Suppl I, JP XVII (2017)

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Add the following:

Goreisan Extract

五苓散エキス

Goreisan Extract contains not less than 0.3 mg and not more than 1.2 mg (for preparation prescribed 1.5 g of Cinnamon Bark) or not less than 0.4 mg and not more than 1.6 mg (for preparation prescribed 2 g of Cinnamon Bark) or not less than 0.5 mg and not more than 2.0 mg (for preparation prescribed 2.5 g of Cinnamon Bark) or not less than 0.6 mg and not more than 2.4 mg (for preparation prescribed 3 g of Cinnamon Bark) of (*E*)-cinnamic acid, per extract prepared with the amount specified in the Method of preparation.

Method of preparation

	1)	2)	3)	4)	5)
Alisma Tuber	5 g	6 g	6 g	4 g	6 g
Polyporus Sclerotium	3 g	4.5 g	4.5 g	3 g	4.5 g
Poria Sclerotium	3 g	4.5 g	4.5 g	3 g	4.5 g
Atractylodes Rhizome	3 g	4.5 g	4.5 g	—	—
Atractylodes Lancea Rhizome	—	—	—	3 g	4.5 g
Cinnamon Bark	2 g	2.5 g	3 g	1.5 g	3 g

Prepare a dry extract or viscous extract as directed under Extracts, according to the prescription 1) to 5), using the crude drugs shown above.

Description Goreisan Extract occurs as a light red-brown to light brown powder, or a black-brown viscous extract. It has a characteristic odor, and a slightly sweet first, bitter, then acrid taste.

Identification (1) Weigh exactly 2.0 g of the dry extract (or 6.0 g of the viscous extract), add 20 mL of water and 2 mL of ammonia solution (28), and shake. Add 20 mL of a mixture of hexane and ethyl acetate (20:1), shake, centrifuge, and separate the supernatant liquid. Add 20 mL of a mixture of hexane and ethyl acetate (20:1) to the residue, shake, centrifuge, and separate the supernatant liquid. Combine these supernatant liquids, evaporate the solvent under reduced pressure, add exactly 2 mL of methanol to the residue, and use this solution as the sample solution. Separately, weigh exactly 10 mg of alisol A for thin-layer chromatography, and dissolve in exactly 10 mL of methanol. Pipet 1 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 2 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a

mixture of ethyl formate, water and formic acid (30:1:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid-acetic acid TS on the plate, heat the plate at 105°C for 5 minutes, allow to cool, and examine under ultraviolet light (main wavelength: 365 nm): one of the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow fluorescent spot from the standard solution, and it is larger and more intense than the spot from the standard solution (Alisma Tuber).

(2) For preparation prescribed Atractylodes Rhizome—Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the solvent under reduced pressure, add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of atractylenolide III for thin-layer chromatography in 2 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (2:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 1-naphthol-sulfuric acid TS on the plate, heat the plate at 105°C for 5 minutes, and allow to cool: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the red to red-purple spot from the standard solution (Atractylodes Rhizome).

(3) For preparation prescribed Atractylodes Lancea Rhizome—Shake 2.0 g of the dry extract (or 6.0 g of the viscous extract) with 10 mL of water, add 25 mL of hexane, and shake. Separate the hexane layer, and evaporate the solvent under reduce pressure, add 0.5 mL of hexane to the residue, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of hexane and acetone (7:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a dark purple spot is observed at an *R*f value of about 0.5. The spot shows a greenish brown color after being sprayed evenly 4-dimethylaminobenzaldehyde TS for spraying, heated at 105°C for 5 minutes, and allowed to cool (Atractylodes Lancea Rhizome).

(4) Perform the test according to the following i) or ii) (Cinnamon Bark).

i) Put 10 g of the dry extract (or 30 g of the viscous extract) in a 300-mL hard-glass flask, add 100 mL of water and 1 mL of silicone resin, connect an apparatus for essential oil determination, and heat to boil under a reflux condenser. The graduated tube of the apparatus is to be previously filled with water to the standard line, and 2 mL of hexane is added to the graduated tube. After heating under reflux for 1 hour, separate the hexane layer, and use the layer as the sample solution. Separately, dissolve 1 mg of (*E*)-cinnamaldehyde for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 50 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane, diethyl ether and methanol (15:5:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 2,4-dinitrophenylhydradine TS on the plate: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-orange spot from the standard solution.

ii) Shake 2.0 g of the dry extract (or 6.0 g of the viscous extract) with 10 mL of water, add 5 mL of hexane, and shake. Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of (*E*)-2-methoxycinnamaldehyde for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (2:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-white fluorescent spot from the standard solution.

Purity **(1)** Heavy metals <*1.07*>—Prepare the test solution with 1.0 g of the dry extract (or an amount of the viscous extract, equivalent to 1.0 g of the dried substance) as directed under Extracts (4), and perform the test (not more than 30 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.67 g of the dry extract (or an amount of the viscous extract, equivalent to 0.67 g of the dried substance) according to Method 3, and perform the test (not more than 3 ppm).

Loss on drying <*2.41*> The dry extract: Not more than 10.0% (1 g, 105°C, 5 hours).

The viscous extract: Not more than 66.7% (1 g, 105°C, 5 hours).

Total ash <*5.01*> Not more than 10.0%, calculated on the dried basis.

Assay Conduct this procedure using light-resistant vessels. Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of (*E*)-cinnamic acid for assay, and dissolve in diluted methanol (1 in 2) to make exactly 100 mL. Pipet 10 mL of this solution, add diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatog-

raphy <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of (*E*)-cinnamic acid in each solution.

$$\text{Amount (mg) of (}E\text{)-cinnamic acid} = M_S \times A_T/A_S \times 1/20$$

M_S: Amount (mg) of (*E*)-cinnamic acid for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 273 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (750:250:1).

Flow rate: 1.0 mL per minute (the retention time of (*E*)-cinnamic acid is about 12 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of (*E*)-cinnamic acid are not less than 5000 and not more than1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of (*E*)-cinnamic acid is not more than 1.5%.

Containers and storage Containers—Tight containers.