

1 Yokukansankachimpihange Extract

2 抑肝散加陳皮半夏エキス
3

4 Yokukansankachimpihange Extract contains not less
5 than 0.6 mg and not more than 2.4 mg of saikosaponin
6 b₂, not less than 10 mg and not more than 30 mg of
7 glycyrrhizic acid (C₄₂H₆₂O₁₆: 822.93), and not less than
8 18 mg and not more than 72 mg of hesperidin, per ex-
9 tract prepared with the amount specified in the Method
10 of preparation.

11 Method of preparation

	1)	2)
Japanese Angelica Root	3 g	3 g
Uncaria Hook	3 g	3 g
Cnidium Rhizome	3 g	3 g
Atractylodes Rhizome	4 g	—
Atractylodes Lancea Rhizome	—	4 g
Poria Sclerotium	4 g	4 g
Bupleurum Root	2 g	2 g
Glycyrrhiza	1.5 g	1.5 g
Citrus Unshiu Peel	3 g	3 g
Pinellia Tuber	5 g	5 g

12
13 Prepare a dry extract or viscous extract as directed under
14 Extracts, according to the prescription 1) or 2), using the
15 crude drugs shown above.

16 **Description** The dry extract: Yokukansankachimpihange
17 Extract is a grayish brown to reddish yellow-brown powder.
18 It has a characteristic odor, and has a sweet and slightly hot
19 taste at first, later bitter.

20 The viscous extract: Yokukansankachimpihange Extract is
21 a brown viscous liquid. It has a characteristic odor, and has a
22 bitter and slightly sweet taste.

23 **Identification (1)** To 2.0 g of the dry extract (or 6.0 g of
24 the viscous extract) add 10 mL of water, shake, then add 10
25 mL of diethyl ether, shake, and centrifuge. Separate the di-
26 ethyl ether layer, add 10 mL of sodium hydroxide TS, shake,
27 then centrifuge, and use the diethyl ether layer as the sample
28 solution. Separately, use (*Z*)-ligustilide TS for thin-layer
29 chromatography as the standard solution. Perform the test
30 with these solutions as directed under Thin-layer Chromatog-
31 raphy <2.03>. Spot 10 μL each of the sample solution and
32 standard solution on a plate of silica gel for thin-layer chro-
33 matography. Develop the plate with a mixture of butyl ace-
34 tate and hexane (2:1) to a distance of about 7 cm, and air-dry
35 the plate. Examine under ultraviolet light (main wavelength:
36 365 nm): one of the several spots obtained from the sample
37 solution has the same color tone and *R_f* value with the blue-
38 white fluorescent spot from the standard solution (Japanese
39 Angelica Root; Cnidium Rhizome).

40 **(2)** To 2.0 g of the dry extract (or 6.0 g of the viscous
41 extract) add 20 mL of water and 2 mL of ammonia TS, shake,

42 then add 20 mL of diethyl ether, shake, and separate the di-
43 ethyl ether layer. Evaporate the solvent under reduced pres-
44 sure (vacuum), add 1 mL of methanol to the residue, and use
45 this solution as the sample solution. Separately, dissolve 1
46 mg each of rhyncophyllin for thin-layer chromatography and
47 hirsutine for thin-layer chromatography in 1 mL of methanol,
48 and use this solution as the standard solution. Perform the test
49 with these solutions as directed under Thin-layer Chromatog-
50 raphy <2.03>. Spot 10 μL of the sample solution and 2 μL of
51 the standard solution on a plate of silica gel with fluorescent
52 indicator for thin-layer chromatography. Develop the plate
53 with a mixture of ethyl acetate, 1-propanol, water and acetic
54 acid (100) (7:5:4:1) to a distance of about 7 cm, and air-dry
55 the plate. Examine under ultraviolet light (main wavelength:
56 254 nm): at least one of the several spots obtained from the
57 sample solution has the same color tone and *R_f* value with
58 one of the two dark violet spots from the standard solution
59 (Uncaria Hook).

60 **(3)** For preparation prescribed Atractylodes Rhizome —
61 To 1.0 g of the dry extract (or 3.0 g of the viscous extract)
62 add 10 mL of water, shake, then add 25 mL of diethyl ether,
63 and shake. Separate the diethyl ether layer, evaporate the sol-
64 vent under reduced pressure (vacuum), then add 2 mL of di-
65 ethyl ether to the residue, and use this solution as the sample
66 solution. Separately, dissolve 1 mg of atractylenoide III for
67 thin-layer chromatography in 2 mL of methanol, and use this
68 solution as the standard solution. Perform the test with these
69 solutions as directed under Thin-layer Chromatography
70 <2.03>. Spot 5 μL each of the sample solution and standard
71 solution on a plate of silica gel for thin-layer chromatography.
72 Develop the plate with a mixture of hexane and ethyl acetate
73 (2:1) to a distance of about 7 cm, and air-dry the plate. Spray
74 evenly 1-naphthol-sulfuric acid TS on the plate, heat at 105°C
75 for 5 minutes, and allow to cool: one of the several spots ob-
76 tained from the sample solution has the same color tone and
77 *R_f* value with the red to red-purple spot from the standard
78 solution (Atractylodes Rhizome).

79 **(4)** For preparation prescribed Atractylodes Lancea Rhi-
80 zome — To 2.0 g of the dry extract (or 6.0 g of the viscous
81 extract) add 10 mL of water, shake, then add 25 mL of hexane,
82 and shake. Separate the hexane layer, evaporate the solvent
83 under reduced pressure (vacuum), then add 2 mL of hexane
84 to the residue, and use this solution as the sample solution.
85 Perform the test with the sample solution as directed under
86 Thin-layer Chromatography <2.03>. Spot 20 μL of the sample
87 solution on a plate of silica gel with fluorescent indicator for
88 thin-layer chromatography. Develop the plate with a mixture
89 of hexane and acetone (7:1) to a distance of about 7 cm, and
90 air-dry the plate. Examine under ultraviolet light (main wave-
91 length: 254 nm): a dark violet spot is observed at an *R_f* value
92 of about 0.5. Spray evenly 4-dimethylaminobenzaldehyde TS
93 for spraying on the plate, heat at 105°C for 5 minutes, and

94 allow to cool: the spot exhibits a greenish brown color (At-
95 ractylodes Lancea Rhizome).

96 (5) To 1.0 g of the dry extract (or 3.0 g of the viscous
97 extract) add 10 mL of water, shake, then add 10 mL of 1-
98 butanol, shake, centrifuge, and use the 1-butanol layer as the
99 sample solution. Separately, dissolve 1 mg of saikosaponin
100 b₂ for thin-layer chromatography in 1 mL of methanol, and
101 use this solution as the standard solution. Perform the test
102 with these solutions as directed under Thin-layer Chromatog-
103 raphy <2.03>. Spot 10 μL of the sample solution and 2 μL of
104 the standard solution on a plate of silica gel for thin-layer
105 chromatography. Develop the plate with a mixture of ethyl
106 acetate, ethanol (99.5) and water (8:2:1) to a distance of about
107 7 cm, and air-dry the plate. Spray evenly 4-dimethylamino-
108 benzaldehyde TS for spraying on the plate, heat at 105°C for
109 5 minutes, and examine under ultraviolet light (main wave-
110 length: 365 nm): one of the several spots obtained from the
111 sample solution has the same color tone and R_f value with the
112 yellow fluorescent spot from the standard solution (Bupleu-
113 rum Root).

114 (6) To 1.0 g of the dry extract (or 3.0 g of the viscous
115 extract) add 10 mL of water, shake, then add 10 mL of 1-
116 butanol, shake, centrifuge, and use the 1-butanol layer as the
117 sample solution. Separately, dissolve 1 mg of liquiritin for
118 thin-layer chromatography in 1 mL of methanol, and use this
119 solution as the standard solution. Perform the test with these
120 solutions as directed under Thin-layer Chromatography
121 <2.03>. Spot 1 μL each of the sample solution and standard
122 solution on a plate of silica gel for thin-layer chromatography.
123 Develop the plate with a mixture of ethyl acetate, methanol
124 and water (20:3:2) to a distance of about 7 cm, and air-dry
125 the plate. Spray evenly dilute sulfuric acid on the plate, heat
126 at 105°C for 5 minutes, and examine under ultraviolet light
127 (main wavelength: 365 nm): one of the several spots obtained
128 from the sample solution has the same color tone and R_f value
129 with the yellow-green fluorescent spot from the standard so-
130 lution (Glycyrrhiza).

131 (7) To 1.0 g of the dry extract (or 3.0 g of the viscous
132 extract) add 10 mL of water, shake, then add 10 mL of 1-
133 butanol, centrifuge, and use the 1-butanol layer as the sample
134 solution. Separately, dissolve 1 mg of hesperidin for thin-
135 layer chromatography in 1 mL of methanol, and use this so-
136 lution as the standard solution. Perform the test with these
137 solutions as directed under Thin-layer Chromatography
138 <2.03>. Spot 20 μL of the sample solution and 10 μL of the
139 standard solution on a plate of silica gel for thin-layer chro-
140 matography. Develop the plate with a mixture of ethyl acetate,
141 acetone, water and acetic acid (100) (10:6:3:1) to a distance
142 of about 7 cm, and air-dry the plate. Spray evenly 2,6-di-
143 bromo-*N*-chloro-1,4-benzoquinone monoimine TS on the
144 plate, and expose the plate to ammonia vapor: one of the sev-
145 eral spots from the sample solution has the same color tone

146 and R_f value with the blue spot from the standard solution
147 (Citrus Unshiu Peel).

148 **Purity (1)** Heavy metals <1.07>—Prepare the test solu-
149 tion with 1.0 g of the dry extract (or an amount of the viscous
150 extract, equivalent to 1.0 g of the dried substance) as directed
151 under Extracts (4), and perform the test (not more than 30
152 ppm).

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

157 **Loss on drying <2.41>** The dry extract: Not more than
158 10.0% (1 g, 105°C, 5 hours).

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

161 **Total ash <5.01>** Not less than 9.0%, calculated on the
162 dried basis.

163 **Assay (1)** Saikosaponin b₂—Weigh accurately about 0.5
164 g of the dry extract (or an amount of the viscous extract,
165 equivalent to about 0.5 g of the dried substance), add 20 mL
166 of diethyl ether and 10 mL of water, and shake for 10 minutes.
167 Centrifuge this solution, remove the diethyl ether layer, then
168 add 20 mL of diethyl ether, proceed in the same manner as
169 above, and remove the diethyl ether layer. To the resultant
170 aqueous layer add 10 mL of methanol, shake for 30 minutes,
171 centrifuge, and separate the supernatant liquid. To the residue
172 add 20 mL of diluted methanol (1 in 2), shake for 5 minutes,
173 centrifuge, and separate the supernatant liquid. Combine the
174 supernatant liquids, add diluted methanol (1 in 2) to make
175 exactly 50 mL, and use this solution as the sample solution.
176 Separately, use saikosaponin b₂ standard TS for assay as the
177 standard solution. Perform the test with exactly 10 μL each
178 of the sample solution and standard solution as directed under
179 Liquid Chromatography <2.01> according to the following
180 conditions, and determine the peak areas, A_T and A_S, of sai-
181 kosaponin b₂ in each solution.

182 Amount (mg) of saikosaponin b₂ = $C_S \times A_T / A_S \times 50$

183 C_S: Concentration (mg/mL) of saikosaponin b₂ in sai-
184 kosaponin b₂ standard TS for assay

185 *Operation conditions*—

186 Detector: An ultraviolet absorption photometer (wave-
187 length: 254 nm).

188 Column: A stainless steel column 4.6 mm in inside diam-
189 eter and 15 cm in length, packed with octadecylsilanized sil-
190 ica gel for liquid chromatography (5 μm in particle diameter).

191 Column temperature: A constant temperature of about
192 40°C.

193 Mobile phase: A mixture of 0.05 mol/L sodium dihydro-
194 gen phosphate TS and acetonitrile (5:3).

195 Flow rate: 1.0 mL per minute.

196 *Systemic suitability*—

197 System performance: When the procedure is run with 10
198 μL of the standard solution under the above operating condi-
199 tions, the number of theoretical plates and the symmetry fac-
200 tor of the peak of saikosaponin b_2 are not less than 5000 and
201 not more than 1.5, respectively.

202 System repeatability: When the test is repeated 6 times
203 with 10 μL of the standard solution under the above operating
204 conditions, the relative standard deviation of the peak area of
205 saikosaponin b_2 is not more than 1.5%.

206 (2) Glycyrrhizic acid—Weigh accurately about 0.5 g of
207 the dry extract (or an amount of the viscous extract, equiva-
208 lent to about 0.5 g of the dried substance), add 20 mL of di-
209 ethyl ether and 10 mL of water, and shake for 10 minutes.
210 Centrifuge this solution, remove the diethyl ether layer, then
211 add 20 mL of diethyl ether, proceed in the same manner as
212 above, and remove the diethyl ether layer. To the resultant
213 aqueous layer add 10 mL of methanol, shake for 30 minutes,
214 centrifuge, and separate the supernatant liquid. To the residue
215 add 20 mL of diluted methanol (1 in 2), shake for 5 minutes,
216 centrifuge, and separate the supernatant liquid. Combine the
217 supernatant liquids, add diluted methanol (1 in 2) to make
218 exactly 50 mL, and use this solution as the sample solution.
219 Separately, weigh accurately about 10 mg of Glycyrrhizic
220 Acid RS (separately determine the water <2.48> by coulo-
221 metric titration, using 10 mg), dissolve in diluted methanol
222 (1 in 2) to make exactly 100 mL, and use this solution as the
223 standard solution. Perform the test with exactly 10 μL each
224 of the sample solution and standard solution as directed under
225 Liquid Chromatography <2.01> according to the following
226 conditions, and determine the peak areas, A_T and A_S , of
227 glycyrrhizic acid in each solution.

$$228 \quad \text{Amount (mg) of glycyrrhizic acid (C}_{42}\text{H}_{62}\text{O}_{16}) \\ 229 \quad = M_S \times A_T / A_S \times 1 / 2$$

230 M_S : Amount (mg) of Glycyrrhizic Acid RS taken, calcu-
231 lated on the anhydrous basis

232 *Operation conditions*—

233 Detector: An ultraviolet absorption photometer (wave-
234 length: 254 nm).

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

238 Column temperature: A constant temperature of about
239 40°C.

240 Mobile phase: Dissolve 3.85 g of ammonium acetate in
241 720 mL of water, and add 5 mL of acetic acid (100) and 280
242 mL of acetonitrile.

243 Flow rate: 1.0 mL per minute.

244 *Systemic suitability*—

245 System performance: Dissolve 5 mg of monoammonium
246 glycyrrhizinate for resolution check in 20 mL of dilute etha-
247 nol. When the procedure is run with 10 μL of this solution
248 under the above operating conditions, the resolution between
249 the peak having the relative retention time about 0.9 to
250 glycyrrhizic acid and the peak of glycyrrhizic acid is not less
251 than 1.5.

252 System repeatability: When the test is repeated 6 times
253 with 10 μL of the standard solution under the above operating
254 conditions, the relative standard deviation of the peak area of
255 glycyrrhizic acid is not more than 1.5%.

256 (3) Hesperidin—Weigh accurately about 0.1 g of the dry
257 extract (or an amount of the viscous extract, equivalent to
258 about 0.1 g of dried substance), add exactly 50 mL of diluted
259 tetrahydrofuran (1 in 4), shake for 30 minutes, centrifuge, and
260 use the supernatant liquid as the sample solution. Separately,
261 weigh accurately about 10 mg of hesperidin for assay, previ-
262 ously dried in a desiccator (silica gel) for 24 hours, dissolve
263 in methanol to make exactly 100 mL. Pipet 10 mL of this
264 solution, add diluted tetrahydrofuran (1 in 4) to make exactly
265 100 mL, and use this solution as the standard solution. Per-
266 form the test with exactly 10 μL each of the sample solution
267 and standard solution as directed under Liquid Chromatog-
268 raphy <2.01> according to the following conditions, and de-
269 termine the peak areas, A_T and A_S , of hesperidin in each solu-
270 tion.

$$271 \quad \text{Amount (mg) of hesperidin} = M_S \times A_T / A_S \times 1 / 20$$

272 M_S : Amount (mg) of hesperidin for assay taken

273 *Operating conditions*—

274 Detector: An ultraviolet absorption photometer (wave-
275 length: 285 nm).

276 Column: A stainless steel column 4.6 mm in inside diam-
277 eter and 15 cm in length, packed with octadecylsilanized sil-
278 ica gel for liquid chromatography (5 μm in particle diameter).

279 Column temperature: A constant temperature of about
280 40°C.

281 Mobile phase: A mixture of water, acetonitrile and acetic
282 acid (100) (82:18:1).

283 Flow rate: 1.0 mL per minute.

284 *System suitability*—

285 System performance: Dissolve 1 mg each of hesperidin for
286 assay and naringin for thin-layer chromatography in diluted
287 methanol (1 in 2) to make 100 mL. When the procedure is
288 run with 10 μL of this solution under the above operating
289 conditions, naringin and hesperidin are eluted in this order
290 with the resolution between these peaks being not less than
291 1.5.

292 System repeatability: When the test is repeated 6 times
293 with 10 μL of the standard solution under the above operating

294 conditions, the relative standard deviation of the peak area of
295 hesperidin is not more than 1.5%.

296 **Containers and storage** Containers—Tight containers.
297