PHYTOCHEMICAL ANALYSIS AND TOXICITY TEST OF SOME SELECTED HERBAL PREPARATION IN MARKET

by

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CERTIFICATE

This is to certify that the dissertation entitled "**The Phytochemical and Cytotoxicity Test of Some Herbal Preparation** is the bonafide record of research work done by Miss Julia binti Abdullah during the period from July 2008 to October 2008 under my supervision.

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LIST OF ABBREVIATIONS

HCl	Hydrochloric acid
NaCl	Sodium chloride
$FeCl_3$	Ferric Chloride
KOH	Potassium hydroxide
NH_4OH	Ammonium solution
FBS	Fetal Bovine Serum
DMSO	Dimethyl sulfoxide
$NaHCO_3$	Sodium bicarbonate
KCl	Potassium chloride
NaOH	Sodium hydroxide
CHCl3	Benzene
EtOAc	Ethyl acetic
n-BuOH	n-butanol
C	Carbon

LIST OF SYMBOLS

ml	mililiter
M	molar
µL	microliter
g	gram
mg	miligram

ABSTRACT

The phytochemical and the cytotoxicity studies of some selected locally available herbal preparation on VERO cell lines were done. Three products have been purchased from the local market namely TA01, EN02 and AM03. Each product contained mixture of herbs. For TA01 product, it was claimed to *Eurycoma longifolia*. Second product, that is EN02, it was claimed to contain *Sauropus androgynus* (cekak manis) and for AM03, it was claim to contain rhizome of Curcuma zedoaria, and the *Elephantopus scaber* (tapak liman). Successive extraction of the herbal products TA01, EN02 and AM03 yields (1.66%, 1.06% and 3.2% in petroleum ether), (10.6%, 32% and 14.0% in methanol) and (9.26%, 4.65% and 9.63% in water) respectively. Phytochemical test was done for all the extracts. Every product was tested positive for the presence of alkaloid, saponin, coumarin, flavonoids, tannin and phenolic compound except for anthraquinones. Cytotoxicity test was done for methanol and water for all of the extract. From the cytotoxicity test, all the herbal preparation has not shown toxic effect on VERO cells. All extracts showed no IC_{50} at concentration of 1.59×10^{-3} µg/ml to 0.1µg/ml.

ABSTRAK

Kajian fitokimia dan ketoksikkan sel beberapa produk herba telah dijalankan ke atas sel VERO. Tiga produk telah dibeli daripada pasaran tempatan dan telah dinamakan sebagai TA01, EN02 dan AM03. setiap produk mengandungi campuran beberapa herba. Bagi produk TA01, ia didakwa mengandungi *Eurycoma longifolia* (Tongkat Ali). Produk EN02 pula mengandungi *Sauropus androgynus* dan produk AM03 pula mengandungi 2 jenis herba iaitu *Elephantopus scaber* (Tapak Liman) dan *Curcuma zedoaria* (turmeric). Proses pengekstrakan secara berperingkat telah dijalankan ke atas ketiga- tiga produk tersebut. TA01, EN02 dan AM03 masing-masing menghasilkan (1.66%, 1.06% and 3.2% dengan petroleum eter), (10.6%, 32% and 14.0% dengan methanol) and (9.26%, 4.65% and 9.63% dengan air). Ujian fitokimia telah dijalankan pada semua ekstrak. Setiap produk memberikan keputusan yang positif terhadap kehadiran coumarin, alkaloid, flavonoids, saponin, tannin, phenol tetapi negatif terhadap kehadiran anthraquinone. Ujian ketoksikkan telah dilakukan pada ekstrak methanol dan ekstrak petroleum eter bagi ketiga-tiga produk. Daripada ujian toksik yang dijalankan, ketiga-tiga produk tidak menunjukkan kesan toksik pada sel VERO. Semua ekstrak tidak menunjukkan IC50 pada kepekatan ekstrak dari 0.1 μg/ml hingga ke 1.59×10^{-3} μg/ml.

1.0 INTRODUCTION

1.1 INTRODUCTION TO HERBAL MEDICINE

Herbal medicine or also known as plant medicine are widely measure in the world today. It is the oldest form of health care known to mankind. Herbs had been used by all cultures throughout history. Herbal medicine is defined as a plant-derived materials used in therapeutic and contains ingredients either raw or processed from one or more plants (Mustafa, 2003). In 20th century, 25% of the prescription drugs dispensed in United State contain at least one active ingredient derived from plant materials. About 80 percent of population employs herbs as their primary medicine and plant based remedies are used for both acute and chronic health problem, use for treating common colds up to controlling blood pressure. Herbal treatments have been found effective for major diseases such as cancer, diabetes mellitus, and cardiovascular disease (Slichenmyer et al., 1990., Holubarsch, 2000., Campbell, 2003). In the United States of America, approximately 18.9% of the population used natural herbs or supplements (Kennedy et al., 2002). In Malaysia the use of Tongkat Ali (*Eurycoma longifolia*), Kacip Fatimah (*Labisia pumila*), Ginseng, Avocado, Aloe Vera, Ginkgo Biloba, gamat preparation etc, has increased tremendously and the demand has led to a parallel increase of these types of products in the market. Figure 1 showing the percentage of population using traditional medicine, selected countries in the Western Pacific Region and republic of China showed 90% of the population using traditional medicine.

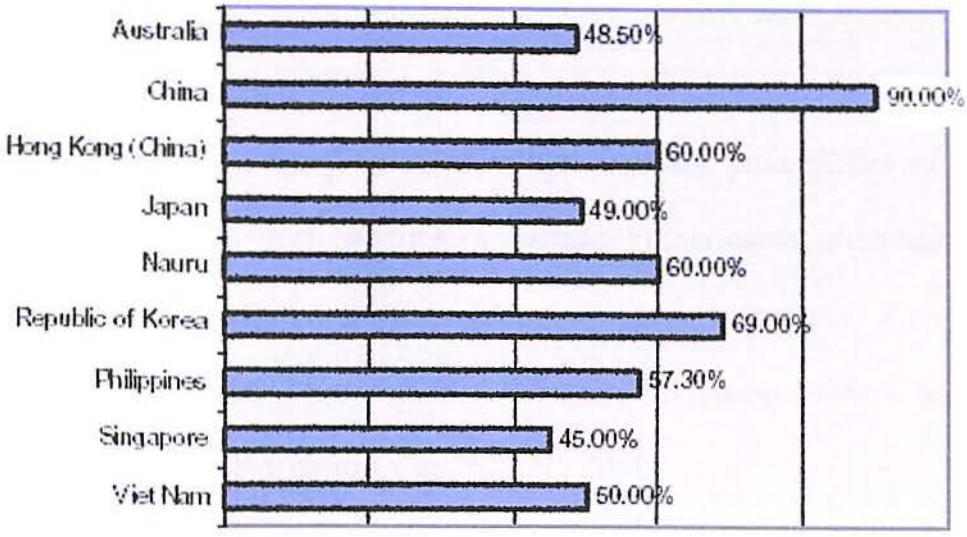

Figure 1: Percentage of population using traditional medicine, selected countries in the Western Pacific Region.
Source by World Health Organization (WHO)

The increase of the usage of herbs in today's medicine have becoming concern due to the studies that have been done which reveals that herbal medicine is not 100% safe to be implement as remedies. Some of the new scientific findings showed that some flavonoids in plants and plant products such as herbal medicines and fruit juices are able to induce or inhibit cytochrome enzymes in human and animals (Mustafa, 2003). Cytochromes are membrane-bound hemoproteins that consists of heme groups and carry out electron transport. They are found in the mitochondrial inner membrane and endoplasmic reticulum of eukaryotes, in the chloroplast of plants and in the photosynthetic microorganism. Cytochrome enzymes are important in production of energy via oxidative phosphorylation. Oxidative phosphorylation is a metabolic pathway that uses energy released by the

oxidation of nutrients to produce adenosine triphosphate (ATP). ATP supplies energy to metabolism.

Many plants are highly toxic; therefore there are probabilities of the herbal medicine adverse effects. There are serious adverse events occur after administration of herbal products have been reported. In the most notorious instance, several women developed rapidly progressive interstitial renal fibrosis after taking Chinese herbs prescribed by the staff of a weight loss clinic (Vickers et al., 2001).

Even though, each product that is being release to the market has stated the content, but we just cannot rely on that fact. This is because the content of those products and its safety are still questionable since the phytochemical analysis and the toxicity test never performed. This is because the black box of herbal-based treatment is the lack of definite and complete information about the composition of the extracts (Zuoli et al., 2007). Krochmal et al. (2004) in their research found that product labels were vary from the information provided, such as serving recommendations and information about the herbs itself.

1.2 INTRODUCTION TO HERBAL PREPARATION

Herbs are available in the form of fresh, dried, tablet or capsules, some found in a bottle in liquid form. As general, herbs can be classified into two categories; wild grown herbs and farm-grown. Wild grown herbs are herbs that grow naturally without human intervention and their growth are resulting from natural selection. There by, these herbs tend to be found in the places that optimized their growth. However these wild grown herbs have their own disadvantage that there is no guarantee the plants have not being exposed to chemicals and also pesticides. Besides, wild grown herbs growing naturally near the country side were exposed to the exhaust fumes from passing traffic. Therefore, the possibility of contamination of wild grown herbs, make them are not 100% safe to be use as remedies. The other choice of herbs use as medicine is the organic herbs that are grown commercially. These herbs are safer to be use since their growth has been monitored. With careful management, organic herb farms can provide a steady supply of quality herbs to the consumer.

Herbs and prepared herbal compounds are available in different forms. There are six type of herb. Firstly, it is known as tincture. Tincture means that its preparation involved the use of alcohol. In this type of preparation, alcohol is employed to extract and concentrate the active properties of the herbs. This is due to the properties of the alcohol itself that is an effective natural preservative. The advantage of tincture is that it is very efficient way of administration of herbal compounds. However the negative part is that tinctures are concentrated, therefore the full taste of the herb comes through very strongly. Secondly, it is known as extracts. These types of preparation can be made with alcohol or the other way

is by leached out the essence of the herbs with water. The advantages and the disadvantages of extract are same as tinctures. It is more concentrated, easily administrated but have strong herbal taste. Other types of herbal preparation are capsules and tablets. Capsules and tablets contain a ground or powdered form of raw herb. Finely milled herbs degrade quickly therefore it is important that the freshly ground herbs promptly encapsulated within 24 hours of being powdered. Both capsules and tablets tend to be less strong and potent than tinctures and extracts. Tea is other types of herbal extract preparation. There are many available herbal teas that are on the shelves of health food stores. These types of herbal preparation have been dried and then some are being pre-bagged for the convenient of the consumers. The administration of herbal teas is very simple, whereby the pre-bagged teas are dip into a hot water and then it can be drink. The other types of herbal preparation are lozenges. Lozenges are herbal based, nutrient rich and naturally sweetened. Lozenges are readily available in most health food shops. Many of lozenges are boosted with natural vitamin C. Mostly, lozenges are prepare for herbs that can be used as cold-fighting formulas, natural cough suppressants and some with decongestant properties. Other types of herbal preparation are ointments, salves and rubs. These types of herbs are being used as topical implementation. For example, calendula ointments are use on broken skin and wounds. Goldenseals are use in treatment of infection, rashes and skin irritations. Goldenseals are being applied on the sites of infection, rash or irritations. In addition, Aloe Vera gels widely use to cool and speed the healing of minor burns including sunburns.

Source: http://www.herbplace.com/alternative_medicine/herbal-medicine.html

A growing number of people who are using herbal products for preventive and therapeutic purposes causing the manufacturers of these products are not required to submit proof of safety and efficacy to the Food Drug Agency (FDA) before marketing. For this reason, the adverse effects and drug interactions associated with herbal remedies are largely unknown. Unlike conventional drugs, herbal products are not regulated for purity and potency. Thus, some of the adverse effects and drug interactions reported for herbal products could be caused by impurities such as allergens, pollen and spores or batch-to-batch variability. In addition, the potency of an herbal product may increase the possibility of adverse effects. Because physicians are likely to encounter patients who are using herbal remedies, they need to be aware of the purported effects of these products. They also need to be cognizant of the adverse effects of herbal remedies and the possibility of deleterious drug interactions. Below is the table showing some of the side effect of herbal product. The table below reveals that the herbal product does cause bad effect to the consumers.

Table 1: Side Effects of Selected Herbal Products

Herbal product	**Side effect**
Ginkgo biloba	Bleeding
St. John's wort	Gastrointestinal disturbances, allergic reactions, fatigue, dizziness, confusion, drymouth, photosensitive
Ephedra (ma huang)	Hypertension, insomnia, arrhythmia, nervousness, tremor, headache, seizure, cerebrovascular event, myocardial infarction, kidney stones
Kava sedation	Oral and lingual dyskinesia, torticollis, oculogyric crisis, exacerbation of Parkinson's disease, painful twisting movement of trunk, rash

Source: http://www.aafp.org/afp/990301ap/1239.html

1.3 INTRODUCTION TO HERBAL PRODUCT OF STUDY

There are three herbal products that are involved in this study and they are bought from several places in Kelantan. They are named as TA01, EN02 and AM03. Generally these products are used for the healthy of the reproductive system. So, mainly, the effects of these products are on the reproductive system of male and female. In these products will be undergone toxicity testing and also phytochemical test to determine the presence of certain metabolites. Meanwhile, toxicity test is done to evaluate the possibility of the herbal to produce adverse effect on the consumers. Toxicity is the ability of a chemical or mixture of chemicals to cause a living organism to undergo adverse effects upon exposure. The toxicity effects can include negative impacts on survival, growth, reproduction and etc. meanwhile, toxicity tests are analytical experiments which attempt to detect or quantify toxicity in a sample by measuring the results exposure produces on standard test organisms.

1.4.1 TK01 product

TK01 product has been claimed by the practitioner that this product is able to boost up energy by increasing body strength and vitality. it was also claimed to have aphrodisiac effect especially towards male. From the label, it has been stated that this product consists of Tongkat Ali or also known as "Pasak Bumi" by the local people. Below is the description on TA01 product which claimed to have Tongkat Ali ingredient.

1.4.1.1 Tongkat Ali

Ethnobotanical

The roots of *Eurycoma longifolia*, commonly known as Tongkat Ali in Malaysia and Pasak Bumi in Indonesia (figure 2). It can be found wildly in Southeast Asian countries and are popularly sought after as an essential ingredient in Malay herbal medicine (Gimlett and Thomson, 1977). Tongkat Ali is flowering plants in the family of Simarobacaea. It is a small green tree that can grow up to 15 meter tall. Tongkat Ali is a slender shrub-tree commonly found as an understroy in the lowland forests at up to 500 m above sea level (Kuo et al., 2003).

Ethnopharmacological uses

The plant is traditionally used as general tonic after childbirth and as an aphrodisiac. It is also used to relieve pains in the bone and also itches. Generally, the Tongkat Ali roots are also well known among various ethnic groups for the treatment of aches. It is also use in persistent fever, fight against tertian malaria and helping in sexual insufficiency. In addition, the roots of *Eurycoma longifolia* are also use in the case of dysentery, glandular swelling and also as health supplements (Perry, 1980).

Pharmacological studies

Eurycoma longifolia has been promoted as the "Malaysian ginseng" due to its aphrodisiac effects (Jaganant, 2000). Other study also shows that *Eurycoma longifolia* can increase testosterone levels in men (Hamzah and Yusof, Oct 2003). The crude ethanolic extract of the root of *Eurycoma longifolia* could decrease the basal release of testosterone but increase the human chorionic gonadotropin (hcG)-induced production of testosterone by rat leydig cells (Lin et al., 2001). Besides, the n-butanol and diethyl ether extract of *Eurycoma longifolia* roots displayed high antimalarial activity (Chan et al., 2004). Some of the constituents have been known to possess antiamoebic (Le and Nguyen, 1970), cytotoxic, antitumoral (Itokawa et al., 1993) and plasmodicidal activities (Chan et al., 1986).

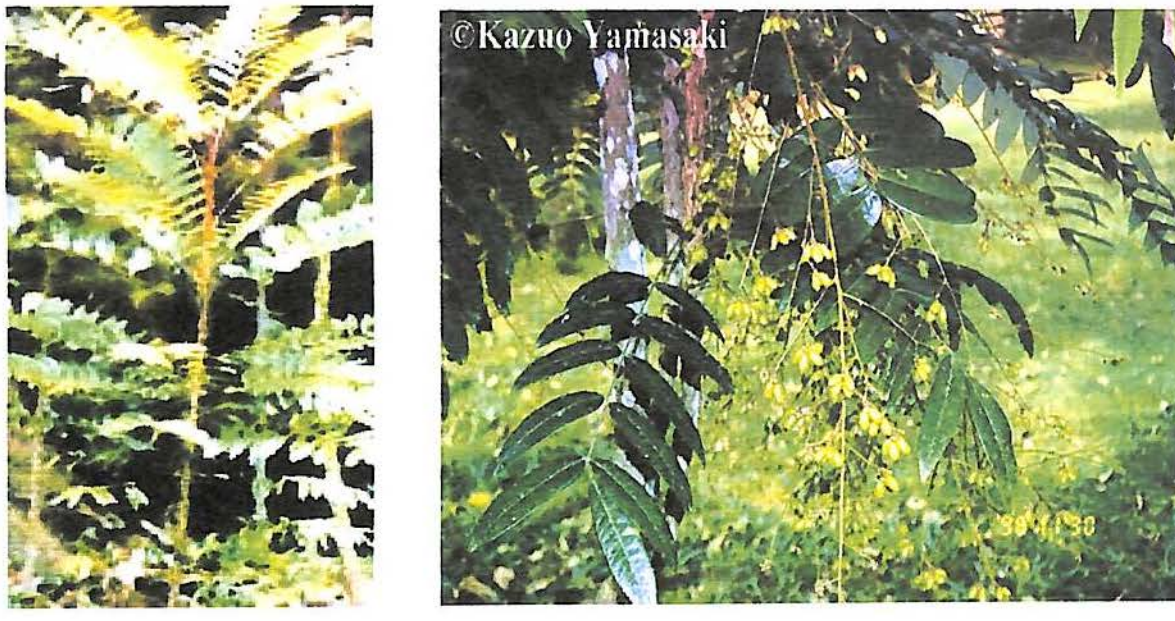

Figure 2: The plant of Tongkat ali

Source: http://www.pharmakobotanik.de/systematik/7_bilder/yamasaki/thai48.jpg

1.4.2 EN02 product

EN02 product is made for the use of female, especially for pregnant mother or women after child birth. It has been claim by the practitioner that it is efficient to be use by women after childbirth. It is also have been claimed that this product is good for keeping women to look young and also good for the healthy of the skin. These products consist of mixture of herbs that is sweat leaf (cekur manis) and some other herbs that have not been mention their names.

1.4.2.1 Sweat leaf (cekur manis)

Ethnobotanical

The scientific name for sweat leaf or cekur manis is *Sauropus androgynus. Saudropus andrygonus* is a member of the Euphorbiaceae family. It is widely distributed in high temperature and humid regions like Malaysia, Indonesia, Southwest China and Vietnam (Yu et al., 2007).

Ethnopharmacological uses

The raw juice of the young sticks and leaves of *Sauropus androgynus* (SA) has been widely used as a natural food for body weight reduction and vision protection in Taiwan and Southeast Asia (Yu et al.,2007).

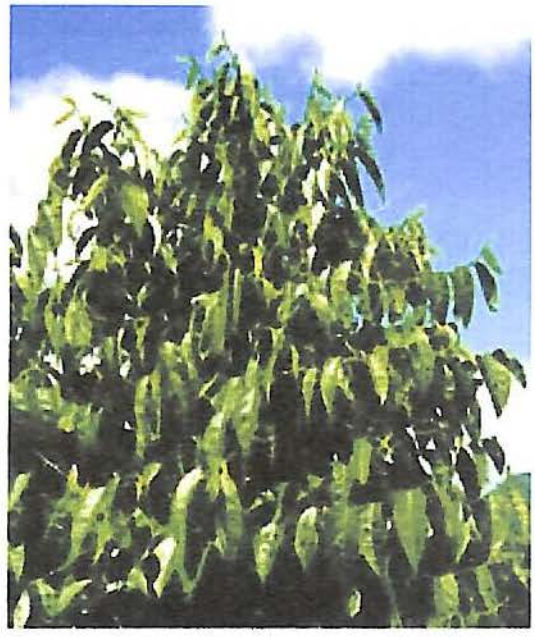

Figure 3: The plant of sweet leaf (Cekur Manis)

Source: http://www.herbsarespecial.com.au/images/herb-images/sweet-leaf.jpg