CRUDE DRUGS CONTAINING FENOLGLYCOSIDES, COUMARINES AND FLAVONOIDS

1. MACROSCOPICAL TESTS

Uvae ursi folium
Juglandis folium
Meliloti herba
Aurantii amari flos
Betulae folium
Crataegi folium cum flore
Fagopyri herba
Ginkgo folium
Hyperici herba
Myrtilli fructus recens
Myrtilli fructus siccus
Ononidis radix
Rutae herba
Sambuci flos
Solidaginis herba
Silybi mariani fructus
Tiliae flos
Verbasci flos

2. MICROSCOPICAL TESTS

Cross section: *Uvae ursi folium*
Tiliae flos
Tiliae argenteae flos
Ginkgo folium

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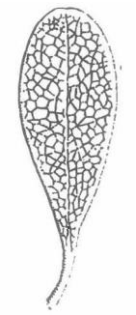
1. MACROSCOPICAL TESTS

Uvae ursi folium
Arctostaphylos uva ursi (L.) Spreng
Ph. Hg. VIII., Ph. Eur.

Bearberry leaves
Ericaceae



The leaves are alternate, entire, 1.2 - 2.2 cm long, obovate to obovate-elliptic from a wedge-shaped base, dark green on the upper surface, pale green and distinctly nerved on the lower surface. Taste, astringent, odour, slight.

Juglandis folium
Juglans regia L.

Walnut leaves
Juglandaceae

Leaves composed of seven to nine leaflets of varying size, averaging 5-10 cm in length and 3-4 cm wide, greenish, parchment-like, turning brown with keeping. Taste bitter and astringent; odor of leaves characteristic and aromatic.

Aurantii amari flos
Citrus aurantium L. ssp. *aurantium*
(*C. aurantium* L. ssp. *amara* Engl.)
Ph. Hg. VIII., Ph. Eur.

Bitter-orange flower
Rutaceae

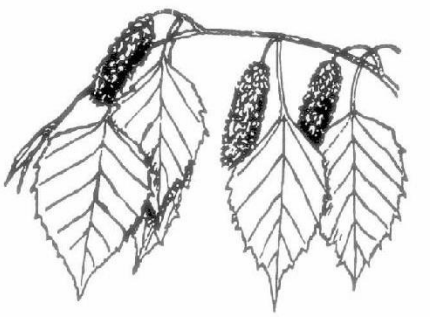
The flower buds are white or yellowish-white and may reach up to 25 mm in length. The dialypetalous corolla is composed of 5 thick, oblong and concave petals dotted with oil glands visible under a hand lens; the short, yellowish-green persistent gamosepalous calyx has 5 spreading sepals, connate at the base and forming a star-shaped structure attached to the yellowish-green peduncle which is about 5 mm to 10 mm long. The flower buds contain at least 20 stamens with yellow anthers and with filaments fused at the base into groups of 4 or 5; the ovary is superior, brownish-black and spherical, consists of 8 to 10 multi-ovular loculi and is surrounded at the base by an annular granular hypogynous disc; the thick, cylindrical style ends in a capitate stigma.

Meliloti herba
Melilotus officinalis L.
Ph. Hg. VIII., Ph. Eur.

Common melilot herb
Fabaceae

A trailing to erect herb with branched stems, 40-90 cm (up to 120) tall. The leaves are trefoil with stipules joined to the stem. The leaflets are ablong-elliptic, 1.5-3 cm long, the margius toothed with weins terminating in the teeth. The flowers are arranged in lax racemes, up to 6 mm long, yellowish, biluterally symmetrical; the standard is as long as the wings and both are longer than the keel. The fruit is a pod, up to 6 mm long, glabrous, wrinkled, ovoid and somewhat compressed, at first green, later brown. Odour, like new - mown hay due to the cumarin.

Betulae folium
Betula pendula Roth
Betula pubescens Ehrh.
Ph. Hg. VIII., Ph. Eur.



Silver birch
Betulaceae

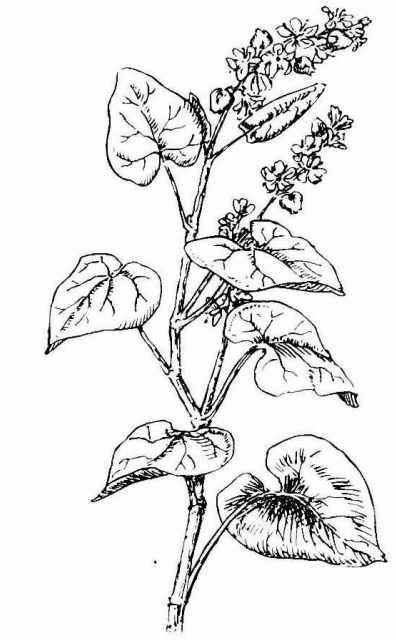
The leaves are alternate, simple, 2-5.5 cm long, ovate to triangular from a broadly wedge-shaped or truncate base, acuminate, glabrous; the margins double-toothed.
Taste astringent and bitter.

Crataegi folium cum flore
Crataegus monogyna Jacq.
Crataegus laevigata (Poir) DC.
(syn. Cr.oxyacantha L.)
Ph. Hg. VIII., Ph. Eur.

Hawthorn
Rosaceae (Maloideae)

The leaves on short shoots simple, 1.5-5.5 cmlong, obovate, broader than long, 3-5 lobed, the lobes short, broad, obtuse, toothed; the leaves on long shoots more deephy lobed and with leaf-like stipules. (Leaves of *Crataegus monogyna* on short sboots are longer than broad, the lobes are triangular, from halfway between margin to midrib.)
The flowers are regular, up to 1.8 cm in diameter, arranged in 5-10 flowered corymbs. The sepals are up to 8 mm long, white.
The anthers are pink or purplish. The styles usually number 2. (In C., monogyna there is usually 1 style).

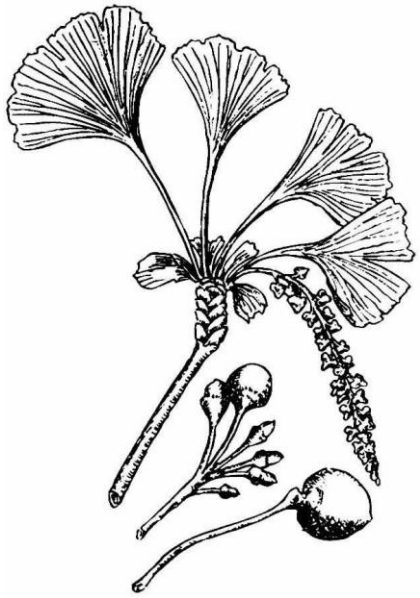
Fagopyri herba
Fagopyrum esculentum Moench.
Ph. Hg. VIII., Ph. Eur.



Buckwheat herb
Polygonaceae

The stem is cylindrical, hollow, finely ridged longitudinally, about 2-6 mm in diameter, brownish-green or reddish, with few branches and thickened at the internodes; the leaves are arranged spirally and have membranous, sheathing stipules; the surface is glabrous except in the region of the stipules, where short, white hairs may occur. The leaves are dark green, paler on the lower surface, up to 7 cm wide and 11 cm long, saggitate or cordate, almost pentagonal with 2 widely rounded lobes; the lower leaves are petiolate, the upper leaves sessile or amplexicaul; the lamina is glabrous and the margin finely sinuate and fringed with minute, reddish-brown projections; similar projections occur on the veins on the upper surface. The inflorescence is a cymose panicle, the individual flowers 1-2 mm long and 6 mm in diameter with 5 free, white or reddish petals.

Ginkgo folium
Ginkgo biloba L.
Ph. Hg. VIII., Ph. Eur.

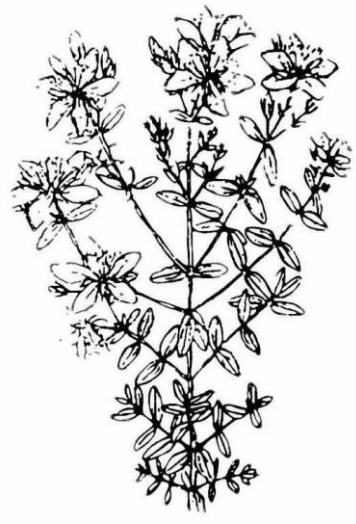


Maidenhair-tree leaves
Ginkgoaceae

Leaves petiolated, glabrous, bilobated, each lobe triangular, up to about 6 cm long and 4 cm wide, with fan-like, fine, prominent, radiate veins and an entire margin.

Hyperici herba
Hypericum perforatum L.
Ph. Hg. VIII., Ph. Eur.

St. John's wort
Hypericaceae



Leaves opposite, sessile, oval to linear, with translucent oil glands on the surface and black dots on the lower surface in some cases. Flowers bright yellow, with numerous stamens, five petala often black dotted along the margins. The stem has two raised lines along the stem. Taste bitter and astringent; odour, aromatic, distinctive

Myrtilli fructus recens
Vaccinium myrtillus L.
Ph. Hg. VIII., Ph. Eur.

Bilberry fruit, fresh
Ericaceae

The fresh fruit is a blackish-blue globular berry about 5 mm in diameter. Its lower end shows a scar or, rarely, a fragment of the pedicel. The upper end is flattened and surmounted by the remains of the persistent style and of the calyx, which appears as a circular fold. The violet, fleshy mesocarp includes 4 to 5 locules containing numerous small, brown, ovoid seeds.

Myrtilly fructus siccus
Vaccinium myrtillus L.
Ph. Hg. VIII., Ph. Eur.

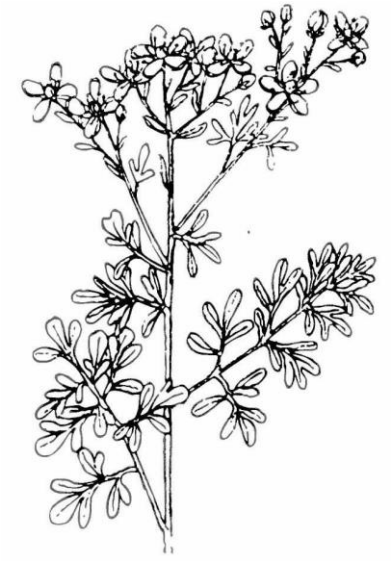
Bilberry fruit, dried
Ericaceae

Dried bilberry is a dark blue, subglobular, shrunken berry about 5 mm in diameter, with a scar at the lower end and surmounted by the persistent calyx, which appears as a circular fold and the remains of the style. The deep violet, fleshy mesocarp contains numerous small, brown, ovoid seeds.

Ononidis radix Rest-harrow root
Ononis spinosa L. Fabaceae
Ph. Hg. VIII., Ph. Eur.

The root is more or less flattenes, twisted and branched, deeply wrinkled and brown in colour. Externic. Taste, sweet and mucilaginous at first, then rather bitter; odour, resembling that of liquorice.

Rutae herba Rue herb
Ruta graveolens L. Rutaceae



The leaves are alternate, up to 15 cm long, 2-3 pinnatisect, glanddotted, the lower ones petiolate, the upper ones usually sessile; the terminal segment is lanceolate to obovate, up to 9 mm wide. The flowers are glandular, arranged in a lax cyme with leaf-like bracts. The sepals number 4, lanceolate, acute. The petals number 4, yellow, oblong-ovate, toothed wary. The stamens number 8. The fruit is a 4-5 lobed capsule, splitting, glabrous.

Sambuci flos Elder flowers
Sambucus nigra L. Caprifoliaceae
Ph. Hg. VIII., Ph. Eur.

The flowers are regular, erranged in large, flat - topped, umber - like inflorescences (up to 21 cm in diameter). The calyx has a very narrow limb. The corolla is 4-5.5 mm in diameter, creamwhite, vith a short tube and a spreading 5-lobed limb. The anthers cream-coloured.
Taste, sweetish.

Silybi mariani fructus
Silibum marianum Gaertn.
Ph. Hg. VIII., Ph. Eur.

Milk thistle fruit
Asteraceae

The drug consists of the obliquely obovoid fruit (achenes) which are 6-7 mm long, up to 3 mm broad, and 1.5 mm thick. The testa is shing brownish black or matt greyish brown, with dark or greyish dots. At the tip, there is a yellowish projecting cartilaginous, swollen ring and at the bottom at the side a canaliculate hilum. Taste oily and bitter, odour scarcely perceptible.

Solidaginis herba
Solidago gigantea Ait
S. canadensis L.,
Their varieties or hybrids and/or mixtures of these.
Ph. Hg. VIII., Ph. Eur

Goldenrod
Asteraceae

The stems are greenish-yellow or greenish-brown, partly tinted reddish, roundish, more or less conspicuously grooved, glabrous and smooth in the lower part, slightly or densely pubescent in the upper part. They are solid with a whitish pith.
The leaves are green, sessile, lanceolate, with a serrate margin, 8-12 cm long and about 1-3 cm wide, the upper surface is green and more or less glabrous, the lower surface is greyish-green and pubescent, especially on the veins. The inflorescence consists of a number of unilateral, curved racemes which together form a pyramidal panicle at the end of the stems.
Each capitulum has an involucre composed of linear-lanceolate, imbricated yellowish-green bracts, surrounding a single row of yellow ligulate florets about the same length as the involucre; yellow, radially arranged tubular florets, as long as, or longer, than the ligulate florets; a brownish inferior ovary surmounted by a white pappus of silky hairs.

Tiliae flos
Tilia cordata Mill.
Tilia platyhpyllos Scop.
Ph. Hg. VIII., Ph. Eur

Lime/linden flowers
Tiliaceae

The flowers are arranged in usually 3 flowered erect or sprending cymes, fragrant, yellowish-white; over half of the length of the pedicel is joined to a large membranous bracteole. The sepals number 5, free.
The petais number 5, free.
The stamens are numerous, exceeding the petals.

Adulteration:

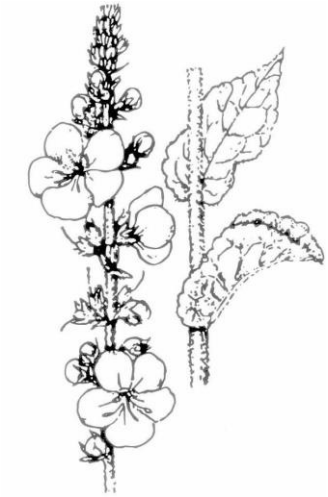
Tiliae argenteae flos
Tilia tomentosa Mönch
(syn.:*T. argentea* Desf.)

Tiliaceae

Verbasci flos
Verbascum phlomoides L.
Verbascum densiflorum Best
Ph. Hg. VIII., Ph. Eur.

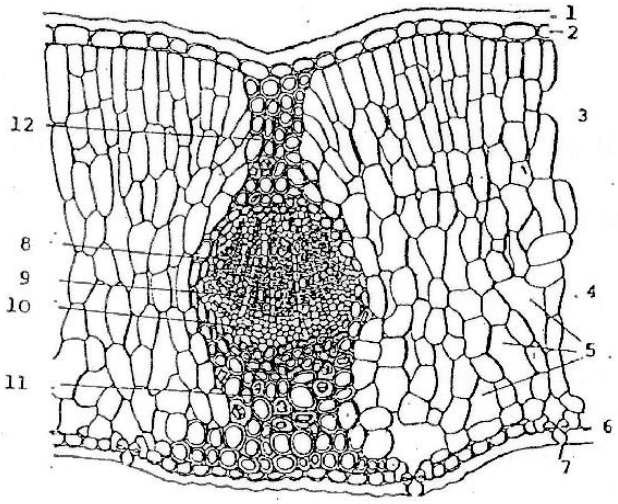
Mullein flower
Scrophulariaceae

Flowers yellow, almost flat, 15-30 mm in diameter. Taste mucilaginous and slightly bitter, odor faint.

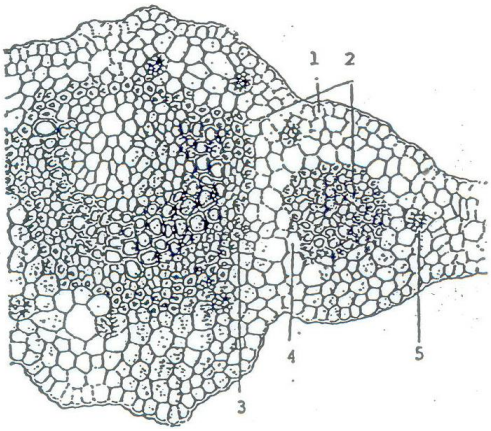


2. MICROSCOPICAL TESTS

Uvae ursi folium cross section

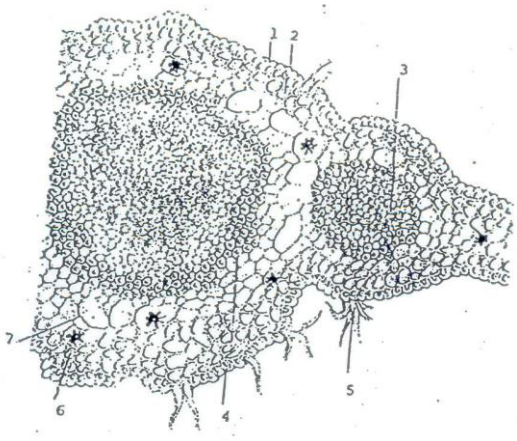


1. thick cuticle
2. upper epidermis
3. multiple palisade mesophyll
4. parenchymatic mesophyl (spongy mesophyll)
5. intercellulares
6. lower epidermis
7. stomata
8. xylem
9. medullary ray
10. phloem
11. $Ca(C00)_2$
12. collenchym



Tiliae flos cross section

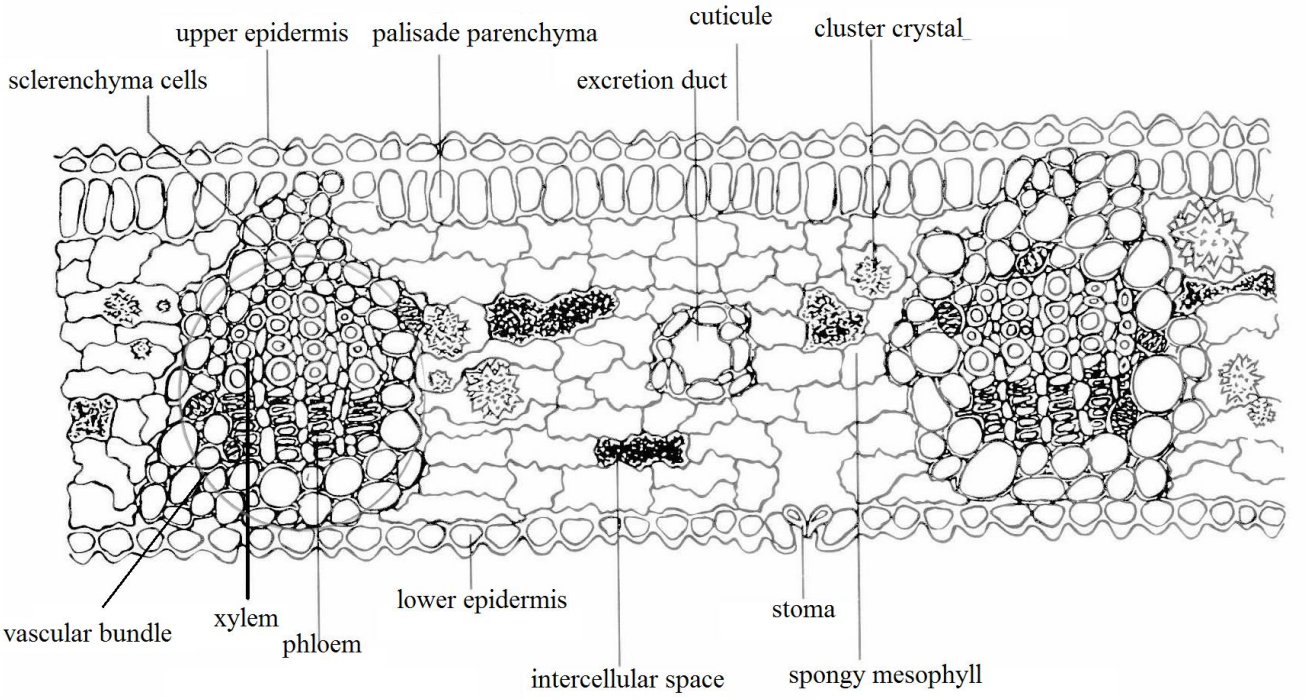
1. epidermis
2. midrid collateral bundle
3. pericyclic fibre
4. nucilage containing idioblast
5. Ca(C00)2 rosette



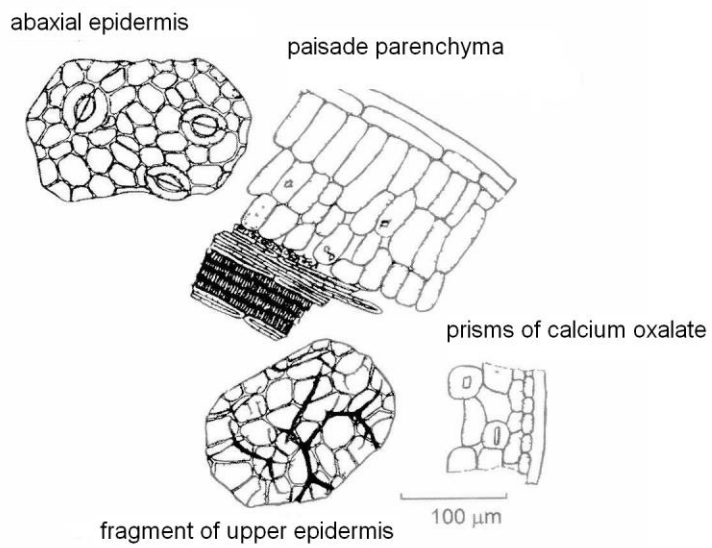
Tiliae argenteae flos cross section

1. epidermis
2. collenchyma
3. midrid collateral bundl
4. pericyclic fibre
5. stellate trichome
6. $Ca(C00)_2$ rosette
7. mucilage containing idioblast

Ginkgo folium cross section



Uvae ursi floium
(powder preparation)



The powder is green to greenish-grey or yellowish-green. Examine under a microscope using chloral hydrate solution R. The powder consists of fragments of epidermises 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 to 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

3. PHYSICOCHEMICAL AND CHEMICAL QUALITY TESTS

3.1. Microsublimation

3.1.1. Hydrochinone in *Uvae ursi folium*

Prepare the sublimate from powdered *Uvae ursi folium* on the usual way (Ph.Hg.). Examine the sublimate through microscope with small magnification without glass cover. Then test the sublimate on the same slide with Fe(III) chloride solution. Brownish, needle crystals appear.

3.1.2. Juglone in *Juglandis folium*

Prepare the sublimate from powdered *Juglandis folium* on the usual way (Ph.Hg.). Examine the sublimate through microscope with small magnification without glass cover. Juglon crystals are yellowish.

3.1.3. Onocol in *Ononidis radix*

Prepare the sublimate from powdered *Ononidis radix* on the usual way (Ph.Hg.). Examine the sublimate through microscope with small magnification without glass cover. Onocol crystals are star-like, colourless, or colourless aggregates.

3.2. Adulteration in *Uvae ursi folium*

Uvae ursi folium may contain leaves of *Buxus sempervirens* L., which contain toxic alkaloids.

Test for alkaloids: 2-3 leaves are powdered and extracted with 5,0 ml of water acidified with few drops of 2M hydrochloric acid by boiling.

After filtration a few drops of potassiomercuric iodide solution (Mayer's reagent) is added to the filtrate. No precipitation can be appear.

3.3. Thin layer chromatography of coumarins in *Rutae herba*

Rutae herba can be characterized by its coumarins and furanocoumarins. Extract 1 g of the powdered drug with 10 ml methanol for 10 min on ultrasonic water bath. Filter the extract, and use 15 µl for TLC investigation.

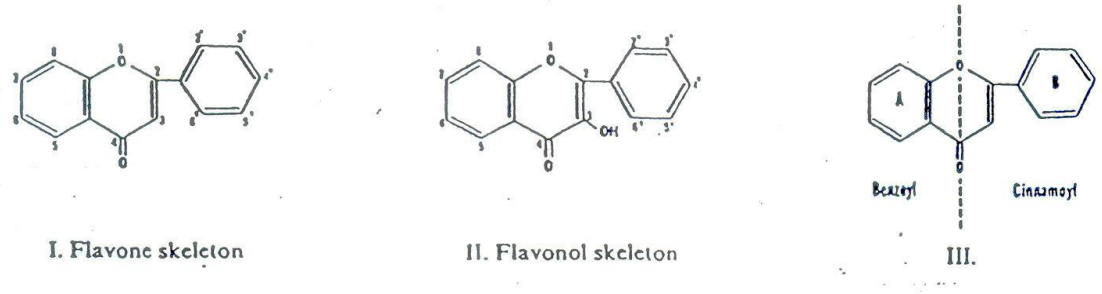
Sorbent:	Silicagel GF254 (Merck 0.2 mm)
Solvent system:	toluene - ether (1:1/saturated with 10 % acetic acid)
Detection:	UV-365 nm
	5 % ethanolic KOH reagent (UV-365 nm)

Reference compounds: umbelliferone-bergaptene

Rutae herba can be characterized by its coumarins. On the TLC plate there are more blue fluorescent zones from the start to Rf 0.9. Red zones are chlorophyll compounds. KOH solution intensifies the blue flourescence (Rf values).

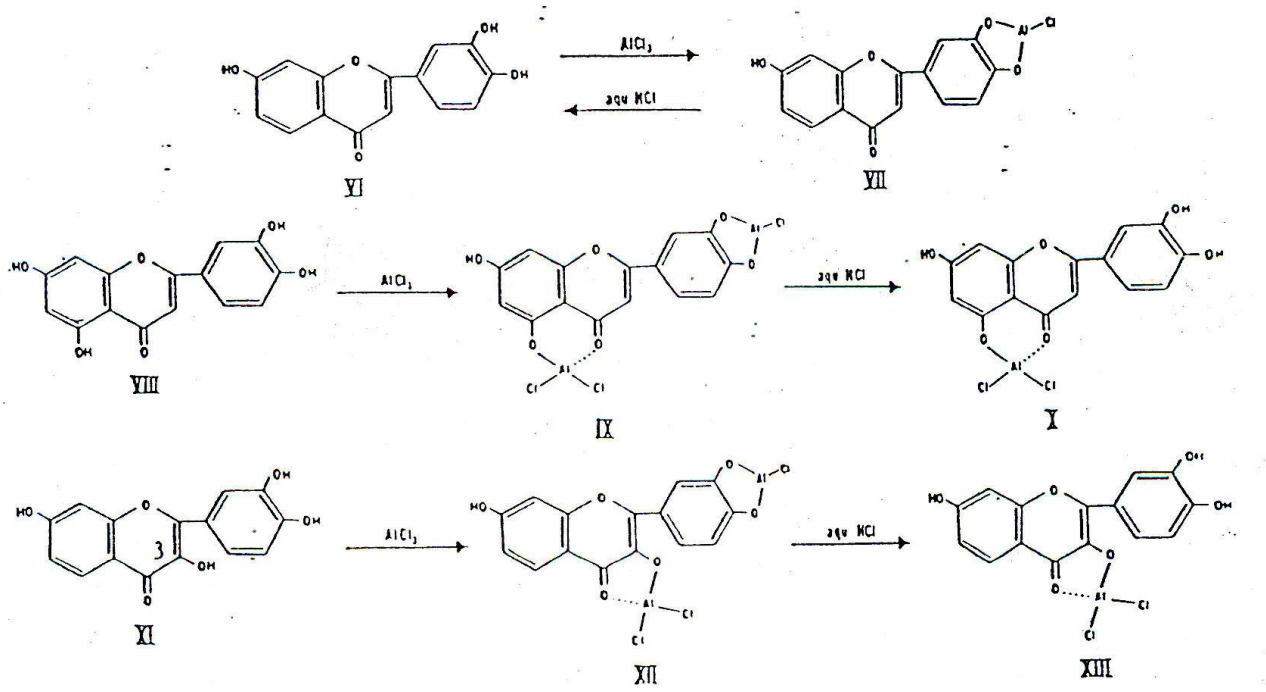
3.4. The UV spectra of rutin and other flavones and flavonoles in methanol

The methanol spectra of flavones and flavonoles exhibit two major absorption peaks in the region 240-400 nm.
These two peaks are commonly referred to as Band I. (usually 300-380 nm) and Band II. (usually 240-280 nm).
Band I. is considered to be associated with absorption due to the B-ring cinnamoyl system, and Band II. with absorption involving the A-ring benzoyl system.



I. Flavone skeleton II. Flavonol skeleton III.

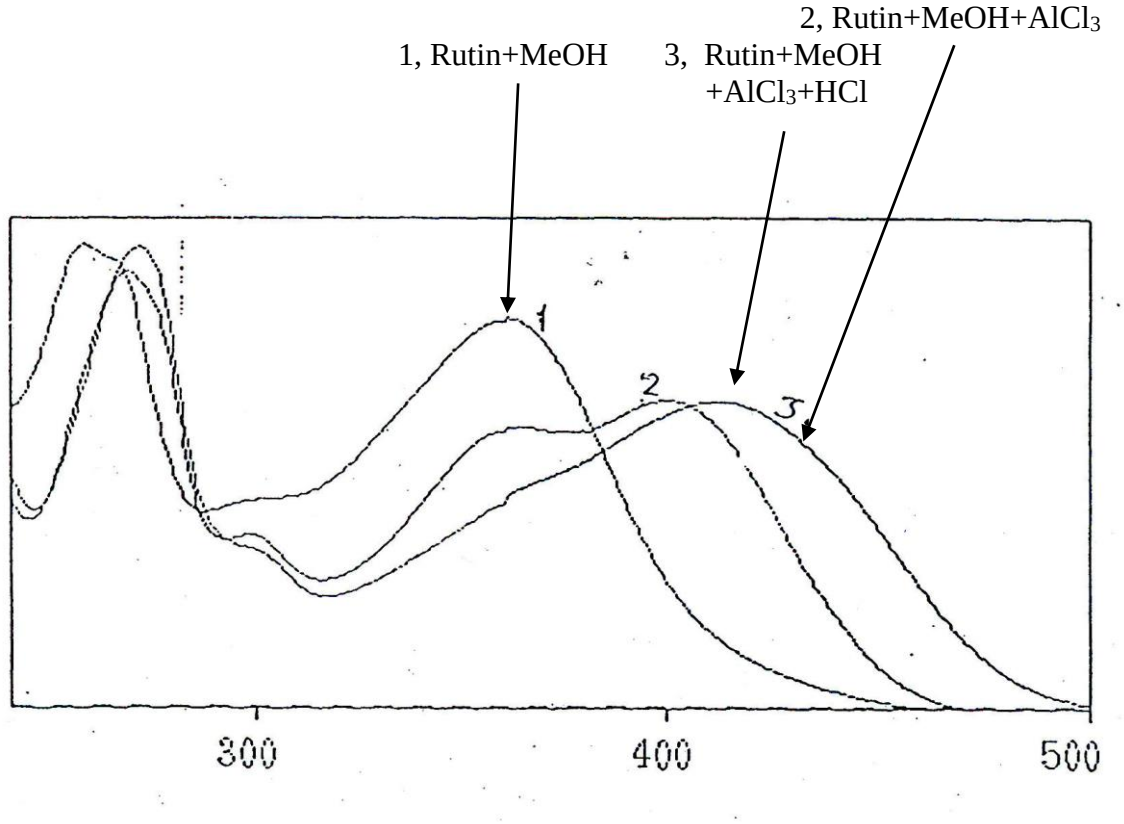
The UV spectra of rutin by the effect of $AlCl_3$ and $AlCl_3$ / HCl on the UV spectrum



Schemes illustrating the types of complexes that $AlCl_3$ could form with certain flavones and flavonols in the presence or absence of acid

Procedure

1. Dilute 1 ml of rutin stock solution with 5 ml of methanol (spectrum in methanol)
2. Mix 3 ml of diluted rutin solution (1) with 0.5 ml of alcoholic $AlCl_3$ (spectrum in methanol by the effect of $AlCl_3$)
3. Mix 3.5 ml of diluted solution of rutin which already contains $AlCl_3$, with 2 drops of hydrochloric acid (spectrum in methanol by the effect of $AlCl_3$/HCl)



3.5. Thin-layer chromatography of flavonoids in various vegetable drugs.

3.5.1. Typical flavonoid drugs.

Drug samples:

Betulae folium (20 µl)	*Hyperici herba* (20 µl)
Crataegi folium cum flore (20 µl)	*Sambuci flos* (20 µl)
Equiseti herba (30 µl)	*Tiliae flos* (25 µl)
Ginkgo folium (40 µl)	

Extract 1g of powdered drug with 10 ml methanol for 10 min on ultrasonic water bath. Filter the extract, and use 20-40 µl for TLC investigation.

Sorbent: Silicagel GF254 (Merck 0.2 mm)
Solvent system: ethylacetate-formic acid-acetic acid-water (100:11:11:26)
Detection: Natural products / polyethylene glycol reagent - UV-365 nm
Reference compounds: rutin, isoquercitrin, chlorogenic acid

Each extract shows a characteristic TLC fingerprint of yellow-orange or yellow green flavonoid glycosides and blue fluorescent phenol carboxylic acids.
The major flavonoids of the individual drugs have to be identified (Rf values)

3.5.2. Flavanolignane containing *Silybi mariani* fructus

Drug sample: *Silybi mariani fructus* (syn. *Cardui mariae fructus*)

Powdered drug (1 g), previously defatted by light petroleum ether is extracted with 10 ml methanol for 10 min. on ultrasonic water bath. The extract is filtered and 20 µl is used for TLC investigation.

Sorbent: Silicagel GF254 (Merck 0.2 mm)
Solvent system:
Detection: Fast blue sall reagent (VIS)
Reference compounds: silybin, silycristin.

Main zones on the chromatoplate become red-brown (VIS) after treatment with fast blue salt reagent.

4. Quantitative determinations

4.1.1 Quantitative determination of arbutin in Uvae ursi folium (DAB 10)

Extract 0.4000 g of crude drug with hot water (50.0 ml) for 30 min by refluxing on boiling water bath. Filter the extract into a 250.00 ml volumetric flask and fill up with water (stock solution).

Transfere 5.00 ml of the extract into a separation funnel and mix it with 45 ml of water, 1 ml of 2 % of 4-aminoantipyrine, 0.5 ml of diluted NH_4OH and 1 ml of 8 per cent $K_3(Fe/CN/_6)$ solution.

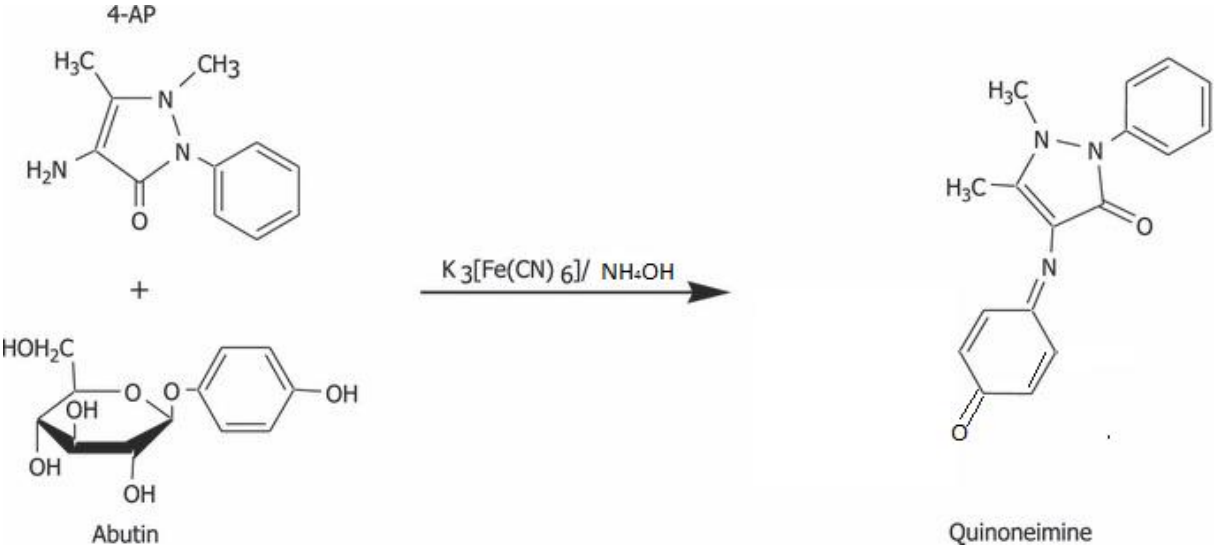
After 5 min extract the reaction mixture with $CHCl_3$ (3x 25 ml). Mix the organic (lower) phases, filter through Na_2SO_4 sicc. into a 100.00 ml volumetric flask and fill up with $CHCl_3$ to 100.00 ml.

Measure the absorbance (1 cm) on 455 nm. The blank solution is water.

Calculate the arbutin content of the crude drug (g/100g)

$A\ ^{1\,\%}_{1\ cm} = 643$

$$\%=\frac{A*50}{643*m}*100=7{,}77*\frac{A}{m}$$ (Ph Eur.: >7%, HPLC method)



4.2.1. Determination of flavonoid content in different crude drugs by spectrophotometry

(Ph. Hg. VIII., Ph. Eur.)

	Ph. Eur. requirement
Betulae folium	>1,5%
Sambuci flos	>0,8%
Solidaginis herba	>2,5%
Urticae folium	-
Myrtilli folium	-
Hyperici herba	-

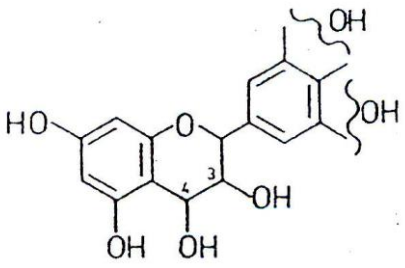
To a 100 ml Erlenmeyer flask introduce 0,5000g of the powdered drug, add 1.0 ml of 0.5 % hexamethylene-tetramine solution, 20 ml of acetone and 2,0 ml of 25 % hydrochoric acid. Hydrolyze the solution for 30 min on boiling water-bath, cool it, and filter into volumetric flask. Reextract the drug with 20 ml of acetone for 10 min. After filtration fill up the combined reaction mixtures to 50.00 ml with acetone.

Take 10.00 ml acetonic extract to a separating funnel containing 20 ml of water, and extrac with 15 ml of ethylacetate (saturated with water) then three times of 10 ml ethylacetate. Wash the combined ethylacetate fractions three times with 50 ml of water, dry over Na_2SO_4 anhydrous and filter to a volumetric flask (50 ml) and fill up with ethylacetate (50.00 ml).

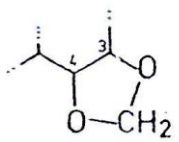
Put 10.00 ml of the solution into a 25.00 ml volumetric flask. Add 1.0 ml of Al-reagent (2 % $AlCl_3$, 6 H_2O in methanolic acetic acid) and fill up the solution to the volume with methanolic acetic acid (5 % v/v solution of acetic acid in methanol). After 30 min. measure the absorbance at 425 nm against the blank prepared without Al-reagent.

Result is calculated in hyperoside (quercetine-3-0-galactoside).

$A^{1\%}_{1\,cm} = 500$



Leukoantocianidin



Main steps of the determination are:

- acidic hydrolyses and extraction (acetone containing hydrochloric acid and hexamethylene tetramine. Hexamethilene tetramine reacts with other phenoloids, like leucoanthocyanins)
- purification (extraction with ethylacetate and washing of acid (pH.)
- complex formation in weekly acidic medium (see 3.4.)

4.2.2 Determination of flavonoid content in Crataegi folium cum florae (Ph. Eur.)

Stock solution. Into a 200 ml flask introduce 0.400 g of the powdered drug (250) (2.9.12) and 40 ml of ethanol (60 per cent V/V) R. Heat in a water-bath at 60 °C for 10 min, shaking frequently. Allow to cool and filter through a plug of absorbent cotton into a 100 ml volumetric flask. Transfer the absorbent cotton with the drug residue back into the 200 ml flask, add 40 ml of ethanol (60 per cent V/V) R and heat again in a water-bath at 60 °C for 10 min, shaking frequently. Allow to cool and filter into the same 100 ml volumetric flask. Rinse the 200 ml flask with a further quantity of ethanol (60 per cent V/V) R, filter and transfer to the same 100 ml volumetric flask. Dilute to 100.0 ml with ethanol (60 per cent V/V) R and filter.

Test solution. Introduce 5.0 ml of the stock solution into a round-bottomed flask and evaporate to dryness under reduced pressure. Take up the residue with 8 ml of a mixture of 10 volumes of methanol R and 100 volumes of glacial acetic acid R and transfer into a 25 ml volumetric flask. Rinse the round-bottomed flask with 3 ml of a mixture of 10 volumes of methanol R and 100 volumes of glacial acetic acid R and transfer into the same 25 ml volumetric flask. Add 10.0 ml of a solution containing 25.0 g/l of boric acid R and 20.0 g/l of oxalic acid R in anhydrous formic acid R and dilute to 25.0 ml with anhydrous acetic acid R.

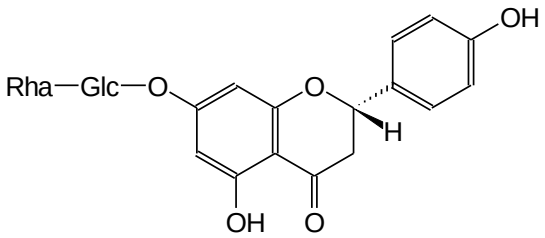
Compensation liquid. Introduce 5.0 ml of the stock solution into a round-bottomed flask and evaporate to dryness under reduced pressure. Take up the residue with 8 ml of a mixture of 10 volumes of methanol R and 100 volumes of glacial acetic acid R and transfer into a 25 ml volumetric flask. Rinse the round-bottomed flask with 3 ml of a mixture of 10 volumes of methanol R and 100 volumes of glacial acetic acid R and transfer into the same 25 ml volumetric flask. Add 10.0 ml of anhydrous formic acid R and dilute to 25.0 ml with anhydrous acetic acid R.

Measure the absorbance (2.2.25) of the test solution after 30 min at 410 nm, by comparison with the compensation liquid.

Calculate the percentage content of total flavonoids, expressed as hyperoside.

4.2.3. Determination of flavonoid content in Aurantii amari flos (Ph. Eur.)

Stock solution. To 0.175 g of the powdered drug (355) (2.9.12) add 95 ml of alcohol (50 per cent V/V) R. Heat on a water-bath under a reflux condenser for 30 min. Allow to cool and filter through a sintered-glass filter (2.1.2). Rinse the filter with 5 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. Into a test tube (10 mm × 180 mm) introduce 0.150 g of powdered (250) (2.9.12) magnesium R, a magnetic stirring bar 25 mm long and 2.00 ml of the stock solution. Maintain the test tube upright, centrifuge at 125 g and carefully add dropwise, especially at the beginning, 2.0 ml of hydrochloric acid R, and then 6.0 ml of alcohol (50 per cent V/V) R. Stopper the tube and mix by inverting.
Compensation solution. Into a second tube, introduce 2.00 ml of the stock solution and carefully add dropwise, especially at the beginning, 2.0 ml of hydrochloric acid R and then 6.0 ml of alcohol (50 per cent V/V) R.
After 10 min, measure the absorbance (2.2.25) of the test solution at 530 nm.
Calculate the percentage content of total flavonoids, expressed as naringin.



Naringin

4.3. Determination of anthocyanin content in Myrtilli fructus recens

Crush 50 g extemporaneously. To about 5.00 g of the crushed, accurately weighed drug, add 95 ml of methanol R. Stir mechanically for 30 min. Filter into a 100.0 ml volumetric flask. Rinse the filter and dilute to 100.0 ml with methanol R. Prepare a 50-fold dilution of this solution in a 0.1 per cent V/V solution of hydrochloric acid R in methanol R.
Measure the absorbance (2.2.25) of the solution at 528 nm, using a 0.1 per cent V/V solution of hydrochloric acid R in methanol R as the compensation liquid.
Calculate the percentage content of anthocyanins, expressed as cyanidin-3-glucoside chloride.