

**MARSH LABRADOR TEA
FOR HOMOEOPATHIC PREPARATIONS**

**LEDUM PALUSTRE
FOR HOMOEOPATHIC PREPARATIONS**

Ledum palustre ad praeparationes homoeopathicas

DEFINITION

Fresh, leafy twig of *Ledum palustre* L.

CHARACTERS

Macroscopic and microscopic characters described under identification tests A and B.

IDENTIFICATION

- A. Reddish-brown, hairy stem. Tough, narrow, sessile, alternate, evergreen leaves measuring up to 35 mm long and 6 mm large. Revolute lamina, underside covered with russet down.
- B. Take a sample of epidermis from the underside of the leaf. Examine under a microscope, using *chloral hydrate solution R*: abaxial epidermis with stomatiferous, polyhedral cells bearing covering and secretory trichomes. Stomata of anomocytic type (2.8.3). Covering trichomes of two types: some short, conic, unicellular with slightly echinulate cell-walls; the others curled on themselves with thick cell-walls reaching 200 µm long. Secretory trichomes of two types; some with bicellular foot, and bi to quadricellular head; the others with unicellular head and globulous multicellular head.

TESTS

Foreign matter (2.8.2): maximum 5 per cent.

Loss on drying (2.2.32): minimum 55.0 per cent determined on 5.0 g of finely-cut drug, by drying in an oven at 100-105 °C for 2 h.

STOCK

DEFINITION

Marsh labrador tea mother tincture complies with the requirements of the general technique for the preparation of mother tinctures (see *Homoeopathic Preparations (1038)* and French Pharmacopoeia Authority Supplement). The mother tincture is prepared with ethanol (65 per cent V/V), using fresh, leafy twig of *Ledum palustre* L.

Content: minimum 0.05 per cent *m/m* of total flavonoids, expressed as quercetin dihydrate.

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

CHARACTERS

Appearance: brown liquid.

IDENTIFICATION

A. Thin layer chromatography (2.2.27).

Test solution. Mother tincture

Reference solution. Dissolve 10 mg of *hyperoside R* and 10 mg of *quercetin dihydrate R* in 20 mL of *ethanol (96 per cent) R*.

Plate: TLC silica gel plate *R*.

Mobile phase: *anhydrous formic acid R*, *water 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 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	
Quercetin dihydrate: an orange zone	An orange zone (quercetin dihydrate) A blue zone
-----	Two to three orange zones
Hyperoside: an orange zone	An orange-yellow zone
-----	One to two blue zones
Reference solution	Test solution

B. Thin layer chromatography (2.2.27).

Test solution. Add 10 mL of *water R* and 5 mL of a saturated solution of *ammonium sulfate R* to 10 mL of mother tincture. Extract with 3 quantities each of 15 mL of *pentane R*. Combine the pentanic phases, allow them to dry on *anhydrous sodium sulfate R* then evaporate under reduced pressure at a temperature below 40 °C. Dissolve the residue in 2 mL of *ethanol (96 per cent) R*.

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

Reference solution. Dissolve 50 µL of *cineole R* and 20 mg of *terpineol R* in 10 mL of *ethanol (96 per cent) R*.

Plate: TLC silica gel plate *R*.

Mobile phase: *isopropyl ester R*, *toluene R* (10:40 V/V).

Application: 20 µL, as bands.

Development: over a path of 10 cm.

Drying: in air.

Detection: spray with *anisaldehyde solution R* and 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	
-----	A purple zone
Cineole: a pinkish-brown zone	A purplish-pink zone
-----	A purplish-pink zone
Terpineol: a brown zone	A purplish-pink zone
-----	A purple zone
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 1.3 per cent *m/m*.

ASSAY

Ultraviolet and visible absorption spectrophotometry (2.2.25).

Stock solution. In a 50.0 mL volumetric flask, place 1.50 g of mother tincture and dilute to 50.0 mL with a mixture of 10 volumes of *methanol R* and 100 volumes of *glacial acetic acid R*.

Test solution. In a 25.0 mL volumetric flask, place 2.0 mL of stock solution, add 15.0 mL of a solution comprising 25.0 g/L of *boric acid R* and 20.0 g/L of *oxalic acid R* in *anhydrous formic acid R* then dilute with 25.0 mL with *glacial acetic acid R*.

Compensation liquid. In a 25.0 mL volumetric flask, place 2.0 mL of stock solution, add 15.0 mL of *anhydrous formic acid R* then dilute to 25.0 mL with *glacial acetic acid R*.

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

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Measure the absorbance of the test solution 30 min after the addition of the last reagent at 438 nm in comparison with the compensation liquid.

Calculate the percentage content m/m of total flavonoids expressed as quercetin dihydrate, from the expression:

$$\frac{A \times 625}{869 \times m}$$

i.e taking the specific absorbance of quercetin dihydrate to be 869.

A = absorbance at 438 nm,

m = mass of the mother tincture sample, in grams.