POTENTIAL OF GELAM (*Melaleuca cajuputi*) LEAF AS NATURAL INSECTICIDE

by

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A report submitted in fulfillment of the requirements for the degree of Bachelor of Applied Science (Natural Resources Science) with Honours

FACULTY OF EARTH SCIENCE
UNIVERSITI MALAYSIA KELANTAN

2017

DECLARATION

I declare that this thesis entitled "Potential of Gelam (*Melaleuca cajuputi*) Leaf as Natural Insecticide"is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

Signature : ______________________________

Name : ______________________________

Date : ______________________________

ACKNOWLEDGEMENT

First and foremost I would like to express my sincere gratitude to my supervisor, Ms. Nivaarani a/p Arumugam and also my co-supervisor, Dr. Suganthi a/p Appalasamy for their valuable guidance, advice, knowledge and encouragement. Their guidance inspired and helped me in completing this report successfully. Also, thank you to Faculty of Earth Science, Universiti Malaysia Kelantan for sponsoring mobility programme to Prince of Songkla University, Pattani for about two month. My greatest gratitude to both Dr. Weeraya Khummueng and Dr. Charoen Pakhathirathien, lecturers of Faculty of Science and Technology, Prince of Songkla University Pattani for their valuable knowledge and also in helping me providing necessary facilities to conduct this research. A warm thank you also to the examiners, Dr. Boon Jia Geng and Dr. Sitti Fatimah Bt Mhd. Ramle who guided me, contribute valuable opinions and respective support during my entire process of completing this final year project.

I also want to acknowledge the inspiration and support from my family. Deepest thanks to both my biggest supportive parents, Mr. Mat Nor Muhamad and Mrs. Mas Kalthom Isa. With their continuous support and advice, this research and report can be completed within a given time.

Last but not least, I would like to thank all the staff members of all laboratories especially Mr. Muhamad B. Isa for his assistance that allow me to conduct my research smoothly. I would also like to thank my friends especially Ms. Visheentha a/p Martin and Ms. Zaween Najjah Mohd Syamsul for their invaluable encouragement and support besides helping me until the completion of my final year project.

Potential of Gelam (*Melaleuca cajuputi*) Leaf as Natural Insecticide

ABSTRACT

An interest in *Melaleuca cajuputi* leaf has been increased due to their benefits especially as potential sources for insects control agents. Cockroaches are one of the rough insects that abundantly found in household areas which can pose various threat to human health and hard to be eliminated. The use of natural insecticides can be an alternative to synthetic insecticides as they are more biodegradable and safer compare to synthetic insecticides which are harmful to human and environment. Therefore, a study was conducted to evaluate the toxicity and repellency of essential oil and extracts from leaves of *Melaleuca cajuputi*. Common household cockroaches were selected for this experiment which is American cockroach (*Periplaneta americana*). The essential oil and hexane crude extract from *Melaleuca* leaves were used against American cockroach. Toxic test in this experiment were subjected to force feeding using 50% concentration at room temperature where the extract or essential oils were mixed with the food. For repellent test, the essential oil and leaves crude extract were tested at three different concentrations which are 0.015g, 0.008g and 0.004g. In the control experiment, the insects were exposed and fed with untreated food and normal tap water. Each test was followed with three replications. Mortality and repellency was recorded within 24 hours with several intervals. The essential oil showed highest mortality values for cockroach (97%) after 24 hours exposure compare to the leaves extract which showed no mortality. Besides, the highest repellency was observed at 0.015g respectively for both crude leaves extract and essential oil. Crude leaves extract was more effective in repellent test after 24 hours exposure at almost all concentration used in this experiment. The percentage repellency of leaves extract and essential oil however exhibited no significant differences with the mortality rate in this study. Therefore, the present study has demonstrated that *Melaleuca cajuputi* have repellent and toxic effects on *Periplaneta americana* thus this can be developed further for pest control and management in future.

Keupayaan Daun Gelam (*Melaleuca cajuputi*) Sebagai Racun Serangga Semulajadi

ABSTRAK

Tumpuan terhadap daun *Melaleuca cajuputi* telah meningkat disebabkan manfaat yang dimiliki terutamanya berpotensi sebagai sumber kepada agen kawalan serangga. Lipas adalah salah satu serangga yang banyak dijumpai di kawasan rumah yang boleh menimbulkan pelbagai ancaman kepada kesihatan manusia dan sukar untuk dihapuskan. Penggunaan racun serangga semula jadi boleh menjadi alternatif kepada racun serangga sintetik kerana ia lebih mesra alam dan lebih selamat berbanding racun serangga sintetik yang berbahaya kepada manusia dan persekitaran. Oleh itu, satu kajian telah dijalankan untuk menilai ketoksikan dan penghalauan serangga daripada minyak pati dan ekstrak dari daun *Melaleuca cajuputi*. Jenis lipas rumah yang biasa telah dipilih untuk eksperimen ini iaitu lipas Amerika (*Periplaneta americana*). Minyak pati dan ekstrak mentah heksana daripada daun *Melaleuca* telah digunakan terhadap lipas Amerika(*Periplaneta americana*). Ujian ketoksikan yang digunakan di dalam eksperimen ini ialah cara paksa makan menggunakan 50% kepekatan iaitu pada suhu bilik di mana ekstrak atau minyak pati telah di campur dengan makanan. Untuk ujian penghalauan, minyak pati dan ekstrak mentah daun telah diuji pada tiga kepekatan berbeza iaitu 0.015g, 0.008g dan 0.004g. Untuk kawalan eksperimen, serangga telah di dedahkan dan diberi makan dengan makanan yang tidak dirawat dan air paip biasa. Setiap ujian diulang sebanyak tiga kali. Kematian dan penangkisan lipas terhadap bahan ujian dicatatkan dalam tempoh 24 jam dengan beberapa selang masa. Minyak pati menunjukkan nilai kematian tertinggi bagi lipas (97%) selepas 24 jam pendedahan berbanding dengan ekstrak daun yang tidak menunjukkan sebarang kematian. Selain itu, kadar penangkisan lipas yang tertinggi telah diperhatikan pada kepekatan 0.015g masing-masing untuk kedua-dua ekstrak daun dan minyak pati. Ekstrak mentah daun adalah lebih berkesan dalam ujian penghalauan serangga selepas di dedahkan selama 24 jam pada semua kepekatan yang digunakan dalam eksperimen ini. Peratus penghalauan oleh ekstrak mentah daun dan minyak pati walaubagaimanpun menunjukan perbezaan yang tidak setara dengan kadar kematian dalam ujian ini. Oleh itu, keputusan kajian ini membuktikan bahawa *Melaleuca cajuputi* mempunyai kesan penghalauan dan ketoksikkan ke atas *Periplaneta americana* seterusnya membolehkan kajian ini dipertingkatkan lagi untuk kawalan dan pengurusan serangga perosak pada masa hadapan.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
B. germanica	*Blatella germanica*
DDT	Dichlorodiphenyltrichloroethane
g	gram
LLE	Liquid-Liquid Extraction
m	meter
mL	millilitre
P.americana	*Periplaneta americana*
SE	standard error
sp.	species
SPE	Solid Phase Extraction
SPSS	Statistical Package for Social Science
UMK	Universiti Malaysia Kelantan

LIST OF SYMBOLS

^{o}C	Degree celcius
%	Percentage

CHAPTER 1

INTRODUCTION

1.1 Background of Study

In this rapid developing world, pests become the most concern issues as they can pose terrifying risks and rendered many problem and diseases. Cockroaches, which one of the most common household pests, are frequently found in baits, storage rooms, kitchens, refuse dumps, pipelines and in damp places in any structure that has food preparation. As a result from their habitation in such places, cockroaches are often correlated with human wastes and certain of diseases thus can lead to public health problems (Beccaloni and Eggleton, 2013). Due to that, cockroaches also have been acquainted as mechanical potential vectors of individual intestinal parasites and animal pathogen as well as a source of human allergens since previous research has acknowledge that cockroach foreign substance is most prevalent in children especially in undeveloped cities for asthma-inducing allergen (Arruda et al., 2001; Busse and Mitchell, 2007). Hence, their control is of great importance and therefore various of insecticides have been produced as one of the alternative to reduce their population.

Insecticides are a type of pesticides generally produced to kill, harm, repel, and disrupt hormones of insect pest in order to counter the damages which also contribute to negative effects in human in numerous ways, most particularly those composed from synthetic substances besides showing toxicity to environment (Khater, 2012a). According to Ujjan et al. (2014), the effect in human due to the usage of synthetic insecticides may include skin and eye irritation, allergic, hormone imbalances and also increased the risk of cancer for long term effects used. Thus, the natural insecticides are worth investigating as it is more biodegradable and do not leave toxic residues rather than those synthetic origins. They are the best option for use in urban area and can play greater role in developing countries as a new form of eco-friendly products for pests control since it is produced originally from plant materials.

Much of the related research to natural insecticides has been on essential oil due to the volatile components in it such as terpenes which may be useful for repelling insects and preventing insect infestation (Ujjan et al., 2014). Neem, Turmeric, Lemongrass and other potential plants are the newer botanical insecticide that has been repeatedly reported to have ability to kill or keep away the pest by processing its plant materials into oil. Gelam (*Melaleuca cajuputi*) leaves actually can also distil to produce oil called 'cajuput' or 'tea-tree oil' but contain different use in which it has medicinal and antiseptic uses especially to treat burns, cramps, skin diseases and various aches (Macdonald and Giesen, 2015). However, there is still lacking of information reported on the efficiency of Gelam leaves to act as natural insecticide although it has been known to have numerous potential active compounds.

So, this research was carried out with the objective to identify the potential of *Melaleuca cajuputi* leaves as natural insecticides as an alternative to reduce the usage of synthetic substances by testing on cockroaches using crude leaves extract and essential oil obtained from crude extraction and steam distillation process.

1.2 Problem Statement

In recent years, the focus and research on *Melaleuca cajuputi* in Myrtacea family has increase among botanical researchers since this plant species has various benefits due to all part of the tree can be obtained and used for many purposes. The leaves beneath it all have great impact as it is previously utilized as ruminant feed besides can be distilled as oil and used in aromatherapy, treat fungal skin infections and helps in joint pain (Widiana et al., 2014). Despite of those potential and importance of this family species has been widely studied with detailed, there still been lacking on research reported that the leaf part of this plants species has potential to act as natural insecticide. Other than that, this plant has been found abundance in Malaysia hence, it is worthwhile to investigate and embark this research. Furthermore, this research also is performed to learn and explore more about this plant.

Cockroaches are one of the creepy insects found abundantly in household area especially American cockroach which undoubtedly can pose serious threat to human health as they mechanically drift and convey many pathogenic reactions (Phillips et al., 2010). Cockroaches also are one of the rough insects that most people find it hard to kill or eliminate it. Hence, to inhibit the parasites of cockroaches and the potential of disease transmittal, products usually depend on synthetic insecticides. However, the usage of these substances can cause to certain adverse effects, including water and soil pollution, insect resistance, toxicity to non-target species and cause environmental pollution (Kumar et al., 2010). Due to that, the public concern has grown unexpectedly, therefore turned the attention of communities and researchers towards plant based insecticides since they consists a range of bioactive chemicals and non toxic materials that are selective and harmless to non-target organisms and the environments (Subramaniam and Sandeep, 2014). As in this study, natural insecticide origin from *Melaleuca cajuputi* leaves was investigated as an alternative source of insect control agents.

1.3 Objectives

The objectives of this research are:

a. To identify the repellency and toxicity of *Melaleuca cajuputi* leaf extract and essential oil against American cockroach.
b. To evaluate the potential of *Melaleuca cajuputi* leaf extract and essential oil as natural insecticide.

1.4 Scope of Study

The focus of this study is to determine the potential of *Melaleuca cajuputi* leaves as natural insecticide against household cockroach, *Periplaneta americana.* The leaves material were going through crude extraction process using hexane as a solvent to produce leaves extract while steam distillation process were regulated to obtain essential oil. The repellent and toxic tests were conducted in order to determine the efficiency of the test materials as insecticides.

1.5 Significance of Study

The purpose of this study is to identify the potential of Gelam leaves extract as natural insecticide. This study is very crucial in order to reduce the practice of synthetic insecticides which is known to have potential in inflicting the environment and human health. At the same time, the pest population that cause diseases also could be control. Although people are aware of insect-transmitted disease, the numbers of illness related to that remain increased. In spite of that, the action is important and should be taken seriously instead of awareness. So, the investigation for safer insecticides on plant origin are intensely needed as the result obtained could establish advantageous information especially to the public and assist in pest management to reduce insect related diseases. Also, this study could inspire the researchers by providing a useful reference and guidance to other related research in the future.

CHAPTER 2

LITERATURE REVIEW

2.1 Pesticides in General

Pesticides are substances that significantly produced for eradicating any associated pests. They are mostly used in plant or crop protection from weeds, fungi and insects for instance and applied especially by farmers. Pesticides also widely used for non-agricultural purposes. The term for pesticide may vary according to the target organism thus includes all of the following: herbicide, insecticide, nematicide, termiticide, piscicide, insect repellent, fungicide and sanitizer (Lah, 2011). Although there are different terms used, the main purposes of the products are to combat the pest problem or unwanted species that are assumed as disturbance. However, the focus in this research is mainly on insecticides.

Insecticides substantively play a greater role especially in the food production in these days besides used in household areas to expel the insects that damage the properties and pose risks to the human health. Insecticides were manufactured in two types depend on the nature of the problem emerge which are synthetic insecticides and natural insecticides (Miller et al., 2010).

2.1.1 Synthetic Insecticides

Synthetic insecticides are widely used by farmers since 1930s to control and battling the insect pests in their crops. Today, synthetic insecticides have been purchased by most peoples around the world as the fastest way to eradicate pests instantly without considering its effects especially to environment and health. Synthetic insecticides such as dichlorodiphenyltrichloroethane (DDT) for example, was the first generation insecticide that has been used especially in urban aerial spray in United States to control mosquito, moth, beetle and others in 1940's before it was banned due to the effect it cause to environment and others (Isman, 2006). There are several classes of synthetic insecticides as shown in the Table 2.1:

Table 2.1: Classes of synthetic insecticides and its area of effects.

Class	Examples	Area of Effect
Organochlorines	DDT, toxaphene, dieldrin, aldrin	Reproductive, nervous, endocrine, and immune system
Organophosphates	Diazinon, glyphosate, malathion	Central nervous system
Carbamates	Carbofuran, aldicarb, carbaryl	Central nervous system
Pyrethroids	Fenpropanthrin, deltamethrin, cypermethrin	Skin and endocrine system

Source: Isman (2006)

According to World Health Organization (2014), it is estimated that there are about three million cases of pesticide poisoning each year which up to 220,000 deaths especially in emerging countries. Young children are oftenly vulnerable to the harmful effects of pesticides. Hence, there are several types of harmful effect that commonly generated by insecticides such in Table 2.1 which are acute effects, delayed effects and allergic effects (Khater, 2012b). Acute effects are basically disorder that appears quickly after disclosure where the responses are tangible and volatile if necessary curative is given on the spot. While, delayed effects involved illnesses that do not appear instantly such as cancer. The last one is allergic effects which are parlous effects that only some people develop in contact to substances.

Today, there are about five hundred species of insects and mites have revived to resistance to some mode of pesticides (Chen et al., 2002). Due to the increasing resistance among these insects, countries has begun to look for a new products that are more powerful and effectives in battling the pests and insects but safer for environment and human.

2.1.2 Natural Insecticides

Natural insecticides usually comprise from plants origin as a defense against insects and become source of insectidal activity. According to Logita (2015), natural insecticides have additional advantage over synthetic insecticides since they are safer, easily biodegradable, innocuous to non-target organisms and do not leave residues besides can minimize costs to the consumers instead of synthetic insecticides. Synthetic insecticides which emerge from chemicals substances are highly toxic and generally lack of selectivity, give impact on the environment, spread of insect resistance and pose harm to human health and safety (Busse and Mitchell, 2007). Long term effects of those substances may include hormones imbalances and increased risk of cancer to the affected organism especially human and fish. Therefore, natural insecticides are comparatively safer to use and are considered as eco- friendly product (Isman, 2006).

Besides, plants are also contain rich sources of bioactive chemicals and may be an alternative source of insect control agents (Asmanizar and Idris., 2012). Some plants materials are extracted and directly applied on the infected crop or target insects and some need to undergo complicated method to obtain required materials such as hydrodistillation and infusion. Meanwhile, phytochemicals which derived from plant sources can act as repellent and these activities have been observed by many researchers (Silva et al., 2013; Suthisut et al., 2011). Rotenone from *Derris* sp., nicotine from tobacco, neem from *Azadiracta indica* and pyrethrins from *Chrysanthemum* sp. have been widely used especially in small-scale industry as well as in commercial agriculture (Schultz et al., 2009). Rotenone and pyrethrins are the most popular traditional botanical insecticides among other natural insecticides that have been used since sixtieth century. Rotenone is a terpene that was applied as a spray on fruits or crop and become potentially toxins to several insects such as aphids, cockroaches and houseflies (Chen et al., 2002). While, pyrethrins can paralyzes the insects in contact within a few seconds only but in mammals, the toxicity is low since the pyrethrins ester can be converted into nontoxic compound in the stomach (Chen et al., 2002).

However, not all natural products are safer since most of those natural products can also contribute to the contact, respiratory or stomach poisons besides effecting beneficial organisms even the toxicity of the products is usually not very high (Khater, 2012b). Despite of that, those natural origin products are highly biodegradable and can become inactive within hours or a few days thus negative impact on beneficial organisms can be reduces and relatively environmentally safe (Khater, 2012b).

Other than that, the type of natural insecticides available nowadays are currently changing and is developed for more better products in order to be the most effective products. Essential oils are now entering in the most market place since they have benefits as a scent in fragrances and perfumes, as flavoring food additives and as medications besides used as insecticides (Isman, 2000).

2.2 Essential Oil from Plants

Essential oils are resilient, natural, complex compound identified by a vigorous odor and produced by aromatic plants as alternate metabolites (Isman, 2000). Essential oils are found in glandular hairs or secretory cavities of plant-cell wall and are present as droplets of fluid in the leaves, stems, bark, flowers, roots or fruits in different plants (Koul et al., 2008). Most essential oils comprise of monoterpenes compounds that contain ten carbon atoms often arranged in a ring or in acyclic form, as well as sesquiterpenes which are hydrocarbons comprising of fifteen carbon atoms (Koul et al., 2008). Higher terpenes may also be present as minor constituents.

Usually, essential oils are obtained by steam hydrodistillation process. Recently, essential oil has earned much attention due to their multi-functional uses including as antimicrobial, antifungal, anti tumour and insectidal agents (Batish et al., 2008). Also, due to the constituents that is primarily lipophilic compounds, essential oil act as toxins, feeding deterrents and oviposition deterrents to a wide variety of insect pests (Koul et al., 2008). Essential oils inherently can be inhaled, ingested or skin absorbed by insects thus altering insects feeding behaviour, growth and development, molting and many more (Manzoor et al., 2012). Insectidal progress of essential oils has been shown against cockroaches (Phillips et al., 2010), mosquitoes (Chaiyasit et al., 2006) and termites (Zhu et al., 2001).

Example for essential oil that commonly reported as repellent by previous research are cedarwood and mint oil. Cedarwood are utilized against termites while mint oil are against both American and German cockroaches (Appel et al., 2001). In spite of that, recent studies also demonstrated that the wide range of insects affected by essential oils from Myrtaceae family. These oils have knockdown and repellent activity, and act as feeding and or oviposition deterrents (Roger et al., 2012). This is proved when the essential oils from *Eucalyptus citriodora*, *Eucalyptus globulus* and *Eucalyptus staigerana* can reduced the percentage of viable eggs and emerged insects of the two coleopterans species (Roger et al., 2012). Apart from that, essential oils have other uses as food additives, flavourings, and components of cosmetics, soaps, perfumes, plastics, and as resins (Koul et al., 2008).

Hence, essential oils are far better than synthetics, but their durability tend to be short-lived in term of effectiveness due to their volatility (Khater, 2012a). Therefore, this research will be conducted to overcome the deficiency of today products for more better result.

2.3 Gelam (*Melaleuca cajuputi*)

Melaleuca cajuputi is a tree which belongs to the family Myrtaceae. Among the Myrtaceous, the genus *Melaleuca*, also recognized as bottle-brush trees generally comprise over two hundred and fifty species of Australian trees and shrubs (Macdonald and Giesen, 2015). The species of *Melaleuca* occurs naturally in peat swamp forests between the old raised sea beaches mostly in Australia, Peninsular Malaysia, Thailand and Singapore. The trees usually have a medium to tall with a narrow and dense crown and can be easily recognised by their distinctive thick papery flaky bark that can be peeled off easily (Zaituliffarizah, 2010). The tree (Figure 2.1) is a large parennial tree which can reach heights of up to forty meters with mature trees having trunks up to 1.2 m in diameter.

Figure 2.1: *Melaleuca cajuputi* tree found in Bachok on 11 April 2016.

Meanwhile, the leaves of *Melaleuca cajuputi* (Figure 2.2) actually resemble those of an *Acacia mangium* (Figure 2.3) which give off a typical 'tea-tree' smell when crushed (Southwell and Lowe, 2000) but their size are more smaller than *Acacia* leaves. While, tiny flowers of Gelam are creamy-yellow emerging on a long spike in the shape of a bottle-brush at the end of the branches.

Figures 2.2: *Melaleuca cajuputi* leaves found in Bachok on 11April 2016.

Figure 2.3: *Acacia mangium* leaves

Source: (Smith, 2016).

Melaleuca have woody capsules (Figure 2.4) for most of their species that discharge seed after the parent branch is wipe out, either by fire or other mean factors. The blooming of serotinous fruits (late-opening capsules) in *Melaleuca* and related genera are usually predicted as an adoption to and protection against fire (Budiadi et al., 2005). However, the abundant production of woody fruits is more closely associated to mineral poor soils (Zaituliffarizah, 2010). Many species that do not have other seed dormancy factors usually yield serotinous fruits and are short-lived in the soil seed bank (Kumar, 2005). There is also some proof that fire could provisionally increase soil nutrients to let germination and survival of woody-fruited species but this is not convincing and conclusive.

Melaleuca cajuputi seed

Figure 2.4: Woody capsules that contain Melaleuca seed found in Bachok on 11 April 2016.

Other than that, *Melaleuca cajuputi* species are usually easily adapted, tolerant and mature well under a several range of environmental circumstances along with those associated with unfavourable to growth such as acidic soil, saline soil, flooded soil and also dry lands (Budiadi et al., 2005). While, in the occurrence of forest fires, *Melaleuca cajuputi* is a species with high resistance to effects of flames and high temperature, particularly the large and mature trees (Kumar, 2005). In cases where forest fires are not resilient, the fibrous bark provides heat insulation and therefore the plant can survive (Kumar, 2005). However, if the forests fire associated with ground fires happen in peat land ecosystems, the tree's root system will be burn and the plant will not survive and die.

2.3.1 Utilization of *Melaleuca cajuputi* in General

Melaleuca cajuputi which abundantly found in Peninsular Malaysia and beyond actually has multi-purpose uses for the people especially that live in and around *Melaleuca cajuputi* forest.

Melaleuca cajuputi's timbers are one of the plant parts that can be utilized as fuel wood by local people which also particularly suitable to produce high quality charcoal (Southwell and Lowe, 2000). Even though the timbers are relatively soft, it is adapted to anaerobic conditions and more resistance to damage cause by water than any other woods available in their habitat. Based on the density of timber, it is proved by Lim and Midon (2001) that *Melaleuca cajuputi* tend to be stronger than light red meranti (*Shorea* sp.), rubberwood (*Hevea brasiliensis*), and mersawa

(*Anisoptera*) but weaker than kempas (*Koompassia malaccensis*) and keruing (*Dipterocarpus* sp.). Therefore, *Melaleuca cajuputi* timber has been widely used in many forms of construction such as construction of fences, boundary markers to demarcate land tenure and used as floorboards and frames (Macdonald and Giesen, 2015).

Melaleuca cajuputi also possess papery bark (Figure 2.5) that can be ripping off easily. The layer of the barks can be calculated to indicate the age of the tree.

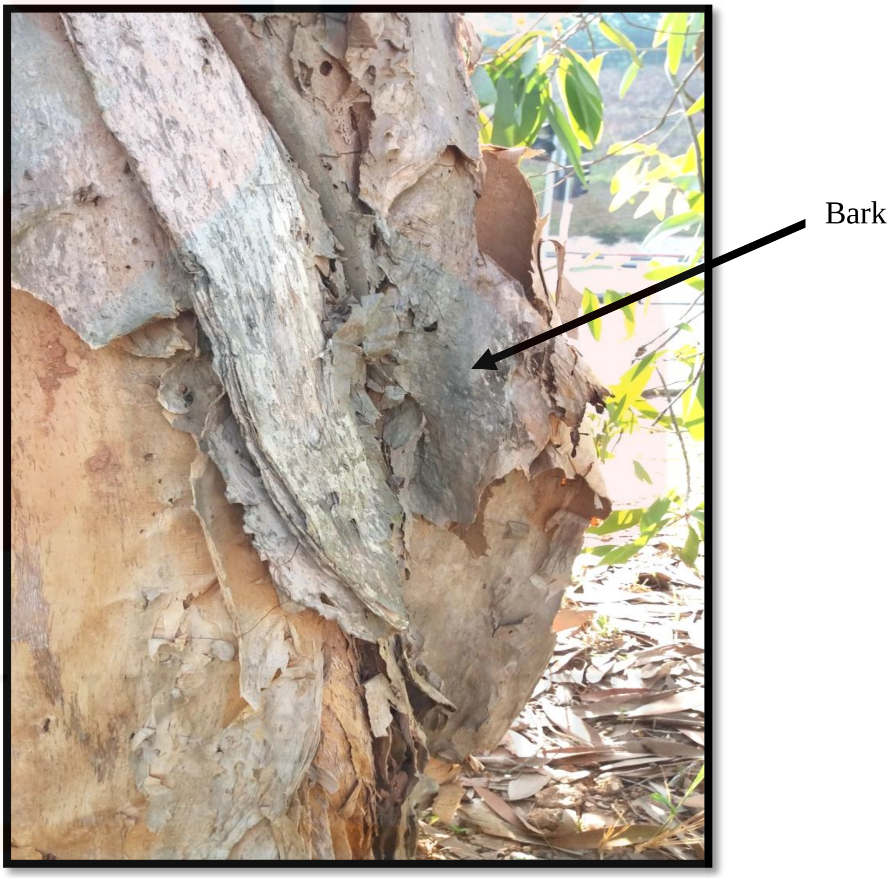

Figure 2.5: *Melaleuca cajuputi* bark found in Bachok on 11 April 2016.

The barks are tenacious, waterproof and contain insulative properties which assist the *Melaleuca cajuputi*'s forest with its certainty against forest fire (Southwell and Lowe, 2000). Despite the ability of being peel and roll off easily, the barks sheet has recognition as a roofing material and adhesive material in padding boat

(Macdonald and Giesen, 2015). Furthermore, the bark's roll also used in seed proliferation, concoction of water and heat resistant materials by local people. Hence, its softness also can be felt and used as stuffing materials for bedstead and pillow (Southwell and Lowe, 2000).

Apart from that, the leaves of *Melaleuca cajuputi* sustain some of the invaluable uses specially respect to its herbal remedial properties. Local people who animate around *Melaleuca cajuputi* forests areas usually boil the plant's leaves and drink them as herbal tea in order to soothe misery and alleviate a number of malady or disease including asthma, intestinal parasites, viruses and other pertinent health problem (Koh et al., 2011).

Melaleuca cajuputi is a very versatile plant thus its leaves are processed on a vast scale in order to yield oil called Cajuput oil that are profitable in a range of personal hygiene products such as shampoo, perfume and skin balm. It also gives strong aroma for medicinal uses and enclosed several active portion which are cineol (60%) and α-terpineol which usually distilled by a known technique called hydro distillation process (Cox et al., 2000). Cajuput oil is further applied in herbal medicinal products to reassured symptoms of coughs, colds, as a cathartic and typical muscle relaxant and sedative besides being use for cancer treatment (Bagg et al., 2006; Garazzo et al., 2011; Macdonald and Giesen, 2015).

Besides, Cajuput oil also has been exposed as a vigorous disinfectant against bacteria and can be used as a mosquito and termite deterrent or repellent (Koh et al., 2011). Cajuput oil that is made from *Melaleuca cajuputi* eventually is better in quality and can reached higher price in contrary of those that are extracted from eucalyptus leaves but the oil also has their own disadvantage especially effect on insect as it only effective for short period of time.

2.4 Cockroaches

Cockroaches are members of the order Blattodea, which known as primitive insects based on fossil evidence since they have exists on earth for over three hundred million of years. They are one of the common and hardy insects which can tolerate and habituate to a wide range of environments and conditions therefore mediated as the most triumphant groups of insects (Jayaram and Robert, 2016). Favourable conditions has actually introduced to their rapid population growth while unfavourable conditions cause adult cockroaches to deteriorate more slowly or die early (Phillips et al., 2010).

Most cockroach species have wings, but have liability to crawl rather than fly. Cockroaches also are omnivorous insects which eventually will scavenge everything ranging from food spills until to faecal matter (Stetson, 2001). Some species can boost their lives and remain active without food for over a month in limited resources condition. Their miscellaneous foraging sources in areas for instance restaurants, pipelines, drains and garbage areas occasionally associate them with disease virus including salmonella, streptococcus and other organisms in league with dysentery, typhoid, hepatitis and tuberculosis (Phillips et al., 2010). The bacteria ingested by cockroaches can actually endure for about a month or even a year in their digestive system. Furthermore, cockroaches also secrete a substance that can stains on surfaces they contact by relaying through vomiting their saliva and defaecate faeces and cast skins besides producing smelling odours (Arruda et al., 2001). Thus the prominent way to prohibit the infestation and transmission of cockroach parasites must include a combination of good hygiene practices and effective insecticides utilization (Beccaloni and Eggleton, 2013).

Moreover, cockroaches are creepy nocturnal insects and are seldomly seen in any quantity during daylight hours since they are sensitive to light (Wilson, 2016). However, in areas where there are massive infestations especially after heavy rain, spot of cockroaches may become more common. Most residents are aware on the health and safety risks related with cockroach infestations, including the allergies and asthma cause by cockroach allergence as infestation may occur when cockroaches gain entry through drain covers, plumbing, under doors and drifting into the premises on raw materials and packaging (Rejitha et al., 2014). But, cockroaches are very hard to be kill even many alternatives have been invented. Current chemical controls especially for cockroaches nowadays are ineffective. This is because, the chemical used sometimes repellent to the cockroaches so they avoid its effects by moving away for a few days and then back again.

Other than that, cockroaches also can evolve resistance to a certain chemical shortly (Beccaloni and Eggleton, 2013). Similar to natural insecticides available today which commonly takes long time to get rid of insects therefore, new insecticides should be explored and produced as an alternative to synthetics especially the one that are green to environment in case to eradicate the pests population. Insectidal chalk is one of the products that are produced and sales commonly in Chinese grocery stores but acquire temporary effect on cockroaches (Stetson, 2001). This product chases the cockroaches away instead of eliminating them (Phillips et al, 2010). American cockroach (Figure 2.6) is one of the largest insects pests that invaded home and commercial properties where the adult could reach the size about thirty-five until fifty-three mm long with wing that prolong over the tip of the abdomen (Stetson, 2001) . They can be distinguished by their unique shiny red-brown colour of exterior body that has an amber look on it compare to the other cockroaches which posses muddy brown colour on exterior body.

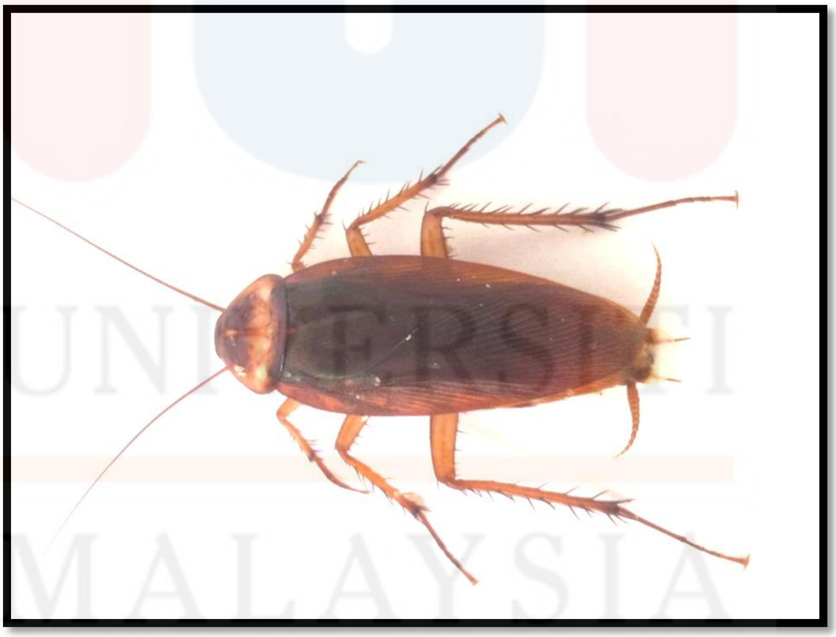

Figure 2.6: *Periplaneta americana* found around hostels and food court on 4 August 2016.

2.4.1 Life Cycle of Cockroach

Following cockroach development, due to their natural behaviour, they reproduce quickly especially in warm and moist condition. Female cockroaches occasionally used pheromones to fascinate their mates while the males practice courtship rituals for instance posturing and stridulation (Stetson, 2001). Cockroaches mostly undergo three life stages (Figure 2.7) which begin with egg, nymph and adult but a few species are known to be parthenogenetic which is emulating without the need of male cockroaches (Wilson, 2016).

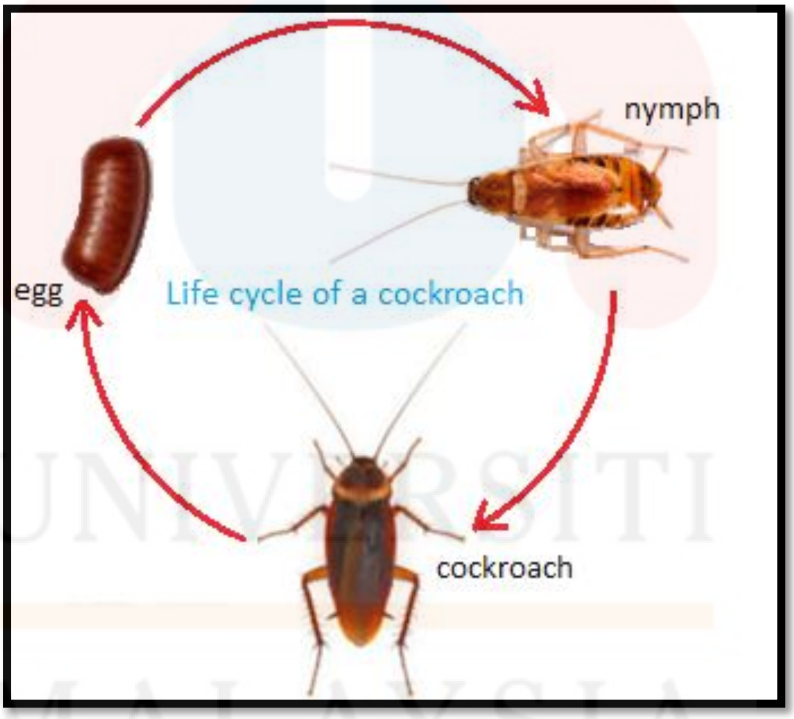

Figure 2.7: Cockroach life cycle

Source: (Jayaram and Robert, 2016)

A female cockroach basically lay between ten to forty eggs at certain time. However, for average, female cockroach can produce around thirty batches of eggs in her lifetime (Jayaram and Robert, 2016). The female cockroaches usually retain their eggs in groups in purse-shapes egg cases called Ootheca (Figure 2.8).

Figure 2.8: Ootheca of different types of cockroach species

Source: (Wilson, 2016)

The egg case are dispose or glue onto a surface preceding to the eggs hatching and enclose with water sufficient for the eggs to develop without acquiring additional water from the substrate (Jayaram and Robert, 2016). After about thirty days, the white colour nymph (Figure 2.9) appear and slowly begin their gradual growth and development then change into greyish-brown and reddish-brown as they encounter metamorphosis by shedding their exoskeleton (Stetson, 2001). The nymphs obtain no wings and possess the same look as adult cockroaches but much smaller in size. However, they will be equipped with wings and reproductive abilities after they have passed their final molt (Stetson, 2001).

Figure 2.9: Cockroach nymph in Ootheca

Source: (Stetson, 2001)

2.5 Extraction in General

Extraction usually involves the segregation and isolation of beneficial active segment of plant or animal tissues from impotent components by having selective solvents in standard extraction procedure (Wells, 2003). Extraction techniques may include liquid-liquid extraction and solid phase extraction. In liquid-liquid extraction (LLE), both phase A and B are originated from liquids that must be immiscible. In practice, one phase is commonly aqueous while the other phase is an organic solvent. While, for solid phase extraction (SPE), the extract can be included in the extraction matter in solid or liquid form. SPE usually used for distilling the analyte completely from the entire sample volume via the sorbent (Hans, 2011). To extract natural products such as plant materials, organic solvents of different polarities are normally selected in modern method instead of water to produce the various solubilities of plant portion. Extraction may include many types of preparations such as fluid extracts, decoctions, percolation, maceration, infusions, powdered extracts and tinctures.

2.5.1 Steam Distillation of Plant

Steam distillation is a method for distilling compounds which are heat-sensitive involves bubbling steam through a heated mixture of the raw material (Forest Research Institute, 2011). Some of the target compound will vaporize (in accordance with its partial pressure) correspond to Raoult's law. The vapor mixture is cooled and condensed, frequently yielding a layer of oil and a layer of water. Thus, steam distillation of various aromatic herbs and flowers can result in two products which is an essential oil as well as a watery herbal distillate (Forest Research Institute, 2011). The essential oils are regularly used in perfumery and aromatherapy while the watery distillates have many applications in aromatherapy, food processing and skin care (Forest Research Institute, 2011).

Steam distillation is a special type of distillation (a separation process) for temperature sensitive materials like natural aromatic compounds. Many organic compounds tend to decompose at high continuous temperatures (Forest Research

Institute, 2011). Therefore, in this study, separation by normal distillation would then not be an option, so water or steam is introduced into the distillation apparatus.

By adding water or steam, the boiling points of the compounds are depressed, allowing them to evaporate at lower temperatures, preferably below the temperatures at which the deterioration of the material becomes substantial and faster besides can prevents damage to the oil (Forest Research Institute, 2011). If the substances to be distilled are very sensitive to heat, steam distillation can also be united with vacuum distillation. After distillation, the vapors are condensed as usual, occasionally yielding a two-phase system of water and the organic compounds, allowing for simple separation (Forest Research Institute, 2011).

Eucalyptus oil and orange oil are obtained by this method on the industrial scale. Steam distillation is also extensively used in petroleum refineries and petrochemical plants where it is commonly referred to as steam stripping (Forest Research Institute, 2011). Other industrial uses of steam distillation include the production of consumer food products such as sprayable or aerosolized condiments such as sprayable mayonnaise.

2.6 Bioassay in General

Bioassay or biological assay is a type of scientific practice which engaging the use of live animal or plant (in vivo) or tissue or cell (in vitro) to investigating the biological activity of a substance such as hormone or drug (Miller et al., 2010).

Usually, bioassay will be operated to identify the effects and causes of target substance on living organism in creating new products or drugs especially in controlling contamination and pollution.

Besides, a bioassay also can be used to identify the experiment of a certain structure of a mixture that can pose disastrous effects whether on environment or on organisms (Miller et al., 2010).

Bioassay can be both qualitative and quantitative where qualitative are applied to reach the physical effects of a materials that maybe cannot be quantified. While, quantitative involve consideration of the dose response curve and how the response changes to the increasing dose level (Miller et al., 2010).

CHAPTER 3

MATERIAL AND METHOD

3.1 Materials

The main instrument that was used in this research is grinder to crush down the leaves into a dry powder, an oven, rotary evaporator, steam distillator and refrigerator. The other apparatus that were needed may include round bottom flask, conical flask, zip lock bag, adjustable pool, rope, plastic container and gloves. Once the leaves extract were produced using hexane as a solvent and the oil obtained from steam distillation process, the bioassay test were prepared. A micro pipette and cotton ball were needed besides Petri dish, biscuits, forceps and plastic jar.

3.2 Methods

This study was conducted at Prince of Songkla University (PSU) Pattani. The flow of the research has been summarized in Appendix A. There were two method used in bioassay testing which are force feed and repellent test to determine the effectiveness of the test materials. The plant materials were processed in the form of crude leaves extract and essential oil.

3.2.1 Collection of Cockroach (*Periplaneta americana*)

In this research, common house cockroaches were used. Required numbers of adult cockroaches were collected stage by stage around Prince of Songkla University Pattani (PSU) hostels and food court using plastic bottle trap (Figure 3.1). The collected cockroaches were kept in plastic bags at room temperature (26^{o}C) and relative humidity (69%). The plastic bags were then perforated for aeration and the cockroaches were fed with some biscuits and water.

Figure 3.1: Plastic bottle trap

3.2.2 Collection of *Melaleuca cajuputi* Leaves for Crude Extraction

The plant material that was used in this research is the leaf part of *Melaleuca cajuputi's* trees. The leaves sample was collected from wild around Bachok area. The dust and sand on the leaves sample were removed to avoid contamination. The leaf samples were then air dried at room temperature (27^{o}C) and weighed before the drying process until completely dried. Next, dried leaves samples subsequently were crushed to a fine powder (Figure 3.2) by using grinder to be used in crude extraction process.

Figure 3.2: *Melaleuca cajuputi*'s leaf fine powder

3.2.3 Crude Extraction Preparation Using Leaves Powder

Crude extraction of *Melaleuca cajuputi* leaves was prepared by modifying the method described based on Logita (2015). Briefly, the *Melaleuca cajuputi* leaves powder was extracted with hexane as a solvent and kept for 24 hours at room temperature (27^{o}C) using glass container tightly closed by aluminium foil. After 24 hours, the extract solution was filtrate using filter funnel and Whatman filter paper No.1. Then, the extract solution was transferred into round bottomed flask after adding sodium sulphate anhydrous to remove remaining water. After filtrated, the solvent used was removed by using rotary evaporator at 48rpm rotation. A solid extract was formed and weighed before kept in refrigerator at 4^{o}C until used for bioassay test. The hexane was poured again into the container using leaves powder that was used before to obtain remaining leaves extract. The process was repeated exactly as above.

3.2.4 Steam distillation Using *Melaleuca cajuputi* Leaves

Steam distillation process was conducted based on the modified method by Manzoor et al. (2012) to obtain essential oil. In this process, a fresh leaf that was collected from the wild of Pattani areas at coordinate 6°49’24.04” N, 101°9’44.4” E was used. The leaves was weighed and washed with water thoroughly to remove dust and other contamination. Then, the leaves was cut into smaller pieces so that the water able to distil all substances in the leaves before filled in 5000 ml round flask with two litres of water. After that, the flask was placed into the heater and the Dean-

Stark trap was set up to start the distillation process. After the distillation finished, the water was removed and the oil obtained was collected. A small amount of sodium sulphate anhydrous was added to remove access water in the oil. Then, the oil was transferred into small container before weighed using the balanced. The essential oil obtained was kept in the refrigerator at 4^{o}C.

3.2.5 Test Jar Preparation for Toxicity and Repellent Test

A plastic jar used in this experiment was based on modified method by Rejitha et al. (2014). The jar (Figure 3.3) posses the following compartment: middle chamber (9 cm diameter) and one plastic tube (12cm length and 3.5 diameter). The end of the plastic tube is fitted with smaller plastic jar than the middle one (11cm height and 7 cm width) which then marked as A and B respectively. B jar taken as a treated jar.

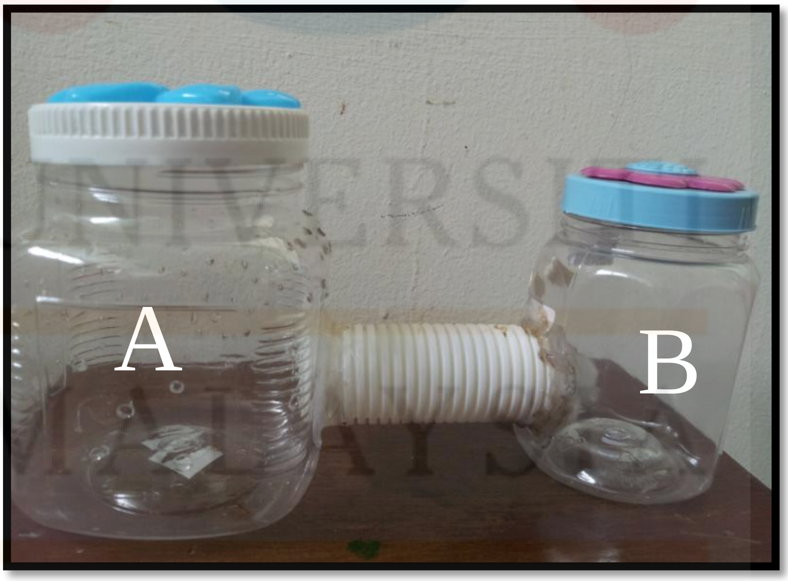

Figure 3.3: A test jar

3.2.6 Bioassay Testing on *Peripleneta americana*

The bioassay test for this experiment involved two types of test which are repellent test and toxic test using essential oil and crude leaves extract separately. Toxicity were determined using force feed method while repellency were determined by the percentage of cockroaches repel to the test materials.

a) Repellent Test Using Essential Oil and Crude Leaves Extract

This test was based on modified method by Tunaz et al. (2008). The experiment was conducted using American cockroaches at maintained room temperature (26^{o}C) and relative humidity (69%). Pure essential oil was applied using micropipette on small cotton ball with different concentration in different test jar which are 0.015 g, 0.008 g and 0.004 g. The food was put in the same placed with the treated cotton ball. Ten cockroaches then were released into the test jar. Repellency (hyperactivity followed by hypertension of legs and abdomen or movement to the untreated compartment) was observed every 1, 3, 6, 9 and 24 hours immediately after the treatment has been started. Tap water was applied on small cotton ball in different test jar as a control for this test before releasing the cockroach. The control was also observed in the same period of time. Three replicate were conducted for each test of different concentration of essential oil separately. The data obtained was calculated using the formula from Thavara et al. (2007) as in Equation 3.1. The experiment was continued by replacing an essential oil with crude leaves extract using the same step as explained above.

$$\text{Repellency (\%)} = 100 - \left[\frac{T \times 100}{N}\right] \text{ ....Equation 3.1}$$

Where, T= cockroaches in treated area

N= total number of cockroaches

b) Toxic Test for Essential Oil and Crude Leaves Extract

Toxic test was conducted by using force feed method on cockroaches following modified method by (Rejitha et al., 2014). Before conducting the experiment, the cockroaches were starved for 48 hours. A plastic test jar was used as a test chamber. The no choice feeding test was conducted by mixing a biscuit powder, water and 1ml of essential oil to make paste before place in the middle of the boxes. Ten cockroaches were released later in the other jar that contained no untreated food. The mortality was recorded at 1 hour, 6 hour and 24 hours (Rejitha et al., 2014). For control, a paste made from a mixing of biscuits powder and tap water were used and placed in the other test jar before ten cockroaches were released. The above set up was replicated three times for each test for treated and controls. For crude leaves extract, the same method was conducted. However, to make a paste, an essential oil was replaced with 1gm of crude leaves extract to mixed with biscuits powder and water. The same control was used and observed. The rate of mortality was corrected by using of Abbott's formula (Abbot, 1925). The mortality was calculated using the formula Umadevi and Sujatha (2013) as in Equation 3.2.

$$\text{\% Mortality} = \frac{\text{number of dead cockroaches}}{\text{total number of insects}} \times 100 \text{ ....Equation 3.2}$$

3.2.7 Statistical Analysis

The statistical analysis was conducted using one-way analysis of variance (ANOVA) and Duncan by adopting an EXCEL and SPSS Version 21 to analyze the repellency while Probit's analysis was used to calculate probit time mortality of cockroach in this research. A p value of < 0.05 was considered to represent significant differences in repellency. The percentage mortality rates were corrected by using Abbott's formula.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Results

4.1.1 Percentage of yield

The result of the leaves extract and essential oil obtained are as shown in Table 4.1 as well as the percentage of yield. Dried leaves were grounded into powder for solvent extraction, while fresh leaves were distilled to obtain essential oil.

Table 4.1 Percentage of yield for *Melaleuca cajuputi* leaves

Type of extracts	Total leaves sample used (g)	Weight of extract obtained (g)	Percentage of yield(%)
Leaves extract	466.30	11.67	2.50
Essential oil	2602.70	8.77	0.34

4.1.2 Mortality of *Periplaneta americana* using essential oil and leaves extract

Toxicity of *Melaleuca cajuputi* leaves was evaluated against adult American cockroach (*Periplaneta americana*) by force feed method within 24 hours exposure. Data presented in Table 4.2 showed the mortality of American cockroach using both essential oil and leaves extract.

Table 4.2: Mortality of *Periplaneta americana* using 50% concentration of Cajuput oil and crude leaves extract.

Treatment	Exposure time (hours)	Average number of dead cockroaches (mean)	Mortality % ± SE
Essential oil	1	0	3.3 ± 0.3
	6	7	70.0 ± 0.5
	24	10	96.7 ± 0.3
Crude leaves extract	1	0	0.0 ± 0.0
	6	0	0.0 ± 0.0
	24	0	0.0 ± 0.0
control	1	0	0.0 ± 0.0
	6	0	0.0 ± 0.0
	24	0	0.0 ± 0.0

Based on the Table 4.2, essential oil shows high percentage mortality of American cockroach compare to leaves extract which showed no mortality within all exposure time at 50% of concentration. Data also reported that no control mortality during the exposure time which is within 24 hours. The lowest mortality percentage (3.3% ± 0.3) was recorded within one hour exposure in adult *P. americana* when applying essential oil.

During the first hour of exposure, all of the cockroaches were hyperactively act and tried to move away from the treated jar as the essential oils produce strong aromatic scent. After a few hours later, they become more resistance as the strong odors vaporized. They started to forage the treated area and the effect started to show up. Rejitha et al. (2014) stated that, the presence of volatile compounds having strong odour could block tracheal respiration of the insects thus leading to their death. The odour from leaves extract is also as strong as essential oil however the odour did not vaporize as quickly as essential oil. Therefore, the effect is slow and the cockroach did not respond or dead relative to the tested materials within exposure time.

Hence, the result in this study also showed that the tested leaves extract was not effective in mortality of *Periplaneta americana*. The results showed that American cockroach was highly resistance against all tested concentration from leaves crude extract. The cockroaches showed hyperactivity during all the exposure period in the untreated jar. However, if the concentration is increased, the extract might be effective against the *Periplaneta americana*.

Therefore, the findings of present study regarding mortality rate are somewhat in conformity with those reported by Yeom et al. (2013) that the effect of Myrtaceae plant essential oils and their constituents against adult male and female

Blatella germanica showed 100% toxicity at concentration 7.5mg/liter of air concentration. While in contact toxicity, they also reported that several Myrtaceae plant produced strong insectidal activity against adult male and female German cockroaches. Of the essential oils constituents, terpinolene, α-terpinene, and terpinen-4-ol demonstrated strong fumigant toxicity against adult male and female *B. germanica*. Eugenol, isoeugenol, methyl eugenol, and terpinen-4-ol showed strong contact toxicity against adult male *B. germanica*.

Despite of that, the probit's analysis was also calculated to determine the probit mortality of *Periplaneta americana* in lethal time 50, LT_{50}. The probit only can be calculated for essential oil since the data collected for leaves extract shows no mortality during all exposure time.

The result from the Table 4.3 shows that 50% of *Periplaneta americana* used in the experiment was dead at time approaching 4 hours and 54 minutes. Appendix B shows the graph that indicates the Probit's mortality of *Periplaneta americana* using essential oil. This can be concluded that, as time exposure increased, the mortality of cockroach will also increase. In the present study, because of time limitation during conducting the experiments, only 50% of concentration were used and tested against American cockroach.

Table 4.3: Probit's of mortality against American cockroach using essential oil

Type of extracts	Exposure time (hrs)	Log time	Probit's mortality	LT_{50} (hrs)
Essential oil	1	0	3.12	4.54
	6	0.78	5.52	
	24	1.38	6.88	

4.1.3 Repellency of essential oil and leaves extract against *Periplaneta americana*

Regarding the repellency of *Melaleuca cajuputi* leaves, the data is summarized in Table 4.4 for the essential oil while Table 4.5 for repellency of leaves extract against *Periplaneta americana* at different concentration (0.015 g, 0.008 g and 0.004 g). Results analyzed are completely based on the repellent activity shown by the cockroaches. Insects which hesitate to move to the treated jar side are considered repellent in the connecting jar of the experimental setup.

Table 4.4 Repellency of essential oil against *Periplaneta americana*

Concentration of essential oil (g)	Exposure time (hours)	Average number of repelled cockroaches (mean)	Observed Repellency % ± SE	Std. Deviation	min	max	mean
0.015	1	10	100±0.0				
	3	10	100±0.0				
	6	10	100±0.0	.000	10	10	10.00
	9	10	100±0.0				
	24	10	100±0.0				
0.008	1	10	100±0.0				
	3	10	100±0.0				
	6	10	100±0.0	1.029	8	10	9.54
	9	10	100±0.0				
	24	7.7	77±0.7				
0.004	1	10	100±0.0				
	3	10	100±0.0				
	6	10	100±0.0	2.370	5	10	8.94
	9	10	100±0.0				
	24	4.7	47±0.7				

Table 4.5 Repellency of leaves crude extract against *Periplaneta americana*

Concentration of leaves crude extract (g)	Exposure time (hours)	Average number of repelled cockroaches (mean)	Observed Repellency % ± SE	Std.deviation	min.	max	mean
0.015	1	10	100±0.0				
	3	10	100±0.0				
	6	10	100±0.0	.000	10	10	10.00
	9	10	100±0.0				
	24	10	100±0.0				
0.008	1	10	100±0.0				
	3	10	100±0.0				
	6	10	100±0.0	.000	10	10	10.00
	9	10	100±0.0				
	24	10	100±0.0				
0.004	1	10	100±0.0				
	3	10	100±0.0				
	6	9.3	93±0.5	.313	9	10	9.86
	9	10	100±0.0				
	24	10	100±0.0				

Both essential oil and leaves extract shows highly percentage of repellency against tested adult cockroach. After 24 hours exposure, both essential oil and leaves extract recorded complete percentage repellency (100% ± 0.0) at a concentration 0.015g of tested materials. While, at 0.008g and 0.004g of concentration, the repellent activity for the essential oil was decreased as the exposure period increased, both recorded 77% ± 0.7 and 47% ± 0.7 after 24 hours, respectively.

Leaves extract eventually had greatest effect compare to the essential oil as it gave excellent level of repellency (100% ± 0.0) at both concentration 0.015g and 0.008g after 24 hours. The lowest percentage of repellency was recorded at 0.004g concentration for leaves extract. Within six hours, the percentage of repellency dropped (93% ± 0.5). Hence, this study shows that the end of 24 hours, the percentage repellency is maximum as this gives maximum time frame for the entire leaves extract used to act upon the cockroaches. Besides, the mean for both essential oil and leaves extract also shows moderate difference. The mean for leaves extract is consistent which are 10.00 at concentration 0.015g and 0.008g hence slightly differ than testing with essential oil which obtained 10.00 and 9.54. At least five cockroaches whose showed no repellency as the concentration is 0.004g for the essential oil while for the leaves crude extract, at least nine cockroaches which showed no repellency effects.

Although the repellency of leaves extract was more effective than Cajuput oil, however the result by ANOVA in Table 4.6 indicated that no statistically significant difference in susceptibility to both Cajuput oil and leaves extract among the adult American cockroach after 24 hours. Duncan results in Table 4.7 also showed no statistically significant differences between all the concentrations used in the experiment for essential oil and leaves extract. The complete table for ANOVA was shown in Appendix C.

Table 4.6 ANOVA for repellency of essential oil and crude leaves extract against American cockroach

		df	Mean square	F	Sig.
Essential oil	Between groups	2	1.413	.635	.547a
	Within groups	12	2.225		
	total	14			
Leaves extract	Between groups	2	.033	1.000	.397a
	Within groups	12	.033		
	total	14			

a = no significant of mean

Table 4.7 Duncan for repellency of essential and crude leaves extract against American cockroach

	Concentration (g)	Subset for alpha=0.05 1	Sig.
Essential oil	0.015	8.94a	.306a
	0.008	9.54a	
	0.004	10.00a	
Leaves extract	0.015	9.86a	.266a
	0.008	10.00a	
	0.004	10.00a	

a = no significant of mean

The essential oil was significantly less protective than leaves extract. Repellency decreased as it was influenced by the concentration used in the study. At lower concentration plant extracts showed attractant and which in turn showed moderate higher repellent activity when the concentration increased (Rejitha et al., 2014). Some study shows that sometimes, the reason for these type of insect behavior might be due to the presence of olfactory stimulus in cockroaches (Rejitha et al., 2014). Other than that, the odour of test materials might be evaporated during the experiment thus the repellency reduced as the time increased.

Melaleuca leaves extract was more effective than Cajuput oil in this case. This is because the extracts is known to be more stable than oils and can be kept for longer time than oils. Also the repellency percentage increased as the concentration increased. After 24 hours, two concentrations of leaves extract shows complete repellency (100%) compare to the essential oil at 0.015g and 0.008g respectively. This is because, essential oil has high vaporization (Koul et al., 2008) compare to the crude leaves extract. Therefore, most of the strong odour has released to the surrounding or in the cotton ball as the exposure time increased and causing the efficacy of essential oil as repellent decreased. At low concentration (0.004g), leaves extract still exhibited high percentage of repellency (93% ± 0.5). It was also seen that the tested insects strongly repelled from the leaves extracts till the termination of experiment. The cockroaches stayed all the time in untreated area and some was surveying the test jar and return back to the untreated place.

Recently, studies regarding the repellency of essential oil and leaves extract derived from *Melaleuca cajuputi* against American cockroach are limited. Appel et al. (2001) reported that mint oil provided complete repellency to adults of American and German cockroaches. Meanwhile, Thavara et al. (2007) mentioned that essential oils

(100%) obtain from kaffir lime leaves the most effective repellency against American and German cockroaches but essential oil from ginger rhizomes showed moderate level of repellency (85%) against *P. americana*. However, Ferrero et al. (2007) reported that *Schinus molle* extract produced significant repellent effect and mortality against *Blatella germanica*.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

In general, both leaves extract and essential oil showed great impacts on American cockroach. The essential oil recorded high mortality compare to leaves extract but both showed no significant different. But the trend might be changed when the concentration and the exposure duration increased. Highest activity was observed at higher dose of concentration of essential oil. Likewise, in repellent test, both essential oil and leaves extract showed excellent repellency at high dose concentration (0.015g) but also has no significant different between both tested leaves material.

Therefore, this study indicates that leaves extract and essential oil might be useful and can be introduced to the field against various insects pest and can be apply in many industrial and commercial for beneficial purpose. Hence the future prospect of the study lies in the fact that more plant species should be identified its component and utilized for management pests. Thus, an eco-friendly approach can be followed for sustainable development of nature despite of cheapness and simplicity of production.

5.2 Recommendations

It is recommended to test the repellent and toxicity efficacy of individual component of the extract and essential oil to identify the most effective ingredient that actually act upon the tested insects such as using Thin Layer Chromagtography (TLC) and Gas Chromatography Mass Spectrometry (GCMS). Moreover, these compounds have to be tested in field condition to evaluate their efficacy and effective durability. In this study, the repellent and toxicity has been tested against only on one species and do not know whether the leaves extract and essential oil used are effectives against other cockroach species or other insect. However, the results indicated that *Melaleuca cajuputi* essential oil and leaves extract are quite effective and highly toxic to American cockroach and both extracts therefore can be developed as fumigants or spray-type control agents against American cockroaches and potentially be used in areas where traditional insecticides use is restricted. The safety of the oil and their active components in human and non target organisms, formulation and their modes of action should be investigated for further practical use of these oils as novel cockroach-control agents or pest management.

However, there are several limitations that must be endured before the essential oil and leaves extract components can successfully be used in the field or industry. Previous research on insecticides have shown to be repellent (Appel et al. (2001) and to have little residual activity (Isman, 2006). Due to the repellency reported, a pest control operator would have to apply the essential oils carefully in the field. Avoiding contact of the spray on or near insecticidal baits, bait stations, and traps would be necessary to preserve their attractiveness to German cockroaches (Appel, 2004). If used cautiously, essential oils could be used as flushing agents and inspection tools for locating infested areas and reducing the availability of suitable harborages.

A microencapsulated formulation might be useful to increase the residual, decrease the repellency, and eliminate the odor of the essential oils (Phillips, 2010). Microencapsulated formulations contain active ingredient in microscopic polymeric capsules that rupture overtime or when stimulated by pressure, such as an insect walking over the capsule (Barcay, 2004). This can be considered to be applying in the industry.

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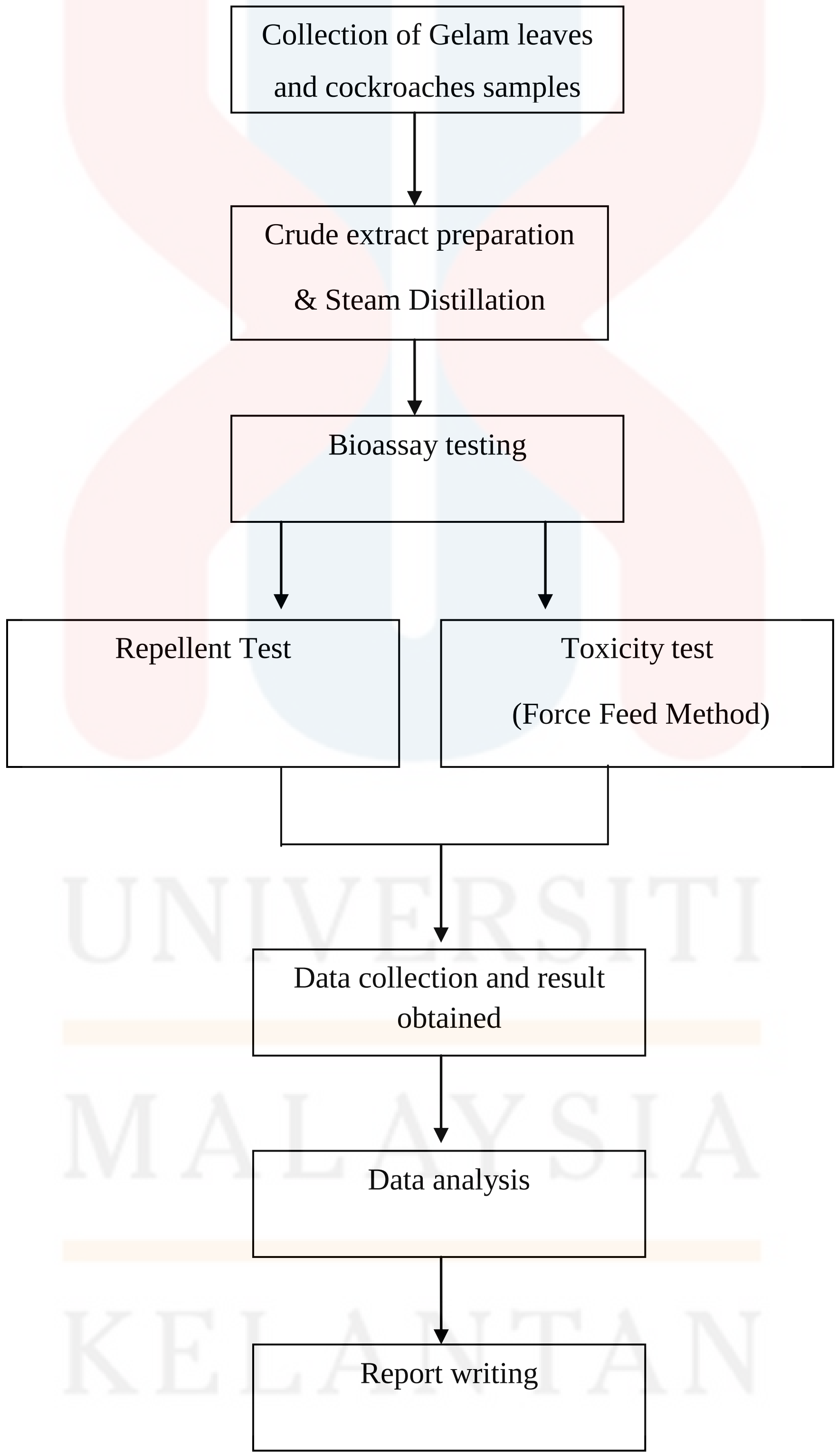
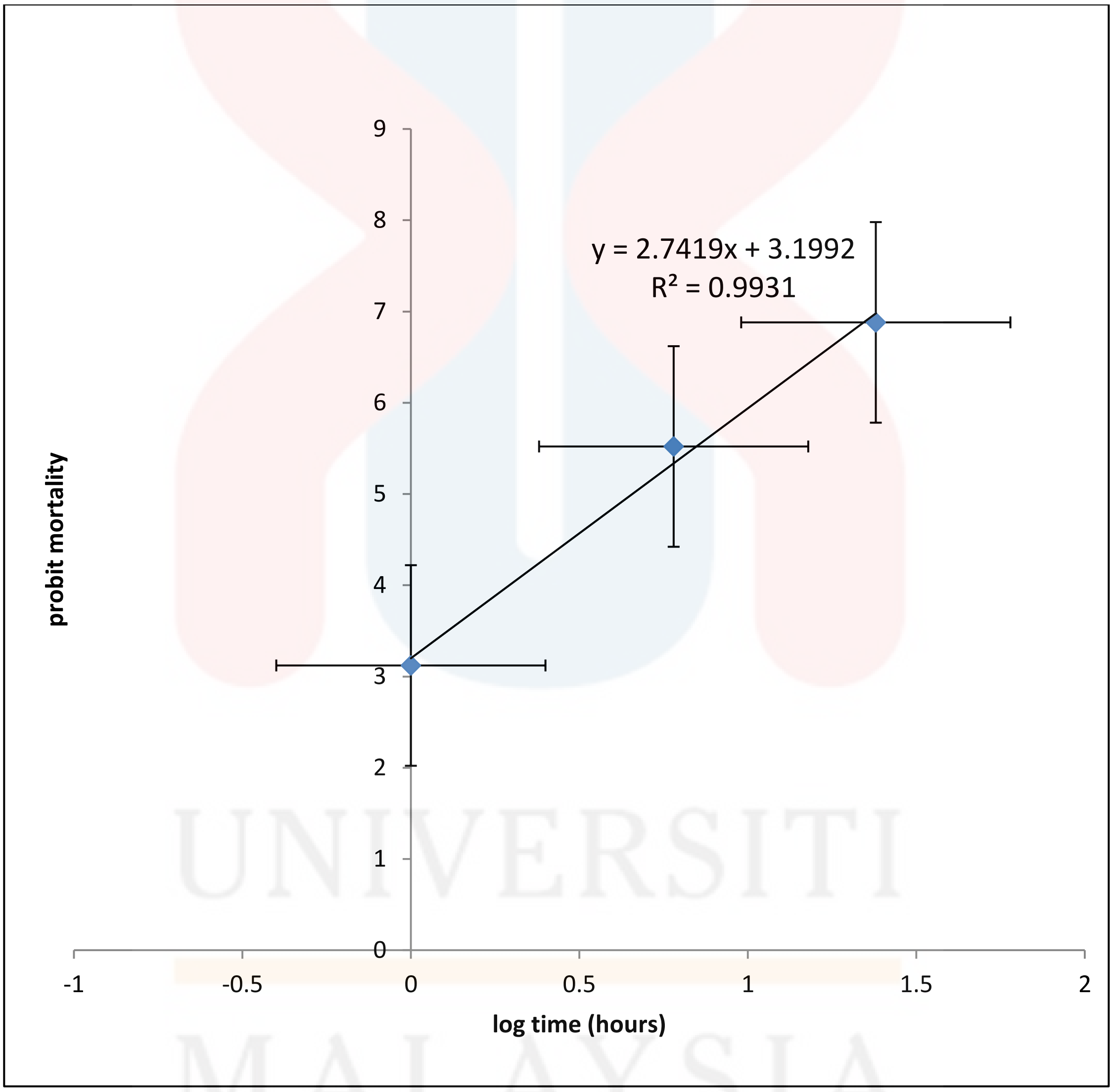
APPENDIX A

RESEARCH FLOWCHART



APPENDIX B

PROBIT'S MORTALITY OF *PERIPLANETA AMERICANA* USING ESSENTIAL OIL



APPENDIX C

ANOVA TABLE FOR REPELLENT TEST USING ESSENTIAL OIL AND LEAF EXTRACT.

repellent		Sum of Squares	df	Mean Square	F	Sig.
essential oil	Between Groups	2.825	2	1.413	.635	.547
	Within Groups	26.704	12	2.225		
	Total	29.529	14			
leaves extract	Between Groups	.065	2	.033	1.000	.397
	Within Groups	.392	12	.033		
	Total	.457	14			