ANTIOXIDANT ACTIVITY, TOTAL PHENOLIC AND FLAVONOID CONTENT OF LEAVE, STEM AND RHIZOME OF *Plagiostachys* spp. (ZINGIBERACEAE)

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I declare that this is the result of my own research except as cited in reference. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any degree.

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ABSTRACT

The total phenolic content, total flavonoid content and the antioxidant activity were studied in *Plagiostachys breviramosa* and *P. roseiflora*. The objectives of this research are to determine the total phenolic and flavonoid content and antioxidant activity of leave, stem and rhizome of the *Plagiostachys breviramosa* and *P. roseiflora*. The total phenolic content was determined according to the Folin-Ciocalteu method while the total flavonoid content of *Plagiostachys* species used Catechin as the standard. The antioxidant activity, the 2, 2-Diphenyl-1-picryl-hydrazyl radical (DPPH) scavenging method was used. *P. breviramosa* rhizome showed the highest value of 156.573 ± 2.847^{b}, 150.667 ± 5.044^{a} and 85.860 ± 0.312^{c} for total phenolic content, total flavonoid content and antioxidant activity respectively. Meanwhile, *P. breviramosa* leave showed the lowest value for total phenolic content, total flavonoid content and antioxidant activity which are 44.950 ± 3.291^{e}, 43.000 ± 0.577^{e} and 54.863 ± 0.979^{a} respectively. As for the *P. roseiflora*, the total phenolic content showed the highest value 172.000 ± 2.074^{a} in leave sample while the lowest value, 7.240 ± 1.356^{f} in stem sample. The total flavonoid content was lowest in stem of *P. roseiflora*, 6.667 ± 0.882^{f} while *P. roseiflora* rhizome has the highest value which is 137.667 ± 0.882^{b}. However, the highest value of antioxidant activity of *P. roseiflora* showed in the rhizome part which is 88.290 ± 1.091^{f} while the leave part has the lowest value which is 56.040 ± 0.156^{d}.

ABSTRAK

Jumlah kandungan fenolik, jumlah kandungan flavanoid dan penentuan aktiviti antioksida telah dikaji pada *Plagiostachys breviramosa* dan *P. roseiflora*. Objektif dalam kajian ini adalah untuk menentukan jumlah kandungan fenolik dan flavanoid serta aktiviti antioksida pada bahagian daun, batang dan rizom *P. breviramosa* dan *P. roseiflora*. Jumlah kandungan fenolik telah ditentukan dengan menggunakan kaedah Folin-Ciocalteu manakala jumlah kandungan flavanoid dalam *Plagiostachys* spesies telah ditentukan dengan menggunakan Catechin sebagai piawai. Demi menentukan aktiviti antioksida, kaedah 2, 2-Diphenyl-1-picryl-hydrazyl radikal (DPPH) telah digunakan. Jumlah kandungan fenolik, flavanoid dan aktiviti antioksida pada bahagian rizom *P. breviramosa* telah menunjukkan nilai yang tertinggi iaitu 156.573 ± 2.847^{b}, 150.667 ± 5.044^{a} dan 85.860 ± 0.312^{c} masing-masing. Manakala daun *P. breviramosa* telah menunjukkan nilai yang terendah iaitu 44.950 ± 3.291^{e}, 43.000 ± 0.577^{e} dan 54.863 ± 0.979^{a} dalam ketiga-tiga eksperimen tersebut. Manakala bagi *P. roseiflora* pula, jumlah kandungan fenolik adalah tertinnggi dalam 172.000 ± 2.074^{a} manakala terendah pada bahagian stemnya iaitu 7.240 ± 1.356^{f}. Bagi jumlah kandungan flavanoid, batang *P. roseiflora* telah menunjukkan nilai terendah 6.667 ± 0.882^{f} manakala nilai yang tertinggi adalah pada bahagian rizomnya iaitu 137.667 ± 0.882^{b}. Aktiviti antioksida dalam rizom *P. roseiflora* adalah tertinggi 88.290 ± 1.091^{f} dan daunnya mempunyai nilai yang terendah iaitu sebanyak 56.040 ± 0.156^{d}.

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LIST OF SYMBOLS

%	Percentage
μgL^{-1}	Microgram per liter
μl	Microliter
cm	Centimeter
g	Gram
L	Liter
M	Molarities
m	Meter
mg	Milligram
mgL^{-1}	Milligram per liter
min	Minute
ml	Millimeter
mM	Millimolar
nm	Nanometer
ppm	Part per million
r. p. m	Revolution per minutes
V	Volume

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CHAPTER 1

INTRODUCTION

1.1 Overview

More than three decades ago, researchers are already struggling to study natural products which have been the major driving force in organic chemistry. The study of natural products in Malaysia began with the establishment of the University of Malaya in Kuala Lumpur. With the human genome being almost completely solved, the mysteries of traditional medicines can now be unraveled. The Malaysian tropical forest is home to large number of plants, many with ethno-botanical uses while a greater biodiversity from other organisms awaits further study (Goh, 2000).

Nowadays, the chemistry of natural products is relatively easy to have but the economic translation to drugs, pesticides and other high-valued products remains difficult and demanding. In recent years, a rich harvest of novel natural products had been made, some of which possess cytotoxic or insecticidal activities. The time to study and develop the natural products on multidisciplinary scale has never been better than at present (Goh, 2000).

Zingiberaceae is one of the significant components of the herbaceous ground flora in the Malaysian tropical forests (Ibrahim & Rahim, 1998). "Gingers" is a general term which is for the species or members of the family Zingiberaceae. This word also truly refers to the edible commerce ginger which is also known as "halia" in Malay language (Larsen *et al.*, 1999). Zingiberaceae is the largest family out of the eight families which comprises the monophyletic tropical order Zingiberales. Family Zingiberaceae form a monophyletic group together with Costaceae, Marantaceae and Cannaceae while the Costaceae is the sister group to Zingiberaceae (Clark *et al.*, 1993).

The family of ginger is widely distributed in the tropics and Indo-Malaysian region and it is thought to be the centre of its diversity (Sakai, 1997). There are about 1,000 of species are occurring in tropical Asia (Larsen *et al.*, 1999). According to Kress *et al.* (2002), there are about 53 genera and over 1300 species are known in this family and about 18 genera and more than 200 species that occurred in Borneo have been reported. Some of the genera such as the genus *Alpinia, Amomum, Boesenbergia, Etlingera, Hornstedtia* and *Plagiostachys* are commonly found in Borneo (Poulsen, 2006). There are many of the wild ginger species, especially the species from Borneo, remain undescribed and to a large extent undocumented (Ibrahim & Rahim, 1998).

There is a characteristic component of the herbaceous ground flora of the wild gingers in Malaysia's forest (Ibrahim & Rahim, 1998). Most of the members of ginger could be easily recognized by its aromatic leave when it is crushed and the arrangement of elliptic leaves in two ranks on the leaf-shoot. Gingers are usually found in lowlands or low hill-forest and there are only a few of them will found on high mountain ridges. They normally found as scattered individuals or in small clumps and seldom found in secondary forest. It usually grows in shade amongst the undergrowth or by the banks of streams or on hill slopes. Only a few species could stand for the full exposure of sun (Khaw, 1995).

Antioxidant is normally considered as fundamental in human life and health. There are many biologically important processes that are strongly influenced by antioxidant and antioxidant systems in human body. Hence, the dietary antioxidant could be important in affecting some process such as ageing and various diseases like cancer, cardiovascular disease, cataracts and brain and immune dysfunction (Pietta, 2000).

Plants are potential sources of natural antioxidants (Ames *et al.*, 1993). These natural protective effects have been attributed to various components such as vitamins C, E and phenolic (Paganga *et al.*, 1999). The interest in plant-derived food additives has grown in recent years. The deterioration of food quality was occurring during the process and storage and it is related to the oxidative process (Halliwell, 1997). Yet, the drawback of antioxidants is they are volatile and easily decompose at high temperature (Martinez-Tome *et al.*, 2001).

Herbal medicine, also called "phytomedicine", is using the therapeutic plants, plant parts or plant derived substances to aid infections, diseases or enhancing overall human's health (Jonas, 1997). There are many species of herbs which have been recognized to have medicinal properties and beneficial impact on human's health. For example, antioxidant activity, digestive stimulation action, anti-inflammatory, antimicrobial, and anti-carcinogenic potential are examples that herbs possessed (Cai *et al.*, 2004).

There are many types of herbs and spices which are usually used as the flavours dishes have been shown the present of phenolic compounds which is known as a good antioxidant activity (Zheng & Wang, 2001). Thus, it may serve as the natural food preservatives. However, herbs and spices are usually containing the essential oils which will show the activity of antioxidant (Ruberto *et al.*, 2000). Herbs are usually used in domains that include medicine, nutrition, flavouring, beverages, dyeing, repellents, fragrances and cosmetics. Therefore, the crude extracts of herbs and spices which are rich in phenolic compounds are getting famous by used in food industry (Djeridane *et al.*, 2006).

1.2 Aim of Research

To determine the antioxidant activity, total content of phenolic and flavonoid of the leave, stem and rhizome in *Plagiostachys breviramosa* and *P. roseiflora*. The antioxidant activity, total content of phenolic and flavonoid of *Plagiostachys breviramosa* and *P. roseiflora* have not yet been studied in the previous research before. Thus, if there is any antioxidant activity present in the sample, the content of phenolic and the flavonoid will be high. Therefore, those species could be use as the traditional medicine herb.

1.3 Research Objectives

The objectives of this research are:

1. To determine the total phenolic and flavonoid content of leave, stem and rhizome of the *Plagiostachys breviramosa* and *P. roseiflora*.
2. To determine antioxidant activity in the leave, stem and rhizome of *Plagiostachys breviramosa* and *P. roseiflora.*

CHAPTER 2

LITERATURE REVIEW

2.1 Antioxidant Activity

The entire living organism has the endogenous defense systems against the oxidative damage such as DNA damage, lipid peroxidation (Lee *et al.*, 2005) and communicational inhabitation of the cells (Singler & Ruch, 1993) due to the reactive oxygen species (ROS). There are two main defense mechanisms of the antioxidant, the antioxidant defense with enzymes and defense with non-enzymatic compounds (Rice-Evans *et al.*, 1997).

In addition, the oxidation of polyunsaturated fatty acids in biological membranes will lead to serious damages in human cell such as coronary atherosclerosis, emphysemas, cancer and cirrhosis. Safe guarding fat which is against oxidation activity is normally done by restricting the access of oxygen or by adding antioxidants. Thus, there are some commonly applied antioxidants which is known as synthetic phenols (Imadia *et al.*, 1983) such as butylhydroxytolutne (BHT) or the butylhydroxyanisole (BHA) that the synthetic antioxidants are normally used to decelerate these processes (Martinez-Tome *et al*., 2001).

Furthermore, antioxidation is an extremely significant activity which can be used as preventive agents against numerous of diseases (Aruoma, 1994). Therefore, more attention is focused on natural antioxidants and these are polyphenols compounds (Yen *et al*., 2003) which will found in all parts of plants (Kim *et al*., 1997). As plants produce a lot of antioxidants to control the oxidative stress, it will represent a natural source of antioxidant and antibacterial activity, which could be observed in several parts of plants such as fruits, roots, leaves and seeds (Policegoudra *et al*., 2007).

Methods for characterization of antioxidants are represented and illustrated since it has been widely speculated that ginger might be beneficial to human health because it exerts "antioxidant activity" (Aruoma *et al*., 1997). The antioxidant activity of the phenolic compounds is due to their ability to scavenge free radicals, donate hydrogen atoms or electron or chelate metal cations (Amarowicz *et al*., 2004).

2.2 Phenolic Compounds

Phenolic compounds which usually ubiquitous in plants such as fruits, vegetables and beverages, are essential part in human diet (Balasundram *et al*., 2006). Thus, the phenolic compounds are secondary metabolites which derivatives from the pentose phosphate, shikimate and phenylpropanoid pathway in plants (Randhir *et al*., 2004). These compounds are one of the most widely occurring groups of phytochemicals and are physiologically and morphologically important in plants. They normally play an important role in growth and reproduction and also providing protection against the pathogens and predators in human health (Bravo, 1998).

Phenolic compounds are exhibiting a wide range of physiological properties such as anti-allergenic, anti-artherogenic, anti-inflammatory, anti-microbial, antioxidant, anti-thrombotic, cardioprotective and vasodilatory effects (Manach *et al*., 2005). According to Parr & Bolwell (2000), the phenolic compounds have associated with the health benefits which were from consuming high levels of fruits and vegetables. Thus, it could be a major determinant of antioxidant potentials of foods and also a natural source of the antioxidants (Parr & Bolwell, 2000).

Structurally, the phenolic compounds are comprised of an aromatic ring which bears one or more hydroxyl substituents and ranged from simple phenolic molecules to highly polymerized compounds (Bravo, 1998). Despite of this structural diversity, this group is often referred as "polyphenols". Phenolic compounds are naturally occur as a conjugate with monosaccharide or polysaccharides that will linked to one or more phenolic group and also may occur as functional derivatives such as esters and methyl esters (Harborne *et al*., 1999). Phenolic compounds were basically categorized into several classes through their structural diversity which result in wide range of phenolic compounds that occur in nature (Harborne *et al*., 1999).

Though phenolic compounds are present in almost every plant and fruits, the major sources of these compounds in human diet are vegetables and beverages (Hertog *et al*., 1993). There is a wide variation between total phenolic contents in different fruits or vegetables, or even the same fruits or vegetables that reported by different authors. These differences may be due to the complexity of these compound groups and also the methods of extraction during the analysis (Bravo, 1998).

Phenolic contents of plant depend on a number of intrinsic (genus, species, cultivars) and extrinsic (agronomic, environmental, handling and storage) factors (Tomás-Barberán & Espín, 2001). On the other hand, phenolic compounds in beverages such as fruit juices, tea and wines were important sources in human diet. Reductions or losses of phenolic compounds have been reported in commercial juices and these have been attributed for commercial processing procedures (Spanos *et al.*, 1990).

2.3 Flavonoid

Flavonoids exist widely in the plant kingdom and they are commonly found in leaves, flowering tissues and pollens of plants (Larson, 1988). There are over 4,000 flavonoids which have been identified and many of them are normally occurred in fruits, vegetables and beverages (Wojdylo *et al.*, 2007). Flavonoids in plants are important in human diet because they may effect on the human nutrition. The well known properties that are found in flavonoids are including free radical scavenging, strong antioxidant activity, inhibition of hydrolytic and oxidative enzymes (phospholipase A_2, cyclooxygenase, lipoxygenase) and anti-inflammatory action (Frankel, 1995).

Flavonoids constitute the largest group of plant phenolic (Harborne *et al.*, 1999). Flavonoids represent a very wide spread group of water-soluble compounds

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