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(2016)**Kamishoyosan Extract**

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Kamishoyosan Extract contains not less than 28 mg and not more than 84 mg of paeoniflorin ($C_{23}H_{28}O_{11}$: 480.46), not less than 25 mg and not more than 75 mg of geniposide, and not less than 12 mg and not more than 36 mg (for preparation prescribed 1.5 g of Glycyrrhiza) or not less than 16 mg and not more than 48 mg (for preparation prescribed 2 g of Glycyrrhiza) of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), per extract prepared with the amount specified in the Method of preparation.

Method of preparation

	1)	2)	3)	4)	5)	6)
Japanese Angelica						
Root	3 g	3 g	3 g	3 g	3 g	3 g
Peony Root	3 g	3 g	3 g	3 g	3 g	3 g
Atractylodes						
Rhizome	3 g	—	3 g	—	3 g	3 g
Atractylodes Lancea						
Rhizome	—	3 g	—	3 g	—	—
Poria Sclerotium	3 g	3 g	3 g	3 g	3 g	3 g
Bupleurum Root	3 g	3 g	3 g	3 g	3 g	3 g
Moutan Bark	2 g	2 g	2 g	2 g	2 g	2 g
Gardenia Fruit	2 g	2 g	2 g	2 g	2 g	2 g
Glycyrrhiza	2 g	2 g	1.5 g	1.5 g	1.5 g	1.5 g
Ginger	1 g	1 g	1 g	1 g	1.5 g	0.5 g
Mentha Herb	1 g	1 g	1 g	1 g	1 g	1 g

Prepare a dry extract or viscous extract as directed under Extracts, according to the prescription 1) to 6), using the crude drugs shown above.

Description Kamishoyosan Extract occurs as a yellow-brown to brown powder or blackish brown viscous extract. It has slightly a characteristic odor, and a sweet, slightly hot, then bitter 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 5 mL of diethyl ether, shake, centrifuge, and use the supernatant liquid 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 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 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots from the sample solution has the same color tone and R_f value with the bluish white fluorescent spot from the standard solution (Japanese Angelica Root).

(2) To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 10 mL of water, shake, then add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of albiflorin 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 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 ammonia solution (28) (6:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS 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 from the sample solution has the same color tone and *R_f* value with the orange fluorescent spot from the standard solution (Peony Root).

(3) For preparation prescribed *Atractylodes 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 5 mL of diethyl ether, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of atractylenolide III 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 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 10 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 from the sample solution has the same color tone and *R_f* value with the red spot 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. Take the hexane layer, add anhydrous sodium sulfate to dry, and filter. Evaporate the filtrate under reduced pressure, add 2 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 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 10 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 0.4. The spot shows a greenish brown color after being sprayed with 4-dimethylaminobenzaldehyde TS for spraying, heated at 105°C for 5 minutes, and allowed to cool (*Atractylodes Lancea Rhizome*).

(5) To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 10 mL of sodium hydroxide TS, shake, then add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of saikosaponin *b*₂ 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 10 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 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 10 mL of water, shake, then add 15 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, add 1 mL of diethyl ether to the residue, and use this solution as the sample solution.

Separately, dissolve 1 mg of paeonol 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 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 diethyl ether (5:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots from the sample solution has the same color tone and *R_f* value with the orange spot from the standard solution (*Moutan Bark*).

(7) To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 10 mL of water, shake, then add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of geniposide 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 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 ammonia solution (28) (6:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots from the sample solution has the same color tone and *R_f* value with the purple spot from the standard solution (*Gardenia Fruit*).

(8) To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 10 mL of water, shake, then add 5 mL of 1-butanol, 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 10 μ L of the sample solution and 5 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, methanol and water (20:3:2) to a distance of about 10 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 from the sample solution has the same color tone and *R_f* value with the yellow-brown spot from the standard solution (*Glycyrrhiza*).

(9) To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 10 mL of water, shake, then add 5 mL of diethyl ether, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of [6]-gingerol 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 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 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots from the sample solution has the same color tone and *R_f* value with the blue-green spot from the standard solution (*Ginger*).

(10) To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 10 mL of diluted phosphoric acid (1 in 30), shake, then add 15 mL of ethyl acetate, shake, centrifuge, and use the supernatant liquid as the sample solution. Sepa-

rately, shake 0.2 g of powdered mentha herb with 10 mL of diluted phosphoric acid (1 in 30), add 15 mL of ethyl acetate, shake, centrifuge, and use the supernatant liquid as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 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, water and formic acid (10:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly vanillin-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 from the sample solution has the same color tone and R_f value with the red-purple spot (around R_f 0.6) from the standard solution (Mentha Herb).

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 the 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 9.0% (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 (1) Paeoniflorin—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 Paeoniflorin RS (separately determine the water <2.48> by coulometric titration, using 10 mg), and 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 paeoniflorin in each solution.

$$\begin{aligned} \text{Amount (mg) of paeoniflorin (C}_{23}\text{H}_{28}\text{O}_{11}) \\ = M_S \times A_T / A_S \times 1/2 \end{aligned}$$

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

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 232 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 20°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (850:150:1).

Flow rate: 1.0 mL per minute (the retention time of paeoniflorin is about 9 minutes).

System suitability—

System performance: Dissolve 1 mg each of Paeoniflorin RS and albiflorin in diluted methanol (1 in 2) to make 10 mL. When the procedure is run with 10 μ L of this solution under the above operating conditions, albiflorin and

paeoniflorin are eluted in this order with the resolution between these peaks being not less than 2.5.

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 paeoniflorin is not more than 1.5%.

(2) Geniposide—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 geniposide for assay, 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 geniposide in each solution.

$$\text{Amount (mg) of geniposide} = M_S \times A_T / A_S \times 1/2$$

M_S : Amount (mg) of geniposide for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 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 water, acetonitrile and phosphoric acid (900:100:1).

Flow rate: 1.0 mL per minute (the retention time of geniposide is about 10 minutes).

System 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 geniposide 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 geniposide 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.

$$\begin{aligned} \text{Amount (mg) of glycyrrhizic acid (C}_{42}\text{H}_{62}\text{O}_{16}) \\ = M_S \times A_T / A_S \times 1/2 \end{aligned}$$

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

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter

ter 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).

System 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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