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Boiogito Extract

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Boiogito Extract contains not less than 4 mg and not more than 16 mg of shinomenine, and not less than 12 and not more than 36 mg of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), per extract prepared with the amount specified in the Method of preparation.

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Method of preparation

	1)	2)	3)
Sinomenium Stem and Rhizome	5 g	5 g	5 g
Astragalus Root	5 g	5 g	5 g
Atractylodes Rhizome	3 g	3 g	—
Atractylodes Lancea Rhizome	—	—	3 g
Ginger	0.8 g	1 g	1 g
Jujube	3 g	3 g	3 g
Glycyrrhiza	1.5 g	1.5 g	1.5 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. Or, prepare a dry extract by adding Light Anhydrous Silicic Acid to an extractive, prepared as directed under Extracts, according to the prescription 3), using the crude drugs shown above.

Description Boiogito Extract is a light yellow-brown to reddish brown powder or blackish brown viscous extract. It has a slightly odor, and a sweet taste at first and then a slight hot and bitter taste later.

Identification (1) To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 15 mL of sodium hydroxide TS, shake, centrifuge, and separate the supernatant liquid. To this liquid add 10 mL of 1-butanol, shake, centrifuge, and separate 1-butanol layer. To this liquid add 10 mL of water, shake, centrifuge, separate the 1-butanol layer, then evaporate the solvent under reduced pressure, dissolve the residue in 1 mL of methanol, and use the solution as the sample solution. Separately, dissolve 1 mg of sinomenine 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, 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 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 obtained from the sample solution has the same color tone and *R_f* value with the red to red-brown spot obtained from the standard solution (Sinomenium Stem and Rhizome).

(2) To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 15 mL of sodium hydroxide TS, shake, centrifuge, and separate the supernatant liquid. To this liquid add 10 mL of 1-butanol, shake, centrifuge, and separate 1-butanol layer. To the aqueous layer add 10 mL of 1-butanol, and proceed in the same manner as above. Combine the 1-butanol layers, add 10 mL of water, shake, centrifuge, separate the 1-butanol layer, and evaporate the solvent under reduced pressure. Dissolve the residue in exactly 1 mL of methanol, and use this solution as the sample solution. Separately, dissolve 1.0 mg of astragaloside IV for thin-layer chromatography in exactly 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 5 μ L 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 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 obtained from the sample solution has the same color tone and *R_f* value with the red-brown spot obtained from the standard solution, and the spot is larger and more intense than the spot from the standard solution (Astragalus Root).

(3) For preparation prescribed Atractylodes Rhizome—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, and shake. Separate the diethyl ether layer, evaporate the solvent under reduced pressure, 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 2 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 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 at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots obtained from the sample solution has the same color tone and *R_f* value with the red to red-purple spot obtained 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. Separate the hexane layer, evaporate the solvent under reduced pressure, then dissolve the residue in 0.5 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 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 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 0.4, and this spot exhibits greenish brown when the plate is sprayed evenly 4-dimethylaminobenzaldehyde TS for spraying, heated at 105°C for 5 minutes, and allowed to cool (Atractylodes Lancea Rhizome).

(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 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the solvent under reduced pressure, then 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 20 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 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°C for 5 minutes, allow to cool, and spray water: one of the spot among 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 obtained from the standard solution (Ginger).

(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 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 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, and heat at 105°C for 5 minutes: 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-brown spot obtained from the standard solution (Glycyrrhiza).

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 11.0% (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) Sinomenine—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, shake, then add 5.0 mL of 0.1 mol/L hydrochloric acid TS, and shake for 10 minutes, centrifuge, and remove the upper layer. Add 20 mL of diethyl ether, proceed in the same manner as described above, and remove the upper layer. To the aqueous layer add 5.0 mL of diluted sodium hydroxide TS (1 in 10) and 10 mL of methanol, shake for 15 minutes, centrifuge, and separate the supernatant liquid. To the residue add 20 mL of diluted methanol (1 in 2), shake for 15 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 sinomenine for assay, previously dried in a desiccator (silica gel) for 24 hours or more, 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 sinomenine in each solution.

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

M_S : Amount (mg) of sinomenine for assay taken

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 30°C.

Mobile phase: To 3 g of sodium lauryl sulfate add 350 mL of acetonitrile, shake, then add 650 mL of water and 1 mL of phosphoric acid.

Flow rate: 1.0 mL per minute (the retention time of

sinomenine is about 18 minutes).

System suitability—

System performance: When the procedure is run according to the conditions above with 10 μL each of the sample solution, the standard solution of sinomenine, and the standard solution of glycyrrhizic acid obtained in Assay (2), peaks of sinomenine and glycyrrhizic acid are observed in the sample solution, glycyrrhizic acid and sinomenine are eluted in this order, and the resolution between these peaks is not less than 4.5. Furthermore, except for the peak of glycyrrhizic acid, distinct peaks are observed before and after of the peak of sinomenine, and the resolutions between sinomenine and these peaks are respectively 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 sinomenine is not more than 1.5%.

(2) 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 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.

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

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 μm 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 μL 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 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.