# PHYTOCHEMICALS AND ANTIOXIDATIVE PROPERTIES OF EDIBLE FERN, STENOCHLAENA PALUSTRIS (BURM. F.) BEDD

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# PHYTOCHEMICALS AND ANTIOXIDATIVE PROPERTIES OF EDIBLE FERN, STENOCHLAENA PALUSTRIS (BURM. F.) BEDD

By

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# LIST OF SYMBOLS AND ABBREVIATIONS

 $\alpha \hspace{1cm} Alpha$ 

 $\beta$  Beta

ε Molar absorptivity

γ Gamma

δ Chemical shift

J Coupling constant

1D One dimension

2D Two dimension

AAE Ascorbic acid equivalent

AlCl<sub>3</sub> Aluminum chloride

AR Analytical grade

bd broad doublet

BHT Butylated hydroxytoluene

ATR Attenuated Total Reflectance

<sup>13</sup>C Carbon

CHCl<sub>3</sub> Chloroform

CH<sub>3</sub>COOH Acetic acid

CD<sub>3</sub>OD Deuterated methanol

COSY Homonuclear Shift Correlation Spectroscopy

DCM Dichloromethane

d Doublet

dd Doublet of doublet

DEPT Distortionless Enhancement by Population Transfer

D<sub>2</sub>O Deuterated water

DPPH 2, 2-α-diphenyl-1-picrylhydrazil

(E) trans configuration

et al. Elsewhere or/and other

ESI Electron-sprayed ionization

EtOAc Ethyl acetate

FRAP Ferric reducing antioxidant power

FT Fourier Transform

GAE Gallic acid equivalent

<sup>1</sup>H Proton

HMBC Heteronuclear Multiple Bond Coherence

HPTLC High Performance Thin-Layer Chromatography

HSQC Heteronuclear Single Quantum Correlation

H<sub>2</sub>SO<sub>4</sub> Sulphuric acid

Hz Hertz

IC<sub>50</sub> 50 % inhibition concentration

i.e. that is

IR Infrared spectroscopy

lit. literature

m Multiplet

M Molar

MeOH Methanol

mM Milimolar

MS Mass spectrometry

MS/MS Tandem mass spectrometry

NMR Nuclear magnetic resonance

p para-substituted

OD Optical density

ppm Parts per million

QE Quarcetin equivalent

Q-TOF Quadrupole- Time of flight

r<sup>2</sup> Coefficient of determination

R<sub>f</sub> Retention factor

s singlet

SD standard deviation

S. palustris Stenochlaena palustris

t triplet

TLC Thin-layer chromatography

TFC Total flavonoid content

TPC Total polyphenol content

UV Ultra-violet

 $\mu g/mL \hspace{1cm} microgram \hspace{1mm} per \hspace{1mm} milliliter$ 

μM micro molar

v/v volume by volume

(Z) cis configuration

### LIST OF PUBLICATIONS

# **Conference Proceedings**

**Nelson Chear Jeng Yeou**, Vikneswaran Murugaiyah & Lai Choon Sheen (2013). Antioxidant activities of *Stenochlaena palustris* and its total phenolic and flavonoid content. Oral Presentation. *International Conference for Young Chemist 2013*, Penang, Malaysia. [Abstract]

Nelson Chear Jeng Yeou, Viknewaran Murugaiyah & Lai Choon Sheen (2015). Antioxidant activities and chemical constituents of *Stenochlaena palustris* (Paku Midin) at two stages of maturity. Oral Presentation. *International Conference of Natural Product 2015*, Johor Bahru, Malaysia [Abstract]

# **Journals**

Yasodha Ponnusamy, **Nelson Jeng-Yeou Chear**, Surash Ramanathan, Vikneswaran Murugaiyah & Choon-Sheen Lai (2013). Antioxidant and antibacterial properties of Malaysian ferns used traditionally against infection. *Journal of Natural Product & Plant Resources*, 3 (6) pp. 14-18. (ISSN: 2231 – 3184).

**Nelson Chear Jeng Yeou**, Khaw Kooi Yeong, Vikneswaran Murugaiyah & Lai Choon Sheen. Cholinesterase inhibitory activity and chemical constituents of *Stenochlaena palustris* fronds at two stages of maturity. *Evidence-based Complementary and Alternative Medicine* [Under review] (IF: 2.175, 2013).

# KANDUNGAN FITOKIMIA DAN AKTIVITI ANTIOKSIDATIF PAKU SAYUR, *STENOCHLAENA PALUSTRIS* (BURM. F.) BEDD

### **ABSTRAK**

Stenochlaena palustris (Nama tempatan: "Paku Midin") merupakan sejenis paku sayur yang popular di Malaysia, Thailand dan Indonesia disebabkan teksturnya rangup apabila dimakan. Selain itu, ia juga digunakan secara tradisional untuk merawat demam dan ulser. Tumbuhan paku ini telah dilaporkan mempunyai kesan antioksidatif yang kuat, akan tetapi maklumat tentang sebatian aktifnya masih kurang. Oleh demikian, projek penyelidikan ini telah dijalankan bagi mengenal pasti sebatian kimia S. palustris yang menpunyai sifat antioksidatif. Dalam kajian ini, pelepah muda dan pelepah matang S. palustris telah dikaji secara berasingan bagi menilai sama ada pelepah yang berbeza kematangan mempunyai kandungan fitokimia yang berlainan. Ekstrak MeOH pelepah matang S. palustris mempunyai kebolehan pemerangkapan radikal DPPH dan kebolehan penurunan FRAP yang dua kali ganda lebih aktif daripada pelepah mudanya. Hal ini mungkin disebabkan kandungan polifenol pelepah matang yang jauh lebih tinggi. Melalui pendekatan pemencilan sebatian kimia yang berpandukan aktiviti pemerangkapan radikal DPPH, satu siri sebatian kimia yang terdiri daripada tujuh glikosida kaempferol telah berjaya dipencilkan, iaitu kaempferol 3-O-α-rhamnosida (A), kaempferol 3-O-(6"-O-E-pkoumaroyl)- $\beta$ -glukosida (**B**), kaempferol 3-O-(3"-O-E-p-koumaroyl)- $\beta$ -glukosida (C), kaempferol 3-O-(3", 6" di-O-E-p-koumaroyl)- $\beta$ -glukosida (**D**), kaempferol 3-O- $(3"-O-E-p-koumaroyl)-(6"-O-E-feruloyl)-\beta-glukosida$  $(\mathbf{E})$ , kaempferol 3-*O*-*β*glukosida (**F**) and kaempferol 3-O-(6"-O-rhamnosil)- $\beta$ -glukosida (**G**). Aktiviti

pemerangkapan radikal DPPH (IC<sub>50</sub>) untuk semua sebatian yang dipencilkan adalah antara 0.13 – 0.88 mM dengan sebatian E menunjukkan aktiviti yang paling tinggi  $(IC_{50} = 0.13 \text{ mM})$ , setanding dengan antioksidan sintetik, BHT  $(IC_{50} = 0.09 \text{ mM})$ . Keputusan ini mencadangkan bahawa kumpulan feruloyl yang terletak pada kedudukan 6"-O residu glukosa adalah struktur kima yang paling penting untuk meningkatkan aktiviti anti-radikal. Walau bagaimanapun, aktiviti anti-radikal untuk sebatian individu adalah agak lemah jika dibandingkan dengan ekstrak MeOH mahupun fraksi aktifnya. Ini menunjukkan bahawa glikosida kaempferol bertindak secara sinergistik dan mengakibatkan aktiviti anti-oksidan yang lebih kuat. Perbandingan kandungan fitokimia dengan menggunakan teknik HPTLC menunjukkan kaempferol 3-O-β-glukosida adalah satu-satunya flavonoid yang muncul pada peringkat awal pertumbuhan pelepah S. palustris. Akan tetapi, sebatian flavonoid yang lebih kompleks akan disintesis daripada kaempferol 3-O-β-glukosida setelah pelepah tumbuhan paku ini mencapai kematangan. Kesimpulannya, kajian ini menujukkan bahawa pelepah matang S. palustris merupakan sumber antioksidan semula jadi yang bagus. Kajian lanjutan harus dijalankan bagi mengimplikasikan penggunaan ekstrak MeOH pelepah matang ataupun sebatian aktifnya sebagai alternatif untuk mengubati penyakit yang berpunca daripada tekanan oksidatif seperti keradangan dan kanser. Akan tetapi, pelepah muda dan pelepah matang S. palustris perlu dikaji secara berasingan disebabkan komposisi fitokimia mereka yang jauh berbeza.

# PHYTOCHEMICALS AND ANTIOXIDATIVE PROPERTIES OF EDIBLE FERN, STENOCHLAENA PALUSTRIS (BURM, F.) BEDD

### **ABSTRACT**

Stenochlaena palustris (local name: "Paku Midin") is a vegetable fern popular in Malaysia, Southern Thailand and Indonesia due to its crispy texture. It is also used traditionally to treat fever and ulcer. The fern has been reported to exhibit significant antioxidant effect, however, there is lack of information concerning the active principles. As such, the present work was carried out to identify the compounds responsible for the antioxidant effect of S. palustris. In order to evaluate whether there are any differences in phytochemicals between the fronds of different maturity, young and mature fronds of S. palustris were studied separately. The MeOH extract of mature fronds was two times more active than the young fronds in term of their radical scavenging and FRAP reducing ability and this could be correlated to its high polyphenols content. Using a DPPH radical scavenging activity-guided isolation approach, a series of seven kaempferol glycosides were isolated, namely kaempferol  $3-O-(6"-O-E-p-coumaroyl)-\beta$ - $3-O-\alpha$ -rhamnopyranoside  $(\mathbf{A}),$ kaempferol glucopyranoside (**B**), kaempferol 3-O-(3"-O-E-p-coumaroyl)- $\beta$ -glucopyranoside (**C**), kaempferol 3-O-(3", 6"-di-O-E-p-coumaroyl)- $\beta$ -glucopyranoside (**D**), kaempferol  $3-O-(3^{\circ}-O-E-p-coumaroyl)-(6^{\circ}-O-E-feruloyl)-\beta-glucopyranoside$  (E), kaempferol 3-O- $\beta$ -glucopyranoside (**F**) and kaempferol 3-O-(6''-O- $\alpha$ -rhamnopyranosyl)- $\beta$ glucopyranoside (G). The DPPH radical scavenging activity (IC<sub>50</sub>) of these compounds ranged from 0.13 to 0.88 mM with compound E showing the highest activity (IC<sub>50</sub> 0.13 mM), comparable to the synthetic antioxidant, BHT (IC<sub>50</sub> 0.09 mM). This suggested that the feruloyl moiety acylated at 6"-O position of the glucose

residue was the most favorable structure for exhibiting antiradical property. However, compared to the MeOH extract, as well as the active fractions from which the compounds were isolated, the antiradical activity of the individual compounds was found to be weaker. This indicated that the kaempferol glycosides worked synergistically to provide stronger antioxidant activity. A comparison of the phytochemicals by HPTLC indicated that kaempferol 3-O- $\beta$ -glucopyranoside was the only flavonoid present in the early developmental stage of *S. palustris* fronds. More complex constituents were synthesized by the plant from kaempferol 3-O- $\beta$ -glucopyranoside as the plant matures. In conclusion, the present study indicated that the not commonly eaten mature fronds are a good source of natural antioxidants. Future research to further support the potential use of mature fronds or its active principles against oxidative stresses related disorders such as inflammation and cancer is warranted but the young and mature fronds should be studied separately due to their differences in phytochemicals compositions.

### CHAPTER 1

# INTRODUCTION

# 1.1 Overview

Oxidative stress is an adverse condition where there is a decrease of natural cell antioxidant capacity or an increased amount of reactive oxygen species (ROS) in human body. When the balance between oxidants and antioxidants in the body is shifted by the overproduction of free radicals, it will lead to oxidative stress, DNA damage and eventually the occurrences of various chronic diseases (Halliwell, 1997; Aruoma, 1998). Antioxidants are compounds that able to inhibit or delay such condition by inhibiting the initiation or propagation of oxidizing chain reactions induced by free radicals (Namiki, 1990). Antioxidants can be easily obtained from our daily diets and they are generally categorized into two basic classes: synthetic and natural. Synthetic antioxidants such as BHT and BHA have been widely used in food industries for decades to preserve the food products by retarding deterioration. However, the use of these compounds has called for restrictions due to their potential carcinogenicity (Kahl & Kappus, 1993). Thus, the strategy now is to search for safer and natural antioxidants, especially from fruits and vegetables to replace the synthetic ones.

In recent years, epidemiological data have consistently showed significant positive associations between the consumption of fruits and vegetables and the reduction of chronic diseases. (Steinmetz & Potter, 1996; Garcia-Closas et al., 1999; Joseph et al., 1999; Dillard & German, 2000; Prior & Cao, 2000; Wargovich, 2000).

Hence, the term 'functional foods' have been attributed to fruits and vegetables, which are capable of delivering additional health benefits on top of fulfilling the basic physiological needs, such as preventing and delaying the occurrence of chronic diseases. The protective action of fruits and vegetables are mainly attributed to the presence of antioxidants such as polyphenols (flavonoids, phenolic acids, lignans, tannins, anthocyanins, catechins, isoflavones) as well as vitamins and pro-vitamins (ascorbic acid,  $\alpha$ -tocopherol and  $\beta$ -carotene) (Wang et al., 1996; Prior & Cao, 2000). Synergistically or additively, these compounds provide bioactive mechanisms to reduce free radical induced oxidative stress. Some examples of vegetables and fruits with strong antioxidant potential and high polyphenol content are white cabbage, mushroom, cauliflower, garlic, broccoli, berries, cucumber, spinach, alfalfa sprouts as well as edible ferns like bracken, cinnamon and ostrich ferns (Willet, 1994; Al-Saikhon et al., 1995; Cao et al., 1996; Gazzani et al., 1998; Gazzani et al., 1998; Velioglu et al, 1998).

Ferns and their allies (Pteridophytes) have long been taken as food or folk medicine by human for the prevention or treatment of various body ailments. Ferns are rich in natural antioxidants and their bioactive components mainly belong to flavonoid, phenolic, alkaloid and terpene families (Lee & Shin, 2010). In Malaysia itself, there are at least 76 species of ferns belonging to 44 genera and 13 families which are known to be traditional medicine or food (Bidin, 1985). However, their phytochemicals and therapeutic properties are rarely explored. Among the local fern species, *Stenochlaena palustris* has recently been identified as a potential new crop owing to its good taste and popularity as a local delicacy (Nicholas et al., 2012). *Stenochlaena palustris* (Burm. F.) Bedd or locally known as 'Paku Midin' is a wild creeping fern which grows naturally in forests, mangroves, estates and swampy areas.

Its fiddleheads and young shoots are often harvested for consumption as a vegetable while the mature fronds are not normally eaten (Piggott, 1988). Besides, the fronds of *S. palustris* are also traditionally used to treat fever, skin diseases, ulcers and stomachache in Southeast Asia. Although this fern possesses parallel functions as a food and traditional medicine, the knowledge about its biological activities and phytochemical contents are still limited. The crude extracts of *S. palustris* were reported to possess antimicrobial activities and several antibacterial acylated flavonol glycosides have been identified (Liu et al., 1999; Sumathy et al., 2010; Zuraini et al., 2010). Numerous studies also consistently showed that the crude extracts of this plant exhibited significant antioxidant properties, however, little is known about the corresponding antioxidant constituents (Chai et al., 2012).

# 1.2 Problem statement

Liu et al. (1999) isolated a number of compounds such as acylated flavonol glycosides, glycosides and steroids from *S. palustris* collected from Papua New Guinea. Some of these compounds have been tested and found to have strong antibacterial activities. However, the antioxidant activities of these compounds remained unknown. It is a known fact that phytochemicals in the same species of plant may vary between different sources due to geographical variations (Cook et al., 2013; Wang et al., 2014). Since *S. palustris* is a popular vegetable among Malaysians and the Malaysian Agricultural Research and Development Institute (MARDI) has identified it as a food crop with export potential to foreign markets (Nicholas et al., 2012), it will be of great interest to determine the phytochemicals present in the Malaysian species and whether these compounds have antioxidant activities.

# 1.3 Objectives

The specific objectives of the present study were:

- 1. To isolate and characterise the chemical constituents of *S. palustris* following a systematic antioxidant activity-guided approach;
- 2. To evaluate the antioxidant potential of the isolated compounds and their structural-activity relationships (SARs);
- 3. To examine whether the isolated antioxidants are found in young and mature stages of the sterile fronds.

### **CHAPTER 2**

### LITERATURE REVIEW

# 2.1 Antioxidant as chemopreventive and therapeutic agent

Free radicals, normally in the form of reactive derivatives of oxygen or also known as Reactive Oxygen Species (ROS) such as O<sub>2</sub>-, H<sub>2</sub>O<sub>2</sub> and OH-, are electron deficient molecules generated continuously inside the human body as byproducts of cellular respiration process. Their levels in human body are governed by numerous endogenous enzymes such as superoxide dismutase, glutathione peroxidase and catalase which act as the body's primary antioxidant defense system (Dimitrios, 2006). At essential level, these ROS radicals are beneficial to human body due to their roles in energy supplying, detoxification, chemical signaling and immune regulation. However, there are other factors that may increase the level of ROS in human body such as exposure to exogenous chemicals, environmental pollutants, radioactive substances, ultraviolet radiation as well as emotional stresses (Halliewell, 1995; Halliewell, 1997; Chitra & Pillai, 2002). When there is an overproduction of these species, causing an imbalance between the ROS generation and the body's antioxidant defense system, it will result in a state of oxidative stress. This is detrimental to biomolecules such as proteins, DNA, lipids and enzymes and will eventually lead to the occurrence of chronic diseases such as inflammation, aging, neurodegenerative diseases, cardiovascular diseases, cancer and etc. (Halliwell, 1997; Aruoma, 1998).

Antioxidants are substances that delay or prevent the oxidation of biomolecules by inhibiting the initiation or propagation of oxidizing chain reactions

caused by free radicals (Namiki, 1990). There are two basic categories of antioxidants, namely natural antioxidants and synthetic antioxidants. Each of these categories can be further classified into hydrophilic and lipophilic antioxidants based on their solubility.

# 2.1.1 Natural antioxidants

Natural antioxidants known as dietary antioxidants are mainly of plant origin. Natural antioxidants can be obtained through daily diet especially from vegetables and fruits which are cheaper and safer than synthetic antioxidants. Two common classes of natural antioxidants are polyphenols such as gallic acid and quercetin, and vitamins / pro-vitamins such as ascorbic acid,  $\alpha$ -tocopherol and  $\beta$ -carotene (Figure 2.1).

α-Tocopherol (Vitamin E)

β-carotene (Pro-vitamin A)

Ascorbic acid (Vitamin C)

Gallic acid

# Quercetin

**Figure 2.1**: Chemical structures of commonly found natural antioxidants:  $\alpha$ -tocopherol,  $\beta$ -carotene, ascorbic acid (vitamins); gallic acid and quercetin (polyphenols).

Polyphenols are a group of hydrophilic secondary metabolites, extensively found in vegetables, fruits and plant derived food products such as red wine and tea. Many of them possess stronger antioxidant capacities than those of vitamins C and E (Ho, 1992; Amié et al., 2003). However, unlike vitamins and pro-vitamins which are scientifically well proven for their beneficial effect towards human health, plant polyphenols have not been completely studied due to the complexity of their chemical nature and vast distribution in plant sources (Dimitrios, 2006). The major subclasses of polyphenols include tannins, flavonoids, phenolic acids as well as lignans.

Flavonoids are phenolic constituents most well known for their antioxidative properties, especially in free radical scavenging (Chen et al, 1990; Amié et al., 2003).

They are naturally occurring plant pigments that bear a benzo-γ-pyrone basic structure, ubiquitous in photosynthesizing cell. In general, the radical-scavenging activity of flavonoids depends on the molecular structure, such as the number and position of the substituted phenolic hydroxyl groups (Bors et al., 1990; Rice-Evans et al., 1996). Several structural criteria needed for a flavonoid molecule to exhibit strong free radical scavenging ability are believed to be:

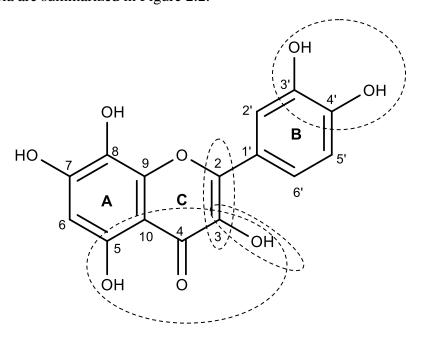
3', 4'-dihydroxyl position due to their electron donating properties and function as radical target site

3-OH moiety of ring C

C2 and C3 double bonds

Both 3-OH, 5-OH in combination with 4-keto group

These criteria are summarized in Figure 2.2.



**Figure 2.2**: Proposed structural criteria for enhanced free radical scavenging capacity of flavonoids (Source: Amié et al., 2003).

The antioxidant mechanism of action of flavonoids, as well as other polyphenols, are thought to be exhibited through the donation of a hydrogen atoms from their hydroxyl groups to the free radical, forming less reactive flavonoid phenoxyl radicals and more stable protonated free radicals (Chen et al, 1990; Amié et al., 2003).

In general, the proposed mechanism of antioxidant action of flavonoids involves three major steps:

# 2.1.2 Synthetic antioxidants

Synthetic antioxidants are mainly used for industrial purposes and can be produced in large quantities. These antioxidants are used as preservatives in food industry in order to prolong the shelf life of foods, especially for those rich in polyunsaturated fats to avoid lipid peroxidation (Wong et al., 2006). Lipid oxidation in food is the major cause of food quality deterioration, nutritional decline, flavor lost and discoloration. Some common synthetic antioxidants widely used in food industry are

propyl gallate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertiary butylhydroquinone as shown in Figure 2.3.

$$HO$$
 $OH$ 
 $OH$ 
 $OH$ 
 $OH$ 
 $OH$ 
 $OH$ 
 $OH$ 

Propyl gallate

Butylated hydroxyanisole (BHA)

Butylated hydroxytoluene (BHT)

Tertiary butylhydroquinone

**Figure 2.3**: Chemical structures of synthetic antioxidants used widely in food industry.

However, the use of these synthetic antioxidants has caused numerous controversies mainly on their safety and potential health risks, particularly the toxicity and carcinogenic effect (Kahl & Kappus, 1993). As such, research intention are now drawn towards the discovery and development of natural antioxidants as substitution for these synthetic antioxidants.

### 2.2 Fern and fern allies

Fern and fern allies consist of approximately 12,000 species of plants that falls under the division of Pteridophyte (Chapman, 2010). Pteridophyte is a general term conventionally used to represent all seedless vascular plants such as ferns and mosses. Unlike mosses, fern species have xylem and phloem as well as roots, stems and leaves, thus are being classified as vascular plant. Physically, fern species are seedless, flowerless and only reproduce via spores. Most of the ferns having unique leaves morphology during their young age and are known as fiddleheads. The fiddleheads will slowly develop into fronds which are delicately divided as they mature.

In addition, there is another small group of plants being classified as fern allies and is often grouped together with ferns under the division of Pteridophyta. Like ferns, fern allies are seedless vascular plant and they also reproduce through spores. However, to the date, there are still a lot of controversial issues on categorising fern allies in the fern genera due to their distinct differences in appearance. Some of the examples of fern allies are club moss, whisk fern and spike moss. In 2006, Smith et al. (2006) proposed a classification scheme of fern and fern allies based on recent molecular systematic studies and morphological data as shown in Figure 2.4.

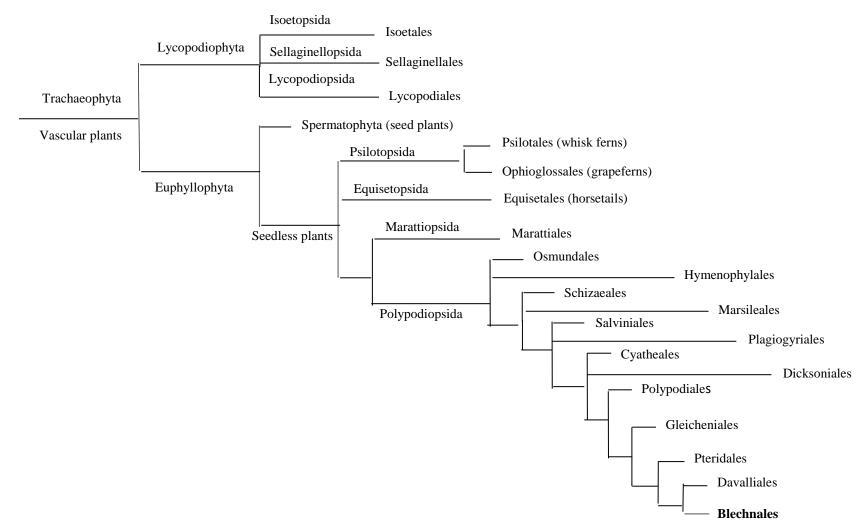
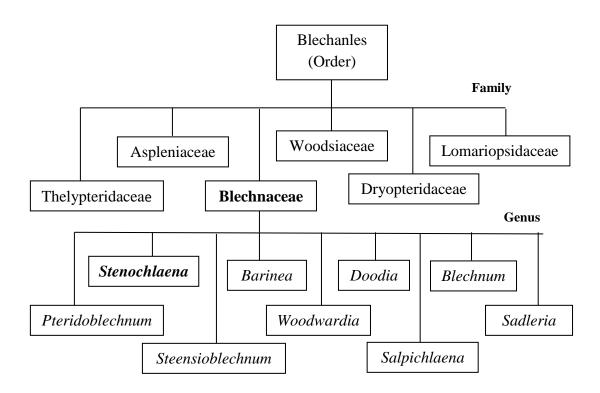


Figure 2.4: Taxonomical scheme of ferns and fern allies from Division to Class to Order (Smith et al., 2006).

# 2.2.1 Blechnaceae family

Blechnaceae is a medium-sized leptosporangiate fern family which belongs to the order of Blechnales. It has a total of nine genera and consists of 220–250 species (Kramer et al., 1990; Smith et al., 2006). Around 80% of the species in this family fall within the *Blechnum* genus and thus making it the largest genus in the Blechnaceae family. Most of members in this family are ground dwelling plants and some of them are climbers as well, such as *Stenochlaena*. A characteristic feature of this family is that the young opening fronds are tinged with red. Geographically, Blechnaceae family is mainly found across the tropical regions of the Southern Hemisphere, especially in Central and South America, Southeast Asia and Oceania (Smith et al., 2006; Schuettpelz & Pryer, 2007; Kuo et al., 2011). The taxonomical classification of Blechnaceae family is shown in Figure 2.5.



**Figure 2.5**: Taxonomical classification of Blechnaceae family from the Order to Genus (Kramer et al., 1990; Smith et al., 2006).

### 2.2.2 Genus of Stenochlaena

Stenochlaena J. Sm. is a small genus in the family of Blechnaceae but is widespread across the tropical and sub-tropical regions. There are no species of Stenochlaena recorded in Americas, including the associated islands and the Caribbeans (Chambers, 2013). There are six members in the genus of Stenochlaena, namely palustris, cumingii, milnei, areolaris, tenuifolia, mildbraedii and a possible extra member, Stenochlaena sp. 'Cameroon'. Most of these species are distributed from the Philippines, through the Luzon Island across Solomon Islands and Papua New Guinea to the northern part of Australia except for Stenochlaena mildbraedill and Stenochlaena tenuifolia. Members in this genus have very long and scandent rhizome, may climb up to the crowns of rainforest tree and at the same time maintain a connection with the ground, or climbs over rocks and forest debris or even grow as a floating mat over water surfaces (Holttum, 1932 and Chambers, 2013). Stenochlaena genus with their geo-distribution is shown in Figure 2.6.

#### Genus of Stenochlaena Stenochlaena Stenochlaena Stenochlaena Stenochlaena tenuifolia Sp. (Cameroon) milnei palustris (Native to (Possibly only (Native to the (Native to Africa) native to Philippines, Indochina, Cameroon) Maluku, Papua Malaysia, and New Guinea and Papuasia) Solomon Islands) Stenochlaena Stenochlaena Stenochlaena cumingii areolaris mildbraedill (Native to the (Native to the (Native to tropical Philippines, Maluku, Philippines and Papua West Africa) Papua New Guinea and New Guinea)

**Figure 2.6**: Members of *Stenochlaena* genus with their geographical distributions (Source: Chambers, 2013).

## 2.3 Fern as a source of food and traditional medicine

Solomon Islands)

Ferns and fern allies are one of the most common wild plants collected by people around the world to be consumed as food source or traditional medicine. Fern parts such as the leaves, stems, rhizomes, young fronds or shoots, and sometimes even the whole plants are used for food or traditional medicine (Liu et al., 2012). Edible ferns play an important role as a cheap and easily available food source especially for the people in Oceania and Asia. A list of edible ferns is given in Table 2.1.

**Table 2.1**: Edible ferns commonly consumed worldwide (Copela, 1942; Wu, 1990; Thakur et al., 1998; Shin, 2010; Liu et al., 2012).

Scientific name	Family name	Countries	Edible parts
Asplenium unilateral	Aspleniaceae	China	Fronds
Acrostichum aureum	Pteridaceae	Asia, Oceania	Fiddlehead
Blechnum orientale L.	Blechnaceae	China, Malaysia	Rhizomes and young red fronds
Ceratopteris thalictroides	Parkeriaceae	Asia, Australia	Young fronds
Cyathea genus	Cyatheaceae	Oceania	Young fronds, terminal bud
Diplazium esculantum	Athyriaceae	Asia, Oceania	Fiddlehead
Diplazium squamigerum	Athyriaceae,	Japan	Fiddlehead
Matteuccia struthiopteris	Onocleaceae	Canada, China, Europe, Malaysia, India, Japan, USA	Fiddlehead
Osmunda japonica	Osmundaceae	East Asia	Fiddlehead
Osmunda regalis	Osmundaceae	Worldwide	Fiddlehead
Pteridium aquilinum var. latiusculum	Pteridiaceae	Worldwide	Fiddlehead
Stenochlaena palustris	Blechnaceae	India, Malaysia, Southern Pacific	Fiddlehead and young red fronds

In Malaysia, four species of ferns are commonly consumed as vegetables, namely *Stenochlaena palustris* (Blechnaceae), *Diplazium esculantum* (Athyriaceae), *Acrostichum aureum* (Pteridaceae) and *Nephrolepis bisserata* (Nephrolepidaceae) However, only *S. palustris* and *D. esculantum* are commonly sold in local markets.

The edible parts of these edible fern species are mainly on their young fronds and fiddleheads (Piggott, 1988; Zuraini et al., 2010)

Besides being as a food source, ferns and fern allies are also used traditionally to cure various types of illnesses and disorders such as skin disorders, relief body heat, inflammation, microbial infection, snake bites, insect bites, urinary tract infection, cancer and others. In Malaysia itself, there are at least 76 species belongs to 44 genera and 13 families of ferns known to be used as traditional medicine, either singly or as an ingredient in a herbal remedy (Bidin, 1986). Some of the well known medicinal ferns used worldwide are listed in Table 2.2.

**Table 2.2**: Traditional medicinal ferns used worldwide (extracted from Ho et al., 2011).

Scientific name	Family name	Countries	Traditional uses	
Acrostichum aureum	Pteridaceae	Polynesia, Fuji	Wound and ulcer healing, as a purgative, treatment of elephantiasis	
Adiantum ceneatum	Adiantaceae	Brazil	Pain killer	
Adiantum lunulatum	Adiantsceae	India	Contraceptive	
Asplenium nidus	Aspleniaceae	Kumaun Himalaya	To cure jaundice, malaria and to remove calculi	
Blechnum occidentale	Blechnaceae	Brazil	Anti-inflammation, urinary infections, pulmonary and liver disease treatments	
Blechnum orientale	Blechnaceae	Tahiti, Cook islands	Tonic and to cure wound cicatrization	
		Kumaun Himalaya	Antihelmintic and typhoid treatment	
Davallia mariesii	Davalliaceae	China	Healing of fractured bones, pain killer, anti- inflammation,	

			anticancer, antiaging, hemostasis
Davallia solida	Davalliaceae	Polynesia	Wound healing, dysmenorrheal, gonorrhea, healing for fractured bones
Dicranopteris linearis	Gleicheniaceae	India	Treatment of sterility.
		Kumaun Himalaya	Laxative, antihelmintic, bacterial infection.
		Polynesia	Gonorrhea treatment, hernia.
Drynaria fortunei	Polypodiacae	China	Lumbago treatment, healing for fractured bones
Huperzia serrata	Lycopodiaceae	China	To treat febrifuge, blood disorder, contusions, strains, myasthenia gravis, schizophrenia.
Lycopodiella cernua	Lycopodiaceae	Kumaun Himalaya	Antitussive, beriberi skin eruption treatment
		Fiji	Rheumatism treatment
Microsorum scolopendria	Polypodiaceae	Polynesia	Skin inflammation, wound healing, purgative
Polypodium leucotomos	Polypodiaceae	South America, India	Antiphlogistic, antitumor, skin inflammation,
Pteridium aquilinium	Pteridaceae	India, Brazil	Astringent, antihelmintic, to relief diarrhea
Pteris semipinnata	Pteridaceae	China	Snake bite
Selaginella tamariscina	Selaginellalaceae	China, Korea	Diuretic, gonorheaa, rhinitis
Stenochlaena palustris	Blechnaceae	Malaysia, Indonesia, Thailand	Skin diseases, fever

Of late, standardized extracts from some of the ferns and fern allies have been developed into pharmaceutical product with reference to their traditional uses. One of the examples of a successful standardized pharmaceutical preparation from fern, "Anapsos" is developed from a medicinal fern, *Polypodium leucotomos*, traditionally used to treat dermatitis in South America. "Anapsos" is a Spanish pharmaceutical formula, registered as "Regender" and "Armaya fuerte" by the Spanish Health Department, containing P. leucotomos as the active ingredient which is effective against dermatological disorders such as psoriasis and atopic dermatitis treatment (Vasange-Tuominen et al., 1994; Sempere-Ortells et al., 2002). Further investigation on its pharmacological activities led to the discovery of new uses of "Anapsos" as photoprotectant, immunomodulator and antioxidant, and thus found its new applications in the field of nutraceuticals and cosmetics. Another pharmaceutical product, "Fernblock" is also an antioxidant formulation developed from the aqueous extract of the aerial part of P. leucotomos, generally used for topical and oral photoprotective purposes or against skin-aging. Several major antioxidative polyphenols were identified in the active extract, namely 3, 4-dihydroxybenzoic acid, 4-hydroxybenzoic acid, vanillic acid, caffeic acid, 4-hydroxycinnamic acid, 4hydroxycinnamoyl-quinic acid, ferulic acid, and five chlorogenic acid isomers (Carcia et al., 2006).

### 2.3.1 Fern as source of natural antioxidants

Ferns and fern allies tend to synthesize various types of secondary metabolites to serve as antioxidant system for self-protection against biotic and abiotic stress conditions (Lee & Shin, 2010). Recently, studies on the antioxidant properties of a number of ferns were reported and most of them exhibited strong radical scavenging activity (Shin & Lee, 2010).

The antioxidant activities of plants are often associated with their high polyphenol content. According to a few studies in which determination of total polyphenol contents of 37 species of ferns and fern allies were carried out, *Polystichum lepidocaulon* and *Polystichum polyblepharum* were reported to have more than 13% of total polyphenols from dried plant extracts of both fronds and rhizomes (Shin & Lee, 2010; Shin, 2010). Besides, the fronds of *Davallia mariesii* and the rhizomes of *Cyrtomium fortune*, *Dicranopteris pedata*, *Athyrium niponicum* and *Dryopteris nipponensis* were also found to contain more than 10% of total polyphenols from dried plant extracts (Ho et al., 2011). These fern species which are rich in polyphenols were found to exhibit strong radical scavenging activities as showed Table 2.3.

**Table 2.3**: Free radical scavenging activities of the MeOH extracts of various fern species compared to ascorbic acid or BHT (Shin, 2010)

Family	Scientific name	Parts	DPPH Scavenging Activity (EC <sub>50</sub> mg/mL)	ABTS Scavenging Activity (IC <sub>50</sub> mg/mL)
	Ascorbic acid		0.03	0.20
	ВНТ		0.12	0.22
Davalliaceae	Davallia mariesii*	Frond	0.05	0.06
		Rhizome	0.08	0.07
Dryopteridaceae	Cyrtomium fortune*	Rhizome	0.03	0.11
	Dryopteris crassirhizoma	Rhizome	0.11	0.11
	Dryopteris nippomemsis*	Frond	0.11	0.14
	• • • • • • • • • • • • • • • • • • • •	Rhizome	0.05	0.06
	Polystichum lepidocaulon*	Frond	0.05	0.09
		Rhizome	0.04	0.04
	Polystichum polyblephanum*	Frond	0.08	0.10
		Rhizome	0.02	0.03
Gleicheniaceae	Dicranopteris pedata*	Rhizome	0.03	0.03
Osmundaceae	Osmunda cinnamomea	Rhizome	0.06	0.08
	Osmunda japonica	Rhizome	0.08	0.10
Parkeriaceae	Adiantum pedatum	Rhizome	0.06	0.07
Polypodicaceae	Pyrossia lingua	Frond	0.11	0.08
Schizaeaceae	Lygodium japonicum	Rhizome	0.07	0.09
Thelypteridaceae	Thelypteris acuminata	Rhizome	0.06	0.06
Woodsiaceae	A. niponicum*	Rhizome	0.04	0.07
	Matteuccia struthiopteris	Rhizome	0.11	0.14
	Onoclea sensibilis var.	Rhizome	0.11	0.15
	interrupta			

<sup>\*</sup>Fern species which are rich in polyphenols

# 2.3.1.1 Polyphenols from ferns

Flavonoids belong to a major subclass of polyphenols well known for their radical scavenging capacity and preventive properties against lipid peroxidation (Chen et al., 1990). Most of the active phytochemicals found in medicinal ferns traditionally used to treat various types of disorders such as inflammation, wound infection, dysentery, rhinitis and osteoporosis were mainly flavonoids.

Four new flavan-4-ol glycosides were reported from *Abacopteris penangiana*, a medicinal fern used for the treatment of upper respiratory tract infection and dysentery. These compounds, namely abacopterins E-H (**1-4**) exhibited significant ABTS radical scavenging activity and the TEAC value ranged from 1.03-1.91 mM (Zhao et al., 2007) (Figure 2.7).

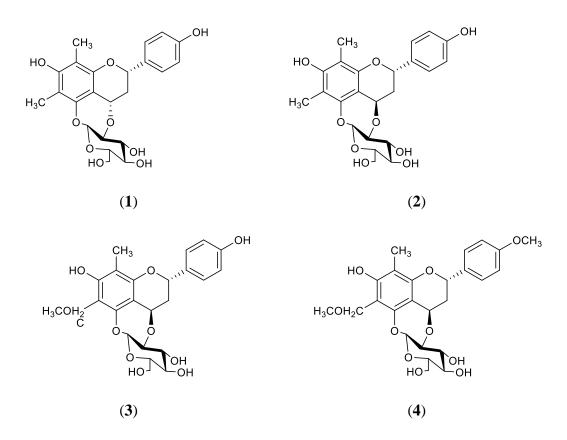


Figure 2.7: Chemical structures of abacopterins E-H (1-4) from A. penangiana.

From another fern, *Pteris ensiformis* traditionally used as herbal tea in Taiwan, two unique flavonol glycosides were reported. These compounds, namely kaempferol  $3\text{-}O\text{-}\alpha\text{-}L\text{-}\text{rhamnopyranoside-}7\text{-}O\text{-}[\alpha\text{-}D\text{-}\text{apiofuranosyl-}(1, 2)\text{-}\beta\text{-}D\text{-}\text{glucopyranoside}]$  (5) and kaempferol  $3\text{-}O\text{-}L\text{-}\text{rhamnopyranoside-}7\text{-}O\text{-}\beta\text{-}D\text{-}\text{glucopyranoside}$  (6) exhibited strong DPPH radical scavenging activities and the TEAC values were 0.58 mM, 0.52 mM, 0.89 mM and 0.85 mM, respectively (Chen et al., 2007) (Figure 2.8).

**Figure 2.8**: Chemical structures of flavonol glycosides (5-6) from *P. ensiformis*.

Another fern widely used in Asia to treat allergic rhinitis, *Selaginella tamariscina* is also well known for its multi-bioactivity biflavonoids in neuroprotecting, anti-inflammation, cell protective against UV-B light damage and anticancer. Numerous unique biflavonoids and flavonoids were isolated from the whole plant of *Selaginella tamariscina*, namely sumaflavone (7), isocryptomerin (8),

amentoflavone (9), heveaflavone (10), 6-(2-hydroxy-5-carboxyphenyl)-apigenin (11) and 3-(4-hydroxyphenyl)-6, 7-dihydroxy coumarin (12) (Lee et al., 2008; Liu et al., 2010) (Figure 2.9).

**Figure 2.9**: Chemical structures of biflavonoids and flavonoids (7-12) from *S. tamariscina*.