Goshuyuto Extract

呉茱萸湯エキス

Goshuyuto Extract contains not less than 0.3 mg (for preparation prescribed 3 g of Evodia Fruit) or not less than 0.4 mg (for preparation prescribed 4 g of Evodia Fruit) of evodiamine, and not less than 0.5 mg and not more than 2.0 mg (for preparation prescribed 1 g of Ginger) or not less than 0.7 mg and not more than 2.8 mg (for preparation prescribed 1.5 g of Ginger) of [6]-gingerol, per extract prepared with the amount specified in the Method of preparation.

Method of preparation

	1)	2)	3)
Evodia Fruit	3 g	4 g	3 g
Ginger	1 g	1.5 g	1.5 g
Ginseng	2 g	3 g	2 g
Jujube	4 g	3 g	4 g

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

Description Goshuyuto Extract occurs as a light brown to light red-yellow powder, or a black-brown viscous extract. It has a slight odor and a hot and bitter taste.

Identification **(1)** To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of sodium hydroxide TS, shake, add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, to 1 g of pulverized evodia fruit 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 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 acetone, 2-propanol, water and formic acid (7:7:1:1) 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 (at an *R*f value of about 0.5) from the standard solution (Evodia Fruit).

(2) To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of water, shake, 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 [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 on the plate, heat at 105℃ 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).

(3) To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of sodium hydroxide TS, shake, add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of ginsenoside Rb_1 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, 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 at 105℃ 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).

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 11.0% (1 g, 105℃, 5 hours).

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

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

Assay **(1)** Evodiamine 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 (7 in 10), shake for 30 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of evodiamine for assay, and dissolve in methanol 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 evodiamine in each solution.

Amount (mg) of evodiamine $= M_S \times A_T / A_S \times 1/4$

M_S: Amount (mg) of evodiamine for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 282 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℃.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (620:380:1).

Flow rate: 1.0 mL per minute (the retention time of evodiamine is about 18 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 evodiamine 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 evodiamine is not more than 1.5%.

(2) [6]-Gingerol—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 (7 in 10), shake for 30 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of [6]-gingerol for assay, dissolve in methanol to make exactly 100 mL. Pipet 5 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 [6]-gingerol in each solution.

Amount (mg) of [6]-gingerol $= M_S \times A_T / A_S \times 1/20$

M_S: Amount (mg) of [6]-gingerol for assay taken

Operating conditions—

Detector, column, column temperature, and mobile phase: Proceed as directed in the operating conditions in (1).

Flow rate: 1.0 mL per minute (the retention time of [6]-gingerol is about 14 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 [6]-gingerol 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 [6]-gingerol is not more than 1.5%.

Containers and storage Containers—Tight containers.

Add the following to 9.41 Reagents, Test Solutions:

Evodiamine for assay $C_{19}H_{17}N_3O$ White to light yellow crystals or crystalline powder. Very slightly soluble in methanol and in ethanol (99.5), and practically insoluble in water.

Identification—Proceed as directed in the Assay: it exhibits a double doublet-like signal equivalent to one proton around δ 2.82 ppm, signals equivalent to four protons which includes a singlet signal around δ 2.91 ppm and a multiplet signal around δ 2.90 ppm – δ 2.98 ppm, a double triplet-like signal equivalent to one proton around δ 3.23 ppm, a double doublet-like signal equivalent to one proton around δ 4.66 ppm, a singlet signal equivalent to one proton around δ 6.16 ppm, a triplet-like signal equivalent to one proton around δ 7.00 ppm, a triplet-like signal equivalent to one proton around δ 7.05 ppm, a doublet-like signal equivalent to one proton around δ 7.08 ppm, a triplet-like signal equivalent to one proton around δ 7.14 ppm, a doublet-like signal equivalent to one proton around δ 7.39 ppm, a doublet-like signal equivalent to one proton around δ 7.51 ppm, a multiplet-like signal equivalent to one proton around δ 7.52 ppm and a doublet-like signal equivalent to one proton around δ 7.83 ppm.

Unity of peak—Dissolve 1 mg of evodiamine for assay in 20 mL of methanol, and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and compare the absorption spectra of at least 3 points including the top of evodiamine peak and around the two middle peak heights of before and after the top: no difference in form is observed among their spectra.

Operating conditions

Column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay (1) under Goshuyuto Extract.

Detector: A photodiode array detector (wavelength: 282 nm, measuring range of spectrum: 220 – 400 nm).

System suitability

System performance: When the procedure is run with 10 μL of the sample solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of evodiamine are not less than 5000 and not more than1.5, respectively.

Assay—Weigh accurately 5 mg of evodiamine for assay and 1 mg of DSS-d_6 for nuclear magnetic resonance spectroscopy using an ultramicrobalance, dissolve them in 1 mL of deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy, and use this solution as the sample solution. Transfer the sample solution into an NMR tube 5 mm in outer diameter, measure ^{1}H-NMR as directed under Nuclear Magnetic Resonance Spectroscopy *<2.21>* and Crude Drugs Test *<5.01>* according to the following conditions, using DSS-d_6 for nuclear magnetic resonance spectroscopy as the internal reference compound. Calculate the resonance intensity A (equivalent to 1 hydrogen) of the signal around δ 6.16 ppm assuming the signal of the internal reference compound as δ 0 ppm.

$$\text{Amount (\%) of evodiamine } (C_{19}H_{17}N_3O) = M_S \times I \times P / (M \times N) \times 1.3521$$

M: Amount (mg) of evodiamine for assay taken

M_S: Amount (mg) of DSS-d_6 for nuclear magnetic resonance spectroscopy taken

I: Signal resonance intensity A based on the signal resonance intensity of DSS-d_6 for nuclear magnetic resonance spectroscopy as 9.000

N: Number of the hydrogen derived from A

P: Purity (%) of DSS-d_6 for nuclear magnetic resonance spectroscopy

Operating conditions

Apparatus: A nuclear magnetic resonance spectrometer having ^{1}H resonance frequency of not less than 400 MHz.

Target nucleus: ^{1}H.

Digital resolution: 0.25 Hz or lower.

Measuring spectrum range: 20 ppm or upper, including between −5 ppm and 15 ppm.

Spinning: off.

Pulse angle: 90°.

^{13}C decoupling: on.

Delay time: Repeating pulse waiting time not less than 60 seconds.

Integrating times: 8 or more times.

Dummy scanning: 2 or more times.

Measuring temperature: A constant temperature between 20℃ and 30℃.

System suitability

Test for required detectability: When the procedure is run with the sample solution under the above operating conditions, the SN ratio of the signal around δ 6.16 ppm is not less than 100.

System performance: When the procedure is run with the sample solution under the above operating conditions, the signals around δ 6.16 ppm is not overlapped with any signal of obvious foreign substance.

System repeatability: When the test is repeated 6 times with the sample solution under the above operating conditions, the relative standard deviation of the ratio of the resonance intensity A to that of the internal reference compound is not more than 1.0%.

Evodia fruit [Same as the namesake monograph]