CRUDE DRUGS CONTAINING ORGANIC AND INORGANIC PLANT ACIDS

1. MACROMORPHOLOGICAL EVALUATION

Equiseti herba Ph. Eur.
Common horsetail
Equisetum arvense L. (Equisetaceae)

The crude drug is formed by the sterile aerial shoots of the plant, greenish-grey, with verticillate branches. The internodes are tubular, the knots are compact. The nodes are rounded by leaves-cod forming vertical, and on the top of the cod brown teeth are seen.

Hibisci sabdariffae flos Ph. Eur.
Roselle
Hibiscus sabdariffa L. (Malvaceae)

The crude drug consists of the inner and outer sepal layer (calyx and epicalyx) of the flower. The calyx and epicalyx are fleshy, dry, easily fragmented and coloured bright-red to deep-purple, somewhat lighter at the base of the inner side.

Rosae pseudofructus Ph. Eur.
Dog rose
Rosa canina L., R. pendulina L. and R. sp. (Rosaceae*)*

It consists of fragments of the fleshy, hollow, receptacle, bearing the remains of the reduced sepals, light pink to orange-pink, with the achenes removed („sine seminibus"). The outer surface is shiny and strongly wrinkled; the lighter inner surface bears abundant bristle-like hairs

Pulmonariae folium
Lung-wort
Pulmonariae officinalis L. (Boraginaceae)

The leaves 10-15 cm long, heart-shaped, sharply pointed, white spotted, with long stalk. They are covered by bristle hairs.

Echinaceae purpureae herba Ph. Eur.
Purple coneflower herb
Echinacea purpurea L. (Asteraceae)

The stem is greenish-red, the leaves are lanceolate-elliptical shaped, serrate. Bracts are in 2-3 rows, violet ligulate and tubular florets are on the capitulum.

Hippophae fructus
common sea buckthorn fruit
Hippophae rhamnoides L. (Eleagnaceae)

The crude drug consists of the false fruit berries generated of the ovary (pericarp), 6-8 mm, oval or roundly, pale yellow to dark orange colour.

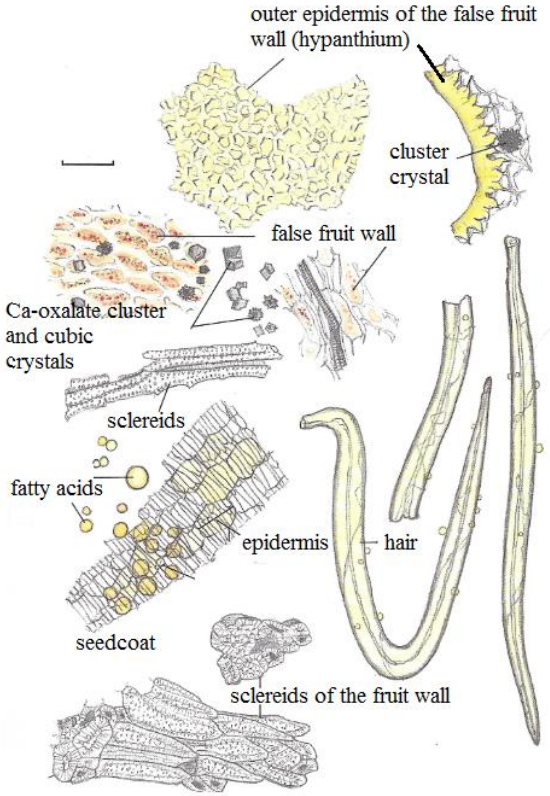
Rosmarini folium Ph. Eur.
Rosemary leaf
Rosmarinus officinalis L. (Lamiaceae)
The leaves are tough, linear o linear-lanceolate, 1-4 cm long and 2-4 mm wide, with recurved edges. The upper surface is dark-green, glabrous, the lower surface is greyish-green and densely tomentose with a prominent midrib.

Melissae folium Ph. Eur.
Melissa leaf
Melissa officinalis L. (Lamiaceae)

Melissa leaf is oval, cordate and up to about 8 cm long and 5 cm wide. The margins are roughly dentate or crenate. The upper surface is bright green and the lower surface is lighter in colour, with conspicuous, raised, reticulate venation.

2. MICROSCOPICAL INVESTIGATION

Rosae pseudofructus



The powder is **orange-yellow** and shows numerous fragments of the receptacle, the outer epidermis with orange-yellow contents and a thick cuticule, the inner epidermis composed of thin-walled cells containing **cluster crystals** and occasional **prisms of Ca-oxalate**; scattered lignified cells (sclereids), isodiametric, with thickened and pitted walls forming the trichome bases; abundant, long, **unicellular trichomes, tapering towards the end**, walls heavily thickened and with waxy cuticule; **numerous oily orange-yellow globules**.

3. QUALITATIVE AND QUANTITATIV DETERMINATION

3.1. Detection of ascorbic acid by TLC (Ph.Eur.)

Crude drug: *Rosae pseudofructus*

1.) Sample preparation:

Test solution: To 5 g of the powdered drug add 25 mL of alcohol (96%). Shake for 25 min. on ultrasonic bath and filter on filter paper. Put 40 µl of this solution on TLC layer.
Reference solution: 10 µl of standard is used (10 mg of ascorbic acid in 5.0 mL of alcohol 60 per cent V/V)

2.) TLC parameters:

Absorbent: Kieselgel 60 F254 (0.2 mm, Merck)

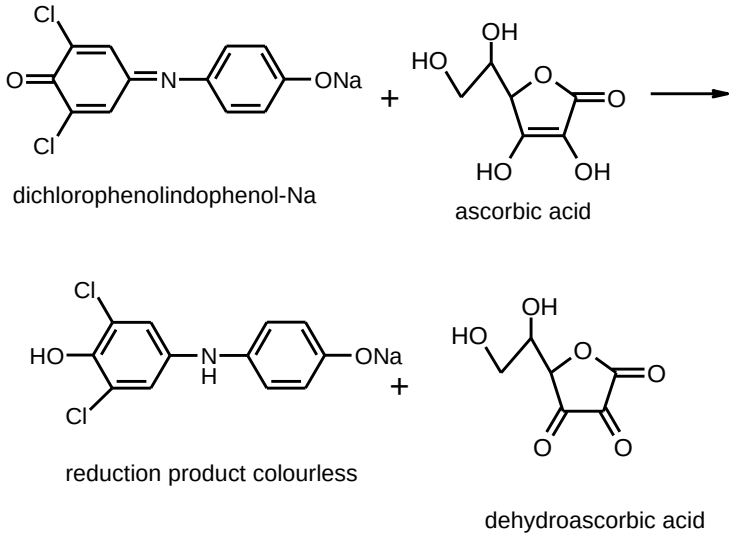
Developing system: acetic acid-acetone-methanol-toluene (5:5:50:40)

Evaluation: a) UV 254

b) Spray reagent: 2,6-dichlorophenolindophenol-Na in alcohol (0.2 g/l)

Reaction mechanism:

The ascorbic acid reduces the coloured reagent, so the pink background turns white.



3.2. Determination of ascorbic acid (vitamin-C) content with spectrophotometry (Ph.Hg.VII.)

Crude drugs: *Rosae pseudofructus, Hippophae fructus, Malpighi fructus* (acerola), *Citrus limon*

1.) Sample preparation:

2.000 g of the crude drug powder - measured with 1 mg of accuracy - is boiled on Bunsen fire with a mixture of 2.0 mL 2 M acetic acid and 60 mL water in a flask of 200 mL with a funnel in the neck. The reaction mixture is boiled for 3 minutes then cooled and filtered through filter paper into a volumetric flask of 200 mL. After repeated washing of the crude drug, the content of the volumetric flask is completed to the sign. 10.0 – 10.00 mL of the solution (extract) is used for making the **test** and the **blind** solution.

Test solution: into volumetric flask of 100 mL volume 2.00 mL of R iron /III/ ammonium-sulphuric solution, 10.0 mL of citric acid solution (of 1 % in water), 10,0 mL of ammonium-acetate solution (of 20 % in water), 0.40 mL α-α'–dipyridyl solution (of 1 % in 96 % ethanol), and 10.0 mL of the stock solution are measured. Shake the reaction mixture and keep on a dark place for 120 minutes, while the red colour is fully developed.

Reference solution: prepared like the test-solution with the difference of using water instead of dipyridyl solution.

2.) Spectrophotometry:

After 120 min. volume of test and reference solution are completed to 100.00 mL with water and absorbance of the test solution is measured in thickness of 1 cm, at 525 nm, against the reference solution.

3.) Determination of the ascorbic acid % in the crude drug

a) Calibration linear:

Make the dilutions of the ascorbic acid of Ph.Eur. quality:

1.5 mg ascorbic acid / 100 mL H_2O saturated with CO_2
2.5 mg ascorbic acid / 100 mL H_2O saturated with CO_2
3.5 mg ascorbic acid / 100 mL H_2O saturated with CO_2
5.0 mg ascorbic acid / 100 mL H_2O saturated with CO_2

Regress a linear based on concentration and absorbance data.

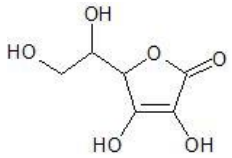
b) Determination of ascorbic in the crude drug:
Read the concentration of ascorbic acid of the investigated solution related to the absorbance measured.

$$\text{ascorbic acid m/m \%} = \frac{2.86 \times A}{\text{mass}_{\text{crude drug}}(g)}$$

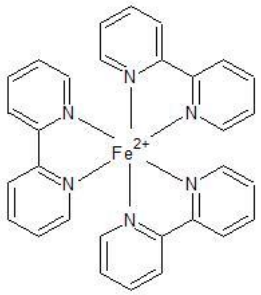
Reaction:

Ascorbic acid reduce Fe(III) to Fe(II), which gives intensive red colour complex with α-α'-dipyridyl:

α-α'-dipyridyl



ascorbic acid



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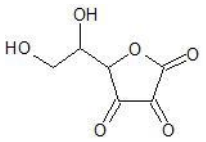

Fe^{2+}-dipyridyl complex (red)

dehydroascorbic acid

Drug	ascorbic acid content (%)
Hippophae fructus	
Rosae pseudo-fructus	
Malpighi fructus	
Citrus limon	

3.3. Determination of total hydroxycinnamic acid derivative content expressed in rosmarinic acid (Ph.Eur.)

Crude drugs: *Melissae folium, Rosmarini folium*

1.) Sample preparation:

Stock solution: add 40 mL alcohol (50% V/V methanol) to 0.100 g powdered crude drug and heat on water bath with reflux cooler for 30 min. After it has cooled down, filter, and wash the flask and the cotton with 5 mL methanol. Put the filtrate in a 50.0 mL volumetric flask and fill up to sign.

To make the **test** and the **blind solution**, 1.0-1.0 mL of the stock solution is used.

Test solution: Put 1.0 mL of the stock solution + 2 mL 0.5 M HCl + 2 mL solution made by solving 10 g $NaNO_2$ and 10 g Na-molybdate in 100 mL water + 2 mL diluted (10% m/V) NaOH solution in a 10.0 mL volumetric flask and fill up to sign with water, homogenize.
Blind solution: 1.0 mL stock solution id diluted in 10.0 mL volumetric flask to sign with water.

2.) Spectrophotometry:

Measure the absorbance of the test solution as soon as possible on 505 nm against the blind solution.

3.) Determination of hydroxycinnamic acid content of the crude drug expressed in rosmarinic acid, m/m%:

$$\%=\frac{1.25*A}{m}$$

A = absorbance on 505 nm
m = mass of the crude drug, g

3.4. Differentiation of *Echinacea* species with TLC

Crude drugs: *Echinaceae angustifoliae radix, E. pallidae radix, E. purpureae radix*

1.) Sample preparation:

1 g powdered crude drug is extracted with 30 mL methanol in ultrasonic bath for 10 min. The extract is filtered on filer-paper into a round flask and evaporated to 5 mL with Rotadest.
10 µl of the solution is put on the TLC layer beside 10 µl reference solution.
Reference solution: echinacoside, chlorogenic acid, cynarine in methanol.

2.) Chromatographic conditions:

Layer: Kieselgel 60 F_{254} (0,2 mm, Merck)
Developing system: toluene : ethyl-acetate : water: formic acid (5:95:10:10)

3.) Evaluation:
Spray reagent: Naturstoff-polyethylene-glycol reagent, detection of spots on UV 365 nm

Identify the unknown *Echinacea* sample according to the components detected in the extract.

	E. angustifolia	***E. pallida***	***E.purpurea***
cynarine	+	-	-
chlorogenic acid	+	+	+
echinacoside	+	+	-

Results in the report:

1. Ascorbic acid TLC results.
    - TLC draws/photo, R_f value
2. Ascorbic acid quantitative determination result
    - Ascorbic acid content (%) of different crude drugs (table)
    - Meet or not with the requirements of Ph. Eur. (Rosae pseudofructus)?
3. Results of total hydroxycinnamic acid derivative content
    - Meet or not with the requirements of Ph. Eur.?
4. Unknown Echinaceae radix TLC results
    - TLC (draw/photo)
    - Result of the identification
5. Microscopic drawings