

**PHYTOCHEMICALS TESTS ON PEANUTS (*Arachis hypogaea* L.)  
OF SABAH FOR SCREENING OF VARIOUS  
CHEMICAL COMPOUNDS**

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**A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE  
REQUIREMENT FOR THE DEGREE OF BACHELOR OF  
FOOD SCIENCE WITH HONOURS IN  
FOOD SCIENCE AND NUTRITION**

PERPUSTAKAAN  
UNIVERSITI MALAYSIA SABAH

**FOOD SCIENCE AND NUTRITION PROGRAMME  
SCHOOL OF FOOD SCIENCE AND NUTRITION  
UNIVERSITY MALAYSIA SABAH  
2006**



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SABAH FOR SCREENING OF VARIOUS CHEMICAL COMPOUNDS

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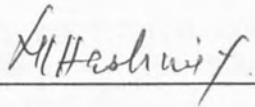
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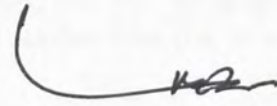
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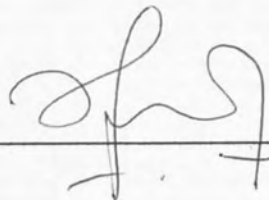
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## ACKNOWLEDGEMENT

First and foremost, I would like to express my thankfulness and appreciation to my thesis supervisor, Dr. Muhammad Iqbal Hashmi. He had helped me a lots by discussing with me and sharing his opinions during the process of completing this research. With his guidance, supports and encouragements had helped me in completing this thesis.

With highest appreciation, I would like to send my regard and appreciation to Dean of School of Food Science and Nutrition, Prof. Madya Dr. Mohd. Ismail Abdullah and all the lecturers that had been helping me in completing my thesis.

Besides that, I would like to thank to the laboratory assistants in School of Food Science and Nutrition, University Malaysia Sabah by aiding me in the process of accomplish this thesis. I also would like to appreciate Mr. Mustaffa, IBTP Science Official in helping me to complete my laboratory works.

Nevertheless, I would like to appreciate my friends, Shakirah, Ting Soon, Tze Yin, Xue ni, Shy Chiou, Chee Ping, Kok Kheng, Alex, Dicken Wong and Mrs Susie who have helped me by giving me encouragements, advices and supports to me when carried out this thesis.

Finally, to my beloved family, thank you for their endless encouragements and supports to me.



## **ABSTRAK**

### **UJIAN FITOKIMIA PADA KACANG TANAH (*Arachis hypogaea* L.) SABAH UNTUK PEMANTAUAN PELBAGAI KOMPONEN BAHAN KIMIA**

*Objektif utama kajian yang dijalankan adalah untuk mengenalpasti secara kualitatif pada kumpulan kelas organik yang hadir dalam kacang tanah di Sabah. Tiga variasi kacang tanah iaitu Bandau Triplets, Kacang Raja dan MGNT VI telah diperolehi dari Jabatan Penyelidikan Agronomi Tuaran sebagai sampel analisis. Oleh kerana komponen utama dalam peanut adalah lemak, jadi penentuan kandungan lemak pada kacang tanah adalah salah satu objektif kajian. Kandungan lemak pada kacang tanah ditentukan secara kuantitatif dengan Kaedah Soxhlet manakala sterol dalam kacang tanah ditentukan secara kualitatif dengan menggunakan silica gel 60 HF254. Ujian fitokimia telah dijalankan dalam kajian ini untuk menguji kehadiran kumpulan alkaloids, flavonoids, saponin, tannin, antakuinon dan komponen fenolik dalam sampel kacang tanah. Kumpulan alkaloids, flavonoids, saponin dan kompone fenolik didapati hadir dalam ketiga-tiga sample kacang tanah manakala tannin dan antrakuinon adalah tidak hadir. Kandungan lemak yang paling tinggi didapati pada varieti Kacang Raja diikuti dengan varieti Bandau Triplets dan MGNT VI. Dalam TLC, komponen adalah di dalam Rf 0.11 dan diidentifikasikan secara kualitatif sebagai komponen sterol. Pembawa larutan yang diguna dalam TLC ialah hexane: diethyl ether: methanol dengan nisbah 25:25:50.*



## ABSTRACT

### **PHYTOCHEMICALS TESTS ON PEANUTS (*Arachis hypogaea* L.) OF SABAH FOR SCREENING OF VARIOUS CHEMICAL COMPOUNDS**

The main objective of this research is to identify qualitatively the different classes of organic compounds in local peanuts. Three varieties of peanuts; Bandau Triplets, Kacang Raja and MGNT VI which obtained from Tuaran Agriculture Research Centre are as the sample in this analysis. Since the main components of peanuts are lipids, therefore lipids determination on peanuts is interested in this research. Total lipids in peanuts are determined quantitatively by using Soxhlet method while sterols in peanuts are identified qualitatively by using thin layer chromatography silica gel 60 HF254. Phytochemical tests is carried out to test the present of alkaloids, flavonoids, saponin, tannins, anthraquinone and phenolic compounds in the peanuts sample. Alkaloids, flavonoids, saponins and phenolic compounds are present in three of the peanut samples while tannins and anthraquinone are absent in the sample. The highest total lipids yield is Kacang Raja follow by Bandau Triplets and MGNT VI. In the TLC sheets, the compound is in R<sub>f</sub> 0.11 and qualitatively identified as sterol compounds. The developing solvent system in the TLC that manages to well separate the compounds is hexane: diethyl ether: methanol with the ratio 25:25:50.



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## ABBREVIATIONS

ANFs	antinutritional factors
AOAC	<i>Association of Official Analytical Chemists</i>
AOCS	American Official Chemical Society
DMAPP	dimethylallyl pyrophosphate
FeCl <sub>3</sub>	ferum chloride
FPP	farnesyl pyrophosphate
GGPP	geranyl geranyl pyrophosphate
H <sub>2</sub> SO <sub>4</sub>	<i>Sulphuric acid</i>
HCl	Hydrochloric acid
IPP	isopentenyl pyrophosphate
K <sub>2</sub> O	Potassium oxide
LD	lethal doses
N <sub>2</sub>	gas ammonia
Nd	Nitrogen deficiency
NH <sub>4</sub> <sup>+</sup>	ammonium
NH <sub>4</sub> OH	ammonium hydroxide
NO <sub>3</sub> <sup>-</sup>	Nitrate
OSC	oxidosqualene cyclase
PAL	phenylalanine ammonia lyase
P <sub>2</sub> O <sub>5</sub>	Phosphorus peroxide
QAs	Quinolizidine alkaloids
TLC	Thin Layer Chromatography
UV	ultraviolet



## SYMBOLS

%	Percentage
cm	Centimeter
g	Gram(s)
kg	Kilogram(s)
mL	Milliliter(s)
mM	miliMolar
mg	Milligram
°C	Degree Celsius



# CHAPTER 1

## INTRODUCTION

### 1.1. Phytochemicals at a glance

Phytochemicals are a major group that believes to contribute health effects to human nutrition. Phytochemicals includes lots of organic compounds and the novel compounds still continued to discover by researcher until this decade. When come to phytochemicals, the first thing come to our mind is the antioxidant properties that characterized them. There are too many phytochemicals group that involved in this properties and therefore it is so familiar to people. Definitely, phytochemicals is not limited to the antioxidant properties only. They still have others health benefits properties such as reduce certain chronic disease such as cardiovascular disease and immune system boost up (Pennington, 2002).

The major groups of phytochemicals that may contribute to the total antioxidant capacity (TAC) of plant foods include polyphenols, carotenoids and the traditional antioxidant vitamins such as vitamin C and vitamin E. The vitamins are, however, not the only phytochemicals that can have a positive effect on the health of consumers. There are other phytochemicals present in plant foods that may have positive effects on the health of consumers and need further investigation. These phytochemicals may be present in small amounts but may be very important to the health of consumers (Lako *et al.*, 2006). Due to small amounts of phytochemicals





can bring up the wonder effect; therefore they are actually made up interest of not only researcher but to those people curious about it.

## **1.2. Interest in Phytochemicals**

Societies nowadays are more health conscious. In terms of nutrition prospects, most of the plants compounds are in the dominant position if compare to animals compounds. Many natural compounds in plants had targeted by researcher in order to find out the benefits of those plants in human nutrition. Medicinal treatments are not any more become the hot topic in this century. The societies had realized that prevention is far better than treatment and natural compounds are more advantageous than synthetic products. Phytochemicals had raised the attention of researcher especially recent year because of the availability of discovered phytochemicals and lots of their health effect towards man.

Phytoestrogen shows health benefits effect on osteoporosis, cardiovascular, thyroid function, central nervous system and immune system (Boyle *et al.*, 2003). Plant phenols may also regenerate other antioxidants and act synergistically with chain-breaking antioxidants under other sets of conditions. Polyphenols such as the flavonoids and anthocyanidins have metal chelating properties and bind otherwise redox-active metal ions. Finally plant phenols protect plants against UV-irradiation and light-induced oxidative damage and may have similar effects in food and beverages (Pedrielli & Skibsted, 2002). Due to the health benefits that provided to human, phytochemicals contents in many food products such as nuts, legumes, cereals and vegetables had been continuously discovered and compared.



Although phytochemicals is well known for its health benefits, but not all phytochemicals are well applied in man. Some natural compounds in food may present in restricted amount or have difficulty in human digestion and absorption tract. Phytosterol is one of the phytochemicals which is not very popular in the past because of the characteristic stated above. Due to poor solubility and bioavailability of the free phytosterols, high doses (up to 25-50 g per day) of phytosterol required for efficacy (Moreau, Whitaker & Hicks, 2002). More over, practical applications of phytosterols in foods are limited due to their crystalline property. The solubility of phytosterols in edible oils is very low while their melting points are rather high which is in the range of 140° C - 150° C (Piironen, Toivo & Lampi, 2000; Vu *et al.*, 2004).

Every element are believed to have dual nature and phytosterol too, theoretically not abandoned. Therefore, phytosterol and other phytochemicals now are gaining the interest of researcher because the application of phytochemicals in man had discovered. The growing interest of phytochemicals and their availability in the functional foods also one of the reason they are interested. There are lots of phytochemicals that can be applied or ready available in foods such as flavonoids, catechins and limonoids. The phytochemicals compounds stated above can easily get from vegetables and fruits. Due to this, interest in phytochemical had brought up by researchers so that people can fully or wisely utilize the foods that available around us.

Synthetic food supplements are widely available in the market and people nowadays are more aware and tend to switch their diet practices to the natural products. Phytochemical is playing an important role because they have potential to replace the synthetic food supplement. Improving the bioavailability of natural food





supplements with nutritional and health benefits (nutraceuticals) are of a great interest to the food industry. Therefore, lots of phytochemicals products had try to incorporate into foods so that the phytochemicals products can be easily reached by people (Spernath *et al.*, 2003).

In Malaysia, research on phytochemicals is not a popular topic. However, people are awake at the benefits of phytochemicals. It is very obvious that the market trend preferences is more to natural products even most of the consumers do not understand the word of phytochemicals. Supplements and sundry goods which phytochemicals added are commonly available in every shopping market and pharmaceuticals shop outlets. For example, toothpaste with tea polyphenol compounds, beverage with flavonoids compounds and so on.

Given the importance of dietary habit and food components to health, the provision of phytochemical and antioxidant information of a range of foods is trying to ready so that they are available to support the future work in assessing the protective status of people from chronic degenerative disorders. The approach used is to be effective and culturally appropriate to the community. Food-based approaches are the essential for sustainable solutions to combat the alarming prevalence of chronic cancer, coronary heart diseases and diabetes (Lako *et al.*, 2006).

### **1.3. Relationship between peanuts and phytochemicals**

Peanuts (*Arachis hypogaea* L.) in Sabah consist of three varieties which are MGNT, Bandau Triplets and Kacang Raja. Bandau Triplets is widely cultivated and mostly found in Tamu market, Sabah. MGNT and Kacang Raja are also available in the



market but in small quantities. Basically, peanut plantations in Sabah are in a small scale (Jabatan Pertanian Sabah, 2003). Peanuts are a favourite foods in Malaysia, as a table snack, just to munch after roasted, added in local foods such as rojak and so on. Peanuts are considered as detrimental to one's health because they contain aflatoxin and are an allergen to most of the people. Indisputable, peanuts do contains some phytochemicals that benefits to health (Anderson & Deskins, 1995).

The phytochemicals function in the peanuts usually is act as antioxidant and applied for oil stability of the products. Naturally-occurring phytochemicals in peanuts such as tocopherols, carotenoids, and polyphenolics, may have a role in slowing or preventing lipid oxidation, due to their antioxidative nature. In model systems, the exogenous addition of phenolic compounds, such as catechin and rosmarinic acid, were shown to enhance oxidative stability during storage of peanut oil and were as effective as common synthetic antioxidants (Chu & Hsu, 1999; Talcott *et al.*, 2005). Flavonoids compound is present in peanuts. Flavonoids compounds in the peanuts are depends on the maturity of the peanuts. Because of the indeterminate fruiting characteristics of peanuts, seeds of varying maturity are on the plant at harvest time which means maturity is depending on the harvest time. From here, we know that harvest time is important for the phytochemical value in peanut (Daigle *et al.*, 1988).

#### 1.4. Objectives

In qualitative analysis, numbers of advance equipment can either included or excluded. Phytochemicals test, saponification extracts and Thin Layer Chromatography identification was carried out in this research. Research carried out for phytochemicals tests on peanuts of Sabah for screening of various chemical compounds. Three types of peanuts; MGNT, Bandau Triplets and Kacang Raja were



obtained from Tuaran Agriculture Research Centre and as the samples in this research. Qualitative analysis is emphasized and simplified method was used to achieve the objectives.

- Estimate total lipids in peanuts using Soxhlet method.
- Qualitative identification of alkaloids, flavonoids, anthraquinone, saponins, tannins and polyphenol compounds in peanuts.
- Identification of sterols in peanuts using TLC.



## CHAPTER 2

### LITERATURE REVIEW

#### 2.1. Discovery of phytochemical

In plant based, wood, rubber, alkaloids, isoprenoids, flower pigments-all of these secondary compounds are important to humans, and some are nearly essential. A number of groups have begun to create transgenic plants in which secondary metabolism is altered, and many of the approaches being taken were discussed at the meeting of the Phytochemical Society of North America (PSNA). Many could be better or more useful if altered in subtle or not-so-subtle ways. Such alterations could eventually result in more effective or less toxic drugs (Chasan, 1994).

Phytochemical are natural products in plants. Although the structures vary immensely in size and complexity, most of them are derived from a limited number of core biosynthetic pathways. The biosynthetic origins of phenylpropanoid (including simple phenolics, flavonoids, anthocyanins and isoflavonoids), polyacetate, terpenoid, and alkaloid classes of metabolites are review in many researches. These compounds reported to be important in plant microbe, plant-animal and plant-plant interactions will be given as examples of each of these classes (Paiva, 2000).

Basically, phytochemicals are bioactive substances of plants that have been associated in the protection of human health against chronic degenerative diseases. Antioxidants are compounds that help delay and inhibit lipid oxidation and when



added to foods tend to minimize rancidity, retard the formation of toxic oxidation products, help maintain the nutritional quality and increase their shelf life (Fukumoto & Mazza, 2000).

### 2.1.1. Alkaloids

Alkaloid is an odorless, intensely bitter, nitrogen based compounds occur naturally in certain plants. Certain alkaloids, strychnine and cocaine are among the most powerful poisons and narcotics known to man (Anderson & Deskins, 1995). Alkaloids are loosely defined as compounds with a nitrogen-containing heterocyclic ring. The rings vary in size and oxidation state, ranging from aromatic to completely reduce. Thousands of alkaloids have been identified and are classified according to the structure of the ring system. Alkaloids are generally synthesized from amino acids (Paiva, 2000). Generally, the alkaloids compounds can extracted and separated easily if compare to others compounds that present in plant (Fasihuddin & Hasmah, 1993).

There is one phenomenon occur in alkaloids which is ergotism. Ergotism is the most notorious mycotoxicosis in human history, which is caused by consumption of grain, usually rye, contaminated with sclerotia of *Claviceps purpurea*. Ergot alkaloids represent a large family of mycotoxins that are derived from both amino acid and isoprenoid precursors, and include clavines, simple derivatives of lysergic acid, and structurally complex ergopeptines such as ergotamine. The core structural feature of ergot alkaloids is the ergoline nucleus, which is formed from 4-(g,g-dimethylallyl)tryptophan. Synthesis of 4-(g,g-dimethylallyl)tryptophan, the branch point step in ergot alkaloid biosynthesis, is catalyzed by the prenyltransferase, 4-





(g,g-dimethylallyl)tryptophan (DMAT) synthase, from dimethylallyl diphosphate and tryptophan (Desjardins & Hohn, 1997).

From figure 2.1, we can find the representative of alkaloids structure which includes pyridine, tropane, and isoquinoline alkaloid classes. Nicotinic acid (derived from aspartic acid and glyceraldehyde-3-phosphate) is a precursor of several pyridine alkaloids. Methylation of the nitrogen produces trigonelline, a compound found in many seeds including leguminous beans, wherein it has been shown to produce significant induction of nodulation-related genes in rhizobia. Nicotine, the infamous alkaloid component of tobacco, is produced by the fusion of nicotinic acid and another nitrogen-containing ring. Nicotine is insecticidal and inhibits feeding of herbivores. Tropinone, a precursor of the tropane alkaloids containing a bicyclic ring system, is derived from ornithine, which is cyclized and modified to form *N*-methylpyrrolinium cation, and malonyl CoA. Scopolamine, a powerful sedative, is formed from the ester of tropine (reduced tropinone) and an aromatic residue; cocaine is similarly formed. Lysine, its precursors and derivatives, can be converted to the six-membered rings, pyridine or piperidine rings, in several alkaloids (Paiva, 2000).





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