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DECLARATION

I hereby declare that the work in this thesis is my own except for quotations and summaries, which have been duly acknowledged, and completed under the supervision of Ms Emily Quek Ming Poh.

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Student ID: I14005491	(SUPERVISOR)

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ABSTRACT

Synthetic insecticides are widely used around the world to reduce the occurrence of pests. However, some of them have caused serious problems to human health and environmental pollution. Therefore, many researchers are focusing on finding natural occurring compounds in plants that have insecticidal properties. *Syzygium campanulatum* is a plant that contains many useful secondary metabolites and is easily found in our country, Malaysia. One of the compounds found in *S. campanulatum* namely farnesol is a sesquiterpene, which has the ability on insect repellency and believe to have the potential in inhibiting the growth of insect cells. Hence, the aims of this research were to extract and detect the presence of farnesol in *S. campanulatum* and to study the effect of the extracted farnesol towards the *Sf9* cells. Non-polar compounds from *S. campanulatum* dried leaf powder were extracted using hexane at the ratio of 2:5 (w/v) and the extract was known as crude leaf extract. Crude leaf extract was separated using silica column chromatography and three solvent systems namely hexane, hexane:ethyl acetate (1:1, v/v), and ethyl acetate. The absorbance readings of each column fraction were measured using UV spectrophotometer with a wavelength range from 270 nm to 310 nm. Further separation of crude leaf extract and the column-isolated fraction were performed using TLC to verify the presence of farnesol. Both crude leaf extract and the column-isolated fraction were then subjected to the inhibition of *Sf9* cell growth for three incubation durations (24-hr, 48-hr and 72-hr) by measuring A_{60}. Hexane-treated *Sf9* cells were used as the positive control in the inhibition study. Hexane was able to extract farnesol based on the presence of one peak identified at 290 nm of the column-chromatogram. Due to the similar peak present in both chromatograms of crude leaf extract and standard farnesol, fraction-14 isolated from crude leaf extract might contain farnesol. Further analysis of TLC results also verified the presence of farnesol spots in both crude leaf extract and fraction-14 when compared to standard farnesol. Hence, both of them were used to inhibit the *Sf9* cell growth at three incubation durations. The growth rates for crude leaf extract and fraction-14 at 24-hr were -17.6×10^{-4} hr^{-1} and -5.8×10^{-4} hr^{-1} respectively. However, the growth rates at 48-hr and 72-hr were remained constant than growth rate at 24-hr which indicated no growth of *Sf9* cells at these incubation durations. In conclusions, farnesol was extracted successfully and able to inhibit the growth of *Sf9* cells.

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LIST OF ABBREVIATIONS

BW	body weight
cm	centimetre
d	day
DCM	dichloromethane
FRIM	Forest Research Institute Malaysia
g	gram
hr	hour
kg	kilogram
L	litre
mg	milligram
min	minute
mL	milliltre
NCBI	National Center for Biotechnology Information
nm	nanometre
R_f	retention factor
rpm	revolutions per minute
S. campanulatum	*Syzygium campanulatum*
TLC	thin layer chromatography
w/v	weight/volume
v/v	volume/volume
%	percentage
°C	degree Celsius
μL	microlitre

CHAPTER 1

INTRODUCTION

There are over one million species of insects in the world's ecosystem. Several serious diseases including Lyme disease, dengue fever, and malaria are transmitted by insects that carry microbes or parasites. Apart from affecting human lives, they also caused impacts on food crops and animals (Niroumand et al., 2014). Insects caused diseases to the crops and livestock, thus reduce the production (Pimentel, n.d.). To overcome the issue caused by insects, insecticide was developed and was the most used pesticide in the world in 2009 as shown in Appendix A. By using insecticide, it increases the production of food crops and reduces the risk of getting diseases that are spread by insects (Ross, 2005) for example: aster yellows, cabbage black ringspot and so on (Meyer, 2003).

Although insecticide decreases farmers' burden, it has potential to end up in small portion with the vegetables and fruits that we consume daily. Furthermore, some of the chemicals present in insecticide have shown undesired side effects to human and cause pollution to the environment (Igbedioh, 1991; Jeyaratnam, 1985). In 1999, Environews Forum reported that there are about 1 million deaths worldwide due to pesticide poisoning. Since using chemical insecticides can cause problems to the environment and human, bio-pesticides have become a better choice as it is safer and eco-friendlier (Rahman et al., 2016). There are various types of compounds present in plant naturally that has the ability to protect against insects. The examples of the compounds are flavonoids, phenols, quinones, terpenoids, and alkaloids (Adeyemi, 2010; Radcliffe's IPM World Textbook, n.d.).

In this research, *Syzygium campanulatum* was used to extract farnesol, as it is one of the plants that contains many secondary metabolites such as flavonoids, terpenoids, lignans, and chalcones (Memon et al., 2015). It is belong to the family Myrtaceae and usually known as wild cinnamon or "kelat paya" in Malay language. *S. campanulatum* originates from South East Asia (Forest Research Institute Malaysia [FRIM], 2014).

Farnesol is a terpene that can be found in various medicinal plants and fruits (Takahashi et al., 2002). According to Ku and Lin (2015), farnesol shows no toxic effect on experimental mice with high dose of 151 mg/kg BW/day for five weeks, which means 151 mg of farnesol is given to the mice per 1 kg of body weight each day. It also shows anti-allergy and anti-inflammatory properties. Based on National Center for Biotechnology Information (NCBI) (n.d.), farnesol has a unique smell, which is mild fresh sweet (Luebke & William, 1985) and is involved in perfume industry. Moreover, farnesol is one of the compound that can be found in the essential oil of lemongrass, which is one of the most common plant people used as mosquito repellent since many years ago (Bhatt, 2013). Thus, farnesol can be potential in insecticidal activity.

In this research, *S. campanulatum* was used as it is easily found in our country and it grows vigorously (Ahmad Nazarudin, Tsan & Mohd Fauzi, 2010). The leaves of *S. campanulatum* were selected to extract its non-polar compound by using one solvent, namely hexane. Next, the isolated farnesol was tested on the growth of the *Spodoptera frugiperda* (*Sf9*) cells. Hence, the objectives of this research were:

i. to identify the presence of farnesol in the column-isolated fractions by measuring absorbance at a wavelength range from 270 nm to 310 nm using UV spectrophotometer.
ii. to verify the identity of farnesol present in crude leaf extract and the column-isolated fraction using thin layer chromatography (TLC).
iii. to examine the effect of crude leaf extract, the column-isolated fraction and hexane towards the growth of *Sf9* cells for three incubation durations (24-hr, 48-hr and 72-hr).

CHAPTER 2

LITERATURE REVIEW

2.1 *Syzygium campanulatum*

S. campanulatum belongs to the Family Myrtaceae, which is the same family with Manuka (Australian National Herbarium, n.d.). *S. campanulatum* has a synonym of *Syzygium myrtifolium* and its common name is "kelat paya" (FRIM, 2014). The word "*Syzygium*" is from a Greek word "*syzygios*", which means it has opposite paired leaves (Chin, 2017). Futhermore, *S. campanulatum* is widely distributed in Borneo, Myanmar, Northeast India, Peninsular Malaysia, Thailand, Sumatra, Singapore and Philippines, so it is easy to get around us. The leaves of *S. campanulatum* are very special as they are red when the leaves are young, and eventually turn into green when it gets mature (NParks Flora & Fauna Web, n.d.). In recent years, researches about *S. campanulatum* have been carried out a lot. Besides, many compounds have been extracted out from the plant based on the research by Memon et al. (2015). Furthermore, this plant is widely planted as landscape and able to adapt harsh environment. Due to vigorous growth, frequent pruning is needed to control excessive growth (Ahmad Nazarudin, Tsan & Mohd Fauzi, 2010). The activity of pruning the leaves causes pruned leaves to become the waste. Hence, one of the reasons of doing this research is to turn the waste into something useful.

2.1.1 Usage of *S. campanulatum*

S. campanulatum has been widely used as medicinal herbs as the compound content is potentially anti-angiogenic and anti-colon cancer (Farsi et al., 2016). According to Aisha (2013), *S. campanulatum* methanolic extract has the property on inhibiting angiogenesis and tumor growth in nude mice. Furthermore, based on Memon et al. (2015), compounds such as secondary metabolite found in *S. campanulatum* have anti-cancer properties.

2.2 FARNESOL

According to NCBI (n.d.), farnesol has an IUPAC name of (2E,6E)-3,7,11-trimethyldodeca-2,6,10-trien-1-ol. Farnesol is a signaling molecule that derived from farnesyl diphosphate. Figure 2.1 shows the structure of farnesol. According to Ku and Lin (2015), farnesol can be found in various fruits, vegetables, and herbs such as peaches, corn, and lemongrass.

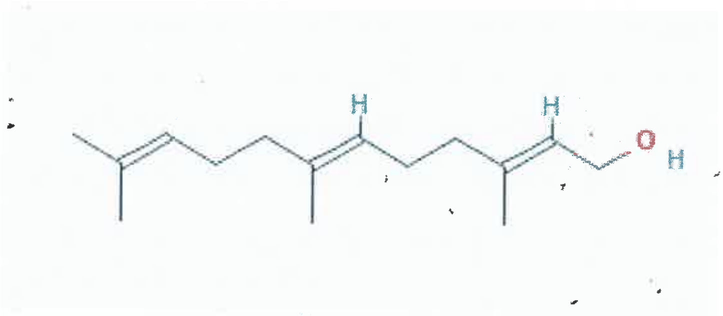

Figure 2.1 Structure of farnesol (Adapted from NCBI, n.d.)

2.2.1 Characteristic of farnesol

According to NCBI (n.d.), farnesol is an organic compound, which has a molecular weight of 222.372 g/mol. Farnesol is a non-polar compound, which contains 15-carbon chain (Song, 2009). Thus, it is three isoprenoid units (Eastman & Kluger, 2015). Due to these characteristics, farnesol is insoluble in water (Jiang, Kempinski & Chappell, 2016). Furthermore, non-polar solutes are highly soluble in non-polar solvent. Hexane is a non-polar solvent with the polarity index of 0.1 and is commonly use as the extraction solvent (Harris, 2015), it was used in this research to extract non-polar compounds from *S. campanulatum* leaf powder. Nonetheless, hexane was also used as a solvent to dilute the 95% (w/v) standard farnesol solution throughout the research due to its non-polar characteristic and its ability to dissolve farnesol. Based on DrugBank (n.d.), the boiling point of farnesol is at 111°C and its melting point is less than 25°C. Also, according to Luebke and William (1985), farnesol has a mild fresh sweet smell.

2.2.2 Application of farnesol

Due to the smell of farnesol, it is involved in several industries such as cosmetics, and perfume industries. According to Cosmetics Info (n.d.), farnesol is used in several daily product such as colognes, cleansing products, face powders, skin cares, and so on. Besides, Food and Drug Administration (FDA) also approved farnesol as a direct adding flavoring agent for food. According to Ku and Lin (2015), farnesol shows no toxic effect on experimental mice with high dose of 151 mg/kg BW/day continuously for five weeks. Moreover, it has anti-inflammatory and anti-allergic properties on allergic asthmatic mice. Based on Dancewicz et al. (2010), farnesol is able to protect plants from the infestation of aphid. In addition, the presence of farnesol in lemongrass has been used as a mosquito repellent (Bhatt, 2013; Wells, n.d.).

2.3 COLUMN CHROMATOGRAPHY

There are a few techniques that can be used to separate mixture compound, for example the high performance liquid chromatographic (HPLC) (Warthen Jr., 2006) and liquid chromatography (Sato, Kageyu, Miyashita & Tanaka, 1981). However, column chromatography is a common technique researchers use to isolate the desired compound from a mixture (Millar, 2012). According to Schroepfer & Gore (1963), farnesol can be isolated by using column chromatography.

Column chromatography is a technique used to isolate compounds based on their polarity or hydrophobicity. There are two phases in column chromatography, which are the stationary phase and the mobile phase. The stationary phase of the column chromatography is in solid form while the mobile phase is in liquid form. The two most common used stationary phases are silica and alumina (University of Toronto, n.d.). Different compounds show different adhesion degree to the stationary phase. Polar compounds, which travel slower has a stronger adhesion to the polar stationary phase. In another word, different polarity compounds will travel at different speed through the polar stationary phase (Khan Academy, n.d.). In this research, silica was used as stationary phase. Furthermore, compared to thin layer chromatography (TLC), column chromatography is able to isolate larger quantity of product, while TLC can only separate a little quantity of compound mixture. Column chromatography is chosen to isolate the farnesol in this research due to this method is a convenient method researchers used to separate terpenes by using solvents such as hexane, pentane or gradient elution as mobile phase (Çitoğlu & Acıkara, 2012).

2.4 *Sf9* CELLS

In this research, *Sf9* cells was used as an insect cell model to test for the insecticidal activity of the extracted farnesol. According to ThermoFisher Scientific (n.d.), *Sf9* is an insect cell line that derived from *Spodoptera frugiperda* and is a suitable host for recombinant protein expression. Furthermore, *Sf9* cells is one of the most common strains that is used to develop new insecticides (Edvotek, n.d.), therefore *Sf9* cell line was used in this research. According to ThermoFisher Scientific (2017), *Sf9* cells have short doubling time, which is 72 hrs thus it is suitable to be used in this research. Besides, *Sf9* cells can grow as either adherent or suspension. By growing the cells in suspension form, the total surface area for the cells to come in contact with farnesol is higher (Doronina, n.d.).

Serum free media (SFM) is the media that was used to culture *Sf9* cells in this research (Griffiths, 2006). By using SFM, the growth of *Sf9* cells can be easily controlled. Besides, it is suitable to be used in this research because it is suitable for adding a factor to see the specialized function (Jha, n.d.).