POLYPHENOL AND FLAVONOID CONTENTS, ANTIOXIDATIVE PROPERTY AND NUTRITIONAL VALUES OF SABAH TEA (*CAMELLIA SINENSIS)*

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I declare that this thesis is my original work except for quotations, excerpts, and summary that has been duly acknowledged.

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ABSTRACT

There were twelve types of extracts (T01 – T12) prepared from tea samples (*Camellia sinensis*) collected from Sabah Tea Plantation which were evaluated for their total polyphenol and flavonoid contents and antioxidative activity. The total polyphenols, as analyzed using the Folin-Ciocalteu method, varied from 410 mg GAE/g to 2138 mg GAE/g in the tea extracts. T01 and T08 (young tea leaves extracts) contained extremely high phenolic compounds (2138 mg GAE/g and 1246 mg GAE/g, respectively) with potent inhibition of free radicals (94% and 77%, respectively at 0.02 mg/mL) in DPPH assay. All the tea extracts showed stronger antioxidative potential compared to BHT (butylated hyroxytoluene, a synthetic antioxidant). T11 and T12 (processed BOPF and Dust tea extracts) contained the highest flavonoids content (29.88 – 35.13 mg QE/g) among all the tea extracts. Seven of the potent antioxidation tea extracts were selected to undergo nutritional values (lipid, caffeine, protein, minerals and phosphorus) determination. The lipid content ranged from 0.5 – 2.25%. T03 (organic open-air young tea leaves extract) showed the highest lipid content while T08 (conventional open-air young tea leaves extract) showed the lowest. T01 and T03 (organic young tea leaves extracts) contained the highest content of caffeine, 8.87% and 13.05%, respectively using UV analysis and 14.31% and 18.55%, respectively using HPLC analysis. Whereas, T02 and T09 (old tea leaves extract) showed the lowest caffeine content. Sodium was the major mineral while copper and zinc were the minor minerals in all the extracts. T03 contained the highest content of all the minerals (2.318 mg Na, 0.042 mg Cu, 0.053 mg Ca, 0.123 mg Fe, 0.028 mg Zn, and 0.766 mg P per gram of tea extract). Moreover, T11 and T12 showed moderate contents in all nutritional tests. In conclusion, organic shaded young tea leaves extract (T01) is the best tea extract to develop as a nutraceutical product due to the highest contents of polyphenols, antioxdative properties and some minerals content. It is also free from pesticide and thus healthier compared to other tea extracts.

ABSTRAK

*Kandungan polifenol dan flavonoid, sifat antioksida dan kandungan nutrisi dalam Teh Sabah (*Camellia sinensis*). Dua belas jenis ekstrak teh (T01 – T12) disediakan daripada Teh Sabah telah dipilih untuk menilai jumlah kandungan polifenol dan flavonoid serta activiti antioksida. Julat kandungan polifenol dari 410 mg GAE/g ke 2138 mg GAE/g dalam ekstrak teh. T01 dan T08 (ekstrak teh bagi daun muda) mengandungi komponen fenol yang tertinggi (masing-masing 2138 mg GAE/g dan 1246 mg GAE/g) dalam keadah Folin-Ciocalteu dengan halangan kepada radikal bebas (masing-masing 94% dan 77% pada 0.02 mg/mL) dalam kaedah DPPH. Semua ekstrak teh menunjukkan keupayaan antioksida yang tinggi dibandingkan dengan BHT, sejenis antioksida sintetik,. Namun begitu, T11 dan T12 (ekstrak teh bagi teh yang telah diproses) menunjukkan kandungan flavonoid (29.88 – 35.13 mg QE/g) yang tertinggi antara semua ekstrak teh. Tujuh ekstrak teh yang mempunyai antioksida yang kuat dipilih untuk menguji kandungan nutrisinya (lemak, kafeina, protein dan logam-logam). T03 (ekstrak teh bagi daun muda organik terbuka) menanda kandungan lemak yang tertinggi manakala T08 (ekstrak teh bagi daun muda tradisional) menanda kandungan yang terendah. T01 dan T03 (ekstrak teh bagi daun muda organik) mengandungi kandungan kafeina yang paling tinggi, masing-masing 8.87% dan 13.05% bagi kaedah UV dan 14.31% dan 18.55% bagi kaedah HPLC. Sebaliknya, T02 dan T09 (ekstrak teh bagi daun tua) menunjukkan kandungan yang terendah. Natrium merupakan logam utama manakala kuprum dan zink merupakan logam yang sedikit dalam ekstrak teh. T03 merupakan ekstrak teh yang mengandungi kandungan logam-logam yang terbanyak (2.318 mg Na, 0.042 mg Cu, 0.053 mg Ca, 0.123 mg Fe, 0.028 mg Zn, and 0.766 mg P per gram ekstrak teh). Tambahan pula, T11 dan T12 menunjukkan kandangan sederhana bagi semua ujian nutrisi. Konklusinya, T01 merupakan ekstrak teh yang terbaik untuk nutraseutikal kerana ia mengandungi kandungan polifenol, activiti antioksida dan beberapa logam yang tinggi. Ia juga bebas daripada pestisid dan lebih menyihatkan badan.*

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°C	Degree Celcius
%	Percentage
Abs	Absorbance
g	Gram
M	Molar
N	Normality
mg	Milligram
mg/L	Milligram per liter
mg/mL	Milligram per milliliter
mol/L	Mole per liter
µL	Microliter
µg	Microgram
mL	Milliliter
UV-Vis	Ultraviolet Visible
v/v	Volume over volume
w/v	Weight over volume

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND OF STUDY

Tea is made from the young tender shoots (flushes) of *Camellia sinensis* (Yao *et al.*, 2006). The tea plant is an evergreen tree belonging to the family of Theaceae (Weisburger, 2007). It can be cultivated in many regions that have a high humidity, fair temperature, and acidic soils, from sea level to high mountains (Dufresne & Farnworth, 2001).

Tea is one of the most widely consumed beverages in the world, next only to water (Ferruzzi & Green, 2006) and well ahead of coffee, beer, wine and carbonated soft drinks (Cheng, 2006) due to its refreshing and mildly stimulant effects (Yao *et al.*, 2006). Considering that an estimated amount of 18-20 billion teacups are consumed daily in the world, its economic and social interest is clear (Fernandez-Caceres *et al.*, 2001). Traditionally, tea was drunk to improve blood flow, eliminate toxins, and to improve resistance to diseases (Dufresne & Farnworth, 2001).

Free radicals occurring in the environment can trigger chain reactions which may cause oxidative damage to sensitive biological structures, such as DNA or cell membranes, and subsequently result in cancer, heart disease, multiple sclerosis and autoimmune diseases (Wang *et al.*, 2000). Active oxygen and free radicals are produced by certain chemical carcinogens and play a role in the carcinogenic process (Yen & Chen, 1995).

Reactive oxygen species (ROS), which include free radicals such as $\cdot O_2^-$ (superoxide anion), $\cdot OH$ (hydroxyl radical), H_2O_2 (hydrogen peroxide) and $^1O_2$ (singlet oxygen) (Wu & Ng, 2007; Yen & Chen, 1995), are considered major causes in the initiation and promotion of cancer. These unstable molecules are by-products of normal metabolism (Yen & Chen, 1995; Borek, 2005). They increase in the body during infection, inflammation and exercise and following exposure to exogenous sources such as pollution, smoking, certain medications (e.g. acetaminophen/paracetamol), and radiation, including UV radiation (Borek, 2005). The ability of free radicals to induce cancer-causing mutations in DNA and oxidize and modify critical regulatory proteins, lipids and other cellular molecules enables them to play a major role in cancer development (Borek, 2005).

ROS also contributes to cellular aging, mutagenesis, carcinogenesis, and coronary heart disease; possibly through destabilization of membranes, damage to biomolecules (e.g., lipid, protein, amino acids, and DNA) (Fang *et al.*, 2002), and oxidation of low-density lipoprotein (LDL) (Heim *et al.*, 2002). Reactive nitrogen species (RNS) also appears to contribute to the pathology of cardiovascular disease. Peroxynitrite, a potent oxidant generated by the reaction of nitric oxide (NO) with superoxide in the vascular endothelium, induces LDL oxidation and proinflammatory cytokine-mediated myocardial dysfunction (Heim *et al.*, 2002).

Fortunately, free radical formation is controlled naturally by various beneficial compounds known as antioxidants (Atoui *et al.*, 2005). Antioxidants are substances that significantly decrease the adverse effects of reactive oxygen species, reactive nitrogen species (Duh *et al.*, 2004) or both on normal physiological functions (Wang *et al.*, 2000; Ferruzzi & Green, 2006). It has been demonstrated that flavonoid compounds in tea have very strong antioxidative and free radical scavenging activities, and were much more effective than vitamins C and E at protecting cells from free radical damage (Wang *et al.*, 2000).

The antioxidative activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers. In addition, they have a metal chelation potential. The antioxidant effect of plant phenolics had been studied in relation to the prevention of coronary diseases and cancer, as well as age-related degenerative brain disorders (Atoui *et al.*, 2005; Ferruzzi & Green, 2006).

Numerous recent papers had reported effects on coronary heart disease and inhibition of carcinogenesis in experimental animals by tea or tea catechins, which constitute up to 30% on a dry weight basis (Borse *et al.*, 2007). These inhibitions had observed at all three levels of cancer progression, namely initiation, promotion, and transformation (Wang *et al.*, 2000; Ramos, 2007; Galati *et al.*, 2006). This raised the possibility that tea drinking may reduce the incidence of both cancer and heart disease in humans (Wang *et al.*, 2000).

Tea also contains minerals, and trace elements that are essential to human health, and, consequently, tea drinking could be an important source of some essential minerals such as manganese, which activates numerous essential enzymes (Fernandez-Caceres *et al.*, 2001). Because of the great importance of the minerals, total polyphenols and flavonoid content, and antioxidative property present in tea, many studies have been carried out to determine their levels in tea leaves and their infusions (Fernandez-Caceres *et al.*, 2001; Atoui *et al.*, 2005).

1.2 OBJECTIVES

The objectives of this study were:

1. To determine the total polyphenols and flavonoids content of tea extracts of *C. sinensis*.
2. To evaluate the antioxidative property of tea extracts of *C. sinensis*.
3. To determine the nutritional values (lipid, caffeine, protein, and minerals) of the potent antioxidation tea extracts (*C. sinensis*).

1.3 SCOPE OF STUDY

There were 12 different kinds of tea samples *(C. sinensis)* selected which were (1) organic, shaded, fresh young and old tea leaves; (2) organic, open air, fresh young and old tea leaves, fermented tea and sorted loose and tea bag tea; (3) non pesticide, open air, fresh young and old tea leaves, fermented tea and sorted loose and tea bag tea. All these tea samples were collected from Sabah Tea Plantation, Ranau, Sabah.

These samples were analyzed to determine the total polyphenols and flavonoids content with Folic-Ciocalteu method (Taga *et al.*, 1984) and $AlCl_3$ colorimetric method (Huang *et al.*, 2006) respectively. The 1,1- diphenyl-2-picrylhydrazyl (DPPH) assay was used to evaluate the antioxidant properties of tea extract (Atoui *et al.*, 2005; Turkmen *et al.*, 2006). The nutritional values which include lipid (Soxhlet extraction system), caffeine (ultraviolet spectrophotometric), and protein (Kjeldahl method) of the potent antioxidation tea extracts were according to AOAC method. Determination of minerals (sodium, copper, calcium, iron, and zinc) was carried out by using Atomic Absorption Spectrophotometer (AAS) according to methodologies by Gallaher *et al.* (2006) except for phosphorus (AOAC method 986.24).

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

According to Chinese legend, tea was discovered accidentally by an emperor 4000 years ago, while according to other sources the Chinese have been drinking tea since 3000 B.C. and over four million acres are devoted to its cultivation (Ferrara *et al.*, 2001).

Tea infusions, consumed by two thirds of the world's population, are obtained from the leaves of one kind of a plant named *Camellia sinensis* (Luczaj & Skrzydlewska, 2005). It is native to Southeast Asia but is currently cultivated in more than 30 countries around the world. Tea is the most consumed beverage in the world with per capita consumption of less than 120 mL in a day (Mukhtar & Ahmad, 2000). About 2.5 million tons of tea is produced in the world every year. Of the total amount of tea produced and consumed in the world, 78% is black, 20% is green, and less than 2% is oolong tea (Mukhtar & Ahmad, 2000; Luczaj & Skrzydlewska, 2005).

Tea is a dietary source of antioxidant nutrients, such as carotenoids, tocopherols, ascorbic acid and non-nutrient phytochemicals generally classified as flavonoids and also regarded as safe by the US food and drug administration (Wu & Wei, 2002). Zuo *et al.* (2002) showed that tea conferred great beneficial effects to the health of consumers, including the effects of reduction of cholesterol, depression of hypertension, anti-oxidation, anti-microbial, protection against cardiovascular disease and cancer. Tea consumption also afforded protection against cancers induced by chemical carcinogens that involve the lung, forestomach, esophagus, duodenum, pancreas, liver, breast, colon, and skin in mice, rats, and hamsters (Mukhtar & Ahmad, 2000).

2.2 TAXONOMY

The taxonomic hierarchy of tea (*C. sinensis*) is classified as (ITIS, 2007):

Kingdom: Plantae

Subkingdom: Tracheobionta

Division: Magnoliophyta

Class: Magnoliopsida

Subclass: Dilleniidae

Order: Theales

Family: Theaceae

Genus: Camellia

Species: *Camellia sinensis*

2.3 FEATURE

C. sinensis (Photo 2.1) is an evergreen tree or shrub that grows to 10 – 15 m high in the wild, and 0.6 – 1.5 m under cultivation. The leaves are short-stalked, light green, coriaceous, alternate, elliptic-obovate or lanceolate, with serrate margin, glabrous, or sometimes pubescent beneath, varying in length from up to 30 cm, and about 2 - 5 cm wide. Young leaves are pubescent. Mature leaves are bright green in colour, leathery, and smooth. Flowers are white or pinkish, fragrant, 2 – 5 cm in diameter, solitary or in clusters of two to four; sepals and petals five to seven, and pedicels 5 – 15 mm long. They have numerous stamens with yellow anthers and produces brownish-red, one-to four-lobed capsules. Each lobe contains one to three spherical or flattened brown seeds; approximately 500 seeds/kg (Ferrara *et al.*, 2001).

Photo 2.1 Feature of *C. sinensis*.

2.4 CLASSIFICATION OF TEA

Tea can be classified principally into three types which differ in how they are produced and in their chemical composition: green (unfermented), oolong (semi-fermented), and black (fully fermented) (Wang *et al.*, 2000).

Green tea is prepared by inactivating the enzymes of the fresh leaves, either by firing or by steaming, to prevent the enzymatic oxidation of catechins (Wang *et al.*, 2000). Therefore green tea contains high concentrations of monomeric polyphenols from the catechins group (Dufresne & Farnworth, 2001).

Black tea undergoes a full fermentation stage before drying and steaming (Zuo *et al.*, 2002). This fermentation process results in the oxidation of simple polyphenols (catechins) to more complex condensed molecules which give black tea its typical colour and strong, astringent flavor (Wang *et al.*, 2000). Black tea is rich in tannins which gives it an astringent quality useful in treating diarrhoea and can help relieve certain types of headaches (Ferrara *et al.*, 2001). Damp black tea bags can also be placed over tired, red eyes or on insect bites to relieve itching and redness (Ferrara *et al.*, 2001).

Oolong tea is prepared by firing the leaves shortly after rolling, and then drying the leaves (Wang *et al.*, 2000). Oolong tea is a partially oxidized product (Luczaj & Skrzydlewska, 2005). The characteristics of oolong tea are between black and green tea (Wang *et al.*, 2000).

2.5 COMPOSITION OF TEA

Fresh tea leaves contain upon average (related to dry substance mass): 36% polyphenolic compounds, 25% carbohydrates, 15% proteins, 6.5% lignin, 5% ash, 4% amino acids, 2% lipids, 1.5% organic acids, 0.5% chlorophyll as well as carotenoids and volatile substances constituting less than 0.1% (Graham, 1992; Luczaj, & Skrzydlewska, 2005) . It must be noted that the chemical composition of tea leaf varies with climatic conditions, season, position on the flushing shoot, age of leaf, and cultural practices (Spiller, 1998).

Data based on the extractable solids present in the beverage for black and green tea is shown in Table 2.1. There is no exact black tea beverage composition because of variability in starting material, manufacturing process, and preparation (Spiller, 1998).

Table 2.1 Composition of green and black tea.

	Green Tea (%)	Black Tea (%)
Catechins	30	9
Theaflavins	-	4
Simple polyphenols	2	3
Flavonols	2	1
Other polyphenols	6	23
Theanine	3	3
Amino acids	3	3
Peptides/proteins	6	6
Organic acids	2	2
Sugars	7	7
Other carbohydrates	4	4
Lipids	3	3
Caffeine	3	3
Other methylxanthines	<1	<1
Potassium	5	5
Other minerals/ash	5	5
Aroma	Trace	Trace

(Source: Spiller, 1998)

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