COMPARISON OF CHEMICAL AND BIOLOGICAL CHARACTERISTICS OF GREEN TEA, OOLONG TEA AND PU-ER TEA

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PERBANDINGAN CIRI-CIRI KIMIA DAN BIOLOGI ANTARA TEH HIJAU, TEH OOLONG DAN TEH PU-ER

ABSTRAK

Teh (Camellia sinensis) merupakan antara minuman yang paling popular dan dinikmati di merata tempat oleh hampir dua pertiga daripada populasi di dunia ini. Kini, pemprosessan teh yang berlainan di merata tempat di dunia ini dapat menghasilkan pelbagai jenis teh seperti teh hijau, teh oolong dan teh pu-er. Dalam kajian ini, lapan jenis sampel teh yang berlainan jenis yang terdapat di pasaran digunakan untuk membuat kajian. Sampel – sampel itu kemudiannya diekstrak dengan menggunakan 70% metanol. Di antara teh sampel yang digunakan laitu teh hijau A (T27), teh hijau B (T28), teh sul xian (T29), teh wang pi (T30), teh mentah pu-er 2003 (T31), teh mentah pu-er 2006 (T32), teh masak pu-er 2005 (T33) dan teh masak pu-er 2007 (T34), teh sampel T34 mempunyai peratusan estrak kasar yang paling tinggi (28.15%). Kesemua estrak metanol teh memberikan jumlah kandungan polifenol antara 343.66 mg GAE/g - 497.33 mg GAE/g. Antaranya, estrak T31 memberikan jumlah kandungan polifenol yang tertinggi (497.33 mg GAE/g). Estrak methanol teh itu dikaji dengan menggunakan RP-HPLC bagi menentukan komponen-komponen utama polifenol seperti katechins (EGCG, ECG), acid galic dan kafeine dalam estrak teh. Dalam penentuan kandungan flavonoid, didapati T32 mengandungi jumlah kandungan yang paling banyak (16.40 mg QE/g). Kesemua estrak methanol teh menunjukkan potensi yang lebih tinggi variasi dari 47.76% - 90.26% berbanding dengan 32.77% BHT (Butylated hydroxy toluene) dalam penyekatan radikal bebas pada kepekanan 0.10 mg/mL. Aktiviti antimikrobal dariipada ekstrak teh telah dikaji dengan terhadap Bacillus cereus, Escherichia coli, Listeria monocytogenes, Yersina enterocolitica, Vibno parahaemolyticus, Enterobacter sakazaki, Salmonella enteritidis, Salmonella typhimurium, dan Staphylococcus aureus dengan menggunakan kaedah peresapan disk. Kesemua estrak metanol teh hanya menunjukkan aktiviti perencatan pertumbuhan yang sederhana terhadap 4 bakteria laitu Bacillus cereus, Listeria monocytogenes, Vibno parahaemolyticus, dan Staphylococcus aureus. Kajian juga menunjukkan bahawa hanya T27, T28, T30 dan T31 memberikan aktiviti yang merencat MKK1 dalam laluan MAPK dan hanya T27 memberi aktiviti positif dalam perencatan pertumbuhan pada MSG5. Kesimpulannya, kajian terkini mendapati teh hijau dan teh oolong tidak mempunyai perbezaan yang tetapi teh pu-er mempunyai perbezaan yang ketara antara teh mentah pu-er dan teh masak pu-er. Keseluruhannya, teh mentah pu-er memberikan keputusan yang lebih baik daripada teh masak pu-er dalam kesemua ujian fitokimia dan biologi yang telah dilakukan. Selain itu, data menunjukkan ekstrak teh mempunyai anti oksida aktiviti yang baik. Oleh itu, teh ekstrak adalah sesuai digunakan sebagai bahan pengawet semulajadi dalam penyedian makanan.
ABSTRACT

Tea (*Camellia sinensis*) is one of the most popular beverages consumed by over two third of the world’s population. Tea is processed differently in different parts of the world to give green tea, pu-er tea or oolong tea. In this study eight commercial tea samples of *C. sinensis* were extracted with 70% methanol. The samples were green tea A (T27), green tea B (T28), sul xian (T29), wang pi (T30), raw pu-er 2003 (T31), raw pu-er 2006 (T32), mature pu-er 2005 (T33) and mature pu-er 2007 (T34). T34 yielded the highest percentage of crude extract (28.15%). The methanolic tea extracts had total polyphenols content within 343.66 mg GAE/g – 497.33 mg GAE/g as analyzed using Folic-Ciocalteu method. Among them, T31 had the highest polyphenols content (497.33 mg GAE/g). The methanolic tea extracts were employed for qualitative analyses using RP-HPLC and were found to have the major phenolic compounds such as catechins (EGCG, ECG), gallic acid and caffeine in the tea extracts. In addition, the methanolic tea extracts exhibited flavanoids content varied from 9.00 mg QE/g – 16.40 mg QE/g as analyzed using aluminium chloride (AlCl₃) colorimetric method. T32 had the highest flavanoids content (16.40 mg QE/g). In DPPH assay, the tea extracts had strongly scavenged activity of DPPH free radicals (varied from 47.76% - 90.26% compared to 32.77% by BHT, a synthetic antioxidant at 0.10 mg/mL). The antimicrobial activity of methanolic tea extracts were evaluated with *Bacillus cereus*, *Escherichia coli*, *Listeria monocytogenes*, *Yersina enterocolitica*, *Vibrio parahaemolyticus*, *Enterobacter sakazaki*, *Salmonella enteritidis*, *Salmonella typhimurium*, and *Staphylococcus aureus* by using disc diffusion method. All the tea samples were found active against four bacteria which were *Bacillus cereus*, *Listeria monocytogenes*, *Vibrio parahaemolyticus*, and *Staphylococcus aureus*. Present study shows that only T27, T28, T30 and T31 had potential inhibitory activity against MKK1 in signal transduction pathway and among them only T27 showed positive inhibition to MSG5. It is concluded that green tea and oolong tea do not show a very distinct difference between them. However, pu-er tea, shows distinct difference between raw pu-er tea and mature pu-er tea. Overall, raw pu-er has better results in all the phytochemical and biological tests that had run compare to mature pu-er. Besides, data in this study shows that phenolic and alkaloid extract of tea displayed good antioxidant activity. Therefore, tea extracts could be a promising source of natural preservatives in foodstuff.
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CHAPTER 1

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Tea plant is a kind of evergreen laurel plant tree and is taxonomically classified as *Camellia sinensis* (L.) O. Kuntze of the family of *Theaceae*. Tea plant has grown widely from tropical to temperate regions in Asia and has been closely associated with people's life since the dawn history. 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 the oldest non-alcoholic caffeine containing beverage in the world. Chinese were the first to use tea as medicinal drink, later as beverage and have been doing so for the past 3000 years (Eden, 1958). Traditionally, tea was drunk to improve blood flow, eliminate toxins, and to improve resistance to diseases (Dufresne & Farnworth, 2001).

There are various types of tea leave sold in the market but they can be differentiated by the content of caffeine, polyphenols, and theanine in the leaves which these characteristics also determine the taste of the tea infusion (Nagata & Sakai, 1984). One of the most important processes in tea manufacturing for drink is fermentation. The degree of fermentation greatly affects the quality and type of tea and according to this degree of fermentation, tea is classified into green tea (unfermented), oolong tea (partially fermented), and pu-er tea (fully fermented).
Green tea is made by inactivating the enzymes in the fresh leaves, either by firing or by steaming, to prevent the enzymatic oxidation of catechins (Wang et al., 2000). Oolong tea is prepared by firing the leaves shortly after rolling, and then drying the leaves. The oxidation is ended by the firing process; hence oolong tea is called semi-fermented tea (Wang et al., 2000). While, black tea is made by a polyphenol oxidase catalysed oxidation of fresh leaf catechins, termed fermentation (Wang et al., 2000).

Tea plant contains various useful chemical compounds and also biological characteristics. In tea plant, the chemical compounds that exist are tea polyphenols, caffeine, theanine, antioxidant, and others (Nagata & Sakai, 1984). The biological activities exist in tea plants are antimicrobial activity, anti-kinase activity and others.

Variation in chemical composition in tea is due to the degree of fermentation of tea leaves, environmental conditions, cultivated varieties and also time decision or season in harvesting tea leaves. The essential variation in chemical composition is the degree of fermentation that affects biological activities in tea. Fermentation of tea leaves will cause reduction in the concentration of catechins (Chou et al., 1999). Catechins are colourless and water-soluble compound which determine the bitterness (taste), colour and aroma of the tea (Wang et al., 2000). During fermentation enzymatic oxidation of catechins takes place leading to formation of a series of coloured chemical compounds, theaflavins (TFs) and thearubigins (TRs) which responsible for the aroma and colour properties of tea (Liang et al., 2003). Besides, concentration of catechins also influences the antimicrobial activity in tea in which the higher concentration of catechin in tea, the stronger antimicrobial activity tea occurs (Cheng et al., 1999).
Non-fermentated green tea has highest catechins concentration displayed light orange-coloured and has strongest antimicrobial activity while fully-fermented pu-er tea displayed dark brown-coloured and has lowest antimicrobial activity. Oolong tea possess characteristic in between green tea and pu-er tea (Cheng et al., 1999).

Many researches has proven that tea regardless is green tea, oolong or pu-er tea contain many phenolic compounds that bring beneficial effects to human health in the past decade (Blot et al., 1996). Nowadays, there are many different kinds of tea products available in the markets, and each is prepared differently. For example, the tea leaves are not fermented or are fermented to various extents. Furthermore, tea products are manufactured with tea leaves prepared at different degree of fermentation will affected the chemical profiles existing in the tea leaves. These variations affect the flavor and aroma of tea products (Kan, 1980), and, therefore, may also affect their biological activities.

1.2 OBJECTIVES OF THE STUDY

The objectives of the study are:

a) To characterize the chemical profiles of green tea, oolong tea and pu-er tea.

b) To evaluate the biological activities of green tea, oolong tea and pu-er tea.

1.3 SCOPE OF STUDY

In the study, tea samples were chosen from among the most well-known and most consumed brands of green tea, oolong tea and pu-er tea available in the market. Samples collected are 2 different brands for green tea, oolong tea, mature pu-er tea and raw pu-er tea respectively from commercialize brand available in market. Then, all these samples were extracted with 70% aqueous methanol.
The tea extracts samples were characterized to determine the chemical profiles such as determination of total polyphenols content and caffeine content using reverse-phase HPLC, determination of flavonoids content according to the Aluminium Chloride (AlCl₃) colorimetric method (Huang et al., 2006) and also determination of antioxidative activity of the tea extracts is tested using 1,1- diphenyl-2- picrylhydrazyl (DPPH) assay (Turkmen et al., 2006) with a minor modification. Tea extracts samples were also screened through some biological activities bioassay screening such as anti-microbial activity screening with 9 targeted bacteria Bacillus cereus, Escherichia coli, Listeria monocytogenes, Yersina enterocolitica, Vibno parahaemolyticus, Enterobacter sakazaki, Salmonella enteritidis, Salmonella typhimurium, and Staphylococcus aureus and also anti-kinase activity screened by using the M KK1 P386 Inhibitor Screening Assay.

Based on the tests that were run on the tea extracts, the chemical profiles and biological activities were obtained and a comparison between the chemical and biological properties of green tea, oo-long tea and pu-er tea has been done based on the result obtained.
2.1 THE BACKGROUND OF TEA

Tea made from the leaves of *C. sinensis* is one of the most popular beverages consumed by over two thirds of the world’s population. Tea is also the oldest non-alcoholic caffeine containing beverage in the world which has attractive aroma, taste and health-promoting effects that why tea is the most popular drink (Lin et al., 2003). As early as 3000 BC, the Chinese used tea as a medicinal drink and by the end of the sixth century as a beverage (Kuroda & Hara, 1999). As far as being tracked, during Tang Dynasty (618 to 906 A.D.) tea is already became a popular drink among the Chinese. The medical use of tea was recorded in the ancient Chinese pharmacopoeia “Ben Cao Gang Mo” written by Shi-Zheng Li in the Ming Dynasty in 16th century (Lin et al., 2003). In 1644, sailors began bringing tea packages from the Far East to the United Kingdom.
The Tea plant *C. sinensis* (L.) Kuntze is a perennial leafy crop where it is grown in about 30 countries worldwide (Graham, 1992). It grows best in tropical and subtropical areas where a warm and humid climate with adequate rainfall, good drainage and slightly acidic soil (Graham, 1999). A Chinese idiom says, “A higher mountain yields higher quality tea,” which indicates that the mountain conditions are optimum to tea plant growth, especially the growth of high-quality flush (Tong *et al.*, 2000). Moreover, tea quality is also determined by the processing techniques employed. Considerable interest has developed in the past decade in unraveling the beneficial health effects of tea, particularly in its polyphenolic components and its antioxidant activity.

### 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 ORIGIN AND FEATURES OF TEA

The original home or 'the primary center of origin' of tea was South-East Asia that is at the point of intersection between the 29° N (latitude) and 98° E (longitude) near the source of the Irrawaddy river at the confluence of North-East India, North Burma, South-West China and Tibet provinces (Mondal, et al., 2004). The cultivated taxa comprise of three main natural hybrids. They are: *C. sinensis* (L.) O. Kuntze or China tea, *C. assamica* (Masters) or Assam tea and *C. assamica* sub spp *lasiocalyx* (Planchon ex Watt.) or Cambod or Southern tea. The *C. sinensis* var. *sinensis* (China tea) is grown extensively in China, Japan and Taiwan, while *C. sinensis* var. *assamica* (Assam tea) predominates in South and Southeast Asia, including Malaysia (Adiwinata et al., 1989) and more recently, Australia (Caffin et al., 2004).

Tea often planted in highlands. In India and Sri Lanka, it is cultivated at elevations up to 2000 m asl (Graham, 1999). In plantations, tea is planted at a density of 5000 – 10,000 plants per hectare and maintained as low shrubs through regular pruning during harvesting. *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 varying in length from up to 30 cm, and about 2 – 5 cm wide. The young leaves are pubescent while the mature leaves are bright green in colour and smooth in texture. Tea plants can grow flowers and the flowers are white or pinkish, fragrant, 2 – 5 cm in diameter, and born singly or pairs at the axils. They have many stamens with yellow anthers and produce one-to four-lobed capsules with brownish-red in colour. Each of this lobe contains one to three spherical or flattened brown seeds; approximately 500 seeds/kg (Ferrara et al., 2001). The fruits are green in colour with 2–3 seeds and start bearing within 5–6 years after planting.
Leaf is the main criterion by which three types of tea are classified (Mondal et al., 2004). Briefly, the Assam tea has biggest leaves (leaf length 16 – 19 cm and width 7 – 9 cm). While china tea has smallest leaves (leaf length 5.5 – 6.1 cm and width 2.2 – 2.4 cm) and the Cambod tea leaves size are in between of Assam and China tea. Today, 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, which is usually consumed in the western countries, 20% is green, which is commonly consumed in Asia countries, and less than 2% is oolong tea which is produced mainly in southern China (Mukhtar & Ahmad, 2000; Luczaj & Skrzydlew ska, 2005).

The feature of *C. sinensis* describe above is shown in Photo 2.1 taken at the Sabah Tea Garden, Ranau, Sabah.

![Photo 2.1 Feature of C. sinensis](image.png)

### 2.4 CLASSIFICATION OF TEA

Tea is now consumed throughout the world not just as a popular beverage, but, because its extracts have been prepared in a variety of physical forms in the range from strong infusion, soft extracts to powders, it is now widely available in a range of food, beverage, toiletries and cosmetic products (Wang et al., 2000). Generally, tea are classified into three major categories which is the non-fermented green tea, the partially fermented oo-long tea and also the fully fermented pu-er tea (Lin et al., 1998).
Tea is made from the tender leaves of two varieties of the plant *C. sinensis*: *assamica* and *sinensis* (Wang *et al.*, 2000). The composition of tea varies with species, season, age of the leaf (plucking position), climate and horticultural practices (Lin *et al.*, 1996). The production stages of various kinds of tea are similar except for fermentation and aroma formation. In recent years tea has been attracted more and more attention because of the reported health benefits, in particularly as an antioxidant, but also as an anticarcinogenic and antiarteriosclerotic agent. It is generally believed that flavanoids are mainly responsible for these actions.

Tea is grown in thousands of tea gardens around the world, resulting in thousands of flavourful variations. The fresh tea leaves are usually used for tea manufacturing and are harvested by hand plucking or mechanical plucking. The well-known high-quality green teas are mostly produced from hand-plucking fresh tea leaves in China (Ho *et al*, 2008). Leaves of the *C. sinensis* plant are dried for stability and shelf life. The mode of processing the fresh tea leaves and their extent of exposure to oxygen determine the types of tea.

One of the most important processes in tea manufacturing for drink is fermentation. The tea leaves contain the polyphenol epigallocatechin and an enzyme (Welsburger, 2000). Therefore, the conversion of tannin in tea leaves is not achieved by microorganisms but by the enzymes present in the leaves, and so, this phenomenon should be called "enzymation," exactly. The so-called fermentation in tea processing is not the anaerobic breakdown of an energy-rich compound (as a carbohydrate to carbon dioxide and alcohol or to an organic acid), but in essence is mainly the oxidative polymerization and condensation of catechins catalyzed by endogenous polyphenol oxidase and peroxidase. The oxidation products such as theaflavins and thearubigins contribute to tea color and the taste of tea (Balentine *et al.*, 1997). The degree of fermentation greatly affects the quality and type of tea. According to the degree of fermentation, tea is classified three main categories which is the non-fermented green tea, the partially fermented oolong tea and the fully fermented pu-er tea (Lin *et al.*, 1998).
2.4.1 GREEN TEA

A distinctive feature of green tea processing is that the tea leaves are never subjected to the condition for causing fermentation. The common young tea shoots required are one bud and two or three leaves, while those for famous high-quality green tea are usually one bud and one leaf, even just one bud (Chen 1986). Shading of the tea plant increases the amount of amino acids, especially of theanine in young shoots, and decreases the polyphenol content (Mukai et al., 1992). The quality of fresh tea leaves plays a key role in the characteristics of green tea.

In order to prevent fermentation (the oxidation of catechins) and formation of the characteristic "green leaf, green liquor" of green tea, the leaves are immediately fixed to inactivate the endogenous enzymes by steamed or pan-fried at 90 – 100 °C. The steaming treatment protects degradation of vitamins and thus, the content of vitamins in green tea is much higher than fermented teas (Yamanishi, 1981). Then, the steamed leaves are rolled to make slender pickle form followed by drying in current air of moderate temperature. The moisture content of tea leaves that is usually 78-80% will decrease to about 10% during rolling process. the rolling process disrupt the leaf tissues and mixes them uniformly in the shape. The drying subsequently applied increases aroma and preservability of the products. The rolled and dried leaves called Aracha are finally fired (roasted) and cut to prepare the final products. The firing treatment is also important to remove the original coarse aroma and add a fired tea flavor (Hara, 1989).

According to the fixation ways, green tea is subdivided into steamed green tea and pan-fried green tea. Steamed green tea is mainly produced and consumed in Japan, while pan-fried green tea is mainly produced in China and exported to the world. The process of manufacturing green tea is summarized in figure 2.1.
2.4.2 OOLONG TEA

Oolong tea, obtained by partially fermented tea leaves and is an intermediate product of green tea and pu-er tea. Oolong tea has an excellent characteristic with the combination of the freshness of green tea and the fragrance of black tea. It is produced by a special process called green leaf shaking (yao qing) and green leaf cooling (liang qing). In this process, the moderately withering green tea leaves are bruised at the edges by hand or mechanical shaking and vibrating. The leaf appearance of oolong tea is featured with the reddish edges and green centers. Oolong tea is produced in China, particularly in Fujian, Guangdong, and Taiwan, and is currently popular in China and Southeastern Asia. It has a good function in helping body building and dieting (Chen, 1986).

Oolong tea is prepared by firing the leaves shortly after rolling, and then drying the leaves. The oxidation is ended by the firing process; hence, oolong tea is called semi-fermented tea. The characteristics of oolong tea are between black and green tea. The manufacturing of oolong tea is summarized in figure 2.2.
REFERENCES


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