

**OLIVE (TREE)
FOR HOMOEOPATHIC PREPARATIONS**

**OLEA EUROPAEA
FOR HOMOEOPATHIC PREPARATIONS**

***Olea europaea* ad praeparationes homoeopathicas**

DEFINITION

Fresh, leafy twig of *Olea europaea* L.

IDENTIFICATION

- A. Tortuous twig with cylindrical section, covered with greyish-white bark, bearing small, greyish, silky, scaly buds and opposite, evergreen entire leaves. Leaf oblong or lanceolate-oval, tapering at the base to a short petiole, 8-10 cm long and 1.5-2 cm large; reflexed lamina with greyish-green, smooth upper side, pitted with white and silky, whitish underside, crossed by a prominent midrib. Clusters of small white, erect flowers at the axil of the leaves.
- B. Take a sample of epidermis from the underside of the leaf. Examine under a microscope using *chloral hydrate solution R*: abaxial epidermis composed of polygonal cells with cell-walls slightly and regularly thickened, covered with a striated cuticle, anomocytic stomata (2.8.3), scutate covering trichomes "escutcheon-like"; of huge size, composed of a central, unicellular pedicel from which about 10-30 thin-walled cells radiate and separate from the subsidiary cells on the escutcheon margins, thus giving it an uneven, jagged appearance.

TESTS

Foreign matter (2.8.2): maximum 5 per cent.

Loss on drying (2.2.32): minimum 30 per cent, determined on 5.0 g of finely-cut drug, by drying in an oven at 105 °C for 2 h.

STOCK

DEFINITION

Olive mother tincture is prepared with ethanol (65 per cent V/V) using the fresh, leafy twig of *Olea europaea* L.

Content: minimum 0.015 per cent *m/m* of luteolin-7-glucoside (C₂₁H₂₀O₁₁; M_r 448.4).

The General Chapters and General Monographs of the European Pharmacopoeia and Preamble of the French Pharmacopoeia apply.

PRODUCTION

Method 1.1.10 (2371). Drug fragmented into about 3 cm-long segments. Maceration time: 3-5 weeks.

CHARACTERS

Appearance: greenish-brown liquid.

IDENTIFICATION

A. Thin layer chromatography (2.2.27).

Test solution. Mother tincture.

Reference solution. Dissolve 5 mg of *rutin R* and 5 mg of *isoquercitroside R* in 10 mL of *ethanol (96 per cent) R*.

Plate: TLC silica gel plate *R*.

Mobile phase: *water R*, *anhydrous formic acid R*, *ethyl acetate R* (10:10:80 V/V/V).

Application: 20 µL, as bands.

Development: over a path of 10 cm.

Drying: in air.

Detection: first spray with a 10 g/L solution of *diphenylboric acid aminoethyl ester R* in *methanol R* then spray with a 50 g/L solution of *macrogol 400 R* in *methanol R*. Allow the plate to dry in air for about 30 min. Examine in ultraviolet light at 365 nm.

Results: see below the sequence of fluorescent zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore other faint, fluorescent zones may be present in the chromatogram obtained with the test solution.

Top of the plate	
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Isoquercitroside: an orange zone	A greenish-blue zone An orange zone (isoquercitroside)
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Rutin: an orange zone	An orange zone (rutin)
Reference solution	Test solution

B. Thin layer chromatography (2.2.27).

Test solution. Mother tincture.

The General Chapters and General Monographs of the European Pharmacopoeia and Preamble of the French Pharmacopoeia apply.

Reference solution. Dissolve 20 mg of *oleuropein R* and 20 mg of *oleanic acid R* in 20 mL of *ethanol (96 per cent) R*.

Plate: TLC silica gel plate R.

Mobile phase: water R, methanol R, methylene chloride R (1.5:15:85 V/V/V).

Application: 20 µL, as bands.

Development: over a path of 10 cm.

Drying: in air.

Detection: spray with *vanillin reagent R*. Heat at 100-105 °C for 10 min. Examine in daylight.

Results: see below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore other faint zones may be present in the chromatogram obtained with the test solution.

Top of the plate	
Oleanolic acid: a purple zone	A purple zone (oleanolic acid)
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Oleuropein: a purplish-pink zone	A purplish-pink zone (oleuropein)
Reference solution	Test solution

TESTS

Ethanol (2.9.10): 60 per cent V/V to 70 per cent V/V.

Dry residue (2.8.16): minimum 2.0 per cent *m/m*.

ASSAY

Liquid chromatography (2.2.29).

Test solution. In a 100.0 mL volumetric flask, place 6.000 g of mother tincture and dilute to 100.0 mL with the mobile layer.

Reference solution. In a 100.0 mL volumetric flask, dissolve 10.0 mg of *luteolin-7-glucoside R* in *methanol (60 per cent V/V) R* and dilute to 100.0 mL with the same solvent. In a 20.0 mL volumetric flask, place 4.0 mL of this solution and dilute to 20.0 mL with *methanol (60 per cent V/V) R*.

Column:

- size: *l* = 0.25 m, Ø = 4.6 mm.
- stationary phase: *octadecylsilyl silica gel for chromatography R* (5 µm).
- temperature: 30 °C.

Mobile phase: *acetonitrile R*, *water R* adjusted to pH 3.0 with *phosphoric acid R* (21:79 V/V).

The General Chapters and General Monographs of the European Pharmacopoeia and Preamble of the French Pharmacopoeia apply.

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 340 nm.

Injection: 20 µL of each solution.

Retention time: *luteolin-7-glucoside*: about 6.5 min.

Calculate the percentage content *m/m* of luteolin-7-glucoside, in the mother tincture, from the expression:

$$\frac{A_1 \times m_2 \times 0.2 \times p}{A_2 \times m_1}$$

A_1 = area of the peak due to luteolin-7-glucoside in the chromatogram obtained with the test solution,

A_2 = area of the peak due to luteolin-7-glucoside in the chromatogram obtained with the reference solution,

m_1 = mass of the mother tincture sample in the test solution, in grams,

m_2 = mass of the sample of luteolin-7-glucoside in the reference solution, in grams,

p = percentage content of luteolin-7-glucoside in *luteolin-7-glucoside R*.

The General Chapters and General Monographs of the European Pharmacopoeia and Preamble of the French Pharmacopoeia apply.