

1 Running title: antioxidant properties and total phenolic contents of wetland medicinal  
2 plants in Taiwan

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4 ***In vitro* antioxidant properties and total phenolic contents of wetland**  
5 **medicinal plants in Taiwan**

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25 **ABSTRACT.** The aim of this study was to examine the possible antioxidant activities  
26 of the methanol and water extracts of 31 medicinal wetland plants in Taiwan. A  
27 number of assays were employed in this experiment to investigate the antioxidant  
28 effects of wetland medicinal plants in Taiwan, including **TEAC assay**, DPPH radical  
29 scavenging, reducing power method, total polyphenol content, total flavonoid content  
30 and total flavonol contents. The results showed that *Rotala rotundifolia* (Wallich ex  
31 Roxb.) Koehne, *Juncus effusus* L. var. *decipiens* Buchen., *Cyperus iria* L., *Salix*  
32 *warburgii* O. Seem., *Lindernia antipoda* (L.) Alston, *Kyllinga brevifolia* Rottb., and  
33 *Typha orientalis* Presl **possessed high** antioxidant activities and high contents of total  
34 polyphenols. The lower correlations between TEAC and total polyphenol content  
35 (water extracts,  $R^2=0.14$ ; methanol extracts,  $R^2=0.23$ ) was found. Therefore, high  
36 phenolic content was not an important factor in determining antioxidant capacities of  
37 these wetland medicinal plants. The results demonstrated that phytochemicals in the  
38 wetland medicinal plants might contribute significantly to the antioxidant activities of  
39 the wetland medicinal plants; however the antioxidant activities were not directly  
40 related to the quantity of polyphenols. Phytochemicals might have additive roles that  
41 contribute significantly to the potent antioxidant activity of wetland medicinal plants.  
42 It indicated that wetland medicinal plants could be used as an easy accessible source  
43 of natural antioxidants in pharmaceutical and medical industries, as well as being  
44 developed into health foods.

45

46 **Keywords:** Wetland medicinal plant ; Antioxidant ; Polyphenol ; Flavonoid; Flavonol.

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48

## 49 INTRODUCTION

50 It is commonly accepted that under situations of oxidative stress, reactive oxygen  
51 species, such as superoxide ( $O_2^-$ ), hydroxyl ( $OH^-$ ), and peroxy ( $OOH$ ,  $ROO$ )  
52 radicals, are generated. These reactive oxygen species play important roles in  
53 degenerative or pathological processes, such as aging (Burns et al., 2001), cancer,  
54 coronary heart disease, Alzheimer's disease (Diaz et al., 1997), neurodegenerative  
55 disorders, atherosclerosis, diabetes, and inflammation (Chen et al., 2006). Several  
56 anti-inflammatory, digestive, anti-necrotic, neuroprotective, and hepatoprotective  
57 drugs have been shown recently to have antioxidant and/or radical scavenging  
58 mechanism as part of their activity (Lin and Huang, 2002). In searching for specific  
59 natural antioxidants and compounds with radical scavenging activity during the last  
60 few years, some had been identified, such as echinacoside in *Echinaceae* root (Hu and  
61 Kitts, 2000), anthocyanin (Espin et al., 2000), phenolic compounds (Rice-Evans et al.,  
62 1997), and the extracts of water spinach and sweet potato tuberous roots (Huang et al.,  
63 2004; Huang et al., 2005).

64 Medicinal plant parts are commonly rich in phenolic compounds, such as  
65 flavonoids, phenolic acids, stilbenes, tannins, coumarins, lignans and lignins. They  
66 have multiple biological effects including antioxidant activity (Packer et al., 1999).  
67 The antioxidant compounds of higher plants have been demonstrated, *in vitro*  
68 experiments, to protect against oxidation damage by inhibiting or quenching free  
69 radicals and reactive oxygen species (Ali et al., 2008). The roles of these compounds  
70 as potential antioxidants can be inferred by their similarity to synthetic antioxidants,  
71 of related structures.

72 The multifarious natural environment of Taiwan harbors abundant plant  
73 resources. However, many species still face endangerment. Therefore, we investigated  
74 wetland medicinal plants and analyzed their antioxidant activities. In the present study,  
75 we collected 31 medicinal wetland plant species that are widely consumed in Taiwan,  
76 and analyzed the antioxidant activities and polyphenol contents of water and  
77 methanolic extracts prepared from these plants.

78

## 79 MATERIALS AND METHODS

### 80 Materials

81 Butylated hydroxytoluene (BHT), Glutathione (GSH),  
82 1,1-Diphenyl-2-picrylhydrazyl (DPPH), 6-Hydroxy-2, 5, 7,  
83 8-tetramethylchroman-2-carboxylic acid (Trolox), potassium peroxodisulfate  
84 ( $K_2S_2O_8$ ), Tris (hydroxymethyl) aminomethane, potassium ferricyanide ( $K_3Fe(CN)_6$ ),  
85 ferric chloride ( $FeCl_3$ ), catechin, 2,2'-azinobis-(3-ethylbenzothiazoline)-6-sulphonic  
86 acid (ABTS), and other chemicals were purchased from Sigma Chemical Co. (St.  
87 Louis, MO, USA). Folin-Ciocalteu solution and 95% ethanol were purchased from  
88 Merck Co. (Santa Ana, CA, USA). Thirty one wetland medicinal plants were  
89 collected from Taichung, Nantou, and Hsinchu counties in Taiwan. They were  
90 identified and authenticated by Dr. Chao-Lin Kuo, Associate professor and Chairman,  
91 Department of Chinese Medicine Resources, China Medical University, Taichung,  
92 Taiwan. The medicinal wetland plants studied were all described in the Catalogue of  
93 Medicinal Plant Resources in Taiwan which was published by Committee on Chinese  
94 Medicine and Pharmacy, Department of Health, Taiwan (Lin 2003).

95

### 96 **Preparation of the methanol extracts of plant materials**

97 Dried whole herbs (100 g for each sample) were macerated with 1L 95% ethanol  
98 for 24 hours at room temperature. Filtration and collection of the extract were done  
99 three times. Then the ethanol extract (3 L) was evaporated to 10 mL and dried in  
100 vacuum at 40°C. The dried extract was weighted and dissolved in 95% ethanol and  
101 stored in -20°C for further use.

102

### 103 **Preparation of the water extracts of plant materials**

104 Dried whole herbs (100 g for each sample) were boiled with 1L distilled water  
105 for 1 hour. Filtration and collection of the extracts were done three times. The  
106 resulting decoction was evaporated to 10 mL and dried in vacuum at 50°C. The dried  
107 extract was weighted and dissolved in distilled water and stored in -20°C for further  
108 use. For each extract, the yield was calculated in percentage on the basis of the dry  
109 weight of the whole herbs used (100 g) and the quantity of dry mass obtained after  
110 extraction (w/w).

111

### 112 **Determination of antioxidant activity by TEAC assay**

113 The TEAC assay was determined according to the method of Ramos et al. (1999).  
114 Aqueous solution of ABTS (7 mM) was oxidized with potassium peroxodisulfate  
115 (2.45 mM) for 16 hours in the dark at room temperature. The ABTS<sup>+</sup> solution was  
116 diluted with 95% ethanol to an absorbance of  $0.75 \pm 0.05$  at 734 nm (Beckman  
117 UV-Vis spectrophotometer, Model DU640B). An aliquot (20  $\mu$ L) of each sample (125  
118  $\mu$ g/mL) was mixed with 180  $\mu$ L ABTS<sup>+</sup> solution and the absorbance was read at 734

119 nm after 1 min. Trolox was used as a reference standard. A standard curve was  
120 constructed for Trolox at 0, 15.625, 31.25, 62.5, 125, 250, 500  $\mu$ M concentrations.  
121 TEAC value was expressed as millimolar concentration of Trolox solution with the  
122 antioxidant equivalent to a 1000 ppm solution of the sample under investigation.

123

#### 124 **Determination of antioxidant activity by DPPH radical scavenging ability**

125 The effects of crude extracts and positive controls (GSH and BHT) on DPPH  
126 radicals were estimated according to the method of Yamaguchi et al. (1998). Aliquot  
127 (20  $\mu$ L) of crude extracts at various concentrations were each mixed with 100 mM  
128 Tris-HCl buffer (80  $\mu$ L, pH 7.4) and then with 100  $\mu$ L of DPPH in ethanol to a final  
129 concentration of 250  $\mu$ M. The mixture was shaken vigorously and left to stand at  
130 room temperature for 20 min in the dark. The absorbance of the reaction solution was  
131 measured spectrophotometrically at 517 nm. The percentage of DPPH decolorization  
132 of the samples was calculated according to the equation: % decolorization = [1- (ABS  
133  $_{\text{sample}} / \text{ABS}_{\text{control}}$ )]  $\times 100$ . IC<sub>50</sub> value was the effective concentration at which DPPH  
134 radicals were scavenged by 50% and was obtained by interpolation from linear  
135 regression analysis. A lower IC<sub>50</sub> value indicated a greater antioxidant activity.

136

#### 137 **Measurement of reducing power**

138 The reducing power of the crude extracts and positive controls (GSH and BHT)  
139 were determined according to the method of Yen and Chen (1995). The samples (0,  
140 31.25, 62.5, 125, 250, 500, and 1000  $\mu$ g/mL) were each mixed with an equal volume  
141 of 0.2 M phosphate buffer, pH 6.6, and 1% potassium ferricyanide. The mixture was  
142 incubated at 50°C for 20 min before an equal volume of 1% TCA was added, and then  
143 centrifuged at 5,000 g for 10 min. The upper layer of the solution was mixed with

144 distilled water and 0.1% FeCl<sub>3</sub> with a ratio of 1: 1: 2, and the absorbance was  
145 measured at 700 nm. Increased absorbance of the reaction mixture indicated an  
146 increase in reducing power.

147

#### 148 **Determination of total polyphenol content**

149 The total polyphenol contents of the crude extracts were determined according to  
150 the method of Ragazzi and Veronese (1973). 20 µL of each extract (125 µg/mL) was  
151 added to 200 µL distilled water and 40 µL of Folin-Ciocalteu reagent. The mixture  
152 was allowed to stand at room temperature for 5 min and then 40 µL of 20 % sodium  
153 carbonate was added to the mixture. The resulting blue complex was then measured at  
154 680 nm. Catechin was used as a standard for the calibration curve. The polyphenol  
155 content was calibrated using the linear equation based on the calibration curve. The  
156 total polyphenol content was expressed as mg catechin equivalent/g dry weight. The  
157 dry weight indicated was the sample dry weight.

158

#### 159 **Determination of total flavonoid content**

160 The total flavonoid contents of the crude extracts were determined according to  
161 the method of Lamaison and Carnet (1990). Aliquots of 1.5 mL extracts were each  
162 added to an equal volume of 2% AlCl<sub>3</sub>·6H<sub>2</sub>O (2g in 100 mL methanol) solution. The  
163 mixture was vigorously shaken, and the absorbance was read after 10 min of  
164 incubation at 430 nm. Rutin was used as the standard for the calibration curve. The  
165 total flavonoid content was calibrated using the linear equation based on the  
166 calibration curve. The total flavonoid content was expressed as mg rutin equivalent/g  
167 dry weight. The dry weight indicated was the sample dry weight.

168

## 169 **Determination of total flavonol content**

170 The total flavonol contents of the crude extracts were determined according to  
171 the method of Arnous et al. (2001). Aliquots of 200  $\mu$ L extracts were each added to 1  
172 mL of 0.1% *p*-dimethylaminocinnamaldehyde (DMACA) in methanol/HCl (3:1, v/v).  
173 The mixture was vigorously shaken, and the absorbance was read after 10 min of  
174 incubation at 640 nm. Catechin was used as the standard for the calibration curve. The  
175 total flavonol content was calibrated using the linear equation based on the calibration  
176 curve. The total flavonol content was expressed as mg catechin equivalent/g dry  
177 weight. The dry weight indicated was the sample dry weight.

178

## 179 **Statistical analysis**

180 Experimental results were presented as the mean  $\pm$  standard deviation (SD) of  
181 three parallel measurements. Statistical analyses were performed by one-way ANOVA,  
182 followed by Dunnett's *t* test. The difference was considered to be statistically  
183 significant when the *p* value was less than 0.05.

184

## 185 **RESULTS**

### 186 **Extraction yields**

187 The yields of water and methanol extracts of the wetland medicinal plants are  
188 presented in Table 1. The yield of water extracts ranged from 4.24 % to 70.18 %, and  
189 the yield of methanol extracts ranged from 0.89 % to 34.03 %. For the water extracts,  
190 *Pistia stratiotes* L. had the highest yield (70.18 %), followed by *Lindernia antipoda*  
191 (L.) Alston. (63.33 %), *Polygonum plebeium* R. Br. (45.99 %), *Alisma orientalis*  
192 (Sam.) Juzep. (43.33 %), and *Torulium odoratum* (L.) S. Hooper (39.83 %). For the  
193 methanol extracts, the highest yield was obtained from *Lindernia antipoda* (L.) Alston



194 (34.03%), followed by *Cyperus alternifolius* L. subsp. *flabelliformis* (Rottb.)  
195 Kukenthal (26.76 %), *Avicennia marina* (Forsk.) Vierh.-root (24.37 %), *Pistia*  
196 *stratiotes* L. (21.58 %) and *Cyperus difformis* L. (17.84 %).

197

198 The yields of water and methanol extracts obtained from the wetland medicinal  
199 plants. The amount of components extracted by water generally was higher as  
200 compared to that extracted by methanol. It is worthy to notice that water extract may  
201 be allow more extracts to form hydrogen bonds with phenolic compounds than the  
202 methanol extract from the wetland medicinal plants.

203

#### 204 **Antioxidant activity estimated by TEAC assay**

205 Trolox equivalent antioxidant capacity (TEAC) assay is often used to evaluate  
206 the total antioxidant power of single compounds and complex mixtures of various  
207 plants (Chang et al., 2007a, b). In this assay, ABTS radical monocation was generated  
208 directly in stable form from potassium peroxodisulfate. The radicals were generated  
209 before the addition of antioxidants to prevent interference of compounds, which  
210 affected radical formation. This modification made the assay less susceptible to  
211 interruptions and prevented overestimation of antioxidant power (Sanchez-Moreno,  
212 2002). The tested samples were not added to the reaction medium until the stable  
213 absorbance was obtained, then their antioxidant activities were measured in terms of  
214 decolorization. It is recommended to be used for plant extracts because the maximum  
215 wavelength absorption of ABTS at 734 nm eliminates color interference in plant  
216 extracts (Awika et al., 2003). The results were expressed as  $\mu\text{M}$  Trolox/mg dry weight  
217 of plant material.

218 In the TEAC assay, the antioxidant capacities of wetland medicinal plants ranged

219 from 7.52  $\mu\text{M}$  to 1753.41  $\mu\text{M}$  Trolox/mg for the water extracts, and 5.69  $\mu\text{M}$  to  
220 2074.35  $\mu\text{M}$  Trolox/mg for the methanol extracts (Table 2). The differences of  
221 antioxidant capacities were very large, up to 233 and 364 fold respectively. Among  
222 the different water extracts, *Rotala rotundifolia* (Wallich ex Roxb.) Koehne possessed  
223 the highest antioxidant capacity ( $1753.41 \pm 76.99$   $\mu\text{M}$  Trolox/mg), followed by  
224 *Juncus effusus* L. var. *decipiens* Buchen. ( $971.14 \pm 49.68$   $\mu\text{M}$  Trolox/mg), *Cyperus*  
225 *iria* L. ( $762.04 \pm 33.80$   $\mu\text{M}$  Trolox/mg), *Salix warburgii* O. Seem. ( $657.57 \pm 18.37$   
226  $\mu\text{M}$  Trolox/mg) and *Kyllinga brevifolia* Rottb. ( $462.67 \pm 9.49$   $\mu\text{M}$  Trolox/mg). For the  
227 methanol extracts, *Juncus effusus* L. var. *decipiens* Buchen. held the highest  
228 antioxidant capacity ( $2074.35 \pm 116.19$   $\mu\text{M}$  Trolox/mg), followed by *Salix warburgii*  
229 O. Seem. ( $931.45 \pm 84.14$   $\mu\text{M}$  Trolox/mg), *Cyperus iria* L. ( $769.41 \pm 53.57$   $\mu\text{M}$   
230 Trolox/mg), *Typha orientalis* Presl ( $651.22 \pm 14.95$   $\mu\text{M}$  Trolox/mg) and *Kyllinga*  
231 *brevifolia* Rottb. ( $342.52 \pm 10.91$   $\mu\text{M}$  Trolox/mg).

232

### 233 **Scavenging activity against 1,1-diphenyl-2-picrylhydrazyl radicals**

234 The relatively stable organic radical DPPH is widely used in modeling systems  
235 to investigate the scavenging activities of several natural compounds, such as  
236 phenolics and anthocyanins, as well as crude mixtures, such as methanol or water  
237 extract of plants. DPPH radical is scavenged by antioxidants through the donation of  
238 electrons forming the reduced DPPH. The color changes from purple to yellow after  
239 reduction, and the accompanying decrease in absorbance can be quantified at  
240 wavelength 517 nm. Table 3 shows the  $\text{IC}_{50}$  values for radical-scavenging activities of  
241 GSH, BHT and different extract fractions of the wetland medicinal plants using the  
242 DPPH colorimetric method.

243 In the DPPH assay conducted on the water extracts, it was found that *Rotala*

244 *rotundifolia* (Wallich *ex* Roxb.) Koehne had the lowest IC<sub>50</sub> value among the  
245 medicinal plants (94.89 ± 0.31 µg/mL), followed by *Salix warburgii* O. Seem. (112.69  
246 ± 0.28 µg/mL), *Lindernia antipoda* (L.) Alston (189.14 ± 4.55 µg/mL), *Cyperus iria* L.  
247 (194.45 ± 0.32 µg/mL), *Avicennia marina* (Forsk.) Vierh.-leaf (271.71 ± 1.28 µg/mL),  
248 and *Polygonum plebeium* R. Br. (301.52 ± 4.62 µg/mL). The positive control  
249 glutathione (GSH) had an IC<sub>50</sub> value of 71.77 ± 2.09 µg/mL.

250 As for the methanol extracts, *Salix warburgii* O. Seem. had the lowest IC<sub>50</sub> value  
251 (59.58 ± 0.33 µg/mL), followed by *Juncus effusus* L. var. *decipiens* Buchen. (108.95 ±  
252 4.47 µg/mL), *Lindernia antipoda* (L.) Alston (144.61 ± 2.53 µg/mL), *Cyperus iria* L.  
253 (167.18 ± 0.64 µg/mL), *Typha orientalis* Presl (208.01 ± 1.46 µg/mL), and *Cyperus*  
254 *imbricatus* Retz. (242.55 ± 3.11 µg/mL). The positive control BHT also had a low  
255 IC<sub>50</sub> value (139.56 ± 2.96 µg/mL). The above IC<sub>50</sub> values showed that *Salix warburgii*  
256 O. Seem. and *Juncus effusus* L. var. *decipiens* Buchen. demonstrated even higher  
257 radical scavenging activities than the positive control in the DPPH assay.

258

### 259 **Measurement of reducing power**

260 We investigated the reducing capacity of wetland medicinal plants by measuring  
261 Fe<sup>3+</sup>-Fe<sup>2+</sup> conversion. The reducing capacity of a compound may serve as an  
262 important indicator of its potential antioxidant activity (Meir et al., 1995). The  
263 antioxidant activities of putative antioxidants have been attributed to various  
264 mechanisms, such as prevention of chain initiation, binding of transition metal ion  
265 catalysts, decomposition of peroxides, prevention of continued proton abstraction, and  
266 radical scavenging (Diplock, 1997). The reducing power of different extract fractions  
267 from the wetland medicinal plants are shown in Table 4. Both reduced GSH and BHT  
268 were used as the positive controls.

269 For the reducing capacity of the water extracts, it was found that *Salix warburgii*  
270 O. Seem. had the highest reducing capacity value among the examined medicinal  
271 plants ( $1.64 \pm 0.01$ ,  $\Delta 700$ ), followed by *Rotala rotundifolia* (Wallich ex Roxb.)  
272 Koehne ( $1.61 \pm 0.05$ ,  $\Delta 700$ ), *Lindernia antipoda* (L.) Alston ( $1.60 \pm 0.07$ ,  $\Delta 700$ ),  
273 *Cyperus iria* L. ( $1.56 \pm 0.01$ ,  $\Delta 700$ ), *Polygonum plebeium* R. Br. ( $1.17 \pm 0.03$ ,  $\Delta 700$ ),  
274 and *Avicennia marina* (Forsk.) Vierh.-leaf ( $1.11 \pm 0.01$ ,  $\Delta 700$ ). The positive control  
275 glutathione (GSH) had a high reducing capacity activity of  $1.80 \pm 0.01$ ,  $\Delta 700$ .

276 For the methanol extracts, *Salix warburgii* O. Seem. had the highest reducing  
277 capacity value ( $1.68 \pm 0.01$ ,  $\Delta 700$ ), followed by *Lindernia antipoda* (L.) Alston ( $1.66$   
278  $\pm 0.01$ ,  $\Delta 700$ ), *Euryale ferox* Salisb. ( $0.76 \pm 0.12$ ,  $\Delta 700$ ), *Marsilea minuta* L. ( $0.69 \pm$   
279  $0.02$ ,  $\Delta 700$ ), *Cyperus iria* L. ( $0.58 \pm 0.03$ ,  $\Delta 700$ ), and *Cyperus difformis* L. ( $0.51 \pm$   
280  $0.02$ ,  $\Delta 700$ ). The positive control BHT also had a quite high reducing capacity ( $0.27 \pm$   
281  $0.02$ ,  $\Delta 700$ ). The results showed that the reducing capacities for radical-scavenging of  
282 all the tested wetland medicinal plants were even higher than the positive controls.

283

#### 284 **Total polyphenol, flavonoid, and flavonol contents of the wetland medicinal** 285 **plants**

286 The total polyphenol, flavonoid, and flavonol contents of the water and methanol  
287 extracts of wetland medicinal plants are shown in Table 5 and 6, respectively. The  
288 total polyphenol content was expressed as  $\mu\text{g}$  of catechin equivalent per milligram of  
289 dry weight. For the water extracts, the total polyphenol content of the wetland  
290 medicinal plants ranged from  $17.91 \mu\text{g}$  to  $565.92 \mu\text{g CE/mg}$ ; as for the methanol  
291 extract, the total polyphenol content ranged from  $7.69 \mu\text{g CE/mg}$  to  $551.50 \mu\text{g CE/mg}$ ,  
292 and the difference of antioxidant capacities was also very large, up to 31 and 71 fold.  
293 It was found that *Rotala rotundifolia* (Wallich ex Roxb.) Koehne had a total

294 polyphenol content of  $565.92 \pm 4.45$   $\mu\text{g CE/mg}$ , followed by *Lindernia antipoda* (L.)  
295 Alston ( $389.25 \pm 19.12$   $\mu\text{g CE/mg}$ ), *Cyperus iria* L. ( $385.67 \pm 5.62$   $\mu\text{g CE/mg}$ ),  
296 *Cyperus iria* L. ( $302.89 \pm 21.19$   $\mu\text{g CE/mg}$ ), *Typha orientalis* Presl ( $230.50 \pm 1.41$   $\mu\text{g}$   
297  $\text{CE/mg}$ ), and *Cyperus difformis* L. ( $162.04 \pm 10.16$   $\mu\text{g CE/mg}$ ) in their water extracts  
298 (Table 5). *Salix warburgii* O. Seem. had a total polyphenol content of  $551.50 \pm 17.57$   
299  $\mu\text{g CE/mg}$ , followed by *Typha orientalis* Presl ( $494.17 \pm 10.13$   $\mu\text{g CE/mg}$ ), *Juncus*  
300 *effusus* L. var. *decipiens* Buchen. ( $489.75 \pm 53.28$   $\mu\text{g CE/mg}$ ), *Lindernia antipoda* (L.)  
301 Alston ( $356.70 \pm 17.32$   $\mu\text{g CE/mg}$ ), *Cyperus iria* L. ( $345.25 \pm 9.81$   $\mu\text{g CE/mg}$ ), and  
302 *Kyllinga brevifolia* Rottb. ( $251.25 \pm 1.90$   $\mu\text{g CE/mg}$ ) in their methanol extracts (Table  
303 6).

304 The total flavonoid content was expressed as  $\mu\text{g}$  of rutin equivalent per milligram  
305 of dry weight. The total flavonoid contents in the water extracts of the wetland  
306 medicinal plants ranged from 3.11 to 53.92  $\mu\text{g RE/mg}$ , and the total flavonoid  
307 contents in their methanol extracts ranged from 1.19 to 72.17  $\mu\text{g RE/mg}$ , furthermore  
308 the difference of antioxidant capacities was also very large, up to 17 and 60 fold  
309 respectively. It was found that *Typha orientalis* Presl had the highest total flavonoid  
310 content ( $53.92 \pm 5.44$   $\mu\text{g RE/mg}$ ), followed by *Rotala rotundifolia* (Wallich ex Roxb.)  
311 Koehne ( $46.24 \pm 0.39$   $\mu\text{g RE/mg}$ ), and *Cyperus iria* L. ( $29.73 \pm 0.51$   $\mu\text{g RE/mg}$ ) in  
312 their water extracts. *Eriocaulon sexangulare* L. had the highest total flavonoid content  
313 ( $74.55 \pm 1.50$   $\mu\text{g RE/mg}$ ), followed by *Polygonum plebeium* R. Br. ( $72.17 \pm 3.33$   $\mu\text{g}$   
314  $\text{CE/mg}$ ), *Typha orientalis* Presl ( $71.89 \pm 0.42$   $\mu\text{g RE/mg}$ ), and *Salix warburgii* O.  
315 Seem. ( $70.34 \pm 2.43$   $\mu\text{g RE/mg}$ ) in their methanol extracts.

316 The total flavonol content was expressed as  $\mu\text{g}$  of catechin equivalent per  
317 milligram of dry weight. The total flavonol content of the water extracts of the  
318 wetland medicinal plants ranged from 0.05 to 14.05  $\mu\text{g CE/mg}$ , and the flavonol

319 content of the methanol extracts ranged from 0.46 to 14.36  $\mu\text{g CE/mg}$  and the  
320 difference of antioxidant capacities was also very large, up to 281 and 31 fold. It was  
321 found that *Cyperus iria* L. had the highest flavonol content ( $14.05 \pm 0.88 \mu\text{g CE/mg}$ ),  
322 followed by *Polygonum plebeium* R. Br. ( $13.47 \pm 1.22 \mu\text{g CE/mg}$ ), and *Salix*  
323 *warburgii* O. Seem. ( $5.54 \pm 0.18 \mu\text{g CE/mg}$ ) for the water extracts. *Cyperus*  
324 *imbricatus* Retz. had the highest flavonol content ( $14.36 \pm 1.28 \mu\text{g CE/mg}$ ), followed  
325 by *Cyperus iria* L. ( $13.41 \pm 0.87 \mu\text{g CE/mg}$ ), and *Typha orientalis* Presl ( $7.04 \pm 0.10$   
326  $\mu\text{g CE/mg}$ ) for the methanol extracts.

327

### 328 **Relationship between total antioxidant activity and total polyphenol content**

329 The correlation coefficients ( $R^2$ ) of total antioxidant activity (TEAC) and total  
330 polyphenol of the water and methanol extracts are shown in Fig. 1A and 1B. The  $R^2$   
331 values of TEAC and total polyphenol content of the water (Fig. 1A) and methanol  
332 (Fig. 1B) extracts were 0.14 and 0.25, respectively. By examining the above statistics,  
333 we could see that there was a low correlation between the TEAC and total polyphenol  
334 contents. The linear regression analysis indicated a low correlation between  
335 antioxidant activity and total polyphenol contents. Different species of wetland  
336 medicinal plants may influence the antioxidant activity as well. Therefore, high  
337 phenolic content was not an only important factor in determining the antioxidant  
338 capacities of these wetland medicinal plants.

339

### 340 **DISCUSSION**

341 From the antioxidant activities evaluated by **TEAC assay**, DPPH radical  
342 scavenging, reducing power, total polyphenol content, total flavonoid content and  
343 flavonol content, among the 31 wetland medicinal plants screened, *Rotala*  
344 *rotundifolia* (Wallich ex Roxb.) Koehne., *Juncus effusus* L. var. *decipiens* Buchen.,  
345 *Cyperus iria* L., *Salix warburgii* O. Seem., *Lindernia antipoda* (L.) Alston, *Kyllinga*  
346 *brevifolia* Rottb., and *Typha orientalis* Presl exhibited best antioxidant activities.

347 *Rotala rotundifolia* (Wallich ex Roxb.) Koehne. has not been reported in any  
348 scientific papers before. This plant possesses antiradiation, anti-inflammatory, and  
349 antibacterial effects. The present study provided valuable preliminary data through  
350 demonstration of its efficient antioxidant capacity. Isolation and characterization of its  
351 individual active components and *in vivo* relevance await further comprehensive  
352 studies.

353 *Juncus effusus* L. var. *decipiens* Buchen. possesses anti-depressant,  
354 anti-inflammatory, and antibacterial effects. To our knowledge, there was no prior  
355 report as to the antioxidant activity of this plant. This study rendered valuable  
356 preliminary data through demonstration of its high antioxidant capacity. To study the  
357 phenolic constituents from the dry stem of *Juncus effusus* L., six phenolic constituents  
358 were purified and identified as 7-carboxy-2-hydroxy-1-methyl-5-vinyl-9,  
359 10-dihydrophenanthrene, 2,3-isopylidene-1-O-ferulic acid glyceride, (2S)-2,  
360 3-isopylidene-1-O-p-coumaroyl glyceride, dehydroeffusal, p-hydroxybenzaldehyde  
361 and luteolin-5,3'-dimethyl ether (Li et al., 2007). Some of them might be antioxidant  
362 components.

363 *Cyperus iria* L. has not been reported in any scientific papers before. This plant  
364 possesses rheumatic, antidiuretic, and anti-inflammatory effects. The present study  
365 showed first hand data on the antixodiant capacity of *Cyperus iria*. Isolation and

366 characterization of its individual active components and *in vivo* relevance of such  
367 activity awaits further comprehensive studies.

368 *Salix warburgii* O. Seem. possesses anticoagulant, anti-inflammatory, and  
369 antibacterial effects. To our knowledge, there was no prior report on the antioxidant  
370 activity of this plant. This paper studied the antioxidant effects of *Salix warburgii* for  
371 the first time; a bioassay-guided *in vitro* screen has revealed that a 70% methanol  
372 extract of the leaves of *Salix matsudana* showed considerable inhibitory activity  
373 against cyclooxygenases (COX-1 and COX-2) (Li et al., 2008). A subsequent  
374 phytochemical study led to the isolation of some compounds: matsudone A, luteolin,  
375 isoquercitrin, 7-methoxyflavone, luteolin 7-O-glucoside, and 4',7-dihydroxyflavone.  
376 These isolated compounds were found to possess activities in inhibiting against  
377 COX-1 or COX-2.

378 *Lindernia antipoda* (L.) Alston possesses analgesic and anti-inflammatory effects.  
379 There has been no prior report regarding the antioxidant activity of this plant. This  
380 study provided valuable data by demonstrating the high antioxidant capacity of  
381 *Lindernia antipoda* for the first time. However, isolation and characterization of its  
382 active components and their *in vivo* relevance await further comprehensive studies.

383 *Kyllinga brevifolia* Rottb. possesses analgesic and anti-inflammatory effects.  
384 Oral administration of doses up to 3.0 g/kg did not provoke any toxic symptoms. The  
385 toxicity of this plant was observed to be dose dependent and its intraperitoneal LD<sub>50</sub>  
386 was found to be 575 mg/kg. It is used in traditional medicine to alleviate stress or as a  
387 sedative agent (Helliön-Ibarrola et al., 1999).

388 *Typha orientalis* Presl is a commonly used Chinese herbal drug which has been  
389 shown to possess blood circulation stimulating, hypertension relieving and nerve  
390 soothing effects. There was no prior report regarding the antioxidant activity of this



391 plant.

392 Phenolic compounds such as flavonoids, phenolic acid and tannins possess diverse  
393 biological activities such as anti-inflammatory, anti-carcinogenic and  
394 anti-atherosclerotic activities. These activities might be related to their antioxidant  
395 activities (Chung et al., 1998; Wong et al., 2006). Both flavonoids and flavonols  
396 belong to polyphenolic compounds. Polyphenolic compounds have an important role  
397 in stabilizing lipid oxidation and are associated with antioxidant activity (Yen et al.,  
398 1993). Phenolic compounds may contribute directly to antioxidative action (Duh et al.,  
399 1999). It is suggested that polyphenolic compounds have inhibitory effects on  
400 mutagenesis and carcinogenesis in humans when as much as 1.0 g is ingested from a  
401 diet rich in fruits and vegetables daily (Tanaka et al., 1998). The antioxidative  
402 activities observed could be ascribed both to the different mechanisms exerted by  
403 different phenolic compounds and to the synergistic effects of different compounds.  
404 The antioxidant assays used in this study measured the oxidative products at the early  
405 and final stages of oxidation. Antioxidants have different functional properties, for  
406 example quercetin, rutin, and catechin can scavenge reactive oxygen species (Liu et  
407 al., 2008); *p*-coumaric acids, on the other hand, inhibits the generation of free radicals  
408 and chain-breaking activity (Laranjinha et al., 1995) and metal chelation (Van-Acker  
409 et al., 1998). These compounds, as well as flavonoids and other organic acids, are  
410 highly effective electron donors. However, the components which were responsible  
411 for the antioxidative activities of the wetland medicinal plants are still currently  
412 unclear. Therefore, further work must be performed to isolate and identify these  
413 components.

414 In conclusion, the results from these *in vitro* experiments, including ABTS  
415 radical monocation scavenging (Table 2), DPPH radical scavenging (Table 3),

416 reducing power method (Table 4), total polyphenol content, total flavonoid content  
417 and total flavonol content (Table 5 and 6), demonstrated that phytochemicals in the  
418 wetland medicinal plants might have significant effects on antioxidant activities.  
419 However, the quantity of polyphenols and flavonoids found in the wetland medicinal  
420 plant extracts were not directly related to their antioxidant activities. The additive  
421 roles of phytochemicals might contribute significantly to the potent antioxidant  
422 activity. Hence, some wetland medicinal plants could be used as an easy accessible  
423 source of natural antioxidants in pharmaceutical and medical industries. For this  
424 reason, further work should be performed to isolate and identify the antioxidative  
425 components of wetland medicinal plants.

426

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434

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531 to their antimutagenicity. *J. Agric. Food Chem.* **46**: 849-854.

532 Table 1. The yield of water and methanol extracts of the wetland medicinal plants

Scientific name	Yield (% w/w) <sup>a</sup>	
	Water extract	Ethanol extract
<i>Acorus gramineus</i> Soland.	12.96	8.37
<i>Avicennia marina</i> (Forsk.) Vierh. -leaf	5.47	5.83
<i>Avicennia marina</i> (Forsk.) Vierh. -root	17.84	24.37
<i>Alisma orientalis</i> (Sam.) Juzep.	43.33	14.84
<i>Alternanthera sessilis</i> (L.) R. Br.	15.91	12.14
<i>Cyperus alternifolius</i> L. subsp. <i>flabelliformis</i> (Rottb.) Kukenthal	11.24	26.76
<i>Commelina communis</i> L.	11.76	14.21
<i>Cyperus difformis</i> L.	23.22	17.84
<i>Cyperus imbricatus</i> Retz.	36.33	9.79
<i>Cyperus iria</i> L.	11.07	7.62
<i>Eichhornia crassipes</i> (Mart.) Solms	14.35	13.05
<i>Echinochloa crus-galli</i> (L.) Beauv.	6.77	6.39
<i>Egeria densa</i> Planch.	20.77	3.42
<i>Euryale ferox</i> Salisb.	14.44	1.43
<i>Eriocaulon sexangulare</i> L.	12.45	2.53
<i>Fimbristylis littoralis</i> Gaud	10.52	3.92
<i>Hedyotis corymbosa</i> (L.) Lam.	21.19	10.66
<i>Hygrophila pogonocalyx</i> Hayata	12.84	6.41
<i>Juncus effusus</i> L. var. <i>decipiens</i> Buchen.	13.04	11.34
<i>Kyllinga brevifolia</i> Rottb.	12.18	4.45
<i>Lindernia antipoda</i> (L.) Alston	63.33	34.03
<i>Marsilea minuta</i> L.	37.03	11.27
<i>Pilea microphylla</i> (L.) Liebm.	4.96	10.76
<i>Phyla nodiflora</i> (L.) Greene	7.86	9.18
<i>Polygonum plebeium</i> R. Br.	45.99	17.31
<i>Pistia stratiotes</i> L.	70.18	21.58
<i>Rotala rotundifolia</i> (Wallich ex Roxb.) Koehne	4.24	12.31
<i>Spirodela punctata</i> G. F. W. Meyer	13.96	11.42
<i>Salix warburgii</i> O. Seem.	14.63	15.44
<i>Typha orientalis</i> Presl	19.7	0.89
<i>Torulium odoratum</i> (L.) S. Hooper	39.83	12.76

533 <sup>a</sup> On dried weight basis.

534

535 Table 2. The TEAC of the water and methanol extracts of the wetland medicinal  
 536 plants.

Scientific name and positive controls	TEAC <sup>a</sup>	
	(µM Trolox/mg ± SD)	
	Water extracted	Methanol extracted
GSH	1827.68 ± 76.84	Not detected
BHT	Not detected	11869.41 ± 34.63
<i>Acorus gramineus</i> Soland.	159.52 ± 2.57	225.65 ± 9.45
<i>Avicennia marina</i> (Forsk.) Vierh. -leaf	376.17 ± 10.42	54.15 ± 2.11
<i>Avicennia marina</i> (Forsk.) Vierh. -root	381.85 ± 13.07	177.00 ± 2.13
<i>Alisma orientalis</i> (Sam.) Juzep.	12.33 ± 3.44	19.08 ± 7.96
<i>Alternanthera sessilis</i> (L.) R. Br.	156.38 ± 9.48	148.46 ± 6.64
<i>Cyperus alternifolius</i> L. subsp. <i>flabelliformis</i> (Rottb.) Kukenthal	47.23 ± 3.66	81.77 ± 4.10
<i>Commelina communis</i> L.	113.02 ± 1.96	96.13 ± 7.69
<i>Cyperus difformis</i> L.	132.00 ± 4.83	15.33 ± 16.20
<i>Cyperus imbricatus</i> Retz.	112.35 ± 4.74	27.35 ± 4.81
<i>Cyperus iria</i> L.	762.04 ± 33.80	769.41 ± 53.57
<i>Eichhornia crassipes</i> (Mart.) Solms	102.13 ± 2.66	143.33 ± 6.39
<i>Echinochloa crus-galli</i> (L.) Beauv.	448.98 ± 6.41	39.56 ± 20.28
<i>Egeria densa</i> Planch.	37.10 ± 2.17	5.69 ± 7.58
<i>Euryale ferox</i> Salisb.	14.58 ± 1.11	350.73 ± 4.13
<i>Eriocaulon sexangulare</i> L.	77.58 ± 3.57	222.65 ± 0.86
<i>Fimbristylis littoralis</i> Gaud	311.73 ± 3.71	87.17 ± 5.02
<i>Hedyotis corymbosa</i> (L.) Lam.	283.58 ± 4.45	56.73 ± 7.18
<i>Hygrophila pogonocalyx</i> Hayata	69.40 ± 0.94	43.38 ± 5.03
<i>Juncus effusus</i> L. var. <i>decipiens</i> Buchen.	971.14 ± 49.68	2074.35 ± 116.19
<i>Kyllinga brevifolia</i> Rottb.	462.67 ± 9.49	342.52 ± 10.91
<i>Lindernia antipoda</i> (L.) Alston	320.35 ± 2.80	311.23 ± 16.05
<i>Marsilea minuta</i> L.	94.27 ± 4.82	196.25 ± 13.50
<i>Pilea microphylla</i> (L.) Liebm.	165.44 ± 0.38	248.17 ± 34.50
<i>Phyla nodiflora</i> (L.) Greene	97.04 ± 1.53	86.21 ± 12.58
<i>Polygonum plebeium</i> R. Br.	364.04 ± 1.07	175.44 ± 9.47
<i>Pistia stratiotes</i> L.	7.52 ± 3.64	34.79 ± 2.63
<i>Rotala rotundifolia</i> (Wallich ex Roxb.) Koehne	1753.41 ± 76.99	159.90 ± 15.52
<i>Spirodela punctata</i> G. F. W. Meyer	360.25 ± 7.70	104.17 ± 5.36
<i>Salix warburgii</i> O. Seem.	657.57 ± 18.37	931.45 ± 84.14
<i>Typha orientalis</i> Presl	344.13 ± 5.48	651.22 ± 14.95
<i>Torulinium odoratum</i> (L.) S. Hooper	32.71 ± 0.71	203.67 ± 52.57

537 <sup>a</sup>Values represented mean ± S.D. of three parallel measurements ( $P < 0.05$ ).

538



539 Table 3. The DPPH radical scavenging activity of the water and methanol extracts of  
 540 the wetland medicinal plants.

Scientific name and positive controls	DPPH radical scavenging activity <sup>a</sup> (IC <sub>50</sub> , µg/mL)	
	Water extract	Methanol extract
GSH	71.77 ± 2.09	Not detected
BHT	Not detected	139.56 ± 2.96
<i>Acorus gramineus</i> Soland.	896.90 ± 7.60	1045.51 ± 0.69
<i>Avicennia marina</i> (Forsk.) Vierh. -leaf	271.71 ± 1.28	>2,000
<i>Avicennia marina</i> (Forsk.) Vierh. -root	404.19 ± 1.18	713.99 ± 0.24
<i>Alisma orientalis</i> (Sam.) Juzep.	>2,000	>2,000
<i>Alternanthera sessilis</i> (L.) R. Br.	844.69 ± 6.42	946.79 ± 8.39
<i>Cyperus alternifolius</i> L. subsp. <i>flabelliformis</i> (Rottb.) Kukenthal	>2,000	>2,000
<i>Commelina communis</i> L.	>2,000	>2,000
<i>Cyperus difformis</i> L.	1125.67 ± 1.22	489.04 ± 3.82
<i>Cyperus imbricatus</i> Retz.	1882.64 ± 3.92	242.55 ± 3.11
<i>Cyperus iria</i> L.	194.45 ± 0.32	167.18 ± 0.64
<i>Eichhornia crassipes</i> (Mart.) Solms	>2,000	>2,000
<i>Echinochloa crus-galli</i> (L.) Beauv.	548.23 ± 4.62	>2,000
<i>Egeria densa</i> Planch.	>2,000	>2,000
<i>Euryale ferox</i> Salisb.	>2,000	307.35 ± 1.61
<i>Eriocaulon sexangulare</i> L.	>2,000	>2,000
<i>Fimbristylis littoralis</i> Gaud	810.61 ± 6.58	>2,000
<i>Hedyotis corymbosa</i> (L.) Lam.	668.89 ± 8.62	>2,000
<i>Hygrophila pogonocalyx</i> Hayata	1520.06 ± 5.25	>2,000
<i>Juncus effusus</i> L. var. <i>decipiens</i> Buchen.	456.88 ± 3.88	108.95 ± 4.47
<i>Kyllinga brevifolia</i> Rottb.	379.52 ± 2.52	523.55 ± 0.091
<i>Lindernia antipoda</i> (L.) Alston	189.14 ± 4.55	144.61 ± 2.53
<i>Marsilea minuta</i> L.	1400.48 ± 3.2	613.76 ± 1.67
<i>Pilea microphylla</i> (L.) Liebm.	>2,000	423.14 ± 5.61
<i>Phyla nodiflora</i> (L.) Greene	>2,000	789.26 ± 5.84
<i>Polygonum plebeium</i> R. Br.	301.52 ± 4.62	>2,000
<i>Pistia stratiotes</i> L.	>2,000	>2,000
<i>Rotala rotundifolia</i> (Wallich ex Roxb.) Koehne	94.89 ± 0.31	721.89 ± 3.91
<i>Spirodela punctata</i> G. F. W. Meyer	432.20 ± 4.63	1094.73 ± 12.61
<i>Salix warburgii</i> O. Seem.	112.69 ± 0.28	59.58 ± 0.33
<i>Typha orientalis</i> Presl	533.59 ± 4.92	208.01 ± 1.46
<i>Torulinium odoratum</i> (L.) S. Hooper	>2,000	>2,000

541 <sup>a</sup> Values represented mean ± S.D. of three parallel measurements ( $P < 0.05$ ).

542

543

544 Table 4. The reducing power of the water and methanol extracts of the wetland  
 545 medicinal plants

546

Scientific name and positive controls	Reducing power $\Delta 700^a$ (Mean $\pm$ SD)	
	Water extract	methanol extract
GSH	1.80 $\pm$ 0.01	Not detected
BHT	Not detected	0.27 $\pm$ 0.02
<i>Acorus gramineus</i> Soland.	0.36 $\pm$ 0.01	0.04 $\pm$ 0.01
<i>Avicennia marina</i> (Forsk.) Vierh. -leaf	1.11 $\pm$ 0.01	0.06 $\pm$ 0.01
<i>Avicennia marina</i> (Forsk.) Vierh. -root	1.010 $\pm$ 0.02	0.36 $\pm$ 0.02
<i>Alisma orientalis</i> (Sam.) Juzep.	0.05 $\pm$ 0.01	0.15 $\pm$ 0.02
<i>Alternanthera sessilis</i> (L.) R. Br.	0.46 $\pm$ 0.01	0.19 $\pm$ 0.02
<i>Cyperus alternifolius</i> L. subsp. <i>flabelliformis</i> (Rottb.) Kukenthal	0.13 $\pm$ 0.01	0.17 $\pm$ 0.01
<i>Commelina communis</i> L.	0.23 $\pm$ 0.01	0.40 $\pm$ 0.01
<i>Cyperus difformis</i> L.	0.39 $\pm$ 0.01	0.51 $\pm$ 0.02
<i>Cyperus imbricatus</i> Retz.	0.27 $\pm$ 0.02	0.41 $\pm$ 0.03
<i>Cyperus iria</i> L.	1.56 $\pm$ 0.01	0.58 $\pm$ 0.03
<i>Eichhornia crassipes</i> (Mart.) Solms	0.17 $\pm$ 0.01	0.26 $\pm$ 0.01
<i>Echinochloa crus-galli</i> (L.) Beauv.	0.63 $\pm$ 0.02	0.03 $\pm$ 0.01
<i>Egeria densa</i> Planch.	0.02 $\pm$ 0.01	0.04 $\pm$ 0.01
<i>Euryale ferox</i> Salisb.	0.07 $\pm$ 0.01	0.76 $\pm$ 0.12
<i>Eriocaulon sexangulare</i> L.	0.09 $\pm$ 0.01	0.32 $\pm$ 0.01
<i>Fimbristylis littoralis</i> Gaud	0.40 $\pm$ 0.01	0.01 $\pm$ 0.01
<i>Hedyotis corymbosa</i> (L.) Lam.	0.552 $\pm$ 0.02	0.06 $\pm$ 0.00
<i>Hygrophila pogonocalyx</i> Hayata	0.310 $\pm$ 0.01	0.09 $\pm$ 0.02
<i>Juncus effusus</i> L. var. <i>decipiens</i> Buchen.	0.56 $\pm$ 0.01	0.26 $\pm$ 0.05
<i>Kyllinga brevifolia</i> Rottb.	0.74 $\pm$ 0.01	0.29 $\pm$ 0.08
<i>Lindernia antipoda</i> (L.) Alston	1.60 $\pm$ 0.07	1.66 $\pm$ 0.01
<i>Marsilea minuta</i> L.	0.27 $\pm$ 0.01	0.69 $\pm$ 0.02
<i>Pilea microphylla</i> (L.) Liebm.	0.08 $\pm$ 0.03	0.29 $\pm$ 0.02
<i>Phyla nodiflora</i> (L.) Greene	0.33 $\pm$ 0.02	0.36 $\pm$ 0.03
<i>Polygonum plebeium</i> R. Br.	1.17 $\pm$ 0.03	0.36 $\pm$ 0.02
<i>Pistia stratiotes</i> L.	0.02 $\pm$ 0.01	0.04 $\pm$ 0.01
<i>Rotala rotundifolia</i> (Wallich ex Roxb.) Koehne	1.61 $\pm$ 0.05	0.25 $\pm$ 0.01
<i>Spirodela punctata</i> G. F. W. Meyer	0.71 $\pm$ 0.05	0.10 $\pm$ 0.04
<i>Salix warburgii</i> O. Seem.	1.64 $\pm$ 0.01	1.68 $\pm$ 0.01
<i>Typha orientalis</i> Presl	0.58 $\pm$ 0.01	0.46 $\pm$ 0.02
<i>Torulinium odoratum</i> (L.) S. Hooper	0.09 $\pm$ 0.01	0.27 $\pm$ 0.05

547

<sup>a</sup> Values represented mean  $\pm$  S.D. of three parallel measurements ( $P < 0.05$ ).

548

549

550 Table 5. Total polyphenol, flavonoid, and flavonol content of the water extracts of the  
 551 wetland medicinal plants <sup>a</sup>.

552

Scientific name	Water extracted		
	Polyphenol <sup>a, b</sup> ( $\mu\text{g CE/mg}$ )	Flavonoid <sup>a, c</sup> ( $\mu\text{g RE/mg}$ )	Flavonol <sup>a, b</sup> ( $\mu\text{g CE/mg}$ )
<i>Acorus gramineus</i> Soland.	91.50 $\pm$ 1.25	13.81 $\pm$ 4.93	2.06 $\pm$ 0.14
<i>Avicennia marina</i> (Forsk.) Vierh. -leaf	109.25 $\pm$ 1.67	27.55 $\pm$ 0.24	0.34 $\pm$ 0.02
<i>Avicennia marina</i> (Forsk.) Vierh. -root	102.1 $\pm$ 7.55	7.78 $\pm$ 0.07	0.42 $\pm$ 0.01
<i>Alisma orientalis</i> (Sam.) Juzep.	37.04 $\pm$ 1.81	3.71 $\pm$ 0.05	1.06 $\pm$ 0.01
<i>Alternanthera sessilis</i> (L.) R. Br.	137.67 $\pm$ 3.81	26.44 $\pm$ 0.53	1.56 $\pm$ 9.11
<i>Cyperus alternifolius</i> L. subsp. <i>flabelliformis</i> (Rottb.) Kukenthal	78.45 $\pm$ 0.93	4.63 $\pm$ 0.05	1.23 $\pm$ 0.02
<i>Commelina communis</i> L.	113.79 $\pm$ 5.76	15.72 $\pm$ 0.20	1.04 $\pm$ 0.01
<i>Cyperus difformis</i> L.	162.04 $\pm$ 10.16	18.96 $\pm$ 0.53	1.15 $\pm$ 0.01
<i>Cyperus imbricatus</i> Retz.	140.87 $\pm$ 7.01	13.70 $\pm$ 0.37	1.31 $\pm$ 0.01
<i>Cyperus iria</i> L.	385.67 $\pm$ 5.62	29.73 $\pm$ 0.51	14.05 $\pm$ 0.88
<i>Eichhornia crassipes</i> (Mart.) Solms	90.12 $\pm$ 7.26	9.97 $\pm$ 0.23	1.14 $\pm$ 0.01
<i>Echinochloa crus-galli</i> (L.) Beauv.	108.33 $\pm$ 9.26	14.88 $\pm$ 1.33	2.93 $\pm$ 1.98
<i>Egeria densa</i> Planch.	24.66 $\pm$ 0.10	5.85 $\pm$ 0.09	1.05 $\pm$ 0.01
<i>Euryale ferox</i> Salisb.	28.16 $\pm$ 0.49	3.28 $\pm$ 0.21	1.93 $\pm$ 0.02
<i>Eriocaulon sexangulare</i> L.	88.62 $\pm$ 0.91	9.57 $\pm$ 0.25	1.25 $\pm$ 0.02
<i>Fimbristylis littoralis</i> Gaud	87.50 $\pm$ 20.02	14.16 $\pm$ 0.36	3.13 $\pm$ 0.08
<i>Hedyotis corymbosa</i> (L.) Lam.	157.50 $\pm$ 7.53	17.04 $\pm$ 0.67	0.71 $\pm$ 0.01
<i>Hygrophila pogonocalyx</i> Hayata	81.25 $\pm$ 9.11	7.86 $\pm$ 0.08	1.22 $\pm$ 0.01
<i>Juncus effusus</i> L. var. <i>decipiens</i> Buchen.	37.33 $\pm$ 10.37	8.79 $\pm$ 2.88	0.38 $\pm$ 0.01
<i>Kyllinga brevifolia</i> Rottb.	215.92 $\pm$ 1.23	9.54 $\pm$ 0.44	1.69 $\pm$ 0.01
<i>Lindernia antipoda</i> (L.) Alston	389.25 $\pm$ 19.12	22.68 $\pm$ 0.57	2.23 $\pm$ 0.02
<i>Marsilea minuta</i> L.	102.41 $\pm$ 6.93	12.42 $\pm$ 0.61	3.97 $\pm$ 0.15
<i>Pilea microphylla</i> (L.) Liebm.	81.67 $\pm$ 3.59	10.00 $\pm$ 0.40	0.05 $\pm$ 0.01
<i>Phyla nodiflora</i> (L.) Greene	137.04 $\pm$ 4.13	16.76 $\pm$ 0.10	1.61 $\pm$ 0.01
<i>Polygonum plebeium</i> R. Br.	64.10 $\pm$ 10.48	17.22 $\pm$ 0.25	13.47 $\pm$ 1.22
<i>Pistia stratiotes</i> L.	17.91 $\pm$ 0.10	3.33 $\pm$ 0.04	0.85 $\pm$ 0.01
<i>Rotala rotundifolia</i> (Wallich ex Roxb.) Koehne	565.92 $\pm$ 4.45	46.24 $\pm$ 0.39	4.50 $\pm$ 0.27
<i>Spirodela punctata</i> G. F. W. Meyer	67.67 $\pm$ 11.58	21.37 $\pm$ 1.27	0.81 $\pm$ 0.02
<i>Salix warburgii</i> O. Seem.	302.89 $\pm$ 21.19	34.20 $\pm$ 1.36	5.54 $\pm$ 0.18
<i>Typha orientalis</i> Presl	230.50 $\pm$ 1.41	53.92 $\pm$ 5.44	2.89 $\pm$ 0.01
<i>Torulinium odoratum</i> (L.) S. Hooper	43.91 $\pm$ 0.55	3.11 $\pm$ 0.04	2.39 $\pm$ 0.04

553 <sup>a</sup> Values represented mean  $\pm$  S.D. of three parallel measurements.

554 <sup>b</sup> Data expressed in  $\mu\text{g}$  catechin equivalent / mg dry weight ( $\mu\text{g CE/mg}$ ).

555 <sup>c</sup> Data expressed in  $\mu\text{g}$  rutin equivalent / mg dry weight ( $\mu\text{g RE/mg}$ ).

556

557 Table 6. Total polyphenol, flavonoid, and flavonol content of the methanol extracts of  
 558 the wetland medicinal plants <sup>a</sup>.  
 559

Scientific name	Methanol extracted		
	Polyphenol <sup>a, b</sup> ( $\mu\text{g CE/mg}$ )	Flavonoid <sup>a, c</sup> ( $\mu\text{g RE/mg}$ )	Flavonol <sup>a, b</sup> ( $\mu\text{g CE/mg}$ )
<i>Acorus gramineus</i> Soland.	160.58 $\pm$ 61.15	19.98 $\pm$ 31.55	1.38 $\pm$ 0.01
<i>Avicennia marina</i> (Forsk.) Vierh. -leaf	7.69 $\pm$ 24.50	18.34 $\pm$ 24.74	1.49 $\pm$ 0.16
<i>Avicennia marina</i> (Forsk.) Vierh. -root	59.76 $\pm$ 3.27	42.08 $\pm$ 1.22	0.46 $\pm$ 0.01
<i>Alisma orientalis</i> (Sam.) Juzep.	37.45 $\pm$ 0.28	6.25 $\pm$ 1.93	0.94 $\pm$ 0.01
<i>Alternanthera sessilis</i> (L.) R. Br.	152.83 $\pm$ 11.30	24.07 $\pm$ 18.78	3.79 $\pm$ 0.38
<i>Cyperus alternifolius</i> L. subsp. <i>flabelliformis</i> (Rottb.) Kukenthal	105.58 $\pm$ 13.19	20.51 $\pm$ 3.02	3.84 $\pm$ 0.05
<i>Commelina communis</i> L.	129.16 $\pm$ 4.99	21.21 $\pm$ 0.24	1.36 $\pm$ 0.01
<i>Cyperus difformis</i> L.	39.01 $\pm$ 6.05	15.31 $\pm$ 0.68	2.29 $\pm$ 0.02
<i>Cyperus imbricatus</i> Retz.	112.07 $\pm$ 10.79	26.11 $\pm$ 9.91	14.36 $\pm$ 1.28
<i>Cyperus iria</i> L.	345.25 $\pm$ 9.81	46.88 $\pm$ 0.47	13.41 $\pm$ 0.87
<i>Eichhornia crassipes</i> (Mart.) Solms	184 $\pm$ 19.12	25.60 $\pm$ 6.30	3.08 $\pm$ 0.06
<i>Echinochloa crus-galli</i> (L.) Beauv.	80.08 $\pm$ 8.80	24.21 $\pm$ 10.61	2.00 $\pm$ 0.38
<i>Egeria densa</i> Planch.	81.79 $\pm$ 18.52	12.5.11 $\pm$ 5.41	2.95 $\pm$ 0.01
<i>Euryale ferox</i> Salisb.	213.58 $\pm$ 7.29	16.70 $\pm$ 2.31	3.43 $\pm$ 0.01
<i>Eriocaulon sexangulare</i> L.	133.02 $\pm$ 5.12	74.55 $\pm$ 1.50	6.15 $\pm$ 0.86
<i>Fimbristylis littoralis</i> Gaud	107.08 $\pm$ 4.12	19.16 $\pm$ 5.42	2.00 $\pm$ 0.37
<i>Hedyotis corymbosa</i> (L.) Lam.	103.67 $\pm$ 1.46	26.02 $\pm$ 6.49	2.57 $\pm$ 0.16
<i>Hygrophila pogonocalyx</i> Hayata	18.05 $\pm$ 8.56	1.19 $\pm$ 0.53	2.54 $\pm$ 1.34
<i>Juncus effusus</i> L. var. <i>decipiens</i> Buchen.	489.75 $\pm$ 53.28	30.16 $\pm$ 5.07	1.60 $\pm$ 0.05
<i>Kyllinga brevifolia</i> Rottb.	251.25 $\pm$ 1.90	41.86 $\pm$ 2.19	3.85 $\pm$ 0.26
<i>Lindernia antipoda</i> (L.) Alston	356.70 $\pm$ 17.32	35.21 $\pm$ 2.42	2.12 $\pm$ 0.01
<i>Marsilea minuta</i> L.	47.81 $\pm$ 9.58	12.05 $\pm$ 3.84	1.45 $\pm$ 0.08
<i>Pilea microphylla</i> (L.) Liebm.	244.08 $\pm$ 14.43	14.78 $\pm$ 3.38	5.98 $\pm$ 0.78
<i>Phyla nodiflora</i> (L.) Greene	29.53 $\pm$ 4.71	11.10 $\pm$ 1.86	2.68 $\pm$ 0.10
<i>Polygonum plebeium</i> R. Br.	44.32 $\pm$ 11.38	72.17 $\pm$ 3.33	2.82 $\pm$ 0.39
<i>Pistia stratiotes</i> L.	51.79 $\pm$ 1.12	20.22 $\pm$ 2.58	4.97 $\pm$ 0.12
<i>Rotala rotundifolia</i> (Wallich ex Roxb.) Koehne	122.92 $\pm$ 3.67	28.14 $\pm$ 5.48	2.43 $\pm$ 0.33
<i>Spirodela punctata</i> G. F. W. Meyer	204.00 $\pm$ 4.39	20.84 $\pm$ 1.53	3.77 $\pm$ 0.29
<i>Salix warburgii</i> O. Seem.	551.50 $\pm$ 17.57	70.34 $\pm$ 2.43	6.62 $\pm$ 0.31
<i>Typha orientalis</i> Presl	494.17 $\pm$ 10.13	71.89 $\pm$ 0.42	7.04 $\pm$ 0.10
<i>Torulinium odoratum</i> (L.) S. Hooper	54.06 $\pm$ 11.80	12.27 $\pm$ 2.27	3.33 $\pm$ 1.56

560 <sup>a</sup> Values represented mean  $\pm$  S.D. of three parallel measurements.

561 <sup>b</sup> Data expressed in  $\mu\text{g}$  catechin equivalent / mg dry weight ( $\mu\text{g CE/mg}$ ).

562 <sup>c</sup> Data expressed in  $\mu\text{g}$  rutin equivalent / mg dry weight ( $\mu\text{g RE/mg}$ ).

563

564 **Figure legend**

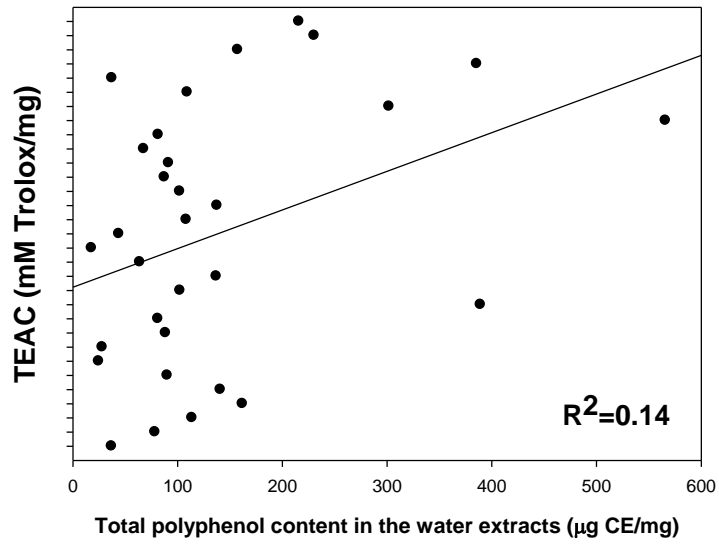
565

566 Figure 1. Correlation coefficients ( $R^2$ ) of TEAC and total polyphenol contents in the  
567 water (A) and methanol (B) extracts of the wetland medicinal plants.

568

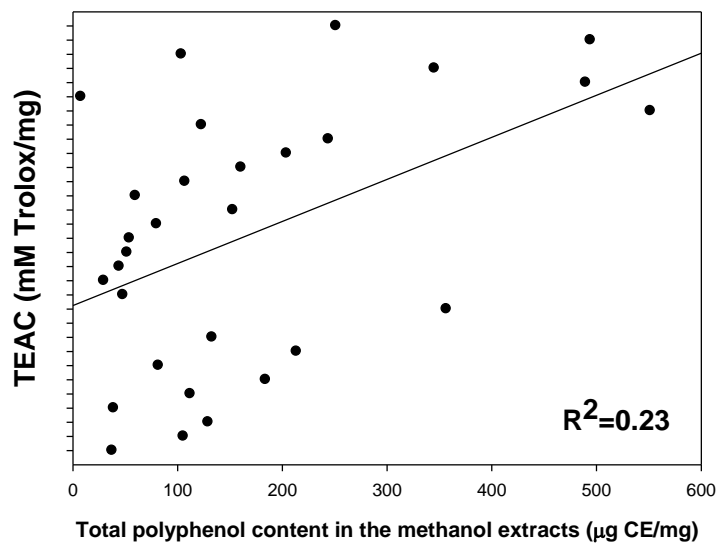
569 Figure 1.

570 A.



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572 B.



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## 臺灣濕地藥用植物之抗氧化活性和總多酚含量

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585

586 本文章研究目的是評估台灣濕地藥用植物之甲醇和水萃取物之抗氧化活性。  
587 評估的項目，包括 ABTS 清除，清除 DPPH 自由基，還原力，總多酚含量，總類  
588 黃酮類含量、總黃酮醇類含量。結果顯示，31 種濕地藥用植物中以水豬母乳、  
589 燈心草、碎米莎草、水柳、泥花草、短葉水蜈蚣和香蒲共七種，其抗氧化物和多  
590 酚類均具不錯之效果和含量。且由抗氧化活性和總多酚含量之線性相關係數結果  
591 得知，水萃取物相關係數為 0.14 和甲醇萃取物相關係數為 0.23。結果顯示，植  
592 物中化學物質含量可能有助於顯著的抗氧化活性，但這種關係並不一定成正比。  
593 濕地藥用植物未來在醫藥和保健食品行業中將可作為一個容易取得的天然抗氧  
594 化劑的來源。

595

關鍵詞：濕地藥用植物；抗氧化劑；多酚類；黃酮類；黃酮醇類