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Rhei radix
Sennae folium et fructus
Aloe

Hyperici herba

2. MICROSCOPIC CHARACTERS

Cross section: Frangulae cortex
Sennae folium

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Sennae folium
Rhei radix

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(Frangulae cortex, Rhei radix)

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(Frangulae cortex, Rhei radix)
1. MACROSCOPIC CHARACTERS

**Frangulae cortex**

*Frangula alnus* Mill.

(syn.: *Rhamnus frangula* L.)

Rhamnaceae

Ph. Eur., Ph.Hg.VIII.

The drug consists of the dried bark of the stems and branches. It comprises quils, double quils, or flats of varying lengths and not more than 2 mm thick. The cut drug consists of flat or slightly inwardly curved pieces. The outer surface is brownish red to grayish brown, shiny to matt, smooth to finely fissured, and has numerous horizontally elongated, whitish lenticels. On careful scraping, a red-coloured tissue is exposed. The inner surface is orange-yellow to brownish and distigthy striated longitudinally. The fracture is irregular, granular on the outside and short and finely fibrous on the inside.

**Odour:** characteristic. **Taste:** mucilaginous and sweetish, somewhat bitter and astringent.
**Rhei radix**  
*Rheum palmatum* L.  
*Rheum officinale* Baillon

Ph. Eur., Ph.Hg.VIII.

The drug consists of the peeled underground organs (turnip-shaped roots with very small rhizomes). The ochre-yellow to brownish pieces are often covered on the outside with powder and they exhibit orange striations or an orange-red mottling. The fracture is granular and crumbly (not fibrous) and reddish brown.

Odour: characteristic and faintly smoky.
Taste: somewhat bitter and harsh.
**Sennae folium**
*Cassia angustifolia* Vahl (Tinnevelly senna) and *Cassia senna* L. (syn.: *Cassia acutifolia* Delile) (Alexandrian or Khartoum senna)

Ph.Eur., Ph.Hg.VIII.

The entire, lanceolate to narrowly lanceolate, pinnate leaflets have a short petiolule and are 2-6 cm long and 7-12 mm wide. The leaflets have an asymmetric base and a thin, stiff and brittle, light green lamina which appears as if glabrous. The leaflets are often marked with transverse or oblique lines.

Odour: faint, characteristic. Taste: to begin with sweetish, then bitter.

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**Sennae fructus**
*Cassia angustifolia* Vahl and *Cassia senna* L.
(syn.: *Cassia acutifolia* Delile)

Ph.Eur., Ph.Hg.VIII.

The flat, compressed, brownish green or grayish green, membranaceous-leathery pods are up to 5 cm long and ca. 20-25 mm (*Cassia senna*) or 15-18 mm (*Cassia angustifolia*) wide and slightly reniform. The two halves of the fruit, which adhere to each other over the whole surface, are difficult to separate. The fruit normally contain 5-7 (*C. senna*) or 7-10 seeds (*C. angustiflora*), which are more or less cordate, whitish to grayish green, and very hard, with a dimpled, reticulately ridged surface.

Odour: faint, characteristic. Taste: mucilaginous and sweetish, then somewhat bitter and harsh.
Aloe
*Aloe barbadensis* Mill.
Aloe species, mainly *A. ferox* Miller.
Ph.Eur., Ph.Hg.VIII.

The drug consists of the juice from the secretory cells of the leaves of the aloe plant which has been concentrated and allowed to solidify.

It is a dark brown, slightly shiny, opaque mass with a waxy conchoidal fracture. The powder is brown and soluble in warm ethanol, partly soluble in boiling water, and practically insoluble in ether and chloroform.


Hyperici herba
*Hypericum perforatum* L.
Hypericaceae (Guttiferae)
Ph.Eur., Ph.Hg.VIII.

The drug consists of the dried flowering tops. Particularly noteworthy are the yellow to yellowish brown flowers, which under certain circumstances are still present in cymes and whose petals are covered with numerous dark spots or streaks; the sepals are lanceolate, sharply pointed, and at the time of flowering twice as long as the ovary. The ca. 50-60 stamens of each flower are usually fused into three groups. The often shrunken and folded oval green to brownish green, glabrous ovate-elliptic leaves are up to 3.5 cm long, with an entire margin and clearly visible translucent dots. The yellowish green, round pieces of stem are hollow and often have two longitudinal ridges opposite each other.
2. MICROSCOPIC CHARACTERS

Frangulae cortex
(Cross section and powdered preparation)

The cortex contains cluster crystals of calcium oxalate, elongated mucilage cavities, but no sclereid. The secondary phloem contains numerous tangentially elongated groups of thick-walled phloem fibres. The medullary rays are mostly two cells wide.

Cross section (Frangulae cortex)

I. outer cortex
II. inner cortex

1. periderm
2. phloem fibre
3. phloem fibre with crystal sheaths
4. medullary ray
5. Ca(COO)₂ cluster crystal

Powdered preparation (Frangulae cortex)

1. medullary ray with cluster crystals
2. parenchyma
3. Ca(COO)₂ cluster and prism crystal
4. periderm
5. phloem fibres in groups with crystal sheaths containing calcium oxalate
**Sennae folium**  
*Cross section and powdered preparation*

The diagnostic features include the unicellular, thick-walled, curved trichomes with a warty cuticle, the isobilateral leaf structure, epidermal cells containing mucilage, vascular bundles incompletely surrounded by a sheath of fibres accompanied by roes of cells with calcium oxalate prisms, and occasional calcium oxalate clusters.

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Cross section (*Sennae folium*)

1. palisade parenchyma  
2. spongy mesophyll  
3. paracytic stomata  
4. vascular bundle  
5. sheath of fibres  
6. fibre  
7. collenchym  
8. calcium oxalate cluster crystal  
9. mucilage cell  
10. unicellular trichome

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**Powdered preparation (*Frangulae cortex*)**

1. mesophyll  
2. upper epidermis with trichome  
3. upper epidermis with stomata  
4. Ca(COO)₂ crystal (cluster)  
5. thick-walled trichome  
6. mucilage cell  
7. crystal sheath  
8. epidermis cell with stomata
**Rhei radix** pulvis (Powdered preparation)

Microscopical examination reveals the large cluster crystals of calcium oxalate and the highly characteristic unlignified reticulately thickened vessels.

![Powdered preparation (Rhei radix)]

1. Ca(COO)\(_2\) cluster crystal
2. starch granules
3. vessel segment
4. parenchyma

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**3. PHYSICO-CHEMICAL AND CHEMICAL STUDIES**

**3.1 Identification of anthraquinone derivatives by simple reactions**

*Crude drug: Frangulae cortex*

With a drop of 6N ammonia solution or R-NaOH, the inner surface takes on a red colour (Bornträger reaction).

**3.2 Identification of emodines**

**3.2.1 Identification of frangula and rheum emodins by microsublimation**

*Crude drugs: Frangulae cortex, Rhei radix*

On microsublimation at 140-160 °C, a yellow crystalline sublimate is obtained, which on addition of potassium hydroxide solution, dissolves to give a red colour.

**3.2.2 Bornträger reaction**

*Crude drugs: Frangulae cortex, Rhei radix, Sennae folium, Sennae fructus*

Extract 0.5 g of powdered drug with 5 ml ethyl-acetate for 5 min by shaking.

After filtration add 5 ml of R-ammonia solution and mix it. The water-phase will turn bright red.

![Anthraquinone derivatives](image)

*The free anthraquinone derivatives (aglycones = emodins = 1,8-dihydroxy-anthraquinone derivatives) are soluble in CHCl\(_3\) and ethyl-acetate. After re-extraction with alkali solution they turn to red due to the mesomeric structures.*
3.2.3 Isolation of frangula-ewoodin, purification, UV-VIS spectra. Preparative layer chromatography.

Heat 1.0 g of powdered drug with 10 ml of R-hydrochloric acid, in a 100 ml flask equipped with a reflux condenser for 20 min on hot water bath. Add through the condenser 50 ml of ethyl-acetate and boil for 30 min. Filter the mixture through a cotton plug into a 250 ml separating funnel, wash the flask and the cotton with 3 x 10 ml of ethyl-acetate. Separate the ethyl-acetate layer, dry on Na₂SO₄ sicc. and evaporate the solvent. Solve the residue in 1.0 ml methanol for layer chromatography (12 cm bands on silica gel G). Solvent system: chloroform-ethylacetate (93:7) Reference solution: frangula emodin.

After evaporation of the solvent remove the orange yellow band corresponding to the reference frangula emodin (Rf ≈0.4) and elute with 20 mL of chloroform-methanol (2:1) solvent by shaking. After filtration evaporate the solvent under reduced pressure and dissolve the residue in 1-2 ml methanol. Prove the identity by spectroscopy (400-800 mm).

Spectral characteristics:

\( \lambda_{\text{max}}: 224, 253, 268, 290, 442 \text{ nm} \)

\( \lambda_{\text{max}}: 250 \text{ nm} \)
Isolation of frangula- emodin by preparative layer chromatography

**Frangulae cortex (TLC)**

*Coating substance:*  
Silica gel 60 F$_{254}$

*Solvent system:*  
chloroform: ethylacetate (93:7)

*Detection:*  
5 % ethanolic KOH reagent (VIS)

*Reference solution:*  
1 = frangula emodin

*Test solution:*  
2 = Isolated frangula emodin  
3 = Hydrolysed frangulae cortex extract, 10μl  
4 = Frangulae cortex extract (MeOH), 20μl
3.3 Identification of hypericin (dianthrone) in *Hyperici herba*.

0.2 g powered drug takes on a green colour with 1 ml of 2% potassium hydroxide.

![Protohypericin and Hypericin](image)

4. QUANTITATIVE DETERMINATIONS (Assay)

4.1 Determination of anthraglycosides (Ph.Eur., Ph.Hg.VIII.)

*(Sennae folium, Sennae fructus)*

*Carry out the assay protected from bright light.*

Place 0.30 g of the powdered drug into a 100 ml flask and add 30.0 ml of water. Place into a water-bath, and heat under a reflux condenser for 15 min (1). Allow to cool, put into a measuring cylinder of 50 ml and complete to the original volume with *water*. Let is sediment and transfer 20.0 ml of the supernatant liquid to a 150 ml separating funnel. Add 0.1 ml of *diluted hydrochloric acid* R and shake with two quantities, each of 15 ml of *chloroform* (2). Allow to separate and discard the chloroform layer. Add 0.10 g of *sodium hydrogen carbonate* and shake for 3 min. Let it sediment and transfer 10.0 ml of the supernatant liquid to a 100 ml round-bottomed flask. Add 20 ml of 0.4 M *ferric chloride solution* and mix it. Heat for 20 min under a reflux condenser in water-bath; add 1 ml of hydrochloric acid and heat for a further 20 min, with frequent shaking, to dissolve the precipitate (3). Cool, transfer the mixture to a separating funnel and shake with three quantities, each of 25 ml of *ethyl-acetate* previously used to rinse the flask (4). Combine the *ethyl-acetate* layers and wash with two quantities, each of 15 ml, of *water*. Transfer the *ethyl-acetate* layers to a volumetric flask and dilute to 100.0 ml with *ethyl-acetate*. Evaporate 10.0 ml carefully to dryness and dissolve the residue in 10.0 ml of a 5 g/l of methanolic magnesium acetate solution. Measure the absorbance at 515 nm using *methanol* as the compensation liquid (5).
Calculate the percentage content of sennoside B from the expression:

\[
\% = \frac{A \times 1.25}{m}
\]

i.e. taking the specific absorbance to be \(A_{1\%}^{1\ cm} = 240\).

\(A\) = absorbance at 515 nm,
\(m\) = mass of the substance to be examined, in grams.

**STORAGE**
Store protected from light and moisture.

1. *Sennosides are water soluble glycosides (-COOH group!)*
2. To remove the free aglycones and the semipolaric substances.
3. *Sennosides are dianthronglycosides. Due to the –C-C- bond oxidative hydrolyses is needed.*
4. *The free aglycones are soluble in chloroform and ethyl-acetate. Note: in Ph.Eur. ether is used for the extraction.*
5. *Mg-complex is formed.*

### 4.2 Determination of anthraglycosides and anthraquinone derivatives

*(Frangulae cortex, Rhei radix)*

Introduce 0.05 g of powdered drug, in a 100 ml flask with stopper and add 6 ml of R-concentrated acetic acid. Heat it on a hot water bath equipped with a reflux condenser, for 15 minutes (1). Add through the condenser 30 ml of ethyl-acetate to the cooled liquid and boil for 15 minutes (2). Filter the mixture through a cotton plug into a 250 ml separatory funnel, wash the flask and the cotton with 2 x 5 ml of ethyl-acetate. Return the cotton into the flask and boil again with a mixture of 2 ml of concentrated R-acetic acid and 30 ml of ethyl-acetate for further 10 minutes. Filter the ethyl-acetate extract through a new cotton plug into the separatory funnel used before. Wash the flask and the cotton again with 2 x 5 ml of ethyl-acetate. Cool it! Add 15 ml of 30 per cent sodium hydroxide solution and 25 ml of ammoniac sodium hydroxide solution (82 volumes of 2M sodium hydroxide solution + 18 volumes of 25 % ammonia) to the combined ethyl-acetate extract (3). Shake the separatory funnel while cooling under the water tap. Transfer the red-coloured alkaline layer after 5 minutes into a 100 ml volumetric flask. Extract the ethyl-acetate fraction with 2 x 20 ml of ammoniac sodium hydroxide solution, allow to stand for 5 minutes and transfer the alkaline layer into the volumetric flask. Heat the volumetric flask for 30 minutes on hot water bath, and fill up to 100 ml with 25 per cent ammonia after cooling.
Measure the absorbance of the solution at 530 nm in a 1 cm cell, against water as the blank. Calculate the total content of anthraquinone and anthranol derivatives.

Explanation:
1. The determination is performed in aglycon form.
2. Emodins can be dissolved in CHCl₃ (extraction)
3. Bornträger reaction.

Calculation:
Calibration curve of 1,8-dihydroxy-anthraquinone is made: absorbance (y) vs. concentration (x, μg/ml); equation: \( x = \frac{A}{0.0472} \)

\[
\begin{align*}
\text{% (total anthraquinone and anthranol derivatives)} & = \frac{0.21 \times A}{m} \\
\end{align*}
\]

\( m = \text{mass (g) of drug (~0.05)} \)
\( A = \text{absorbance} \)

4.3 Calculation of anthraglycosid-doses based on the results of assay

Anthraglycoside-containing drugs are stimulant laxatives; as they act directly on the intestinal mucosa by influencing several pharmacological targets.

The long-term use or extreme doses of anthranoids may result a (reversible) pigmentation of the colon (Pseudomelanosis coli), which is due to the incorporation of metabolites of the anthranoids. It is thought to be associated with an increased risk of colon carcinoma.

The recommended doses of hydroxyanthracene derivatives are:

- 30 mg / die (laxatives)
- 15 mg / die (slimming cure)

for maximum of 2 weeks. After 2-3 months the cure can be repeated.

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Results presented in the report:

1. Results of the identification tests and TLC
2. Isolated frangula-edomin (VIS spectra)
3. Results of the assay (Sennae folium/fructus and Frangulae cortex of Rhei radix)
4. Calculation of doses based on the results of assay.