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Flavonoid concentrations and bioactivity of flavonoid extracts from 19 species of ferns from China

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ABSTRACT

Ferns are famous traditional medicinal plants and 300 fern species have been used as phytomedicines by Southwestern of China. However, there is little information on the phytochemicals and pharmacological characteristics of most fern species. The total flavonoid concentrations, antioxidant activity, anticancer activity, and acetylcholinesterase inhibition of extracts from 19 species of ferns were investigated. The total flavonoid concentrations ranged from 8.6 to 306.4 mg/g (w/w). The antioxidant activity including DPPH free radical scavenging, ABTS radical scavenging, and superoxide anion scavenging, reducing power and ferric reducing antioxidant potential (FRAP) of flavonoid extracts from these ferns showed a weak linear relationship with their total flavonoid concentrations. *Selaginella frondosa* with very low total flavonoid concentration (13.4 mg/g) showed highest inhibition against A549 cells and it is necessary to further investigate the anti-cancer compounds in *S. frondosa*. The extracts from *Davallia cylindrica* Ching and *Stenoloma chusanum* Ching significantly inhibited AChE activity, which illustrated that it is worthy of attention to investigate flavonoids from *D. cylindrica* and *S. chusanum* as AChE inhibitors.

1. Introduction

Ferns are famous traditional medicinal herbs and there are about 61 families, 223 genera, about 2600 species in China. Moreover, 300 fern species are used as medicinal herbs mainly in Southwestern of China (Ching, 1978). Specially, there are about 1500 species of ferns found in Yunnan province of China (Sun et al., 2003). There is however, few data on the phytochemicals and pharmacological characteristics of most ferns species.

The total flavonoid concentrations, radical scavenging activity, antioxidant activity and anticancer potential of 72 fern species from China have been recently investigated (Cao et al., 2012a). It shows that all ferns contain flavonoids in all parts (leaves, stems, rachis and roots). The total flavonoid concentrations in 50% ethanol extracts from these ferns ranged from 0.55 mg/g (w/w) to 306.4 mg/g (w/w). The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging potential of these extracts improved with the increasing concentrations of total flavonoid. Moreover, 19 fern species such as *Selaginella frondosa*, *Stenoloma chusanum*, and *Woodwardia magnifica*, which are famous for traditional application, obviously showed antioxidant activity and anticancer activity (Zheng et al., 2009; Chang et al., 2005).

Many flavonoids, especially flavonols, have been isolated and characterized from ferns (Harborne and Williams, 1988; Cao et al., 2013a,b; Zhang et al., 2012). The epidemiological and medical evidence has suggested that the flavonoids play a key role in preventing and managing of modern diseases (Candiracci et al., 2012; Murthy et al., 2012; Xiao and Kai, 2012; Andrae-Marobela et al., 2013; Xiao et al., 2013a,b; Delmas and Xiao, 2012; Xiao, 2013). Despite interest being shown in their antioxidant activity, flavonoids also are found to be beneficial to cancer, diabetes, and cardiovascular diseases. However, there is few data on the biological activity and phytochemical components of most fern species. The objective of current research is to investigate the total flavonoid concentrations, antioxidant activity, anticancer activity and acetylcholinesterase (AChE) inhibition of flavonoid extracts from 19 fern species.

2. Materials and methods

2.1. Chemicals and materials

Rutin (>98%) was purchased from Sigma Co. (MO, USA). DPPH, 2,2′-azinobis-(3-ethylbenzoth-iazoline-6-sulfonic acid) (ABTS),

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Table 1
Information of the samples and total flavonoid concentrations in 19 species of ferns.

Family	Species	Date of sampling	Collecting locations	Habitat	Concentrations (mg/g)
Selaginellaceae	*Selaginella frondosa*	2011-10-14	Xishuangbanna, Yunnan	Half-shade Under-forest	13.4
Lindsaeaceae	*Stenoloma chusanum*	2011-10-16	Xishuangbanna, Yunnan	Half-shade Slope-growing	306.4
Sinopteridaceae	*Pellaea smithii*	2011-10-18	Kunming, Yunnan	Shine Rock-gap	129.8
Athyriaceae	*Allantodia doederleinii*	2011-10-13	Xishuangbanna, Yunnan	Shade Under-forest	36.5
	Athyrium nipponicum	2011-10-18	Xishuangbanna, Yunnan	Shade Under-forest	8.6
	Diplazium donianum	2011-10-13	Kunming, Yunnan	Half-shade Slope	45.1
	Dryoathyrium boryanum	2011-10-13	Xishuangbanna, Yunnan	Half-shade Under-forest	145.8
Blechnaceae	*Woodwardia japonica*	2011-07-15	Lin'an, Zhejiang	Half-shade Under-forest	195.3
	W. magnifica	2011-10-13	Xishuangbanna, Yunnan	Half-shade Under-forest	258.7
	Blechnum orientale	2011-10-13	Xishuangbanna, Yunnan	Half-shade Under-forest	19.1
	Brainea insignis	2011-10-14	Xishuangbanna, Yunnan	Half-shade Under-forest	59.5
Dryopteridaceae	*Dryopteris erythrosora*	2011-10-13	Xishuangbanna, Yunnan	Half-shade Under-forest	143.2
	D. sparsa	2011-10-18	Kunming, Yunnan	Shade Under-forest	31.6
	D. reflexosquamata	2011-10-18	Kunming, Yunnan	Shade Under-forest	44.2
	D. lacera	2011-07-15	Lin'an, Zhejiang	Shade Under-forest	54.7
Bolbitidaceae	*Egenolfia sinensis*	2011-10-14	Xishuangbanna, Yunnan	Shine Slope	150.1
Davalliaceae	*Davallia cylindrica*	2011-10-13	Xishuangbanna, Yunnan	Shine Attachment	164.4
	Araiostegia yunnanensis	2011-10-13	Xishuangbanna, Yunnan	Shine Attachment	84.9
Polypodiaceae	*Pyrrosia gralla*	2011-10-13	Xishuangbanna, Yunnan	Shine Attachment	81.4

nitrotetrazolium blue chloride (NBT), phenazine methosulfate (PMS) were purchased from Aladdin Reagent Int. (Shanghai, China). Nicotinamide adenine dinucleotide (NADH) was obtained from Sangon Biotech Co. Ltd. (Shanghai, China). All other reagents and solvents were analytical grade and all aqueous solutions were prepared using newly double-distilled water.

2.2. Preparation of flavonoid extracts from plants

In 19 species of ferns, 13 species and 4 species were collected from Xishuangbanna and Kunming in Yunnan province (China) during October 13th to 18th of 2011, and others were collected from Lin'an in Zhejiang province (China) on July 15th of 2011. The aerial parts of the fresh plants were selected as samples for further study. At least five or more individuals per species were sampled. Three individuals or fronds of the plant were made as specimen and stored for checking. Other samples were mixed for flavonoids analysis. The samples were firstly dried at 60–75 °C for 1–2 days. Then the dried plants were powdered and filtered through 40-mesh screen. The dried sample (1.00 g) was extracted with 50% ethanol (25 ml) at 50 °C for 2 h. Then, the ultrasound-assisted extraction was carried out for further 20 min. The full extraction process was performed twice. Then, the extracts were filtered and concentrated to dry by evaporating with a rotary evaporator. The residue was dissolved with 50 ml methanol, filtered and kept at −20 °C before further test (Zhang et al., 2012).

2.3. Determination of total flavonoids concentration

The total flavonoid concentrations were measured by a colorimetric assay. The extract in methanol (2.0 ml) was added to a 10-ml flask, and 5% $NaNO_2$ solution (0.3 ml) was added. The mixture was allowed to stand for 6 min at 20 °C. And then 5% $Al(NO_3)_3$ solution (0.3 ml) was added to the flask and kept for 6 min at 20 °C. Finally, 4% NaOH solution (4.4 ml) was added and kept for 12 min at 20 °C. Absorbance at 510 nm was recorded on a TU-1810 UV-spectrophotometer (Beijing, China), and the total flavonoid concentrations were estimated using a calibration curve.

2.4. Antioxidant assay

2.4.1. DPPH free radical scavenging activity

DPPH free radical scavenging activity of flavonoid extracts from 19 fern species was measured by means of the absorbance of DPPH at 517 nm (Om et al., 2008; Ren et al., 2013). Briefly, 1.0 ml sample was added to 1.0 ml of DPPH solution (0.2 mM in 50% methanol). The decrease in the solution absorbance at 517 nm was measured after 30 min incubation. The DPPH free radical scavenging activity was calculated using the following formula:

$$\text{Scavenging percentage}\,(\%) = \left(\frac{1 - A_1}{A_0}\right) \times 100 \quad (1)$$

where A_0 is the absorbance of the control only without sample, and A_1 is the absorbance of sample. Each sample from Section 2.2 with