CHEMICAL COMPOSITION, ANTIOXIDANT AND ANTIBACTERIAL ACTIVITY OF ESSENTIAL OIL FROM LEAF OF *SYZYGIUM POLYANTHUM* (Wight) Walp.

NUR AMALINA BINTI AHMAD

MASTER OF SCIENCE (BIOTECHNOLOGY)

UNIVERSITI MALAYSIA PAHANG

CHEMICAL COMPOSITION, ANTIOXIDANT AND ANTIBACTERIAL ACTIVITY OF ESSENTIAL OIL FROM LEAF OF *SYZYGIUM POLYANTHUM* (Wight) Walp.

NUR AMALINA BINTI AHMAD

Thesis submitted in fulfilment of the requirements
for the award of the degree of
Master of Science in Biotechnology

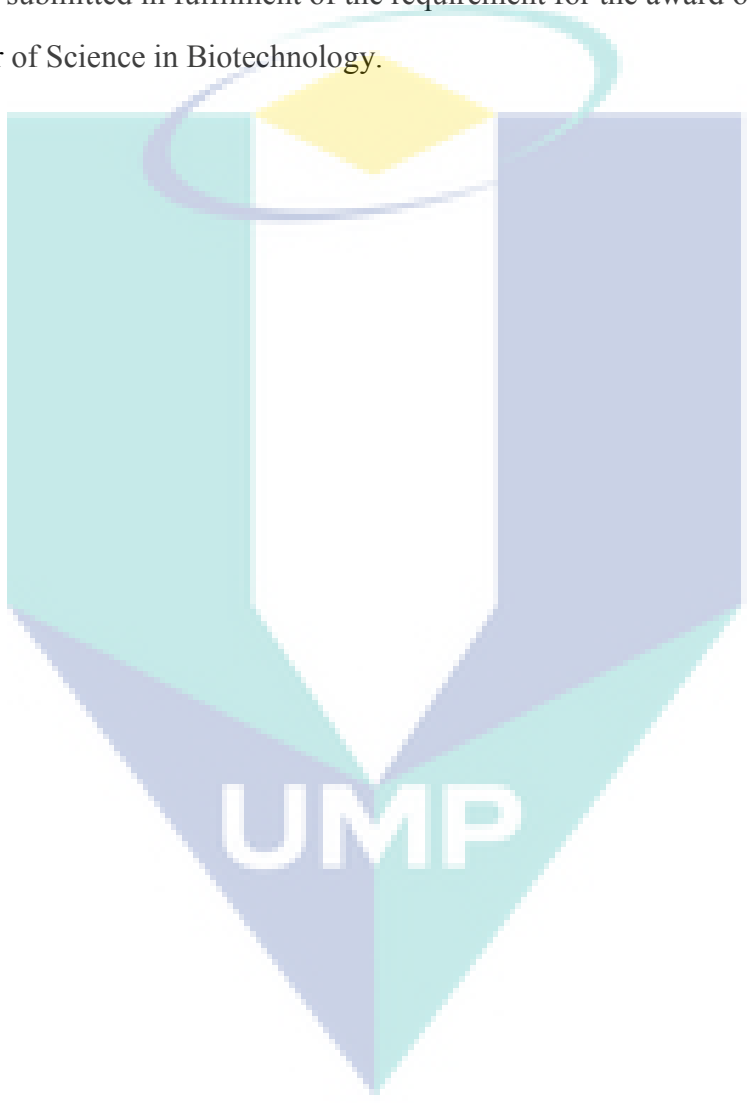
Faculty of Industrial Sciences and Technology
UNIVERSITI MALAYSIA PAHANG

APRIL 2014

STATEMENT OF AWARD FOR DEGREE

1. Master of Science (by Research)

Thesis submitted in fulfilment of the requirement for the award of the degree of Master of Science in Biotechnology.



SUPERVISOR'S DECLARATION

We hereby declare that we have checked this thesis and in our opinion, this thesis is adequate in terms of scope and quality for the award of the degree of Master of Science in Biotechnology.

Signature:

Name of Supervisor: DR. NATANAMURUGARAJ GOVINDAN

Position: SENIOR LECTURER

Date:

Signature:

Name of Co-Supervisor: PROFESSOR DR. MASHITAH MOHD YUSOFF

Position: DEAN, FACULTY OF INDUSTRIAL SCIENCES AND TECHNOLOGY

Date:

STUDENT'S DECLARATION

I hereby declare that the work in this thesis is my own except for the quotations and summaries which have been duly acknowledged. The thesis has not been accepted for any degree and is not concurrently submitted for the award of any other degree.

Signature :

Name : NUR AMALINA BINTI AHMAD

ID Number : MKT 11001

Date :

DEDICATION

Dedicated to my beloved husband, father, mother, sister and son

ACKNOWLEDGEMENTS

Bismillahirrahmanirrahim.

In the name of Allah, selawat and salam to our prophet Muhammad (s.a.w) and his friends. Alhamdulillah, finally I managed to finish my project at the given time. I would like to thank Universiti Malaysia Pahang providing the research allowance, research grant (RDU110379 and GRS120301) and opportunity, I will treasure all the time when I was in the Universiti Malaysia Pahang.

I would like to take this opportunity to express my sincere appreciation to my project supervisor, Dr. Natanamurugaraj Govindan for his advices, supports, guidance, encouragement and contribution to my project.

I am heartily grateful to my co-supervisor Professor Dr. Mashitah Mohd Yusoff, Dean of the Faculty, for her encouragement and interest in this project. I would also like to express my thanks to Dr. Mohd Hasbi Ab Rahim, Deputy Dean of Research and Postgaduate, Dr. Saiful Nizam Tajuddin, Deputy Dean of Academics and Student Affairs, Dr. Gaanty Pragas Maniam, Deputy Director of the Central Laboratory and all the staffs in the Faculty of Industrial Sciences and Technology (FIST) for allowing me to conduct my project in their laboratory. I really appreciate their support and help. Indeed, their invaluable assistance and efforts did much to ensure the smooth running of this project.

Special thanks are dedicated to my beloved parents, Ahmad Saad and Azizah Ismail, my beloved husband Ramlee Ibrahim and not to forget my elder sister, Noor Azrin Haniza for their endless support and encouragement. Finally yet importantly, I would like to thank all my postgraduate laboratory friends and everyone for their constructive criticisms, encouragement and precious help to complete my project.

ABSTRACT

Syzygium polyanthum (Wight) Walp. is a plant widely consumed as raw vegetables or used in culinary in Malaysia. This study was performed to determine the chemical composition, antioxidant and antibacterial activity of *Syzygium polyanthum* essential oil from Malaysia. The essential oil was extracted from the leaf by hydrodistillation method. The percentage of the essential oils obtained, for the fresh and the dried leaf were 0.67 % and 1.50 %, respectively. Analysis by GCMS revealed a total of 26 and 29 compounds, representing 71.71 % and 90.92 % of the overall compositions, were detected in the fresh and the dried leaf essential oils. Both oils were characterized by high amount of monoterpene hydrocarbons with α-pinene was the major compounds for the fresh leaf (28.78 %) and the dried leaf (34.15 %). The antioxidant activities of the fresh and dried leaf oils were evaluated using three different assay; DPPH free radical scavenging, β-carotene bleaching and ferrous-ion chelating. Results showed that the oils exhibited a potential antioxidant activity. The antibacterial activity was evaluated using the disc diffusion and microdilution methods against three gram-positive bacteria (*Bacillus subtilis, Enterococcus faecalis*) and three gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa, Salmonella typhii*). The oils exhibited antibacterial activity against all of the tested bacteria. The minimum inhibitory concentration (MIC) values for the fresh leaf oil were in the range of 0.03 mg/ml to 0.25 mg/ml while the MIC values for the dried leaf oil were in the range of 0.03 mg/ml to 0.12 mg/ml. The chemical composition, antioxidant and antibacterial activity of essential oil of *S. polyanthum* from Malaysia is presented here for the first time.

ABSTRAK

Syzygium polyanthum (Wight) Walp. adalah sejenis tumbuhan yang digunakan secara meluas sebagai ulaman atau digunakan dalam masakan di Malaysia. Kajian ini telah dijalankan untuk menentukan komposisi kimia dan aktiviti antioksida dan antibakteria minyak pati *Syzygium polyanthum* dari Malaysia. Minyak pati ini diekstrak dari daun melalui kaedah penyulingan hidro. Peratusan minyak pati yang diperolehi adalah 0.67 % (daun segar) dan 1.50 % (daun kering). Analisis oleh GCMS mendedahkan sejumlah 26 dan 29 sebatian, yang mewakili 71.71 % dan 90.92 % daripada komposisi keseluruhan, dikesan dalam minyak pati daun segar dan daun kering. Kedua-dua minyak telah disifatkan oleh hidrokarbon monoterpene dengan α-pinene adalah sebatian utama untuk daun segar (28.78 %) dan daun kering (34.15 %). Aktiviti antioksida minyak dari daun segar dan kering diuji menggunakan tiga uji kaji berbeza; penghapusan radikal bebas DPPH, pelunturan β-karotena dan pengkelat ion ferus. Keputusan menunjukkan bahawa minyak pati mempamerkan potensi terhadap aktiviti antioksidan. Aktiviti antibakteria dinilai menggunakan menggunakan penyebaran cakera dan kaedah microdilution terhadap tiga bakteria gram-positif (*Staphylococcus aureus*, *Bacillus subtilis, Enterococcus faecalis*) dan tiga bacteria gram-negatif (*Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi*). Minyak pati ini mempamerkan aktiviti antibakteria terhadap semua bakteria yang diuji. Nilai MIC daripada minyak daun segar adalah di antara 0.03 mg/ml hingga 0.25 mg/ml manakala nilai MIC untuk minyak daun kering di antara 0.03 mg/ml hingga 0.12 mg/ml. Komposisi kimia, aktiviti antioksida dan aktiviti antibakteria untuk minyak pati dari *Syzygium* polyanthum dari Malaysia dilaporkan disini buat pertama kalinya.

TABLE OF CONTENTS

CHAPTER 4 RESULTS AND DISCUSSIONS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS

α	Alpha
β	Beta
cm	Centimetre
^{o}C	Degree Celsius
δ	Delta
=	Equals
Fe^{2+}	Ferrous-ion
γ	Gamma
g	Gram
>	Greater-than Sign
≥	Greater-than or Equal to Sign
<	Less-than Sign
L	Liter
m	Meter
µl	Microliter
mg	Milligram
ml	Mililiter
mm	Millimeter
mM	Millimolar
x	Multiplication Sign
nm	Nanometer
-	Negative Sign

%	Percentage
±	Plus-minus Sign
+	Positive Sign

LIST OF ABBREVIATIONS

A	Absorbance
ANOVA	Analysis of Variance
ATTC	American Type Culture Collection
BC	Before Century
CFU	Colony-forming Unit
GCMS	Gas Chromatography Mass Spectrometry
KI	Kovats Index
MAE	Microwave-assisted Extraction
NCCLS/CLSI	National Committee for Clinical Laboratory Standards
NIST	National Institute of Standards and Technology
RI	Retention Indices
SD	Standard Deviation
SPSS	Statistical Package for Social Science
SPME	Solid-phase Microextraction
USNRC	United States Nuclear Regulatory Commission

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND OF STUDY

In this modern era, people are more aware of their health status that leads them to find natural-based products to substitute the role of various synthetic products which are available in market. According to the World Health Organization (WHO), about 60-80 % of people from developing countries consume traditional medicine for treatment of various diseases (Prabuseenivasan *et al.*, 2006). Therefore, herbs and medicinal plants are gaining interest because of their potential to produce natural products, which are known to be good sources to provide better health. Continuous research and studies of potential herbs and medicinal plants are important as natural products from plant origin will continue to be in demand.

Malaysia, a tropical Asian country, is gifted with a rich diversity of flora and fauna, particularly, herbs and medicinal plants. Malay people, especially, like to consume traditional vegetables, also known as *ulam* in their daily diet. This is due to the taste (Abas *et al*., 2006) and aroma which enhance their appetite and promote better health. There are more than 120 species of traditional *ulam*, varying from shrubs to large trees, which can be found in Malaysia. They are usually grown at home and can also be found abundantly in local market. One of the favorite *ulam* that have been consumed for ages is *Syzygium polyanthum* (Wight) Walp. *Syzygium polyanthum* (Wight) Walp. (syn. *Eugenia lucidula* miq and *Eugenia polyantha* Wight) is a member of Myrtaceae, mainly distributed in Malaysia, Indonesia, Myanmar, Thailand, Indochina and also in Suriname (Wartini, 2009). This deciduous tropical tree (Raden *et al*., 2009) is called differently in each country or province: serai kayu, salam, kelat (Malaysia), meselangan (Sumatra), salam (Java, Sunda, Madura), gowok (Sunda), manting (Java) or kastolam (Kangean) (Dalimartha, 2005 and Suganda and Ruslan, 2007). In Indonesia, it

is commonly called as Indonesian bay leaf or Indonesian laurels (Heyne, 1987) because the function in cooking resembles the original European bay leaf (*Laurus nobilis* L.).

Fresh and dried aromatic leaf of *S. polyanthum* are useful in culinary (Katzer, 2004) because of its scent, colour and flavour. It is often used as flavouring spice for meat, fish, and vegetable dishes or in rice (De Guzman and Siemonsma, 1999). In Malaysia, the leaf are an important ingredient in *nasi kerabu* and *kerabu perut* or eaten raw as *ulam*. It is believed that the leaf can treat haemorrhoids, stomach-ache, diarrhea (Kloppenburg-Versteegh, 1983), diabetes, itchiness, gastritis, astringent and scabies (Wijayakusuma, 1995), hypertension, high cholesterol (Suharti *et al.*, 2008) and others.

Figure 1.1: *Syzygium polyanthum* (Wight) Walp.

The presence of the essential oils and their compositions determine the characteristic aroma of the plant and flavour of the condiments (Chalchat and Ozcan, 2008). Essential oil (or volatile oil) is a concentrated hydrophobic mixture of chemical compounds with distinctive odor and flavor, which can be obtained from different parts, namely leaf, stems, barks, flowers, fruits, seeds and rhizomes (Munir and Oliver, 2007) varying according to plant species. Extraction is by physical means only (Ferhat, 2007), either by suppression, fermentation or extraction (Prabuseenivasan *et al.*, 2006). The common methods used are conventional hydro-distillation and steam-distillation and further analyzes using gas chromatography mass spectrometry (GCMS) for the chemical constituents. *Syzygium polyanthum* also produces essential oil.

Previous studies on the essential oils of *S. polyanthum* leaf shows that the main components are citric acid, eugenol, methyl chavicol (Sumono and Agustin, 2008). The essential oil of the leaf obtained from steam-distillation was found to contain cis-4-decenal (27.12 %), octanal (11.98 %), α-pinene (9.09 %), farnesol (8.84 %), β-ocimene (7.62 %) and nonanal (7.60 %) (Wartini, 2009). The essential oil is also reported to have potential use in dentistry (Sumono and Agustin, 2008). There are other previous studies which report that *S. polyanthum* also possesses biological activities such as antioxidant (Wong *et al.*, 2006 and Raden *et al.*, 2009), antibacterial (Hendradjatin, 2004 and Sumono and Agustin, 2008), antimicrobial (Srimuwarni *et al.*, 2005), anti-inflammation (Wientarsih *et al.*, 2007) and antifungal (Guynot *et al.*, 2005 and Noveriza and Miftakhurohmah, 2010).

.

1.2 STATEMENT OF PROBLEM

The compositions of essential oil from *S. polyanthum* have been reported in earlier studies, but no studies have been done on the essential oil of the species grown in Malaysia. The lack of information on the phytochemical aspects of the tree is in great contrast with the widespread use in Malaysian cuisine. Thus, it would be an interesting subject for study. Based on these facts, it is our interest to perform the screening and analysis of the chemical compounds of the essential oil from *S. polyanthum* leaf grown in Malaysia and its antioxidant and antibacterial properties in order to provide information for potential utilization in future.

1.3 RESEARCH OBJECTIVES

1. To extract the essential oil from fresh and dried leaf of *Syzygium polyantum* (Wight) Walp. using hydrodistillation method.

2. To analyze the chemical compositions of the essential oil from fresh and dried leaf of *Syzygium polyantum* (Wight) Walp. using Gas Chromatography Mass Spectrometry (GCMS).

3. To determine the antioxidant and antibacterial activities of the essential oil extracted from fresh and dried leaf of *Syzygium polyantum* (Wight) Walp.

CHAPTER 2

LITERATURE REVIEW

2.1 HERBS AND MEDICINAL PLANTS

2.1.1 General

The oldest form of maintaining good health was practiced by the Egyptians, Chinese, Greeks and Romans back in 1500 B.C, where they utilized the traditional herbs and medicinal plants for medicine and also remedies for various usages (Juri *et al*., 1999). The natural products possessed by these plants are believed to improve and maintain health because they have minimal side effects, effective and safe (Katarina, 2005) compared to chemically synthesized and pure drugs. The return of herbalism and ancient medicines in this modern world are prior to the recognition that chemicals or synthetic products may cause harmful side effect in the long term. Currently, studies on medical plants are extensively done in order to improve the productivity of medicinal plants (Runham, 1996). Various scientific tests have been performed to prove that many traditional herbs possess interesting healing properties. With the rise of scientific tests on herbs and medicinal plants for their potential natural products, the limitation of sources is overcome and we can assess plants from around the world (Katarina, 2005).

2.1.2 Herbs and Medicinal Plants in Malaysia

Malaysia, as a tropical rainforest country, is gifted with great diversity of flora and fauna. About 12,000 species of flowering plants can be found in Malaysian rainforest and about 1,300 species are acknowledged as medicinal plants. Various chemical structures possessed by herbs and medicinal plants that can be found in Malaysian flora make natural products the excellent candidate for any screening interest (Ismail, 2000). The rapid increase of interest in herbs and herbal products, not only amongst scientists but also consumers has lead to the increased in demand, thus

increasing the price of raw materials and production cost. The utilization of local plants, either wild or introduced, by herbal industries has risen. This will give benefit to the local farmers and also provides profit to the country (Khatijah and Ruzi, 2006).

2.2 FAMILY *MYRTACEAE*

The family Myrtaceae comprises of around 121 genera and over 3800-5800 species (Stefanello *et al.*, 2011). It is widely distributed in Australia, South East Asia and America (tropical to southern temperate) and small occurrence in Africa. The genera *Eugenia, Syzygium,* and *Myrcia* (c. 1000, 1050, and 770 species, respectively) are among the predominant in the tropical rainforest trees, with the first two genera sharing much morphological similarity that has lead to their repeated synonymy (Lucas *et al.*, 2011) . The distinguished features of the family are leaf containing oil glands, half inferior to inferior ovary, numerous stamens, internal phloem, and xylem vessels with vestured pits (Wilson *et al*, 2001). Plants of Myrtaceae are evergreen trees or shrubs, usually with presence of essential oil in foliage, branches and flowers. All over the world, Myrtaceae is used as medicinal hygenic, edible and ornamental purposes (Ghannadi and Dezfuly, 2011). The uses of Myrtaceae as traditional medicine have led to the screening of some species for the essential oil and their biological activities (Stefanello *et al.*, 2011).

2.2.1 The Genus *Syzygium*

Syzygium, the genus of flowering plants, is one of the important genus in Myrtaceae family, mostly distributed in the tropical and sub-tropical region of the world, with the greatest diversity of species taking place in South East Asia such as Indonesia, Malaysia and also in East India. About 175 species can be found in Sabah and Sarawak (Ashton, 2006). It is represented by around 140 genera and 1100 species (Ayyanar and Babu, 2012), occurring as evergreen trees, shrubs and suffrutices.

Several species of the genus *Syzygium* were formerly classified as *Eugenia* and many references may still list these species as *Eugenia.* Most *Eugenia* species originated from America and the West Indies whereas *Syzygium* species originated from the Indo-Malaysian region. S*yzygium cuminutesi, Syzygium aromaticum, Syzygium polyanthum, Syzygium jambos, Syzygium malaccense,* and *Syzygium aqueum* are some key species of the genus. Several species of the *Syzygium* are grown and consumed for their edible fruits (*Syzygium jambos, Syzygium aqueum, Syzygium samarangense)* and some are used in traditional medicine to treat inflammation (Chauduri *et al.*, 1990), asthma (Alma *et al.*, 2007), various allergic disorders by oral administration (Kim *et al,* 1998), sore throat, bronchitis, thirst, dysentery and ulcers (Ayyanar and Babu, 2012). Some species also exhibited antiseptic properties and the commercial antiseptic from this genus is Clove (Mohanty and Cock, 2010).

Phytochemical studies on *Syzygium* species have led to the identification and isolation of main compounds such as eugenol, eugenol acetate, β-caryophyllene (Alma *et al.*, 2007), flavonoids, caryophyllene (Gao *et al.*, 2012) and others. Several species were reported to have antioxidant, antibacterial (Tsakala *et al.*, 1996; Corine *et al.*, 2000 and Shafi *et al*., 2002), antifungal (Chandrasekaran and Venkatesalu, 2004), anti-inflammatory activities (Muruganandan *et al.*, 2001). The chemical compositions of the essential oils and their biological activities from some *Syzygium* species have been previously evaluated, however little is known for the Malaysian *S. polyanthum* species.

2.2.2 Essential Oils of *Syzygium* Species

Table 2.1: Summary on major chemical constituents of essential oils from the leaf of some *Syzygium* species

Species	Region	Extraction method	Major Compounds	References
S. aromaticum (L.) Merr and Perry	Madagascar	Hydro distillation	Eugenol (82%), β-caryophyllene (13%), α-humulene (1.5%), eugenyl acetate (0.4%) and caryophyllene oxide (0.5%)	Srivastava *et al.* (2005)
S. samarangense (Blume) Merr. And L.M. Perry	India	Steam distillation	β-salinene (11.61%), α-salinene (11.40%), γ-terpinene (10.68%), β-caryophyllene (10.20%) and β-gurjunene (9.48%)	Reddy and Jose (2011)
S.polyanthum (Wight) Walp.	Indonesia	Steam distillation	*Cis-4-decenal* (27.12%), octanal (11.98%), α-pinene (9.09%), farnesol (8.84%), β-ocimene (7.62%) and nonanal (7.60%)	Wartini (2009)
S. cuminui L.	Egypt	Hydro distillation	α-pinene (17.53%), α-terpineol (16.67%),alloocimene (13.55%), α-bornyl acetate (6.27%) and caryophyllene (5.41%)	Elansary *et al.* (2012)
S. malacenses Merr. et Perry	Nigeria	Hydro distillation	p-cymene(13.5%),β-caryophyllene (9%), β-pinene (8%), α-terpineol (7.5%) and α-pinene (7.3%)	Karioti and Skaltsa (2007)
S.guineense (Willd.) DC. var *guineense*	Benin	Hydro distillation	α-cadinol(12.7%), cis-calamenen-10-ol (7.1%), epi-α-muurolol (5.7%), caryophyllene oxide (5.5 %) and cubenol (5.3 %)	Noudogbessi *et al.* (2008)

2.3 *SYZYGIUM POLYANTHUM* (WIGHT) WALP.

2.3.1 Introduction

Syzygium polyantum (Wight) Walp., (synonym *Eugenia polyantha*) is known as *Serai Kayu* or *Salam*, *Daun Kelat, Kelat Samak, Kelat Putih, Kelat Merah, Serah, Daun Salam Manting, Mantang, Ubah Laut, Pokok Palong, Jambu Hutan* in Malaysia and bay-leaf in Indonesia. It is native to tropical Asian countries which are Malaysia, Indonesia (Java, Sumatra, Kalimantan), Myanmar (Burma), Indo-China and Thailand. This medium-sized tree can reach up to 30 to 60 m high and grows wild in the rainforests but it can also be grown in gardens (Sumono and Agustin, 2008). Wee

(2003) reported that this plant can attract birds when fruiting and this helps to spread the seeds to other places. The chemical properties of *S. polyanthum* are the factors that contribute to the uses of this plant in dentistry for therapy and treatment (Utami and Lentera, 2005).

2.3.2 Description of Plant

Table 2.2: Description of *Syzygium polyanthum*

Plant Parts	Morphology and Descriptions
Leaf 	Leaf is opposite, simple, with 2.5-8 cm long and flat margins. The tip is blunt and the base of the leaf stretches along length and tights (Utami and Lentera, 2005) with presence of small oil glands (De Guzman, 1999). The dried brown leaf are aromatic, slightly sour and astringent.
Flowers 	The small flowers are in loose bunches that arise from twigs behind the leaf (Wee, 2003). The flowers are cream and later turning pink or reddish and have fragrance with the fruits around.
Fruits 	Fruit is a one-seed berry (12 mm). It is green and turns red to brown when mature (POM, 2004). The seeds are small (9-10 mm). The ripe fruits are quite sweet mixed sour.
Bark 	The grey round bark is fissured and scaly.

2.3.3 Uses

Culinary

Despite having a slightly sour taste and astringent, the fresh and dried leaf are often used as flavor condiment and food additive (Dalimartha, 2005) especially in Malaysian and Indonesian culinary. The crushed or ground leaf releases more desired fragrance than the whole leaf. In order to release the fragrance, it is suggested to leave the leaf to cook for some time. Ripe fruits are edible, although slightly astringent. In Northern Thailand, they consume and sell fresh ripe fruits during fruiting season (Suksri *et al.*, 2005).

Medicine

The leaf and bark are used traditionally to treat diarrhea (Very *et al.*, 2000). The chemical compounds present in the leaf have the effect of inhibiting the growth of bacteria that can cause diarrhea (Very *et al*., 2000 and Setiawaty, 2003). Poultices (combination of crushed leaf, bark and roots) are applied for curing itches and scabies. The leaf is used to stop gastritis, diabetes mellitus and can treat patient with high uric acid. The roots and fruits can be used to neutralize drunken people (Sumono and Agustin, 2008). The plant also has other benefits such as diuretic and analgesic effect (Utami and Lentera, 2005). *Syzygium polyanthum* is stated to have less side effect than synthetic drugs. It can be consumed as drugs by boiling or crushed as ointment and applied to the affected skin (Dalimartha, 2005).

Tannin or dyeing stuff

The bark consists of tannin, often used for dyeing fishing-nets to enhance the strength of the nets (Suksri *et al.*, 2005), bamboo matting and others.

Timber

Timber (trade name) of *S. polyanthum* belongs to the trade group *kelat*, which is a medium-weight to heavy hardwood. It is used for house building and furniture (Noorma Wati, 1995).

2.3.4 Phytochemicals

The many uses of *S. polyanthum* in traditional treatment are contributed mainly by the presence of the secondary metabolites possessed by the plant, referred to as essential oils and plant extracts. The essential oil of *S. polyanthum* can be obtained from the leaf, stems, fruits and barks. Previous studies have reported the composition of *S. polyanthum* essential oil in samples collected from Indonesia. The leaf contained essential oils, triterpenoids, saponins, flavonoids and tannins (Davidson and Branen, 1993).

The percentage yields and the main chemical compounds of the essential oil from previous studies of *S. polyanthum* have been reported previously. The highest yield was reported by Agusta (2000) with 1.0 % oil while Sumono and Agustin (2008) reported the essential oil yield of the dried leaf is about 0.17 %. The evaluation by Sembiring *et al*. in 2003 showed that the essential oil content of Indonesian bay leaf in Sukabumi (0.02 %) was higher than in Bogor (0.01 %).

Wartini (2009) reported that the essential oils obtained from steam-distillation without using n-hexane as solvents contained 27 compounds while the essential oil extracted from steam distillation using n-hexane yielded 25 compounds. The main compounds were *cis*-4-decenal, octanal, α-pinene, farnesol, β-ocimene, α-caryophyllene, trans-caryophyllene, citronellol and nerolidol. Another study reported that citric acid, eugenol and methyl chavicol (Sumono and Agustin 2008) were the main compounds of *S. polyanthum* oils. Sembiring (2003) have reported the presence of octanal, 3, 7-dimethyl-1-octene and cyclohexane while Agusta (2000) also reported 3, 7-dimethyl-1-octene but with the presence of n-decanal, patchoulin, D-nerolidol and caryophyllene oxide. Unfortunately, no eugenol, citric acid and methyl chavicol have

been identified in the present study and most of the previous studies. Eugenol is the major compound in the essential oils of clove, a member of the genus *Syzygium*. The main class of compounds listed above was aliphatic compounds and oxygenated sesquiterpenes.

Davidson and Branen (1993) reported that besides essential oils, the leaf also contained triterpenoids, saponins, flavonoids and tannins. The pytochemical analysis of leaf and stems of *S. polyanthum* done by Liliwirianis *et al.* in 2011 have reported the presence of alkaloid, saponin, steroid, phenolic and flavonoid with absence of terpenoid in both leaf and stems. It has also been reported that this plant consists of tannins (Dalimartha, 2005). Earlier research on the chemical constituents of the essential oil from Indonesian *S. polyanthum* has already been reported but no previous investigations have been done on the essential oil constituents of *S. polyanthum* cultivated in Malaysia.

2.3.5 Bioactivities

Some past studies supported the worth of the traditional use of *S. polyanthum*, providing several biologically active constituents especially main constituents in the essential oils. Studies involving bioactivities of the essential oil and plant extracts such as the antioxidant, antibacterial and antifungal properties from *S. polyanthum* have been previously demonstrated. Antioxidant activities of aqueous *S. polyanthum* leaf showed relatively high radical scavenging activity and high total polyphenol content (Wong *et al.*, 2006). Raden *et al.* (2009) reported that three bark extracts using methanol, methanol-water and water exhibit potential antioxidant activities, ranking in order: methanol-water extract > water extract > methanol extract. They proved that the total phenolic content has positive correlation with total antioxidative capacity in bark extracts.

The chemical compositions of *S. polyanthum* leaf also have the ability in the inhibition of pathogenic bacteria such as *Salmonella* sp., *Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas fluorescens and Bacillus subtilis* (Setiawan, 2002). Infusion and decoction of *S. polyanthum* leaf showed inhibition towards *E.coli* and *V. cholera* (Hendradjatin, 2004 and Srimurwarni *et al.*, 2005) and the extract of *S.*

polyanthum showed inhibition against *Candida albicans* growth in acrylic resin denture base (Sumono and Agustin, 2008). *Streptococcus* sp. colony was reduced in samples rinsed with (100 %, 75 % and 50 %) *S. polyanthum* solution. Noveriza and Miftakhurohmah (2010) demonstrated that the methanol extract of the leaf inhibited the growth of *Fusarium oxysporum* in solid media while for liquid media, the extract decreased conidia production and germination and hifa weight significantly. The essential oils of the leaf also possess antifungal properties. This was confirmed by Guynot *et al*. in 2005, where the essential oils inhibited the growth of the common fungus associated with the production of bakery which are *Eurotium* spp., *Aspergillus* spp. and *Penicillium* spp.

Tannin is believed to be responsible for the antibacterial activity of *S. polyanthum.* The mechanisms of inhibition of the bacteria growth involved precipitation forming and denaturing of the bacteria protein. The leaf of *S. polyanthum* also have potential in reducing cholesterol. Suharti *et al.* in 2008 concluded that *S. polyanthum* leaf meal could replace the use of antibiotic in broiler ration and reduces carcass cholesterol. The leaf of *S. polyanthum* showed strong *in vitro* anti-tumor promoting activity when assayed using Raji cells (Ali *et al.*, 2000) and significantly reduced the triglyceride serum level in hyperlipidemic rats (Hardhani and Suhardjono, 2008). Studies on fruit extract revealed that the fruit diet decreases blood glucose level, total cholesterol, triglycerides, cholesterol LDL and increases cholesterol HDL level of wistar mice (Ariviani, 2012).

2.3.6 Past Studies

The overview of past studies on the chemical compositions and bioactivities of *Syzygium polyanthum* is shown in Table 2.3.

Table 2.3: Past studies on the chemical compositions and bioactivities of *Syzygium polyanthum*

YEAR	RESEARCH	FOCUS
2002	Setiawan, C.P. 2002. Effect of chemical and physical treatment on the antimicrobial activity of leaf (*Syzygium polyanthum* (wight) Walp.). Thesis. Faculty of Agricultural Technology. Bogor Agricultural University, Bogor	Antimicrobial activity
2003	Sembiring, S., Winarti, C. and Baringbing, B. 2003. Identification of chemical compounds of salam leaf (*Eugenia polyantha*) essential oil from Sukabumi and Bogor. *Bulletin TRO*, July: 10.	Chemical composition
2003	Setiawaty, R. 2003. Study the influence of leaf extract (*Syzygium polyanthum* (Wight) Walp.). of the working power of yogurt starter. Thesis. Faculty of Veterinary Medicine, Bogor Agricultural University, Bogor.	Bioactivity
2005	Guynot, M.E., Marln, S., Setu, L., Sanchis, V. and Ramos, A.J. 2005. Screening for Antifungal activity of some essential oils against common spoilage fungi of bakery products. *Food Science and Technology International,* **11**(1): 25-32.	Antifungal activity
2005	Srimurwarni, Djokobaskoro, A. and Sasmeita, W. 2005. In vitro test on effects of antimicrobial of salam leaf (*Eugenia polyantaha* Wight) decoction against *Escherichia coli* bacteria from patients with urinary tract infection. *Working Paper*. University of Brawijaya: 11 March.	Antimicrobial activity
2006	Wong, S.P., Leong, L.P., Koh, J.H.W. 2006. Antioxidant activities of aqueous extracts of selected plants. *Food Chemistry*, **9**9:775-783.	Antioxidant activity
2008	Sumono, A. and Agustin, W.S.D. 2008. The use of bay laf (Eugenia polyantha Wight) in dentistry. *Dentistry Journal*, **41**(3): 147-150.	Dentistry
2008	Suharti, S., Banowati, A., Hermana, W. and Wiryawan, K.G. 2008. Cholestrol composition and contents of broiled chicken diarrhea given salam leaf (*Synzigium polyanthum* Wight) flour in feed. *Poultry Media*, **31**(2): 138-145.	Bioactivity

Table 2.3: Continued

YEAR	RESEARCH	FOCUS
2009	Raden, A.A.L., Sanro, T. and Kazutaka, I. 2009. *In vitro* antioxidative activities and polyphenol content of *Eugenia polyantha* Wight grown in Indonesia. *Pakistan Journal of Biological Sciences*, **12**(24): 1564-1570.	Antioxidant activity
2009	Wartini, N.M. 2009. Chromatogram sequencing of flavor extract of Salam leaf (*Eugenia polyantha* Wight) from steam distillation using n-hexane and without using n-hexane. *Agrotechnology*, **15**(2): 72-77.	Chemical composition
2009	Hendradtajin, A.A. 2004. In vitro antibacterial effect of infusion of bay leaf (*Eugenia polyantha* Wight) against enteropathogen *Vibrio choleare* dan *Escherichia coli*. *Bandung Medical Journal*, **36**(2).	Antibacterial activity
2010	Noveriza, R. and Miftakhurohmah. 2010. Effectiveness of methanol extract of salam leaf (*Eugenia polyantha*) and kaffir lime leaf (*Cytrus histrix*) as antifungal against *Fusarium oxysporum* growth. *Littri Journal*, **16**(1): 6-11.	Antifungal activity
2011	Liliwirianis, N., Nor Lailatus, W.M., Wan Zuraida, W.M.Z., Kassim, J. and Syaikh, A.K. 2011. Premilinary Studies on Phytochemical Screening of Ulam and Fruit from Malaysia. E-Journal of Chemistry, **8**(S1): 85-88.	Phytochemical
2012	Har, L. W. and Ismail, I. S. 2012. Antioxidant activity, total phenolics and total flavonoids of *Syzygium polyanthum* (Wight) Walp leaf. International Journal of Medicinal and Aromatic Plants, **2**(2): 219-228.	Bioactivities
2012	Perumal, S., Roziahanim, M., Piaru, S. P. 2012. Potential antiradical activity and cytotoxicity assessment of *Ziziphus mauritiana* and *Syzgium polyanthum*. International Journal of Pharmacology, **8**(6): 535-541.	Antiradical and cytotoxicity

2.4 ESSENTIAL OIL

2.4.1 Introduction

Almost all plant species from various families possess natural products termed as essential oil or plant oil (volatile, ethereal oils; Guenther, 1948). In the 16th century, Paracelsus von Hohenheim, who came with the idea of *quinta essentia*, believed that the essence has a major role in medicinal properties. Essential oil is produced only in a small amount (approximately 0.001 to 20 % of fresh weight) but composed of highly concentrated chemical compounds (Runham, 1996). This aromatic essential oil is commonly extracted from plant part such as leaf, flowers, stems, buds, seeds, twigs, rhizomes or bark. The essential oils are produced usually in secretory or specialized tissues, such as glandular trichomes, oils cells, resin ducts, cavities, canals or epidermic cells (Bakkali *et al*, 2008). In order to obtain the oils, the plant parts sometimes need to be crushed, dried or distilled using different techniques.

Essential oil is very concentrated and hundreds of constituents can be found where each compound carries its special aroma and therapeutic properties of the plant. Because of their volatile properties, the quality and quantity of the essential oil depends on many factors including seasonal, geographical and climate conditions (Cheh, 2005), plant parts and their maturity, chemotype and extraction techniques. Other factors such as post-harvest handling and treatment may also contribute to the differences. Essential oils have lower density than water, thus they are usually not mixing well with water (hydrophobic) but with carrier oil (vegetable oil) such as evening primrose oil, sunflower oil and others. People give special interest to essential oils either for their therapeutic properties or for the fragrance alone.

2.4.2 Uses

Essential oils have been used for thousand of years, giving benefit either to plants or humans. Essential oil is vital for the plant life as they play several functions for the plants survival. The occurrence of various compounds in the essential oil help to fight against parasites and infections, to attract insects for pollination, to survive against

predation by microorganism, insects and herbivores; many essential oils have anti-bacterial, anti-fungal and anti-parasitic properties (Dupler and Odle, 2005). In concern of the goodness of using natural products, usage of essential oil is rapidly increasing and commercially employed in perfumes and cosmetics industries, bath products, for flavoring and functional ingredients in food industry, medicinal purposes, pharmaceutical, flavoring and possess a wide range of pharmacological properties (Yentema *et al.*, 2007).

One of the many advantages of essential oil is that they are eco-friendly; their usage as pesticides is promising. There are growing interests in essential oils in food, pharmaceutical and cosmetic industries and therefore, a systematic examination on the plant extracts is important. Compounds such as carvacrol can be used in non-alcoholic beverages and in baked goods. Cinnamaldehyde is used in ice cream products and chewing gum. Thymol and beta ionone are also used as flavoring agents in foods. In aromatherapy, the usage of essential oils is highly in demand. They believe that each of the oil carries special aroma for mind and body healing.

2.4.3 Method of Extraction

Essential oils occurred in different parts of plants, usually in leaf, seeds, flowers, roots, and barks. For more than 2000 years, distillation method has been used in the production of essential oil especially in the East (Guenther, 1948) and in the 9th century, the Arabs have taken this method to a new level where Ibn al-Baitar, a physician, pharmacist and chemist of Andalusia (1188-1248) was the first to use the method (Brill, 1913-1936) while Villanova (ca. 1235–1311) is the first to describe the distillation of essential oil in written form (Guenther, 1948). Different extraction methods have widely been used to isolate the essential oil including hydro distillation, steam distillation, steam and water distillation, expression, solvent extraction and microwave-accelerated distillation.

Hydrodistillation is the conventional extraction method of essential oil, yet the most preferable and common approach of isolating the essential oil from a wide range of plant species. This method is operated by immersing the plant material completely in

water, to facilitate free circulation of the plant during distillation, with the resultant steam being captured and condensed. Water acts as a regulator to maintain the temperature, thus preventing the plant from being over boiled. The oil will always be above the water (as water and oil cannot mix well). Separation of the oil and water are either by dissolving the oil with anhydrous sodium carbonate or by using rotary evaporator to evaporate the water under reduced pressure, obtaining solid compound. *Hydrosol*, the water obtained from distillation is also important and can be used as it contains some remaining plant essence. The popular one is none other than rose hydrosol, which is commonly used for its mild antiseptic and soothing properties, as well as its pleasing floral aroma. (Cheh, 2005). But not all plants produce pleasant hydrosol, thus is not considered to be commercialized.

2.4.4 Compound Analysis

Analytical analysis in chemistry includes the study of the separation, identification, and quantification of the chemical constituents of natural and artificial materials (Holler *et al.*, 1996). Qualitative analysis provides information on the chemical species in the test sample while quantitative analysis verifies the quantity of these components.

Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS is a common technique used to analyze the essential oil which is very volatile and polar (Rubiolo *et al*., 2010). The compositions of various essential oil using gas chromatography coupled to mass spectrometry have been published widely (Delaquis *et al.,* 2002). The separation of the compounds from a mixture is done by gas chromatography while mass spectrometry acts in distinguishing each of the compound individually.

2.4.5 Chemical Compositions

Phytochemistry is the study of phytochemicals that are derived from plants and often described as secondary metabolites of plants. Essential oil is one of the phytochemicals that give characteristics to the aroma of a particular plant. Their chemical compositions and concentrations of individual compounds are variable (Lee *et al.*, 2004) and significantly depend on the plant species, geographical and environmental conditions and post-harvest handling. Dupler and Odle (2005) stated that essential oils could be very complex chemically, with some essential oils with more than 200 identified chemical substances In certain plants, one to two main constituents may predominate and constitute up to 85 % of the essential oil at fairly high concentration, whereas in others there is a balance of various components (Bakkali *et al.*, 2008). Essential oils consist of two main classes of compounds, the hydrocarbon (mainly composed of terpenes) and oxygenated compounds (alcohols, aldehyde, ketones, acids, esters, phenols, oxides).

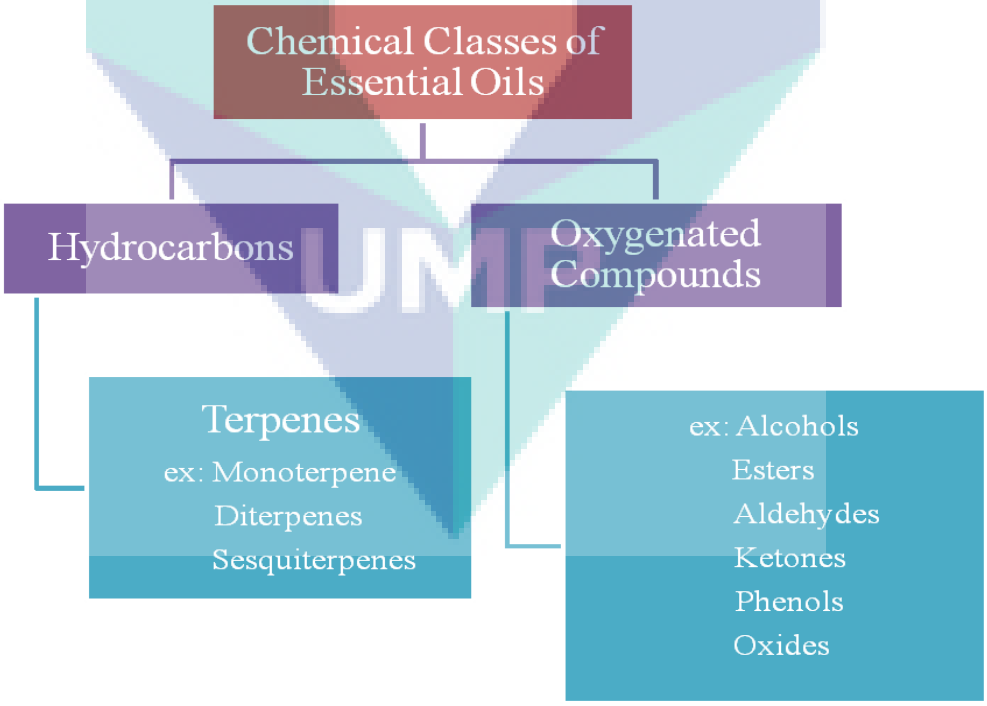


Figure 2.1: Chemical classes of essential oils (Radulescu *et al.*, 2004)

2.4.6 Bioactivities

Many previous studies have confirmed that essential oil plays an important role in biological activities because it is environmentally-friendly, readily available with less or no harmful effect compared to the synthetic products. The bioactivity properties such as antioxidant, antibacterial, antifungal and anti-inflammatory, of the essential oils are strictly related to their various chemical compositions. Thus, it is crucial to characterize different type of medicinal plants for their bioactivity potential.

Essential oils from many herbs and medicinal plants show antibacterial activities that vary depending on species, sub-species and plant varieties. Due to their bioactivity properties, essential oils are certainly promising (Damjanovic-Vratnica *et al.*, 2011) because they are the new options to replace synthetic products for medicinal and healing treatment and also for cosmetics and food industry. The presence of key compounds (one or two main compounds) is usually the main reason for all bioactivity of the essential oil but other compounds may also contribute which can be related by their synergistic or antagonistic outcome among compounds (Xianfei *et al.*, 2007).

Antioxidant Activity

Recently, the essential oils from herbs, medicinal and aromatic plants have been increasingly assessed for antioxidant activity. The evaluation for potential antioxidative activity is important since the food, pharmaceutical and cosmetic industries extensively use the essential oils (Wei and Shibamoto, 2007). Antioxidants act as compounds that can hinder or delay the oxidation of other molecules. They can either inhibit the initiation or inhibit the proliferation of oxidizing chain reactions initiated by free radicals. There are many assays that can be used to test, calculate and compare the antioxidant properties of foods and plant species in order to provide understanding on the functional properties of the foods and plants.

Antibacterial Activity

Antibacterial compounds are those that hinder the growth of microorganisms. De la Croix was the very first person to conduct the bacterial activity of the essential oil in 1881. Essential oil from many herbs and medicinal plants showed antibacterial activity that varies depending on species, sub-species and plant varieties. Review on the potential role of the essential oil as antimicrobial agents from various plants have been widely done (Sadashiva *et al.*, 2010). Phenolic compounds and alcohols are related to positive antimicrobial acitivity.

2.5 BIOASSAYS

Biological assay (shorten as Bioassay) involves specific experiment which is performed to quantify the effect of a substance on living organism either by direct measurement (*in vivo* counting) or by analysis and evaluation of materials excreted or removed (*in vitro*) from the subject (USNRC, 2012). Bioassay can be qualitative or quantitative, where qualitative assay measures the physical outcome while quantitative assay measures the biological reaction of a mixture which depends on the concentration or power. Many bioassays have been developed in order to determine the biological activity of the essential oils.

2.5.1 Antioxidant Activity Assay

There are many methods to evaluate the antioxidant potential of essential oils. Some chemical compounds may act as a primary antioxidant while others may serve as secondary antioxidants or co-antioxidants. The mode of action played by the essential oils or plant extracts are either by prevention of lipid peroxidation or free radical scavenging and some others may inhibit metal ion chelating (Maria, 2010).

DPPH Free Radical Scavenging (FRS)

2, 2-diphenyl-1-picrylhydrazyl or DPPH is a common method to evaluate antioxidant. It is simple but also rapid with high sensitivity (Maria, 2010). The cost is also inexpensive. Only small amount of samples are needed to measure the antioxidant capacity. The change of color from purple to yellow indicates that the desired compounds can donate hydrogen to a stable free-radical DPPH, thus showing a decrease in absorbance at 517 nm.

β-carotene Bleaching (BCB)

The β-carotene bleaching assay is a combination of oxidation of β-carotene and linoleic acid. The presence of antioxidant prevents the free radical linoleic acid from oxidizing β-carotene rapidly, preventing discoloration of orange color (Maria, 2010).

Ferrous ion Chelating (FIC)

Transition metal ions can trigger lipid peroxidation. This assay helps in chelating of these transition metals. Ferrozine will form complexes with Fe^{2+}. In the presence of antioxidant, the red color of ferrozine- Fe^{2+} will diminish, thus giving a lower absorbance at 562 nm (Maria, 2010).

2.5.2 Antibacterial Activity Assay

Disc Diffusion Method

The disc-diffusion method is widely used to screen for antibacterial and antifungal properties of plants. Essential oils extracted from plants have also been tested for antimicrobial activity. In the disc-diffusion method, the ability of antimicrobial agents to inhibit desired bacterial strains is assessed (Chung *et al.*, 2004).

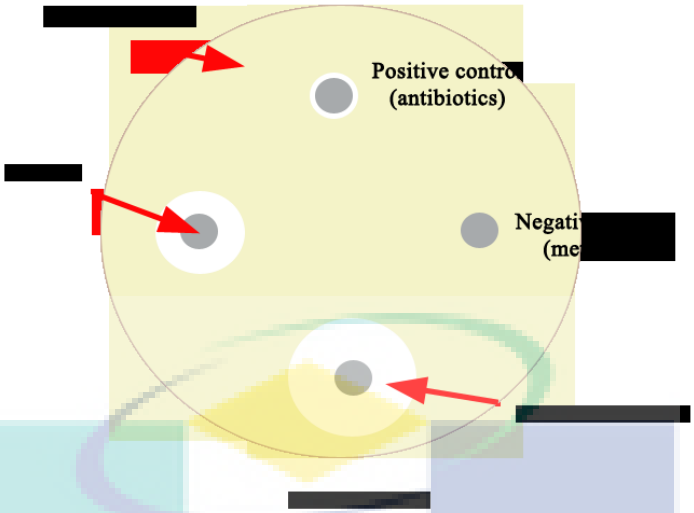


Figure 2.2: Disc diffusion method

Minimum Inhibitory Concentration (MIC)

MIC is another quantitative method and the most basic laboratory measurement for the measurement of antimicrobial activity. MIC can be defined as the lowest concentration needed for the samples to inhibit the growth of test microorganism. It can be determined either by agar or broth dilution method. Extracts are serially diluted and loaded onto wells bored using cork borer. It is the minimum concentration that showed positive activity using the disc diffusion method. A lower MIC is an indication of a better antimicrobial agent.

Minimum Bactericidal Concentration (MBC)

MBC can best be defined as the lowest concentration of antimicrobial agents that can kill particular strains (Medical Microbiology, 1993). It can be screened from broth dilution taken directly from MIC tests that shows no growth (French, 2006).

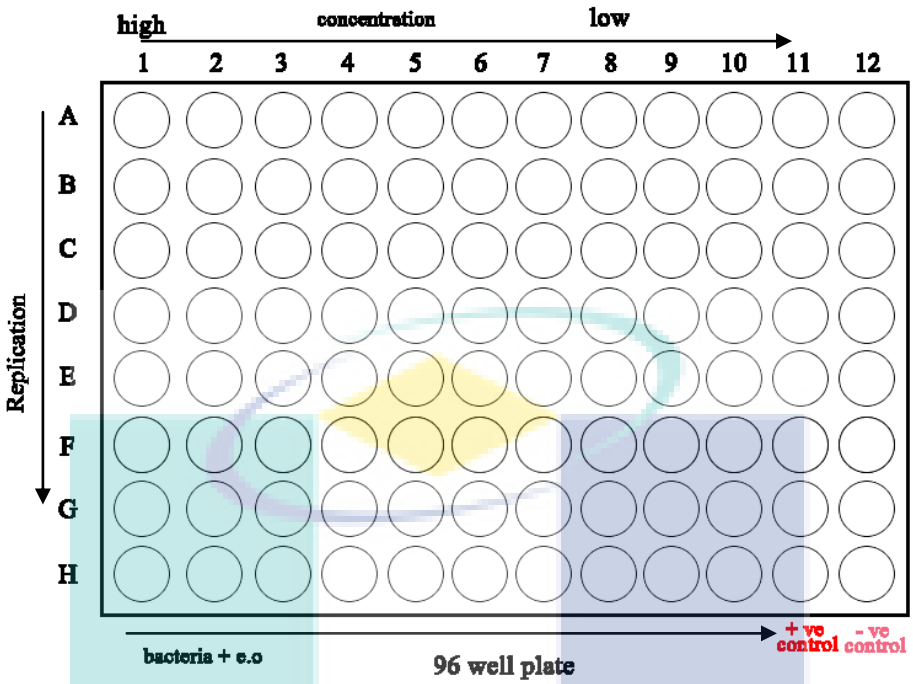


Figure 2.3: 96 well-plate method

2.5.3 Statistical Analysis

Statistical analysis aims at calculating the probability between two sets of data which may have significant differences or correlation. Analysis of variance (ANOVA), a t-test or a non-parametric method is a set of measurement to calculate and compare mean and standard deviation (Festing *et al.*, 2002). Software such as SPSS and Microsoft Excel are used to assess the data.

CHAPTER 3

MATERIALS AND METHODS

3.1 PLANT MATERIAL

Fresh samples of *Syzygium polyanthum* (Wight) Walp. leaf were collected from Kuantan, Pahang. Voucher specimens (SK2180/13) have been deposited at the herbarium of Institute of Bioscience, Universiti Putra Malaysia, Serdang, Selangor, Malaysia.

3.2 REAGENTS AND CHEMICALS

All the chemicals used in this research were purchased from Merck (Germany) and Sigma-Aldrich (USA). The chemicals were of analytical grade.

3.3 EXTRACTION OF ESSENTIAL OIL

3.3.1 Preparation of Sample

The fresh leaf was cleaned with excess tap water to remove dirt. The fresh leaf were chopped into small pieces and immediately used while for the dried sample, the leaf (300 g) was dried under sunlight for 2 days to remove moisture and blend into powder before used.

3.3.2 Hydrodistillation Extraction

The plant samples (100 g) were weighed and subjected to hydrodistillation for 4 hours using all glass Clevenger-type apparatus (British Pharmacopoeia, 1980). The oil was collected and stored in dark glass vials (to avoid direct light) in the fridge (4 ^{o}C) until further analysis.

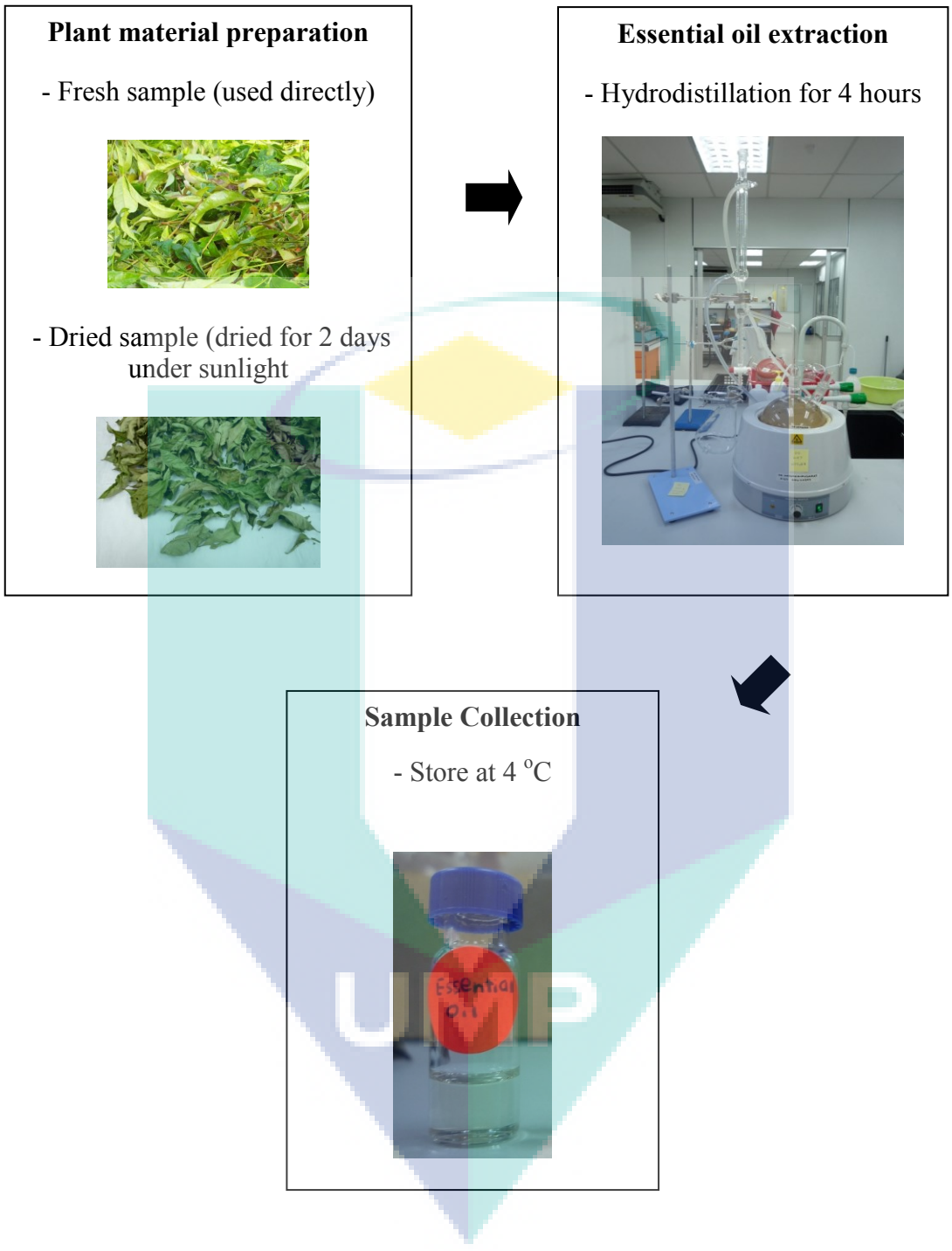


Figure 3.1: Sample preparation and hydrodistillation extraction

3.3.3 Determination of Yield

The yield of the essential oil in the collecting tube was measured and calculated based on the net weight of the plant material. In order to measure the moisture content of the plant, the sample was dried for 2 days until the weight was constant.

Calculation of the moisture content of the sample

$$M_n = [(W_w - W_d)/W_w)] \times 100$$

M_n = moisture content (%) of material n
W_w = wet weight of the sample
W_d = weight of the sample after drying

Calculation of the dry and net weight of the sample

$$D_w = 100 - M_n$$
$$N_w = D_w \times \text{Sample (g)}$$

D_w = dry weight of sample
N_w = net weight of sample

Calculation of percentage yields based on the dry weight of the sample

$$\% \text{ Yield} = \text{Oil Collected (g)}/ N_w \text{ (g)} \times 100$$

3.3.4 Compound Analysis

Gas Chromatography Mass Spectrometry (GCMS)

The essential oil of *Syzygium polyanthum* (Wight) Walp. was analyzed on a gas chromatograph (Agilent 7890) - mass spectrometer (Agilent 5975 C), inert XL MSD with Triple-Axis Detector, Agilent 7693 auto sampler, equipped with a HP-5MS capillary column (30 m×0.25 mm x 0.25 μm diameter). The injector and detector temperature were set at 220 ^{o}C and 300 ^{o}C. The oven temperature was kept at 50 ^{o}C for 3 minutes, and then gradually raised to 160 ^{o}C at 3 ^{o}C/minutes, held for 10 minutes and finally raised to 280 ^{o}C at 3 ^{o}C/minutes.

Identification of Compounds

The chemical constituents were identified by comparing the mass spectra with those of National Institute of Standards and Technology (NIST-08) library data.

3.4 DETERMINATION OF ANTIOXIDANT ACTIVITY

The antioxidant activities of the essential oil from fresh and dried leaf were determined using three different assays namely DPPH free radical scavenging, β-carotene bleaching (BCB) and ferrous-ion chelating (FIC) assay.

3.4.1 Free Radical Scavenging Assay

Free radical scavenging activity of the essential oils was evaluated according to the method proposed by Chan *et al.* (2008) using DPPH with slight modifications.

Preparation of test samples

Different dilutions of the samples were prepared in test tubes by diluting test samples in ethanol in the range of 0.13 to 2.00 mg/ml.

Preparation of DPPH solution

A stock solution of DPPH was prepared in a volumetric flask by dissolving 0.01 g DPPH in 100 ml ethanol. The flask used was covered with aluminum foil to prevent direct light.

Preparation of DPPH working solution

The stock solution of 20 ml was taken into volumetric flask and 80 ml ethanol was added to make the volume 100 ml.

Protocol for estimation of DPPH scavenging activity

Different dilutions of the samples (1.5 ml) were added to 1 ml of ethanolic solution of 2, 2-diphenyl-1-picrylhydrazyl (0.01 g/100 ml ethanol) in test tubes. The mixture was shaken vigorously and left in the dark for 30 minutes at room temperature. The absorbance of mixture was read at 517 nm using UV-visible spectrophotometer (Thermo Scientific) against a blank of absolute ethanol (95 %) without DPPH. The commercial butylated hydroxytoluene (BHT) was used as a positive control.

The inhibition percentage was calculated using formula:

$$\% \text{ of inhibition} = [1 - A_{sample} / A_{control}] \times 100$$

$A_{control}$ = absorbance of DPPH solution without essential oil

A_{sample} = absorbance of DPPH solution with essential oil

3.4.2 β-carotene Bleaching Method

β-carotene bleaching of the essential oils was evaluated according to the method of Chan *et al.* (2008) with slight modifications.

Preparation of test samples

Different dilutions of the samples were prepared in test tubes by diluting test samples in ethanol in the range of 0.13 to 2.00 mg/ml.

Preparation of β-carotene stock solution

A stock solution of β-carotene was prepared in a test tube by dissolving 10 mg of β-carotene in 10 ml chloroform.

Preparation of β-carotene working solution

The stock solution (1 ml) was added to 20 µl of linoleic acid and 200 µl Tween 40 in a volumetric flask. Then 100 ml of distilled water was added slowly and the resulting mixture was vigorously shaken for 30 minutes. The solution was evaporated under vacuum in a rotary evaporator at 40 ^{o}C to remove chloroform.

Protocol for estimation of β-carotene bleaching

Different dilutions of the samples (0.5 ml) in test tubes were added to 2.0 ml of the previously prepared working solution. The test tubes, including BHT as positive controls and blank were incubated in a hot water bath at 50 ^{o}C for 2 hours. The absorbance of each sample was read at 470 nm using UV-visible spectrophotometer (Thermo Scientific) against a blank.

β-carotene bleaching (%) of the samples and BHT were calculated using the following equation:

$$I\% = (A_{\beta\text{-carotene after 2 h assay}} / A_{\text{initial } \beta\text{-carotene}}) \times 100$$

$A_{\beta\text{-carotene after 2 h assay}}$ = absorbance of β-carotene after 2 hours assay remaining in the samples

$A_{\text{initial } \beta\text{-carotene}}$ = absorbance of β-carotene at the beginning of the experiments

All tests were carried out in triplicate and the bleaching percentages were evaluated as means ±SD of the triplicate.

3.4.3 Ferrous-ion Chelating Assay

Ferrous-ion chelating of the essential oils was estimated according to the method of Dinis *et al.* (1994) with slight modifications.

Preparation of test samples

Different dilutions of the samples were prepared in test tubes by diluting test samples in solvent in the range of 0.13 to 2.00 mg/ml.

Preparation of ferrous chloride (2 mM) solution

A stock solution of ferrous chloride was prepared in a volumetric flask by dissolving 0.04 g of ferrous chloride in 100 ml ethanol (95 %). The flask used was covered with aluminum foil to prevent direct light.

Preparation of Ferrozine (5 mM) solution

A stock solution of ferrozine was prepared in a volumetric flask by dissolving 0.25 g of ferrozine in 100 ml ethanol (95 %). The flask used was covered with aluminum foil to prevent direct light.

Protocol for estimation of ferrous-ion chelating assay

Different dilutions of the samples (0.5 ml) were added to 0.05 ml ferrous chloride solution (2 mM) in test tubes. The mixtures were shaken vigorously and incubated for 5 minutes at room temperature. Ferrozine (5 mM; 0.1 ml) was added followed by the addition of 4 ml of ethanol and left to stand at room temperature for 10 minutes. The absorbance of mixture was read at 562 nm using UV-visible spectrophotometer (Thermo Scientific) against a blank. The commercial butylated hydroxytoluene (BHT) was used as positive control.

The chelating activity was given by the following equation:

$$\text{Chelating ability (\%)} = (1 - A_{sample}/A_{control}) \times 100\ \%$$

$A_{control}$ = absorbance of the control

A_{sample} = absorbance of the sample

The control contains only ferrous chloride and ferrozine.

3.4.4 Statistical Analysis

Results were analyzed by using analysis of variance (ANOVA) to see the significance of the data. Statistical analysis was carried out using the SPSS Statistical Software version 16.0 and Microsoft Excel.

3.5 SCREENING OF ANTIBACTERIAL ACTIVITY

The essential oils from the fresh and dried leaf of *Syzygium polyanthum* were tested for their antibacterial activity against human pathogenic bacteria using disc diffusion and micro dilution method.

3.5.1 Strains of Bacteria

The bacteria strains used in this study are known to be the cause of disease in human. Three strains of gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa, Salmonella typhii*) and three strains of gram-positive bacteria (*Staphylococcus aureus, Bacillus subtilis, Enterococcus faecalis)* were used in the antibacterial assay. All of these bacteria were obtained from the Microbiology Laboratory of Faculty Industrial Sciences and Technology, Universiti Malaysia Pahang.

3.5.2 Preparation of Sample

Preparation of inocula

The bacteria were grown and maintained on Nutrient Broth (NB) slants. They were then stored at 4 ^{o}C under aerobic conditions. The bacteria were cultured overnight at 35 ^{o}C in nutrient broth which was then adjusted to obtain turbidity comparable to that of 0.5 Mc Farland standards (10^6 CFU/ml) for further use.

Preparation of discs

The essential oil (10 µl) at different concentration was impregnated on sterile paper discs (6 mm). Disc impregnated with ethanol were used as negative control while streptomycin sulfate and tetracycline were used as positive reference standard.

3.5.3 Disc Diffusion Assay

The Kirby-Bauer disc diffusion susceptibility test was employed in order to determine the sensitivity or resistance of selected bacterial strains. The assay is based on the method described by Damjanovic-Vratnica *et al.* (2011) with a slight modification. Mueller-Hinton agar (20 ml) was poured into the petri plates and allowed to solidify. The inoculums were spread evenly over the surface of the media using a sterile cotton swab.

The prepared discs were then applied firmly using sterile forceps and pressed gently to ensure contact with the inoculated agar surface. The plates were incubated for 24 hours at 37 ^{o}C. For each petri dish, only maximum of 5 discs were applied. The inhibition zone (no growth) were observed and compared to the reference standard. The measurement was in mean diameter (mm). The oils inducing zone > 3 mm diameters around the discs were considered as positive antibacterial inhibition. All tests were performed in duplicate.

3.5.4 Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) was determined using the serial dilution method, using 96-well micro-plates according to protocol by NCCLS (2004) and Damjanovic-Vratnica *et al.* (2011). All tests were performed in Mueller-Hinton Broth. To each well, 50 µl of MHB was added followed by 50 µl of bacterial suspension (10^6 CFU/ml). Aliquots (100 µl) of different concentrations ranging from 0.03 mg/ml to 1.00 mg/ml (two-fold serial dilution) of the samples were introduced to a sterile 96-well micro-plate. Two columns in each plate were used as controls: one column with ethanol as negative control and one column containing streptomycin sulfate as positive standard in this antibacterial activity test. The plates were covered and mixed well. After an incubation period at 37 ^{o}C for 24 hours, turbidity was taken as indication of growth, thus the lowest concentration which remained clear after macroscopic evaluation was taken as the minimum inhibitory concentration (MIC). The growth was observed using micro-plate reader to confirm the bacterial activity. The MIC will be recorded as the mean concentration of triplicates.

3.5.5 Minimum Bactericidal Concentration (MBC)

MBC were determined for all wells used in MIC test that did not show any turbidity with the bacteria. An aliquot of the suspension (1.0 ml) was spread onto Mueller-Hinton Agar (MHA) using hockey stick and incubated at 37 ^{o}C for 24 hours. The MBC was the lowest concentration in which initial inoculums were killed at the least one log cycle or more.

3.5.6 Statistical Analysis

Results were analyzed by using analysis of variance (ANOVA) to see the significance of the data. Statistical analyses were carried out using the SPSS Statistical Software version 16.0 and Microsoft Excel.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 ESSENTIAL OIL EXTRACTION

In this study, the fresh and dried leaves (100 g) of *S. polyanthum* were subjected to hydrodistillation extraction for 4 hours. The amount of the essential oils does not increase and became constant over the 4 hours. The physicochemical properties of the essential oils were shown in table 4.1.

Table 4.1: The physicochemical properties of the fresh and dried leaf of *Syzygium polyanthum* essential oils

Physicochemical Properties	Fresh Leaf Oil	Dried Leaf Oil
Yield (%)	0.67	1.50
Physical Appearance	Colourless	Light yellow
Smell	Pungent smell, slightly sour	Pungent smell, slightly sour

The leaf dried under sunlight had a higher yield of essential oil with 1.50 % while the fresh leaf afforded oil at a yield of 0.67 %. There are several studies that have been done on the essential oil of *Syzygium polyanthum* grown in Indonesia. The highest yield was reported by Agusta (2000) with 1.0 % oil extracted using hydrodistillation method. In the study by Sembiring (2003), the plant species was taken from two different regions in Indonesia and was extracted using water-steam distillation. The yield of the essential oil from the two regions differed slightly with 0.02 % (Bogor) and 0.01 % (Sukabumi). It is worth noting that even the same plant species can produce different yields of the essential oil depending on their geographical, seasonal and environmental factors as well as the extraction method.

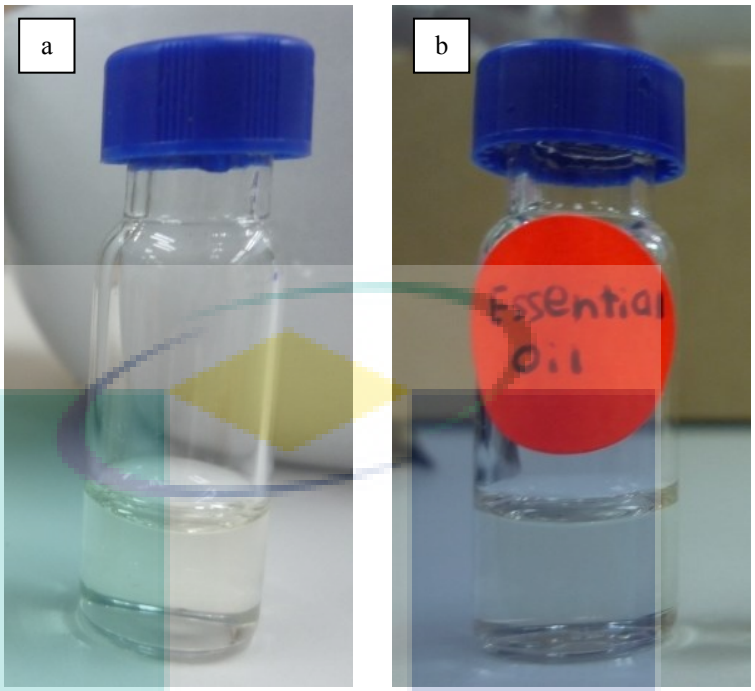


Figure 4.1: Essential oils of *Syzygium polyanthum* leaf: (a) Fresh Leaf - colourless, (b) Dried leaf - light yellow

4.2 CHEMICAL COMPOSITIONS OF THE ESSENTIAL OILS

This present study provides information on the chemical constituents of the essential oil from the leaf of *S. polyanthum* from Peninsular Malaysia. It is important to note that this is the first report to evaluate the chemical compositions of *S. polyanthum* grown in Malaysia. The essential oil identified using GC-MS with HP-5 MS column. The gas chromatograms of the essential oils studied are attached in Appendix B. The list of compounds identified is presented in Table 4.2.

Table 4.2: Chemical compositions of the fresh and the dried leaf oils of *Syzygium polyanthum*

	Compounds	RT[a]	Fresh Leaf (%) [b]	Dried Leaf (%) [b]	Method of Identification
1.	α-Pinene	9.26	28.78	34.15	MS
2.	Camphene	9.64	-	0.23	MS
3.	Ocimene	10.22	1.18	-	MS
4.	β-Pinene	11.16	-	0.83	MS
5.	Octanal	11.69	-	29.18	MS
6.	*p*-Cymene	12.21	-	0.28	MS
7.	Limonene	12.33	1.42	1.36	MS
8.	β-Ocimene	12.71	0.70	0.21	MS
10.	γ-Terpinene	13.31	0.38	0.11	MS
11.	Neo-Allo-Ocimene	13.52	0.52	-	MS
12.	α-Terpinolene	14.22	0.49	0.10	MS
13.	α-Pinene Epoxide	14.51	-	1.01	MS
14.	Linalool	14.65	-	0.39	MS
15.	α-Campholenal	15.41	-	0.15	MS
16.	Octanoic acid	17.22	-	0.61	MS
17.	α-Terpineol	17.37	-	0.12	MS
18.	*cis*-4-Decenal	17.49	-	0.23	MS
19.	Decanal	17.83	1.73	1.52	MS
21.	α-Cubebene	22.55	-	2.79	MS
20.	Neryl Acetate	23.11	0.12	-	MS
22.	α-Guaiene	24.16	-	0.11	MS
23.	Copaene	24.26	3.47	-	MS
24.	α-Caryophyllene	24.55	9.11	1.40	MS
25.	γ-Selinene	25.11	0.58	0.25	MS
26.	4, 11-Selinadiene	25.30	-	0.17	MS
27.	β-Selinene	25.38	5.62	2.88	MS
28.	α-Selinene	25.60	-	2.57	MS
29.	α-Muurolene	25.71	-	0.25	MS
30.	Caryophyllene	25.81	0.22	-	MS
31.	α-Panasinsen	26.11	0.45	0.53	MS
32.	δ-Cadinene	26.27	1.72	0.67	MS
33.	Nerolidol	27.27	5.22	6.68	MS
34.	Alloaromadendrene	27.46	0.12	-	MS
35.	Selinene	28.91	5.85	-	MS
36.	δ-Cadinol	29.00	0.23	0.20	MS
37.	γ-Cadinene	29.43	0.25	-	MS
38.	(E,Z)-Farnesol	30.79	2.98	1.94	MS
39.	3,7-Cycloundecadien-1-ol,1,5,5,8-tetramethyl	32.45	0.10	-	MS
40.	β-Eudesmol	34.10	0.18	-	MS
41.	9, 12-Octadecadienoic Acid (Z, Z)	47.06	0.20	-	MS
42.	Octadecanoic Acid	49.44	0.09	-	MS
	Identified compounds (%)		71.71	90.92	
	Monoterpene hydrocarbons		33.47	37.27	
	Oxygenated monoterpenes		-	1.67	
	Sesquiterpene hydrocarbons		27.39	11.62	
	Oxygenated sesquiterpenes		8.71	8.82	
	Others		2.14	31.54	

[a]Retention time on a HP-5 column
[b]Relative percentage of the identified volatiles based on GC-MS

In this study, different chemical compounds have been identified in the essential oil extracted from the leaf of *S. polyanthum*. As shown in table 4.2, 26 and 29 compounds, representing about 71.71 % and 90.92 % of the essential oil from the fresh and dried leaf were identified. Both oils were characterized by high amount of monoterpene hydrocarbons. The major compounds for the fresh leaf oil were α-pinene (28.78 %), α-caryophyllene (9.11 %), selinene (5.85 %) and the major compounds for the dried leaf oil were α-pinene (34.15 %), octanal (29.18 %) and nerolidol (6.68 %). Based on the result, it can be assumed that the essential oil from *S. polyanthum* leaf belongs to α-pinene chemotype.

Among the 55 compounds identified, 14 of them were common in both of the two essential oils. The percentage of α-pinene, α-panasinsen and nerolidol increased in the dried leaf oil while limonene, β-ocimene, γ-terpinene, α-terpinolene, decanal, α-caryophyllene, γ-selinene, β-selinene, δ-cadinene, δ-cadinol and (E,Z)-farnesol have higher percentages in the fresh leaf oil.

There is an occurrence of similar compounds from the previous study (Agusta, 2000; Sembiring, 2003 and Wartini, 2009) which were nerolidol, octanal and *cis*-4-decenal. Nerolidol is a sesquiterpenes, which occurs naturally in many plants and flowers. *Cis*-4-decenal is an aldehyde with citrus-like odour. Octanal, also known as caprylic aldehyde produces fruit-like odor which is colorless or light yellow in color. These compounds are often used as a flavoring agent in the food industry. From this, it could be assumed that the aroma of *S. polyanthum* leaf could be attributed by the presence of nerolidol, *cis*-4-decanal and octanal but may be contributed by other compounds as well.

4.3 DETERMINATION OF ANTIOXIDANT ACTIVITY

Antioxidant activity is a complex process usually occurring through several mechanisms (Bamoniri *et al.*, 2010): free radical scavenging, lipid peroxidation or metal chelating. Due to the multifaceted photochemical, the antioxidant activities of essential oils/extracts should not be estimated by a single method only, but at least two or more systems should be employed in order to determine the antioxidant properties (Schlesier *et al.*, 2002). In view of these, the potential antioxidant properties of *S. polyanthum* essential oil were assessed by three testing methods, DPPH, β-carotene/linoleic acid and metal-chelating assays.

In vitro antioxidants assays in foods and biological systems can be divided into two groups: evaluation of lipid peroxidation and measurement of free radical scavenging ability. Antioxidant can be classified as primary, secondary or co-antioxidant based on their mode of action. Primary antioxidants are able to donate a hydrogen atom rapidly and form a stable, new radical. Secondary antioxidants are involved in eliminating the initiators by retarding the radical initiation without generating new radical species (Aazaa *et al.*, 2011).

4.3.1 Free Radical Scavenging Activity (DPPH)

The essential oil was subjected to DPPH assay to test its ability to scavenge the DPPH free radical. In order to measure the effect of sample concentration on radical scavenging power using DPPH assay, 5 different concentrations of the samples were used (0.13, 0.25, 0.500, 1.00 and 2.00 mg/ml). The results on the ability of the samples to scavenge the free-radical are shown in Table 4.3 and Figure 4.2 compared with BHT as control standard.

Table 4.3: Antioxidant activity of the fresh and the dried leaf essential oils of *Syzygium polyanthum* compared to BHT defined as inhibition percentage in DPPH radical scavenging assay

Sample	% Inhibition of DPPH scavenging activity				
	0.13 mg/ml	**0.25 mg/ml**	**0.50 mg/ml**	**1.00 mg/ml**	**2.00 mg/ml**
Fresh Leaf Oil	90.95[a]	91.14[a]	91.79[b]	91.88[b]	92.16
Dried Leaf Oil	91.79	92.07	93.10[a]	93.19[ab]	93.38[b]
BHT	88.06	88.34	88.99	89.55	91.60

Values of absorbance are means ±SD (n=3). Values followed by the same letters (a-b) are not statistically different as $p < 0.05$ as measured by the Tukey HSD test

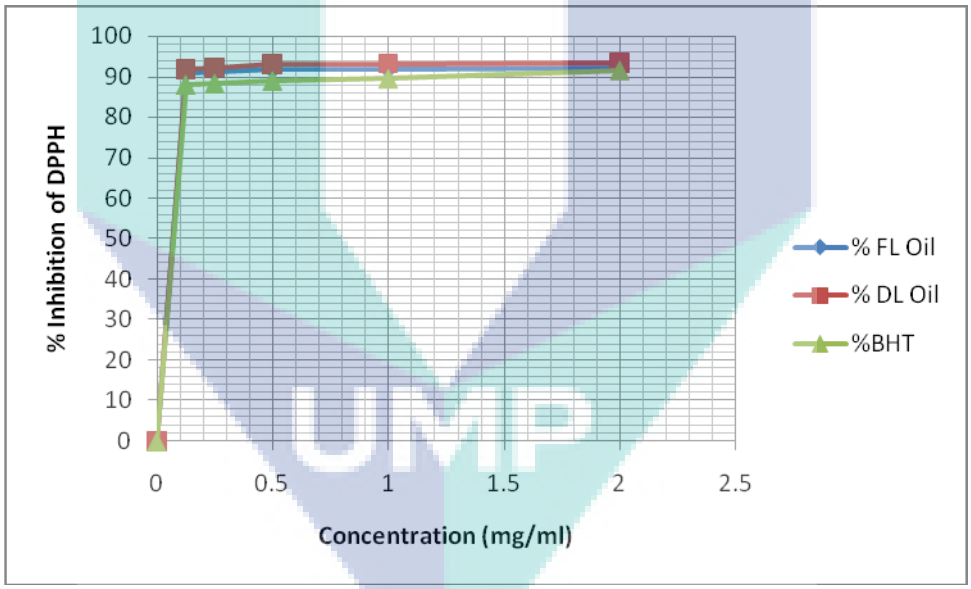


Figure 4.2: The DPPH radical scavenging activity of the fresh and the dried leaf essential oils of of *Syzygium polyanthum* compared to BHT

As seen in Table 4.3, the DPPH radical scavenging of the essential oil increased from 90.95 % to 92.16 % for the fresh leaf oil and 91.79 % to 93.38 % for the dried leaf oil, with the concentration of 0.12 to 2.00 mg/ml. This showed that the percentage inhibition is in increasing order with the increase in concentration. BHT showed lower inhibition from 88.06 % to 91.60 % with the same concentration.

Figure 4.2 shows the DPPH radical scavenging (%) activity for both the fresh and dried leaf oil have higher antioxidant activity as compared to BHT at all concentration levels. The highest activity of the essential oil was 92.16 % (fresh leaf) and 93.38 % (dried leaf) compared to BHT with 91.60 %. The results showed that the investigated *S. polyanthum* oil demonstrated a potential antioxidant ability to reduce DPPH but better activity when compared to that of the standard.

Free radicals are known to be the main cause in biological damages associated with chronic diseases such as cancer and heart diseases (Hajji *et al.*, 2010). The DPPH free radical scavenging assay is widely used and offers rapid techniques (Amarowicz *et al.*, 2004) to evaluate the ability of plant sample to scavenge free radicals.

The DPPH (2, 2-diphenyl-1-picrylhydrazyl) is a molecule that contain a stable free radical usually used to determine the ability of compounds to scavenge free radicals (Shafique *et al.*, 2011). In the presence of antioxidant, which can donate an electron to DPPH, the purple color of DPPH is reduced to yellow color and the change is viewed spectrometrically at absorbance 517 nm (Tepe *et al.*, 2005).

4.3.2 β-carotene Bleaching Activity

In order to confirm its ability to prevent lipid peroxidation, the essential oil was tested through β-carotene/linoleic acid assay. The anti-bleaching activity of β-carotene was studied by monitoring the color intensity at 470 nm for every 30 minutes for 2 hours. Results are summarized in Table 4.4.

Table 4.4: Antioxidant activity of the fresh and the dried leaf oil of *Syzygium polyanthum* compared to BHT defined as percentage of β-carotene bleaching

Sample	% of β-carotene Bleaching at 2.00 mg/ml				
	0 min	30 min	60 min	90 min	120 min
Fresh Leaf Oil	100	91.44	91.39	91.10	90.58
Dried Leaf Oil	100	98.39	96.33	93.29	91.15
BHT	100	93.84	89.88	86.68	86.26

Values of absorbance are means ±SD (n=3). Values are statistically different as $p < 0.05$ as measured by the Tukey HSD test

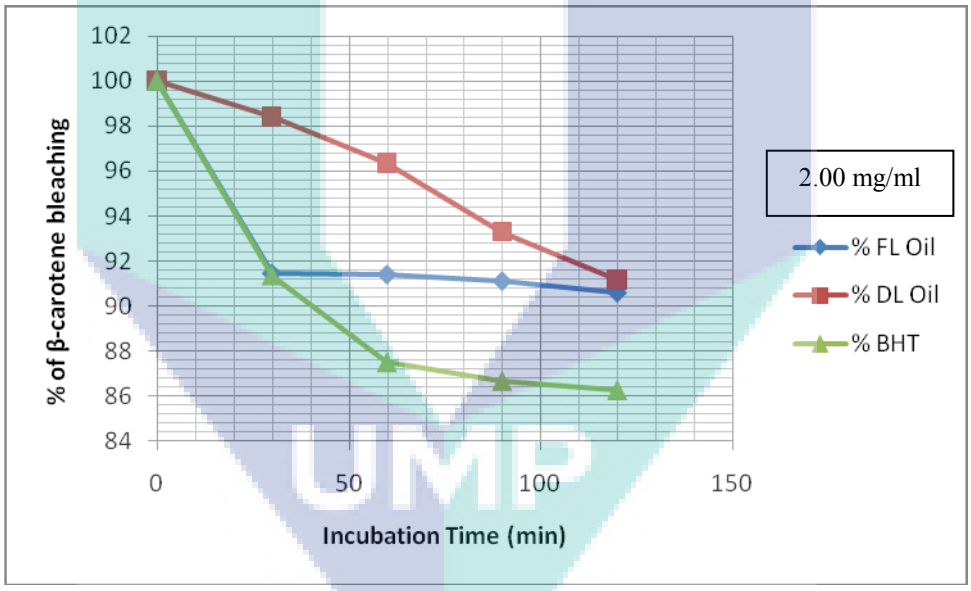


Figure 4.3: β-carotene bleaching activity of the fresh and the dried leaf oils of *Syzygium polyanthum* compared to BHT based on incubation time at 2.00 mg/ml

The essential oil of fresh and dried leaf of *S. polyanthum* showed potential ability to prevent the bleaching of β-carotene. The inhibition of β-carotene bleaching of the essential oil was compared to the standard, BHT. Table 4.4 shows the decrease in absorbance during the coupled oxidation of β-carotene and linoleic acid. The bleaching activity decreased with increasing oil concentration. Slower decrease of the absorbance of β-carotene indicates a lower rate of linoleic acid oxidation and higher antioxidant

activity with the presence of essential oil. The initial concentration of β-carotene was considered as 100 %.

At a concentration of 2.00 mg/ml, in the first 30 minutes, the fresh and dried samples showed 91.44 % and 98.39 % bleaching respectively, compared to BHT with 93.84 %. After 1 hour of incubation, percentage decrease was found to be 91.39 % and 96.33 % for the fresh and dried leaf oils and 89.88 % for BHT. During the second hours, it had reached to 90.58 % and 91.15 % for oil and 86.26 % for BHT, respectively. The plotted graph of β-carotene degradation (%) against incubation time (minutes) is presented in Figure 4.3. From the results evaluated, BHT shows a high percentage of β-carotene at initial time but failed to prevent the oxidation as the decrease rate of β-carotene become higher during incubation time up to 2 hours.

While for the fresh leaf essential oil, the initial rate of β-carotene was lower compared to BHT but the essential oil managed to slow down the oxidation rate of β-carotene during the incubation time. The dried leaf oil showed high initial concentration of β-carotene until the end of the incubation time. The high antioxidant activity of the essential oil of *S. polyanthum* indicates a possible ability of the oil to prevent lipid peroxidation. The essential oil which shows the lowest degradation rate of β-carotene exhibits the highest antioxidant activity.

This assay is often used for the determination of antioxidant activity because it is frequently involved in emulsion of foods (Gopalakrishnan *et al.*, 2012). In this system, free radicals produced by oxidation of linoleic acid at 50 ^{o}C in an emulsion are responsible for the loss of the orange colour of β-carotene, which is monitored spectrophotometrically at 470 nm. In the presence of antioxidant agents, the bleaching of β-carotene was slowed down (Prakash *et al.*, 2012). β-carotene/linoleic acid assay is classified as hydrogen atom transfer (HAT) based antioxidant tests. The antioxidant activity of essential oil or extracts may be associated with the presence of molecules with functional groups such as allylic or benzylic which are able to donate hydrogen (Larson, 1997).

4.3.3 Ferrous-ion Chelating Activity

The ability of the essential oil from the fresh and dried leaf of *Syzygium polyanthum* to chelate the ferrozine/Fe^{2+} complexes is determined using this assay. Five different concentrations of (0.13, 0.25, 0.50, 1.00 and 2.00 mg/ml) were used to test the chelating activity of the essential oil. The ferrozine/Fe^{2+} complexes have a maximum absorbance at 562 nm and decrease in absorbance indicates stronger chelating activity. Results are summarized in Table 4.5 and Figure 4.4.

Table 4.5: Antioxidant activity of the fresh and the dried leaf of *Syzygium polyanthum* essential oils compared to BHT defined as inhibition percentage in ferrous-ion chelating

Sample	**% Inhibition of ferrous ion chelating**				
	0.13 mg/ml	**0.25 mg/ml**	**0.50 mg/ml**	**1.00 mg/ml**	**2.00 mg/ml**
Fresh Leaf Oil	44.58	53.63	70.48	80.36	83.49
Dried Leaf Oil	45.12	53.79	78.86	84.74	91.66
BHT	55.88	57.55	57.71	60.59	61.30

Values of absorbance are means ±SD (n=3). Values followed by the same letters (a-b) are not statistically different as $p < 0.05$ as measured by the Tukey HSD test

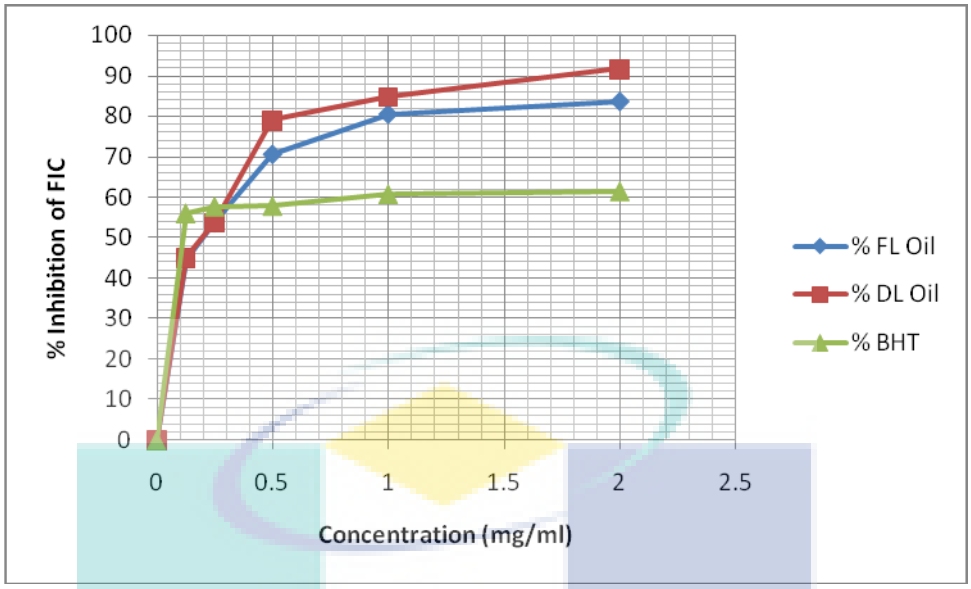


Figure 4.4: Metal-chelating activity of the fresh and the dried leaf essential oils of *Syzygium polyanthum* compared with BHT

As seen in Table 4.5, the metal chelating activity of the fresh and dried leaf essential oils increased with increasing concentrations used in the test. The essential oils of *S polyanthum* showed better chelating activity than BHT with inhibition percentage from 44.58 % to 83.49 % for the fresh leaf oil and 45.12 % to 91.66 % for the dried leaf oil. The inhibition percentage for BHT was from 55.88 % to 61.30 %. The plotted graph of inhibition (%) against concentration (mg/ml) is shown in Figure 4.4. The dried leaf oil has the highest chelating activity with 91.66 % compared to the fresh leaf oil (83.49 %) and the standard (61.30 %).

Fe^{2+} ions are the most powerful prooxidant among various species of metal ions (Oke *et al.*, 2009). They possess the ability to move single electrons which allows the formation and propagation of many radical reactions even starting with relatively non-reactive radicals. Ferrozine can quantitatively chelate with Fe^{2+} and form complex with a red color. The reaction is restricted with the presence of other chelating agents and the red colur of the ferrozine- Fe^{2+} complexes (maximum absorbance at 562 nm) is reduced. Measurement of the color reduction estimates the chelating activity to compete with ferrozine for the ferrous ions (Ćavar *et al.*, 2012). The transition metals were involved in both initiation and propagation of lipid peroxidation (Hsiao *et al.*, 1996). The metal

chelating ability might be involved in antioxidant activity and influenced other functions that contribute to antioxidant activity (Moure *et al.*, 2006).

No earlier reports are available with regards to the antioxidant activity of the essential oils of *S. polyanthum* which can be used to compare the results of our present analysis. However, there are some previous studies which reported the antioxidant activity for extracts of *S. polyanthum*. Aqueous extract of *S. polyanthum* showed relatively high free radical scavenging activity and chelating of cupric ion (Wong *et al.*, 2006). Antioxidant activities of three bark extracts (methanol, methanol-water and water) showed that the methanol-water was found to have high antioxidant activity of free radical scavenging (ED_{50} = 0.18 mg/ml) and prevention of β-carotene bleaching with 85.7 % at 100 µg/ml (Raden *et al.*, 2009). Har and Ismail (2012) reported that the methanolic extract of the leaf showed weak antioxidant activity with IC_{50} values of 90.85 µg/ml compared to the standard quercetin (24.09 µg/ml).

Some other members of the genus *Syzygium* have been subjected to antioxidant activity evaluation of their essential oils. The percentage of inhibition in DPPH free radical assay for clove essential oil was considered high with 93.0 % to 94.0 % from concentration 5 g/L to 50 g/L. Clove essential oil has been reported previously as one of the strongest antioxidants, even higher than some synthetic antioxidants such as BHT and BHA (Jitrovetz *et al.*, 2006; Misharina and Samusenko, 2008 and Wei and Shibamoto, 2010). The strong activity of clove was mainly due to the presence of eugenol, a phenolic compound, and the main constituent of the essential oil which is known to have antioxidant activity (Wei and Shibamoto, 2010).

The antioxidant activity of *S. samarangense* and *S. cuminutesi* was evaluated using DPPH free radical system. The IC_{50} for *S. samarangense* leaf oil was 2740.93 ± 122.77 µg/ml. The percentage of total antioxidant capacity (TAA) of *S. cuminutesi* essential oil was 11.13 ± 0.20 %.

4.3.4 Relationship between Chemical Compounds and Antioxidant Activity

In general, the oxygenated monoterpene and monoterpene hydrocarbons are the principle antioxidant compounds in the plants (Cao *et al.*, 2009). The GCMS analysis of the present study revealed that monoterpene hydrocarbons present in higher amounts, representing 33.47 % (fresh leaf oil) and 37.27 % (dried leaf oil) of overall oil with α-pinene as major compounds (fresh oil- 28.78 %; dried oil-34.15 %). In contrast, several authors reported that the antioxidant activity of α-pinene (Lado *et al.*, 2004 and Wang *et al.*, 2008) and *p*-cymene (Lee and Shibamoto, 2001) did not show appreciable antioxidant activity.

Previously, some other studies have reported that γ-terpinene (Ruberto and Barrata, 2000 and Tepe and Sokmen, 2007) and limonene have a high radical scavenging activity (Wei *et al.*, 2007 and Yang *et al.*, 2010). It is well-known that the presence of sesquiterpene hydrocarbons and some oxygenated sesquiterpene could attribute to weak antioxidant activity (Ricci *et al.*, 2005 and Cavar *et al.*, 2008). The lower antioxidant activity is probably due to the absence of phenolic compounds (Gholivand *et al.*, 2010). Though, it is very difficult to claim that the antioxidant activity of the essential oil may be due to one or two major compounds because minor compounds could attribute to the activity. As essential oil is a complex mixture of various compounds, there might be synergistic or antagonistic interactions among the compounds (Okoh *et al.*, 2011).

The present results suggested that both fresh and dried leaf oils exhibit moderate antioxidant activity with a dose-dependent in three testing assays. The moderate activities of *S. polyanthum* oils are associated with the absence of phenolic compounds that possess strong antioxidant activity. However, the presence of α-pinene as a major compound could be responsible for the antioxidant activity observed. This is in accordance to previous work by Wang *et al.* (2008) suggesting that α-pinene possesses antioxidant activity in the DPPH system. Results obtained confirm the importance of essential oils as natural antioxidants and their role in the protection of human health. To the best of the author's knowledge, this is the first study reporting on antioxidant

activity using DPPH, β-carotene/linoleic acid and metal-chelating assays, on essential oils of *S. polyanthum* grown in Malaysia.

4.4 SCREENING OF ANTIBACTERIAL ACTIVITY

The potential antibacterial activities of *S. polyanthum* essential oils against selected bacterial strains were qualitatively and quantitatively evaluated by the presence/absence of inhibition zones, diameter of zone inhibition and values of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The main factors that are responsible for the antibacterial activity are the type, compositions and concentrations of the essential oil, the type of microorganism, pH value and the ability of the antibacterial agents to diffuse through the agar uniformly and the thickness of the agar layer (Burt, 2004).

4.4.1 Selected Bacteria Strains

Three gram-positive bacteria (*Staphylococcus aureus* ATCC BAA1026, *Bacillus subtilis* ATCC 11774, and *Enterococcus faecalis* ATCC 14506) and three gram-negative bacteria (*Pseudomonas aeruginosa* ATCC 15442, *Escherichia coli* ATCC 10536, *Salmonella typhi* ATCC 13311) were chosen for the antibacterial activity test for the essential oil. All of the strains are known to cause diseases in human.

Staphylococcus aureus is frequently found as part of the normal skin flora and nasal passages (Cole *et al.*, 2001). It can cause a range of illness, from minor skin infections (pimple) to life-threatening diseases (pneumonia, meningitis). *Bacillus subtilis* is known to cause disease in immunodeficiency patients and rarely associated with food poisoning. *Enterococcus faecalis* can be found in human gastrointestinal tracts and other mammals (Ryan and Ray, 2004). It can cause life-threatening infections in humans and has been frequently found in root canal-treated teeth (Molander *et al.*, 1998). The gram-negative *E. coli* can be found in human intestine and affect lower urinary tract while *S. typhi* is associated with food borne diseases and typhoid fever. *Pseudomonas aeruginosa* is a common bacterium that can cause disease in animals, including humans (Wikipedia, 2012).

4.4.2 Disc Diffusion Assay

The disc diffusion method using filter paper was employed to screen the potential antibacterial activity of *S. polyanthum* leaf oil. The method was first introduced by Kirby and Bauer in 1966. The disc diffusion results for the fresh and dried leaf oils are shown in Table 4.6 and 4.7.

Table 4.6: Mean radius of inhibition zones (mm) of the fresh leaf essential oil tested against six bacteria strains

Bacteria	**Strain**	**Inhibition zone (mm)**				
		I	II	III	TE	SM
Gram-positive						
S. aureus	ATCC BAA1026	27.3±0.6^{a}	23.7±1.2^{ab}	21.0±1.0^{b}	29.3±1.2	11.3±1.5
B. subtilis	ATCC 11774	17.7±0.6^{a}	13.0±1.0^{ab}	8.70±0.6^{b}	20.7±1.2	11.0±0.6
E. faecalis	ATCC 14506	14.7±0.6^{a}	11.3±0.6^{ab}	7.30±0.6^{b}	29.3±0.6	11.0±1.4
Gram-negative						
P. aeruginosa	ATCC 15442	16.3±0.6^{a}	8.0±1.0^{ab}	6.70±0.6^{b}	17.3±1.2	8.0±1.0
E. coli	ATCC 10536	19.7±0.6^{a}	17.7±0.6^{ab}	11.0±1.0^{b}	29.3±1.2	8.5±0.7
S. typhi	ATCC 13311	19.0±1.0^{a}	17.0±1.0^{ab}	11.3±1.2^{b}	29.3±1.2	9.3±1.4

Values are means ± standard deviation of triplicates. Values are means ± standard deviation of triplicates. Values followed by the same letters (a-b) are not statistically different as $p < 0.05$ as measured by the Tukey HSD test. *I (8.00 mg/ml), II (4.00 mg/ml, III (2.00 mg/ml); TE - tetracycline; SM - streptomycin

Table 4.7: Mean radius of inhibition zones (mm) of the dried leaf essential oil tested against six bacteria strains

Bacteria	Strain	Inhibition zone (mm)				
		I	II	III	TE	SM
Gram-positive						
S. aureus	ATCC BAA1026	29.7±0.6^{a}	27.3±1.2^{ab}	23.7±1.5^{b}	29.3±1.2	11.3±1.5
B. subtilis	ATCC 11774	19.3±1.2^{a}	14.3±1.2^{ab}	10.7±1.2^{b}	20.7±1.2	11.0±0.6
E. faecalis	ATCC 14506	16.3±1.2^{a}	13.0±1.7^{ab}	9.7±0.6^{b}	29.3±0.6	11.0±1.4
Gram-negative						
P. aeruginosa	ATCC 15442	18.7±1.2^{a}	9.3±1.2^{ab}	8.7±0.6^{b}	17.3±1.2	8.0±1.0
E. coli	ATCC 10536	21.0±1.0^{a}	19.3±1.2^{ab}	13.7±1.6^{b}	29.3±1.2	8.5±0.7
S. typhi	ATCC 13311	21.3±1.6^{a}	19.0±1.0^{ab}	13.7±1.6^{b}	29.3±1.2	9.3±1.4

Values are means ± standard deviation of triplicates. Values followed by the same letters (a-b) are not statistically different as $p < 0.05$ as measured by the Tukey HSD test**.** *I (8.00mg/ml), II (4.00mg/ml, III (2.00mg/ml); TE - tetracycline; SM - streptomycin

From the tables above, the antibacterial activity of the essential oils (10 µl/disc) from the fresh and dried leaf of *S. polyanthum* showed positive activity against all gram-positive and gram-negative bacteria. Variable zones of inhibition were noted in the fresh leaf oil (6.7 mm to 27.3 mm) and the dried leaf oil (8.7 mm to 29.7 mm) depending on the concentration of the oil (8.00 mg/ml > 4.00 mg/ml > 2.00 mg/ml). The susceptibility of the organisms in the disc diffusion assay was *S. aureus* > *S.typhi* > *E. coli* > *B. subtilis* > *E. faecalis* > *P. aeruginosa* for the fresh leaf and dried leaf oils.

The positive controls used in this assay were tetracycline and streptomycin. Tetracycline was found to be more susceptible than streptomycin with zone of inhibition ranging from 17.3 mm to 29.3 mm. The negative control (ethanol) showed no antibacterial activity. At a concentration of 2.00 mg/ml, *S. aureus* showed the largest inhibition zone for both fresh leaf oil (21.0 mm) and dried leaf oil (23.7 mm) while the smallest inhibition zone was recorded by *P. aeruginosa* which were 6.7 mm (fresh leaf oil) and 8.70 mm (dried leaf oil).

Based on the screening of antibacterial activity using disc diffusion method, *S. aureus* was found to be the most sensitive strains towards the oil while *P. aeruginosa* was the most resistant bacteria as the oil showed weak inhibition towards it. The weak activity observed in *P. aeruginosa* might be due to the inability of the antibacterial compounds to diffuse through the agar. The presence of outer membrane barrier in *P. aeruginosa* made it very resistant (Skočibušić *et al.*, 2006) to all known antibiotics (Aburjai and Darwish, 2001) and antimicrobial agents as well as synthetic drugs.

The essential oil that produces inhibition zone diameters > 3mm around the disc was considered as antibacterial (Konaté *et al.*, 2011). Antibacterial activity is classified as strong (≥ 20mm), moderate (< 20-12mm) and weak (< 12mm). The antibacterial activity of the essential oil was compared with that standard antibiotics, tetracycline and streptomycin and ethanol as negative control. The advantages of this method are it is simple, does not require any special equipment, cheap and practical.

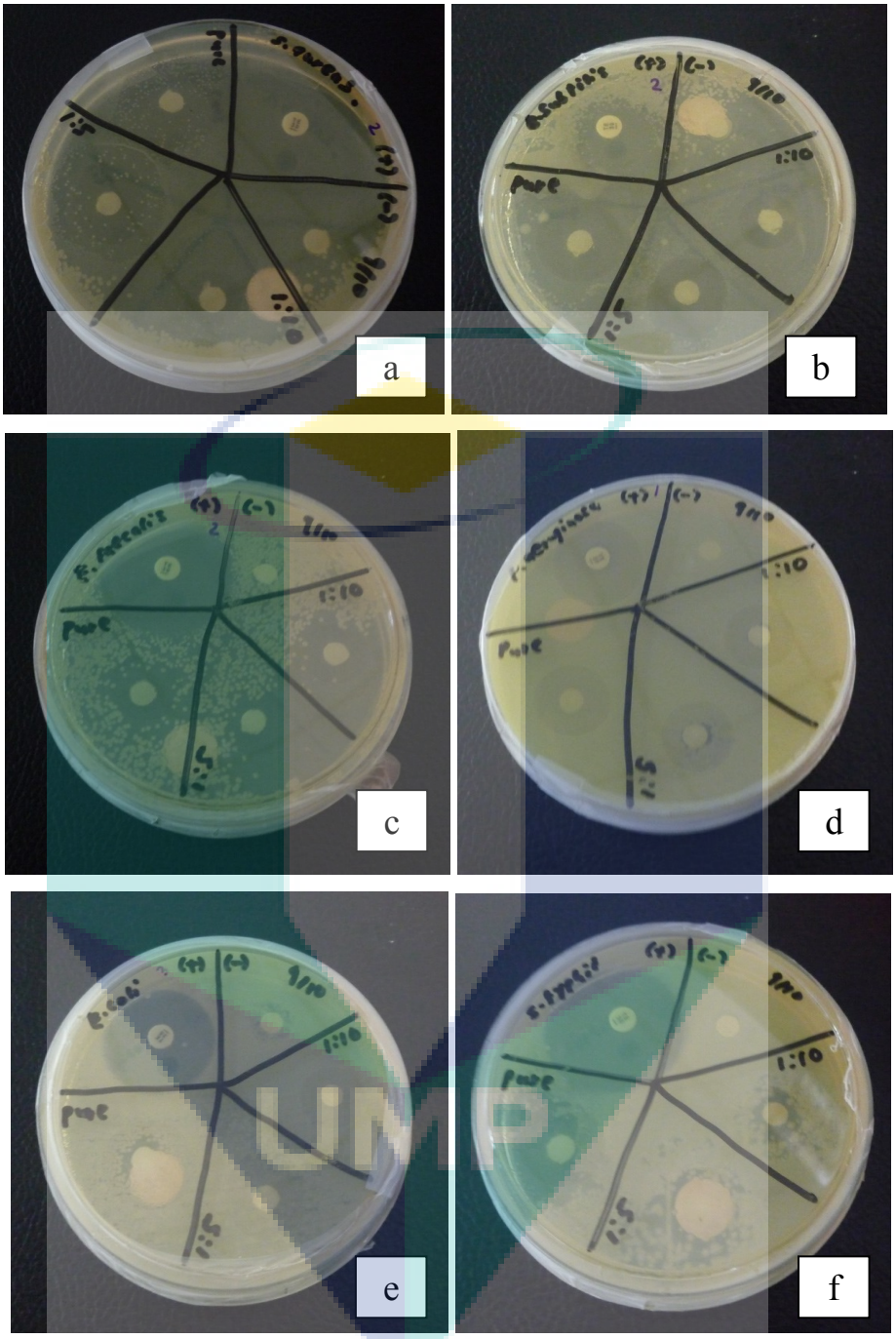


Figure 4.5: Inhibition zones at I, II and III of *S. polyanthum* fresh leaf essential oil against *S.aureus* (a), *B. subtilis* (b), *E. faecalis* (c), *P. aeruginosa* (d), *E. coli* (e) and *S. typhi* (f). (+) = positive control, (-) = negative control

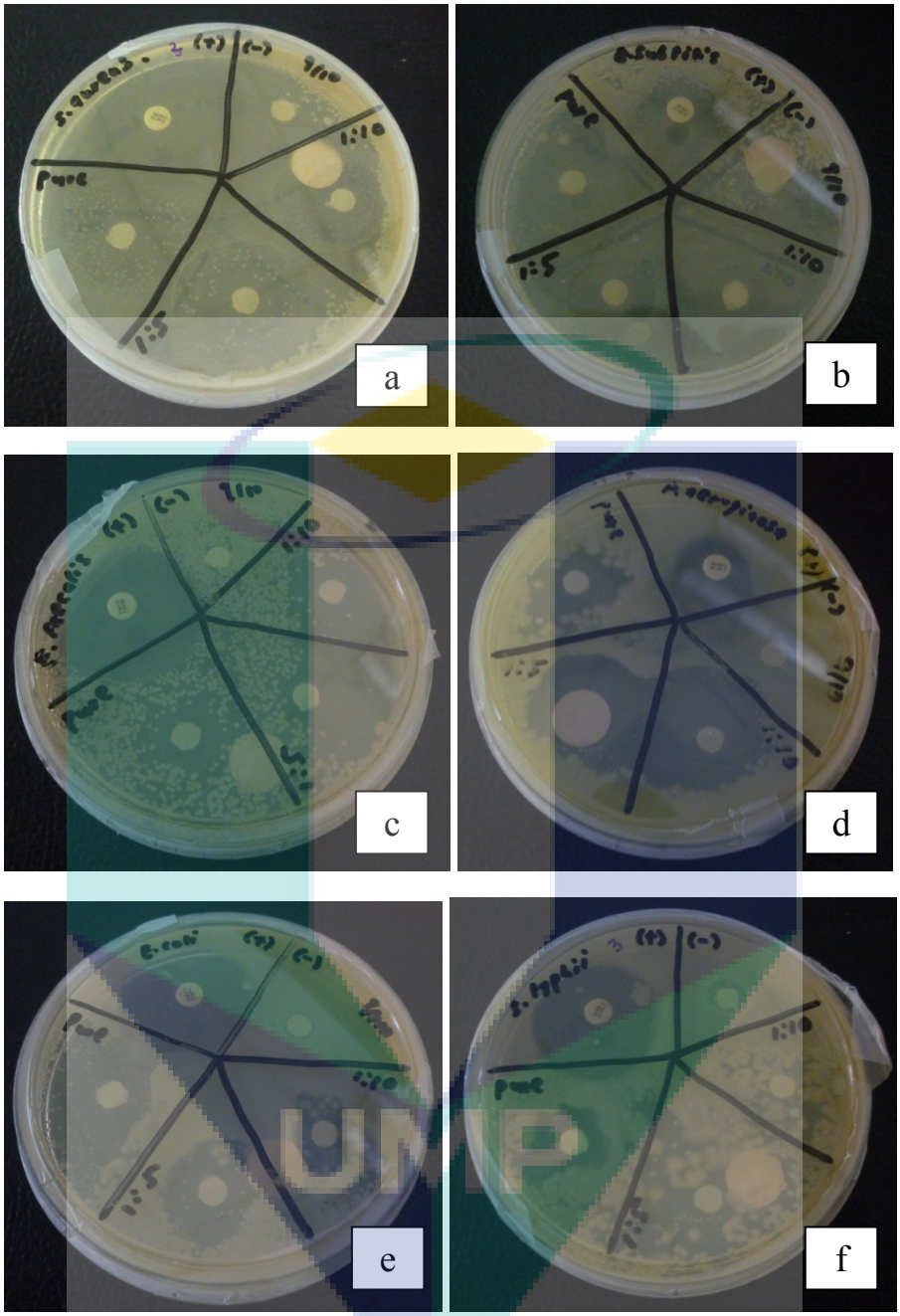


Figure 4.6: Inhibition zones at I, II and III of *S. polyanthum* dried leaf essential oil against *S.aureus* (a), *B. subtilis* (b), *E. faecalis* (c), *P. aeruginosa* (d), *E. coli* (e) and *S. typhi* (f). (+) = positive control, (-) = negative control

4.4.3 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Minimum inhibitory concentration (MIC) can be described as the lowest concentration of the test samples where the absence of growth is observed (Glowniak *et al.*, 2006). The MIC of the essential oil was evaluated using micro-dilution as recommended by NCCLS (2004) in 96-well plate with different concentration of oil ranging from 1.00 mg/ml to 0.03 mg/ml. The MBC/MIC ratio is determined where if MBC/MIC = 1 or 2, it is bactericidal and if MBC/MIC = 4 or 16, it is considered as bacteriostatic (Berche *et al.*, 1988). Results are shown in Table 4.8.

Table 4.8: MIC and MBC of the fresh and dried leaf essential oils from *Syzygium polyanthum*

Bacteria	**Strain**	**Essential oil (mg/ml)**			
		Fresh Leaf		Dried Leaf	
		MIC	MBC	MIC	MBC
Gram-positive					
S. aureus	ATCC BAA1026	0.03	0.06	0.03	0.06
B. subtilis	ATCC 11774	0.13	0.13	0.06	0.06
E. faecalis	ATCC 14506	0.06	0.13	0.06	0.13
Gram-negative					
P. aeruginosa	ATCC 15442	0.25	0.50	0.13	0.25
E. coli	ATCC 10536	0.06	0.13	0.06	0.13
S. typhii	ATCC 13311	0.13	0.13	0.12	0.13

Values of absorbance are means ±SD (n=3). Values are statistically different as $p < 0.05$ as measured by ANOVA test

The essential oils showed positive activity against all selected organism in disc diffusion assay. Following this, MIC and MBC assays were carried out using the same six bacteria strains but with different concentration of the oils.

The MIC and MBC values of S. *polyanthum* oils against the selected bacteria strains are shown in Table 4.8. The MIC and MBC values of the fresh leaf essential oil ranged from 0.03 mg/ml to 0.25 mg/ml and 0.06 mg/ml to 0.50 mg/ml while for the dried leaf essential oil was 0.03 mg/ml to 0.13 mg/ml and 0.06 mg/ml to 0.25 mg/ml. The strongest activity was seen against *S. aureus* (MIC 0.03 mg/ml) for both fresh and dried leaf oil while *P. aeruginosa* recorded the weakest activity with MIC values of 0.25 mg/ml for the fresh leaf oil and 0.13 mg/ml for the dried leaf oil. Generally, larger inhibition zone correlated with lower MIC values (Damjanovic-Vratnica *et al.*, 2011). It can be seen that *S. aureus* showed the largest inhibition zone with 23.7 mm and the strongest MIC value of 0.03 mg/ml for the dried leaf oil.

MBC/MIC ratio

From calculation, MBC/MIC ratio for for the fresh leaf oil was *S. aureus* (0.5), *B. subtilis* (1), *E. faecalis* (0.5), *P. aeruginosa* (0.5), *E. coli* (1) and *S. typhi* (0.5). Meanwhile, for dried leaf oil MBC/MIC ratio was *S. aureus* (0.5), *B. subtilis* (1), *E. faecalis* (0.5), *P. aeruginosa* (0.5), *E. coli* (0.5) and *S. typhi* (1). According to the calculation, all bacteria strains were considered as bactericidal with ratio 0.5 to 1.

In general, a few reports on the antibacterial activitiy of *S. polyanthum* extracts have been published but none have reported on the essential oil activity. Compounds found in the leaf of *S. polyanthum* (essential oils, saponin, tannins, triterpenoids and flavonoids) have the ability to inhibit growth of pathogenic bacteria such as *Salmonella* sp., *B. cereus*, *B. subtilis*, *S. aureus*, *E. coli* and *P. fluorescens* (Setiawan, 2002). A study done by Hendradjatin in 2004 revealed that infusion of *S. polyanthum* leaf have effective effect against *E. coli* and *V. cholera*. The zone of inhibition and MIC values for *E. coli* was 20.7 mm, MIC 12.5 % while for *V. cholera* was 23.3 mm, MIC 3.12 %. The number of *E. coli* colonies was reduced when treated with 3 % of *S. polyanthum* leaf flour (Hermana *et al.*, 2008).

Although this is the first report on antibacterial activity of the essential oil of *S. polyanthum*, some other members of the genus *Syzygium* have been subjected to antibacterial/antimicrobial activity evaluation. Previous reports on the antibacterial and antimicrobial activity on some *Syzygium* species showed the inhibitory activity against several bacteria. *S. aromaticum* or clove is well-known for its powerful essential oils associated with the presence of eugenol. The essential oils of clove inhibited almost all gram-positive and gram-negative bacteria (Srivastava *et al.*, 2005). *S. samarangense* leaf oil is effective against all gram-positive and gram-negative bacteria with *E. coli* showing the largest zone of inhibition of 25 mm while *S. cuminutesi* showed strong inhibition against the tested bacterial strains with the largest zone of inhibition 17 mm for *S. lutea*.

4.4.4 Susceptibility of Gram-positive and Gram-negative Bacteria

The potential antimicrobial and antibacterial activity of essential oils have been acknowledged and scientifically proven and confirmed (Sadashiva *et al.*, 2010). Various methods have been proposed and employed to evaluate antibacterial activity and the selection of test microorganisms varied between publications.

Generally, most studies report that plant essential oils are more active against gram-positive bacteria than gram-negative bacteria (Hussain *et al.*, 2011 and Tg Siti Amirah *et al.*, 2012). However, there a few studies that reported that gram-positive bacteria have been found to be less (Randrianarivelo *et al.*, 2009 and Bachir Raho and Benali, 2012) or equally sensitive to gram-negative bacteria (Obame *et al.*, 2008 and Ghalem and Mohamed, 2009; Martins *et al.*, 2012). The difference in sensitivity between gram-positive and gram-negative bacteria could be due to the morphological differences with the presence or absence of outer phospholipidic membrane (Nostro *et al.*, 2000).

Syzygium polyanthum oils showed activity against all gram-negative bacteria, namely *P. aeruginosa*, *E. coli* and *S. typhi*. This result is important because gram-negative bacteria are usually resistant towards essential oils. This could be explained by the presence of lipoproteins and lipopolysaccharides in gram-negative bacteria which

restrict the penetration of compounds through the bacterium cell as they formed a barrier to hydrophobic compounds. The result obtained in this study suggests that *S. polyanthum* oil may have the ability to alter the outer membrane of the gram-negative bacteria.

Screening and evaluation of various essential oils for their antibacterial properties have been done extensively. The mechanism of action of the essential oils is not yet known but may be due to their important characteristic which is hydrophobic that they can interact with membrane lipids, disturbing the membrane structure and make it permeable (Martins *et al*., 2012).

4.4.5 Relationship between Chemical Compounds and Antibacterial Activity

The presence of active compounds in essential oils mainly monoterpene, sesquiterpene and their oxygenated compounds, related alcohols, phenols and other hydrocarbons (Sacchetti *et al.*, 2004 and Berger, 2007) have strong potential to possess antifungal and antibacterial properties.

Several authors (Aggarwal *et al.*, 2002 and Hussain *et al.*, 2008) stated that oxygenated terpenes also exhibit potential antibacterial activity. Phenolic compounds have the strongest antimicrobial activity (Sivropoulou *et al.*, 1995) followed by aldehyde, ketones and alcohols (Tepe *et al.*, 2006). Some monoterpene and sesquiterpene were found to lower antimicrobial activity but others still possess antibacterial properties (Chalchat *et al.*, 1997).

Some previous studies have reported regarding the relationship between the major chemical compounds in the essential oil and the antibacterial activity. Compounds such as 1, 8-cineole, eugenol, citronellol, limonene, linalool, *p*-cymene, α-terpineol, terpinen-4-ol, α-terpinolene, α-pinene, β-pinene, β-caryophyllene, α-phellandrene, camphene and caryophyllene oxide were found to be effective against various bacteria strains (Sivasothy *et al.*, 2011). Aldehyde compounds such as decanal and dodecanal also possess antibacterial properties (Sadashiva *et al.*, 2010). Camphene was found to be effective against *S. aureus* and *E. coli* (Chalchat *et al.*, 1997).

Alpha-pinene, which appears to be the major and most abundant compound in our present study, is well known to have good antibacterial activity. This is in agreement with the study by Obame *et al*. (2008) which revealed that the presence of terpinene-4-ol and α-pinene in high content contributes to the antimicrobial activity of their works. The compound has also been previously reported to have antibacterial and antifungal acitivities (Ghalem and Mohamed 2009 and Devi *et al.*, 2010). The α-pinene successfully reduced the growth of *Erwinia amylovira* while β-pinene inhibits the growth of bacteria in high population (Scotichini and Rossi, 1991).

Even though the major compounds are usually responsible for the antibacterial activity of many essential oils, some studies have reported that the whole essential oils have higher antibacterial activity compared to the major compounds or combination of the major compounds. This revealed that minor compounds with lower percentage are also responsible for the activity.

For some cases, some compounds (such as *p*- cymene) that exist in essential oil do not possess antibacterial properties but they are able to integrate into the membrane layer which enable the active antibacterial compounds to diffuse through it (Mihajilov-Krstev *et al.*, 2010). Essential oils when used as whole, may lead to indifferent, additive, synergistic or antagonistic effects (Sibanda *et al.*, 2004; and Xianfei *et al.*, 2007). Thus, it is difficult and yet not well-known which exact compounds or mixtures of them are mainly responsible for their antibacterial activity.

From the findings, the positive antibacterial activity of *S. polyanthum* essential oil could be attributed to the presence of monoterpene hydrocarbons and sesquiterpene hydrocarbons with α-pinene and β-selinene as major compounds. It is worth to point out that minor compound such as linalool, α-terpineol, *p*-cymene, γ-terpinene and α-terpineol may be contributing as well. To the best of the author's knowledge, this is the first study to report on the antibacterial activity of essential oil of *S. polyanthum* grown in Malaysia.

CHAPTER 5

CONCLUSION

In this study, the main focus is on the essential oil extracted by hydrodistillation from the fresh and dried leaf of *S. polyanthum* grown in Peninsular Malaysia and evaluation of their chemical composition, antioxidant and antibacterial activity, which have not been or rarely reviewed. The percentage of the essential oils obtained, for the fresh and the dried leaf were 0.67 % and 1.50 %, respectively. Analysis by GCMS revealed a total of 26 and 29 compounds, representing 71.71 % and 90.92 % of the overall compositions were detected in the fresh and the dried leaf essential oils. Both oils were characterized by high amount of monoterpene hydrocarbons. The major compounds for the fresh leaf were α-pinene (28.78 %), α-caryophyllene (9.11 %), selinene (5.85 %) and the major compounds for the dried leaf were α-pinene (34.15 %), octanal (29.18 %), nerolidol (6.68 %). Based on the results, it can be assumed that the essential oil from *S. polyanthum* leaf belongs to α-pinene chemotype. Among the 55 compounds identified, 14 of them were common in both essential oils. The percentage of α-pinene, α-panasinsen and nerolidol increased in the dried leaf oil while limonene, β-ocimene, γ-terpinene, α-terpinolene, decanal, α-caryophyllene, γ-selinene, β-selinene, δ-cadinene, δ-cadinol and (E,Z)-farnesol have higher percentages in the fresh leaf oil.

The antioxidant activity of the essential oils from leaf of *S. polyanthum* was evaluated by three different assays, namely DPPH, β-carotene bleaching and ferrous-ion chelating assays. In DPPH assay, the inhibition percentage of the essential oil increased from 90.95 % to 92.16 % for the fresh leaf oil and 91.79 % to 93.38 % for the dried leaf oil, with concentration from 0.13 to 2.00 mg/ml. BHT showed lower inhibition from 88.06 % to 91.60 % with the same concentration. The essential oils of *S. polyanthum* demonstrated high ability to prevent the bleaching of β-carotene. For concentration 2.00 mg/ml, the fresh and dried leaf oils showed bleaching of 90.58 % and 91.15 % compared to BHT (86.26 %). The essential oils of *S polyanthum* showed better chelating activity than BHT with inhibition percentage from 44.58 % to 83.49 % for the

fresh leaf oil and 45.12 % to 91.66 % for the dried leaf oil. The inhibition percentage for BHT was from 55.88 % to 61.30 %.

The essential oils of *S. polyanthum* leaf showed inhibition against six bacteria strains, with three gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*, *Enterococcus faecalis*) and three gram-negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhi*). At a concentration of 2.00 mg/ml, *S. aureus* showed the largest inhibition zone for both fresh leaf oil (21.0 mm) and dried leaf oil (23.7 mm) while the smallest inhibition zone was recorded by *P. aeruginosa* which were 6.7 mm (fresh leaf oil) and 8.70 mm (dried leaf oil). Based on the screening of the antibacterial activity using the disc diffusion method, *S. aureus* was found to be the most sensitive strains towards the oils while *P. aeruginosa* was the most resistant bacteria as the oils showed weak inhibition towards it. The MIC and MBC values of the fresh leaf essential oil ranged from 0.03 mg/ml to 0.25 mg/ml and 0.06 mg/ml to 0.50 mg/ml while for the dried leaf essential oil , the values ranged from 0.03 mg/ml to 0.13 mg/ml and 0.06 mg/ml to 0.25 mg/ml. The strongest activity was seen against *S. aureus* (MIC 0.03 mg/ml) for both fresh and dried leaf oil while *P. aeruginosa* recorded the weakest activity with MIC values of 0.25 mg/ml for the fresh leaf oil and 0.13 mg/ml for the dried leaf oil.

The essential oil compositions of *S. polyanthum* have been previously reported in Indonesia but with limited data. Therefore, to the best of the author's knowledge, data presented here can be assumed as the first to report on this plant which grows in Peninsular Malaysia. Only extraction of the leaf has been done and no previous report or research has been done on isolation of the essential oil from other parts of the *S. polyanthum*. This gives an opportunity to further the interest in profiling the essential oils from other parts of the plant in the future as the other parts of the plants also have special traditional uses to treat diseases. Overall, this study presents valuable information on the compositions, antioxidant and antibacterial activity of *S. polyanthum* essential oils.

REFERENCES

Aazza, S., Lyoussi, B. and Miguel, M.G. 2011. Antioxidant activity of some Morrocan hydrosols. *Journal of Medicinal Plants Research*, **5**(30): 6688-6696.

Abas, F., Lajis, N.H., Israf, D.A., Khozirah, S. and Kalsom, Y.U. 2006. Antioxidant and nitric oxide activities of Malay traditional vegetables. *Food Chemistry*, **95**: 566-573.

Aburjai, T. and Darwish, R.M. 2001. Screening of antibiotic resistant inhibitors from local plant materials against two different strains of *Pseudomonas aeruginosa*. *Journal of Ethnopharmacology*, **76**: 39-44.

Aggarwal, K.K., Khanuja, S.P.S., Ahmad, A., Santha Kumar, T.R., Gupta, V.K. and Kumar, S. 2002. Antimicrobial activity profiles of the two enantiomers of limonene and carvone isolated from the oils of *Mentha spicata* and *Anethum sowa*. *Flavour and Fragrance Journal*, **17**: 59-63.

Agusta, A. 2000. *Indonesian tropical plants essential oils*. Indonesia: Institute of Technology Bandung.

Ali, A.M. , Mooi, L.Y., Yih Yih, K., Norhanom, A.W., Mat Saleh, K., Lajis, N.H., Yazid, A.M., Ahmad, F.B.H. and Prasad, U. 2000. Anti-tumor Promoting Activity of Some Malaysian Traditional Vegetable (Ulam) Extracts by Immunoblotting Analysis of Raji Cells. *Natural Product Sciences*, **6**(3): 147-150.

Alma, M.H., Ertas, M., Nitz, S. and Kollmannsberger, H. 2007. Chemical composition and content of essential oil from the bud of cultivated Turkish Clove (*Syzygium aromaticum* L.). *BioResources*, **2**(2): 265-269.

Amarowicza, R., Peggb, R.B., Rahimi-Moghaddam, P., Barid, B. and Weilc, J.A. 2004. Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. *Food Chemistry*, **84**: 551-562.

Ariviani, S. 2012. Effect of salam (bay) (*Syzygium polyanthum* [Wight.] Walp) fruit extract diet toward blood. Abstract. Journal of Agricultural Technology. University of Sebelas Maret, Surakarta, Indonesia: 29 March.

Ashton, P.S. 2006. New Syzygium (Myrtaceae) from northern Borneo. *Kew Bulletin*, **61**: 107-144.

Ayyanar, M., Babu, P.S. 2012. *Syzygium cumini* (L.) Skeels: A review of its phytochemical constituents and traditional uses. *Asian Pacific Journal of Tropical Biomedicine*, 240-246.

Bachir Raho, G. and Benali, M. 2012. Antibacterial activity of the essential oils from the leaves of *Eucalyptus globulus* against *Escherichia coli* and *Staphylococcus aureus*. *Asian Pacific Journal of Tropical Biomedicine*, 739-742.

Bakkali, F., Averbeck, S., Averback, D., Idaomar, M.M. 2008. Biological effects of essential oils - a review. *Food Chemistry Toxicology*, **46**: 446-475.

Bamoniri, A., Ebrahimabadi, A.H., Mazoochi, A., Behpour, M., Kashi, F.J. and Batooli, H. 2010. Antioxidant and antimicrobial activity evaluation and essential oil analysis of *Semenovia tragioides* Boiss. from Iran. *Food Chemistry*, **122**: 553-558.

Bauer, A.W., Kirby, W.M.M., Sherris, J.C. and Turck, M. 1966. Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology*, **45**(4): 493-496.

Berche, P., Gaillard, J.L. and Simonet, M. 1988. Nosocomial infections caused by bacteria and their prevention in bacteriology. *Edition Flammarion Medicine Sciences*, 64-71.

Berger, R.G. 2007. *Bioactivity of essential oils and their components. In: Flavors and fragrances: Chemistry, bioprocessing and sustainability*. Germany: Springer.

British Pharmacopoeia. 1980. London: HMS Office.

Burt, S. 2004. Essential oils: Their antibacterial properties and potential applications in foods-a review. *International Journal of Food Microbiology*, **94**: 223-253.

Cao, L., Si, J.Y., Liu, Y., Sun, H., Jin, W., Li, Z., Zhao, X.H. and Pan, R.I. 2009. Essential oil composition, antimicrobial and antioxidant properties of *Mosla chinensis* Maxim. *Food Chemistry*, **115**: 801-805.

Cavar, S., Maksimović, M., Solic, M.E., Mujkic, A.J. and Besta, R. 2008. Chemical composition and antioxidant and antimicrobial activity of two *Satureja* essential oil. *Food Chemistry*, **3**: 648-653.

Cavar, S., Maksimović, M., Vidic, V. and Parić, C. 2012. Chemical composition and antioxidant and antimicrobial activity of essential oil of *Artemisia annua* L. from Bosnia. *Industrial Crops and Products*, **37**: 479-485.

Chalchat, J.C., Garry, R.P., Menut, C., Lamaty, G., Malhurct, R. and Chopineau, J. 1997. Correlation between chemical composition and antimicrobial activity of some African essential oils. *Journal of Essential Oil Research*, **9**: 67-75.

Chalchat, J.C. and Ozcan, M.M. 2008. Comparative essential oil composition of flowers, leaves and stems of basil (*Ocimum basilicum* L.) used as herb. *Food Chemistry*, **110**: 501-503.

Chan, E.W.C., Lim, Y.Y., Wong, L.F., Lianto, F.S., Wong, S.K., Lim, K.K., Joe, C.E., Lim, T.Y. 2008. Antioxidant and tyrosine inhibition properties of leaves and rhizomes of ginger species. *Food Chemistry*. **109**: 477-483.

Chandrasekaran, M. and Venkatesalu, V. 2004. Antibacterial and antifungal activity of *Syzygium jambolanum* seeds. *Journal of Ethnopharmacology*, **91**: 105-8.

Chaudhuri, A.K.N., Pal, S., Gomes, A. and Bhattacharya, S. 1990. Anti-inflammatory and related actions of *Syzygium cumini* seed extract. *Phytotherapy Research*, **4**: 5.

Cheh, M.R. 2005. The making of essential oils-steam distillation, absolutes, and co2's explained (online). http://naturalingredient.org/Articles/cech2.html. (9 September 2011).

Chung, P.Y., Chung, L.Y., Ngeow, Y.F., Goh, S.H. and Imiyabir, Z. 2004. Antimicrobial activities of Malaysian plant species. *Pharmaceutical Biology,* **42**: 292–300.

Cole, A.M., Tahk, S., Oren, A., Yashioka, D., Kim, Y.H., Park, A. and Ganz, T. 2001. Determinants of *Staphyloccocus aureus* nasal carriage. *Clinical and Diagnostic Laboratory Immunology*, **8**(6): 1064-1069.

Corine, D.D., Michel, D. and Quetin-Leclercq, J. 2000. Antimicrobial activity of bark extracts of *Syzygium jambos* (L.) Alston (Myrtaceae). *Journal of Ethnopharmacology*, **71**: 307-313.

Dalimartha, S. 2005. *Salam (Syzygium polyanthum* Wight*)* (online). http://www.pdpersi.pdpersi.co.id. (29 April 2008).

Damjanovic-Vratnica, B., Dakov, T., Sukovic, D. and Damjanovic, J. 2011. Antimicrobial Effect of Essential Oil Isolated from Eucalyptus globules Labill. From Montenegro. *Czech Journal of Food Science*, **29**(3): 277-284.

Davidson, P.M. and Branen, A.L. 1993. *Antimicrobials in food*. New York: Marcel Dekker Inc.

De Guzman, C.C. and Siemonsma, J.C. 1999. *Plant Resources of South-East Asia No. 13 Spices*. Leiden, the Netherlands: Backhuys Publisher.

Delaquis, P.J., Stanich, K., Girard, B. and Mazza, G. 2002. Antimicrobial activity of individual and mixed fractions of dill, cilantro, coriander and eucalyptus essential oils. *International Journal of Food Microbiology,* **74**(1-2): 101-109.

Devi, K.P., Nisha, S. A., Sakhivel, R. and Pandian, S.K. 2010. Eugenol (an essential oil of clove) acts as an antibacterial agent against *Salmonella typhi* by disrupting the cellular membrane. *Journal of Ethnopharmacology*, **130**: 107-115.

Dinis, T.C.P., Madeira, V.M.C., Almeida, L.M. 1994. Action of phenolic derivatives (acetaminophen, salicylate and 5-amino salicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. *Archives of Biochemistry and Biophysics*, **315**: 161-169.

Dupler, D., and Odle, T.G. 2005. Essential oils. In: J. L. Longe, *The Gale Encyclopedia of Alternatives Medicine.* Farmington Hills, Michigan: Thomson/Gale.

Elansary, H.O., Salem, M.Z.M., Ashmawy, N.A. and Yacout, M.M. 2012. Chemical composition, antibacterial and antioxidant activities of leaves essential oils from *Syzygium cumini* L., *Cupressus sempervirens* L. and *Lantana camara* L. from Egypt. *Journal of Agricultural Science*, **4**(10): 144-152.

Festing, M.F.W., Overend, P., Gaines Das, R., Cortina Borja, M. and Berdoy, M. 2002. *The Design of Animal Experiments*. London: Laboratory Animals Ltd.

French, G.L. 2006. Bactericidal agents in the treatment of MRSA infections--the potential role of daptomycin. *Journal of Antimicrobial Chemotherapy,* **58**(6): 1107–1117.

Gao, Y., Hu, Q., Xican L. 2012. Chemical composition and antioxidant activity of essential oil from *Syzygium samarangense* (BL.) Merr.et Perry flower-bud. *Spatula DD,* **2**(1): 23-33.

Ghalem, B.R. and Mohamed, B. 2009. Essential oil from gum of *Pistacia atlantica* Desf.: Screening of antimicrobial activity. *African Journal of Pharmacy and Pharmacology*, **3**(3): 087-091.

Ghannadi, A. and Dezfuly, N. 2011. Essential oil analysis of the leaves of Persian true Myrtle. *International Journal of Medicinal and Aromatic Plants*, **1**(2): 48-50.

Gholivand, M.B., Rahimi-Nasrabadi, M., Batooli, H. and Ebrahimabadi, A.H. 2010. Chemical composition and antioxidant activities of the essential oil and methanol extracts of *Psammogeton canescens*. *Food and Chemical Toxicology*, **48**: 24-28.

Glowniak, P., Los, R., Skalicka-Wozniak, K., Widelski, J., Burczyk, J. and Malm, A. 2006. Activity of *Crithmum maritimum* L. (*Apiaceae*) against Gram-positive bacteria. *Annales universitatis Mariae Curie-Sklodowska,* **19**(2): 17.

Gopalakrishnan, V.K., Vishnupriya, M., Nishaa, S., Sasikumar, J. M., Teepica, P.D.D. and Hephizibah, C.P. 2012. Chemical composition and antioxidant activity of essential oil from *Curcuma amada* Roxb. *International Research Journal of Pharmacy*, **3**(6).

Guenther, E. 1948. *The Essential Oils*. New York: Van Norstrand Co.

Guynot, M.E., Marln, S., Setu, L., Sanchis, V. and Ramos, A.J. 2005. Screening for Antifungal activity of some essential oils against common spoilage fungi of bakery products. *Food Science and Technology International,* **11**(1): 25-32.

Hajji, M., Jarraya, R., Lassoued, I., Masmoudi, O., Damak, M. and Nasri, M. 2010. GC/MS and LC/MS analysis, antioxidant and antimicrobial activities of various solvent extracts from *Mirabilis jalapa* tubers. *Process Biochemistry*, **45**: 1486-1493.

Har, L.W. and Ismail, I.S. 2012. Antioxidant activity, total phenolics and total flavonoids of *Syzygium polyanthum* (Wight) Walp leaves. *International Journal of Medicinal and Aromatic Plants*, **2**(2): 219-228.

Hardhani, A.S. and Suhardjono. 2008. *The Effect of Orally Administered Bay Leaves (Eugenia polyantha) extract on Triglyceride Serum Level in Hyperlipidemic Male Wistar Rats*. Thesis. Faculty of Medical, University of Diponegoro, Semarang, Indonesia.

Hendradtajin, A.A. 2004. In vitro antibacterial effect of infusion of bay leaf (*Eugenia polyantha* Wight) against enteropathogen *Vibrio choleare* dan *Escherichia coli*. *Bandung Medical Journal*, **36**(2).

Hermana, W. Puspitasari, I.N., Wiryawan, K.G. and Suharti, S. 2008. Salam leaf flour provision (*Syzygium polyanthum* (Wight) Walp.) in rations in *Escherichia coli* to antibacterial material organ in broiler chicken. *Medical Pet*, **31**: 63-70.

Heyne K. 1987. *Useful plants of Indonesia*. Indonesia: Department of Research and Development of Forestry.

Holler, F. James, Skoog, Douglas A. and West, D.M. 1996. *Fundamentals of analytical chemistry*. Philadelphia: Saunders College Publishing.

Hsiao, K., Chapman, P., Nilsen, S., Eckman, C., Harigaya, Y., Younkin, S., Yang, F. and Cole, G. 1996. Correlative memory deficits, Aß elevation, and amyloid plaques in transgenic mice. *Science*, **274**: 99-102.

Hussain, A.I., Anwar, F., Sherazi, S.T.H. and Przybylski, R. 2008. Chemical composition, antioxidant and antimicrobial activities of basil (*Ocimum basilicum*) essential oils depends on seasonal variations. *Food Chemistry*, **108**: 986-995.

Hussain, A.I., Anwar, F., Nigam, P.S., Sarker, S.D., Moore, J.E., Rao, J.R. and Mazumdar, A. 2011. Antibacterial activity of some *Lamiaceae* essential oils using resazurin as an indicator of cell growth. *LWT- Food Science and Technology*, **44**: 1199-1206.

Ismail, S. 2000. *Traditional vegetables 'ulam' and flavours*. Malaysia: Universiti Kebangsaan Malaysia.

Jitrovetz, L., Buchbauer, G., Stoilova, I., Stoyanova, A., Krastanov, A. and Schmidt, E. 2006. Chemical composition and antioxidant properties of clove leaf essential oil. *Journal of Agriculture and Food Chemistry*, **54**: 6303-6307.

Juri, M.L., Ahsanul, K., Sidek, A.W., Radziah, O.A. and Foziah, A. 1999. Medicinal Plants - Quality herbal products for healthy living. *CFFPR '99 Series*. Jun: 22-23.

Karioti, A. and Skaltsa, H. 2007. Analysis of the leaf oil of *Syzygium malaccense* Merr. et Perry from Nigeria. *Journal of Essential Oil Research*, **19**: 313-315.

Katarina, E. 2005. A (very) short history of herbs (online): http://www.huntingtonbotanical.org/Rose/Subrosa/43/herbs. htm. (23 April 2012).

Katzer, G. 2004. Indonesian bay leaf (*Eugenia polyantha* Wight). *Gernot Katzer's Spice Dictionary (online).* http://www.ang.kfunigraz.ac.at/~Katzer/engl/genericframe. html. (4 March 2004*)*.

Khatijah, H. and Ruzi, M. 2006. *Anatomical atlas of malaysian medicinal plants*. Bangi: UKM Publisher.

Kloppenburg-Versteegh J. 1983. *A complete guide on Indonesian plants and their property as traditional drugs*. Yogyakarta: Welfare Fund Foundation.

Lado, C., Then, M., Varga, I., Szoke, E. and Szentmihalyi, K. 2004. Antioxidant property of volatile oils determined by the ferric reducing ability. *Z. Naturfor*, **59**: 354-358.

Larson, R.A. 1997. *Naturally occurring antioxidants*. Boca Raton, Florida, USA: CRC Press.

Lee, K.G. and Shibamoto, T. 2001. Antioxidant property of aroma extract isolated from clove buds [*Syzygium aromaticum* (L.) Merr. Et Perry]. *Food Chemistry*, **74**: 443-448.

Lee, K.W., Everts, H. and Beynen, A.C. 2004. Essential Oils in Broiler Nutrition. *International Journal of Poultry Science.* **3**(12): 738-752.

Liliwirianis, N., Nor Lailatus, W.M., Wan Zuraida, W.M.Z., Kassim, J. and Syaikh, A.K. 2011. Premilinary Studies on Phytochemical Screening of Ulam and Fruit from Malaysia. *E-Journal of Chemistry*, **8**(S1): 85-88.

Lucas, E.J., Matsumoto, K., Harris, S.J., Nic Lughadha, E.M., Bernardini, B., and Chase, M.W. 2011. Phylogenetics, Morphology, and evolution of the large genus Myrcia s.l. (Myrtaceae). *International Journal of Plant Science*, **172**(7): 915.

Maria, G.M. 2010. Antioxidant and anti-inflammatory activites of essential oils: A short review. *Molecules*, **15**: 9252-9287.

Martins, M.R., Tinoco, M.T., Almeida, A.S. and Cruz-Morais, J. 2012. Chemical composition, antioxidant and antimicrobial properties of three essential oils from Portuguese flora. *Journal of Pharmacognosy*, **3**(1): 39-44.

Medical Microbiology. 1993. Mims and Playfair , Mosby Europe.

Mihajilov-Krstev, T., Radnovic, D. and Kitić, D. 2010. Antimicrobial activity of *Satureja* L. essential oils against phytopathogenic bacteria *Erwinia amylovora*. *Biologica Nyssana*, **1**(1-2): 95-98.

Misharina, T. and Samusenko, A. 2008. Antioxidant properties of essential oils from lemon, grapefruit, coriander, clove and their mixture. *Applied Biochemistry and Microbiology*, **44**: 438-442.

Mohanty, S. and Cock, I.E. 2010. Bioactivity of *Syzygium jambos* methanolic extracts: Antibacterial activity and toxicity. *Pharmacognosy Research*, **2**: 4-9.

Molander, A., Reit, C., Dahlen, G. and Krist, T. 1998. Microbiological status of root-filled teeth with apical periodontitis. *International Endodontic Journal,* **31**(1).

Moure, A., Dominguez, H. and Parajo, J. C. 2006. Antioxidant properties of ultrafiltration recovered soy protein fractions from industrial effluents and their hydrolysates. *Process Biochemistry*, **41**(2): 447–456.

Munir, A. and Oliver, H. 2007. Development of a solar distillation system for essential oils extraction from herbs. *Proceedings of the International Agricultural Research Conference,* 1-4.

Muruganandan, S., Srinivasan, K., Chandra, S., Tandan, S.K., Lal, J. and Raviprakash, V. 2001. Anti- inflammatory activity of *Syzygium cumini* bark. *Fitoterapia*. **72**: 369-375.

NCCLS/CLSI. 2004. *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically*. Document M7-A6. USA: National Committee for Clinical Laboratory Standards.

Noorma Wati, H. 1995. *Syzygium Gaertner*. Lemmens, R.H.M.J., Soerianegara, I. and Wong, W.C. (Eds.): Plant Resources of South-East Asia No 5(2). Timber trees: Minor commercial timbers. Bogor, Indonesia: Prosea Foundation.

Nostro, A., Germanò, M.P.D., Angelo, V., Marino, A. and Cannatelli, M.A. 2000. Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. *Applied Microbiology*, **30**: 379-384.

Noudogbessi, J.P., Yedomonhan, P., Dominique, C.K.S., Chalchat, J.C. and Figueredo, G. 2008. Chemical composition of essential oil of *Syzygium guineense* (willd.) D.C. var. guineense (Myrtaceae) from Benin. *Records of Natural Products*, **2**(2): 33-38.

Noveriza, R. and Miftakhurohmah. 2010. Effectiveness of methanol extract of salam leaf (*Eugenia polyantha*) and kaffir lime leaf (*Cytrus histrix*) as antifungal against *Fusarium oxysporum* growth. *Littri Journal*, **16**(1): 6-11.

Obame, L.C., Edou, P., Bassolé, I.H.N., Koudou, J., Agnaniet, H., Eba, F. and Traoe, A.S. 2008. Chemical composition, antioxidant and antimicrobial properties of the essential oil of *Dacryodes edulis* (G. Don) H. J. Lam from Gabon. *African Journal of Microbiology Research*, **2**: 148-152.

Oke, F., Aslim, B., Ozturk, S. and Altundag, S. 2009. Essential oil composition, antimicrobial and antioxidant activities of *Satureja cuneifolia* Ten. *Food Chemistry*, **112**: 874-879.

Okoh, O.O., Sadimenko, A.P. and Afolayan, A.J. 2011. Antioxidant activities of Rosmarinus officinalis L. essential oil obtained by hydro-distillation and solvent free microwave extraction. *African Journal of Biotechnology*, **10**(20): 4207-4211.

Perumal, S., Roziahanim, M., Piaru, S. P. 2012. Potential antiradical activity and cytotoxicity assessment of *Ziziphus mauritiana* and *Syzgium polyanthum*. International Journal of Pharmacology, **8**(6): 535-541.

POM. 2004. Nine superior medicinal plants resulting from clinical experiments by POM (online). http://www.beritabumi.or.id./artikes13. (22 July 2004).

Prabuseenivasan, S., Jayakumar, M. and Ignacimuthu, S. 2006. In vitro antibacterial activity of some plant essential oils. *BMC Complementary and Alternative Medicine*, **6**: 39.

Prakash, B., Singh, P., Mishra, P.K. and Dubey, N.K. 2012. Safety assessment of *Zanthoxylum alatum* Roxb. essential oil, its antifungal, antiaflatoxin, antioxidant activity and efficacy as antimicrobial in preservation of *Piper nigrum* L. fruits. *International Journal of Food Microbiology*, **153**: 183-191.

Raden, A.A.L., Sanro, T. and Kazutaka, I. 2009. *In vitro* antioxidative activities and polyphenol content of *Eugenia polyantha* Wight grown in Indonesia. *Pakistan Journal of Biological Sciences*, **12**(24): 1564-1570.

Radulescu, V., Chiliment, S. and Orea, F. 2004. Capillary gas chromatography-mass spectrophotometry of volatile and semi volatile compounds of *Salvia officinalis*. *Journal of Chromatography*, **1027**: 121-126.

Randrianarivelo, R., Sarter, S., Odoux, E., Brat, P., Lebrun, M., Romestand, B., Menut, C., Andrianoelisoa, H.S., Raherimandimby, M. and Danthu, P. 2009. Composition and antimicrobial activity of essential oils of *Cinnamosma fragrans*. *Food Chemistry*, **114**: 680-684.

Reddy, L.J. and Jose, B. 2011. Chemical composition and antibacterial activity of the volatile oil from the leaf of *Syzygium samarangense* (Blume) Merr. and L. M. Perry. *Asian Journal of Biochemical and Pharmaceutical Research*, **3**(1): 263-269.

Ricci, D., Fraternale, D., Giamperi, L., Bucchini, A., Epifano, F., Burini, G. and Curini, M. 2005. Chemical composition, antimicrobial and antioxidant activity of the essential oil of *Teucrium marum* (Lamiaceae). *Journal of Ethnopharmacology*, **98**: 195-200.

Ruberto, G., Baratta, M.T., Deans, S.G. and Dorman, H.J.D. 2000. Antioxidant and antimicrobial activity of *Foeniculum vulgare* and *Crithmum maritimum* essential oils. *Medical Plants*, **66**: 687-693.

Rubiolo, P., Sgorbini, B., Liberto, E., Cordero, C. and Bicchi, C. 2010. Essential oils and volatiles: sample preparation and analysis. *Flavour and Fragrance Journal*, **25**: 282-290.

Runham, S.R. 1996. An updated review of the potential uses of plants grown for extracts including essential oils and factors affecting their yield and composition. Technical Reports. Alternative Crops Unit: MAFF ST0105.

Ryan, K. J., Ray, C. G. 2004. *Sherris Medical Microbiology*. 4th ed. New York: McGrawHill.

Sacchetti, G., Medici, A., Maietti, S., Radice, M., Muzzoli, M., Manfredini, S., Braccioli, E. and Bruni, R. 2004. Composition and functional properties of the essential oil of Amazonian Basil, *Ocimum micranthum* Willd., *Labiatae* in comparison with commercial essential oils. *Journal of Agriculture Food Chemistry*, **52**(11): 3486-3491.

Sadashiva, C.T., Sharanappa, P., Remashree, A.B., Raghu, AV., Udayan, P.S. and Balachandran, I. 2010. Chemical Composition and Antimicrobial Activity of the Essential Oil from Bark of Pittosporum dasycaulon Miq. *Advances in Biological Research*, **4**(6): 301-304.

Schlesier, K., Harwat, M., Bohm, V. and Bitsch, R. 2002. Assessment of antioxidant activity by using different in vitro methods. *Free Radical Research*, **36**: 177-187.

Scortichini, M. and Rossi, M.P. 1999. Prelimanary in-vitro evaluation of the antimicrobial activity of terpenes and terpenoids towards *Erwinia amylovora* (Burrill) Winslow *et al*. *Journal of Applied Bacteriology*, **71**: 109-112.

Sembiring, S., Winarti, C. and Baringbing, B. 2003. Identification of chemical compounds of salam leaf (*Eugenia polyantha*) essential oil from Sukabumi and Bogor. *Bulletin TRO*, July: 10.

Setiawan, C.P. 2002. Effect of chemical and physical treatment on the antimicrobial activity of leaves (*Syzygium polyanthum* (wight) Walp.). Thesis. Faculty of Agricultural Technology. Bogor Agricultural University, Bogor.

Setiawaty, R. 2003. Study the influence of leaf extract (*Syzygium polyanthum* (Wight) Walp.). of the working power of yogurt starter. Thesis. Faculty of Veterinary Medicine, Bogor Agricultural University, Bogor.

Shafi, P.M., Rosamma, M.K., Jakil, K. and Reddy, P.S. 2002. Antibacterial activity of *Syzygium cumuni* and *S. travancoricum* leaf essential oils. *Fitoterapia,* **73**: 414-416.

Shafique, M., Khan, S.J. and Khan, N.H. 2011. Study of antioxidant and antimicrobial activity of sweet basil (*Ocimum basilicum*) essential oil. *Pharmacologyonline*, **1**: 105-111.

Sibanda, S., Chigwada, G., Poole, M., Gwebu, E.T., Noletto, J.A. and Schmidt, J.M. 2004. Composition and bioactivity of the leaf essential oil of *Heteropyxis dehniae* from Zimbabwe. *Journal of Ethnopharmacology*, **92**(1): 107-111.

Sivasothy, Y., Chong, W.K., Hamid, A., Eldeen, I.M., Sulaiman, S.F. and Awang, K. 2011. Essential oils of *Zingiber officinale var. rubrum Theilade* and their antibacterial activities. *Food Chemistry*, **124**: 514-517.

Sivropoulou, A., Kokkini, S., Lanaras, T. and Arsenakis, M. 1995. Antimicrobial activity of mint essential oils. *Journal of Agricultural and Food Chemistry*, **43**: 2384-2388.

Srimurwarni, Djokobaskoro, A. and Sasmeita, W. 2005. In vitro test on effects of antimicrobial of salam leaf (*Eugenia polyantaha* Wight) decoction against *Escherichia coli* bacteria from patients with urinary tract infection. *Working Paper*. University of Brawijaya: 11 March.

Srivastava, A.K., Srivastava, S.K. and Syamsundar, K.V. 2005. Bud and leaf essential oil composition of *Syzygium aromaticum* from India and Madagascar. *Flavour and Fragrance Journal*, **20**: 51-53.

Stefanello, M.E.A., Pascoals, A.C.R.F. and Salvador, M.J. 2011. Essential oils from neotropical Myrtaceae: chemical diversity and biological properties. *Chemistry and Biodiversity*, **16**: 9827-9837.

Suganda, A.G. and Ruslan, K., dkk. 2007. *Serial Drug Plants: Salam*. Jakarta: Food and Drugs Administrator.

Suharti, S., Banowati, A., Hermana, W. and Wiryawan, K.G. 2008. Cholestrol composition and contents of broiled chicken diarrhea given salam leaf (*Synzigium polyanthum* Wight) flour in feed. *Poultry Media*, **31**(2): 138-145.

Suksri, S., Premcharoen, S., Thawatpan, C. and Sangthongprow, S. 2005. Ethnobotany in Bung Khong Long non-hunting area, Northeast Thailand. *Kasetsart Journal (Natural Science)*, **39**: 519-533.

Sumono, A. and Agustin, W.S.D. 2008. The use of bay laf (Eugenia polyantha Wight) in dentistry. *Dentistry Journal*, **41**(3): 147-150.

Tepe, B., Sokmen, M., Akpulat, H.A., Daferera, D., Polissiou, M. and Sokmen, A. 2005. Antioxidative activity of the essential oils of *Thymus sipyleus* subsp. *sipyleus* var. *sipyleus* and *Thymus sipyleus* subsp. *sipyleus* var. *rosulans*. *Journal of Food Engineering*, **66**: 447-454.

Tepe, B., Akpulat, H.A., Sokmen, M., Daferera, D., Yumrutas, O. and Aydin, E. 2006. Screening of the antioxidative and antimicrobial properties of the essential oils of *Pimpinella anisetum* and *Pimpinella flabellifolia* from Turkey. *Food Chemistry*, **97**: 719-724.

Tepe, B. and Sokmen, A. 2007. Screening of the antioxidative properties and total phenolic contents of three endemic *Tanacetum* subspecies from Turkish flora. *Bioresource Technology*, **98**: 3076-3079.

Tg. Siti Amirah, T. K., Abd Samah, O., Taher, M., Susanti, M. and Qaralleh, H. 2012. Antimicrobial activity and essential oils of *Curcuma aeruginosa*, *Curcuma mangga* and *Zingiber cassumunar* from Malaysia. *Asian Pacific Journal of Tropical Medicine*, 202-209.

Tsakala, T.M.O. Penge, O. and John, K. 1996. Screening of *In vitro* antibacterial activity from *Syzygium guineense* (Willd) hydrosoluble dry extract. *Annals Pharmceutiques Francaises*, **54**: 276-279.

UNSRC. 2012. United States Nuclear Regulatory Commission (online). http://www.nrc.gov/ (10 December, 2012).

Utami, P. and Lentera, T. 2005. *Drug plants for rheumatic and uric acid*. Jakarta: Agromedia Pustaka.

Very, H.M., Ascetic, E.A.M. and Damayanti, E.K. 2000. *Dictionary of plant diseases and drug Indonesia (Etnofi tomedika I)*. Jakarta: Populat Literature Torch.

Wartini, N.M. 2009. Chromatogram sequencing of flavor extract of Salam leaf (*Eugenia polyantha* Wight) from steam distillation using n-hexane and without using n-hexane. *Agrotechnology*, **15**(2): 72-77.

Wang, W., Wu, N., Zu, Y.G., Fu, Y.J. 2008. Antioxidative activity of *Rosmarinus officinalis* L. essential oil compared to its main components. *Food Chemistry*, **108**: 1019-1022.

Wee, Y.C. 2003. *Tropical trees and shrubs - A selection for urban plantings*. Singapore: Sun Tree Publishing.

Wei, A., Shibamoto, T. 2007. Antioxidant activities and volatile constituents of various essential oils. *Journal of Agricultural and Food Chemistry*, **54**: 1737–1742.

Wei, A. and Shibamoto, T. 2010. Antioxidant/lipoxygenase inhibitory activities and chemical compositions of selected essential oils. *Journal of Agriculture and Food Chemistry*, **58**: 7218-725.

Wijayakusuma, H. 1995. *Medicinal plants in Indonesia*. Jakarta: Pustaka Kartini.

Wientarsih, I., Iskandar, M. and Saputra, G.H. 2007. The Effect of bay leaves infusum (*Syzigium polyanthum* (Wight)) on anti inflammation in white rat Sprague-dawley. *Proceedings of Journal of Agriculture and Rural Development in the Tropics and Subtropics, Empowering of Society through the Animal Health and Production activities with the appreciation to the indigenous knowledge, Southeast Asia Germany Alumni Network (SEAG),* **90**: 102-109.

Wikipedia. 2013. Pseudomonas aeruginosa (online). http://en.wikipedia.org/wiki/Pseudomonas_aeruginosa (January 2, 2013).

Wilson, P.G., O'Brien, M.M., Gadek, P.A. and Quinn, C.J. 2001. Myrtaceae revisited: a reassessment of infrafamilial groups. *American Journal of Botany*, **88**: 2013-2025.

Wong, S.P., Leong, L.P. and Koh, J.H.W. 2006. Antioxidant activities of aqueous extracts of selected plants. *Journal of Food Chemistry*, **7**: 58-56.

Xianfei, X., Xiaoqiang, C., Shunying, Z. and Guolin, Z. 2007. Chemical composition and antimicrobial activity of essential oils of *Chaenomeles speciosa* from China. *Food Chemistry*, **100**(4): 1312-1315.

Yang, S.A., Sang-Kyung, J., Eun-Jung, L., Chang-Hyun, S. and In-Seon, L. 2010. Comparative study of the chemical composition and antioxidant activity of six essential oils and their components. *Natural Product Research***, 24**(2): 140-151.

Yentema, O., Alioune, O. and Dorosso, S.A. 2007. Chemical composition and physical characteristic of the essential oil of *Cymbopogon schoenanthus* (L.) spring of Burkina Faso. *Journal of Applied Sciences*, **7**(4): 503-506.

APPENDIX A

SPSS ANALYSIS

Determination of Antioxidant Activity (DPPH Free Radical Scavenging)

i) Fresh Leaf Oil

```
ONEWAY inhibition BY concentration
  /POSTHOC=TUKEY DUNCAN ALPHA(0.05).
```

Oneway

Descriptives

inhibition

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	3	90.9500	.09000	.05196	90.7264	91.1736	90.86	91.04
2	3	91.1700	.05196	.03000	91.0409	91.2991	91.14	91.23
3	3	91.9800	.18000	.10392	91.5329	92.4271	91.80	92.16
4	3	91.9767	.09504	.05487	91.7406	92.2128	91.88	92.07
5	3	92.3167	.14295	.08253	91.9616	92.6718	92.16	92.44
Total	15	91.6787	.55255	.14267	91.3727	91.9847	90.86	92.44

ANOVA

inhibition

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4.129	4	1.032	71.027	.000
Within Groups	.145	10	.015		
Total	4.274	14			

ii) Dried Leaf Oil

```
ONEWAY inhibition BY concentration
   /POSTHOC=TUKEY DUNCAN ALPHA(0.05).
```

Oneway

Descriptives

inhibition

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	3	90.9500	.09000	.05196	90.7264	91.1736	90.86	91.04
2	3	91.1700	.05196	.03000	91.0409	91.2991	91.14	91.23
3	3	91.9800	.18000	.10392	91.5329	92.4271	91.80	92.16
4	3	91.9767	.09504	.05487	91.7406	92.2128	91.88	92.07
5	3	92.3167	.14295	.08253	91.9616	92.6718	92.16	92.44
Total	15	91.6787	.55255	.14267	91.3727	91.9847	90.86	92.44

ANOVA

inhibition

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4.129	4	1.032	71.027	.000
Within Groups	.145	10	.015		
Total	4.274	14			

iii) BHT

```
ONEWAY inhibition BY concentration
  /POSTHOC=TUKEY DUNCAN ALPHA(0.05).
```

One Way

Descriptives

inhibition

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	3	88.0900	.05196	.03000	87.9609	88.2191	88.06	88.15
2	3	88.4333	.09504	.05487	88.1972	88.6694	88.34	88.53
3	3	88.9933	.09504	.05487	88.7572	89.2294	88.90	89.09
4	3	89.5533	.09504	.05487	89.3172	89.7894	89.46	89.65
5	3	91.6033	.09504	.05487	91.3672	91.8394	91.51	91.70
Total	15	89.3347	1.28441	.33163	88.6234	90.0459	88.06	91.70

ANOVA

inhibition

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	23.018	4	5.755	740.933	.000
Within Groups	.078	10	.008		
Total	23.096	14			

Determination of Antioxidant Activity (β-Carotene Bleaching)

i) Fresh Leaf Oil

```
ONEWAY bleaching BY time
   /POSTHOC=TUKEY DUNCAN ALPHA(0.05).
```

Oneway

Descriptives

bleaching

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	3	1.0000E2	.00000	.00000	100.0000	100.0000	100.00	100.00
2	3	91.4433	.02517	.01453	91.3808	91.5058	91.42	91.47
3	3	91.3333	.07371	.04256	91.1502	91.5164	91.25	91.39
4	3	91.0400	.05196	.03000	90.9109	91.1691	91.01	91.10
5	3	90.5533	.02517	.01453	90.4908	90.6158	90.53	90.58
Total	15	92.8740	3.70200	.95585	90.8239	94.9241	90.53	100.00

ANOVA

bleaching

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	191.848	4	47.962	2.551E4	.000
Within Groups	.019	10	.002		
Total	191.867	14			

ii) Dried Leaf Oil

```
ONEWAY bleaching BY time
   /POSTHOC=TUKEY DUNCAN ALPHA(0.05).
```

Oneway

Descriptives

bleaching

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	3	1.0000E2	.00000	.00000	100.0000	100.0000	100.00	100.00
2	3	98.3833	.04041	.02333	98.2829	98.4837	98.34	98.42
3	3	96.2767	.05508	.03180	96.1399	96.4135	96.22	96.33
4	3	93.2833	.04041	.02333	93.1829	93.3837	93.24	93.32
5	3	91.1533	.05508	.03180	91.0165	91.2901	91.10	91.21
Total	15	95.8193	3.35315	.86578	93.9624	97.6762	91.10	100.00

ANOVA

bleaching

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	157.392	4	39.348	2.108E4	.000
Within Groups	.019	10	.002		
Total	157.411	14			

iii) BHT

```
ONEWAY bleaching BY time
   /POSTHOC=TUKEY DUNCAN ALPHA(0.05).
```

Oneway

Descriptives

bleaching

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	3	1.0000E2	.00000	.00000	100.0000	100.0000	100.00	100.00
2	3	91.3600	.03000	.01732	91.2855	91.4345	91.33	91.39
3	3	87.4933	.01155	.00667	87.4646	87.5220	87.48	87.50
4	3	86.6833	.02517	.01453	86.6208	86.7458	86.66	86.71
5	3	86.2367	.06807	.03930	86.0676	86.4058	86.16	86.29
Total	15	90.3547	5.33194	1.37670	87.4019	93.3074	86.16	100.00

ANOVA

bleaching

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	398.001	4	99.500	7.897E4	.000
Within Groups	.013	10	.001		
Total	398.014	14			

Determination of Antioxidant Activity (Ferrous-ion Chelating)

i) Fresh Leaf Oil

```
ONEWAY inhibition BY concentration
  /POSTHOC=TUKEY DUNCAN ALPHA(0.05).
```

Oneway

Descriptives

inhibition

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	3	44.5800	.04000	.02309	44.4806	44.6794	44.54	44.62
2	3	53.6433	.02309	.01333	53.5860	53.7007	53.63	53.67
3	3	70.5067	.04619	.02667	70.3919	70.6214	70.48	70.56
4	3	80.3300	.08888	.05132	80.1092	80.5508	80.23	80.40
5	3	83.5300	.04000	.02309	83.4306	83.6294	83.49	83.57
Total	15	66.5180	15.65109	4.04109	57.8507	75.1853	44.54	83.57

ANOVA

inhibition

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3429.366	4	857.341	3.114E5	.000
Within Groups	.028	10	.003		
Total	3429.393	14			

ii) Dried Leaf Oil

```
ONEWAY incubation BY concentration
  /POSTHOC=TUKEY DUNCAN ALPHA(0.05).
```

Descriptives

incubation

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	3	45.1600	.04000	.02309	45.0606	45.2594	45.12	45.20
2	3	53.8500	.06557	.03786	53.6871	54.0129	53.79	53.92
3	3	78.9000	.04000	.02309	78.8006	78.9994	78.86	78.94
4	3	84.7667	.04619	.02667	84.6519	84.8814	84.74	84.82
5	3	91.6200	.04000	.02309	91.5206	91.7194	91.58	91.66
Total	15	70.8593	18.73987	4.83861	60.4815	81.2371	45.12	91.66

ANOVA

incubation

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4916.534	4	1229.134	5.471E5	.000
Within Groups	.022	10	.002		
Total	4916.556	14			

iii) BHT

```
ONEWAY inhibition BY concentration
  /POSTHOC=TUKEY DUNCAN ALPHA(0.05).
```

Oneway

Descriptives

inhibition

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	3	55.9500	.06557	.03786	55.7871	56.1129	55.88	56.01
2	3	57.5900	.04000	.02309	57.4906	57.6894	57.55	57.63
3	3	57.7700	.06557	.03786	57.6071	57.9329	57.71	57.84
4	3	60.6200	.05196	.03000	60.4909	60.7491	60.59	60.68
5	3	61.3567	.06658	.03844	61.1913	61.5221	61.30	61.43
Total	15	58.6573	2.09112	.53992	57.4993	59.8154	55.88	61.43

ANOVA

inhibition

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	61.184	4	15.296	4.412E3	.000
Within Groups	.035	10	.003		
Total	61.219	14			

Screening of Antibacterial Activity (Disc Diffusion Assay)

i) Fresh Leaf Oil

```
ONEWAY diameter BY concentration
  /POSTHOC=TUKEY DUNCAN ALPHA(0.05).
```

Oneway

Descriptives

diameter

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	6	19.1167	4.39928	1.79600	14.4999	23.7334	14.70	27.30
2	6	15.1167	5.54235	2.26266	9.3003	20.9330	8.00	23.70
3	6	11.0000	5.24519	2.14134	5.4955	16.5045	6.70	21.00
Total	18	15.0778	5.86902	1.38334	12.1592	17.9964	6.70	27.30

ANOVA

diameter

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	197.654	2	98.827	3.821	.046
Within Groups	387.917	15	25.861		
Total	585.571	17			

ii) Dried Leaf Oil

```
ONEWAY diameter BY concentration
  /POSTHOC=TUKEY DUNCAN ALPHA(0.05).
```

Oneway

Descriptives

diameter

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	6	21.050	4.6051	1.8800	16.217	25.883	16.3	29.7
2	6	17.033	6.2921	2.5687	10.430	23.636	9.3	27.3
3	6	13.367	5.4650	2.2311	7.631	19.102	8.7	23.7
Total	18	17.150	6.0902	1.4355	14.121	20.179	8.7	29.7

ANOVA

diameter

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	177.223	2	88.612	2.932	.084
Within Groups	453.322	15	30.221		
Total	630.545	17			

iii) Standards

ONEWAY diameter BY standards

Oneway

Descriptives

diameter

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	6	25.8667	5.42648	2.21535	20.1719	31.5614	17.30	29.30
2	6	9.8500	1.43492	.58580	8.3441	11.3559	8.00	11.30
Total	12	17.8583	9.18066	2.65023	12.0252	23.6914	8.00	29.30

ANOVA

diameter

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	769.601	1	769.601	48.855	.000
Within Groups	157.528	10	15.753		
Total	927.129	11			

Screening of Antibacterial Activity (MIC/MBC)

i) Fresh Leaf Oil

ONEWAY values BY test

Oneway

Descriptives

values

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	6	.1093750	.07843688	.03202172	.0270605	.1916895	.03125	.25000
2	6	.1770833	.16015943	.06538481	.0090063	.3451603	.06250	.50000
Total	12	.1432292	.12532510	.03617824	.0636014	.2228569	.03125	.50000

ANOVA

values

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.014	1	.014	.865	.374
Within Groups	.159	10	.016		
Total	.173	11			

ii) Dried Leaf Oil

ONEWAY values BY test

Oneway

Descriptives

values

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	6	.0781250	.03827328	.01562500	.0379597	.1182903	.03125	.12500
2	6	.1250000	.06846532	.02795085	.0531501	.1968499	.06250	.25000
Total	12	.1015625	.05827327	.01682204	.0645374	.1385876	.03125	.25000

ANOVA

values

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.007	1	.007	2.143	.174
Within Groups	.031	10	.003		
Total	.037	11			

APPENDIX B

GAS CHROMATOGRAMS

B1: Fresh Leaf Oil

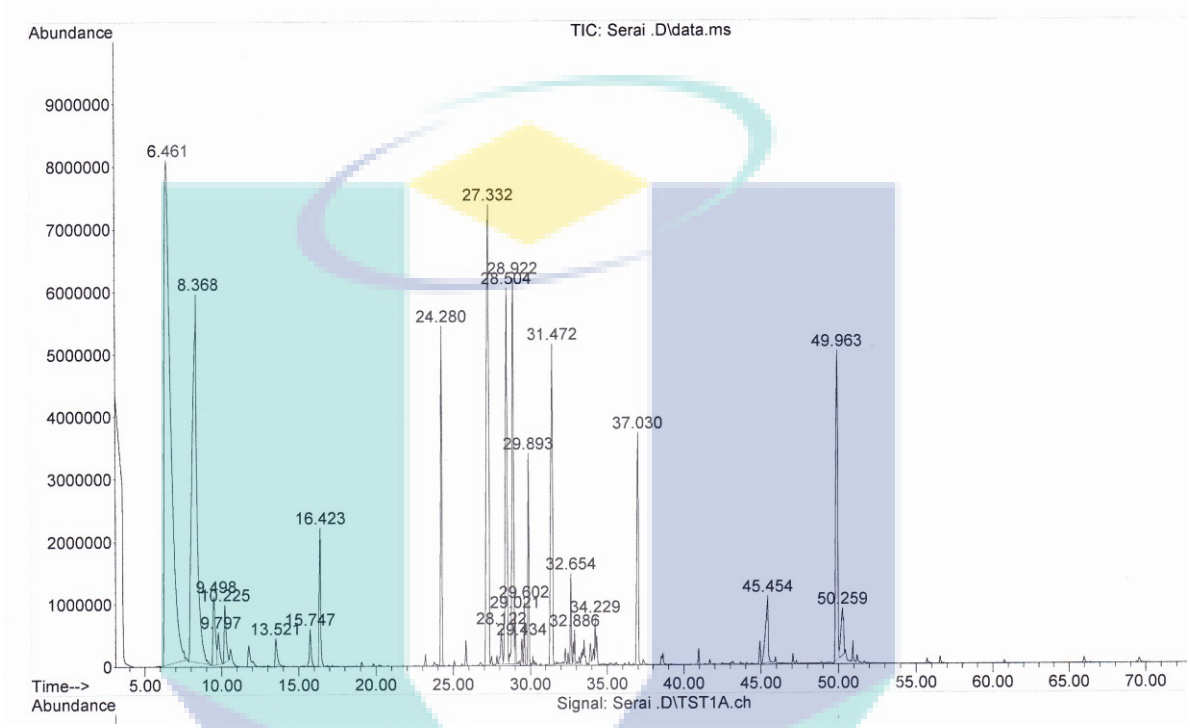


Figure B.1: Gas chromatogram of the fresh leaf oil of *Syzygium polyanthum*

B2: Dried Leaf Oil

Figure B.2: Gas chromatogram of the dried leaf oil of *Syzygium polyanthum*

APPENDIX C

LIST OF PUBLICATIONS

C.1: Nur Amalina, A., Natanamurugaraj, G., Mashitah, M.Y., Nurul Ashikin, A.K. 2012. Chemical Composition, Antioxidant and Antibacterial Activity of Essential Oilsfrom Leaves Of *Syzygium Polyanthum* (Wight) Walp. *Proceedings of National Conference for Postgraduate Research 2012 (NCON-PGR 2012)*, 8-9 September 2012, Universiti Malaysia Pahang (UMP), Gambang, Malaysia.

C.2: Nur Amalina, A., Natanamurugaraj, G., Mashitah, M.Y. ,Nurul Ashikin, A.K. 2012. Chemical Composition, Antioxidant and Antibacterial Activities Of *Syzygium polyanthum* (Wight) Walp. Essential Oils. *Proceedings of International Conference on Postgraduate Education 2012 (ICPE-5 2012)*, 18-19 December 2012, Universiti Teknologi Malaysia (UTM), Skudai, Malaysia.

C.3: Nur Amalina, A., Natanamurugaraj, G., Mashitah, M.Y., Nurul Ashikin, A.K. 2013. Chemical Composition, Antioxidant and Antibacterial Activities of Syzgium polyanthum (Wight) Walp. Essential Oils. *Proceedings of International Conference on Natural Products 2013 (ICNP 2013)*, 4-6 March 2013, Shah Alam Convention Centre (SACC), Shah Alam, Selangor.


UMP