid fruit with a hard, thick wall containing a single, elongated, pale brown, smooth and shiny seed. The false fruit of *Crataegus laevigata* is up to 13 mm long. It contains 2-3 stony fruits, ventrally flattened, with short hairs at the top. Frequently, in the centre of the disc of the false fruit occur the remains of the 2 styles.

B. Reduce to a powder (355) (2.9.12). The powder is greyish-red. Examine under a microscope using chloral hydrate solution R. The powder shows the following diagnostic characters: covering trichomes from inside the disc which are long, unicellular, frequently bent, tapering to a point, with smooth, much thickened and lignified walls; parenchymatous receptacle fragments, the outer layer with red colouring matter, some cells of the inner layers containing small cluster crystals of calcium oxalate; occasional fragments including groups of sclereids and vascular strands with associated files of cells containing prisms of calcium oxalate; pericarp fragments consisting of large thick-walled sclereids with numerous pits, some of which are conspicuously branched; a few fragments of the testa having an epidermal layer composed of hexagonal, mucilaginous cells beneath which is a yellowish-brown pigment layer containing numerous elongated prisms of calcium oxalate; thin-walled parenchyma of the endosperm and cotyledons containing aleurone grains and globules of fixed oil.

Mullein Flower (Ph Eur monograph 1853)

DEFINITION

Dried flower, reduced to the corolla and the androecium, of *Verbascum thapsus* L., *V. densiflorum* Bertol. (*V. thapsiforme* Schrad), and *V. phlomoides* L.

IDENTIFICATION

A. The corolla of *V. thapsus* is about 20 mm in diameter, pale yellow, yellow to brown, funnel-shaped with 5 slightly unequal and spreading lobes. The corolla lobes are densely hairy on the outer surface, glabrous on the inner surface, with a fine network of light brown veins. There are 5 stamens, alternating with the petal lobes, 2 of these are long, with glabrous filaments, the other 3 shorter, with densely tormentose filaments. The anthers are attached transversely. In *V. phlomoides* the corolla is up to about 30 mm in diameter, bright yellow to orange, and the anthers are obliquely attached to the filaments. The corolla of *V. densiflorum*, about 30 mm in diameter, is almost flat and deeply divided into 5 slightly unequal lobes, with rounded apices.

B. Reduce to a powder (355) (2.9.12). The powder is yellow or yellowish-brown. Examine under a microscope using chloral hydrate solution R. The powder shows many covering trichomes from the corolla, whole and fragmented; there are pluricellular, of the candelabra type with a central uniseriate axis from which whorls of branch cells arise at the position of the cross walls and at the apex. The covering trichomes from the stamen filaments are unicellular, long, thin-walled and tubular, sometimes with a club-shaped tip; they have a distinctly granular or striated surface. Numerous pollen grains, ovoid with a finely granular exine with 3 pores. Fragments of the fibrous layer of the anther with thickened walls giving a characteristic star-shaped appearance. Yellow fragments of the petals in the surface view, the epidermal cells polygonal and isodiametric; fragments of the mesophyll consisting of irregular parenchymatous cells and scattered spiral vessels.
**Ginkgo Leaf (Ph Eur monograph 1828)**

**DEFINITION**
Whole or fragmented, dried leaf of *Ginkgo biloba* L.

**Content**
Not less than 0.5 per cent of flavonoids, calculated as flavone glycosides (M, 757) (dried drug).

**CHARACTERS**
Greyish or yellowish-green or yellowish-brown.

**IDENTIFICATION**

A. The upper surface of ginkgo leaf is slightly darker than the lower surface. The petioles of the leaf are about 4 cm to 9 cm long. The lamina is about 4 cm to 10 cm wide, fan-shaped, usually bilobate or sometimes undivided. Both surfaces are smooth, and the venation dichotomous, the veins appearing to radiate from the base; they are equally prominent on both surfaces. The distal margin is incised, irregularly and to different degrees, and irregularly lobate or emarginate. The lateral margins are entire and taper towards the base.

B. Reduce to a powder (355) (2.9.12). The powder is greyish or yellowish-green or yellowish-brown. Examine under a microscope using chloral hydrate solution R. The powder shows irregularly-shaped fragments of the lamina in surface view, the upper epidermis consisting of elongated cells with irregularly sinuous walls, the lower epidermal cells smaller, with a finely striated cuticle and each cell shortly papillose; stomata about 60 µm, large, deeply sunken with 6 to 8 subsidiary cells, are more numerous in the lower epidermis; abundant large cluster crystals of calcium oxalate of various sizes in the mesophyll; fragments of fibro-vascular tissue from the petiole and veins.
1., TLC (Elder flower, Hawthorn leaf and fruit, Mullein flower, Ginkgo leaf)

Extraction:

- 0.5 g drug
- extraction with 5 mL MeOH
- filter
- apply to TLC (30 μL)

Standard: -, identification by reference Rf values (see Table in Pr. 6.)

Eluent: EtOAc + HCOOH + AcOH + H2O (100+11+11+27)

Stationary phase: Silicagel G

Dry the plate until it is solvent-free.

Visualization, examination:
1. examine in UV 365 nm, without spraying
2. Spray with 1% AlCl₃ (in EtOH), examine in UV 365 nm
8. Practice
Coumarin-containing plant drugs
Lovage root, Angelica root

Lovage Root (Ph Eur monograph 1233)

DEFINITION
Whole or cut, dried rhizome and root of Levisticum officinale Koch.

Content
Minimum 4.0 mL/kg of essential oil for the whole drug and minimum 3.0 mL/kg of essential oil for the cut drug (dried drug).

IDENTIFICATION
A. The rhizome and the large roots are often split longitudinally. The rhizome is short, up to 5 cm in diameter, light greyish-brown or yellowish-brown, simple or with several protuberances; the roots, showing little ramification, are the same colour as the rhizome; they are usually up to 1.5 cm thick and up to about 25 cm long; the fracture is usually smooth and shows a very wide yellowish-white bark and a narrow brownish-yellow wood.

B. Reduce to a powder (355) (2.9.12). The powder is brownish-yellow. Examine under a microscope using chloral hydrate solution R. The powder shows the following diagnostic characters: cork cells, polygonal or rounded in surface view, with brown contents; abundant parenchyma, mostly thin-walled and rounded but some with thicker walls; groups of small, reticulately thickened vessels embedded in small-celled, un lignified parenchyma; fragments of larger vessels with reticulate thickening, up to 125 µm in diameter; fragments of secretory canals up to 180 µm wide. Examine under a microscope using a 50 per cent V/V solution of glycerol R. The powder shows starch granules, simple, rounded or ovoid, up to about 12 µm, and numerous larger, compound granules, many with several components.

Angelica Root (Ph Eur monograph 1857)

DEFINITION
Whole or cut, carefully dried rhizome and root of Angelica archangelica L. (Archangelica officinalis Haffm.).

Content
Minimum 2.0 mL/kg of essential oil (dried drug).

CHARACTERS
Bitter taste.

IDENTIFICATION
A. The rhizome is greyish-brown or reddish-brown, with transversely annulated thickenings. The base bears greyish-brown or reddish-brown, cylindrical, longitudinally furrowed, occasionally branched roots often with incompletely encircling, transverse ridges. The apex sometimes shows remnants of stem and leaf bases. The fracture is uneven. The transversely cut surface shows a greyish-white, spongy, distinctly radiate bark, in which the secretory channels are visible as brown spots, and a bright yellow or greyish-yellow wood which, in the rhizome, surrounds the greyish or brownish-white pith.

B. Reduce to a powder (355) (2.9.12). The powder is brownish-white. Examine under a microscope using chloral hydrate solution R. The powder shows the following diagnostic characters: fragments of cork consisting of several layers of thin-walled greyish-brown or reddish-brown cells; fragments of large, yellowish-brown secretory channels; fragments of medullary rays 2 or 4 cells wide; fragments of xylem with medullary rays and radially arranged, lignified vessels with reticulate thickenings. Examine under a microscope using a 50 per cent V/V solution of glycerol R. The powder shows numerous, simple starch granules 2-4 µm in diameter.
1., TLC (Lovage root, Angelica root)

Extraction:

- 1 g drug
- extraction with 5 mL MeOH (until boiling)
- filter
- apply to TLC (20 μL)

Standard: -

Stationary phase: Silicagel G

Eluent: Et₂O + toluene (1+1), saturated with 10% AcOH (50+50+50 mL)

Dry the plate until it is solvent-free.

Visualization, examination:

1., examine in UV 254 nm, without spraying
2., examine in UV 356 nm, without spraying
3., spray with 1% KOH (EtOH), examine in UV 356 nm

Reference data:

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9. Practice

Analysis of plant drugs containing tannic and other polyphenolic substances

Agrimony (Ph Eur monograph 1587)

DEFINITION

Dried flowering tops of Agrimonia eupatoria L.

Content
Minimum 2.0 per cent of tannins, expressed as pyrogallol (C₆H₆O₃; M, 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 imparipinnate 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 tormentose 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 to grey. Examine under a microscope using chloral hydrate solution R. The powder shows numerous straight or bent, unicellular, long thick-walled (about 500 µm) trichomes finely warty and sometimes spirally marked; fragments of parenchyma with prisms and cluster crystals of calcium oxalate; fragments of leaf epidermis with sinuous walls, those of the lower epidermis with abundant stomata, mostly anomocytic but occasionally anisocytic; ovoid to subspherical-pollen grains, with 3 pores and a smooth exine; fragments of glandular trichomes with a multicellular uniseriate stalk and a spherical unicellular or quadricellular head; groups of fibres and spiral and barded-fitted vessels from the stem.

Bearberry Leaf (Ph Eur monograph 1054)

DEFINITION

Whole or cut, dried leaf of Arctostaphylos uva-ursi (L.) Spreng.

Content
Minimum 7.0 per cent of anhydrous arbutin (C₁₂H₁₆O₇; M, 272.3) (dried drug).

IDENTIFICATION

A. The leaf, shiny and dark green on the adaxial surface, lighter on the abaxial surface, is normally 7-30 mm long and 5-12 mm wide. The entire leaf is obovate with smooth margins, somewhat reflexed downwards, narrowing at the base into a short petiole. The leaf is obtuse or retuse at its apex. The lamina is thick and coriaceous. The venation, pinnate and finely reticulate, is clearly visible on both surfaces. The adaxial surface is marked with sunken veinlets, giving it a characteristic grainy appearance. Only the young leaf has ciliated margins. Old leaves are glabrous.

B. Reduce to a powder (355) (2.9.12). The powder is greenish-grey or yellowish-green. Examine under a microscope using chloral hydrate solution R. The powder shows the following diagnostic characters: fragments of epidermis, which, seen in surface view, show polygonal cells covered by a thick smooth cuticle, and with straight, thick and irregularly pitted walls; anomocytic stomata (2.8.3), surrounded by 5-11 subsidiary cells and scars of hair bases only on the abaxial epidermis; fragments of palisade parenchyma, with 3 or 4 layers of cells of unequal lengths, and spongy parenchyma; groups of lignified fibres from the pericycle, with rows of cells containing prisms of calcium oxalate; occasional conical, unicellular covering trichomes.
Oak Bark (Ph Eur monograph 1887)

DEFINITION
Cut and dried bark from the fresh young branches of *Quercus robur* L., *Q. petraea* (Matt.) Liebl. and *Q. pubescens* Willd.

Content
Minimum 3.0 per cent of tannins, expressed as pyrogallol (C₆H₆O₃; Mr 126.1) (dried drug).

IDENTIFICATION
A. The bark occurs in channelled or quilled pieces, not more than 3 mm thick. The outer surface is light grey or greenish-grey, rather smooth, with occasional lenticels. The inner surface is dull brown or reddish-brown and has slightly raised longitudinal striations about 0.5-1 mm wide. The fracture is splintery and fibrous.

B. Reduce to a powder (355) (2.9.12). The powder is light brown or reddish-brown and fibrous. Examine under a microscope using chloral hydrate solution R. The powder shows the following diagnostic characters: groups of thick-walled fibres surrounded by a moderately thickened parenchymatous sheath containing prism crystals of calcium oxalate; fragments of cork composed of thin-walled tabular cells filled with brownish or reddish contents; abundant sclereids, isolated and in groups, some large with thick, stratified walls and branching pits, others smaller and thinner-walled with simple pits, often with dense brown contents; fragments of parenchyma containing cluster crystals of calcium oxalate; occasional fragments of sieve tissue, thin-walled, some showing sieve areas on the oblique end-walls.
1., TLC #1 (Agrimony, Oak Bark)

Extraction:
- 0.5 g drug
- extraction with 5 mL MeOH (short boiling)
- filter
- evaporate to dryness
- dissolve (500 μL MeOH)
- apply to TLC (10 μL)

Standard: gallic acid (1mg/mL, MeOH), (10 μL)

Stationary phase: Silicagel G (UV 254, with fluorescein)

Eluent: ethyl-formiate + HCOOH + water (8+1+1)

Dry the plate until it is solvent-free.

Visualization:
- 1., examine in UV 254 nm, without spraying
- 2., spray with 10% FeCl₃, examine in VIS
2., TLC #2 (Bearberry leaf)

Extraction:
- 1 g drug
- extraction with 5 mL MeOH (short boiling)
- filter
- evaporate to 1 mL
- bring to 10 mL with water
- extraction with toluene (2x5 mL), discard toluene
- extraction with EtOAC (2x5 mL)
- collect EtOAc fractions, filter through Na$_2$SO$_4$
- evaporate to dryness
- dissolve in MeOH (500 μL)

- apply to TLC (5, 10 and 20 μL)

Standard: -

Stationary phase: Silica gel G

Eluent: toluene + EtOAc (60+40)

Dry the plate until it is solvent-free.

Visualization, examination:
Spray with 1:1 mixture of 1% FeCl$_3$ solution and 1% K$_3$[Fe(CN)$_6$]. Spray reagent should be prepared before use and used immediately. Examine in VIS.

Reference value: hydroquinone, 0.50, blue.

3., Detection of proanthocyanidines (Bearberry leaf)
- dip small pieces of the drug in alcoholic vanillin-HCl solution (50 mg vanillin / 5 mL EtOH + 35 mL 25% HCl)
- positive reaction: red discoloration along the edges of the plant drug pieces