

# 1 Mashiningan Extract

2 麻子仁丸エキス

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4 Mashiningan Extract contains not less than 14 mg  
5 and not more than 56 mg of paeoniflorin ( $C_{23}H_{28}O_{11}$ :  
6 480.46), not less than 1.3 mg of magnolol, and not less  
7 than 3.0 mg (for preparation prescribed 3.5 g of Rhu-  
8 barb) or not less than 3.5 mg (for preparation prescribed  
9 4 g of Rhubarb) of sennoside A ( $C_{42}H_{38}O_{20}$ : 862.74),  
10 per extract prepared with the amount specified in the  
11 Method of preparation.

## 12 Method of preparation

	1)	2)
Hemp Fruit	5 g	4 g
Peony Root	2 g	2 g
Immature Orange	2 g	2 g
Magnolia Bark	2 g	2 g
Rhubarb	4 g	3.5 g
Apricot Kernel	2 g	2.5 g

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

17 **Description** Mashiningan Extract occurs as a deep reddish  
18 yellow to very dark reddish yellow powder. It has a charac-  
19 teristic odor, and has a bitter taste and an astringent taste later.

20 **Identification** (1) Shake 0.5 g of Mashiningan Extract  
21 with 10 mL of methanol, centrifuge, and use the supernatant  
22 liquid as the sample solution. Separately, shake 0.3 g of pow-  
23 dered Hemp Fruit with 3 mL of methanol, centrifuge, and use  
24 the supernatant liquid as the standard solution. Perform the  
25 test with these solutions as directed under Thin-layer Chro-  
26 matography <2.03>. Spot 20  $\mu$ L of the sample solution and 10  
27  $\mu$ L of the standard solution on a plate of silica gel for thin-  
28 layer chromatography. Develop the plate with a mixture of  
29 hexane and ethyl acetate (2:1) to a distance of about 7 cm,  
30 and air-dry the plate. Spray evenly vanillin-sulfuric acid-eth-  
31 anol TS for spraying on the plate, and heat the plate at 105°C  
32 for 5 minutes, and allow to cool: one of the several spots ob-  
33 tained from the sample solution has the same color tone and  
34 *R<sub>f</sub>* value with the blue- purple to dark purple spot (*R<sub>f</sub>* value  
35 of about 0.5) from the standard solution (Hemp Fruit and  
36 Apricot Kernel).

37 (2) Shake 1.0 g of Mashiningan Extract with 10 mL of  
38 water, add 10 mL of 1-butanol, shake, centrifuge, and use the  
39 1-butanol layer as the sample solution. Separately, dissolve 1  
40 mg of Paeoniflorin RS or paeoniflorin for thin-layer chroma-  
41 tography in 1 mL of methanol, and use this solution as the  
42 standard solution. Perform the test with these solutions as di-  
43 rected under Thin-layer Chromatography <2.03>. Spot 10  $\mu$ L  
44 of the sample solution and 5  $\mu$ L of the standard solution on a

45 plate of silica gel for thin-layer chromatography. Develop the  
46 plate with a mixture of ethyl acetate, methanol and ammonia  
47 solution (28) (6:3:2) to a distance of about 7 cm, and air-dry  
48 the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid  
49 TS on the plate, and heat the plate at 105°C for 2 minutes:  
50 one of the several spots obtained from the sample solution  
51 has the same color tone and *R<sub>f</sub>* value with the red-purple to  
52 purple spot from the standard solution (Peony Root).

53 (3) Shake 1.0 g of Mashiningan Extract with 10 mL of  
54 water, add 10 mL of 1-butanol, shake, centrifuge, and use the  
55 1-butanol layer as the sample solution. Separately, shake 1.0  
56 g of pulverized Immature Orange with 10 mL of methanol,  
57 centrifuge, and use the supernatant liquid as the standard so-  
58 lution. Perform the test with these solutions as directed under  
59 Thin-layer Chromatography <2.03>. Spot 10  $\mu$ L of the sample  
60 solution and 5  $\mu$ L of the standard solution on a plate of silica  
61 gel for thin-layer chromatography. Develop the plate with a  
62 mixture of ethyl acetate, 1-propanol, water and acetic acid  
63 (100) (7:5:4:1) to a distance of about 10 cm, and air-dry the  
64 plate. Spray evenly 2,6-dibromo-*N*-chloro-1,4-benzoquinone  
65 monoimine TS on the plate, and allow to stand in ammonia  
66 gas: two consecutive spots at *R<sub>f</sub>* values of about 0.7 obtained  
67 from the sample solution have respectively the same color  
68 tone and *R<sub>f</sub>* value with the blue-green spot and the blue spot  
69 immediately below it from the standard solution (Immature  
70 Orange).

71 (4) Shake 1.0 g of Mashiningan Extract with 10 mL of  
72 water, add 10 mL of diethyl ether, shake, centrifuge, and use  
73 the diethyl ether layer as the sample solution. Separately, dis-  
74 solve 1 mg of magnolol for thin-layer chromatography in 1  
75 mL of methanol, and use this solution as the standard solution.  
76 Perform the test with these solutions as directed under Thin-  
77 layer Chromatography <2.03>. Spot 10  $\mu$ L of the sample so-  
78 lution and 5  $\mu$ L of the standard solution on a plate of silica  
79 gel with fluorescent indicator for thin-layer chromatography.  
80 Develop the plate with a mixture of ethyl acetate and hexane  
81 (1:1) to a distance of about 7 cm, and air-dry the plate. Ex-  
82 amine under ultraviolet light (main wavelength: 254 nm): one  
83 of the several spots obtained from the sample solution has the  
84 same color tone and *R<sub>f</sub>* value with the dark purple spot from  
85 the standard solution (Magnolia Bark).

86 (5) Shake 1.0 g of Mashiningan Extract with 10 mL of  
87 water, add 10 mL of diethyl ether, shake, centrifuge, and use  
88 the diethyl ether layer as the sample solution. Separately, dis-  
89 solve 1 mg of rhein for thin-layer chromatography in 10 mL  
90 of acetone, and use this solution as the standard solution. Per-  
91 form the test with these solutions as directed under Thin-layer  
92 Chromatography <2.03>. Spot 10  $\mu$ L of the sample solution  
93 and 5  $\mu$ L of the standard solution on a plate of silica gel for  
94 thin-layer chromatography. Develop the plate with a mixture  
95 of ethyl acetate, methanol and water (20:3:2) to a distance of  
96 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).

(6) Shake 0.5 g of Mashiningan Extract with 10 mL of methanol, centrifuge, and use the supernatant liquid 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 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, 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-methoxybenzaldehyde-sulfuric acid TS on the plate, heat the plate at 105°C for 10 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 green to green-brown spot from the standard solution (Apricot Kernel).

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

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

**Total ash** <5.01> Not more than 9.0%.

**Assay (1)** Paeoniflorin—Weigh accurately about 0.5 g of Mashiningan Extract, add exactly 50 mL of diluted methanol (1 in 2), shake for 30 minutes, and centrifuge. Pipet 5 mL of the supernatant liquid, flow through in a column packed with 2 g of polyamide for column chromatography, elute with 20 mL of water, then add 1 mL of acetic acid (100) and water to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Paeoniflorin RS (separately determine the water <2.48> by coulometric titration, using 10 mg), 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 paeoniflorin in each solution.

$$\begin{aligned} &\text{Amount (mg) of paeoniflorin (C}_{23}\text{H}_{28}\text{O}_{11}) \\ &= M_S \times A_T / A_S \times 5 / 8 \end{aligned}$$

$M_S$ : Amount (mg) of Paeoniflorin RS taken, 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 per minute.

*System suitability—*

System performance: Dissolve 1 mg each of Paeoniflorin RS 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 paeoniflorin 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 paeoniflorin is not more than 1.5%.

(2) Magnolol—Weigh accurately about 0.5 g of Mashiningan Extract, add exactly 50 mL of diluted methanol (7 in 10), shake for 15 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 10 mg of magnolol for assay, and dissolve in diluted methanol (7 in 10) to make exactly 100 mL. Pipet 5 mL of this solution, add diluted methanol (7 in 10) 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 magnolol in each solution.

$$\text{Amount (mg) of magnolol} = M_S \times A_T / A_S \times 1 / 8$$

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

*Operating conditions—*

Detector: An ultraviolet absorption photometer (wavelength: 289 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 water, acetonitrile and acetic acid (100) (50:50:1).

195 Flow rate: 1.0 mL per minute.

196 *System suitability—*

197 System performance: Dissolve 1 mg each of magnolol for  
198 assay and honokiol in diluted methanol (7 in 10) to make 10  
199 mL. When the procedure is run with 10  $\mu$ L of this solution  
200 under the above operating conditions, honokiol and magnolol  
201 are eluted in this order with the resolution between these  
202 peaks being not less than 2.5.

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

207 **(3) Sennoside A**—Weigh accurately about 0.2 g of  
208 Mashiningan Extract, add exactly 50 mL of diluted methanol  
209 (1 in 2), shake for 30 minutes, and centrifuge. Pipet 10 mL of  
210 the supernatant liquid, pour it into a column about 10 mm in  
211 inside diameter (previously prepared by packing 0.36 g of  
212 strongly basic ion-exchange resin for column chromatog-  
213 raphy, and washing with 10 mL of methanol and 10 mL of  
214 diluted methanol (1 in 2)) to elute, wash out the column with  
215 10 mL of diluted methanol (1 in 2), then elute with a mixture  
216 of water, methanol and formic acid (25:25:1) to obtain ex-  
217 actly 5 mL of the effluent, and use this liquid as the sample  
218 solution. Separately, weigh accurately about 10 mg of Sen-  
219 noside A RS (separately determine the water <2.48> by cou-  
220 lometric titration, using 10 mg), dissolve in 10 mL of diluted  
221 tetrahydrofuran (1 in 2), and add diluted methanol (1 in 2) to  
222 make exactly 100 mL. Pipet 25 mL of this solutions, add di-  
223 luted methanol (1 in 2) to make exactly 100 mL, and use this  
224 solution as the standard solution. Perform the test with ex-  
225 actly 10  $\mu$ L each of the sample solution and standard solution  
226 as directed under Liquid Chromatography <2.01> according  
227 to the following conditions, and determine the peak areas,  $A_T$   
228 and  $A_S$ , of sennoside A in each solution.

$$\begin{aligned} 229 \quad & \text{Amount (mg) of sennoside A (C}_{42}\text{H}_{38}\text{O}_{20}\text{)} \\ 230 \quad & = M_S \times A_T / A_S \times 1/16 \end{aligned}$$

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

233 *Operating conditions—*

234 Detector: An ultraviolet absorption photometer (wave-  
235 length: 340 nm).

236 Column: A stainless steel column 4.6 mm in inside diam-  
237 eter and 15 cm in length, packed with octadecylsilanized sil-  
238 ica gel for liquid chromatography (5  $\mu$ m in particle diameter).

239 Column temperature: A constant temperature of about  
240 30°C.

241 Mobile phase: A mixture of water, acetonitrile and phos-  
242 phoric acid (2460:540:1).

243 Flow rate: 1.0 mL per minute.

244 *System suitability—*

245 System performance: When the procedure is run with 10  
246  $\mu$ L of the standard solution under the above operating condi-  
247 tions, the number of theoretical plates and the symmetry fac-  
248 tor of the peak of sennoside A are not less than 5000 and not  
249 more than 1.5, respectively.

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

254 **Containers and storage** Containers—Tight containers.

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