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1 Preliminary assessment of antioxidant activity of young edible
2 leaves of seven *Ficus* species in the ethnic diet in
3 Xishuangbanna, Southwest China

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10 **ABSTRACT**

11 The young leaves of seven *Ficus* species including *F. virens* var. *sublanceolata*, *F. auriculata*, *F.*
12 *vasculosa*, *F. callosa*, *F. virens* var. *verins*, *F. racemosa* and *F. oligodon* are traditionally consumed by
13 local people in Xishuangbanna. The dry young leaves were studied for their total phenolic and
14 flavonoid contents. The 90% ethanolic extracts of the young leaves were screened for potential
15 antioxidant capacity by employing different *in vitro* assays including ABTS^{•+} and DPPH[•] radical
16 scavenging capacities, ferric reducing power and lipid peroxidation inhibition properties. Extracts from
17 *F. virens* var. *sublanceolata* and *F. auriculata* showed higher antioxidant activity in all the systems than
18 other species. We concluded that these two species are promising sources of natural dietary
19 antioxidants. According to our statistical analysis, the total phenolic and flavonoid contents appear to
20 be responsible, at least in part, for the extracts' excellent antioxidant capacity and all the extracts
21 exhibited dose-dependent antioxidant activity.

22 **Keywords:** *Ficus* species; Phenolics; Flavonoids; Free radicals; Antioxidant activity; Reducing power;
23 Lipid peroxidation

24 **1. Introduction**

25 The use of *Ficus* species as food or pharmacological agents to improve human health has a history of
26 about ten thousand years. Some of the species are used in Ayurvedic and Traditional Chinese Medicine
27 according to Lansky, Paavilainen, Pawlus and Newman (2008). These researchers have reported the
28 antioxidant actions of *Ficus* components and the contemporary ethnopharmacological uses of *Ficus*
29 species against cancer and inflammation. Species including *F. benghalensis*, *F. carica*, *F. microcarpa*
30 and *F. racemosa* have been reported. However, the antioxidant profile of most species belonging to
31 *Ficus* have remained unexamined and lack extensive documentation.

32 Xishuangbanna in southwest China, bordered with Laos and Myanmar, is one of the biodiversity
33 hotspots for conservation priorities (Myers, Mittermeier, Mittermeier, Da Fonseca, & Kent, 2000). It is
34 inhabited by 13 ethnic groups with a total population of about 1 million in 2010. These ethnic groups
35 traditionally lived on gathering and hunting in the tropical forest for their subsistence, and accumulated
36 rich knowledge about uses of wild plants as vegetables. In recent decades, wild vegetables have
37 become an important source of cash income for local communities, due to their high nutritional value,
38 special local flavour and free from chemical residues. According to Xu, Tao, Liu, Yan, and Dao (2004),
39 the income generated from wild vegetable market accounted for 30.9% of the total income from
40 vegetable sales. They also reported that over 300 vascular plant species and varieties in Xishuangbanna
41 are consumed as wild vegetables by the ethnic groups. Statistical analysis showed, at genus level, *Ficus*
42 contains the most abundant edible species, including the seven species we intend to study, namely *F.*
43 *virens* Ait var. *sublanceolata* (Miq.) Corner, *F. auriculata* Lour., *F. vasculosa* Wall ex Miq., *F. callosa*
44 Willd., *F. virens* Ait var. *verins*, *F. racemosa* L. and *F. oligodon* Miq.. The young leaves' nutrient
45 contents have already been reported (Xu, Liu, Xiao, Wu, Dao, & Cai, 2005). However, apart from *F.*

46 *racemosa* no scientific literature exists on the antioxidant capacity of these plants. It is known that a
47 dietary or pharmaceutical supplement of extrinsic antioxidant compounds to neutralize excessive
48 harmful free radicals is important for maintaining human health. For example, fruits of *Ficus carica*
49 (figs) have high phenolic antioxidant concentrations, and antioxidants from figs can protect
50 lipoproteins in human plasma from oxidation and produce a significant increase in plasma antioxidant
51 capacity after consumption (Vinson, Zubik, Bose, Samman, & Proch, 2005). In this research, we
52 measured the total phenolic and flavonoid contents as well as total antioxidant activity of the seven
53 *Ficus* species mentioned above. Due to the complex nature of phytochemicals, the antioxidant activity
54 of plant extracts cannot be evaluated by only a single method. Therefore, commonly accepted assays,
55 including methods on ABTS^{•+} and DPPH[•] radical scavenging capacities, ferric reducing power and
56 linoleic acid peroxidation inhibition properties, were employed to evaluate the total antioxidant effects.
57 The study may give rise to further research on the application of these edible plants in pharmaceuticals
58 and functional foods.

59 **2. Materials and methods**

60 *2.1. Chemicals*

61 2,2-azobis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) was purchased from Bio Basic inc.
62 (Markham, Ontario, Canada). Folin-Ciocalteu phenol reagent, α,α -diphenyl- β -picrylhydrazyl (DPPH[•])
63 and linoleic acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Rutin,
64 trichloroacetic acid (TCA), ferric chloride, ferrous chloride, aluminium chloride, ammonium
65 thiocyanate, ethylenediamine tetracetic acid (EDTA), sodium nitrite, potassium ferricyanide, gallic acid
66 and other chemicals used were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai,
67 China). All reagents were of analytical grade.

68 2.2. *Plant material and extraction*

69 All of the seven young leaf samples were collected from three separate locations in Xishuangbanna
70 Tropical Botanical Garden, Chinese Academy of Science (Yunnan, China). The leaves were air-dried in
71 shade and pulverized with a household food processor. After that, the dried powder was weighed and
72 extracted with 90% aqueous ethanol for about 36 hrs in a shaker (120 rpm) at 45°C. The residues were
73 re-extracted twice under the same conditions to obtain ethanol extracts. The resulting extracts were
74 pooled and centrifuged at 4000 g for 15 min. The collected supernatants were evaporated *in vacuo* at
75 45°C to dryness, weighed and stored in a desiccator in the dark for further use.

76 2.3. *Determination of ethanol extractable total phenolics and flavonoids*

77 2.3.1. *Contents of ethanol extractable total phenolics*

78 Total phenolic contents (TPC) were determined with Folin-Ciocalteu reagent (Karadeniz, Burdurlu,
79 Koca, & Soyer, 2005; Kaur & Kapoor, 2002). A 0.5 ml aliquot of a methanolic solution of each extracts
80 (10 mg/ml) was taken in a 10 ml colorimeter tube and 7 ml of distilled water and 0.5 ml of
81 Folin–Ciocalteu reagent were added. The solution was well mixed and allowed to stand at room
82 temperature for 3 min. Two ml of 20% aqueous sodium carbonate was added and the contents were
83 mixed thoroughly. Absorbance of the resultant solution was measured at 738 nm after 1 h. The TPC
84 was calculated from a standard calibration curve based on gallic acid.

85 2.3.2. *Contents of ethanol extractable flavonoids*

86 Flavonoid contents of the samples were determined according to Karadeniz et al. (2005). One ml of
87 methanolic solution of each extract (10 mg/ml) was placed in a 10 ml colorimeter tube, then 5 ml of
88 distilled water and 0.3 ml of 5% aqueous NaNO₂ were added. The solution was mixed well and allowed
89 to stand at room temperature for 5 min. Then 0.6 ml of 10% aqueous AlCl₃·6H₂O was added. After 6

90 min, 2 ml of 1 M NaOH was added and the final volume was made up to 10 ml with distilled water.
91 The solution was mixed thoroughly and the absorbance was read at 510 nm. Flavonoid content was
92 calculated from the standard calibration curve based on rutin.

93 2.4. Radical scavenging assays

94 2.4.1 Scavenging effect on ABTS^{•+} radicals

95 The assay was a slightly modified version of Subhasree, Baskar, Keerthana, Susan and Rajasekaran
96 (2009). Seven mM ABTS solutions were prepared and reacted with 2.45 mM aqueous ammonium
97 persulfate solution. The mixture was then kept for 12-16 hrs in the dark at room temperature, to
98 produce a dark green solution containing ABTS^{•+} radical cations. The initial absorbance at 745 nm was
99 read. The ABTS^{•+} solution was diluted with ethanol to an absorbance of about 0.7 (\pm 0.02) at 745 nm
100 and equilibrated at 30 °C. Different concentrations of the methanolic solution of each extract (0.1 – 0.5
101 mg/ml) were prepared and 0.5 ml of the extract was mixed with 4.5 ml of ABTS^{•+} working standard in
102 a 5 ml colorimeter tube. The absorbance was measured exactly 1 min after initial mixing and then for 6
103 additional min. The final absorbance was noted. Gallic acid was employed as a reference and the
104 percentage inhibition was calculated according to the formula:

$$105 \text{ ABTS}^{\bullet+} \text{ radical scavenging (\%)} = [(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}] \times 100$$

106 Where A_{sample} is the absorbance of the solution when the extract/reference has been added at a
107 particular level, and A_{control} is the absorbance of the ABTS^{•+} working standard without extract added. All
108 analyses were run in triplicates and mean values were calculated.

109 2.4.2. Scavenging effects on DPPH[•] radicals

110 The ability of prepared extracts to scavenge DPPH[•] radicals was determined by the method described
111 by Brandwilliams, Cuvelier and Berset (1995) with slight modifications. One ml methanol solution of

112 each extract in different concentrations (0.1 – 0.5 mg/ml) was added to 9 ml methanolic solution of
113 DPPH^{*} (6×10^{-5} M). The solution was well mixed and then left at room temperature for 15 min in the
114 dark. The absorbance of the resulting solution was read at 517 nm. Gallic acid was employed as a
115 reference and the radical scavenging activity was calculated as a percentage of DPPH^{*} discolouration
116 using the equation:

$$117 \quad \text{DPPH}^* \text{ radical scavenging (\%)} = [(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}] \times 100$$

118 Where A_{sample} is the absorbance of the solution when the extract/reference has been added at a
119 particular level, and A_{control} is the absorbance of the DPPH^{*} solution without extract added. All analyses
120 were run in triplicate and mean values were calculated.

121 *2.5. Determination of the reducing power*

122 The reducing power of the leaf extracts was tested according to the method described by Siddhuraju,
123 Mohan and Becker (2002). One-half ml of methanol solution from each extract in different
124 concentrations (0.05, 0.10, 0.15, 0.20 and 0.25 mg/ml) as well as gallic acid were mixed with 2 ml of
125 phosphate buffer (0.2 M, pH 6.6) and 2 ml of aqueous potassium ferricyanide (1%). Afterwards, the
126 mixture was incubated at 50 °C for 20 min and then 2 ml of 10% trichloroacetic acid was added. The
127 mixture was centrifuged at 3000 rpm for 10 min. Five ml of the upper layer of the solution was mixed
128 with 5 ml of distilled water and 0.5 ml of aqueous FeCl₃ (1%). The absorbance was read at 700 nm.
129 Analyses were triplicated.

130 *2.6. Antioxidant activity against lipid peroxidation*

131 The antioxidant activity of the 90% aqueous ethanol extracts against lipid peroxidation was
132 measured using the thiocyanide method as described previously by Kikuzaki and Nakatani (1993).
133 Each extract (0.5 mg) in 0.5 ml of absolute ethanol was mixed with 0.5 ml absolute ethanol solution of

134 linoleic acid (2.51%), 1 ml of 0.05 M phosphate buffer (pH 7), and 0.5 ml of distilled water. The
135 reaction mixture was then incubated in the dark at 40°C for autoxidation. Aliquots of 0.1 ml were taken
136 at every 12 h during incubation and the degree of oxidation was measured by sequentially adding
137 ethanol (9.7 ml, 75%), aqueous ammonium thiocyanate (0.1 ml, 30%) and aqueous ferrous chloride
138 (0.1 ml, 0.02 M in 3.5% HCl). After the mixture has stood for 3 min, the peroxide value was
139 determined by monitoring absorbance at 500 nm until the absorbance of the control (containing all
140 reagents except the test sample) reached the maximum. Gallic acid was employed as a reference and
141 the antioxidant activity was expressed as percent inhibition relative to control using the equation:

142 Lipid peroxidation inhibition (%) = $[1 - (\text{sample absorbance at 48 h} - \text{sample absorbance at 0 h}) / (\text{control absorbance at 48 h} - \text{control absorbance at 0 h})] \times 100$
143

144 2.6. Statistical analysis

145 The statistical analyses were performed using the statistical package SPSS (Statistical Package for
146 Social Science, SPSS Inc., Chicago, IL). Analyses of variance were performed by ANOVA procedures
147 and significance of each group was verified with one-way analysis of variance followed by Duncan's
148 multiple range test ($P < 0.05$). The 50% inhibitory concentration (IC_{50}) was calculated according to
149 Concentration-Effect regression line. Correlation analysis was carried out to determine the relationship
150 between the antioxidant activity and the TPC or flavonoid content. Values obtained are means of three
151 replicate determinations \pm standard deviation.

152 3. Results and discussion

153 3.1. Total phenolic and flavonoid contents

154 The grouped bar chart (Fig. 1) indicates the extractable total phenolic and flavonoid levels in the
155 dried leaf samples. The TPC was determined from the regression equation of the calibration curve

156 obtained from gallic acid ($y = 0.0890x + 0.0037$, $r > 0.999$). The content of flavonoid was determined
157 from the regression equation of the calibration curve obtained from rutin ($y = 0.0115x + 0.0033$, $r >$
158 0.999). The result showed that *F. virens* var. *sublanceolata* possesses the highest levels of total
159 phenolics (17.44 mg/g) and flavonoids (3.87 mg/g), while the lowest amounts of total phenolics and
160 flavonoids were found in *F. racemosa* (7.83 mg/g) and *F. oligodon* (1.05 mg/g), respectively.

161 In this research, all of the dry leaves had a content of 7.83 – 17.44 mg/g phenolics and 1.05 – 3.87
162 mg/g flavonoids. The values are comparable to those of the root extract of *F. racemosa* (Sharma &
163 Gupta, 2008) and the bark, fruits and leaf extracts of *F. microcarpa* L. f. (Ao, Li, Elzaawely, Xuan, &
164 Tawata, 2008). Additional work is necessary to determine the specific chemical constituents of the leaf
165 extracts mentioned above. Li, Leach, Myers, Lin, Leach and Waterman (2004) and Veerapur et al.
166 (2009) have previously reported that the specific natural antioxidant compounds in the ethanol extracts
167 of *F. racemosa* include racemosic acid, bergenin, tannins, kaempferol, rutin, bergapten, psoralenes,
168 ficusin, coumarin and phenolic glycosides.

169 3.2. Free radical scavenging capacity

170 The abilities for each concentration of the extract samples to scavenge ABTS^{•+} and DPPH[•] radicals
171 are shown in Fig. 2 and Fig. 3, respectively. These extracts significantly inhibited the activities of
172 ABTS^{•+} and DPPH[•] radicals in a dose-dependent manner ($P < 0.05$) and almost complete inhibition
173 (97.68%) of ABTS^{•+} radicals was observed for 0.5 mg/ml of young leaf extract of *F. virens* var.
174 *sublanceolata*. Similar ABTS^{•+} and DPPH[•] free radical scavenging activities have previously been
175 documented for the ethanol extract of *Ficus carica* L. fruits and methanol extract of *Ficus microcarpa*
176 L. fil. leaves (Ao et al., 2008; Yang, Yu, Ou, Ma, Liu, & Ji, 2009).

177 The IC_{50} values were calculated and tabulated in Table 1 to facilitate the comparison of the free

178 radical scavenging activities (FRSA) of different samples. The IC_{50} value is the concentration of the
179 samples required to scavenge 50% of the free radicals present in the system. It was calculated using the
180 Concentration-Scavenging activity curve ($r > 0.99$). Lower IC_{50} implies a higher FRSA. As shown in
181 Table 1, the IC_{50} of *F. virens* var. *sublanceolata* and *F. auriculata* extracts were lower than those of
182 other species, suggesting that both ABTS^{•+} and DPPH[•] radical scavenging by extracts from *F. virens* var.
183 *sublanceolata* and *F. auriculata* were more effective than other species. The gallic acid reference
184 displayed excellent FRSA (data was not shown in Fig. 2 and Fig. 3) and its IC_{50} values were computed
185 (Table 1). In the report by Orhan, Kartal, Abu-Asaker, Senol, Yilmaz and Sener (2009), it was also
186 shown that gallic acid exhibited a particular high radical scavenging activity compared with plant
187 extracts.

188 3.3. Reducing power

189 The relative ferric reducing power of extracts from young leaves of the seven *Ficus* species as well
190 as gallic acid reference are tabulated in Table 2. A higher absorbance of the reaction mixture indicated
191 greater reducing power. Various concentrations of each sample were used for the assay and all of the
192 samples appeared to exert effective reducing power in a dose-dependent manner ($P < 0.05$). However,
193 reducing power exhibited by the extracts from *F. virens* var. *sublanceolata* and *F. auriculata* was again
194 noteworthy under these experimental concentrations. Similar observations of antioxidant properties in
195 terms of dose dependency and reducing power activity have been reported for solvent extracts of the
196 aerial root of *F. bengalensis* L. and the stem bark of *F. racemosa* L. (Manian, Anusuya, Siddhuraju, &
197 Manian, 2008). The notable reducing power of gallic acid was also documented by Orhan et al. (2009).

198 In the presence of the extracts, Fe^{3+} is reduced to Fe^{2+} and forms a green and blue-coloured ferrous
199 cyanide complex, which showed maximum absorbance at 700 nm. The reducing power of a bioactive

200 substance is closely related to its antioxidant ability (Orhan et al., 2009). Considering the effective
201 reducing power of the samples tested, the antioxidant compounds of all the extracts should function as
202 good electron and hydrogen-atom donors and therefore be able to terminate radical chain reaction by
203 converting free radicals and reactive oxygen species to more stable products (Siddhuraju et al., 2003).

204 3.4. Peroxidation inhibition activity in a linoleic acid system

205 The extracts from *F. virens* var. *sublanceolata*, followed by *F. callosa* and *F. auriculata*, conferred
206 substantial inhibition against the peroxidation of linoleic acid, whereas *F. virens* var. *verins* and *F.*
207 *oligodon* offered less effective inhibition as depicted in Fig. 4 (values for *F. vasculosa* and *F. racemosa*
208 are not shown). Gallic acid was also compared along with the extracts at a concentration of 8 µg/ml. All
209 of the five species' extracts showed excellent inhibition at 1 mg/ml in the range of 41.40 – 83.80% after
210 incubation for 48 hrs. Peroxidation inhibition efficacy has also been reported for solvent extracts of the
211 aerial root of *F. bengalensis* L. and the stem bark of *F. racemosa* L. (Manian et al., 2008).

212 Some natural antioxidants from plants have been shown to prevent lipid peroxidation-mediated cell
213 injury. The lipid peroxidation inhibition activities of our plant samples expose the complexity of the
214 extracts composition (aqueous versus hydrophobic compounds) as well as potential interaction between
215 the extract and emulsion components, oil, water or lipid-air interfaces (Koleva, van Beek, Linssen, de
216 Groot, & Evstatieva, 2002). However, the inhibition effect of all the extracts in our study was
217 significantly lower than the gallic acid reference at the specified experimental concentrations. This
218 might partly be due to the fact that gallic acid (hydrophobic antioxidant) is extraordinarily effective in
219 an oil-in-water emulsion system. By moving to the oil phase, the hydrophobic antioxidants readily
220 attain adequate concentrations to protect oil at the oil-water interface (Frankel & Meyer, 2000).

221 3.5. Correlation analysis

222 Phenolics are important nutritional ingredients in edible plants and are confirmed to possess a wide
223 range of therapeutic uses. Several studies suggest that increased dietary intake of natural phenolic
224 antioxidants can decrease the risk of coronary heart disease (Middleton, Kandaswami, & Theoharides,
225 2000; Stampfer, Hennekens, Manson, Colditz, Rosner, & Willett, 1993) because of their antioxidant
226 activities, which mainly arise from the presence of hydroxyl groups. The flavonoids are a large family
227 of low molecular weight polyphenolic compounds and it has long been recognized that many
228 flavonoids widely distributed in edible plants are strong antioxidants which possess effective ROS
229 scavenging capacity due to their phenolic hydroxyl groups (Cao, Sofic, & Prior, 1997). These
230 compounds can modulate lipid peroxidation involved in atherogenesis, thrombosis and carcinogenesis,
231 and their known properties include strong antioxidant activity, inhibition of hydrolytic and oxidative
232 enzymes as well as anti-inflammatory action (Siddhuraju et al., 2003).

233 In our study, the results obtained by correlation analysis indicate that the content of the extractable
234 total phenolic have a statistically significant influence on the observed antioxidant capacities
235 determined by different systems ($P < 0.01$), and the ABTS^{•+} radicals scavenging effects ($P < 0.05$),
236 ferric reducing power ($P < 0.01$) and peroxidation inhibition properties ($P < 0.05$) of the leaf extracts
237 correlate well with their contents of extractable flavonoids. Research on the leaf extracts of *F. carica* L.
238 showed similar results (Konyalioglu, Saglam, & Kivcak, 2005). While an insignificant relationship was
239 observed between the flavonoid content and the DPPH[•] radical scavenging activity, this might partly be
240 attributed to the presence of glycoside in the flavonoids that could decrease the DPPH[•] radical
241 scavenging ability by affecting the donation of hydrogen (vonGadow, Joubert, & Hansmann, 1997). On
242 the other hand, other compounds besides phenolics and flavonoids may contribute to these activities.
243 Take *F. auriculata* as an example, the young leaves of *F. auriculata* contain considerably high amount

244 of selenium and it is known that selenium acts as an integral constituent of the antioxidative enzyme
245 glutathione peroxidase (GSH-Px), which detoxifies hydrogen peroxide and organic lipid peroxides. The
246 extracts' effective antioxidant activities may also attribute to their higher vitamin E contents compared
247 with regular vegetables (Xu et al, 2005). In addition, the observed total antioxidant effect may be
248 higher than the simple summation of antioxidant donation of every individual compound because of
249 synergetic effects (Dasgupta & De, 2004).

250 Significant correlations were also observed between antioxidant capacities determined by different
251 systems ($P < 0.01$), indicating that these four methods have satisfactory correlations for the
252 examination of antioxidants.

253 **4. Conclusions**

254 For the first time, we evaluated the antioxidant effects of the edible young leaves of *F. virens* var.
255 *sublanceolata*, *F. auriculata*, *F. vasculosa*, *F. callosa*, *F. virens* var. *verins*, *F. racemosa* and *F. oligodon*
256 along with their total phenolic and flavonoid contents. Our results showed that the edible young leaves
257 of the seven *Ficus* species possess abundant antioxidants at various concentrations, and the ethanol
258 extracts of *F. virens* var. *sublanceolata* and *F. auriculata* showed considerable high antioxidant
259 potential compared with other species tested.

260 Recently, seven different antioxidant compounds, including protocatechuic acid, chlorogenic acid,
261 catechin, epicatechin etc., have been reported in methanolic extracts of the stem bark of *Ficus*
262 *microcarpa* L. fil. All the reported compounds showed strong antioxidant activity (Ao, Higa, Ming,
263 Ding, & Tawata, 2010). Joseph et al. (2010) overviewed the phytopharmacological properties of *F.*
264 *racemosa* and reported much evidence on its considerable antioxidant ability. Meanwhile there has
265 been no previous study of antioxidant effects of the other six species examined here. Some examples,

266 such as young leaves of *F. virens* var. *sublanceolata* and *F. auriculata* have however been found to be
267 more potent than *F. racemosa*. Hence, further research is essential to establish the antioxidant potential
268 of some underexploited *Ficus* species, especially those being used traditionally for pharmacological or
269 dietetic purposes. The one with considerable nutrient content and antioxidant potential should be given
270 preferential consideration for functional food or local special products development, which aims to
271 meet the demands for pollutant free, health improving and house special dishes of the residents and
272 millions of tourists.

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280 **References**

- 281 Ao, C., Higa, T., Ming, H., Ding, Y. T., & Tawata, S. (2010). Isolation and identification of antioxidant
282 and hyaluronidase inhibitory compounds from *Ficus microcarpa* L. fil. bark. *Journal of Enzyme*
283 *Inhibition and Medicinal Chemistry*, 25, 406-413.
- 284 Ao, C. W., Li, A. P., Elzaawely, A. A., Xuan, T. D., & Tawata, S. (2008). Evaluation of antioxidant and
285 antibacterial activities of *Ficus microcarpa* L. fil. extract. *Food Control*, 19, 940-948.
- 286 Brandwilliams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free-radical method to evaluate
287 antioxidant activity. *Food Science and Technology-Lebensmittel-Wissenschaft & Technologie*, 28,

- 288 25-30.
- 289 Cao, G. H., Sofic, E., & Prior, R. L. (1997). Antioxidant and prooxidant behavior of flavonoids:
290 Structure-activity relationships. *Free Radical Biology and Medicine*, 22, 749-760.
- 291 Dasgupta, N., & De, B. (2004). Antioxidant activity of *Piper betle* L. leaf extract *in vitro*. *Food*
292 *Chemistry*, 88, 219-224.
- 293 Finkel, T., & Holbrook, N. J. (2000). Oxidants, oxidative stress and the biology of ageing. *Nature*, 408,
294 239-247.
- 295 Joseph, B., & Raj, S. J. (2010). Phytopharmacological properties of *Ficus racemosa* Linn - an overview.
296 *International Journal of Pharmaceutical Sciences Review and Research*, 3, 134-138.
- 297 Karadeniz, F., Burdurlu, H. S., Koca, N., & Soyer, Y. (2005). Antioxidant activity of selected fruits and
298 vegetables grown in Turkey. *Turkish Journal of Agriculture and Forestry*, 29, 297-303.
- 299 Kaur, C., & Kapoor, H. C. (2002). Anti-oxidant activity and total phenolic content of some Asian
300 vegetables. *International Journal of Food Science and Technology*, 37, 153-161.
- 301 Kikuzaki, H., & Nakatani, N. (1993). Antioxidant effects of some ginger constituents. *Journal of Food*
302 *Science*, 58, 1407-1410.
- 303 Koleva, I. I., van Beek, T. A., Linssen, J. P. H., de Groot, A., & Evstatieva, L. N. (2002). Screening of
304 plant extracts for antioxidant activity: a comparative study on three testing methods. *Phytochemical*
305 *Analysis*, 13, 8-17.
- 306 Konyalioglu, S., Saglam, H., & Kivcak, B. (2005). Alpha-Tocopherol, flavonoid, and phenol contents
307 and antioxidant activity of *Ficus carica* leaves. *Pharmaceutical Biology*, 43, 683-686.
- 308 Lansky, E. P., Paavilainen, H. M., Pawlus, A. D., & Newman, R. A. (2008). *Ficus* spp. (fig):
309 Ethnobotany and potential as anticancer and anti-inflammatory agents. *Journal of*

- 310 *Ethnopharmacology*, 119, 195-213.
- 311 Li, R. W., Leach, D. N., Myers, S. P., Lin, G. L. D., Leach, G. J., & Waterman, P. G. (2004). A new
312 anti-inflammatory glucoside from *Ficus racemosa* L. *Planta Medica*, 70, 421-426.
- 313 Manian, R., Anusuya, N., Siddhuraju, P., & Manian, S. (2008). The antioxidant activity and free radical
314 scavenging potential of two different solvent extracts of *Camellia sinensis* (L.) O. Kuntz, *Ficus*
315 *bengalensis* L. and *Ficus racemosa* L. *Food Chemistry*, 107, 1000-1007.
- 316 Middleton, E., Kandaswami, C., & Theoharides, T. C. (2000). The effects of plant flavonoids on
317 mammalian cells: Implications for inflammation, heart disease, and cancer. *Pharmacological*
318 *Reviews*, 52, 673-751.
- 319 Myers, N., Mittermeier, R. A., Mittermeier, C. G., Da Fonseca, G. A. B., Kent, J., 2000. Biodiversity
320 hotspots for conservation priorities. *Nature* 403, 853-858.
- 321 Orhan, I., Kartal, M., Abu-Asaker, M., Senol, F. S., Yilmaz, G., & Sener, B. (2009). Free radical
322 scavenging properties and phenolic characterization of some edible plants. *Food Chemistry*, 114,
323 276-281.
- 324 Sharma, S. K., & Gupta, V. K. (2008). *In vitro* antioxidant studies of *Ficus racemosa* Linn. root.
325 *Pharmacognosy Magazine*, 4, 70-74.
- 326 Siddhuraju, P., & Becker, K. (2003). Antioxidant properties of various solvent extracts of total phenolic
327 constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera* Lam.)
328 leaves. *Journal of Agricultural and Food Chemistry*, 51, 2144-2155.
- 329 Siddhuraju, P., Mohan, P. S., & Becker, K. (2002). Studies on the antioxidant activity of Indian
330 Laburnum (*Cassia fistula* L.): a preliminary assessment of crude extracts from stem bark, leaves,
331 flowers and fruit pulp. *Food Chemistry*, 79, 61-67.

- 332 Stampfer, M. J., Hennekens, C. H., Manson, J. E., Colditz, G. A., Rosner, B., & Willett, W. C. (1993).
333 Vitamin-E consumption and the risk of coronary-disease in women. *New England Journal of*
334 *Medicine*, 328, 1444-1449.
- 335 Subhasree, B., Baskar, R., Keerthana, R. L., Susan, R. L., & Rajasekaran, P. (2009). Evaluation of
336 antioxidant potential in selected green leafy vegetables. *Food Chemistry*, 115, 1213-1220.
- 337 Veerapur, V. P., Prabhakar, K. R., Parihar, V. K., Kandadi, M. R., Ramakrishana, S., Mishra, B., Rao, B.
338 S. S., Srinivasan, K. K., Priyadarsini, K. I., & Unnikrishnan, M. K. (2009). *Ficus racemosa* stem
339 bark extract: A potent antioxidant and a probable natural radioprotector. *Evidence-Based*
340 *Complementary and Alternative Medicine*, 6, 317-324.
- 341 Vinson, J. A., Zubik, L., Bose, P., Samman, N., & Proch, J. (2005). Dried fruits: Excellent *in vitro* and
342 *in vivo* antioxidants. *Journal of the American College of Nutrition*, 24, 44-50.
- 343 vonGadow, A., Joubert, E., & Hansmann, C. F. (1997). Comparison of the antioxidant activity of
344 aspalathin with that of other plant phenols of rooibos tea (*Aspalathus linearis*), alpha-tocopherol,
345 BHT, and BHA. *Journal of Agricultural and Food Chemistry*, 45, 632-638.
- 346 Xu, Y. K., Tao, G. D., Liu, H. M., Yan, K. L., & Dao, X. S. (2004). Wild vegetable resources and
347 market survey in Xishuangbanna, southwest China. *Economic Botany*, 58, 192-212.
- 348 Xu, Y. K., Liu, H. M., Xiao, C. F., Wu, Z. L., Dao, X. S., & Cai, C. T. (2005). The nutrient contents of
349 six Fig species and its evaluation as woody vegetables. *Journal of Wuhan Botanical Research*, 23,
350 85-90.
- 351 Yang, X. M., Yu, W., Ou, Z. P., Ma, H. L., Liu, W. M., & Ji, X. L. (2009). Antioxidant and immunity
352 activity of water extract and crude polysaccharide from *Ficus carica* L. fruit. *Plant Foods for Human*
353 *Nutrition*, 64, 167-173.

354 Fig. 1 Levels of total phenolic and flavonoid present in young leaves of the seven *Ficus* species. Values
355 are the mean of triplicate determinations \pm SD
356 **FVS**, *F. virens* var. *sublanceolata*; **FC**, *F. callosa*; **FV**, *F. vasculosa*; **FA**, *F. auriculata*; **FVV**, *F. virens*
357 var. *verins*; **FR**, *F. racemosa*; **FO**, *F. oligodon*. For total phenolics, results are expressed as mg of gallic
358 acid equivalent per g of dry material. For flavonoids, results are expressed as mg of rutin equivalent per
359 g of dry material. The bars with different lowercases are significantly different ($P < 0.05$).

360 Fig. 2 ABTS^{•+} radical scavenging activities of young leaf extracts of the seven *Ficus* species. Values are
361 the mean of triplicate determinations \pm SD
362 **FVS**, *F. virens* var. *sublanceolata*; **FC**, *F. callosa*; **FV**, *F. vasculosa*; **FA**, *F. auriculata*; **FVV**, *F. virens*
363 var. *verins*; **FR**, *F. racemosa*; **FO**, *F. oligodon*. The bars with different letters are significantly different
364 ($P < 0.05$).

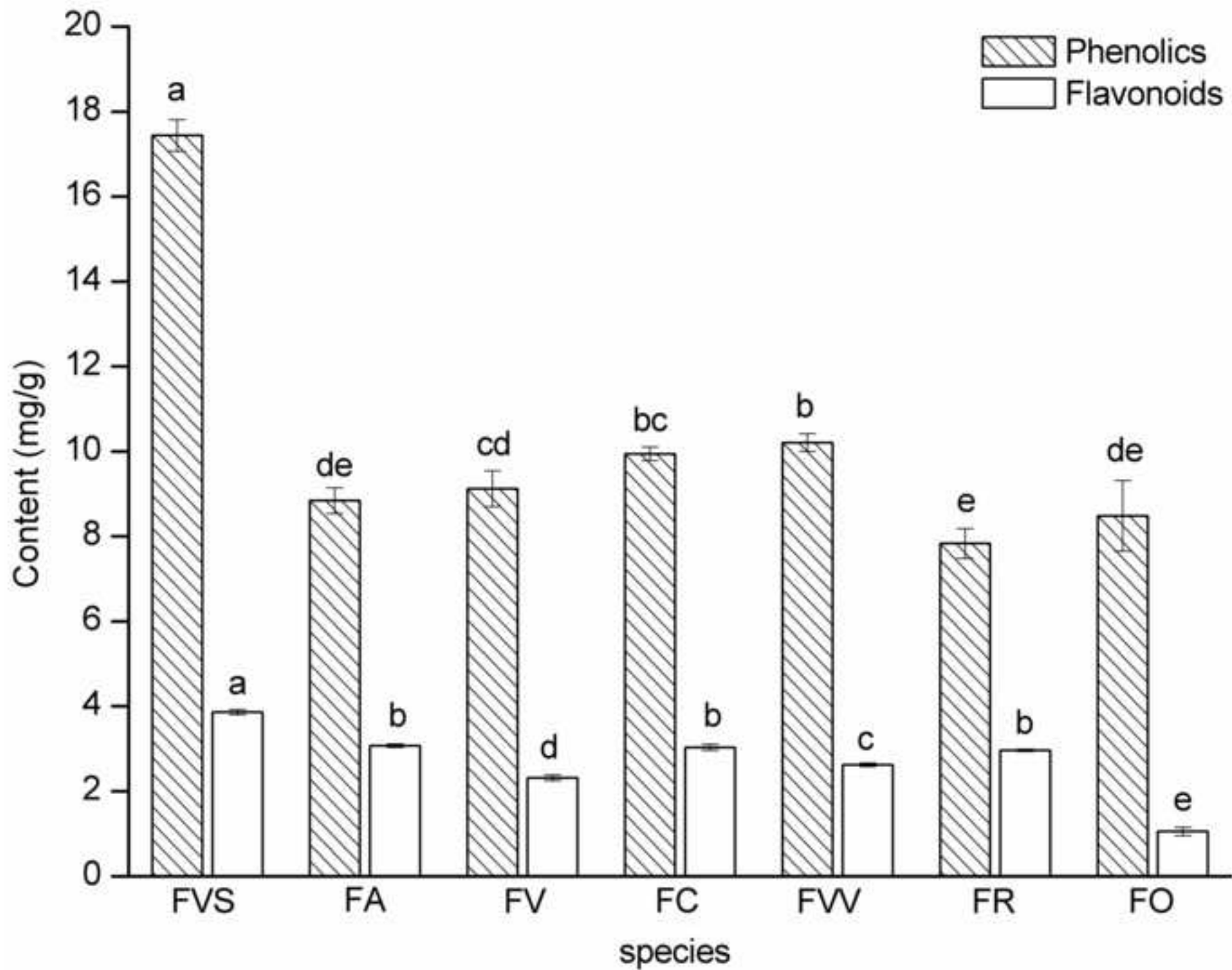
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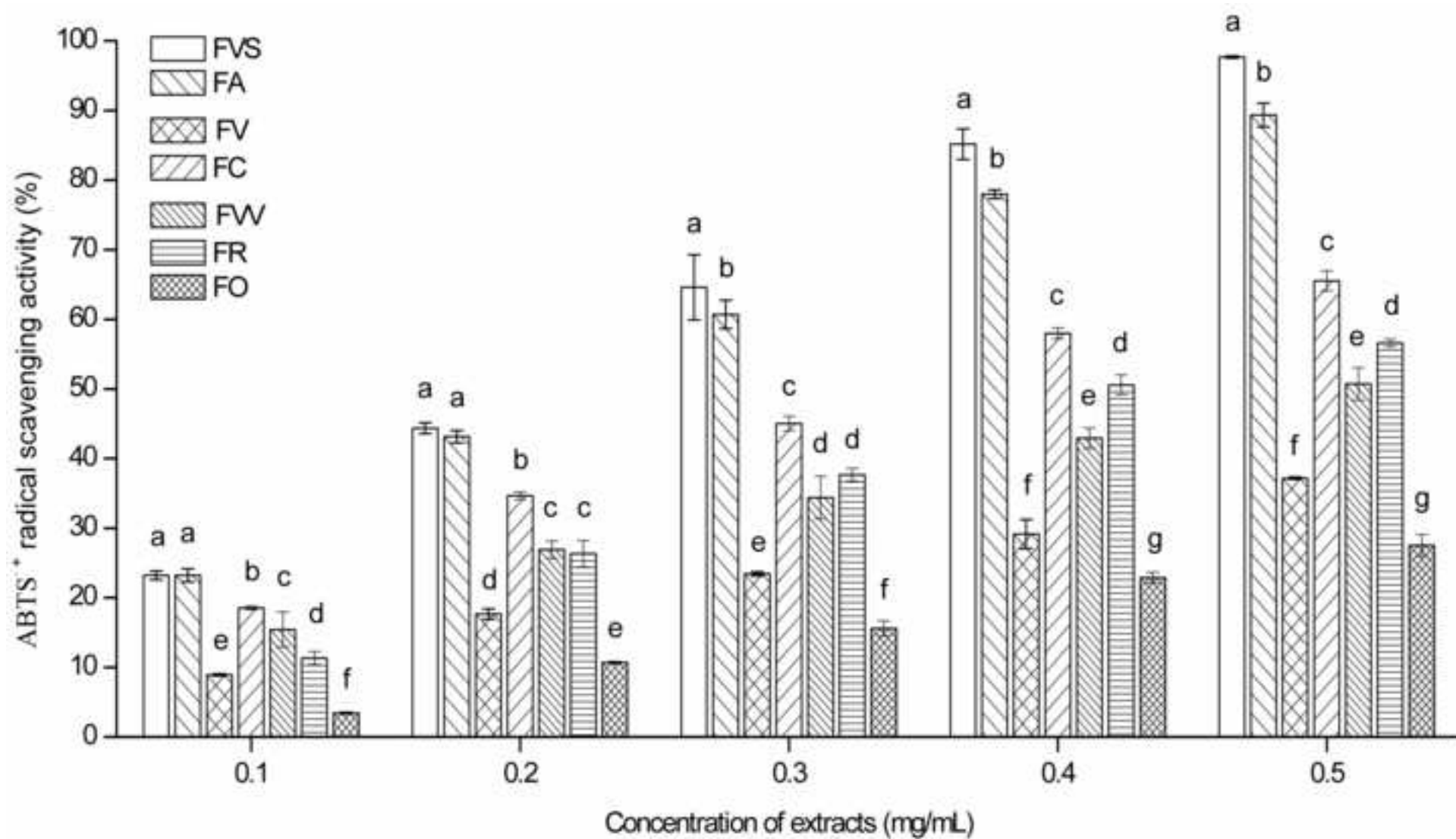
365 Fig. 3 DPPH^{*} radical scavenging activities of young leaf extracts of the seven *Ficus* species. Values are
366 the mean of triplicate determinations \pm SD
367 **FVS**, *F. virens* var. *sublanceolata*; **FC**, *F. callosa*; **FV**, *F. vasculosa*; **FA**, *F. auriculata*; **FVV**, *F. virens*
368 var. *verins*; **FR**, *F. racemosa*; **FO**, *F. oligodon*. The bars with different letters are significantly different
369 ($P < 0.05$).

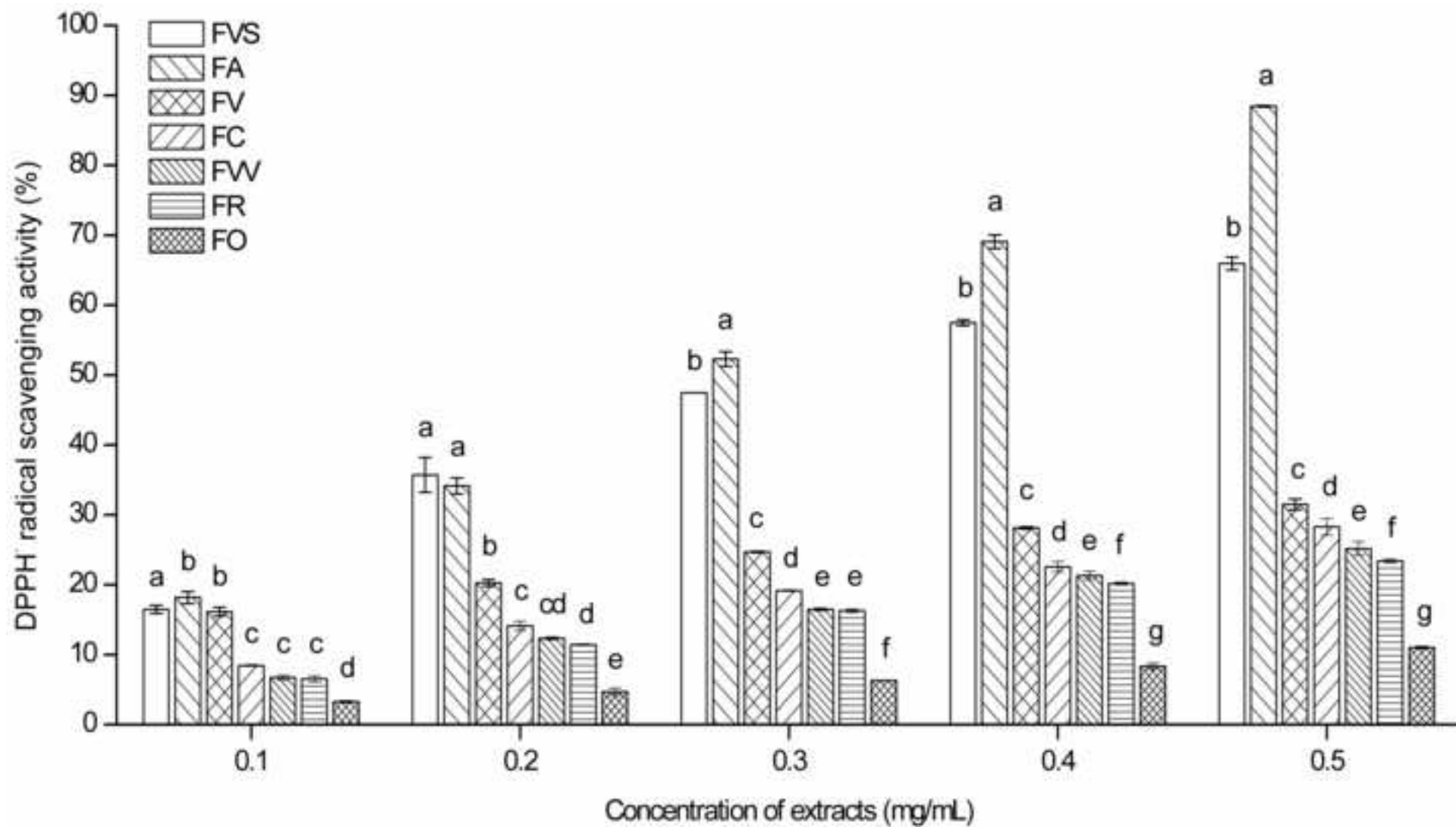
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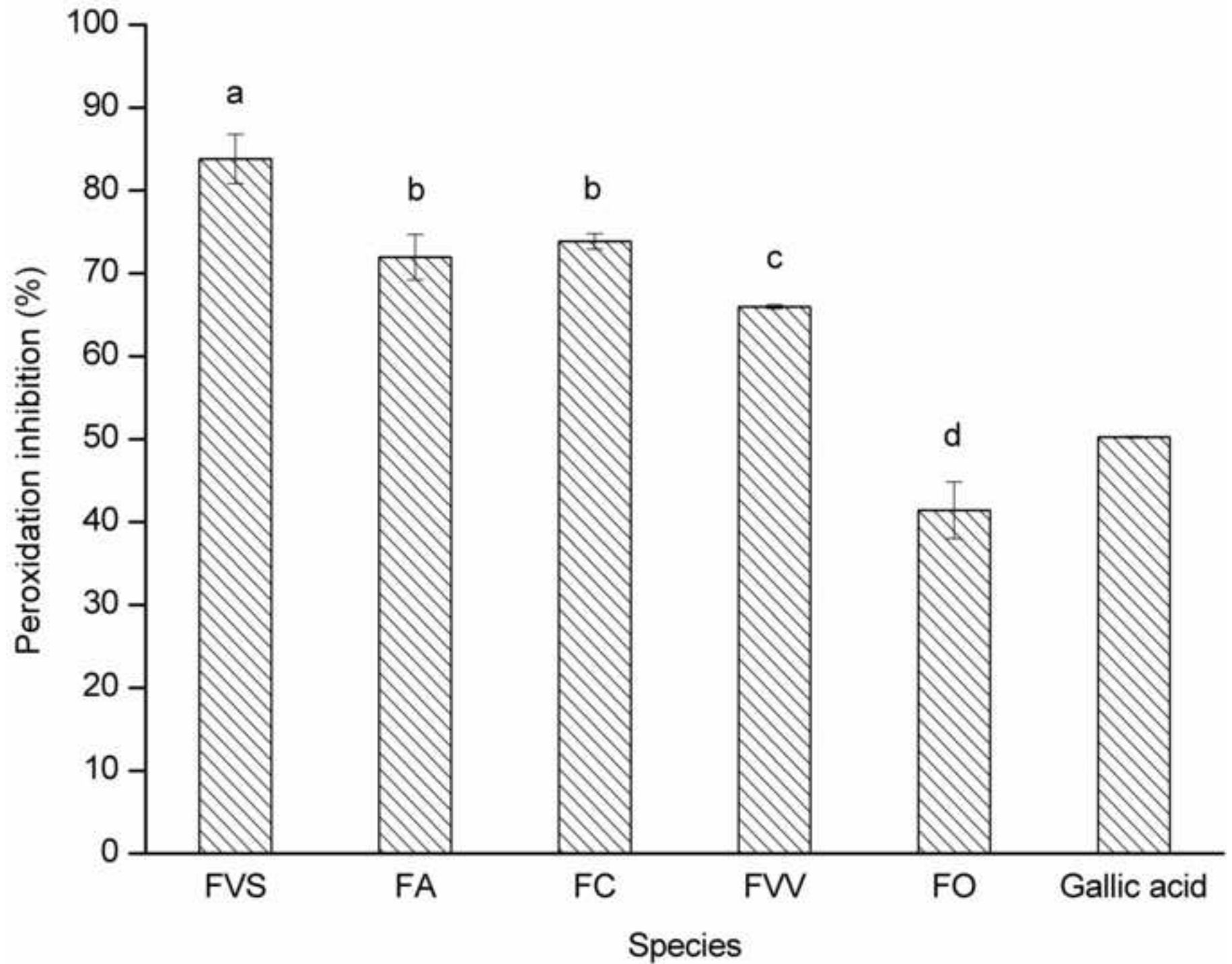
370 Fig. 4 Peroxidation inhibition by young leaf extracts of five *Ficus* species as well as gallic acid. Values
371 are the mean of triplicate determinations \pm SD
372 **FVS**, *F. virens* var. *sublanceolata*; **FC**, *F. callosa*; **FA**, *F. auriculata*; **FVV**, *F. virens* var. *verins*; **FO**, *F.*
373 *oligodon*. Extracts at a concentration of 1 mg/ml. Gallic acid at a concentration of 8 μ g/ml. The bars
374 with different letters are significantly different ($P < 0.05$).

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376 **Table 1**377 **Comparison of the IC_{50} values for radical scavenging assays of young leaf extracts of the seven *Ficus* species as well as gallic acid**

Species	IC_{50} values of each free radical scavenging assay (mg/ml)	
	ABTS ^{•+}	DPPH [•]
FVS	0.23	0.34
FA	0.25	0.29
FV	0.69	0.97
FC	0.35	0.95
FVV	0.48	1.03
FR	0.42	1.11
FO	0.86	2.54
Gallic acid [^]	2.76	5.04

378 **FVS, *F. virens* var. *sublanceolata*; FC, *F. callosa*; FV, *F. vasculosa*; FA, *F. auriculata*; FVV, *F. virens* var. *verins*; FR, *F. racemosa*; FO, *F. oligodon*.**379 [^] IC_{50} values in $\mu\text{g/ml}$.

380 Table 2

381 Ferric reducing power of young leaf extracts of the seven *Ficus* species as well as gallic acid

Extract concentration ^A	Absorbances							Gallic acid concentration ^B	Absorbances
	FVS	FA	FV	FC	FVV	FR	FO		
0.05	0.13±	0.11±	0.07±	0.08±	0.06±	0.05±	0.04±	1.6	0.12±0.000
	0.003 a	0.006 b	0.005 d	0.005 c	0.001 d	0.001 e	0.001 f		
0.10	0.25±	0.19±	0.12±	0.15±	0.13±	0.11±	0.08±	3.2	0.21±0.008
	0.006 a	0.002 b	0.001 e	0.003 c	0.001 d	0.003 e	0.003 f		
0.15	0.38±	0.28±	0.16±	0.22±	0.20±	0.16±	0.13±	4.8	0.30±0.009
	0.000 a	0.004 b	0.002 e	0.003 c	0.004 d	0.001 e	0.005 f		
0.20	0.52±	0.36±	0.22±	0.28±	0.25±	0.21±	0.18±	6.4	0.41±0.008
	0.013 a	0.011 b	0.005 e	0.002 c	0.006 d	0.006 e	0.003 f		
0.25	0.66±	0.45±	0.26±	0.33±	0.32±	0.26±	0.22±	8.0	0.53±0.011
	0.021 a	0.008 b	0.001 d	0.002 c	0.003 c	0.001 d	0.004 e		

382 FVS, *F. virens* var. *sublanceolata*; FC, *F. callosa*; FV, *F. vasculosa*; FA, *F. auriculata*; FVV, *F. virens* var. *verins*; FR, *F. racemosa*; FO, *F.*383 *oligodon*. Values are means of triplicates ± SD at 700 nm. Means with different letters in the same row are significantly different (P < 0.05).384 ^A Concentrations in mg/ml. ^B Concentrations in µg/ml.

385

386 **Highlights**

387 ► Young leaves of seven *Ficus* species contain considerable phenolics and flavonoids ► Extracts of the
388 young leaves exhibit excellent *in vitro* antioxidant capacities ► Phenolics and flavonoids act as
389 important antioxidants ► Young leaves of two *Ficus* species show notable antioxidant potential

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