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PERPUSTAKAAN UTAMA

ANTIOXIDANT PROPERTIES OF SOME LOCAL MEDICINAL PLANTS AND FRUITS

LAM POOI WAN

THIS DISSERTATION IS PRESENTED AS PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF BACHELOR OF SCIENCE WITH HONOURS

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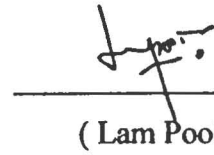

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CERTIFICATION

It is hereby certified that we have read the dissertation entitled Antioxidant Properties of Some Local Medicinal Plants and Fruits and it fulfils the requirement for the degree of Bachelor of Science with honours.

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ABSTRACT

The leaves of *Camellia sinensis* plant (black tea and green tea), *Mentha arvensis* (pudina) and peels/fleshes of *Citrus limon* (lemon) were extracted via reflux and the respective crude extracts were then fractionated using solvent-solvent extraction with 4 different polarity of solvents and yielded petroleum ether (PE), chloroform(CL), n-butanol (BU) and methanol aqueous (AQ) extracts. Phytochemical screenings carried out on sixteen extracts showed that 12 extracts (75 %) contained each of alkaloids and saponins whereas flavonoids were found to occur in 11 extracts (68.8 %). 6 extracts (37.5 %) contained each of tannins and polyphenols substances, whilst anthraquinones present in 10 extracts (62.5 %). Antioxidant properties of CL, BU and AQ extracts of each samples were evaluated using autoxidation linoleic acid assay to determine semiquantitatively of the inhibitory capacity against linoleic acid oxidation relative to butylated hydroxytoluene (BHT). This study indicated that the three extracts of each samples appeared as moderate antioxidants compared to BHT. The relative absorbance values of all these 3 extracts almost similar to each other indicating that they possessed a similar efficiency in inhibitory activity against linoleic acid peroxidation.

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ABSTRAK

Daun-daun dari tumbuhan *Camellia sinensis* (teh hitam dan hijau), *Mentha arvensis* (pudina) dan kulit/isi dari *Citrus limon* (lemon) diekstrak melalui refluks dan ekstrak-ekstrak mentah yang terhasil ini kemudian dipisahkan melalui ekstraksi cecair-cecair dengan menggunakan 4 pelarut yang berlainan kepolaran dan menghasilkan ekstrak petroleum eter (PE), klorofom (CL), n-butanol (BU) dan akues metanol (AQ). Penyaringan fitokimia yang dijalankan ke atas 16 ekstrak menunjukkan 12 ekstrak daripadanya (75 %) mengandungi alkaloid dan saponin masing-masing manakala flavonoid didapati wujud dalam 11 ekstrak (68.8 %). Sebanyak 6 ekstrak (37.5 %) mengandungi tannin dan sebatian polifenol sementara antrakuinon didapati hadir dalam 10 ekstrak (62.5 %). Sifat-sifat anti-oksida dinilai ke atas ekstrak-ekstrak CL, BU dan AQ dari setiap sampel dengan menggunakan kaedah auto-oksidaan asid linoleik untuk menentukan kemampuan ekstrak-ekstrak dalam perencatan terhadap pengoksidaan asid linoleik secara semi-kuantitatif dan relatif kepada butylated hydroxytoluene (BHT). Kajian ini menunjukkan ketiga-tiga ekstrak dari setiap sampel muncul sebagai anti-oksida yang sederhana di mana aktiviti anti-oksidaannya kurang cekap berbanding dengan BHT. Nilai kerelatifan ketiga-tiga ekstrak dalam semua sampel adalah hampir sama antara satu sama lain menunjukkan mereka memiliki kecekapan yang hampir sama dalam aktiviti perencatan atas pengoksidaan asid linoleik .

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CHAPTER 1

INTRODUCTION

1.1 Background Of The Study

The natural world once served as the source of all medicinal agents, with higher plants constituting by far the principle of these. Balandrin *et al.* (1993) noted that natural products today still represent over 50 % of all drugs in clinical use, with higher plant-derived natural products representing 25 % of the total. In view of this point, the commercial value of drug products still derived directly from higher plants is considerable and should not be underestimated. For instance, in 1980 American consumers paid about $8 billion for prescription drugs derived solely from plant sources (Balandrin *et al.*, 1993). Undoubtedly, plant-derived drugs represent a stable markets upon which both physicians and patients rely and its contributions in worldwide market are difficult to estimate, but undoubtedly amount to many additional billions of dollar (Principe, 1989).

From numerous literatures, there are available evidences concerning the participation of free radicals in the etiology and physiopathology of human diseases, such as cancer, neurodegenerative disorders, cardiovascular and oxidative stress dysfunctions. Cancer and heart disease, for example, are two of the leading causes of death in Malaysia

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as evidenced by some frightening statistics from the National Cancer Registry (NCR) Report, 2002 that a total of 26,089 cancers were diagnosed among all residents in Peninsular Malaysia in the year 2002, as means 1 in 5.5 Malaysians can be expected to get cancer in their lifetime. Since cancer mortality is rising with prolongation of the life span, it is important to detect human carcinogens and to eliminate them. One of the important possible way to decrease human cancer mortality is through cancer chemoprevention (Hirose *et al.*, 1994).

The role of free radicals in several humankinds' diseases and the potential antioxidant protective effect of natural compounds on affected tissues are topics of high current interest. To consider a natural compound or a drug as an antioxidant substance it is necessary to investigate its antioxidant properties *in vitro* and then to evaluate its antioxidant functions in biological systems (Repetto and Llesuy, 2002). Owing to this necessity, investigations have suggested that spices and herbs have been recognized as sources of natural antioxidants that can protect from free radicals-induced oxidative stress and thus play an important role in the chemoprevention of diseases resulting from lipid peroxidation (Hirose *et al.*, 1994). The medicinal properties of medicinal plants are mainly attributed to the presence bioactive compounds especially the most publicized flavonoids (Hirose *et al.*, 1994) .

Flavonoids and other polyphenols belong to the popular phytochemicals, chemicals derived from plant material with potentially beneficial effects on human health. There has been an increasing interest in the research of flavonoids from dietary sources, due to a

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growing evidences of the versatile health benefits of flavonoids through epidemiological studies (Yao *et al.* 2004). Yet over the years they have been found to be an important part of the human diet and are considered to be an active compound in some medicinal plants (Repetto and Llesuy, 2002). Among the principal properties that may account for the potential health benefits of flavonoids is their antioxidant properties. Several *in vitro* studies have demonstrated that flavonoids can scavenge superoxide, hydroxyl and peroxyl radicals (Repetto and Llesuy, 2002). In addition to these important effects, they have also affect some processes of intermediary metabolism and inhibit lipid peroxidation in different systems. These factors making plants with flavonoids were chosen for further investigation.

However, according to Yao *et al.* (2004), there is still difficulty in accurately measuring the daily intake of flavonoids because of the complexity of existence of flavonoids from various food sources, the diversity of dietary culture, and the occurrence of a large amount of flavonoids itself in nature. Nevertheless, research on the health aspects of flavonoids for humans is expanding rapidly. They also mentioned that many flavonoids are shown to have antioxidative activity, free-radical scavenging capacity, coronary heart disease prevention, and anticancer activity. As such research progresses, further achievements will undoubtedly lead to a new era of flavonoids in either foods or pharmaceutical supplements.

Likewise, despite many important past contributions from the plant-derived natural products serve as medicinal agents for treating humankind's diseases, however, a great

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number of plant species have never been described and remain unknown to science, and relatively few have been surveyed systematically to any extent for biologically active chemical constituents. It is often the case that even plants that are considered to have been "investigated" have been screened for only a single type (or, at best, a few types) of biological activity (Balandrin *et al.*, 1993). Thus, it is reasonable to expect that new plant sources of valuable and pharmaceutically interesting materials remain to be discovered and developed.

Aside from these, herbal products and medicines are somewhat unique from nutriceuticals and functional foods in that many have been used for centuries in other societies as medicinal treatments or for disease prevention. As a result, the need for scientific proof of positive effects would seem to many to be reductant. However, there are several issues with this. Particularly, the active compounds are often assumed, rather than determined. If wrongly identified, an active compounds then isolated to give a more potent product may not bring about the purported effect. Owing to this reason, it is important that the active compounds in the plants which suspected have biological activity be identified, which is a partially motive for the present study to be carried out.

1.2 Objective Of The Study

The aims of this study are:

(a) To screen the phytochemical contents of the plant extracts

(b) To evaluate antioxidation activity of some plant extracts relative to a synthetic antioxidant, BHT.

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1.3 Scope Of The Study

In this study, tea (black and green tea), *citrus limon* (lemon), *citrus aurantifolia* (lime), *mentha arvensis* (pudina) and *mentha piperita* (peppermint) were chosen for antioxidation activity evaluation. The plant extracts were screened for its alkaloids, saponins, flavonoids, tannins (including polyphenol substances) and anthraquinones contents. Antioxidation activity of the plant extracts were studied semiquantitatively relative to BHT using UV spectrophotometer and the autoxidation linoleic acid assay in an alcohol-aqueous system to measure anti-oxidation activity against lipid peroxidation (linoleic acid).

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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 research remains one of the main means of discovering bioactive compounds (Ghisalberti, 1993) in which isolation from higher plants provided novel, clinically active drugs. The key to the success of discovering naturally occurring therapeutic agents rests on bioassay-guided fractionation and purification procedure.

Malaysia is blessed with enormous biodiversity resources, many of them are said to be medicinal but only a few have been investigated fully for their potential. The huge diversity of the Malaysian flora means that we can expect well diversed chemical structures from their secondary metabolites, and chemical diversity is one of the plus factors that makes myriad natural products excellent candidates in any screening programme for drugs discovery development. Screening of both synthetic organic compounds and extracts of natural products has had an impressive history of identifying active agents. For example, there are about 50 commercially available

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anticancer drugs (excluding endocrines) which have been approved to date by the United States Food Development Association (USFDA), and significantly, the drugs based on natural products represent almost 1/3 of these total approved agents (http://www.arbec.com.my/biotech).

The development of medicinal plants into therapeutic drugs takes several years and millions of dollars are needed, hence making the process very capital-intensive, high risks and the success rate are not very good. Despite all this, natural products drugs discovery programmes are still in existence all over the world, mainly because of the high chemical diversity from natural products as compared to synthetics, the potential of these natural products is largely unknown (http://www.arbec.com.my/biotech.).

Since little is known about the etiology of many human, animal, and plant diseases, it is difficult to design potentially active molecules for their treatment, and therefore leads from natural sources will continue to be sought (Ghisalberti, 1993).

2.2 Phytochemicals

Phytochemicals (phyto is Greek for plants), meaning "plant chemicals" are naturally occurring chemicals found in foods like fruits, vegetables and beverages such as teas and wines in which our bodies may use it as part of their disease-fighting arsenals. Phytochemicals differ from vitamins and minerals in that they have no known nutritional value (Dao *et al.*, 1996). More than 4,000 substances have been identified in which one huge class is the polyphenols, which include the current publicized

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flavonoids . The other phytochemicals that appear within the major class are alkaloids, saponin, tannin, anthraquinone, etc.

Phenolic compounds, or polyphenols, exhibit a wide range of biological effects as a consequence of their antioxidant properties with mechanisms involving both free radical scavenging and metal chelation (Urquiaga and Leighton, 2000). The antioxidation activity of phenolic compounds is mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Osawa, 1994). Polyphenols like flavonoids can exert their antioxidative action by hydrogen atom donation to free radicals (Madsen *et al*, 2000). In details, the chemical reaction (2.1) showed the site of attack by peroxyl radical is the hydroxyl group of the phenol (ArOH) and the oxy radicals could abstract an H atom from ArO-H bonds,

$$ArOH + ROO\bullet \rightarrow ROOH + ArO\bullet \qquad (2.1)$$

Plants vary in composition of phytochemicals with concomitant protective functions. Investigations have suggested that diets rich in polyphenolic compounds are associated with longer life expectancy (Hertog and Hollman, 1996). 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). One reason scientists are so excited about phytochemicals is their apparent ability to stop a cell's conversion from healthy to a multi-stages cancerous (Dao *et al.*, 1996).

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2.3 Importance Of Antioxidants

Antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidizing chain reactions (Velioglu *et al.*, 1998; Klein and Kurilich, 2000). The Food and Nutrition Board of the National Academy of Science (1998) defined a dietary antioxidant as a substance in foods that significantly decreases the adverse effects of reactive oxygen species, reactive nitrogen species, or both on normal physiological function in humans.

Free radicals are highly reactive natural by-product of normal cell processes with incomplete electron shells which make them very unstable and thus, highly reactive. Exposure to various environmental factors, including tobacco smoke and UV radiation, may lead to free radical formation (Mojžišová and Kuchta, 2001). When the balance between the production and elimination of free radicals is impaired, an oxidative stress develops (Mojžišová and Kuchta, 2001), which is detrimental to life. Figure 2.1a and 2.1b show a comparison between normal healthy blood cell and cell which was experienced free radical damage.

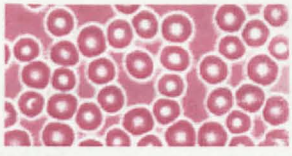

Figure 2.1a Healthy blood cell

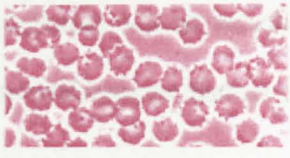

Figure 2.1b Free radical damage

(http://www.nzymes.com/Articles/antioxidants.htm)

Antioxidants are often described as "mopping up" free radicals (as shown in Figure 2.2), meaning they neutralize the electrical charge and scavenge free radicals

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via donation of an electron or a hydrogen atom, or by deactivation of prooxidant metal ions and singlet oxygen (Shahidi, 2002). Morello *et al.* (2002) stated that the primary role of antioxidants is to prevent degredation induced by free radical reactions by hydrogen abstraction and metal ion assisted electron transfer. The antioxidant donates hydrogen atoms to the free radicals, thus inhibiting the propagation of the autocatalytic chain reaction.

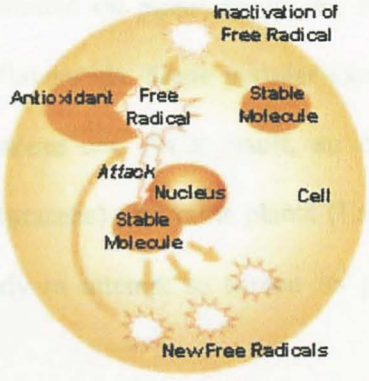

Figure 2.2 Process of antioxidants "mopping up" free radicals in cell (http://165.123.33.33/yr2005/July/research_960930.html)

Nzaramba (2004) stated that aerobic organisms are protected by an array of defense systems against oxidative stress. Various antioxidants with versatile functions constitute an elegant, yet complex, defense network to cope with such oxidative stress. Under certain circumstances, however, the natural defenses can be insufficient, and administration of exogenous antioxidants as food constituents or therapeutic agents may be beneficial.

Antioxidants are closely related with the prevention of degenerative illness such as cardiovascular, neurological diseases, cancer and oxidative stress dysfunctions (Szőllősi and Varga, 2002). Concurrently, an increasing number of epidemiological

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studies have shown an inverse correlation between the consumption of antioxidants and the incidence of various diseases such as cancer and heart disease (Block *et al.*, 1992; Hertog *et al.*, 1993).

Since flavonoids were already (generally) proven by numerous reports to be an effective antioxidants, inadvertently, a trend was set early on whereby most of the phytochemical work concentrated on plants belonging to certain families or genus only because they contain flavonoids. Some popular examples are the species from Theaceae, Rutaceae, Lamiaceae etc. As a result, an outstanding interest in teas (Theaceae), lemon, lime (Rutaceae) and mint plants (Lamiaceae) were chosen as a sample for the present study in attempt to screen its phytochemical contents and antioxidation activities.

2.4 Tea (Black and Green Tea)

2.4.1 Background

Tea was first cultivated in China and then in Japan. With the opening of ocean routes to the East by European traders, during the fifteenth to seventeenth centuries, commercial cultivation gradually expanded to Indonesia and then to the Indian subcontinent, including Sri Lanka. According to Ho *et al.* (1994), a monograph in Alternative Medicine Review (2000), Lai *et al.* (2001) and Kris-Etherton and Keen (2002), tea is one of the most widely consumed beverages in the worldwide, second only to water, and its medicinal properties have been widely explored. Three billion kilograms of tea are produced each year (Kris-Etherton and Keen, 2002). Black and

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