CRUDE DRUGS CONTAINING TANNINS

TOPICS

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1. MACROMORPHOLOGICAL EVALUATION

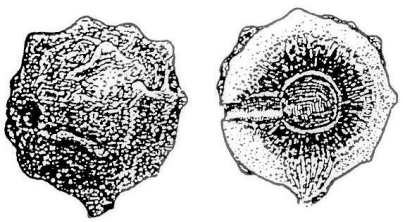
Agrimoniae herba — Rosaceae
Agrimonia eupatoria L. — Agrimony
Ph.Hg. VIII. Ph. Eur.

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 — Venetian sumac
Cotinus coggygria Scop. — Anacardiaceae

The drug consists of the green leaves of the shrub. These are wide, ovate or obovate, entire edge leaves, with pointless top, bald on the upper and lanate on the lower epidermis. Autumn leaves are red. The drug is odourless, the taste is characteristic, astringent.

Galla — Gall
Quercus infectoria Oliv. — Fagaceae
(Cynips gallae tinctoriae Oliv
Cynipidae)



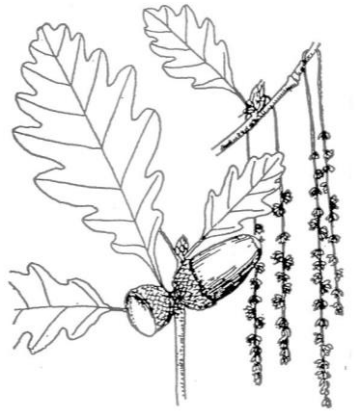
Aleppo galls are globular in shape and from 10-25 mm in diameter. They have a short, basal stalk and numerous rounded projections on the surface. Galls are hard and heavy, usually sinking in water. (White galls show a circular tunnel through which the insect has emerged. Galls without this opening have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

Cerasi stipes
Cerasus avium L. (syn. *Prunus avium*)
Cerasus vulgaris Mill. (syn. *Prunus cerasus*)

Cherry peduncle
Rosaceae

Quercus cortex
Quercus robur L.
Quercus petraea Liebl.
Ph.Hg. VIII. Ph. Eur.

Oak bark, Dryer's oak
Fagaceae



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.

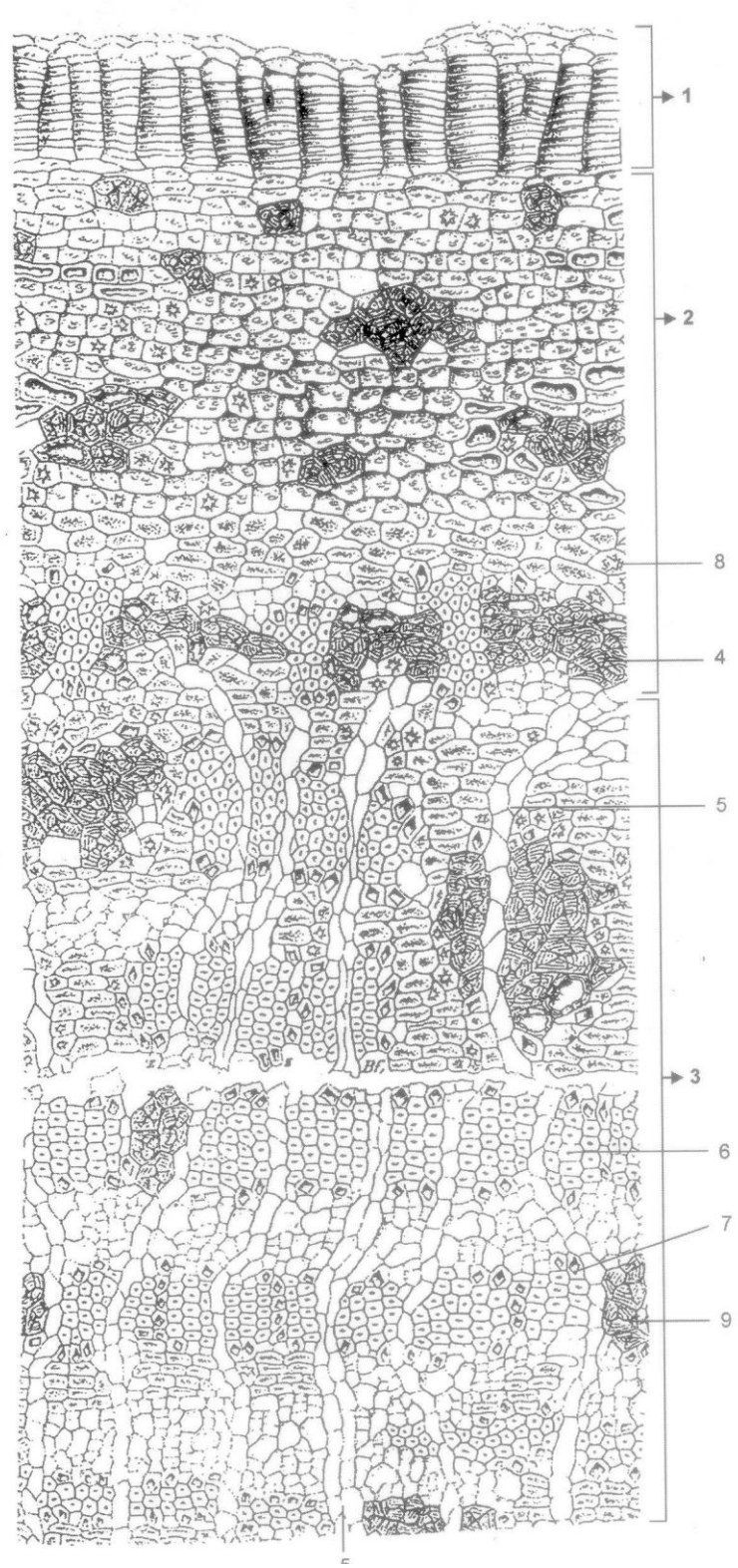
Ratanhiae radix
Krameriatrianda Ruiz et Pavon
Ph.Hg. VIII. Ph. Eur.

Ratanhia root, Rhatany
Caesalpiniaceae

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.

2. MICROSCOPICAL EVALUATION

Quercus cortex transverse section

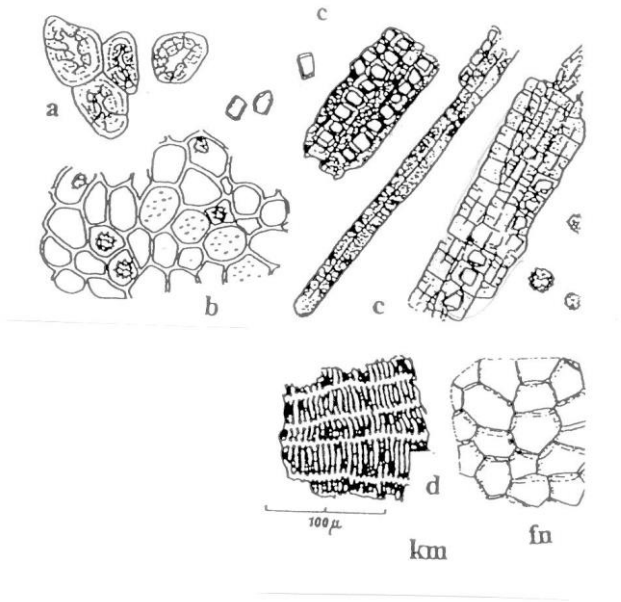


1. cork/periderm
2. cortex
3. secondary phloem
4. sclerenchyma ring
5. medullary ray
6. fibre group
7. group of fibre with calcium oxalate prism sheats
8. rosette
9. sclereid

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

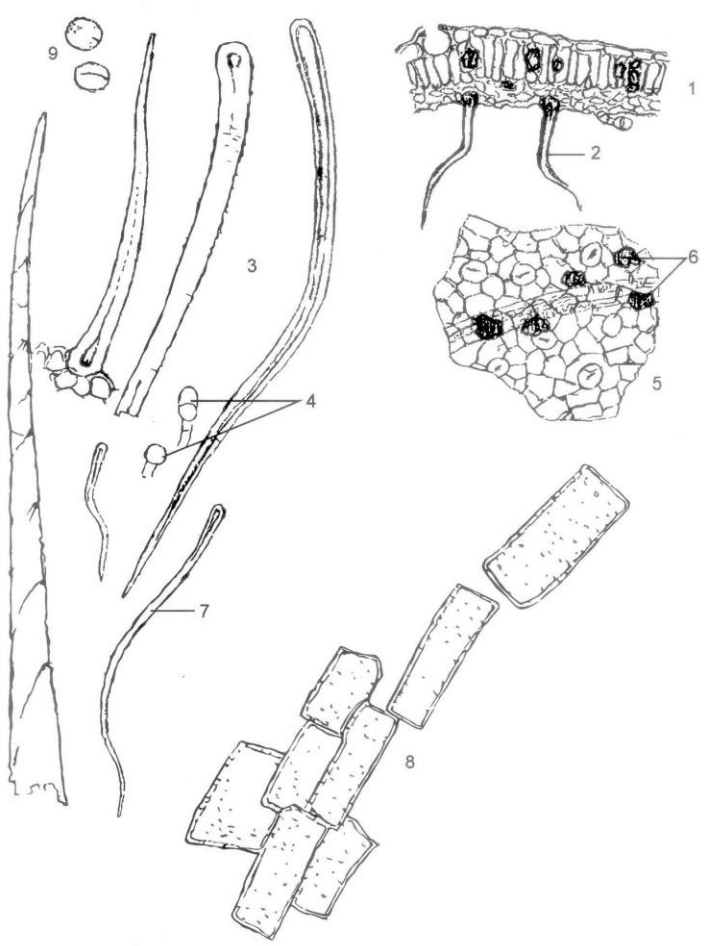
The powder shows the following diagnostic characters: group of fibres with calcium oxalate prism sheats, reddish-brown fragments of cork, sclereids, fragments of parenchyma containing calcium oxalate cluster crystals.



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. leaf 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

Quercus cortex, Cotini folium, Cerasi tipes, Agrimoniae herba, Galla

Sample preparation:
Boil 1 g of the powdered drug 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 tests described below.

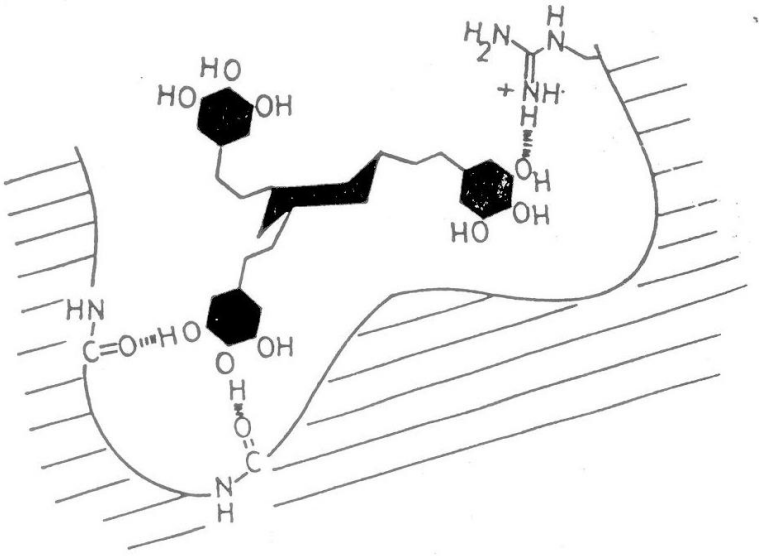
3.1. General test tube reactions

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 exaction is not stoichiometric and not specific, polyphenols, other than tannins also react. With alkaloids, tannins form addition compounds insoluble in water.



Polyphenol-protein complex

3.2. Distinctive reaction using ferric chloride solution

Quercus cortex, Cotini folium

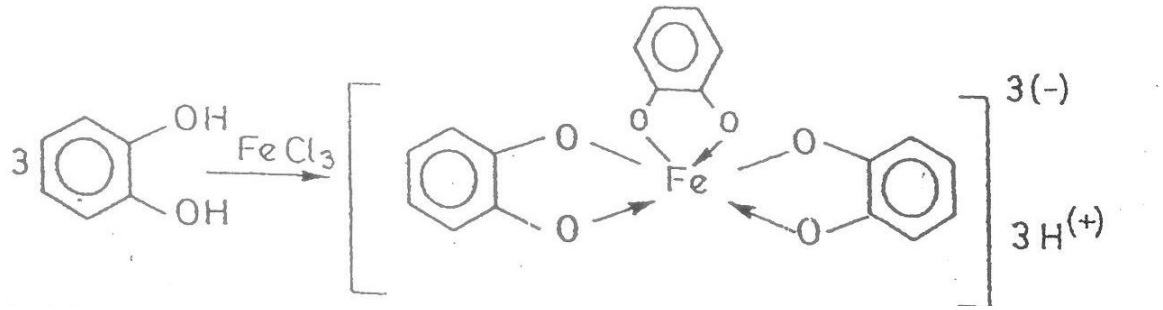
To 5 ml of the filtrate add 1 to 2 drops of R-iron (III) chloride solution. The liquid turns bluish-black and the supernatant liquid above the slowly depositing **bluish-black precipitate** becomes dirty green.

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 becomes colourless.

Explanation:

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



*The colour of the complex depends on the number and the position of the phenolic groups. Gallotannins and ellagitannins (**hydrolyzable** or **pyrogallol-type tannins**) give bluish-black colour and precipitates, and **condensed** or **catechin type 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. Isolation and TLC investigation of tannin mixture from *Cotini folium*

Boil 5 g powdered drug with 50 ml of water in an Erlenmeyer flask of 100 ml 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 with 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:

Sorbent: 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 $FeCl_3$ solution

Test: 0.5 % methanolic gallic acid solution, 2 µl

Sample: ethanolic extract, 2-5 µl

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

Sample: *Rosmarini folium* 0.2 g

Melissae folium 2.00 g

Boil the powdered drugs with 10 ml of methanol of 80 % for 15 minutes on water bath using reflux condenser (!). Filter it and use the filtrate to the investigation.

TLC parameters:

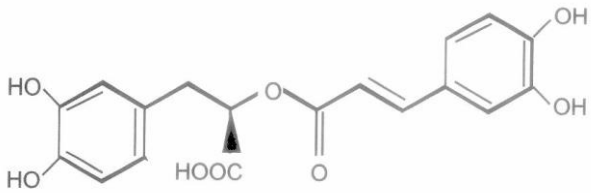
Sorbent: Kiesegel-G (Merck 0.2 mm) 100 x 60 mm

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

Dying reagent: 3% methanolic $FeCl_3$ 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

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.
To the stated amount of the powdered drug in a 250 ml Erlenmayer flask add 150 ml of *water* . Heat on a water-bath for 30 min. Cool under running water and transfer quantitatively to a 250 ml volumetric flask. Rinse the Erlenmayer flask and collect the washings in the volumetric flask, then dilute to 250.0 ml with *water*. Allow the solids to settle and filter the liquid through a filter paper. Discard the first 50 ml of the filtrate.

Total polyphenols. Dilute 5.0 ml of the filtrate to 25.0 ml with *water*. Mix 2.0 ml of this solution with 1.0 ml of *phosphomolybdotungstic reagent R* and 10.0 ml of *water* and dilute to 25.0 ml with a 290 g/l solution of *sodium carbonate R*. After 30 min measure the absorbance at 760 nm (A_1), 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 for 30 min in ultrasonic bath. Filter and dilute 5.0 ml of the filtrate to 25.0 ml with *water*. Mix 2.0 ml of this solution with 1.0 ml of *phosphomolybdotungstic reagent R* and 10.0 ml of *water* and dilute to 25.0 ml with a 290 g/l solution of *sodium carbonate R*. After 30 min measure the absorbance at 760 nm (A_2), using *water R* as the compensation liquid.

Standard. Dissolve immediately before use 50.0 mg of *pyrogallol R* in *water* and dilute to 100.0 ml with the same solvent. Dilute 5.0 ml of the solution to 100.0 ml with *water*. 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_3), using *water* as the compensation liquid.
Calculate the percentage content of tannins expressed as pyrogallol from the expression:

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

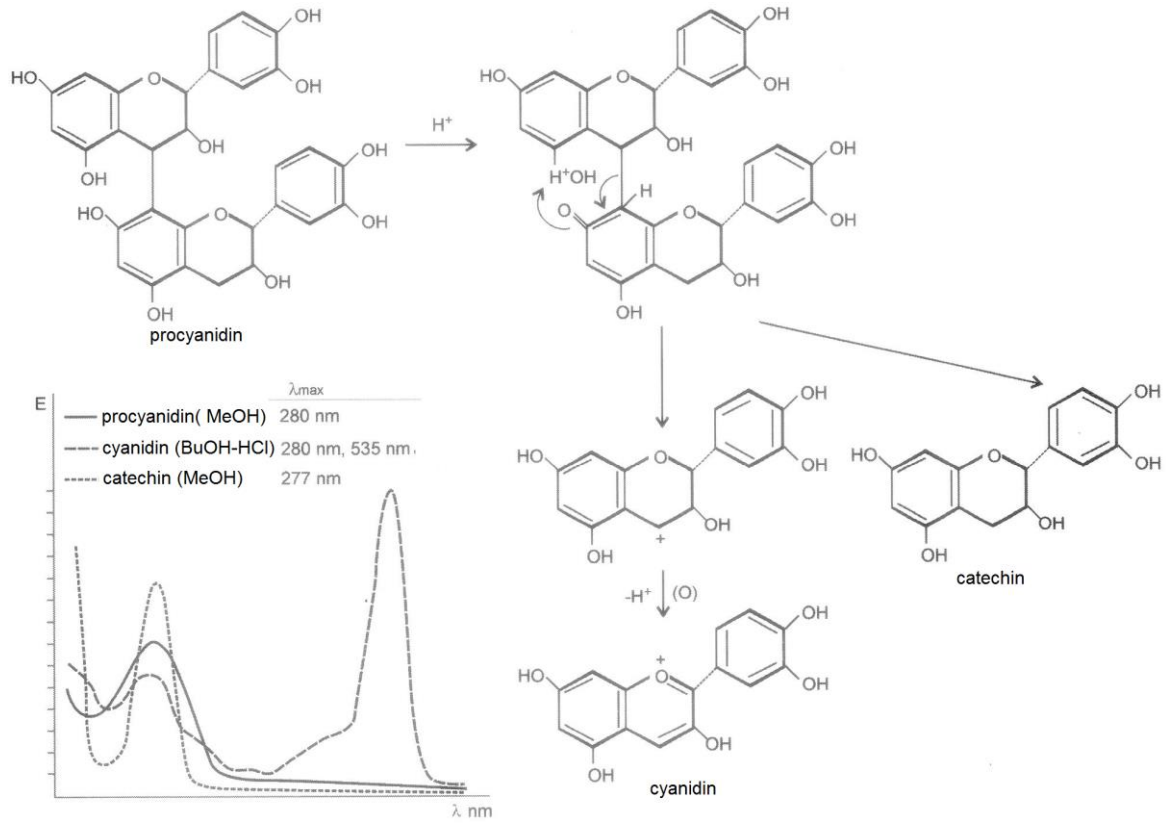
m_1 = mass of the sample to be examined, in grams,
m_2 = mass of pyrogallol, in grams.

Remark: During laboratory practice, instead of measuring the absorbance of pirogallol, 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 % $Fe(NH_4)(SO_4)$ 12 H_2O in 2N 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 $A^{1\%}_{1cm} = 75$ (cyanidine chloride).
Calculation: % = 250A/75m = A/3m, m = mass weight, A = absorbance value



Decomposition of procyanidin of B-2 type, catalyzed by acid and the UV-VIS spectra of the products arised.

Explanation: the reaction is based on the oxidative, acid-catalysed degradation of the B-type catechins (procyanidins-B), and the production of a rich colored cyaniding-chlorid afterwards.

4.3. Determination of total hydroxycinnamic acid derivatives content (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* and then add 2 ml of *dilute sodium hydroxide solution R* and dilute to 10.0 ml with *water*; mix.
Compensation solution. Dilute 1.0 ml of the stock solution to 10.0 ml with *water*.
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:

$$\frac{A \times 2.5}{m}$$

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

A = absorbance of the test solution at 505 nm,
m = mass of the substance to be examined, in grams.