QUANTIFICATION OF FARNESOL FROM RED AND GREEN LEAVES OF *Syzygium campanulatum*

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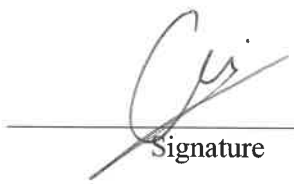
Yap Qing Yang	
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'*Success is not accident. It's hard work, perseverance, learning, study, sacrifice and most of all, love of what you are doing or learning to do'* said Pele. During this 6 weeks of final year project period, with tears and sweat, and finally came to an end. First, I would like to express my deepest gratitude to my supervisor, Miss Emily Quek Ming Poh for share her patience guidance, encouragement, enthusiastic support, and useful experience to me throughout this research period. It is my honour to work with her guidance and be her collaborator. Next, I would like to thank my partner, Lim She Yan for her encouragement, guidance and support throughout this period. Moreover, I would like to show my sincere gratitude to all my lecturers for their assistances and especially Dr Choong Chieh Wean, Dr Geeta Selvarajah, Ms Liow and Ms Chong Mee Yoke for contributing their precious time to make sure my research go on wheels. Furthermore, I would like to thank the lab assistants for sharing their laboratory equipment handling techniques and support during my research period. Last but not least, I would like to be deeply grateful to my family members, especially my mother, for their support in pecuniary and mentally.

ABSTRACT

S. campanulatum, also known as "kelat paya" can grow young leaves with various colors. Due to this characteristic, *S. campanulatum* is commonly used in landscaping. *S. campanulatum* under family of Myrtaceae and genus of *Syzygium* which is rich in secondary metabolites among which is farnesol. Farnesol produces a floral scent and widely is used as an ingredient in fragrance. Farnesol was found to have anti-fungal, anti-microbial and anti-tumour properties. The aims of this study were to determine the suitable wavelength to detect the maximum absorbance of farnesol in *S. campanulatum* extracts, to qualify the farnesol using four extraction solvents; namely hexane, methanol, acetone and sterile dH_2O in red leaf of *S. campanulatum* and to compare and quantify of the farnesol content between red and green leaves of *S. campanulatum*. Wavelength scan showed the optimal wavelength to detect farnesol was 290 nm. TLC was used to separate farnesol from other compounds in crude leaf extracts of *S. campanulatum.* The distance travelled by the sample corresponding to farnesol standard solution indicated the presence of farnesol. Apart from comparing distance travel by compound, colour development after stained with *p*-anisaldehyde also used to verify isolation of farnesol. The silica was scrapped off from TLC plate and re-extracted with hexane and this extract was known as TLC-isolated extracts. The A_{290} of TLC-isolated extracts of red and green leaves were analysed and interpolated their concentration using farnesol standard. The hexane extract of red leaf contained higher concentration of farnesol compared with hexane extract of green leaf. *t*-test result showed there was significant different between the mean of farnesol content of red leaf and green leaf of *S. campanulatum.*

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LIST OF ABBREVIATIONS

A_{290}	absorbance at 290 nm
cm	centimeter
DMC	dimethyl cardamonin
dH_2O	Deionized water
EPA	Environmental Protection Agency
FRIM	Forest Research Institute Malaysia
g	gram
L	liter
min	minute
mg/mL	milligram/milliliter
mL	milliliter
NCBI	National Center for Biotechnology Information
nm	nanometer
PMA	Phosphomolybdic Acid
R_f	Retardation factor
S. campanulatum	*Syzygium campanulatum*
TLC	thin layer chromatography
UV	ultraviolet
w/v	weight/volume
°C	degree Celsius
%	percentage

CHAPTER 1

INTRODUCTION

Plants contain various types of metabolites such as phenolic (Dai & Mumper, 2010), terpenoid and flavonoid compounds. Since ancient time, plants has been used to prevent diseases (Uzoekwe & Mohammed, 2016) and used as drug to treat several diseases (Petrovska, 2012). Large amount of phytochemical compounds, for example, terpenes, alkaloids and phenolic compounds are present in leaves (Nwere & Nwafor 2014; Harini, Jerlinshowmya & Geetha, 2014). Secondary metabolites in leaf extracts have various applications include anti-microbial (George, Obot, Ikot, Akpan & Obi-Egbedi, 2010), anti-cancer (Dai & Mumper, 2010; Wahle, Brown, Rotondo & Heys, 2010), anti-tumour, cholesterol-lowering, immuno-suppressant, anti-protozoal, anti-viral and anti-ageing activities (Vaishnav & Demain, 2011).

Syzygium campanulatum, commonly known as "kelat paya" or wild cinnamon, originated from South East Asia (Forest Research Institute Malaysia [FRIM], 2014). *S. campanulatum* have young leaves with various colours ranging from red, pink, yellow, orange and maroon (Waysidetrees, 2011). With these colourful leaf characteristics, *S. campanulatum* are commonly used for landscaping by pruning the plant into various shapes of beautiful topiaries (Morad, 2011). *S. campanulatum* can produce either white or red flowers and fruits with dark red when ripe (Chong, 2014).

S. campanulatum belongs to family Myrtaceae and genus *Syzygium*. Species belonging to this genus are rich in secondary metabolites such as terpenoids (Wong & Lai, 1996) and flavonoids (Memon et al, 2015) that have the ability to treat gastrointestinal disorders, hemorrhage, dysentery, inflammation and diabetes (Rezende, Borges, Alves, Ferri & Paula, 2013). Secondary metabolites in *S. campanulatum* leaf extracts possessed anti-cancer, anti-fungal, anti-biofilm formation application. Aisha, Ismail, Abu-Salah,

Siddiqui, Ghafar and Majid (2013) discovered methanolic extracts of *S. campanulatum* leaves have ability to inhibit angiogenesis and tumour growth.

S. campanulatum containing various secondary metabolites such as terpenoids, flavonoids and alkaloids. Farnesol is secondary metabolite found naturally produced by many organisms such as fungus *Candida albicans* (Cugini, Calfee, Farrow, Morales, Pesci & Hogan, 2007) and plants such as peaches, tomatoes, corns, lemon grass and chamomiles (Ku & Lin, 2015). This terpene is used as fragrance ingredient as it produced the mild and fresh sweet floral smell and plays an important role in the production of hair and skin care products, lotions, deodorants, moisturizers and shaving products (Cosmeticsinfo, 2016). Apart from fragrance ingredient, farnesol is having an interesting application and gradually being concerned due to their anti-microbial properties by acting as quorum sensing molecule that can inhibit the formation of biofilm (Jabra-Rizk, Meillerm, James & Shirtliff, 2006; Kaneko, Togashi, Hamashima, Hirohara & Inoue, 2011), as cell apoptosis inducer (Joo, Liao, Collins, Grissom & Jetten, 2007; Kaneko et al, 2011) and as both anti-inflammatory and anti-allergic molecules (Ku & Lin, 2015).

The plant used in this research, *S. campanulatum* can grow vigorously and produce large amount of leaves. Young leaf of *S. campanulatum* are red and it grow into green when mature. To date, phytochemical extraction from red leaf of *S. campanulatum* has not been studied previously. Farnesol is a secondary metabolite which has the great potential on medical and industrial applications. Therefore, with the interest on the farnesol content in *S. campanulatum*, the objectives of this project were;

1. to determine the suitable wavelength to detect the maximum absorbance of farnesol in *S. campanulatum* extract..
2. to qualify the farnesol extraction by four extraction solvents; namely hexane, methanol, acetone and water in red leaves of *S. campanulatum* using TLC.
3. to quantify and compare the farnesol content between red and green leaves of *S. campanulatum*.

CHAPTER 2

LITERATURE REVIEW

2.1 FARNESOL

Farnesol, the IUPAC name (2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trien-1-ol (National Center for Biotechnology Information [NCBI], n.d.; Abcam, n.d.) has other synonyms such as farnesyl alcohol, 6,10-trimethyl-2,6,10-dodecatrien-12-ol and 10-dodecatrien-1-ol,3,7,11-trimethyl-6 (Chemical Book, n.d.). Farnesol is found widely in vegetables such as corns and tomatoes, fruits such as peaches, and herbs such as chamomiles and lemon grass (Ku & Lin, 2015). Farnesol have high industrial value, for example, currently in market, 5 g of farnesol worth about RM125 (Sigma-Aldrich, 2017).

2.1.1 Characteristics of farnesol

Farnesol, $C_{15}H_{26}O$ is an acyclic sesquiterpene primary alcohol made up by three consecutive isoprene units as shown in Figure 2.1. Farnesol is a slightly yellow or colourless liquid with molecular weight of 222.372 g/mol (NCBI, nd). It has hydrophobic characteristic with boiling point 110-113 °C and melting point lower than 25 °C.

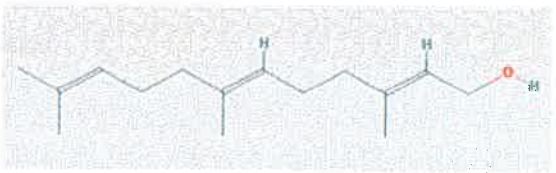

Figure 2.1. The structure of farnesol

2.1.2 Importance and applications of farnesol

Farnesol is widely used as a fragrance ingredient due to its delicate sweet floral scent. Farnesol is formulated in cosmetics and personal care products such as eye lotions, aftershave lotions, face powders, foot powders, deodorants, fragrances, moisturizers,

cleansing products, hair care products, shaving products and skin care products (Cosmeticsinfo, 2016). Farnesol is involved in isoprenoid metabolism and acts as an important intracellular metabolite (Thai et al., 1999). Research showed farnesol has anti-inflammation potential by testing on allergic asthmatic mice (Ku & Lin, 2015). Apoptosis is the ability to undergo programmed cell death by disassembly of cell, degradation of mitochondrial and caused inhibition of growth, farnesol was discovered its ability of inducing apoptosis in mammalian cells (Shirtliff et al., 2009). Research has showed that farnesol have anti-fungal ability by inducing apoptosis in fungal species, such as *S. cerevisiae*, *P. brasiliensis* and *A. nidulans* (Shirtliff et al., 2009; Brilhante et al., 2013). Interestingly, farnesol produced by *C. albicans* has an apoptotic pathway and affected tumor cells (Shirtliff et al., 2009). Therefore, farnesol was formulated into the nebulized aerosol and used to induce the apoptosis on human lung cancerous cells *in vitro* (Finlay, Wang, Chen & Roa, 2003).

Study showed farnesol involved in quorum-sensing by showing inhibition of the formation of biofilm (Brilhante et al., 2013; Jeon et al., 2011, Pammi, Liang, Hicks, Barrish & Versalovic, 2011). There was an interesting application of farnesol in controlling spider mites, farnesol was found present in the female mites to attract male mites for mating purpose. Therefore, farnesol are applied and mixed to the miticide to attract the male mites to contact with the miticides and enhance the miticide's effect in killing the mites (Environmental Protection Agency [EPA], n.d.).

2.2 *S. campanulatum*

S. campanulatum, or synonyms known as *S. myrtifolium* or *Eugenia oleina* (Roskov et al., 2017) originates from South East Asia with various synonyms such as *"kelat paya", "ubah laut", chinese red wood, red lips and* wild cinnamon (FRIM, 2014). *Syzygium* have the meaning of 'yoked together' and this name comes from the Greek words 'syn' which means together and 'zygon' which means yoke (Scientist Sees Squirrel, 2016) and the name is chosen because of the coupled leaves and branches. This genus is comprised about 1200 species (Floral of China, 2007), all the species under this genus have the similar

characteristics such as leaves arranged oppositely to each other, produced fleshy fruits and flowers with prominent stamens (Australiantreeimages, 2016). *Syzygium* can grow in various types of habitat such as swamp forest, coastal forest, bamboo forests, monsoons, peat swamp, lowland forest, montane forest, savanna and shrub vegetation (Parnell, Craven & Briffin, 2007), as well as in extreme habitats like limestone and ultramafic (Mudiana, 2016). *S. campanulatum* are widely spread in Southeast Asia, Australia, South Asia and New Caledonia, and found in Africa, Malagasy and other southwestern region of Pacific Islands, New Zealand and Hawaii (Mudiana, 2016).

2.2.1 Characteristics of *S. campanulatum*

S. campanulatum is an evergreen dicotyledon plant and it can grow to 20 m tall. Their foliages are elliptical shape and arranged oppositely (Nparks Flora & Fauna Web, n.d.). One of the interesting facts is after pruning this plant, the young shoot can grow into various colours ranging from maroon, yellow and orange as shown in Figure 2.2. Like other angiosperms, *S. campanulatum* can produce flowers if they are left alone to grow into trees (Aisha et al., 2013). They are two different types of *S. campanulatum*, one of the varieties their shoot with red leaves can produce red flower clusters, the other varieties their shoot with yellow leaves can produce whitish powderpuff flowers (Waysidetrees, 2011). Fruits of *S. campanulatum* are produced in April-May and December-January (Aisha et al., 2013), the berries-like fruits ripe from green to dark red or black (Trees of UM, 2015).