CRUDE DRUGS CONTAINING TANNINS

TOPICS

1. MACROMORPHOLOGICAL EVALUATION

- Agrimonieae herba
- Cotini folium
- Galla
- Quercus cortex
- Ratanhiae radix
- Tormentillae rhizome et radix
- Cerasi stipes
- Crataegi folium cum floriae
- Crataegi fructus
- Melissae folium
- Rosmarini folium

2. MICROSCOPICAL EVALUATION

- Transverse section: Quercus cortex
- Powder preparation: Quercus cortex
- Powder preparation: Agrimonieae herba

3. PHYSICOCHEMICAL AND CHEMICAL TESTS

3.1. General test tube reactions

3.2. Distinctive reaction using ferric chloride solution
- Quercus cortex, Cotini folium, Cerasi stipes, Agrimonieae herba

3.3 Test for proanthocyanidins
- Agrimonieae herba, Crataegi summitas

3.4. Isolation and TLC investigation of tannin mixture from Cotini folium

3.5. TLC detection of Lamiaceae depsides (e.g. rosmarinic acid)

4. QUANTITATIVE DETERMINATIONS

4.1. Determination of tannins in herbal drugs (Ph. Eur.)

4.2. Determination of procyanidin content by method based on Bate-Smith reaction

4.3. Total hydroxycinnamic derivatives (PH. Eur.)
1. MACROMORPHOLOGICAL EVALUATION

Agrimoniae herba
_Agrimonia eupatoria_ L.
Ph.Hg. VIII. Ph. Eur.

The drug consists of the flowering herb. The stem yielding the drug is angular, or almost cylindrical, hairy having longer internodes on the upper part. The lower leaves are lamella-like, upper leaves are pinnate; leaf compounds consisting of 5-9 larger and 6-10 smaller leaflets. The leaflets are sessile, longish egg-shapes, roughly dentate, with winged nervure, their upper surface is dark green and often hairy, the abaxial surface is yellowish green with thick pubescences and downs, with tiny gland among them. The bracts are 1-1.2 cm long, egg-shaped having 1-5 rough teeth. Its inflorescence is simple or rarely ramifying, multiflowered longish spike with hairy rachis, the flowers can be found on 1-3 mm long pedicle on the axil of the tiny bracts of 3 prisms. The receptacle is hard, calycular short coniform, hairy having 10 deep longitudinal striae, on its top multicircular wreath of hairs can be found which later transform to hooked thorns. It sepals are small, triangular, egg-shaped, yellow with entire top. The stamens are shorter than the petals; their number is 10-20. It two styles are divergent; the stigma is kidney-shaped. The calyx of the receptacle is grown together with the fruit and becomes larger at ripening. The fruit consists of single acorns. The odor of the drug is light, pleasant, the taste astringent, spicy, tart.

_Cotini folium_
_Cotinus coggyria_ Scop.

Venetian sumac
Anacardiaceae

_Galla_
_Quercus infectoria_ Oliv.
(Cynips gallae tinctoriae Oliv
Cynipidae)

Gall
Fagaceae

Aleppo galls are globular in shape and from 10-25 mm in diameter. They have a short, basal stalk and numerous rounded projections on the surface. Galls are hard and heavy, usually sinking in water. (White galls show a circular tunnel through which the insect has emerged. Galls without this opening have insect remains in the small central cavity). Odourless. Taste is very astringent

_Cerasi stipes_
_Cerasus avium_ L. (syn. _Prunus avium_) Rosaceae
_Cerasus vulgaris_ Mill. (syn. _Prunus cerasus_)
**Quercus cortex**  
*Quercus robur* L.  
*Quercus petraea* Liebl.  
Ph.Hg. VIII. Ph. Eur.

Oak bark is 1 to 3 cm wide, 15 to 20 cm long, through-shaped or rashly tubular. Bright silver grey external surface. Few white cork verrucae, rarely lichen colonies. Internal surface reddish or yellowish brown, roughly striated lengthways. The hand magnifier shows in the cross-section cork bark portions, a light-coloured sclerenchyma ring and fibre groups. Stringy fracture. Odourless. Taste is tart, astringent.

---

**Ratanhia radix**  
*Krameriatranda Ruiz et Pavon*  
Ph.Hg. VIII. Ph. Eur.

The root branches may be as long as 1m 15 to 20 cm in length, the drug pieces are hard, lignified, difficult to break, cylindrical, curved, hardly ramified, 1 to 2 mm thick. The cork is comparatively smooth, dark brownish red in colour, sometimes transversally cracked, or detached in squamae. The bark is reddish, the sapwood of the xylem part is light-coloured, refuse-yellow, the heartwood brownish red. The main part of the root consists of the xylem that is 5 to 6 times as thick as the bark. The woody part is almost tasteless. Stringy fracture. Odourless taste, intensely tart, astringent.

---

**Tormentillae rhizome et radix**  
*Potentilla erecta* (L.) Rauschal  
Ph.Hg. VIII. Ph. Eur.

The rhizome is 6-8 cm long, 2-3 cm thick; it stands in the ground vertically or diagonally. It is covered by woody fibers, rout sometimes ramifying. Outside it is dark red, dish-brown; inside it is light white spotty when fresh, and blood-red after drying. The drug is odourless, with strongly astringent tart.
2. MICROSCOPICAL EVALUATION

Quercus cortex transverse section

1. cork
2. cortex
3. secondary phloem
4. sclerenchyma ring
5. medullary ray
6. fibre group
7. group of fibre with calcium oxalate prism sheaths
8. rosette
9. sclereid
The outer layer of young oak bark consists of small flat cork-cells. The cortex exhibits a ring, but slightly interrupted, of thick-walled cells (sclereids) and isolated shining bundles of liber fibres. The secondary phloem exhibits tangential bands of fibres, surrounded by cells which contain a prism of calcium oxalate forming a crystal sheat to each group of fibres. The medullary rays are one – two cell wide and somewhat wavy. Sclereid groups and cells with cluster crystals of calcium oxalate can be found in both the cortex and the secondary phloem.

**Quercus cortex pulvis**

The powder shows the following diagnostic characters: group of fibres with calcium oxalate prism sheats, reddish-brown fragments of cork, sclereids, fragments of parenchyma containing calcium oxalate cluster crystals.

**Powder preparation of oak bark**

a. sclereids  
b. parenchyma with rosettes and starch granules  
c. groups of fibres with calcium oxalate prism sheats  
d. fragments of cork in surface view (fn), in sectional view (km)
Agrimoniae herba pulvis

1. cross section
2. unicellular covering trichome
3. covering trichome types
4. glandular trichomes
5. upper epidermis
6. cluster crystals of Ca(COO)$_2$
7. rat tail shaped trichome
8. brick-shaped cells from the stem
9. pollen
3. PHYSICOCHEMICAL AND CHEMICAL TESTS

Sample preparation:
Boil 1 g of the powdered drug (V) with 100 ml of water for 5 minutes (put funnel into the neck of the flask) and filter the extract through cotton plug. Use the filtrate for the test described below.

3.1. General test tube reactions

To 2-3 ml of the filtrate add 1 to 2 drops of
• 1 % solution of gelatine
• 1 % solution of caffeine in 2 % hydrochloric acid
• basic lead acetate

Explanation:
Tannins give precipitate with heavy metals, alkaloids and proteins. Precipitation reaction with mineral salts (Cu (II) salts) is almost quantitative, therefore it can be used for determination of tannins. Complexation with proteins results disturbance or precipitate. The exactation is not stoichiometric and not specific, polyphenols, other than tannins also react. With alkaloids, tannins form addiction compounds insoluble in water.
3.2. Distinctive reaction using ferric chloride solution

*Quercus cortex, Cotini folium*

To 5 ml of the filtrate add 1 to 2 drops of R-iron (III) chloride solution. The liquid turns bluish-black and the supernatant liquid above the slowly depositing bluish-black precipitate becomes colourless.

*Cerasi tipes, Agrimoniae herba*

To 5 ml of the filtrate add 1 to 2 drops of R-iron (III) chloride solution. The liquid turns green and after standstill, the depositing residue turns quickly brown, while the supernatant liquid assumes a dirty green colour.

*Explanation:*

*Compounds containing phenolic –OH groups give the reaction. The structure of the complex is the next:*

![Chemical structure](image)

*The colour of the complex depends on the number and the position of the phenolic groups. Gallotannins and ellagitannins give bluish-black colour and precipitates, and condensed tannins give greenish-brown precipitate. The reaction proceeding in neutral or weekly acidic medium is a complexation of electrondonor OH groups in ortho position and Fe$^{3+}$ ions. It is a non-specific reaction.*

3.3 Test for proanthocyanidins

*Agrimoniae herba, Crataegi folium cum florae, Crataegi fructus*

Boil 0.5 g of powdered drug (V) with 10.0 ml of 2 M hydrochloric acid on hot water bath for 30 minutes using reflux condenser. After cooling and filtration, shake the wateric solution out with 10.0 ml of n-butanol The butanol phase turns to red.
Decomposition of procianidin of B-2 type, catalyzed by acid and the UV-VIS spectra of the products arised.

3.4. Isolation and TLC investigation of tannin mixture from *Cotini folium*

Boil 5 g powdered drug with 50 ml of water in an Erlenmeyer flask for five minutes (put the funnel into the neck). Filter the extract and add 5 g NaCl. Filter the mixture and shake the aqueous solution three times, each of 15 ml, of ethyl acetate. Dry the combined ethyl acetate layers over anhydrous sodium sulphate and concentrate to 5-10 ml in vacuum. Add to the concentrated solution 15 ml of dried chloroform. Yellowish-white precipitate deposits (tannin mixture). Collect the precipitate into a filtration paper previously measured analytically. Dry the tannin mixture in oven at 100 °C for 30 minutes. Calculate its weight.

*Explanation:* Tannins are soluble in hot water. NaCl added to the extract decreases the solubility of tannins in water (salting out effect) helping in this way their dissolution in the organic phase. From their concentrated ethyl acetate solution, tannins are precipitated by chloroform. (Tannins are insoluble in chloroform).

TLC parameters:

*Adsorbent:* Kiesegel-G (Merck 0.2 mm) 100 X 60 mm

*Developing system:* chloroform – methyl ethyl ketone – formic acid (6+9+6)
Dying reagent: 3% methanolic FeCl3 solution

Test: 0.5 % methanolic gallic acid solution, 2 μl

Sample: ethanolic extract, 2-5 μl

3.5. TLC detection of Lamiaceae depsides (e.g. rosmarinic acid)

Sample: *Rosmarini folium* 0.2 g

*Melissae folium* 2.00 g

Boil portions of the powdered drugs mentioned above with 10 ml of methanol of 80 % for 15 minutes on water bath using reflux condenser (!). After filtration shake it out with 10 ml of CCl₄ for removing chlorophyll. Separate the two phases and use different portions of the upper phase to the investigation.

*TLC parameters:*

*Adsorbent:* Kiesegel-G (Merck 0.2 mm) 100 X 60 mm

*Developing system:* toluene – ethyl acetate – formic acid (25+20+6)

Dying reagent: 3% methanolic FeCl₃ solution

*Sample application:* on plate apply portions of extracts or test as follows:

1. *Rosmarini folium* extract 20 μl
2. Rosmarinic acid standard (1 μg/ml) 5 μl
3. *Melisse folium* extract 20 μl

![Rosmarinic acid](image)
4.1. Determination of tannins in herbal drugs (Ph. Eur.)

Sample: *Agrimoniae herba* 1.000 g  
*Quercus cortex* 0.700 g

*Carry out all the extraction and dilution operations protected from light.*

In the case of a herbal drug or a dry extract, to the stated amount of the powdered drug (180) (2.9.12) or the extract in a 250 ml round-bottomed flask add 150 ml of *water R*. Heat on a water-bath for 30 min. Cool under running water and transfer quantitatively to a 250 ml volumetric flask. Rinse the round-bottomed flask and collect the washings in the volumetric flask, then dilute to 250.0 ml with *water R*. Allow the solids to settle and filter the liquid through a filter paper 125 mm in diameter. Discard the first 50 ml of the filtrate.

In the case of a liquid extract or a tincture, dilute the stated amount of the liquid extract or tincture to 250.0 ml with *water R*. Filter the mixture through a filter paper 125 mm in diameter. Discard the first 50 ml of the filtrate.

*Total polyphenols.* Dilute 5.0 ml of the filtrate to 25.0 ml with *water R*. Mix 2.0 ml of this solution with 1.0 ml of *phosphomolybdotungstic reagent R* and 10.0 ml of *water R* and dilute to 25.0 ml with a 290 g/l solution of *sodium carbonate R*. After 30 min measure the absorbance (2.2.25) at 760 nm (*A*) using *water R* as the compensation liquid.

*Polyphenols not adsorbed by hide powder.* To 10.0 ml of the filtrate, add 0.10 g of hide powder CRS and shake vigorously for 60 min. Filter and dilute 5.0 ml of the filtrate to 25.0 ml with *water R*. Mix 2.0 ml of this solution with 1.0 ml of *phosphomolybdotungstic reagent R* and 10.0 ml of *water R* and dilute to 25.0 ml with a 290 g/l solution of *sodium carbonate R*. After 30 min measure the absorbance (2.2.25) at 760 nm (*A*), using *water R* as the compensation liquid.

*Standard.* Dissolve immediately before use 50.0 mg of *pyrogallol R* in *water R* and dilute to 100.0 ml with the same solvent. Dilute 5.0 ml of the solution to 100.0 ml with *water R*. Mix 2.0 ml of this solution with 1.0 ml of *phosphomolybdotungstic reagent R* and 10.0 ml of *water R* and dilute to 25.0 ml with a 290 g/l solution of *sodium carbonate R*. After 30 min measure the absorbance (2.2.25) at 760 nm (*A*), using *water R* as the compensation liquid.

Calculate the percentage content of tannins expressed as pyrogallol from the expression:

\[
\frac{62.5(A_1 - A_2)m_2}{A_3 \times m_1}
\]

\[m_1 = \text{mass of the sample to be examined, in grams,}
\]
\[m_2 = \text{mass of pyrogallol, in grams.}
\]

Remark: During laboratory practice, instead of measuring the absorbance of pyrogallol, use \(A_3=0.36\) for calculation.
4.2. Determination of procyanidin content by method based on Bate-Smith reaction

Sample: *Agrimoniae herba*
*Cerasi stipes*

Boil 0.1 g of freshly powdered crude drug for 60 minutes with 20 ml of a mixture of n-butanol + cc HCl (95+5) and 0.5 ml of 2 % Fe(NH₄)(SO₄) 12 H₂O in 2 n HCl (protect from light!). Cool and filtrate the reaction mixture into a volumetric flask of 25.00 ml volume and with butanol containing hydrochloric acid fill it up to the mark. 0.5 ml of this solution dilute to 5.0 ml.
Determine the light absorption at 550 nm, in 1 cm thickness against butanol-hydrochloric acid solution $A_{1 \% 1 \text{cm}}^{1 \%} = 75$ (cyanidine chloride).
Calculation: $\% = \frac{250A}{75m} = A/3m$, $m =$ mass weight, $A =$ absorbance value

4.3. Total hydroxycinnamic derivatives (PH. Eur.)

Sample: *Rosmarini folium*
*Melissae folium*

*Stock solution.* To 0.200 g of the powdered drug (355) (2.9.12) add 80 ml of *alcohol (50 per cent V/V)* R. Boil in a water-bath under a reflux condenser for 30 min. Allow to cool and filter. Rinse the filter with 10 ml of *alcohol (50 per cent V/V)* R. Combine the filtrate and the rinsings in a volumetric flask and dilute to 100.0 ml with *alcohol (50 per cent V/V)* R.
*Test solution.* To 1.0 ml of the stock solution add 2 ml of *0.5 M hydrochloric acid*, 2 ml of a solution prepared by dissolving 10 g of *sodium nitrite* R and 10 g of *sodium molybdate* R in 100 ml of *water* R and then add 2 ml of *dilute sodium hydroxide solution* R and dilute to 10.0 ml with *water* R; mix.
*Compensation solution.* Dilute 1.0 ml of the stock solution to 10.0 ml with *water* R.
Measure immediately the absorbance (2.2.25) of the test solution at 505 nm.
Calculate the percentage content of total hydroxycinnamic derivatives, expressed as rosmarinic acid, from the expression:

\[
A \times \frac{2.5}{m}
\]

i.e. taking the specific absorbance of rosmarinic acid to be 400.

\[
A = \text{absorbance of the test solution at 505 nm,}
\]

\[
m = \text{mass of the substance to be examined, in grams.}
\]