MACROSCOPIC INVESTIGATIONS

Schisandrae chinensis fructus – Schisandra fruit Ph. Eur.

Schisandra chinensis (Turcz.) Baillon. (Schisandraceae)

The berry is more or less spherical, up to 8 mm in diameter; red, reddish-brown or blackish outer surface; strongly shrivelled pericarp; presence of 1-2 reniform, yellowish-brown, lustrous seeds, with thin seed-coat.

Bardanae radix – burdock root

Arctium lappa L. (Asteraceae)

Greyish-brown outer surface, whitish inside. The harvest occurs three to four months after the seeding until late autumn, when the roots become too fibrous.

Meliloti herba – melilot Ph. Eur.

Melilotus officinalis (L.) Lam. (Leguminosae)

The stem is green, cylindrical, glabrous and finely ridged. The leaves are alternate, petiolate and trifoliate with 2 lanceolate stipules. The inflorescence is racemose with numerous pale yellow flowers.

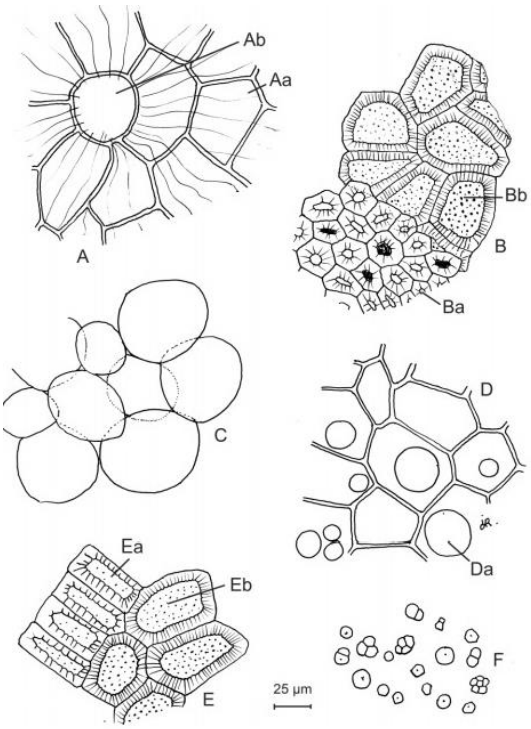
Curcumae longae rhizoma – turmeric rhizome Ph. Eur.

Curcuma longa L. (Zingiberaceae)

Whole, cured (by boiling or steaming), dried rhizome of the plant with roots and outer surface removed.

The rhizome is ovate, oblong-ovoid, pyriform or cylindrical, often shortly branched, up to 6 cm long and 15 mm thick. The primary rhizome shows scars from the lateral branches. The surface is slightly dusty, spotted and brownish-yellow, yellow or brownish-grey, finely striated. The fracture is granular, smooth, non-fibrous, slightly glossy, uniformly orange-yellow; it shows a narrow cortex that is darker on the outside.

MICROSCOPIC INVESTIGATIONS



Schisandrae pulvis

A: reddish-brown fragments of pericarp, consisting of 1 layer of thin-walled epicarp cells (surface view); Aa: wrinkled cuticule; Ab: oil cell sparsely

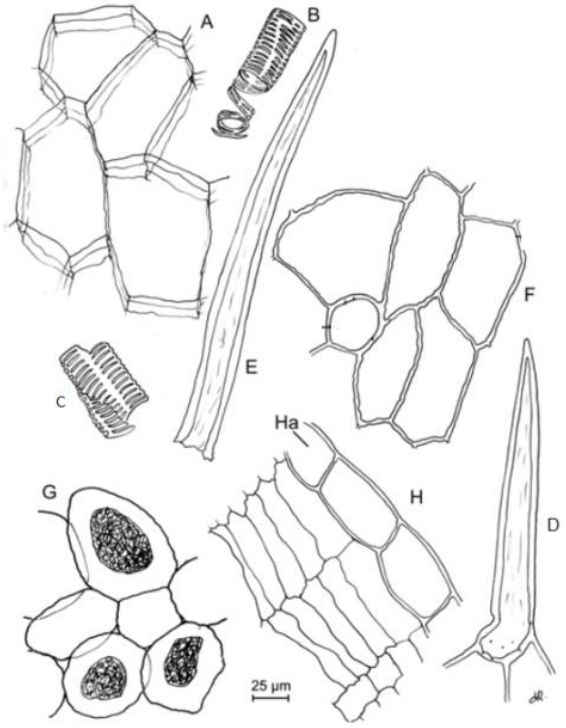
B: fragments of the testa of the seed with sclereids, Ba: surface view, Bb: thick-walled, finely channelled sclereids, polygonal in surface view

C: ovoid, more-or-less flattened mesocarp cells

D: fragments of endosperm containing oil droplets and aleurone grains

E: fragments of the testa; Ea: palisade arrangement in side view; Eb: thick-walled, finely channelled sclereids

Curcumae pulvis



A: rare fragments of brown cork – surface view

B, C: reticulate or pitted vessels

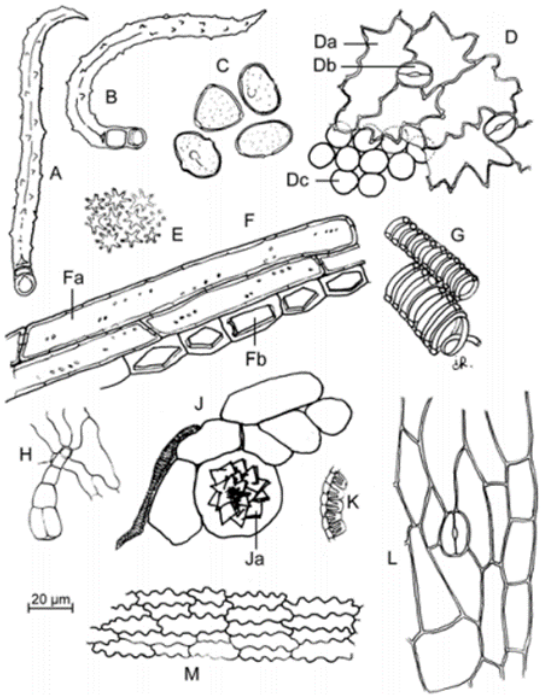
D, E: unicellular trichomes

F: fragments of the epidermis

G: fragments of parenchyma sometimes coloured yellow by curcumin

H: rare fragments of brown cork – side view, Ha: epidermis

Meliloti pulvis



A, B: uniseriate covering trichomes with 2 basal cells and a long terminal cell, bent at right angles, with a warty cuticle

C: spherical or ovoid pollen grains with 3 germinal pores and a smooth exine

D: fragments of the leaf lamina in surface view showing slightly sinuous epidermal cells; Db: numerous stomata, mostly anomocytic with Da: subsidiary cells; Dc: palisade parenchyma

E: fragments of the fibrous layer of the anthers in surface view

F,G: fragments of vascular tissue from the stem including large vessels [G], sometimes associated with unlignified septate fibres [Fa] and a sheath of parenchymatous cells containing prisms of calcium oxalate [Fb]

H: occasional glandular trichomes with a short, 2- or 3- celled stalk and ovoid, biseriate head with 4 indistinct cells

J: fragments of including some cells which may occasionally contain cluster crystals of calcium oxalate [Ja]

K: fragments of the fibrous layer of the anthers in transverse section

L: fragments of the stem epidermis with elongated, straight-walled cells and anomocytic stomata

M: fragments of the petals composed of cells with wavy walls

CHEMICAL INVESTIGATIONS

1. Investigation of turmeric rhizome samples according to Ph. Eur. methods

Curcumae longae rhizoma

Curcumae xanthorrhizae rhizoma

1.1. Extraction: Disperse 0.200 g of the powdered herbal drug in 25 mL of methanol. Heat under a reflux condenser for 1.5 h. Cool and filter (through filter paper) into a volumetric flask, rinse the flask and filter with methanol and dilute to 50.0 mL (stock solution). (*Protect from light*)

1.2. TLC investigation of curcuminoids

Test solution: **stock solution -** 15 µl

Reference solution: 2 mg curcuminoid-mixture dissolved in 1 ml methanol, 10 µl is used.

Plate: silica gel 60 F_{254}

Mobile phase: glacial acetic acid R, toluene R (1:4 V/V).

Development: over a path of 4 cm

Detection: examine in ultraviolet light at 365 nm. Curcuminoids show intensive green fluorescence.

Identificate the sample!

Curcumae longae rhizoma: 3 intensive spots can be seen, bis-desmethoxy-curkumin (Rf: 0,2); desmethoxy-curkumin (Rf: 0,3); curcumin, Rf: 0,4.

Curcumae xanthorrhizae rhizoma: 2 intensive spots. Bis-desmethoxy-curkumin is absent.

1.3. Quantitative determination of dicinnamoyl methane derivatives

Dilute 1.0 mL of the **stock solution** to 25.0 mL with methanol. Measure the absorbance at 425 nm using methanol as the compensation liquid.

Calculate the percentage content of dicinnamoyl methane derivatives, expressed as curcumin. Does it meet the requirements?

$A_{1\%,1\ cm\ (curcumin)}$: 1607

Curcumin shows keto-enol tautomer transformation. Enol form has strong intramolecular hydrogen bond. (If curcumin is dissolved, intermolecular H-bond is also possible to form.) Because of the aromatic ring, the unsaturated bonds of the C7 chain and the intramolecular H- bond of the enol form,there is an extended conjugated system in curcumin, which cause broad absorption maximum in 410-430 nm.

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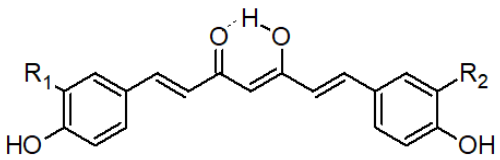


Curcumin: R_1, R_2 = OCH_3
Desmethoxy-curcumin: R_1 = OCH_3, R_2 = H
Bis-desmetoxy-curcumin: R_1, R_2 = H

2. Investigation of glycosidase enzymes in burdock friut.

Measure 0,1-0,1 g of crude drug in 2 short test tubes. Add 0,5 ml water to one of them and leave it for 15 min., shaking sometimes. Fill up to 4 ml both test tubes (add 3,5 and 4 ml) with methanol. Shake on ultrasonic bath for 15 min. Leave the test tubes to sediment. Use 50-60 µl of the upper part of the solutions and 20-20 µl of the standards (pinoresinol, arctigenine) on the TLC plate.

Plate: silica gel 60 F_{254} lemez

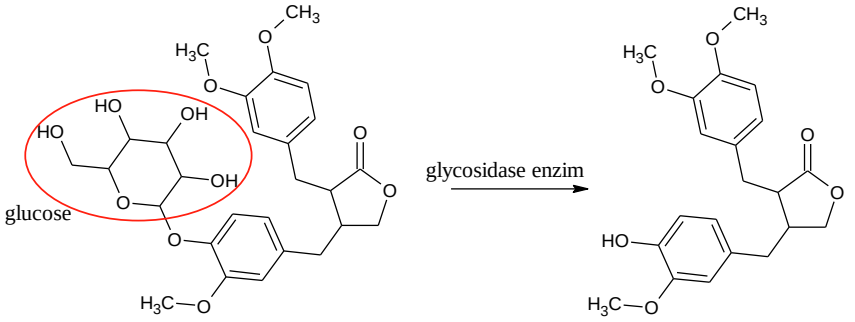
Mobile phase: petrolether : acetone (10:6, v/v) (20 min.)

Detection: A) UV light (254 nm)

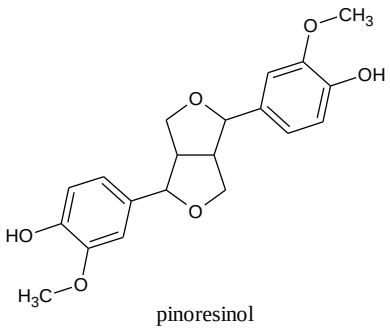
B) cc. H_2SO_4 10% metanolic solution, heating on 120 °C-on for 5 min.

Evaluation: *fruit of Arctium lappa contain arctiin glycoside, which is transformed to arctigenin aglycon in aqueous medium due to the glycosidase enzimes present in the fruit. Arctigenin can be found only in the sample treated with water, while in the methanolic extract glycosidase enzymes are blocked. Carduus nutans (musk thistle) contain pinoresinol, a furofurane type lignan aglycon, which can be detected in both samples,*

Which of the two plants you had?



arctiin (glycoside) arctigenine (aglycon)



pinoresinol

3. Invesigation of dibenzo-cycloocadiene lignans in *Schisandrae fructus*

Measure 1,25 g of *Schisandra chinensis* (chinese magnolia-vine), add 5 ml methanol and shake for 10 min. on ultrasonic bath. Content of 1 capsule of *Shisandra* containing supplement product is used for making extract in the same way (5 ml methanol, 5 min. ultrasonic bath). Extracts are filtered into a little test tube.

Dibenzo-cyclooctadiene lignans (20-20 µl of both extract) are used on TLC layer, beside schizandrin standard (20 µl) (in lines!).

Plate: silica gel 60 F_{254}

Mobile phase: petrolether : ethyl-acetate (1:1, v/v) (20 min.)

Detection: A) UV light (254 nm)

B) cc. H_2SO_4 10% metanolic solution, heating on 120 °C-on for 5 min.

4. TLC investigation of coumarins

Rutae herba, Angelicae radix

Test solution: 1 g drug powder + 5 ml methanol, in ultrasonic bath, for 10 min. Filter on filter paper and put 25 µl of the solution on the layer.

Reference solution: 1-1 mg umbelliferone, xanthotoxine and bergapten in 1 ml methanol, 10 µl is used.

Plate: silica gel 60 F_{254} lemez

Mobile phase: toluene-ether (1:1, v/v) saturated with 10% acetic acid

Development: over a path of 8 cm

Detection: A) UV light (365 nm)

B) 5% KOH/ethanol and UV (365 nm)

Coumarins, furocumarins and furanoquinolin alkaloids give intensive blue fluorescence which become more intensive after spraying with KOH.

Report:

1. Qualification of unknown curcuma sample according to the Ph.Eur.: TLC and spectrophotometric quantitative determination (R_f values, result, limits)
2. Investigation of unknown burdock/thistle fruit based on the identification of aglycons by TLC. R_f values, result.
3. Investigation of *chinese magnolia-vine* by TLC. What the plant and the food supplement product contains? (R_f values)
4. Identification of coumarins by TLC (R_f values).