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ANTIOXIDANT ACTIVITY OF SABAH TEA LEAVES

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DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT FOR THE DEGREE BACHELOR OF FOOD SCIENCE WITH HONOURS

SCHOOL OF FOOD SCIENCE AND NUTRITION
UNIVERSITI MALAYSIA SABAH
2009

DECLARATION

I hereby declare that the material in this thesis is my own except for the quotations, excerpt, equations, summaries and references, which have been duly acknowledged.

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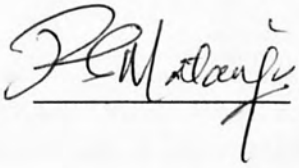

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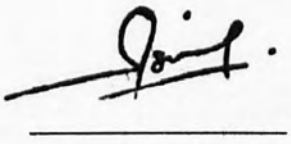

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ABSTRACT

In this study the antioxidant activity of methanol and hot water extracts of Sabah fresh tea leaves at different ages of shoot, young and old leaves were analyzed and their total phenolic and flavonoid contents were measured spectrophotometrically. The antioxidant activity of tea samples was determined by ferric reducing antioxidant power (FRAP) assay, DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging assay and $ABTS^{\cdot+}$ radical scavenging assay. The correlation that exists between total phenolic and antioxidant activity and also total flavonoid and antioxidant activity was determined by obtaining the correlation coefficient values. Outcomes of this study showed that methanol showed higher extraction efficiency than hot water. Methanol extract of tea leave samples at different ages contained relatively higher content of total phenolic and flavonoids than hot water extract. Total phenolic content in methanol extract of tea leaves of different ages ranged from 428.38 ± 17.93mg GAE/L to 231.95 ± 17.96mg GAE/L. Total flavonoid content of tea leaves of different ages in methanol extract ranged from 4.85 ± 0.04mg QEQ/ml to 2.07 ± 0.03mg QEQ/ml. A very strong antioxidant activity was also exhibited by methanol extract of tea leave samples. The highest total phenolic content, total flavonoid content and antioxidant activity were revealed by the methanol extract of shoot followed by young leaves and lastly old leaves. Methanol extract of shoot had FRAP value of 471.89 ± 35.33 μMol Fe2SO4.7H2O /L, IC_{50} value of 0.73 ± 0.61mg/ml, AEAC value of 9269.90 ± 11291.06 mgAA/100g and TEAC value of 1.29 ± 0.08 mgTROLOX Equivalent/ml. The correlation between total flavonoid content and antioxidant activity was found to be stronger in tea leave samples as compared to the correlation between total phenolic content and antioxidant activity.

ABSTRAK

AKTIVITI ANTIOKSIDAN DAUN TEH SABAH

Dalam kajian ini, aktiviti antioksidan ekstrak methanol dan air panas daun teh Sabah pada tiga peringkat kematangan, pucuk, daun muda dan daun matang telah dianalisa dan kandungan jumlah fenolik dan kandungan jumlah flavonoid turut ditentukan. Aktiviti antioksidan ekstrak daun teh telah ditentukan melalui kaedah penurunan ferric – tripyridyltriazine, kaedah perencatan radikal DPPH (2, 2-diphenyl-1-picrylhydrazyl) dan kaedah perencatan radikal $ABTS^{+}$. Kandungan jumlah fenolik dan kandungan jumlah flavonoid ditentukan dengan kaedah spektrofotometri. Korelasi yang wujud antara jumlah fenolik dan aktiviti antioksidan dan jumlah flavonoid dan aktiviti antioksidan ditentukan melalui nilai koefisien korelasi. Hasil kajian ini menunjukkan bahawa methanol mempunyai keberkesanan pengekstrakan yang tinggi berbanding dengan air panas. Ekstrak methanol daun teh pada ketiga-tiga peringkat kematangan didapati mengandungi kandungan jumlah fenolik dan kandungan jumlah flavonoid yang lebih tinggi berbanding dengan ekstrak air panas. Kandungan jumlah fenolik dalam ekstrak methanol daun teh pada tiga kematangan yang berbeza adalah dari 428.38 ± 17.93mg GAE/L hingga 231.95 ± 17.96mg GAE/L. Kandungan jumlah flavonoid dalam ekstrak methanol daun teh pada tiga kematangan yang berbeza adalah dari 4.85 ± 0.04mg QEQ/ml hingga 2.07 ± 0.03mg QEQ/ml. Aktiviti antioksidan yang lebih tinggi turut ditunjukkan oleh ekstrak methanol daun teh. kandungan jumlah fenolik, kandungan jumlah flavonoid dan aktiviti antioksidan yang paling tinggi telah diberikan oleh ekstrak methanol pucuk diikuti oleh daun muda dan akhirnya daun matang. Pucuk dalam ekstrak methanol mempunyai nilai FRAP 471.89 ± 35.33 µMol Fe2SO4.7H2O /L, nilai IC_{50} 0.73 ± 0.61mg/ml, nilai AEAC 9269.90 ± 11291.06 mgAA/100g dan nilai TEAC 1.29 ± 0.08 mgTROLOX Equivalent/ml. Didapati korelasi antara kandungan jumlah flavonoid dan aktiviti antioksidan pada daun teh lebih tinggi berbanding dengan korelasi antara kandungan jumlah fenolik dan aktiviti antioksidan.

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LIST OF ABBREVIATIONS AND SYMBOL

C.sinensis	*Camellia sinensis*
EC	Epicatechin
EGC	Epigallocatechin
ECG	Epicatechin gallate
EGCG	pigallo catechin gallate
TPC	Total Phenolic Content
TFC	Total Flavonoid Content
AOA	Antioxidant Activity
DPPH	2,2-diphenyl-1-picrylhydrazyl
FRAP	Ferric Reducing Antioxidant Power
TEAC	Trolox Equivalent Antioxidant Capacity
TF	Theaflavins
TR	Thearubigins
asl	above sea level
GAE	Gallic Acid Equivalent
QEQ	Quarcetine Equivalent
IC	Inhibitory Concentration
AEAC	Ascorbic acid Equivalent Antioxidant Capacity
%	Percentage

LIST OF UNITS

g	Gram
mg	Miligram
µl	Microlitre
ml	Mililitre
nm	Nanometer
°C	Degree Celsius
L	Litre
ppm	Parts per million
mM	Milimolar
µM	Micromolar
cm	Centimeter

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CHAPTER 1

INTRODUCTION

1.1 Background of the study

Tea is becoming more popular due to its attributed popularity of sensory properties, relatively low retail prices, stimulating effect and potential health benefits. Consumption of tea as beverage become wider with more varieties of flavoured brand teas that include tea bags, instant tea, pot bags, tin cans and plastic bottles being produced for the convenience of customer (Helen, 2006).

Tea is the most widely consumed beverage worldwide (Benzie & Szeto 1999; Lin *et al.*, 1996) which is originated from China, dates back several thousands years. Currently, the tea plant *Camellia sinensis* (L.) Kuntze is grown in about 30 countries worldwide (Graham, 1992). The tea plant is previously known as *Thea sinensis* and later changed to *Camellia sinensis* (Burkill, 1993). Tropical and subtropical areas with adequate rainfall, good drainage and slightly acidic soil are best for tea plantation (Graham, 1999).

Tea plant is normally planted in high lands. In plantations, tea is planted at a density of 5000-10000 plants per hectare and maintained as low shrubs of 1-1.5m in height through regular pruning during harvesting. Manual plucking of the terminal bud

and two youngest leaves gives the finest quality of tea (Caffin *et al.*, 2004). Tea leaves are dark green, alternate and oval with serrated edges and blossoms are white, fragrant and appear in cluster and single.

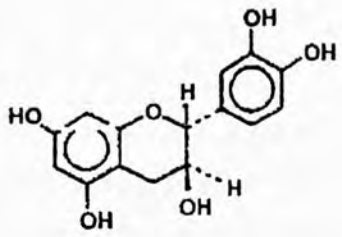
India, China, Jepun, Sri Lanka and Indonesia are the biggest tea producers in the world. There are two varieties of tea; *C.sinensis* var. *sinensis* (China tea) is grown extensively in China and Japan while, *C.sinensis* var. *assamica* is largely cultivated in south and Southeast Asia, including Malaysia (Sang *et.al*, 2002). In Malaysia tea is widely planted in Cameron Highlands, Pahang and Ranau, Sabah.

There are three major categories of tea; green tea, black tea and oolong tea which are derived from the tea leaves of *Camellia sinensis*. These tea varieties are differing in terms of processing methods. Green tea (non-fermented) is derived directly from drying and steaming of fresh tea leaves; Oolong tea is prepared when fresh tea leaves are subjected to partial fermentation before drying and finally black tea undergoes full fermentation before drying and steaming (Polovka *et al.*, 2003). Of the total amount of teas produced and consumed in the world, 78% are black tea, 20% are green tea and 2% are oolong tea.

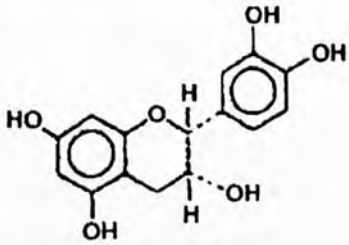
There are three major chemical constituents in tea: polyphenols, caffeine and amino acid. A chemical composition, concentration and mutual relation of major components in tea leaves influence the properties of tea. Tea polyphenol which comprises of catechins, flavonoids, theaflavin and thearubigins (Wang & Helliwell, 2001) are the most important group of tea components and have a wide range of pharmaceuticals properties (Frei & Higdon, 2003). Tea polyphenols also plays an important role in determining quality of tea.

Catechins are known to be non-volatile taste compounds of tea (Nwuha *et al.*, 1999) and presented at 8-15% of dry leaf weight. The most important catechins are epicatechins (EC), epigallocatechins (EGC), epicatechin gallate (ECG) and epigallo catechins gallate (EGCG). Fresh tea leaves are very rich in catechins, which constitute up to 30% of dry weight. Content of catechins in tea leaves varies with few factors such

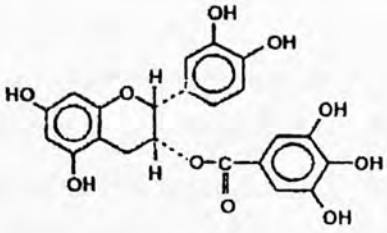
as climate, season, horticultural practices, leaf age and variety. The amount of catechins was in the order: green tea > oolong tea > fresh tea leaves > black tea (Lin *et al.,* 2003). The greatest amount of catechins in green tea is 26.7% followed by oolong tea is 23.2% and black tea is 4.3% (Yen & Chen, 1995). Chemical structure of catechins in *Camellia sinensis* is showed in Figure 1.1.



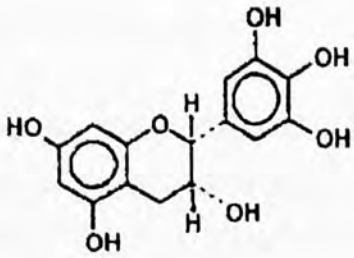
Catechins



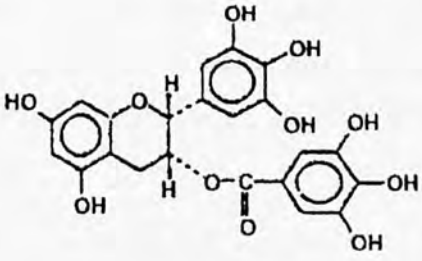
Epicatechins



Epicatechin gallate



Epigallo catechin



Epigallo catechin gallate

Figure 1.1 Chemical structures of catechins in *Camellia Sinensis* (Almajano *et al.*, 2007).

Flavonoids on the other hand are biologically active polyphenolic compounds which widely distributed in plants. Flavonols, flavones, flavanones, flavan-3-ols, and anthocyanidins are the five subclasses of flavonoid. Tea contains high levels of flavonoids, including flavanols and flavan-3-ols, which comprise 30 percent of the dry weight of the leaf. Based on the USDA database for flavonoid content of foods, the average total amount of flavonoids in a serving (235 ml) of brewed tea, is 159±43 mg for green tea and 178±45 mg for black tea (Holden *et al.*, 2002).

Flavonoids and other polyphenolic compounds in tea are effective free-radical scavengers (Salah *et al.*, 1995) and also effective metal chelation. In other words, these polyphenolic compounds exhibit powerful antioxidant activity by scavenging free oxygen radicals and metal ions and preventing these radicals from damaging healthy cells (Frei & Higdon, 2003). Antioxidant compounds which naturally found in tea play an important role as a health-protecting factor. Many previous studies suggest that antioxidants reduce the risk for chronic diseases including cancer and heart disease.

Antioxidant compounds like polyphenols scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative diseases.

Despite of antioxidant capability, tea polyphenols also exhibit anticarcinogenic and antimutagenic properties as well. The antioxidant activity can be determined by applying few commonly used spectrophotometric assays (DPPH, FRAP and TEAC) on the tea extracts.

1.2 Significance of the study

Various experiments or studies have been conducted in determining the polyphenol content and antioxidant activity in tea leaves. However, there are only few published

records around Sabah concerning the relevant study about the polyphenol content and antioxidant especially in Sabah tea leaves at different maturity stages. Tea is a popular beverage which widely consumed by people due its various health benefits. The tea sample is also easily obtained locally, as Sabah is the state producer of its own tea leaves and tea powder. The main aspect that has been emphasized in this study is the knowledge and confirmation of total polyphenol and antioxidant activity of Sabah fresh tea leaves at different maturity stages.

1.3 OBJECTIVE

I. To investigate the effect of different extracting solvents (hot water and methanol) on Total Phenolic Content, Total Flavonoid Content and Antioxidant Activity of Sabah tea leaves at different ages.

II. To determine and compare the Antioxidant Activity of Sabah tea leaves at three maturities (shoots, young leaves and old leaves) by using DPPH assay, FRAP assay and TEAC assay.

III. To study the correlations between the total phenolic content and antioxidant activity and total flavonoid content and antioxidant activity of tea leaves.

CHAPTER 2

LITERATURE REVIEW

2.1 Tea

Tea is a well known beverage which consumed by two thirds of the world's population. Tea is simply considered as tasteful drink with its pleasant aroma and flavour. Tea is obtained from the leaves of a plant known as *Camellia sinensis.* Historically, tea (C. *sinensis*) is originated from China since 3000BC. Tea is belongs to the Theaceae family and comes from two main varieties: C. *sinensis var sinensis* and C. *sinensis var assamica.* Tea is also popular as a medicinal drink in many regions due to its many beneficial health effects such as a good remedy for headache, body pain, digestion, depression, and detoxification, as an energizer and to prolong life (Shi *et al.,* 2005). Currently China, Japan, India, Sri Lanka and Indonesia are the five major tea producers in the world.

Table 2.1 Differences between the two major tea varieties.

Variety	Growth habitat	Leaf features	Leaf angle
China *Camellia sinensis* var sinensis (L)	Dwarf, slow growing, shrub like.	Small, erect narrow, serrate, dark green	< 50°
Assam *Camellia sinensis* var assamica	Tall, quick growing.	Large, horizontal, broad, moslty non-serrated, light green.	> 70°

Source: Banerjee (1992)

2.2 Tea Classification

Tea can be classified into three main groups: green tea, black tea and oolong tea. These tea varieties are obtained from the same leaves of C. *sinensis* but only differ in their processing method in terms of fermentation (Polovka *et al.,* 2003).

Green tea is obtained from the fresh young leaves which do not undergo fermentation (unfermented tea); Oolong tea is produced when fresh tea leaves are subjected to partial fermentation before drying; whereas black tea undergoes full fermentation before drying and steaming. In other words, the procedure of tea fermentation, which is an oxidative process, generally divides the teas into three main groups as mentioned and substantially affects the tea composition. Colour, aroma and flavour properties of these teas also differ from one another due to difference in their tea composition (Polovka *et al.,* 2003).

Of these teas, green tea has been called the second-most consumed beverage in the world, after water (Shi *et al.,* 2005). Asian people more commonly consume green and oolong tea while black tea is most popular in the United States. Green tea has been consumed widely for many years in India, China, Japan, and Thailand. In traditional

Chinese and Indian medicine, practitioners used green tea as a stimulant, diuretic (to promote the excretion of urine), astringent (to control bleeding and help heal wounds), to improve heart health, treating flatulence (gas), regulating body temperature and blood sugar, promoting digestion, and improving mental processes. Extensive studies on green tea in people, animals, and laboratory experiments suggest that green tea also may be useful for health conditions such as cancer, atherosclerosis, high cholesterol, diabetes, liver disease and weight loss (Shi *et al.,* 2005).

2.3 Tea Processing

The conversion of fresh tea leaves from the carefully nurtured tea plants into green tea, black tea or oolong tea is a very specialized process in order to maintain the final quality of the tea. The whole process is consisting of six major steps: harvesting, withering, rolling, fermentation, drying and sorting (Desa Tea Sdn.Bhd., 2008).

Harvesting is a process whereby the tea leaves and a bud are selectively harvested by using shears or mechanized cutter by tea harvesters. After that, withering is the initial stage of processing, where the freshly cut shoots are spread evenly in special racks, and conditioned air flow through and around the leaf to absorb the moisture and concentrate the juices in the leaf. In the rolling process, the withered leaf is passed through machines which twist and squeeze the juices to the surface of the leaf. This method is also popularly known as *Rotovane.* Rolling the tea leaves gives them their future form (Desa Tea Sdn.Bhd., 2008).

The rolling process is followed by fermentation which is an oxidization process where the tea juice in the presence of air oxidizes. The period of fermentation varies depending on the external conditions and there is a distinct change in colour and aroma. Fermentation is essential for the teas to be palatable. Only the black tea undergoes full fermentation while the oolong tea undergoes partial-fermentation. During fermentation, the leaves absorb oxygen which activates enzymes to create essential oils and causes chemical changes, oxidizing some of the polyphenols or what is known as

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