



Total flavonoid contents, antioxidant potential and acetylcholinesterase inhibition activity of the extracts from 15 ferns in China



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ABSTRACT

Fern species have been classified as traditional Chinese medicinal herbs. However, the phytochemicals and bioactivities of most fern species were rarely explored. Herein, we examined the total flavonoid contents, *in vitro* antioxidant potential and acetylcholinesterase inhibitory activity of the extracts from 15 fern species in Yunnan Province of China. The total flavonoid contents ranged from 4.67 to 125.88 mg/g (w/w, dry weight). Their antioxidant potentials were evaluated by the radical scavenging, superoxide anion scavenging, and reducing power, which indicated that most of the extracts from these fern species showed very strong radical and superoxide anion scavenging ability. However, among these 15 fern species, only five extracts from *Pyrrosia petiolosa*, *Polypodiodes amoena*, *Araïostegia imbricate*, *Pseudodrynaria coronans*, and *Pilea nummulariifolia* showed acetylcholinesterase inhibitory activity. The polyphenol profiles of *Lemmaphyllum carnosum* with highest total flavonoid content were identified as dehydroagastol, bufotalin, chlorogenic acid, monogalloyl-glucose, luteolin 7-O-β-D-glucoside, kaempferol 3-O-α-L-rhamnopyranoside, myricetin 3-O-rhamnoside, quercetin 3-O-galactoside, apigenin 7-O-glucoside, and Woorenoside II.

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1. Introduction

From the beginning of 21st century, the natural products have been considered as a source of potential medicines (Cerella et al., 2014; Gechev et al., 2014; Georgiev, 2014; Schnekenburger et al., 2014). Various phytochemicals from nature, such as polysaccharides, alkaloids, flavonoids, and stilbenoids have obtained a considerable attention due to their biological benefits (Georgiev, 2014; Lanzotti, 2014; Xiao, 2015). Up to now, there is limited information available in the literature concerning the chemical composition and bioactivity of wild plants, that might represent innovative sources of novel medicines. There are more than 1500 fern species in Yunnan province of China (Sun et al., 2003). The major bioactive components in ferns are polyphenols, terpenoids, and alkaloids (Harborne and Williams, 1988; Cao et al., 2014; Cao et al., 2013a, 2013b; Xia et al., 2014). Among them, the flavonoids have attracted great interest (Zhao et al., 2010).

Most of the flavonoids can be classified to several groups such as isoflavonoids, flavanoids, flavones and anthocyanidins. Over 15,000 natural flavonoids have been identified and most of them existed as the glycoside forms (Xiao et al., 2014, 2013a,b, 2015). Flavonoid C-glycosides showed significant anticancer (Nagaprashantha et al., 2011) and antitumor, antioxidant, hepatoprotective (Xiao et al., 2013a,b, 2015), anti-inflammatory (Liu et al., 2012), anti-diabetic (Xiao and Högger, 2015), neuroprotective (Jiang et al., 2012) and antifungal activities (Yao et al., 2004).

However, the benefits and phytochemistry of most fern species are still not clear. In our previous report (Xia et al., 2014), the total flavonoid contents, antioxidant and anticancer activities, and acetylcholinesterase (AChE) inhibition potential of 19 fern extracts from China were investigated in detail. It was found that the ferns with more flavonoids content showed higher radical scavenging potential (Xia et al., 2014). Herein, we investigated the total flavonoid contents, and bioactivities of the extracts from 15 fern species in China. Specially, *Lemmaphyllum carnosum* (Fig. 1) with highest total flavonoid content was found to have excellent antioxidant and AChE inhibition activity. We further screened the flavonoid profile of *L. carnosum*.

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Fig. 1. *Lemnaphyllum carnosum*.

2. Materials and methods

2.1. Chemicals and materials

Please find this part in our previous report (Cao et al., 2013a; Xia et al., 2014).

2.2. Preparation of plant extracts

Fifteen fern species were collected in Yunnan province of China and identified by Professor Jianguo Cao in College of Life & Environment Science of Shanghai Normal University. The bioactive components were extracted by our previous report (Xia et al., 2014).

2.3. Determination of total flavonoids

The total flavonoid contents in ferns were measured by our previous report (Xia et al., 2014).

2.4. Antioxidant activity assay

2.4.1. DPPH radical scavenging activity

DPPH free radical scavenging activity of flavonoid extracts from 15 fern species was measured by our previous report (Xia et al., 2014).

2.4.2. ABTS assay

The ABTS assay was carried out according to our previous report (Xia et al., 2014).

2.4.3. Reducing power assay

The reducing power of flavonoids extract was quantified according to our previous report (Xia et al., 2014).

2.4.4. Superoxide anion (O_2^-) scavenging activity

Superoxide anion scavenging activity of these extracts was measured according to our previous report (Xia et al., 2014).

2.5. LC-DAD-ESI/MS analysis

The flavonoids profile in the extract were identified by LC-DAD-ESI/MS system according to our previous report (Cao et al., 2013a).

2.6. AChE inhibitory activity

AChE inhibition potential of these extracts from ferns were evaluated according to our previous report (Xia et al., 2014).

3. Results and discussion

3.1. Total flavonoid contents in 15 fern species

The total flavonoid contents in these 15 fern species were expressed as rutin equivalent in mg/g material (w/w, dry weight). The extracts from these 15 ferns showed an important variation in their total flavonoid contents, which were ranged from 4.7 to 125.9 mg/g (Table 1). *L. carnosum* (125.9 mg/g) and *Araiostegia imbricata* (105.9 mg/g) appeared very high total flavonoid contents. *Pyrrhosia petiolosa* (80.6 mg/g), *Pseudodrynaria coronans* (78.9 mg/g), *Hicriopteris glauca* (77.6 mg/g), *Polypodiodes amoena* (71.4 mg/g), *Athyrium pachyphyllum* (68.2 mg/g), *P. nummulariifolia* (59.6 mg/g) contained more than 50 mg/g flavonoids. *Selaginella uncinata* exhibits a lowest total flavonoids content (4.7 mg/g), which is about 27-fold lower than that of *L. carnosum* (125.9 mg/g).

Recently, we have investigated the total flavonoid contents in about 100 fern species from China (Cao et al., 2014a; Cao et al., 2014b; Cao et al., 2014a). It was found that there is not an obvious relationship between the total flavonoid contents and their families. It means even in the same family, the flavonoid contents in different species significantly varied. Among these 100 fern species tested, *Stenoloma chusanum* Ching appears the highest total flavonoid content (306.4 mg/g) (Cao et al., 2012). As for Polypodiaceae plants in current research, *P. lingua* (26.5 mg/g), *P. nummulariifolia* (59.6 mg/g), *P. petiolosa* (80.6 mg/g), *P. amoena* (71.4 mg/g) and *P. muda* (40.7 mg/g) showed significant different total flavonoid contents.

However, it was illustrated that the total flavonoid contents in ferns are related to their environment. It was found that ferns growing under the sunshine showed higher total flavonoid contents than that growing under the shade. In current research, *Microsorium punctatum* which belongs to shade plant was found to contain very low total flavonoid content (4.9 mg/g). Higher temperature may be one of the important factors affecting the metabolism of flavonoids in plants, because thermal stress induces the production of phenolic compounds by activating their biosynthesis as well as inhibiting their oxidation, such as flavonoids and phenylpropanoids (Panagopoulos et al., 1992; Cen and Bomman, 1993). Moreover, solar radiation not only stimulates but also induces the flavonoids compounds (McClure, 1975; Wellmann, 1983; Zaprometov, 1988).

3.2. DPPH free radical scavenging activity of the extracts from 15 fern species

The extracts from these 15 ferns exhibited a significant variation in their reducing power (Table 2). The IC_{50} values of DPPH free radical scavenging potential were ranged from 0.1 to 4.2 mg/L. The extract from *L. carnosum* with an IC_{50} value of 0.1 mg/L showed the highest DPPH free radical scavenging activity. The extract from *P. coronans* also appeared high DPPH free radical scavenging potential with the IC_{50} of 0.4 mg/L. The extract from *P. lingua*, *M. punctatum*, *S. uncinata*, *S. biformis* showed poor DPPH free radical scavenging activity that their inhibition ratio were less than 50%. The DPPH free radical scavenging potential of the extracts from these 15 fern species exhibited a remarkable reciprocal relationship with the total flavonoid contents (Fig. 2). The extracts from 15 ferns with higher contents of total flavonoids showed higher DPPH free radical scavenging potential.

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