TOXICITY, PHYTOCHEMICAL SCREENING AND CHEMICAL COMPOSITION OF *Acalypha indica*

BY

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ABSTRACT

Natural products such as herbs have been extensively explored due to its medicinal properties that exist within the plants. This study aimed to evaluate the chemical composition and phytochemical content of *Acalypha indica* dried plant and extract. It is also aimed to collect recent data in acute and subchronic toxicity study based on haematological, biochemistry and histological examinations on normal rats. The chemical composition analysis was conducted based on method adopted from Association of Official Analytical Chemist (AOAC) (2003) and calculation by difference. The phytochemical screening was performed according to standard method. In acute toxicity study, 24 Sprague Dawley rats aged 8 weeks were randomly divided into two groups, 6 animals per gender. The treated group was given a single dosage of 5,000 mg/kg body weight of dried *A. indica* extract while control group received water vehicle and observed for 14 days. In subchronic toxicity study, 48 Sprague Dawley rats aged 8 weeks were randomly divided into four groups, 6 animals per gender. A control group received water vehicle while three treated groups received the extract at dosage of 100, 200 and 300 mg/kg body weight of dried *A. indica* extract. The sample was administered daily by oral gavage for 90 days. The samples used for chemical analysis and phytochemical screening were dried root, dried leaves, dried stem, dried whole plant and the whole plant extract. The chemical composition analysis showed that the dried leaves contained the significant moisture ($9.50\pm0.02\%$), ash ($12.83\pm0.03\%$) and protein ($23.98\pm0.10\%$) content as compared to other samples ($p<0.05$). The root contained the most significant carbohydrate ($80.13\pm0.22\%$), crude fibre ($42.05\pm0.36\%$) and gross energy (347.50 ± 0.50Kcal/100g) content as compared to other samples ($p<0.05$) while the root had the lowest water activity (0.51 ± 0.00 A_w) as compared to other samples ($p<0.05$). The phytochemical screening revealed the absence of alkaloids in all samples while saponins presence in whole plant extract and tannins in the dried whole plant, whole plant extract and dried leaves. The triterpenes and steroid were presence in all samples except the whole plant extract, while flavonoid was presence in all samples analysed. In acute toxicity study, the blood and histopathological result showed no sign of toxicity. In subchronic toxicity study, histopathology slide of the kidney, heart and liver revealed certain cellular damage in both control and treatment groups and it is suggested due to infection and stressful environment. However, the treatment groups showed better cell condition. Based on these results, it can be concluded that *A. indica* did not show any toxic effect in acute and subchronic toxicity study. LD_{50} for *A. indica* extract was more than 5000mg/kg body weight. Thus, this study proved that *A. indica* extract might be safe for human consumption when administered orally.

البحث خلاصة

تم استكشاف المستحضرات الطبيعية مثل الأعشاب بشكل كبير نظرا للفوائد الطبية التي تحتويها. هدفت هذه الدراسة لتقييم المحتوى الكيميائي والنباتي لمستخلصات والعينات المجففة من نبتة أكاليفا إنديكا (*Acalypha indica*). هدفت هذه الدراسة أيضا لجمع أحدث المعلومات عن دراسات السمية الحادة والسمية الدون مزمنة والمعتمدة على الفحوص الدموية، والبيوكيميائية، والتشريحية التي أجريت على فئران عادية. تم إجراء تحليل المحتوى الكيميائي بالاعتماد على طريقة جمعية الكيميائيين التحليليين الرسمية (AOAC) (2003) وتم حسابها بالاختلاف. في دراسة السمية الحادة تم تقسيم 24 فأرا من نوع سبراغ داولي،، إلى مجموعتين، في كل مجموعة 6 أزواج من جنسين مختلفين. تم إعطاء المجموعة المعالجة جرعة واحدة من مستخلصات الأكاليفا إنديكا المجففة، مقدارها 5000 مغ/كج من وزن الفأر، أما مجموعة الضابطة فأعطيت ماء فقط، وبعد ذلك تمت مراقبة الفئران لمدة 14 يوم. في دراسة السمية الدون مزمنة، تم تقسيم 48 فأر من نوع سبراغ إلى 4 مجموعات، ستة أزواج من جنسين مختلفين في كل مجموعة. تلقت المجموعة الضابطة ماء فقط، أما المجموعات الثلاثة الأخرى فعولجت بالمستخلصات الجافة للنبتة على الجرعات 100، و 200، و 300 مغ/كج بشكل يومي على مدى 90 يوما. العينات التي استعملت في تحليل المحتوى الكيميائي والمسح الكيميائي النباتي كانت من الجذور المجففة، والأوراق المجففة، ومجففات ومستخلصات النبتة إجمالا. أظهر تحليل المحتوى الكيميائي أن الأوراق المجففة احتوت على كميات كبيرة من الرطوبة (9.50±0.02%)، والرماد (12.83±0.03%)، والبروتين (23.98±0.10%) مقارنة بالعينات الأخرى($p<0.05$). احتوت الجذور على أعلى كمية من الكاربوهيدرات (80.13±0.22%)، والألياف الخامة (42.05±0.36%)، والطاقة الكلية (347.98±0.50 كيلو كالوري/100 غ) مقارنة بالعينات الأخرى ($p<0.05$)، وفي الوقت نفسه كان لدى الجذور أدنى نشاط مائي (0.51+0.00 A_w) مقارنة بالعينات الأخرى ($p<0.05$). أظهر المسح الكيميائي النباتي انعدام القلويدات في كل العينات، ووجود الصابونيات في مستخلصات النبتة إجمالا، وأيضا وجود التنيك في كل من مجففات ومستخلصات النبتةإجمالا. تواجد ثلاثي التيربينوالستيرويد في كل العينات ما عدا مستخلصات النبتة، أما الفلافونويد فكان موجودا في كل العينات المفحوصة. ومع ذلك، فقد أظهرت مجموعات المعالجة حالات أفضل للخلايا. بناء على هذه النتائج، من المستنتج أن أكاليفا إنديكا لم تنتج أيت مؤشرات للسمية في دراسات السمية الحادة والدون مزمنة. مقدار LD_{50}(الجرعة المميتة للنصف) للأكاليفا إنديكا كان أكثر من 5000 مغ/كج. وبالتالي فقد أثبتت هذه الدراسة أن مستخلصات الأكاليفا إنديكا آمنة للاستهلاك البشري.

APPROVAL PAGE

I certify that I have read this study and that in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Health Sciences.

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DECLARATION

I hereby declare that this thesis is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

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LIST OF ABBREVIATIONS

ALT	Alanine transferase
ALP	Alkaline phosphatase
AOAC	Association of Official Analytical Chemists
AST	*Aspartate transferase*
GGT	Gamma glutamate transferase
Hand E	Haematoxylin and Eosin
HDL	High density lipoprotein
LD_{50}	Median lethal dose
LDL	Low density lipoprotein
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
MCV	Mean corpuscular volume
NOAEL	No Observable Adverse Effect Level
OECD	Organization of Economic Co-operation and Development
PCV	Hematocrit
RBC	Red blood cell
RDW	Red blood cell distribution width
S.D	Standard deviation
S.E.M	Mean standard error
SPSS	Statistical Package for the Social Science
TCM	Traditional and Complementary Medicine
TM	Traditional Medicine
WBC	White blood cell
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION

1.1 PROBLEM STATEMENT

Malaysia has been ranked 12th in the world as megadiversity countries by National Biodiversity Index (Malaysia Biodiversity Information System, 2016). The variety of animal, plant and microorganisms species within the small country contributed to the biodiversity of this tropic kingdom (Ministry of Natural Resources and Environment of Malaysia, 2010). Moreover, this country located near to the Equatorial line and result in warm-humid climate that is suitable for the growth and spread of plant.

According to Jamal, Barkat and Amid (2010), only 1,300 of herbs from 12,000 plants species have been discovered to have medicinal benefits. Herbs have been used as a medicine since ages especially by indigenous people living in rural area. They rely upon natural sources to survive and mainly obtained the food and medicine from the forest or farm (Yahya and Mohd Ali, 2012). Besides that, as Malaysia is a multiracial, multiethnic and multi-religious nation, there were vast knowledge of traditional practices using herbs as an alternative treatment to improve health condition.

Currently, the popularity of alternative medicines such as homeopathy, acupuncture as well as herbal medicines had increasing globally due adverse effect of conventional medicines and expensive cost of treatment (Gratus, Wilson, Greenfield, Damery, Warmington, Grieve, Steven, and Routledge, 2009). Moreover, the resistance of microorganisms towards conventional drugs initiated the researcher to explore the natural medicines from herbs. Likewise, the natural product research has

gain the interest of pharmaceutical companies to discover the active ingredients in plants that can reduce or treat illness (Sekar, Kolanjinathan, Saranraj and Gajendiran, 2012).

It is well known that most of the herb has medicinal benefits however; limited scientific research had been conducted to study the properties of the herbs. Lack of data regarding the safety consumption of medicinal plant might expose the society to the risk of toxicity. Majority of the public assumed that herbal product was non toxic as it came from natural sources. Nevertheless, not all natural substances could be considered as safe and effective (Bandaranayake, 2006). Therefore, by conducting toxicity study, the society may understand the potential adverse effect of medicinal herb (Parasuraman, 2011).

1.2 SIGNIFICANCE OF STUDY

Recent research interest has been directed towards exploring the potential of Malaysian medicinal herb, *Acalypha indica*. This study is essential to find the nutraceutical value that this herb could possess. The nutrient values of this herb may supply as guideline for development of product in the future. Furthermore, by analyzing the nutrient composition of the herb, the consumer will be perceived on nutrient content that had been incorporated in their daily meal. Besides that, the analysis of chemical constituent may enrich the information regarding chemical composition of *A. indica* plant in Malaysian food composition database.

The exploration of medicinal herbs has started since ages but most of them were not scientifically proven. Hence, current research help to provide data on the toxicity information of standardized extract of *A. indica* will be documented and can be used for national references particularly in developing herbal products. The

approach of understanding the toxicity effect of the herb, might determine the potential adverse effect on health as well as anticipating the dose that is safe to be consumed. In addition, herbal medicine is cost-effective and potentially safe to be consumed as a supplement and alternative treatment. In addition, there were scarce knowledge regarding the toxicity study of *A.indica*

1.3 HYPOTHESIS

Acalypha indica is safe for human consumption and contain chemicals as well as phytochemicals that have medical value.

1.4 OBJECTIVE

1.4.1 General Objective

The overall objective of this research project is to investigate the safety consumption of *Acalypha indica* and identify its chemicals composition and phytochemicals.

1.4.2 Specific Objectives

1. To evaluate the chemical composition of dried *Acalypha indica* parts of plant.
2. To determine the phytochemical contents of *Acalypha indica* dried plant and extract.
3. To investigate the toxicity effects of *Acalypha indica* dried whole plant extract based on haematological and biochemical parameters, and histological examinations on normal rats.

CHAPTER TWO

LITERATURE REVIEW

2.1 TRADITIONAL AND COMPLEMENTERY MEDICINES (TCM)

Traditional and Complementary Medicine (TCM) has been growing rapidly worldwide. According to World Health Organization (WHO) (2000), traditional medicine (TM) can be described as the application of health practices, understanding and beliefs via plant, animal or mineral based medicines to sustain health, to cure illness or to prevent diseases. WHO further defined complementary medicine (CM) as wide array of health care practices apart from traditional medicines used in country's tradition and not incorporated with the mainstream health-care system (WHO, 2000). Thus, TCM includes products, practices and practitioners (WHO, 2013). In Malaysia, any practices that aid to improve health of individuals such as multiracial traditional medicines, homeopathy and complementary therapies other than dominant health care system can be classified under TCM (Traditional and Complementary Medicine Division, 2011).

People opt for TCM due to its variability, availability, safety, affordability and convenience. The variability of TCM mainly divided into two approaches; natural products or mind and body practices (National Center for Complementary and Integrative Health, 2016). Several examples of natural products are herbs, product from plants, vitamin and mineral. Acupuncture, massage therapy, tai chi and yoga are few instances of mind and body practices that are popular globally.

In developing countries, 80% of the societies depend on TCM as their health care needs and the trend grows to high income countries (WHO, 2002). The unlimited resources of TCM especially medicinal herbs make it convenient to be used and at the

same time less expensive compared to modern remedies. Furthermore, due to many adverse side effects of pills and tablets, people feel safer and more comfortable to use the TCM.

TCM is a vital part of the practice of ancient civilization to enhance the health and well-being of public in countries such as India, China, Malaysia, and throughout the world (Sooi and Keng, 2013). A survey conducted in Sabah reported that TCM has significant influence in healthcare needs among the local population (Chang, Tam, and Mohd Suki, 2015). Currently, TCM has been integrated into Malaysia mainstream healthcare system by establishing the TCM service in selected government hospital (Raja Ikram and Abd Ghani, 2015). Among services provided are acupuncture, Malay massage, herbal therapy and Malay postnatal treatment.

2.2 HERBAL MEDICINE

Herbal medicine together with other pharmaceutical-type preparations such as homeopathy and dietary supplements is classified under TCM (Jayaraj, 2010). In tropical countries, conventional medicines are not available to most of the rural community. Various diseases have been cured using herbs for instance infection, malaria, tuberculosis, and pneumonia. Numerous tropical herbal medicine species have shown in vivo and in vitro biological activities against these health problems (Tapsell, Hemphill, Cobiac, Patch, Sullivan, Fenech, Roodenrys, Keogh, Clifton, Williams, Fazio, and Inge, 2006).

The interest to explore on herbal medicines increased due to the escalate consumption of herbal products. The use of herbal medicines as an alternative treatment generally relies on magical or spiritual beliefs, or folklore by elderly in society. Furthermore, herbal medicines are more engaging than modern medicines

because it contain various chemical compounds that may help the body back to homeostasis (Wachtel-Galor and Benzie, 2011). The gradual healing process can develop a dynamic equilibrium that assist the organ to adapt with stress later.

Herbal medicine also known as botanical medicine mainly uses parts of plant for remedy. Plant's root, stem, leaves, flowers or seeds are prepared in several forms such as powder, extract, tea or capsules to sustain health and to prevent or heal illness (Bandaranayake, 2006). However, this natural remedy also may contribute to adverse effect especially if the herbs contain toxic substances. Thus, safety evaluation of herbal medicines can be conducted to attain comprehensive information regarding the safe level of consumption and its effect on body function (Ahmad and Othman, 2013).

2.2.1 Herbal Medicine in Malaysia

Malaysia is a blessed country which has rainforest rich with plant species which have been used by Orang Asli and the Malays as source of food and medicine (Wiart, 2006). About 1200 species of plant in Peninsular Malaysia has medical value and only few of them have been proven scientifically (Wahid, Kamaruzzan, Shariff and Selamat, 2015). The diversity of Malaysian population contributes to wide option of alternative medicines that being passed from one generation to other generation. They belief that natural sources of medicines are safe and convenient as some of them can be found in their garden as well as backyard.

Nowadays, the consumption of herbal product not only focused in the village. A survey reported that wealthier and educated people preferred natural product due to less adverse effect (Aziz and Tey, 2009). In addition, herbal remedies were the most commonly used TCM to treat health problems and to maintain wellness (Siti, Tahir,

Farah, Fazlin, Sondi, Azman, Maimunah, Haniza, Siti Haslinda, Zulkarnain, Zakiah, and Zaleha, 2009). By integrating the knowledge passed from the ancestor with modern technology, herbal products has been commercialized and become one of the main industries in this country.

The trend of going back to nature bloom worldwide and the value of herbal industry in Malaysia was about RM17 billion in 2013 and by 2020, it is estimated to reach RM32 billion (Hafizudin, 2015). Consequently, this expanding market creates more opportunity for farmers and young generations. Moreover, a variety of herbal medicine products may be exported to other countries and at the same time competing with international products. The invention of a quality herbal product requires extensive research and development (R & D). Therefore, scientific research may validate and confirm the benefits of the herb, which has been used through generations.

2.2.2 Scientific Research of Natural Products and Herbal Medicines

Before synthetic drugs were invented, natural products have been a primary source of medicine. Among natural products used as a treatment of human illness were plants, animals and microorganism (Aslam, Ahmad, and Mamat, 2015). Relatively, plants specifically herbs evolved together with human evolution and still applied until today. Herbs that exists in traditional or folk medicine has become a precursor to development of current drug including stress reliever, pain killer, anti diabetic and others depending on type of herbs used (Tiwari, Kumar, Kaur, Kaur, and Kaur, 2011). Nowadays, pharmaceutical companies still extract and isolate crude herbal medicines from raw herbs to formulate the pharmaceutical products (Ahmad et al., 2013)

Scientists conducted ethnobotanical study to investigate medicinal plants consumed by the natives to understand its healing mechanism, enhance the preparations and lastly develop a standard herbal medicine (Ganesan, 2008). Identification and study of secondary metabolites in herbs is one of fundamental research conducted as it mainly responsible to bioactive properties of the plant (Janna, Khairul and Maziah, 2007). Some examples of secondary metabolites are flavonoid, triterpenes, alkaloids, steroid, glycosides and phenolic compounds.

According to Abdelgadir and Van Staden (2013), alkaloids has antibacterial, anticancer and antimalarial properties. Moreover, phenolic compounds are the most active substances present in plant and possess antiallergic and antidiabetic activities (Hardainiyan, Nandy, and Saxena, 2015). Several studies have shown antioxidant activity of flavonoid in plant and it may prevent damage on cells caused by free radical molecules such as free radical species (ROS), hydrogen peroxide (H_2O_2) and the superoxide anion radical (O_2^-) (Habila and Bello, 2010; Hazra, Biswas, and Mandal, 2008; Shanmugapriya, Ramanathan, and Thirunavukkarasu, 2011). Thus, scientific research on bioactive constituents may provide evidence to the curative effect of plants especially herbal medicines.

2.3 EUPHORBIACEAE FAMILY

Euphorbiaceae family is an extensive family consist of 300 genera and about 7500 species (Wiart, 2006) which traditionally utilized by people as wound healing and antibacterial medicine. Generally, the plants in this family can be identified by its 3-locular capsules as well as stipules and spikes of small flowers (Coviello, Haring, Wellons, Vaidya, Lehtimäki, Keildson, Lunetta, He, Fornage, Lagou, Mangino, Onland-Moret, Chen, Eriksson, Garcia, Liu, Koster, Lohman and Lyytikäinen, 2012).

Besides that, they are widely distributed almost in all habitats including low and upper land and on most kind of soil.

Several plants of this family are known as traditional healer especially in poor and developing countries. A review on antifertility potential from plants revealed that 19.7 % of Euphorbiaceae members possess antifertility effect on female (Kumar, Kumar, and Prakash, 2012). Apart from that, *Euphorbia hirta* L (Euphorbiaceae). was found to exhibit wound healing properties and might treat injuries such as burn, bruises and cut (Akinrinmade and Oyeleye, 2010; Jaiprakash and Reddy, 2006; Upadhyay, Chattopadhyay, Goyary, Mazumder and Veer, 2014) .

Furthermore, previous study has reported that *Jatropha gossypiifolia* L . (Euphorbiaceae) and *Emblica officinalis* L. (Euphorbiaceae) have antidote properties to neutralize snake venoms (Félix-Silva, Souza, Menezes, Cabral, Câmara, Silva-Junior, Rocha, Rebecchi, Zucolotto, and Fernandes-Pedrosa., 2014; Rita, Animesh, Aninda, Benoy, and Sandip, 2011). Many other plant members of this family have been used for treatment of diseases yet to be scientifically proven by the researcher. Further study to investigate the medicinal properties of these plants will be beneficial for the community throughout the world

2.4 ACALYPHA L. GENUS

The genus Acalypha is the third largest genus in Euphorbiaceae family comprises of 500 species (Barberá, Velayos, and Aedo, 2013). This genus consists of herbs, shrubs and tree, typically distributed in tropics region including Peninsular Malaysia, Papua New Guinea, Mexico and East Africa (Sagun, Levin, and van Welzen, 2010). The Acalypha species is the most diverse and complex genus but not much research has been conducted to investigate its bioactive substances and pharmacological properties.

The members of the genus Acalypha L. have been studied for its pharmacological activities including antimicrobial, antioxidant and cytotoxicity study (Canales, Hernández, Rodriguez-Monroy, Flores, Jiménez-Estrada, Hernández, Durán, Hernández-Moreno, Trejo, Hernández, Ramírez, Orozco, Eleno, and Martínez, 2011; Mothana, Abdo, Hasson, Althawab, Alaghbari and Lindequist, 2010). *Acalypha wilkesiana* is one of the Acalypha species that has gained lot of attention due to its medicinal benefits. Several research conducted on this herb suggested that it may assist to inhibit cancer cells such as brain and lung cancer (Cardiel and Muñoz, 2012; Hungeling, Lechtenberg, Fronczek, and Nahrstedt, 2009; Lim, Ting, Bradshaw, Zeenathul, Wiart, Khoo, Lim, and Loh, 2011). Furthermore, a study on Malaysian Acalypha species, *Acalypha simensis* reported a novel purification of acalyphaser A. that may associate with anticancer activity (Kambara, Yamada, Tsujioka, Matsunaga, Tanaka, Ali, Wiart, Yusof, Hassan, Hanifah, Fauzi, Mazlan, Jay, Kunishima, and Akaho, 2006).

To date, limited research found in literature review to investigate the genus of Acalypha L., despite the fact that significant studies on the pharmacological properties have been undertaken. Further elucidation on the mechanism of biological activity shall enhance the understanding regarding its healing effect.

2.5 *Acalypha indica Linn.*

Acalypha indica belongs to Euphorbiaceae, a large family of flowering plants. Majority of the species is distributed in the southern China, tropical and South Africa, South East Asia, Sri Lanka, Pakistan and Yemen (Kamalakannan, Murugan, and Barnard, 2011). It is locally known as “kucing galak” or “kuppaimeni” in India and “t’ie han tsai” in China (Gnanasangeetha and Thambavani, 2014). In Malaysia, *A.*