PHYTOCHEMICAL AND BIOLOGICAL STUDIES ON SWEET POTATO (*IPOMOEA BATATAS*)

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DECLARATION

I hereby declare that this dissertation is based on my original work, except for quotations and summaries each of which have been fully acknowledged.

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ABSTRACT

Sweet potato has received a broad attention because it is an important resource of food and there is an abundance of pharmacologically active ingredients in it. Three genotypes (storage roots and leaves) of sweet potato commercially available in Sabah (orange and purple fleshed) were studied for its phytochemical contents, antioxidation, antimicrobial and anti-kinase (MAPK) properties. Methanol crude extracts were obtained which were further partitioned into petroleum ether, chloroform and butanol extracts using solvent-solvent extractions. Phytochemical screenings demonstrated that the petroleum ether and chloroform extracts of all the genotypes contained saponins, tannins and anthraquinones whereas the butanol extract contained more classes of compounds (saponins, tannins, flavonoids and anthraquinones). The petroleum ether extract of storage roots (purple fleshed, Tambunan) showed a very potent antioxidation activity with relative antioxidation value of 1.025 (compared to fullness BHT, a synthetic antioxidant) as evaluated using the ferric thiocyanate (FTC) method. Petroleum ether extracts showed moderate to strong antioxidation activities. All the extracts demonstrated moderate antimicrobial activities against *S. aureus* (S 277) and *B. cereus* (B 43/04B) as evaluated a disc diffusion method. Relatively, the butanol extract was the most potent antimicrobial agent among all the extracts. However, no inhibition against *E. coli* (E 91/026) was observed. It was interesting to found out that all the butanol extracts of the storage roots showed very potent MAPK kinase inhibition as evaluated using a yeast screening system (yeast growing zone was 17 mm). However, inhibition of GSK-3ß was only detected in the petroleum ether extract of the leaves with 15 mm inhibition zone. Hence, sweet potato can be used as an easy accessible source of natural antioxidants, as a food supplement, or in the pharmaceutical and medical industries.

*KAJIAN FITOKIMIA DAN AKTIVITI BIOLOGI KE ATAS KELEDEK (*IPOMOEA BATATAS*)*

ABSTRAK

Keledek semakin mendapat perhatian yang meluas kerana ia merupakan sumber makanan yang penting serta kaya dengan kandungan farmakologikal yang aktif. Sifat tiga jenis (umbi dan daun) keledek di Sabah (berisi jingga dan ungu) telah dikaji bagi kandungan fitokimia, anti-pengoksidaan, antimikrob dan anti-kinase (MAPK). Ekstrak petroleum ether, ekstrak butanol dan ekstrak kloroform telah dihasilkan melalui pengestrakan pelarut. Penyaringan fitokimia mendapati bahawa eksrak petroleum-ether dan ekstrak kloroform mengandungi saponin, tanin dan antrakuinon. Manakala, kandungan bahan kimia yang didapat dalam ekstrak butanol pula adalah saponin, flavonoid, tanin dan antrakuinon. Bagi aktiviti anti-pengoksidaan (FTC), ekstrak petroleum-ether dari umbi keledek yang berisi ungu (Tambunan) telah memberi kesan anti-oksida yang terbaik di mana kerelatifan ekstrak petroleum-ether terhadap BHT adalah paling rendah dan hampir dengan nilai BHT, iaitu 1.025. Ekstrak petroleum ether menunjukkan ciri perencat serderhana terhadap aktiviti antimikrob. Eksrak-ekstrak dari keledek menunjukkan bahawa ciri perencat serdehana terhadap S. aureus *(S 277) dan* B. cereus *(B 43/04B). Secara bandingan, keputusan menunjukkan bahawa ekstrak butanol mempunyai ciri perencat antimikrob yang paling kuat daripada ekstrak lain . Tetapi, semua ekstrak tidak berpotensi merencat terhadap* E. coli *(E 91/026). Bagi sistem penyaringan perencat MAPK kinase, ketiga-tiga ekstrak butanol dari ubmi keledek didapati perencat sebesar 17 mm. Manakala, sistem penyaringan GSK-3ß hanya didapati satu perencat pada ekstrak petroleum-ether dari daun keledek dimana diameternya adalah sebesar 15 mm. kesimpulannya, keledek boleh diguna sebagai bahan anti-oksida semulajadi, makanan dan di industri perubatan.*

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LIST OF SYMBOLS AND ABBREVIATIONS

NA	Nutrient Agar
PDA	Potato Dextrose Agar
MAPK	Mitogen-activated protein kinase
GSK	Glycogen synthase kinase
DMSO	Dimethyl sulfoxide
E. coli	*Escherichia coli*
S. aureus	*Staphylococcus aureus*
B. cereus	*Bacillus subtilis*
CH	Chloroform
PE	Petroleum Ether
BUT	n-Buthanol

CHAPTER 1

INTRODUCTION

1.1 Background

Since the beginning of civilization, human have succeeded in using plants in various ways, such as for building shelters, food and also as a source for medicine (Ahmad & Raji, 1992). Exploration of the plant kingdom has been made in search of chemical compounds of medicinal values and has been going on ever since, resulting in many empirical discoveries uncovering the main sources of botanical drugs. Remedies for various ailments normally involve the use of roots, leaves and bark of the plants (Ahmad & Raji, 1992).

In recent years, there has been a global trend toward the use of natural phytochemical, as antioxidants and functional ingredients, which are present in natural resources such as vegetable, fruits, oilseeds and herbs (Huang *et al.*, 2006). The use of traditional medicine is widespread, and plants still represent a large source of natural antioxidants that might serve as leads for the development of novel drugs (Barton *et al*., 1999). Several anti-inflammatory, digestive, anti-necrotic, neuroprotective, and hepatoprotective drugs have recently been shown to have an anti-oxidation and radical-scavenging mechanism as part of their activity (Huang *et al*., 2004). Phytochemicals and antioxidant constituents in plant material have raised interest among scientists, food manufacturers, producers, and consumers for their roles in the maintenance of human health (Milner, 1999). Numerous epidemiological studies suggest that diets rich in phytochemicals and antioxidants execute a protective role in health and disease (Lako *et al*., 2006).

Moreover, fruits and vegetables in the diet have been found in epidemiology studies to be protective against several chronic diseases. Frequent consumption of fruits and vegetables is associated with a lowered risk of cancer, cardiovascular disease, cataracts, heart disease, hypertension and stroke (Lako *et al*., 2006). The risk of macular degeneration and stroke is diminished in people consuming large amounts of fruits and vegetables. Over 170 epidemiological cancer studies have been showed that there is a lower risk with increasing intake of fruit and vegetables. It is generally assumed that the vitamin and pro-vitamin antioxidants in these foods (ascorbic acid, tocopherols, and carotenoids) account for the beneficial effects (Vinson *et al*., 1998).

However, belief in the medicinal power of foods is not a recent event but has been a widely accepted philosophy for generations (Milner, 1999). Moreover, researchers have found that some foods have functional properties because they provide physiological benefits which may enhance health and reduce the risk of developing chronic diseases. Functional foods are a very important part of wellness and are defined as those which are whole, fortified, enriched or enhanced, providing health benefits beyond basic nutrition when consumed as part of regular, varied diet (Simmons, 2005). The term "functional food" is surfacing as a generic descriptor of the benefits that accompany ingesting foods that go beyond those accounted for merely by the nutritive provided. The Institute of Medicine of the National Academy of Sciences, United State has expanded this definition to include "any food or food ingredient that may provide a health benefit beyond the traditional nutrients it contains" (Milner, 1999).

Sweet potato has received a broad attention because it is an important resource of food and there is an abundance of pharmacologically active ingredients in it. So far, sweet potato has been widely used as a food staple, vegetable, and animal feed for industrial starch extraction and various processed products (Huang *et al*., 2003; Srisuwan *et al*., 2006). At the same time, more modern research show that sweet potato had higher levels of both carbohydrate and dietary fiber than potato and also had a stronger anti-oxidation activity than most other vegetables in a typical Western diet (Guan *et al*., 2006).

A lot of works have been performed on the health-related function of sweet potato, and several important biological activities are attributed to sweet potato (Zhao *et al*., 2005). Sweet potato (*Ipomoea batatas* L.), in which vitamin C, chlorogenic acid, caffeic acid, quercetin, and rutin are abundant, is one of the functional food products aimed at introducing human dietary ingredients that aid specific body functions in addition to being nutritious (Guan *et al*., 2006). Extracts from sweet potato show strong radical scavenging and anti-mutagenic activities, significantly reduced high blood pressure and carbon tetrachloride-induced liver injury, anti-inflammatory, antimicrobial, and antihypertensive activities, and ultraviolet protection effects (Aruoma, 1998; Hou *et al.*, 2001). Furthermore, sweet potato was recently identified to possess a postprandial anti-hyperglycemic (anti-diabetic) effect in rats through retardation of maltase activity (Guan *et al.*, 2006; Konczak-Islam *et al.*, 2003).

Sweet potato is nutritionally valuable, with higher levels of both carbohydrate and dietary fiber than potato (*Solanum tuberosum*), and a strong anti-oxidation activity that has been claimed to surpass most other vegetables in a typical Western diet. Hydroxycinnamic acids (HCA) are the main phenolic antioxidants in most commercially available sweet potato varieties, which can vary in storage root size, shape, flavor, texture, and colour, with the most common being white-, cream-, yellow-, or orange-fleshed. Several varieties of sweet potato have been developed with intense purple coloration, and conferred by high anthocyanin content in Japan and New Zealand (Philpott *et al.*, 2004).

1.2 Objectives of the study

1. To determine phytochemicals in sweet potato (*Ipomoea batatas*).
2. To screen the extracts for anti-kinase, anti-oxidation and anti-microbial activities.

1.3 Scope of study

Three varieties of sweet potato tuberous roots and leaves were selected in these studies which were purple fleshed sweet potato and orange fleshed sweet potato. These sweet potatoes were originated from Ranau and Tambunan, Sabah. Tuberous roots and leaves of those variety sweet potatoes were selected in this study because no report on the anti-oxidation, anti-microbial and anti-kinase activities of those varieties are presently available. Solid-solvent extraction and solvent-solvent extraction were carried out for bioactive compounds extraction from sweet potato. Phytochemical test and biological activity test for sweet potato (*Ipomoea batatas*) were carried out in this study. Screening of alkaloid, flavonoid, saponin, tannin and anthraquinon were included in the phytochemical study. Biological activities included anti-oxidation test, anti-microbial test and MAPK kinase and GSK-3ß test which showed medicinal use bioactive compounds in sweet potato.

CHAPTER 2

LITERATURE REVIEW

2.1 Phytochemistry

The subject of phytochemistry, or plant chemistry, has developed in recent year as a distinct discipline, somewhere in between natural product organic chemistry and plant biochemistry and is closely related to both. It is concerned with the enormous variety of organic substances that are elaborated and accumulated by plants and deals with the chemical structures of these substances, their biosynthesis, turnover and metabolism, their natural distribution and their biological function (Harbone, 1998).

In all these operations, methods are needed for separation, purification and identification of the many different constituents present in plants. Phytochemistry is directly related to the successful exploitation of known techniques, and the continuing development of new techniques to solve outstanding problems as they appear. One of the challenges of phytochemistry are to carry out all the above operations on vanishingly small amounts of material (Harbone, 1998).

2.2 Phytochemicals

Phytochemicals from fruits and vegetables have been shown to exert varied beneficial biological actions (Simmons, 2005). Phytochemicals are biologically-active, non-nutritive secondary metabolites which provide plants with colour, flavour and natural toxicity to pests (Johnson & Williamson, 2003). 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).

The classification of this huge range of compounds is fall into three main groups which are phenolic compound, glucosinolates, and carotenoids. Many thousands of phenolic compounds have been identified. They include monophenols, the hydroxycinnamic, acid group which contain caffeic and ferulic acid, flavonoids and glycosides, phytoestrogens and tannins (Johnson & Williamson, 2003).

2.3 Chemical compounds from plants

2.3.1 Phenols

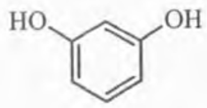
The vast majority of the plant-based aromatic natural products are phenols. Numerous categories of these compounds are derived from phenol which includes simple phenols, phenylpropanoids, flavonoids, tannins and quinines (Kaufman *et al.*, 1999).

Phenolic compounds are usually susceptible to different factors (e.g., acidic solution and high temperature) during the extraction process. Drying at room temperature may enhance the enzymatic degradation and thus lower the amount of phenolics in the samples. Increasing the temperature above 60 ^{0}C lowering the phenolic amount considerably. At high temperatures, certain phenolics may decompose or combine with the other plant components (Miean & Mohamed, 2001).

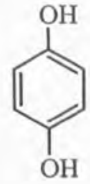
In addition, other phenolic and polyphenolic compounds are present in plants such as cinnamic acid derivatives, for example, chlorogenic acid, and isomers of flavones known as isoflavones. Many of these phenols have been found to be more powerful anti-oxidation activity than vitamins C, E, and carotene using an in *vitro* model for heart disease, namely the oxidation of lower density lipoproteins (Vinson *et al.*, 1998).

2.3.2 Simple phenols

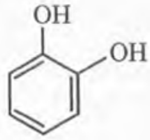
Most of the simple phenols are monomeric components of the polymeric polyphenols and acids which make up plant tissues, including lignin, melanin, flavolan and tannins. These individual components are obtained by acid hydrolysis of plant tissues. The components include *p*-hydroxybenzoic acid, protocatechuic acid, vanillic, syringic and gallic acid. Free phenols which do not require degradation of cell-wall polymers are relatively rare in plants. Hydroquinone, catechol, orcinol, and other simple phenols are found in relatively low concentrations. Some examples are shown in Figure 2.1.



Resorcinol



Hydroquinone

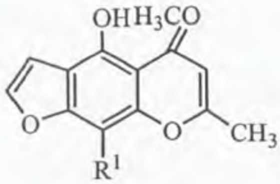


Catechol

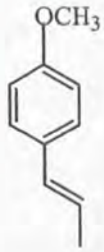
Figure 2.1 Some phenolic plant natural products (Kaufman *et al.*, 1999).

2.3.3 Phenols ethers

Many of the phenols also exist as their methyl ether; a few are shown in Figure 2.2. Khellin and visnagin are the active coumarin derivatives of the ammi visnaga fruit (*Ammi visnaga*). Trans Anethole is chiefly responsible for the taste and smell of anise seeds (*Pimpinella anisum*).



Khellin: $R^1 = OCH_3$
Visnagin: $R^1 = H$



trans-Anethole

Figure 2.2 Phenol ethers (Kaufman *et al.*, 1999).

2.3.4 Phenylpropanoids

As the name implies, the phenylpropanoids contain a three-carbon side chain attached to a phenol. Common examples include the hydroxycoumarins, phenylpropenes and the lignans. Anethole and myristicin, the principles of nutmeg, are also representative

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