Pelargonium root

EUROPEAN PHARMACOPOEIA 8.0

DEFINITION
Dried, usually fragmented, underground organs of Pelargonium sidoides DC and/or Pelargonium reniforme Curt. Content: minimum 2.0 per cent of tannins, expressed as pyrogallol (C₆H₆O₃; M, 126.1) (dried drug).

IDENTIFICATION
A. The root is covered with dark, partly reddish-brown, longitudinally fissured bark. The transverse section shows, underneath the cork layer, yellow or white wood, which clearly shows partly brownish medullary rays.
B. Reduce to a powder (355 (2.9.12)). The powder is brownish-red. Examine under a microscope using chloral hydrate solution R. The powder shows the following diagnostic characters: multilayer cork cells consisting of almost uniform, rectangular cells; fragments of parenchyma underneath the cork containing sclereids with a wide lumen; numerous calcium oxalate cluster crystals. Examine under a microscope using a 50 per cent V/V solution of glycerol R. The powder shows simple starch granules without striations or cracks.
C. Thin-layer chromatography (2.2.27). Test solution. To 0.5 g of the powdered herbal drug (355) (2.9.12) add 10 mL of methanol R, shake for 15 min and filter.

TESTS
Loss on drying (2.8.17): maximum 5.0 per cent, determined on 0.500 g.

ASSAY
Stock solution. To 50 mg of the extract to be examined add ethanol (60 per cent V/V) R. Shake, filter and dilute to 100.0 mL with ethanol (60 per cent V/V) R.

Test solution. Introduce 5.0 mL of the stock solution into a round-bottomed flask and evaporate to dryness under reduced pressure. Take up the residue with 8 mL of a mixture of 10 volumes of methanol R and 100 volumes of glacial acetic acid R and transfer into a 25 mL volumetric flask. Rinse the round-bottomed flask with 3 mL of a mixture of 10 volumes of methanol R and 100 volumes of glacial acetic acid R and transfer into the 25 mL volumetric flask. Add 10.0 mL of a solution containing 25.0 g/L of boric acid R and 20.0 g/L of oxalic acid R in anhydrous formic acid R and dilute to 25.0 mL with anhydrous acetic acid R.

Compensation liquid. Introduce 5.0 mL of the stock solution into a round-bottomed flask and evaporate to dryness under reduced pressure. Take up the residue with 8 mL of a mixture of 10 volumes of methanol R and 100 volumes of glacial acetic acid R and transfer into a 25 mL volumetric flask. Rinse the round-bottomed flask with 3 mL of a mixture of 10 volumes of methanol R and 100 volumes of glacial acetic acid R and transfer into the 25 mL volumetric flask. Add 10.0 mL of anhydrous formic acid R and dilute to 25.0 mL with anhydrous acetic acid R.

After 30 min, measure the absorbance (2.2.25) of the test solution at 401 nm. Calculate the percentage content of total flavonoids, expressed as vitexin, from the following expression:

\[
A \times \frac{0.8}{m}
\]

i.e. taking the specific absorbance of vitexin to be 628.

\[A = \text{absorbance at 401 nm},\]

\[m = \text{mass of the extract to be examined, in grams}.\]

PELARGONIUM ROOT

Pelargonii radix

Top of the plate

<table>
<thead>
<tr>
<th>Hyperoside: a yellowish-orange fluorescent zone</th>
<th>A green fluorescent zone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rutin: a yellowish-orange fluorescent zone</td>
<td>A yellow fluorescent zone</td>
</tr>
<tr>
<td></td>
<td>A green fluorescent zone</td>
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</tbody>
</table>

Reference solution Test solution

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See the information section on general monographs (cover pages)
**Results:** see below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other blue fluorescent zones may be present in the chromatogram obtained with the test solution.

<table>
<thead>
<tr>
<th>Top of the plate</th>
<th>Reference solution</th>
<th>Test solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scopoletin: a very bright blue fluorescent zone</td>
<td>A blue fluorescent zone</td>
<td>A weak blue fluorescent zone (scopoletin)</td>
</tr>
<tr>
<td>Esculin: a very bright blue fluorescent zone</td>
<td>A blue fluorescent zone</td>
<td>A weak blue fluorescent zone</td>
</tr>
<tr>
<td></td>
<td>A blue fluorescent zone</td>
<td></td>
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</tbody>
</table>

**TESTS**

**Loss on drying** (2.2.32): maximum 12.0 per cent, determined on 1.000 g of the powdered herbal drug (355) (2.9.12) by drying in an oven at 105 °C.

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

**Ash insoluble in hydrochloric acid** (2.8.1): maximum 3.0 per cent.

**ASSAY**

**Tannins** (2.8.14). Use 0.750 g of the powdered herbal drug (180) (2.9.12).

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**PEPPER**

*Piperis fructus*

**DEFINITION**

Dried, ripe or nearly ripe fruit of *Piper nigrum* L. with an unbroken pericarp (black pepper) or with the outer layers of the pericarp removed (white pepper).

**Content:**

- **essential oil**: minimum 25 mL/kg (anhydrous drug);
- **piperine** (C_{17}H_{19}NO_{3}; M, 285.3): minimum 3.0 per cent (anhydrous drug).

**IDENTIFICATION**

**A. White pepper.** Spheroid berries, 3-5 mm in diameter, slightly flattened at one pole and with a small protuberance at the other, with smooth, externally matt, brownish-grey, greyish-white or pale yellowish-white surface, with numerous pale, linear striations between apex and base.

**Black pepper.** Spheroid berries, 3-6 mm in diameter, externally blackish-brown, with raised reticulare wrinkles, bearing fine remains of the style at the apex and a scar of the peduncle at the base. The texture is hard, the epicarp can be stripped, the endocarp is greyish-white or pale yellow. The fracture is greyish-white, starchy, possessing a small space at the centre.

**B. Microscopic examination (2.8.23).**

**White pepper.** The powder is light grey. Examine under a microscope using *chloral hydrate solution* R. The powder shows the following diagnostic characters (Figure 2477.-1): fragments of the endocarp in surface view, consisting of more or less polygonal sclereids about 20-30 μm in diameter, which have irregularly thickened walls [Ac, C, Fa] and which may or may not be associated with the testa [A, F], consisting of a layer of indistinct, reddish-brown pigmented cells constituting the ‘pigmented layer’ [Ab, Fb] and a layer of very thin-walled polygonal cells constituting the ‘hyaline layer’ [Aa]; fragments of the endocarp, in transverse section [G], showing sclereids with thickened inner walls on the 3 lower sides [Ga], usually associated with the testa (pigmented layer [Gb] and hyaline layer [Gc]); fragments of the parenchyma of the mesocarp [D] containing large oil cells 50-75 μm in diameter [Da]; numerous thin-walled, ovoid or polygonal cells of the parenchyma of the seed [E]; rare, elongated sclereids, with thickened walls, from the fruit peduncle [B]; a few fragments of vascular tissue with narrow spiral vessels [J]. Examine under a microscope using a 50 per cent *V/V* solution of *glycerol* R. Rounded, compound starch granules [H], about 30 μm in diameter, made up of tiny individual granules, ovoid or polyhedral by compression, free [Hb] or included in the parenchymatous cells of the seed [Ha].

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

**Test solution.** To 0.5 g of the powdered herbal drug (355) (2.9.12) add 5 mL of *methanol* R. Sonicate for 10 min, centrifuge and use the supernatant.

**Reference solution.** Dissolve 10 mg of *borneol* R and 15 mg of *piperine* R in 10 mL of *methanol* R.

**Plate:** TLC silica gel *F_{254}*, plate R (5-40 μm) [or TLC silica gel *F_{254}*, plate R (2-10 μm)].

**Mobile phase:** ethyl acetate R, cyclohexane R (30:50 *V/V*).