Tokakujokito Extract

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Tokakujokito Extract contains not less than 38 mg and not more than 152 mg of amygdalin, not less than 1 mg and not more than 4 mg of (*E*)-cinnamic acid, not less than 3 mg of sennosides A ($C_{42}H_{38}O_{20}$: 862.74) or not less than 9 mg of rhein, and not less than 10 mg and not more than 30 mg 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)
Peach Kernel	5 g	5 g	5 g
Cinnamon Bark	4 g	4 g	4 g
Rhubarb	3 g	3 g	3 g
Glycyrrhiza	1.5 g	1.5 g	1.5 g
Anhydrous Sodium Sulfate	1 g	0.9 g	
Sodium Sulfate			2 g

Prepare a dry extract as directed under Extracts, according to the prescription 1) to 3), 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 prescription 2), using the crude drugs shown above.

Description Tokakujokito Extract occurs as a green-yellow-brown to dark brown powder. It has characteristic odor and, salty, slightly astringent, and then slightly sweet taste.

Identification **(1)** To 1.0 g of Tokakujokito Extract add 10 mL of water, shake, then add 10 mL of 1-butanol, shake, centrifuge, and use the 1-butanol layer as the sample solution. Separately, dissolve 2 mg of amygdalin 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 1-propanol, ethyl acetate and water (4:4:3) 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 10 minutes: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the green-brown spot from the standard solution (Peach Kernel).

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

(i) Put 10 g of Tokakujokito Extract in a 300-mL of hard-glass flask, add 100 mL of water and 1 mL of silicone resin, connect the 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 this solution 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 40 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. Spray evenly 2,4-dinitrophenylhydrazine 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) To 2.0 g of Tokakujokito Extract add 10 mL of water, shake, then add 5 mL of hexane, shake, centrifuge, and use the hexane layer 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 40 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 bluish white fluorescent spot from the standard solution.

(3) To 1.0 g of Tokakujokito Extract add 10 mL of water, shake, then add 10 mL of diethyl ether, shake, centrifuge, and use the diethyl ether layer as the sample solution. Separately, dissolve 1 mg of rhein for thin-layer chromatography in 10 mL of acetone, 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, methanol and water (20:3:2) 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 orange fluorescent spot from the standard solution (Rhubarb).

(4) To 1.0 g of Tokakujokito Extract add 10 mL of water, shake, then 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).

Purity **(1)** Heavy metals <*1.07*>—Prepare the test solution with 1.0 g of Tokakujokito Extract as directed in Extracts (4), and perform the test (not more than 30 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.67 g of Tokakujokito Extract according to Method 3, and perform the test (not more than 3 ppm).

Loss on drying <*2.41*> The dry extract: Not more than 8.0% (1 g, 105°C, 5 hours).

Total ash <*5.01*> Not less than 20.0% and more than 40.0%.

Assay **(1)** Amygdalin—Weigh accurately about 0.5 g of

Tokakujokito Extract, add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, and filter. Pipet 5 mL of the filtrate, elute through a column prepared previously with 2 g of polyamide for column chromatography using water to make exactly 20 mL of effluent , and use this solution as the sample solution. Separately, weigh accurately about 10 mg of amygdalin for assay, previously dried in a desiccator (silica gel) for 24 hours or more, and dissolve in diluted methanol (1 in 2) to make exactly 50 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 Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of amygdalin in each solution.

$$\text{Amount (mg) of amygdalin} = M_S \times A_T/A_S \times 4$$

M_S: Amount (mg) of amygdalin for assay taken

Operation conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 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 45°C.

Mobile phase: A mixture of 0.05 mol/L sodium dihydrogen phosphate TS and methanol (5:1).

Flow rate: 0.8 mL per minute (the retention time of amygdalin is about 12 minutes).

Systemic 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 amygdalin are not less than 5000 and not more than 1.5, 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 amygdalin is not more than 1.5%.

(2) (*E*)-Cinnamic acid—Conduct this procedure using light-resistant vessels. Weigh accurately about 0.5 g of Tokakujokito Extract, add 20 mL of diethyl ether and 10 mL of water, shake for 10 minutes, centrifuge, and separate the diethyl ether layer. To the aqueous layer add 20 mL of diethyl ether, proceed in the same manner as above, and repeat this procedure two more times. Combine all the extracts, evaporate the solvent under low pressure (in vacuo), dissolve the residue in 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 (*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 Chromatography <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, calculated on the basis of the content obtained by qNMR

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 (800:200:1).

Flow rate: 1.0 mL per minute (the retention time of (*E*)-cinnamic acid is about 22 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 than 1.5, 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%.

(3) Sennoside A—Weigh accurately about 0.5 g of Tokakujokito Extract, add 20 mL of ethyl acetate and 10 mL of water, shake for 10 minutes, centrifuge, remove the ethyl acetate layer, then add 20 mL of ethyl acetate, proceed in the same manner as above, and remove the ethyl acetate layer. To the aqueous layer obtained 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, weigh accurately about 5 mg of Sennoside A RS (separately determine the water <2.48> by coulometric titration, using 10 mg), dissolve in diluted methanol (1 in 2) to make exactly 200 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 Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of sennoside A in each solution.

$$\text{Amount (mg) of sennoside A } (C_{42}H_{38}O_{20}) = M_S \times A_T/A_S \times 1/4$$

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

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 340 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 50°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (840:160:1).

Flow rate: 1.0 mL per minute (the retention time of sennoside A is about 20 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 sennoside A are not less than 5000 and not more than 1.5, 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 sennoside A is not more than 1.5%.

(4) Rhein—Weigh accurately about 0.5 g of Tokakujokito Extract, add 80 mL of water, shake, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add 20 mL of iron (III) chloride TS, heat under a reflux condenser for 30 minutes, add 3 mL of hydrochloric acid, and heat in addition under a reflux condenser for 30 minutes. After cooling, extract three times with 25 mL each of diethyl ether, combine all the extracts, evaporate the solvent under low pressure (in vacuo), dissolve the residue to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 5 mg of rhein for assay, and dissolve in acetone to make exactly 100 mL. Pipet 10 mL of this solution, add methanol to make exactly 50 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 Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of rhein in each solution.

$$\text{Amount (mg) of rhein} = M_S \times A_T/A_S \times 4/5$$

M_S: Amount (mg) of rhein for assay taken, calculated on the basis of the content obtained by qNMR

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 278 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (650:350:1).

Flow rate: 1.0 mL per minute (the retention time of rhein is about 17 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 rhein are not less than 5000 and not more than 1.5, 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 rhein is not more than 1.5%.

(5) Glycyrrhizic acid—Weigh accurately about 0.5 g of Tokakujokito Extract, add 20 mL of ethyl acetate and 10 mL of water, and shake for 10 minutes. After centrifugation remove the ethyl acetate layer, add 20 mL of ethyl acetate, proceed in the same manner as described above, and remove the ethyl acetate 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 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 glycyrrhizic acid in each solution.

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

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

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: 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 μL 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 μL 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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