CHEMICAL COMPOSITION AND ANTIBACTERIAL ACTIVITY OF ESSENTIAL OIL FROM *CYMBOPOGON CITRATUS* AND *CYMBOPOGON NARDUS*

SITI FIQRYYAH BINTI MUSA

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

Faculty of Industrial Sciences and Technology
UNIVERSITI MALAYSIA PAHANG

APRIL 2014

CHEMICAL COMPOSITION AND ANTIBACTERIAL ACTIVITY OF ESSENTIAL OIL FROM *CYMBOPOGON CITRATUS* AND *CYMBOPOGON NARDUS*

SITI FIQRYYAH BINTI MUSA

MASTER OF SCIENCE

UNIVERSITI MALAYSIA PAHANG

EXAMINERS APPROVAL DOCUMENT

UNIVERSITI MALAYSIA PAHANG

INSTITUTE OF POSTGRADUATE STUDIES

We certify that the thesis entitled "Chemical Composition and Antibacterial Activity of Essential Oil From *Cymbopogon citratus* and *Cymbopogon nardus*" is written by Siti Fiqryyah binti Musa. We have examined the final copy of this thesis and in our opinion; it is fully adequate in terms of scope and quality for the award of the degree of Master of Science in Industrial Chemistry. We herewith recommend that it be accepted in fulfilment of the requirements for the degree of Master of Science specializing in Industrial Chemistry.

Name of External Examiner Signature

Institution:

Name of Internal Examiner Signature

Institution:

STATEMENT OF AWARD FOR DEGREE

Thesis submitted in fulfilment of the requirements for the award of the degree of Master of Science in Industrial Chemistry.

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 Masters in Science of Industrial Chemistry.

Name of Supervisor : Dr. Azhari Hamid Nour Abduel Rahman

Position : Senior Lecturer

Date :

Name of co-supervisor: Prof. Mashitah binti Mohd. Yusoff

Position : Dean of Faculty of Industrial Sciences and Technology

Date :

STUDENT'S DECLARATION

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

Name : Siti Fiqryyah binti Musa

ID Number : MKD 10005

Date :

Dedicated to “Abang”, “Ayah”, “Mak”, Hani, Izz and Hada

ACKNOWLEDGEMENTS

Thanks to Allah the Almighty who give me, His blessing in finishing and completing my research. I am grateful and would like to express my sincere gratitude to my supervisor, Dr. Azhari Hamid Nour Abduel Rahman for his encouraged ideas, invaluable guidance, continuous encouragement and constant support in making this research possible. I am truly grateful for his progressive vision about my training in science, his tolerance of my naïve mistakes, and his commitment to my future career. My gratitude also goes to my co-supervisor, Prof. Dr. Mashitah binti Mohd Yusoff for her suggestions and co-operation throughout the study.

My sincere thanks go to all my labmates; Sis Faridah, Sis Hazrul, Sis Radhiah, Asma, Fatehah and staffs of Faculty of Industrial Sciences and Technology, UMP, especially Ida, Ben, Laili, and Farid, who have helped me in many ways and made my stay at UMP pleasant and unforgettable.

My sincere indebtedness and gratitude to my beloved husband, Mohamad Nasir bin Mat Daud who believe in me and gave me all the love, support, sacrifices, useful advices, prayers and always patience with me. Thank you so much. I am also grateful to my lovely children, Hani, Izz and Hada for their sacrifices; patience and understanding that were inevitable to make this work possible. My thanks also go to my parents, Musa bin Yusoff and Wan Halimah binti Wan Ismail; also to my mother in law, Fatimah binti Hussin. I cannot find the appropriate words that could describe my appreciation for their devotion, support and faith in my ability to attain my goals.

Last but not least to all the person that I did not mentioned above. Thank you for helping me and giving me the opportunity to learn and experience a lot of things to become a better person in the future. May Allah the Almighty bless you. Amin.

ABSTRACT

The bacterial resistance has created a major health issue worldwide whereby the pathogens becoming resistant even to the most recently approved antibiotics. Essential oils have showed many biological activities such as antibacterial, antifungal, antiviral, antioxidant and insecticidal. This study was conducted to analyse the chemical composition of the essential oils of *Cymbopogon citratus* and *Cymbopogon nardus*; and to study their antibacterial activities in alone and in combination. Essential oils obtained by steam distillation were analysed by gas chromatography-mass spectrometry (GC-MS); while the antibacterial activity of the essential oils were evaluated against five bacteria namely *Enterococcus faecalis* ATCC 14506, *Staphylococcus aureus* BAA-1026, *Bacillus Subtilis* ATCC 11774, *Escherichia coli* ATCC 10536, and *Salmonella typhimurium* ATCC 14506 by using disk diffusion and broth microdilution methods. To determine the antibacterial effects of essential oils in combination, the broth microdilution checkerboard method was utilized. From the results, it is observed that the major compounds contained in essential oils of *Cymbopogon citratus*, and *Cymbopogon nardus* were geranial (33.01%) and elemol (44.14%), respectively. The result of antibacterial activity indicated that *Cymbopogon citratus* possessed a good and wide spectrum of antibacterial activity against all the tested bacteria; whereas *Cymbopogon nardus* only showed stronger antibacterial activity against Gram-positive bacteria than Gram-negative bacteria. Gram-positive bacteria were more sensitive to the investigated oils than Gram-negative bacteria; in which *Staphylococcus aureus* was the most sensitive strain tested, with the lowest MIC value (0.47µl/ml). The *Cymbopogon nardus* had showed greater bactericidal activity against all Gram-positive bacteria compared to *Cymbopogon citratus*. The result of antibacterial activity of essential oils in combination showed that the combination were less effective compared to when each of the essential oils was used individually; the antagonism responses were obtained against all the tested bacteria except for *Enterococcus faecalis* bacteria which showed indifference response. The results presented may suggest that the essential oils of *Cymbopogon citratus* and *Cymbopogon nardus* could be employed as a potential source of antibacterial ingredients for food and pharmaceutical industry; however, it is recommended for not mixing these both essential oils as they have not given positive results for antibacterial activity.

ABSTRAK

Kerintangan bakteria telah mewujudkan satu masalah kesihatan utama di seluruh dunia di mana patogen menjadi kebal walaupun antibiotik yang baru ditemui. Minyak pati telah menunjukkan pelbagai aktiviti biologi seperti antibakteria, antikulat, anti-virus, anti-oksida dan anti-serangga. Kajian ini dijalankan untuk menganalisis komposisi kimia minyak pati *Cymbopogon citratus* dan *Cymbopogon nardus*, dan untuk mengkaji aktiviti antibakteria mereka secara bersendirian dan kombinasi. Minyak pati yang diperolehi daripada penyulingan stim dianalisis oleh kromatografi gas- spektrometri jisim (GC-MS), manakala aktiviti antibakteria telah dinilai terhadap lima jenis bakteria iaitu *Enterococcus faecalis* ATCC 14506, *Staphylococcus aureus* BAA - 1026 , *Bacillus subtilis* ATCC 11774 , *Escherichia coli* ATCC 10536 , dan *Salmonella typhimurium* ATCC 14506 dengan menggunakan kaedah penyebaran cakera dan kaedah kaldu mikrocairan. Untuk menentukan kesan antibakteria minyak pati dalam gabungan, kaedah kaldu mikrocairan dam telah digunakan. Keputusan yang diperolehi menunjukkan bahawa sebatian utama yang terkandung di dalam *Cymbopogon citratus* dan *Cymbopogon nardus* adalah geranial (33.01%) dan elemol (44.14%), masing-masing. Hasil keputusan daripada ujian aktiviti antibakteria pula menunjukkan bahawa *Cymbopogon citratus* memberikan spektrum yang baik dan meluas terhadap semua bakteria yang diuji; manakala *Cymbopogon nardus* hanya menunjukkan aktiviti antibakteria yang kuat terhadap bakteria Gram positif daripada bakteria Gram-negatif. Bakteria gram-positif adalah lebih sensitif kepada minyak pati yang diuji daripada bakteria Gram-negatif, di mana *Staphylococcus aureus* merupakan bakteria yang paling sensitif, dengan nilai MIC terendah, 0.47µl/ml. *Cymbopogon nardus* juga telah menunjukkan aktiviti bakteria lebih berkesan terhadap semua bakteria Gram-positif berbanding Cymbopogon *citratus*. Hasil daripada aktiviti antibakteria minyak pati dalam gabungan menunjukkan bahawa kombinasi kurang berkesan berbanding apabila setiap minyak pati digunakan secara sendirian; tindak balas antagonistik telah diperolehi terhadap semua bakteria yang diuji kecuali kepada *Enterococcus faecalis* yang menunjukkan tindak balas sebaliknya. Kajian ini mencadangkan bahawa minyak pati *Cymbopogon citratus* dan *Cymbopogon nardus* boleh digunakan sebagai salah satu sumber bahan antibakteria dalam industri makanan dan farmaseutikal. Walau bagaimanapun, ia tidak digalakkan untuk mencampurkan kedua-dua minyak pati ini untuk aktiviti antibakteria memandangkan keputusan yang diperolehi adalah kurang berkesan.

TABLE OF CONTENTS

		Page
SUPERVISOR'S DECLARATION		iii
STUDENT'S DECLARATION		iv
ACKNOWLEDGEMENTS		vi
ABSTRACT		vii
ABSTRAK		viii
LIST OF TABLES		xii
LIST OF FIGURES		xiii
LIST OF SYMBOLS		xv
LIST OF ABBREVIATIONS		xvii
CHAPTER 1	**INTRODUCTION**	
1.1	Research Background	1
1.2	Problem Statement	3
1.3	Objectives of the Research	4
1.4	Scopes and Limitation of the Study	4
1.5	Significance of the Study	4
CHAPTER 2	**LITERATURE REVIEW**	
2.1	Historical Review on Aromatic Substances and Essential Oils	6
	2.1.1 The Use of EO in Ancient Time	6
	2.1.2 The EOs in Europe	8
	2.1.3 The EOs in Malaysia	9
2.2	Systematic Investigation of Constituents from Essential Oils	10
2.3	Chemistry of Essential Oils	12
	2.3.1 Terpenes Group	14
	2.3.2 Phenylpropenes	25

2.4	Genus *Cymbopogon*	28
	2.4.1 *Cymbopogon citratus*	29
	2.4.2 *Cymbopogon nardus*	31
2.5	Use of Gas Chromatography-Mass Spectrometry (GC-MS) In Analysis of EOs Chemical Composition	34
2.6	Bacteria	35
	2.6.1 Cell size and shape	36
	2.6.2 Bacterial Diversity	37
	2.6.3 Bacteria Cell Wall	37
2.7	Essential Oil as Antibacterial	41
	2.7.1 Methods Used For Antibacterial Activity Testing	41
2.8	Essential Oils in Combination and Antibacterial Activity	43
	2.8.1 Definitions of Antibacterial Interactions In Vitro	44
	2.8.2 Interaction Test Methods	44
	2.8.3 Interpretation of the Results	46
2.9	Mechanism of Action of EOs Against Bacterial Cell	47
CHAPTER 3	**MATERIALS AND METHODS**	
3.1	Materials	51
3.2	Plants Material	51
3.3	Essential Oils Extraction	51
3.4	Gas Chromatography-Mass Spectrometry (GC-MS)	52
3.5	Antibacterial Aspects	53
	3.5.1 Culture and Media Preparation	53
	3.5.2 Inoculum Preparation	54
	3.5.3 Disk Diffusion Assays	54
	3.5.4 Determination of Minimum Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC)	55
	3.5.5 Checkerboard Assay	56
CHAPTER 4	**RESULTS AND DISCUSSION**	
4.1	Chemical Composition of Essential Oils	58
	4.1.1 Total Yields	58
	4.1.2 GC-MS Analysis of Essential Oils	59

4.2	Antibacterial Activity of *C. citratus* and *C. nardus* EOs	64
4.3	Interaction between Essential Oils in Combination	70
CHAPTER 5	**CONCLUSION AND RECOMMENDATIONS**	
5.1	Conclusion	74
5.2	Recommendation for Future Studies	75
REFERENCES		76
APPENDICES		
A1	GC-MS Chromatogram of Essential Oil of *Cymbopogon citratus*	90
A2	GC-MS Chromatogram of Essential Oil of *Cymbopogon nardus*	91
B1	GC-MS Library Search Report of Essential Oil of *Cymbopogon citratus*	92
B2	GC-MS Library Search Report of Essential Oil of *Cymbopogon nardus*	96
C1	Raw Schematic Result of Minimum Inhibitory Concentration (MIC) of *Cymbopogon citratus* Against Tested Bacteria (In Triplicate)	99
C2	Raw Schematic Result of Minimum Inhibitory Concentration (MIC) of *Cymbopogon nardus* Against Tested Bacteria (In Triplicate)	101
D	Raw Schematic Result of Minimum Inhibitory Concentration (MIC) of *Cymbopogon citratus* and *Cymbopogon nardus* Essential Oils in Combination Against Tested Bacteria (In Triplicate)	104
E	Interpretation of the Result of Checkerboard Assay	112
F	Conference	113

LIST OF TABLES

Table No.	Title	Page
2.1	Examples of monoterpenes	17
2.2	Examples of sesquiterpenes	19
2.3	Examples of terpenoids classified in the different functional groups	21
2.4	Examples of phenylpropenes presented in EOs	27
2.5	Gram-positive and Gram-negative cell wall composition	41
2.6	Calculation of Fractional Inhibitory Concentration (FIC) Index for Combination of Two Antibacterial	46
4.1	Chemical compositions of *C. citratus* and *C. nardus* essential oils identified by GC-MS	59
4.2	Antibacterial activity of *C. citratus* and *C. nardus* essential oils (Inhibition zone diameter, minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC))	66
4.3	Checkerboard assay of the combination of *C. citratus* and *C. nardus* essential oils against tested bacteria	71

LIST OF FIGURES

Table No.	Title	Page
1.1	Growth in the sales of herbal medicine of nine representative countries from 1999-2001(Bhutan, Canada, the Czech Republic, Iran, Madagascar, Malaysia, Pakistan, Sudan and Sweden)	2
1.2	History of antimicrobial agent development vs. subsequent acquaintance of resistance by microorganisms	3
2.1	Biological pathway of terpenes and phenylpropenes	13
2.2	Examples of terpenes	14
2.3	Mevalonic Acid	15
2.4	Synthesis of mono- and sesquiterpenes	16
2.5	Phenylpropyl building unit	25
2.6	Shikimic acid	26
2.7	Phenylalanine	26
2.8	Cinnamic acid	26
2.9	4-coumaric acid	26
2.10	Picture of *Cymbopogon citratus* (a) plant (b) leaves and (d) stem/stalks	29
2.11	Chemical structure of (a) geranial and (b) neral	31
2.12	Picture of *Cymbopogon nardus* (a) plant (b) leaves and (d) stem/stalks	32
2.13	Structure of citronellal	32
2.14	Gas Chromatography-Mass Spectrometry	34
2.15	The difference between eukaryotic cell and prokaryotic cell	36
2.16	Shapes of some different bacteria	36
2.17	The difference between gram positive and gram negative	

	bacteria	38
2.18	The peptidoglycan structure	39
2.19	(a) A glycerol based-teichoic acid and (b) A ribitol based-teichoic acid	40
2.20	Example of broth microdilution checkerboard	45
2.21	Method of interpretation by Eliopoulus: ☐ non-turbid well ▨ turbid well	47
2.22	Schematic overview of the hypothesized activity of carvacrol	49
2.23	Scanning electron micrographs of *E. coli* cells: (A) untreated (magnification×30,000); (B) treated with combinations of oregano oil and basil oil at MIC value for 3 hours (magnification x 20,000)	50
2.24	Scanning electron micrographs of *S. aureus* cells: (A) untreated (magnification×100,000); (B) treated with combinations of basil oil and bergamot oil at MIC value for 3 hours (magnification x 50,000)	50
3.1	The steam distillation used to obtain essential oils	52
3.2	Isolated pure colonies	53
3.3	Genesys 20 Visible Spectrophotometer Thermo Scientific brand	54
3.4	Diameter zone of inhibition	55
3.5	Template of microdilution broth susceptibility assay	56
3.6	Template of broth microdilution checkerboard	57
4.1	Colours of essential oils : (a) *C. citratus* –pale yellow (b) *C. nardus*-colourless	58
4.2	Chemical structure of (a) geranial (b) neral and (c) β-gurjunene	61
4.3	Chemical structure of (a) elemol (b) α-cadinol and (c) citronellal	63
4.4	Inhibition zone diameter of essential oils from (a) *C. nardus* against *S. aureus* and (b) *C. citratus* against *E. faecalis*	64

LIST OF SYMBOLS

%	Percent
<	less than
>	greater than
≤	less than or equal to
≥	greater than or equal to
µl	Microliter
µl/ml	Microliter per mililiter
µm	Micrometer
cfu/ml	Colony forming unit per mililiter
eV	Electron volt
g	Gram
h	Hour
m	Meter
M	Molarity
m/z	Mass to charge ratio
mg/ml	Miligram per milimeter
min	Minutes
ml	Mililiter
ml/min	mililiter per minute
mm	Milimeter
nm	Nano meter
ºC	Degree celcius
pH	A Measure of Acidity or Basicity

rpm	rotation per minute
s	Second
v/v	Volume per volume
w/w	Weight per weight
α	Alpha
β	Beta
γ	Gamma

LIST OF ABBREVIATIONS

ATP	Adenosine triphosphate
BC	Before Christ
C. citratus	*Cymbopogon citratus*
C. nardus	*Cymbopogon nardus*
CLSI	Clinical and Laboratory Standards Institute
-CoA	-coenzyme A
DMAPP	Dimethylallyl pyrophosphate
DMSO	Dimethylsulfoxide
EOs	Essential Oils
FIC	Fractional Inhibitory Concentration
FICI	Fractional Inhibitory Concentration Indices
FPP	Farnesyl pyrophosphate
GC-MS	Gas Chromatography-Mass Spectrometry
GPP	Geranyl pyrophosphate
IPP	Isopentenyl pyrophosphate
KDO	Ketodeoxyoctonate
LPS	Lipopolysaccharide
MBC	Minimum Bactericidal Concentration
MHB	Mueller Hinton broth

MIC	Minimum Inhibitory Concentration
MVA	Mevalonic acid
OD	Optical density
PP	Pyrophosphate
RM	Ringgit Malaysia
WHO	World Health Organisation

CHAPTER 1

INTRODUCTION

1.1 RESEARCH BACKGROUND

Over the last 50 years, there is a growing study on plant secondary metabolites (Bourgaud et al., 2001). These plant secondary metabolites are known to contribute a major role in the adaptation of plants to their ecological interactions. For instance, they could act as protector against herbivory and microbial infection (which also been described as antibiotic, antifungal, and antiviral), as attractants for pollinators and seed dispersing animals, and as allelopathic agents (allelochemicals that influence competitions among plant species). In addition, they also contain important UV absorbing compounds to prevent serious leaf damage from the light (Bourgaud et al., 2001; Croteau et al., 2000 and Hyldgaard et al., 2012).

The plant secondary compounds consist of three major groups: phenolics, terpenes and steroids, and alkaloids. These groups are classified according to their biosynthetic pathways; phenolics and alkaloids are derived from shikimic acid pathway while terpenes and steroids from acetyl-CoA mevalonic acid pathway (Croteau et al., 2000; Bourgaud et al., 2001 and Ramawat et al., 2009).

Many biological activities showed by plant secondary metabolites have long been used in traditional medicine (Bourgaud et al., 2001). Traditional medicine is defined as the sum total of the knowledge, skills and practices based on the theories, beliefs and experiences indigeneous to different cultures used in the health maintenance,

prevention of diseases and improvement of physical and mental diseases (Ramawat et al., 2009). One of the types of traditional medicine is herbal medicine; it is also known as medicinal plants (Effendy et al., 2012).

Malaysia is gifted with a wide variety of herbal medicine and these medicine have served as the primary healthcare for locals since ages (Mustaffa et al., 2011 and Effendy et al., 2012). From a global survey report by WHO, it shows that Malaysia was one of the nine countries that contributed a large amount of sales in herbal medicine worldwide between the year of 1999 to 2001 (Figure 1.1). In 2008, the Malaysian market for herbal and natural products was estimated to growth approximately RM10 billion with the raise of 8% rate per year (Effendy et al., 2012). At the same time, referring to World Bank report, they predicted that during 2050, the global market for herbal products would be about 5 US trillion dollars (Rasadah and Ali, 2008).

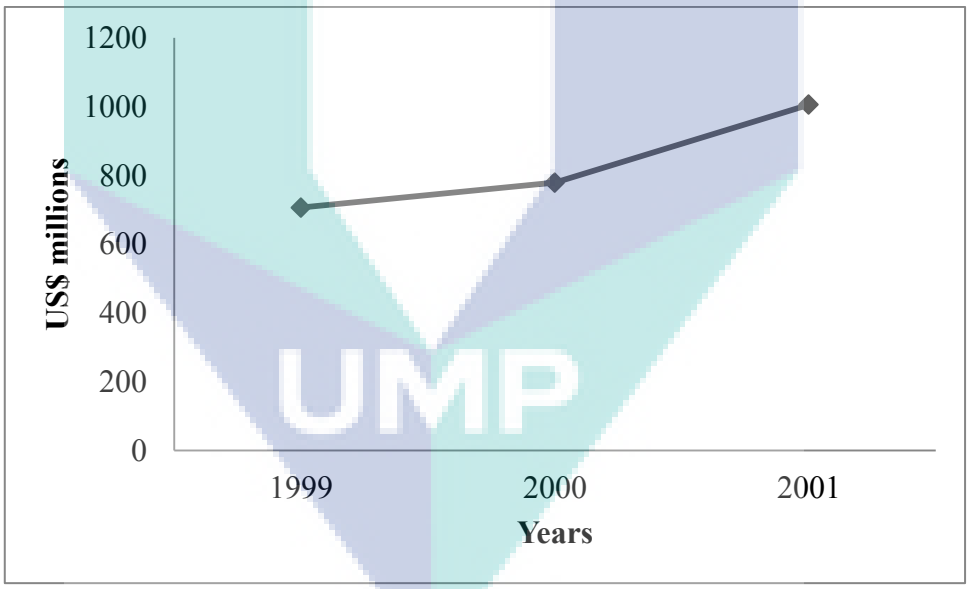


Figure 1.1: Growth in the sales of herbal medicine of nine representative countries from 1999-2001 (Bhutan, Canada, the Czech Republic, Iran, Madagascar, Malaysia, Pakistan, Sudan and Sweden)

Adapted from: Effendy et al. (2012)

In relation to this, the government urged researchers, academicians and industry operators to grab the opportunity by speeding up their research and development activities in medicinal plants to find new leads and could market them worldwide (Rasadah and Ali, 2008).

1.2 PROBLEM STATEMENT

The extensive use of antibiotics in human medicine, in animal production and as growth promoters in agriculture has led to the increase of bacterial resistance (Palaniappan and Holley, 2010). This bacterial resistance has created a major health issue worldwide whereby the pathogens becoming resistant even to the most recently approved antibiotics (Figure 1.2) (Huh and Kwon, 2011). These resistant organisms may be transferred to humans in two ways; either directly via the food chain or indirectly as a result of spread of animal waste in fields (Palaniappan and Holley, 2010).

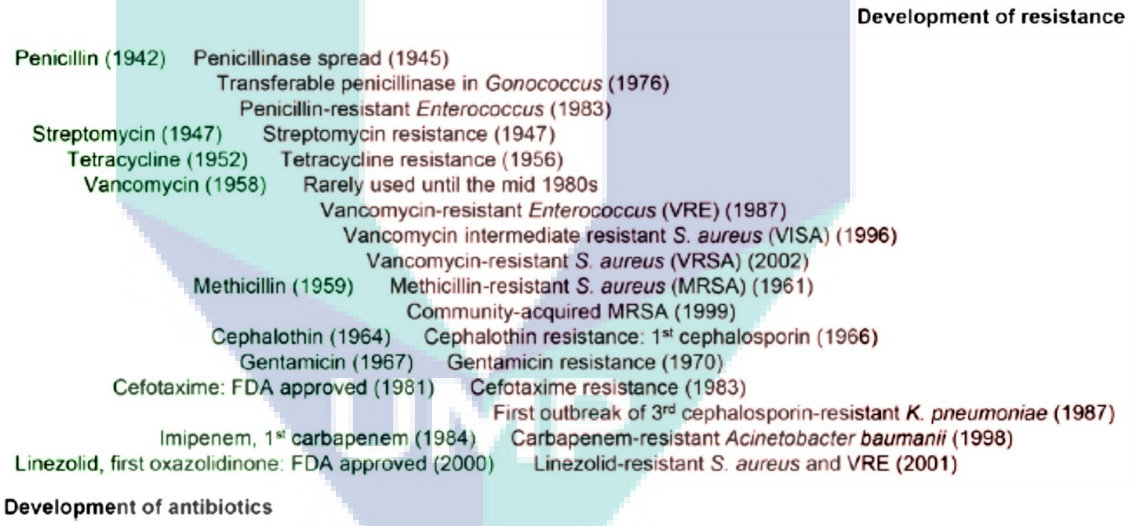


Figure 1.2: History of antimicrobial agent development vs. subsequent acquaintance of resistance by microorganisms

Source: Huh and Kwon (2011)

Due to the increasing dilemma of antibiotic resistance, adverse effects and high costing have led researchers to explore natural resources especially plant materials as an alternative source of antimicrobials (Zaidi et al., 2009). In relation to this, the study about essential oils from various plants in Malaysia has been done extensively by the

researchers to discover their beneficial potential. Many of the essential oils from the plants have shown their potential as antimicrobials (Hossain et al., 2011; Ibrahim et al., 2009; Maizura et a., 2008). However, as far as the articles could be ascertained, there is no yet study about antibacterial activity of essential oils in combination from Malaysian medicinal plants. Therefore, this study will highlight the antibacterial activity of essential oils from Malaysian medicinal plants, *Cymbopogon citratus* and *Cymbopogon nardus* independently and in combination.

1.3 OBJECTIVES OF THE RESEARCH

The objectives of this study are:

i. To analyse the chemical composition of *Cymbopogon citratus* and *Cymbopogon nardus* essential oils by using Gas Chromatography-Mass Spectrometry (GC-MS).
ii. To study the antibacterial activity of the essential oils *Cymbopogon citratus* and *Cymbopogon nardus*
iii. To study the antibacterial activity of the essential oils of *Cymbopogon citratus* and *Cymbopogon nardus* in combination through broth microdilution checkerboard assay.

1.4 SCOPES AND LIMITATION OF THE STUDY

This study focuses on the screening for antibacterial agents of essential oils from the Malaysian medicinal plants, *Cymbopogon citratus* and *Cymbopogon nardus*. These species are well known in Malaysia and have been used by Malaysian herbal manufacturers to produce a wide variety of health related products. This study is limited to the chemical identification and antibacterial activity from the medicinal plants.

1.5 SIGNIFICANCE OF THE STUDY

The results of this study would contribute a new, inexpensive and alternative antibacterial agent from Malaysian medicinal plants. In addition, it is hoped that this research will help the medicinal plant research and development to gain an insight into

the effectiveness of our local herbal traditional formulations as most of them are prepared in combination of more than one ingredient.

CHAPTER 2

LITERATURE REVIEW

2.1 HISTORICAL REVIEW ON AROMATIC SUBSTANCES AND EOs

The study of essential oil (EO) is a long history; it was started since antiquity until this modern world. This study is never ending; every time there are new findings are discovered. The researchers work very hard in order to maximise the use of EOs (Surburg and Panten, 2006).

2.1.1 The use of EO in ancient time

It is known that the aromatic sources which originated from spices, resins from animals and plants have been used enormously since ancient times for perfumery, flavour purposes and in health care system (Buckle, 2003 and Surburg and Panten, 2006). Dates back to the fourth century Before Christ (BC), Hippocrates, the well-known father of medicine in Greek employed the burning of aromatic substances to prevent from contagious diseases; he also suggested the Greeks and Romans to add aromatic oils in their bath houses for their health (Worwood, 1991).

In another reports, it was stated that the Egyptians has written the oldest documentation of therapeutic treatments and pharmaceutical plant preparations namely 'Papyrus Ebers' in 1500 BC. Correspondingly, the aromatic substances were discovered in Tutankhamen's tomb; this showed that their priests had used aromatic substances to embalm the pharaoh's body from decaying. The Babylonians are also one of the earliest people who used those sources in their daily lives. They favoured to use oils of myrrh

(*Commiphora* spp.), frankincense (*Boswellia* spp.) and cedarwood to treat various diseases (Marshall, 2004 and Worwood, 1991).

Instead of those peoples, the Arabs, Indians, and Chinese also have been reported to use the aromatic subtances. For instance, in China, it was reported that the first text of procedure on herbal medicine preparation was found around 2800 BC. To treat the transdermal illness, the Chinese absorbed a cloth in herbs and put it on the skin because they believed that the benefits contained in the herbs may permeable through the skin (Buckle, 2003 and Worwood, 1991).

In India, the Ayurvedic medicine had been practised approximately in 2000 BC; this was found in their first Sanskrit medical treatises, *Caraka Samhita* and *Sushrata Sambita.* The manuscripts described the use of 700 plants and many of them are aromatics such as ginger, coriander, myrrh, cinnamon and sandalwood (Buckle, 2003).

In Arabia, the Arabs had improved the use of herbal and aromatic medicine by introducing new aromatics such as senna, camphor, tamarind, nutmeg, and cloves to the list of medicinal plants. The Arabs also recommended to add in the rose and orange-blossom water in giving the anaesthetic effect (Buckle, 2003). The famous medical textbook, *Canon of Medicine* written by Ibn Sina or Avicenna was translated from Arabic to Latin and had spread to Europe in the twelfth century. This *Canon* lists 760 medicinal plants and the drugs that can be derived from them. This knowledge has led Europe to apply it in treating the disease caused by bad odours by using aromatics waters like "eau de cologne" (Buckle, 2003 and Worwood, 1991).

Consequently, the importance of aromatic natural products has resulted in the discovery of the technique for its preparation. Hence, the distillation technique to obtain EOs has been introduced in 9th century A. D. and it was reported that the person who is responsible to this was Ibn Sina. He called the distillation apparatus as *alembic* (Buckle, 2003; Burt, 2004 and Surburg and Panten, 2006).

2.1.2 The EOs in Europe

The use of EOs in aromatherapy is very well known in a part of country such as in United Kingdom, United States and France. In the United Kingdom, the EOs are commonly been applied in the massage to reduce stress and in other health care system; while in France, the EOs are diluted in vegetable oil and be given orally in a gelatin capsule by a medical or herbal doctor. This oral application is effective to treat gastrointestinal problem and to fight an acute or chronic infection (Buckle, 2003).

In the United Kingdom, the effort to evaluate the EOs scientifically has been started in the nineteenth century and many of these results have been recorded in *Materia Medica and Therapeutics* (1882) published by William Whitla. As the idea to identify and isolate therapeutic components of the plants become crucial later, in the late 1890s the specific components in essential oil such as geraniol and citronellol have been successfully identified (Buckle, 2003).

In France, the efforts to use EOs in the health care and disease treatments have been introduced by the first pioneers of modern aromatherapy: Gattefosse (a chemist), Valnet (an army physician), and Maury (a nurse) (Buckle, 2003).

Rene-Maurice Gattefosse was the person who had introduced the word *aromatherapy*. He was very interested in the research of topical application of EOs after he accidentally used one rinse of essential oil of lavender *(Lavandula augustifolia)* to treat the wounds that infected with gas gangrene when he was injured in a fire. Surprisingly, the wounds that had been treated with the essential oil had healed. This incident has brought him to do more research on EOs all his life (Buckle, 2003 and Worwood, 1991).

His research became beneficial when the EOs of thyme, chamomile, clove, and lemon were used on infected wounds, gangrene treatment and as sterilizer for surgical instruments in World War I and World War II (Buckle, 2003).

On the other hand, Jean Walnet had spent much of his life researching aromatherapy and he really believed the powerful of EOs to keep away from accidents and incidents. He also had applied the use of EOs when he served as a commander of an

advanced surgical when he was in Indochina. He had written a book of classic aromatherapy entitled *The Practice of Aromatherapy* and it has been translated into many languages such as English, German, Italian, Spanish, and Japanese (Buckle, 2003).

Marguerite Maury has given her contribution to the public by categorizing the use of EOs into various clinical departments: surgery, radiology, dermatology, gynecology, general medicine, psychiatry, spa treatment, physiotherapy, sports and cosmetics. Her efforts paid off when she had won two international prizes in the research of EOs and dermatology; and her book, *Le Capital Jeunesse* has been translated into English (Buckle, 2003).

2.1.3 The EOs in Malaysia

Malaysia, which is located in the Southeast Asia and on the equator, only facing with hot and humid throughout the year. This region, which also surrounded with oceans receives rainfall about 200 centimetres (79 inches) and the temperatures varying from 20ºC to 35 ºC (70º to 100ºFahrenheit) each year. This equatorial climate has categorised Malaysian forests as tropical rainforests (Bodeker, G. et al., 2009).

The rainforests is invaluable gift to Malaysia as it contains with an extremely rich biodiversity. Due to its extent of the biological diversity, Malaysia has been recognized as one of 12 global mega diversity areas in the world (Syukor, A.R.A. et al., 2008). Regarding to this, the Malaysian people are very fortunate because this rainforests sources are very close at their hand. Plants can be picked, mashed, cooked, consumed and applied at all times of the year (Bodeker, G. et al., 2009).

In particular, they used this source in their traditions to improve their health and beauty. The term *ramuan* is used in Malay language which refers to a healing mixture of medicinal plant and plants part. This *ramuan* is considered as a force of healing, beauty and vitality. Another terms that are also commonly used by the villages are: *rempah ratus* (a term refers to a polyherbal preparation from a hundred kinds of medicinal plants and spices), *ramuan akar kayu* (plant roots mixture) and *ramuan asli* (original plants mixture). This *ramuan* was inherited from generation to generation of their ancestors (Bodeker, G. et al., 2009).

Generally, they used this *ramuan* in their daily lives; for example, for facial and skin care, in traditional dental care, in bridal grooming, in pre-natal and post-natal care, for nursing mothers, and in traditional herbals for male vitality. Commonly, they used the *ramuan* preparation in form of herbal masks and scrubs, flower baths, scented steams and herbal oils (Bodeker, G. et al., 2009).

There are many plants used by Malaysian in their practices, such as: mashed noni fruit (*Morinda citrifolia*), oil of coconut milk (*Cocos nucifera*), keremak leaves (*Alternanthera sessilis* L.), buah keras (*Aleurites moluccana*) and pandan leaves (*Pandanus odorous*) have been used in the hair care; while a warm herbal bath consisted of sweet lemongrass (*Cymbopogon nardus),* betel leaves (Piper betle), pandan leaves and slices of ginger, *asam keping* (*Garcinia atroviridis*) were used in women personal hygiene. Other than that, for postpartum remedies, the specialist herbs which are commonly used by Malays are *Kacip fatimah* (*Labisia pumila*), mas cotek (*Ficus deltoidea*) senduduk (*Melastoma malabathricum*) and many more (Bodeker, G. et al., 2009 and Jamal et al., 2011).

However, from the reviews, it can be observed that the use of essential oils from the plants in Malaysia is not too familiar among the old folks; it only could be found widely in this modern era after scientific studies are beginning to validate the efficacy of some of these traditional formulations and the country is becoming more aware of the therapeutic and commercial potential of the *ramuan* tradition. Hence, nowadays, the researchers in Malaysia are very exciting to find new scents and properties of essential oils from the Malaysian rainforest plants it is always on growing (Bodeker, G. et al., 2009).

2.2 SYSTEMATIC INVESTIGATIONS OF CONSTITUENTS FROM EOs

By the 13th century, the pharmacies started to produce the EOs and describe their pharmacological effects in pharmacopoeias. This effort was the beginning to the systematic development of the EOs (Surburg and Panten, 2006). However, the first systematic investigation of constituents from essential has been performed by M. J. Dumas, a French chemist. He analysed some hydrocarbons, oxygen, and sulphur- and nitrogen-containing constituents (Kubeczka, 2010). In 1834, he and PELIGOT had

isolated cinnamaldehyde from cinnamon oil and followed by the isolation of benzyldehyde from bitter almond oil by LIEBIG and WOHLER in 1837 (Surburg and Panten, 2006).

Later, a new part of the chemical industry in the history of natural fragrance materials was opened when the fragrance and flavour chemicals could be produced synthetically and industrially. This shift began with the production of acid esters of several alcohols (in 1845 and 1850), followed by methyl salicylate (1859), benzaldehyde (1870), vanillin (1874) and coumarin (1878) (Surburg and Panten, 2006)

In particular, the study about EOs has brought to a number of scientists be honoured for Nobel Prize. It was began with Otto Wallach (German chemist) who was honoured for Nobel Prize in Chemistry "in recognition of his outstanding research in organic chemistry and especially in the field of alicyclic compounds" in 1910 (Surburg and Panten, 2006). He has dedicated his life to study about terpenes, which enormously found as the major constituents in EOs. His book, *Terpene und Campher* was a compilation of his 180 articles and the knowledge on terpenes (Kubeczka, 2010). His efforts have resulted in the most important finding in the study of terpenes, which is the discovery of isoprene rule. This rule explained that the terpene compounds were constructed from isoprene unit (C_5H_8) which joined together in a repetitive head-to-tail manner (Carson and Hammer, 2011 and Kubeczka, 2010).

Instead of Wallach, Leopold Ruzicka also was awarded the Nobel Prize in Chemistry (1939) for his outstanding investigations in structure elucidation for his work on "polymethylenes and higher terpenes". This followed by D.H.R Barton (English chemist) who was awarded the Nobel Prize in Chemistry in1969 for his discovery on the structure of caryophyllene which has a 4- and 9-membered ring (Carson and Hammer, 2011 and Kubeczka, 2010).

Other chemists who also showed their contributions were F.W Semmler and G. Wagner (1899); they discovered about rearrangement for the elucidation of chemical constitution on some of acyclic monoterpenes like geraniol, linalool, and citral. This investigation was continued by H. Meerwein (1914) and later he generalized it as Wagner-Meerwein rearrangement. Furthermore, J. Read, W. Huckel, H. Schmidt, W. Treibs, and V. Prelog had explained the stereochemical structures that occur on

menthols, carvomenthols, borneols, fenchols, pinocampheols and the related ketones (Kubeczka, 2010).

As a consequence, these systematic investigations done by the researchers have led to a lot of structure elucidations on terpenes. Moreover, nowadays, with the existence of many types of modern equipment and the use of sophisticated chromatographic and spectroscopic techniques may allow the research on it could be done extensively; thereby, the interesting components may be produced synthetically (Kubeczka, 2010).

2.3 CHEMISTRY OF EOS

EOs consist of complicated natural mixtures which may contain up to 100 components (Carson and Hammer, 2011). However, the common numbers of components reported are between 20-60 (Bakkali et al., 2008 and Carson and Hammer, 2011). These components are comprised of terpenes as the main group while phenylpropenes and other compounds also present in smaller or in trace amounts (Bakkali et al., 2008 and Benchaar and Greathead, 2011). Some cases showed that the major compounds can constitute up to 85% whereas other components are present as trace (Burt, 2004 and Espina et al., 2011). Terpenes group arises from mevalonic pathway while phenylpropenes come from the shikimic or shikimate pathway (Figure 2.1) (Benchaar and Greathead, 2011; Dewick, 2002 and Hyldgaard et al., 2012).

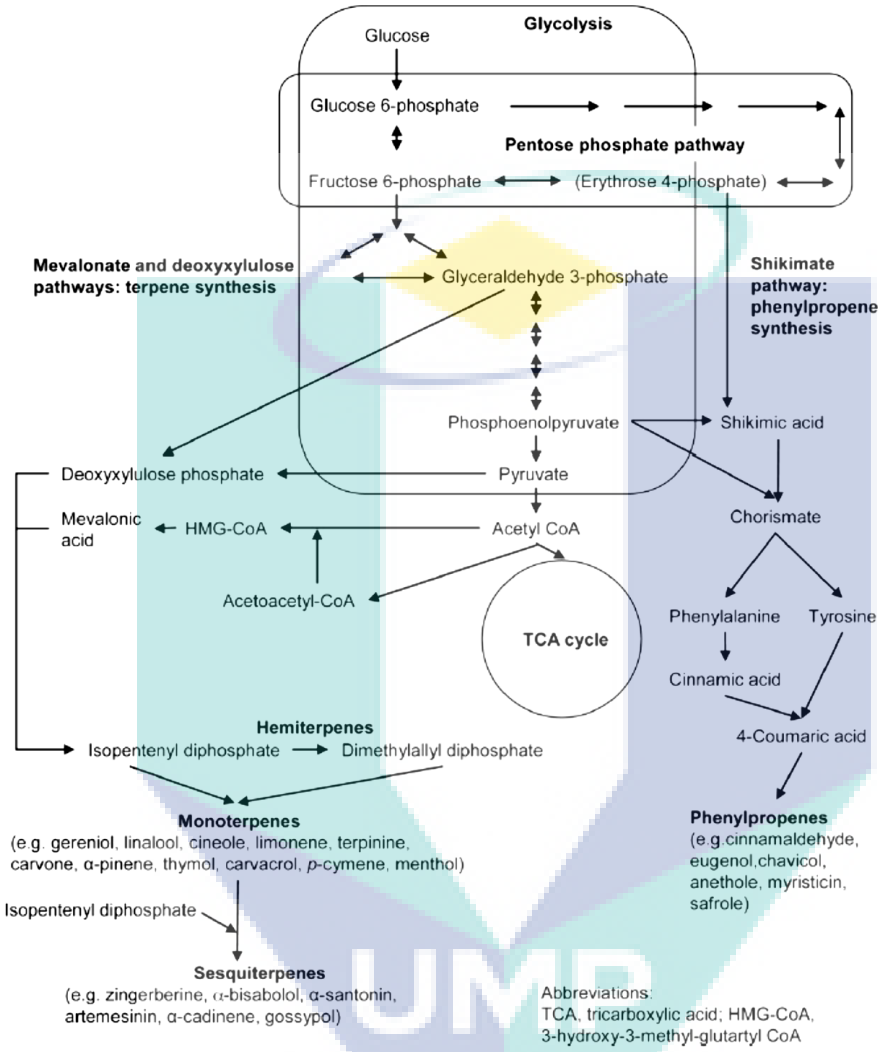


Figure 2.1: Biological pathway of terpenes and phenylpropenes

Source : Benchaar and Greathead (2011)

2.3.1 Terpenes Group

Terpenes form the largest group of natural compounds with more than 30,000 structures idendtified (Breitmaier, 2006 and Carson and Hammer, 2011;). Terpenes in EOs consist of the combination of isoprene units (2-Methyl-1,3-butadiene); which contain five carbon atoms per unit $(C_5)_n$. This unit may combine from head-to-head, tail-to-tail, or head-to-middle joining to form monoterpenes (C_{10}), sesquiterpenes (C_{15}), diterpenes (C_{20}), sesterterpenes (C_{25}), triterpenes(C_{30}) and tetraterpenes (C_{40}). However, hemiterpenes may also exist (Figure 2.2) (Bauer et al., 2001 and Breitmaier, 2006).

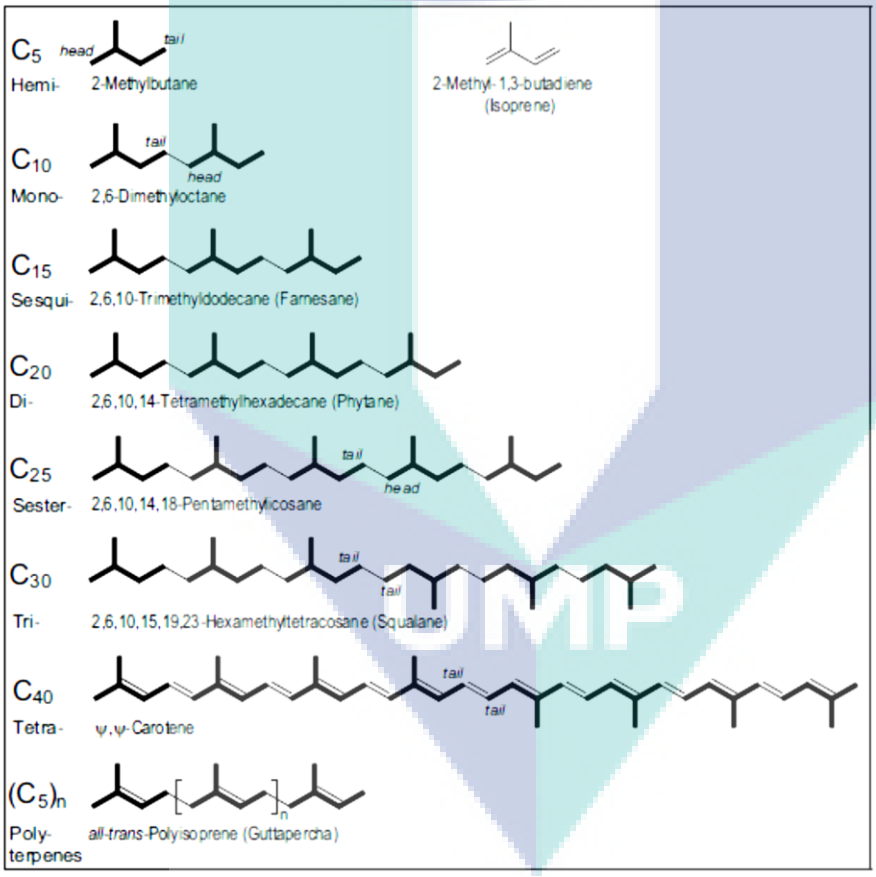


Figure 2.2: Examples of terpenes

Source: Breitmaier (2006)

(i) Biosynthesis of Terpenes

Terpenes group are arisen from mevalonic pathway; this pathway is started from mevalonic acid (MVA) (Figure 2.3), a compound substance that is essential to a plant's life. Mevalonic acid which contains six carbon atoms will convert to a-five carbon structure through isoprene arrangement. This unit will subsequently make up hemi-, mono-, sesqui-, and diterpenes (1, 2, 3, and 4 isoprene unit respectively). Most of the EOs are commonly composed of monoterpenes (constitute up to 90%) and sesquiterpenes. Molecular structure of terpenes arranged in chain and ring. All terpenes end in suffix –ene except if they are joining to form terpenoids (Buckle, 2003; Breitmaier, 2006 and Sell, 2010).

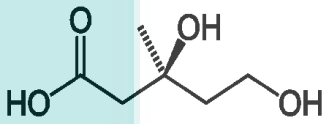


Figure 2.3: Mevalonic Acid

The biosynthesis of terpenes begins with the formation of isopentenyl pyrophosphate (IPP) from mevalonate/mevalonic acid. This mevalonic acid derived from acetyl-CoA (produced in mitochondria). Subsequently, IPP is next converted to its isomer, dimethylallyl pyrophosphate (DMAPP) by isopentenyl PP isomerase. Next, geranyl pyrophosphate (GPP) is generated during a condensation reaction between IPP and DMAPP (IPP + DMAPP). Then, farnesyl PP is formed from condensation reaction between GPP and IPP catalysed by geranyl transferase. The GPP is responsible to produce monoterpenes while sesquiterpenes is generated from farnesyl PP (Figure 2.4) (Breitmaier, 2006; McKee and McKee, 2003; Ramawat et al., 2009 and Sell, 2010).

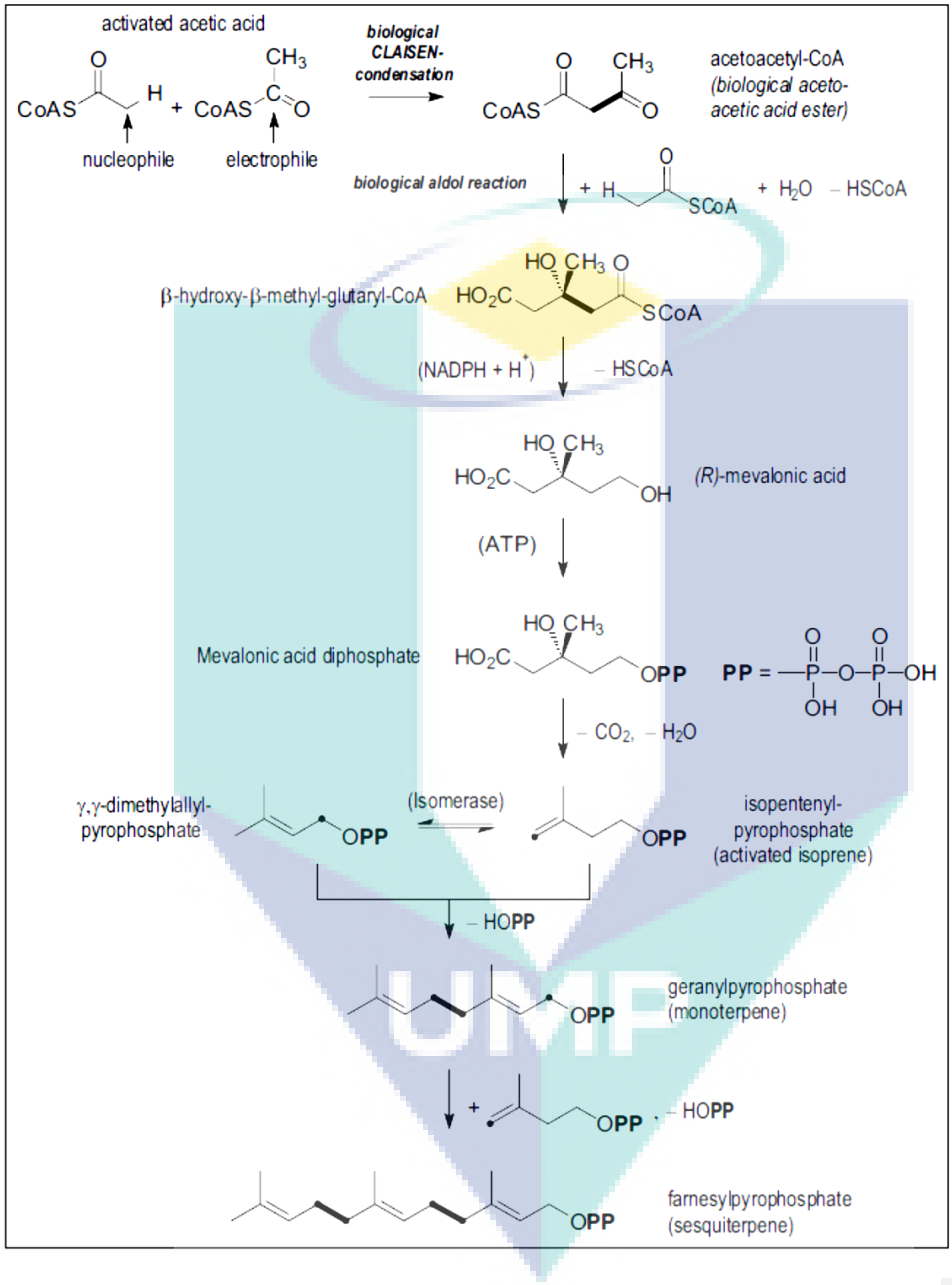


Figure 2.4: Synthesis of mono- and sesquiterpenes

Source: Breitmaier (2006)

(ii) Monoterpenes

Monoterpenes are formed from the joining of two C_5 isoprene units that make up a selection with the molecular formula $C_{10}H_{16}$ (Carson and Hammer, 2011). Generally, they constitute the highest composition of the EOs with a variety of structures formed whether with substitutions, cyclizations and/or isomerizations. They may be in form of cyclic or acyclic. Types of cyclic monoterpenes may be as monocyclic, bicyclic, and even tricyclic compounds (Buckle, 2003 and Sell, 2010). Table 2.1 shows some examples of monoterpenes.

Table 2.1: Examples of monoterpenes

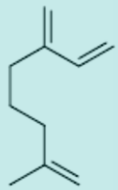
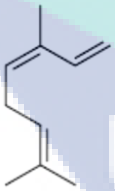
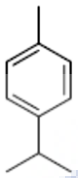
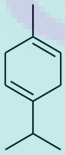
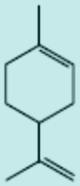
Monoterpenes	Structure	Plants	References
i) acyclic a) myrcene b) ocimene	 Myrcene	*Salvia potentillifolia*	(Duru et al,. 2009)
	 Ocimene	*Ocimum basilicum L.*	(Telci and Bayram, 2006)

Table 2.1: Continued

ii) monocyclic a) p-cymene b) terpinene c) limonene	 p-cymene	*Salvia montana*	(Djenane et al., 2011)
	 Terpinene	*Ocimum gratissimum*	(Cimanga et al., 2002)
	 Limonene	*Lemon (Citrus lemon)*	(Espina et al., 2011)
iii) bicyclic a) sabinene b) pinene	 Sabinene	*Nigella sativa*	(Bourgou et al. 2012)
	 Pinene	*Juniperus excels*	(Salehi et al. 2010)

Acyclic monoterpenes generally formed from head-to-head arrangement of isoprene units, while cyclic monoterpenes are derived from a cyclization process by enzymes known as monoterpene cyclises. This process occurred via the universal intermediate, α- terpinyl cation (Carson and Hammer, 2011). Subsequent cyclisation of monocyclic monoterpenes will produce bicyclic monoterpenes and tricyclic monoterpenes. However, the tricyclic monoterpenes are very rare to be found in EOs. Two important examples found in EOs are pinene oxide and tricyclene (Buckle, 2003; Breitmaier, 2006 and Carson and Hammer, 2011).

(iii) Sesquiterpenes

Sesquiterpenes are comprised of three isoprene units attached to form a skeleton with the molecular formula of $C_{15}H_{24}$. They are the second most common constituents found in EOs after monoterpenes. Sesquiterpenes are less volatile compared to monoterpenes because of their larger structures. The sesquiterpenes are derived from FPP by various cyclization process which often followed by skeletal rearrangement. Like monoterpenes, sesquterpenes also may form structure of linear, branched or cyclic (Buckle, 2003; Breitmaier, 2006; Carson and Hammer, 2011). Table 2.2 shows some examples of sesquiterpenes.

Table 2.2: Examples of sesquiterpenes

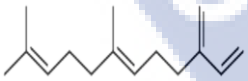
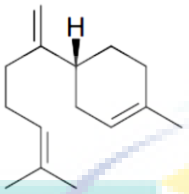
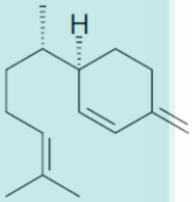
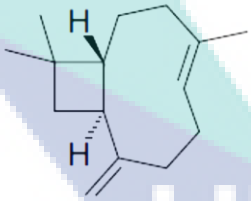
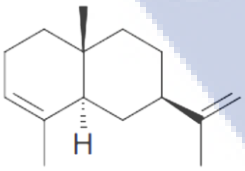
Sesquiterpenes	**Structure**	**Plants**	**References**
i) acyclic a) β-farnesene	 β-farnesene	ginger (*Zingiber officinale* Roscoe)	(Natta et al., 2008)

Table 2.2: Continued

ii) monocyclic a) β-bisabolene b) β-sesquiphellandrene	 β-bisabolene  β-sesquiphellandrene	*Alpinia conchigera* ginger (*Zingiber officinale* Roscoe.)	(Ibrahim et al., 2009) (Natta et al., 2008)
iii) bicyclic a) β-caryophyllene b) α-eudesmene (α-selinene)	 β-caryophyllene  α-eudesmene (α-selinene)	*Lycopus lucidus* *Cymbopogon nardus*	(Yu et al., 2011) (Silva et al., 2011)

(iv) Diterpenes and Norterpenes

Diterpenes formed by head-to-tail combinations of four isoprene units followed by rearrangement and/or substitutions. The general molecular formula for it is $C_{20}H_{32}$. Therefore, they are much heavier than mono- and sesquiterpenes. Thus, this property has made it rarely found in EOs because bigger amount of energy is needed to separate them from plant parts by steam distillation. However, they may be found in solvent extracts. A common diterpenes found in many EOs is phytol and plaunutol (Bakkali et al. 2008; Ramawat et al., 2009; Carson and Hammer, 2011).

(v) Terpenoid

Terpenoid is formed when one oxygen atom is attached into the terpenes structure. Terpenoids may be generated from different functional group such as alcohols, aldehydes, ketone, esters, and phenols (Bakkali et al. 2008). Table 2.3 shows examples of terpenoids classified in the different functional groups.

Table 2.3: Examples of terpenoids classified in the different functional groups

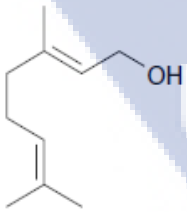
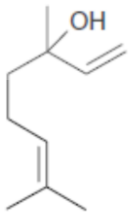
Types of terpenoids	Structures	Plants	References
i) monoterpene alcohols alcohols a) geraniol b) linalool c) citronellol	 geraniol	Palmarosa oil	(Chen and Viljoen, 2010)
	 linalool	*Ocimum basilicum L.*	(Hussain et al., 2008)

Table 2.3: Continued

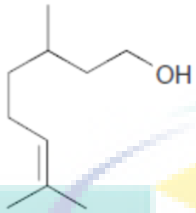
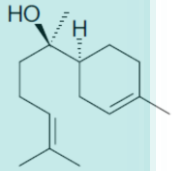
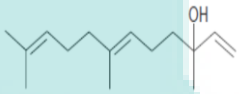
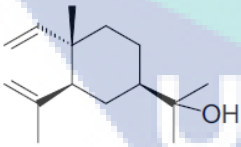
ii) sesquiterpene alcohols a) α-bisabolol b) nerolidol c) β-elemol	 citronellol	*Cymbopogon nardus*	(Nakahara et al., 2003)
	 α-bisabolol	*Semenovia tragioides*	(Bamoniri et al., 2010)
	 nerolidol	*Aniba canelilla*	(Taveira et al., 2003)
	 β-elemol	*Cymbopogon jawarancusa*	(Dar et al., 2011)

Table 2.3: Continued

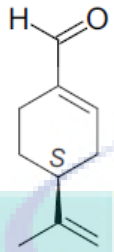
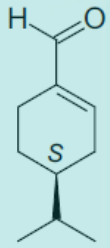
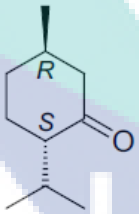
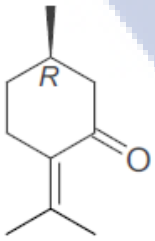
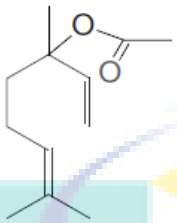
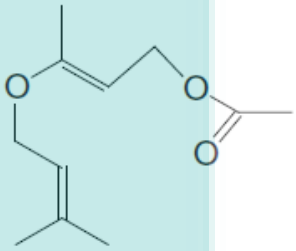
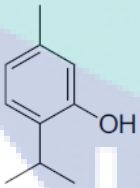
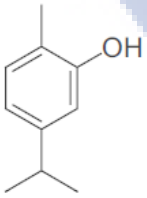
ii) aldehydes mono a) perillaaldehyde b) phellandral	 perillaaldehyde  phellandral	Perilla oil *Phellandrium aquaticum*	(Inouye et al., 2001) (Breitmaier, 2006)
iii) ketones a) menthone b)pulegone	 menthone  pulegone		(Teixeira et al., 2012) (Teixeira et al., 2012)

Table 2.3: Continued

iv) esters a) linalyl acetate b) geranyl acetate	 linalyl acetate	*Lavandula angustifolia*	(Buckle, 2003)
	 geranyl acetate	*Cymbopogon martini*	(Buckle, 2003)
v) Phenols a) Thymol b) Carvacrol	 thymol	*Thymus vulgaris*	(Bagamboula et al., 2004)
	 carvacrol	*Thymus vulgaris*	(Bagamboula et al., 2004)

2.3.2 Phenylpropenes

The aromatic compounds are derived from the phenylpropenes, which composed of the C_6C_3 skeleton (six carbon aromatic ring with a three-carbon side chain) (Figure 2.3). Generally, they occur less frequently or less abundantly than terpenes or terpenoids in EOs (Bakkali et al. 2008). However, some of the EOs in which phenylpropenes do occur contain significant percentages of them, such as eugenol in clove oil (*Syzygium aromaticum*), constituted of 75 to 90% of the oil (Dewick, 2002), *Cinnamomum cassia* (cinnamon) which is rich in cinnamaldehyde (75.3%) (Hyldgaard et al. 2012) , and methyl chavicol (75.23%) presented in *Artemisia dracunculus* (Oussalah et al. 2006). It is reported that plant families in which phenylpropenes present more commonly are Apiaceae (Umbilliferae), Lamiaceae, Myrtaceae, Piperaceae and Rutaceae (Carson and Hammer, 2011).

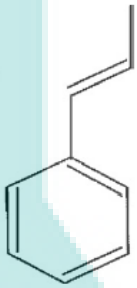


Figure 2.5: Phenylpropyl buiding unit

Source : Benchaar and Greathead (2011)

(i) Biosynthesis of Phenylpropenes

Phenylpropenes are synthesized *via* the the shikimate pathway (Figure 2.1). This pathway is responsible for the synthesis of many of the phenolic compounds in plants and produces the aromatic amino acids phenylalanine, tyrosine and tryptophan (Carson and Hammer, 2011 and Ramawat et al., 2009).

The name of this pathway arises from shikimic acid, which is one of the pathway intermediates (Figure 2.4). Phenylalanine (Figure 2.5), which is produced from chorismate and shikimic acid, generates cinnamic acid from deamination reaction. It may also be synthesized from deamination of tyrosine. After hydroxylation, the

cinnamic acid changes to yield 4-coumaric acid (Figure 2.6), which further synthesize the phenylpropenes (e.g cinnamaldehyde, eugenol, anethole, myristin and safrole) (Benchaar and Greathead, 2011 and Dewick, 2002). Table 2.4 shows some examples of phenylpropenes presented in EOs.

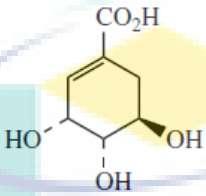


Figure 2.6: Shikimic acid

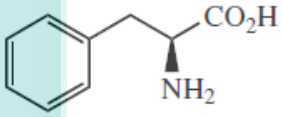


Figure 2.7: Phenylalanine

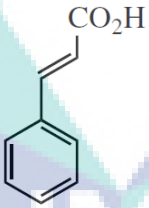


Figure 2.8: Cinnamic acid

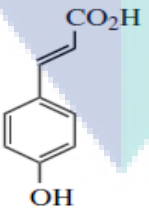
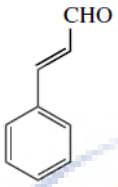
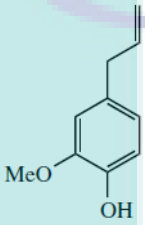
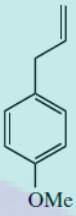
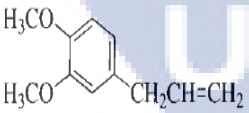
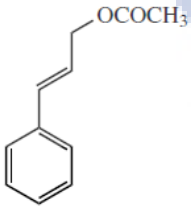


Figure 2.9: 4-coumaric acid

Source: Dewick (2002)

Table 2.4: Examples of phenylpropenes presented in EOs

Polypropenes	Structures	Plants	References
i)cinnamaldehyde ii)eugenol iii)estragole (methyl chavicol) iv)methyl eugenol v)cinnamyl acetate	 cinnamaldehyde	*Cinnamomum zeylandicum*	(Burt, 2004)
	 eugenol	oil of clove	(Devi et al., 2010)
	 estragole	*Pimpinella anisum* seeds	(Al-bayati, 2008)
	 methyl eugenol	*Aniba canilella*	(Taveira et al., 2003)
	 cinnamyl accetate	bark of cinnamon (*Cinnamomum zeylanicum)*	(Dewick, 2002)

2.4 GENUS *Cymbopogon*

The genus *Cymbopogon* (grasses), which is a family of Gramineae has about 180 species, subspecies, varieties and subvarieties (Bertea and Maffei, 2010). This type of grasses grows in tropical and subtropical regions around the world from mountains to grasslands to arid zones (Akhila, 2010; Bertea and Maffei, 2010; Figueirinha et al., 2008 and Machado et al. 2012). Among the species, it has been reported that 52 are exist in Africa, 45 in India, six in Australia and South America respectively, four in Europe, two in North America and the rest are grown in South Asia (Khanuja et al., 2005).

This genus *Cymbopogon* was named by Sprengel in 1815. Eventhough there was a suggestion to classify this genus as a subgenus to *Andropogon*, Stapf (1906) remained the genus as its original name because of the difference in the plant traits found between the *Andropogon* and *Cymbopogon* (Bertea and Maffei, 2010 and Khanuja et al. 2005).

Cymbopogon plants are aromatic perennial grasses, can grow up one to two meters high with narrow and long leaves which generally recognized by the appearance of silica thorns aligned on the leaf edges (Bertea and Maffei, 2010). Specifically, the most valuable source for industries that produced from this genus *Cymbopogon* is its EOs. These EOs are used in beverages, foodstuffs, fragrances, household products, personal care products, pharmaceuticals, and in tobacco (Akhila, 2010).Therefore, there is a large worldwide demand for these EOs (Pandey, 2010 and Tiwari, 2010).

Generally, the EOs of the *Cymbopogon* species mainly constituted of mono- and sesquiterpenoids. For instance, they are well-known as a source of commercially valuable compounds such as geraniol, geranyl acetate, citral (neral and geranial), citronellal, and piperitone; which commonly used in perfumery and allied industries or as precursor for the synthesis of other products (Pandey, 2010).

Among the species, five of them have been identified to produce three oils of main commercial importance which are: lemongrass from *C. citratus* of Malaysian origin (known as West Indian lemongrass) and *C. flexuosus* from India, Sri Lanka, Burma and Thailand (known as East Indian origin); palmarosa oil from *C. martini*;

citronella oil from *C. nardus* (Sri Lanka), and *C. winterianus* (Java) (Akhila, 2010 and Tiwari, 2010).

2.4.1 *Cymbopogon citratus*

C. citratus is one of the commercial importances for its essential oil (Figure 2.10). *C. citratus*, which is known as lemongrass was originated from Malaysia and has been recognized as West Indian lemongrass (Akhila, 2010). In Malaysia, it is called as 'serai'. Lemongrass is a tufted perennial grass with numerous stiff leafy stems arising from short rhizomatous rootstocks. Commonly, the aboveground parts (may come from leaves and flowering tops) will contain the essential oil. The essential oil usually contained in oil glands, veins or hairs that are often very fragile. This species prefer to grow in a warm climate with well-distributed rainfall and well-drained soil (Akhila, 2010; Ong and Nordiana, 1999 and Pandey, 2010).

(a) (b) (c)

Figure 2.10 : Picture of *Cymbopogon citratus* (a) plant (b) leaves and (d) stem/stalks

This lemongrass is widely used in Asian cooking, in folk-medicine to treat gastrointestinal disturbances; also known as antispasmodic, analgesic, anti-inflammatory, anti-pyretic, diuretic and anti-sedative (Bassolé et al. 2011; Naik et al. 2010 and Tiwari, 2010). A study by Ong and Mardiana (1999) on Malay ethno-medico in Machang, Kelantan, Malaysia reports that the villagers used pounded leaves of *C. citratus* to relieve the headache while its decoction is drunk to treat stomachache and cracks in the feet. In addition, the Sundanese community in West Java, Indonesia also uses its decoction for muscle pain relief (Roosita et al., 2008). Instead of traditional

medicine, it also has been used widely in soap, perfumery, cosmetics and the beverages industry (Rauber et al., 2005a and Saddiq and Khayyat, 2010).

The name of lemongrass is due to the lemony odor and flavor of the essential oil, which highly contains in citral compound. It is mobile and pale yellow in colour. The lemongrass also has been used in teas, soups, and curries (Saddiq and Khayyat, 2010). Because of *C. citratus* essential oil has lower citral content and lower solubility in alcohol, it is less favoured compared to East Indian type (*C. flexuosus*). The lower solubility is because of myrcene content which may undergo diene-condensation and polymerization on aging. However, after World War II, it became important because the latter was difficult to obtain (Akhila, 2010 and Tiwari, 2010).

Chemical composition and oil content of. *C. citratus* may be influenced by several factors: physiological variations, environmental conditions, geographic variations, genetic factors and also harvest time and technique. Harvesting time with correct handling procedure and the right postharvest management (predistillation handling, drying, storage and distillation) will enhance the essential oil content and the citral composition (Khanuja et al., 2005 and Pandey, 2010). It was reported that the oils yield will increase after one year until the third year and started to decline after these. It also may sustain on average, for 3-5 years (Pandey, 2010).

It has been reported by many literatures that citral (3,7-dimethyl-2,6-octadienal) is the main component contained in this essential oil. Citral consists of two isomers namely geranial (trans-citral, citral A) and neral (cis-citral, citral B) (Figure 2.11). It may present 65-85% from its total chemical composition with the ratio of 60:40. The content of citral will determine the quality of the essential oil (Bassolé et al. 2011; Moyler, 2010; Nguefack et al., 2012; Rauber et al., 2005 and Santin et al., 2009).

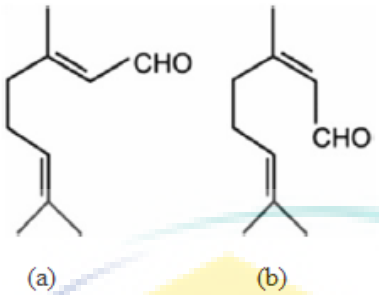


Figure 2.11: Chemical structure of (a) geranial and (b) neral

Source: Saddiq and Khayyat (2010)

Other components that may contain in this species are geraniol, myrcene, and geranyl acetate (Nguefack et al. 2009; Nguefack et al. 2012 and Yang et al., 2009). The citral compound isolated from its essential oil is vital as the precursor to synthesize beta-ionone. Beta-ionone is further used to produce vitamin A and for synthesis of a number of aroma chemicals that widely used in perfumery and cosmetics (Pihlasalo et al., 2007 and Yang et al., 2009).

2.4.2 *Cymbopogon nardus*

Cymbopogon nardus is a perennial grass cultivated in Southeast Asia. In Malaysia, it is known as 'serai wangi'. *C. nardus* is a drought-resistant hardy grass and has a hairy and fibrous shallow root system with long linear lanceolate leaves (Figure 2.12). It produces large, fawn-colored inflorescence with white, hairy, star-like spiked flowers. It needs pH soil of 7.5-8.5 for an ideal cultivation and the temperatures ranging from 10ºC-36ºC with annual rainfall around 1000-1500mm and ample sunshine are congenial for its growth (Akhila, 2010; Nakahara et al., 2003 and Pandey, 2010).

(a) (b) (c)

Figure 2.12: Picture of *Cymbopogon nardus* (a) plant (b) leaves and (d) stem/stalks

The essential oil from *C. nardus* is classified as citronella oils as well as *C. winterianus*. The name of citronella oils is given because EOs from both of these plants produce citronellal compound (Figure 2.13), which is important for industrial use (Akhila, 2010 and Tiwari, 2010). Citronella oil is an important source for perfumery chemicals such as citronellol, citronellal, and geraniol, which are widely used in perfumery, soaps, detergents, industrial polishes, cleaning compounds, and their industrial products (Silva et al., 2011). In industry, citronellal is used as a starting material for further derivatives such as hydroxycitronellal and citronellol. These derivatives are added in perfumery industry and personal care products. Instead of that, citronellal is also important in the synthesis of pheromones, alicyclic, cyclic and polycyclic compounds (Lenardão et al., 2007 and Pandey, 2010) .

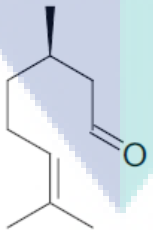


Figure 2.13: Structure of citronellal

However, to differentiate between them in the trade, *C. nardus* is classified as Ceylon citronella oil while *C. winterianus* is called as Java citronella oil. The classification is given because there is a major difference between these oils in proportion geraniol and citronellal. Generally, *C. winterianus* contains higher percentage of citronellal and geraniol compared to *C. nardus* (Khanuja et al. 2005; Pandey, 2010 and Tiwari, 2010). They are also distinguished morphologically by the shape and length of their leaves. There is also a study reported that the presence of phenolic derivatives (methyl eugenol and methyl isoeugenol) is the most significant difference between the Ceylon-type and Java-type oils. The presence of elemol in the Ceylon-type has been suggested to be formed as an artefact (Akhila, 2010).

The main component contained in *C. nardus* is citronellal (Clemente et al. 2010; Lenardão et al. 2007 and Rodríguez et al., 2012). However, it may also contain minor components like geranial, neral, geraniol, linalool, limonene and camphene (Clemente et al. 2010; Nakahara et al. 2003; Oussalah et al. 2007 and Silva et al., 2011). *C. nardus* have been reported to possess biological activities including antibacterial, antifungal, anticancer, pesticidal, antihelmintic, antiviral, antigenotoxic, antioxidant, mosquito repellent, mosquito larvicidal, anti-inflammatory, analgesic, and hypoglycaemic (Bahtiar et al., 2011; Clemente et al., 2010; Nakahara et al., 2003; Nerio et al., 2010; Silva et al., 2011 and Tyagi et al., 1998).

The Malay ethno-medico botany in the Machang district, Kelantan state, Malaysia have used this plant to make hair shampoo and as water bath to refresh the body and get rid of body smell (Ong and Nordiana 1999). The Medicinal Plants Research in FRIM (Forest Research Institutes Malaysia) has marked the *C. nardus* as a potential plant to be commercialized as cosmeceutical products and they have studied about it scientifically and processed using modern techniques to ensure a good quality and safely used for consumers (Rasadah and Ali, 2008).

2.5 USE OF GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GCMS) IN ANALYSIS OF ESSENTIAL OILS CHEMICAL COMPOSITION

Nowadays, Gas Chromatography-Mass Spectrometry (GC-MS) is the most frequent instrument used by the researchers for the analysis of essential oils (Kubezcka, 2010) (Figure 2.14). This instrument has been acknowledged as a well-established technique because of the development of easy-to-handle powerful systems concerning sensitivity, data acquisition and processing, and above all their relatively low cost (Kubezcka, 2010).

Figure 2.14: Gas Chromatography-Mass Spectrometry

Gas chromatography (GC) is an excellent tool for the separation, characterization, and quantitative analysis of essential oils. The composition of essential oils which mainly constituted of monoterpenes and sesquiterpenes contained of many geometric, positional, optical isomers and most of them are unstable; this is the advantage of using gas chromatography because it could separate many of these components. In addition, it has advantages of being fast, of using an inert atmosphere, and of requiring small sample sizes (Burchfiled and Storrs, 1970).

In particular, GC resembles as column chromatography in principle; but there are three differences of GC compared to column chromatography: first, the partitioning processes for the compounds to be separated in GC are carried out between a moving gas phase and a stationary liquid phase; second is that the temperature of the gas system can be controlled because the column is contained in an insulated oven; and the third is

the concentration of any given compound in the gas phase is a function of its vapour pressure only (Pavia et al., 2013).

Regarding to mass spectrometry (MS), it is a sophisticated instrumental technique that produces, separates, and detects ions in a gas phase (Christian, 2004). At its simplest, MS measures the molecular weight, and therefore the formula, of a molecule (McMurry, 2011). Generally, there are three basic parts of MS: an ionization source in which sample molecules are given an electrical charge; a mass analyzer in which ions are separated by their mass-to-charge ratio, *m/z*; and a detector in which the ions are observed and counted (McMurry, 2011).

In short, the GC-MS applications today mostly utilize one dimensional capillary GC with quadrapole MS detection and electron ionization. However, there are considerable numbers applying different types of mass spectrometers and ionization techniques. In chemical composition analysis, mass spectral libraries (e.g., NIST/EPA/NIH 2005; WILEY Registry 2006; MassFinder 2007; and diverse printed versions such as Jennings and Shibamoto, 1980; Joulain and Konig, 1998; and Adams, 1989, 1995, 2007 inclusive of retention indices) are provided (Kubezcka, 2010).

2.6 BACTERIA

Bacteria are the smallest free-living organisms and classified under prokaryotes group. Unlike with eukaryotic cells (which contained organelles like nucleus, mitochondria, and lysosomes), the prokaryotes normally contain a single chromosome which is not separated from the other cell contents by membrane (Figure 2.15). The functions of the organelles in prokaryotic cell like bacteria have been substituted by the plasma membrane and the cell wall. Both plasma membranes and cell walls play important role in order to maintain the cell integrity and to control the activities occurred in and out of the cells and between its internal compartments (Talaro and Talaro, 2002 and Thomas, 2007). Some bacteria are known as pathogens which can cause many diseases in humans but some also have shown their importance in industrial, pharmaceutical and medical use (Denyer et al., 2004).

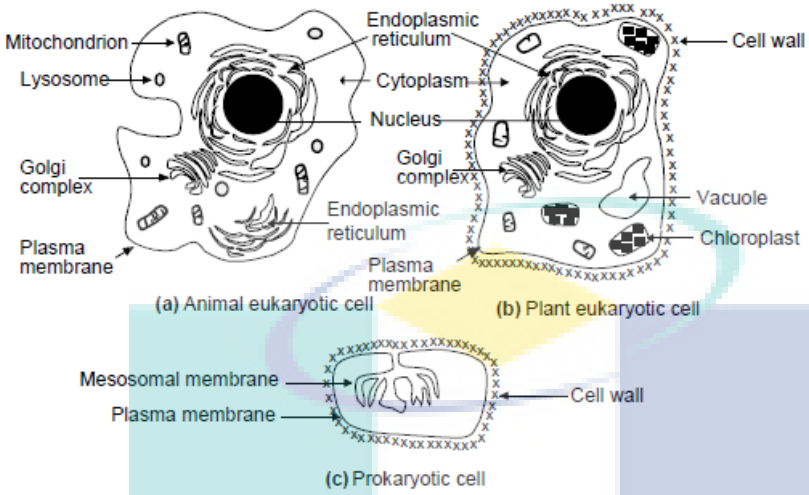


Figure 2.15: The difference between eukaryotic cell and prokaryotic cell

Source: Thomas (2007)

2.6.1 Cell size and shape

As the bacteria are the smallest free-living organisms, their size being measured in micrometres (microns). Their size may vary from 0.1 to 10µm; but most of the bacteria are commonly 1-10µm in size. Most spherical bacteria have diameters of 0.2 to 2µm, and rod-shaped cells are generally 0.2 to µm wide and 1 to 10µm long. They may exist in a few forms such as spheres; also called as cocci (singular: coccus), rods; are called bacilli (singular: bacillus), bent or curved rods, and spirals; are called spirilla if the cells are rigid and spirochets if they are more flexible and undulating (Figure 2.16) (Allison and Gilbert, 2004 and Neidhardt, 2004).

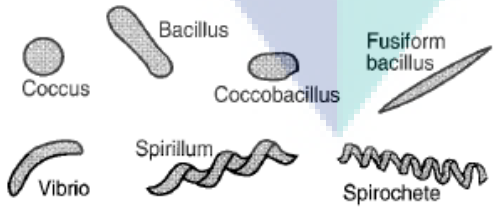


Figure 2.16: Shapes of some different bacteria

Source: Neidhardt (2004)

2.6.2 Bacterial Diversity

Bacteria are generally able to survive independently from other cells in order to carry on their life processes of growth, energy generation and reproduction. For instance, they can live in a multiplicity of environments ranging from hot sulphur springs (65ºC) to deep freezers (-20ºC), from high (pH 1) to low (pH 13) acidity and high (0.7M) to low osmolarity (water). Moreover, they can grow in both nutritionally rich (compost) and nutritionally poor (distilled water) situations. Therefore, they are regarded as ubiquitous because no natural environment that is free from bacteria However, most of the well-characterized bacteria live best at temperature 25ºC-45ºC and pH growth optima between 7.4 and 7.6 but may grow suboptimally at pH values of 5-8.5 (Allison and Gilbert, 2004).

2.6.3 Bacteria Cell Wall

The bacteria are divided into two groups namely Gram-positive and Gram-negative. This classification is according to their reaction to a staining procedure which has been developed by Christian Gram in 1884 (Denyer et al., 2004). The Gram stain procedure uses a series of stains and reagents in order to distinguish the bacteria based on the cell wall structure. A film of bacteria dried on a microscope slide with a solution of crystal violet, followed by a solution of iodine; were then washed with an alcohol solution (Denyer et al., 2004).

Bacteria which lose the crystal-violet iodine complex and rendered colourless are called Gram-negative whereas Gram-positive cells retain the dye. On the other hand, if the red coloured dye, carbol fuchsin is used, under the light microscope, the Gram-negative cells will appear red while Gram-positive cells are purple. The differences are reflected from the differences in the wall structure. The Gram-positive cell wall primarily consists of a single type of molecules whereas the Gram-negative cell wall is a multilayered and quite complex (Figure 2.17) (Allison and Gilbert, 2004). The primary function of the cell wall is to provide a strong, rigid structural component that can prevent the osmotic pressures caused by high chemical concentrations of inorganic ions in the cell from swelling and bursting (Allison and Gilbert, 2004 and Thomas, 2007)

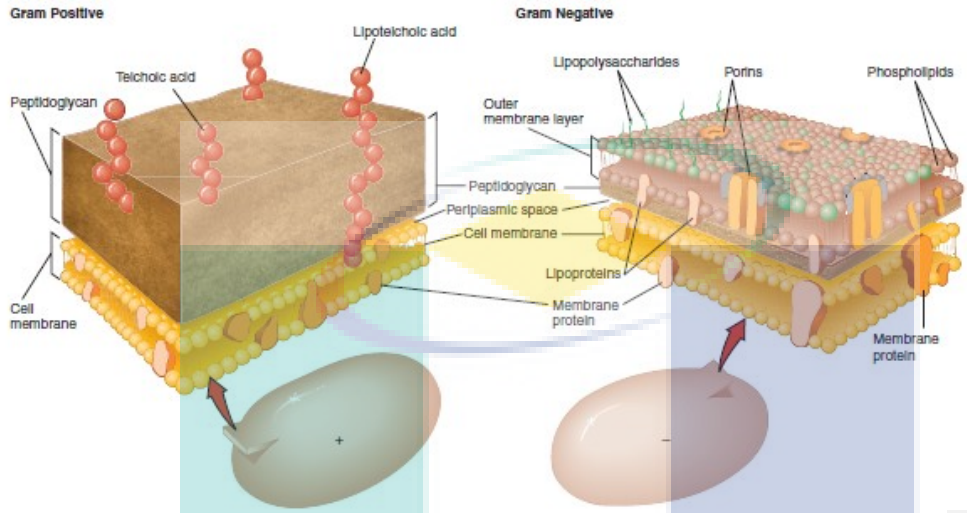


Figure 2.17: The difference between gram positive and gram negative bacteria

Source: Talaro and Talaro (2002)

Both Gram-positive and Gram-negative bacteria have structural component called peptidoglycan (also called murein or glycopeptide). The peptidoglycan is a large molecule containing glycan (polysaccharide) chains that are cross-linked by short peptide bridges. The polysaccharide chains consist of alternating 1-4-liked β-*N*-acetylmuramic acid (NAM) and β-*N*-acetylglucosamine (NAG) units, while the tetrapeptide chains ahich consisting of L-alanine, D-alanine, D-glutamic acid and either lysine or diaminopimelic acid (DAP) are attached at NAM units of the polysaccharide chains (Figure 2.18) (Allison and Gilbert, 2004 and Thomas, 2007).

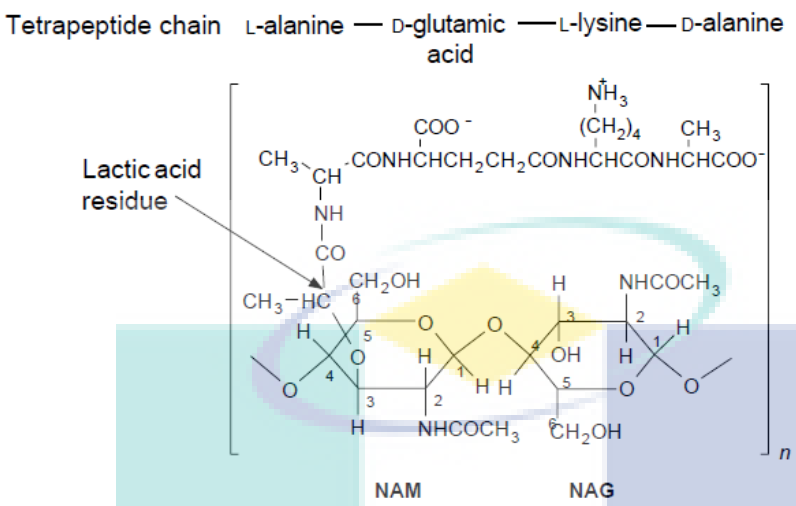


Figure 2.18: The peptidoglycan structure

Source: Thomas (2007)

(i) Cell Walls in Gram-positive bacteria

The cell walls in Gram-positive bacteria are quite thick (20-80 nm) and consist of between 60% and 80% peptidoglycan. Gram-positive walls normally covered by by *teichoic acid*s as the exterior surface (Figure 2.19). Teichoic acids consist of either ribitol phosphate or glycerol phosphate molecules that are attached to peptidoglycan by phosphate diester bridges. In some gram-positive bacteria, the glycerol-teichoic acids which are bound to membrane lipoids are called lipoteichoic acids. The functions of these acids are as receptors to bacteriophages and for antigenic properties (Allison and Gilbert, 2004; Neidhardt, 2004 and Thomas, 2007).

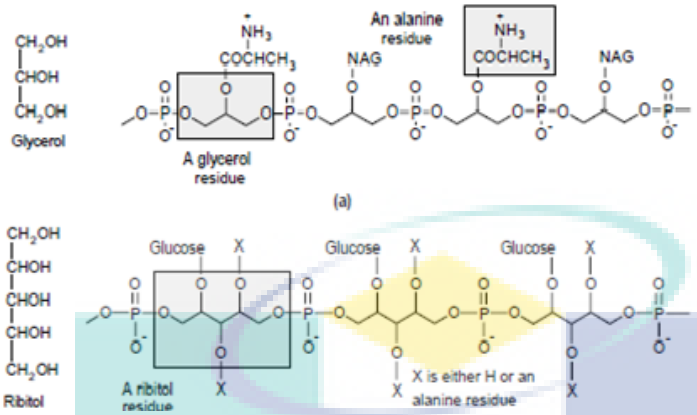


Figure 2.19: (a) A glycerol based-teichoic acid and (b) A ribitol based-teichoic acid

Source: Thomas (2007)

(ii) Cell walls in Gram-negative bacteria

The cell walls or envelope of Gram-negative bacteria are quite complicated compared to Gram-positive bacteria because even though they contain less peptidoglycan (10-20 % of wall), but they are coated by a second membrane structure outside the peptidoglycan layer. This outer membrane consisting of proteins, lipopolysaccharide (LPS) and phospholipids. The lipid-polysaccharide structure is separated from plasma membrane by aqeous compartment known as *perisplasmic space* (Figure 2.17) (Neidhardt, 2004 and Talaro and Talaro, 2002). The molecule of LPS consists of lipid A, core polysaccharide and O-specific polysaccharide. Lipid A is linked to the core polysaccharide by a molecule namely ketodeoxyoctonate (KDO). The LPS structure is an important molecule because it determines the antigenicity of the Gram-negative and it is very toxic to animal cells (Allison and Gilbert, 2004). Table 2.5 shows the major differences in wall composition between Gram-positive and Gram-negative cells.

Table 2.5: Gram-positive and Gram-negative cell wall composition

Feature	Gram-positive cells	Gram-negative cells
Peptidoglycan	60-80%	10-20%
Teichoic acid	Present	Absent
Lipoteichoic acid	Present	Absent
Lipoprotein	Absent	Present
Lipopolysaccharide	Absent	Present
Protein	c. 15%	c. 60%
Lipid	c. 2%	c. 20%

2.7 EOs AS ANTIBACTERIAL

Many studies have proved that EOs showed many biological activities such as antibacterial, antifungal, antiviral, antioxidant and insecticidal. Generally, the efficacy of these biological activities may be attributed to the chemical composition consisted in the EOs. The chemical composition that involved may be contributed either from the content of the major or minor compounds or the combination of both of them (Bakkali et al., 2008; Bassolé and Juliani 2012; Burt 2004 and Fadli et al., 2012). However, many factors may influence the chemical composition of the EOs such as types of plants, different plant parts, methods of extraction and types of solvent used, climate changes, and soils of geographical (Hussain et al., 2008; Telci and Bayram 2006 and Wang et al., 2012).

2.7.1 Methods Used For Antibacterial Activity Testing

Although there are no standardised methods developed for antibacterial assessment of essential oil, the Clinical and Laboratory Standards Institute (CLSI) method for antibacterial susceptibility testing, which is mainly aimed at the testing of antibiotics has been modified for testing EOs (Burt, 2004 and Qaiyumi, 2007). The commonly used methods include disk diffusion, macro- and microbroth dilution and agar well dilution (Burt, 2004; Hanlon, et al., 2007; Pillai, et al., 2005; Qaiyumi, 2007 and Wenger, 2007). However, the published data are still quite difficult to compare because of a few factors such as the method used to extract the essential oil from plant

material, the volume of inoculum, growth phase, culture medium used, pH of the media and incubation time and temperature (Burt, 2004 and Tajkarimi et al., 2010). In spite of these, most of the researchers prefer to use the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) evaluation in order to determine the performance of the EOs (Bassolé and Juliani, 2012; Burt, 2004 and Tajkarimi et al., 2010).

MIC is the lowest concentration of an antibacterial agent that will inhibit the growth of the organism being tested (visually no growth by comparing with the growth control); the lower the MIC, the greater the antibacterial activity (Qaiyumi, 2007). The MIC result may determine the concentration of antibacterial agent needed to inhibit the pathogen. On the other hand, MBC is the lowest concentration of the antibacterial agent at which incubated microorganism was 99.9% killed. It is determined after a certain volume of clear wells from MIC results are spreading on agar media (Qaiyumi, 2007).

As a screening of antibacterial activity of EOs, the disc diffusion method is the most often technique used by the published data for the preliminary study. In this method, the paper discs are impregnated with EOs and are placed on the inoculated surface of an agar plate. After incubation, the zone of inhibition or the clear zone with no bacteria growth is measured (Burt 2004; Hussain et al., 2008 and Tajkarimi et al., 2010). It follows the concept that the impregnated compound will diffuse through the agar and shows its activity against the inoculated bacteria. It also depends on the rate of diffusion of the compound and the cell growth. The larger the diameter of the inhibition zone, the stronger activity that antibacterial agent has (Isenberg, 2004).

Commonly, after the screening by disc diffusion method, the positive results of EO which show zone of inhibition are further been examined for their strength of antibacterial activity with broth dilution method. In this method, a few concentration of EOs are used in order to determine the MIC and MBC. The determination of optical density (OD) (turbidity) for bacterial concentration by spectrophotometer is the frequent method used by the researchers (Burt, 2004 and Pillai, et al., 2005).

2.8 EOs IN COMBINATION AND ANTIBACTERIAL ACTIVITY

The use of antibiotic combinations is very common in the practice of today's clinical medicine. This is due to the resistance of the identified pathogen to inhibition and/or killing by conventional doses of single antimicrobials, but more susceptible to inhibition or killing by the combination. In addition, its combination may provide broader-spectrum empiric coverage in the treatment of seriously ill or immunocompromised patients (Cottarel and Wierzbowski, 2007; Eliopoulos and Eliopoulos, 1988; Isenberg, 2004; Pillai, et al., 2005 and Verma, 2007).

Generally, there are few rationales for the use of the antimicrobial combinations: expansion of antimicrobial spectrum in the treatment of mixed (polymicrobial) infections, minimization of drug toxicity as a result of reduced dosage, minimization of the emergence of the drug-resistant subpopulations of the pathogenic organism, and antimicrobial synergism (Cottarel and Wierzbowski, 2007; Eliopoulos and Eliopoulos, 1988; Hemaiswarya et al., 2008; Isenberg, 2004; Pillai, et al., 2005 and Verma, 2007).

In connection with that, recently, there is growing interests among the researchers to study the EOs in combination against the antibacterial activity (Bassolé et al. 2010; Bassolé and Juliani 2012; Burt, 2004; Moon et al., 2011; Nguefack et al., 2012 and Tajkarimi et al., 2010). Instead of those similar reasons in antibiotic combinations, the employ of EOs in combination specifically purposed to minimise their concentrations when used alone and to reduce the sensory effect (such as color, flavor and texture) of the products. This is because the antimicrobial effect of EOs in food which is only attainable when used in higher concentrations may cause the organoleptic impact; which changing the natural taste of the food greater than the acceptable flavour thresholds (Delaquis et al., 2002; Goñi et al., 2009; Gutierrez et al., 2008 and Hyldgaard et al. 2012).

In general, the EO in combination are being studied in a few modes: a number of studies have focused on the synergistic activity of the EO in combination with antibiotic in order to minimize the side effects of the antibiotic (Hemaiswarya et al., 2008; Hemaiswarya and Doble, 2009; Moon et al., 2011; Rosato et al., 2008; Silva et al., 2011 and Toroglu, 2007), combinations of EOs with other food additives compounds (e.g.,

sodium chloride, sodium nitrite and nisin) and combination with other EOs to reduce the minimum effective dose of EOs (Burt, 2004 and Tajkarimi et al., 2010).

2.8.1 Definitions of Antibacterial Interactions in Vitro

To determine the antibacterial interactions of the EOs, there are few terms used: synergism, indifference and antagonism. Synergism is a positive interaction; the combined effect of the antimicrobial agents is significantly greater than the sum of individual results. Conversely, antagonism is negative effect; the combination of the antimicrobial agents less effective compared to when each of them is used independently. On the other hand, indifference means no interaction occured between the combinations; the antimicrobial effect used alone is the same when used in combination (Bassolé and Juliani, 2012; Isenberg, 2004; Meletiadis et al., 2010 and Pillai et al., 2005).

2.8.2 Interaction Test Methods

In fact, there is still no standardised methods developed to assess the interaction of EOs in combination; therefore, many researchers have applied the methods that are used to evaluate the interaction between the drugs as suggested by the Clinical and Laboratory Standards Institute (CLSI) (Burt, 2004). The most frequently used techniques to assess drug interactions is checkerboard titration; hence, this technique has been employed similarly in assessing EOs interactions. This technique has a few advantages: easy to understand, interpretation of the results by using simple mathematics, the equipments required readily available in the microbiology laboratory and many studies have suggested that this technique has advantage of synergistic therapy in the treatment of neurotropenic patients with Gram-negative septicemia (Isenberg, 2004; Pillai, et al., 2005 and Verma, 2007).

(i) Checkerboard Titration

The term “checkerboard” represents the pattern of multiple dilutions (of tubes or microtiter wells or agar plates) of the two antibacterial agents that are being tested. The concentrations employed are equal to, above and below their minimal inhibition concentrations (MICs). It was suggested to test each of antibacterial agents in the range of four to five dilutions lower than the minimal inhibitory concentration (MIC) to at

least twice higher than the MIC (if antagonism is suspected) (Pillai, et al., 2005 and Verma, 2007). Twofold dilutions of each antibacterial agent are used. Each row has a constant amount of one antibacterial agent and twofold doubling dilutions of the second antibacterial agent. In other words, the first row has doubling dilutions of antibacterial A and the columns have twofold doubling dilutions of the second antibacterial B (Figure 2.20). Thus, each well contains a unique combination of the two antibacterial agents being tested (Pillai et al., 2005). As controls, each antibacterial agent alone that is without any amount of the second antibacterial is added in a row (or column). Nowadays, to save the costs and time consuming, microtiter trays have been used frequently (Pillai et al., 2005).

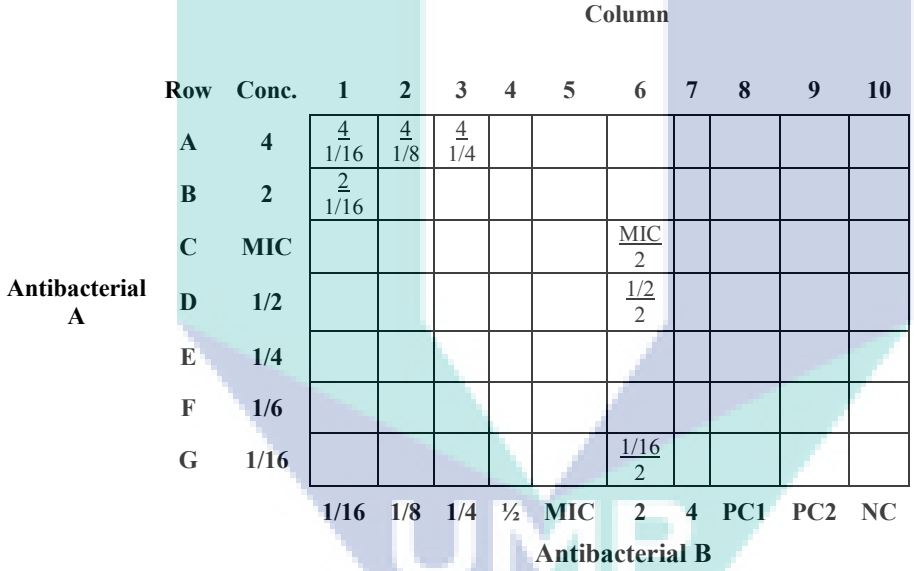


Figure 2.20: Example of broth microdilution checkerboard

Source: Eliopoulus (1988) and Charles Bonapace (2000)

2.8.3 Interpretation of the Results

The results from the checkerboard studies are calculated mathematically and expressed in terms of fractional inhibitory concentration (FIC) index (Table 2.6). This method is the most commonly used in the literature to report the results of studies with antibacterial combination (Bonapace et al., 2002 and Pillai, et al., 2005). In this method, the FIC of each antibacterial agent is derived from the MIC of the antibacterial in combination divided by the MIC of the antibacterial used alone. The FIC index is equal to the sum of FICs for each antibacterial (Pillai, et al., 2005). If the FIC index is ≤ 0.5, the antibacterial combination is interpreted as being synergistic; between 1 and 4 as indifferent ($1 < FIC \leq 4$); and >4 as antagonistic (Pillai, et al., 2005).

Table 2.6: Calculation of the Fractional Inhibitory Concentration (FIC) Index for Combination of Two Antibacterial

$\frac{MIC_A \text{ combination}}{MIC_A \text{ alone}} + \frac{MIC_B \text{ combination}}{MIC_B \text{ alone}} = FIC_A + FIC_B = \text{FIC index}$

Briefly, the FIC index was calculated for each drug using the column and row with the lowest concentrations in which there was no turbidity (no visual growth) observed in the entire row and column (Bonapace et al., 2000 and Eliopoulos and Eliopoulos,1988).

For example, in Figure 2.21, it shows that MIC in combination for antibacterial A is 1.0 while MIC in combination for antibacterial B is 0.5. Here, the MIC alone for each of the antibacterial is 1.0. Therefore, the $FIC_A = 1.0/1.0$ and $FIC_B = 0.5/1.0$. Thus, FIC index = FIC_A (1.0) + FIC_B (0.5) = 1.5. Thus, in this case the interaction between antibacterial A and antibacterial B is interpreted as indifference which means no interaction occur between the combinations; the antimicrobial effect used alone is the same when used in combination (Bonapace et al., 2002).

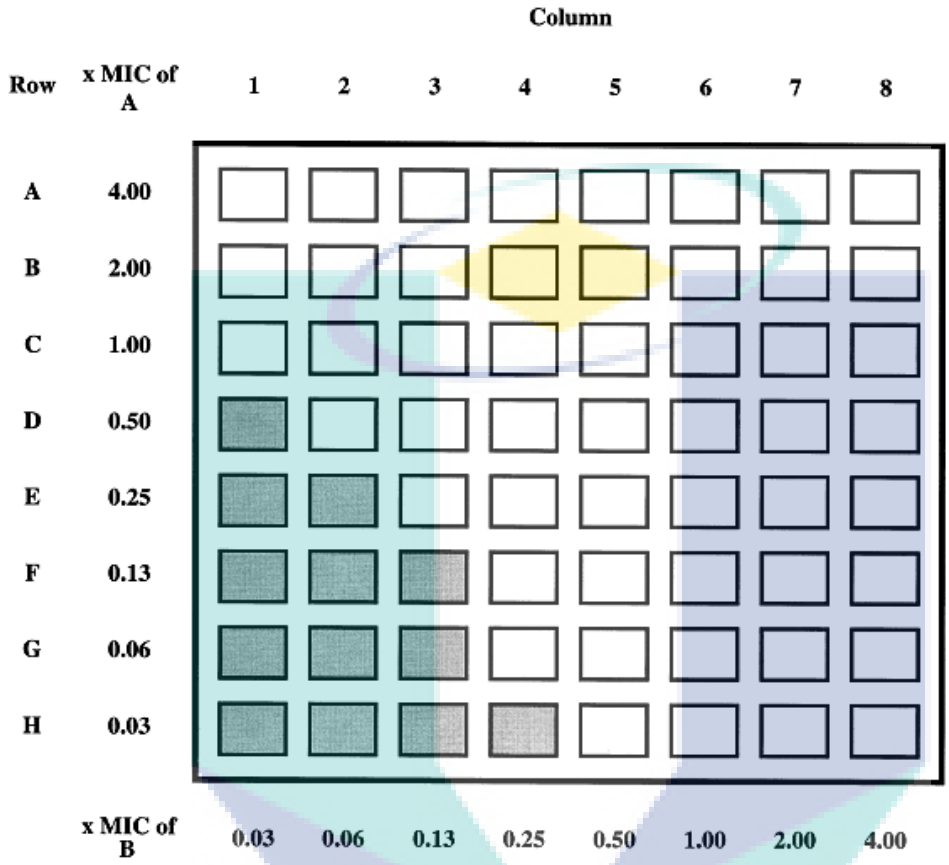


Figure 2.21: Method of interpretation by Eliopoulus: □ non-turbid well ▨ turbidwell

Source: Bonapace et al. (2000)

2.9 MECHANISM OF ACTION OF EOs AGAINST BACTERIAL CELL

Although there are many studies showing the effectiveness of EOs and their components as antibacterial agent in the past, but their mechanisms of action against the bacteria remain obscure (Burt, 2004 and Devi et al., 2010). Generally, there are five main mechanisms by which antibacterial agents act (such as antibiotic): inhibition of cell metabolism, inhibition of bacterial cell wall synthesis, interaction with the plasma membrane, disruption of protein synthesis and inhibition of nucleic acid transcription and replication (Patrick, 2009). These mechanisms will lead to the loss of vital cellular cell and subsequent death of the cell (Thomas, 2007).

The functional group contained in a compound would determine the type of intramolecular forces (electrostatic or ionic forces, van der Waals forces, dispersion forces, repulsive forces) formed between the biological membrane of bacterial cell; these will identify the mechanisms involved and their effectiveness against antibacterial activity (Patrick, 2009).

Essential oil, which contains of different group of compounds, might act synergistically at several targets in the bacteria cell (Burt, 2004). Hyldgaard et al. (2012) have overviewed mode of action of some EOs in crude. In the study of thirteen types of plant which EOs are derived, they concluded some mechanisms involved are: membrane permeability, potassium and ATP leakage (cell lysis), histidine decarboxylase and respiration activity inhibition, leakage and coagulation of cytoplasmic content and cell wall disruption. These actions were contributed from various types of functional groups presented in the crude EOs.

The study of individual constituents of EOs also been carried out and it was reported that carvacrol and thymol possessed the strongest antibacterial activity (Burt, 2004; Dorman and Deans, 2000; Hyldgaard et al., 2012; Ultee et al., 1999 and Ultee et al., 2002). The mode of action of carvacrol and thymol have been discovered by Ultee et al. (1999) and Ultee et al. (2002) and they found that the presence of the hydroxyl group and a system of delocalized electrons exhibit a significant role in the antimicrobial activity of carvacrol. The position of the hydroxyl group in the benzene ring does not affect the activity.

They hypothesized that carvacrol destabilizes the cytoplasmic membrane by acting as a proton exchanger with another ion (such as a potassium ion) in cytoplasm. This action may reduce the pH gradient across the cytoplasmic membrane and would result in the collapse of proton motive force and depletion of the ATP pool, which lead to cell death. This model is shown in Fig. 2.22. The destabilization process of the membrane also might be supported by the presence of cymene which acts synergistically with carvacrol by expanding the membrane; thereby causing to phospholipids gap and ion leakage.

Comment [V1]: insert the process figure

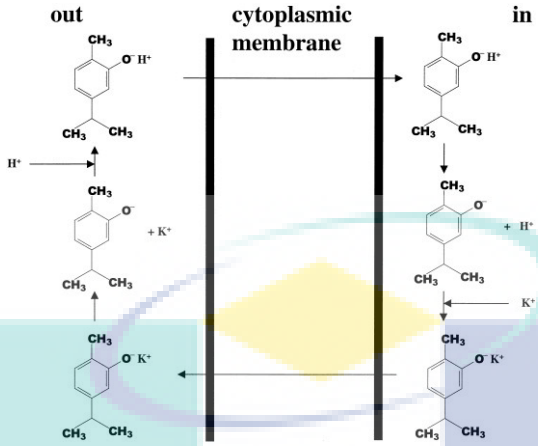


Figure 2.22: Schematic overview of the hypothesized activity of carvacrol

Source: Ultee et al. (2002)

In other study by Gill and Holley (2004) suggested that the bactericidal activity of eugenol and cinnamaldehyde against bacteria *L. monocytogenes* and *L. sakei* may be attributed to the inhibition of energy generation (inhibition of glucose uptake or utilization of glucose) and effects on membrane permeability. The failure to generate energy may cause inability of the cells to reproduce or adapting their metabolism to antimicrobial effects.

Whereas Sikkema et al. (1995) in their review of mechanisms of action of lipophilic compounds (terpenes, aromatics, cycloalkanes, alkanes, alcohols, and phenols) have concluded that the accumulation of these compounds in the cytoplasmic membrane of microorganisms have affected the structural and functional properties of these membranes. This effect may be attributed to the interactions of these compounds with the membrane and with membrane constituents; which perturb in the lipid part and proteins embedded in the membrane. As a result, the membrane loses its integrity and allowing the protons and ions to leaking. The disruption also can be observed from an increase in membrane fluidity and expansion of the membrane.

Recently, in the study of antimicrobial mechanisms of EO combinations (oregano–basil for *E. coli*, basil–bergamot for *S. aureus*, oregano–bergamot for *B. subtilis* and oregano–perilla for *S. cerevisiae*) by Lv et al. (2011), they have demonstrated the result of electron micrographs of damaged cells and the significant

increase of the cell constituents' release; these results proved that all essential oil combinations affected the cell membrane integrity. Some of the figures of electron micrographs are shown in the Figures 2.23 and 2.24 below.

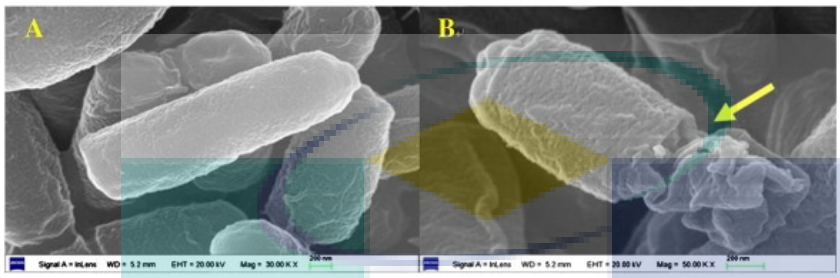


Figure 2.23: Scanning electron micrographs of *E. coli* cells: (A) untreated (magnification×30,000); (B) treated with combinations of oregano oil and basil oil at MIC value for 3 hours (magnification x 20,000)

Source: Lv et al. (2011).

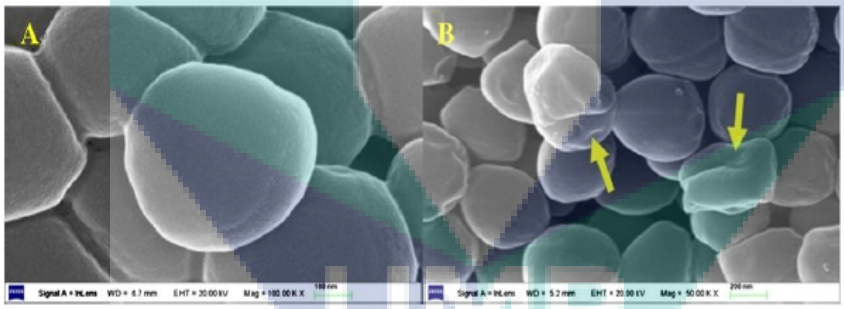


Figure 2.24: Scanning electron micrographs of *S. aureus* cells: (A) untreated (magnification×100,000); (B) treated with combinations of basil oil and bergamot oil at MIC value for 3 hours (magnification x 50,000)

Source: Lv et al. (2011).

CHAPTER 3

MATERIALS AND METHODS

3.1 MATERIALS

Dichloromethane, anhydrous sodium sulphate, nutrient agar and nutrient broth were manufactured by Merck, Germany; while Mueller Hinton agar and Mueller Hinton broth were manufactured by Oxoid, England. Dimethyl sulfoxide was manufactured by R & M Chemicals, United Kingdom; and Ampicillin was manufactured by Sigma-Aldrich, USA. The bacteria *Enterococcus faecalis* ATCC 14506, *Staphylococcus aureus* BAA-1026, *Bacillus Subtilis* ATCC 11774, *Escherichia coli* ATCC 10536, and *Salmonella typhimurium* ATCC 14506 were obtained from MicroBioLogics, USA.

3.2 PLANT MATERIALS

The aerial parts of *Cymbopogon citratus* and *Cymbopogon nardus* were collected during July 2011 from a garden at Gambang area, Kuantan, Pahang. Plants were identified by Dr. Sugumaran A/L Manickam, the coordinator of Rimba Ilmu, Institute of Biological Sciences, University of Malaya, Kuala Lumpur, Malaysia.

3.3 ESSENTIAL OILS EXTRACTION

Fractions of 700g of fresh plant material were subjected to steam distillation (Figure 3.1) for four hours to give a mixture of water/essential oil. This mixture was collected and dichloromethane (DCM) was used to extract the essential oil from the water layer. The essential oils obtained were then dried with anhydrous sodium sulphate

and were kept in airtight containers in a refrigerator at 4ºC. The yields were calculated according to the weight of the plant material before distillation (expressed in percent, w/w of the fresh plant material).

Figure 3.1: The steam distillation used to obtain essential oils.

3.4 GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS)

The GC-MS analysis was conducted using Agilent 7890 GC (Figure 2.14) equipped with Agilent 5975 C, inert XL MSD with Triple-Axis Detector, Agilent 7693 auto sampler, and Agilent 190915-433 (30m x 250 µm x 0.25 µm diameter) capillary column. The GC-MS conditions were as follows:

Injector temperature	240ºC in split mode 1:10
Injection volume	3µl via auto sampler
GC condition	50ºC then ramped at a flow rate of 3 ºC/min to 240 ºC
Column flow	Helium at 1ml/min
Solvent delay	5 minutes
MS	Electron impact mode (70eV) and scanned from m/z 20 to 500 at one scan per second at temperature 280ºC

The components were identified by comparison of their mass spectra with those of NIST08 library data of the GC–MS system (Appendix A and Appendix B).

3.5 ANTIBACTERIAL ASPECTS

3.5.1 Culture and media preparation

The essential oils were tested against five bacteria namely *Enterococcus faecalis* ATCC 14506, *Staphylococcus aureus* BAA-1026, *Bacillus Subtilis* ATCC 11774, *Escherichia coli* ATCC 10536, and *Salmonella typhimurium* ATCC 14506. The stock cultures in KWIK-STIK were obtained from MicroBioLogics, USA and were subcultured onto nutrient agar plate (Merck) and incubated in an incubator at 37ºC. Isolated pure colonies (Figure 3.2) were then selected and transferred onto slant nutrient agar (Merck) there after kept in refrigerator at 4ºC for stock culture maintenance. For antibacterial assays of disk diffusion, minimum inhibitory concentration, minimum bactericidal concentration and fractional inhibitory concentration, the media used are Mueller Hinton broth (Oxoid) and Mueller Hinton agar (Oxoid). All media were prepared according to the instructions provided by the supplier i.e weighed, dissolved in distilled water and autoclaved at 121ºC for 15 min.

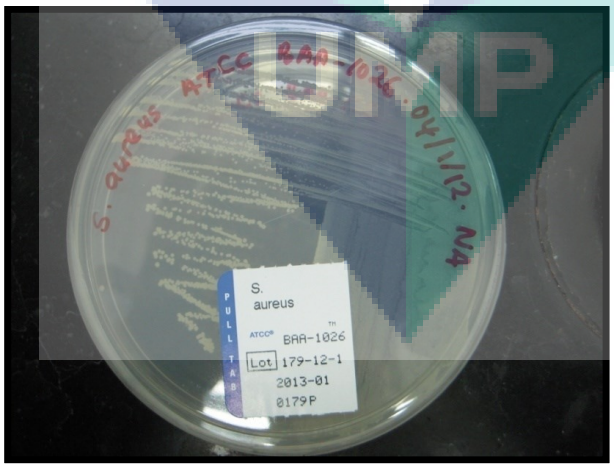


Figure 3.2: Isolated pure colonies

3.5.2 Inoculum preparation

About three to five colonies of the same morphological type from agar plate culture was touched by using a loop and was suspended 4-5 ml of Mueller-Hinton broth. It was incubated at 37ºC for overnight. To be used for antibacterial assays, the turbidity of the growth cells was adjusted to the 0.5 McFarland standard using nutrient broth. This will contain an approximate inoculum size of 1 x 10^8 CFU/ml. The inoculum adjustment was done with a spectrophotometer (Figure 3.3) at 600 nm wavelength to give Absorbance = 0.132. For MIC and FIC determination, the inoculums size used were 1 x 10^6 CFU/ml.

Figure 3.3 Genesys 20 Visible Spectrophotometer Thermo Scientific brand

3.5.3 Disk diffusion assays

The test was performed in sterile Petri dishes (90mm diameter) containing solid and sterile Mueller–Hinton agar medium. A sterile cotton swab was dipped into adjusted inoculum (1 x 10^8 CFU/ml) and rotated it several times. The excess inoculum is removed by pressing the swab against the well of the tube above the liquid. The swab then was streaked carefully over the entire sterile agar surface to obtain an even growth in three different directions. Finally, the rim of the agar was swabbed. The inoculated plate was allowed to stand for 3-5min before applying the essential oil-impregnated disks; this is to allow for any excess surface moisture to be absorbed. After that, the essential oils of *C. citratus* and *C. nardus* were absorbed on sterile paper discs (10µl per Whatman disc of 6mm diameter), and were placed on the surface of the media

previously inoculated with bacterial suspension. A negative control filter paper disk (water) was placed per Petri dish in order to avoid a possible additive activity while Ampicillin (10mg/ml) was used as positive control. Every plate was sealed with laboratory film to avoid evaporation, and then incubated aerobically upside down at 37°C for 18 hours. The results were recorded in triplicate and the mean of diameter zone of inhibitions were expressed in mm (Figure 3.4).

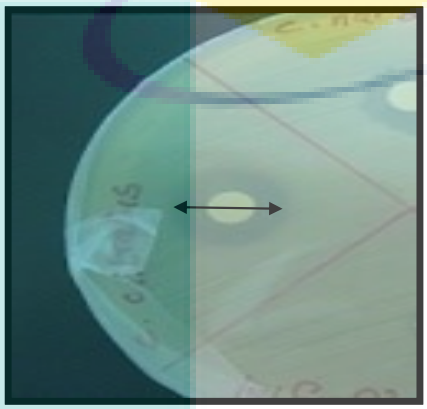


Figure 3.4: Diameter zone of inhibition

3.5.4 Determination of minimum inhibitory concentration (MIC) and minimal bactericidal concentration (MBC)

A microdilution broth susceptibility assay for bacteria was done as recommended by Clinical and Laboratory Standards Institute (CLSI) for the determination of the minimum inhibitory concentration. All tests were performed in Mueller Hinton broth (MHB) supplemented with 10% (v/v) DMSO (Bamoniri et al. 2010) to increase oil solubility. Serial two fold solutions of *C. citratus* and *C. nardus* essential oils (from 0.47µl/ml to 30µl/ml for gram positive and from 3.74µl/ml to 240 µl/ml for gram negative bacterial) were prepared in a 96-well plate (100µl per well). 100µl of diluted bacterial suspension with final concentration of 1 x 10^6 cfu/ml was added to each well to give final volume 200µl. Positive controls were wells with a bacterial suspension and a bacterial suspension with 10% DMSO (Klančnik et al., 2010) while negative control was 10% DMSO and essential oils (Teixeira et al. 2012) (Figure 3.5). Contents of each well were mixed on a plate shaker at 250 rpm for 20s and

incubated at 37ºC for 24h .The lowest concentration of each essential oil showing visually no growth (by comparing with the growth controls) was taken as its minimal inhibitory concentration. The minimal bactericidal concentration (MBC) was determined by spreading 100µl of clear wells on to Mueller Hinton Agar and incubated at 37ºC for 24h. The MBC was defined as the lowest concentration of the essential oil at which incubated microorganism was 99.9% killed. All determinations were performed in triplicate.

	Column									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Row	**0.47**	**0.94**	**1.88**	**3.75**	**7.5**	**15**	**30**	**PC1**	**PC2**	**NC**
A										
B										
C										

Figure 3.5: Template of microdilution broth susceptibility assay

3.5.5 Checkerboard assay

The broth microdilution checkerboard method, which was frequently used to assess interactive inhibiton *in vitro*, was employed to determine the antimicrobial effects of selected essential oil combinations obtained in antimicrobial activity testing (Lv et al. 2011). The assay was arranged as follows: EOa (*Cymbopogon citratus*) was diluted two-fold in vertical orientation, while EOb (*Cymbopogon nardus*) was diluted two-fold in horizontal orientation. The concentration of EOa and EOb were prepared corresponding to 1/16, 1/8, ¼, ½, MIC, 2 and 4 of the MIC values (which has been determined previously), respectively (Isenberg, 2004). A 50µl of each of essential oils was added to the wells regarding to the orientation. Then, 100µl of fresh bacterial suspension (~10^6) were transferred to each wells (Figure 3.6). Contents of each well were mixed on a plate shaker at 250rpm for 20s and incubated at 37ºC for 24h. All determinations were performed in triplicate.

Column

Comment [A1]: the font size not 12

Row	Conc.	1	2	3	4	5	6	7	8	9	10
A	4										
B	2										
C	MIC										
D	1/2										
E	1/4										
F	1/8										
G	1/16										
		1/16	1/8	1/4	½	MIC	2	4	PC1	PC2	NC

Figure 3.6: Template of broth microdilution checkerboard

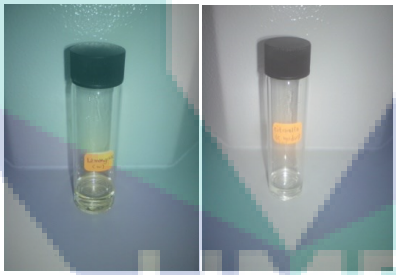
CHAPTER 4

RESULTS AND DISCUSSION

4.1 CHEMICAL COMPOSITION OF ESSENTIAL OILS

4.1.1 Total Yields

The fresh stalks of the plants were subjected to steam distillation and the total yields of volatile chemicals obtained for *C. citratus* and *C. nardus* were 0.46% (w/w) and 0.1% (w/w), with pale yellow-coloured and colourless, respectively (Figure 4.1).



(a) (b)

Figure 4.1: Colours of essential oils : (a) *C. citratus* –pale yellow (b) *C. nardus*-colourless

It was found that the oil percentages of *C. citratus* were between 0.3%- 1.6% which is in agreement with previous work (Bassolé et al., 2011; Khanuja et al., 2005; Pandey, 2010 and Santin et al. 2009) . Meanwhile, *C. nardus* were in the range of 0.1% to 1.10% are in agreement with the work by Castro et al. (2010), Khanuja et al. (2005) and Nakahara et al. (2003). The difference in the content and yield of the oils may be due to several factors such as climatic conditions of the place of cultivation, time of

harvesting, maturity of the grass, nature of material being distilled (fresh or dry) and the method of distillation (Pandey, 2010).

4.1.2 GC-MS Analysis of Essential Oils

Table 4.1 shows the chemical composition of essential oils in *C. citratus* and *C. nardus*. The details of this chemical composition data were shown in the Appendix A1, Appendix A2, Appendix B1 and Appendix B2. From the GC-MS result, it revealed that oxygenated monoterpene compounds were the higher contents in *C. citratus* (54.35%); this followed by sesquiterpene hydrocarbons (28.53%), oxygenated sesquiterpene hydrocarbons (11.42%) and monoterpene hydrocarbons (1.33%).

Table 4.1: Chemical compositions of *C. citratus* and *C. nardus* essential oils identified by GC-MS.

Compounds	**Chemical Formula**	**Retention Time (RT)**	**Composition *C. citratus***	**Retention Time (RT)**	**Composition *C. nardus***
Monoterpene hydrocarbons			**(%)**		**(%)**
α-pinene	$C_{10}H_{16}$	10.36	0.48	-	-
β-ocimene	$C_{10}H_{16}$	10.76	0.23		-
Limonene	$C_{10}H_{16}$	-	-	9.95	0.22
α-myrcene	$C_{10}H_{16}$	-	-	25.58	4.86
β-thujene	$C_{10}H_{16}$	8.59	0.62	-	-
Santolina triene	$C_{10}H_{16}$	-	-	25.13	0.19
Oxygenated monoterpenes			**(%)**		**(%)**
Neral/ β-citral	$C_{10}H_{16}O$	19.54	21.34	-	-
Geranial/α-citral	$C_{10}H_{16}O$	21.05	33.01	=-	-
Santolina epoxide	$C_{10}H_{16}O$	-	-	35.50	2.77
Citronellal	$C_{10}H_{18}O$	-	-	15.43	7.39

Table 4.1: Continued.

β-citronellol	$C_{10}H_{20}O$	-	-	18.78	2.48
Lavandulol	$C_{10}H_{18}O$	-	-	19.86	0.84
Sesquiterpene hydrocarbons			(%)		(%)
β-gurjunene	$C_{15}H_{24}$	34.77 35.17	20.90	-	-
α-amorphene	$C_{15}H_{24}$	-	-	30.36	0.31
β-cubebene	$C_{15}H_{24}$	30.37	0.35	29.11	2.78
α-muurolene	$C_{15}H_{24}$	-	-	29.84	0.41
δ-cadinene	$C_{15}H_{24}$	30.76	0.90	30.85	4.64
β-maaliene	$C_{15}H_{24}$	-	-	35.16	6.05
aristolene	$C_{15}H_{24}$	26.52	0.80	-	-
α-bergamotene	$C_{15}H_{24}$	27.23	0.53	-	-
γ-selinene	$C_{15}H_{24}$	29.19	0.80	-	-
β-bourbonene	$C_{15}H_{24}$	32.83	0.72	-	-
α-selinene	$C_{15}H_{24}$	37.38	1.35	-	-
isoaroma-dendrene (V)	$C_{15}H_{24}$	35.49	2.18	-	-
Oxygenated sesquiterpenes			(%)		(%)
Germacrene-D-4-ol	$C_{15}H_{26}O$	-	-	32.97	0.92
α-cadinol	$C_{15}H_{26}O$	36.06	8.05	36.14	12.77
Trans-farnesol	$C_{15}H_{26}O$	38.32	1.23	-	-
Trans-farnesal	$C_{15}H_{24}O$	38.96	0.32	-	-
Elemol	$C_{15}H_{26}O$	15.74	0.58	32.35	44.14
β-eudesmol	$C_{15}H_{26}O$	34.05	1.82	-	-
Others					
Geranyl formate	$C_{11}H_{18}O_2$	-	-	25.38	3.83

Table 4.1: Continued.

2-propenyl, cyclopentane	C_8H_{14}	-	-	37.15	0.31
2,6-Nonadienal	$C_9H_{14}O$	-	-	38.30	0.70
2-pentene, 3-methyl	C_6H_{12}	-	-	38.95	0.20
Citronellyl butyrate	$C_{14}H_{26}O_2$	-	-	24.00	2.33

The major components contained in this oil were (a) geranial (33.01%), (b) neral (21.34%), and (c) β-gurjunene (20.90%) (Figure 4.2), while other compounds such as α-cadinol (8.05%), trans-farnesol (1.23%), β-eudesmol (1.82%), α-selinene (1.35%) and isoaromadendrene (2.18%) existed as minor compounds (>1%).

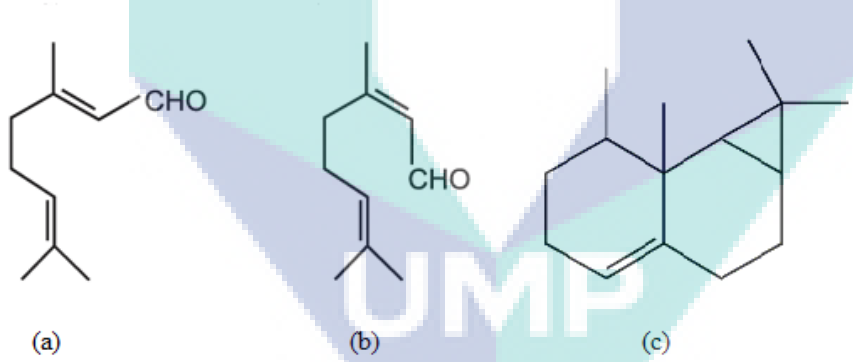


Figure 4.2: Chemical structure of (a) geranial (b) neral and (c) β-gurjunene

Geranial and neral (namely trans-citral, citral A and cis-citral, citral B, respectively) are two isomeric acyclic monoterpene aldehydes from citral (3,7-dimethyl-2,6-Octadienal). Citral, which possess a strong lemon aroma has been used widely in food, cosmetics, detergents, perfumery and pharmaceutical industries as flavouring or scenting agents (Pihlasalo, Klika, Murzin & Nieminen 2007a; Yang, Xi, Li & Qu 2009a; Lalko & Api 2008a; Rauber et al. 2005). It is also commercially used in

the production of vitamin A, ionones and methylionones (Díez et al. 2010; Yang, Xi, Li & Qu 2009b; Pihlasalo, Klika, Murzin & Nieminen 2007b). However, it has been identified as a potential contact allergen as it may react with skin proteins to form an immunogen because of its capability to penetrate through both animal and human skin (Lalko & Api 2008b).

According to Khanuja et al. (2005), the rich citral content in *C. citratus* oil has made it to be clustered in 'Citrati' series. This series of cluster analysis was grouping based on the dominant components (as markers) contained in essential oils of different *Cymbopogon* species. To the best of our knowledge, the chemical composition of *C. citratus* essential oil has been reported by many other studies (Bassolé et al., 2011; Khanuja et al., 2005; Machado et al., 2012; Maciel et al., 2010; Nguefack et al., 2012 and Oussalah et al., 2007). Most of these studies have shown that geranial and neral (citral) were the main constituents of *C. citratus*.

This study also showed that β-gurjunene had appeared as one of the major components presented in this oil. The presence of β-gurjunene was very rare compared to the other findings which reported that other major compound that commonly existed along with citral was β-myrcene (Akhila, 2010; Bassolé et al., 2011 and Machado et al., 2012).

On the other hand, in *C. nardus* essential oil, oxygenated sesquiterpene compounds existed as the higher contents (57.83%), followed by sesquiterpene hydrocarbons (14.19%), oxygenated monoterpenes (13.48%) and monoterpene hydrocarbons (5.27%). Elemol (44.14%) had showing the most dominant constituent followed by α-cadinol (12.77%) and citronellal (7.39%) (Figure 4.3); whereas β-citronellol (2.48%), β-cubebene (2.78%), δ-cadinene (4.64%), β-maaliene (6.05%), citronellyl butyrate (2.33%), geranyl formate (3.83 %), α-myrcene (4.86%), and santolina epoxide (2.77%) were the minor constituents.

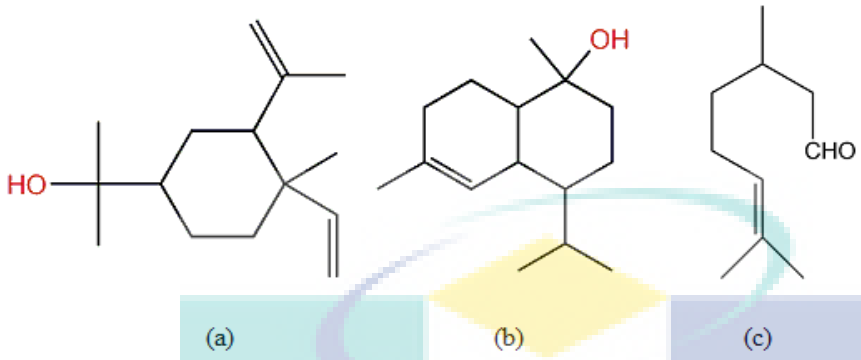


Figure 4.3: Chemical structure of (a) elemol (b) α-cadinol and (c) citronellal

The existence of elemol in this essential oil has been suggested to be formed as an artifact to Ceylon-type (Akhila, 2010). The major compositions of elemol and α-cadinol presented in this study were different from that previously reported by Mahalwal and Ali (2002), Maciel et al. (2010), Santin et al. (2009), Kanko et al. (2004), Oliveira et al. (2011), Rodríguez et al. (2012) and Sakulku et al. (2009), who found that citronellal, geraniol and citronellol were the major compounds contained in this essential oil. Although there was a difference, the presence of citronellal has been recognized as a marker compounds (chemotypes) in the *C. nardus* and its content varied among the cultivated varieties (Khanuja et al., 2005 and Nakahara et al.,2003).

Furthermore, according to Castro et al. (2010), the factor of different harvesting times would affect the content and chemical composition of *C. nardus* essential oil. Their study has shown that there was variation in the number of compounds of the *C. nardus* essential oil presented in the five harvest seasons in Brazil and the percentage of major compounds (citronellol, geraniol and elemol) also different as the seasons changed. In addition, several studies of another plants which were affected by different harvesting times also showing the similar effect (Blank et al., 2011; Dar et al., 2011; Kamatou et al., 2008 and Salehi et al., 2010).

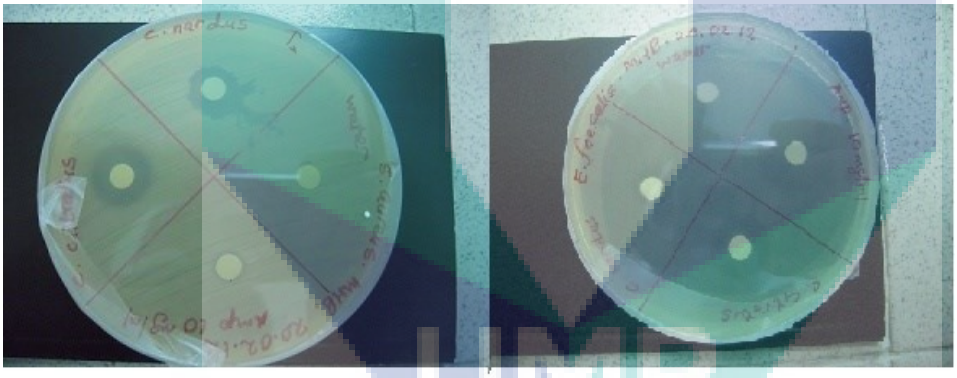
As a consequence, instead of different harvesting time factor, there are many other causes might be considered to the variety of the chemical composition in these *C. citratus* and *C. nardus* essential oils. Soil types (Taveira et al., 2003), plant parts, geographical sources and methods of extraction (Cao et al., 2009; Hussain et al., 2008;

Natta et al., 2008; Oussalah et al., 2007; Tepe, 2005 and Wang et al., 2012) may also contribute to this result.

4.2 ANTIBACTERIAL ACTIVITY OF *C. CITRATUS* AND *C. NARDUS* EOs

The antibacterial activity of *C. citratus* essential oils were evaluated against pathogenic strains of Gram positive (*E. faecalis*, *S. aureus*, *B. subtilis*) and Gram negative (*E. coli*, *S. typhimurium*) bacteria qualitatively and quantitatively by the presence or absence of inhibition zones, zone diameters (disc diffusion method), minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values (broth microdilution method).

To determine inhibition zone diameter, the disc of essential oils which showed clear zone (no bacteria growth around the disc) were measured in milimeter by a ruler including the diameter of the disc (Figure 4.4).



(a) (b)

Figure 4.4: Inhibition zone diameter of essential oils from (a) *C. nardus* against *S. aureus* and (b) *C. citratus* against *E. faecalis*

The minimal inhibition concentration (MIC) of essential oils were determined from the lowest concentration of essential oils at which no growth occurs (absence of turbidity) in the broth (Appendix C); while minimal bactericidal concentration (MBC) were the lowest concentration of essential oils that produce ≥99.9% kill of bacteria after plating out the wells showing no growth onto agar. The essential oils were tested in the range of concentration 0.47µl/ml to 30µl/ml. Generally, the larger inhibition zone diameter correlated with lower MIC, and vice versa.

Table 4.2 shows the antibacterial activity of *C.citratus* and *C. nardus* essential oils. From preliminary screening by disc diffusion method, the essential oils were found to be active against all the tested strains, by producing inhibition zone diameters varying from 7 to 22mm. Generally, the essential oils possessed stronger antibacterial activity against Gram-positive bacteria than against Gram-negative bacteria. According to the result of MIC determination, it was revealed that *S. aureus* and *B. subtilis* were the most sensitive bacteria with similar and the lowest MIC values for *C. citratus* (1.88µl/ml) and *C. nardus* (0.47µl/ml) while *E. coli* and *S. typhimurium* were the most resistant bacteria with the highest MIC values for both essential oils (7.5µl/ml; 7.5µl/ml and 120µl/ml; 120µl/ml, respectively).

While between the essential oils, it was observed that *C. citratus* essential oil had exhibited a good antibacterial activity against a wide range of bacteria (gram positive and gram negative). This activity was shown by the large inhibition zone diameter ranges and low MIC values (11.0-17mm and 1.88-7.5µl/ml, respectively). However, *C. nardus* only showed a good antibacterial activity against gram positive bacteria compared to gram negative bacteria; this difference had been shown by the small inhibition zone diameters of bacteria *E. coli* and *S. typhimurium* (7.0 and 8.0 mm) and high MIC values, which were 120µl/ml and 240µl/ml, respectively.

Table 4.2: Antibacterial activity of *C. citratus* and *C. nardus* essential oils (Inhibition zone diameter, minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC))

Test bacteria	Inhibition zone diameter (mm)				MIC and MBC (µl/ml)			
	C. citratus	*C. nardus*	Ampicillin	Water	*C. citratus*		*C. nardus*	
					MIC	MBC	MIC	MBC
E. faecalis ATCC 14506	15.0 ± 4.4	12.3 ± 2.5	21.0 ± 1.0	6.0 ± 0.0	3.75	7.5	1.88	1.88
S. aureus BAA-1026	15.3 ± 5.5	13.3 ± 4.0	7.3 ± 0.6	6.0 ± 0.0	1.88	3.75	0.47	1.88
B. subtilis ATCC 11774	17.0 ± 2.6	22.0 ± 2.0	20.3 ± 0.6	6.0 ± 0.0	1.88	3.75	0.47	1.88
E. coli ATCC 10536	11.0 ± 1.0	7.0 ± 0.0	19.3 ± 2.1	6.0 ± 0.0	7.5	7.5	120	>240
S. typhimurium ATCC 14506	11.3 ± 0.6	8.0 ± 1.0	29.0 ± 1.0	6.0 ± 0.0	7.5	7.5	120	240

In the case of antibiotic Ampicillin, it showed a strong antibacterial activity against all the tested bacteria except for *S. aureus*, with the smallest inhibition zone diameter (7.3 mm). The most sensitive bacterium to this antibiotic was *E. coli*, with inhibition zone diameter, 29.0 mm. It was found that both essential oils showed better antibacterial activity against *S. aureus* than the antibiotic, with the larger inhibition zone diameter and vice versa.

The data obtained from MBC showed that *C. nardus* possessed greater bactericidal activity (lowest MBC value 1.88µl/ml) against all Gram-positive bacteria compared to *C. citratus*. However, the MBC values of *C. citratus* (ranging 3.75 - 7.5µl/ml) against all the tested strains had been confirmed with MIC values that it was a wide spectrum antibacterial. Whereas *E. coli* was the most resistant bacteria for *C. nardus* with the MBC value higher than 240µl/ml. The results also showed that the essential oils possessed similar MBC and MIC values against *E. faecalis* (*C. nardus*; 1.88µl/ml); *E. coli* and *S. typhimurium* (*C. citratus*; 7.5µl/ml).

Many studies have reported that antibacterial activity is closely related to the chemical composition contained in the essential oils (Al-Bayati, 2008; Djenane et al., 2011; Kelen and Tepe, 2008; Lang and Buchbauer, 2012; Natta et al., 2008; Shirugumbi et al., 2010 and "Skocˇibus et al., 2006). The chemical composition in essential oils composed of different classes of compounds such as hydrocarbons (terpenes and sesquiterpenes) and oxygenated compounds (alcohols, esters, ethers, aldehydes, ketones, lactones, phenols and phenol ethers) (Nerio et al. 2010). This difference of chemical structures in the components may contribute to their efficacy on antibacterial action (Khunkitti, 2010).

In this study, the result of a good and wide spectrum of antibacterial activity of *C. citratus* essential oil was in parallel with other studies (Aiemsaard et al., 2011; Cimanga et al., 2002; Maizura et al., 2008 and Naik et al., 2010). Bassolé et al. (2011) also reports that *C. citratus* have shown the highest activity against wide range of bacteria such as *E. faecalis*, *L. monocytogenes*, *S. enteric*, *S. typhimurium* and *D. dysenteria* whereas Wannissorn et al. (2005) have demonstrated that *C. citratus* is one of the essential oils tested which showing a promising antibacterial activity against zoonotic enteropathogens (such as *Salmonella spp.*, *E. coli 0157*, *C. jejunii* and *Cl.*

Comment [S1]: rearrange the citations

perfringens); hence, they conclude that this essential oil could be applied as an alternative to antibiotic used in animal feed.

Other study by Inouye et al. (2001) reports that lemongrass is one of 14 essential oils tested showing high activity by inhibiting five strains of respiratory tract pathogens (*H. influenzae* ATCC 33391, *S. pyogenes* ATCC 12344, Penicillin-susceptible *S. pneumoniae* IP-692, penicillin- resistant *S. pneumoniae* PRC-53, *S. aureus* FDA 209P JC). Beside of good antibacterial activity, *C. citratus* essential oil also has been reported to exhibit other bioactivities such as antifungal (Palhano et al. 2004; Tzortzakis & Economakis 2007; Sacchetti et al. 2005; Nguefack et al. 2009; Nguefack et al. 2012), insect repellent (Nerio et al. 2010), and anti-Leishmania (Machado et al. 2012; Santin et al. 2009).

Several studies have shown that the major components in the essential oils play big role of the effectiveness of bioactivities (Bajpai et al. 2012; Duru et al. 2004; Nejad Ebrahimi et al. 2008; Bagamboula et al. 2004). According to Bassolé and Juliani (2012), EOs containing aldehydes or phenols, such as cinnamaldehyde, citral, carvacrol, eugenol or thymol as major components show the highest antibacterial activity, followed by EOs containing terpene alcohols. In this study, it could be assumed that the good and wide spectrum of antibacterial activity by *C. citratus* may be caused by its rich in contents of aldehyde (citral; 54.35%) and alcohols (α-cadinol, trans-farnesol, β-eudesmol; 11.10%). It has been reported by many studies that citral has exhibited significant antimicrobial or bioactivities properties and possessed widest spectrum of activity when tested separately (Zore et al. 2011; Saddiq & Khayyat 2010; Santin et al. 2009; Belletti et al. 2010; Palhano et al. 2004; Machado et al. 2012; Aiemsaard et al. 2011; Fisher & Phillips 2008; Park et al. 2009; Burt 2004; Ultee et al. 2002).

Comment [S2]: you can rearrange the sentence structure

Comment [S3]: relate to c. nardus major cmpds;elemol, but it has been assisted by citronellol which has been recognized as antibacterial.

Comment [S4]: recheck the sentence used

On the other hand, as far as the literature survey could ascertain, there are little of studies regarding the antibacterial activity of *C. nardus*; most of the studies of this essential oil are focusing on its effectiveness as antifungal and insect repellent (Nakahara et al. 2003; Tyagi et al. 1998; Nerio et al. 2010)). However, this study had revealed that *C. nardus* EO also could exhibit high bacteriostatic and bactericidal activity against the gram-positive bacteria. It was reported that alcohols group were known to possess bactericidal rather than bacteriostatic activity against vegetative cell (Ultee et al., 2002). This antibacterial activity of *C. nardus* may be due to the greater

amount of terpene alcohols and aldehydes [such as elemol (44.14%), α-cadinol (12.77%), β-citronellol (2.48%) and citronellal (7.39%)] presented in its essential oil(Imaël Henri Nestor Bassolé & Juliani 2012; Ait-Ouazzou et al. 2011).

Comment [S5]: support with another citations

In other study by Wannissorn et al. (2005), it showed that *C. nardus* exhibited a moderate antibacterial activity against *S. typhimurium*, *Cl. Perfringens*, *C. jejuni* and weak antibacterial activity against *E.coli* and *S. enteritidis*. *C. nardus* also has been reported to possessed antiproliferative (Bakkali et al. 2008), and antiviral activity (Bahtiar et al. 2011). It was found that citronellal and citronellol compounds which contained in this essential oil have led to many bioactivities (Lenardão et al. 2007; Nerio et al. 2010; Zore et al. 2011; Nakahara et al. 2003).

Although the biological activity of essential oil was attributed to the major compounds, there is a possibility that the synergism may occur from minor compounds presented (Rocha et al. 2009; Burt 2004; Bakkali et al. 2008; Imaël Henri Nestor Bassolé & Juliani 2012; de Azeredo et al. 2011; Bajpai et al. 2012). According to Maksimovi, (2012), the various type of chemical structures in essential oils may play a role in mechanism of action against bacteria; in which the distribution of the oil in the cell sections would determine the different types of radical reactions produced. Hence, in gaining more useful knowledge for biological purposes, they recommended to study the entire oil than some of its components.

Generally, most studies investigating essential oils as antibacterial agent have reported that gram-negative bacteria are more resistant than gram-positive bacteria (Ait-Ouazzou et al., 2011; Al-bayati, 2008; Burt, 2004; Duru et al., 2009; Holley and Patel, 2005; Tajkarimi et al., 2010 and Tenore et al., 2011). This resistance may be due to the presence of an outer lipid bilayer attached to the cell wall (Figure 2.12) (Burt 2004; Bolla et al. 2011; Al-bayati 2008) in which the antibacterial agent needs to penetrate both the outer membrane and the periplasmic space before it can interfere with cell wall synthesis; comparing to gram-positive bacteria, the outer surfaces are only covered by a thin layer of teichoic acids (Thomas, 2007). It has been observed by many studies that *S. aureus* and *B. subtilis* are more susceptible to EOs while *E. coli* and *P. aeruginosa* are the most resistant bacteria (Bamoniri et al., 2010; Cao et al., 2009; Shirugumbi et al., 2010 and Tajkarimi et al., 2010).

4.3 INTERACTION BETWEEN ESSENTIAL OILS IN COMBINATION

The interaction between the combinations of the essential oils was evaluated by checkerboard broth microdilution method. The interaction (synergism, indifference or antagonism) was assessed by fractional inhibitory concentration (FIC) indices: synergism (FIC < 0.5), indifference (1 < FIC ≤ 4) or antagonism (FIC > 4) (Isenberg, 2004). Synergism is a positive interaction; the combined effect of the antimicrobial agents is significantly greater than the sum of individual results. While antagonism is negative effect; the combination of the antimicrobial agents less effective compared to when each of them is used independently. Indifference means no interaction occur between the combinations; the antimicrobial effect used alone is the same when used in combination (Aurelio, 2005).

According to the results in Table 4.3, MIC in combination of each essential oils showed greater values compared to MIC alone (Table 4.2); this increasing had resulted in greater FICI values; therefore, contributing to antagonism and indifference responses. These responses had shown that the combinations were less effective compared to when each of the essential oils were used independently. To the best of our knowledge, the antibacterial activity of *C. citratus* and *C. nardus* essential oils in combination against these bacteria have not been reported before.

As can be seen the FICI of antagonism response were ranging from 5.0 to 12.0, with the greatest FICI value (12.0) was on the combination against *S. aureus* bacterium; while combination of essential oils against *S. typhimurium* was the lowest FICI value (5.0). The indifference response of essential oils combination against *E. faecalis* with FICI value 4.0 was yielded from a similar FIC value (2.0) each of *C. citratus* and *C. nardus* essential oils.

Table 4.3: Checkerboard assay of the combination of *C. citratus* and *C. nardus* essential oils against tested bacteria

Test bacteria	$MIC_{combination}$		FIC		FICI	OUTCOME
	C. citratus	*C. nardus*	*C. citratus*	*C. nardus*		
E. faecalis ATCC 14506	7.5	3.75	2.0	2.0	4.0	Indifference
S. aureus BAA-1026	7.5	3.75	4.0	8.0	12.0	Antagonism
B. subtilis ATCC 11774	6.25	1.88	3.3	4.0	7.3	Antagonism
E. coli ATCC 10536	30	240	4.0	2.0	6.0	Antagonism
S. typhimurium ATCC 14506	30	120	4.0	1.0	5.0	Antagonism

$MIC_{combination}$ and FIC are average of triplicate tests

The antagonistic results between the combination of *C.citratus* and *C. nardus* essential oils in this study are in agreement with Nguefack et al. (2012), who found that the antagonistic effects have been observed between the combinations of *Cymbopogon citratus-Ocimum gratissimum* and *Cymbopogon citratus-Thymus vulgaris* fractions. The results of these combinations might be reflected from the chemical composition of combined essential oils and possible interaction that may occur between their major components (Imaël Henri Nestor Bassolé et al. 2010). In the case of Nguefack et al. (2012), they have suggested that the chemical reaction might occur between the fractions with alcohol function (such as terpinene 4-ol, borneol, linalool) or phenol group (thymol, carvacrol) in *Ocimum gratissimum* and *Thymus vulgaris*, respectively with fraction that enriched in citral (geranial and neral) in *Cymbopogon citratus*. This situation is related to this study; in which the major compounds contained in *C. citratus* and *C. nardus* were citral and elemol, respectively.

When the hydroxyl group of alcohols and phenols react with carbonyl function whether in neutral or acidic medium, acetals will be yielded (Nguefack et al. 2012). Acetals are the compounds with blocked hydroxyl group and carbonyl function. This blocking hydroxyl group and carbonyl function may cause to the less activity against the bacterial cell (Dorman and Deans, 2000 and Nguefack et al., 2012) .

The study by Ultee et al. (2002) have revealed that the lack of hydroxyl group may resulted in a weak antimicrobial activity. In their study at certain concentration, the carvacrol methyl ether and cymene (absent in hydroxyl group) did not show any antibacterial activity against *Bacillus cereus* bacterium compared to carvacrol and thymol, which possess hydroxyl group and a system of delocalized electrons.

As a result, these compounds do not have ability to release a proton and are therefore not antimicrobial. The proton of original hydroxyl group is involved as a hydrogen bond donor and if it is removed, the hydrogen bond is lost. The extra bulk of methyl group (in carvacrol methyl ether) would hinder the close approach that was previously attainable and should disrupt hydrogen bonding. Although the hydrogen bonding is not completely prevented, but it is expected to be weakened (Patrick, 2009). Hence, weak activity has been observed against the bacteria.

Therefore, according to those reasons, this study is assumed that the citral compounds (which representing terpenes with an aldehyde function) in *C. citratus* might react with elemol (alcohol group) compounds in *C. nardus* which lead to the form of acetals thus, may weakening the antibacterial activity when in combination. This weak activity could be seen from the greater MIC results when in combination; as a consequence, the antagonistic results had been observed almost the bacteria tested.

Regarding to the chemical reaction occurred between those major compounds (citral and elemol) which lead to the weak antibacterial activity, it could be suggested that other minor components presented in the essential may supported the overall antibacterial activity in combination (Ultee et al. 2002). This can be seen in the study by Salih et al. (2000) who have reported that hydroxycinnamic acids showed antimicrobial activities even though their esters were not active. These minor compounds may take part in penetration of the membrane, which resulted in cell damaged (Goñi et al. 2009).

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

From this study, it can be concluded that the essential oils of *Cymbopogon citratus* and *Cymbopogon nardus* showed antibacterial activity against all the tested strains (*E. faecalis*, *S. aureus*, *B. subtilis*, *E. coli* and *S. typhimurium*). However, between the essential oils, *C. citratus* essential oil had exhibited a good antibacterial activity against a wide range of bacteria (gram positive and gram negative) compared to *C. nardus*.

On the other hand, among the bacteria, it can be seen that *S. aureus* and *B. subtilis* were the most sensitive bacteria with similar and the lowest MIC values for *C. citratus* (1.88 µl/ml) and *C. nardus* (0.47 µl/ml) while *E. coli* and *S. typhimurium* were the most resistant bacteria with the highest MIC values for both essential oils (7.5 µl/ml; 7.5 µl/ml and 120 µl/ml; 120 µl/ml, respectively). Generally, the gram-negative bacteria were more resistant than gram-positive bacteria.

The result of the essential oils in combination shows that antibacterial activity was less effective compared to when each of the essential oils were used independently. Therefore, it is suggested for not mixing these both essential oils as they have not given positive results for antibacterial activity.

5.2 RECOMMENDATION FOR FUTURE STUDIES

It is proven that most of Malaysian medicinal plants showed many biological activities. From this study, a few recommendations may be highlighted for the future studies. First, since the number of essential oils samples only consisted of two types of plant, it is recommended to add few other plants so that the results would be more comparable in order to identify the best antibacterial agent independently or in combination. This is corresponding to our commercial local herbal formulations/ traditional medicines today which are mostly prepared in combination of several ingredients (natural products-based).

Furthermore, instead of the essential oils, it is also suggested to extend the methods of extraction such as in aqueous or in solvent extracts. Therefore, there will be more comparability between the results. In addition, for chemical composition analysis of essential oils become more accurate, Kovats Index method is proposed to be applied. Finally, other than antibacterial testing, it is also recommended to extend the biological activities in testing the essential oils independently and in combination as antifungal, antivirus or as insecticides.

With all of these recommendations, it is hoped that there is improvement in testing the essential oils of the plants in future.

REFERENCES

Aiemsaard, J., Aiumlamai, S., Aromdee, C., Taweechaisupapong, S., and Khunkitti, W. 2011. The effect of lemongrass oil and its major components on clinical isolate mastitis pathogens and their mechanisms of action on *Staphylococcus aureus* DMST 4745.*Research in Veterinary Science*, **91**(3): 31-37.

Ait-Ouazzou, A., Cherrat, L., Espina, L., Lorán, S., Rota, C., and Pagán, R. 2011. The antimicrobial activity of hydrophobic essential oil constituents acting alone or in combined processes of food preservation. *Innovative Food Science and Emerging Technologies*, **12**(3): 320-329.

Akhila, A. 2010. Chemistry and Biogenesis of Essential Oil from the Genus Cymbopogon. In A. Akhila, *Essential oil-bearing grasses : the genus Cymbopogon.* Boca Raton: Taylor and Francis Group.

Al-bayati, F. A. 2008. Synergistic antibacterial activity between *Thymus vulgaris* and *Pimpinella anisum* essential oils and methanol extracts. *Journal of Ethnopharmacology*, **116:** 403-406.

Allison, D., and Gilbert, P. 2004. Bacteria. In S. P. Denyer, N. A. Hodges, and S. P. Gorman, *Pharmaceutical Microbiology 7th edition* (pp. 23-43). Massschussets: Blackwell Publishing.

Bagamboula, C., Uyttendaele, M., and Debevere, J. 2004. Inhibitory effect of thyme and basil essential oils, carvacrol, thymol, estragol, linalool and p-cymene towards *Shigella sonnei* and *S. flexneri*. *Food Microbiology*, **21**(1): 33-42.

Bahtiar, A. a., Ibrahim, N., and Ahmad, I. 2011. Antiviral Activity of *Cymbopogon nardus* (L.) Rendle Fractions Against HSV-1. *Antiviral Research*, **90**(2), A54.

Bajpai, V. K., Baek, K.-H., and Kang, S. C. 2012. Control of *Salmonella* in foods by using essential oils: A review. *Food Research International*, **45**(2): 722-734.

Bakkali, F., Averbeck, S., Averbeck, D., and Idaomar, M. 2008. Biological effects of essential oils--a review. *Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association*, **46**(2): 446-475.

Bamoniri, A., Ebrahimabadi, A. H., Mazoochia, 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**(3): 553-558.

Bassolé, I H N, Lamien-meda, A., Bayala, B., Obame, L. C., Ilboudo, A. J., Franz, C., and Novak, J. 2011. Phytomedicine Chemical composition and antimicrobial activity of *Cymbopogon citratus* and *Cymbopogon giganteus* essential oils alone and in combination. *Phytomedicine*, **18**: 1070-1074.

Bassolé, Imaël Henri Nestor, and Juliani, H. R. 2012. Essential oils in combination and their antimicrobial properties. *Molecules (Basel, Switzerland)*, **17**(4): 3989-4006.

Bassolé, Imaël Henri Nestor, Lamien-Meda, A., Bayala, B., Tirogo, S., Franz, C., Novak, J., and Nebié, R. C. 2010. Composition and antimicrobial activities of *Lippia multiflora Moldenke*, *Mentha x piperita* L. and *Ocimum basilicum* L. essential oils and their major monoterpene alcohols alone and in combination. *Molecules (Basel, Switzerland)*, **15**(11): 7825-7839.

Bauer, K., Garbe, D., and Surburg, H. 2001. *Common Fragrance and Flavor Materials: 4th Edition.* Weinheim: WILEY-VCH Verlag GmbH.

Belletti, N., Kamdem, S. S., Tabanelli, G., Lanciotti, R., and Gardini, F. 2010. Modeling of combined effects of citral, linalool and beta-pinene used against *Saccharomyces cerevisiae* in citrus-based beverages subjected to a mild heat treatment. *International journal of food microbiology*, **136**(3): 283-289.

Benchaar, Chaouki, and Henry Greathead. 2011. Essential oils and opportunities to mitigate enteric methane emissions from ruminants. *Animal Feed Science and Technology*. 166-167: 338-355.

Bertea, C. M., and Maffei, M. E. 2010. The Genus Cymbopogon: Botany, Including Anatomy, Physiology, Biochemistry, and Molecular Biology. In A. Akhila, *Essential oil-bearing grassess: the genus Cymbopogon* (pp. 1-24). Boca Raton: Taylor and Francis Group.

Blank, A. F., Cavalcanti, T., Sant, P., Santos, P. S., Arrigoni-blank, M. F., Paula, A., and Cesar, H. 2011. Chemical characterization of the essential oil from patchouli accessions harvested over four seasons. *Industrial Crops and Products* (Article in Press).

Bolla, J.-M., Alibert-Franco, S., Handzlik, J., Chevalier, J., Mahamoud, A., Boyer, G., and Kieć-Kononowicz, K.2011. Strategies for bypassing the membrane barrier in multidrug resistant Gram-negative bacteria. *FEBS letters*, **585**(11): 1682-1690.

Bodeker, G., Salleh, H., Hashim, R.S., Jaenicke, C., Gruenwald, J., and Abidin, Z.Z. 2009. Health and Beauty from the Rainforest: Malaysian Traditions of Ramuan. Kuala Lumpur: Didier Millet.

Bourgaud, F., Gravot, A., Milesi, S., and Gontier, E. 2001. Production of plant secondary metabolites: a historical perpsective. *Plant Science,* **161:** 839-851.

Bourgou, S., Pichette, A, Lavoie, S., Marzouka, B. and Legault, J. 2012. Terpenoids isolated from Tunisian *Nigella sativa* L. essential oil with antioxidant activity and the ability to inhibit nitric oxide production. *Flavour and Fragrance Journal*.**27**(1): 69-74.

Bonapace, C. R., Bosso, J. A., Friedrich, L. V., and White, R. L. 2002. Comparison of methods of interpretation of checkerboard synergy testing. *Diagnostic Microbiology and Infectious Disease*. 44: 363-366.

Bonapace, C. R., White, R. L., Friedrich, L. V., and Bosso, J. A. 2000. Evaluation of antibiotic synergy against *Acinetobacter baumannii* : a comparison with Etest , time-kill, and checkerboard methods. *Diagnostic Microbiology and Infectious Disease*.**38:** 43-50.

Breitmaier, E. 2006. *Terpenes*. Engelfriedshalde 46, Tubingen, Germany: WILEY-VCH Verlag GmbH and Co.

Buckle, J. 2003. *Clinical Aromatherapy: Essential Oils in Practice 2nd Edition*. Philadelphia: Elsevier Science.

Burt, S. 2004. Essential oils: their antibacterial properties and potential applications in foods--a review. *International journal of food microbiology*, **94**(3): 223-53.

Burchfield, H.P. and Storrs, E.E. 1970. *Biochemical Applications of Gas Chromatography 4th Edition*. United States of America: Academic Press Inc.

Cao, L., Si, J. Y., Liu, Y., Sun, H., Jin, W., Li, Z., and Zhao, X. H.2009. Essential oil composition , antimicrobial and antioxidant properties of *Mosla chinensis* Maxim. *Food Chemistry*, **115**(3): 801-805.

Carson, C. F., and Hammer, K. A. 2011. Chemistry and Bioactivity of Essential Oils. In H. Thormar, *Lipids and Essential Oils as Antimicrobial Agents* (pp.203-238). Chichester, West Sussex, United Kingdom: John Wiley and Sons.

Castro, H. G. D., Borges, V., Perini, D. M., and Rodrigues, G. 2010. Evaluation of content and composition of the essential oil of *Cymbopogon nardus* (L.) in different harvest times. *Revista Ciência Agronômica*. **41**(2): 308-314.

Chen, W., and M. Viljoen. 2010. Geraniol — A review of a commercially important fragrance material. *South African Journal of Botany*.**76**(4): 643-651.

Christian, G. D. 2004. *Analytical Chemistry* 6th Edition.USA. John Wiley & Sons, Inc.

Cimanga, K., Kambu, K., Tona, L., Apers, S., De Bruyne, T., Hermans, N., and Totté, J. 2002. Correlation between chemical composition and antibacterial activity of essential oils of some aromatic medicinal plants growing in the Democratic Republic of Congo. *Journal of ethnopharmacology*, **79**(2): 213-220.

Clemente, M. A., de Oliveira Monteiro, C. M., Scoralik, M. G., Gomes, F. T., de Azevedo Prata, M. C., and Daemon, E. 2010. Acaricidal activity of the essential oils from *Eucalyptus citriodora* and *Cymbopogon nardus* on larvae of *Amblyomma cajennense* (Acari: Ixodidae) and *Anocentor nitens* (Acari: Ixodidae). *Parasitology research*. **107(**4): 987-92.

Cottarel, G. and Wierzbowski, J. 2007. Combination drugs, an emerging option for antibacterial therapy. *Trends in biotechnology*.**25**(12): 547-55.

Croteau, R., Kutchan, T.M., and Lewis, N.G., 2000. Natural products (secondary metabolites). In: Buchanan, B., Gruissem, W., Jones, R. (Eds.), Biochemistry and Molecular Biology of Plants. American Society of Plant Physiologists.

Dar, M. Y., Shah, W., Rather, M., Qurishi, Y., Hamid, A., and Qurishi, M. 2011. Chemical composition, in vitro cytotoxic and antioxidant activities of the essential oil and major constituents of *Cymbopogon jawarancusa* (Kashmir). *Food Chemistry*, **129**(4), 1606-1611.

de Azeredo, G. A., Stamford, T. L. M., Nunes, P. C., Gomes Neto, N. J., de Oliveira, M. E. G., and de Souza, E. L. 2011. Combined application of essential oils from *Origanum vulgare* L. and *Rosmarinus officinalis* L. to inhibit bacteria and autochthonous microflora associated with minimally processed vegetables. *Food Research International*, **44**(5), 1541-1548.

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): 101-9.

Devi, K. P., Nisha, S. A., Sakthivel, 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**(1): 107-115.

Denyer, S., Hodges, N., and Gorman, S. 2004. Introduction to Pharmaceutical Microbiology. In S. P. Denyer, N. A. Hodges, and S. P. Gorman, *Pharmaceutical Microbiology 7th edition* (pp. 3-8). Massachussetts: Blackwell Publishing.

Dewick, P. M. 2002. The Shikimate Pathway: Aromatic Amino Acids and Phenylpropanoids. In *Medicinal Natural Products* (pp. 121-166). John Wiley and Sons, Ltd.

Djenane, D., Yangüela, J., Montañés, L., Djerbal, M., and Roncalés, P.2011. Antimicrobial activity of *Pistacia lentiscus* and *Satureja montan*a essential oils against *Listeria monocytogenes* CECT 935 using laboratory media : Efficacy and synergistic potential in minced beef. *Food Control*, **22**(7): 1046-1053.

Dorman, H. J., and Deans, S. G. 2000. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *Journal of Applied Microbiology*, **88**(2), 308-316.

Duru, M. E., Mercan, N., and Harmandar, M. 2009. Antioxidant , anticholinesterase and antimicrobial constituents from the essential oil and ethanol extract of *Salvia potentillifolia*, **116**: 470-479.

Duru, M. E., Oztürk, M., Uğur, A., and Ceylan, O. 2004. The constituents of essential oil and in vitro antimicrobial activity of *Micromeria cilicica* from Turkey. *Journal of ethnopharmacology*, **94**(1): 43-48.

Díez, V. K., Apesteguía, C. R., and Di Cosimo, J. I. 2010. Synthesis of ionones on solid Brønsted acid catalysts: Effect of acid site strength on ionone isomer selectivity. *Catalysis Today*, **149**(3-4): 267-274.

Effendy, N. M., Mohamed, N., Muhammad, N., Mohamad, I. N., andShuid, A. N. 2012. *Eurycoma longifolia* : Medicinal Plant in the Prevention and Treatment of Male Osteoporosis due to Androgen Deficiency. *Hindawi Based Corporation*.**2012**: 1-9.

Eliopoulos, G M, and Eliopoulos, C.T. 1988. Antibiotic Combinations : Should They Be Tested?. *Clinical Microbiology Review*.**1**(2): 139-156.

Espina, L., Somolinos, M., Lorán, S., Conchello, P., García, D.,and Pagán, R. 2011. Chemical composition of commercial citrus fruit essential oils and evaluation of their antimicrobial activity acting alone or in combined processes. *Food Control*.**22**(6): 896-902.

Fadli, M., Saad, A., Sayadi, S., Chevalier, J., Mezrioui, N., Pagès, J., and Hassani, L. 2012. Antibacterial activity of *Thymus maroccanus* and *Thymus broussonetii* essential oils against nosocomial infection - bacteria and their synergistic potential with antibiotics. *Phytomedicine : International Journal Of Phytotherapy And Phytopharmacology*.**19**(5): 464-71.

Figueirinha, A., Paranhos, A., Pérez-Alonso, J. J., Santos-Buelga, C., and Batista, M. T. 2008. *Cymbopogon citratus* leaves: Characterization of flavonoids by HPLC–PDA–ESI/MS/MS and an approach to their potential as a source of bioactive polyphenols. *Food Chemistry*.**110**(3): 718-728.

Fisher, K., and Phillips, C. 2008. Potential antimicrobial uses of essential oils in food: is citrus the answer? *Trends in Food Science and Technology*.**19**(3): 156-164.

Gagan Shah, Richa Shri, Vivek Panchal, Narender Sharma, Bharpur Singh, and A.S. Mann. 2011. Scientific basis for the therapeutic use in *Cymbopogon citratus*, stapf (Lemon grass). *J Adv Pharm Technol Res*. **2**(1): 3-8.

Gill, A. O., and Holley, R. A. 2004. Mechanisms of Bactericidal Action of Cinnamaldehyde against *Listeria monocytogenes* and of Eugenol against *L . monocytogenes* and *Lactobacillus sakei*.*Applied And Environmental Microbiology*. **70**(10): 5750–5755.

Goñi, P., López, P., Sánchez, C., Gómez-Lus, R., Becerril, R., and Nerín, C. 2009. Antimicrobial activity in the vapour phase of a combination of cinnamon and clove essential oils. *Food Chemistry*, **116**(4): 982-989.

Gutierrez, J, Barry-Ryan, C., and Bourke, P. 2008. The antimicrobial efficacy of plant essential oil combinations and interactions with food ingredients. *International Journal of Food Microbiology***124**(1): 91-7.

Hammer, C. F. 2011. Chemistry and Bioactivity of Essential Oils. In H. Thormar, *Lipids and Essential Oils as Antimicrobial Agents* (pp. 203-238). Chichester, West Sussex, United Kingdom: John Wiley and Sons Ltd.

Hanlon, A., Taylor, M., and Dick, J. D. 2007. Agar Dilution Susceptibility Testing. In R. Schwalbe, L. Steele-Moore, and A. C. Goodwin, *Antimicrobial Susceptibility Testing Protocols.* Boca Raton: CRC Press, Taylor and Francis Group.

Hemaiswarya, S., and Doble, M. 2009. Synergistic interaction of eugenol with antibiotics against Gram negative bacteria. *Phytomedicine : International Journal of Phytotherapy and Phytopharmacology.***16**(11): 997-1005.

Hemaiswarya, S., Kruthiventi, A.K. and Doble, M. 2008. Synergism between natural products and antibiotics against infectious diseases. *Phytomedicine : International Journal of Phytotherapy and Phytopharmacology.***15**(8): 639-52.

Holley, R.andPatel, D. 2005. Improvement in shelf-life and safety of perishable foods by plant essential oils and smoke antimicrobials. *Food Microbiology*.**22**(4): 273-292.

Hossain, M. A., Shah, M.D., Sang, S.V., and Sakari, M. 2011. Chemical composition and antibacterial properties of the essential oils and crude extracts of Merremia borneensis. *Journal Of King Saud University - Science*.

Huh, A. J., and Kwon, Y. J. 2011. "Nanoantibiotics": a new paradigm for treating infectious diseases using nanomaterials in the antibiotics resistant era. *Journal of controlled release : official journal of the Controlled Release Society*. **156**(2); 128-45.

Hussain, A. I., Anwar, F., Hussain Sherazi, S. T., and Przybylski, R. 2008. Chemical composition, antioxidant and antimicrobial activities of basil (*Ocimum basilicum*) essential oils depends on seasonal variations. *Food Chemistry*, **108**(3): 986-995.

Hyldgaard, M., Mygind, T., and Meyer, R. L. 2012. Essential oils in food preservation: mode of action, synergies, and interactions with food matrix components. *Frontiers in microbiology*, **3:** 12.

Ibrahim, H., Aziz, A.N, Syamsir, D. R., Ali, N.A.M., Mohtar, M., Ali, R.M., Awanga, K. 2009. Essential oils of *Alpinia conchigera* Griff . and their antimicrobial activities.*Food Chemistry*.**113**(2): 575-577.

Inouye, S., Takizawa, T., and Yamaguchi, H. 2001. Antibacterial activity of essential oils and their major constituents. JAC: 565-573.

Isenberg, H.D. 2004. *Clinical Microbiology Procedures Handbook*. Washington, DC: ASM Press.

Jamal, J. A., Ghafar, Z. A., and Husain, K. (2011). Medicinal Plants used for Postnatal Care in Malay Traditional Medicine in the Peninsular Malaysia. *Pharmacognosy Journal*.**3**(24): 15-24.

Kamatou, G. P. P., Zyl, R. L. V., Vuuren, S. F. V., Figueiredo, A. C., and Barroso, J. G. 2008. Seasonal variation in essential oil composition, oil toxicity and the biological activity of solvent extracts of three South African *Salvia* species. *South African Journal Of Botany*, **74**: 230-237.

Kanko, C., Sawaliho, B. E.-H., Kone, S., Koukoua, G., and N'Guessan, Y. T. 2004. Étude des propriétés physico-chimiques des huiles essentielles de Lippia multiflora, *Cymbopogon citratus*, *Cymbopogon nardus*, *Cymbopogon giganteus*. *Comptes Rendus Chimie*.**7**(10-11): 1039-1042.

Kelen, M., and Tepe, B. 2008. Chemical composition , antioxidant and antimicrobial properties of the essential oils of three *Salvia* species from Turkish flora. *Bioresource Technology*, **99**: 4096-4104.

Khanuja, S. P. S., Shasany, A. K., Pawar, A., Lal, R. K., Darokar, M. P., Naqvi, and Rajkumar, S. 2005. Essential oil constituents and RAPD markers to establish species relationship in *Cymbopogon* Spreng. (Poaceae). *Biochemical Systematics and Ecology*,**33**(2): 171-186.

Khunkitti, W. 2010. In vitro antimicrobial and antioxidant activities of some *Cymbopogon* species. In: Akhila, A. (Ed.), Essential Oil-Bearing Grasses. Boca Raton: Taylor and Francis Group. pp. 167–183.

Klančnik, A., Piskernik, S., Jeršek, B., and Možina, S. S. 2010. Evaluation of diffusion and dilution methods to determine the antibacterial activity of plant extracts. *Journal of Microbiological Methods*. **81**(2): 121-126.

Kubeczka, K.H. 2010. History and Sources of Essential Oil Research. In Baser,K. H., and Buchbauer,G.*Handbook of Essential Oils : Science, Technology, and Applications.* (pp. 3-38). Boca Raton: Taylor and Francis Group.

Lalko, J., and Api, A. M. 2008. Citral: identifying a threshold for induction of dermal sensitization. *Regulatory toxicology and pharmacology : RTP*.**52**(1): 62-73.

Lang, G., and Buchbauer, G. 2012. A review on recent research results (2008-2010) on essential oils as antimicrobials and antifungals. *Flavour and Fragrance Journal*, **27**(1): 13-39.

Lenardão, E. J., Botteselle, G. V., de Azambuja, F., Perin, G., and Jacob, R. G. 2007. Citronellal as key compound in organic synthesis. *Tetrahedron*, **63**(29): 6671-6712.

Lv, F., Liang, H., Yuan, Q., and Li, C. 2011. In vitro antimicrobial effects and mechanism of action of selected plant essential oil combinations against four food-related microorganisms. *Food Research International*, **44**(9): 3057-3064.

Machado, M., Pires, P., Dinis, M., Santos-Rosa, M., Alves, V., Salgueiro, L., and Cavaleiro, C. 2012. Monoterpenic aldehydes as potential anti-*Leishmania* agents: activity of *Cymbopogon citratus* and citral on *L. infantum*, *L. tropica* and *L. major*. *Experimental parasitology*, **130**(3): 223-231.

Maciel, M., Oliveira, M. D., Florisvaldo, D., Cardoso, G., Alves, E., and Hilsdorf, R. 2010. Disinfectant action of *Cymbopogon* sp . essential oils in different phases of biofilm formation by *Listeria monocytogenes* on stainless steel surface. *Food Control*, **21**(4), 549-553.

Mahalwal, V. S. and Ali, M. 2003. Volatile constituents of *Cymbopogon nardus* (Linn.) Rendle. *Flavour and Fragrance J*. 18: 73–76.

Maizura, M., Fazilah, A., Norziah, M. H. and Karim, A. A. 2008. Antibacterial Activity of Modified Sago Starch-Alginate Based Edible Film Incorporated with Lemongrass (*Cymbopogon citratus*) Oil, **15**(2): 233-236.

Maksimovi, M., ´Cavar, S., Vidic, D., and Pari´c, A.2012. Chemical composition and antioxidant and antimicrobial activity of essential oil of *Artemisia annua L*. from Bosnia. *Industrial Crops and Products*. 37: 479-485.

Marshall, S. 2004. Myrrh: Magi, medicine and mortality. *Pharmaceutical Journal*.**273** (7330): 919-920.

McKee, T., and McKee, J. R. 2003. *Biochemistry: The Molecular Basis of Life 3rd ed.* New York: McGraw-Hill .

McMurry, J. 2011. *Fundamental of Organic Chemistry 7th Edition*. USA. Brooks/Cole Cencage Learning.

Mirghani, M.E.S., Liyana, Y. and Parveen, J. 2012. Bioactivity analysis of lemongrass (*Cymbopogon citratus*) essential oils. *International Food Research Journal*. **19**(2): 569-575.

Moon, S., Kim, H. and Cha,J. 2011. Synergistic effect between clove oil and its major compounds and antibiotics against oral bacteria. *Archives of Oral Biology*.**56**(9): 907-916.

Moyler, D. A. 2010. Citral from Lemongrass and Other Natural Sources: Its Toxicology. In A. Akhila, *Essential oil-bearing grasses: the genus Cymbopogon* (pp. 223-237). Boca Raton: CRC Press, Taylor and Francis Group.

Mustafa, F., Indurkar, J., Ali, N.I.M., Hanapi, A., Shah, M., Ismail, S.and Mansor, S.M. 2011. A Review of Malaysian Medicinal Plants with Potential Antidiabetic Activity. *Journal of Pharmacy Research*.**4**(11): 4217-4224.

Naik, M. I., Fomda, B. A., Jaykumar, E., and Bhat, J. A. 2010. Antibacterial activity of lemongrass (*Cymbopogon citratus*) oil against some selected pathogenic bacteria. *Asian Pacific Journal of Tropical Medicine*, **3**(7): 535-538.

Nakahara, K., Alzoreky, N. S., and Yoshihashi, T. 2003. Chemical Composition and Antifungal Activity of Essential Oil from *Cymbopogon nardus* (Citronella Grass). *Sciences-New York*, **37**: 249-252.

Natta, L., Orapin, K., Krittika, N. and Pantip, B. 2008. Essential oil from five *Zingiberaceae* for anti food-borne bacteria. *International Food Research Journal*.**15**(3).

Neidhardt, F. C. 2004. The Bacterial Cell. In K. J. Ryan, and C. G. Ray, *Sherris Medical Microbiology: An Introduction to Infectious Diseases 4th edition.* (pp. 27-75). United States of America: McGraw-Hill.

Nejad Ebrahimi, S., Hadian, J., Mirjalili, M. H., Sonboli, and Yousefzadi, M. 2008. Essential oil composition and antibacterial activity of *Thymus caramanicus* at different phenological stages. *Food Chemistry*, **110**(4): 927-931.

Nerio, L. S., Olivero-Verbel, J., and Stashenko, E. 2010. Repellent activity of essential oils: A review. *Bioresource technology*, **101**(1): 372-378.

Nguefack, J., Dongmo, J. B. L., Dakole, C. D., Leth, V., Vismer, H. F., Torp, J., and Guemdjom, E. F. N.2009. Food preservative potential of essential oils and fractions from *Cymbopogon citratus*, *Ocimum gratissimum* and *Thymus vulgaris* against mycotoxigenic fungi. *International journal of food microbiology*, **131**(2-3), 151-156.

Nguefack, J., Tamgue, O., Dongmo, J. B. L., Dakole, C. D., Leth, V., Vismer, H. F.,and Zollo, P. H. A.2012. Synergistic action between fractions of essential oils from *Cymbopogon citratus*, *Ocimum gratissimum* and *Thymus vulgaris* against *Penicillium expansum*. *Food Control*, **23**(2): 377-383.

Oliveira, M.M.M., Brugnera, D.F., Cardoso, M.G., Guimarães, L.G.L., and Piccoli, R. H. 2011. Yield, chemical composition and antilisterial activity of essential oils from *Cymbopogon* species. *Revista Brasileira de Plantas Medicinais*. **13**(1): 8-16.

Ong, H. C., and Nordiana, M. 1999. Malay ethno-medico botany in Machang, Kelantan, Malaysia. *Fitoterapia*. **70**(5): 502-513

Oussalah, M., Saucier, L., and Lacroix, M. 2007. Inhibitory eVects of selected plant essential oils on the growth of four pathogenic bacteria : *E . coli* O157 : H7 , *Salmonella Typhimurium*, *Staphylococcus aureus* and *Listeria monocytogenes*. *Heart*, **18**: 414-420.

Palaniappan, K., and Holley, R. A. 2010. International Journal of Food Microbiology Use of natural antimicrobials to increase antibiotic susceptibility of drug resistant bacteria. *International Journal of Food Microbiology*.**140(**2-3): 164-168.

Palhano, F. L., Vilches, T. T. B., Santos, R. B., Orlando, M. T. D., Ventura, J. A., and Fernandes, P. M. B. 2004. Inactivation of *Colletotrichum gloeosporioides* spores by high hydrostatic pressure combined with citral or lemongrass essential oil. *International Journal of Food Microbiology*.**95**(1): 61-66.

Pandey, A. 2010. The *Cymbopogons*: Harvest and Postharvest Management. In A. Akhila, *Essential oil-bearing grasses: The genus Cymbopogon* (pp. 107-133). Boca Raton: Taylor and Francis Group.

Park, M. J., Gwak, K. S., Yang, I., Kim, K. W., Jeung, E. B., Chang, J. W., and Choi, I. G. 2009. Effect of citral, eugenol, nerolidol and alpha-terpineol on the ultrastructural changes of *Trichophyton mentagrophytes*. *Fitoterapia*, **80**(5): 290-296.

Patrick, G.L. 2009. *An Introduction to Medicinal Chemistry*.United States: Oxford University Press Inc., New York.

Pavia, D.L, Lampman, G.M., and Kriz, G.S. 2013. *A Microscale Approach to Organic Laboratory Techniques*. USA. Brooks/Cole Cencage Learning.

Pihlasalo, J., Klika, K. D., Murzin, D. Y., and Nieminen, V. 2007. Conformational equilibria of citral. *Journal of Molecular Structure: THEOCHEM*, **814**(1-3): 33-41.

Pillai, S. K., Robert C. Moellering, J., and Eliopoulus, G. M. 2005. Antimicrobial Combinations. In V. Lorian, *Antibiotics in Laboratory Medicine - 5th Edition.* Walnut Street, Philadelphia, USA: Lippincott Williams and Walkins.

Qaiyumi, S. 2007. Macro- and Microdilution Methods of Antimicrobial Susceptibility Testing. In R. Schwalbe, L. Steele-Moore, and A. C. Goodwin, *Antimicrobial Susceptibility Testing Protocols.* Boca Raton: CRC Press, Taylor and Francis Group .

Ramawat, K., Dass, S., and Mathur, M. 2009. The Chemical Diversity of Bioactive Molecules and Therapeutic Potential of Medicinal Plants. In K. Ramawat, *Herbal Drugs: Ethnomedicine to Modern Medicine* (pp. 7-32). Heidelberg: Springer-Verlag Berlin Heidelberg.

Rasadah, M., and Ali, A. 2008. Nutraceutical and Cosmetic Products Developed from Malaysian Biodiversity Resources. Kuala Lumpur.

Rauber, C. D. S., Guterres, S. S., and Schapoval, E. E. S. 2005. LC determination of citral in *Cymbopogon citratus* volatile oil. *Journal of pharmaceutical and biomedical analysis*, **37**(3): 597-601.

Rocha, G., Steffen, G., Almeida, D., Aparecida, M., Regitano, B., Arce, D., and Fa, M. 2009. Activity of essential oil and its major compound, 1,8-cineole, from *Eucalyptus globulus* Labill., against the storage fungi *Aspergillus flavus* Link and *Aspergillus parasiticus* Speare. *Journal of Stored Products Research*. **45**:108–111.

Rodríguez Quintanilla, R., Ruiz Nova, C., Arias Moyano, G., Castro Salazar, H., Martínez, J., and Stashenko, E. 2012.Comparative study of the essential oil compositions of four *Cymbopogon* (Poaceae) species grown in Colombia. *Boletin Latinoamericano y del Caribe de Plantas Medicinales y Aromaticas*.**11**(1): 77-85.

Roosita, K., Kusharto, C. M., Sekiyama, M., Fachrurozi, Y., and Ohtsuka, R. 2008. Medicinal plants used by the villagers of a Sundanese community in West Java, Indonesia. *Journal of ethnopharmacology*. **115**(1): 72-81.

Rosato, A., Vitali, C., Gallo, D., Balenzano, L., and Mallamaci, R. 2008. The inhibition of Candida species by selected essential oils and their synergism with amphotericin B. *Phytomedicine : International Journal of Phytotherapy and Phytopharmacology*. **15**(8): 635-8.

Salih, A. G., le Quere, J.M., and Drilleau, J.F. Action des acides hydroxycinnamiques libres et estérifiés sur la croissance des bactéries lactiques. *Sciences des aliments*.**20**(6): 537-560.

Sacchetti, G., Maietti, S., Muzzoli, M., Scaglianti, M., Manfredini, S., Radice, M., and Bruni, R. 2005. Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. *Food Chemistry*, **91**(4): 621-632.

Saddiq, A., and Khayyat, S. 2010. Chemical and antimicrobial studies of monoterpene: Citral. *Pesticide Biochemistry and Physiology*, **98**(1): 89-93.

Sakulku, U., Nuchuchua, O., Uawongyart, N., and Puttipipatkhachorn, S. 2009. Characterization and mosquito repellent activity of citronella oil nanoemulsion. *International Journal of Pharmaceutics*, **372:** 105-111.

Salehi, P., Mirza, M., Calagari, M., and Adams, R. P. 2010. Effects drying and harvest season on the essential oil composition from foliage and berries of *Juniperus excelsa*. *Industrial Crops and Products*, **32**(2), 83-87.

Santin, M. R., dos Santos, A. O., Nakamura, C. V., Dias Filho, B. P., Ferreira, I. C. P., and Ueda-Nakamura, T. 2009. In vitro activity of the essential oil of *Cymbopogon citratus* and its major component (citral) on *Leishmania amazonensis*. *Parasitology research*, **105**(6): 1489-96.

Sell, C. 2010. Chemistry of Essential Oils. In K. H. Baser, and G. Buchbauer, *Handbook of Essential Oils: Science, Technology and Application* (pp. 121-150). Boca Raton: Taylor and Francis Group

Shirugumbi, M., Bhimashya, S., Madhava, P., Nagella, P., Reddy, H., and Niranjana, H. 2010. Essential oils of *Lavandula bipinnata* and their antimicrobial activities. *Food Chemistry*, **118**(3): 836-839.

Shukor, A.R.A., Yusoff, A.M. M, Norowi, H.M. and Shukor, N.M. Contribution of Biodiversity Towards Sustainable Agriculture Development. Kuala Lumpur.

Sikkema, J., de Bont, J., and Poolman, B. 1994. Interactions of cyclic hydrocarbons with biological membranes. *The Journal of biological chemistry*, **269**(11): 8022-8028.

Sikkema, J., de Bont, J., and Poolman, B. 1995. Mechanisms of membrane toxicity of hydrocarbons. *Microbiological reviews*, **59**(2): 201-22.

Silva, C. F., Moura, F. C., Mendes, M. F., and Pessoa, F. L. P. 2011. Extraction of Citronella (*Cymbopogon nardus*) Essential Oil Using Supercritical CO_2 : Experimental Data And Mathematical Modeling. Brazilian Journal of Chemical Engineering. **28**(02): 343-350.

Skocˇibus, M., and Nada Bezic, V. D. 2006. Phytochemical composition and antimicrobial activities of the essential oils from *Satureja subspicata* Vis . growing in Croatia. *Food Chemistry*, **96:** 20-28.

Surburg, H., and Panten, J. 2006. *Common Fragrance and Flavour Materials: Preparation, Properties and Uses.* Weinheim: Wiley-VCH

Tajkarimi, M. M., Ibrahim, S., and Cliver, D. O. 2010. Antimicrobial herb and spice compounds in food. *Food Control*, **21**(9): 1199-1218.

Talaro, K. P., and Talaro, A. (2002). *Foundations in Microbiology.* McGraw-Hill.

Taveira, F. S. N., Lima, W. N. D., and Andrade, E. H. A. 2003. Seasonal essential oil variation of *Aniba canelilla*. *Biochemical Systematics and Ecology*, **31**: 69-75.

Telci, I., Bayram, E., Yilmaz, G., and Avci, B. 2006. Variability in essential oil composition of Turkish basils (*Ocimum basilicum* L.). *Biochemical Systematics and Ecology*.**34**: 489-497.

Tenore, G. C., Ciampaglia, R., Arnold, N. A., Piozzi, F., Napolitano, F., Rigano, D., and Senatore, F. 2011. Antimicrobial and antioxidant properties of the essential oil of *Salvia lanigera* from Cyprus. *Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association*, **49**(1), 238-243.

Tepe, B. 2005. Food Chemistry Antimicrobial and antioxidant activities of the essential oil and various extracts of *Salvia tomentosa* Miller (Lamiaceae). *Food Chemistry*, **90**: 333-340.

Teixeira, B., Marques, A., Ramos, C., Batistaa, I., Serrano, C., Matos, O., Neng, N.R., Nogueira, J.M.F., Saraiva, J. A., and Nunes, M.L. 2012. European pennyroyal (*Mentha pulegium*) from Portugal: Chemical composition of essential oil and antioxidant and antimicrobial properties of extracts and essential oil. *Industrial Crops and Products*.**36**(1): 81-87.

Thomas, G. 2007. *Medicinal Chemistry: An Introduction 2nd edition.* Chichester, West Sussex, England: John Wiley and Sons.

Toroglu, S. 2007. In vitro antimicrobial activity and antagonistic effect of essential oils from plant species. *Journal of Environmental Biology/Academy of Environmental Biology, India*. **28**(3): 551-9.

Tiwari, R. 2010. The Trade in Commercially Important *Cymbopogon* Oils. In A. Akhila, *Essential oil-bearing grasses: the genus Cymbopogon* (pp. 151-167). Boca Raton: CRC Press, Taylor and Francis Group.

Tyagi, B. K., Shahi, K., and Kaul, B. L. 1998. Evaluation of repellent activities of *Cymbopogon* essential oils against mosquito vectors of Malaria, Filariasis and Dengue Fever in India. *Phytomedicine*, **5**(4), 324-329.

Tzortzakis, N. G., and Economakis, C. D. 2007. Antifungal activity of lemongrass (*Cympopogon citratus* L.) essential oil against key postharvest pathogens. *Innovative Food Science and Emerging Technologies*, **8**(2): 253-258.

Ultee, A., Kets, E. P., and Smid, E. J. 1999. Mechanisms of action of carvacrol on the food-borne pathogen *Bacillus cereus*. *Applied Environmental Microbiology*, **65**(10): 4606-4610

Ultee, A., Bennik, M. H. J., and Moezelaar, R. 2002. The Phenolic Hydroxyl Group of Carvacrol Is Essential for Action against the Food-Borne Pathogen *Bacillus cereus*Applied Environmental Microbiology. **68**(4): 1561-1568.

Verma, P. 2007. Methods for Determining Bactericidal Activity and Antimicrobial Interactions: Synergy Testing, Time-Kill Curves, and Population Analysis. In R. Schwalbe, L. Steele-Moore, and A. C. Goodwin, *Antimicrobial Susceptibility Testing Protocols.* Boca Raton: CRC Press, Taylor and Francis Group.

Wang, H., Liu, Y., Wei, S., and Yan, Z. 2012. Comparative seasonal variation and chemical composition of essential oils from the leaves and stems of *Schefflera*heptaphylla using microwave-assisted and conventional hydrodistillation. *Industrial Crops and Products*, **36**(1): 229-237.

Wannissorn, B., Jarikasem, S., Siriwangchai, T., and Thubthimthed, S. 2005. Antibacterial properties of essential oils from Thai medicinal plants. *Fitoterapia*, **76**(2): 233-236.

Wenger, A. 2007. Disk Diffusion Test and Gradient Methodologies. In R. Schwalbe, L. Steele-Moore, and A. C. Goodwin.*Antimicrobial Susceptibility Testing Protocols.* Boca Raton: CRC Press, Taylor and Francis Group.

Worwood, V. A. 1991. *The Complete Book of Essential Oils and Aromatherapy.* Novato: New World Library.

Yang, Z., Xi, J., Li, J., and Qu, W. 2009. Biphasic effect of citral, a flavoring and scenting agent, on spatial learning and memory in rats. *Pharmacology, Biochemistry, and Behavior*, **93**(4): 391-396.

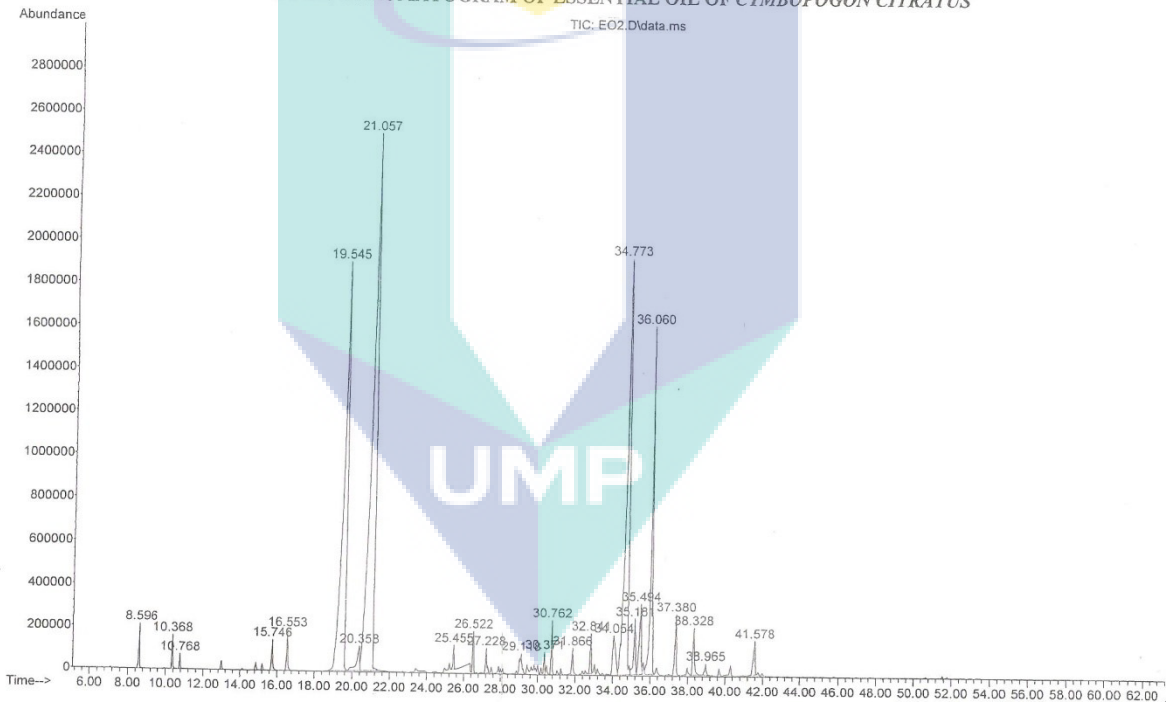
Yu, J., Lei, J., Zhang, X., Yud, H., Tian, D., Liao, Z., and Zoud, G. 2011. Anticancer, antioxidant and antimicrobial activities of the essential oil of *Lycopus lucidus* Turcz . var . hirtus Regel. *Food Chemistry*.**126**(4): 1593-1598.

Zaidi, S. F. H., Yamada, K., Kadowaki, M., Usmanghani, K., and Sugiyama, T. 2009. Bactericidal activity of medicinal plants, employed for the treatment of gastrointestinal ailments, against *Helicobacter pylori*. *Journal of Ethnopharmacology*, **121**(2), 286-91.

Zore, G. B., Thakre, A. D., Jadhav, S., and Karuppayil, S. M. 2011. Terpenoids inhibit *Candida albicans* growth by affecting membrane integrity and arrest of cell cycle. *Phytomedicine : International Journal of Phytotherapy and Phytopharmacology*, **18**(13): 1181-1190.

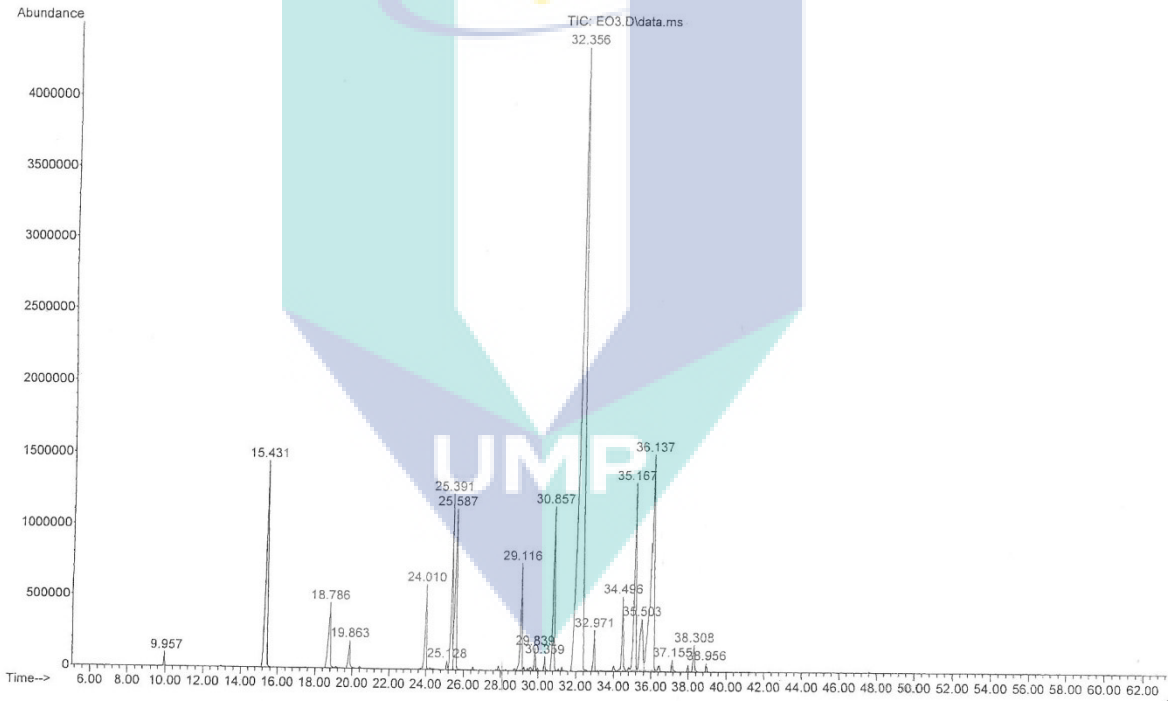
APPENDIX A1

GC-MS CHROMATOGRAM OF ESSENTIAL OIL OF *CYMBOPOGON CITRATUS*



APPENDIX A2

GC-MS CHROMATOGRAM OF ESSENTIAL OIL OF *CYMBOPOGON NARDUS*



APPENDIX B1

GC-MS LIBRARY SEARCH REPORT OF ESSENTIAL OIL OF *CYMBOPOGON CITRATUS*

RT	Area%	Library/ID	Ref#	CAS#	Qual
8.594	0.62	C:\Database\NIST08.L			
		Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	15704	003387-41-5	27
		Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	15710	003387-41-5	27
		Ethanone, 1-cyclopropyl-2-(4-pyridinyl)-	30954	006580-95-6	27
10.368	0.48	C:\Database\NIST08.L			
		1R-.alpha.-Pinene	15517	007785-70-8	87
		1,3,7-Octatriene, 3,7-dimethyl-	15573	000502-99-8	50
		1R-.alpha.-Pinene	15515	007785-70-8	43
10.769	0.23	C:\Database\NIST08.L			
		1,3,6-Octatriene, 3,7-dimethyl-, (Z)-	15615	003338-55-4	93
		1,3,6-Octatriene, 3,7-dimethyl-	15570	013877-91-3	93
		1,3,7-Octatriene, 3,7-dimethyl-	15573	000502-99-8	50
15.747	0.58	C:\Database\NIST08.L			
		1,4-Heptadiene, 3-methyl-	5908	001603-01-6	43
		3,4-Nonadiene	10503	037050-03-6	38
		2,5-Heptadiene, (E,E)-	2846	039619-60-8	38
16.554	0.78	C:\Database\NIST08.L			
		1,4-Heptadiene, 3-methyl-	5908	001603-01-6	53
		2,3-Hexadiene, 2-methyl-	2862	029212-09-7	42
		Cyclopropane, 1-chloro-2-ethenyl-1-methyl-	8227	062337-93-3	38
19.546	21.34	C:\Database\NIST08.L			
		2,6-Octadienal, 3,7-dimethyl-, (Z)	24760	000106-26-3	86
		2,6-Octadienal, 3,7-dimethyl-	24719	005392-40-5	72
		1,7-Nonadiene, 4,8-dimethyl-	25009	062108-28-5	40
20.359	1.08	C:\Database\NIST08.L			
		2,6-Octadien-1-ol, 2,7-dimethyl-	26277	022410-74-8	72
		2,6-Octadien-1-ol, 3,7-dimethyl-, (E)-	26337	000106-24-1	64
		2,6-Octadien-1-ol, 3,7-dimethyl-, formate, (E)-	45866	000105-86-2	64
21.057	33.01	C:\Database\NIST08.L			
		2,6-Octadienal, 3,7-dimethyl-, (E)	24763	000141-27-5	94
		2,6-Octadienal, 3,7-dimethyl-	24715	005392-40-5	90
		2,6-Octadienal, 3,7-dimethyl-, (E)	24753	000141-27-5	90
25.457	0.39	C:\Database\NIST08.L			
		1,3-Butadiene, 2-methyl-	457	000078-79-5	58
		1,3-Pentadiene	439	000504-60-9	53
		1,3-Pentadiene, (Z)-	449	001574-41-0	53

26.521	0.80	C:\Database\NIST08.L			
		(-)-Aristolene	62266	006831-16-9	55
		Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-,[1R-(1R*,4Z,9S*)]-	62433	000118-65-0	45
		Bicyclo[3.1.0]hexane, 6-methylene-	2609	054211-16-4	43
27.231	0.53	C:\Database\NIST08.L			
		trans-.alpha.-Bergamotene	62324	013474-59-4	78
		1,3,6,10-Dodecatetraene, 3,7,11-trimethyl-, (Z,E)-	62350	026560-14-5	38
		1,3,6,10-Dodecatetraene, 3,7,11-trimethyl-, (Z,E)-	62352	026560-14-5	35
29.119	0.80	C:\Database\NIST08.L			
		Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethylidene)-, (4aR-trans)-	62451	000515-17-3	60
		Spiro[5.5]undec-2-ene, 3,7,7-trimethyl-11-methylene-, (-)-	62383	018431-82-8	52
		1-(3,5-Dimethyl-1-adamantanoyl)semicarbazide	109301	351327-47-4	43
30.372	0.35	C:\Database\NIST08.L			
		1H-Cyclopenta[1,3]cyclopropa[1,2]benzene, octahydro-7-methyl-3-methylene-4-(1-methylethyl)-, [3aS-(3a.alpha.,3b.beta.,4.beta.,7.alpha.,7aS*)]-	62563	013744-15-5	94
		Bicyclo[4.4.0]dec-1-ene, 2-isopropyl-5-methyl-9-methylene-	62379	150320-52-8	78
		1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-, [s-(E,E)]-	62421	023986-74-5	55
30.761	0.90	C:\Database\NIST08.L			
		Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-	62439	000483-76-1	94
		Naphthalene, 1,2,4a,5,8,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, [1S-(1.alpha.,4a.beta.,8a.alpha.)]-	62497	000523-47-7	93
		Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-	62441	000483-76-1	64
31.866	0.58	C:\Database\NIST08.L			
		Cyclohexanemethanol, 4-ethenyl-.alpha.,.alpha.,4-trimethyl-3-(1-methylethenyl)-, [1R-(1.alpha.,3.alpha.,4.beta.)]-	76577	000639-99-6	68
		3,7-Cyclodecadiene-1-methanol, .alpha.,.alpha.,4,8-tetramethyl-, [s-(Z,Z)]	76548	021657-90-9	64
		3,7-Cyclodecadiene-1-methanol, .alpha.,.alpha.,4,8-tetramethyl-, [s-(Z,Z)]	76549	021657-90-9	64
32.838	0.72	C:\Database\NIST08.L			
		Cyclobuta[1,2:3,4]dicyclopentene, decahydro-3a-methyl-6-methylene-1-(1-methylethyl)-, [1S-(1.alpha.,3a.alpha.,3b.beta.,6a.beta.,6b.alpha.)]-	62561	005208-59-3	50
		Cyclohexene, 3-(1-methylethyl)-	10585	003983-08-2	47
		Cyclohexene, 3-(1-methylethyl)-	10591	003983-08-2	47

051 1.82 C:\Database\NIST08.L

2-Naphthalenemethanol, decahydro-.alpha.,.alpha.,4a-trimethyl-8-methylene-, [2R-(2.alpha.,4a.alpha.,8a.beta.)]-	76573	000473-15-4	83
2-Naphthalenemethanol, 1,2,3,4,4a,5,6,8a-octahydro-.alpha.,.alpha.,4a,8-tetramethyl-, (2.alpha.,4a.alpha.,8a.alpha.)-	76582	079254-46-9	81
2-Naphthalenemethanol, 1,2,3,4,4a,5,6,8a-octahydro-.alpha.,.alpha.,4a,8-tetramethyl-, [2R-(2.alpha.,4a.alpha.,8a.beta.)]-	76588	000473-16-5	64

34.772 19.39 C:\Database\NIST08.L

1H-Cyclopropa[a]naphthalene, 1a,2,3,5,6,7,7a,7b-octahydro-1,1,7,7a-tetramethyl-, [1aR-(1a.alpha.,7.alpha.,7a.alpha.,7b.alpha.)]-	62555	017334-55-3	90
1H-Cycloprop[e]azulene, 1a,2,3,4,4a,5,6,7b-octahydro-1,1,4,7-tetramethyl-, [1aR-(1a.alpha.,4.alpha.,4a.beta.,7b.alpha.)]-	62548	000489-40-7	58
1,2,4-Metheno-1H-indene, octahydro-1,7a-dimethyl-5-(1-methylethyl)-, [1S-(1.alpha.,2.alpha.,3a.beta.,4.alpha.,5.alpha.,7a.beta.,8S*)]-	62557	022469-52-9	52

35.179 1.51 C:\Database\NIST08.L

1H-Cyclopropa[a]naphthalene, 1a,2,3,5,6,7,7a,7b-octahydro-1,1,7,7a-tetramethyl-, [1aR-(1a.alpha.,7.alpha.,7a.alpha.,7b.alpha.)]-	62555	017334-55-3	95
Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1R-(1.alpha.,7.beta.,8a.alpha.)]-	62508	004630-07-3	70
Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-	62441	000483-76-1	64

35.493 2.18 C:\Database\NIST08.L

(-)-Isoaromadendrene-(V)	62320	1000156-14-3	50
Spiro[5.5]undec-2-ene, 3,7,7-trimethyl-11-methylene-, (-)-	62382	018431-82-8	46
1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene-	62389	072747-25-2	46

36.060 8.05 C:\Database\NIST08.L

.alpha.-Cadinol	76472	000481-34-5	89
Cyclohexane, 1-ethenyl-1-methyl-2-(1-methylethenyl)-4-(1-methylethylidene)-	62428	003242-08-8	49
(-)-Isoaromadendrene-(V)	62320	1000156-14-3	43

37.382 1.35 C:\Database\NIST08.L

Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2.alpha.,4a.alpha.,8a.beta.)]-	62519	000473-13-2	78
1H-Benzocycloheptene, 2,4a,5,6,7,8,9,9a-octahydro-3,5,5-trimethyl-9-methylene-, (4aS-cis)-	62459	003853-83-6	46
1-Naphthalenol, decahydro-1,4a-dimethyl-7-(1-methylethylidene)-, [1R-(1.alpha.,4a.beta.,8a.alpha.)]-	76561	000473-04-1	44

95

38.326	1.23	C:\Database\NIST08.L				
		2,6,10-Dodecatrien-1-ol, 3,7,11-tr imethyl-, (E,E)-	76507	000106-28-5	72	
		Hexadeca-2,6,10,14-tetraen-1-ol, 3 ,7,11,16-tetramethyl-, (E,E,E)-	129367	007614-21-3	59	
		Hexadeca-2,6,10,14-tetraen-1-ol, 3 ,7,11,16-tetramethyl-, (E,E,E)-	129366	007614-21-3	50	
38.967	0.32	C:\Database\NIST08.L				
		2,6,10-Dodecatrienal, 3,7,11-trime thyl-, (E,E)-	74801	000502-67-0	64	
		2,6,10-Dodecatrienal, 3,7,11-trime thyl-, (Z,E)-	74802	004380-32-9	64	
		2,6,10-Dodecatrien-1-ol, 3,7,11-tr imethyl-, (E,E)-	76507	000106-28-5	59	
41.576	0.96	C:\Database\NIST08.L				
		2-Butyl-3-methyl-5-(2-methylprop-2 -enyl)cyclohexanone	76517	1000281-10-7	20	
		3-Buten-2-one, 3-methyl-4-(1,3,3-t rimethyl-7-oxabicyclo[4.1.0]heptan -1-yl)-	76358	097371-44-3	18	
		Propanedioic acid, bromo-, dimethy l ester	67311	000868-26-8	14	

96

APPENDIX B2

GC-MS LIBRARY SEARCH REPORT OF ESSENTIAL OIL OF *CYMBOPOGON NARDUS*

RT	Area%	Library/ID	Ref#	CAS#	Qual
9.956	0.22	C:\Database\NIST08.L			
		Limonene	15482	000138-86-3	84
		4-Cyclopropylnorcarane	15536	1000222-73-9	72
		1,3,6-Heptatriene, 2,5,6-trimethyl	15604	042123-66-0	62
15.432	7.39	C:\Database\NIST08.L			
		6-Octenal, 3,7-dimethyl-, (R)-	26261	002385-77-5	95
		1-Hexene, 3,3-dimethyl-	6666	003404-77-1	50
		.alpha.-Santoline alcohol	26236	090823-36-2	42
18.785	2.48	C:\Database\NIST08.L			
		6-Octen-1-ol, 3,7-dimethyl-, (R)-	27811	001117-61-9	93
		2,7-Octadiene, 4-methyl-	10536	1000061-78-0	50
		Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-, [1R-(1.alpha.,2.alpha.,5.alpha.)]-	16808	004863-59-6	43
19.861	0.84	C:\Database\NIST08.L			
		4-Hexen-1-ol, 5-methyl-2-(1-methylethenyl)-	26367	058461-27-1	87
		2,6-Octadien-1-ol, 2,7-dimethyl-	26277	022410-74-8	72
		2,6-Octadien-1-ol, 3,7-dimethyl-, formate, (E)-	45866	000105-86-2	64
24.009	2.33	C:\Database\NIST08.L			
		Butanoic acid, 3,7-dimethyl-6-octenyl ester	79692	000141-16-2	87
		6-Octen-1-ol, 3,7-dimethyl-, propanoate	68693	000141-14-0	83
		Cyclohexane, 1-methyl-4-(1-methylethenyl)-, trans-	16797	001124-25-0	78
25.131	0.19	C:\Database\NIST08.L			
		Santolina triene	15513	002153-66-4	58
		1,7-Octadiene, 2-methyl-6-methylene-	15610	001686-30-2	52
		1,7-Octadiene, 2-methyl-6-methylene-	15607	001686-30-2	52
25.388	3.83	C:\Database\NIST08.L			
		2,6-Octadien-1-ol, 3,7-dimethyl-, formate, (E)-	45864	000105-86-2	80
		2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (E)-	56335	000105-87-3	72
		2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (E)-	56334	000105-87-3	64
25.589	4.86	C:\Database\NIST08.L			
		1,7-Octadiene, 2-methyl-6-methylene-	15610	001686-30-2	62
		1,7-Octadiene, 2-methyl-6-methylene-	15607	001686-30-2	58

97

		e- Cyclohexane, 1-ethenyl-1-methyl-2, 4-bis(1-methylethenyl)-	62372	110823-68-2	52
29.119	2.78	C:\Database\NIST08.L 1H-Cyclopenta[1,3]cyclopropa[1,2]benzene, octahydro-7-methyl-3-methylene-4-(1-methylethyl)-, [3aS-(3a.alpha.,3b.beta.,4.beta.,7.alpha.,7aS*)]-	62563	013744-15-5	97
		1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-, [s-(E,E)]-	62421	023986-74-5	58
		Bicyclo[4.4.0]dec-1-ene, 2-isopropyl-5-methyl-9-methylene-	62379	150320-52-8	58
29.840	0.41	C:\Database\NIST08.L Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1.alpha.,4a.alpha.,8a.alpha.)-	62470	031983-22-9	97
		Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1.alpha.,4a.alpha.,8a.alpha.)-	62471	031983-22-9	70
		Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-	62409	000483-75-0	70
30.361	0.31	C:\Database\NIST08.L Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-	62415	000483-75-0	97
		Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1.alpha.,4a.alpha.,8a.alpha.)-	62530	030021-74-0	95
		1H-Cyclopenta[1,3]cyclopropa[1,2]benzene, octahydro-7-methyl-3-methylene-4-(1-methylethyl)-, [3aS-(3a.alpha.,3b.beta.,4.beta.,7.alpha.,7aS*)]-	62563	013744-15-5	94
30.858	4.64	C:\Database\NIST08.L Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-	62439	000483-76-1	94
		Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-	62438	000483-76-1	93
		Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-	62440	000483-76-1	91
32.358	44.14	C:\Database\NIST08.L Cyclohexanemethanol, 4-ethenyl-.alpha.,.alpha.,4-trimethyl-3-(1-methylethenyl)-, [1R-(1.alpha.,3.alpha.,4.beta.)]-	76577	000639-99-6	93
		3,7-Cyclodecadiene-1-methanol, .alpha.,.alpha.,4,8-tetramethyl-, [s-(Z,Z)]	76549	021657-90-9	81
		3,7-Cyclodecadiene-1-methanol, .alpha.,.alpha.,4,8-tetramethyl-, [s-(Z,Z)]	76548	021657-90-9	74
32.970	0.92	C:\Database\NIST08.L 1-Hydroxy-1,7-dimethyl-4-isopropyl-2,7-cyclodecadiene	76518	072120-50-4	97
		Trifluoroacetyl-.alpha.-fenchol	97474	031076-73-0	49
		Cyclobuta[1,2:3,4]dicyclopentene, decahydro-3a-methyl-6-methylene-1-	62561	005208-59-3	47

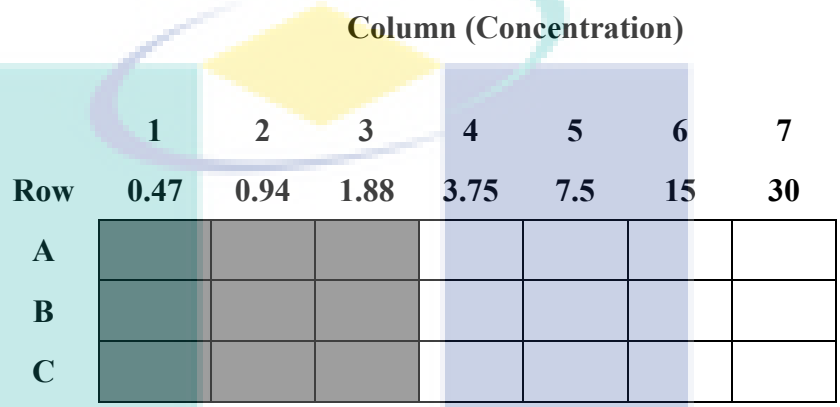
98

		(1-methylethyl)-, [1S-(1.alpha.,3a .alpha.,3b.beta.,6a.beta.,6b.alpha .)]-			
34.498	1.85	C:\Database\NIST08.L			
		1-Methylene-2b-hydroxymethyl-3,3-d imethyl-4b-(3-methylbut-2-enyl)-cy clohexane	76551	1000144-10-6	53
		1,6,10-Dodecatriene, 7,11-dimethyl -3-methylene-	62348	077129-48-7	53
		3,7,11-Trimethyl-3-hydroxy-6,10-do decadien-1-yl acetate	122700	1000144-12-7	53
35.167	6.05	C:\Database\NIST08.L			
		1H-Cyclopropa[a]naphthalene, 1a,2, 3,3a,4,5,6,7b-octahydro-1,1,3a,7-t etramethyl-, [1aR-(1a.alpha.,3a.al pha.,7b.alpha.)]-	62534	000489-29-2	98
		2-Naphthalenemethanol, 1,2,3,4,4a, 5,6,7-octahydro-.alpha.,.alpha.,4a ,8-tetramethyl-, (2R-cis)-	76559	001209-71-8	95
		Naphthalene, 1,2,4a,5,8,8a-hexahyd ro-4,7-dimethyl-1-(1-methylethyl)- , (1.alpha.,4a.beta.,8a.alpha.)-(. +/-.)-	62506	005951-61-1	92
35.505	2.77	C:\Database\NIST08.L			
		Santolina epoxide	24667	060485-45-2	43
		Bicyclo[4.1.0]hept-2-ene	2589	002566-57-6	30
		Naphthalene, 2,3,4,4a,5,6-hexahydr o-1,4a-dimethyl-7-(1-methylethyl)-	62412	000473-14-3	30
36.140	12.77	C:\Database\NIST08.L			
		.alpha.-Cadinol	76472	000481-34-5	70
		Epiglobulol	76464	1000150-05-1	46
		1,5,6,7-Tetramethylbicyclo[3.2.0]h ept-6-en-3-one	32994	120345-87-1	45
37.153	0.31	C:\Database\NIST08.L			
		Cyclopentane, 2-propenyl-	5905	003524-75-2	43
		4-Hexen-1-ol, 2-ethenyl-2,5-dimeth yl-	26325	050598-21-5	37
		Cyclopropane, 1,1-dimethyl-2-(1-me thyl-2-propenyl)-	10609	074779-84-3	35
38.309	0.70	C:\Database\NIST08.L			
		2,6-Nonadienal, (E,E)-	17307	017587-33-6	80
		2,6,10-Dodecatrien-1-ol, 3,7,11-tr imethyl-, (E,E)-	76507	000106-28-5	72
		2,6,10-Dodecatrien-1-ol, 3,7,11-tr imethyl-, acetate, (E,E)-	108859	004128-17-0	53
38.955	0.20	C:\Database\NIST08.L			
		2-Pentene, 3-methyl-, (E)-	1505	000616-12-6	50
		Furan, 2,3-dihydro-3-methyl-	1438	001708-27-6	49
		6,11-Dimethyl-2,6,10-dodecatrien-1 -ol	65612	1000196-53-3	47

APPENDIX C1

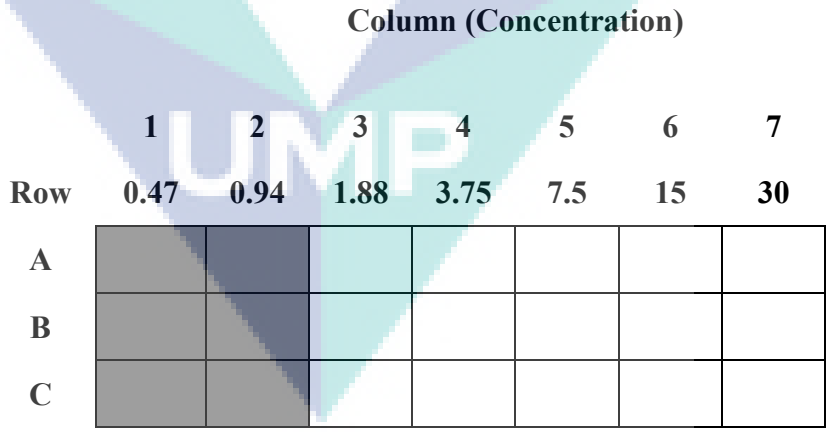
RAW SCHEMATIC RESULT OF MINIMUM INHIBITORY CONCENTRATION (MIC) OF *CYMBOPOGON CITRATUS* AGAINST TESTED BACTERIA (IN TRIPLICATE)

(i) BACTERIA: *E. faecalis*



MIC : 3.75

(ii) BACTERIA: *S. aureus*



MIC : 1.88

Turbidity

Concentration in µl/ml

(iii) BACTERIA: *B. subtilis*

Column (Concentration)

Row	1 0.47	2 0.94	3 1.88	4 3.75	5 7.5	6 15	7 30
A							
B							
C							

MIC : 1.88

(iv) BACTERIA: *E. coli*

Column (Concentration)

Row	1 3.75	2 7.5	3 15	4 30	5 60	6 120	7 240
A							
B							
C							

MIC : 7.5

(v) BACTERIA: *S. typhimurium*

Column (Concentration)

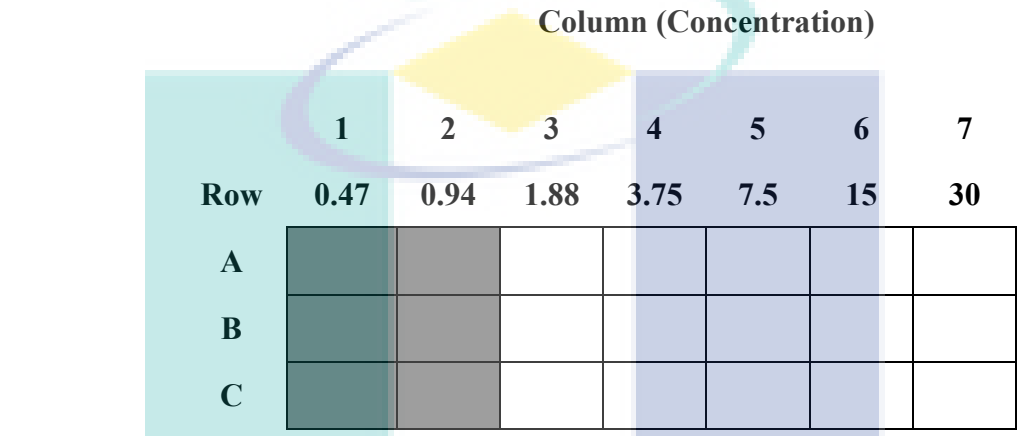
Row	1 3.75	2 7.5	3 15	4 30	5 60	6 120	7 240
A							
B							
C							

MIC : 7.5

APPENDIX C2

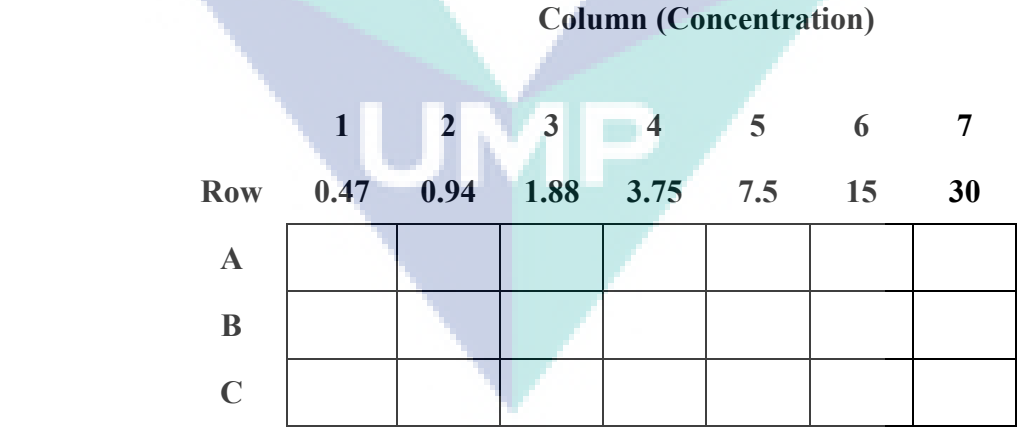
RAW SCHEMATIC RESULT OF MINIMUM INHIBITORY CONCENTRATION (MIC) OF *CYMBOPOGON NARDUS* AGAINST TESTED BACTERIA (IN TRIPLICATE)

(i) BACTERIA: *E.faecalis*



MIC : 1.88

(ii) BACTERIA: *S. aureus*



MIC : 0.47

Turbidity

Concentration in µl/ml

(iii) BACTERIA: *B. subtilis*

Column (Concentration)

Row	1 0.47	2 0.94	3 1.88	4 3.75	5 7.5	6 15	7 30
A							
B							
C							

MIC : 0.47

(iv) BACTERIA: *E.coli*

Column (Concentration)

Row	1 3.75	2 7.5	3 15	4 30	5 60	6 120	7 240
A							
B							
C							

MIC : 120

(v) BACTERIA: *S.typhimurium*

Column (Concentration)

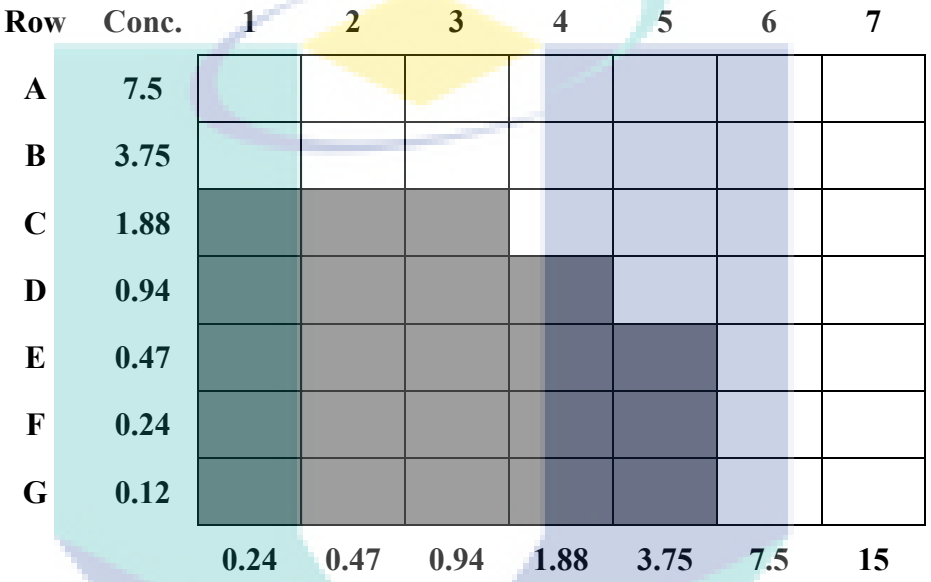
	1	2	3	4	5	6	7
Row	**3.75**	**7.5**	**15**	**30**	**60**	**120**	**240**
A							
B							
C							

MIC : 120

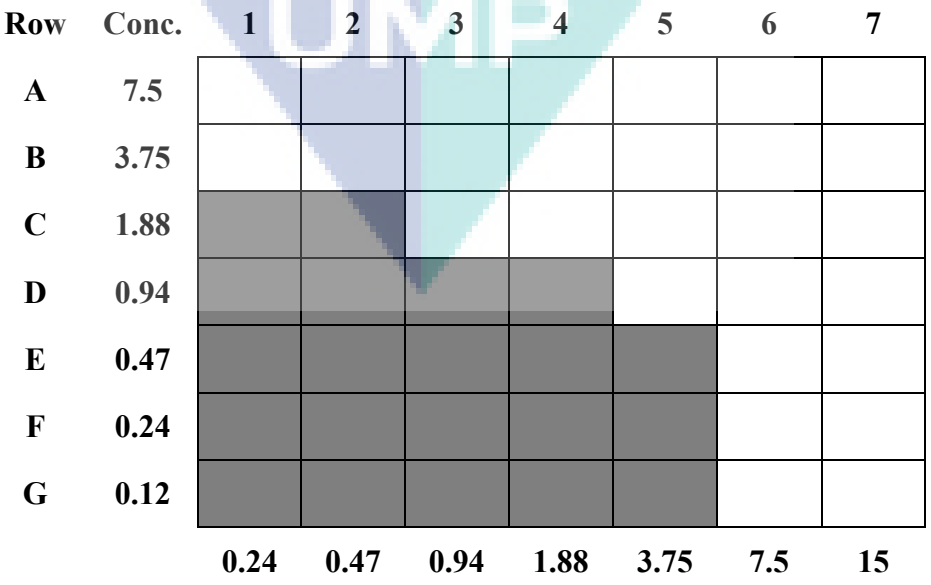
APPENDIX D

RAW SCHEMATIC OF MINIMUM INHIBITORY CONCENTRATION (MIC) OF *CYMBOPOGON CITRATUS* AND *CYMBOPOGON NARDUS* EOs IN COMBINATION AGAINST TESTED BACTERIA (IN TRIPLICATE)

(i) BACTERIA : *E. faecalis* (a)



(b)



(c) **Column**

Row	Conc.	1	2	3	4	5	6	7
A	**7.5**							
B	**3.75**							
C	**1.88**							
D	**0.94**							
E	**0.47**							
F	**0.24**							
G	**0.12**							
		0.24	**0.47**	**0.94**	**1.88**	**3.75**	**7.5**	**15**

Vertical orientation : EOa (*Cymbopogon citratus*)

Horizontal orientation : EOb (*Cymbopogon nardus*)

(ii) BACTERIA : *S. aureus*

(a)

Column

Row	Conc.	1	2	3	4	5	6	7
A	1.88							
B	0.94							
C	0.47							
D	0.24							
E	0.12							
F	0.06							
G	0.03							
		0.12	0.24	0.47	0.94	1.88	3.75	7.5

(b)

Column

Row	Conc.	1	2	3	4	5	6	7
A	1.88							
B	0.94							
C	0.47							
D	0.24							
E	0.12							
F	0.06							
G	0.03							
		0.12	0.24	0.47	0.94	1.88	3.75	7.5

(c) **Column**

Row	Conc.	1	2	3	4	5	6	7
A	1.88							
B	0.94							
C	0.47							
D	0.24							
E	0.12							
F	0.06							
G	0.03							
		0.12	0.24	0.47	0.94	1.88	3.75	7.5

(iii) **BACTERIA : *B. subtilis***

(a) **Column**

Row	Conc.	1	2	3	4	5	6	7
A	1.88							
B	0.94							
C	0.47							
D	0.24							
E	0.12							
F	0.06							
G	0.03							
		0.12	0.24	0.47	0.94	1.88	3.75	7.5

(b)

Column

Row	Conc.	1	2	3	4	5	6	7
A	1.88							
B	0.94							
C	0.47							
D	0.24							
E	0.12							
F	0.06							
G	0.03							
		0.12	0.24	0.47	0.94	1.88	3.75	7.5

(c)

Column

Row	Conc.	1	2	3	4	5	6	7
A	1.88							
B	0.94							
C	0.47							
D	0.24							
E	0.12							
F	0.06							
G	0.03							
		0.12	0.24	0.47	0.94	1.88	3.75	7.5

(iv) BACTERIA : *E. coli*

(a)

Column

Row	Conc.	1	2	3	4	5	6	7
A	480							
B	240							
C	120							
D	60							
E	30							
F	15							
G	7.5							
		0.94	1.88	3.75	7.5	15	30	60

(b)

Column

Row	Conc.	1	2	3	4	5	6	7
A	480							
B	240							
C	120							
D	60							
E	30							
F	15							
G	7.5							
		0.94	1.88	3.75	7.5	15	30	60

(c) **Column**

Row	Conc.	1	2	3	4	5	6	7
A	480							
B	240							
C	120							
D	60							
E	30							
F	15							
G	7.5							
		0.94	1.88	3.75	7.5	15	30	60

(v) **BACTERIA : *S. typhimurium***

(a) **Column**

Row	Conc.	1	2	3	4	5	6	7
A	480							
B	240							
C	120							
D	60							
E	30							
F	15							
G	7.5							
		0.94	1.88	3.75	7.5	15	30	60

(b)

Column

Row	Conc.	1	2	3	4	5	6	7
A	480							
B	240							
C	120							
D	60							
E	30							
F	15							
G	7.5							
		0.94	1.88	3.75	7.5	15	30	60

(c)

Column

Row	Conc.	1	2	3	4	5	6	7
A	480							
B	240							
C	120							
D	60							
E	30							
F	15							
G	7.5							
		0.94	1.88	3.75	7.5	15	30	60

APPENDIX E

INTERPRETATION OF THE RESULT OF CHECKERBOARD ASSAY

Bacteria	MIC (µl/ml)		MIC_{combi} (µl/ml)						FIC								FIC_{index}			FICI
	C. citratus	*C. nardus*	*C. citratus*			*C. nardus*			*C. citratus*			Mean	*C. nardus*			Mean				
E. faecalis	3.75	1.88	7.5	7.5	7.5	3.75	3.75	3.75	2	2	2	2.0	1.99	1.99	1.99	2.0	3.99	3.99	3.99	**4.0**
S. aureus	1.88	0.47	7.5	7.5	7.5	3.75	3.75	3.75	3.99	3.99	3.99	4.0	7.98	7.98	7.98	8.0	12	12	12	**12.0**
B. subtilis	1.88	0.47	7.5	7.5	3.75	1.88	1.88	1.88	3.99	3.99	1.99	3.3	4	4	4	4.0	7.99	7.99	5.99	**7.3**
E. coli	7.5	120	30	30	30	240	240	240	4	4	4	4.0	2	2	2	2.0	6	6	6	**6.0**
S. typhimurium	7.5	120	30	30	30	120	120	120	4	4	4	4.0	1	1	1	1.0	5	5	5	**5.0**

$$\frac{MIC_A \text{ combination}}{MIC_A \text{ alone}} + \frac{MIC_B \text{ combination}}{MIC_B \text{ alone}} = FIC_A + FIC_B = \text{FIC index}$$

CONFERENCES

Siti Fiqryyah Musa, Azhari H. Nour and Mashitah M.Yusoff. 2011. Effect of Seasons on Essential Oil Composition of Three Types of *Ocimum* Species. Regional Annual Fundamental Science Symposium 2011. Johor Bharu, Johor.

Siti Fiqryyah Musa, Azhari H. Nour and Mashitah M.Yusoff. 2012. Chemical composition and antibacterial activity of *Ocimum basilicum*, *Cymbopogon citratus* and *Cymbopogon nardus* essential oils. 17th National Conference on Medical And Health Sciences. Kota Bharu, Kelantan.