CHEMICAL COMPOSITION AND BIOACTIVITIES OF *CITHAREXYLUM SPINOSUM* AND *OSMANTHUS FRAGRANS* FLOWERS

AE MAR

DOCTOR OF PHILOSOPHY

IN

APPLIED CHEMISTRY

SCHOOL OF SCIENCE

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Ae Mar

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Degree	Doctor of Philosophy (Applied Chemistry)
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ABSTRACT

The chemical composition of essential oil and various solvent extracts of *C. spinosum* and *O. fragrans* flowers were reported. Essential oil was extracted with hydrodistillation method. The fresh *C. spinosum* and *O. fragrans* flowers were macerated with various solvents including *n*-hexane, dichloromethane, and ethanol to produce crude extracts. The chemical composition of *C. spinosum* were determined by gas chromatography-mass spectrometory (GC-MS) with 151 identified volatile constituents. This included 86, 41, 83, 59 compounds from flowers oil, hexane extract, dichloromethane extract, and ethanol extract respectively. Maltol and piperitone were the dominant compounds, followed by 2*Z*,6*E*-farnesyl acetate, methyl benzoate, indole, and benzyl benzoate in essential oil of *C. spinosum*. The major components of hexane extract were methyl benzoate followed by maple furanone, isolongifolan-7α-ol, amorpha-4,9-dien-14-al, β-chenopodiol and allyl anthranilate. Benzyl acetate, piperitone, maple furanone, isopropyl isobutyrate, 2*Z*,6*E*-farnesyl acetate, and furfural were found as the major constituents in dichloromethane extract. In contrast, *cis*-α-ambrinol, 2*Z*,6*E*-farnesyl acetate, maltol,

thujopsan-2β-ol, dihydroeudesmol, and dihydroeremoligenol were identified as the principle components in ethanol extract.

In total, 76, 79, 95 and 106 volatile components were detected in essential oil, hexane, dichloromethane and ethanol extract of *O. fragrans*, respectively. The prominent scent components of the essential oil were α-terpinyl isobutanoate, geranial, γ-decalactone, anisyl methyl ketone, *E*-β-ionone, *cis*-verbenyl acetate, pentyl salicylate, β-biotol, and linalool isovalerate. The major volatile compounds of hexane extract were *E*-isoeugenyl benzyl ether, *Z*-β-damascenone, *E,Z*-geranyl linalool, *E*-β-ionone, γ-decalactone while anisyl methyl ketone, benzyl cinnamate, γ-decalactone and *E*-β-ionone were considered as the principle components of dichloromethane extract. Ethanol extract presented α-patchoulene, pentadecanol, pentyl salicylate and geranyl benzoate as major constituents. Thus, quantitation of each compound could be related to environmental conditions and plant habitat.

Highly odor volatile compounds of fresh *C. spinosum* and *O. fragrans* flowers were extracted by using solid phase microextraction prior analysis by gas chromatography-mass spectrometry (SPME-GC-MS). Three fibers including PDMS, CAR/PDMS and DVB/CAR/PDMS were chosen for extraction of *C. spinosum* and *O. fragrans* odor constituents. Fifty-two odor volatile components were identified among these fibers. Twenty-two compounds were detected with PDMS fiber. The key odor volatiles were methyl benzoate, phenyl ethyl alcohol and 2-phenyl ethyl acetate while thirty-four constituents were found when using CAR/PDMS fiber with the major compounds of octen-3-ol, methyl benzoate, phenyl ethyl alcohol and 2-phenyl ethyl acetate. For DVB/CAR/PDMS fiber, thirty-six compounds were investigated. The major volatiles were octen-3-ol, methyl benzoate, phenyl ethyl alcohol, methyl salicylate and 2-phenyl ethyl acetate. Different contents of volatile components of *C. spinosum* flowers were related to composites on each fiber. The DVB/CAR/ PDMS fiber was considered to be the best fiber for extraction of odor volatiles of fresh *C.*

spinosum flowers due to the highest number of volatile components compared to other fibers. It is noted that the solid phase microextraction technique is more sensitive to extract the volatile components which played the significant role as the key scent in *C. spinosum* flower.

Highly volatile compounds of *O. fragrans* flowers were investigated using SPME-GC-MS method. Twenty-nine compounds were detected with PDMS fiber. The key odor volatiles were naphathalene, heptadecane, *E*-β-ionone, and isobazzanene while thirty-seven constituents were found when using CAR/PDMS fiber with the major compounds of isobazzanene, octane, 3*Z*-hexenol, 3-3-methyl-3-butenyl-methyl butanoate, *E*-β-ocimene, *E*-β-ionone, γ-delactone. Using DVB/CAR/PDMS fiber, we obtained 59 identified volatiles. The major volatiles were isobazzanene, *E*-β-ionone, γ-decalactone, undecane, *E*-β-ocimene, *cis*-linalool oxide, 3-3-methyl-3-butenyl- methyl butanoate, and *trans*-linalool oxide.

All extracts and essential oil of *C. spinosum* and *O. fragrans* flowers were tested for their antibacterial activities. The flower oil of *C. spinosum* had the greatest antibacterial activity against all bacterial strains (MIC values of 31.2 µg/mL), while the other solvent extracts had MIC values ranging from 31.2 to 1000 µg/mL. For *O. fragrans* flowers, the best antibacterial and antifungal activity was obtained from the exthanol extract of *O. fragrans* flowers followed by dichloromethane and hexane extracts, respectively. The presence of various terpene components with their derivatives such as α-patchoulene, γ-decalactone, *E*-β-ionone, γ-gurjunene, hinesol acetate, geraniol and *epi*-cubenol and 1,8-cineole were contributed to promote the antimicrobial activities.

Keywords: *Citharexylum spinosum*/*Osmanthus fragrans*/Volatile compounds/ SPME-GC MS/ Antibacterial/Antifungal

TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

TABLE OF CONTENTS (continued)

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES (continued)

LIST OF FIGURES (continued)

ABBREVIATIONS AND SYMBOLS

C. spinosum	*Citharexylum spinosum*
O. fragrans	*Osmanthus fragrans*
mm	millimeter
cm	centimeter
µm	micrometer
nm	nanometer
m	meter
g	gram
kg	kilogram
mL	milliliter
µL	microliter
µg/mL	microgram per millimeter
mg/mL	milligram per millimeter
g/L	gram per liter
v/v	volume per volume
%w/w	percent weight per weight
s	second
min	minute
h	hour
i.d.	internal diameter
°C	degree Celsius
°C/min	degree Celsius per minute
Hz	Hertz
V	voltage
eV	Electron voltage
m/z	mass-to-charge ratio

ABBREVIATIONS AND SYMBOLS (continued)

ESI-MS	electrospray ionization mass spectrometry
NMR	nuclear magnetic resonance
α	alpha
β	beta
γ	gamma
δ	delta
o	ortho
m	meta
p	para
t	tertiary
EI	electron ionization
HPLC	High Performance Liquid Chromatography
GC	Gas Chromatography
GC–MS	Gas Chromatography–Mass Spectrometry
GC–FID	Gas Chromatography–Flame Ionization Detector
LC-MS	Liquid Chromatography-Mass Spectrometry
SPME	Solid–Phase Microextraction
HS	headspace
DVB/CAR/PDMS	Divinylbenzene/carboxen/polydimethylsiloxane
PDMS/DVB	Polydimethylsiloxane/divinylbenzene
CAR/PDMS	Carboxen/polydimethylsiloxane
CW/DVB	Carbowax/divinylbenzene
CW/TPR	Carbowax/templated resin
CAR/TAR	Carboxen - Templated resin
PDMS	Polydimethylsiloxane
PA	Polyacrylate

ABBREVIATIONS AND SYMBOLS (continued)

DCM	dichloromethane
HEX	hexane
ETH	ethanol
ESS	essential oil
RT	retention time
%RA	relative peak area percentage
RI	retention index (retention indices)
CO_2	carbondioxide
MM	molecular mass
IC50	the half maximal inhibitory concentration
MIC	minimal inhibitory concentration
NB	nutrient broth
NA	nutrient agar
PDA	potato dextrose agar
TLC	thin layer chromatographic
CFU/mL	colony forming unit per milliliter
B. subtilis	*Bacillus subtilis*
B. cereus	*Bacillus cereus*
E. coli	*Escherichia coli*
S. aureus	*Staphylococcus aureus*
Ps. aeruginosa	*Pseudomonas aeruginosa*
M. luteus	*Micrococcus luteus*
S. typi	*Salmonella typhi*
En. aerogenes	*Enterobacter aerogenes*
C. albicans	*Candida albicans*
C. utilis	*Candida utilis*

ABBREVIATIONS AND SYMBOLS (continued)

MBC	minimal bactericidal concentratio
MFC	minimal fungicidal concentration
± SD	standard deviation

CHAPTER 1

INTRODUCTION

1.1 Plants of This Work

The *Citharexylum* genus, typically containing aromatic, flowering trees and shrubs, includes approximately 100 species which are found in Bermuda, West Indies, and the southern United States through Mexico to South America (Whistler, 2000). *O. fragrans* (Sweet Osmanthus) is a novel ornamental plant belonging to the Oleaceae family and extensively cultivated in Asia, especially in Southern China, Taiwan, Southern Japan (Hu, Liang, Guo, Li & Wang, 2010).

1.1.1 *Citharexylum spinosum*

The taxonomy and classification of *C. spinosum* is expressed below (Starr, Starr, & Loope, 2003);

Kingdom:	Plantae
Oder:	Lamiales
Family:	Verbenaceae
Genus:	*Citharexylum*
Species:	*C. spinosum*

Figure 1.1 Flowers of *C. spinosum*

Citharexylum spinosum, also known as *C. quadrangulare* or *C. fruticosum*, belongs to the family Verbenaceae, which includes common plants known as Florida Fiddlewood, Spiny fiddlewood, Chinese rose, and Bungasari (Thai). Common name of Fiddlewood is derived from the use of its wood, making stringed instruments by the people of the Caribbean (Whistler, 2000) .Normally, it is planted as a street tree which is a popular ornamental, principally in tropical and subtropical areas of the world as an ornamental plant because of the redolent of white flowers and attractive leafage. This lovely small tree has attractive and green leaves characteristically turn russet gold through autumn and winter (Starr et al., 2003). *C. spinosum*, an aromatic plant that may grow up to 15 m, has small, white flowers in hanging axillary and terminal racemes and panicles (Whistler, 2000). Their flowers blossom throughout the year, but especially during the rainy season. *C. spinosum* flowers release a sweet fragrance that spreads out over a wide area. The essential oil of *C. spinosum* flowers are used in traditional remedies and aromatherapy in Thailand (Starr et al., 2003). It is also used in traditional medicine as a diuretic and antipyretic, for the treatment of liver disorders, and for its antiulcer, antihypertensive, and hepatoprotective effects (Tomlinson & Fawcett, 1972).

1.1.2 *Osmanthus fragrans*

The taxa and classification of *O. fragrans* is presented below (Gilman & Watson, 1994);

Kingdom: Plantae

Odor: Lamiales

Family: Oleaceae

Genus: *Osmanthus*

Species: *O. fragrans*

Figure 1.2 Flowers of *O. fragrans*

According to the flower's color, varieties of *O. fragrans* can be divided into three groups such as *O. fragans* Luteus (gold-orange), *O. fragrans* Albus (silver-white) and *O. fragrans* Aurantiacus (reddish) (Hu et al., 2010). Sweet aroma from their white blossom flower is released from October through winter sometimes as long as until April or May (Gilman & Watson, 1994). In China, *O. fragrans* is deliberated as one of the four famous traditional flowers and is widely cultivated as an ornamental and economic plant until now (Wang, Pan, Tang & Huang, 2006). Moreover, *O. fragrans* is not only symbols of social civilization, but also rare materials for the study of ancient climate, germplasm resources and cultivar breeding as there is a woody plant and has a long life cycle, in China (Duan et al., 2015). *Osmanthus* flowers contain a large amount of aromatic chemicals, which are derived

from carotenoid pigment (Li & Huang, 2011). *O. fragrans* flowers have been used in many applications such as an ingredient for tea, and other beverages and a food additives (Yang & Zhu, 2000; Yang, Yao & Qian, 2005). In addition, *O. fragrans* essential oil has been used in the most expensive perfumes and cosmetics because it has an extremely powerful ornamental fragrance (Jin, Zheng & Jin, 2006). Besides, the *O. fragrans* wood can be used as the excellent materials of furniture and architecture for its compact texture (Liu et al., 2008). Pharmacological investigation showed that *O. fragrans* (flowers, seeds, root) can also be used as a folk medicine to improve overall lung health, to treat menstrual pain as well as regular pain and tooth aches, to remove many toxins from the body, and for the treatment of liver and stomachache (Peng & Ji, 2003).

1.2 Background of Volatile Compounds and Essential oil

Various classes of natural compounds are produced by plants. These components contain the different purposes according to functional group appeared in their structure. At least 1,700 compounds are in a class of volatile components (Dicke & Loreto, 2010). These volatile compounds such as terpenoids, fatty acid derivatives, indole and benzenoids play important role in plant reproduction by the attraction of insects (Brown, 2002; Knudsen & Tollsten, 1993; Verdonk, 2006). Moreover, isolated aroma compounds are used as ingredients in perfume and food industries (Verdonk, 2006).

An essential oil is a volatile oil, ethereal oil or aetherolea, and also known as a concentrated hydrophobic liquid containing true essence compounds and volatile aroma compounds of plants. In the sensation, oil is "essential" that it conveys a distinctive odor, or essence, of the plant. Essential oils are not similar to perfume oils or fragrance oils because those oils do not proffer the worth of therapeutic benefits that essential oils offer by containing artificially created fragrances or substances and diluting with carrier oils (Baser & Buchbauer, 2009). Moreover, Bedi and Vyas (2008) stated that the aromatic-ring structure of essential oil should not be confused with the linear carbon-hydrogen structure of the fatty oils, which are composed of a

mixture of lipids. Although essential oils contain sulfur and nitrogen atoms, the fatty oil do not have. The main constituents of volatile oils are monoterpenes, sesquiterpines which are hydrocarbons with the general formula $(C_5H_8)_n$ and oxygenating compounds such as esters, aldehydes, ketones, alcohols, phenol oxides. The aromatic or odoriferous volatile compounds of oils are derived from various parts of plants (berries, seeds, wood, bark, rhizome, leaves, resin, flowers, peel and root) as such or less frequently may result from the degradation of glycerides by enzyme action (Elsharkawy, 2014).

The various applications of essential oils may be found in many industries, for instance; in the comestic industry, as ingredients of fragrances, decorative cosmetic, fine fragrances, and flavoring, in the food industry, as aromas and flavors, in the pharmaceutical industry, as active components of medicines and as antibacterials/antimicrobials and in aromatherapy. These products supply directly to improve our health, happiness and general well-being. Undoubtedly, essential oils have the following effects on external application: hyperaemic, antiinflammatory, antiseptic, granulation stimulating, deodorizing, insecticide and repellent actions (Kumar & Tripathi, n.d.).

1.3 Background of Extraction Methods

The isolation of compounds from aroma plants by many different methods. The initial efforts of extraction were used alcohol and a fermentation process (Rao, 2006). The primary methods for the extraction of essential oil are cold pressing, maceration, hydrodistillation, extraction with organic solvents and extraction with compressed CO_2 (Norsuzieana, 2009). The extraction technique of oils from plants is important because some solvent using processes can adversely affect the therapeutic value. Some plants, and specially flowers, do not give themselves to steam distilling because of their delicate structure. The oils will be showed as 'absolutes' but it is not technically considered as the essential oils they can still be of therapeutic properties. In particular, Jasmine oil and Rose oil are delicate flowers whose oils are often produced as 'absolutes' form (Rao, 2006).

1.3.1 Maceration

In the small scale of the general maceration process, a moderately coarse powder material or properly crushed plant material placed in a closed vessel where filled with selected solvent called menstruum. The mixture is allowed to stand for over one week at room temperature, with occasional agitation. When equilibrium has been reached, the mixture is then strained off and the damp solid material, called marc, is pressed to recover as much occluded solution as possible. The solution may be cloudy with colloidal and small substances, thus, sufficient time is (perhaps several weeks) need for settling and clotting. The settled matter is filtered through a filter press or any other suitable equipment (Handa, n.d.). From the concentrate, the oil is extracted by pure alcohol. This is very much the same technique used in solvent extraction (Yalavarthi & Thiruvengadarajan, 2013).

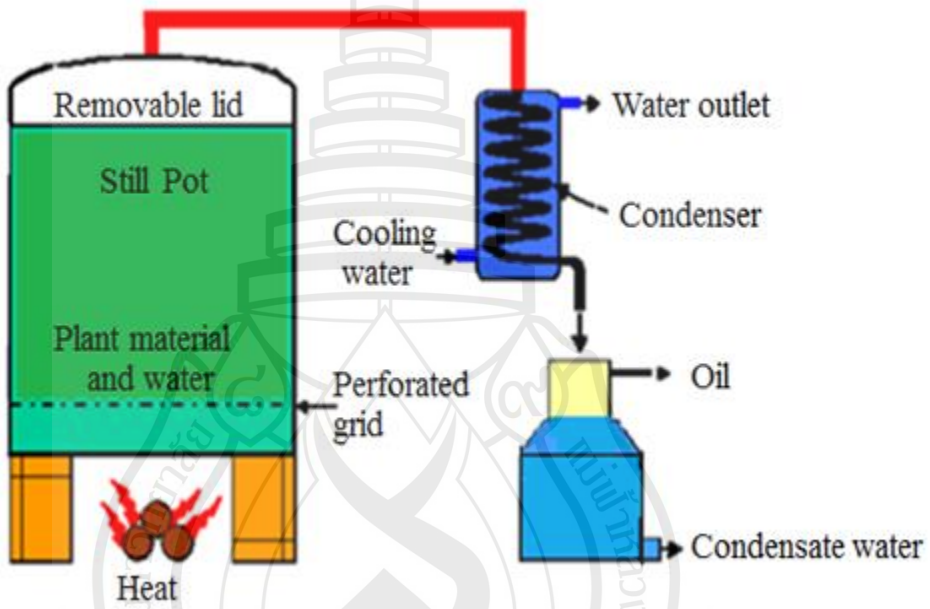
1.3.2 Hydrodistillation

There are three types of hydrodistillation for extracting essential oils from plant materials (Handa, n.d.) :

1.3.2.1 Water Distillation

In the case of water distillation, instead of the steam input, the botanic material is completely soaked in water, which is heated by direct fire, steam jacket, closed steam jacket, closed steam coil or open steam coil. The main characteristic of this process is that there is direct contact between plant materials and boiling water. The surrounding water acts as a barrier to prevent overheating. The heat released from the boiling water penetrates to the plant cells and extracts the odorous molecules in the plant cells. These volatile aroma compounds and water form an azeotropic mixture, which can be evaporated together at the same pressure and then condensed and separated in a separating funnel owing to their different density and immiscibility (Ranjitha & Vijiyalakshmi, 2014). One distinct advantage of water distillation is that it allows to process finely power material or part of plants by contact with live steam, would otherwise form lumps through which the steam cannot penetrate. Moreover, water distillation is simple, inexpensive, easy to handle and suitable for field operation. In many countries, these are still widely used with portable equipment to extract the essential oil from plants. This is not considered the best method for

extraction because it may not be complete extraction. Besides, oil components like esters are partly hydrolyzed and sensitive substances like aldehydes susceptible to polymerize. The other risk is that the still can run dry, or be overheated, burning the aromatic and resulting in an essential oil with a burnt smell. This hydrodistillation method seems to operate best for powders such as spice powders, ground wood, etc. and very hard materials like roots, wood, or nuts (Handa, n.d.).



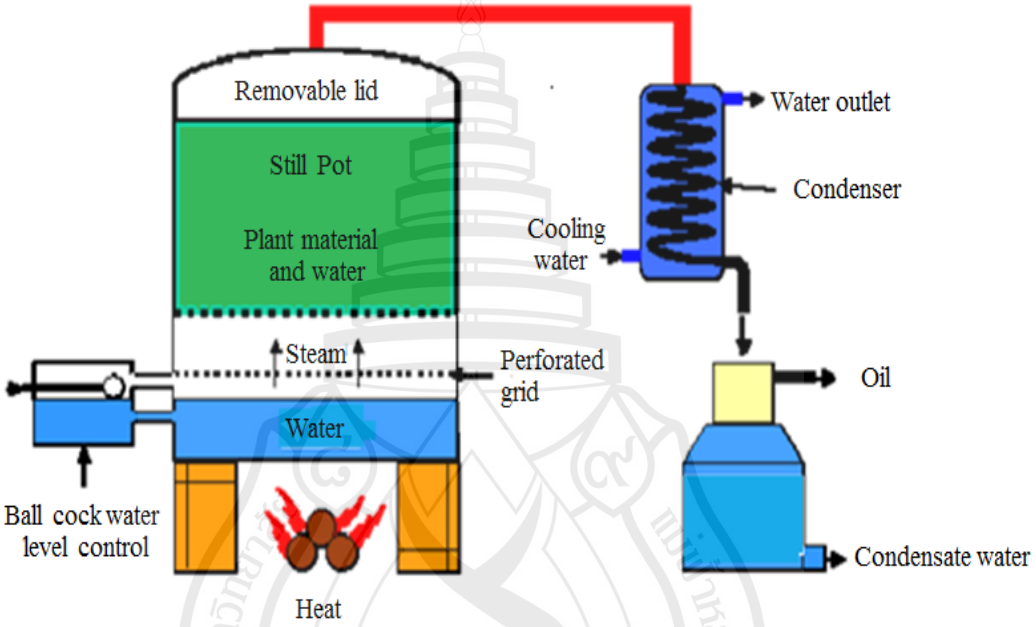
Source Douglas, Heyes & Smallfield (2005).

Figure 1.3 Diagrammatic Representation of Water Distillation Unit

1.3.2.2 Water and Steam Distillation

To overcome the drawback of water distillation, water and steam distillation is developed by modification technique and widely used in rusticity areas. Besides, it does not mean a great deal more investment consumption than water distillation. In this method, the wet and saturated steam can be generated either in a vast boiler or within the still, although without direct contact with plant material. Generally, the equipment of this method is very similar to the using apparatus for

water distillation, but the water beneath the perforated grid is heated by open fire which gives saturated and wet steam which bring up the essential oil with it through the plant material vaporizing. According to their very simple process, cheap and easy operation field distillation units is the famous technique for extracting essential oil producers in developing countries (Handa, n.d.).



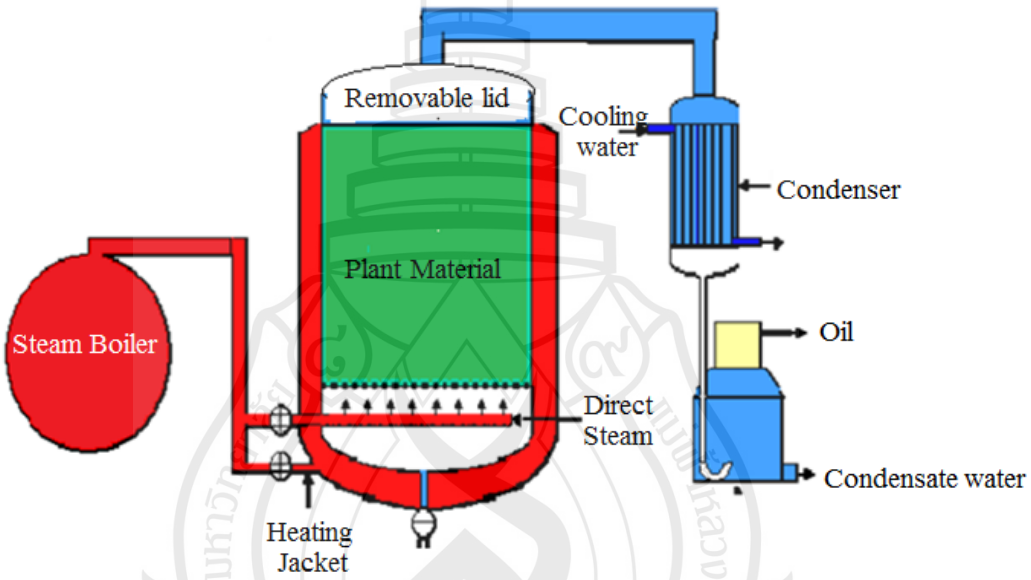
Source Douglas, Heyes & Smallfield (2005).

Figure 1.4 Diagrammatic Representation of Water & Steam Distillation Unit

1.3.2.3 Direct Steam Distillation

According to the name suggests, direct steam distillation is the process of distilling botanical material by steam generated outside the still in a supporter steam generator commonly referred to as a boiler. In this method, the botanical plant material is supported on a perforated grid above the steam inlet. The hot steam is extracted the aromatic molecules from the plant material. The steam is produced at greater pressure than the atmospheric pressure and, consequently, boils at above 100 °C which is used to the remove the essential oil from the plant material. An obvious

advantage of steam distillation is that the amount of steam can be easily controlled. The plant material is heated not much than 100 °C and, therefore, it should not accept thermal decomposition. For the production of essential oils on a large scale, steam distillation is the most widely accepted method in the world. A distinct hindrance of steam distillation is the quite high capital cost needed to construct such a facility so that only bigger producers can afford to own such units (Handa, n.d.).



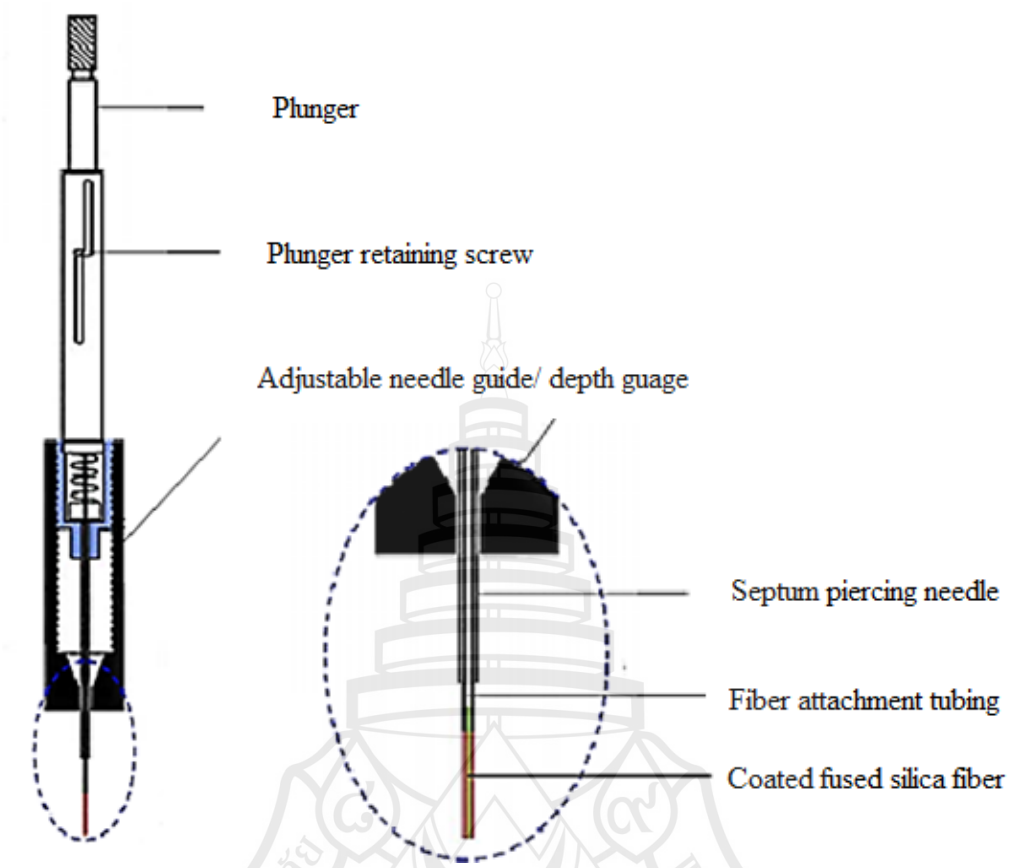
Source Douglas, Heyes & Smallfield (2005).

Figure 1.5 Diagrammatic Representation of Steam Distillation Unit

1.4 Background of Instruments

1.4.1 Solid Phase Microextraction (SPME**)**

Since 1990, Dr. Janusz Pawliszyn and his colleagues from the University of Waterloo (Ontario, Canada) were devised a new extraction technique; solid-phase microextraction (SPME) to investigate volatile constituents from plant tissues (Majors, 2013). SPME is an innovative, versatile, economical, and is a fast technique as preconcentration of analyte is done in a single step and sample separation steps are reduced or even no more required (Kumar & Tripathi, n.d.; Ruoff, 2003). This technique is environmental friendly as reduce solvent consumption and shorter analysis time (Ruoff, 2003). The device provides fiber holder and fiber assembly with built-in fiber inside the needle, which looks like a modified syringe which can be moved between two positions, inside and outside the needle (Kumar & Tripathi, n.d.) in Figure 1.3 (Zhang & Pawliszyn, 1993). The SPME fiber (1 cm long, 0.11 mm diameter) itself is a thin fused-silica optical fiber, coated with an adsorbent polymer film (such as polydimethylsiloxane (PDMS)) (Ruoff, 2003; Shirey, 1999). The coated fibers act like a sponge, and use to isolate and concentrate analytes into a range of coating materials. After extraction, the fibers are convey to an analytical instrument such as Gas Chromatography (GC), Gas Chromatography-Mass Spectrometry (GC-MS), High-Performance Liquid Chromatography (HPLC), and Liquid Chromatography-Mass Spectrometry (LC-MS) (Kataoka, Lord & Pawliszyn, 2000), for separation and quantification of the target analytes (Keene, 2009).

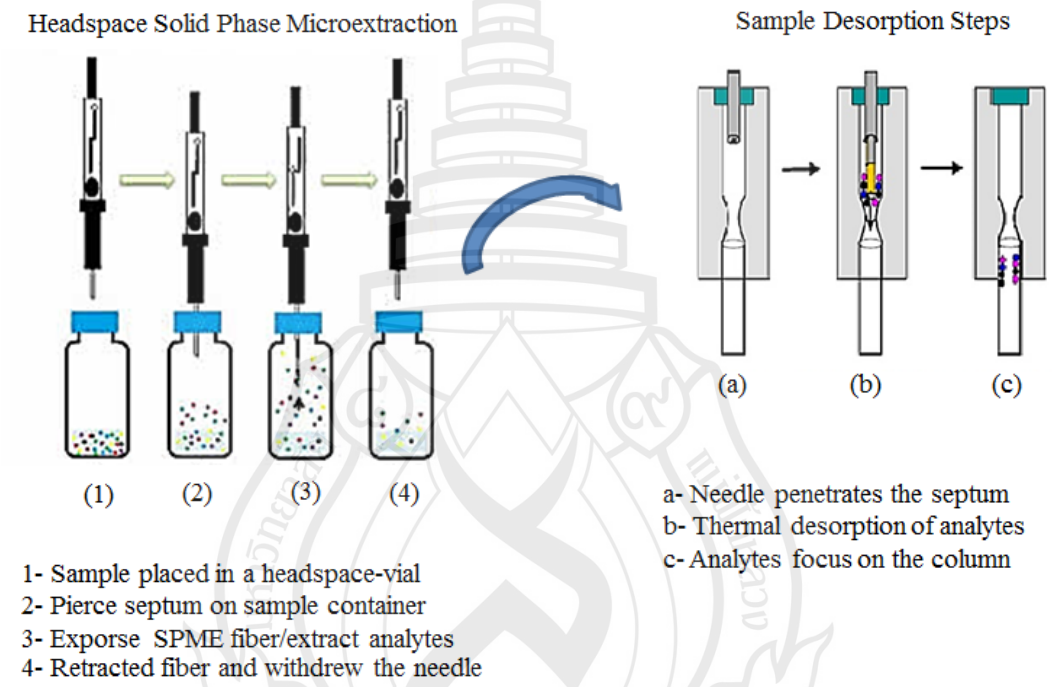


Source Zhang & Pawliszyn (1993).

Figure 1.6 Design of Commercial SPME Fiber Holder

There are two types of SPME utilizations, gaseous sample (headspace (HS)) or liquid sample. In either case the SPME needle is inserted through a septum into the headspace with the appropriate position, the needle coating the fiber is retracted and the fiber is exposed to the environment. The fiber coatings look like a sponge, concentrate the analytes by adsorption/desorption process (Vas & Vekey, 2004).The extraction of SPME is similar to the principle of chromatography, based on gas–liquid or liquid–liquid partitioning (Ulrich, 2000). The efficiencies of SPME extraction are strongly influenced by many factors such as film thickness, agitation of the sample, extraction temperature, but sampling times are usually in the order of a few minutes. After sampling, draw the fused silica into the metal needle for mechanical protection,

and another step is desorption of the analytes from the fiber coating in the injection port of an analytical instrument, where the adsorbed analytes are thermally desorbed and delivered to a capillary GC column (Vas & Vekey, 2004). The absorption and desorption procedures for SPME is presented in Figure 1.4 (Wang, Tang, Chen, Feng & Li, 2012).



Source Wang, Tang, Chen, Feng, & Li (2012).

Figure 1.7 Absorption and Desorption Procedures for SPME

1.4.2 Extraction Mode of SPME

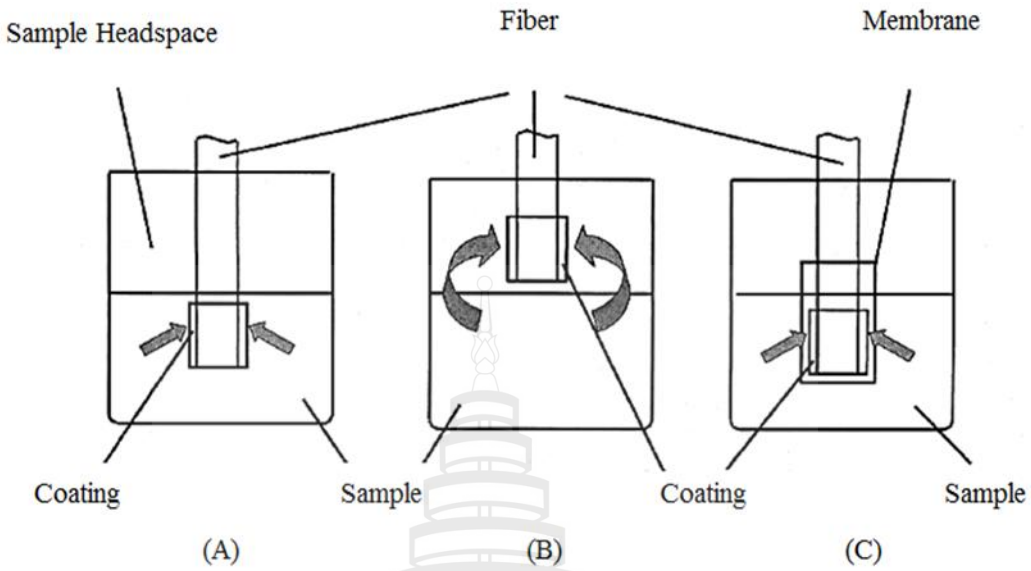
There are three different extraction methods for SPME such as direct extraction, headspace extraction, and membrane extraction (Pawliszyn, 1997; Ruoff, 2003). Figure 1.5 illustrates the differences between these three extraction modes.

1.4.2.1 Direct extraction SPME: For this extraction mode (Fig.1.5 A), the coated fiber is injected into the sample and the analytes transported directly from the

sample matrix; liquid or gaseous, to the extraction phase. For a liquid sample, agitation is used to increase adsorption of the analytes and to greatly reduce adsorption time. In the natural convection of gas, stirring is quite unnecessary because it is enough for rapid extraction (Pawliszyn, 1997; Ruoff, 2003).

1.4.2.2 Headspace extraction SPM: In the headspace mode (Fig. 1.5 B), the analytes are extracted from the liquid samples containing components that may damage or contaminate the fiber (e.g., soil particles, sugars or protein). According to ensure extraction by the coating polymer, the analytes are removed from the sample matrix to the headspace first, and then from there to adsorbent layer as the indirect extraction from the matrix. For the headspace samples, faster transport rates are attained and volatile analytes are extracted faster than semi- volatiles (Baser & Buchbauer, 2009). In general, the extraction times for volatiles are much shorter for headspace extraction mode than in direct extraction mode under similar agitation condition (Pawliszyn, 1997; Ruoff, 2003).

1.4.2.3 Membrane protected SPME: The third mode (SPME with membrane protection, Fig. 1.5 C), is an appropriate method for the extraction of analytes that are adverse effects caused the fiber by too dirty matrixes or high molecular-weight compounds. The time of extraction (until equilibrium) may be decreased with use of thin membrane and increase of extraction temperature (Pawliszyn, 1997; Ruoff, 2003).



Source Pawliszyn (2000).

Figure 1.8 Modes of SPME operation: direct SPME (A), headspace SPME (B) Membrane protected SPME (C)

1.4.3 Coating materials

Nowadays, SPME fibers coated with different polymeric films are commercially available (see Table 1.1). There are mainly two types of coating films: solid porous phases and liquid or crystalline phases. The strength of the coating is investigated by its capability to tie the fiber and by the crosslinking within the polymer. The stationary phases are immobilized by non-bonded, partial cross-linked and high cross linked (bonded) phases. The non-bonded phases do not compose of any crosslinking agents that mean to swell in organic solvents. This phases are stable with some water-miscible organic solvents (up to 20% organic content), but slight swelling may occur when used non-polar solvents (Vas & Vekey, 2004). The cross-linked phases are not bound to the fused silica core. Bonded phases, however, are both cross-linked and linked to the supporting fiber. These coatings conduct the best thermal stability and compatible with the organic solvents except for some non-polar solvents (hexane, dichloromethane). The phase will become very difficult to bond the

phase, if the phase is thick. Consequently, a fiber coated with 7μm polydimethylsiloxane (PDMS) is the only available bonded phase (Pawliszyn, 1997; Ruoff, 2003).

As shown in Table 1.1, the most common types of coating fibers are currently available for the extraction of analytes. They consist of one or two polymers; PDMS, PA, CAR-PDMS, PDMS-DVB and CW-DVB, for example. Works by Pravina, 2008 have shown that the effect of the fiber for an analyte depends on different properties or thickness of coating fibers and the ‘like dissolves like' principle .

Polydimethylsiloxane (PDMS), Polyacrylate (PA), and Carbowax (CW) are represented the liquid phase coatings. Among them, PDMS is a non-polar coating and more suitable for the extraction of hydrocarbons (Ruoff, 2003). Polyacrylate (PA) and CW are more polar coating fiber, which are designed to extract more-polar analytes, especially phenols, alcohols, and carboxylic acid (Pravina, 2008; Ruoff, 2003). The remaining mixed coating fibers such as Carboxen - Divinylbenzene (CAR/DVB), Carboxen - Templated resin (CAR/TPR), Carboxen - Polydimethylsiloxane (CAR/PDMS), and Polydimethylsiloxane- divinylbenzene (PDMS/DVB), have high retention capacity because of the adsorption and distribution effect. For the extraction of volatile low molecular compounds and polar analytes, PDMS-DVB fiber can be used. Moreover, CAR-PDMS fiber is better than a 100 μm PDMS fiber and similar fibers in extraction efficiency, but it is a poorer and consume more time to achieve equilibrium extraction (Kataoka et al., 2000; Pravina, 2008). Therefore, Ruoff (2003) proposed that phases consisting of blends of different coatings have many advantages over single phase coatings, especially when extracting complex analyte mixture. In general, a thick polymer coat is suitable for volatile compounds and semi-volatile compounds require a thin coat. Furthermore, the use of a thicker coated fiber takes a more time to achieve equilibrium, but it might provide higher sensitivity as a result of the large amount of the analytes that can be extracted (Kataoka et al., 2000).

Table 1.1 Summary of commercially available SPME fibers

Fibre coating	Film Thickness (μm)	Polarity	Coating method	Maximum operating temperature (°C)	Technique	Compounds to be analysed
PDMS	100	Non-polar	Non-bonded	280	GC/HPLC	Volatiles
PDMS	30	Non-polar	Non-bonded	280	GC/HPLC	Non-polar semivolatiles
PDMS	7	Non-polar	Bonded	340	GC/HPLC	Medium-to-non-polar semivolatiles
PDMS/DVB	65	Bipolar	Cross-linked	270	GC	Polar volatiles
PDMS/DVB	60	Bipolar	Cross-linked	270	HPLC	General purposes
PDMS/DVB*	65	Bipolar	Cross-linked	270	GC	Polar volatiles
PA	85	Polar	Cross-linked	320	GC/HPLC	Polar semivolatiles (phenols)
CAR/PDMS	75	Bipolar	Cross-linked	320	GC	Gases and volatiles
CAR/PDMS*	85	Bipolar	Cross-linked	265	GC	Gases and volatiles
CW/DVB	65	Polar	Cross-linked	265	GC	Polar analytes (alcohols)
CW/DVB*	70	Polar	Cross-linked	265	GC	Polar analytes (alcohols)
CW/TPR	50	Polar	Cross-linked	240	HPLC	Surfactants
DVB/PDMS/CAR*	50/30	Bipolar	Cross-linked	270	GC	Odours and flavors

Note. *Stableflex type is on a 2 cm length fiber.

Source Vas & Vekey (2004).

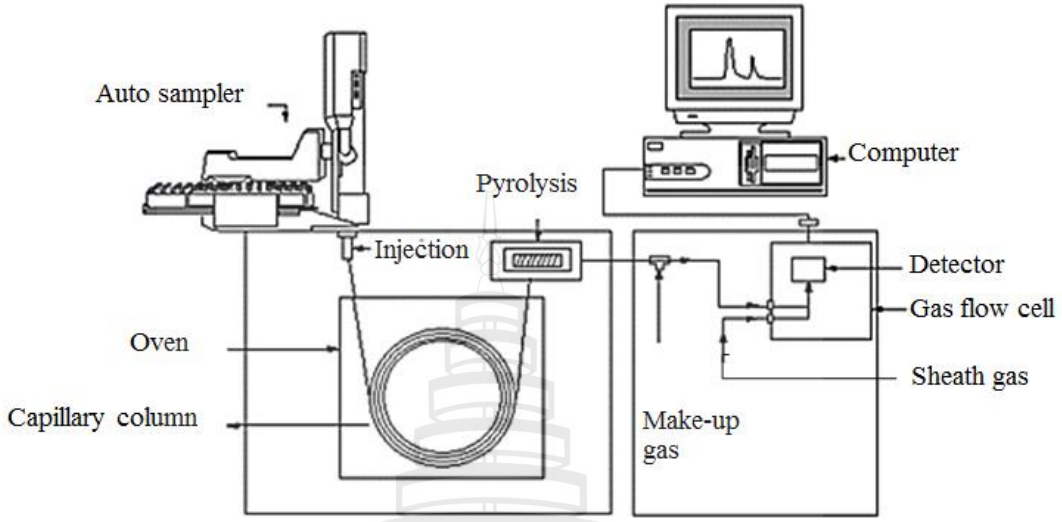
1.4.3 Gas Chromatograph-Mass Spectrometry (GC-MS)

GC-MS is a hyphenated analytical method that combines the character of gas/liquid chromatography and mass spectrometry to determine different components in a mixture of organic compounds. Since 1975, GC-MS was developed to separate, identify, and quantify of organic compounds in complex mixtures as one of the most sensitive and selective analytical methods. It provides a two-dimensional identification consisting of both a GC retention time and a mass spectrum for every component of the mixture. The dual effect is especially efficacious in isomer differentiation where good GC separation frequently compensates for the difficulties encountered as the result of indeterminate mass spectra (Harvey, 2012).

GC is one kind of chromatography in which the mobile phase is a carrier gas, generally an inactive gas such as nitrogen or an inert gas such as helium and the stationary phase is a microscopic layer of liquid or polymer on an inert solid support, inside glass or metal tubing, called a column. The capillary column contains a stationary phase; a fine solid support coated with a nonvolatile liquid. The sample is swept through the column by a stream of carrier gas; helium. The components of the sample are split up from each other because some components take longer to pass through the column than others (Hussain & Maqbool, 2014).

The detector for the GC is MS. Although GC can divide volatile and semi-volatile compounds with great resolution, it cannot determine them. Meanwhile, MS can accurately identify most compounds because it produces detailed structural information on most compounds, but it cannot readily separate them. Since the sample presents the end of the GC column it is separated by ionization and the fragments are ordered by mass to form a fragmentation pattern. The fragmentation pattern for a given component of sample is unique in a like manner with the retention time (RT) and, hence, is an identifying characteristic of that component. It is so special that it is often attributed the molecular fingerprint (Hussain & Maqbool, 2014).

An analytical chemist can both qualitatively and quantitatively determine a solution containing a number of chemicals by combining these two techniques. The applications for GC-MS are numerous. They are widely used in the medical, pharmacological, environmental, and law enforcement fields (Hussain & Maqbool, 2014).



Source Sanchez-Rodas, Corns, Chen & Stockwell (2010).

Figure 1.9 Schematic Diagram of GC-MS System

1.5 Background of Antimicrobial Activities

Human illness has been caused generally from microbial contamination. Pathogens from the microbes are estimated to cause approximately 5 million illnesses, 46,000 hospitalizations, and 1458 deaths each year (Crump, Griffin & Angulo, 2002). Infectious diseases caused by pathogenic microorganisms are still a major problem in the world, even in well-developed countries, like USA where development of medical care and treatment has been improved (Mead et al., 1999; Gulluce et al., 2007). Extracts of medicinal and aromatic plants have been used for resistance microbial pathogens causing human diseases (Okunowo, Oyedeji, Afolabi & Matanmi, 2013; Roby, Sarhan, Selim & Khalel, 2013; Shittu et al., 2008). Aromatic plants have been a source for new drugs which their chemical composition is still largely unexplored. Among the estimated 250,000-500,000 plant species, phytochemical screening of only a small percentage of total plants has been investigated (Mahesh & Satish, 2008).

Plants and their essential oils may be considered the most popular traditional antimicrobial agents throughout the world due to their health benefits, pleasant aromas, and minimal side effects (Umetali, 2012).

1.5.1 General screening assay

There are currently available assays for the detection of antimicrobial activity of natural products. The standard ordinary screening assays are diffusion assays, dilution assays and bioautographic assays (Sumthong, 2007).

1.5.1.1 Diffusion assays

The most widely used method is disc diffusion test.

1. Paper disc diffusion assays: The assays are generally used to determine the antibacterial and antifungal activities of crude extracts and essential oils by examining the rate of growth inhibition (Ahmad Fadzli, 2006; Pyun & Shin, 2006; Quiroga, Sampietro & Vattuone, 2001; Sumthong, 2007). However, the diffusion method is unsuitable to assay nonpolar samples or samples that do not easily diffuse into the agar if the inhibition zone has to be measured (Cos, Vlietinck, Berghe & Maes, 2006; Sumthong, 2007). For this test, the inoculum concentration of bacterial or fungi is between 104 -108 CFU (Colony Forming Units)/mL. The inoculi are dispersed on the agar surface or blend with the agar media (Eldeen, Elgorashi & Van Staden, 2005; Pyun & Shin, 2006; Sumthong, 2007). Whatman No.4 or No.1, 5 mm or 8 mm diameter, sterile filter paper are the most frequently used (Ahmad Fadzli, 2006; Moreno, Isla, Cudmani, Vattuone & Sampietro, 1999; Pyun & Shin, 2006; Quiroga et al., 2001; Sumthong, 2007).

2. Well diffusion assay: The well diffusion assay is appropriate for aqueous extracts because of the difficulties to dry on paper discs (Fazeli et al., 2007; Magaldi et al., 2004; Sumthong, 2007; Tadeg, Mohammed, Asres & Gebre-Mariam, 2005; Valgas, Souza, Smânia & Smânia Jr, 2007; Vlietinck et al., 1995). By using a cork borer, wells with 8 mm diameter are cut in the agar plate and 100 μL of sample is loaded into the well (Fazeli et al., 2007; Patton, Barrett, Brennan & Moran, 2006; Sumthong, 2007). Microbial cell suspension is used in a similar way to the disc diffusion assay and the diameter of inhibition zone is measured after incubation (Sumthong, 2007).

1.5.1.2 Dilution assays

Dilution assay is one of the standard methods used for comparing the inhibition efficiency of antimicrobial agents. The test samples or extracted compounds are blended with proper media that has been inoculated with the test microorganism. It can be performed in liquid media (broth dilution assay) or in solid media (agar dilution assay). The growth inhibition is declared as Minimal Inhibitory Concentration (MIC) which is stated as the lowest concentration ability to inhibit any visible microbial growth. The Minimal Bactericidal or Fungicidal Concentration (MBC or MFC) can be investigated by plating-out samples of completely inhibited dilution cultures and appraising growth after incubation (Cos et al., 2006; Salie, Eagles & Leng, 1996; Sumthong, 2007; Yin & Tsao, 1999). The concentration of of bacterial or fungal inoculum cultures is between 104 -108 CFU/mL (Camporese et al., 2003; Karaman et al., 2003; Sumthong, 2007).

1.5.1.3 Bioautographic assays

According to Cos and co-workers (2006), the bioautography restricts antimicrobial activity on TLC chromatogram by using three different approaches: (a) direct bioautography, where the microorganism multipies directly on the thin-layer chromatographic (TLC) plate, (b) contact bioautography (biogram assay), where the antimicrobial compounds are transferred from the TLC plate to an inoculated agar plate by direct contacting and (c) the agar overlay bioautography, where agar media is applied directly onto the TLC plate . In this assay, an inoculum of about 106 CFU/mL is adequate for bacteria and fungi (Moreno et al., 1999).

1.6 Microbes

There are various types of bacterial and fungi that some of them are herewith presented as below;

1.6.1 *Escherichia coli*

According to Garcia (2009), *Escherichia coli* is a Gram negative rod shaped and Gram-negative (bacillus) belongs to the family Enterobacteriaceae. Most *E. coli* are harmless and normal commensals found in the intestinal tract. These commensal

E. coli strains rarely cause disease except in immune compromised hosts or where the normal gastrointestinal barriers are breached — as in peritonitis, for example. The pathogenic *E. coli* can cause diarrhea or hemorrhagic colitis in humans.

1.6.2 *Salmonella typhi*

Salmonella is a rod-shaped gram-negative facultative anaerobe bacterium belonging to the Enterobacteriaceae family. *S. typhi* causes a bacterial infection of the intestinal tract and occasionally the bloodstream, which is called typhoid fever (Mcclelland et al., 2001; Zhang, Jeza & Pan, 2008).

1.6.3 *Enterobacter aerogenes*

Enterobacter aerogenes is a genus of a common Gram-negative, facultative anaerobic, rod-shaped, nonspore-forming bacteria belonging to the family *Enterobacteriaceae.* It is a nosocomial and pathogenic bacterium that causes opportunistic infections. *E. aerogenes* is generally found in the human gastrointestinal tract, various wastes, hygienic chemicals, and soil (Davin-Regli, 2015).

1.6.4 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is an aerobic Gram-negative bacillus considered to be an opportunistic pathogen. *Ps. aeruginosa* can survive low oxygen conditions, grow low levels of nutrients and survive in temperatures ranging from 4-42 °C. It can cause pneumonia, urinary tract infections and also causing high morbidity and mortality in patients with cystic fibrosis owe to chronic infections that finally cause pulmonary damage and respiratory insufficiency (Ochoa et al., 2013).

1.6.5 *Bacillus subtilis*

Bacillus subtilis has been a rod model for Gram-positive bacteria for more than a century. *B. subtilis* is a ubiquitous naturally occurring saprophytic bacterium that is commonly recovered from soil, water, air, and decomposing plant material. It is considered a benignant organism because it does not possess bad traits of character that cause harmful disease. Most common incidences of *B. substilis* symptoms are

vomiting in 1-2 hours and diarrhea after about 8 hours (Matarante, Baruzzi, Cocconcelli & Morea, 2004; Barbe et al., 2009).

1.6.6 *Bacillus cereus*

Bacillus cereus is a Gram-positive, spore-forming, facultative anaerobic bacterium which is associated with food poisoning in humans. *B. cereus* is commonly found in the soil and the food industry, in such foods as herbs, spices, milk and vegetables. It can cause two types of toxins – emetic (vomiting) and diarrhoeal, and illness (Ehling-Schulz et al., 2006).

1.5.7 *Micrococcus luteus*

Micrococcus luteus, a member of the high G+C cohort of Gram-positive, non-pathogenic, nonsporing, catalase-positive microbe (Arunkumar, Thanalakshmi, Kumar, & Premkumar, 2013). They grow in circular, entire, convex and creamy yellow pigmented colonies having diameters of approximately 4mm after 2-3 days at 37°C that may be isolated from human skin, soil and water (Mukamolova et al., 2002). It has been related to a pathogen in meningitis, intracranial abscess, arthritis, pneumonia catheter-related bacteremia in paint undergoing hemodialysis or leukemia treatment (Tsai et al., 2010; Liu, 2011).

1.6.8 *Staphylococcus aureus*

Staphylococcus aureus is a Gram-positive bacterium that can grow in the form of spherical shaped and approximately 1 μm in diameter. Commonly, It is often found as a commensal associated with skin, skin glands, and mucous membranes, particularly in the nose of healthy individuals. *S. aureus* can cause infection in human as staphylococcal food poisoning, a form of gastroenteritis with rapid onset of symptoms (Lowy, 1998).

1.6.9 *Candida albicans*

Candida albicans is a dimorphic fungus that is capable growing in a yeast phase or a hyphal (filamentous) phase depends on the environmental conditions (McCreath, Specht & Robbins, 1995; Soll, 1985). It can be found in warm-blooded

animals and humans as a commensal organism, but can act as an opportunistic pathogen in immune compromised hosts (Matthews, 1993; McCreath et al., 1995; Odds, 1994). *C. albicans* is the most virulent among the Candida species, and can cause several forms of candidiasis in debilitated human (Mahmoudabadi, Zarrin & Miry, 2010). It has been linked to a few infectious diseases such as infancy, pregnancy, diabetes, prolonged broad spectrum antibiotic administration, steroidal chemotherapy as well as AIDS (Manohar et al., 2001).

1.6.10 *Candida utilis*

Candida utilis is a strain of yeast that can be utilized as a food substance, either as a food supplement or substitute in the diet. Other names that are commonly used for this same strain of yeast are Torula yeast, Torula utilis, and Torulopsis utilis (Rentschler, 1971). *C. utilis* has represented an industrially important yeast for more than sixty years ago, been classified as GRAS (generally recognized as safe) by regulatory authorities. One of the advantages of this yeast is that it can easily adapt to a number of different carbon and nitrogen sources, such as pentose sugars, organic acids, alcohols, urea, ammonium salts, pyrimidine, and various amino acids (Boze, Moulin & Galzy, 1992). The incidence of *C. utilis* infection is lesser but it has been found to candidemia in neonates, and individuals who have undergone invasive medical procedures or with existing health conditions (González, Elizondo & Ayala, 2008; Lukic-Grlić, Mlinarić-Missoni, Škaric, Važic-Babić & Svetec, 2011; Luzzati et al. 2013; Montagna et al. 2014; Pfaller, Moet, Messer, Jones & Castanheira, 2011; Presterl, Daxböck, Graninger & Willinger, 2007; Tortorano et al. 2004), including keratitis (Alkatan, Athmanathan & Canites, 2012; Shih, Sheu, Chen, & Lin, 1999), urinary tract infection (Dorko and Pilipčinec, 2002; Hazen, Theisz & Howell, 1999), vaginitis (Al Akeel, El Kersh, Al Sheikh & Al Ahmadey, 2013), dental thrush (Song, Sun, Støre, Hansen & Olsen, 2009), and fungaemia (Dekeyser et al. 2003).

CHAPTER 2

LITERATURE REVIEW

2.1 *Citharexylum spinosum*

The investigation of chemical composition and some biological activities of *C. spinosum* flowers have very rarely been reported. Few studies on the chemical composition and some biological activities of *C. spinosum* flowers were presented therein;

Balazs, Toth, Duddeck, and Soliman (2006) studied the lignan glycosides and iridoid from *C. spinosum* by ESI-MS spectra, as well as by one- and two- dimensional NMR. The results shown that one new iridoid glucoside, 7-β-O-acetate of lamiide, follow by four known compounds such as lamiide, lamiidoside, duranterectoside C, 8-epiloganin and one known lignan glucoside (+)-lyonirenisol-3a-O-β-D-glucopyranoside were isolated from the aerial parts of *C. spinosum*.

Khan and Siddique (2012) investigated the bioactive compounds in the methanolic extract of *C. spinosum* leaves and determined the antioxidant activities against the nephrotoxicity induced CCl_4 in rat. The major compounds of the chloroform extract of *C. spinosum* leaves (CSCE) were flavonoids, terpenoids, alkaloids and very low amount of saponins. Among them total flavonoids estimated were (12.7 ± 14.6) as rutin equivalent mg/g of the extract. According to the results, they recommended that CSCE possesses powerful antioxidant and nephroprotective properties.

El Ayeb-Zakhama and co-workers (2015) investigated the phytochemical and the phytotoxic activity of three extracts: methanol–water, ethyl acetate and butanol from *C. spinosum* L. flowers. In this investigation, the ethyl acetate extract showed the highest reduction by 100% inhibiting the growth of lettuce at 600ppm. They

remarked that durantoside-I tetraacetylated was the most inhibitory compound for the growth of lettuce seedling.

Mansour, Salem, Khamis, and Ali (2015) tested the natural durability of *C. spinosum* and *Morus alba* woods against three mold fungi; *Penicillium selerotigenum*, *Paecilomyces variotii*, and *Aspergillus niger*. The methanol extract from *C. spinosum* wood showed remarkable inhibition against the growth of P. variotii at a concentration of 8 μg/mL. The results of this study clearly showed the changes that occur in wood samples as a result of fungal infestation.

2.2 *Osmanthus fragrans*

Since the noble ornamental tree growing specifically in China, *O. fragrans* is widely used to produce special drugs, high-grade essence and aromatic tea from its flowers. Because of their specific, delicate fragrance, this plant has been studied by many researchers (Huang & Huang, 1949; Huifang, Hong, Yuan, & Caiyun, 2005; Liu & Xiang, 2002; Yingxiong, Shaoqing, Yuelei, Xiao, & Guanghong, 2004; Zhu & Zeng, 2000). However, there is no report on the study of the antimicrobial activities of *O. fragrans* flowers. Few studies have reported on their chemical composition, bioactivity and antioxidant activities of *O. fragrans* leaves and pulp (Hu, Liang, Guo, Li, & Wang, 2010; Kumar & Tripathi, n.d.; Wang et al., 2009; Wu, Chang, Chen, Fan, & Ho, 2009).

Ding and co-workers (1989) studied the aroma constituents of three different varieties of *O. fragrans* flowers : *thunbergii* (golden flower), *latifolius* (silver flower), and *aurantiacus* (reddish flower).Headspace volatiles of fresh flowers were trapped using XAD-4 and analysed by gas-chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) and the volatile constituents of petroleum ether extract of fresh *O. fragrans* flowers were identified by LC, GC, GC-MS and GC-FtIR. Ionones, α- and β-ionols, megastigmas, theaspirans, damascones and terpenic alcohols were found to greatly contribute to the fragrance of the flowers.

Ômura, Honda, and Hayashi (2000) examined that the floral scent of *Osmanthus fragrans* discourages foraging behavior of cabbage butterfly, *Pieris rapae* by

means of chemical analyses, behavioral bioassays (proboscis extension reflex (PER)), and electroantennographic (EAG) experiments. Fractionation of the extract by silica gel column chromatography yielded an active fraction (based on proboscis extension reflex (PER) performance), which, upon GC and GC-MS analyses, was found to consist of γ-decalactone, β-ionone, and linalool oxide (LO) isomers (furanosides and pyranosides) as major components together with small amounts of linalool and α-ionone. In the flower-visiting tests, γ-decalactone showed notable repellency, whereas β-ionone had no repellent effect. On the other hand, the butterfly showed strong to weak EAG responses to LO furanosides, *cis*-LO pyranoside, γ-decalactone, and β-ionone in decreasing order of intensity. The results clearly indicated that the repellency of a compound is correlated with its deterrent effect on PER but not necessarily with antennal sensitivity.

Wang and co-workers (2006) described the isolation and identification of the characteristic of melanin from *O. fragrans'* seeds. The aims of this research were to extract and obtain melanin from *O. fragrans's* seeds using alkaline extraction, acid hydrolysis, and repeated precipitation, and to study the color value of the melanin. This work indicated that the melanin derived directly from *O. fragrans'* seeds were resemblance to the qualities of typical melanin by physical and chemical characteristics of the melanin.

Lee, Lin, and Yang (2007) investigated free radical scavenging and neuroprotection effects of *Osmanthus fragrans*. In this study, they were assessed the free radical scavenging activities of the ethanol crude extract from dried *O. fragrans* with the stable free radical the 1,1-diphenyl-2-picryl-hydrazyl (DPPH), scavenged the hydroxyl anion, investigated the ferric reducing/antioxidant power (FRAP) and measured lipid-peroxidation inhibition in rat tissues. They found that OFE contained a high amount of total flavonoid and polyphenol, displayed FRAP assay with IC_{50} values of 0.23μg/mL, and 7.74 μg/mL. Moreover, they also found that the scavenging ability of DPPH and hydroxyl anion was similar activity with IC_{50} values of 10 μg/mL. In rat brain, liver, heart and kidney mitochodrias, OFE inhibited lipid peroxidation initiated by ferrous chloride with IC_{50} values ranging from

46 to 97 µg/mL. According to all of IC_{50} values of each assay, the ability of OFE was OH scavenging< DPPH scavenging< FRAP< Fe2+chelating.

Liu and Liu's colleagues (2008) firstly reported the bioactivity of the components from *O. fragrans'* leaf, especially biomedical constituents from acetone ultrasonic extractive of *O. fragrans* leaf by using Pyrolysis- gas chromatograph/ mass spectrometer (Py-GC/MS). The principle chemicals found in the extract were friedelan-3-one (38.87%), lup-20(29)-en-3-one (12.46%), 22,23-dihydro-stigmasterol (6.23%), urs-12-ene (5.45%), 4-hydroxy-3-methoxy- benzoic acid (2.08%), stigmasterol (1.96%), cyclotetracosane (1.92%), acetone (1.59%), beta-amyrin (1.57%), benzoic acid,4-hydroxy- (1.32%), 1,6-anhydro-.beta.-D-glucopyranose (1.32%), 3-(2-cyano-2-phenylethenyl-) toluene, (1.22%), taraxasterol (1.20%), 1,2-propanediamine (1.07%), hexadecanoic acid(1.00%), 4,6,22-trien-3.alpha.-ol- ergosta (1.00%), 1,2-benzenediol (0.91%), vitamin E (0.90%), campesterol (0.89%), etc. According to their results, the *O. fragrans* leaf rich in biomedical constituents and it can be used as a natural top value-added product.

Hu and co-workers (2009) extracted essential oil of *O. fragrans* flowers by using steam distillation and analyzed by gas chromatography-mass spectrometry (GC-MS) supported with the heuristic evolving latent projections (HELP), an effective chemometric resolution method. 1, 2-epoxy linalool and nonanal were the major constituents of the *O. fragrans* Lour. *var. thunbergii* Mak. (TM) and *O. fragrans* Lour. *var. aurantiacus* Mak. (AM). A total of 52 and 45 components of the essential oils of TM and AM were identified, accounting for 95.67 % and 92.28% total contents of the essential oils of TM and AM, respectively.

Hui, Zu-guang, and De-long (2009) investigated the fingerprint of different varieties of *O. fragrans* Lour. by HS-SPME GC-MS. In this study *Z* -ocimene, 4-hexenyl acetate, *cis*-linalool oxide, *trans*-linalool oxide, β-linalool, α-ionone, β-ionone, γ-decalactone were the same volatiles released from fresh flowers of three different *O. fragrans* Lour; *O. fragrans var.thunbergii*, *O. fragrans*, *O. fragrans f.aurantiacus.* The results indicated that headspace SPME-GC/MS afforded a simple sampling method for identification of the main volatile fragrance from different varieties of fresh *O. fragrans* Lour. flowers.

Wang, Li, Jin, Li, Zhang, and Yu (2009) proposed a method; headspace solid-phase microextraction (HS-SPME), for quick analysis of volatile profiles from the four different stages of living *Osmanthus* flowers. Linalool and its oxide, α-ionone, β-ionone, nerol, γ-decalactone, 9,12,15-octadecatrienoic acid, and hexadecanoic acid were reported as major components. The highest concentration of most of the primary components was found in the initial flowering stage. Therefore, they reported that the initial flowering stage was the best time to harvest *O. fragrans* by their results.

Wang and co-workers (2010) investigated the components of *O. fragrans* and tested the antioxidant capacity of the extracts from the pulp of *O. fragrans*. They were achieved the ethanolic extracts of *O. fragrans'* pulp demonstrated significant antioxidant activities. Moreover, their results suggested that *O. fragrans'* pulp could be a favorite source of natural antioxidant, and that MSF and salidroside may be beneficial candidates for further development as natural antioxidants.

Baldermann and co-workers (2010) studied the functional characteristics of a carotenoid cleavage dioxygenase 1 and its relation to the carotenoid accumulation and volatile emission during the floral development of *O. fragrans* Lour. In this research, they exhibited that the carotenoid was the forerunner of important fragrance compounds in the flowers of *Osmanthus fragrans* Lour. var. *aurantiacus*. Furthermore, they revealed that the higher amount of α-ionone and β-ionone of the total sensory evaluation of the aroma of the flowers is the reason for increased attraction of the fragrance of Osmanthus by human.

Hu, Liang, Guo, Li, and Wang (2010) analyzed the chemical composition of the essential oils of the *O. fragrans* tea (OFT), *O. fragrans* (OF) and green tea (GT) was identified by gas chromatography-mass spectrometry (GC-MS) with the help of heuristic evolving latent projections (HELP), and a powerful chemometric resolution method (CRM). A total of 67compounds was identified from the essential oil of the OFT, which represented 90.83%of identified components. Seventy-three components were detected in the OFT's essential oil, representing 93.65% of the total and 53 constituents representing 89.97% of the total contents of the essential oil of GT were also investigated.

Li and Huang (2011) extracted essential oil of three *O. fragrans* Albus Group; Xianning, Hangzhou, and Anhui province, by supercritical CO_2 fluid extraction method (SCFE), and also isolated *Osmanthus* extractum by digesting with petroleum ether and analysed chemical components by GC-MS. The principle chemicals found in the essential oil were ionone and ionol (36.99%), linalool (13.11%); ionone and ionol contained in extractum were as high as up to 33.33%, while linalool up to 21.92%. They also proposed that Xianning province fragrance quality is better than the quality of Hangzhou and Anhui districts *O. fragans* Albus group fragrance.

Hu, B., Guo, X., Xiao and Luo (2012) had extracted the essential oil from four groups of *O. fragrans* Lour. flowers (*Thunbergii, Aurantiacus, Latifolius*, and *Sempeiflorens*) by using by simultaneous distillation extraction (SDE), and the chemical constituents of the essential oils were evaluated by using GC-MS. The major compounds from the analysis of the essential oil of four different *O. fragrans* were ketones (90.05%), alcohols (95.07%), esters (86.76%), aldehydes (80.21%) and acids, respectively.

Hung, Tsai, and Li (2012) presented the qualitative phenolic antioxidant analysis of aqueous and crude methanol extracts of *O. fragrans* flowers. In this study, the crude methanol extract had high antioxidant activity with IC_{50} values of 12.8 µg/mL in the scavenging of DPPH and IC_{50} values 16.6 µg/mL in the scavenging of H_2O_2. Besides, the crude extract also had high total phenolic content with 291.3 mg/g because it's composed of the natural antioxidants such as phenolic, ascorbic acid, tocopherols, and carotenes. Furthermore, they investigated the strong antioxidant activity of the aqueous solution ($CHCL_3$ sub-extract) of *O. fragrans*. The results pointed, the aqueous had also strong antioxidant activity with IC value 20.9µg/mL.

Hung and co-workers (2013) studied the antioxidant ability of the ethanolic extract of *O. fragrans* flowers (OFE) in vivo and also evaluated its antioxidant activity and therapeutic effect on an allergic airway inflammation in mice. OFE was also therapeutically efficacy in a mouse model of ovalbumin-induced allergic airway inflammation and OFE had the powerful antioxidative state in the mice. Therefore, they suggested that OFE may be useful as an antiallergic agent according to the ability of the *O. fragrans* flowers.

Yang and co-workers (2013) studied the isolation and determination of Irdoid Glycosides from the seeds of *O. fragrans* by HPLC. The main constituents; nuzhenide and GI-3 were used as successfully as chemical markers to determine the optimal time of *O. fragrans'* seeds for collection. The results showed that the last ten days of April is the best time to collect the seeds because *nuzhenide* and *GI-3* reached the highest level.

Xin, Wu, Zhang, Wang, Li, Yang, and Li (2013) characterized the volatile components from four cultivar groups of sweet Osmanthus flowers (*Osmanthus fragrans* Lour.), including Thunbergii, Latifolius, Aurantiacus and Semperflorens Groups by using Headspace-solid-phase microextraction (HS-SPME) and gas chromatography-mass spectroscopy (GC-MS). A total of 72 volatile compounds was identified from all cultivars by GC-MS analysis. In this report, β-ionone, *cis*-linalool oxide (furan), *trans*-linalool oxide (furan) and linalool were the dominant volatiles in tested cultivars, while *E* -2-hexenal, *Z* -3-hexen-1-ol and hexanal were found abundantly in all of Osmanthus cultivars. This principal component analysis showed that *cis*- and *trans*-linalool oxide (furan)were abundant in the Aurantiacus Group, whereas *E* -2-hexenal and *Z* -3-hexen-1-ol were found with high level in Latifolius group.

Cai and co-workers (2014) studied the aroma-active compounds in three sweet Osmanthus (*O. fragrans*: creamy-white (‘Houban Yingui’, HBYG), yellow (‘Liuye Jingui’, LYJG), and orange (‘Gecheng Dangui’, GCDG)) cultivars by GC-olfactometry and GC-MS. HBYG, exhibiting more herb odors, correlated with *cis*-β-ocimene and *trans*-β-ocimene, while *trans*-β-ionone, α-ionone, and hexyl butanoate were responsible for the woody/violet/fruity notes of LYJG. In GCDG, the more floral odors can be related to *cis*-linalool oxide, *trans*-linalool oxide, and linalool.

Han and co-workers (2014) analysed the metabolites of carotenoid and the expression of carotenoid-biosynthetic genes in three different *O. fragrans* cultivars substantially variety in flower coloration: 'Zi Yingui' (butter yellow), 'Jingui' (golden yellow), and 'Chenghong Dangui' (orange-red). The results of high-performance liquid chromatography (HPLC) analysis indicated that the small amount of β-carotene was found in 'Zi Yingui' petals, 'Jingui' petals have high levels of lutein, as well as

low levels of α-carotene and β-carotene, and 'Chenghong Dangui' petals accumulate considerable concentrations of α-carotene and β-carotene. These results indicated that the *O. fragrans* cultivars vary in pigment composition and concentrations due to differential expressions of the downstream genes in the carotenoid synthesis pathway as well as the genes in the carotenoid degradation pathway.

Mu, Li, Wang, Yang, Sun, and Xu (2014) reported the sequence of transcriptome and analysis of sweet Osmanthus (*O. fragrans* Lour.). In this report, transcriptome sequencing of *O. fragrans* was carried out by using the Illumina HighSeqTM2000 sequencing platform. This result allowed to the identification of genes associated with several major metabolic pathways and provided a useful public information platform for sequence functional genomic studies in *O. fragrans* Lour.

Ouyang, Wei, Wang, and Pan (2015) investigated the antioxidant activity and phytochemical composition of ethanol extracts and its fraction of *O. fragrans'* pulps by using 1, 1-diphenyl-2picrylhydrazyl (DPPH) and 2, 2-azinobis (3-ethylbenzothiazline-6-sulfonic acid) (ABTS) radical scavenging capacity assay, and ferric reducing antioxidant potential (FRAP) assay. *n*-Butanol fraction shows the best antioxidant activity, according to the antioxidant experiments of crude ethanol extract and its fractions, salidroside and phillyrin may be the highest contributors and responsible for the antioxidant activity in the *n*-butanol fraction.

Liu and co-workers (2015) studied the chemical structures of components of *O. fragrans var*. *aurantiacus* flowers. Here, they described the structure of isolated three new megastigmane glycosides named floraosmanosides I, II, and III, and a new γ-decalactone labeled floraosmanolactone I and the effects of inhibition in the constituents of nitric oxide (NO) production.

2.3 Objectives, Scopes and Expected Outcome of Research

To investigate the chemical compositions of *C. spinosum* and *O. fragrans* flowers obtained from various extraction methods.

To analyze highly volatile odor components of *C. spinosum* and *O. fragrans* flowers by SPME method by using three different fibers including (PDMS), (CAR/PDMS) and (DVB/CAR/PDMS).

To determine antimicrobial activities from *C. spinosum* and *O. fragrans* flowers obtained from various extraction methods.

The first scope is to extract and investigate the chemical constituents of essential oils, hexane, dichloromethane, and ethanol extracts of *C. spinosum* and *O. fragrans* flowers by using GC-MS technique.

The second scope is to consider HS-SPME method to be an alternative to analyze the odor components of fresh *C. spinosum* and *O. fragrans* flowers .The SPME device consists of a fuse silica fiber, such as100 μm PDMS fiber, 75 μm CAR/PDMS fiber and 30 μm DVB/CAR/PDMS.

The last scope is to identify the antimicrobial activities of essential oils and all extracts of both flowers. In this context, the antibacterial activities of the essential oil and all extracts were evaluated by the diameter of inhibition and MIC values compared with those obtained from the standard drugs, tetracycline and penicillin.

The expected outcome of this research is expected that the essential oil and all extracts of *C. spinosum* and *O. fragrans* flowers can be contributed to a modern of application; food, cosmetics, and pharmaceuticals, in this world.

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Plant Materials

Fresh *C. spinosum* (Verbenaceae family) flowers and *O. fragrans* flowers (Oleaceae family) were collected in July 2013 and in January 2014, from Mae Fah Luang University, Chiang Rai, Thailand. The voucher herbarium specimens (QBG No. 32612) and (QBG No. 60012) for *C. spinosum* and *O. fragrans* were discriminated and deposited at the Queen Sirikit Botanical Garden, Mae Rim, Chiang Mai, Thailand.

3.2 Hydrodistillation

The isolation of *C. spinosum* flowers (150 g) was performed in a Clevenger-type apparatus for 2 h to obtain the flower's oil. After isolation, the distillate was made collection in a conical flask which was then dried over anhydrous sodium sulfate and concentrated by the way of the vacuum rotary evaporator. The oil content (w/w%) was estimated on the fresh sample weight basis. The essential oils were then stored in sterile bottles, under refrigerated conditions, until further analysis.

The essential oil from fresh *O. fragrans* flowers (150 g) was also extracted by hydrodistillation with a Clevenger-type apparatus for 2 h. The mixture of oil and water was dehydrated by anhydrous sodium sulphate and filtered. The distillate was then concentrated by using a rotary evaporator at 40 °C, before the resulting extract oil was kept at 4 °C for additional study. The yield percent of essential oil (w/w%) was calculated as the weight of oils divided by the weight of the fresh sample flower.

3.3 Maceration

Fresh *C. spinosum* flowers (170 g) were macerated individually with 500 mL of hexane, dichloromethane, and ethanol. Each extraction was accomplished at room temperature for over one week. Solutions were then passed through filter paper and evaporated under vacuum using a rotary evaporator. All crude extracts were stored at 4 °C. Extract yields of yellow residue 0.18 g, green-brown residue; 0.61 g, and black residue; 1.5 g were obtained for hexane, dichloromethane, and ethanol, respectively.

The extract of fresh *O. fragrans* flowers was also obtained by using the maceration of 170 g of this flower with hexane, dichloromethane, and ethanol (500 mL each). The extraction process was done over the period of seven days at room temperature. After filtration and evaporation of all solvent under reduced pressure yielded *O. fragrans* crude extracts of hexane (yellow extract; 0.38 g), dichloromethane (green extract; 0.49 g) and ethanol (dark-brown extract; 1.62 g), respectively.

The extracts were then stored at 4 °C in the dark until further inspection.

3.4 Identification of the Chemical Composition

3.4.1 Gas Chromatography-Mass Spectrometry (GC-MS) Technique for Essential Oils and Crude Extracts Analysis

The chemical compositions of the essential oil and crude extracts were investigated using a gas chromatograph-mass spectrometer (Agilent 6890 and HP5973 mass-selective detector, Agilent Technologies, USA) equipped with a fused-silica capillary column, HP-5MS (5% phenyl-polymethylsiloxane), with dimensions of 30 m × 0.25 mm i.d. × 0.25 mm film thickness (Agilent Technologies). The GC-MS was operated under a temperature program that started at 60 °C and was ramped up to 240 °C at 3 °C/min. The injection temperature was 250 °C. The injection volume was 1 μL in the split mode with a split ratio of 50:1. The quadrupole temperature was 150 °C and the transfer-line temperature 280 °C. Helium was used as a carrier gas and was maintained at a constant pressure. Identification of the volatile

constituents was performed by comparing their mass spectra with those of the database using W8N08 and NIST 98 mass spectral libraries. The identification was also confirmed by comparison of their Kováts retention indices relative to C_8-C_{22} *n*-alkanes, and comparison of the mass spectra of individual components with the reference mass spectra in the Wiley 275 and NIST05 databases.

Kováts retention index was calculated by the following formula:

RI =100[C+[[RtX-RtC]/ [Rt(C+1)-RtC]]]

RI =Retention index

C =The number of carbon atom of the n-alkanes peak that before sample peak

Rt_X =Retention time of sample peak

Rt_C =Retention time of n-alkanes before sample peak

$Rt_{(C+1)}$ =Retention time of n-alkanes after sample peak

3.4.2 Solid Phase Microextraction (SPME)

3.4.2.1 SPME condition: A manual SPME holder and three commercial SPME fibers, of retention power and different polarity, coated with; 100µm polydimethylsiloxane (PDMS), 75 µm carboxen/polydimethyl siloxane (CAR/PDMS) and 50/30 µm divinylbenzene/carboxen/ polydimethylsilocyne (DVB/CAR/PDMS), were obtained from Supelco (Bellefonte, PA, USA). The SPME fibers were conditioned before use by heating them in the injection port of the chromatographic: 250 ºC for 30 min for PDMS, 300 ºC for 1 h for CAR/PDMS and 270 ºC for 1 h for DVB/CAR/PDMS as recommended by the manufacturer. A fiber cleaning step of 10 min at the conditioning temperature with split value opened was performed in the GC injector after every chromatographic run to ensure of non-contaminant remained in a fiber coating. After conditioning, a blank test was performed under the same experimental conditions to check possible carry-over.

3.4.2.2 Gas Chromatography-Mass Spectrometry (GC-MS) for SPME: The volatile constituents of *C. spinosum* and *O. fragrans* flowers extracted from all SPME fibers were analyzed by using a Hewlett Packard model HP6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA). The GC was equipped with an HP-5MS

(5% phenylpolymethylsiloxane) capillary column (30 m × 0.25 mm i.d., film thickness 0.25µm; Agilent Technologies, USA). The initial oven temperature was at 60 °C and then increased by 3 °C/min to 280 °C. The temperatures of the injector and detector were 250 and 280 °C, respectively. Purified helium was used as the carrier gas at a flow rate of 1mL/min. EI mass spectra were collected at 70 eV ionization voltages over the range of *m/z* 29-300. The electron multiplier voltage was 1150 V. The ion source and quadrupole temperatures were set at 230 °C and 150 °C, respectively. Compounds were identified by comparing their mass spectra with those contained in the NIST05 database, and confirmed by comparison of the retention times of the separated constituents with those of the authentic samples, Kovát retention indices (RIs), relative to *n*-alkanes, with the RIs reported in the literature.

Kováts retention index was also calculated as the same formula mentioned above.

3.4.2.3 SPME preparation and sampling: For each adsorption, 25 g of fresh flowers of *C. spinosum* was added to 250 mL-headspace vial. The vial was closed with the septum and a plastic cap and equilibrated for 30 min in a 60°C water bath before the SPME fiber was inserted into the vial. After that, the fiber was exposed to the headspace for extraction of volatile constituents for 30 min before desorption of volatile compounds in the injection port of GC-MS for 5 min.

The adsorption, desorption and analysis of the fresh *O. fragrans* volatiles could be carried out as the same performance mentioned previously.

3.5 The Investigation of Selected Biological Activities

All bacterial and fungi strains were obtained from the Thailand Institute of Scientific and Technological Research, including four Gram-positive bacteria (*Micrococcus luteus* TISTR884, *Staphylococcus aureus* TISTR 1466, *Bacillus subtilis* TISTR008 and *Bacillus cereus* TISTR 687) and four Gram-negative bacteria (*Salmonella typhi* TISTR 292, *Enterobacter aerogenes* TISTR1540, *Pseudomonas aeruginosa* TISTR 781 and *Escherichia coli* TISTR 780) and fungi included two yeast species (*Candida albicans* TISTR5779 and *Candida utilis*

TISTR5773). The antimicrobial activities of the oils and the extracts of both sample flowers had been analysed in our microbiological laboratory.

3.5.1 Analysis of Antibacterial Activity

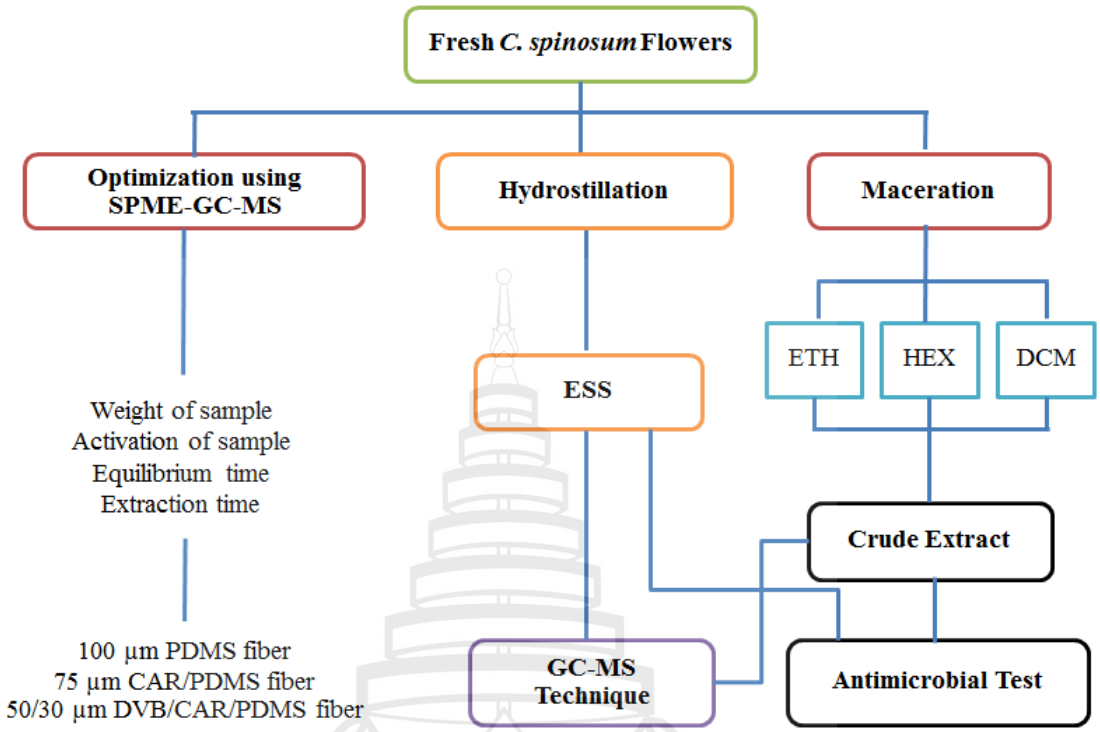
The essential oil and various extracts were tested for their antibacterial activities using the disc diffusion technique. *M. luteus*, *S. aureus*, *B. subtilis*, *B. cereus*, *S. typhi*, *En. aerogenes*, *Ps. aeruginosa* and *E.coli* were testing of the antibacterial activity of *C. spinosum* essential oil and crude extract. The inhibition of pathogenic bacteria, *S. typhi*, *Ps. aeruginosa*, *E. coli*, *M. luteus*, *S. aureus*, and *B. cereus*, by crude extract of *O. fragrans* had been tested. Two yeast species: *C. albicans* and *C. utilis* strains were brought for the investigation of minimal inhibition concentration (MIC) values inhibited by the *O. fragrans* crude extract. The standard discs containing tetracycline and penicillin were used as positive control while all used solvents including ethanol, dichloromethane, and *n*-hexane were used as negative control.

3.5.1.1 Paper disc diffusion method: All extracts and essential oils of and *C. spinosum* flowers were tested for their antibacterial activity by disc diffusion method. To prepare the testing bacteria, a single colony of each bacterial culture was transferred to 3 mL nutrient broth (NB) pH 6.9 (HiMedia Laboratories Pvt. Ltd, Mumbai, India) and incubated for overnight at 37 °C and each bacterial culture was then spread on the surface of the nutrient agar medium (NA) obtaining from 8.0 g/L of NB and 15.0 g/L of agar (Union Science Co. Ltd, Chiang Mai, Thailand) using sterile cotton swab. Subsequently, filter paper discs (6 mm in diameter (Whatman No.1, Maidstone, UK)) were placed on surface of each inoculated plate. The crude extracts were prepared at two fold concentrations (31.25, 62.5, 125, 250, 500, and 1,000 µg/mL). A small amount, 20 µL, of each was then added into a disc plate using a sterile micropipette. These plates were then incubated overnight at 37 °C, but *M. luteus* was incubated at 30 °C. The diameter of the clear zone around each disc plate was measured in mm after incubation and was expressed as the mean value +/- the standard deviation (± SD). This experiment was performed 3 times on each extract and essential oil.

The minimium inhibitory concentration (MIC) of antibacterial activity of *O. fragrans* extract could be carried out as the same procedure mentioned above.

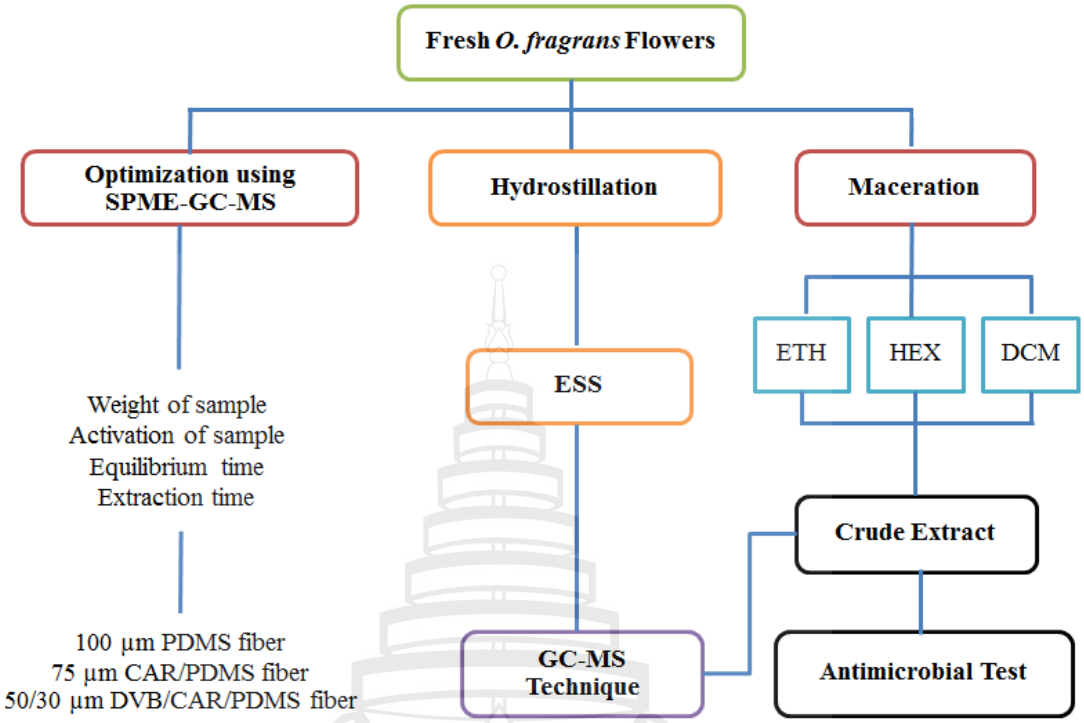
3.5.2 Analysis of Antifungal Activity

The antifungal activity of *O. fragrans* flowers was also tested by disc diffusion method. Initially, yeast cultures were grown on Potato Dextrose Agar (PDA) medium from Criterion Chemical LLC, Newark, New Jersey, for a certain period (generally one week) at the optimum temperature (30 ºC) for growth. After that, a single colony of each yeast was transferred to 3 mL nutrient broth and incubated for overnight at 37 °C and the yeast cultures were then spread on the surface of the PDA plates using sterile cotton swab. The plant extracts were also prepared two fold concentrations (31.25, 62.5, 125, 250, 500, and 1,000 µg/mL) like antibacterial activity. These plant extracts were tested against the yeast culture as described above. The growth inhibition of each yeast strain was calculated as the following formula; Percentage of inhibition (%) = [diameter of clear zone (mm) - diameter of paper disc (mm))/ diameter of clear zone (mm)] ×100.



Note. ESS, essential oil; ETH, ethanol extract; HEX, hexane extract; DCM, dichloromethane extract.

Figure 3.1 Flowchart of All Experiment Processes of *C. spinosum*



Note. ESS, essential oil; ETH, ethanol extract; HEX, hexane extract; DCM, dichloromethane extract.

Figure 3.2 Flowchart of All Experiment Processes of *O. fragrans*

CHAPTER 4

RESULTS AND DISCUSSION

4.1 The Determination of Chemical Composition from *C. spinosum* and *O. fragrans* Flowers Essential Oils and Extracts

Compound identification of the essential oils and extracts of *C. spinosum* and *O. fragrans* flowers was carried out by gas chromatography mass spectrometry (GC-MS). Confirmation of several compounds was accomplished by comparing mass spectra with those of the database using W8N08 and NIST 98 mass spectral libraries.

4.1.1 *C. spinosum* Essential Oil and Extracts

The essential oil and extracts obtained from hydrodistillation and maceration were produced yellowish colored oils with a strong pleasant odor. Different yields were obtained according to each extraction solvent and method, as shown in Table 4.1. Higher yields were detected with ethanol and dichloromethane maceration compared to hexane maceration and the essential oil. A total of 151 volatile components were identified from all extracts by GC-MS analysis. The identified constituents, their percentages, and retention indices are listed in Table 4.2. Figure 4.1-4.4 show the chromatogram of extracts and essential oil of *C. spinosum*. Significant qualitative and quantitative variations of most identified constituents were detected among these extracts.

A total of 86 compounds were identified from the essential oil, which represented 94.2% of the identified components. Maltol (14.7%) and piperitone (13.83%) were the dominant compounds, followed by 2*Z*, 6*E*-farnesyl acetate (6.82%), methyl benzoate (6.62%), indole (6.59%), and benzyl benzoate (4.61%) (see Figure 4.5). The whole essential oil or its main component, maltol, is a natural compound and used as a food additive owing to its flavor and antioxidant properties

(Samejo, Ndukwe, Burdi, Bhanger, & Khan, 2009). Moreover, Abdolpour and co-workers (2007) have been determined that monoterpens, including piperitone (second dominant compound of this essential oil), can be used for the treatment of urine tract infection.

Forty-one components were detected in the hexane extract, representing 77.2% of the total. The major components were methyl benzoate (28.39%) and maple furanone (6.43%), followed by isolongifolan-7α-ol (4.95%), amorpha-4, 9-dien-14-al (4.04%), β- chenopodiol (2.98%), and allyl anthranilate (2.41%). Eighty-three compounds were detected in the dichloromethane extract, representing 88.5% of the identified compounds, including methyl benzoate (26.78%), piperitone (12.52%), maple furanone (8.09%), isopropyl isobutyrate (4.73%), 2*Z*, 6*E*-farnesyl acetate (2.14%), and furfural (1.70%) as the major constituents. In contrast, 59 volatiles were identified in the ethanol extract, representing 86.7% of the chemical constituents. It was found that *cis*-α-ambrinol (25.34%) was a key constituent, followed by 2*Z*, 6*E*-farnesyl acetate (4.64%), maltol (4.41%), thujopsan-2β-ol (4.31%), dihydroeudesmol (4.25%), dihydroeremoligenol (4.11%), and vetivenic acid (3.16%). The chemical structure of major identified compounds of essential oil and all extracts are presented in Fig 4.5-4.8.

In the present investigation, monoterpenes and sesquiterpenes and their derivatives predominated in the essential oil and dichloromethane extracts of *C. spinosum* flowers. Among identified compound, benzene acetaldehyde, methyl benzoate, dimethylacetal-ρ-Anisaldehyde, *E*-methyl cinnamate, sesquicinol-2-one, geranyl benzoate were found in flower's oil and all extracts. In general, the application of essential oils and extracts such as the biocide action are depend on the composition of these oils and extracts (Chamorro, Zambón, Morales, Sequeira, & Velasco, 2012).

Table 4.1 Weight and Yield Percent of *C. spinosum* Essential Oil and Extracts

Type of extraction	Weight of sample (g)	Weight of Products (g)	Yield Percent (%)
Hydrostillation	150	0.08	0.05
Ethanol	170	1.50	0.90
Dichloromethane	170	0.61	0.37
Hexane	170	0.18	0.11

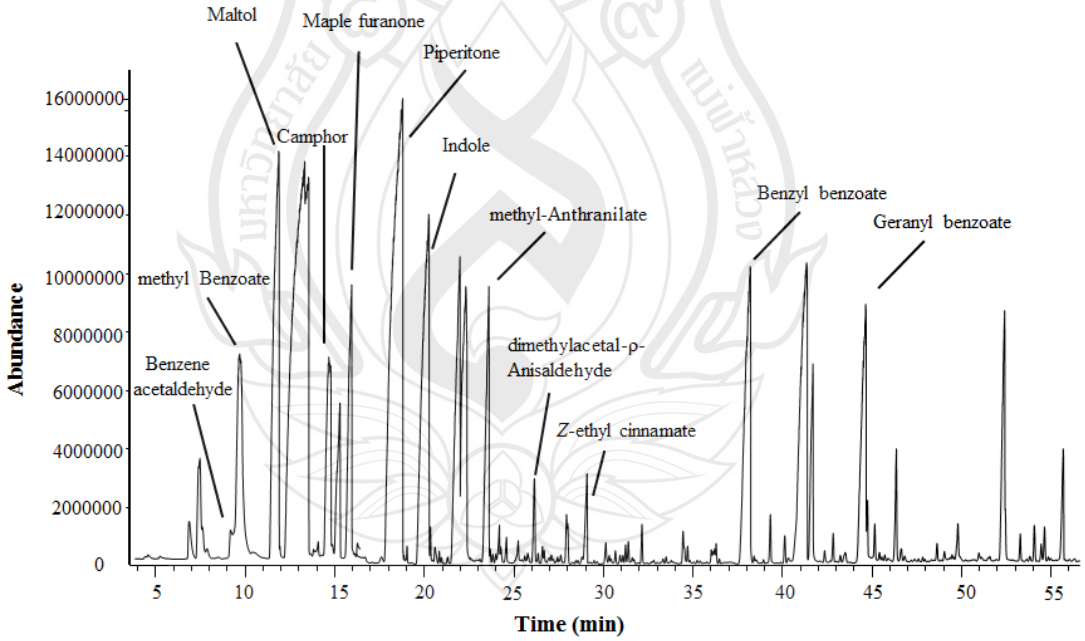


Figure 4.1 GC-MS Chromatogram of *C. spinosum* Essential Oil

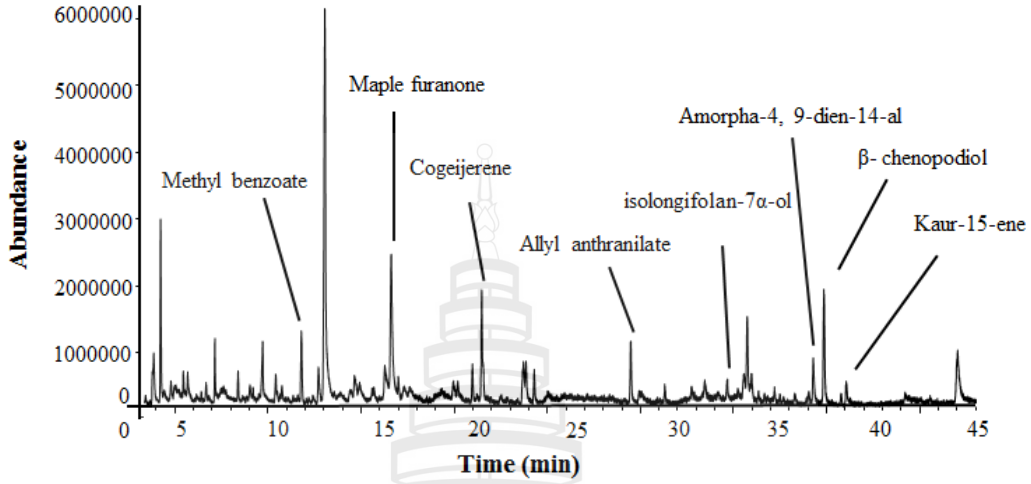


Figure 4.2 GC-MS Chromatogram of *C. spinosum* Hexane Extract

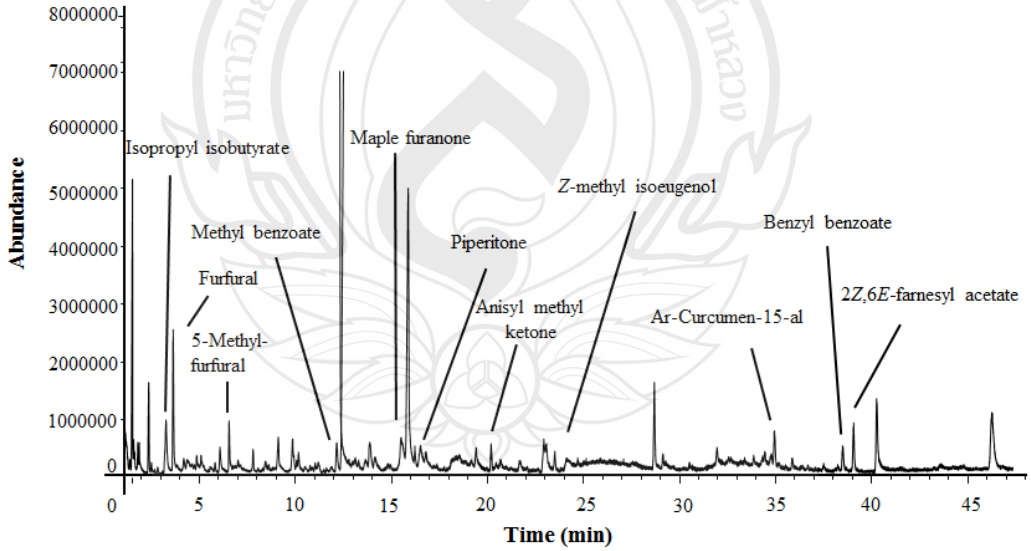


Figure 4.3 GC-MS Chromatogram of *C. spinosum* Dichloromethane Extract

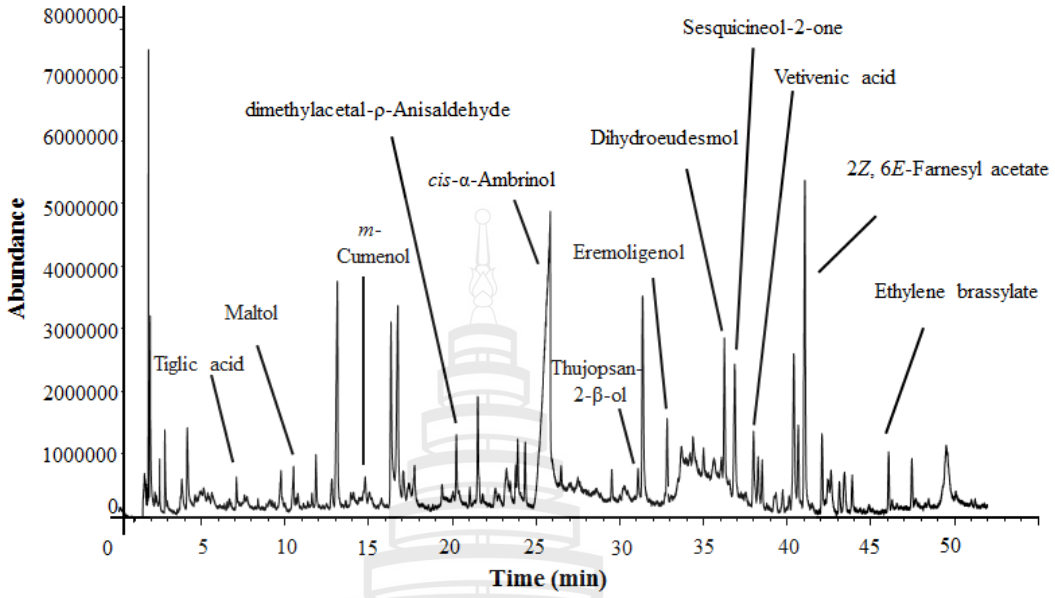


Figure 4.4 GC-MS Chromatogram of *C. spinosum* Ethanol Extract

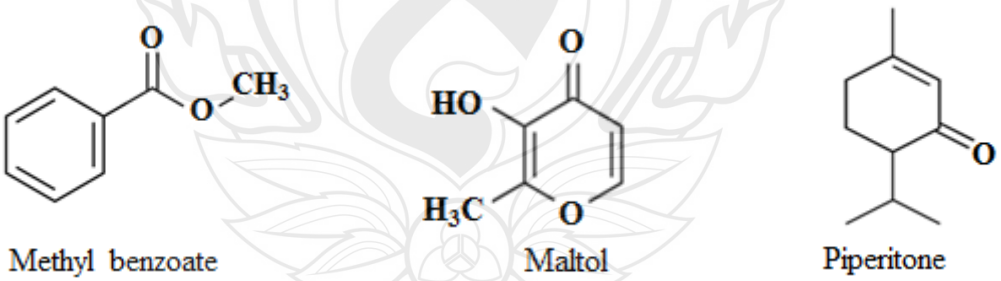


Figure 4.5 The Chemical Structure of Major Components in Essential of *C. spinosum* Flowers

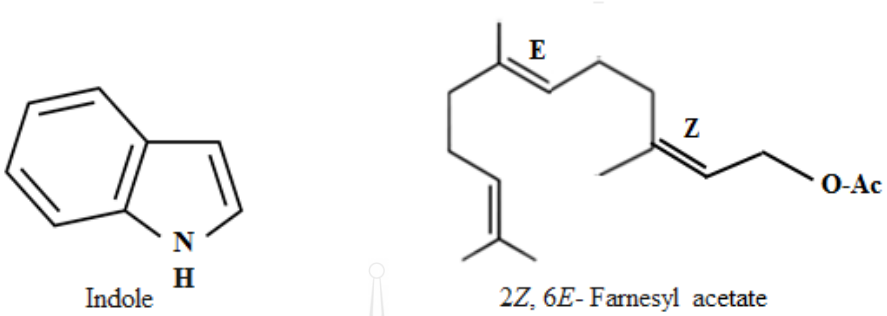


Figure 4.5 The Chemical Structure of Major Components in Essential of *C. spinosum* Flowers (continued)

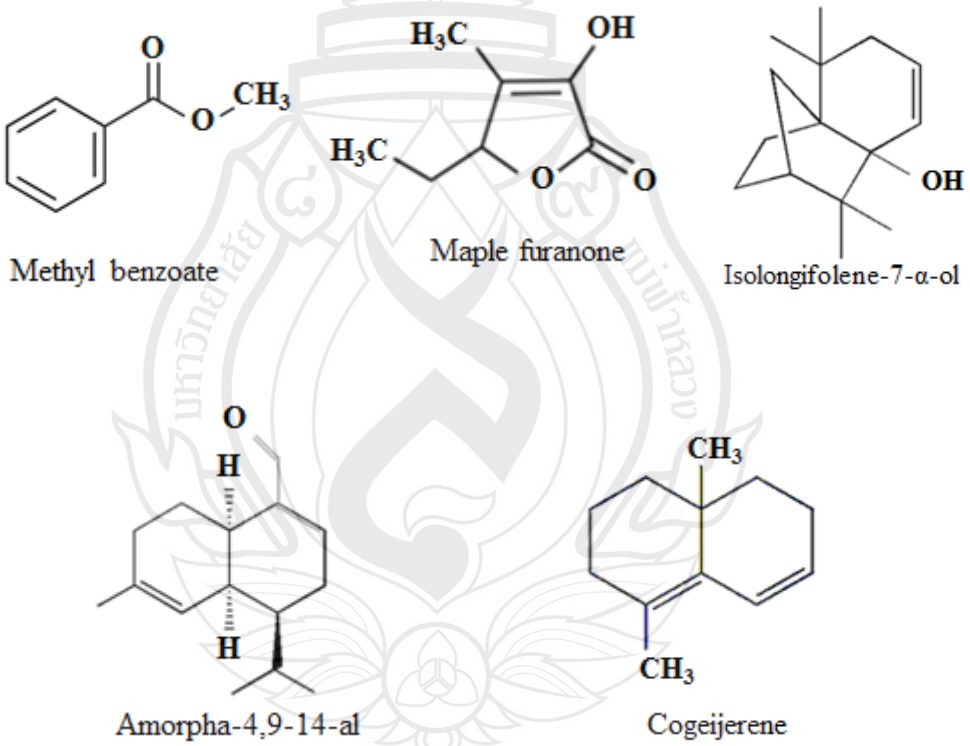


Figure 4.6 The Chemical Structure of Major Components in Hexane Extract of *C. spinosum* Flowers

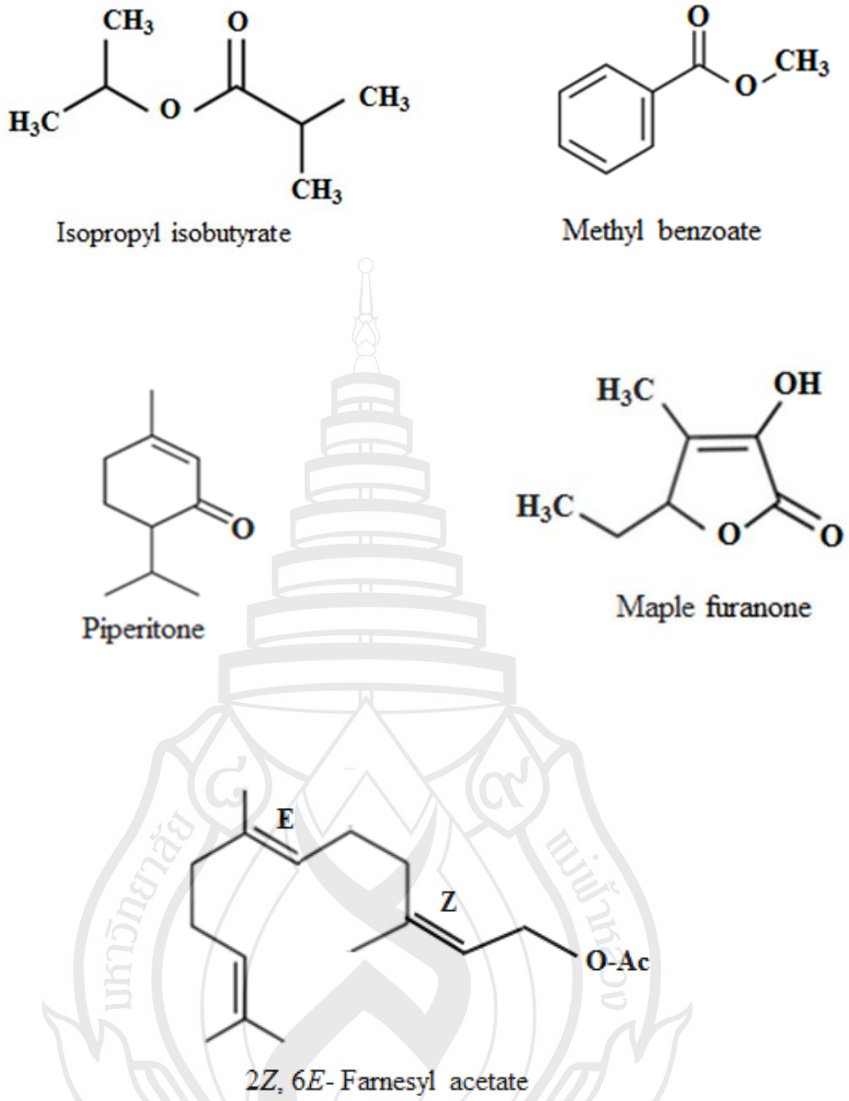


Figure 4.7 The Chemical Structure of Major Components in Dichloromethane Extract of *C. spinosum* Flowers

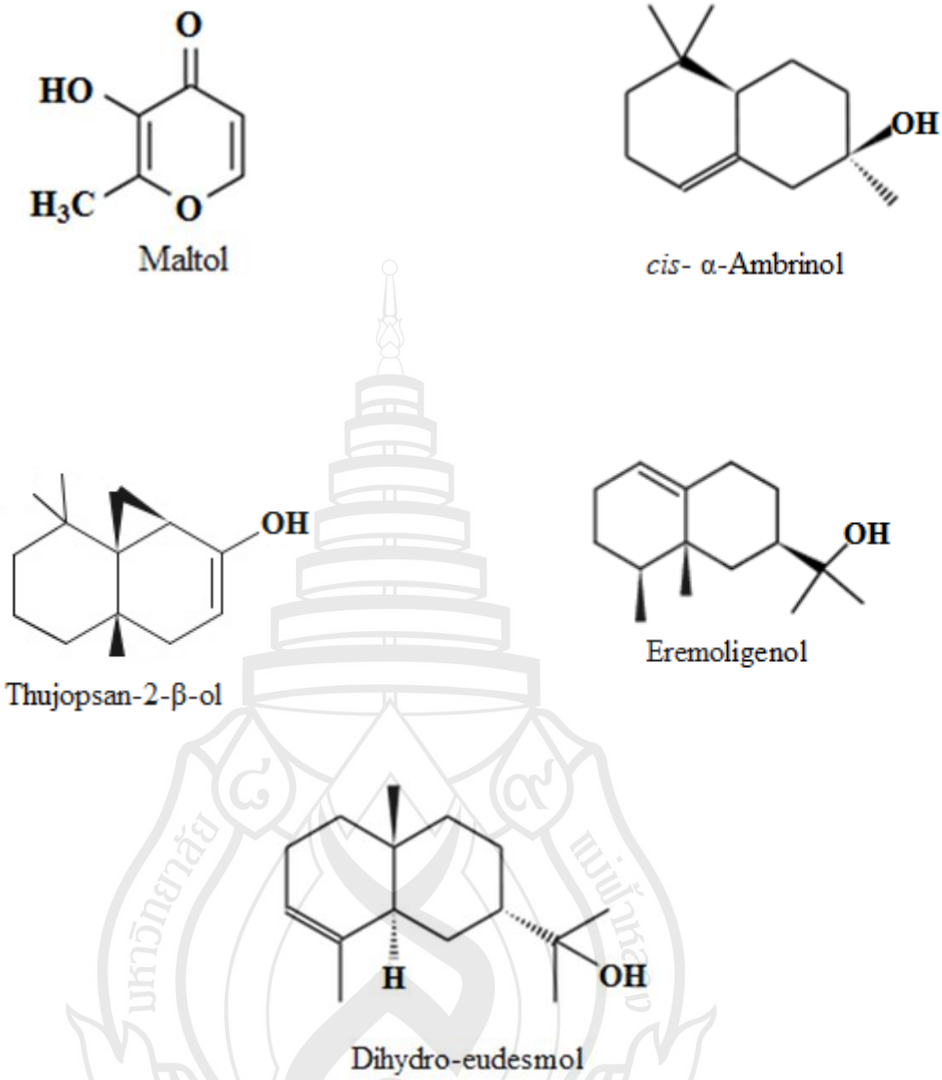


Figure 4.7 The Chemical Structure of Major Components in Ethanol Extract of *C. spinosum* Flowers

Table 4.2 Chemical Constituents and their Relative Peak Area Percentage of the Essential Oil and Various Extracts of *C. spinosum* Flowers

No.	Compound	KI[a]	Extract			
			ESS[b]	HEX[c]	DCM[d]	ETH[e]
1	Isopentyl formate	791	-	-	-	0.16
2	Isopropyl isobutyrate	793	-	-	4.73	-
3	Furfural	836	-	-	1.70	0.60
4	Furfuryl alcohol	884	-	-	-	0.99
5	Tiglic acid	912	-	-	0.35	1.63
6	Isocitronellene	923	-	-	0.49	-
7	Ethyl tiglate	936	-	-	0.95	-
8	*2E*-Heptenal	954	-	-	-	0.12
9	Benzaldehyde	960	0.36	-	-	0.11
10	5-Methyl-fufural	964	-	-	1.40	0.18
11	Isopropyl tiglate	976	-	-	0.49	-
12	*cis*-Pinane	986	-	0.44	-	-
13	Limonene	1029	-	0.56	0.27	-
14	Benzyl alcohol	1031	0.23	-	-	0.20
15	Benzene acetaldehyde	1042	3.90	0.53	0.57	0.16
16	Acetophenone	1065	0.17	-	-	0.21
17	Methyl benzoate	1090	6.62	28.39	26.78	0.13
18	Ipsenol	1100	-	-	-	0.27
19	2-Methyl butyl isovalerate	1104	-	0.79	-	-
20	Maltol	1108	14.70	-	0.57	4.41
21	Ipsdienol	1145	-	1.01	-	-
22	Camphor	1146	3.68	-	0.20	0.16
23	Furfuryl butanoate	1173	-	-	0.37	0.31
24	Benzene acetic acid methyl ester	1178	1.46	-	-	0.58
25	Maple furanone	1184	2.77	6.43	8.09	-
26	Methyl salicylate	1191	-	-	-	0.12

Table 4.2 (continued)

No	Compound	KI[a]	Extract			
			ESS[b]	HEX[c]	DCM[d]	ETH[e]
27	Safranal	1196	0.12	-	-	-
28	Verbanol	1197	-	-	-	0.19
29	*trans*-Carveol	1216	-	-	-	0.35
30	*m*-Cumenol	1227	-	-	0.76	1.88
31	*nor*-Davanone	1231	-	0.33	-	-
32	Piperitone	1254	13.83	-	12.52	-
33	Carvenone	1258	-	0.42	-	-
34	2-Phenyl ethyl acetate	1258	-	0.80	-	-
35	Citronellyl formate	1273	0.15	-	0.28	-
36	δ-Octalactone	1278	-	0.69	-	-
37	Cogeijerene	1285	-	2.24	-	-
38	Indole	1291	6.59	-	0.53	0.43
39	*Z*-Methyl cinnamate	1299	0.09	2.06	-	-
40	Dihydro carveol acetate	1307	-	0.37	-	-
41	ρ-Vinyl-guaiacol	1309	0.10	-	0.57	1.02
42	Anisyl formate	1332	-	0.68	-	-
43	*cis*-Piperitol acetate	1334	-	0.37	-	-
44	*Z*-Isosafrole	1336	-	-	0.95	-
45	Methyl-anthranilate	1337	3.81	0.51	0.23	-
46	Dimethylacetal-ρ-anisaldehyde	1364	3.73	0.57	0.36	3.02
47	Linalool isobutanoate	1375	-	0.48	-	-
48	*E*-Isosafrole	1375	-	0.85	-	-
49	*Z*-Ethyl cinnamate	1377	2.41	-	0.56	0.85
50	*E*-Methyl cinnamate	1378	0.08	0.76	0.19	0.45
51	Anisyl methyl ketone	1382	-	-	1.28	-
52	Skatole	1383	-	-	0.52	-
53	Vanillin	1394	0.08	-	0.33	0.36

Table 4.2 (continued)

No	Compound	KI[a]	Extract			
			ESS[b]	HEX[c]	DCM[d]	ETH[e]
54	Sibirene	1400	0.08	-	0.09	-
55	Methyl eugenol	1403	-	2.31	-	-
56	Cyclosechellene	1407	-	0.57	-	0.90
57	*Z*-Isoeugenol	1407	0.12	-	0.44	-
58	Acora-3,7(14)-diene	1408	-	0.57	-	-
59	α-Thujaplicin	1411	-	0.52	0.62	-
60	β-Funebrene	1414	0.05	-	0.08	-
61	β-Ylangene	1420	0.06	-	0.05	0.63
62	*cis*-Carvyl propanoate	1422	0.09	-	0.11	-
63	Isoamyl benzoate	1435	0.09	-	0.09	-
64	α-Guaiene	1439	0.14	-	0.19	-
65	*cis*-α-Ambrinol	1441	-	0.70	-	25.34
66	Pinonic acid	1442	-	-	-	0.45
67	Isoamyl octanoate	1445	-	0.46	-	-
68	*E*-Cinnamyl acetate	1446	0.16	0.57	0.22	-
69	*bis*-Ether,davana	1452	-	0.83	-	-
70	*Z*-Methyl Isoeugenol	1453	0.08	-	1.41	-
71	*E*-Cinnamic acid	1454	-	-	-	2.95
72	Geranyl acetone	1455	0.43	0.65	0.83	-
73	*allo*-Aromadendrene	1460	0.09	-	0.05	-
74	*E*-Ethyl cinnamate	1467	0.06	-	0.12	-
75	*cis*-Thujopsadiene	1467	0.05	-	0.17	-
76	Geranyl propanoate	1477	-	-	-	0.64
77	β-Thujaplicin	1477	-	-	0.22	-
78	γ-Muurolene	1479	0.08	-	-	-
79	Amorpha-4,7(11)-diene	1481	0.10	-	-	-
80	α-Cyclogeranyl acetate	1482	0.12	-	-	-

Table 4.2 (continued)

No	Compound	KI[a]	Extract			
			ESS[b]	HEX[c]	DCM[d]	ETH[e]
81	α-Amorphene	1484	0.09	-	-	-
82	Germacrene D	1485	0.05	-	-	-
83	Aristolochene	1488	0.09	-	-	-
84	Benzyl tiglate	1497	0.12	-	-	-
85	α-Selinene	1498	0.18	-	-	-
86	Pentadecane	1500	-	0.51	-	-
87	Allyl anthranilate	1501	-	2.41	-	-
88	Epizonarene	1501	0.11	-	-	-
89	γ-Patchoulene	1502	0.12	-	-	-
90	Artedouglasia oxide C	1523	-	-	0.41	-
91	*cis*-Calamenene	1529	0.14	-	-	-
92	β-Thujaplicinol	1538	0.60	-	-	-
93	δ-Cuprenene	1543	-	-	0.72	0.65
94	*cis*-Cadinene ether	1553	0.11	-	-	-
95	Caryophylleneyl alcohol	1572	0.11	-	-	-
96	α-Cedrene epoxide	1575	0.29	-	-	0.48
97	Silphiperfol-5-en-3-one A	1575	-	-	0.54	-
98	Thujopsan-2-β-ol	1589	0.10	-	-	4..31
99	Allo-cedrol	1589	0.14	-	0.08	-
100	Fokienol	1596	0.12	-	0.11	-
101	Sesquithuriferol	1605	0.16	-	0.09	-
102	Isomyristicin	1617	0.15	-	0.58	-
103	Isolongifolan-7-*α*-ol	1619	0.19	4.95	0.17	-
104	β-Cedrene epoxide	1622	0.17	-	0.09	-
105	2-*epi*-α-Cedren-3-one	1627	-	-	-	0.99
106	1-*epi*-Cubenol	1628	0.23	-	0.11	-
107	Eremoligenol	1631	0.27	-	0.12	4.13

Table 4.2 (continued)

No	Compound	KI[a]	Extract			
			ESS[b]	HEX[c]	DCM[d]	ETH[e]
108	Hinesol	1641	0.28	-	0.74	-
109	Cubenol	1646	-	-	1.32	-
110	α-Cadinol	1654	0.21	0.86	0.08	-
111	Allohimachalol	1662	0.15	-	0.14	-
112	Dihydroeudesmol	1662	-	-	-	4.25
113	*E*-Amyl cinnamaldehyde	1668	0.17	-	0.68	-
114	*epi*-Zizanone	1670	0.12	-	0.08	-
115	Occidenol	1677	-	-	-	1.43
116	2*Z*,6*Z*-Farnesol	1698	-	-	0.27	-
117	Sesquicineol-2-one	1702	0.28	0.80	0.12	1.73
118	Amorpha-4,9-dien -14-al	1704	-	4.04	-	-
119	2-Propyl chromone	1706	-	-	0.79	0.76
120	ar-Curcumen-15-al	1713	0.15	-	1.04	1.71
121	14-Hydroxy-α-Humulene	1714	0.17	-	0.08	-
122	Longifolol	1714	0.19	-	0.11	-
123	*Z*-Nuciferal	1714	0.12	-	0.19	-
124	*Z*-β-Santalol	1716	0.18	-	0.21	-
125	*Z*-α-Atlantone	1718	0.22	-	0.65	-
126	Chamazulene	1731	-	-	-	1.75
127	*E*-Coniferyl alcohol	1734	-	-	1.03	0.37
128	Ligustilide	1736	-	-	-	0.89
129	Cuparenal	1753	-	-	-	0.58
130	Benzyl benzoate	1760	4.61	-	1.09	0.52
131	β-Acoradienol	1763	0.08	-	-	0.51
132	β-Chenopodiol	1792	-	2.98	0.37	-
133	β-Eudesmol acetate	1792	0.23	-	0.78	0.22
134	α-Eudesmol acetate	1795	-	-	0.79	-

Table 4.2 (continued)

No	Compound	KI[a]	Extract			
			ESS[b]	HEX[c]	DCM[d]	ETH[e]
135	*iso*-Acorone	1811	-	-	-	0.72
136	Vetivenic acid	1811	-	-	-	3.16
137	2*Z*,6*E*-Farnesyl acetate	1822	6.82	-	2.14	4.64
138	Benzyl salicylate	1865	1.56	-	0.54	0.60
139	5*Z*,9*E*-Farnesyl acetone	1889	0.26	-	0.11	0.46
140	Geranyl benzoate	1959	3.88	1.17	0.62	0.48
141	Columellarin	1953	0.17	0.59	-	-
142	Isophyllocladene	1967	0.06	-	0.08	0.52
143	Sclarene	1974	0.05	0.87	0.11	-
144	Bifloratriene	1978	0.24	-	-	-
145	*E*,*Z*-Geranyl linalool	1987	0.58	-	-	0.31
146	Kaur-15-ene	1997	0.19	1.53	0.18	-
147	Ethylene brassylate	2015	-	-	-	2.43
148	Warburganal	2024	0.22	-	1.19	-
149	6*Z*,10*E*-Pseudo phytol	2030	0.33	-	0.08	-
150	13-*epi*-Manool	2060	0.19	-	-	-
151	Nezukol	2133	2.30	-	-	0.31
	Number of compounds		**86**	**41**	**83**	**59**
	Sum		**94.23%**	**77.17%**	**88.48%**	**86.71%**

Note. [a]Kovát indices on DB-5 column, [b]Essential oil, [c]Dichloromethane extract, [d]Hexane extract, [e]Ethanol extract

4.1.2 *O. fragrans* Essential Oil and Extracts

The flowers of *O. fragrans* afforded a yellowish colored essential oil with a percentage yield of 0.002% (w/w) (calculated on a dry weight basis). *O. fragrans* flower extracts obtained by maceration with different solvents including hexane, dichloromethane and ethanol yielded 0.22%, 0.28% and 0.95% w/w, respectively Table 4.3. A total of 245 constituents of the flower oil and extracts were established. Essential oil, different extracts and their contents analyzed by GC-MS are summarized in Table 4.4. The typical GC-MS chromatograms of the oils and extracts are shown in Figure 4.9-4.12.Most volatile compounds in all extracts belong to monoterpenes, oxygenated monoterpenes and sesquiterpenes.

The GC-MS analysis of the essential oil led to the identification of 76 constituents representing 98.9% of the total oil. The most prominent compound is α-terpinyl isobutanoate (13.83%), geranial (12.32%), γ-decalactone (10.04%), anisyl methyl ketone (7.89%), *E*-β-ionone (4.55%), *cis*-verbenyl acetate (4.18%), pentyl salicylate (3.93%), β-biotol (3.72%), and linalool isovalerate (3.33%). The quantitative and qualitative results are presented in detail in Table 4.4. Seventy-nine volatiles were identified in the hexane extract of *O. fragrans* flowers representing 92.08% of the total peak area with the major components of *E*-isoeugenyl benzyl ether (23.77%), *Z*-β-damascenone (13.19%), *E, Z*-geranyl linalool (10.02%), *E*-β-ionone (7.89%), γ-decalactone (6.33%), geranyl benzoate (4.72%), nezukol (4.53%) and geraniol (2.43%) respectively. Individually, *O. fragrans* flower extract from dichloromethane solvent yield ninety-four identified components representing 94.78% with dominant components consisting of anisyl methyl ketone (25.7%), benzyl cinnamate (12.21%), γ-decalactone (6.33%), *E*-β-ionone (5.79%), respectively. The minor components of the dichloromethane extract including γ-gurjunene (3.33%), pentyl salicylate (2.65%), α-patchoulene (2.64%), geraniol (2.44%), *trans*-carvone oxide (2.12%) were also identified. Most volatile components identified were similar to previous reports although the different quantities of compounds were detected. Ethanol extract contained 105 volatile constituents accounting for 82.39%. The major constituents were α-patchoulene (20.57%), γ-decalactone (7.06%) and pentyl salicylate (4.58%). Geranyl benzoate (2.95%), pentadecanol (2.71%), 6*R*, 7*R*-bisabolone (2.68%), octadecene (2.37%), *Z*-isoeugenyl benzyl ether (2.32%), *epi*-α-

bisabolool acetate (2.24%) and *m*-cumenol **(**2.05%) were also found to be minor components of the ethanol extract. The structural types of major compounds of oil and extracts were given in Figure 4.13-4.16.

Hui and co-workers (2009) demonstrated that *Z*-ocimene, 4-hexenyl acetate, *cis*-linalool oxide, *trans*-linalool oxide, β-linalool, α-ionone, β-ionone, γ-decalactone were major volatiles released from fresh *O. fragrans* flowers. Wang et al. (2009) reported that *O. fragrans* flowers of Wuhan (China) was dominated by linalool and its oxide, α-ionone, β-ionone, nerol, γ-decalactone, 9,12,15-octadecatrienoic acid and geranyl benzoate. Beta-linalool, geranyl benzoate, and 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3-buten-2-ol and 1,2-epoxylinalool were reported as major constituents in *O. fragrans* flowers by Hu et al.(2010) and Xin et al. (2013) also reported that β-ionone, *cis*-linalool oxide (furanoid), *trans*-linalool oxide (furanoid) and linalool as the main constituents. Tucker et al. (2001) and Guyot-Dederck et al. (2005) verified that lactone compounds were considered to be the origin of the characteristics coconut odor. As the results, δ-decalactone and γ-decalactone were detected as the principle components in the hexane and dichloromethane extracts of *O. fragrans* which could be responsible for the coconut aroma of the *O. fragrans* flowers. According to literature data, it was obvious that the chemical composition of *O. fragrans* differs according to developmental stage, extraction method, conditions of the analysis (Kim & Lee, 2004; Sangwan, Farooqi, Shabih, & Sangwan, 2001). The chemical composition could be due to several environmental conditions such as climatic, seasonal, geographical and genetic variations (Perry et al., 1999). Moreover, Karousou et al. (2005) noted that plant habitat affected on the content of volatile constituents.

Table 4.3 Weight and Yield Percent of *O. fragrans* Essential Oil and Extracts

Type of extraction	Weight of sample (g)	Weight of Products (g)	Yield Percent
Hydrostillation	170	0.003	0.002
Ethanol	170	1.619	0.952
Dichloromethane	170	0.492	0.289
Hexane	170	0.378	0.222

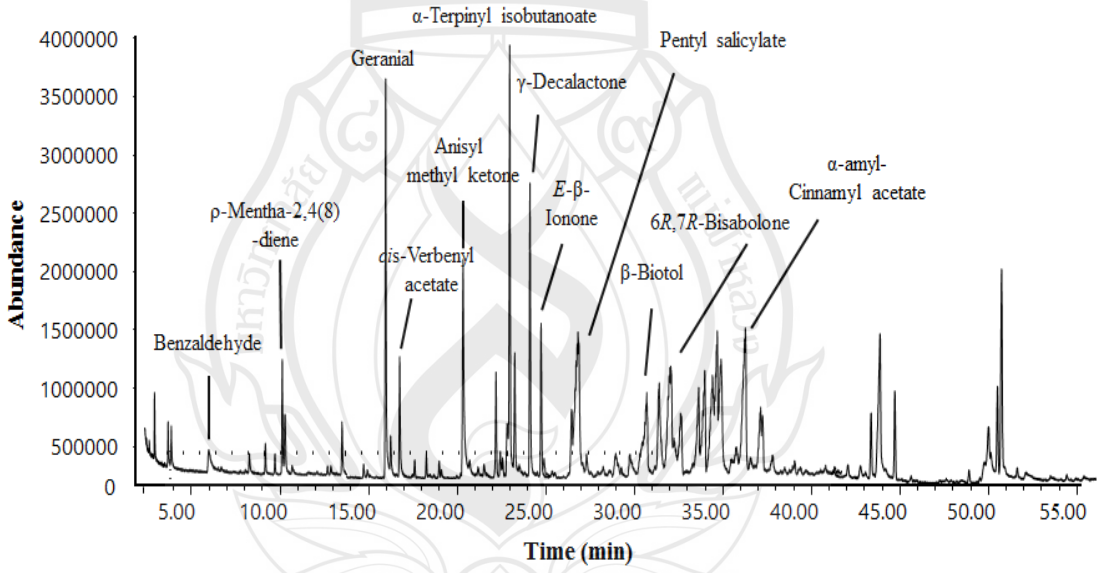


Figure 4.9 GC-MS Chromatogram of *O. fragrans* Essential oil

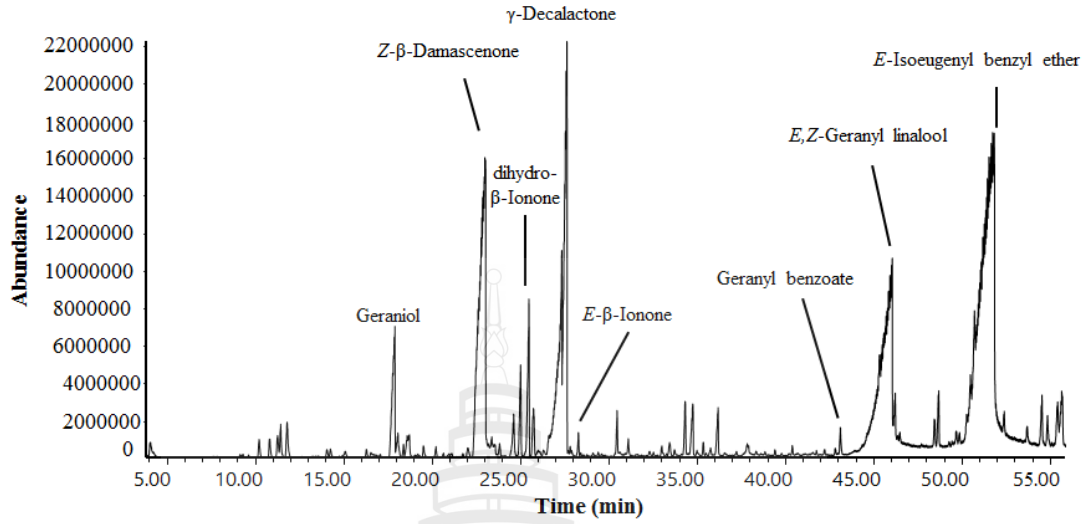


Figure 4.10 GC-MS Chromatogram of *O. fragrans* Hexane Extract

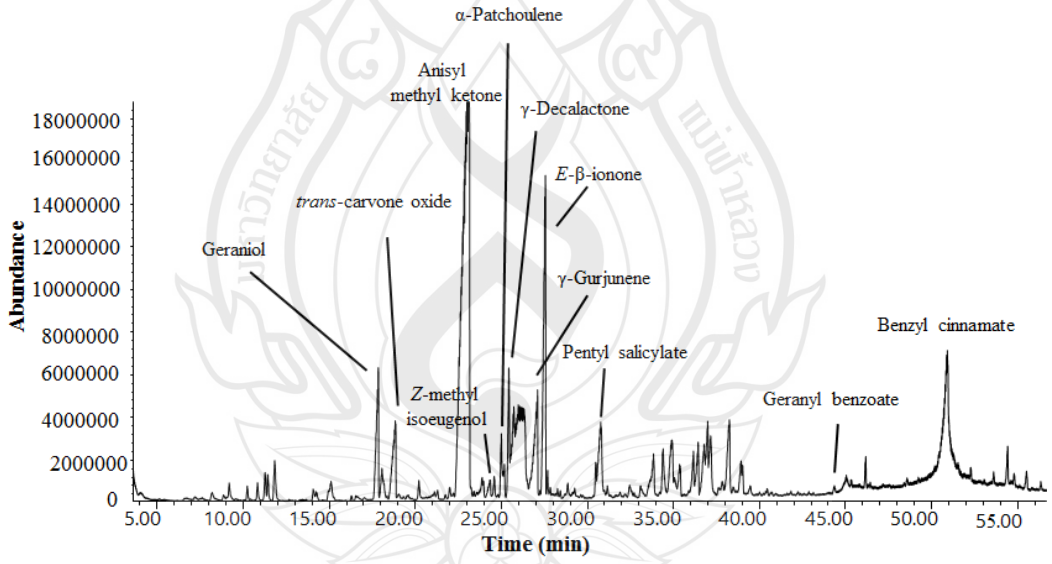


Figure 4.11 GC-MS Chromatogram of *O. fragrans* Dichloromethane Extract

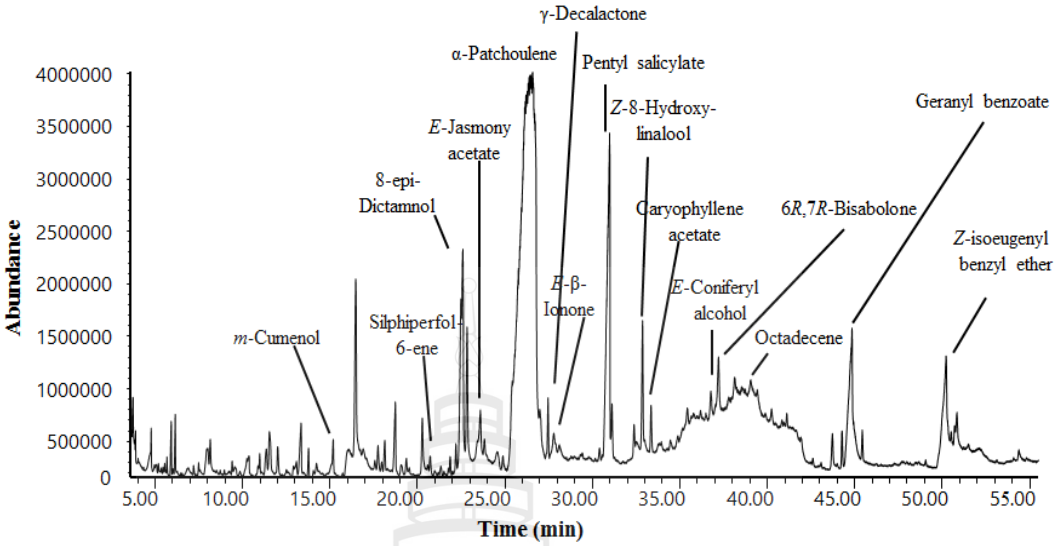


Figure 4.12 GC-MS Chromatogram of *O. fragrans* Ethanol Extract

Figure 4.13 The Chemical Structure of Major Components in Essential Oil of *O. fragrans* Flowers

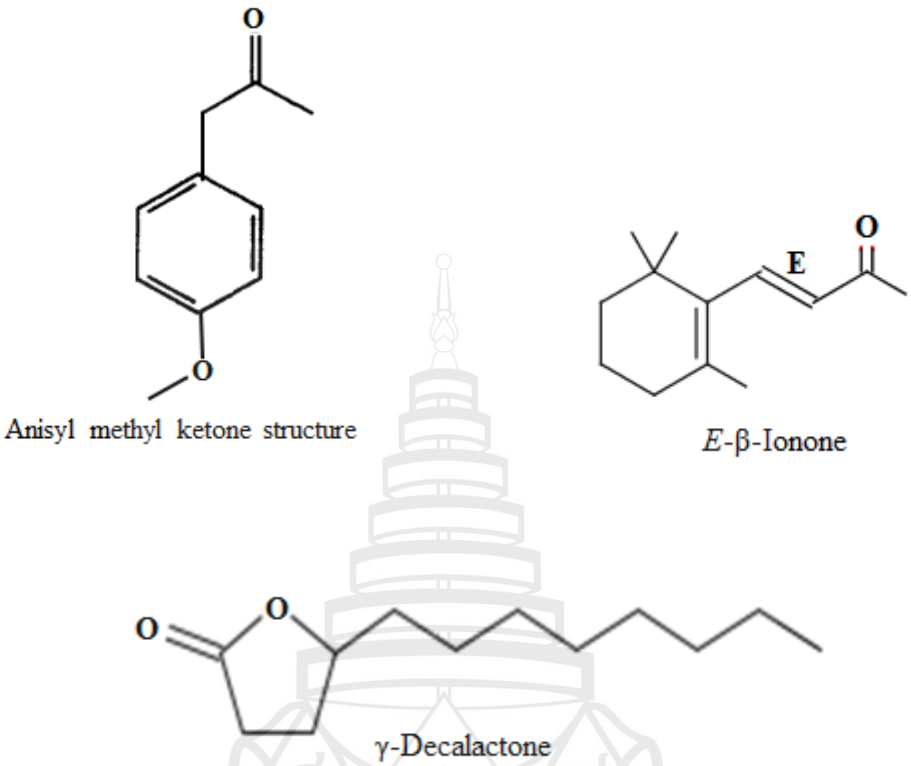


Figure 4.13 The Chemical Structure of Major Components in Essential Oil of *O. fragrans* Flowers (Continued)

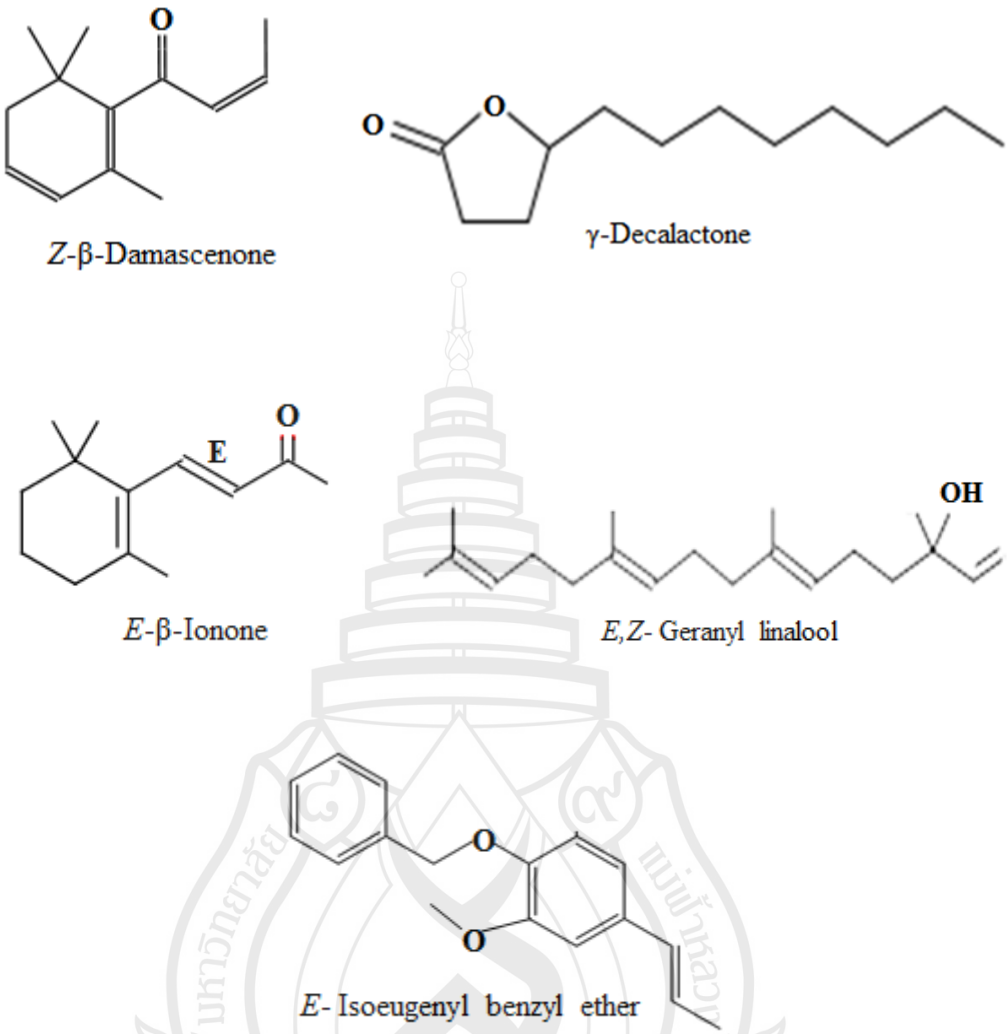


Figure 4.14 The Chemical Structure of Major Components in Hexane Extract of *O. fragrans* Flowers

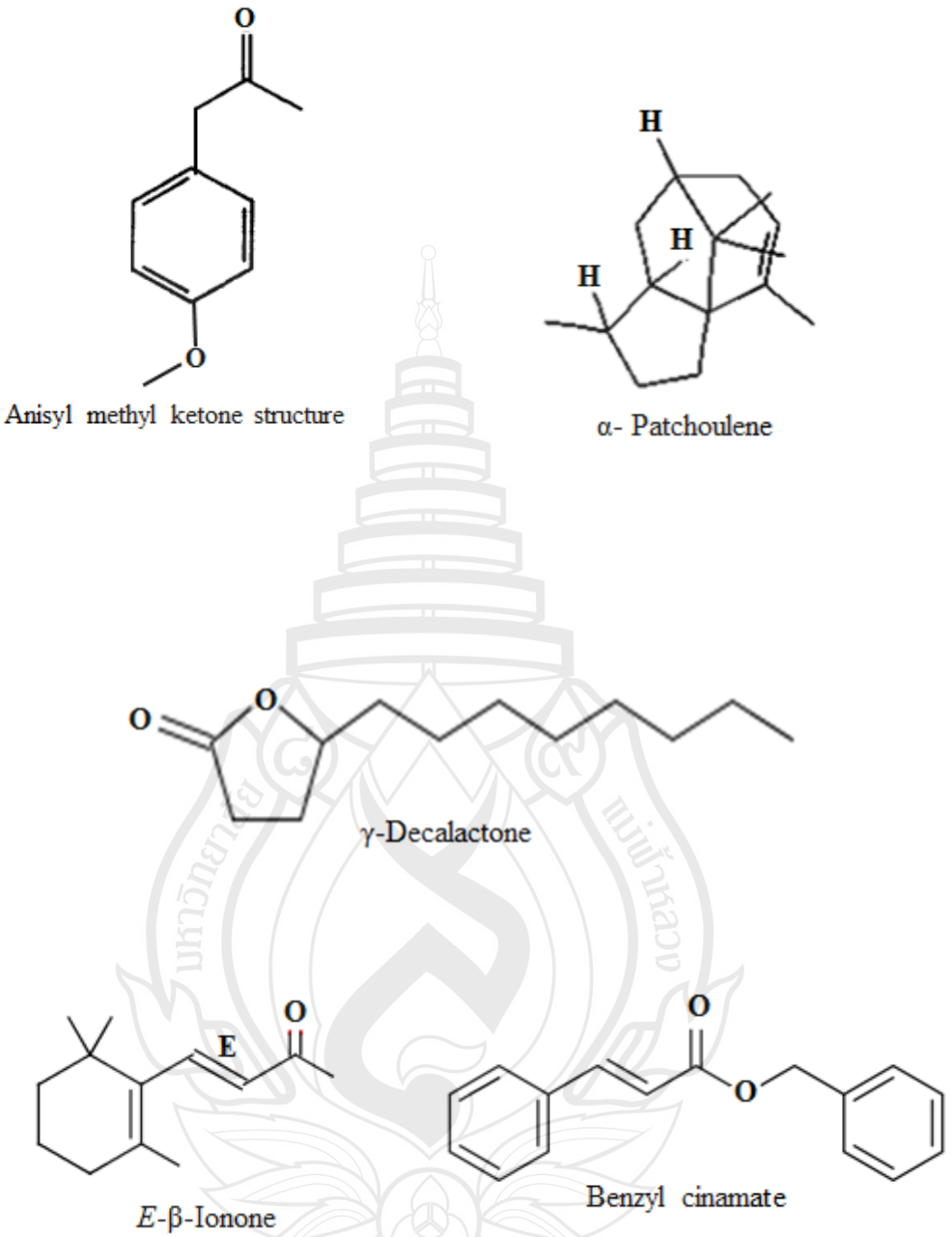


Figure 4.15 The Chemical Structure of Major Components in Dichloromethane Extract of *O. fragrans* Flowers

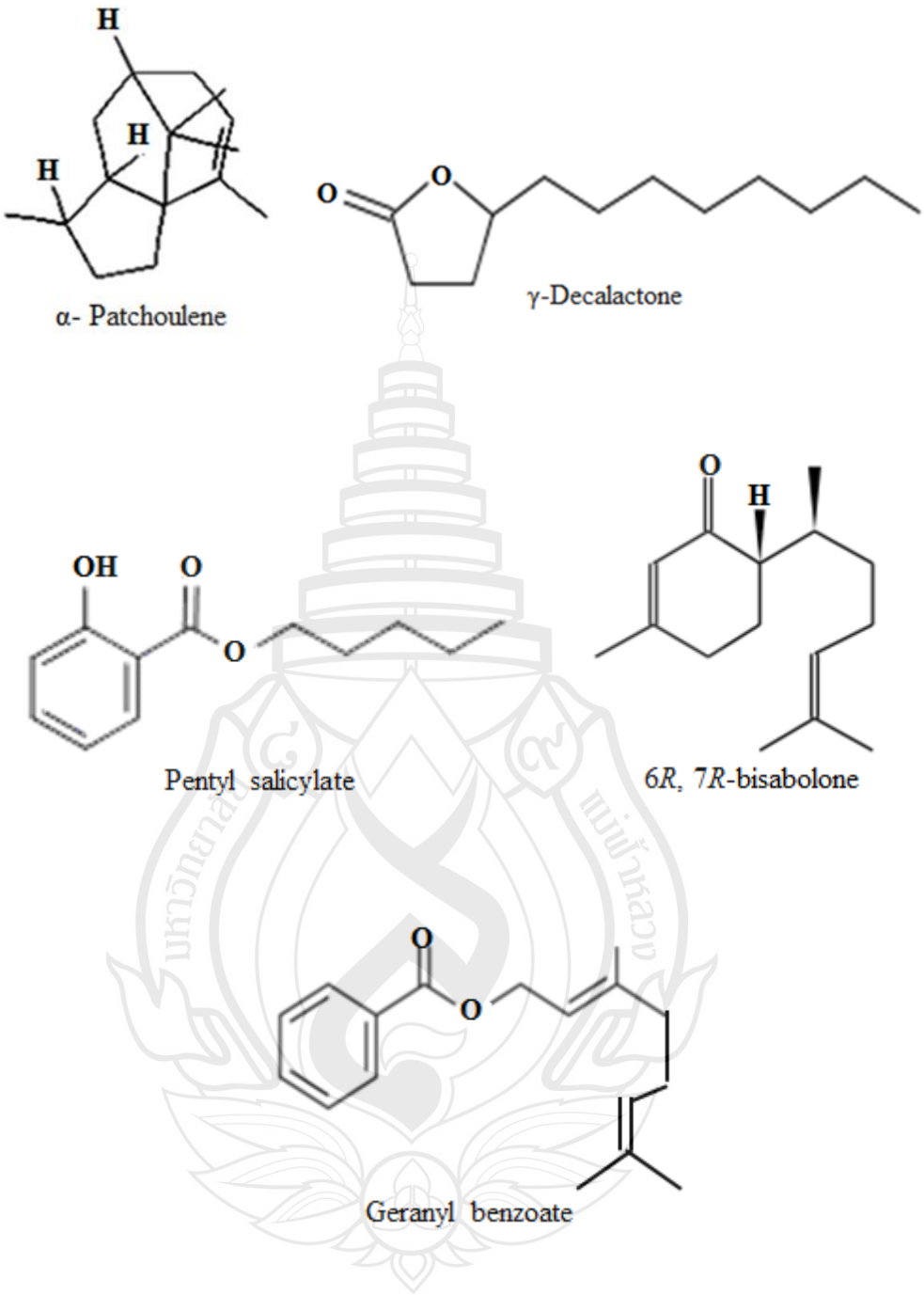


Figure 4.16 The Chemical Structure of Major Components in Ethanol Extract of *O. fragrans* Flowers

Table 4.4 Chemical Constituents and their Relative Peak Area Percentage of the Essential Oil and Various Extracts of *O. fragrans* Flowers

No.	Compound	KI[a]	Exract			
			ESS[b]	HEX[c]	DCM[d]	ETH[e]
1	Butyl actate	811	-	-	-	0.24
2	4-Hydroxyl-4-methyl-2-pentanone	839	-	-	-	0.05
3	Isopropyl butanoate	844	-	-	0.05	-
4	3*E*-Hexenol	853	-	0.3	-	-
5	Methyl tiglate	877	1.10	-	-	-
6	5-Methyl-3*E*-hexen-2-one	894	-	-	-	0.28
7	Propyl butanoate	899	-	-	-	0.03
8	Heptanal	902	-	-	-	0.06
9	2-Ethoxy ethyl acetate	904	-	-	0.02	0.05
10	Isobutyl isobutyrate	911	-	-	-	0.02
11	Amyl acetate	914	-	-	-	0.03
12	3*Z*-Hexenyl formate	917	1.12	-	-	-
13	Isocitronellene	920	-	-	-	0.05
14	Acetonyl acetone	924	1.24	-	-	-
15	Cumene	929	-	-	-	0.05
16	4-Methyl-3-heptanone	931	-	-	-	0.20
17	*exo*-5-Norbornen-2-ol	948	-	-	-	0.04
18	β-Citronellene	950	-	-	-	0.24
19	α-Fenchene	952	-	-	-	0.01
20	Glycerol	957	-	-	-	0.01
21	Benzaldehyde	960	2.03	-	0.03	-
22	5-Methyl-furfural	964	-	-	-	0.15
23	*endo*-2-Norborneol	985	-	-	0.05	0.06
24	Myrcene	991	-	-	-	0.06
25	δ-2-Carene	1002	-	-	0.04	-
26	Pentyl propanoate	1007	-	-	-	0.35

Table 4.4 (continued)

No.	Compound	KI[a]	Exract			
			ESS[b]	HEX[c]	DCM[d]	ETH[e]
27	Hexyl acetate	1009	-	-	0.2	-
28	2*E*-Hexenyl acetate	1013	-	-	-	0.31
29	α-Terpinene	1018	-	-	-	0.05
30	Benzyl alcohol	1031	-	-	0.09	-
31	1,8-Cineole	1031	-	-	0.04	-
32	Lavender lactone	1039	-	-	-	0.07
33	Benzene acetaldehyde	1042	0.01	0.04	0.28	-
34	2-Heptyl acetate	1043	-	-	-	0.12
35	γ-Hexalactone	1047	-	-	0.05	-
36	*E*-β-Ocimene	1050	-	0.04	-	-
37	Dihydro-tagetone	1052	-	0.04	-	-
38	Ethyl levulinate	1056	-	-	-	0.04
39	5*Z*-Octenol	1070	0.50	-	-	-
40	*cis*-Linalool oxide (furanoid)	1072	0.59	0.19	0.18	0.16
41	Allyl hexanoate	1082	-	-	-	0.15
42	*trans*-Linalool oxide(furanoid)	1086	-	0.22	0.24	0.10
43	ρ-Mentha-2,4(8)-diene	1088	2.64	-	-	-
44	Methyl benzoate	1090	2.94	-	-	-
45	Linalool	1096	-	0.26	0.35	0.24
46	Tetrahydro linalool	1100	-	0.34	0.29	0.46
47	Phenyl ethyl alcohol	1107	-	0.46	0.63	0.24
48	6-Camphenol	1110	-	-	-	0.06
49	Myrcenol	1122	-	-	0.02	0.05
50	*trans*-Rose oxide	1125	-	-	-	0.06
51	Butyl tiglate	1131	-	-	-	0.42
52	ρ-Mentha-1,5-dien-8-ol	1170	0.01	0.1	0.18	0.13
53	*cis*-Linalool oxide (pyranoid)	1174	0.01	0.11	0.15	0.04

Table 4.4 (continued)

No.	Compound	KIa	Exract			
			ESS[b]	HEX[c]	DCM[d]	ETH[e]
54	Santolinyl acetate	1174	-	-	0.03	-
55	*cis*-Linalool oxide (pyranoid)	1174	0.01	-	-	-
56	*cis*-Pinocarveol	1184	-	0.15	-	-
57	2-Allyl-Phenol	1191	1.95	-	-	-
58	Methyl salicylate	1191	-	-	0.55	-
59	Dihydro citronellol	1194	-	-	-	0.42
60	*p*-Cymen-9-ol	1202	-	-	-	0.03
61	2*E*,4*E*-Nonadienol	1219	-	0.1	0.06	0.68
62	*m*-Cumenol	1227	-	-	-	2.05
63	Nerol	1229	-	0.13	0.18	-
64	Citonet	1237	-	-	-	0.70
65	Ascaridole	1237	0.01	-	-	-
66	*E*-Ocimenone	1238	0.03	0.04	0.04	-
67	Perilla ketone	1248	-	-	-	0.15
68	Geraniol	1252	-	2.43	2.44	0.23
69	Piperitone	1252	-	-	-	0.12
70	*cis*-Myrtanol	1253	-	0.39	-	-
71	γ-Octalactone	1254	-	-	0.73	-
72	Linalool acetate	1257	1.88	-	-	0.23
73	*cis*-Carvone oxide	1263	-	0.32	-	-
74	Geranial	1267	12.32	0.12	0.04	-
75	*trans*-Carvone oxide	1276	-	0.25	2.12	0.54
76	*cis*-Verbenyl acetate	1283	4.18	-	0.18	-
77	*iso*-Bornyl acetate	1286	-	0.04	-	-
78	Bornyl acetate	1288	-	-	-	0.13
79	Limonen-10-ol	1289	-	-	-	0.14
80	*cis*-α-Necrodol acetate	1299	-	-	0.11	-

Table 4.4 (continued)

No.	Compound	Kia	Exract			
			ESS[b]	HEX[c]	DCM[d]	ETH[e]
81	*iso*-Menthyl acetate	1305	0.01	-	-	-
82	ρ-Vinyl-guaiacol	1309	-	0.15	0.28	0.48
83	2-Adamantanone	1311	-	-	-	0.08
84	δ-Terpinyl acetate	1317	0.52	-	-	-
85	*neo*-Verbanol acetate	1321	0.52	0.06	-	-
86	8-Hydroxy-*neo*-menthol	1330	-	-	0.11	0.15
87	Piperonal	1332	-	-	-	0.05
88	Anisyl formate	1332	-	0.07	-	-
89	Piperitenone	1343	-	-	-	0.02
90	Verbanol acetate	1343	-	-	0.13	-
91	*trans*-Piperitol acetate	1346	0.01	-	-	0.07
92	Citronellyl acetate	1352	0.02	-	-	-
93	α-Longipinene	1356	-	0.05	0.06	0.04
94	Eugenol	1359	-	0.17	0.18	-
95	*neoiso*-Dihydro carveol acetate	1360	-	-	-	0.04
96	Neryl acetate	1361	-	-	-	0.09
97	*Z*-β-Damascenone	1364	-	13.19	-	-
98	*cis*-Carvyl acetate	1367	-	-	0.09	0.03
99	Cyclosativene	1371	-	-	-	0.21
100	Silphiperfol-6-ene	1379	-	-	-	1.64
101	8-*epi*-Dictamnol	1380	-	-	-	1.29
102	Geranyl acetate	1381	-	0.54	-	-
103	Anisyl methyl ketone	1382	7.89	-	25.7	-
104	*E*-β-Damascenone	1384	-	-	0.12	-
105	*E*-Jasmony acetate	1389	-	-	-	1.23
106	*E*-Jasmone	1391	-	0.18	0.1	0.51
107	β-Longipinene	1400	-	-	0.43	-

Table 4.4 (continued)

No.	Compound	KI[a]	Exract			
			ESS[b]	HEX[c]	DCM[d]	ETH[e]
108	Sibirene	1400	-	-	0.19	-
109	Methyl-cresol acetate	1403	-	0.61	-	-
110	Dodecanal	1408	-	-	0.45	-
111	β-Ionol	1413	-	-	0.34	0.53
112	*trans*-α-Ambrinol	1416	0.01	-	-	-
113	Mefranal	1425	0.02	-	-	-
114	*E*-α-Ionone	1430	2.73	0.98	0.92	0.18
115	Dihydro-β-ionone	1436	-	1.75	-	-
116	Nezukone	1437	-	-	-	0.03
117	α-Guaiene	1439	2.09	-	-	-
118	*cis*-Prenyl limonene	1445	1.08	-	-	-
119	Bakerol	1446	-	0.66	-	-
120	Vanillyl alcohol	1447	0.01	-	-	-
121	*Z*-Methyl isoeugenol	1453	-	-	1.64	-
122	α-Patchoulene	1456	-	0.19	2.64	20.57
123	Cabreuva oxide B	1464	-	0.13	-	-
124	γ-Decalactone	1466	10.04	6.31	6.33	7.06
125	Linalool isovalerate	1468	3.33	-	-	-
126	α-Terpinyl isobutanoate	1473	13.83	-	-	-
127	γ-Gurjunene	1479	-	-	3.33	-
128	*E*-β-Ionone	1488	4.55	7.89	5.79	0.55
129	Drim-8(12)-ene	1491	-	0.04	0.14	-
130	δ-Decalactone	1494	-	0.09	0.15	0.86
131	Raspberry ketone methyl eter	1494	-	0.07	-	-
132	Piperonyl acetate	1503	-	0.2	0.11	0.68
133	Methyl isovalerate	1518	-	0.04	0.08	-
134	*E*-γ-Bisabolene	1531	-	0.06	0.34	0.17

Table 4.4 (continued)

No.	Compound	KI[a]	Exract			
			ESS[b]	HEX[c]	DCM[d]	ETH[e]
135	Dauca-4(11),8-diene	1534	-	-	0.28	-
136	Dihydro-eugenol acetate	1537	-	0.05	0.1	0.27
137	*trans*-Dauca-4(11),7-diene	1557	-	-	-	0.14
138	Dimethyl-ionone	1567	-	-	-	0.3
139	α-Cedrene epoxide	1575	-	0.42	0.03	-
140	Pentyl salicylate	1576	3.93	-	2.65	4.58
141	*cis*-β-Elemenone	1589	-	0.19	0.23	0.48
142	Salvial-4(14)-en-1-one	1594	-	-	0.07	-
143	*E*-Carpacin	1594	-	-	0.13	-
144	Guaiol	1600	-	0.07	-	-
145	*trans*-β-Elemenone	1602	0.01	-	-	-
146	Humulene epoxide II	1607	-	-	0.08	-
147	β-Oplopenone	1607	-	-	0.29	-
148	β-Atlantol	1608	-	-	-	0.72
149	β-Biotol	1613	3.72	-	-	-
150	*cis*-Isolongifolanone	1613	-	0.05	-	-
151	Butyl anthranilate	1618	-	-	0.08	-
152	*epi*-Cedrol	1619	0.01	-	-	-
153	*Z*-8-Hydroxy-linalool	1620	-	-	-	1.55
154	*iso*-Butyl cinnamate	1624	-	-	-	0.67
155	*trans*-Isolongifolanone	1626	0.01	-	-	-
156	1-*epi*-Cubenol	1628	-	-	0.32	-
157	α-Acorenol	1633	0.01	-	-	-
158	Gossonorol	1637	0.04	-	-	-
159	β-Acorenol	1637	0.24	0.12	-	-
160	*epi*-α-Muurolol	1641	-	0.03	-	-
161	6-Hydroxy-isobornyl isobutanoate	1644	0.29	-	-	-

Table 4.4 (continued)

No.	Compound	KI[a]	Exract			
			ESS[b]	HEX[c]	DCM[d]	ETH[e]
162	α-Muurolol	1644	-	0.18	-	-
163	Vulgarone B	1651	0.03	-	-	-
164	Atractylone	1658	-	0.09	1.22	0.51
165	Gymnomitrol	1660	0.01	-	-	-
166	Allohimachalol	1662	0.25	-	-	-
167	Salicylic acid, hexyl ester	1674	0.02	-	-	-
168	8-Hydroxy- isobornyl isobutanoate	1675	0.01	-	-	-
169	β-Bisabolol	1675	-	0.57	1.00	-
170	Khusilol	1676	0.01	-	-	-
171	Drimenol	1676	0.01	-	-	-
172	*E*-Asarone	1676	-	-	1.94	-
173	*cis*-14-nor-Muurol-5-en-4-one	1689	-	0.69	-	-
174	*Z*-α-trans-Bergamotol	1690	0.01	-	-	-
175	Germacrone	1693	0.11	-	-	-
176	Deodarone	1698	-	-	0.62	-
177	*n*-Heptadecane	1700	0.65	-	-	-
178	Caryophyllene acetate	1700	-	0.12	-	1.12
179	Amorpha-4,9-dien-14-al	1704	0.04	-	-	-
180	Mayurone	1710	-	-	0.08	-
181	1-Phenyl-hepta-1,3,5-triyne	1721	0.02	-	-	-
182	β-Davanone-2-ol	1721	-	0.1	0.86	-
183	*iso*-Longifolol	1729	-	0.55	0.97	-
184	Zerumbone	1733	-	0.07	-	-
185	*E*-Coniferyl alcohol	1734	-	-	1.14	1.30
186	Eremophilone	1736	-	-	1.25	-
187	Oplopanone	1740	-	0.06	-	-
188	6*R*,7*R*-Bisabolone	1742	2.55	-	1.26	2.68

Table 4.4 (continued)

No.	Compound	KI[a]	Exract			
			ESS[b]	HEX[c]	DCM[d]	ETH[e]
189	*E*-Pseudoisoeugenyl isobutyrate	1742	-	0.34	-	-
190	Mint sulfide	1748	0.01	-	0.16	-
191	*iso*-Baeckeol	1754	-	-	0.33	-
192	α-Amyl-cinnamyl acetate	1758	2.09	-	-	1.95
193	β-Acoradienol	1763	0.01		-	-
194	Pentadecanol	1774	-	-	-	2.71
195	*E*-α-Atlantone	1778	-	-	1.75	-
196	Hinesol acetate	1784	-	-	0.18	-
197	Papuanone	1787	-	-	-	1.36
198	β-Bisabolenol	1789	-	0.08	-	-
199	Octadecene	1790	-	0.05	-	2.37
200	Drimenone	1792	-	-	0.99	-
201	*n*-Octadecane	1800	0.05	-	-	-
202	*epi*-α-Bisabolool acetate	1803	-	-	-	2.24
203	Vetivenic acid	1811	0.02	-	-	-
204	Cryptomeridiol	1813	-	0.05	0.26	-
205	Avocadynofuran	1820	-	-	0.07	-
206	β-Vetivone	1823	0.01	-	-	
207	*E*-Nerolidy isobutyrate	1826	1.42	-	0.03	-
208	Acorone	1826	-	-	0.12	-
209	*Z*-Nuciferol acetate	1831	-	0.03	-	1.13
210	Cyclopentadecanolide	1833	0.59	-	-	-
211	Hillyl acetate	1838	-	0.09	-	-
212	α-o-Methyl pipitzol	1841	-	-	-	1.06
213	Cubitene	1878	-	-	0.09	0.58
214	8*S*,13-Cedranediol	1897	-	0.06	0.06	-
215	*n*-Nonadecane	1900	0.01	-	-	-

Table 4.4 (continued)

No.	Compound	KI[a]	Exract			
			ESS[b]	HEX[c]	DCM[d]	ETH[e]
216	*epi*-Laurenene	1900	-	-	-	0.11
217	Dihydro-columellarin	1900	-	0.07	-	0.05
218	5*E*,9*E*-Farnesyl acetone	1913	0.09	-	0.07	0.28
219	Methyl hexadecanoate	1921	-	0.27	0.03	-
220	Carissone	1927	-	-	-	0.05
221	Methyl hexadecanoate	1931	0.08	-	-	-
222	Procerin	1931	-	-	-	0.20
223	Columellarin	1953	0.16	-	0.19	-
224	Geranyl benzoate	1959	-	4.72	1.44	2.95
225	Hexadecanoic acid	1960	1.06	-	-	-
226	Manool oxide	1987	-	-	-	0.41
227	*E*,*Z*-Geranyl linalool	1987	0.01	-	-	-
228	Ethyl hexadecanoate	1988	0.12	-	-	-
229	*E*,*Z*-Geranyl linalool	1988	-	10.02	0.49	-
230	*Z*,*E*-Geranyl linalool	1998	-	0.69	0.42	-
231	Catalponol	2024	-	0.65	-	-
232	Manool	2057	-	0.7	0.68	-
233	6*E*,10*E*-Pseudo phytol	2059	0.05	-	-	-
234	13-*epi*-Manool	2060	-	-	0.76	-
235	*E*-Methyl-isoprenyl cinnamate	2066	-	-	-	0.09
236	*n*-Octadecanol	2077	0.26	-	-	-
237	*Z*-isoeugenyl benzyl ether	2077	-	-	-	2.32
238	Abietadiene	2085	-	0.44	-	-
239	Methyl linoleate	2087	0.15	-	-	-
240	Benzyl cinnamate	2092	-	0.43	12.21	0.34
241	Methyl linoleate	2100	0.29	0.75	-	-
242	Laurenan-2-one	2116	-	1.35	-	0.30

Table 4.4 (continued)

No.	Compound	KI[a]	Exract			
			ESS[b]	HEX[c]	DCM[d]	ETH[e]
243	*E*-isoeugenyl benzyl ether	2133	-	23.77	-	0.77
244	Linoleic acid	2133	0.88	-	-	-
245	Nezukol	2133	-	4.53	0.83	-
	Number of compounds		**76**	**79**	**95**	**106**
	Sum of %		**98.96**	**91.95**	**94.78**	**82.39**

Note. [a]Kovát indices on DB-5 column, [b]Essential oil, [c]Dichloromethane extract, [d]Hexane extract, [e]Ethanol extract

4.2 The Identification of Chemical Composition of Fresh *C. spinosum* and *O. fragrans* Flowers by Using SPME-GC-MS Technique

The extraction technique of volatile compounds from *C. spinosum* and *O. fragrans* flowers performed the usage of Head Space Solid Phase Micro Extraction, which is solvent free method. The analysis of constituents has been accomplished by gas chromatography which is fused with mass spectrometer as a detector.

4.2.1 Chemical Composition of Fresh *C. spinosum* Flower by Using SPME-GC-MS Technique

Volatile constituents of *C. spinosum* flowers were extracted by using three different SPME fibers including PDMS, CAR/PDMS and DVB/CAR/PDMS prior analysis by GC-MS. The fingerprints of volatile components of *C. spinosum* flowers obtained from various fibers are present in Figure 4.17-4.19. All volatile components, their percentage and retention indices are listed in Table 4.5. Fifty-two odor volatile components were identified among all fibers. The structures of main identified volatile compounds are shown in Figure 20.

Twenty-two compounds were detected with PDMS fiber. The key odor volatiles, approximately 82.78% were represented by the dominant components of 2-phenyl ethyl acetate (31.76%), methyl benzoate (21.67%) and phenyl ethyl alcohol (5.95%). Small amount of methyl salicylate (3.06%), benzyl acetate (3.01%), indole (3.00%), benzyl benzoate (2.55%) and phenyl ethyl octanoate (2.16%) were also detected. Thirty-four volatile constituents representing 81.54% were identified by CAR/PDMS fiber. Eight major compounds were detected including methyl benzoate (33.01%), phenyl ethyl alcohol (10.42%), phenyl ethyl acetate (8.72%), octen-3-ol (6.89%), methyl salicylate (3.45%), 2-heptanol (2.54%), benzyl acetate (1.83%) and 2-*E*-cinnamaldehyde (1.69%). For DVB/CAR/PDMS fiber, 36 constituents representing 88.67% of *C. spinosum* flowers were investigated. It was found that 36.15% of their percentage was represented by methyl benzoate. Other components such as phenyl ethyl alcohol (10.26%), phenyl ethyl acetate (9.89%), octen-3-ol (9.56%), methyl salicylate (8.01%), benzyl acetate (2.03%), *trans*-pinane (1.06%)

and 2-*E*-methyl cinnamate (1.01%) were considered to be major constituents.

In general, SPME process is based on the partition of analytes between the coating phase, sample headspace and sample. The completed extraction is happened when the analyte concentration reaches to distribution equilibrium between these three phases (Klensporf & Jeleñ, 2005). The choice of the fiber coating is played important role to obtain best trapping. Moreover, Characteristic of the target analytes is affected on the extraction. It was found that different numbers and contents of volatile components of *C. spinosum* flowers were related to composites on each fiber. Most monoterpenic components were extracted by CAR/PDMS and DVB/CAR/PDMS fibers compared to those extracted by PDMS fiber. In contrast, PDMS fiber presented higher number of sesquiterpenes than those obtained by other fibers. Fiber containing more than one composite provided higher number of volatiles as can be found on CAR/PDMS and DVB/CAR/PDMS fibers. DVB/CAR/PDMS was suitable for trapping the volatile components of *C. spinosum* flowers as compared to other fibers. The DVB/CAR/PDMS coated-fiber was improved to extract successfully volatile components within a broad range of chemical characteristics and volatility. PDMS is a non-polar coating well which is suitable for extraction of non-polar analytes such as many volatile flavor compounds (Kataoka, Sumida, Nishihata, & Makita, 1995). In general, a thick film fiber is used for volatile compounds while semi-volatile compounds are extracted completely by a thin film fiber (Hinshaw, 2003). In addition, Ruoff (2003) reported that various classes of compounds in the complex samples are extracted greater when using fiber consisting of different composites compared to single composite coatings fiber. Shirey (1999) also reported that mixed phase coatings fiber contain complementary properties compared to single phase films which is enable the absorption of a broad range of analytes with different chemical characteristics. The volatile compound including methyl benzoate, 2-phenyl ethyl acetate, phenyl ethyl alcohol, octen-3-ol and methyl salicylate were considered to be the key constituents of *C. spinosum* flowers. It is noted that the solid phase microextraction technique is more sensitive to extract the highly volatile components which played the significant role as the key scent in *C. spinosum* flower.

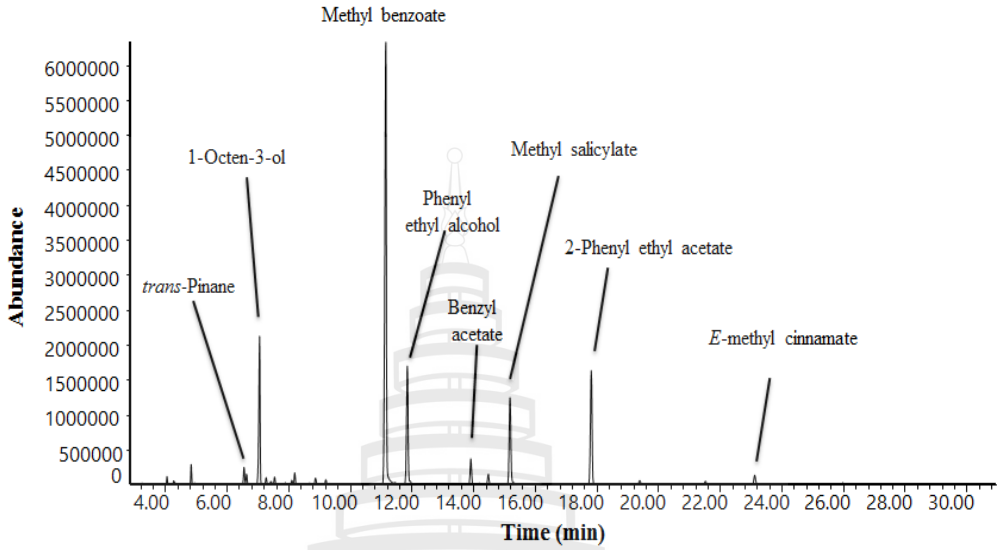


Figure 4.17 Chromatogram of Fresh *C.spinosum* flowers by SPM-GC-MS Technique (DVB/CAR/PDMS fiber Assembly)

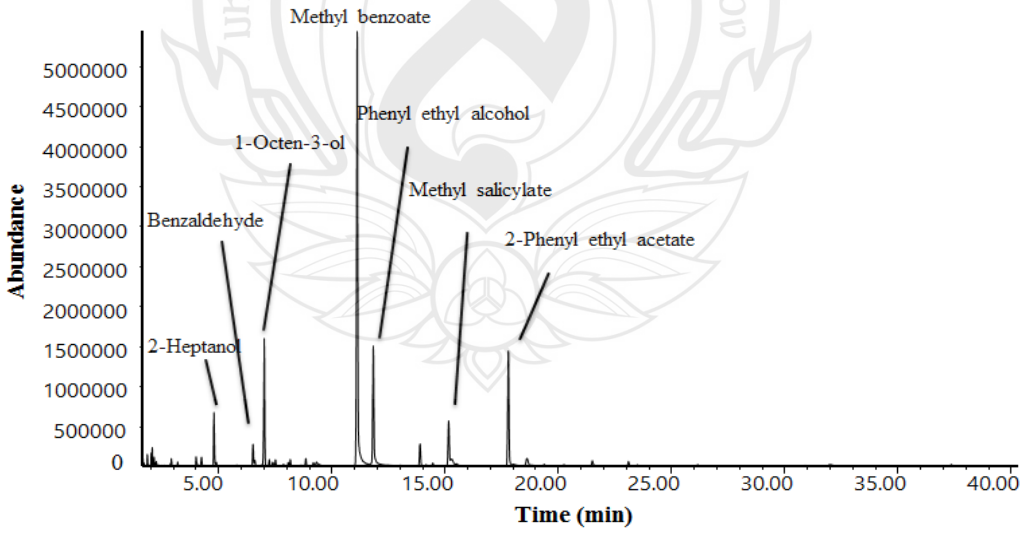


Figure 4.18 Chromatogram of Fresh *C. spinosum* flowers by SPME-GC-MS Technique (CAR/PDMS fiber Assembly)

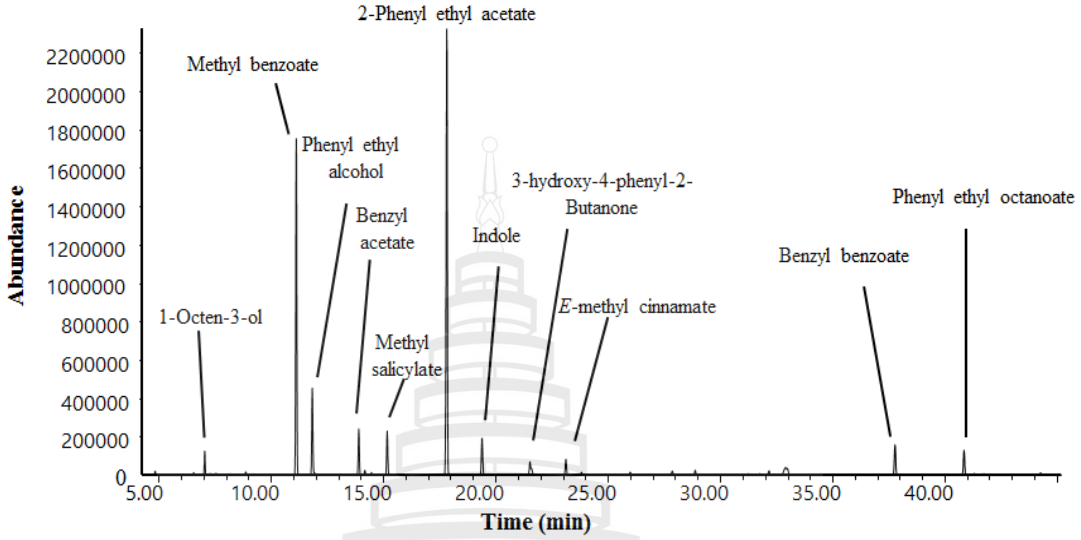


Figure 4.19 Chromatogram of Fresh *C. spinosum* flowers by SPME-GC-MS Technique (PDMS fiber Assembly)

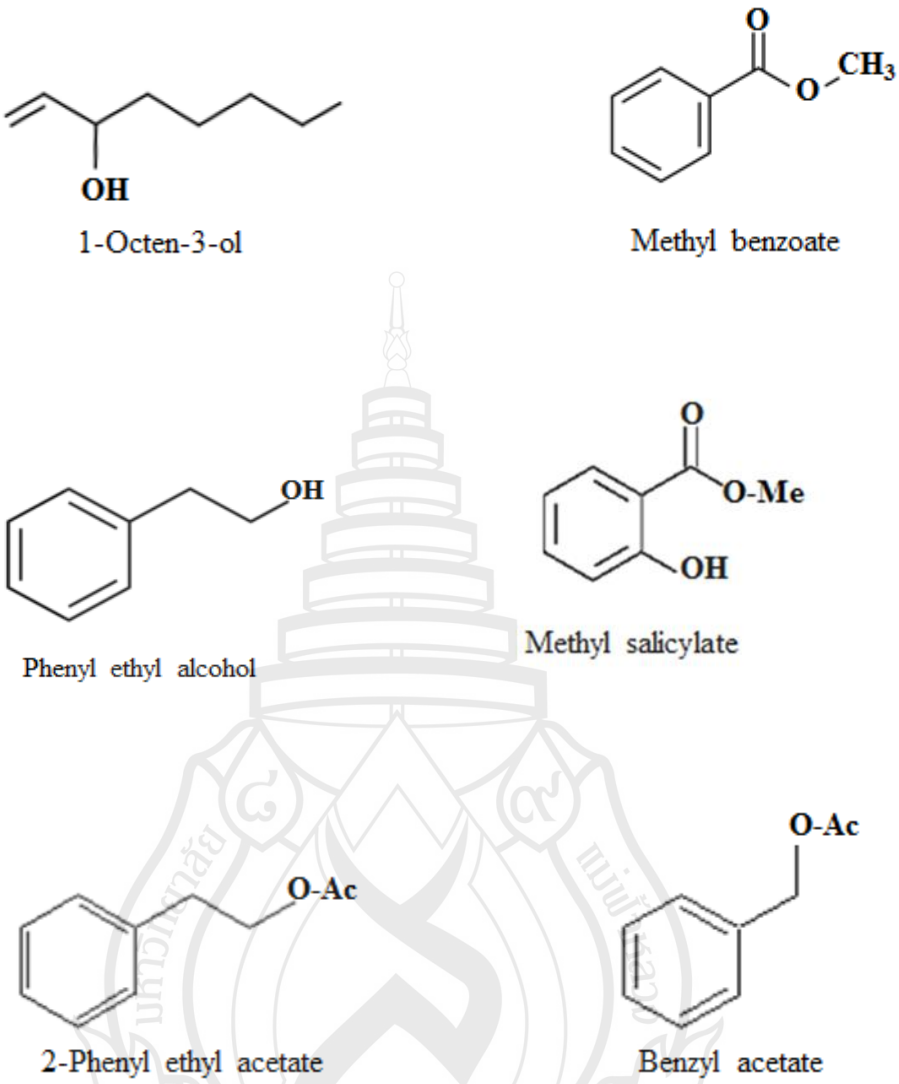


Figure 4.20 The Chemical Structure of Major Volatile Compounds of Fresh *C. spinosum* Flowers by SPME-GC-MS Technique (PDMS, CAR/PDMS, and DVB/CAR/PDMS fiber Assembly)

Table 4.5 Volatile Flavor Compounds in *C. spinosum* Extracted by SPME-GC-MS with DVB/CAR/PDMS, CAR/PDMS, and PDMS fiber

No.	Compound	KI[a]	Extract		
			A[b]	B[c]	C[d]
1	Hexanal	801	-	0.2	-
2	2*E*-hexenal	855	0.44	0.59	-
3	Methyl tiglate	870	0.30	-	-
4	Isopropyl-2-methyl butyrate	885	-	0.59	-
5	3-Mehylthio-Propanal	890	0.95	-	0.19
6	2-Heptanol	896	0.97	2.54	
7	Tricyclene	926	0.09	-	-
8	*trans*-Pinane	960	1.06	-	-
9	Benzaldehyde	975	0.69	1.22	0.16
10	1-Octen-3-ol	979	9.56	6.89	1.31
11	3-Octanone	983	0.48	0.44	-
12	2-Pentyl furan	988	-	0.36	-
13	Furfuryl acetate	990	0.32	-	-
14	3-Octanol	991	-	0.43	-
15	3*E*-Hexenyl acetate	1002	-	0.18	-
16	dehydroxy-*cis*-Linalool oxide	1005	0.17	-	-
17	Hexyl acetate	1006	0.31	0.26	-
18	2*E*,4*E*-Heptadienal	1007	0.78	0.45	-
19	Limonene	1029	0.14	-	-
20	Benzyl alcohol	1031	0.52	0.57	0.25
21	Z-β-Ocimene	1037	0.12	-	-
22	Benzene acetaldehyde	1042	0.39	1.02	-
23	Methyl benzoate	1090	36.15	33.31	21.67
24	Phenyl ethyl alcohol	1107	10.26	10.42	5.95
25	Benzyl acetate	1162	2.03	1.83	3.01
26	Ethyl benzoate	1173	0.20	-	0.38
27	Benzene acetic acid,methyl ester	1178	0.95	0.54	0.27
28	Methyl salicylate	1191	8.01	3.45	3.06
29	3*Z*-Hexenyl 2-methyl butanoate	1232	0.15	-	-

Table 4.5 (continued)

No.	Compound	KI[a]	Extract		
			A[b]	B[c]	C[d]
30	2*Z*-Hexenyl isovalerate	1244	0.14	-	-
31	2-Phenyl ethyl acetate	1258	9.89	8.72	31.76
32	*E*-Cinnamaldehyde	1270	-	1.69	-
33	Indole	1291	0.49	0.42	3.00
34	*Z*-Methyl cinnamate	1299	-	0.53	-
35	Methyl-Anthranilate	1337	0.48	-	-
36	3-hydroxy-4-phenyl-2-Butanone	1342	-	0.89	1.73
37	Benzyl butanoate	1346	-	-	1.73
38	*E*-methyl cinnamate	1378	1.01	0.69	1.27
39	β-Panasinsene	1382	-	0.36	-
40	Phenyl ethyl isobutanoate	1393	-	-	0.34
41	Geranyl acetone	1455	0.23	0.43	0.36
42	Phenyl ethyl 2-methylbutanoate	1487	-	0.22	-
43	Benzyl tiglate	1497	-	-	0.66
44	*cis*-Calamenene	1529	0.15	-	0.51
45	*n*-Hexyl benzoate	1580	-	0.27	-
46	Dodecyl acetate	1607	0.18	0.75	0.46
47	2,7*Z*-Bisaboladien-4-ol	1619	0.22	-	-
48	Gymnomitrol	1660	-	0.40	-
49	Sesquicineol-2-one	1702	-	0.20	-
50	Benzyl benzoate	1756	0.04	0.20	2.55
51	*E*-Nuciferol	1760	0.33	-	-
52	Phenyl ethyl octanoate	1847	0.47	0.30	2.16
	Number of Components		**36**	**34**	**22**
	Sum of %		**88.67**	**81.5**	**82.78**

Note. [a]Kovát retention indices on DB-5 column, [b]DVB/CAR/PDMS fiber, [c]CAR/PDMS fiber, [d]PDMS fiber

4.2.2 The Identification of Chemical Composition of Fresh *O. fragrans* Flowers by Using SPME-GC-MS Technique

In this study different volatile compounds were analysed by the three different SPME fibers; PDMS, CAR/PDMS, and DVB/CAR/PDMS. These three commercially available SPME fibers, with single and two or three different coating polymers were compared for assessing their concentration capacity**.** The fingerprints of volatile components of *O. fragrans* flowers with different fibers are shown in Figure 4.21-4.23. A total of 79 volatile compounds were identified, representing more than 95% of the total extract, in the fresh *O. fragrans* flowers by three different types of fibers (see Table 4.6). This includes 57, 37, and 29 compounds by DVB/CAR/PDMS, CAR/PDMS, and PDMS respectively. The key aroma compound structures of all fibers are shown in Figure 24.

Twenty-nine compounds were detected with PDMS fiber. The key odor volatiles were naphathalene (82.08%), *n*-heptadecane (2.52%), *E*-β –ionone (2.16%), and isobazzanene (1.3%) while thirty-seven constituents were found when using CAR/PDMS fiber with the major compounds of isobazzanene (19.78%), n-octane (10.52%), 3*Z*-hexenol (9.64%), 3-3-methyl-3-butenyl-methyl butanoate (8.49%), *E*-β-ocimene (7.67%), *E*-β-ionone (6.42%), γ-delactone (5.52%). The DVB/CAR/PDMS fiber extracted much more volatiles, and fifty-nine relative intense peaks were observed in the chromatogram. The major volatiles were isobazzanene (16.92%), *E*-β-ionone (15.01%), γ-decalactone (11.72%), undecane (8.13%), *E*-β-ocimene (7.23%), *cis*-linalool oxide (4.36%), 3- 3-methyl-3-butenyl- methyl butanoate (3.54%), and *trans*-linalool oxide (3.22%).

The absorption efficiency of each fiber was determined on the basis of two main factors, the total MS-detector peak area and the number of identified compounds (Soto, Maldonado, Jofré, Galmarini, & Silva, 2015). DVB/CAR/PDMS and CAR/PDMS fibers were more extracted most monoterpenic components than PDMS fiber. Most sesquiterpenic compounds were extracted by PDMS fibers compared to those extracted by DVB/CAR/PDMS and CAR/PDMS fibers. Interestingly, PDMS gave a better yield in terms of volatile constituents such as naphthalene when it came to extract volatile compounds from *O. fragrans*. The result is agreement with Kataoka's, who have proposed since 2000 that non-polar polydimethylsiloxane

(PDMS) fiber is preferred for the extraction of non-polar analytes such as very volatile flavor compounds. The volatile components of CAR/PDMS fiber were dominated by monoterpenes, with sesquiterpenes constituting < 37%, the principal contributors being variously *n*-octane, 3*Z*-hexenol, limonene, *E*-β –ocimene, *cis*-linalool oxide, *trans*-linalool oxide, *n*-nonanal, and 3-3-methyl-3-butenyl-methyl butanoate. The major sesquiterpenes were *E*-α-ionone, isobazzanene, bakerol, γ-decalactone, and *E*-β-ionone. Works by Bichhici.et al (2000) have shown that more than one coating of SPME fibers were the most effective for studying medicinal and aromatic plants. In this study, the DVB/CAR/PDMS fiber permitted the identification of a larger number of constituents of the sample flower than CAR/PDMS and PDMS fibers (see Table 1). Besides, the main ingredients of *O. fragrans* are ionone, alcohol, fatty acid and their esters which determine the quality of *O. fragrance* (Li & Huang, 2011). γ-decalactone was also found in major amount in DVCB/CAR/PDMS and CAR/PDMS fibers but minor amount in PDMS fiber. This compound is reported to be responsible for the coconut flavor (Guyot-Declerck, François, Ritter, Govaerts, & Collin, 2005; Tucker, Maciarello, & Alkire, 2001). In contract, the fiber coating composed of three different polymers, proved to extract successfully volatile components with broad range of chemical characteristics and volatility.

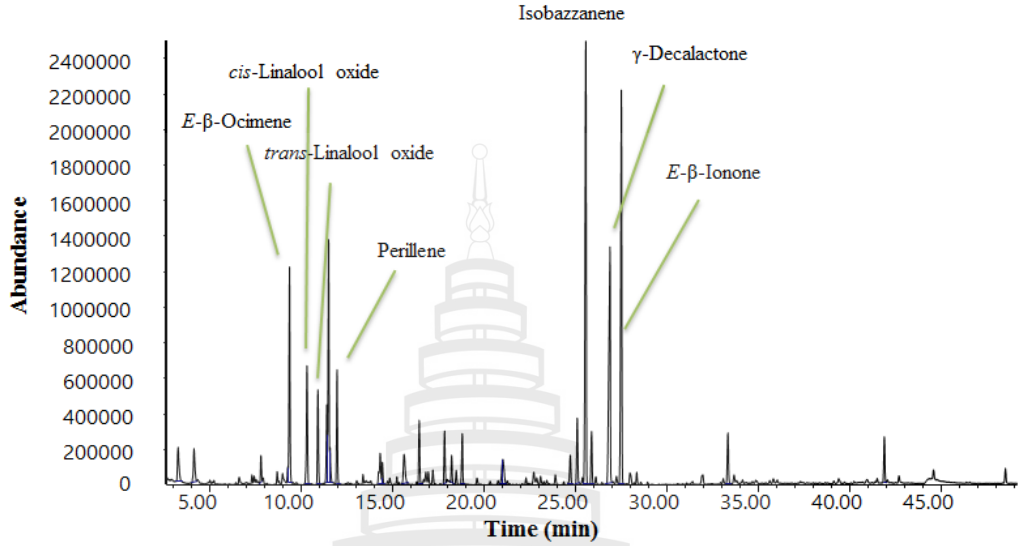


Figure 4.21 Chromatogram of Fresh *O. fragrans* flowers by SPME-GC-MS Technique (DVB/CAR/PDMS fiber Assembly)

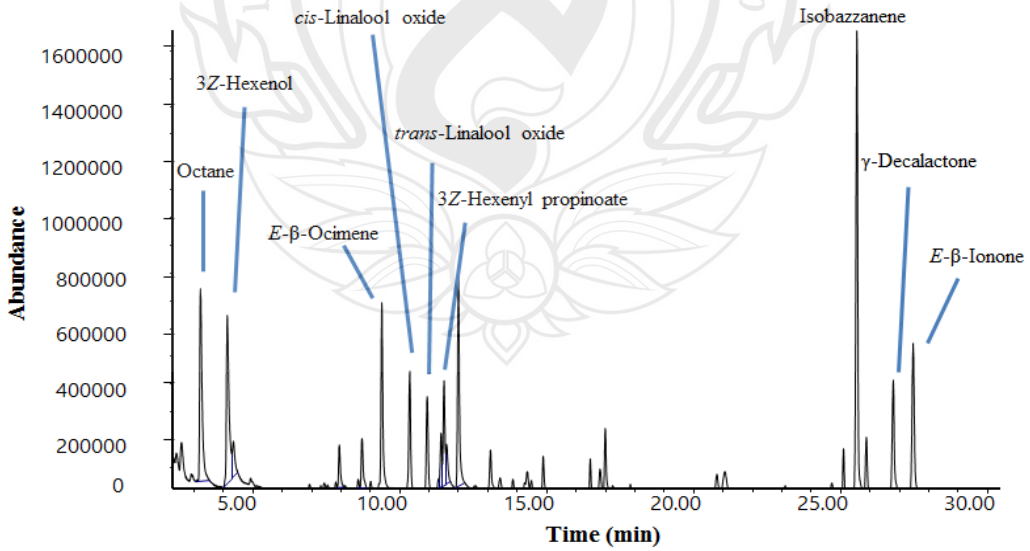


Figure 4.22 Chromatogram of Fresh *O. fragrans* flowers by SPME-GC-MS Technique (CAR/PDMS fiber Assembly)

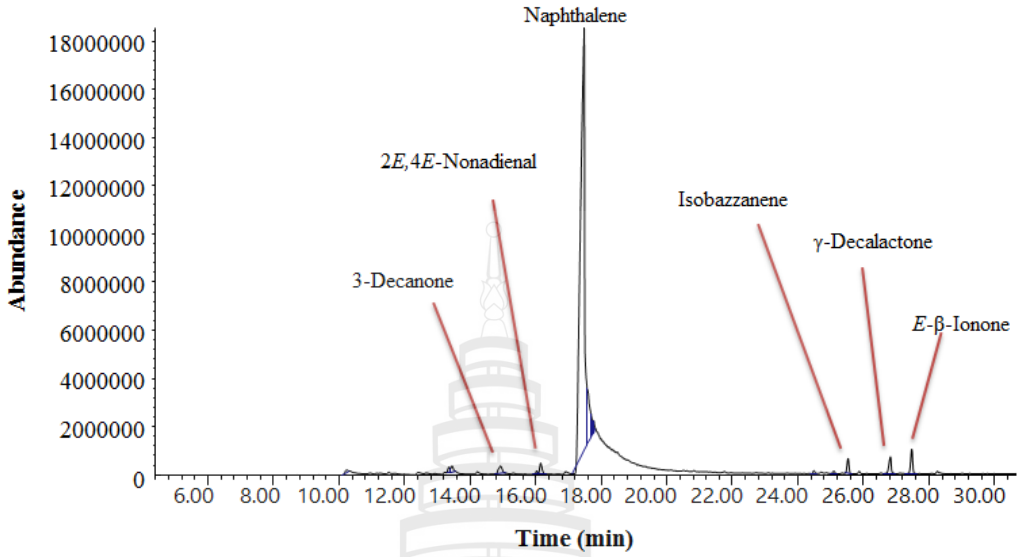


Figure 4.2 Chromatogram of Fresh *O. fragrans* flowers by SPME-GC- MS Technique (PDMS fiber Assembly)

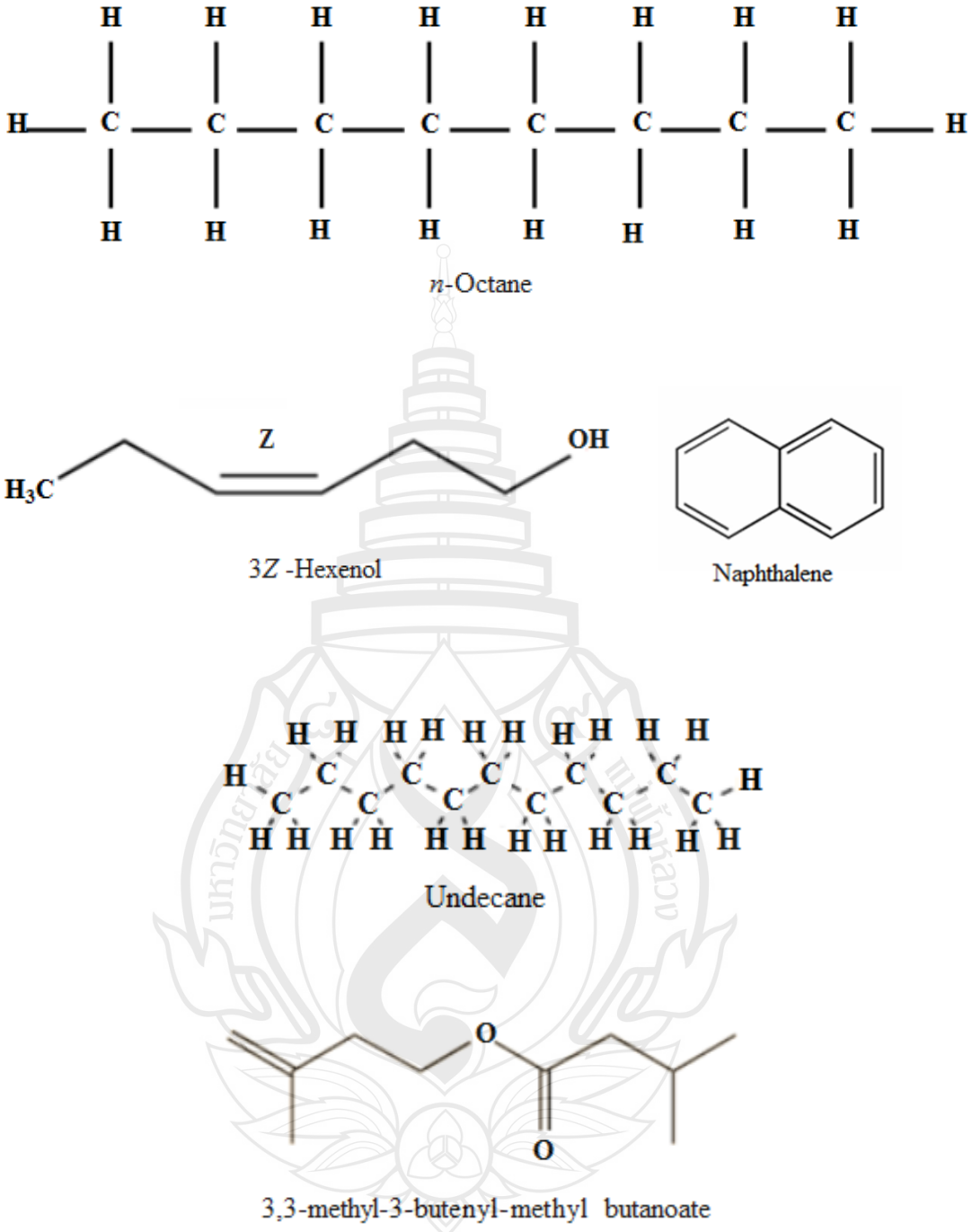


Figure 4.24 The Chemical Structure of Major Volatile Compounds of Fresh *O. frgrans* Flowers by SPME-GC-MS Technique (PDMS, CAR/PDMS, and DVB/CAR/PDMS fiber Assembly)

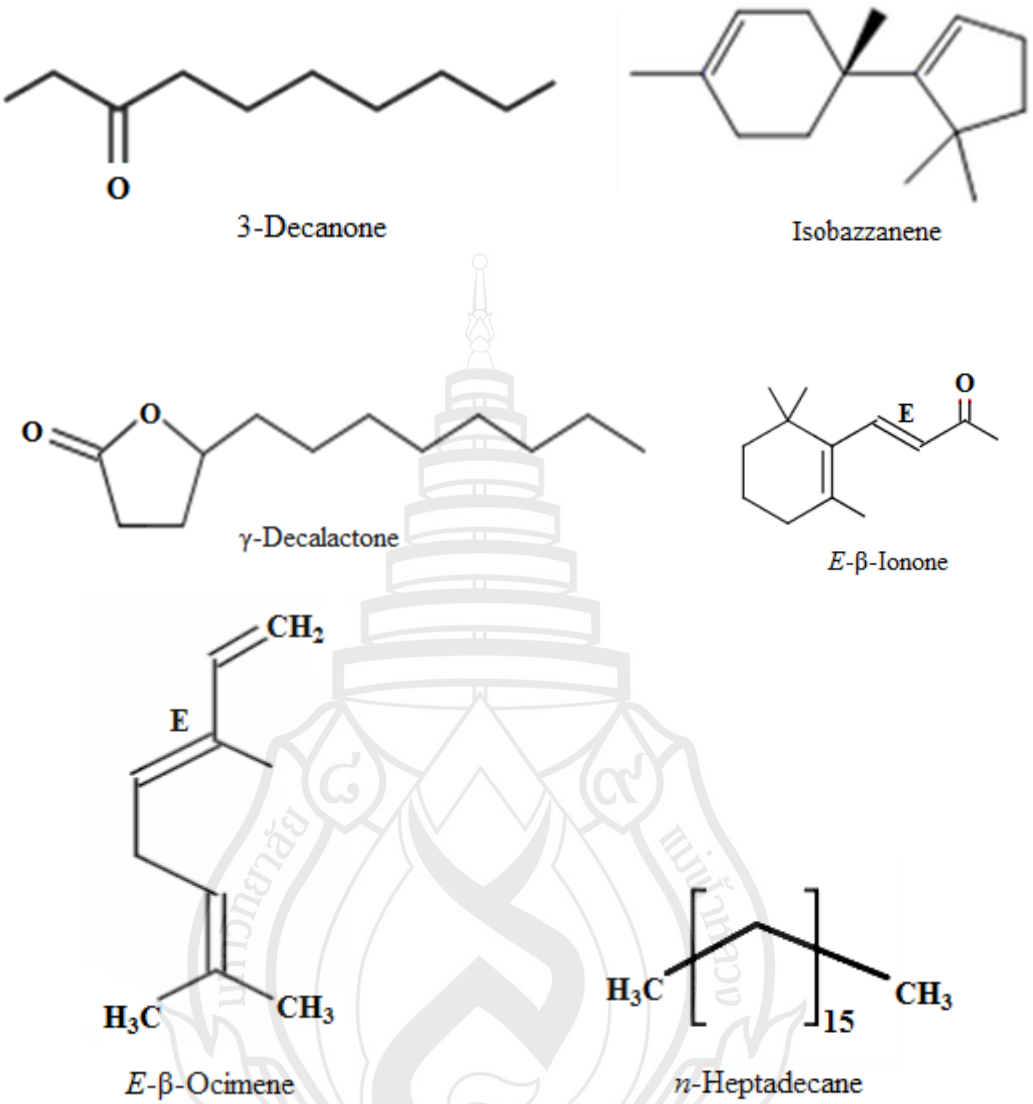


Figure 4.24 The Chemical Structure of Major Volatile Compounds of Fresh *O. frgrans* Flowers by SPME-GC-MS Technique (PDMS, CAR/PDMS, and DVB/CAR/PDMS fiber Assembly) (Continued)

Table 4.6 Organic Volatile Flavor Compounds in *O. fragrans* Extracted by SPME GC-MS with DVB/CAR/PDMS, CAR/PDMS, and PDMS fiber

No.	Compound	KI[a]	Extract		
			A[b]	B[c]	C[d]
1	Octane	800	-	10.42	-
2	4*E*-Octene	802	1.79	-	-
3	3*Z*-Hexenol	859	1.69	9.64	-
4	Heptanal	902	0.85	-	-
5	Benzaldehyde	960	t	-	t
6	Sabinene	975	-	t	-
7	6-Methyl-5-hepten-2-one	985	t	t	-
8	2-Pentyl furan	988	t	t	-
9	6-Methyl-5-hepten-2-ol	991	-	t	-
10	Octanal	998	t	t	-
11	3*Z*-Hexenyl acetate	1005	-	1.64	-
12	ρ-Cymene	1024	-	t	-
13	Limonene	1029	t	2.12	t
14	Sylvestrene	1030	t	-	-
15	*E*-β-Ocimene	1050	7.23	7.67	t
16	*cis*-Linalool oxide(furanoid)	1072	4.36	4.44	-
17	2-methoxyethyl-Benzene	1080	-	-	0.23
18	*trans*-Linalool oxide(furanoid)	1086	3.22	3.14	-
19	Linalool	1096	2.55	1.96	-
20	Undecane	1100	8.13	3.89	t
21	3- 3-methyl-3-butenyl- Methyl butanoate	1114	3.54	8.49	
22	dehydro-Sabina ketone	1120	-	-	t
23	3*Z*-Hexenyl isobutanonate	1146	-	1.67	-
24	Camphene hydrate	1149	t	t	-
25	2*E*,4*E*-Hexadienol propanoate	1158	-	-	0.43
26	Nerol oxide	1159	-	-	0.56
27	Isoborneol	1160	-	t	-
28	*cis*-Linalool oxide (pyranoid)	1174	t	-	-

Table 4.6 (continued)

No.	Compound	KI[a]	Extract		
			A[b]	B[c]	C[d]
29	*cis*-Pinocamphone	1175	-	t	-
30	*trans*-Linalool oxide (pyranoid)	1176	0.76	t	t
31	*iso*-Menthol	1182	-	-	t
32	Thuj-3-en-10-al	1184	1.6	-	-
33	3*Z*-Hexenyl butanoate	1186	t	1.37	-
34	3-Decanone	1187	-	-	1.14
35	*trans*-4-Caranone	1196	t	-	-
36	Safranal	1196	-	-	0.26
37	Camphor	1219	1.81	-	1.11
38	2*E*,4*E*-Nonadienol	1219	2.15	1.29	-
39	Citronellol	1225	t	-	-
40	3*Z*-Hexenyl, 2-methyl butanoate	1232	-	t	-
41	Pulegone	1233	-	-	t
42	3*Z*-Hexenyl, 3-methyl butanoate	1235	-	2.52	-
43	2*E*-Hexyl butanoate	1242	t	-	-
44	Hexyl isovalerate	1244	-	t	-
45	Geraniol	1252	1.77	t	-
46	γ-Octalactone	1254	-	t	-
47	Naphthalene	1267	-	-	82.08
48	Geranial	1268	t	-	-
49	Hydroxy citronellal	1288	0.92	-	-
50	2-pentyl-Cyclopent-2-en-1-one	1289	1.76	-	-
51	Sesamol	1312	t	-	-
52	Anisyl formate	1332	1.07	-	-
53	α-Longipinene	1352	0.85	t	-
54	Silphiperfol-4,7(14)-diene	1360	t	-	-
55	Anisyl methyl ketone	1382	t	-	-
56	β-Cedrene	1420	-	t	-
57	β-Ylangene	1420	1.22	-	0.23

Table 4.6 (continued)

No.	Compound	[a]KI	Extract		
			A[b]	**B**[c]	**C**[d]
58	*E*-α-Ionone	1430	2.37	1.85	0.23
59	Neryl acetone	1436	t	-	-
60	Isobazzanene	1438	16.92	19.78	1.30
61	Bakerol	1446	1.92	2.30	t
62	Geranyl acetone	1455	t	-	-
63	γ-Decalactone	1466	11.72	5.52	1.75
64	*E*-β-Ionone	1488	15.01	6.42	2.16
65	*n*-Pentadecane	1500	t	-	-
66	α-Muurolene	1500	t	-	-
67	Decyl propanoate	1501	t	-	t
68	*E*,*E*-α-Farnesene	1505	t	-	-
69	*Z*-γ-Bisabolene	1515	t	-	-
70	7-*epi*-α-Selinene	1522	t	-	-
71	5-oxy-Isobornyl isobutanoate	1603	t	-	t
72	Benzophenone	1627	t	-	t
73	Mustakone	1677	2.06	-	0.67
74	α-Cadinol	1654	t	-	t
75	Valerianol	1658	t	t	t
76	Gymnomitrol	1660	t	-	t
77	*n*-Heptadecane	1700	t	-	2.52
78	10-nor-Calamenen-10-one	1702	t	-	-
79	8*S*,14-Cedranediol	1889	1.64	-	-
	Number of component		**57**	**37**	**29**
	Sum of %		**98.91**	**96.40**	**92.15**

Note. [a]Kovát retention indices on DB-5 column, [b]DVB/CAR/PDMS fiber, [c]CAR/PDMS fiber, [d]PDMS fiber, t-trace amount <0.1%

4.3 Biological Activities of *C. spinosum* and *O. fragrans*

Screening plants oils and extracts for their antimicrobial activities was conducted using the paper disc diffusion assay. The inhibition zone of bacterial (including the diameter of paper disc) were measured in mm after incubation and were expressed as the mean value +/- the standard deviation (± SD).

4.3.1 Antibacterial activities of essential oil and extracts of *C. spinosum*

The antibacterial activities of the essential oil and all extracts were evaluated by the diameter of inhibition and MIC values compared with those obtained from positive control, tetracycline and penicillin. The antibacterial activities of all samples and drugs on Gram-negative and Gram-positive bacteria are summarized in Figure 4.25 and 4.26, while MIC values of all samples are shown in Table 4.7. The essential oil and the various extracts provided various efficiencies of antibacterial activity depending on the tested bacterial strains. The essential oil had the greatest antibacterial activity against all Gram-negative bacteria as compared with the other extracts. The essential oil had the largest inhibition zones against *E. coli*, *Ps. aeruginosa*, *S. typhi,* and *En. aerogenes* measured at 14.8, 13.9, 12.1, and 8.0 mm, respectively (see Figure 4.15 and 4.16). The ethanol extract showed higher antibacterial activity than the dichloromethane extract while no inhibition was obtained with the hexane extract. Among all Gram-negative bacteria, *En. aerogenes* was the least sensitive strain to the essential oil and all solvent extracts while *E. coli* revealed the modest sensitivity. These results are confirmed by the MIC values in Table 4.8. All Gram-negative bacteria were inhibited by the essential oil and ethanol extract at the lowest MIC value of 31.2 µg/mL, while the dichloromethane extract presented higher MIC values ranging from 31.2 to 500 µg/mL (see Table 4.7). It is interesting to note that the essential oil exhibited the strongest inhibitory effects against all Gram-positive bacterial strains. An increased inhibition zone of the essential oil was observed with *B. subtilis*, followed by *B. cereus*, *S. aureus*, and *M. luteus*, measured at 18.3, 15.9, 15.5, and 14.9 mm, respectively. Similar inhibition zone on *B. cereus* and *M. luteus* strains was detected in the dichloromethane and ethanol extracts. The hexane extract inhibited only two

Gram-positive bacteria, *S. aureus* and *B. subtilis*. As a result, *S. aureus* was more sensitive to the essential oil and various extracts followed by *B. subtilis*, *B. cereus*, and *M. luteus*, respectively. The lowest MIC value (31.2 µg/mL) was observed for the essential oil, dichloromethane, and ethanol extracts, while the hexane extract showed higher MIC values at concentrations of 500 and 1000 µg/mL for *S. aureus* and *B. subtilis*, respectively. The essential oil showed antibacterial activity which was more selective for Gram-positive bacterial strains than Gram-negative bacterial strains.

Normally, Gram-positive bacteria are more sensitive to the essential oil than Gram-negative bacteria, which contain lipopolysaccharides in their external walls which limit access to compositions of the essential oil (Burt, 2004). This is the first report on the antibacterial activities of *C. spinosum* flowers in which their antibacterial activities may be attributed to the major component, maltol (Aoyagi, Kimura & Murata, 1974). Another major compound, piperitone, also has antibacterial properties, as reported by Shahverdi and co-workers (2004). The antibacterial properties of *C. spinosum* flowers resulted from various oxygenated monoterpene compounds such as camphor, citronellyl formate, ethyl cinnamate, methyl eugenol, methyl isoeugenol, and isoeugenol. These components were identified as possessing antibacterial activity in previous reports. It is noted that the antibacterial activities of *C. spinosum* flowers were related significantly to qualitative and quantitative variations in chemical compositions seen in each extraction method.

In summary, *C. spinosum* flowers were studied as part of a report on their chemical compositions and antibacterial and antioxidant activities. The essential oil possesses important antibacterial potential, especially against Gram-positive bacteria. *C. spinosum* flower oil is rich in maltol, piperitone, and indole, which may play important roles as antibacterial compounds. Other minor constituents, such as camphor, citronellyl formate, ethyl cinnamate, methyl eugenol, and isoeugenol may also have important antibacterial properties. The highest antioxidant activity was detected in the essential oil, when compared with the other extracts. The results of this work indicate that the essential oil of *C. spinosum* flowers may be considered an alternative drug for the screening and development of natural bactericides.

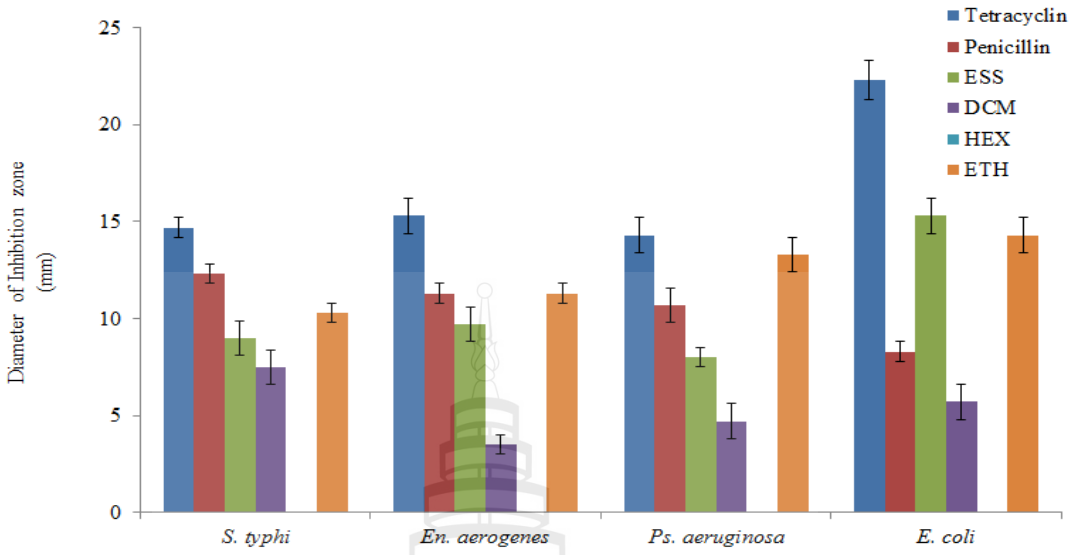


Figure 4.25 Antibacterial Activities of *C. spinosum* Essential Oil and Various Extract and Drugs on Gram-Negative Bacteria

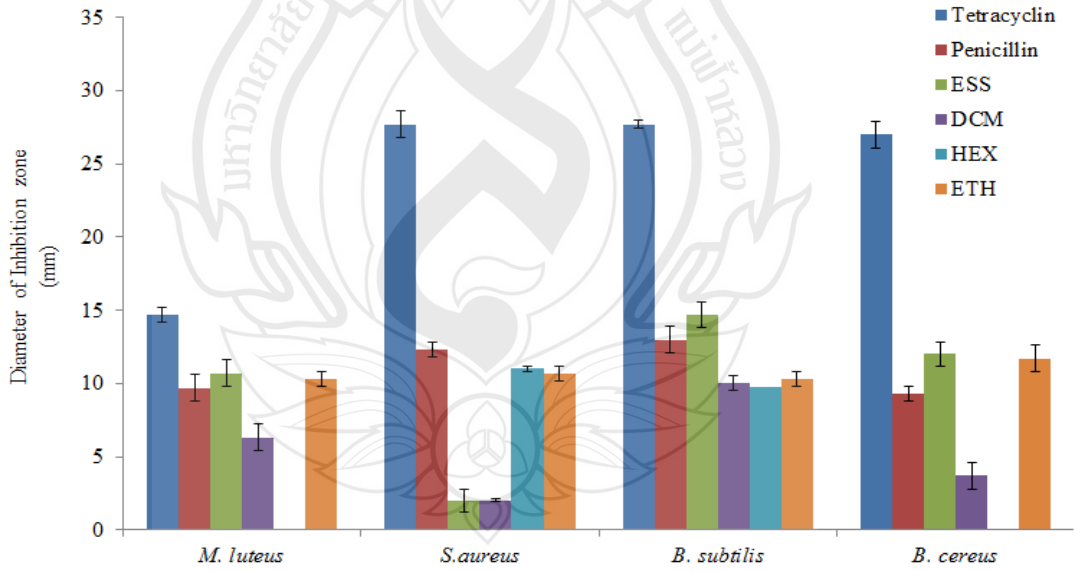


Figure 4.26 Antibacterial Activities of *C. spinosum* Essential Oil and Various Extracts and Drugs on Gram-Positive Bacteria

Table 4.7 MIC (µg/mL) and Zone Diameter (mm ± SD) of Essential Oil and Various Extracts Obtained from *C. spinosum* Flowers

Bacteria	Minimum inhibitory concentration (µg/mL)			
	ESS[a]	**DCM**[b]	**HEX**[c]	**ET**[d]
S. typhi	31.25	31.25	-	31.25
	(6 ± 3.6)	(6.7 ± 0.9)		(3.7 ± 1.3)
En. aerogenes	31.25	62.50	-	31.25
	(3 ± 0.8)	(1.7 ± 0.9)		(3.0 ± 0.8)
Ps. aeruginosa	31.25	31.25	-	31.25
	(2.3 ± 1.7)	(2.0 ± 0.0)		(5.7 ± 1.3)
E. coli	31.25	500	-	31.25
	(4.3± 0.9)	(6.7± 2.5)		(3.7 ± 1.3)
M. luteus	31.25	31.25	-	31.25
	(1.7 ± 0.9)	(7.7 ± 1.7)		(7.0 ± 0.8)
S. aureus	31.25	62.50	500	31.25
	(1.5 ± 0.5)	(2.7 ± 1.3)	(8.5 ± 0.4)	(4.0 ± 0.8)
B. subtilis	31.25	31.25	-	31.25
	(4.0 ± 1.4)	(5.5 ± 0.5)		(4.3 ± 0.5)
B. cereus	31.25	62.50	1000	31.25
	(8.0 ± 1.6)	(1.7 ± 0.5)	(9.8 ± 0.2)	(5.3 ± 1.3)

Note. [a]Essential oil ; [b]Dichloromethane extract ; [c]Hexane extract ; [d]Ethanol extract ; - Antibacterial activity not detected

4.3.2 Microbial Activities of *O. fragrans* Flower Extracts

The antibacterial and antifungal activities of all extracts against all the species tested were evaluated by zone diameter and the minimal inhibitory concentration (MIC) values compared with those obtained from the standard drugs; tetracycline and penicillin as shown in Table 4.9. As the results, the ethanol extracts of *O. fragrans* flowers showed significant antibacterial activity against all bacterial around 10-12 mm diameter except *M. luteus* bacteria *as* compared to other extracts at a concentration of 1000 µg/mL. The greatest antibacterial activity from ethanol extract was detected in *M. luteus* with 20 mm diameter while lowest activity was recorded in *Ps. aeruginosa* at 10 mm diameter. The antibacterial effect of the ethanol extract was higher than those found on penicillin which inhibited two Gram-positive bacteria (*Ps. aeruginosa* and *E. coli*) and one Gram-negative bacteria*; B. cereus*. The dichloromethane extract of *O. fragrans* flowers was evaluated as moderate antibacterial activity against all pathogens. All extracts possessed antibacterial activities and demonstrated MIC effect at 31.25-1000 µg/mL as shown in Table 4.9. The dichloromethane extract showed antibacterial activity with a MIC of 31.25-500 µg/mL. Extract form hexane maceration exhibited inhibitory activity at 31.25-1000 µg/mL with only 4 pathogenic bacteria including *S. typhi*, *Ps. aeruginosa, S. aureus* and *B. cereus* while ethanol extract provided MIC value at 31.25 µg/mL for all bacteria. Tetracycline and penicillin presented an inhibitory effect at 3.91-7.81 and 3.91-15.62 µg/mL, respectively. The ethanol extract showed greater antibacterial activity than all the extracts. It can be observed that the hexane extract provided the lowest bactericidal effect (MIC = 1000 µg/mL) against *S. typhi* and *Ps. aeruginosa.*

The observed difference in antibacterial capacity of *O. fragrans* flower extracts against susceptible bacteria was expected due to variation in volatile components of each extract that previously reported for the this species (Kumar & Ganjewala, 2007; Tsai, Chien, Lee, & Tsai, 2007).

The antifungal activity as percentage of growth inhibition of three extracts of *O. fragrans* flowers are summarized in Table 4.8. All the extracts tested exhibited different degree of antifungal activity against *C. albicans* and *C. utilis*. It was found that ethanol and dichloromethane extracts inhibited all pathogenic fungi while hexane extract couldn't inhibit both strains. As a result, ethanol extract at the

concentration of 1000 µg/mL showed the strongest effects inhibited the growth of *C. albicans* (47.3%) and *C. utilis* (50.0%). The flower extracts obtained by dichloromethane at the concentration of 1000 µg/mL exhibited the moderate antifungal properties against all tested fungi ranging from 26.0% to 32.7%. Hexane extracts were found that no inhibition effect was demonstrated among two yeasts. MICs from antifungal activities of all extracts are present in Table 4.8. As in the case of antifungal activity, all extracts exhibited antifungal potential with MIC of 31.25 µg/mL.

Antifungal potential which was related to growth inhibition assay percentage could be presented as follows: hexane extract<dichloromethane extract<ethanol extract. Thus it can be seen that the antifungal activity was the same as in the case of those antibacterial activity. The most sensitive fungus to the ethanol extract of *O. fragrans* flowers was *C. utilis* with 45.7% of growth inhibition.

The stronger activity of the ethanol extract of *O. fragrans* flower against all the susceptible bacteria and fungi may be due to the presence of a high concentration of α-patchoulene. The antimicrobial properties of α-patchoulene were described previously (Yang, Zhang, Yang, & Yu, 2013). Gamma-decalactone, another major compound, was considered to be bactericide and fungicide that reported by Chambers et al. (2013). Other minor components such as *E*-β-ionone, γ-gurjunene, hinesol acetate, geraniol and *epi*-cubenol were expected to have antibacterial and antifungal properties (Taban, Masoudi, Chalabian, Delnavaz, & Rustaiyan, 2009; Dorman & Deans, 2000; Jojireddy & Jose, 2010; Singh et al., 2013). Some researchers (Jalsenjak, Peljnajk, & Kustrak, 1987; Sivropoulou, Nikolaou, Papanikolaou, Kokkini, & Arsenakis, 1997; Sur, Tuiljipa, & Sur, 1991) reported that great antibacterial and antifungal capacity was resulted by 1,8-cineole. In addition, diversity of major and minor constituents present in the extracts due to the synergistic effects could be affected on the consideration to account for their biological activity (Giweli et al., 2013).

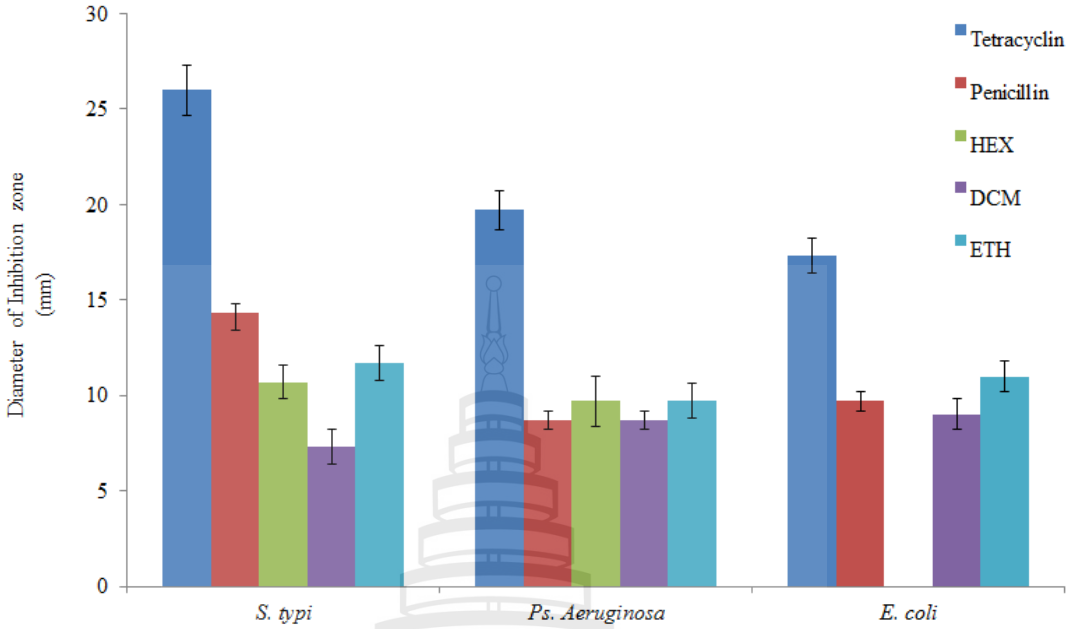


Figure 4.27 Antibacterial Activities of *O. fragrans* Various Extracts and Drugs on Gram-negative Bacteria

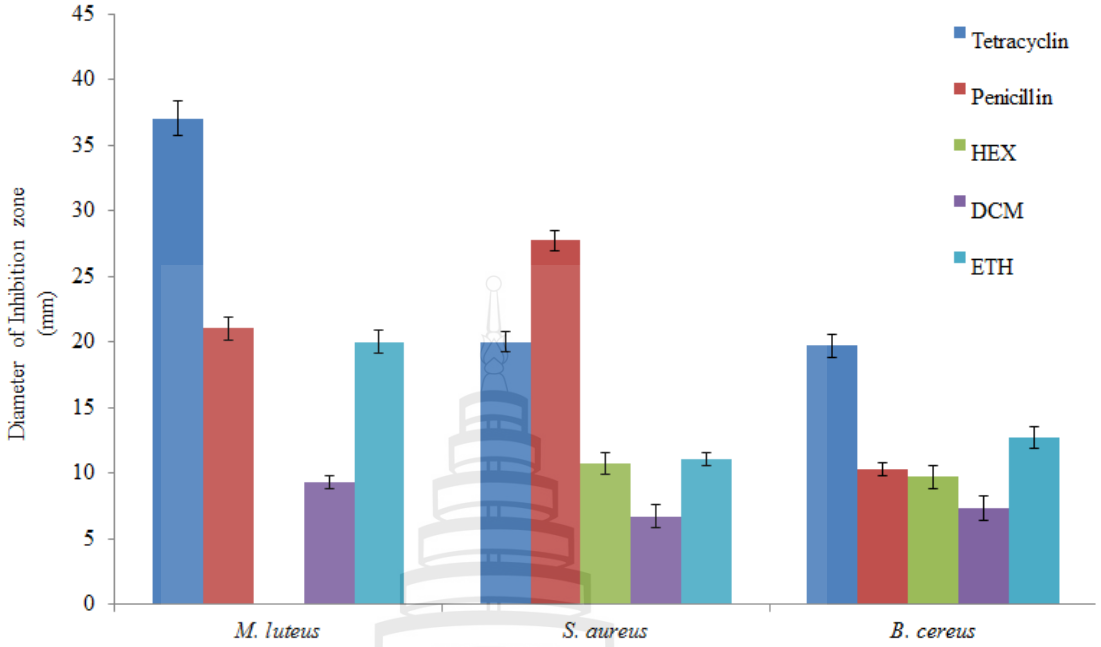


Figure 4.28 Antibacterial Activities of *O. fragrans* Various Extracts and Drugs on Gram-positive Bacteria

Table 4.8 Percentage of Growth Inhibition to Fungal Pathogens by Different Extracts of *O. fragrans* Flowers (10µL corresponding to a concentration of 1000 µg/mL)

Pathogens	**Radical growth inhibition (%)**				
	tetracycline	**penicillin**	**DCM**[a]	**HEX**[b]	**ETH**[c]
C. albicans	-	58.0 ± 4.1	32.7 ± 6.1	-	47.3 ± 7.1
C. utilis	-	59.3 ± 3.2	27.7 ± 3.8	-	50.0 ± 8.2

Note. [a]Dichloromethane extract ; [b]Hexane extract ; [c]Ethanol extract ; - Antifungal activity not detected

Table 4.9 MIC (μg/mL) and Zone Diameter (mm ± SD) of Various Extracts Obtained from *O. fragrans* Flowers

Pathogenes	**Minimum inhibitory concentration (μg/mL)**		
	DCM[a]	**HEX**[b]	**ETH**[c]
S. typhi	31.25	1000	31.25
	(6.0 ± 1.3)	(10.7 ± 0.9)	(10.3 ± 0.5)
Ps. aeruginosa	31.25	10000	31.25
	(7.0 ± 0.8)	(9.7 ± 3.3)	(9.33 ± 0.5)
E. coli	62.50	-	31.25
	(7.7± 0.5)	-	(11.0 ± 0.8)
M. luteus	62.50	-	31.25
	(12.3 ± 2.1)	-	(9.3 ± 0.9)
S. aureus	31.25	500	31.25
	(6.3 ± 0.5)	(10.7 ± 0.9)	(9.7 ± 1.7)
B. cereus	31.25	31.25	31.25
	(7.7 ± 0.9)	(8.7 ± 0.9)	(11.3 ± 0.5)

Note. [a]Dichloromethane extract ; [b]Hexane extract ; [c]Ethanol extract ; - Antibacterial activity not detected

Table 4.10 MIC (μg/mL) and radical growth inhibition (% ± SD) of Various Extracts Obtained from *O. fragrans* Flowers

Pathogens	Minimum inhibitory concentration (μg/mL)		
	DCM[a]	**HEX**[b]	**ET**[c]
C. albicans	31.25	-	31.25
	(16.0 ± 2.8)	-	(41.7 ± 2.4)
C. utilis	31.25	-	31.25
	(27.7 ± 3.8)	-	(45.7 ± 8.0)

Note. [a]Dichloromethane extract ; [b]Hexane extract ; [c]Ethanol extract ; - Antifungal activity not detected

CHAPTER 5

CONCLUSION

5.1 *Citharexylum spinosum* flower

The chemical composition of essential oil and dichloromethane extracts of *C. spinosum* flowers illustrated a similarity in the terpenoids profile, however, those that are responsible for the odor tent to contain two or three isoprene units (monoterpenes and sesquiterpenes). Thus, our results suggested that the flowers oil and dichloromethane were strongly recommended for volatile constituent's extraction from *C. spinosum* flowers. According to the advantage of SPME, DVB/CAR/PDMS was considered as the best fiber among three different fibers for extraction volatile compounds with a broad range of chemical characteristics and volatility. In summary, the highest antimicrobial activity was detected in the essential oil observed with measured at 8.7 mm (lowest MIC 31.25µg/mL), when compared with the other extracts. In addition, dichloromethane and ethanol extracts were found to have marked inhibitory effect on *M. luteus* measured at 6.7 mm, 7 mm clear zone inhibition, respectively (least MIC 31.25 µg/mL). But, the hexane extract showed highly against on *S. aureus* measured at 8.5 mm with the least MIC being 500 µg/mL. The results of this work indicate that the essential oil of *C. spinosum* flowers may be considered an alternative drug for the screening and development of natural bactericides.

5.2 *Osmanthus fragrans* flower

The chemical compounds of flowers oil and extracts can be used to decide the quality of oil and extracts, because the results found that the essential oil and extracts of *O. fragrans* flowers were made up of a complex mixture of monoterpenes, sesquiterpenes, their oxygenated compounds. DVB/CAR/PDMS was considered as the best fiber for extraction volatile compounds with a broad range of chemical characteristics and volatility. Results obtained from disc diffusion method, followed by measurements of MIC, showed that ethanol extract inhibited on *B. cereus* and *C. utilis* (inhibition zone diameter 11.3 mm, 45.7 %) with the lowest MIC value 31.25 µg/mL. Meanwhile, the highest activities (inhibition zone diameter 6.3 mm, 27.7 %) were observed in the dichloromethane extract against the *B. cereus* bacteria and *C. utilis* fungi strain with the least MIC value 31.25 µg/mL. Hexane extract, however, showed activity (inhibition zone diameter 8.7 mm) against only *B. cereus* with the lowest MIC value 31.25 µg/mL. From the point of antimicrobial activity view, the ethanol extract possesses important antibacterial and antifungal potential. The results of this work indicate that the ethanol extract of *O. fragrans* flowers may be considered an alternative drug for the screening and development of natural bactericides and fungicides. Thus, in order to the reported results indicate that *O. fragrans* can be used in herbal formulas without any toxic effects and be used safely in the development of health foods.

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APPENDICES

APPENDIX A

INHIBITION ZONES OF BACTERIA BY ESSENTIAL OIL OF *CITHAREXYLUM SPINOSUM* FLOWERS

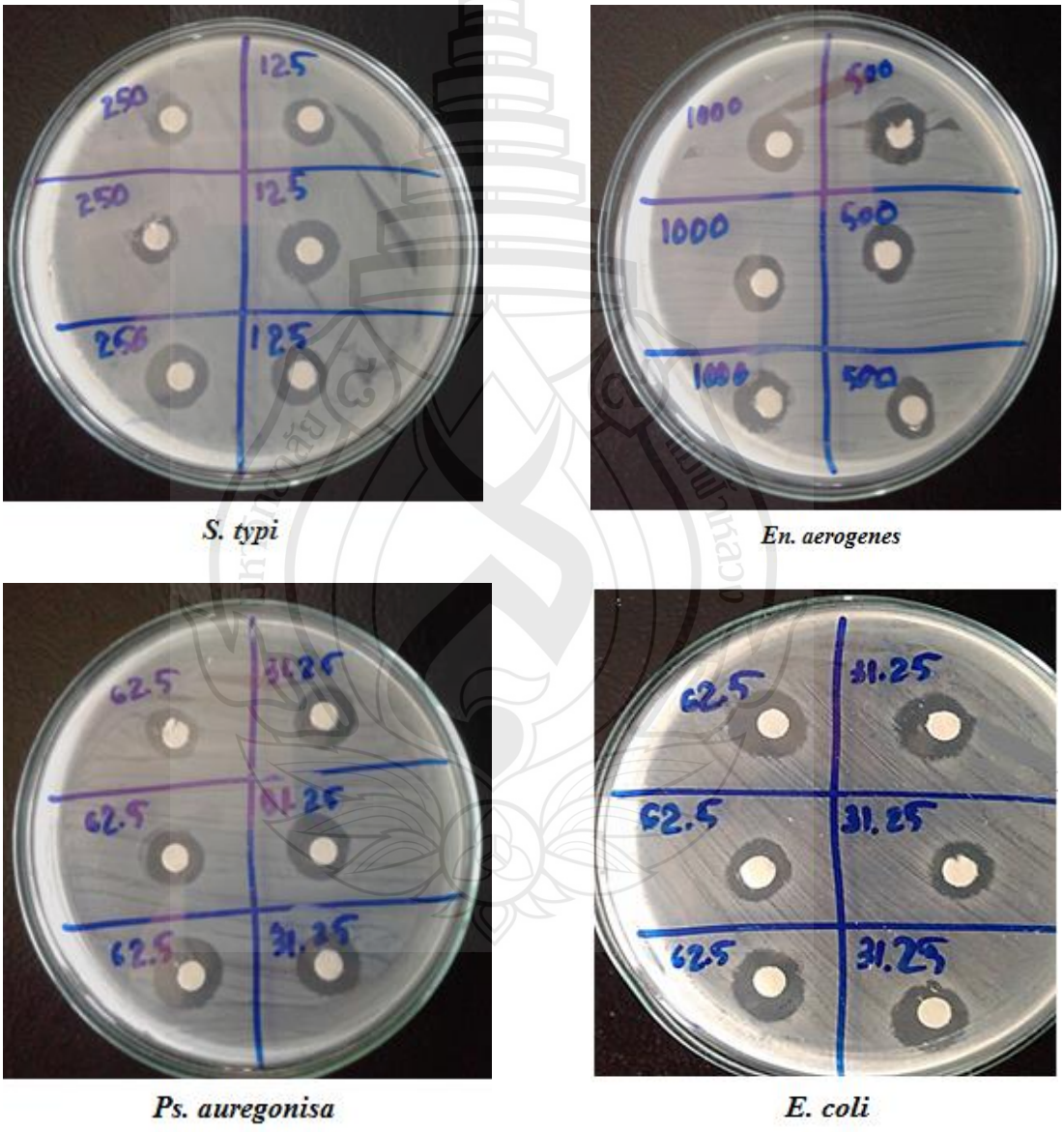
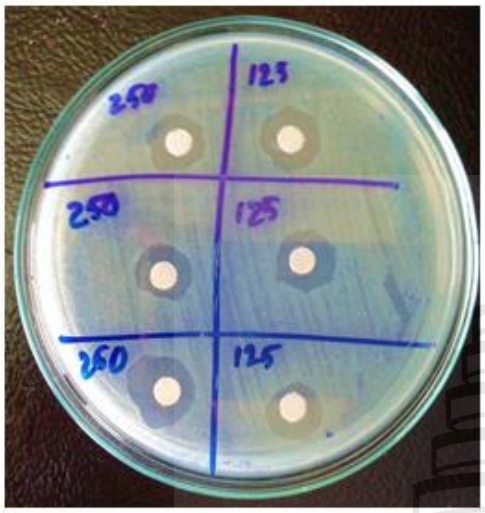
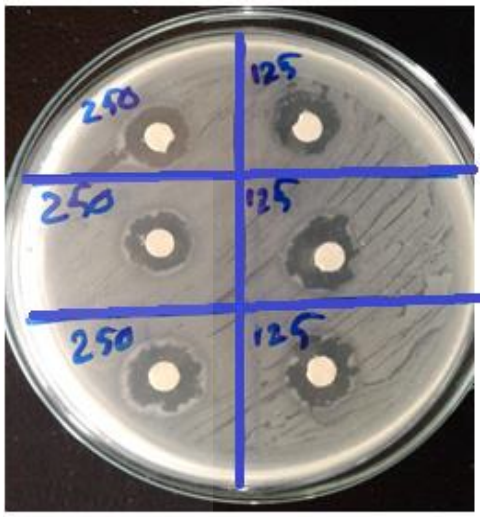


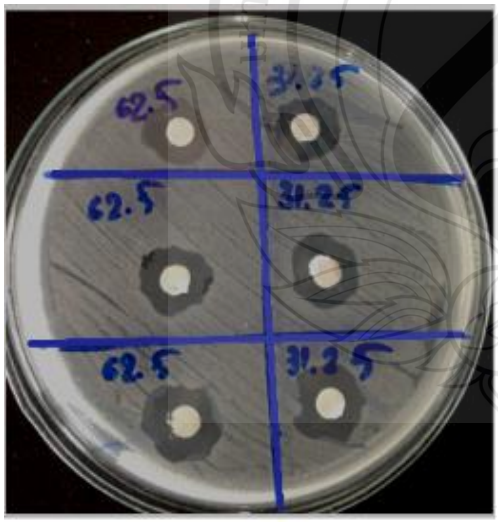
Figure A1 Inhibition Zones of Bacteria by Essential Oil of *Citharexylum Spinosum* Flowers



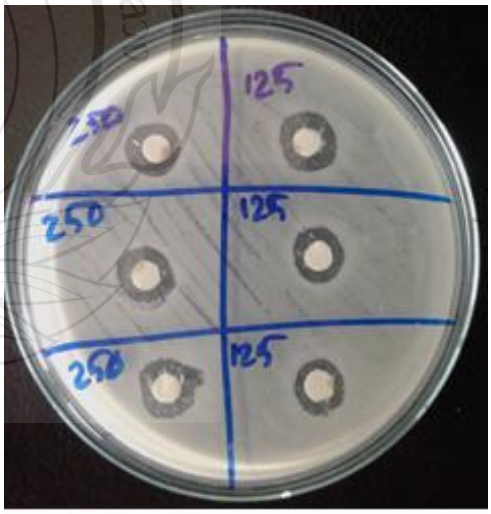
M. luteus



S. aureus



B. subtilis



B. cereus

Figure A1 (continued)

APPENDIX B

INHIBITION ZONES OF BACTERIA BY ETHANOL EXTRACT OF *CITHAREXYLUM SPINOSUM* FLOWERS

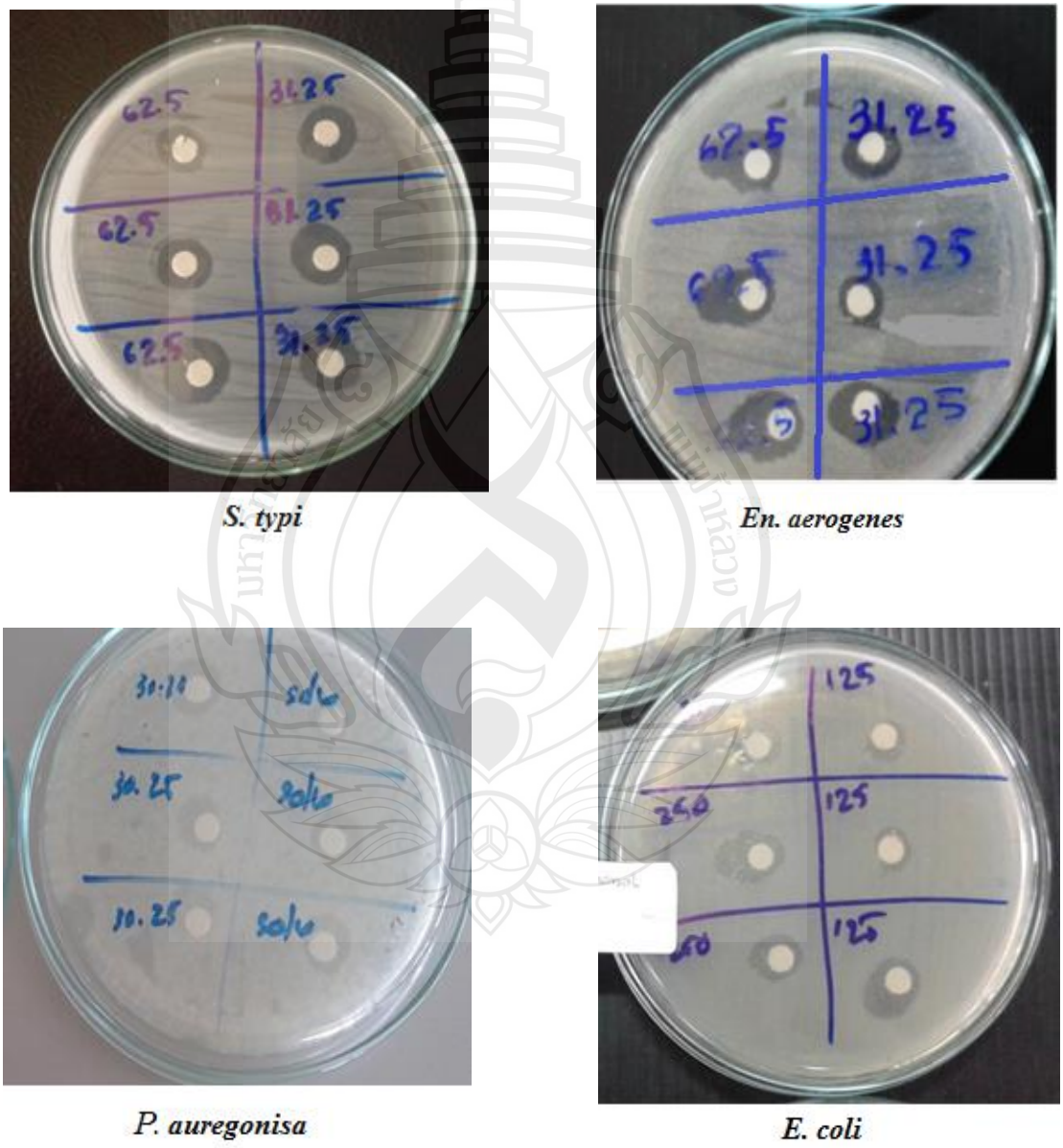
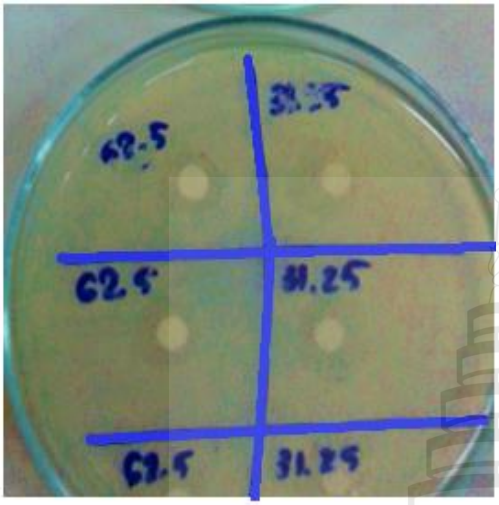
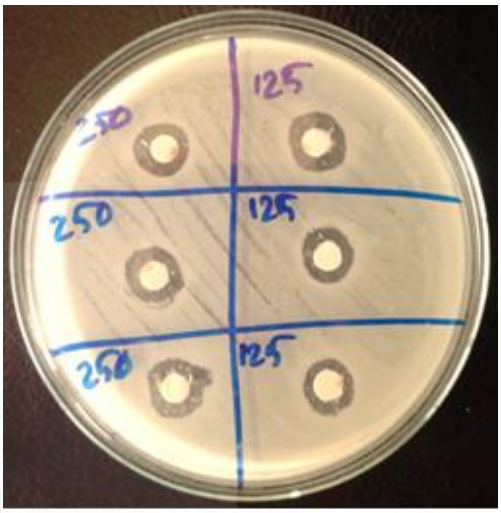


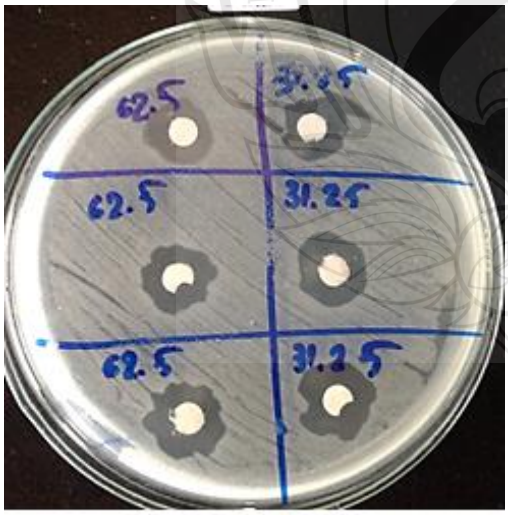
Figure B1 Inhibition Zones of Bacteria by Ethanol Extract of *Citharexylum Spinosum* Flower



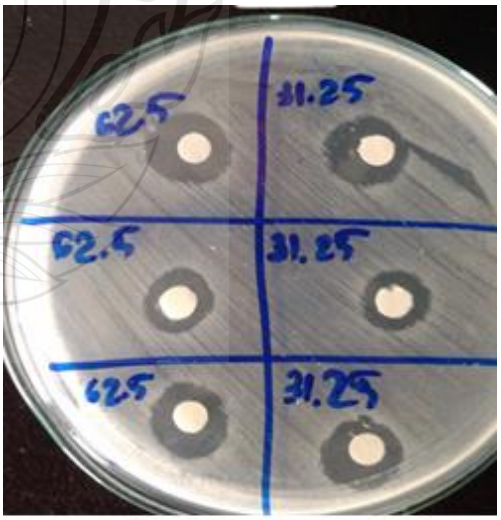
M. luteus



S. aureus



B. subtilis



B. cereus

Figure B1 (continued)

APPENDIX C

INHIBITION ZONES OF BACTERIA BY DICHLOROMETHANE EXTRACT OF *CITHAREXYLUM SPINOSUM* FLOWERS

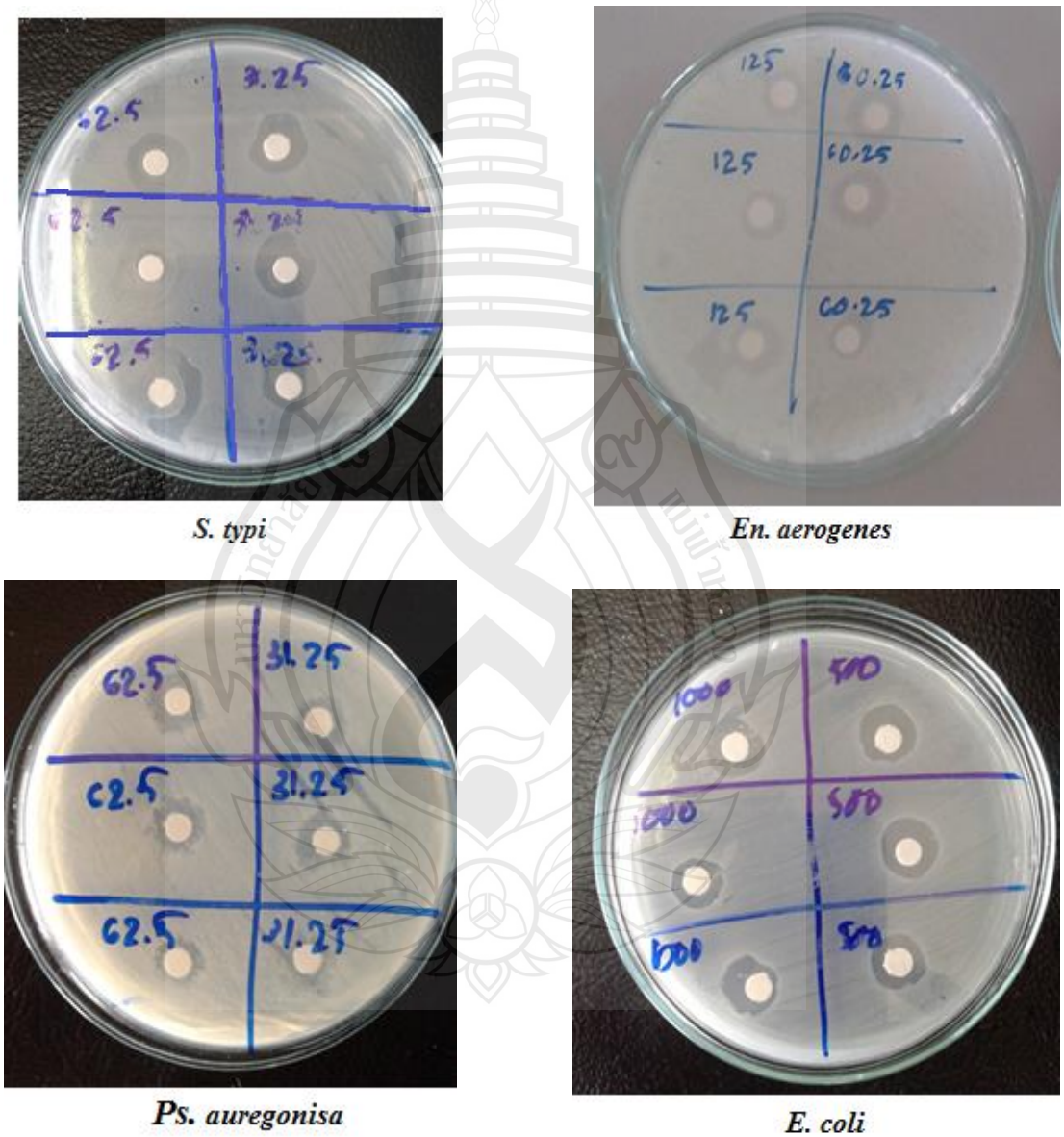
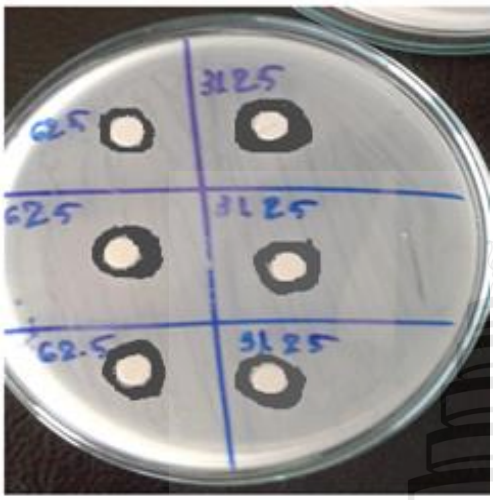
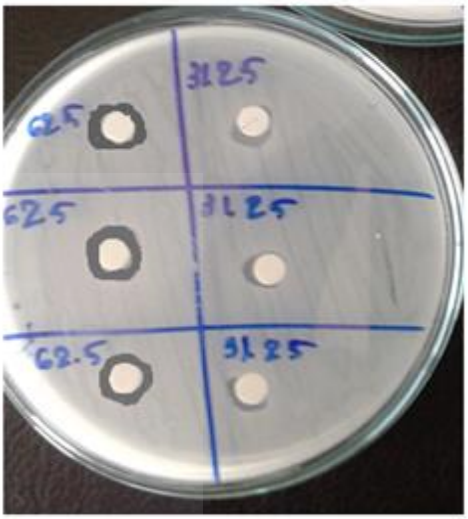


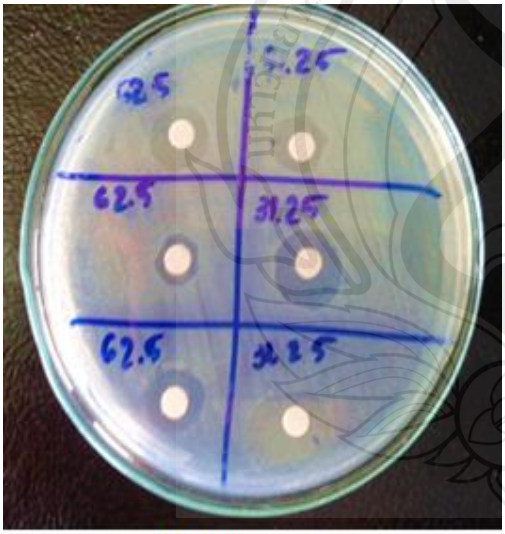
Figure C1 Inhibition Zones of Bacteria by Dichloromethane Extract of *Citharexylum Spinosum* Flowers



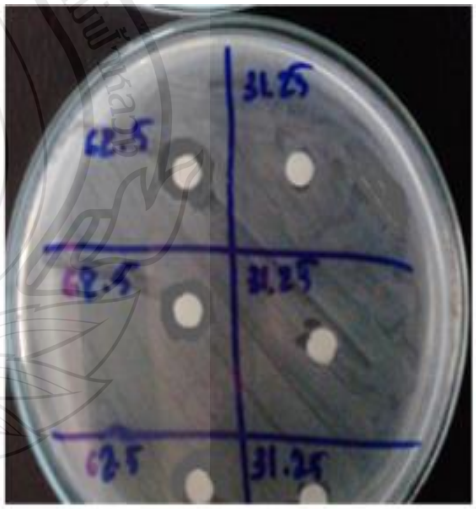
B. subtilis



B. cereus



M. luteus



S. aureus

Figure C1 (Continued)

APPENDIX D

INHIBITION ZONES OF BACTERIA BY HEXANE EXTRACT OF *CITHAREXYLUM SPINOSUM* FLOWERS

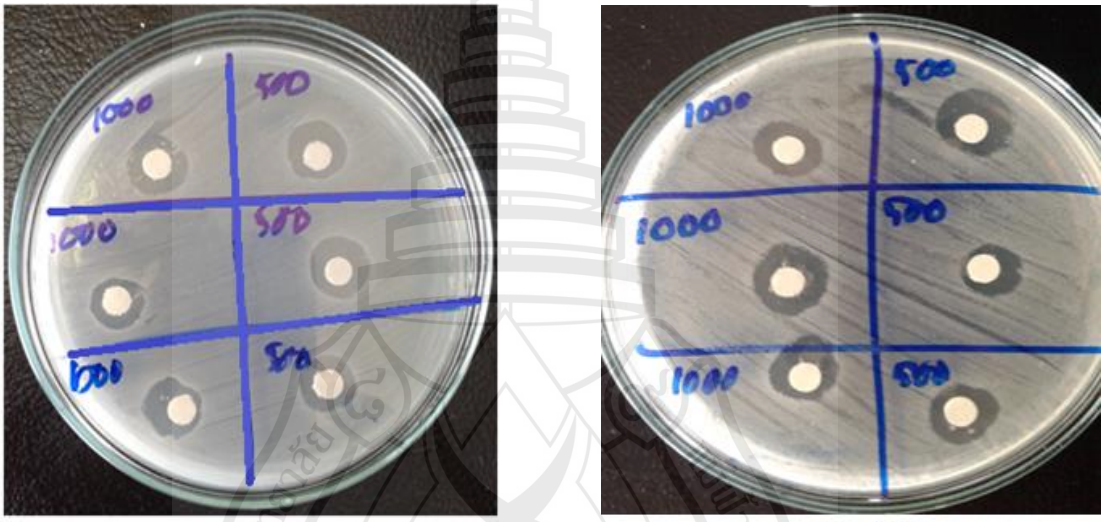
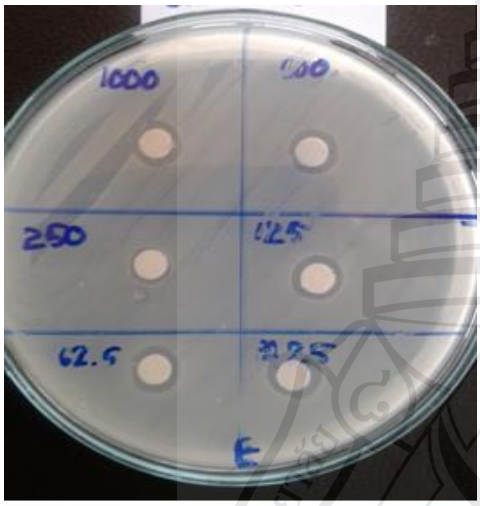


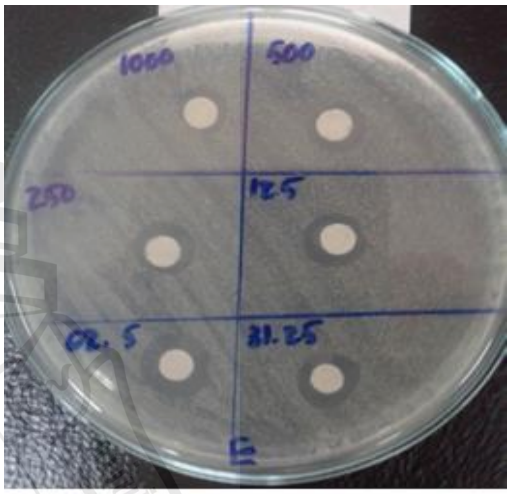
Figure D1 Inhibition Zones of Bacteria by Hexane Extract of *Citharexylum Spinosum* Flowers

APPENDIX E

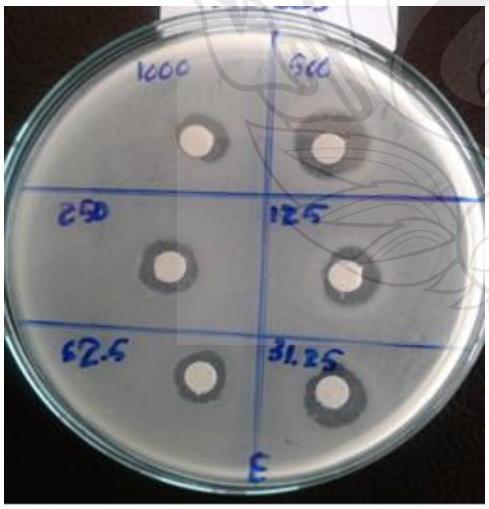
INHIBITION ZONES OF BACTERIA AND FUNGI BY ETHANOL EXTRACT OF *OSMANTHUS FRAGRANS* FLOWERS



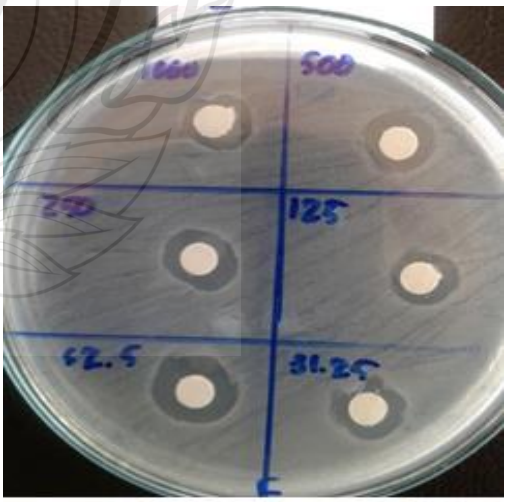
S. aureus



M. luteus

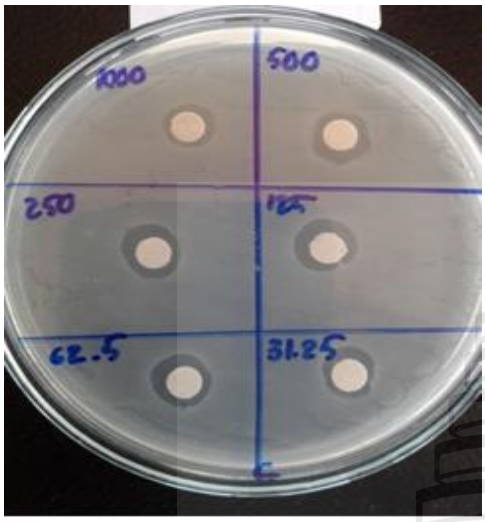


B. cereus

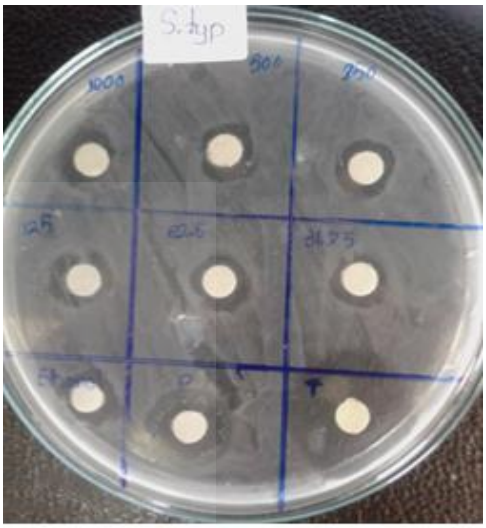


E. coli

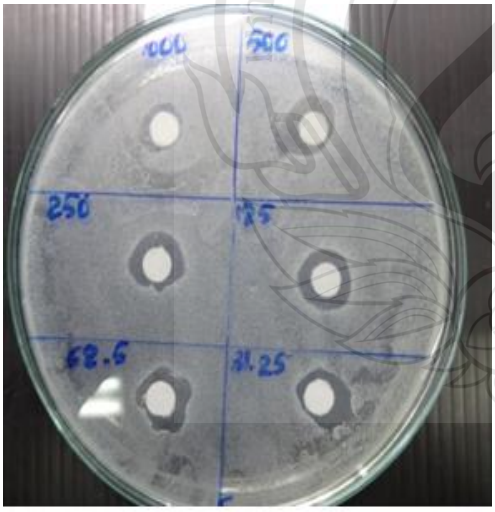
Figure E1 Inhibition Zones of Bacteria and Fungi by Ethanol Extract of *Osmanthus fragrans* Flowers



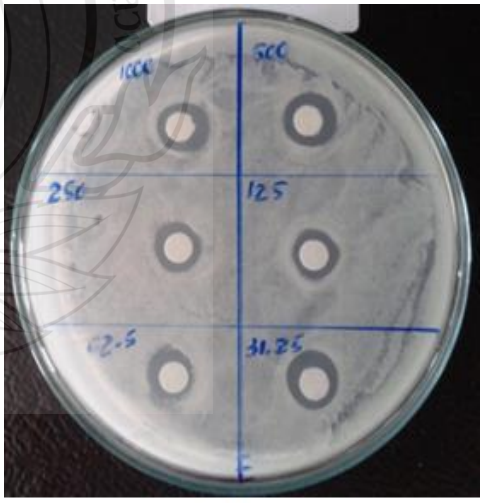
Ps. aeruginosa



S. typi



C. utilis



C. albican

Figure E1 (continued)

APPENDIX F

INHIBITION ZONES OF BACTERIA AND FUNGI BY DICHLOMETHANE EXTRACT OF *OSMANTHUS FRAGRANS* FLOWERS

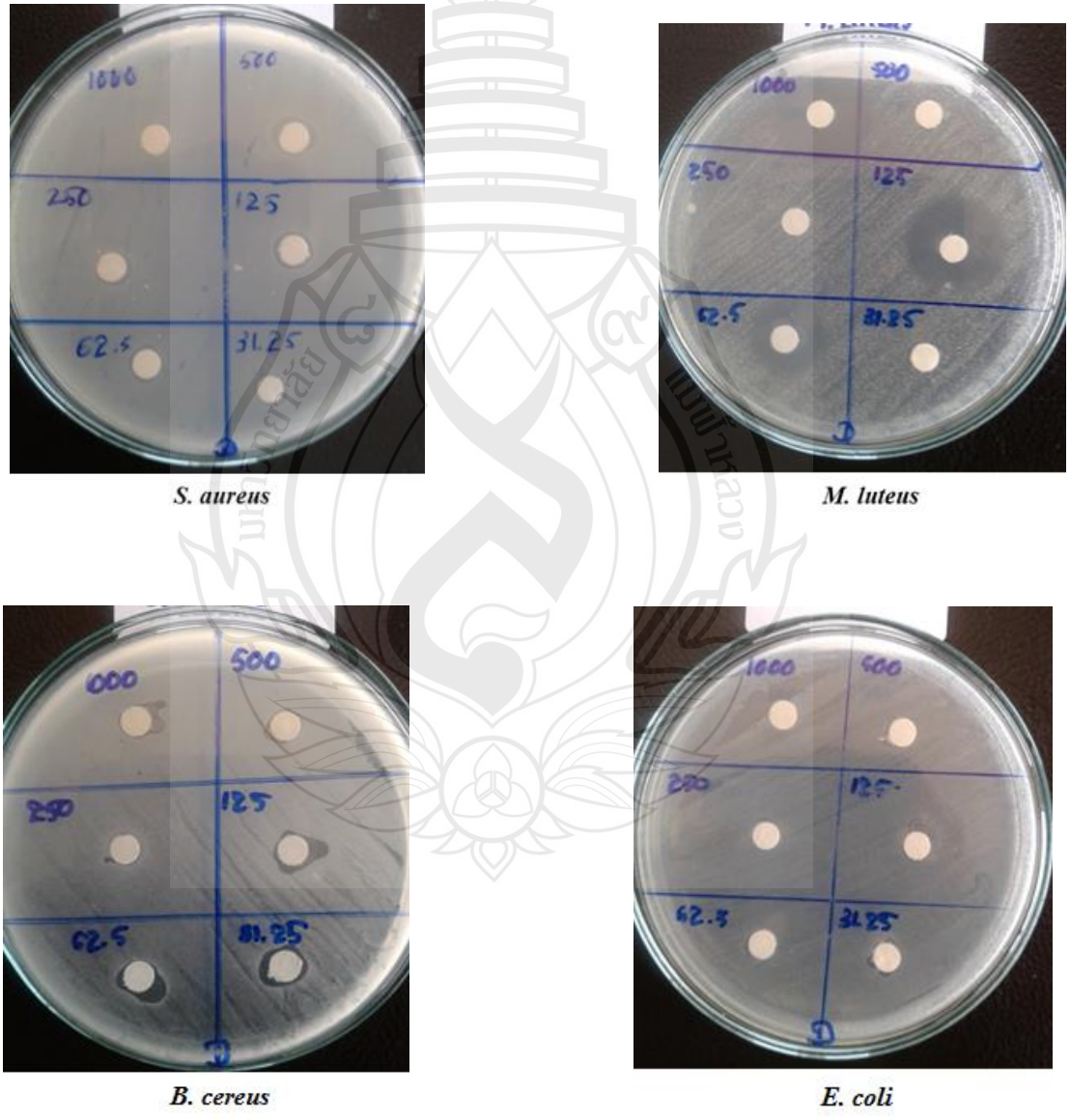
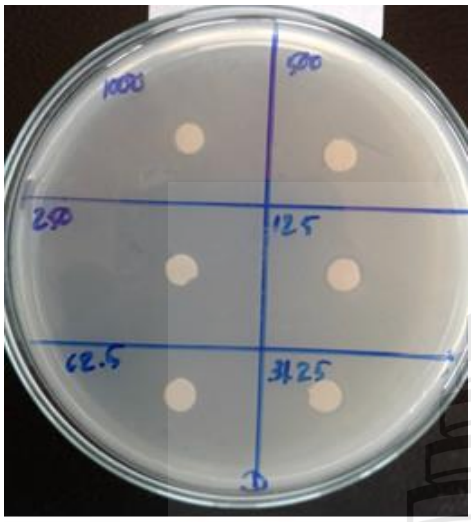
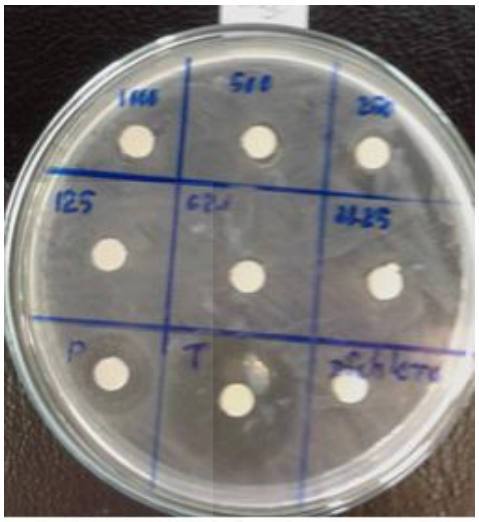


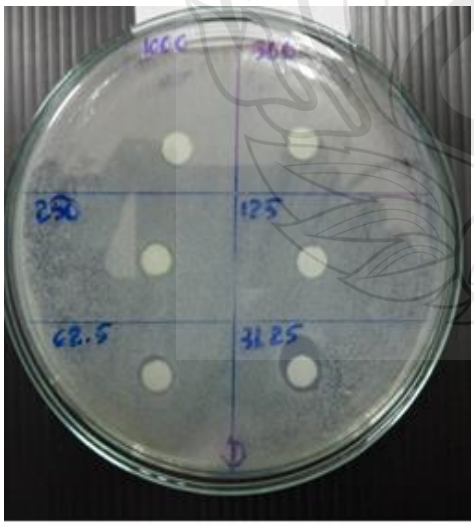
Figure F1 Inhibition Zones of Bacteria and Fungi by Dichloromethane Extract of *Osmanthus fragrans* Flowers



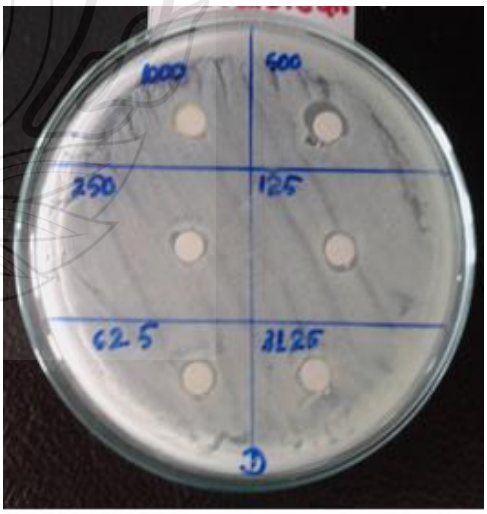
Ps. aeruginosa



S. typi



C. utilis



C. albican

Figure F1 (continued)

APPENDIX G

INHIBITION ZONES OF BACTERIA AND FUNGI BY HEXANE EXTRACT OF *OSMANTHUS FRAGRANS* FLOWERS

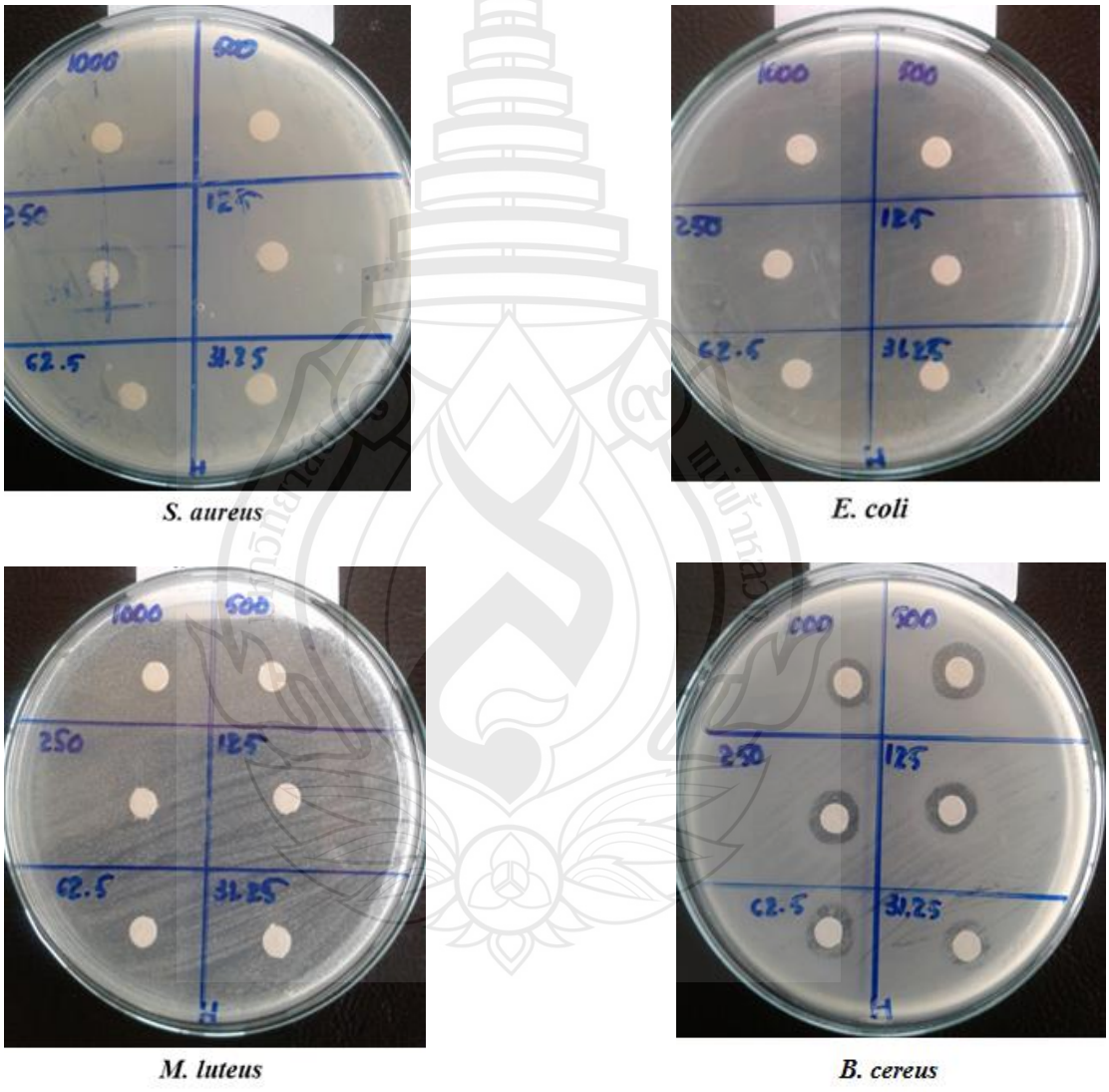
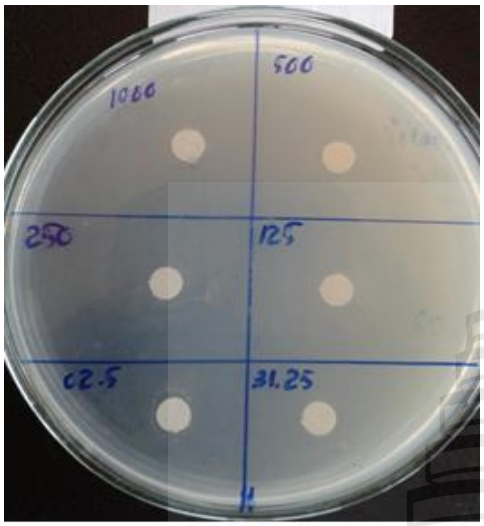
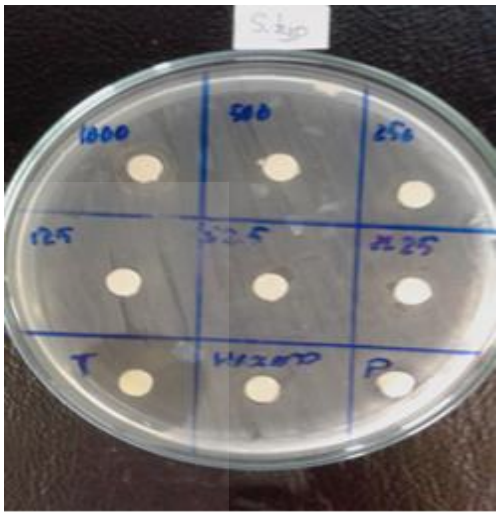


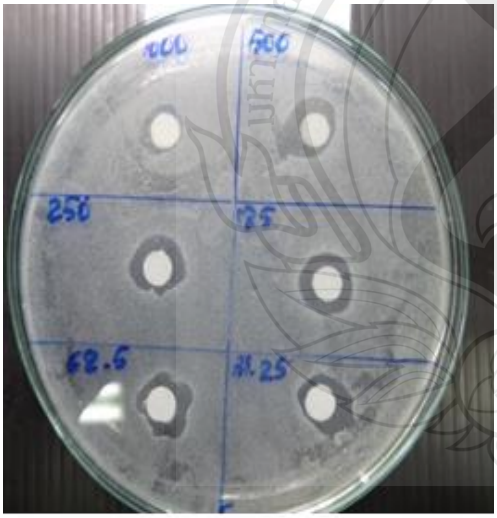
Figure G1 Inhibition Zones of Bacteria and Fungi by Hexane Extract of *Osmanthus fragrans* Flowers



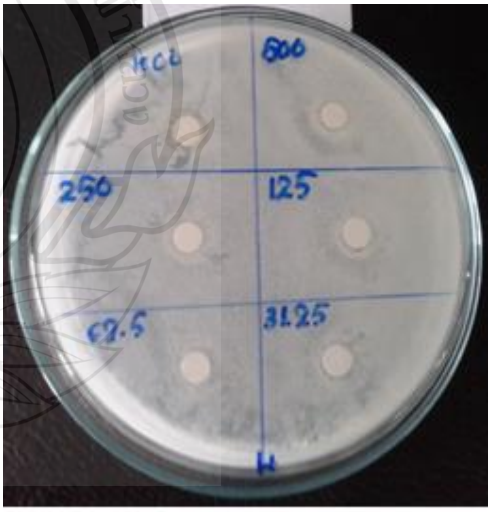
Ps. aeruginosa



S. typi



C. utilis



C. albican

Figure G1 (continued)

CURRICULUM VITAE

CURRICULUM VITAE

NAME	Ms. Ae Mar
DATE OF BIRTH	11 February 1979
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PUBLICATIONS

Mar, A., & Pripdeeveech, P. (2014). **Chemical composition and antibacterial activity of essential oil and extracts of *Citharexylum spinosum* flowers from Thailand** [International Journal]. Natural Product and Communications. **Vol.9**. Issue 5, **Pages** 597-736.

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