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corrected 6.0**RHATANY TINCTURE****Ratanhiaie tinctura****DEFINITION**

Tincture produced from *Rhatany root* (0289).

Content: minimum 1.0 per cent *m/m* of tannins, expressed as pyrogallol (C₆H₆O₃; M_r 126.1).

PRODUCTION

The tincture is produced from 1 part of the herbal drug and 5 parts of ethanol (70 per cent *V/V*) by a suitable procedure.

CHARACTERS

Appearance: reddish-brown liquid.

IDENTIFICATION

Thin-layer chromatography (2.2.27).

Test solution. The tincture to be examined.

Reference solution. Dissolve 5 mg of *thymol R* and 20 mg of *dichlorophenolindophenol, sodium salt R* in 20 mL of *ethanol* (60 per cent *V/V*) *R*.

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

Mobile phase: *methylene chloride R*.

Application: 10 µL [or 4 µL] as bands of 8 mm [or 8 mm].

Development: over a path of 15 cm [or 6 cm].

Drying: in air.

Detection: treat with a 5 g/L solution of *fast blue B salt R*, allow to dry in air and 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	
Thymol: a brownish-yellow zone	A violet zone
	An orange zone
	A bluish-grey zone
Dichlorophenolindophenol: a greyish-blue zone	An intense violet zone
Reference solution	Test solution

TESTS

Ethanol (2.9.10): 63 per cent *V/V* to 67 per cent *V/V*.

Methanol and 2-propanol (2.9.11): maximum 0.05 per cent *V/V* of methanol and maximum 0.05 per cent *V/V* of 2-propanol.

ASSAY

Tannins (2.8.14). Use 2.500 g of the tincture to be examined.

RHUBARB**Rhei radix****DEFINITION**

Rhubarb consists of the whole or cut, dried underground parts of *Rheum palmatum* L. or of *Rheum officinale* Baillon or of hybrids of these two species or of a mixture. The underground parts are often divided; the stem and most of the bark with the rootlets are removed. It contains not less than 2.2 per cent of hydroxyanthracene derivatives, expressed as rhein (C₁₅H₈O₆, M_r 284.2), calculated with reference to the dried drug.

CHARACTERS

Characteristic, aromatic odour.

IDENTIFICATION

- A. The appearance is variable: disc-shaped pieces up to 10 cm in diameter and 1 cm to 5 cm in thickness; cylindrical pieces; oval or planoconvex pieces. The surface has a pinkish tinge and is usually covered with a layer of brownish-yellow powder. It shows, especially after moistening, a reticulum of darker lines. This structure causes the marbled appearance of the drug. The fracture is granular. The transverse section of the rhizome shows a narrow outer zone of radiating brownish-red lines. These medullary rays are crossed perpendicularly by a dark cambial ring. Inside this zone is a ring of small star-spot formations of anomalous vascular bundles. The root shows a more radiate structure.
- B. Microscopic examination (2.8.23). The powder is orange to brownish-yellow. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters: large calcium oxalate cluster crystals, which may measure more than 100 µm, and their fragments; reticulately thickened non-lignified vessels measuring up to 175 µm. Numerous groups of rounded or polygonal, thin-walled parenchyma cells. Sclereids and fibres are absent. Examine under a microscope using a 50 per cent *V/V* solution of *glycerol R*. The powder shows simple, rounded or compound (2 to 4) starch granules with a star-shaped hilum.

- C. Examine by thin-layer chromatography (2.2.27), using a suitable silica gel as the coating substance.

Test solution. Heat 50 mg of the powdered herbal drug (180) (2.9.12) in a water-bath for 15 min with a mixture of 1 mL of *hydrochloric acid R* and 30 mL of *water R*. Allow to cool and shake the liquid with 25 mL of *ether R*. Dry the ether layer over *anhydrous sodium sulfate R* and filter. Evaporate the ether layer to dryness and dissolve the residue in 0.5 mL of *ether R*.

Reference solution. Dissolve 5 mg of *emodin R* in 5 mL of *ether R*.

Apply separately to the plate as bands 20 µL of each solution. Develop over a path of 10 cm using a mixture of 1 volume of *anhydrous formic acid R*, 25 volumes of *ethyl acetate R* and 75 volumes of *light petroleum R*. Allow the plate to dry in air and examine in ultraviolet light at 365 nm. The chromatogram obtained with the reference solution shows in its central part a zone of orange fluorescence (emodin). The chromatogram obtained with the test solution shows: a zone due to emodin; above the emodin zone, two zones of similar fluorescence (physcione and chrysophanol, in order of increasing *R_F* value); below the emodin zone, also two zones of similar fluorescence

(rhein and aloe-emodin, in order of decreasing R_f value). Spray with a 100 g/L solution of *potassium hydroxide R* in *methanol R*. All the zones become red to violet.

- D. To about 50 mg of the powdered herbal drug (180) (2.9.12) add 25 mL of *dilute hydrochloric acid R* and heat the mixture on a water-bath for 15 min. Allow to cool, shake with 20 mL of *ether R* and discard the aqueous layer. Shake the ether layer with 10 mL of *dilute ammonia R1*. The aqueous layer becomes red to violet.

A = absorbance at 515 nm,
 m = mass of the herbal drug used, in grams.

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TESTS

Rheum rhaponticum. Examine by thin-layer chromatography (2.2.27), using *silica gel G R* as the coating substance.

Test solution. To 0.2 g of the powdered herbal drug (180) (2.9.12) add 2 mL of *methanol R* and boil for 5 min under a reflux condenser. Allow to cool and filter. Use the filtrate as the test solution.

Reference solution. Dissolve 10 mg of *rhaponticin R* in 10 mL of *methanol R*.

Apply separately to the plate, as bands not more than 20 mm by 3 mm, 20 μ L of each solution. Develop over a path of 12 cm using a mixture of 20 volumes of *methanol R* and 80 volumes of *methylene chloride R*. Allow the plate to dry in air and spray with *phosphomolybdic acid solution R*. The chromatogram obtained with the test solution does not show a blue zone near the line of application (*rhaponticin*) corresponding to the zone in the chromatogram obtained with the reference solution.

Loss on drying (2.2.32). Not more than 12.0 per cent, determined on 1.000 g of the powdered herbal drug (180) (2.9.12) by drying in an oven at 105 °C.

Total ash (2.4.16). Not more than 12.0 per cent.

Ash insoluble in hydrochloric acid (2.8.1). Not more than 2.0 per cent.

ASSAY

Carry out the assay protected from bright light.

Introduce 0.100 g of the powdered herbal drug (180) (2.9.12) into a 100 mL flask. Add 30.0 mL of *water R*, mix and weigh. Heat in a water-bath under a reflux condenser for 15 min. Allow to cool, add 50 mg of *sodium hydrogen carbonate R*, weigh and adjust to the original mass with *water R*. Centrifuge and transfer 10.0 mL of the liquid to a 100 mL round-bottomed flask with a ground-glass neck. Add 20 mL of *ferric chloride solution R1* and mix. Heat under a reflux condenser on a water-bath for 20 min, add 1 mL of *hydrochloric acid R* and heat for a further 20 min, shaking frequently. Cool, transfer to a separating funnel and shake with three quantities, each of 25 mL, of *ether R* previously used to rinse the flask. Combine the ether extracts and wash with two quantities, each of 15 mL, of *water R*. Filter the ether extracts through a plug of absorbent cotton into a volumetric flask and dilute to 100.0 mL with *ether R*. Evaporate 10.0 mL 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 515 nm, using *methanol R* as the compensation liquid.

Calculate the percentage content of rhein from the expression:

$$\frac{A \times 0.64}{m}$$

i.e. taking the specific absorbance of rhein to be 468, calculated on the basis of the specific absorbance of barbaloin.

RIBWORT PLANTAIN

Plantaginis lanceolatae folium

DEFINITION

Whole or fragmented, dried leaf and scape of *Plantago lanceolata L. s.l.*

Content: minimum 1.5 per cent of total *ortho*-dihydroxycinnamic acid derivatives expressed as acteoside ($C_{29}H_{36}O_{15}$; M_r 624.6) (dried drug).

IDENTIFICATION

- A. The leaf is up to 30 cm long and 4 cm wide, yellowish-green to brownish-green, with a prominent, whitish-green, almost parallel venation on the abaxial surface. It consists of a lanceolate lamina narrowing at the base into a channelled petiole. The margin is indistinctly dentate and often undulate. It has 3, 5 or 7 primary veins, nearly equal in length and running almost parallel. Hairs may be almost absent, sparsely scattered or sometimes abundant, especially on the lower surface and over the veins. The scape is brownish-green, longer than the leaves, 3-4 mm in diameter and is deeply grooved longitudinally, with 5-7 conspicuous ribs. The surface is usually covered with fine hairs.
- B. Microscopic examination (2.8.23). The powder is yellowish-green. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters (Figure 1884.-1): fragments of epidermis, composed of cells with irregularly sinuous anticlinal walls, the fragments of the upper epidermis of the lamina (surface view [H], transverse section [D]) are accompanied by palisade parenchyma [Da, Ha], and those of the lower epidermis (surface view [G]) show stomata (2.8.3) mostly of the diacytic type [Ga] and sometimes of the anomocytic type [Gb]; the multicellular, uniseriate, conical covering trichomes are highly characteristic, whole [C] or mostly fragmented [A], with a basal cell larger than the other epidermal cells followed by a short cell supporting 2 or more elongated cells with the lumen narrow and variable, occluded at intervals corresponding to slight swellings in the trichome and giving a jointed appearance, the terminal cell has an acute apex and a filiform lumen; the glandular trichomes have a unicellular, cylindrical stalk and a multicellular, elongated, conical head consisting of several rows of small cells and a single terminal cell [B, Gc]; dense groups of lignified fibro-vascular tissue with narrow, spirally and annularly thickened vessels and slender, moderately thickened fibres [F]; fragments of the scape [E] with cells with thickened walls and a coarsely ridged cuticle, stomata [Ec], multicellular, uniseriate covering trichomes [Eb] and glandular trichomes [Ea] of the type previously described.