

## SUPPLEMENTARY MATERIAL

# Anti-allergic and Antioxidant Potential of Polyphenol-Enriched Fractions from *Cyclopia subternata* (Honeybush) Produced by a Scalable Process

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**Table S1** Adsorption capacity (mg/g) of XAD 1180N and HP20 macroporous resins for *Cyclopia subternata* phenolic compounds as a function of sample concentration (mg/mL)

Resin	Sample concentration (mg/mL)	Adsorption capacity (mg g <sup>-1</sup> )										
		IDG	IMG	PDG	HPDG	Eriocitrin	Hesperidin	Mangiferin	Isomangiferin	Vicenin-2	Scolymoside	<i>p</i> -Coumaric acid
XAD 1180N	1	0.447 <sup>i</sup> ± 0.004	0.148 <sup>j</sup> ± 0.001	0.279 <sup>h</sup> ± 0.002	0.064 <sup>k</sup> ± 0.000	0.099 <sup>j</sup> ± 0.001	0.163 <sup>j</sup> ± 0.001	0.333 <sup>k</sup> ± 0.002	0.092 <sup>i</sup> ± 0.001	0.035 <sup>j</sup> ± 0.000	0.169 <sup>k</sup> ± 0.001	0.005 <sup>j</sup> ± 0.000
	2	0.873 <sup>g</sup> ± 0.005	0.269 <sup>j</sup> ± 0.001	0.518 <sup>g</sup> ± 0.002	0.125 <sup>i</sup> ± 0.000	0.185 <sup>h</sup> ± 0.001	0.295 <sup>j</sup> ± 0.001	0.600 <sup>j</sup> ± 0.002	0.172 <sup>h</sup> ± 0.001	0.067 <sup>i</sup> ± 0.000	0.318 <sup>j</sup> ± 0.001	0.008 <sup>h</sup> ± 0.000
	3	1.303 <sup>f</sup> ± 0.002	0.409 <sup>h</sup> ± 0.001	0.803 <sup>f</sup> ± 0.003	0.194 <sup>g</sup> ± 0.001	0.290 <sup>g</sup> ± 0.001	0.476 <sup>f</sup> ± 0.002	0.991 <sup>g</sup> ± 0.003	0.292 <sup>f</sup> ± 0.001	0.111 <sup>g</sup> ± 0.000	0.541 <sup>g</sup> ± 0.002	0.014 <sup>f</sup> ± 0.000
	5	2.137 <sup>e</sup> ± 0.014	0.688 <sup>e</sup> ± 0.004	1.365 <sup>d</sup> ± 0.010	0.336 <sup>e</sup> ± 0.002	0.492 <sup>e</sup> ± 0.003	0.796 <sup>d</sup> ± 0.006	1.683 <sup>e</sup> ± 0.011	0.494 <sup>d</sup> ± 0.003	0.182 <sup>e</sup> ± 0.001	0.947 <sup>e</sup> ± 0.007	0.024 <sup>d</sup> ± 0.000
	7.5	3.812 <sup>b</sup> ± 0.010	0.996 <sup>d</sup> ± 0.002	2.026 <sup>c</sup> ± 0.005	0.512 <sup>c</sup> ± 0.001	0.726 <sup>d</sup> ± 0.002	1.178 <sup>c</sup> ± 0.003	2.516 <sup>d</sup> ± 0.006	0.752 <sup>c</sup> ± 0.002	0.268 <sup>c</sup> ± 0.001	1.458 <sup>d</sup> ± 0.004	0.035 <sup>c</sup> ± 0.000
	10	5.080 <sup>a</sup> ± 0.010	1.284 <sup>a</sup> ± 0.000	2.663 <sup>b</sup> ± 0.005	0.679 <sup>a</sup> ± 0.001	0.956 <sup>b</sup> ± 0.002	1.529 <sup>b</sup> ± 0.003	3.313 <sup>b</sup> ± 0.005	1.007 <sup>a</sup> ± 0.001	0.354 <sup>a</sup> ± 0.001	1.938 <sup>b</sup> ± 0.004	0.046 <sup>a</sup> ± 0.000
HP20	1	0.448 <sup>i</sup> ± 0.001	0.153 <sup>j</sup> ± 0.001	0.267 <sup>i</sup> ± 0.001	0.058 <sup>j</sup> ± 0.000	0.094 <sup>j</sup> ± 0.000	0.151 <sup>k</sup> ± 0.001	0.303 <sup>j</sup> ± 0.001	0.086 <sup>j</sup> ± 0.000	0.034 <sup>j</sup> ± 0.000	0.154 <sup>j</sup> ± 0.001	0.004 <sup>j</sup> ± 0.000
	2	0.838 <sup>h</sup> ± 0.008	0.276 <sup>j</sup> ± 0.001	0.526 <sup>g</sup> ± 0.001	0.114 <sup>j</sup> ± 0.000	0.187 <sup>h</sup> ± 0.001	0.301 <sup>h</sup> ± 0.001	0.616 <sup>j</sup> ± 0.002	0.171 <sup>h</sup> ± 0.000	0.068 <sup>j</sup> ± 0.000	0.332 <sup>j</sup> ± 0.001	0.007 <sup>j</sup> ± 0.000
	3	1.300 <sup>f</sup> ± 0.019	0.426 <sup>g</sup> ± 0.009	0.809 <sup>f</sup> ± 0.005	0.188 <sup>h</sup> ± 0.001	0.286 <sup>g</sup> ± 0.002	0.466 <sup>g</sup> ± 0.003	0.948 <sup>h</sup> ± 0.007	0.269 <sup>g</sup> ± 0.002	0.106 <sup>h</sup> ± 0.001	0.521 <sup>h</sup> ± 0.003	0.012 <sup>g</sup> ± 0.001
	5	2.506 <sup>d</sup> ± 0.015	0.678 <sup>f</sup> ± 0.010	1.294 <sup>e</sup> ± 0.008	0.312 <sup>f</sup> ± 0.002	0.458 <sup>f</sup> ± 0.003	0.729 <sup>e</sup> ± 0.004	1.542 <sup>f</sup> ± 0.011	0.446 <sup>e</sup> ± 0.003	0.169 <sup>f</sup> ± 0.001	0.865 <sup>f</sup> ± 0.005	0.021 <sup>e</sup> ± 0.000
	7.5	3.769 <sup>c</sup> ± 0.018	1.045 <sup>c</sup> ± 0.000	2.024 <sup>c</sup> ± 0.009	0.502 <sup>d</sup> ± 0.002	0.730 <sup>c</sup> ± 0.003	1.182 <sup>c</sup> ± 0.006	2.604 <sup>c</sup> ± 0.000	0.753 <sup>c</sup> ± 0.000	0.263 <sup>d</sup> ± 0.000	1.519 <sup>c</sup> ± 0.007	0.037 <sup>b</sup> ± 0.000
	10	5.069 <sup>a</sup> ± 0.005	1.275 <sup>b</sup> ± 0.010	2.704 <sup>a</sup> ± 0.003	0.669 <sup>b</sup> ± 0.001	0.973 <sup>a</sup> ± 0.001	1.569 <sup>a</sup> ± 0.002	3.356 <sup>a</sup> ± 0.000	0.978 <sup>b</sup> ± 0.000	0.344 <sup>b</sup> ± 0.000	2.025 <sup>a</sup> ± 0.002	0.045 <sup>a</sup> ± 0.000

Different letters in the same column indicate significant differences ( $p < 0.05$ ). Data are given as mean ± standard deviation ( $n = 3$ ). *Abbreviations*: IDG, 3-β-D-glucopyranosyl-4-O-β-D-glucopyranosyliriflophenone; IMG, 3-β-D-glucopyranosyliriflophenone; HPDG, 3',5'-di-β-D-glucopyranosyl-3-hydroxyphloretin; PDG, 3',5'-di-β-D-glucopyranosylphloretin.

**Table S2** Adsorption ratios (%) for *Cyclopia subternata* phenolic compounds on XAD 1180N and HP20 macroporous resins as a function of sample concentration (mg/mL)

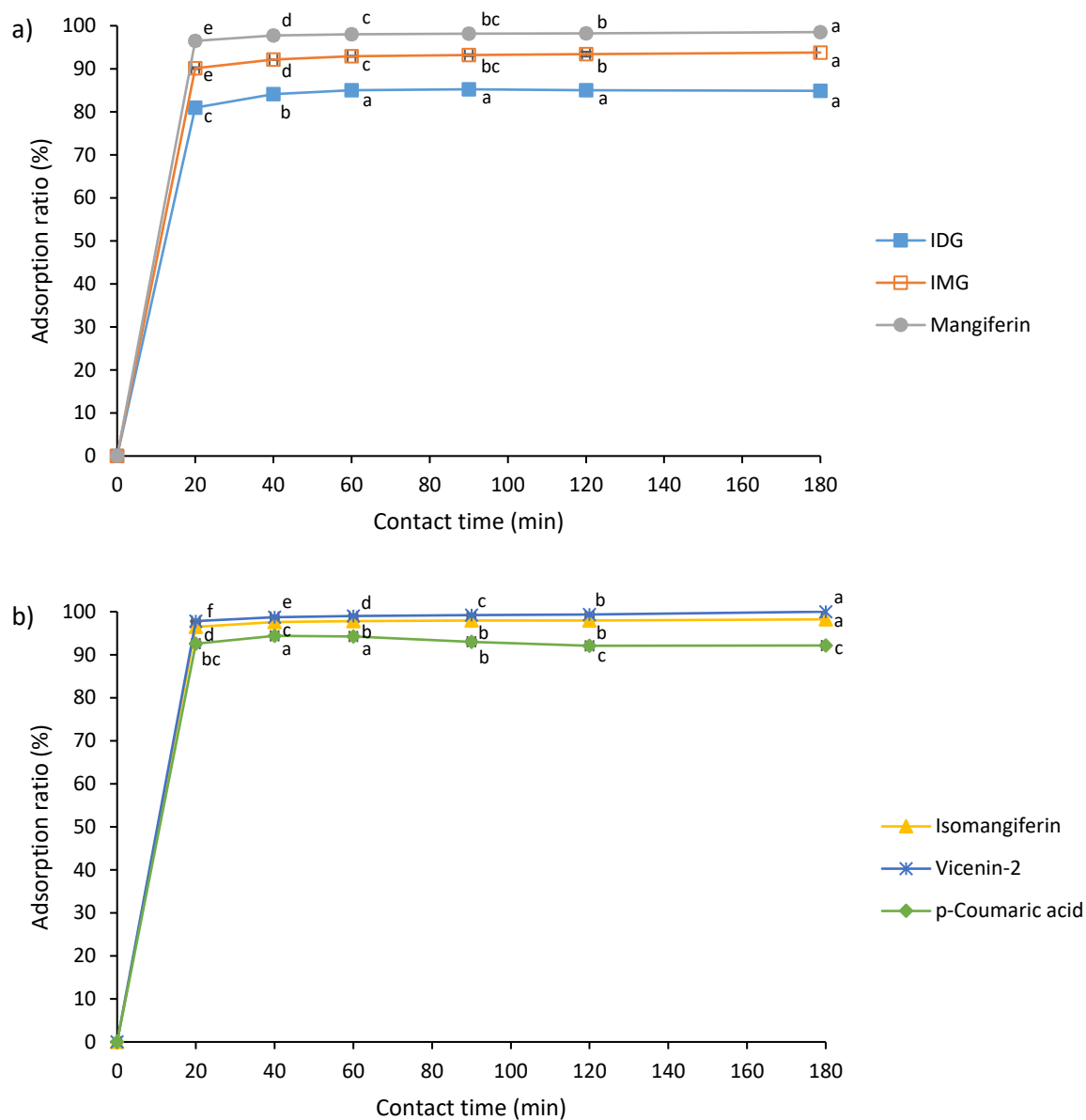
Macroporous resin	Sample concentration (mg/mL)	Adsorption ratio (%)				
		IDG <sup>a</sup>	IMG <sup>b</sup>	Mangiferin	Isomangiferin	<i>p</i> -Coumaric acid
XAD 1180N	1	88.5 <sup>a</sup> ± 0.9	100.0 <sup>a</sup> ± 0.0	99.3 <sup>a</sup> ± 0.1	100.0 <sup>a</sup> ± 0.0	91.9 <sup>b</sup> ± 0.6
	2	87.5 <sup>a</sup> ± 0.3	96.7 <sup>b</sup> ± 0.1	99.1 <sup>b</sup> ± 0.0	99.1 <sup>b</sup> ± 0.0	92.3 <sup>ab</sup> ± 0.1
	3	86.0 <sup>b</sup> ± 0.2	96.3 <sup>c</sup> ± 0.1	99.0 <sup>b</sup> ± 0.0	99.0 <sup>b</sup> ± 0.0	91.7 <sup>bc</sup> ± 0.3
	5	83.3 <sup>c</sup> ± 0.1	94.9 <sup>d</sup> ± 0.1	98.8 <sup>c</sup> ± 0.0	98.7 <sup>c</sup> ± 0.0	91.6 <sup>bc</sup> ± 0.0
	7.5	78.5 <sup>d</sup> ± 0.4	93.1 <sup>f</sup> ± 0.1	98.4 <sup>d</sup> ± 0.0	98.3 <sup>d</sup> ± 0.0	90.4 <sup>cd</sup> ± 0.2
	10	72.2 <sup>f</sup> ± 0.6	90.6 <sup>g</sup> ± 0.2	97.8 <sup>e</sup> ± 0.1	97.6 <sup>f</sup> ± 0.1	89.3 <sup>de</sup> ± 0.3
HP20	1	87.6 <sup>a</sup> ± 0.5	100.0 <sup>a</sup> ± 0.0	99.1 <sup>ab</sup> ± 0.1	100.0 <sup>a</sup> ± 0.0	93.5 <sup>a</sup> ± 0.4
	2	84.3 <sup>c</sup> ± 0.9	100.0 <sup>a</sup> ± 0.0	98.8 <sup>c</sup> ± 0.1	98.5 <sup>c</sup> ± 0.1	80.7 <sup>g</sup> ± 1.2
	3	84.5 <sup>c</sup> ± 2.1	97.1 <sup>b</sup> ± 0.7	98.8 <sup>c</sup> ± 0.2	98.6 <sup>c</sup> ± 0.3	88.4 <sup>e</sup> ± 2.2
	5	76.8 <sup>e</sup> ± 1.1	93.5 <sup>ef</sup> ± 0.5	97.8 <sup>e</sup> ± 0.2	97.6 <sup>f</sup> ± 0.2	86.5 <sup>f</sup> ± 0.8
	7.5	77.1 <sup>de</sup> ± 0.0	93.7 <sup>e</sup> ± 0.0	98.3 <sup>d</sup> ± 0.0	98.0 <sup>e</sup> ± 0.0	89.3 <sup>de</sup> ± 0.3
	10	62.5 <sup>g</sup> ± 0.9	86.4 <sup>h</sup> ± 0.4	95.6 <sup>f</sup> ± 0.0	94.6 <sup>g</sup> ± 0.0	85.6 <sup>f</sup> ± 1.3

Different letters in the same column indicate significant differences ( $p < 0.05$ ). Data are given as mean ± standard deviation ( $n = 3$ ). *Abbreviations:* IDG, 3-β-D-glucopyranosyl-4-O-β-D-glucopyranosyliriflophenone; IMG, 3-β-D-glucopyranosyliriflophenone.

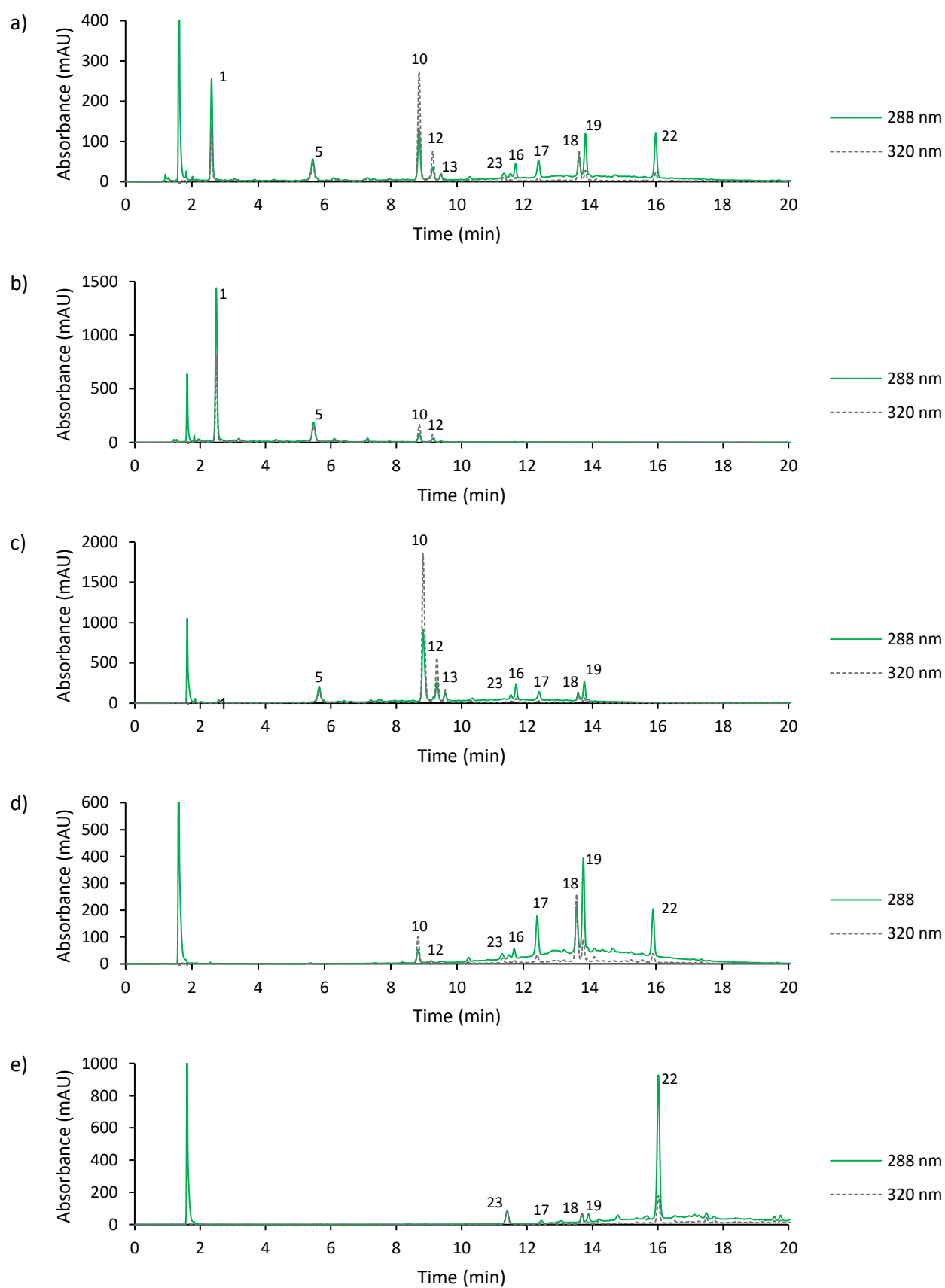
**Table S3** Phenolic compounds identified in a green *Cyclopia subternata* hot water extract and four enriched fractions using high-resolution mass spectrometry in the negative ionisation mode

Nr	t <sub>R</sub> (min)	λ <sub>max</sub> (nm)	[M-H] <sup>-</sup> (m/z)	Error (ppm)	Formula	MS <sup>E</sup> fragments (m/z)	Compounds	Extract	F1	F2	F3	F4
1	2.7	295	569.1533	4.7	C <sub>25</sub> H <sub>29</sub> O <sub>15</sub>	479, 449*, 317, 287, 167, 125	3-β-D-glucopyranosyl-4-O-β-D-glucopyranosylriflophenone	+	+	+	+	
2	3.5	321	423.0914	-3.1	C <sub>19</sub> H <sub>19</sub> O <sub>11</sub>	303*, 193	3-β-D-glucopyranosylmaclurin	+	+	+		
3	3.8	276, 310, 356	583.1272	-4.6	C <sub>25</sub> H <sub>27</sub> O <sub>16</sub>	493, 463, 331, 301, 273, 153*	tetrahydroxyxanthone-di-O,C-hexose	+	+			
4	5.4	260, 303, 366	583.1299	0.0	C <sub>25</sub> H <sub>27</sub> O <sub>16</sub>	463, 457, 421, 331, 301, 275, 245, 163*, 119	tetrahydroxyxanthone-di-O,C-hexose	+	+	+		
5	5.8	294	407.0980	0.5	C <sub>19</sub> H <sub>19</sub> O <sub>10</sub>	317, 287*, 245, 193	3-β-D-glucopyranosylriflophenone	+	+	+		+
6	6.0	no peak	285.0612	0.7	C <sub>12</sub> H <sub>13</sub> O <sub>8</sub>	153	dihydroxybenzoic acid-O-pentose	+	+			
7	6.5	262, 317, 279	871.2034	0.8	C <sub>51</sub> H <sub>35</sub> O <sub>14</sub>	751, 557, 457, 421, 331, 301, 269*, 225, 163, 119, 89	aspalathin derivative of (iso)mangiferin	+	+	+		
8	7.0	no peak	457.1350	0.9	C <sub>20</sub> H <sub>25</sub> O <sub>12</sub>	417, 163*, 119	p-coumaric acid-O-pentose-O-hexose	+	+	+		
9	7.1	290	595.1641	-3.7	C <sub>27</sub> H <sub>31</sub> O <sub>15</sub>	487, 457, 415, 385, 355, 163, 119*, 89	eriodictyol-O-(hexose-O-deoxyhexose)	+	+	+		
10	8.4	239, 257, 317, 366	421.0768	-0.7	C <sub>19</sub> H <sub>17</sub> O <sub>11</sub>	403, 331, 301*, 259	mangiferin	+	+	+	+	+
11	8.7	259, 328	437.0703	-3.9	C <sub>19</sub> H <sub>17</sub> O <sub>12</sub>	421*, 291, 231	pentahydroxyxanthone-C-hexose	+			+	
12	8.9	255, 316, 366	421.0764	-1.7	C <sub>19</sub> H <sub>17</sub> O <sub>11</sub>	331, 301*, 271	isomangiferin	+	+	+	+	
13	9.0	270, 327	593.1503	-0.5	C <sub>27</sub> H <sub>29</sub> O <sub>15</sub>	473, 421, 383, 353, 331, 301*, 271	vicenin-2	+	+	+	+	
14	9.9	236, 277, 328	437.0727	1.6	C <sub>19</sub> H <sub>17</sub> O <sub>12</sub>	317, 289*, 271, 269, 175, 163, 137, 125, 119, 89	pentahydroxyxanthone-C-hexose	+		+	+	
15	10.0	232, 282	449.1066	-4.0	C <sub>21</sub> H <sub>21</sub> O <sub>11</sub>	287, 151*, 135	eriodictyol-O-hexose	+			+	
16	11.2	284	613.1766	-0.5	C <sub>27</sub> H <sub>33</sub> O <sub>16</sub>	433, 403, 395, 373*, 331, 287, 239, 209, 179, 167, 163, 135, 119	3',5'-di-β-D-glucopyranosyl-3-hydroxyphloretin	+	+	+	+	
17	11.9	284	595.1651	-2.0	C <sub>27</sub> H <sub>31</sub> O <sub>15</sub>	387, 287, 271, 151*, 135	eriocitrin	+		+	+	+
18	13.0	254, 349	593.1501	-0.8	C <sub>27</sub> H <sub>29</sub> O <sub>15</sub>	285	scolymoside	+		+	+	+
19	13.2	285	597.1793	-4.4	C <sub>27</sub> H <sub>33</sub> O <sub>15</sub>	477, 459, 417, 387, 357*, 315, 239, 209, 167	3',5'-di-β-D-glucopyranosylphloretin	+		+	+	+
20	14.1	278	579.1713	-0.2	C <sub>27</sub> H <sub>31</sub> O <sub>14</sub>	271	naringenin-O-(hexose-O-deoxyhexose)	+				+
21	15.0	267, 343	577.1567	1.7	C <sub>27</sub> H <sub>29</sub> O <sub>14</sub>	433, 269*, 125	isorhoifolin	+			+	+
22	15.3	283, 326sh	609.1819	0.0	C <sub>28</sub> H <sub>33</sub> O <sub>15</sub>	301	hesperidin	+			+	+

\* Indicates base peak ion. *Abbreviations*: F1, fraction 1; F2, fraction 2; F3, fraction 3; F4, fraction 4; sh, shoulder; t<sub>R</sub>, retention time.



**Figure S1** Adsorption ratios of 3- $\beta$ -D-glucopyranosyl-4-O- $\beta$ -D-glucopyranosylriflophenone (IDG), 3- $\beta$ -D-glucopyranosylriflophenone (IMG) and mangiferin (a) and isomangiferin, vicenin-2 and *p*-coumaric acid (b) on XAD 1180N macroporous resin as a function of contact time (min), using static adsorption. Data are given as mean  $\pm$  standard deviation ( $n = 3$ ). Different letters at individual time intervals on the adsorption ratio graphs indicate significant differences ( $p < 0.05$ ).



**Figure S2** HPLC-DAD chromatograms of a green *Cyclopia subternata* hot water extract (a) and fractions 1-4 (b-e) obtained from large-scale fractionation on XAD 1180N. Peak labels correspond to Table S3.