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Kamikihito Extract

加味帰脾湯エキス

Kamikihito Extract contains not less than 0.8 mg and not more than 3.2 mg of saikosaponin b₂, not less than 27 mg and not more than 81 mg of geniposide, and not less than 6 mg and not more than 18 mg of glycyrrhizic acid (C₄₂H₆₂O₁₆: 822.93), per extract prepared with the amount specified in the Method of preparation.

Method of preparation

	1)	2)	3)	4)
Ginseng	3 g	3 g	3 g	3 g
Atractylodes Rhizome	3 g			3 g
Atractylodes Lancea Rhizome		3 g	3 g	
Poria Sclerotium	3 g	3 g	3 g	3 g
Jujube Seed	3 g	3 g	3 g	3 g
Longan Aril	3 g	3 g	3 g	3 g
Astragalus Root	2 g	3 g	2 g	3 g
Japanese Angelica Root	2 g	2 g	2 g	2 g
Polygala Root	1.5 g	2 g	1 g	2 g
Bupleurum Root	3 g	3 g	3 g	3 g
Gardenia Fruit	2 g	2 g	2 g	2 g
Glycyrrhiza	1 g	1 g	1 g	1 g
Saussurea Root	1 g	1 g	1 g	1 g
Jujube	1.5 g	2 g	1 g	2 g
Ginger	0.5 g	1 g	1 g	0.5 g
Moutan Bark				2 g

Prepare a dry extract or viscous extract as directed under Extracts, according to the preparation 1) to 4), using the crude drugs shown above. Or, prepare a dry extract by adding Light Anhydrous Silicic Acid to an extractive prepared as directed under Extracts, according to the preparation 2, using the crude drugs shown above.

Description Kamikihito Extract is a light yellow-brown to brown powder or black-brown viscous extract. It has a slightly odor, and a slightly sweet, hot and bitter taste.

Identification (1) To 3.0 g of the dry extract (or 9.0 g of the viscous extract) add 15 mL of sodium hydroxide TS, shake, centrifuge, and separate the supernatant liquid. To the liquid add 10 mL of 1-butanol, shake, centrifuge, and separate the 1-butanol layer. To this layer add 10 mL of water, shake, centrifuge, and separate the 1-butanol layer. Evaporate the solvent under low pressure (in vacuo), dissolve the residue in 2 mL of methanol, and use the solution as the sample solution. Separately, dissolve 1 mg of ginsenoside Rb₁ 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 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel 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. Spray evenly vanillin-sulfuric acid-ethanol TS for spraying 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 blue-purple spot from the standard solution (Ginseng).

(2) For preparation prescribed *Atractylodes Rhizome*—To 3.0 g of the dry extract (or 9.0 g of the viscous extract) add 15 mL of water, shake, then add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the solvent under low pressure (in vacuo), then dissolve the residue in 2 mL of diethyl ether, and use the solution 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 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 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 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*—To 3.0 g of the dry extract (or 9.0 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 low pressure (in vacuo), then dissolve the residue in 2 mL of hexane, and use the solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 10 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 violet spot is observed at an *R_f* value of about 0.5, and this spot exhibits greenish brown when the plate is sprayed evenly 4-dimethylaminobenzaldehyde TS for spraying, heated the plate at 105°C for 5 minutes, and allowed to cool (*Atractylodes Lancea Rhizome*).

(4) To 3.0 g of the dry extract (or 9.0 g of the viscous extract) add 15 mL of sodium hydroxide TS, shake, centrifuge, and separate the supernatant liquid. To the liquid add 10 mL of 1-butanol, shake, centrifuge, and separate 1-butanol layer. To the 1-butanol layer add 10 mL of water, shake, centrifuge, separate the 1-butanol layer, and evaporate the solvent under low pressure (in vacuo). Dissolve the residue in 2 mL of methanol, and use this solution as the sample solution. Separately, dissolve 1 mg of astragaloside IV 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 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 2-propanol, water and ammonia solution (28) (9:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid-ethanol TS for spraying 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 blue-green to blue-purple spot from the standard solution (*Astragalus Root*).

(5) To 3.0 g of the dry extract (or 9.0 g of the viscous ex

tract) add 15 mL of water, shake, then add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the solvent under low pressure (in vacuo), then add 2 mL of diethyl ether to the residue, and use the solution as the sample solution. Separately, use (*Z*)-ligustilide TS for thin-layer chromatography as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 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 butyl acetate and hexane (2:1) to a distance of about 7 cm, and air-dry the plate. Examine the plate 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 (*Japanese Angelica Root*).

(6) To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 30 mL of 1 mol/L hydrochloric acid TS, and heat for 10 minutes. After cooling, to 10 mL of this solution add 10 mL of ethyl acetate, shake, centrifuge, and use the ethyl acetate layer as the sample solution. Separately, to 2.0 g of a powder of polygala root add 30 mL of 1 mol/L hydrochloric acid TS, and heat for 10 minutes. After cooling, to 10 mL of this solution add 10 mL of ethyl acetate, shake, centrifuge, and use the ethyl acetate layer 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 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, 1-propanol and acetic acid (100) (7:5:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, heat the plate at 105°C for 1 minute, and observe while hot: one of the several spots obtained from the sample solution has the same color tone and *R_f* value with the purplish red spot (at an *R_f* value of about 0.5) from the standard solution (*Polygala Root*).

(7) To 3.0 g of the dry extract (or 9.0 g of the viscous extract) add 15 mL of sodium hydroxide TS, shake, centrifuge, and separate the supernatant liquid. To the liquid add 10 mL of 1-butanol, shake, centrifuge, and separate the 1-butanol layer. To this layer add 10 mL of water, shake, centrifuge, and separate the 1-butanol layer. Evaporate the solvent under low pressure (in vacuo), dissolve the residue in 2 mL of methanol, and use this solution 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 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 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 the plate at 105°C for 5 minutes, 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 (*Bupleurum Root*).

(8) To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of water, shake, add 10 mL of 1-butanol, shake, centrifuge, and use the 1-butanol layer 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 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 ethyl acetate, methanol, ammonia solution (28) (6:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat the plate at 105°C for 5 minutes: one of the several spots obtained from the sample solution has the same color tone and R_f value with the dark purple spot from the standard solution (Gardenia Fruit).

(9) To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of water, shake, add 10 mL of 1-butanol, shake, centrifuge, and use the 1-butanol layer 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 1 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 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, heat the plate at 105°C for 5 minutes, 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-green fluorescent spot from the standard solution (Glycyrrhiza).

(10) To 3.0 g of the dry extract (or 9.0 g of the viscous extract) add 15 mL of water, shake, add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the solvent under low pressure (in vacuo), dissolve the residue in 2 mL of diethyl ether, and use this solution as the sample solution. Separately, to 1.0 g of a powder of saussurea root add 10 mL of methanol, 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 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and acetone (7:3) to a distance of about 7 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid-ethanol TS for spraying on the plate, heat the plate at 105°C for 2 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 blue spot from the standard solution (Saussurea Root).

(11) To 3.0 g of the dry extract (or 9.0 g of the viscous extract) add 15 mL of water, shake, add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the solvent under low pressure (in vacuo), dissolve the residue in 2 mL of diethyl ether, and use this solution 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 μL of the sample solution and 5 μL of the 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 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat the plate at 105°C for 5 minutes, allow to cool, and spray water: one of the several spots obtained from the sample solution has the same color tone and R_f value with the blue-green to grayish green spot from the standard solution (Ginger).

(12) For preparation prescribed Moutan Bark—To 3.0 g of the dry extract (or 9.0 g of the viscous extract) add 15 mL

of water, shake, add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the solvent under low pressure (in vacuo), dissolve the residue in 2 mL of diethyl ether, 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 20 μL of the sample solution and 10 μL of the 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 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-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 orange spot from the standard solution (Moutan Bark).

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 8.0%, calculated on the dried basis. However, for the dry extract prepared by adding Light Anhydrous Silicic Acid, between 9.0% and 18.0%.

Assay (1) 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. Centrifuge, remove the diethyl ether layer, then add 20 mL of diethyl ether, proceed in the same manner as above, and remove the diethyl ether layer. To the 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, and 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. Separately, use saikosaponin b_2 standard TS for assay as the standard solution. Perform the test with exactly 10 μL 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.

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

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

Operating 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 μm 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 (retention time of saikosaponin b_2 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 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%.

(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, calculated on the basis of the content obtained by qNMR

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 (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 20 mL of diethyl ether and 10 mL of water, and shake for 10 minutes. After centrifugation, remove the diethyl ether layer, add 20 mL of diethyl ether, proceed in the same manner as described above, and remove the diethyl ether layer. To the aqueous layer add 10 mL of methanol, shake for 30 minutes, centrifuge, and take the supernatant liquid. To the residue add 20 mL of diluted methanol (1 in 2), shake for 5 minutes, centrifuge, and take the supernatant liquid. Combine these supernatant liquids, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution 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 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: Dissolve 3.85 g of ammonium acetate in 720 mL of water, and add 5 mL of acetic acid (100) and 280 mL of acetonitrile.

Flow rate: 1.0 mL per minute (the retention time of glycyrrhizic acid is about 15 minutes).

System suitability—

System performance: Dissolve 5 mg of monoammonium glycyrrhizinate for resolution check in 20 mL of dilute ethanol. When the procedure is run with 10 μ L of this solution under the above operating conditions, the resolution between the peak having the relative retention time of about 0.9 to glycyrrhizic acid and the peak of glycyrrhizic acid is not less than 1.5.

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 glycyrrhizic acid is not more than 1.5%.

Containers and storage Containers—Tight containers.

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