# ANTIOXIDATIVE AND ANTIMICROBIAL ACTIVITIES OF BLACK TEA

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## ABSTRACT

Chemistry and biological activities of black tea are not well defined, thus, it should be investigated to better understand their properties and efficiencies. Dried materials of Boh Black Tea, Lipton Black Tea and Sabah Black Tea from the leaves of Camellia sinensis plant were extracted via reflux using solid-solvent extraction with 70% methanol in distilled water to obtain crude extracts. Total phenolic contents, antioxidative activity and antimicrobial activities of the tea extracts were investigated. The total phenolic content, determined according to the Folin-Ciocalteu method, varied from 83.6709 to 101.3817 mg GAE/g dry weight. Antioxidative activities of the extracts were measured using the Ferric Thiocynate (FTC) method to determine semiquantitatively of the inhibitory capacity against linoleic acid oxidation relative to butylated hydroxytoulene (BHT). The results showed that the three extracts appeared as strong antioxidants comparable to BHT. The relative absorbance values of these three extracts almost similar to each other indicating that they possessed a similar efficiency in inhibitory activity against linoleic acid peroxidation. Results showed that there was a significant positive relationship between the total phenolic contents and anti-oxidation activities of all extracts tested. Antimicrobial activity test was carried out using a disc diffusion method against five bacteria species. The results showed that all of the three extracts showed strong inhibiton activity against S. aureus S 277, B. cereus B 43/04 B (10-15 mm) and moderate range of diameter inhibition zone against S. typhimurium S 1000 and E.coli E 91/026 (1-3 mm). However all of the samples had no bacteriostatic inhibiting activity against P. aeruginosa AT CC 10145. Conclusively, from this study, it is known that black tea could be as effective as green tea as a healthy beverage.



#### AKTIVITI ANTI-OKSIDA DAN AKTIVITI ANTI-BAKTERIA TEH HITAM

#### ABSTRAK

Aktiviti-aktiviti biologi dan kimia teh hitam adalah kurang jelas dikenali. Maka, ia harus dikaji untuk memahami ciri-ciri dan keberkesanannya dengan lebih mendalam. Tiga jenis serbuk kering Teh hitam Boh, Teh hitam Lipton dan Teh hitam Sabah yang berasal dari tumbuhan Camellia sinensis diekstrak melalui refluks dengan kaedah ekstraksi pepejalcecair dengan menggunakan 70% metanol dalam air suling untuk mendapatkan ekstrakekstrak mentah. Kajian jumlah kandungan polifenol, aktiviti anti-oksida dan aktiviti antibakteria telah dijalankan. Jumlah kandungan polifenol dinilai berdasarkan kaedah Folin-Ciocalteu menunjukkan keputusan berbeza daripada 83.6709 kepada 101.3817 mg GAE/g berat kering. Kajian aktiviti anti-oksida telah dijalankan melalui kaedah Ferric Thiocynate (FTC) untuk menentukan kemampuan ekstrak-ekstrak dalam perencatan terhadap pengoksidaan asid linoleik secara semi-kuantitatif dan relatif kepada 'butylated hydroxytoluene' (BHT). Keputusan menunjukkan ketiga-tiga ekstrak adalah anti-oksida yang kuat di mana aktiviti anti-oksidanya kurang sedikit daripada BHT. Nilai kerelatifan ketiga-tiga ekstrak adalah hampir sama antara satu sama lain menunjukkan mereka memiliki keupayaan yang hampir sama dalam aktiviti perencatan atas pengoksidaan asid linoleik. Kajian aktiviti anti bakteria dijalankan dengan menggunakan kaedah peresapan cakera ke atas lima spesies bakteria. Ketiga-tiga ekstrak menunjukkan aktiviti perencatan yang kuat terhadap penumbuhan bakteria jenis S. aureus S 277 dan B. cereus B 43/04B (10-15 mm) tetapi hanya menunjukkan aktiviti perencatan yang lemah terhadap S. typhimurium S 1000 dan E.coli E 91/026 (1-3 mm). Akan tetapi ia tidak menunjukkan aktiviti penyekatan penumbuhan bakteria jenis P. aeruginosa AT CC 10145. Kesimpulannya, melalui kajian ini, didapati bahawa teh hitam dapat bertindak sebagai minuman sihat seperti teh hijau dengan berkesan.



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## Photo 2.1 Manufacturing process of tea

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# LIST OF SYMBOLS AND ABBREVIATIONS

>	inequality sign; greater than
<	inequality sign; less than
=	equal sign
%	percentage
°C	degree celcius
mg	milligram
mL	milliliter
mgmL <sup>-1</sup>	milligram per milliliter
μg	microgram
nm	nanometer
Fe	iron
Mg	magnesium
HCl	hydrochloric acid
FTC	Ferric Thiocyanate
BHT	butylated hydroxytoluene
DMSO	dimethyl sulphoxide
NA	nutrient agar
GAE	gallic acid equivalents



## **CHAPTER 1**

## INTRODUCTION

## 1.1 Background of The Study

Dietary habits play an important role in animal and human health, and in the development of a variety of diseases, including cancer and heart disease. The use of dietary substances is receiving increasing attention as a practical approach for reducing the risk of developing these diseases (Caffin *et al.*, 2004).

Consumer awareness and concerns over the potential risks of synthetic food additives to human health have renewed the interests in using naturally occurring alternatives as their dietary substances (Harbowy & Balentine, 2004). A number of substances contained in foodstuffs, for instance, antioxidant compounds, seem to exert an effective protective action. In addition to fresh vegetables, also other foods contain antioxidants such as fruits, red wine, coffee, cocoa and herbal products. And this is true of tea, above all types of tea (Caffin *et al.*, 2004).



Today, tea chemistry has led both consumers and researches to debate numerous issues and to probe for a deeper understanding of the nature of this beverage. With the growing popularity of tea and increased awareness of the potential health benefits associated with tea consumption, tea chemistry promises to endure as a growing and vibrant field (Harbowy & Balentine, 2004).

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). Traditional Chinese medicine has recommended tea for body aches and pains, digestion, enhancement of immune defences, detoxification, as an energizer and to prolong life. Now many of these health benefits are confirmed (Smith *et al.*, 2002).

Today, India has the largest total consumption of tea (540 000 metric tons, 620 g per capita) and three billion kilograms of tea are produced each year (Kris-Etherton & Keen, 2002). With the increasing consumption of tea, quality control of tea becomes more and more important nowadays, for example, many national and international authorities are setting criteria for quality factors (Cowan, 1999).

The rapidly increasing number of reports on biological effects of tea and its biochemical components reflects the growing interest of scientists in the pharmacological potential of tea plant extracts (He *et al.*, 2006). Among a large variety of biological activities, antioxidant, anti-inflammatory and anticarcinogenic properties are the most



prominent features of tea. Whereas epidemiological evidence in humans for an inhibitory activity of the popular beverage on carcinogenesis is still debated, experimental studies clearly demonstrate chemoprevention of cancer development, growth delay in existing solid tumors, and prohibition of metastasis formation by tea (Hara *et al.*, 1995).

Recently, many researchers have carried out studies into health effects of tea, including the effects of reduction of cholesterol, depression of hypertension, antimicrobial and protection against cardiovascular disease (Ferrara *et al.*, 2001). To understand the mechanisms involved in these beneficial effects, a great deal of scientific efforts has been contributed to isolate and identify active components in various tea samples. Polyphenols, especially catechins and phenolic acids, have been considered as a main player in these beneficial effects on the human health (Bailey *et al.*, 1990).

An interest in studying polyphenols is due to the fact that many polyphenolscontaining products display a pronounced preventive activity against a number of degenerative diseases. In particular, tea has been reputed for years to prevent tumours and oncologic disease (Yang *et al.*, 2000). An increased interest in studying polyphenols began in the early 1990s, when tea was recognized as an agent preventing atherosclerosis (so-called "French paradox"). Despite the abundance of published work devoted to the antioxidant activity of tea polyphenol, only a few studies present rational and reproducible information (Harbowy & Balentine, 1997).



Black tea is a major form of tea consumed, but its chemistry, biological activities, and chemopreventive properties are not well defined and its health advantages are less studied than those of green tea. Black tea is assumed as less beneficial compared to green tea, however, reports have demonstrated that black tea could be as effective as green tea in cancer chemoprevention. Because information on the biological activities of black tea is not well documented, thus, black tea should be investigated and studied to better understand their properties, safety and efficiency (Liang *et al.*, 1999).

Although a considerable body of information provides evidence supporting the preventive potential of tea against cancer, the outlook for its uses in the future is still uncertain. Therefore, a proper understanding of the mechanisms by which tea polyphenols reduce the risk of diseases is necessary to devise strategies for a better health (Wright, 2005).

## 1.2 Objectives of The Study

The main objectives of this study were

- (a) To determine polyphenol content in black tea extracts.
- (b) To evaluate antioxidative and antimicrobial properties of the black tea extracts.



## 1.3 Scope of The Study

In this study, three different brands of black tea (*Camellia sinensis L.*) are extracted and tested for their polyphenol content. The brands of black tea selected in this study are: Lipton black tea, Boh black tea and Sabah black tea. The commercial Lipton black tea bag and Boh black tea bag are purchased from local market while Sabah black tea bags is obtained from Sabah Tea Garden, Ranau, Sabah.

The total polyphenol content of methanolic extracts of black tea extracts is tested using Folin-Ciocalteu method. Antioxidative and antimicrobial activities will be also tested on tea extracts using Ferric thiocyanate (FTC) method and disc diffusion methods against *Staphylococcus aureus* S 277, *Escherichia coli* E91/026, *Bacillus cereus* B43/04B, *Pseudomonas aeruginosa* AT CC 10145 and *Salmonella typhimurium* S1000 respectively.



## **CHAPTER 2**

## LITERATURE REVIEW

## 2.1 Natural Products

Natural products chemistry has always been concerned with nature and natural phenomena and, as a consequence, biologically active metabolites. Natural products are naturally occurring organic compounds that posses a variety of biological activities and have long served as sources of therapeutic drugs and lead structures (Ghisalberti, 1993). It is isolated from higher plants which had provided novel, clinically active drugs. The key to the success of discovering naturally occurring therapeutic agents rests on bioassay-guided fractionation and purification procedures. Screening of both synthetic organic compounds and extracts of natural products has had an impressive history of identifying active agents (Dao *et al.*, 1996).

Malaysia is blessed with enormous biodiversity resources with about 12 000 species of flowering plants of which about 1300 are said to be medicinal, and only about a hundred have been investigated fully for their potential (Mann *et al.*, 1996). The huge diversity of the Malaysian flora implies that we can expect well diversed chemical



structures from their secondary metabolites. In addition, chemical diversity is one of the additional factors that make myriad natural products excellent candidates in any screening programme for drugs discovery development (Mann *et al.*, 1996).

The development of medicinal plants including tea into therapeutic drugs take several years and millions of dollars are needed, thus making the process very capitalintensive and high risks. Beside all this, natural products drugs discovery programmes are still exist all over the world, mainly due to the high chemical diversity from natural products as compared to synthetics, and the potential of these natural products is largely unknown (Dao *et al.*, 1996).

Overall, potentially active compounds are found difficult to be designed for their treatment due to the limited known about the etiology of many human, animal, and plant diseases. And thus leads from natural sources will continue to be sought (Klein & Kurilich, 2000).

## 2.2 Phytochemistry

Phytochemistry, or plant chemistry is a study on plant in between natural products organic chemistry and plant biochemistry (Dao *et al.*, 1996). Nowadays, the research on phytochemistry has become significantly important, especially the research of phytochemicals determination in medicinal plants due to the growing interest and concern of peoples on health. The importance of phytochemistry on medicinal plants is that the



uses of separated compound as a therapeutic agent, for instance morphine, an analgesic that is much more stronger than aspirin (Bruice, 2001).

#### 2.3 Phytochemicals

Phytochemicals exist as long as plants exist but their existence are only known about hundred years. During 19th and 20th century, the main strategy of the scientists was to discover the active ingredients, which had medicinal or pesticidal properties. At the same time, other scientist were conducting epidemiological studies to determine the relationship between the consumption of phytochemicals and human health (Dao *et al.*, 1996).

Phytochemicals (phyto is Greek for plants), meaning "plant chemicals" are naturally occurring chemicals found in foods like vegetables, fruits and beverages such as teas and wines in which our body may use it as part of their disease-fighting arsenals. Phytochemicals are sometimes referred to as phytonutrients and these terms are often used interchangeably (Benaveto-Garcisa *et al.*, 1997). Most broadly defined, it could be said to be any chemical or nutrient derived from a plant source. Despite, it have a more limited definition in common usage. They are usually used to refer to compounds found in plants which are not required for normal functioning of the body but which nonetheless have a beneficial effect on health or an active role in the amelioration of disease (Harborne, 1973).



Phytochemiclas can be used as an initial material for useful drugs synthesis. For instance hormone adrenal cortex, which are synthesized from sapogenin steroid that acquired from plants. The compound from plants can also be the pharmacology active compound models in drugs synthesis (Harborne, 1973).

Phytochemicals differ from vitamins and minerals in that they have no known nutritional value (Dao *et al.*, 1996). Phytochemicals in plants can be roughly classified into primary and secondary constituents depending on their roles in plant metabolism. The primary constituents involving plant metabolisms such as sugar, amino acid and chlorophyll while secondary constituents including alkaloids, terpendoids, phenolic compounds and other substances that vary from plant to plant. These constituents usually do not play essential roles in plant metabolism, yet they are contributed in protecting the plant from environment pressure or controlling plant growth (Harborne, 1973).

More than 4000 substances have been identified in which one of the huge class is polyphenols, which include the current publicized flavonoids. The other phytochemicals that appear within the major class are alkaloids, saponins, tannins, anthrequinone, etc. these compounds have many health-related properties such as anticancer, antiviral and anti-inflammatory activities, effects on capillary fragility, and ability to inhibit human platelet aggregation (Benaveto-Garcisa *et al.*, 1997). These concominant protective functions of phytochemicals are the reasons why scientists are so excited about it.



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