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Add the following:

Shimbuto Extract

真武湯エキス

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Shimbuto Extract contains not less than 26 mg and not more than 78 mg of peoniflorin ($C_{23}H_{28}O_{11}$: 480.46), not less than 0.5 mg and not more than 2.0 mg (for preparation prescribed 0.8 g of Ginger) or not less than 0.6 mg and not more than 2.4 mg (for preparation prescribed 1 g of Ginger) or not less than 0.9 mg and not more than 3.6 mg (for preparation prescribed 1.5 g of Ginger) of [6]-gingerol, and not less than 0.7 mg (for preparation prescribed 1 g of Processed Aconite Root 1) of total alkaloids (as benzoylmesaconine hydrochloride and 14-anisoylaconine hydrochloride) or not less than 0.2 mg (for preparation prescribed 1 g of Powdered Processed Aconite Root 1) of total alkaloids (as benzoylmesaconine hydrochloride and 14-anisoylaconine hydrochloride, or as benzoylmesaconine hydrochloride and benzoylhypaconine hydrochloride) or not less than 0.1 mg (for preparation prescribed 1 g of Powdered Processed Aconite Root 2) of total alkaloids (as benzoylmesaconine hydrochloride and 14-benzoylhypaconine hydrochloride) or not less than 0.1 mg (for preparation prescribed 0.5 g of Powdered Processed Aconite Root 1) of total alkaloids (as benzoylmesaconine hydrochloride and 14-anisoylaconine hydrochloride, or as benzoylmesaconine hydrochloride and benzoylhypaconine hydrochloride) per the extract prepared as directed in the Method of preparation.

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Method of preparation Prepare a dry extract or viscous extract as directed under Extracts, with 5 g of Poria Sclerotium, 3 g of Paeony Root, 3 g of Atractylodes Rhizome, 1 g of Ginger and 1 g Processed Aconit Root 1 of “Processed Aconit Root”, or with 5 g of Poria Sclerotium, 3 g of Paeony Root, 3 g of Atractylodes Lancea Rhizome, 1 g of Ginger and 1 g of Powdered Processed Aconit Root 1 of “Powdered Processed Aconit Root”, or with 5 g of Poria Sclerotium, 3 g of Paeony Root, 3 g of Atractylodes Rhizome, 0.8 g of Ginger and 1 g of Powdered Processed Aconit Root 2 of “Powdered Processed Aconit Root”,

or with 4 g of Poria Sclerotium, 3 g of Paeony Root, 3 g of Atractylodes Lancea Rhizome, 1.5 g of Ginger and 0.5 g of Powdered Processed Aconit Root 1 of "Powdered Processed Aconit Root".

Description Shimbuto Extract occurs as light yellow-brown to brown powder. It has slightly a characteristic odor and a hot and bitter taste.

Identification (1) To 2.0 g of Shimbuto Extract, add 10 mL of water, shake, then add 5 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 μ 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-methoxybenzaldehyde-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_f value with the purple spot from the standard solution (Peony Root).

(2) (For preparation prescribed Atractylodes Rhizome) To 1.0 g of Shimbuto Extract, add 10 mL of water, shake, then add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer 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 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 5 μ 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 and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly diluted sulfuric acid on the plate, heat at 105°C for 5 minutes, and 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 R_f value with the bluish white fluorescent spot from the standard solution (Atractylodes Rhizome).

(3) (For preparation prescribed Atractylodes Lancea Rhizome) To 2.0 g of Shimbuto Extract, add 10 mL of water, shake, then add 25 mL of hexane, and shake. Take the hexane layer, add anhydrous sodium sulfate to dry, and filter. Evaporate the filtrate under reduced pressure, add 2 mL of hexane to the residue, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 20 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 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a dark purple spot is observed at around R_f 0.4. The spot shows a greenish brown color after being sprayed evenly 4-dimethylaminobenzaldehyde TS for spraying, heated at 105°C for 5 minutes and allowed to cool (Atractylodes Lancea Rhizome).

zome).

(4) To 1.0 g of Shimbuto Extract, add 10 mL of water, shake, then add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer 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 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 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heated at 105°C for 5 minutes and allowed to cool: one of the spot among the several spots from the sample solution has the same color tone and R_f value with the blue-green spot from the standard solution (Ginger).

(5) To 3.0 g of Shimbuto Extract, add 20 mL of diethyl ether and 2 mL of ammonia TS, shake for 10 minutes, centrifuge, and take the supernatant liquid. Evaporate the supernatant liquid under reduced pressure, add 1 mL of acetonitrile to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of benzoylemesaconine hydrochloride for thin-layer chromatography in 10 mL of ethanol (99.5), 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 10 μ L of the 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) (4:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spraying on the plate, and air-dry the plate. Then spray evenly sodium nitrite TS on the plate: one of the spot among the several spots from the sample solution has the same color tone and R_f value with the yellow-brown spot from the standard solution (Processed Aconite Root or Powdered Processed Aconite Root).

Purity (1) Heavy metals <1.07> Prepare the test solution with 1.0g of Shimbuto Extract 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 Shimbuto Extract according to Method 3, and perform the test (not more than 3 ppm).

(3) Aconitum diester alkaloids (aconitine, jesaconitine, hy-paconitine and mesaconitine)—Weigh accurately 1.0 g of Shimbuto Extract, add 20 mL of diethyl ether, shake, then add 3.0 mL of 0.1 mol/L hydrochloric acid TS and shake for 10 minutes. Centrifuge this solution, remove the upper layer, then add 20 mL of diethyl ether, proceed in the same manner as described above, and remove the upper layer. To the water layer, add 1.0 mL of ammonia TS and 20 mL of diethyl ether, shake for 30 minutes, centrifuge, and take the supernatant liquid. To the water layer, add 1.0 mL of ammonia TS and 20 mL of diethyl ether, and repeat the above process twice more. Combine all the supernatant liquids, and evaporate to dryness under reduced pressure. Dissolve the residue with exactly 10 mL of a

mixture of phosphate buffer solution for processed aconite root and acetonitrile (1:1). Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, pipet 1 mL of aconitum diester alkaloids standard solution for purity, add a mixture of phosphate buffer solution for processed aconite root and acetonitrile (1:1) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 40 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions: the heights of the peaks corresponding to aconitine, jesaconitine, hypaconitine and mesaconitine from the sample solution are not higher than the respective heights corresponding to aconitine, jesaconitine, hypaconitine and mesaconitine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 231 nm for aconitine, hypaconitine and mesaconitine; 254 nm for jesaconitine).

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 phosphate buffer for processed aconite root and tetrahydrofuran (183:17).

Flow rate: 1.0 mL per minute (the retention time of mesaconitine is about 31 minutes).

System suitability—

System performance: When the procedure is run with 20 μ L of aconitum diester alkaloids standard solution for purity under the above operating conditions, using 254 nm, mesaconitine, hypaconitine, aconitine and jesaconitine are eluted in this order, and each resolution between their peaks is not less than 1.5 respectively.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, using 231 nm, the relative standard deviation of the peak height of mesaconitine is not more than 1.5%.

Loss on drying <2.41> Not more than 7.0% (1 g, 105°C, 5 hours).

Total ash <5.01> Not more than 10.0%.

Assay (1) Peoniflorin—Weigh accurately about 0.5 g of Shimbuto Extract, 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 Peoniflorin Reference Standard (separately determine the water), and 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 peoniflorin.

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

W_S : Amount (mg) of Peoniflorin Reference Standard, calcu-

lated 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 μ 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 per minute (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 μ L of this standard 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 μ 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%.

(2) [6]-gingerol—Weigh accurately about 0.5 g of Shimbuto Extract, add exactly 50 mL of diluted methanol (7 in 10), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of [6]-gingerol for component determination, dissolve in diluted 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 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 [6]-gingerol.

$$\text{Amount (mg) of [6]-gingerol} = W_S \times (A_T / A_S) \times (1/20)$$

W_S : Amount (mg) of [6]-gingerol for component determination

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 μ m in particle diameter).

Column temperature: A constant temperature of about 30°C.

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

Flow rate: 1.0 mL per minute (the retention time of [6]-gingerol is about 15 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 [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%.

(3) Total alkaloids—Weigh accurately about 1 g of Shimbuto Extract, add 20 mL of diethyl ether, shake, then add 3.0 mL of 0.1 mol/L hydrochloric acid TS, and shake for 10 minutes. Centrifuge this solution, remove the upper layer, then add 20 mL of diethyl ether, proceed in the same manner as described above, and remove the upper layer. To the water layer, add 1.0 mL of ammonia TS and 20 mL of diethyl ether, shake for 30 minutes, centrifuge, and take the supernatant liquid. To the water layer, add 1.0 mL of ammonia TS and 20 mL of diethyl ether, and repeat the above process twice more. Combine all the supernatant liquids, and evaporate to dryness under reduced pressure. Dissolve the residue with a mixture of phosphate buffer solution for processed aconite root and acetonitrile (1:1) to make exactly 10 mL. Centrifuge this solution, and use the supernatant liquid as the sample solution. Perform the test with exactly 20 μL each of the sample solution and the aconitum monoester alkaloids standard solution TS for component determination as directed under Liquid Chromatography <2.01> according to the following conditions. Determine the peak areas of benzoylmesaconine, benzoylhypaconine and 14-anisoylaconine, A_{TM} and A_{SM} , A_{TH} and A_{SH} , as well as A_{TA} and A_{SA} , in each solution, respectively.

$$\begin{aligned} \text{Amount (mg) of benzoylmesaconine hydrochloride} \\ = C_{\text{SM}} \times (A_{\text{TM}} / A_{\text{SM}}) \times 10 \end{aligned}$$

$$\begin{aligned} \text{Amount (mg) of benzoylhypaconine hydrochloride} \\ = C_{\text{SH}} \times (A_{\text{TH}} / A_{\text{SH}}) \times 10 \end{aligned}$$

$$\begin{aligned} \text{Amount (mg) of 14-anisoylaconine hydrochloride} \\ = C_{\text{SA}} \times (A_{\text{TA}} / A_{\text{SA}}) \times 10 \end{aligned}$$

C_{SM} : Concentration (mg/mL) of benzoylmesaconine hydrochloride for component determination in aconitum monoester alkaloids standard solution TS for component determination

C_{SH} : Concentration (mg/mL) of benzoylhypaconine hydrochloride for component determination in aconitum monoester alkaloids standard solution TS for component determination

C_{SA} : Concentration (mg/mL) of 14-anisoylaconine hydrochloride for component determination in aconitum monoester alkaloids standard solution for TS component determination

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 231 nm for benzoylhypaconine and benzoylmesaconine; 254 nm for 14-anisoylaconine).

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 phosphate buffer solution for processed aconite root and tetrahydrofuran (183:17).

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

System suitability—

System performance: When the procedure is run with 20 μL of the aconitum monoester alkaloids standard solution TS for component determination under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of benzoylmesaconine are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the aconitum monoester alkaloids standard solution TS for component determination under the above operating conditions, the relative standard deviation of the peak areas of benzoylmesaconine, benzoylhypaconine and 14-anisoylaconine is not more than 1.5%.

Container and storage Containers—Tight containers.

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