BUSH CLOVER, FRESH FOR HOMOEOPATHIC PREPARATIONS

LESPEDEZA CAPITATA RECENS FOR HOMOEOPATHIC PREPARATIONS

Lespedeza capitata recens ad praeparationes homoeopathicas

DEFINITION

Fresh, flowering, aerial part of *Lespedeza capitata* Michx.

IDENTIFICATION

A. Ramified stems all over the upper part of the plant; alternate, compound leaves with three entire leaflets, elliptical-ovate, more or less acuminate, 4.5 cm long and about 1.8 cm large; both sides covered with silky hairs, more numerous on the underside. Petiole shorter than the petiolule of the middle leaflet. Numerous inflorescences arranged in sub-globular spikes; compact and borne by a short peduncle; very numerous and tight flowers, measuring about 1 cm long; calyx presenting 5 almost equal teeth; creamy white corolla, sometimes tinged with purple. Ten stamens; 9 united into a tube opening backwards and 1 free, posterior stamen.

B. Examine under a microscope a sample of abaxial epidermis of the leaflet, using *chloral hydrate solution R*. The epidermis of the lamina is composed of polygonal cells with straight cell-walls; paracytic stomata (*2.8.3*), very numerous unicellular covering trichomes measuring up to 500 µm long, stiff; with thinly echinulate cell-walls and acuminate end, lying parallel to the epidermis and all oriented towards the same direction. Frequently, cells of palisade parenchyma accompany the lower epidermis of the lamina. The epidermic cells of the midrib are elongated, more or less rectangular and accompanied by sheath calcium oxalate prisms.

TESTS

Loss on drying (*2.2.32*): minimum 50.0 per cent, determined on 5.0 g of finely-cut drug, by drying in an oven at 105 °C for 2 h.

STOCK

DEFINITION

Bush clover, fresh mother tincture is prepared with ethanol (55 per cent *V/V*), using the fresh, flowering, aerial part of *Lespedeza capitata* Michx.

Content: minimum 0.050 per cent *m/m* of total flavonoids, expressed as isoorientin ($C_{21}H_{20}O_{11}$; 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 5-7 cm long segments. Maceration time: 3-5 weeks.

CHARACTERS

Appareance: orange-brown liquid.

IDENTIFICATION

Thin-layer chromatography (*2.2.27*).

Test solution. Mother tincture.

Reference solution. Dissolve 2.5 mg of *orientin R,* 2.5 mg of *isoorientin R* and 10.0 mg of *rutin R* in 10 mL of *methanol R.*

Plate: *TLC silica gel plate R* (5-40 µm) [or *TLC silica gel plate R* (2-10 µm)].

Mobile phase: *glacial acetic acid R, anhydrous formic acid R, water R, ethyl acetate R* (11:11:27:100 *V/V/V/V*).

Application: 20 µL [or 5 µL] as bands.

Development: over a path of 12 cm [or 7 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 the 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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Orientin: a yellow zone	A yellow zone (orientin)
Isoorientin: a yellow zone	A yellow zone (isoorientin)
Rutin: an orange zone	An orange zone (rutin)
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	A yellow zone
Reference solution	**Test solution**

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TESTS

Ethanol (*2.9.10*): 50 per cent *V/V* to 60 per cent *V/V.*

Dry residue (*2.8.16*): minimum 1.8 per cent *m/m*.

ASSAY

Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Stock solution. In a 20.0 mL volumetric flask, place a sample *m* accurately weighed, of about 6.500 g of mother tincture and dilute to 20.0 mL with *glacial acetic acid R*.

Test solution. Place 1.0 mL of stock solution into a 25.0 mL volumetric flask. Add 10 mL of a mixture of 10 volumes of *methanol R* and 100 volumes of *glacial acetic acid R*. Add 10 mL of a 25.0 g/L *boric acid R* and 20.0 g/L *oxalic acid R* solution in *anhydrous formic acid R*, and dilute to 25.0 mL with *glacial acetic acid R*.

Compensation liquid of the test solution. Place 1.0 mL of stock solution into a 25.0 mL volumetric flask. Add 10 mL of a mixture of 10 volumes of *methanol R* and 100 volumes of *glacial acetic acid R.* Add 10 mL of *anhydrous formic acid R*, and dilute to 25.0 mL with *glacial acetic acid R.*

Reference stock solution. In a 100.0 mL volumetric flask, place 10.0 mg of *isoorientin R* and dilute to 100.0 mL with a mixture of 10 volumes of *methanol R* and 100 volumes of *glacial acetic acid R*. In a 50.0 mL volumetric flask, place 10.0 mL of this solution and dilute to 50.0 mL with the same solvent.

Reference solution. Place 10.0 mL of reference stock solution into a 25.0 mL volumetric flask, add 10.0 mL of a 25.0 g/L *boric acid R* and 20.0 g/L *oxalic acid R* solution in *anhydrous formic acid R.* Dilute to 25.0 mL with *glacial acetic acid R.*

Compensation liquid of the reference solution. Place 10.0.mL of reference stock solution into a 25.0 mL volumetric flask, add 10.0 mL of *anhydrous formic acid R*, and dilute to 25.0 mL with *glacial acetic acid R.*

Thirty min after the addition of the last reagent, measure the absorbance of the test solution at 410 nm, in comparison with the compensation liquid of the test solution, and the absorbance of the reference solution in comparison with the compensation liquid of the reference solution.

Calculate the percentage content *m/m* of total flavonoids, expressed as isoorientin, from the expression:

$$\frac{A_1 \times m_2 \times 40}{A_2 \times m_1}$$

A_1 = absorbance of the test solution,
A_2 = absorbance of the reference solution,
m_1 = mass of the mother tincture sample in the test solution, in grams,
m_2 = mass of *isoorientin R* sample in the reference solution, in grams.

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