

# Suppl I, JP XV (2007)

## Kakkonto Extract

葛根湯エキス

### **Change to read:**

Kakkonto Extract contains not less than 9 mg and not more than 27 mg (for preparation prescribed 3 g of Ephedra Herb) or not less than 12 mg and not more than 36 mg (for preparation prescribed 4 g of Ephedra Herb) of total alkaloids [ephedrine ( $C_{10}H_{15}NO$ : 165.23) and pseudoephedrine ( $C_{10}H_{15}NO$ : 165.23)], not less than 14 mg and not less than 42 mg (for preparation prescribed 2 g of Peony Root) or not less than 21 mg and not more than 63 mg (for preparation prescribed 3 g of Peony Root) of peoniflorin ( $C_{23}H_{28}O_{11}$ : 480.46), and not less than 19 mg and not more than 57 mg of glycyrrhizic acid ( $C_{42}H_{62}O_{16}$ : 822.93) per the extract prepared as directed in the Method of preparation.

**Method of preparation** Prepare a dry extract or viscous extract as directed under Extracts, with 8 g of Pueraria Root, 4 g of Ephedra Herb, 4 g of Jujube, 3 g of Cinnamon Bark, 3 g of Peony Root, 2 g of Glycyrrhiza and 1 g of Ginger, or with 4 g of Pueraria Root, 4 g of Ephedra Herb, 3 g of Jujube, 2 g of Cinnamon Bark, 2 g of Peony Root, 2 g of Glycyrrhiza and 1 g of Ginger, or with 4 g of Pueraria Root, 3 g of Ephedra Herb, 3 g of Jujube, 2 g of Cinnamon Bark, 2 g of Peony Root, 2 g of Glycyrrhiza and 2 g of Ginger.

**Description** Kakkonto Extract occurs as a light brown to black-brown powder or viscous extract. It has a characteristic odor, and a sweet first, then hot, and slightly bitter taste.

**Identification** (1) 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 Puerarin Reference Standard 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  $\mu$ 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 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 *Rf* value with the bluish white fluorescent spot from the standard solution (Pueraria Root).

(2) 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 ephedrine hydrochloride 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  $\mu$ 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 1-butanol, water and acetic acid (100) (7:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly ninhydrin 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 *Rf* value with the red-purple spot from the standard solution (Ephedra Herb).

(3) Put 10 g of the dry extract (or 30 g of the viscous extract) in a 300-mL 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 the layer 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 20  $\mu$ L of the sample solution and 2  $\mu$ L 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 10 cm, and air-dry the plate. Spray evenly 2,4-dinitrophenylhydrazine TS on the plate: one of the spot among the several spots from the sample solution has the same color tone and *Rf* value with the yellow-orange spot from the standard solution (Cinnamon Bark).

(4) 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 Peoniflorin Reference Standard 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  $\mu$ 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 10 cm, and air-dry the plate. Spray evenly 4-methoxybezaldehyde-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*<sub>f</sub> value with the purple spot from the standard solution (Peony Root).

(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 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  $\mu$ 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 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*<sub>f</sub> value with the yellow-brown spot from the standard solution (Glycyrrhiza).

(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 25 mL of diethyl ether, shake, and take the diethyl ether layer. Evaporate the layer under reduced pressure, dissolve the residue in 2 mL of diethyl ether, and use the 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  $\mu$ L of the sample solution and 5  $\mu$ L 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 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*<sub>f</sub> value with the blue-green spot from the standard solution (Ginger).

**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 in the Extracts (4) under General Rules for Preparations, 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 more than 10.0%, calculated on the dried basis.

**Assay (1)** Total alkaloids (ephedrine and pseudoephedrine)—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 ephedrine hydrochloride for assay, previously dried at 105°C for 3 hours, 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 50 mL, and use this solution as the standard solution. Perform the test with exactly 10  $\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine the peak areas, *A*<sub>TE</sub> and *A*<sub>TP</sub>, of ephedrine and pseudoephedrine with the sample solution, and the peak area, *A*<sub>S</sub>, of ephedrine with the standard solution.

Amount (mg) of total alkaloids [ephedrine (C<sub>10</sub>H<sub>15</sub>NO) and pseudoephedrine (C<sub>10</sub>H<sub>15</sub>NO)]

$$= W_S \times \{(A_{TE} + A_{TP})/A_S\} \times 0.819 \times (1/10)$$

*W*<sub>S</sub>: Amount (mg) of ephedrine hydrochloride for assay

*Operating 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  $\mu$ m in particle diameter).

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

Mobile phase: A mixture of a solution of sodium lauryl sulfate (1 in 130), acetonitrile and phosphoric acid (650:350:1).

Flow rate: 1.0 mL/min. (the retention time of ephedrine is about 27 minutes.)

*System suitability—*

System performance: Dissolve 1 mg each of ephedrine hydrochloride for assay and pseudoephedrine hydrochloride in diluted methanol (1 in 2) to make 10 mL. When the procedure is run with 10  $\mu$ L of this solution under the above operating conditions, pseudoephedrine and ephedrine are eluted in this order with the resolution between these peaks being not less than 1.5.

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

(2) Peoniflorin—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, and filter.

Pipet 5 mL of the filtrate, flow through in a column packed with 2 g of polyamide for column chromatography, elute with water to make exactly 20 mL of eluate, and use this as the sample solution. Separately, weigh accurately about 10 mg of Peoniflorin Reference Standard (separately determine the water), and dissolve in diluted methanol (1 in 2) to make exactly 100 mL. Pipet 5 mL of this solution, add diluted methanol (1 in 2) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10  $\mu$ 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 peoniflorin.

$$\text{Amount (mg) of peoniflorin (C}_{23}\text{H}_{28}\text{O}_{11}\text{)} \\ = W_S \times (A_T/A_S) \times (1/2)$$

$W_S$ : Amount (mg) of Peoniflorin Reference Standard, 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  $\mu$ 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/min. (the retention time of peoniflorin is about 9 minutes.)

*System suitability—*

System performance: Dissolve 1 mg each of Peoniflorin Reference Standard and albiflorin in diluted methanol (1 in 2) to make 10 mL. When the procedure is run with 10  $\mu$ L of this solution under the above operating conditions, albiflorin and peoniflorin 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  $\mu$ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of peoniflorin 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 Reference Standard (separately determine the water) 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  $\mu$ 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.

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

$W_S$ : Amount (mg) of Glycyrrhizic Acid Reference Standard, 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  $\mu$ 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/min. (the retention time of glycyrrhizic acid is about 12 minutes.)

*System suitability—*

System performance: When the procedure is run with 10  $\mu$ 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  $\mu$ 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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