PRODUCTION AND CHARACTERIZATION OF PU ERH TEA FROM LOCAL TEA LEAVES

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I declare that the works presented in this dissertation are based on my own research. Sources of finding reviewed herein have been duly acknowledged.

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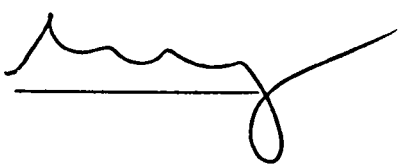

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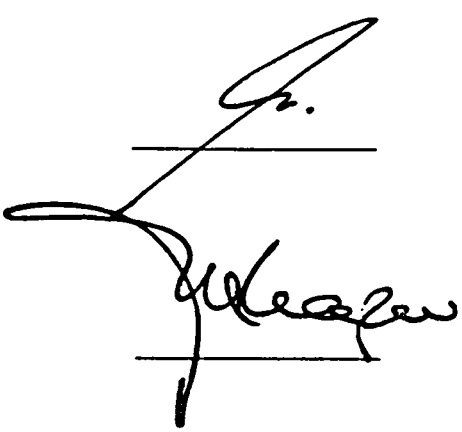

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PENYEDIAAN DAN PENCIRIAN TEH PU ERH TEMPATAN

ABSTRAK

Teh Pu erh telah menjadi salah satu minuman kegemaran para pengguna disebabkan oleh ciri-ciri kesihatan seperti, keupayaan anti oksida, penurunan cholesterol, penurunan tekanan darah and perlindungan tisu berantara. Sejumlah empat teh Pu erh komersial (T31, T32, T33, T34) dibandingkan dengan teh buatan tempatan Pu erh muda and matang (T35,T36). Jumlah kandungan polifenol, flavonoid, aktiviti anti oksida, antimikrobial dan perencat kinase telah dinilaikan bagi setiap teh Pu erh. T36 mencatat peratusan hasil ekstrak yang paling tinggi manakala T34 mempunyai jumlah kandungan flavonoid yang paling tinggi dalam ekstrak dengan menggunakan cara $AlCl_3$. Nilai IC_{50} bagi T35 dan T36 dalam kajian ini ialah 0.032 mg/mL dan 0.034 mg/mL. Keputusan ini menunjukkan potensi teh Pu erh yang lebih tinggi berbanding dengan BHT (Butylates hydroxyl toluene) dalam penyekatan radikal bebas pada kepekatan 0.10 mg/mL. Semua teh Pu erh telah menunjukkan aktiviti antimikrobial terhadap *Staphylococcus aureus and Bacillus cereus, Vibrio parahaemolytics, Listeria Monocytogenes.* Manakala hanya the Pu erh muda dan matang buatan tempatan (T35, T36) menyekat penumbuhan *Enterobacter sakazaki.* Acid gallic, catechin, caffeine, EGCG, dan ECG telah dikesan dalam semua teh Pu erh muda (T31, T35, T36) dengan menggunakan RP-HPLC. Oleh itu, the Pu erh muda adalah sumber agen antioxidan yang kuat dan berpotensi untuk mencegah penyakit yang berkaitan dengan radikal bebas.

ABSTRACT

Pu erh teas are of interest to the consumers because of it antioxidant properties, ability to reduce cholesterol, lower blood pressure and protect connective tissue. Four commercial Pu erh teas, raw Pu erh 2003 (T31), raw Pu erh 2006 (T32), mature Pu erh 2005 (T33) and mature Pu erh 2007 (T34) were used to compare with the freshly prepared raw and mature Pu erh produced by using Sabah tea leaves (T35, T36). The total polyphenol and flavanoid, DPPH radical scavenging, antimicrobial and anti kinases effects were determined to examine the tea infusion quality. T36 possessed the highest percentage yields of crude extract (6.048 g) and T34 possessed the highest total flavonoids content ($AlCl_3$ method) in crude extract in tea samples (12.9 mg QE/g). The IC_{50} value of T35 and T36 on DPPH radical scavenging was found to be 0.032 mg/mL and 0.034 mg/mL respectively, which were significantly higher than Butylated hydroxyl toluene (IC_{50} value0.13 mg/mL). All the Pu erh tea extracts inhibited the growth of *Staphylococcus aureus and Bacillus cereus, Vibrio parahaemolytics, Listeria Monocytogenes*. However, only freshly prepared raw and mature Pu erh tea (T35, T36) inhibited the growth of *Enterobacter sakazaki*. Gallic acid, catechin, caffeine, EGCG and ECG were detected in tea extracts of all the raw Pu erh (T31,T32,T35) as analyzed using RP-HPLC. Therefore, raw pu erh is a powerful source of antioxidant beverage and may be used in preventing free radical-related diseases.

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LIST OF SYMBOLS AND ABBREVIATIONS

=	Equal
>	More than
<	Less than
±	Plus, minus
~	Approximately
%	Percentage
°	Degree
°C	Degree Celsius
AD	Anno Domini
BC	Before Christ
BOPF	Broken Orange Pekoe Fannings
cm	centimetre
Da	Dalton
DAD	Diode-Array Detector
etc	etcetera
g	gram
h	hour
i.e.	That is
kg	kilogram
LC_{50}	Lethal Concentration of 50
mg	milligram
mg/g	milligram per gram
mg/mL	milligram per milli litre
min	minute
mL	milli Litre
mm	millimetre
nm	nanometre
ppm	Part per million
µg	microgram
µL	micro litre
N	North
S	South
US	United State

CHAPTER 1

INTRODUCTION

1.1 Background study

Pu erh tea, a well-known traditional Chinese tea is originated from the districts of Xi-shuang-ban-na, Si mao, and Lan-chuang-jiang valley, Yunnan Province, China. The famous tea mountains in Yunnan are Youle Shan, Nannuo Shan, Yibang Shan and Bada Shan. There are two major types of Pu erh which are raw (sheng) and mature (shu) Pu erh, depending on the processing method and the degree of fermentation. Within the raw Pu erh category, we find various grades of loose leaf sun-dried green teas made with Yunnan's broad leaf varietals. Mature Pu erh's referred to as "the cooked type" due to the fact that dried green tea leaves are transformed via natural fermentation. Yunnan province produces the vast majority of pu erh tea. Tea mountains with adequate rainfall, good drainage and high elevation met the requirement for tea plantation (Graham, 1999). Sabah Mount Kinabalu which has similar climate and geographical condition with Yunnan tea mountains shows the potential of producing Pu erh tea from local tea leaves.

1.2 Pu erh tea and health

Pu erh tea is of interest to the consumers because of its quality and health care function and distinct from other kinds of tea. Unlike other teas, the quality of Pu erh is not easy to evaluate, it is highly affected by the production process which involves fermentation. Sano *et al.* (1986) shows that longer the fermentation period of the Pu erh tea has better function in lipid reducing than the younger preparation.

Researches had been done on the polyphenol fraction in Pu erh tea on its heath beneficial properties, such as suppressing fatty acid synthase expression (Chiang *et al.*, 2006), acting as an inhibitor of lipid and non-lipid oxidative damage (Duh *et al.*, 2004) and also exhibiting metal-binding ability, reducing power, scavenging effect for free radical (Jie *et al.*, 2006) and potential antimicrobial effect (Wu *et al.*, 2007).

1.3 Objectives

The production of high quality of Pu erh tea requires precise information on the diversity available and also the biochemicals which contributes toward the tea quality. Pu erh tea is characterized by physiochemical. The objectives of this study are:

a) To produce Pu erh tea from local Sabah tea leaves.
b) To analyse the polyphenol and flavonoids content of Pu erh tea produced from local Sabah tea leaves.
c) To evaluate the antioxidant activity of Pu erh tea produced from Sabah tea leaves.
d) To evaluate the antimicrobial activity of Pu erh tea against foodborne microbial.
e) To evaluate antikinases inhibition activity of Pu erh tea.
f) To compare the locally produced Pu erh tea with the Yunnan Pu erh tea leaves.

1.4 Scope of study

Fresh tea leaves were plucked from tea plants (*Camellia sinensis*) at Sabah Tea Plantation, Ranau, Sabah and were produced into raw (sheng) Pu erh and mature (shu) Pu erh. The milled leaves sample was extracted with 70% aqueous methanol (200 mL) (Sharma *et al.*, 2005). The extracted samples were cooled and filtered. The filtrate was evaporated with a rotary evaporator around 40 – 42 °C and then concentrated to powder using freeze dryer.

The sample extracts were analyzed to determine the total polyphenols and flavonoids content with classical Folin and Ciocalteo method (Turkmen *et al.*, 2006)

using gallic acid (standard phenolic compound) as a calibrant and $AlCl_3$ colorimetric method (Huang *et al.*, 2006) respectively.

Three types of bioactivities of Pu erh tea tested included antioxidant, antimicrobial, and antikinase activity. The antioxidative activity was evaluated using a free radical 1,1-diphenly-2-picrylhydrazyl (DPPH) inhibition assay (Katalinic *et al.*, 2006). The bacteria used in the antimicrobial test were *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella typhimurium*, *Salmonella enteritidis*, *Enterobacter sakazaki*, *Listeria Monocytogenes*, *Yersina Enterocolitica*, *Vibrio parahaemolyticus* and *Escherichia coli*. Antikinases activity was elevated by $MKK1^{P386}$ screening system. The antikinases screening systems involved genetically modified yeast strains, targeting on protein kinases.

The main constituents of Pu erh tea, four major catechin, gallic acid and caffeine are analyzed by using HPLC (Zuo *et al.*, 2002).

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Tea was discovered around 5000 to 6000 years ago during the "Shen-Nong" era of ancient China (Zhen *et al*, 2002). The centre of origin of tea is in southwest of China. Tea began as a medicine and grew to beverage. The world first commentary on tea was written by Lu Yu entitled *The Book of Tea* which was later exerting a profound influence after its appearance. Tea drinking spread widely and rapidly and its health benefits were documented. As listed in one of the greatest masterpieces of traditional Chinese medicine pharmacology Compendium of Materia Medica (Ben Cao Gang Mu), tea acts as a medicine as an antidote to herbal poisons (Zhen *et al.*, 2002).

Tea production including cultivation, harvesting and processing had developed rapidly since in Tang Dynasty (618 – 906 A.D). Tea is made by processing the tea leaves (*Camellia sinensis* O. kun-tze var. assamica Kitamura) (Jen *et al.*, 2008). Tea is classified into several types according to different processing method, typically green tea (nonferment tea), oolong tea (partially fermented tea), black tea (fully fermented tea) and Pu erh tea (produce by microbial fermentation of green tea) (Jen *et al.*, 2008).

Pu erh tea is one of China's most unusual tea with long history which is originated from the districts of Xi-shuang-ban-na, Si-mao, and Lan-chuang-jiang valley, Yunnan Province, China. Pu erh tea is named after an important town in southern Yunnan and also the largest market for tea trade located just north of Simao in the Xi-shuang- ban- na district of Yunnan. The ancient tea-horse road is a significant part of the international trade of tea in history, which starts from Sichuan and Yunnan Province in Southwest China, runs along the eastern foothills and deep canyons of several major rivers in between the six famous tea mountains.

2.2 Taxanomy

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 Morphology

C. sinensis can be a shrub or evergreen tree up to 16 m tall. Leaves alternate, exstipulate, lanceolate to obovate, up to 30 cm long, 2–5 cm broad, pubescent, sometimes becoming glabrous, serrate, acute or acuminate; flowers 1–3, in axillary or subterminal cymes, deflexed, 2–5 cm broad, aromatic, white or pinkish, actinomorphic, sepals and petals 5–7, pedicels 5–15 mm long; stamens numerous; ovary 3–5 carpellate, each carpel 4–6 ovulate; capsules depressed-globose, brownish, lobate, to 2 cm broad, valvate, with 1–3 subglobose seeds in each lobe; approximately 500 seeds/kg (Duke & Atchley, 1984). Sabah tea leaves were shown in Photo 2.1.

Photo 2.1: Fresh tea leaves (*C. sinensis*) in Sabah tea plantation.

2.4 Classification of Pu erh tea

Pu erh tea can be classified in many ways by its shapes, processing methods, region, cultivation, grade, and season. Pu erh tea is commercially sold in three types of products: loose pu erh tea, pressed pu erh tea and pu erh tea bags (Jen *et al.*, 2008). However, Pu erh tea is compressed into a variety of shapes. The classification of Pu erh tea is shown in Table 2.1(Liu, 2005).

Table 2.1 Classification of Pu erh tea

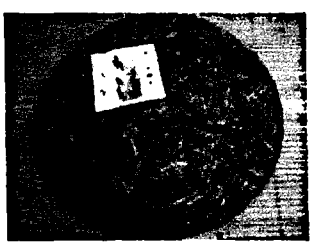
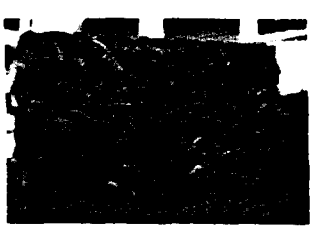
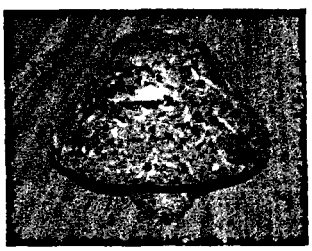
Name	Description
 Cake (Bingcha)	A round, flat, disc shaped tea which its size ranges from 100 g to 5 kg. It is also known as seven units cake tea (qi zi bing cha) due to the fact that seven of the cake are packaged together at a time for sale or transport.
 Bowl (Tuocha)	A convex knob-shaped tea with size ranging from 3 g to 3 kg.
 Brick (Zhuancha)	A thick rectangular block of tea ranging from 100g to 1 kg.

Table 2.1 continue

 Square (Fangcha)	A flat square of tea, usually in 100 g or 200g sizes which often contain words that are pressed into the square.
 Mushroom (Jincha)	A mushroom shape of pressed pu erh tea, usually in 250g or 300g.
 Gold melon (jingua)	A pumpkin-like shape tea.

Pu erh tea can be classified into two main types according to its degree of fermentation. A green tea like pu erh tea which is commonly known as raw Pu erh is process without fermentation or storage under high humidity. The fermented Pu erh is produced by microbial fermentation of piled fresh loose tea and stored at room temperature which is known as mature Pu erh (Jen *et al.*, 2008).

There are three different tea cultivation methods which is important in classifying Pu erh tea. Some tea plants are planted in a plantation bushes (guanmu) which the seeds or cutting of wild tea are planted in relatively low altitudes and flatter terrain. Wild arbor trees are tea plants from older plantation that were cultivated in the previous generation that have gone feral due to lack of care. Wild tree (gushu) is tea from wild tree which grown without human intervention and are at the highest valued Pu erh teas. These teas have deeper and more complex flavors (Liu, 2005).

2.5 Chemical composition of Pu erh tea and its bioactivity

The composition of tea varies with species, season, and horticultural conditions and particularly with degree of fermentation during the manufacturing process (Chen *et al.*, 2009). Pu erh teas undergo a full fermentation stage by using microorganism like *Aspergillius niger*. During the fermentation, tea catechins in Pu erh are oxidized and condensed to other large polyphenolic molecules such as theaflavins and thearubigins. This oxidation gives a low content of catechin in Pu erh tea compare to green tea and semi-fermented oolong tea (Zuo *et al.*, 2002).

The microorganism in Pu erh tea oxidized tea polyphenols more completely than the enzymatic oxidation process which occurs in black tea, resulting in lower concentration of tea polyphenols and tea catechins (Xie *et al.*, 2009). During black tea processing, fresh tea leaves are rolled and cut before drying so that tea polyphenols in tea leaves come into contact with the tea polyphenol oxidizes and then oxidized in the consequences of fermentation process. However, during Pu erh tea fermentation process, fresh tea leaves are fixed by heat in a drum to inactivate polyphenol oxidizes. The fixed tea leaves is then rolled and partially dried and piled up in humid conditions for a week. The tea polyphenol is more intensively oxidized by the action of microorganism (Mo *et al.*, 2008).

2.5.1 Tea polyphenol

Tea polyphenols, previously called tea tannins, account for 30% of the dry weight of the fresh tea leaves. Catechins are predominant form of polyphenols which account for 12 – 24% of dry weight. Besides catechins, flavonol, and their glycosides, anthocyanidin and leucoanthocyanidin, phenolic acid and despides also present. These phenolic compounds are directly or indirectly associated with the characteristics of tea, including its color, taste, and aroma. The polyphenol contents known to be present in Pu erh tea samples, such as gallic acid, (-) epicatechin (EC), (-) epigallocatechin (EGC), (-) epigallocatechin gallate (EGCG) and epicatechin gallate (ECG), catechin (C), myricetin, quercetin, and kaempferol. (Lu *et al.*, 2008).

2.5.1(a) Tea flavonoids

Tea flavonoids are largely responsible for the distinctive taste and color of tea. The common flavonoids in tea are the flavan-3-ols (flavanols or flavans). The flavan-3-ols subclasses are ranked by degree of polymerization. The catechins are monomers which consists of (-) epicatechin (EC), (-) epigallocatechin (EGC), (-) epigallocatechin gallate (EGCG) and epicatechin gallate (ECG). However, the theaflavins are dimmers, such as theflavin, theflavin 3-gallate, theaflavin 3'-gallate, theaflavin 3,3'-digallate), and the derived tannins the arubigins are oligomers of unknown structure.Other flavonoids, including the flavonols (quercetion, kaempferol, mycertin) and flavones (apigenin and luteolin), are also present but lesser amount then flavor-3-ols (Peterson *et al.*, 2004). Flavonoids are colourless, water-soluble compound which impart bitterness and astringency to green tea infusion (Wang *et al.*, 2000). Total flavonoids content could be used to indicate the quality potential of tea, with high content being related to high quality (Magoma *et al.*, 2000). The characteristics of manufactures tea mostly are associated directly or indirectly with modifications to the flavonoids. During green tea manufacture, most catechins and other polyphenols are preserved owing to inactivation of the andogenous enzymes by dry heating or steaming at the initial step.

A vast body of scientific researches and studies has suggested that catechins are responsible for the majority of the potential health benefits attribute to tea consumption. Among the catechins, EGCG is most effective in reacting with most reactive oxygen species. This chemical structure contributes to effective antioxidant activity of catechins including the vicinal dihydroxyl or trihydroxyl structure which can chelate metal ions and prevent the generation of free radicals. This structure also allows electron delocalization, conferring high reactivity of quench free radicals. Free radicals occurring in the environment can trigger chain reaction which may cause oxidative damage to sensitive biological structures, such as DNA or cell membranes, and subsequently result in cancer, heart diseases. Studies in animal models suggest that EGCG may reduce some of the harmful effects following to exposure of UV radiation, and also inhibit cervical cancer cell growth (Nagle *et al.*, 2006). Epicatechin gallate (ECG) is reported to inhibit growth of some of the cancer cell lines (Vergote *et al.*, 2002) but not their normal counterparts.

Other catechins are also present in smaller amounts: gallocatechin, epigallocatechin digallate, 3-methylepicatechin gallate, catechin gallate, and gallocatechin gallate (Luczaj *et al.*, 2005). Figure 2.1 shows the chemical structure of major flavonoids in tea.

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