APHRODISIAC PROPERTIES AND TOXICOLOGICAL EVALUATION OF *Aquilaria malaccensis* AND *Aquilaria subintegra* (AGARWOOD) LEAVES AQUEOUS EXTRACT IN ICR MICE

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ABSTRACT

This study aimed to investigate the properties of aphrodisiac and to evaluate the toxicological effect of leaves aqueous extract from *Aquilaria malaccensis* (AEAM) and *Aquilaria subintegra* (AEAS) against ICR mice. The aphrodisiac properties, which include sexual behaviour, orientation activity, number of offspring, male to female ratio of offspring, the percentage of pregnancy and testosterone level. Meanwhile, toxicological evaluation consists of acute and sub-acute study. In aphrodisiac study, the treated groups received 50, 100, 200, 500, and 1000 mg/kg body weight (b.w.) of extracts for 21 consecutive days. In acute toxicity study, the treated animals received a single dose at 2000 mg/kg b.w., while in sub-acute toxicity study, the treated groups received 50, 100, 150, 200, 500, and 1000 mg/kg b.w. of extracts for 21 consecutive days compared to control groups that received normal saline only. Statistical analyses were performed using t-test and one-way ANOVA and also considered significantly different at $p<0.05$. Results showed insignificant difference ($p>0.05$) in sexual behaviour and orientation activity of treated mice as compared to the controls. The treatment groups that received higher doses (500 and 1000 mg/kg) of both extracts found to be inactive due to the sedative effect. However, higher aphrodisiac properties were displayed by AEAM treated mice compared to AEAS. The number of offspring in AEAS was higher compared to AEAM. Toxicological evaluation showed that there was a significant increase ($p<0.05$) in sperm motility in most of the treated groups as compared to the control group. Percentage of abnormal sperm was found to be significantly lower ($p<0.05$) in the treatment group compared with the control groups in the sub-acute study. Besides, the sub-acute study also found to cause a slight reduction in body weight of mice due to stress. Therefore, this study found that both agarwood leaves aqueous extracts lack of aphrodisiac properties in mice due to the sedative effect. Nevertheless, both extracts were non-toxic based on the toxicological parameters and beneficial to enhance sperm quality as well as can increase breeding rate in mice. In conclusion, *A. malaccensis* and *A. subintegra* aqueous leaves extract have a good potential as an alternative treatment to enhance sperm quality and treatment of sleeping disorder. The implication of this study that it provides scientific data on the advantages of agarwood leaves usage that can be a reference in the pharmaceutical field.

CIRI-CIRI AFRODISIAK DAN PENILAIAN TOKSIKOLOGI EKSTRAK AKUEUS DAUN *Aquilaria malaccensis* DAN *Aquilaria subintegra* (KARAS) TERHADAP MENCIT ICR

ABSTRAK

Kajian ini bertujuan untuk meneliti ciri-ciri afrodisiak dan menilai kesan ketoksikan ekstrak akueus daun *Aquilaria malaccensis* (AEAM) dan *Aquilaria subintegra* (AEAS) terhadap mencit ICR. Ciri-ciri afrodisiak merangkumi tingkah laku seksual, aktiviti orientasi, bilangan anak, nisbah bilangan anak jantan kepada betina, peratusan tahap kehamilan dan tahap testosteron. Sementara itu, penilaian toksikologi terdiri daripada kajian akut dan sub-akut. Dalam kajian afrodisiak, kumpulan rawatan telah menerima 50, 100, 200, 500, dan 1000 mg/kg berat badan (b.w.) ekstrak selama 21 hari berturut-turut. Dalam kajian ketoksikan akut, haiwan yang dirawat telah menerima dos tunggal sebanyak 2000 mg/kg b.w., manakala dalam kajian ketoksikan sub-akut, kumpulan rawatan telah menerima 50, 100, 150, 200, 500, dan 1000 mg/kg b.w. ekstrak selama 21 hari berturut-turut berbanding kumpulan kawalan yang menerima *normal saline* sahaja. Analisis statistik telah dilakukan menggunakan ujian-t dan ujian ANOVA satu hala dan juga dianggap berbeza secara signifikan pada $p<0.05$. Dapatan kajian menunjukkan perbezaan yang tidak signifikan ($p>0.05$) bagi tingkah laku seksual dan aktiviti orientasi kumpulan rawatan berbanding dengan kawalan. Kumpulan rawatan yang menerima dos-dos yang tinggi (500 dan 1000 mg/kg) bagi kedua-dua ekstrak didapati tidak aktif disebabkan oleh kesan sedatif. Walau bagaimanapun, mencit dalam kumpulan rawatan AEAM mempamerkan ciri-ciri afrodisiak yang lebih tinggi berbanding AEAS. Bilangan anak dalam kumpulan rawatan AEAS adalah lebih tinggi berbanding AEAM. Penilaian toksikologi mendapati bahawa terdapat peningkatan yang signifikan ($p<0.05$) dalam pergerakan sperma bagi kebanyakan kumpulan rawatan berbanding kumpulan kawalan. Peratusan sperma yang tidak normal didapati menurun dengan signifikan ($p<0.05$) bagi kumpulan rawatan berbanding kumpulan kawalan dalam kajian sub-akut. Di samping itu, kajian sub-akut juga didapati telah menyebabkan sedikit penurunan berat badan mencit disebabkan stres. Oleh itu, kajian ini mendapati bahawa kedua-dua ekstrak akueus daun karas tidak mempunyai ciri-ciri afrodisiak pada mencit disebabkan oleh kesan sedatif. Walau bagaimanapun, kedua-dua ekstrak ini tidak toksik berdasarkan parameter toksikologi dan bermanfaat untuk meningkatkan kualiti sperma serta dapat meningkatkan kadar pembiakan mencit. Kesimpulannya, ekstrak akueus daun *A. malaccensis* dan *A. subintegra* berpotensi sebagai rawatan alternatif untuk meningkatkan kualiti sperma dan rawatan gangguan tidur. Implikasi kajian ini ialah ia menyediakan data saintifik tentang kelebihan penggunaan daun karas yang boleh dijadikan rujukan dalam bidang farmaseutikal.

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LIST OF ABBREVIATIONS

AEAM	*A. malaccensis* Leaves Aqueous Extract
AEAS	*A. subintegra* Leaves Aqueous Extract
ANOVA	Analysis of Variance
ATP	Adenosine Triphosphate
b.w.	Body Weight
cGMP	Cyclic Guanosine Monophosphate
CNS	Central Nervous System
ED	Erectile Dysfunction
ELISA	Enzyme-linked Immunosorbent Assay
EL	Ejaculation Latency
FSH	Follicle Stimulating Hormone
GnRH-LH	Gonadotropin Releasing Hormone - Luteinizing Hormone
ICP-MS	Inductively Coupled Plasma Mass Spectrometer
ICR	Institute of Cancer Research
IF	Intromission Frequency
IL	Intromission Latency
IUCN	International Union for Conservation of Nature
ng/ml	Nanogram/milliliter
LH	Luteinizing Hormone
LD_{50}	The Median Lethal Dose
MEBF	Methanol Extract of *B. frondosa* Bark

MF	Mount Frequency
ML	Mount Latency
MSD	Male Sexual Dysfunction
NO	Nitric Oxide
JPSM	Jabatan Perhutanan Semenanjung Malaysia
MARDI	Malaysian Agricultural Research and Development Institute
OECD	Organization of Economic Cooperation and Development
PBS	Phosphate Buffer Saline
PDE-5	Phosphodiesterase Type-5 Synthetic
PHF	Polyherbal Formulations
PUFA	Polyunsaturated Fatty Acid
ROS	Reactive Oxygen Species
ROW	Relative Organ Weight
SEM	Standard Error Mean
spp.	Species
SPSS	Statistical Packages For The Social Science
SOL	Solignum®
WHO	World Health Organization

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CHAPTER 1

INTRODUCTION

1.1 Introduction

This chapter elucidated the several subtopics that become baseline and backbone of the current research that comprises of the research background, the problem statement which important to discuss the related issues of the study field, research objectives, research questions, research limitation and in addition the significance of the study.

1.2 Research Background

The World Health Organization (WHO) estimated about 80% of the populace in developing countries still rely on traditional plant medicine for their main healthcare requirements (Ifeoma, 2008; Bagewadi, Siddanagouda, & Baligar, 2012; Dahham et al., 2016). The utilization of medicinal plants have become more well known among the community because it is natural, cheap, have fewer side effects, easy to access and has good therapeutic outcome (Kotta, Ansari, & Ali, 2013; Porwal, Khan, & Maheshwari, 2017). The potential of tropical rainforest as the main sources of the medicinal plants for various ethnic groups in Malaysia, particularly in treating various diseases is undeniable and this practice is well known among the populace (Samuel et al., 2010).

Malaysia has become the reservoir for numerous medicinal plants (A'attiyyah et al., 2017) where approximately 8300 species are found in Peninsular Malaysia and 12000 species are recorded in Sabah and Sarawak areas (Saw, Chua, Suhaida, Yong, & Hamidah, 2010). Basically, the different parts of medicinal plants such as the roots, leaves, barks, flowers fruits, seeds, rhizomes, tubers, bulbs, and stems are used to treat many ailments by traditional practitioners for decades (Ong, Rosnaini Mat Zuki, & Pozi Milow, 2011). However, they discovered that the most common plant parts used in the medicinal plant preparation by Malaysian traditional practitioners are leaves, roots and the whole plant. Previous studies that were done by Samuel et al. (2010) and Ong et al. (2011) successfully listed about 62 and 56 plant species respectively from different study areas that beneficial to treat various disease and already practiced

among Malaysian populace for ages ago. Thus, the effectiveness and popularity of medicinal plants depend on the user's experiences and Malaysian folks' belief.

The most common research trends in Malaysia involve the investigation of medicinal plants with antimicrobial, anticancer, antidiabetic, antiproliferative, antioxidant, anti-inflammatory as well as anti-nociceptive properties (A'attiyyah et al., 2017). In modern days, anti-infertility become another investigation field that gains popularity among Malaysian researchers. Interest in treating male infertility by using medicinal plants is rising in both developing and developed countries (Nantia, Moundipa, Monsees, & Carreau, 2009; Sharma, Bhardwaj, Arif, Khan, & Singh, 2014). Researchers are now attempting to formulate drugs from plant origin to overcome infertility issues as these drugs are believed to be safer than any other drugs that are already available nowadays.

There are various species of medicinal plants such as *Eurycoma longifolia* (Norhazlina Abdul Wahab, Norfilza M. Mokhtar, Wan Nurul Heriza A. Halim, & Das, 2010; Mahmoud & Mahanem Mat Noor, 2013), *Ficus deltoidea* (Nurdiana Samsulrizal, Zanariah Awang, Mohd Luqman Hakim Mohd Najib, & Mohd Idzham Abu Zarin, 2011), *Nigella sativa* (Saheera Kamarzaman, Munirah Sha'ban, & Suzanah Abdul Rahman, 2013; Suzanah Abdul Rahman, Noor Faridah Shaik Dawood, Shaik Sadak Basha, & Saheera Kamarzaman, 2013), *Lunasia amara* (Nor-Raidah & Mahanem, 2015), *Gynura procumbens* (Pusparanee Hakim, Halimah Abdullah Sani, & Mahanem Mat Noor, 2008), *Chlorophytum borivilianum* (Giribabu, Kumar, Rekha, Muniandy, & Salleh, 2014), *Phaleria macrocarpa* (Saadat Parhizkar, Maryam Jamielah Yusoff, & Mohammad Aziz Dollah**,** 2013), *Hylocereus*

costaricensis (Farid Abdul Aziz & Mahanem Mat Noor, 2010) and *Hibiscus sabdariffa* (Muhd Hanis Md Idris, Siti Balkis Budin, Mohamad Osman, & Jamaludin Mohamed, 2012) that have been investigated by local researchers due to their capability to enhance sex drive and likewise, they can overcome infertility problem particularly among Malaysians.

To date, there are still a lot of medicinal plants that has not been explored and investigated for its aphrodisiac purposes which essential to overcome infertility problem, especially among men. One of them is agarwood which belongs to the Thymelaeaceae family in the *Aquilaria* genus. There are more than fifteen different species of *Aquilaria* around the world where the population is dense in Asia region (Kakino et al., 2010a; Sattayasai, Bantadkit, Aromdee, Lattmann, & Airarat, 2012). Agarwood is high price resinous heartwood that is broadly used in the Middle East, South Asia, Japan, and China as traditional medicines, incense and perfume (Xu et al., 2013). Nowadays, the Middle East countries become the major importers for agarwood oil in the world (Nor Azah Mohd Ali, Nurlaila Ismail, & Mohd Nasir Taib, 2012).

The utilization of agarwood as an aphrodisiac agent already stated by several previous studies (Naef, 2011; Chetpattananondh, 2012; Selvan, Nandini, & Kaushik, 2014; Alam et al., 2015) and this plant leaves part likewise recommended by local communities as an aphrodisiac agent that has great potential to treat infertility problem. Aphrodisiac can be defined as any food or medication that useful in stimulating the sexual desire, induces general desire and increases sexual pleasure as well as performance (Zade & Dabhadkar, 2013; Veeresh, Kumar, & Gupta, 2017).

In general, the utilization of agarwood previously was focusing more on agarwood production in the form of agarwood sawdust, agarwood woodchips or woodblocks and agarwood oil (Ismail Muhammad & Mohd Zin Jusoh, 2011). But, nowadays many researchers are more creative in order to construct an amazing innovation that can be produced from other parts of agarwood plant especially from the agarwood leaves. Principally, harvesting of the resin from the agarwood tree regularly involves chopping off the tree where the leaves and other parts of the tree are considered as waste (Azrina, Nor Azah, Siti Humeirah, Mailina, & Muhammad Haffiz, 2014). For a few years ago, the utilization of agarwood leaves as a healthy herbal tea already recorded in Vietnam, Cambodia, and Thailand (Kamonwannasit et al., 2013). Lately, the utilization of this herbal drink that also called as 'teh gaharu' or 'teh karas' is gaining popularity among Malaysians due to its vast range of pharmacological values that are good for human health (Aimi Zafirah Adam, Lee, & Rozi Mohamed, 2017).

Many previous studies have proved the therapeutic purposes of agarwood leaves which have strong potential in treating Alzheimer's disease (Bahrani, Mohamad, Paydar, & Rothan, 2014), good as laxative agent (Hara et al., 2008; Kakino et al., 2010a; Kakino et al., 2010b), antipyretic and anti-inflammatory agents (Zhou, Wang, Suolangjiba, Kou, & Yu, 2008; Rahman, Eswaraiah, & Dutta, 2016), antimicrobial (Dash, Patra, & Panda, 2008), anti-hyperglycemic activity (Pranakhon, Pannangpetch, & Aromdee, 2011; Nur Liyana Zulkifle, Nor Adila Mhd Omar, Saiful Nizam Tajuddin, & Mohd Rosly Shaari, 2013; Sxureha Yunus, Nurul Asyikin Md Zaki, & Ku Halim Ku Hamid, 2015), antioxidant agent (Huda, Munira, Fitrya, & Salmah, 2009; Nik Noor Asma Nik Wil, Nor Adila Mhd Omar, Noorhuda

Awang@Ibrahim, & Saiful Nizam Tajuddin, 2014; Begum, 2016; Habsah Alwi et al., 2017) and anti-lipase activity (Zainal, Musa, Idris, & Hamid, 2018; Mastura Ibrahim et al., 2018) scientifically. So far there have been no studies reported on agarwood leaves extracts that may act as aphrodisiac booster and can enhance fertility effect scientifically although it has been used traditionally for years. Therefore, the scientific study is needed to verify this agarwood usage.

1.3 Problem Statement

In developing countries such as Malaysia, the inability to afford modern medical therapy for infertility problem has forced patients to seek traditional plant medicine in order to find cure for their sickness. Traditional plant medicine or also known as phytotherapy or phytomedicine with aphrodisiac properties provides a safer way to counteract various problems associated with male infertility (Mathur, 2012). This type of remedy is gaining popularity among the community because it is natural, cheap, has fewer side effects, easily accessible and has the good therapeutic outcome (Patel et al., 2011; Kotta et al., 2013; Veeresh et al., 2017).

Recently, there is a continuing worldwide green revolution which mainly believes that the utilization of plant medicine is safer and less harmful to the human body rather than synthetic drugs (Parekh & Chanda, 2006). There are numerous plant medicine that already been reported by previous researchers to have positive aphrodisiac effects (Patel et al., 2011) particularly *E. longifolia* that is known as "Tongkat Ali" which is widely used in Malaysia to treat infertility issues by increase

sexual activity and produce strong sex drive (Redzuan Nul Hakim Abdul Razak et al., 2017). Another potential plant for aphrodisiac and fertility purpose that is still not investigated belongs to the genus of *Aquilaria* which are *Aquilaria malaccensis* and *Aquilaria subintegra*.

The phytochemical constituents found by previous researchers in *Aquilaria* leaves extract are flavonoid glycosides (Yang, Feng, Yang, Zhao, & Liu, 2012; Chen et al., 2012a), 2-(2- phenylethyl) chromenes (Yang et al., 2012), lignans (Peng et al., 2011), genkwanin 5-*O*-*β*-primeveroside and mangiferin (Hara et al., 2008; Kakino et al., 2010b; Ito et al., 2012), iriflophenone 2-*O*-*α*-rhamnoside and iriflophenone 3,5-*C*-*β*-diglucoside (Hara et al., 2008), Iriflophenone 3-*C*-*β*-glucoside (Pranakhon, Aromdee, & Pannangpetch, 2015), benzophenone glucoside (Qi, Lu, Liu, & Yu, 2009) and also diterpenoids (Chen et al., 2012b). Besides, the chemical constituents that consist of alkaloids, tannins, saponins, flavonoids, and terpenoids are also present in the *Aquilaria* leaves extract (Khalil, Rahim, Taha, & Abdallah, 2013; Fatmawati & Hidayat, 2016).

Traditionally, the utilization of agarwood as an aphrodisiac agent already claimed by several previous studies specifically from the heartwood (Naef, 2011; Chetpattananondh, 2012; Selvan et al., 2014; Alam et al., 2015) as well as from agarwood leaves (Ratna Sari Dewi 2016; Ompusunggu, 2018 in Sukandar, 2010). Aphrodisiac can be defined as any food or medicine that is useful for stimulating sexual desire (Zade & Dabhadkar, 2013; Veeresh et al., 2017), inducing general desire and increasing sexual pleasure and also performance (Zade & Dabhadkar, 2013) and these substances commonly are obtained from plants, animals or minerals (Singh et

al., 2013a). An aphrodisiac also can be defined as any substance that enhances sexual activity as well as sexual pleasure (Yakubu et al., 2007; Mahajan, Mahajan, & Mahajan, 2012). Walton (1966) in Yakubu et al. (2007) claimed that the aphrodisiac term is derived from 'Aphrodite', the Greek goddess of adoration, beauty, and fertility.

There are some phytochemicals that possess aphrodisiac properties which are saponins (Gauthaman, Ganesan, & Prasad, 2003; Yakubu et al., 2005), alkaloids (Zamblé et al., 2009) as well as flavonoids (Yakubu & Akanji, 2011; Silva, Borges, & Velozo, 2012) since these phytochemicals have androgen enhancing and antioxidant properties. Thus, the presence of various phytochemical constituents reported by previous researchers specifically saponins, alkaloids, and flavonoids as well as the claimed that made by previous researchers additionally become the primary backbone for the aphrodisiac investigation specifically from agarwood leaves. Besides, the beneficial usage of agarwood leaves from *A. malaccensis* and *A. subintegra* that were chosen for the present study has still not been explored sufficiently by researchers especially via *in vivo* study using suitable animal models.

According to Mat Saat Mahmood (personal communication, July 22, 2016) and Mohd Noor Jusoh (personal communication, May 24, 2017), majority of Malay community believe that aqueous extracts of the agarwood leaves possess aphrodisiac properties that could reduce infertility problems in men. Both personal communication previously. Locally, the plant is prepared as a decoction by boiling the leaves in water and then cooled before consumption. Jones and Kinghorn (2006) also claimed that the preparation of plant medicines commonly through infusions by

soaking in water or decoction by boiling the plant material in the water. Thus, in the present study water was chosen in agarwood leaves extraction in order to mimic the way it is prepared by the local community for aphrodisiac purposes. This plant preparation is in accordance with the study that was done by Ugwah-Oguejiofor et al. (2011) while working on *Ficus platyphylla* to promote fertility effects in rats.

Apart from its claim for improvement of sexual functions in men, the agarwood leaves have currently been considered as an energizer and vitalizer in the indigenous medicine (Ratna Sari Dewi, 2016; Sukandar (2010) in Ompusunggu, 2018) specifically in Malaysia. Although agarwood leaves have been used among the communities for reproduction booster, there is still no scientific report on agarwood leaves verifying its usage as the sexual tonic and fertility stimulant. Keeping in view the growing popularity and market interest for the plant-based drug (Nirmal et al., 2013; Gunjan et al., 2015), this study was undertaken to provide scientific support for its purported folkloric usage since there is no scientific information available in the literature on the effect of *A. malaccensis* and *A. subintegra* leaves aqueous extracts on human fertility and reproductive function.

Therefore, this study was designed to investigate the effect of aphrodisiac properties from oral administration of *A. malaccensis* and *A. subintegra*'s leaf aqueous extract in ICR mice. This study was also done in order to scientifically elucidate the claims made by Ratna Sari Dewi (2016), Sukandar (2010) in Ompusunggu (2018) and also several personal communications mentioned previously on the agarwood leaves which possess aphrodisiac properties that benefit in treating sexual disorder among men nowadays. The main parameters that were evaluated in

this study include the toxicity study in term of body weight (b.w.), relative organ weight and spermatotoxicity; orientation behaviour activity; sexual behaviour activity; estimation of testosterone level; percentage of pregnancy, the number and male to female ratio of offsprings. Additionally, this study also evaluates the distinction between *A. malaccensis* and *A. subintegra* leaves aqueous extract effects on aphrodisiac parameters which consist of spermatotoxic evaluation, orientation behaviour, sexual behaviour, testosterone level, the percentage of pregnancy, number and male to female ratio of offspring in ICR mice. The results of this study are hoped to prove that the agarwood leaves have a good potential as traditional medicine that is rich with fertility enhancer properties in the future. Besides promoting the utilization of phytomedicine for replacement of modern medical and synthetic drugs, this study also benefits to reduce biomass waste for the better environment.

1.4 Objectives

This study aligned several objectives, which are:

i) To evaluate the effects of the acute and sub-acute toxicity studies on body weight, relative organ weight, mortality rate and sperm parameters (count, motility and abnormality) of two species of agarwood leaves aqueous extracts in male ICR mice.

ii) To analyse the effects of two species of agarwood leaves aqueous extracts towards sexual behaviour, orientation activity, testosterone level,

percentage of pregnancy, number and male to female ratio of offspring in ICR mice.

iii) To compare the distinction between two species of agarwood leaves aqueous extracts in spermatotoxic evaluation, orientation behaviour, sexual behaviour, testosterone level, percentage of pregnancy, number and male to female ratio of offspring in ICR mice.

1.5 Research Question

This researched aimed to answer the following reseach questions:

i) What are the effects of the acute and sub-acute toxicity studies towards body weight, relative organ weight, mortality rate and sperm parameters (count, motility and abnormality) of two species of agarwood leaves aqueous extracts in male ICR mice?

ii) What are the effects of two species of agarwood leaves aqueous extracts towards orientation activity, sexual behaviour, testosterone level, the percentage of pregnancy, and the number of offspring in ICR mice?

iii) What are the differences between the two species of agarwood leaves aqueous extracts towards spermatotoxic evaluation, sexual behaviour, orientation behaviour, testosterone level, the percentage of pregnancy, the number and male to female ratio of offspring in ICR mice?

1.6 Research Limitation

This research focussed only on the leaves extracts of *A. malaccensis* and *A. subintegra* species only. Thus, the result obtained through this experiment cannot be applied to other parts of this plant such as flower, root, fruit, bark, seed and wood. Besides, the data obtained in this study also cannot be generalized to other studies due to the different localization of the plant, albeit belonging to the same species. This situation may be due to the different concentration of phytochemicals as well as mineral content in the plant species that grow in a different location which also may affect its biological activity towards the experimental animal.

Moreover, the solvent used in the present study may also influenced the finding of the presence of phytochemical constituents in the plant samples. Thus, the findings cannot be generalized to other studies that used different type of solvents such as ethyl acetate, acetone, alcohol (methanol and ethanol), chloroform and hexane. The choice of the solvent usually influences the final plant product because it might contain traces of residual solvent. Therefore, the solvent that was chosen for plant extraction should be non-toxic and should not interfere with the bioassay.

1.7 Significance of Study

The main rationale for conducting this research is to verify the claim of agarwood as aphrodisiac particularly by local community in Malaysia. By doing more research on the benefits of the agarwood usage, more systematic data and information can be easily obtained and become the important guide for future investigation. With all research and sources available, Malaysia is capable in producing a better quality plant based products. Nowadays, the selection for medicinal plants to be investigated scientifically is based on its ethnopharmacological information, folklore, or traditional uses.

Additionally, the effort to discover the benefits derived from medicinal plants, especially agarwood, is vital for producing a better quality of plant-based drug for human healthcare purposes. The most important role of plants in modern medicine to guide the discovery and development of the new plant derived drug rather than direct utilization for therapeutic proposes that already practiced by local people for ages ago. This is most important as in traditional medicine practices; the medicinal plants are normally used without consideration of quality or quantity, unlike dose-dependent commercial drugs that are currently in the market. Therefore toxicity studies are needed to determine the safety and effectiveness of agarwood leaves extract utilization as the aphrodisiac enhancer as claimed by local community. The toxicity studies done in this present research such as acute (single dose) as well as sub-acute (repeated dose) are able to provide the sufficient database that aids correct decision making concerning the toxicology properties and able to establish the safe limits for dose level selection that can be used for future experiment.

Besides, the findings of this research also lead to the discovery of the benefits of phytochemical constituents that are present in both agarwood species and might shorten the time required to develop the new plant derived drug specifically from leaf part of these plants. Despite just focusing on aphrodisiac activities investigation, this study also helps the future researchers to obtain information on the benefits of agarwood leaves usage specifically to nourish sperm cells as well as enhance the production of sperm. Moreover, the safe dose level of agarwood extracts also can be estimated and well planned by other researchers that used the same species of plant in *Aquilaria* genus. As a conclusion, this study also can become baseline information and perfect guideline for the future researchers specifically in the similar field of the investigation.