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Yokukansan Extract

抑肝散エキス

Yokukansan Extract contains not less than 0.15 mg of total alkaloids (rhynchophylline and hirsutine), not less than 0.6 mg and not more than 2.4 mg of saikosaponin b₂, and not less than 12 mg and not more than 36 mg of glycyrrhizic acid (C₄₂H₆₂O₁₆: 822.93), per extract prepared with the amount specified in the Method of preparation.

Method of preparation

	1)	2)
Japanese Angelica Root	3 g	3 g
Uncaria Hook	3 g	3 g
Cnidium Rhizome	3 g	3 g
Atractylodes Rhizome	4 g	—
Atractylodes Lancea Rhizome	—	4 g
Poria Sclerotium	4 g	4 g
Bupleurum Root	2 g	2 g
Glycyrrhiza	1.5 g	1.5 g

Prepare a dry extract or viscous extract as directed under Extracts, according to the prescription 1) or 2), using the crude drugs shown above.

Description Yokukansan Extract is a light brown to grayish brown powder or a blackish brown viscous extract. It has a slightly odor, and a slightly bitter and acid taste.

Identification (1) To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 10 mL of water, shake, then add 10 mL of diethyl ether, shake, and centrifuge. Separate the diethyl ether layer, add 10 mL of sodium hydroxide TS, shake, centrifuge, separate the diethyl ether layer, and use this layer as the sample solution. Separately, dissolve 1 mg of (Z)-ligustilide for thin-layer chromatography in 10 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 10 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 butyl acetate and hexane (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 spot among the several spots obtained from the sample solution has the same color tone and R_f value with the bluish white fluorescent spot obtained from the standard solution (Japanese Angelica Root; Cnidium Rhizome).

(2) To 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 TS, shake, then add 20 mL of diethyl ether, shake, and separate the diethyl ether layer. Evaporate the solvent under reduced pressure, add 1 mL of methanol to the residue, and use the solution as the sample solution. Separately, dissolve 1 mg each of rhynchophyllin for thin-layer chromatography and hirsutine 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 with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, 1-propanol, water and acetic acid (100) (7:5:4:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): at least one of the spot among the several spots obtained from the sample solution has the same color tone and R_f value with one of the two dark violet spots obtained from the standard solution (Uncaria Hook).

(3) For preparation prescribed Atractylodes Rhizome—To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of water, shake, then add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the solvent under reduced pressure, dissolve the residue in 2 mL of diethyl ether, and use this solution as the sample solution. Separately, dissolve 1 mg of atractylenoide 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 μ L 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 acetate and hexane (1:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 1-naphthol-sulfuric acid TS on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots obtained from the sample solution has the same color tone and *R_f* value with the red to purple-red spot obtained from the standard solution (*Atractylodes Rhizome*).

(4) For preparation prescribed *Atractylodes Lancea Rhizome*—To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 10 mL of water, shake, then add 25 mL of hexane, and shake. Separate the hexane layer, evaporate the solvent under reduced pressure, dissolve the residue in 2 mL of hexane, 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 μ L 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 violet spot is observed at an *R_f* value of about 0.4. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat at 105°C for 5 minutes, and allow to cool: the spot exhibits a greenish brown color (*Atractylodes Lancea Rhizome*).

(5) To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of water, shake, then add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of saikosaponin b₂ 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 10 μ L of the sample solution and 2 μ L of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and water (8:2:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R_f* value with the yellow fluorescent spot obtained from the standard solution (*Bupleurum Root*).

(6) To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of water, shake, then add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of liquiritin 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 5 μ L 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 acetate, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R_f* value with the yellow-brown spot obtained from the standard solution (*Glycyrrhiza*).

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 less than 10.0%, calculated on the dried basis.

Assay (1) Total alkaloids (rhyncophylline and hirsutine)—Weigh accurately about 1 g of the dry extract (or an amount of the viscous extract, equivalent to about 1 g of the dried substance), add 20 mL of diethyl ether, shake, then add 3 mL of 1 mol/L hydrochloric acid TS and 7 mL of water, and shake for 10 minutes, centrifuge, and remove the diethyl ether layer. To the aqueous layer add 20 mL of diethyl ether, and proceed in the same manner as above. To the resultant aqueous layer add 10 mL of sodium hydroxide TS and 20 mL of diethyl ether, shake for 10 minutes, centrifuge, and separate the supernatant liquid. To the residue add 20 mL of diethyl ether, and repeat the process above two more times. Combine all the supernatant liquids, evaporate the solvent under reduced pressure at not more than 40°C, dissolve the residue in the mobile phase to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 5 mg each of rhyncophylline for assay and hirsutine for assay, dissolve in a mixture of methanol and diluted acetic acid (7:3) to make exactly 100 mL. Pipet 10 mL of this solution, add the mixture of methanol and diluted acetic acid (7:3) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, *A_{TR}* and *A_{TH}*, and *A_{SR}* and *A_{SH}*, of rhyncophylline and hirsutine in each solution.

Amount (mg) of total alkaloids (rhyncophylline and hirsutine)

$$= (M_{SR} \times A_{TR}/A_{SR} + M_{SH} \times A_{TH}/A_{SH}) \times 1/50$$

M_{SR}: Amount (mg) of rhyncophylline for assay taken

M_{SH}: Amount (mg) of hirsutine for assay taken

Operation conditions—

Detector: An ultraviolet absorption photometer (wavelength: 245 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 μ m in particle diameter).

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

Mobile phase: To 1 g of sodium lauryl sulfate add 600 mL of methanol, shake, then add 400 mL of water and 1 mL of phosphoric acid.

Flow rate: 1.0 mL per minute (the retention times of rhyncophylline and hirsutine are about 17 minutes and about 47 minutes, respectively).

Systemic suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peaks of rhyncophylline and hirsutine are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of rhyncophylline and hirsutine is not more than 1.5%, respectively.

(2) Saikosaponin b_2 —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 20 mL of diethyl ether and 10 mL of water, and shake for 10 minutes. After centrifugation, remove the upper layer, add 20 mL of diethyl ether, proceed in the same manner as above, and remove the upper layer. To the resultant aqueous layer add 10 mL of methanol, shake for 30 minutes, centrifuge, and separate the supernatant liquid. To the residue add 20 mL of diluted methanol (1 in 2), shake for 5 minutes, centrifuge, separate the supernatant liquid, combine all the supernatant liquids, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the sample solution. Use saikosaponin b_2 standard TS for assay as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of saikosaponin b_2 in each solution.

Amount (mg) of saikosaponin b_2 = $C_S \times A_T/A_S \times 50$

C_S : Concentration (mg/mL) of saikosaponin b_2 in saikosaponin b_2 standard TS for assay

Operation conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 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 μ m in particle diameter).

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

Mobile phase: A mixture of 0.05 mol/L sodium dihydrogen phosphate TS and acetonitrile (5:3).

Flow rate: 1.0 mL per minute (the retention time of saikosaponin b_2 is about 12 minutes).

Systemic suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of saikosaponin b_2 are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of saikosaponin b_2 is not more than 1.5%.

(3) Glycyrrhizic acid—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 Glycyrrhizic Acid RS (separately determine the water <2.48> by coulometric titration, using 10 mg), dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of glycyrrhizic acid in each solution.

Amount (mg) of glycyrrhizic acid ($C_{42}H_{62}O_{16}$)
= $M_S \times A_T/A_S \times 1/2$

M_S : Amount (mg) of Glycyrrhizic Acid RS taken, calculated on the anhydrous basis

Operation conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 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 μ m in particle diameter).

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

Mobile phase: A mixture of diluted acetic acid (31) (1 in 15) and acetonitrile (13:7).

Flow rate: 1.0 mL per minute (the retention time of glycyrrhizic acid is about 12 minutes).

Systemic suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of glycyrrhizic acid are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glycyrrhizic acid is not more than 1.5%.

Containers and storage Containers—Tight containers.

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