

Phenolic contents and antioxidant properties of *Stenochlaena palustris*, an edible medicinal fern

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ABSTRACT. *Stenochlaena palustris* is an edible fern that is used as vegetable and in traditional medicine. This study assessed the total polyphenol, flavonoid, hydroxycinnamic acid and anthocyanin contents, as well as radical scavenging, ferric reducing and metal chelating activities of the extracts of *S. palustris*. The four extracts analysed were prepared from the young sterile, mature sterile, young fertile, and mature fertile fronds of the fern. The extract of mature sterile frond had the highest contents of total polyphenols (51.69 mg/g dry matter), flavonoids (58.05 mg/g dry matter), and hydroxycinnamic acids (48.80 mg/g dry matter). The extract of the edible young sterile frond contained 20-fold more anthocyanins (51.32 mg/100 g dry matter) compared with the other extracts. Overall, total polyphenol contents correlated strongly and positively with radical scavenging activity ($R^2=0.968$) and ferric reducing activity ($R^2=0.960$), but only moderately with metal chelating activity ($R^2=0.446$). Anthocyanin content and high specific metal chelating activity of the edible young sterile frond highlight the potential of the fern as a functional food. On the other hand, the mature sterile frond, with its high phenolic contents, potent effectiveness as reductants and year-round availability, represents a potential source of natural antioxidants.

Keywords: Antioxidant; DPPH; FRAP; Metal chelating; Phenolic compound; *Stenochlaena palustris*.

INTRODUCTION

A broad range of human diseases and pathological conditions are associated with free radical damage. Numerous studies have also established the relationships between consumption of antioxidant-rich food and prevention of human diseases (Rathore et al., 2011). Ferns have been used by mankind as food and medicine since ancient times (Lee and Shin, 2010). Bioactive components of ferns mainly belong to the phenolic, flavonoid, alkaloid and terpenoid families (Ho et al., 2010). Flavonoids and other phenolic compounds have been demonstrated to be potent antioxidants (Dai and Mumper, 2010; Procházková et al., 2011). Hence, one of the functional properties of ferns that are pertinent to human health is their antioxidant activities (Lee and Shin, 2010).

Stenochlaena palustris (Burm. F.) Bedd is an edible fern that occurs in India through Southeast Asia to Polynesia and Australia. The plant produces fertile fronds that bear spores and sterile fronds that do not (Giesen et al., 2006). The reddish, young sterile fronds of the fern are harvested from the wild and consumed as vegetable in

countries such as Malaysia, Indonesia, Thailand, and the Philippines (Ahmad and Holdsworth, 1994; Giesen et al., 2006; “*Stenochlaena palustris*”, 2010; Antonio et al., 2011; Ong et al., 2011). Fiddleheads of the fern are also used as vegetable in the South Pacific region and in India (Lee and Shin, 2010). In Indonesia and Malaysia, the fern is commonly sold on local markets (Voon and Kueh, 1999; Giesen et al., 2006).

Analysis of nutrient composition found *S. palustris* to be a good source of phosphorus and potassium. Moreover, the nutritional contents of the fern is comparable or superior to some common leafy and fruit vegetables (Voon and Kueh, 1999). In addition to its use as vegetable, leaves of the fern are used in the traditional medicine of several countries to treat fever, skin diseases, ulcers and stomach-ache (Compendium of medicinal plants used in Malaysia, 2002; Benjamin and Manickam, 2007). The fern's parallel functions as food and medicine prompted us to speculate its role as a potential low-cost functional food, especially in the communities of developing countries.

Bioactivity and phytochemical investigations of *S. palustris* are scarce. Antibacterial properties of acylated flavonol glycosides isolated from the fern (Liu et al., 1999) and antifungal properties of methanolic leaf extract of the fern (Sumathy et al., 2010) were previously reported. However, little is known about the antioxidant activity of

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the fern, although free radical scavenging activity and anti-lipid peroxidation activity of a methanolic leaf extract of the fern have been very briefly reported (Bunyaphrathasara et al., 2003). In all these studies, it is unclear whether the material analysed was the young sterile leaves commonly used as vegetable. It is also unclear how the reported antioxidant properties relate to the phenolic contents of *S. palustris*. The mode of action of phenolic antioxidants involves multiple mechanisms and extends beyond radical scavenging (Dai and Mumper, 2010; Procházková et al., 2011; Rathore et al., 2011). Hence, in this study, we investigated the radical scavenging, ferric reducing and metal chelating activities of the fern, distinguishing between edible and non-edible leaves. We also analysed these activities as a function of the abundance of phenolic constituents in the leaf extracts.

MATERIALS AND METHODS

Plant materials

Wild plants of *Stenochalena palustris* were collected from a swampy land near the university campus in mid-August 2011. The plant was authenticated by one of the co-authors (H.-C. Ong). Voucher specimens were deposited at Department of Chemical Science, Universiti Tunku Abdul Rahman.

Preparation of aqueous extracts

Sterile and fertile fronds of the fern, both mature and young, were separately cleaned and oven-dried for 48 hours at 45°C. Extracts of young sterile, mature sterile, young fertile and mature fertile fronds were prepared from pulverised samples with autoclaved deionised water at a 1:20 (dry weight: volume) ratio at 90°C for 60 min (Kumaran and Joel karunakaran, 2006). Extracts were clarified by vacuum-filtration and centrifugation at 9000 rpm and 4°C for 10 min. The supernatant obtained, taken as 50 mg/mL, was immediately aliquoted (500 µL each) and stored at -20°C until used.

Determination of total polyphenol, flavonoid, hydroxycinnamic acid and anthocyanin contents

Total polyphenol and flavonoid contents of the extracts were determined as previously described (Chai and Wong, 2012). Total polyphenol contents were expressed as mg gallic acid equivalents (GAE)/g dry matter, calculated from a standard curve prepared with 0-100 mg/L gallic acid. Total flavonoid contents were expressed as mg catechin equivalents (CE)/g dry matter, calculated from a standard curve prepared with 0-500 µg/mL catechin. Total hydroxycinnamic acid contents were determined using Arnov reagent (Matkowski et al., 2008) and expressed as mg caffeic acid equivalents (CAE)/g dry matter. A standard curve was prepared with 0-200 µg/mL caffeic acid. Total anthocyanin contents of the extracts were evaluated using the pH differential method (Giusti and Wrolstad,

2001). Total anthocyanin contents were expressed as mg cyanidin-3-glucoside equivalents (CGE)/100 g dry matter, calculated using the molar extinction coefficient of 26900 M⁻¹cm⁻¹.

Determination of antioxidant activities

1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and Ferric Reducing Antioxidant Power (FRAP) of the extracts were assessed as previously described (Chai and Wong, 2012). In the DPPH assay, Trolox was used as the positive control. EC₅₀ values were also computed, which represent concentrations of extracts required to scavenge 50% of DPPH radicals. In the FRAP assay, ferric reducing activity was expressed as FRAP values (mM Fe²⁺ equivalents), calculated from a standard curve prepared with 0-0.40 mM FeSO₄·7H₂O. Trolox was used as the positive control.

Metal chelating activity of the extracts was assessed as described in Chew et al. (2009) with minor modifications. Briefly, a mixture of 200 µL FeSO₄ (0.10 mM), 200 µL of leaf extract and 400 µL of ferrozine (0.25 mM) was allowed to react at room temperature for 10 min. Absorbance was then read at 562 nm. Disodium salt of EDTA was used as positive control in the assay.

Determination of specific antioxidant activities

Specific antioxidant activity (the ratio of total antioxidant activity per total soluble phenolics) can be used to estimate the effectiveness of a specific phenolic mixture in an extract/sample in scavenging free radicals (Jacobovelázquez and Cisneros-Zevallos, 2009). In this study, we adopted this concept to determine the specific radical scavenging, metal chelating and ferric reducing activities of the extracts on the basis of their total polyphenol contents. These specific activities were determined from the slopes of correlations of radical scavenging, metal chelating, and ferric reducing activities against total polyphenol contents. Specific radical scavenging activity was expressed as mg Trolox equivalents, calculated from a Trolox standard curve (0-10 µg/mL). Specific ferric reducing activity was expressed as µmol Fe²⁺ equivalents. Specific metal chelating activity was expressed as µg EDTA equivalents, calculated from an EDTA standard curve (0-20 µg/mL).

Data analysis

All experiments were conducted in triplicates and data reported are mean ± standard errors. Statistical analysis was carried out using SAS (Version 9.2). Data were analysed by the ANOVA test and means of significant differences were separated using Fisher's Least Significant Difference test at the 0.05 level of probability.

RESULTS AND DISCUSSION

Relative abundance of total polyphenols in the four leaf extracts in descending order was as follows: mature sterile frond > young sterile frond, young fertile frond > mature

Table 1. Total contents of polyphenols, flavonoids, hydroxycinnamic acids and anthocyanins of the leaf extracts of *S. palustris*.

Extract	Total polyphenols ^{ab} (mg GAE/g)	Total flavonoids ^{ac} (mg CE/g)	Total hydroxycinnamic acids ^{ad} (mg CAE/g)	Total anthocyanins ^{ae} (mg CGE/100 g)
Young sterile frond	42.58 ± 1.01	46.59 ± 0.07	32.24 ± 0.16	51.32 ± 2.95
Mature sterile frond	51.69 ± 1.28	58.05 ± 0.30	48.80 ± 0.18	2.56 ± 0.80
Young fertile frond	41.68 ± 0.19	57.21 ± 0.41	38.93 ± 0.41	2.67 ± 0.77
Mature fertile frond	18.78 ± 0.51	18.95 ± 0.26	15.26 ± 0.12	2.67 ± 0.33

^aData are mean ± SE values ($n=3$).

^bData expressed as mg gallic acid equivalents/g dry matter (mg GAE/g).

^cData expressed as mg catechin equivalents/g dry matter (mg CE/g).

^dData expressed as mg caffeic acid equivalents/g dry matter (mg CAE/g).

^eData expressed as mg cyanidin-3-glucoside equivalents/100 g dry matter (mg CGE/100 g).

fertile frond (Table 1). Total polyphenol content of the edible young sterile frond was higher compared with the aqueous extracts of 25 edible tropical plants (Wong et al., 2006b), 28 Chinese medicinal plants consumed as health tonic or anti-ageing remedies (Wong et al., 2006a), and 84 other anticancer Chinese medicinal plants (Cai et al., 2004). The mature sterile frond contained the highest total polyphenol contents among the four extracts, surpassing those of the aqueous extracts of 25 edible tropical plants (Wong et al., 2006b) and 104 anticancer Chinese medicinal plants (Cai et al., 2004). Our results suggest that the young and mature sterile fronds were promising sources of phenolic compounds. Considering the protective values of polyphenol-rich diets against the development of cancers, diabetes, osteoporosis, cardiovascular diseases and neurodegenerative diseases (Kondratyuk and Pezzuto, 2004; Pandey and Rizvi, 2009), our result points to possible health benefits associated with consumption of this fern as vegetable.

Flavonoids and hydroxycinnamic acids are two classes of phenolic compounds that are nutritionally important (Manach et al., 2004). Total flavonoid contents of the four extracts decreased in the following order: mature sterile frond, young fertile frond > young sterile frond > mature fertile frond (Table 1). Flavonoid contents of the mature sterile, young fertile and young sterile fronds were all higher compared with the aqueous extracts of some vegetables (Dasgupta and De, 2007; Sumazian et al., 2010), medicinal plants (Bouayed et al., 2007) and two nutraceutical herbs, *Camellia sinensis* and *Toona sinensis* (Chen et al., 2007). Thus, when consumed as vegetable, the young sterile fronds may represent a good source of dietary flavonoids.

Anthocyanins, a subclass of flavonoids, are phytopigments that could impart colours in plants (Ignat et al., 2011). Anthocyanin content of the young sterile frond extract was about 20-fold higher compared with the other extracts, consistent with the distinct red hue in the young sterile frond (Table 1). Anthocyanins also comprised a substantially higher proportion of the flavonoid pool of the young sterile frond extract in comparison with other

extracts. Anthocyanins possess potent antioxidant, anti-carcinogenic and anti-inflammatory activities (Horbowicz et al., 2008; Ignat et al., 2011). Correlation between anthocyanin contents and antioxidant activity has also been reported for pigmented vegetables (Li et al., 2012). Hence, anthocyanins are likely an important contributor of the health benefits of the edible young sterile frond. Compared with other fruits and vegetables, the anthocyanin content of the young sterile frond was modest (Horbowicz et al., 2008; Li et al., 2012). However, following optimisation of anthocyanin extraction, the young sterile frond may become a promising source of natural food colourants with health benefits and this deserves further investigation.

Hydroxycinnamic acids are a class of phenolic acids commonly found in a broad range of edible plants and often at high concentrations (Manach et al., 2004). Relative abundance of total hydroxycinnamic acids in the extracts decreased in the following order: mature sterile frond > young fertile frond > young sterile frond > mature fertile frond (Table 1). The ratio of total hydroxycinnamic acids: total polyphenol contents of the mature sterile frond (0.94) was also higher compared with young sterile frond (0.76). Thus, the abundance of hydroxycinnamic acid contents and their proportion in the total polyphenol pool increased during the maturation of the sterile frond of *S. palustris*.

Radical scavenging activities of the four extracts clearly increased in a concentration-dependent manner. These activities, in descending order, were as follows: mature sterile frond > young sterile frond, young fertile frond > mature fertile frond (Figure 1). For radical scavenging activity, EC₅₀ values of the extracts and Trolox were 72.51 ± 0.69 (mature sterile frond), 99.21 ± 0.17 (young fertile frond), 101.22 ± 0.70 (young sterile frond), 180.29 ± 6.92 µg dry matter/mL (mature fertile frond), and 6.59 ± 0.05 µg/mL (Trolox), respectively. The EC₅₀ value of the edible young sterile frond was lower compared with nine leafy vegetable (Dasgupta and De, 2007), eight tonic Chinese medicinal herbs (Guo et al., 2008), as well as four medicinal ferns (Chang et al., 2007). These results imply that both edible (young sterile frond) and non-edible leaves of the fern possess potent radical scavenging properties.

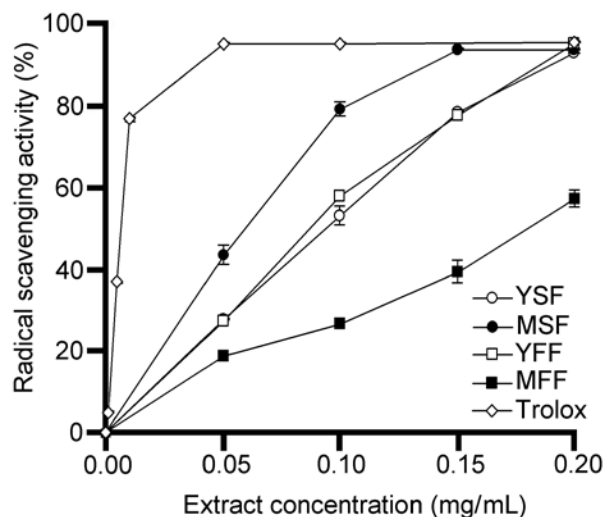


Figure 1. DPPH radical scavenging activities of the leaf extracts of *S. palustris*, compared with Trolox. YSF, young sterile frond; MSF, mature sterile frond; YFF, young fertile frond; MFF, mature fertile frond. Data are mean \pm SE values ($n=3$).

In our DPPH assay, the response curve for Trolox (positive control) levelled off close to but below 100% at 0.05 mg/mL and higher concentrations (Figure 1). The plateau in the response curve and our observation of complete loss of purple colour in the DPPH solution implied that complete scavenging of DPPH by Trolox had been achieved in our assay system. The inability to achieve 100% scavenging activity in a DPPH assay was also observed by others (Miliauskas et al., 2004; Barreira et al., 2009). These authors suggested that the residual yellow colour resulting from the decolorisation of a purple DPPH solution renders it impossible to obtain a “100% scavenging activity” when compared to the colourless methanol solution (blank) (Miliauskas et al., 2004; Barreira et al., 2009).

Ferric reducing abilities of all four extracts increased linearly over the concentration range tested (Figure 2). At extract concentration 0.5 mg/mL, FRAP value of young sterile frond was similar to that of young fertile frond, but was 2-fold higher compared with mature fertile frond. FRAP values of the extracts, expressed on a dry matter basis, were 72.36 ± 0.52 (mature sterile frond), 45.07 ± 0.74 (young fertile frond), 41.92 ± 0.90 (young sterile frond), 20.99 ± 0.45 mmol $\text{Fe}^{2+}/100$ g dry matter (mature fertile frond), respectively. These values were all higher compared with 27 Chinese medicinal plants (Wong et al., 2006a).

The relative radical scavenging and ferric reducing activities of the extracts paralleled the relative abundance of total polyphenols, flavonoids, and hydroxycinnamic acids, suggesting that these activities were attributable to phenolic contents. Correlation analyses revealed strong, positive relationships between total polyphenol content and radical scavenging activity ($R^2 = 0.968$, $p < 0.05$) and between total polyphenol content and ferric reducing activity ($R^2 =$

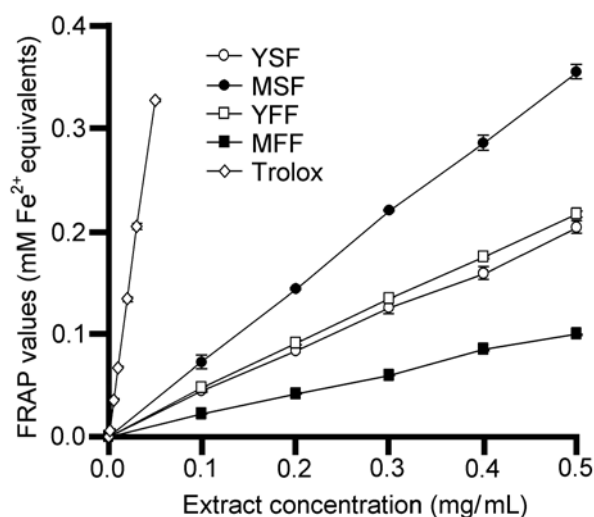


Figure 2. Ferric reducing activities of the leaf extracts of *S. palustris*, compared with Trolox. YSF, young sterile frond; MSF, mature sterile frond; YFF, young fertile frond; MFF, mature fertile frond. Data are mean \pm SE values ($n=3$).

0.960, $p < 0.05$). Thus, regardless of leaf type or developmental stage, variation in polyphenol contents accounted for at least 96% of the variation in the radical scavenging and ferric reducing activities among the leaf extracts. Antioxidant properties of hydroxycinnamic acids have been previously demonstrated (Silva et al., 2000; Gulcin, 2006; Maurya and Devasagayam, 2010). Hence, a higher hydroxycinnamic acid content may have contributed to the greater antioxidant activities of mature sterile frond when compared with young sterile frond. On the other hand, anthocyanins were likely the key flavonoid compounds responsible for the radical scavenging and ferric reducing activities detected in the extract of young sterile frond.

Besides being direct scavengers of free radicals, phenolic compounds may also prevent oxidative stress by acting as metal chelators, restricting metal-induced free radical formation (Dai and Mumper, 2010; Procházková et al., 2011). In this study, iron chelating activities of the four extracts increased in a concentration-dependent manner (Figure 3). At extract concentration 5 mg/mL, the anthocyanin-rich young sterile frond had the highest iron chelating activity (79%), whereas mature sterile frond the lowest (18%). Our result implies that anthocyanins may have enhanced the metal chelating ability of the young sterile frond extract.

A weak correlation was found between polyphenol content and metal chelating activity among the four extracts ($R^2 = 0.446$, $p < 0.05$). Such weak correlation between the two aforementioned parameters was previously reported (Hinneburg et al., 2006; Lim et al., 2009; Rumbaoa et al., 2009). Our observation implies that differences in the metal chelating activities among the extracts cannot be adequately explained by quantitative difference in their total polyphenol contents. Considering that the specific

Table 2. Specific antioxidant activities of the leaf extracts of *S. palustris*.

Extract	Specific radical scavenging activity ^{a,b} (mg TE/mg GAE)	Specific ferric reducing activity ^{a,c} ($\mu\text{mol Fe}^{2+}$ /mg GAE)	Specific metal chelating activity ^{a,d} ($\mu\text{g EDTA}$ /mg GAE)
Young sterile frond	1.801 \pm 0.013	9.58 \pm 0.20	101.22 \pm 1.42
Mature sterile frond	2.071 \pm 0.020	13.87 \pm 0.18	16.54 \pm 1.71
Young fertile frond	1.997 \pm 0.004	10.56 \pm 0.06	82.14 \pm 2.66
Mature fertile frond	2.299 \pm 0.088	10.92 \pm 0.06	176.37 \pm 6.51

^aData are mean \pm SE values ($n=3$).

^bData expressed as mg Trolox equivalents/mg gallic acid equivalents (mg TE/mg GAE).

^cData expressed as $\mu\text{mol Fe}^{2+}$ equivalents/mg gallic acid equivalents ($\mu\text{mol Fe}^{2+}$ /mg GAE).

^dData expressed as $\mu\text{g EDTA}$ equivalents/mg gallic acid equivalents ($\mu\text{g EDTA}$ /mg GAE).

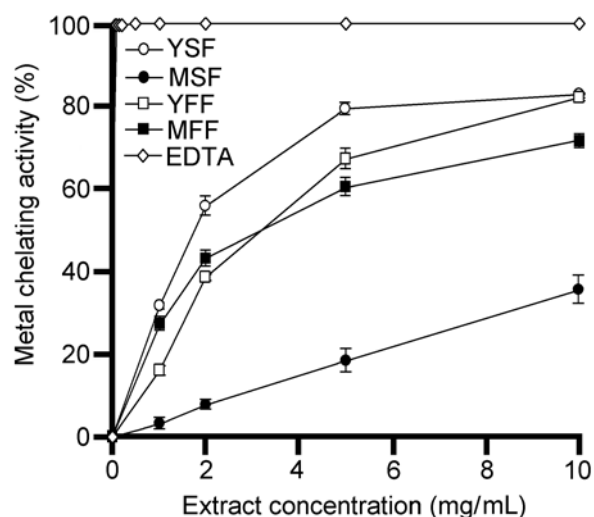


Figure 3. Metal chelating activities of the leaf extracts of *S. palustris*, compared with EDTA. YSF, young sterile frond; MSF, mature sterile frond; YFF, young fertile frond; MFF, mature fertile frond. Data are mean \pm SE values ($n=3$).

phenolic profile in each extract was likely to be qualitatively different from the others, one plausible explanation for such a discrepancy is that the phenolic mixture in each extract varied in its effectiveness as antioxidant (Jacobovelázquez and Cisneros-Zevallos, 2009). To further assess the antioxidant properties of the specific phenolic mixture in each extract, we compared their specific radical scavenging, metal chelating and ferric reducing activities (Table 2).

The phenolic profile in the mature fertile frond was the most effective in scavenging radicals and chelating metal ions, while that of the mature sterile frond was the most effective in reducing ferric ions. The four extracts exhibited only moderate difference (about 1.4-fold difference between the highest and lowest values) in specific radical scavenging and ferric reducing activities. Thus, the phenolic mixtures in the four extracts did not differ markedly in their effectiveness as radical scavengers and reductants. Considerable difference (11-fold) was found between extracts with the highest (mature fertile frond) and lowest

(mature sterile fronds) specific metal chelating activities. The phenolic mixture in the extract of young sterile frond was the second most effective, being 6-fold higher than that of the mature sterile frond, which had the lowest specific metal chelating activity.

While the mature fertile frond possessed the highest specific radical scavenging and metal chelating activities, its low phenolic contents and infrequent availability may present a challenge to its exploitation as a source of natural antioxidant. Conversely, the high phenolic contents and specific ferric reducing activity of mature sterile frond, in addition to its year-round availability, may render it a promising source of antioxidants to be used in foodstuff or healthcare products. The potent specific metal chelating activity of the edible young sterile frond, together with its high contents of phenolic constituents, including anthocyanins, highlights its potential benefits as a source of dietary antioxidants when consumed as vegetable. In addition, the anthocyanins of the young sterile frond may be exploited as a source of natural food colourants with health benefits.

In conclusion, our study revealed that *S. palustris* represents a promising source of phenolic antioxidants, although the effectiveness of the specific phenolic mixture in each leaf extract varied according to the type of leaf used. High contents of phenolic constituents, including anthocyanins, and high specific metal chelating activity in the edible young sterile frond of *S. palustris* highlight the potential of the fern as a functional food. On the other hand, the mature sterile frond, with its high phenolic contents, potent effectiveness as reductants and year-round availability, is a potential source of phenolic antioxidants for further exploitation. Based on our findings, future studies to characterise the phenolic profiles of both young and mature sterile fronds of *S. palustris* are warranted.

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Stenochlaena palustris，一種可食用之藥用蕨類的酚類含量及抗氧化物特性

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Stenochlaena palustris 是一種可當蔬菜和用於傳統醫學之一種食用蕨類。本研究分析了總多酚，類黃酮素，氫氧肉桂酸及花青素含量；還有 *S. palustris* 抽出物之消除根基、還原鐵離子以及螯合金屬離子的能力。所使用之四種抽取物乃從蕨類之幼不育葉、成熟不育葉、幼孢子囊莖、及成熟孢子囊莖四部份各自抽取所得。成熟不育葉之抽取物含最高量之總多酚 (51.69 mg/g 乾物)，類黃酮素 (58.05 mg/g 乾物)，及氫氧肉桂酸 (48.80 mg/g 乾物)，幼不育葉之抽取物含多出 20 倍之花青素，相比於其他三種抽取物。總合言之，總多酚量密切地且正面地和根基清除能力相關 ($R^2=0.968$)，還原鐵離子的能力相關 ($R^2=0.960$)；但是僅中等地和金屬離子之螯合能力正相關 ($R^2=0.446$)，因可食用之幼不育葉含高量之花青素及高比金屬離子螯合能力突顯出本文所介紹之蕨類作為功能性食物之潛在價值。另一方面，因成熟不育葉具高多酚量，強力之還原力，以及整件可供給的優點；代表了有潛力之天然抗氧化物之來源。

關鍵詞：抗氧化物；DPPH；FRAP；金屬離子螯合；多酚類；*Stenochlaena palustris*。