ASSAY

Carry out the determination of tannins in herbal drugs (*2.8.14*). Use 1.000 g of powdered drug (180) (*2.9.12*).

01/2008:1387
corrected 6.0

ALCHEMILLA

Alchemillae herba

DEFINITION

Whole or cut, dried, flowering, aerial parts of *Alchemilla vulgaris* L. *sensu latiore*.

Content: minimum 6.0 per cent of tannins, expressed as pyrogallol ($C_6H_6O_3$; M_r 126.1) (dried drug).

IDENTIFICATION

A. The greyish-green, partly brownish-green, radical leaves which are the main part of the drug are reniform or slightly semicircular with a diameter generally up to 8 cm, seldom up to 11 cm and have 7 to 9, or 11 lobes and a long petiole. The smaller, cauline leaves, which have a pair of large stipules at the base, have 5-9 lobes and a shorter petiole or they are sessile. The leaves are densely pubescent especially on the lower surface and have a coarsely serrated margin. Young leaves are folded with a whitish-silvery pubescence; older leaves are slightly pubescent and have a finely meshed venation, prominent on the lower surface. The greyish-green or yellowish-green petiole is pubescent, about 1 mm in diameter, with an adaxial groove. The apetalous flowers are yellowish-green or light green and about 3 mm in diameter. The calyx is double with 4 small segments of the epicalyx alternating with 4 larger sepals, subacute or triangular. They are 4 short stamens and a single carpel with a capitate stigma. The greyish-green or yellowish-green stem is pubescent, more or less longitudinally wrinkled and hollow.

B. Reduce to a powder (355) (*2.9.12*). The powder is greyish-green. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters: unicellular, narrow trichomes up to 1 mm long partly tortuous, acuminate, and bluntly pointed at the apex, with thick lignified walls, somewhat enlarged and pitted at the base; fragments of leaves with 2 layers of palisade parenchyma, the upper layer of which is 2-3 times longer than the lower layer and with spongy parenchyma, containing scattered cluster crystals of calcium oxalate, up to 25 µm in diameter; leaf fragments in surface view with sinuous or wavy epidermal cells, the anticlinal walls unevenly thickened and beaded, anomocytic stomata (*2.8.3*); groups of vascular tissue and lignified fibres from the petioles and stems, the vessels spirally thickened or with bordered pits; occasional thin-walled conical trichomes, about 300 µm long; thin-walled parenchyma containing cluster crystals of calcium oxalate; spherical pollen grains, about 15 µm in diameter, with 3 distinct pores and a granular exine; occasional fragments of the ovary wall with cells containing a single crystal of calcium oxalate.

C. Thin-layer chromatography (*2.2.27*).

Test solution. To 0.5 g of the powdered drug (355) (*2.9.12*) add 5 mL of *methanol R* and heat in a water-bath at 70 °C under a reflux condenser for 5 min. Cool and filter.

Reference solution. Dissolve 1.0 mg of *caffeic acid R* and 1.0 mg of *chlorogenic acid R* in 10 mL of *methanol R*.

Plate: *TLC silica gel plate R*.

Mobile phase: *anhydrous formic acid R, water R, ethyl acetate R* (8:8:84 *V/V/V*).

Application: 20 µL of the test solution and 10 µL of the reference solution, as bands.

Development: over a path of 10 cm.

Drying: at 100-105 °C for 5 min.

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

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

Top of the plate	
	2 red fluorescent zones (chlorophyll)
Caffeic acid: a light blue florescent zone	1 or 2 intense light blue fluorescent zones
	One or several intense green or greenish-yellow fluorescent zones
Chlorogenic acid: a light blue fluorescent zone	An intense yellow or orange fluorescent zone
Reference solution	**Test solution**

TESTS

Loss on drying (*2.2.32*): maximum 10.0 per cent, determined on 1.000 g of powdered drug (355) (*2.9.12*) by drying in an oven at 105 °C for 2 h.

Total ash (*2.4.16*): maximum 12.0 per cent.

ASSAY

Carry out the determination of tannins in herbal drugs (*2.8.14*). Use 0.50 g of the powdered drug (355) (*2.9.12*).

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corrected 6.0

ALOES, BARBADOS

Aloe barbadensis

DEFINITION

Concentrated and dried juice of the leaves of *Aloe barbadensis* Miller.

Content: minimum 28.0 per cent of hydroxyanthracene derivatives, expressed as barbaloin ($C_{21}H_{22}O_9$; M_r 418.4) (dried drug).

CHARACTERS

Appearance: dark brown masses, slightly shiny or opaque with a conchoidal fracture, or brown powder.

Solubility: partly soluble in boiling water, soluble in hot ethanol (96 per cent).

IDENTIFICATION

A. Thin-layer chromatography (*2.2.27*).

Test solution. To 0.25 g of the powdered drug add 20 mL of *methanol R* and heat to boiling in a water-bath. Shake for a few minutes and decant the solution. Store at about 4 °C and use within 24 h.

Reference solution. Dissolve 25 mg of *barbaloin R* in *methanol R* and dilute to 10 mL with the same solvent.

Plate: *TLC silica gel G plate R*.

Mobile phase: *water R, methanol R, ethyl acetate R* (13:17:100 *V/V/V*).

Application: 10 µL, as bands of 20 mm by maximum 3 mm.

Development: over a path of 10 cm.

Drying: in air.

Detection A: spray with a 100 g/L solution of *potassium hydroxide R* in *methanol R* and examine in ultraviolet light at 365 nm.

Results A: the chromatogram obtained with the test solution shows in the central part a yellow fluorescent zone (barbaloin) similar in position to the zone due to barbaloin in the chromatogram obtained with the reference solution and in the lower part a light blue fluorescent zone (aloesine).

Detection B: heat at 110 °C for 5 min.

Results B: in the chromatogram obtained with the test solution, a violet fluorescent zone appears just below the zone due to barbaloin.

B. Shake 1 g of the powdered drug with 100 mL of boiling *water R*. Cool, add 1 g of *talc R* and filter. To 10 mL of the filtrate add 0.25 g of *disodium tetraborate R* and heat to dissolve. Pour 2 mL of this solution into 20 mL of *water R*. Yellowish-green fluorescence appears which is particularly marked in ultraviolet light at 365 nm.

C. To 5 mL of the filtrate obtained in identification test B add 1 mL of freshly prepared *bromine water R*. A brownish-yellow precipitate is formed and the supernatant liquid is violet.

TESTS

Loss on drying (*2.2.32*): maximum 12.0 per cent, determined on 1.000 g of the powdered drug by drying in an oven at 105 °C.

Total ash (*2.4.16*): maximum 2.0 per cent.

ASSAY

Carry out the assay protected from bright light.

Introduce 0.300 g of powdered drug (180) (*2.9.12*) into a 250 mL conical flask. Moisten with 2 mL of *methanol R*, add 5 mL of *water R* warmed to about 60 °C, mix, then add a further 75 mL of *water R* at about 60 °C and shake for 30 min. Cool, filter into a volumetric flask, rinse the conical flask and filter with 20 mL of *water R*, add the rinsings to the volumetric flask and dilute to 1000.0 mL with *water R*. Transfer 10.0 mL of this solution to a 100 mL round-bottomed flask containing 1 mL of a 600 g/L solution of *ferric chloride R* and 6 mL of *hydrochloric acid R*. Heat in a water-bath under a reflux condenser for 4 h, with the water level above that of the liquid in the flask. Allow to cool, transfer the solution to a separating funnel, rinse the flask successively with 4 mL of *water R*, 4 mL of *1 M sodium hydroxide* and 4 mL of *water R* and add the rinsings to the separating funnel. Shake the contents of the separating funnel with 3 quantities, each of 20 mL, of *ether R*. Wash the combined ether layers with 2 quantities, each of 10 mL, of *water R*. Discard the washings and dilute the organic phase to 100.0 mL with *ether R*. Evaporate 20.0 mL of the solution carefully to dryness on a water-bath and dissolve the residue in 10.0 mL of a 5 g/L solution of *magnesium acetate R* in *methanol R*. Measure the absorbance (*2.2.25*) at 512 nm using *methanol R* as the compensation liquid.

Calculate the percentage content of hydroxyanthracene derivatives, as barbaloin, from the following expression:

$$\frac{A \times 19.6}{m}$$

i.e. taking the specific absorbance of barbaloin to be 255.

A = absorbance at 512 nm,

m = mass of the substance to be examined, in grams.

STORAGE

In an airtight container.

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corrected 6.0

ALOES, CAPE

Aloe capensis

DEFINITION

Concentrated and dried juice of the leaves of various species of *Aloe*, mainly *Aloe ferox* Miller and its hybrids.

Content: minimum 18.0 per cent of hydroxyanthracene derivatives, expressed as barbaloin ($C_{21}H_{22}O_9$; M_r 418.4) (dried drug).

CHARACTERS

Appearance: dark brown masses tinged with green and having a shiny conchoidal fracture, or greenish-brown powder.

Solubility: partly soluble in boiling water, soluble in hot ethanol (96 per cent).

IDENTIFICATION

A. Examine the chromatograms obtained in the test for Barbados aloes.

Results: the chromatogram obtained with the test solution shows in the central part a yellow fluorescent zone (barbaloin) similar in position to the zone due to barbaloin in the chromatogram obtained with the reference solution and in the lower part 2 yellow fluorescent zones (aloinosides A and B) and 1 blue fluorescent zone (aloesine).

B. Shake 1 g of the powdered drug with 100 mL of boiling *water R*. Cool, add 1 g of *talc R* and filter. To 10 mL of the filtrate add 0.25 g of *disodium tetraborate R* and heat to dissolve. Pour 2 mL of the solution into 20 mL of *water R*. A yellowish-green fluorescence appears which is particularly marked in ultraviolet light at 365 nm.

C. To 5 mL of the filtrate obtained in identification test B add 1 mL of freshly prepared *bromine water R*. A yellow precipitate is formed. The supernatant liquid is not violet.

TESTS

Barbados aloes. Thin-layer chromatography (*2.2.27*).

Test solution. To 0.25 g of the powdered drug add 20 mL of *methanol R* and heat to boiling in a water-bath. Shake for a few minutes and decant the solution. Store at about 4 °C and use within 24 h.

Reference solution. Dissolve 25 mg of *barbaloin R* in *methanol R* and dilute to 10 mL with the same solvent.

Plate: *TLC silica gel G plate R*.

Mobile phase: *water R, methanol R, ethyl acetate R* (13:17:100 *V/V/V*).

Application: 10 µL, as bands of 20 mm by maximum 3 mm.

Development: over a path of 10 cm.

Drying: in air.

Detection: spray with a 100 g/L solution of *potassium hydroxide R* in *methanol R*. Heat at 110 °C for 5 min and examine in ultraviolet light at 365 nm.

Results: the chromatogram obtained with the test solution shows no violet fluorescent zone just below the zone due to barbaloin.

Loss on drying (*2.2.32*): maximum 10.0 per cent, determined on 1.000 g of the powdered drug by drying in an oven at 105 °C.

Total ash (*2.4.16*): maximum 2.0 per cent.

ASSAY

Carry out the assay protected from bright light.

Introduce 0.400 g of powdered drug (180) (*2.9.12*) into a 250 mL conical flask. Moisten with 2 mL of *methanol R*, add 5 mL of *water R* warmed to about 60 °C, mix, then add a further 75 mL of *water R* at about 60 °C and shake for 30 min. Cool,