

1 Goshuyuto Extract

2 呉茱萸湯エキス

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4 Goshuyuto Extract contains not less than 0.3 mg
5 (for preparation prescribed 3 g of Evodia Fruit) or not
6 less than 0.4 mg (for preparation prescribed 4 g of
7 Evodia Fruit) of evodiamine, and not less than 0.5 mg
8 and not more than 2.0 mg (for preparation prescribed
9 1 g of Ginger) or not less than 0.7 mg and not more
10 than 2.8 mg (for preparation prescribed 1.5 g of
11 Ginger) of [6]-gingerol, per extract prepared with the
12 amount specified in the Method of preparation.

13 Method of preparation

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| | 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 |

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

19 **Description** Goshuyuto Extract occurs as a light brown
20 to light red-yellow powder, or a black-brown viscous ex-
21 tract. It has a slight odor and a hot and bitter taste.

22 **Identification** (1) To 1.0 g of the dry extract (or 3.0 g
23 of the viscous extract) add 10 mL of sodium hydroxide TS,
24 shake, add 5 mL of 1-butanol, shake, centrifuge, and use
25 the supernatant liquid as the sample solution. Separately, to
26 1 g of pulverized evodia fruit add 10 mL of methanol, shake,
27 centrifuge, and use the supernatant liquid as the standard
28 solution. Perform the test with these solutions as directed
29 under Thin-layer Chromatography <2.03>. Spot 1 μL each
30 of the sample solution and standard solution on a plate of
31 silica gel for thin-layer chromatography. Develop the plate
32 with a mixture of acetone, 2-propanol, water and formic
33 acid (7:7:1:1) to a distance of about 7 cm, and air-dry the
34 plate. Examine under ultraviolet light (main wavelength:
35 365 nm): one of the several spots obtained from the sample
36 solution has the same color tone and *R_f* value with the blue-
37 white fluorescent spot (at an *R_f* value of about 0.5) from the
38 standard solution (Evodia Fruit).

39 (2) To 1.0 g of the dry extract (or 3.0 g of the viscous
40 extract) add 10 mL of water, shake, add 25 mL of diethyl
41 ether, and shake. Separate the diethyl ether layer, evaporate
42 the solvent under reduced pressure, add 2 mL of diethyl
43 ether to the residue, and use this solution as the sample so-
44 lution. Separately, dissolve 1 mg of [6]-gingerol for thin-
45 layer chromatography in 1 mL of methanol, and use this
46 solution as the standard solution. Perform the test with

47 these solutions as directed under Thin-layer Chromatog-
48 raphy <2.03>. Spot 10 μL of the sample solution and 5 μL
49 of the standard solution on a plate of silica gel for thin-layer
50 chromatography. Develop the plate with a mixture of ethyl
51 acetate and hexane (1:1) to a distance of about 7 cm, and
52 air-dry the plate. Spray evenly 4-dimethylaminobenzalde-
53 hyde TS on the plate, heat at 105°C for 5 minutes, allow to
54 cool, and spray water: one of the several spots obtained
55 from the sample solution has the same color tone and *R_f*
56 value with the blue-green to grayish green spot from the
57 standard solution (Ginger).

58 (3) To 1.0 g of the dry extract (or 3.0 g of the viscous
59 extract) add 10 mL of sodium hydroxide TS, shake, add 5
60 mL of 1-butanol, shake, centrifuge, and use the supernatant
61 liquid as the sample solution. Separately, dissolve 1 mg of
62 ginsenoside Rb₁ for thin-layer chromatography in 1 mL of
63 methanol, and use this solution as the standard solution.
64 Perform the test with these solutions as directed under
65 Thin-layer Chromatography <2.03>. Spot 5 μL each of the
66 sample solution and standard solution on a plate of silica
67 gel for thin-layer chromatography. Develop the plate with
68 a mixture of ethyl acetate, 1-propanol, water and acetic acid
69 (100) (7:5:4:1) to a distance of about 7 cm, and air-dry the
70 plate. Spray evenly vanillin-sulfuric acid-ethanol TS for
71 spraying on the plate, heat at 105°C for 5 minutes, and al-
72 low to cool: one of the several spots obtained from the sam-
73 ple solution has the same color tone and *R_f* value with the
74 blue-purple spot from the standard solution (Ginseng).

75 **Purity** (1) Heavy metals <1.07>—Prepare the test solu-
76 tion with 1.0 g of the dry extract (or an amount of the vis-
77 cuous extract, equivalent to 1.0 g of the dried substance) as
78 directed under the Extracts (4), and perform the test (not
79 more than 30 ppm).

80 (2) Arsenic <1.11>—Prepare the test solution with 0.67
81 g of the dry extract (or an amount of the viscous extract,
82 equivalent to 0.67 g of the dried substance) according to
83 Method 3, and perform the test (not more than 3 ppm).

84 **Loss on drying** <2.41> The dry extract: Not more than
85 11.0% (1 g, 105°C, 5 hours).

86 The viscous extract: Not more than 66.7% (1 g, 105°C, 5
87 hours).

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

90 **Assay** (1) Evodiamine Weigh accurately about 0.5 g
91 of the dry extract (or an amount of the viscous extract,
92 equivalent to about 0.5 g of the dried substance), add ex-
93 actly 50 mL of diluted methanol (7 in 10), shake for 30
94 minutes, filter, and use the filtrate as the sample solution.
95 Separately, weigh accurately about 10 mg of evodiamine
96 for assay, and dissolve in methanol to make exactly 200 mL,

97 and use this solution as the standard solution. Perform the
98 test with exactly 10 μL each of the sample solution and
99 standard solution as directed under Liquid Chromatog-
100 raphy <2.01> according to the following conditions, and de-
101 termine the peak areas, A_T and A_S , of evodiamine in each
102 solution.

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

104 M_S : Amount (mg) of evodiamine for assay taken

105 *Operating conditions*—

106 Detector: An ultraviolet absorption photometer
107 (wavelength: 282 nm).

108 Column: A stainless steel column 4.6 mm in inside
109 diameter and 15 cm in length, packed with
110 octadecylsilanized silica gel for liquid chromatography (5
111 μm in particle diameter).

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

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

115 Flow rate: 1.0 mL per minute (the retention time of
116 evodiamine is about 18 minutes).

117 *System suitability*—

118 System performance: When the procedure is run with 10
119 μL of the standard solution under the above operating
120 conditions, the number of theoretical plates and the
121 symmetry factor of the peak of evodiamine are not less than
122 5000 and not more than 1.5, respectively.

123 System repeatability: When the test is repeated 6 times
124 with 10 μL of the standard solution under the above
125 operating conditions, the relative standard deviation of the
126 peak area of evodiamine is not more than 1.5%.

127 (2) [6]-Gingerol—Weigh accurately about 0.5 g of the
128 dry extract (or an amount of the viscous extract, equivalent
129 to about 0.5 g of the dried substance), add exactly 50 mL
130 of diluted methanol (7 in 10), shake for 30 minutes, filter,
131 and use the filtrate as the sample solution. Separately,
132 weigh accurately about 10 mg of [6]-gingerol for assay, dis-
133 solve in methanol to make exactly 100 mL. Pipet 5 mL of
134 this solution, add methanol to make exactly 50 mL, and use
135 this solution as the standard solution. Perform the test with
136 exactly 10 μL each of the sample solution and standard so-
137 lution as directed under Liquid Chromatography <2.01> ac-
138 cording to the following conditions, and determine the peak
139 areas, A_T and A_S , of [6]-gingerol in each solution.

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

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

142 *Operating conditions*—

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

145 Flow rate: 1.0 mL per minute (the retention time of [6]-
146 gingerol is about 14 minutes).

147 *System suitability*—

148 System performance: When the procedure is run with 10
149 μL of the standard solution under the above operating
150 conditions, the number of theoretical plates and the
151 symmetry factor of the peak of [6]-gingerol are not less
152 than 5000 and not more than 1.5, respectively.

153 System repeatability: When the test is repeated 6 times
154 with 10 μL of the standard solution under the above
155 operating conditions, the relative standard deviation of the
156 peak area of [6]-gingerol is not more than 1.5%.

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

158 **Add the following to 9.41 Reagents,**
159 **Test Solutions:**

160 **Evodiamine for assay** $\text{C}_{19}\text{H}_{17}\text{N}_3\text{O}$ White to light
161 yellow crystals or crystalline powder. Very slightly soluble
162 in methanol and in ethanol (99.5), and practically insoluble
163 in water.

164 *Identification*—Proceed as directed in the Assay: it ex-
165 hibits a double doublet-like signal equivalent to one proton
166 around δ 2.82 ppm, signals equivalent to four protons
167 which includes a singlet signal around δ 2.91 ppm and a
168 multiplet signal around δ 2.90 ppm – δ 2.98 ppm, a double
169 triplet-like signal equivalent to one proton around δ 3.23
170 ppm, a double doublet-like signal equivalent to one proton
171 around δ 4.66 ppm, a singlet signal equivalent to one proton
172 around δ 6.16 ppm, a triplet-like signal equivalent to one
173 proton around δ 7.00 ppm, a triplet-like signal equivalent
174 to one proton around δ 7.05 ppm, a doublet-like signal
175 equivalent to one proton around δ 7.08 ppm, a triplet-like
176 signal equivalent to one proton around δ 7.14 ppm, a dou-
177 blet-like signal equivalent to one proton around δ 7.39 ppm,
178 a doublet-like signal equivalent to one proton around δ 7.51
179 ppm, a multiplet-like signal equivalent to one proton
180 around δ 7.52 ppm and a doublet-like signal equivalent to
181 one proton around δ 7.83 ppm.

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

191 *Operating conditions*

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

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

197 System suitability

198 System performance: When the procedure is run with 10
199 μL of the sample solution under the above operating con-
200 ditions, the number of theoretical plates and the symmetry
201 factor of the peak of evodiamine are not less than 5000 and
202 not more than 1.5, respectively.

203 Assay—Weigh accurately 5 mg of evodiamine for assay
204 and 1 mg of DSS- d_6 for nuclear magnetic resonance spec-
205 troscopy using an ultramicrobalance, dissolve them in 1 mL
206 of deuterated dimethylsulfoxide for nuclear magnetic reso-
207 nance spectroscopy, and use this solution as the sample so-
208 lution. Transfer the sample solution into an NMR tube 5
209 mm in outer diameter, measure ^1H -NMR as directed under
210 Nuclear Magnetic Resonance Spectroscopy <2.21> and
211 Crude Drugs Test <5.01> according to the following condi-
212 tions, using DSS- d_6 for nuclear magnetic resonance spec-
213 troscopy as the internal reference compound. Calculate the
214 resonance intensity A (equivalent to 1 hydrogen) of the sig-
215 nal around δ 6.16 ppm assuming the signal of the internal
216 reference compound as δ 0 ppm.

217 Amount (%) of evodiamine ($\text{C}_{19}\text{H}_{17}\text{N}_3\text{O}$)
218
$$= M_S \times I \times P / (M \times N) \times 1.3521$$

219 M : Amount (mg) of evodiamine for assay taken

220 M_S : Amount (mg) of DSS- d_6 for nuclear magnetic reso-
221 nance spectroscopy taken

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

225 N : Number of the hydrogen derived from A

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

228 Operating conditions

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

231 Target nucleus: ^1H .

232 Digital resolution: 0.25 Hz or lower.

233 Measuring spectrum range: 20 ppm or upper, including
234 between -5 ppm and 15 ppm.

235 Spinning: off.

236 Pulse angle: 90° .

237 ^{13}C decoupling: on.

238 Delay time: Repeating pulse waiting time not less than
239 60 seconds.

240 Integrating times: 8 or more times.

241 Dummy scanning: 2 or more times.

242 Measuring temperature: A constant temperature between
243 20°C and 30°C .

244 System suitability

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

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

253 System repeatability: When the test is repeated 6 times
254 with the sample solution under the above operating condi-
255 tions, the relative standard deviation of the ratio of the reso-
256 nance intensity A to that of the internal reference com-
257 pound is not more than 1.0%.

258 **Evodia fruit** [Same as the namesake monograph]

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