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วิทยานิทเป็นที่วนหนึ่งของการทึกษาตามหลักสูตรปริญญาเกล้งศาสตรมหาบัญทิต สาขาวิชาเกล้บเวท กาดวิชาเกล้บเวท บัญทิตวิทยาลัย กุฬาลงกรณ์มหาวิทยาลัย ปัการทึกษา 2542 ISBN 974-639-539-4

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# องค์ประกอบทางเคมีของน้ำมันระเหยจากพืชในวงศ์ LAMIACEAE ของไทย

นางสาวดาวจันทร์ ชูโชติ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต สาขาวิชาเภสัชเวท ภาควิชาเภสัชเวท บัณฑิตวิทยาลัย จุฬาลงกรณ์มหาวิทยาลัย ปีการศึกษา 2542

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# CHEMICAL COMPOSITION OF ESSENTIAL OILS FROM THAI LAMIACEOUS PLANTS

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for the Degree of Master of Science in Pharmacy

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Graduate School

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จากการศึกษาพืชในวงศ์ Lamiaceae ของไทย จำนวน 10 ต้น ในแง่ของปริมาณและชนิดของ องค์ประกอบของน้ำมันระเหย โดยใช้วิธีการกลั่นด้วยไอน้ำและเทคนิคทางโครมาโทกราฟี/แมสสเปกโทเมตรี ผลการศึกษาพบว่ามีความหลากหลายขององค์ประกอบทางเคมีและปริมาณโดยพบว่าองค์ประกอบส่วนใหญ่อยู่ ในกลุ่มออกซิจิเนเตคโมโนเทอร์ป็นและในแง่ปริมาณจะอยู่ในช่วงร้อยละ 0.01-0.9 นอกจากนี้ยังได้ทำการศึกษา พืชต่างประเทศในวงศ์ Lamiaceae ที่นำมาปลูกในไทยอีกจำนวน 10 ต้น ซึ่งได้ทำการเปรียบเทียบในด้าน ปริมาณและองค์ประกอบ พบว่าองค์ประกอบส่วนใหญ่ที่วิเคราะห์ได้ไม่มีความแตกต่างกันมากนักแต่ปริมาณจะต่ำกว่า และเมื่อนำน้ำมันระเหยไปทดสอบฤทธิ์ในการต้านจุลชีพ พบว่าน้ำมันระเหยส่วนใหญ่มีฤทธิ์ต้าน แบคทีเรียและไม่มีฤทธิ์ต้านเชื้อรา

ภาควิชา	เภสัชเวท
สาขาวิชา	กลัชเวท
ปีการศึกษา	2542 .

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ลายมือชื่อนิสิต <u>ภากัพ</u> ลายมือชื่ออาจารย์ที่ปรึกษา	High Blosof
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RUANGRUNGSI, Ph.D. THESIS CO-ADVISOR : ASSOC. PROF. WANCHAI DEEKNAMKUL, Ph.D., ASSIST. PROF. NONGLUKSNA SRIUBOLMAS, Ph.D. 213 pp
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Contents and compositions of essential oils from ten species of Thai Lamiaceous plants were investigated. The results obtained from GC/MS analysis showed diversity of their components. In this study, oxygentated monoterpenes are commonly found in essential oils of these particular species. Variations of the contents were found to be between 0.01 to 0.9 %. Essential oils of ten species of Western Lamiaceous plants cultivated in Thailand were also studied. Results have shown similarity of their constituents, but with less amount. Screening for antimicrobial activity of essential oil from particular plants against Staphylococcus aureus ATCC 29213, Enterococcus faecalis ATCC 29212, Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853, Bacillus subtilis ATCC 6633, Candida albicans ATCC 10231 and Microsporum gypseum was carried out, and it was found that most of the essential oils from Thai Lamiaceous plants exhibited antibacterial activity, but none of them demonstrated antifungal activity.

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#### **ABBREVIATIONS**

AOAC = Association of official unalytic chemists

cm = Centimeter

°C = Degree celsius

Fig = Figure

g = Gram

GC = Gas chromatography

GC-MS = Gas chromatography-Mass spectrometry

h = hour

HPLC = High performance liquid chromatography

i.d. = Internal diameter

m = Meter

MeOH = Methanol

mg = Milligram

min = Minute

ul = Microliter

ml = Millilter

mm = Millimeter

MW = Molecular weight

No. = Number

RT = Retention time

sp = Species

v/w = Volume by weight

wt = Weight

#### CHAPTER I

#### INTRODUCTION

Lamiaceae is a family in the order Lamiales, generally regarded as one of the most highly evolved of all dicotyledon families. The Lamiaceae is closely related to the Verbenaceae, primary a woody tropical family, generally without essential oils and usually without a deeply four-lobed ovary. The small aquatic family Callitrichaceae is also considered to be an ally of the Lamiaceae (Heywood, 1978).

The family Lamiaceae consists of 160 genera and about 3000 species distributed over the whole earth, but especially abundant in the Mediteranean region, the Orient and the mountains of the subtropics (Bailey, 1949).

#### The description of plants in the Lamiaceae is as below (Keng, 1978)

Unarmed, erect, mostly aromatic (sometimes fetid-aromatic) herbs, sometimes woody at the base; stem mostly quadrangular, sometimes conspicuously noded. Leaves decussate, rarely whorled, mostly simple, rarely lobed or pinnate, Indumentum of simple, capitate-glandular or stellate hairs, or a exstipulate. combination. (Extra-Mal. sometimes woody, climbing, spiny and with spiral leaves.) Flowers bisexual, mostly zygomorphic, axillary, in pairs, or in short, fascicled cymes forming verticillasters or in cincinni, in many cases compound into spurious spicate, racemose, capitate or paniculate, essentially cymose, in florescences. Calyx persistent, ± regular or unequally 4-5-toothed or-lobed, tubular or 2-lipped, sometimes with an appendage. Corolla tube long or short, sometimes with a hair-ring within, limb 5-, rarely 4-lobed, mostly 2-lipped and personate, lobes imbricate in bud. **Stamens** usually 4 and didynamous, inserted on the corolla tube, sometimes the upper (posterior) pair imperfect, rarely the lower pair barren (Mosla), filaments sometimes hairy, rarely connate at base; anthers linear to round, cells parallel or divaricate, sometimes confluent, rarely one cell barren (Anisomeles), or disjoined by a slender connective (Salvia), basifixed. Disk usuaslly prominent, regular of irregular. Ovary superior,

consisting of 2 carpels, each of which is 2-celled by intrusion of the ovary wall. Style simple, mostly gynobasic; stigma usually 2-fid, often with unequal arms. **Ovules** solitary, anatropous. **Fruit** consisting of 4 dry or rarely fleshy (*Gomphostemma*), 1-seeded schizocarpous nutlets which remain enclosed in the persistent calyx; the scar of attachment usually small and basal but sometimes sublateral and large; pericarp smooth or sculptured, endocarp sometimes hard; exocarp sometimes becoming gelatinous when moistened. **Seed** small, erect or  $\pm$  transverse (*Scutellaria*),  $\pm$  exalbuminous; seed-coat usually much deteriorated as to be almost negligible.

#### Lamiaceous Plants in Thailand

The occurrence of Lamiaceous plants in Thailand has been reported by Tem Smitinand in the Thai Plant Names (1980). These plants include various species as shown below.:-

#### Achyrospermum

A. wallichianum Benth, ex Hook, f.

Lacal name: สาขาบ Saa hom (Loei); ชีบบาโก Hompaa (Chiang Mai).

#### Anisochilus

A. carnosus Wall.

Local name: midium Huu Suea khao (Prachuap khiri khan)

A. harmandii Doan

Local name : คุมวย Khuu muai (Chong Trat) ; ครูมวย Khruu muai (Trat)

#### Ceratanthus (

C. amamensis G. Taylor

Local name: 🌇 Khaao kam (Sakon Nakhon).

Coleus

C. amboinicus Lour.

Local name : เมื่อมหูเสีย Niam huu suea (Central) ; หอมด่วนหลวง Hom duan

Luang, ทอมดีวนานชื่อ Hom duan huu Suea (Northern); Indian Borage.

C. atropurpureus Benth.

Local name : ภาษ์ผสมแล้ว Ruesee phasom Laeo (Central)

C. blumei Benth.

Local name : ว่ามเดือดแห้ง Waan Lueat haeng (Chiang Mai)

C. blumei var. verschaffeltii Lem.

Local name : ภูทิเผสมแล้ว Ruesee phasom laeo (Central)

C. parvifolius Benth.

Local name : มันขี้หนู Man khee nuu, มันหนู Man nuu (Peninsular) ; อุปิกะสิง U-

bee Ka-ling (Malay-Nara-Thiwat)

*C. tuberosus* Benth = *C. parvifolius* Benth.

Dysophylla

D. auricularia Bl.

Local name: สามแร็จสามกา Saapraeng saapkaa (Surat Thani)

D. cruciata Benth.

Local name : เมื่อมคอกฐป Niam dok thuup, แทนทานค่าง Nae haang khaang (Loei).

D. stellata Benth.

Local name : เมื่อม Niam (kan-chanaburi) ; เมื่อมลง Niam dong (Ratchaburi).

Elsholtzia

iz. kachinensis Prain

Local name : ผักสัวนุคอย Phak luan doi, ผักเสียน Phak luean (Chiang Mai)

Epimeredi

E. indicus Roth = Anisomeles indica (L.) Kuntze

Local name : กอมกับห้อง Komko huai (Chiang Mai) ; ฮากเฮีย Saapsuea (Saraburi) ; หญ้วฝรั่ง Yaa farang (Pra chin Buri)

Eurysolon

E. gracilis Prain

Local name : กมวดผมว Nuat maeo (Loei)

Genisporum

G. coloratum Ktze.

Local name : ผักอิหลึ่งป่า Phak ee lueng paa, ห้อมป่า Hom paa (Chiang Mai).

Gomphostemma

G. intermedium Craib

Local name: himmi Haat kop (Chiang Mai)

G. oblongum wall.

Local name : กลอนคู่ Klon duu (Trang) ; ขนทนอน Khon non (Surat Thani)

G. pholomides Prain

Local name: พอมฮอก Hom hok (Chiang Mai)

G. strobilinum Wall. var. variegatum Craib

Local name : ว่าขบกกุ้ม Waan nok khom (Chiang Mai)

Hyptis

H. brevipes Poit.

Local name : จัดรพระอิษทร์ Chat pra in (Southern)

H. suaveolens Poit.

Local name : mm Kaaraa (Surat thani) ; личётыт Maeng lak khaa (Chumphon).

Isodon

1. coetsa Kudo

Local name : ที่ออกน้ำ Pluak nam (Chiang Mai)

*i. sīriatus* Kudo

Local name : หญ้าข้าวดอก Yaa khaao tok, หญ้าปลวกดิน Yaa pluak Din, ใหวดิน Wai din (Chiang Mai)

I. ternifolius Kudo

Local name : ผักยินสิบ Phak ee luen (Chiang Mai)

Leonotis

L. nepetifolia R. Br.

Local name . พัตรพระอินทร์ Chat phra in (Central)

Leonurus

L. sibiricus Linn.

Local name : நிறையா Khon chaa thet (Ratchaburi), சீரை Saa saa (Nakhon Phanom) ; ர்ஸ் Saa nam (Loei) ; Mother worth

Leucas

L aspera Lin

Local name : คักทั่วโด Phak hua to, กญ้ากั่วโด Yaa hua to (Ratchaburi, kanchanaburi) ; กญ้าเกเล้า Yaa nok khao (Chiang Mai)

L. chinensis R. Br.

Local name : บ้ำลับโฟ Nam dap fai (Ratchaburi)

L. ciliata Benth.

Local name : หญ้าทั่งเสีย Yaa hua suea, ฮังเตม Hang taen (Loei)

L. zevlanica R. Br.

Local name : விளை Thian taak (Chaathaburi) ; விளி Yaa prik (Nakhon Si Thammarat). Mentha

M. arvensis Linn. var javanica Hook.

Local name : ມີນທີ່ມີນຳຄານີເພື່ອ Min indoneesia (Bangkok)

M. arvensis Linn. var piperascens Malinvaud

Local name : บ้าพับหม่อง Nam man mong สะระยบน์ผู้ปุ่น Saranae yeepun (Bangkok) ; Japanese Mint.

M. cordifolia Opiz

Local name : มักเงาะ Mak ngok, สะแม่ Sanae (Peninsular) ; สะระแหน่ Saranae, สะระแหน่สวน Saranae suan (Central) ; หอมค่วน Hom duan (Northern) ; Kitchen Mint.

M. javanica Bl.

Local name : สะระแทม์ทูวน Saranae yuan (Central)

M. piperita Linn.

Local name : กปกโยร์ชินต์ Pepper min (Bangkok) ; Peppermint

M. pulegium Linn.

Local name : แบงลักท้ำ , สะระแทท์พูวบ Saranae yuan (Bangkok).

M. spicata Linn.

Local name : สเปรียนต์ Sapae min (Bangkok) ; Common Spear Mint.

Mesona

M. chinensis Benth.

Local name: คาก็วง Chao kuai (chinese-Bangkok)

Microtoena

M. cymosa Prain = M. insuavis Prain ex Brig.

M. insuavis Prain ex Brig.

Local name: Anha Kham pong (Chiang Mai)

Mosla

M. dianthera Maxim.

Local name : ผักฮ้าน Phak haan (Chiang Mai)

M. cochinchinense Merr.

Local name : ทางเสีย Haang suea ทางเสียลาย Haang suea llaai (Loei)

Ocimum.

O. basilicum Linn.

Local name : ห่อกวยชวย Ho - kuai - suai, ห่อวอชุ Ho-Wo-su (Karen Mae Hong Son) ; โพระพา Horaphaa (General) ; อื่มกิมชาว Im - khim-khaao (Shan-Mae-Hang Son) ; Common Basil, Sweet Basil.

O. canum Sims

Local name : ก้อมก้องาว komko khaao (Northern) ; มังลัก Mang lak, แมงลัก Maeng lak (Central) ; Hairy Basil.

O. gratissimum Linn.

Local name : กะเพราญวน Ka phrao yuan (Bangkok) ; จันหน้ชี้ใก่ Chan kheekai, ก็เชมต้น Niam ton (Mae Hong Son) ; จันหน้ทอม Chan hom, เนียม Niam (Chiang Mai) ; ชี่หร่า Yeerea, โทระพาช้าง Horaphaa chaang (Central) ; สะหลี่ดี Sa-lee-dee (karen-Mae Hong Son).

O. kirimandcharicum Gverke

Local name: กะเพรานขก Ka phrao khack (Bangkok)

O. sanctum Linn.

Local name : กอมก้อ Komko, กอมก้อดง komko dong (Chiang Mai) ; กะเพรา kaphrao, กะเพราชาว ka phrao khaao, กะเพรานดง ka phrao daeng (Central) ; ห่อกวอชู Ho-kwo-suu, ห่อดูปดู Ho-tuu-pluu (karen-Mae Hong Son) ; อิ๋มคิมหล้า Im khim lam (shan-Mae Hong Son) ; Holy Basil.

O. sanctum Linn. var. hirsutum Back.

Local name: กะเพราชน Ka phrao khon (Central)

Orthosiphon

O. aristatus Mig.

Local name : บางรักป่า Baang rak paa (Prachuap khiri khan) ; หญ้าหนวดแมว Yaa nuat maeo (Chai nat) ; ชี่ตู้ดง Ee-tuu-dong. (Phetchabun)

O. grandiflorus Bolding

Local name : หอักแบน Pha-yap mek (Bangkok) ; หญ้าหนาดแบว Yaa nuat maeo (Central, Eastern, Chanthaburi)

O. rubicandus Benth

Local name : แข้งงาน้อย Khaeng khaa noi, หนวดเสียเขียว Nuat suea khieo (Loei)

Perilla

P. frutescens Britt.

Local name : งาร์เกียน Ngaa khee mon (Northern) งามน Ngaa-mon (Shan-Mae Hong Son) ; แง Ngae (Khan chanaburi) บอ No (Karen-Mae Hang Son) ; น่อง Nong (Karen-kan chanaburi)

P. ocymoides Linn = P. frutescens Britt

Pogostemon

P. cablin Benth.

Local name : வியாய் Phim sen (Bangkok)

P. glaber Roxb.

Local name : กับมกับคง kom ko Dong (Chiang Mai)

P. menthoides Bl.

Local name : ภาราค้า Phrao dam (Ranong) ; ทางเกียลอย Hom po doi (Chiang Mai)

P. plectranthoides Dest.

Local name . เพียมงางช้าง Niam nguang chaang (Central) ; ชั้ม Om (Chiang Mai)

Salvia

S. coccinea juss.

Local name : ประทัพเด็ก Pra that Lek (Bangkok) ; Scarlet Sage

S splendens ker-Gawl.

Local name : ประทักเล็ก Prathat Lek (Bangkok); Scarlet Sage

Scutellaria

S. incurva Wall.

Local name : ก้ามปู Kaampuu (Pra-chin Buri) ; หญ้าคางเดียย Yaa khaang luet (Chiang Mai)

#### Description and uses of selected Thai plants in this study

#### Coleus amboinicus Lour

Shrub, 2-3 feet; branches tomentosely pubescent, or hispid; leaves petiolate broad, ovate, crenated, rounded at the base, or cuneate, very thick, hispid on both surfaces, or clothed with white villi, very fragrant, floral leaves hardly equal in length to the calyx; racemes simple; whorls 20-30 flowered or more; calyx tomentose; tube of corolla about twice as long as the calyx, defracted at the middle; throat dilated; lower lip a little dilated, boat shaped; flowers smallish, pale blue. This plant has a pleasant aromatic odour and pungent taste (Drury, 1873).

The fresh leaves are frequently eaten, and mixed with various articles of food, drink or medicine. It possesses antibacterial, antifebrile and antitussive properties. They are used in treating coryza, influenza, hyperthermia, diaphoretic pyrexia and asthma (Medicinal Plants in Viet Nam, 1990). The Malay use its juice, or a decoction of it, though in a less degree, for pains in the neighbourhood of the stomach and heart (Burkill, 1935). In the Philippines macerated leaves are used with burns and also for bites of centipedes and scorpions, furthermore for dyspepsia, asthma, and as a medicine after childbirth (Keng, 1978).

### Hyptis suaveolens Poit

A rigid, sweetly aromatic herb, sometimes attaining a height of 7 ft. Leaves broadly ovate, very variable, tomentose, flowers small, blue, in unilateral axillary or terminal clusters often arranged in panicles, nutlets blackish brown, ovoid, compressed. (Council of Scientific and Industrial Research, 1959)

*H. suaveolens* is considered to be stimulant, carminative, sudorific and lactogogue. An infusion of the plant is used in catarrhal conditions. The leaf juice is taken in cases of colic and stomachache (Burkill, 1935).

In the Philippines, the leaves and top are considered to be antispasmodic and used in antirheumatic and antisudorific baths and the root is chewed with betel nuts as a stomachic (Council of Scientific and Industrial Research, 1959)

Mentha arvensis Linn var piperascens Malinvaud.

The entire plant of *Mentha arvensis* var *piperascens* Malinvaud is good to prevent frostbite, fatigue, and the common cold. This variety is the source of Japanese mint oil containg 80-90% menthol (Perry, 1980).

### Mentha cordifolia Opiz

Leaves all or for the greater part with a broadly cuneate, rounded, tuncate or shallowly cordate base, ovate oval oblong (small ones sometimes suborbicular), usually with a broadly rounded or obtuse top, not rarely serratedentate form the very base, thickly herbaceous, with rather much sunken nerves on the upper surface, consequently subcorrugate, subgrabrous or on the nerves shortly hairy, very densely gland-dotted on the lower surface; stem quadrangular, not grooved, very thinly short-hairy on glabrous (Backer and Bakhuizen, 1965)

#### Ocimum basilicum L.

An erect, almost glabrous herb, 30-90 cm. high, native of Central Asia and North-West India, Leaves ovate-lanceolate, acuminate, toothed or entire, glabrous on both surfaces, glandular; flowers white or pale purple, in simple or much-branched racemes, often thyrsoid; nutlets ellipsoid, black, pitted (Medicinal Plants in Vietnam, 1990).

The oil possesses insecticidal and insect repellent properties; it is effective against houseflies and mosquitoes (Council of Scientific & Industrial Research, 1966)

The leaves of *O. basilicum* are considered to be tonic, carminative and digestive; a decoction of them is used to wash ulcers, and also is prescribed for vomiting; the seeds are recommended to treat eye troubles. In Indo-china: the ashes of the roots are a suggested remedy for skin diseases. The seed have a mucilaginous seed coat which, in water, becomes a jelly with demulcent, stimulant, diuretic and diaphoretic properties (Perry, 1980).

#### Ocimum canum Sims

An erect, much-branched herb, found widely in the tropics to the Old World, and introduced into America. Leaves elliptic-lanceolate, entire or faintly toothed, almost glabrous, gland dotted, flowers small, white, pink or purplish, in more are less closely set whorls in spiciform racemes; nutlets narrowly ellipsoid, punctulate, black. (Burkill, 1935).

The plant is used as a pot-herb. It possesses aromatic, carminative, diaphoretic and stimulant properties. A decoction of the plant is taken for coughs, that of leaves for dysentery; it is also used as a mouth wash for relieving toothache. The juice of leaves is given to children for cold, catarrh and bronchitis; paste of leaves is used as an

external application for parasitical skin affections. The volatile oil from the whole plant inhibits the growth of tubercular bacilli in a dilution of 1:50,000; the leaf oil shows antibacterial activity against Mycobacteria (Council of Scientific & Industrial Research, 1966).

# Ocimum gratissimum Linn.

Much-branched perennial shrub, 1-1.5 m. high. Stems quadrangular, pubescent, woody at the base. Leaves opposite, apiculate, pubescent on both surfaces; margins coarsely toothed. Inflorescence axillary or terminal in simple or branched whorled raceme; flowers white. Nutlets subglobose, rugose. All parts of the plant are strongly scented. The whole plant is used in treating sunstroke, headache and influenza. It is also considered to be diaphoretic. It serves also as material for the extraction of essential oil and eugenol. Eugenol is used widely in odontology and for the synthesis of vanillin (Medicinal Plants in Viet Nam, 1990).

Oil of *Ocimum gratissimum* acts as a local anaesthetic and is useful as an external application for inflamed joints. It is considered digestive, tonic, stimulant, demulcent, diuretic, antiemetic and antiseptic. The leaf juice is given in stomachache. The seeds of the piant are given in headache (Council of Scientific and Industrial Research, 1966).

#### Ocimum sanctum L.

Erect, small plant, annual or perennial, about 1 m. in height. Stems and branchlets purple, pubescent. Leaves opposite, usually purplish-brown, long-petioled; margins slightly denticulate, pubescent on both surfaces. Inflorescence in closed-whorled terminal raceme; flowers small, lilac or white. Nutlets sub-globose, slightly compressed. All parts of the plant are sweet-scented (Medicinal Plants in Vietnam, 1990).

The oil is reported to possess antibacterial and insectisidal properties. It inhibits the in vitro growth of *Mycobacterium tuberculosis* and *Micrococcus pyogenes* var. *aureus*. It has marked insecticidal activity against mosquitoes (Council of Sciences and Industrial Research, 1966).

The entire plant, except for the roots, possesses antibacterial, antifebrile and demulcent properties. It is prescribed for coryza, fever, headache, colic, diarrhoea, chest pains, vomitting chilblains, oedema and epistaxis (Medicinal Plants in Viet Nam, 1990)

The root is used in a decoction for fever, flowers are given with honey for bronchitis and seeds are mucilaginous, demulcent which are used like the seeds of *O. basilicum* (Burkill, 1935).

#### Perilla frutescens Britt

An annual herb, the stem branching, tomentose, 0.5-1.5 m. high. Leaves opposite, lengthily petiolate, oval, acuminate, pubescent, dentate, crenelate, limb 14 cm. long by 6 cm. wide, green occasionally marked reddish brown. Inflorescence an axillary and terminal raceme, 6-20 cm. long; September-October. Flower small; 3-8; calyx campanulate, teeth 5; corolla campanulate, white or violet, 5-lobed, stamens 4. Fruit a collection of globular nutlets, 2 mm. in diameter, reticulate, light brown. Southern China, Taiwan, Japan, northern Vietnam, Laos. Thailand, India, Burma. The leaves and seed are officinal. The taste is pungent the oder aromatic. The seeds are the source of a drying oil resembling linseed oil and comprising glycerides of linoleic, oleic, and palmitic acids (Keys, 1976).

The fruit is effective against cough in doses of 3 to 5 g per day in the form of a decoction (Medicinal Plants in Viet Nam, 1990).

## Pogostemon cablin Benth.

Suffruticose, 2-3 feet, pubescent; stems ascending; leaves petioled, rhomboovate, slightly obtuse, crenato-dentate; spikes terminal and axillary, densely crowed with flowers interrupted at the base; calyx hirsute; segments lanceolate; filaments bearded; flowers white, with red stamens and yellow anthers (Drury, 1873).

The leaves of *P. cahlin* yield the Patechouli Oil which is used in perfumery and medicine. The oil is almost a perfume by iteslf and is one of the finest fixatives for heavy perfumes. The dried leves are used for scenting wardrobes. The leaves and tops are added in both for their antirheumatic action (Council of Scientific and Industrial Research, 1969).

In the Philippines, an infusion of the fresh leaves is given in mentstruation and emmenagogue (Perry, 1980).

These are still some essential oil containing plants of Lamiaceae in the rain forests of Thailand which have not been investigated. These include plant species of the genera Achyrospermum, Anisochilus, Ceratanthus, Coleus, Dysophylla, Elsholtzia, Genisporum, Gomphostemma, Hyptis, Isodon, Leonotis, Leonurus, Leucas, Mentha, Microtoena, Mosla, Ocimum, Orthosiphon, Pogostemon, Salvia, Scutellaria.

The main objectives in this investigation are as follows:

- 1. to screen for new essential oils from little studied Thai Lamiaceous plants, the family Lamiaceae growing in the forests.
  - 2. to evaluate the potential of the isolated essential oils for commercial use.
  - 3. to study the antimicrobial activity of essential oils.

Additionally, this work also included Lamiaceous plants from Western countries, which are cultivated in Chiangmai by The Royal Project Foundation, namely, *Melissa officinalis* L., *Mentha piperita* L., *Mentha spicata* L., *Origanum majorana* L., *Origanum vulgare* L., *Rosmarinus officinalis* L., *Salvia officinalis* L., *Thymus* sp.1 (summer Thyme), *Thymus* sp.2 (winter Thyme) and *Thymus vulgaris* L. All of them are called Savory herbs. The aim is to analyze the chemical composition and percent of the oil for as spices produced in Thailand comparing with the imported one.

## **CHAPTER II**

# HISTORICAL

### 1. Chemical Constituents of Lamiaceae

Volatile or essential oils, as their name implies, are volatile in steam. They are natural products which are commercially used in pharmaceutical products. For examples, 4-terpineol is used to as antiseptic. Citronellal and geraniol are used in combination as mosquito repellent. Most of the plants in this family secrete characteristic volatile oils from their glandular hairs. The following reviews focus only on Thai Lamiaceous plants of which the chemical compositions of essential oils have been previously worked on. List of compounds are shown below.

**Table 1** Chemical constituent of Thai Lamiaceous plants

Plant part	Chemical constituent	Reference
Leaves	Coleus amboinicus Lour.	Haque, 1988; Morton, 1992;
	Monoterpene	Prudent et al., 1995; Ameenah,
	lpha-phellandrene	Mala, and Fawzia, 1995; Pino,
	$\delta$ -3-carene	Garcia, and Martinez, 1996
	$\alpha$ -terpinene	
	<i>p</i> -cymene	
	limonene	
	$\gamma$ -terpinene	
	lpha-pinene	
	sabinene	
	myrcene	
	α-thujene	

Table 1 Chemical constituent of Thai Lamiaceous plants (continued)

Plant part	Chemical constituent	Reference
	camphene	
	verbenone	
	eta-pinene	
	eta-phellandrene	·
	cis-sabinene hydrate	
	terpinolene	
•		
	Oxygenated monoterpene	
<b>]</b>	linalool	
	camphor	
	terpinen-4-ol	
	geraniol	
	thymol	
	carvacrol	
	$\alpha$ -terpineol	
	Sesquiterpene	·
	lpha-copaene	·
	eta-caryophyllene	
	lpha-humulene	
	$\delta$ -muurolene	
	eta-selinene	
	$\alpha$ -selinene	
	$\delta$ -cadinene	
	<i>α-cis</i> -bergamotene	
	( $E$ )- $β$ -bergamotene	
	α-amorphene	
	$(Z)$ - $\beta$ -farnesene	

Table 1 Chemical constituent of Thai Lamiaceous plants (continued)

Plant part	Chemical constituent	Reference
	lpha-guaiene	
	lpha-muurolene	
	(E)-β-farnesene	
	$\beta$ -sesquiphellandrene	·
	<i>trans-</i> $\alpha$ -bergamotene	
	$(E,E)$ - $\alpha$ -farnesene	
	$\beta$ -bisabolene	
	Oxygeneted sesquitarnens	
	Oxygenated sesquiterpene γ-cadinol	
	$\alpha$ -cadinol	
	(Z) E-farnesol	
	caryophyllene oxide	
	humulene oxide II	
	aromadendrene oxide	
	aromadend only	
	Phenylpropanoid	
	eugenol	
	methyleugenol	
	Aliphatic Alcohols	
	1-octen-3-ol	
	1-0ctcn-3-01	
Leaves	<i>Hyptis suaveolens</i> Poit.	Iwu, Ezeugwu, and Okunji, 1990
	Monoterpene	; Ahmed, Scora, and Ting, 1994
	tricyclene	
	$\alpha$ -pinene	
	camphene	

Table 1 Chemical constituent of Thai Lamiaceous plants (continued)

Plant part	Chemical constituent	Reference
	sabinene	
	eta-pinene	
	myrcene	
	lpha-pheliandrene	
	$\delta$ -3-carene	
	$\alpha$ -terpinene	
	<i>p</i> -cymene	
	limonene	
	$(Z)$ - $\beta$ -ocimene	
	γ-terpinene	
	<i>p</i> -cymemene	
	terpinolene	
	thujane	
	Oxygenated monoterpene	
	1, 8-cineole	
<u>.</u>	linalool	
	camphor	
	borneol	
	terpinen-4-ol	
	$\alpha$ -terpineol	
	thymol	
	trans-pinene hydrate	
	3,7-Dimethyl-1, 6-octadien 3 ol	
	3, cyclohexen-1 -carboxaldehyde	
	Sesquiterpene	
	lpha-copaene	

Plant part	Chemical constituent	Reference
	$\beta$ -bourbonene	
	eta-elemene	
	$\beta$ -caryophyllene	
	trans- $\alpha$ -bergamotene	·
:	lpha-humulene	
	γ-muurolene	
	valencene	
	germacrene B	
	lpha-muurolene	
	$\alpha$ -caryophyllene	
	Oxygenated sesquiterpene	
	spathulenol	
	globulol	
	$\alpha$ -cadinol	
	muurolol	
	bergamotol	
	lpha-caryophyllene	
	Diterpene	
	rimuene	
	$5-\beta$ , $8-\beta$ , H, $9-\beta$ , H, 10	
	lpha-Labd - 14-ene	
Fresh	Mentha arvensis var	Retamar. and De-Riscala, 1980;
flowering	<i>piperascens</i> Malinyaud	Pino, Rosado, and Fuentes, 1995
stems and	Monoterpene	
leaves	$\alpha$ -pthene	
	sabinene	

Table 1 Chemical constituent of Thai Lamiaceous plants (continued)

Plant part	Chemical constituent	Reference
	$\beta$ -pinene	
	myrcene	
	<i>p</i> -cymene	
	limonene	
	camphene	
	Oxygenated monoterpene	
	menthol	
	piperitone	
	pulegone	
	neomethyl acetate	
	menthyl acetate	
	isomenthyl acetate	
	menthone	
	isomenthone	
	Sesquiterpene	
	$\beta$ -bourbonene	
	$\beta$ -caryophyllene	
	$\alpha$ -humulene	
	γ-muurolene	
	γ-elemene	
	$\delta$ -cadinene	
	Oxygenated sesquiterpene	
	caryophyllene oxide	

Plant part	Chemical constituent	Reference
Aerial part	Ocimum basilicum Linn.	Ozek <i>et al.</i> , 1995
	Monoterpene	
	$\alpha$ -pinene	
	camphene	·
	eta-pinene	
	sabinene	
	myrcene	
	$\alpha$ -terpinene	
	limonene	
	γ-terpinene	
	(E)-β-ocimene	
	p-cymene	
	terpinolene	
	carvone	
	Oxygenated monoterpene	
	1,8-cineole	
	camphor	
	linalool	
	$\alpha$ -terpineol	
	geraniol	
	linalyl acetate	
	bornyl acetate	
	Sesquiterpene	
	lpha-cubebene	
	lpha-copaene	
	$\beta$ -bourbonene	

Plant part	Chemical constituent  Chemical constituent	Reference
	eta-cutebene	
	$\beta$ -elemene	
	$\alpha$ -guaiene	
	$\beta$ -caryophyllene	·
	$\alpha$ -humulene	
	$(Z)$ - $\beta$ -farnesene	
	germacrene D	
	γ-guaiene	
	γ-elemene	
	germacrene A	
	$\delta$ -cadinene	
	trans- $\alpha$ -bergamotene	
	Oxygenated sesquiterpene	
	spathulenol	
	<i>T</i> -cadinol	
	$\beta$ -eudesmol	
	Phenyl propanoid	
	methyl eugenol	
	eugenol	
	(E)-anethole	
	(E)-methylcinnamate	
	(Z)-methylcinnamate	
Fresh	Phenyl propanoid	D: 1004
flowerering	methyl chavicol	Pino <i>et al.</i> , 1994
:		

Table 1 Chemical constituent of Thai Lamiaceous plants (continued)

Plant part	Chemical constituent	Reference
Aerial part	Ocimum canum Sims.	Xaasan <i>et al.</i> , 1981
	Monoterpene	
	$\alpha$ -pinene	
	camphene	·
	$\beta$ -pinene	
	sabinene	
	$\alpha$ -phellandrene	
	myrcene	
	limonene	
	eta-terpinene	
	<i>p</i> -cymene	
	γ-terpinene	
	terpinolene	
	Oxygenated monoterpene	
	camphor	
	terpinen-4-ol	
	borneol	
	isoborneol	
	myrtenol	
	Sesquiterpene	}
	cai y ophyllene	
	$\beta$ -elemene	
	humulene	
	$\beta$ -selinene	
	α-selinene	

Plant part	Chemical constituent	Reference
	Oxygenated monoterpene	
Leaves and	linalool	Ntezurubanza, Scheffer, and
flowers		Looman, 1985
		C also a said Cafeerana 1071
Leaves	Ocimum gratissimum Linn.	Sainsbury and Sofowora, 1971;
		Zamureenko <i>et al.</i> ,1986;
	Monoterpene	Ntezurubanza <i>et al.</i> , 1987;
	$\alpha$ -terpinene	Pino, Rosado, and Fuentes, 1996
	$\alpha$ -thujene	
	$\alpha$ -pinene	
	camphene	
	$\beta$ -pinene	
	myrcene	
	<i>p</i> -cymene	
	$(Z)$ - $\beta$ -ocimene	
	limonene	
	γ-terpinene	
	trans-sabinene hydrate	
	terpinolene	
	sabinene	
	$\delta$ -3-carene	
	cis-sabinene hydrate	
	Oxygenated monoterpene	
	thymol	
	1, 8-cineole	
	camphor	
	linalool	
	$\alpha$ -terpineol	
	terpinen-4-ol	

Plant part	Chemical constituent  Chemical constituent	Reference
	carvacrol	
	borneol	
	p-cymen-8-ol	
	Sesquiterpene	
	$\beta$ -caryophyllene	
	lpha-humulene	
	eta-selinene	
	longifolene	
	clovene	
	$\beta$ -bourbonene	
	$\beta$ -elemene	
	trans- $\beta$ -bergamotene	
	γ-cadinene	
	lpha-copaene	
	germacrene D	
	$\delta$ -cadinene	
	Oxygenated sesquiterpene	
	caryophyllene oxide	
	humulene oxide II	
	$\beta$ -caryophyllene epoxide	
	Phenylpropanoid	
	methyl eugenol	
	eugenol	

Table 1 Chemical constituent of Thai Lamiaceous plants (continued)

Plant part	Chemical constituent	Reference
Flowers	Monoterpene	Pino <i>et al.</i> , 1996
	$\alpha$ -thujene	
	$\alpha$ -pinene	
	camphene	
	$\beta$ -pinene	
	myrcene	
	lpha-phellandrene	
	<i>p</i> -cymene	
	limonene	
	γ-terpinene	
	trans-sabinene hydrate	
	terpinolene	
	Oxygenated monoterpene	
	linalool	
	borneol	
	terpinen-4-ol	
	lpha-terpineol	
	p-cymen-8-ol	
	thymol	
	carvacrol	·
	Sesquiterpene	
	eta-bourbonene	
	eta-elemene	
	eta-caryophyllene	
	<i>trans-<math>\beta</math></i> -bergamotene	
	lpha-humulene	
	$\beta$ -selinene	·

Table 1 Chemical constituent of Thai Lamiaceous plants (continued)

Plant part	Chemical constituent	Reference
	γ-cadinene	
	Oxygenated sesquiterpene	
	caryophyllene oxide	·
	humulene oxide II	
seed	Phenylpropanoid	Dro and Hefendehl, 1974
	methyl eugenol	, , , , , , , , , , , , , , , , , , , ,
Leaves	Perilla frutescens Britt	Kang et al., 1992; Nguyen et al.,
	Monoterpene	1995
	ocimene	
	sabinene	
	eta-pinene	
	myrcene	
	pseudolimonene	
	limonene	
	terpinolene	
	perillaldehyde	
	$\alpha$ -terpinyl acetate	
	piperitone	
	camphene	
	$\alpha$ -pinene	
	$\alpha$ -phellandrene	
	<i>p</i> -cymene	
	carvone	

Table 1 Chemical constituent of Thai Lamiaceous plants (continued)

Plant part	Chemical constituent	Reference
	Oxygenated monoterpene	
	1,8-cineole	
	linalool	
	limonene oxide	
	perillyl alcohol	
	Sesquiterpene	
	$\beta$ -caryophyllene	
	lpha-caryophyllene	
	$\alpha$ -bergamotene	
	farnesene	
	aromadendrene	
	lpha-copaene	
	$\beta$ -bourbonene	
	eta-elemene	
	$\alpha$ -humulene	
	germacrene D	
	$\delta$ -cadinene	
	(Z)-nerolidal	
		,
	Oxygenated sesquiterpene	
	caryophyllene oxide	
Leaves	Pogostemon cablin Benth	Nguyen, Leelereq, Tran, and La
	Sesquiterpene	Dinh; 1989; Nguyen et. al; 1990
	lpha-patchoulene	
	$\alpha$ -patchoulene	

Plant part	Chemical constituent	Reference
	$\beta$ -patchoulene	***
	$\delta$ -patchoulene	
	eta-caryophyllene	
	lpha-quaiene	
	$\delta$ -quaiene	
	seychellene	
	lpha-bulnesene	
	$\delta$ -cadinene	
	Oxygenated monoterpene	
	patchouli alcohol	

### **CHAPTER III**

### MATERIALS AND METHODS

#### 3.1 Plant Materials

The plant materials were collected from various locations in Thailand and at different periods of time as shown in Table 1. Authentication was achieved through comparison with herbarium specimens at the Botany Section, Technical Division, Department of Agriculture, Ministry of Agriculture and Coorperative, Thailand.

**Table 2** Cultivating locations of collected plants and harvesting times.

Number	Name	Place	Harvesting times
1	Coleus amboinicus Lour.	Faculty of Pharmaceutical	December 1996
		Science Chulalongkorn	
		University, Bangkok	
2	Hyptis suaveolens Poit	Pak Thong Chai	December 1996
		Nakorn Ratchasima,	
3	<i>Mentha arvensis</i> L. var	Chiang Mai	October 1997
	piperascens Malinvaud		
4	<i>Mentha cordifolia</i> Opiz	Nakornprathom	April 1997
5	Ocimum basilicum L.	Pakkred Nonthaburi	August 1996
6	Ocimum canum Sims.	Pakkred Nonthaburi	August 1996
7	Ocimum gratissimum L.	Bangkok	June 1997
8	Ocimum sanctum L.	Pakkred Nonthaburi	September
			1996
9	Perilla frutescens Britt.	Chiang Mai	May 1997
10	Pogostemon cablin Benth	Faculty of Pharmaceutical	April 1997
		Sciences Chulalongkorn	
		University	

### 3.2 Essential oil content and composition

### 3.2.1 <u>Essential oil content determination</u>

Essential oil was determined by the method described in the Association of Official Analytical Chemists (method 962.17, AOAC, 1990). One hundred and fifty grams of each sample was put into a 1000 ml round bottom flask. The tridistilled water was added into the flask to about half full. The flask was connected to the apparatus for the determination of volatile oil (Fig. 1). The content of the flask was distilled until two consecutive readings taken at one hour interval showed no change in oil content (about four hours). After cooling, the oil volume was measured, calculated and expressed as millilitre of the oil per one hundred grams of sample. The essential oil obtained was then collected and stored at 4°C until being analyzed for its chemical composition by GC-MS.

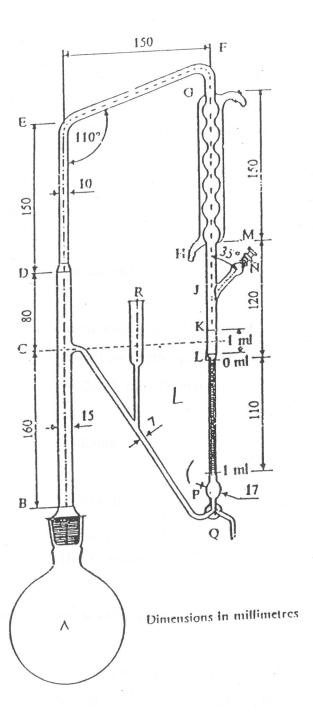


Figure 1 Apparatus for volatile oil content determination.

### 3.2.2 Gas chromatography-mass spectrometry

For identification of the composition of essential oil, a gas chromatography coupled with a mass spectrometer (GC-MS) was used. The essential oil was diluted to 1:100 in methanol before being injected into GC-MS system. The condition of GC-MS was described below. The spectra were recorded and compared with the terpene library (Adam, 1995).

### **GC-MS Condition**

Instrument model	Varian Saturn 3
Column	fused silica capillary column (30 m x 0.25 mm.i.d.)
	coated with DB-5 (J&W) film thickness 0.25 $\mu m$ .
Column programming	60-240°C rate 3°C/min
Injector temperature	240°C
Helium carrier gas	1 ml/min
Split ratio	100:1
Accelerating voltage	1700 volts
Sample size	ا پرا
Solvent	HPLC grade methanol

### 3.3 Scanning electron microscopic examination (Weakly, 1972)

The leaf was cut into small pieces (3x5 mm<sup>2</sup>). Then, they were fixed in a primary fixative (2.5% glutaraldehyde in 0.1 M phosphate buffer pH 7.2) at room temperature for 1 h or kept in refrigerator overnight. After that they were washed 3 times with buffer, 10-15 min in each and were treated with secondary fixative, 1% osmium tetroxide in 0.1 M phosphate buffer pH 7.2, for 1-2 h. The specimens were dehydrated through an ascending series of ethanol 30%, 50%, 70%, 90%, and absolute ethanol. Each dehydration step took 10-15 min. Finally they were dehydrated in the

critical point dryer. the specimen was fixed to a stub and coated with a thin film of gold by Sputter coater.

The glandular trichomes responsible for the essential oil accumulation were examined in a Jeol JSM-5410LV scanning electron microscope.

#### 3.4 Determination of antimicrobial activities of essential oils

### 3.4.1 Agar diffusion assay

The preliminary study of antimicrobial activities of essential oil was done by using the agar diffusion method (Edwin *et al.*, 1985; Lorian, 1991)

#### 3.4.1.1 Preparation of sample

Each essential oil was diluted to a final concentration of 10% in 0.1% sterile Tween 80.

### 3.4.1.2 Preparation of the inoculum

The bacterial strains used were as follows:

- Staphylococcus aureus ATCC 29213
- Enterococcus faecalis ATCC 29212
- Bacillus subtilis ATCC 6633
- Escherichia coli ATCC 25922
- Pseudomonas aeruginosa ATCC 27853

Preparations of bacterial inocula were done according to the standard method (Lorian, 1991). Each bacterial strain was cultured overnight on Trypticase Soy Agar (TSA) plate at 37°C. Four well isolated colonies of the overnight grown culture were inoculated into a 5-int Trypticase Soy Broth (TSB) and incubated at 37°C for 2-3 h. The turbidity of inoculum was adjusted with sterile normal saline solution to match a 0.5 turbidity standard of Mc Farland No 1.

The fungal strains used in this study were as follows:

- Candida albicans ATCC 10231
- *Microsporum gypseum* (clinical isolate)

Candida albicans ATCC 10231 was grown on Sabouraud Dextrose Agar (SDA) slant at 30°C for 24 h. The inoculum was prepared by suspending the culture in sterile normal saline solution and turbidity of the inoculum was adjusted to match a 0.5 turbidity standard of Mc. Farland No 1.

Spores of *Microsporum gypseum* grown on SDA slant at 30°C for 4 days were washed from the slant culture with sterile 0.05% Tween 80. The turbidity of the spore suspension was adjusted to match 0.5 turbidity standard of Mc. Farland No 1.

## 3.4.1.3 Preparation of test plates

- For testing bacteria:

Mueller Hinton Agar (MHA) was melted and allowed to cool at 45°C - 50°C in a water bath. Then 25 ml of the melted agar medium was dispensed into sterile glass petri dishes, with internal diameters of 9 cm, to yield a uniform depth of 4 mm. The agar was allowed to harden on a flat level surface. The plates were dried for 1 h at 37°C

### - For testing fungi:

Sabouraud Dextrose Agar (SDA) was used and prepared as described above.

### 3.4.1.4 <u>Inoculation of agar plates</u>

A sterile cotton swab was dipped in each inoculum and the excess was removed by rotating the swab several times against the inside wall of the tube above the fluid level. The entire surfaces of the MHA plate and the SDA plate for testing bacteria and fungi, respectively, were inoculated by streaking with the swab for 3 times and each time the plate was rotated 60 degrees.

### 3.4.1.5 Assay procedure

A 50 µl of each 10% oil sample or diluent (0.1% sterile Tween 80) was delivered to each hole (6 mm dia.) in the inoculated medium. This was done in triplicate. After maintaining at room termperature for 1 h, the bacterial and fungal plates were incubated at 37°C overnight and 30°C for 48-72 h, respectively. The oil samples showing inhibition zone were examined further for their minimal inhibitory concentrations (MIC).

### 3.4.2 Determination of Minimal Inhibitory Concentration (MIC).

Determination of MIC of essential oil was done by the broth microdilution test (Lorian, 1991).

### 3.4.2.1 Preparation of the inoculum

The inoculum was prepared as described above in 4.1.2. The turbidity of the 0.5 McFarland turbidity standard No 1, provides approximatedly 1 x  $10^8$  CFU/ml. The inoculum was further diluted 1:200 in Mueller Hinton broth.

### 3.4.2.2 Preparation of the essential oil dilutions

Each oil sample was mixed with equal volume of dimethyl sulfoxide (DMSO) and diluted with Mueller-Hinton brotin (MHB) in a two fold dilution to give the concentrations ranging from 10% to 0.639% v/v.

### 3.4.2.3 Assay procedure

A 100 µl of each concentration was dispensed to the corresponding well of sterile multiwell microdilution plate (96-Flat-shaped wells). A 100 µl of diluted inoculum was added into each well. After incubating the tray at 37°C for 24 h, the lowest concentration of the oil sample that showed growth inhibition was considered as the MIC. This was done in duplicate. The corresponding concentrations of DMSO were also tested for their antibacterial activities. Inhibitory effect of DMSO was examined by measurement of culture turbidity in each well using microplate reader (Bio-Rad, model 450).

#### CHAPTER IV

#### RESULTS

## 4.1 Chemical Composition of Essential Oil from Thai Lamiaceous plants

### 4.1.1 Essential Oil Composition of Coleus amboinicus Lour.

The essential oil from the leaves of *Coleus amboinicus* was isolated by hydrodistillation. The oil yield was found to be 0.1 % (v/w) of fresh weight. Analysis of the essential oil by GC/MS showed 17 seperate peaks (Fig. 2). These peaks were identified as 8 monoterpenes, 3 oxygenated monoterpenes, 4 sesquiterpenes, and 1 oxygenated sesquiterpene (Table 3). Among these, carvacrol (77.95 %) appeared to be the major compenent, followed by (E)-caryophyllene (6.19%).

In terms of relative amount, the oxygenated monoterpenes appeared to be the major terpenoid group, accounting for 79 % of the essential oil (Fig. 3). Sesquiterpenes, monoterpenes and oxygenated sesquiterpenes were present in lesser amount with 14 %, 6 % and 1 %, respectively.

In terms of structure, the major components, carvacrol, belongs to the oxygenated monoterpenoid group of menthane, while (E)-caryophyllene belongs to the sesquiterpenoid group of caryophilane.

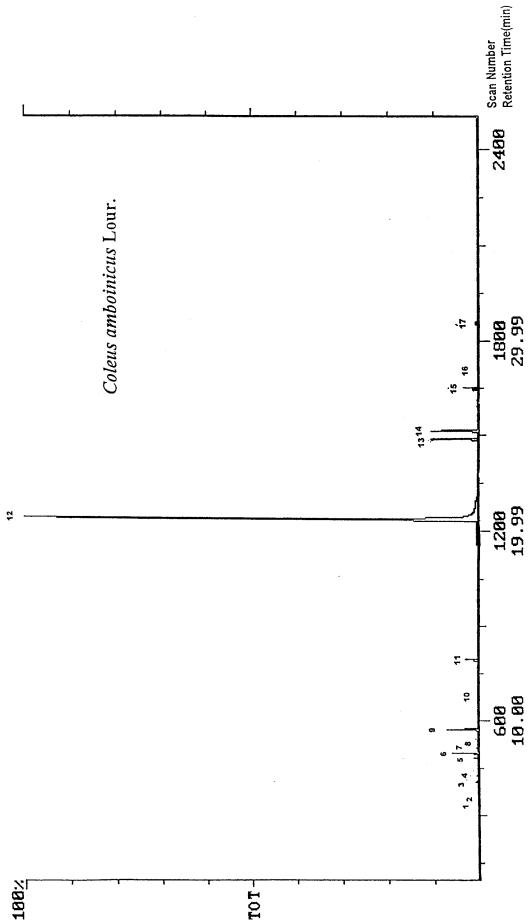
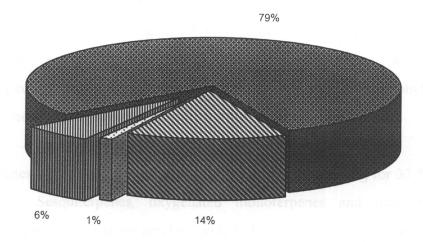


Figure 2 GC chromatogram of the essential oil from Coleus amboinicus leaves

 Table 3 Essential oil composition of Coleus amboinicus leaves

Number of peak	Compound	Retention time ( min )	% Area
	Monoterpene		
1	tricyclene	5.28	0.05
2	$\alpha$ -thujene	5,50	t
4	myrcene	6.90	0.13
5	$\delta$ -2-carene	7.12	0.41
6	o-cymene	7.75	2.50
7	limonene	8.01	0.11
9	γ-terpinene	9.08	2.89
10	terpinolene	10.13	t'
	Oygenated monoterpene		
8	1,8-cineole	8.13	0.19
11	terpin-4-ol	13.94	0.88
12	carvacrol	20.33	77.95
	Sesquiterpene		
13	(E)-caryophyllene	24.38	6.19
14	$\alpha$ -trans-bergamotene	25.05	5.70
15	$(Z)$ - $\alpha$ -bisabolene	27.58	1.78
16	lpha-cadinene	28.88	0.08
	Oxygenated sesquiterpene		
17	caryophyllene oxide	31.10	0.62
	Long chain hydrocarbon		
3	1-octen-3-ol	6.79	0.48



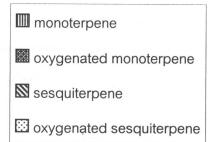


Figure 3 The percentage of various terpenoid groups found in the essential oil of *Coleus amboinicus* leaves

### 4.1.2 Essential Oil Composition of Hyptis suaveolens Poit.

The leaves of *Hyptis suaveolens*, were found to contain essential oil at 0.1 % (v/w) of fresh weight. By GC/MS analysis of essential of it was found that the essential oil had at least 43 peaks in its GC chromatogram (fig. 4). These peaks were identified as 9 monoterpenes, 3 oxygenated monoterpenes, 17 sesquiterpenes, 5 oxygenated sesquiterpenes and 2 diterpenes (Table 4). Among these, 1,8-cineole (21.70 %) appeared to be the major component, followed by (*E*)-caryophyllene (17.87 %) and sabinene (16.92 %).

Monoterpenes were found to be the major component, accounting for 33 % of the essential oil. Sesquiterpenes, oxygenated monoterpenes and oxygenated sesquiterpenes were present in a lesser amount, with 31 %,22 % and 2 %, respectively (Fig. 5).

Structurally, the major component, 1,8-cineole and sabinene, belong to the monoterpenoid group of menthane and thujane, respectively, while (E)-caryophyllene belongs to the sesquiterpenoid group of caryophilane.

sabinene (*thujane*)

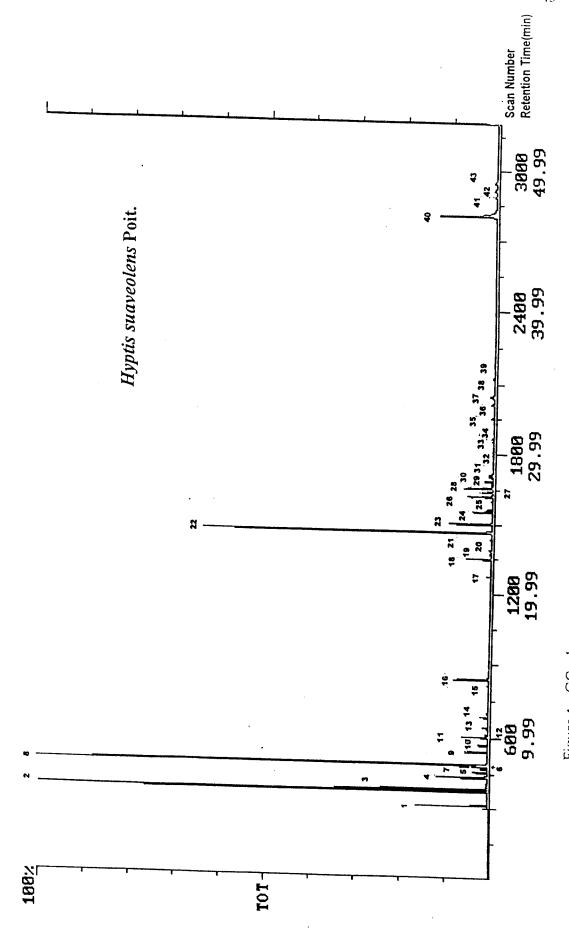


Figure 4 GC chromatogram of the essential oil from Hyptis suaveolens leaves

 Table 4 Essential oil composition of Hyptis suaveolens leaves

Number	Compound	Retention time	% Area
of peak	Monoterpene	(min)	
1	tricyclene	5.28	2.66
2	sabinene	6.35	16.92
3	$\beta$ -pinene	6.41	6.63
4	$\alpha$ -phellandrene	7.26	2.19
5	$\delta$ -3-carene	7.40	0.68
6	o-cymene	7.75	0.32
7	limonene	8.02	1.31
9	γ-terpinene	9.08	1.16
11	terpinolene	10.13	1.48
	oxygenated monoterpene		
8	1,8-cineole	8.13	21.70
14	exo-fenchol	11.39	0.46
15	borneol	13.61	0.12
	Sesquiterpene		
17	$\alpha$ -cubebene	21.28	0.26
18	$\alpha$ -copaene	22.50	1.60
19	$\beta$ -bourbonene	22.58	0.14
20	$\beta$ -cubebene	23.06	0.17
21	β-elemene	23.09	0.16
22	(E)-caryophyllene	24.38.	17.87
23	$\alpha$ -trans-bergamotene	25.01	2.81
24	α-humulene	25.91	1.25
25	allo-aromadendrene	26.08	0.40

Table 4 Essential oii composition of Hyptis suaveolens leaves (continued)

Number of peak	Compound	Retention time (min)	% Area
26	germacrene D	26.56	1.71
27	$\beta$ -selinene	26.80	0.92
29	germacrene A	27.89	0.10
30	$\alpha$ -bulnesene	28.00	0.65
31	γ-cadinene	28.04	0.32
32	$\delta$ -cadinene	28.56	0.32
33	$\alpha$ -cadinene	28.88	0.11
	Oxygenated sesquiterpene		
34	caryophyllene oxide	31.10	0.20
35	longiborneol acetate	32.56	0.25
36	<i>epi-α</i> -cadinol	33.55	0.36
37	$\alpha$ -eudesmol acetate	34.13	0.61
38	Z-α-trans-bergamotol acetate	35,36	0.13
	Diterpene		
41	abietatriene	48.10	0.22
43	abietadiene	49.13	0.43
	Miscellaneous		
10	unknown	9.49	Q.55
12	unknown	10.20	0.19
13	uńknown	10.68	0.39
16	unkuown	13.98	2.24
39	unknown	35.49	0.13
40	unknown	46.73	7.22
42	unknown	48.51	0.38

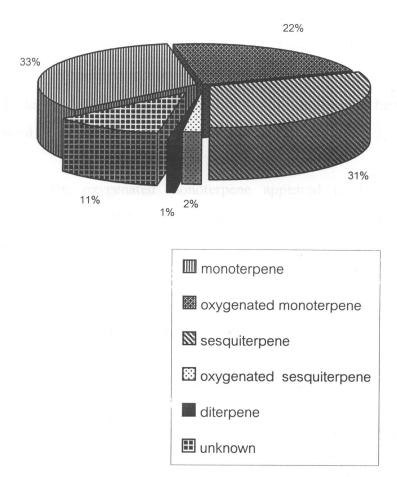


Figure 5 The percentage of various terpenoid groups found in the essential oil of *Hyptis suaveolens* leaves

# 4.1.3 Essential Oil Composition of *Mentha arvensis* L. var. *piperascens* Malinvaud

The essential oil hydrodistilled from *Mentha arvensis* L. var. *piperascens* leaves was found to have the yield of 0.9 % (v/w) of fresh weight. Analysis of the essential oil by GC/MS showed 25 distinct peaks (fig 6). These peaks were identified as 5 monoterpenes, 10 oxygenated monoterpenes, 6 sesquiterpenes and 2 oxygenated sesquiterpenes (Table 5). Among these, menthol (79.44 %) was found to be the major component, followed by menthone (9.44 %) and limonene (2.85 %).

Quantitatively, the oxygenated monoterpene appeared to be the major terpenoid group, accounting for 93 % of the essential oil. Monoterpenes were present in a lesser amount, at 4 % (Fig 7).

Structurally, the major component, menthol and menthone, belong to the oxygenated monoterpenoid group of menthane.

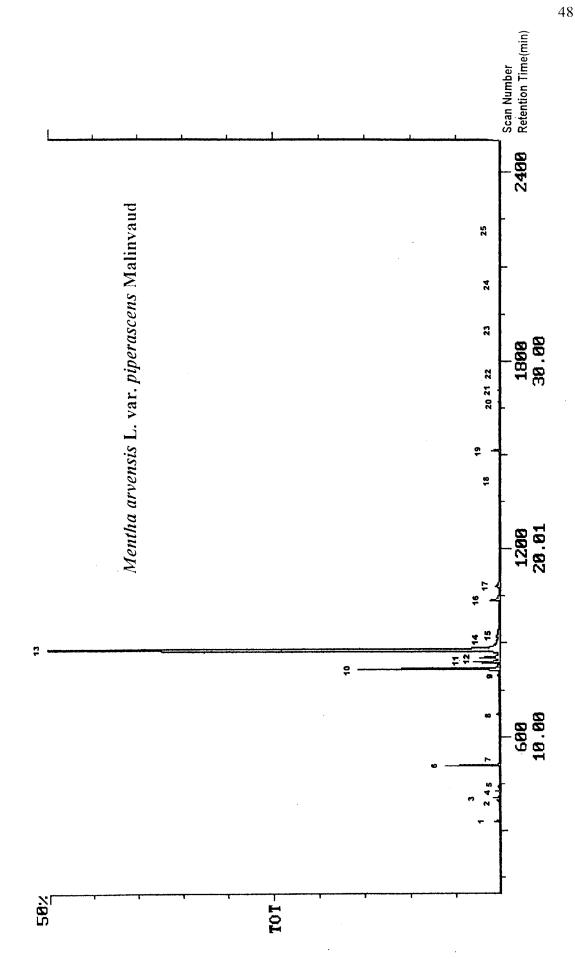


Figure 6 GC chromatogram of the essential oil from Mentha arvensis L.var. piperascens leaves

 Table 5
 Essential oil composition of Mentha arvensis var. piperascens leaves

Number of peak	Compound	Retention time (min)	% Area
P	Monoterpene	(11111)	
1	lpha-thujene	5.50	0.24
2	sabinene	6.35°	0.14
3	$\beta$ -pinene	6.41	0.31
4	myrcene	6.90	0.24
6	limonene	8.01	2.85
	oxygenated monoterpene		
7	1,8-cineole	8.13	0.11
8	linalool	10.63	0.29
()	<i>neo-iso-</i> isopulegol	13.30	t
10	menthone	13.62	9.44
12	neo-menthol	14.23	1.52
13	menthol	14.64	79.44
14	isomenthol	15.07	0.45
15	isobornyl formate	15.35	0.42
16	pulegone	17.23	1.13
17	piperitone	17.95	0.64
	Sesquiterpene		
18	$\beta$ -bourbonene	22.58	t
19	9-epi-(E)-caryophyllene	25.43	0.55
20	γ-muurolene	26.43	0.10
21	bicyclogermacrene	27.61	0.15
22	$\delta$ -cadinene	28.56	t
23	lpha -cadinene	30.01	t
			-
		<u>                                     </u>	

Table 5 Essential oil composition of Mentha arvensis var. piperascens leaves (continued)

Number of peak	Compound	Retention time (min)	% Area
	Oxygenated sesquiterpene		
24	<i>epi-α</i> -cadinol	33.55	t
25	lpha -cadinol	34.04	t ·
	Long chain hydrocarbon		
5	3-octanol	7.37	0.08
	Miscellaneous		
11	unknown	14.01	1.82

t = trace

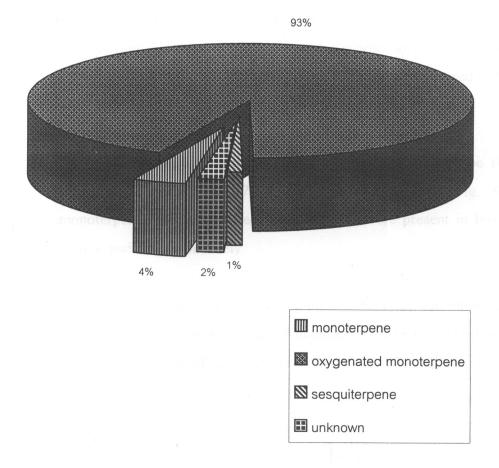


Figure 7 The percentage of various terpenoid groups found in the essential oil of *Mentha arvensis* var. *piperascens* leaves

#### 4.1.4 Essential Oil Composition of Mentha cordifolia Opiz

The essential oil from the leaves of *Mentha cordifolia* Opiz was isolated by hydrodistillation. The oil yield was found to be 0.01 % (v/w) of fresh weight. Analysis of the essential oil by GC/MS showed 37 peaks (Fig 8). These peaks were identified as 5 monoterpenes, 7 oxygenated monoterpenes, 11 sesquiterpenes and 5 oxygenated sesquiterpenes (Table 6). Among these, piperitenone oxide (73.20 %) appeared to be the major component, followed by  $\gamma$ -muurolene (5.80 %) and limonene (4.14 %).

In terms of relative amount, oxygenated monoterpenes appeared to be the major terpenoid group, accounting for 76 % of the essential oil (Fig. 9). Sesquiterpenes, monoterpenes and oxygenated sesquiterpenes were present in lesser amount, with 13%, 6%, and 2%, respectively.

In terms of structure, the major components, piperitenone oxide and limonene, belong to the oxygenated monoterpenoid group of menthane, whereas  $\gamma$ -muurolene belongs to the sesquiterpenoid group of cadinane, respectively.

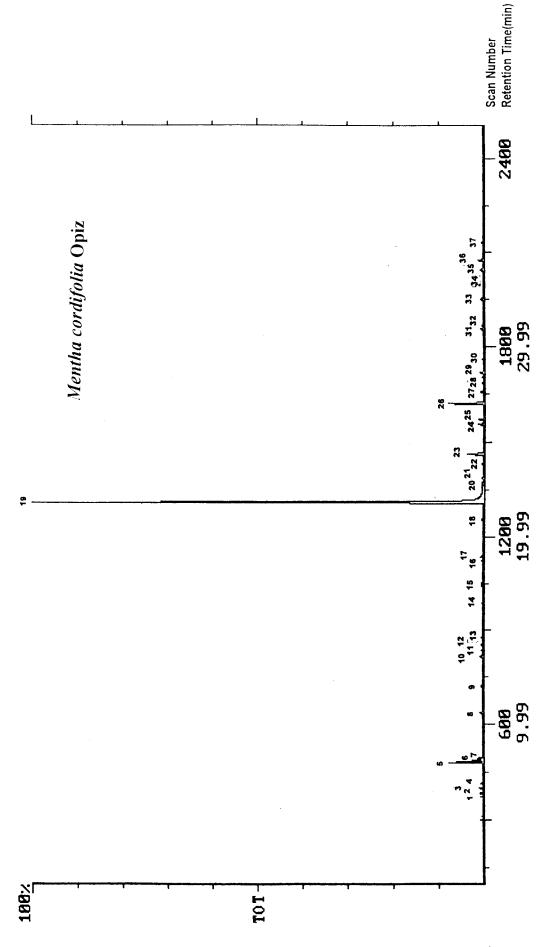


Figure 8 GC chromatogram of the essential oil from Mentha cordifolia leaves

 Table 6
 Essential oil composition of Mentha cordifolia leaves

Number of peak	Compound	Retention time (min)	% Area
	Monoterpene		
ı	sabinene	6.35	0.25
2	$\beta$ -pinene	6.41	0.34
3	myrcene	6.90	0.58
5	limonene	8.01	4.14
7	$(Z)$ - $\beta$ -ocimene	8.23	0.74
	Oxygenated monoterpene		
6	1,8-cineole	8.13	1.32
8	linalool	10.63	0.49
10	borneol	13.58	0.50
11	terpin-4-ol	13.96	0.11
13	$\alpha$ -terpineol	14.21	0.17
18	piperitenone	20.86	0.20
19	piperitenone oxide	21.83	73.20
	Sesquiterpene		
20	eta-bourbonene	22.58	0.46
21	eta-elemene	23.11	0.37
22	lpha-gurjunene	23.86	0.18
23	(E)-caryophyllene	24.36	2.47
24	lpha-humulene	25.91	0.85
25	cis-muurola-4(14), 5-diene	26.10	0.82
26	$\gamma$ -muurolene	26.43	5.80
27	bicyclogermacrene	27.61	0.37
28	germacrene A	27.89	0.26
29	<i>cis</i> -calamenene	28.61	0.84
30	lpha-cadinene	28.88	0.18

 Table 6
 Essential oil composition of Mentha cordifolia leaves (continued)

Number of peak	Compound	Retention time (min)	% Area
	Oxygenated sesquiterpene		
31	spathulenol	30.91	0.42
32	caryophyllene oxide	31.10	0.17
33	1 <i>-epi</i> -cubenol	32.48	0.61
34	<i>epi-<math>\alpha</math></i> -cadinol	33.56	0.12
35	lpha-cadinol	34.04	0.65
	Long Chain hydrocarbon		
4	3-octanol	7.37	0.42
	Miscellaneous		
9	unknown	12.01	0.28
12	unknown	14.28	0.31
14	unknown	16.41	0.35
15	unknown	17.38	0.23
16	unknown	18.66	0.12
17	unknown	18.91	0.41
36	unknown	34.56	1.04
37	unknown	35.49	0.23

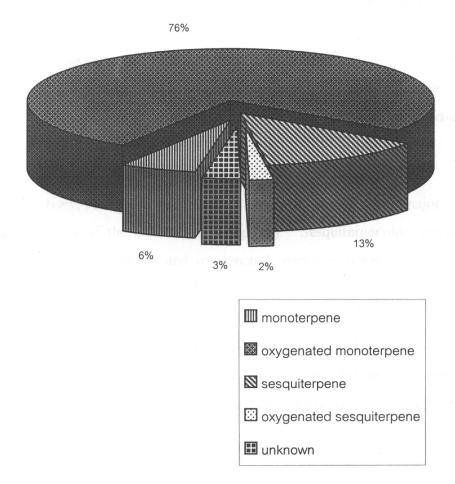


Figure 9 The percentage of various terpenoid groups found in the essential oil of *Mentha cordifolia* leaves

# 4.1.5 Essential Oil Composition of Ocimum basilicum L.

The yield of the essential oil isolated from *Ocimum basilicum* leaves was found to be 0.1 % (v/w) of fresh weight. GC/MS analysis of the essential oil showed that there were 24 components in the oil (Fig. 10). These peaks were identified as 3 monoterpenes, 3 oxygenated monoterpenes, 11 sesquiterpenes, 3 oxygenated sesquiterpenes and 2 non-terpenoid components (Table 7). Among these, methyl chavicol (88.40 %) appeared to be the major component, followed by  $epi-\alpha$ -cadinol (2.38 %) and  $\alpha$ -trans-bergamotene (1.98 %).

Quantitatively, the phenylpropanoid appeared to be the major group, accounting for 89 % of the essential oil (Fig. 11). Sesquiterpenoids, oxygenated sesquiterpenoids, monoterpenoids and oxygenated monoterpenoids were present in lesser amount, at 5 % 3 %, 2 % and 1 %, respectively.

Structurally, the major component, methylchavicol, belongs to the phenylpropanoid group, whereas epi- $\alpha$ -cadinol and  $\alpha$ -trans-bergamotene belong to the sesquiterpenoid group of cadinane and bicyclic sesquiterpene, respectively.

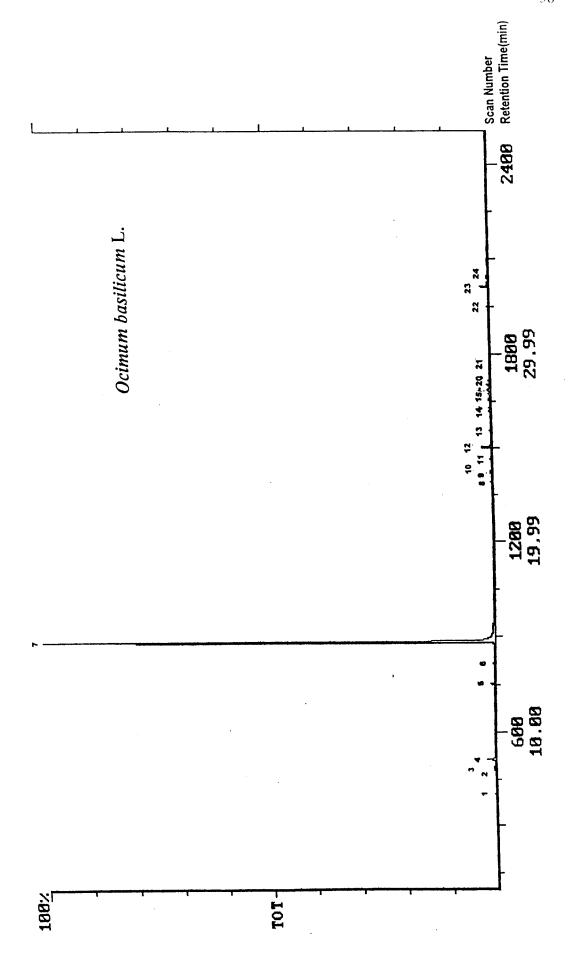


Figure 10 GC chromatogram of the essential oil from Ocimum basilicum leaves

Table 7 Essential oil composition of Ocimum basilicum leaves

Number of peak	Compound	Retention time ( min )	% Area
ОГРешк	Monoterpene		
1	myrcene	6.90	0.11
2	limonene	8.01	0.15
4	(E)- $\beta$ -ocimene	8.59	1.29
	Oxygenated monoterpene		
3	1,8-cineole	8.13	0.31
5	camphor	12.56	0.71
6	borneol	13.61	0.16
	sesquiterpene		
8	$\beta$ -elemene	23.13	0.15
11	(E)-caryophyllene	24.38	0.18
12	α-trans-bergamotene	25.01	1.98
13	$\alpha$ -humulene	25.91	0.25
14	germacrene D	26.56	0.38
15	$(Z)$ - $\alpha$ -bisabolene	27.59	0.13
16	$\beta$ -bisabolene	27.78	0.35
17	germacrene A	27.88	0.27
18	$\alpha$ -bulnesene	28.00	0.41
19	$\gamma$ -cadinene	28.04	0.54
20	$\delta$ -cadinene	28.58	0.17
	Oxygenated sesquiterpene		
22	<i>l-epi</i> -cubenol	32.48	0.24
23	<i>epi-α</i> -cadinol	33.54	2.38
24	$\alpha$ -cadinol	34.04	0.41

Table 7 Essential oil composition of Ocimum basilicum leaves (continued)

Number of peak	Compound	Retention time ( min )	% Area
•	Phenyl propane		
7	methy chavicol	14.73	88.40
9	methyl eugenol	23.66	0.75
	Miscellaneous		
10	unknown	24.18	0.14
21	unknown	29.19	0.14

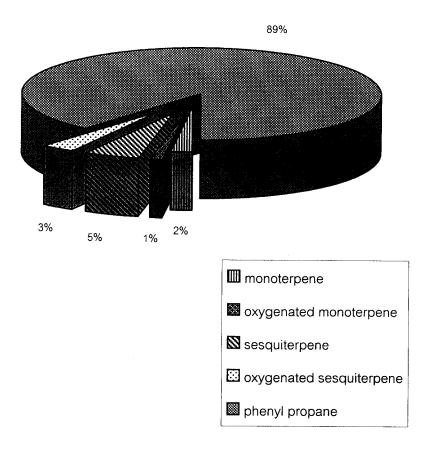


Figure 11 The percentage of various terpenoid groups found in the essential oil of *Ocimum basilicum* leaves

## 4.1.6 Essential Oil Composition of Ocimum canum Sims.

By hydrodistillation, the yield of the essential oil from *Ocimum canum* leaves was found to be 0.2 % (v/w) of fresh weight. Analysis of the essential oil by GC/MS showed 35 separate peaks (Fig.12). These peaks were identified as 5 monoterpenes, 12 oxygenated monoterpenes, 11 sesquiterpenes, 2 oxygenated sesquiterpenes and 1 non-terpenoid component (Table 8). Among these, geranial (22.36 %) appeared to be the major component, followed by geranyl acetate (13.10 %) and (*E*)-caryophyllene (9.55 %).

These major components were in the oxygenated monoterpenoid group, accounting for 49 % of the essential oil (Fig.13). Sesquiterpenes, monoterpenes and oxygenated sesquiterpenes were present in lesser amount, at 26 %, 3 %,1 %, respectively.

In terms of structure, the major components, geranial and geranyl acetate, belong to the oxygenated monoterpenoid group of acyclic monoterpenoid whereas (E)-caryophyllene belongs to sesquiterpenoid group of caryophilane.

(E)-caryophyllene (caryophilane)

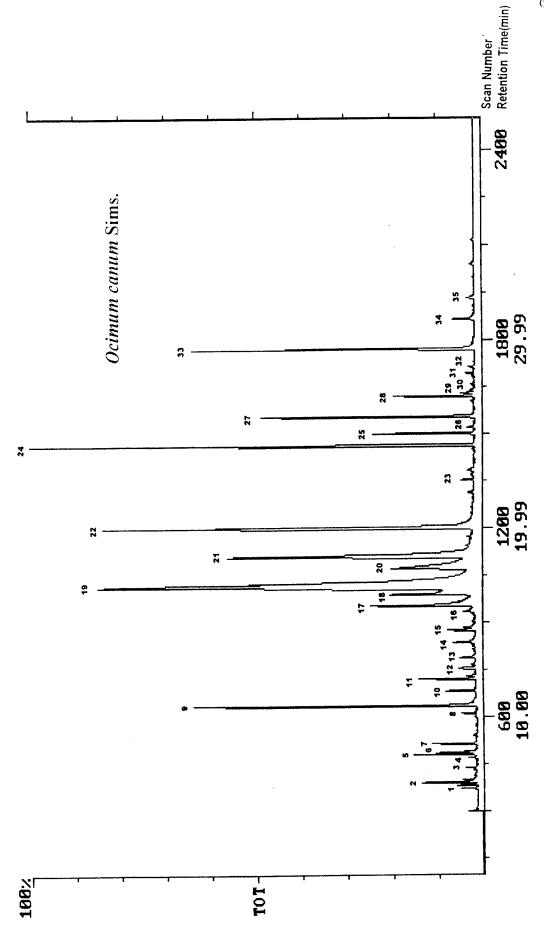


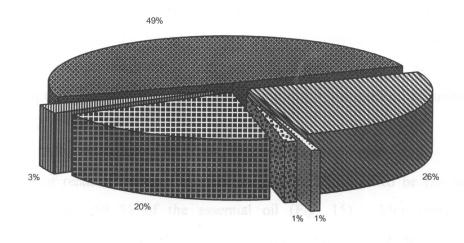
Figure 12 GC chromatogram of the essential oil from Ocimum canum leaves

Table 8 Essential oil composition of Ocimum canum leaves

Number of peak	Compound	Retention time (min)	% Area
	Monoterpene		
1	sabinene	6.35	0.26
3	$\delta$ -3-carene	7.40	0.14
4	o-cymene	7.75	0.13
5	limonene	8.01	1.11
7	(E)- $β$ -ocimene	8.60	0.78
	Oxygenated monoterpene		
6	1,8-cineole	8.13	0.63
8	fenchone	10.08	0.21
9	linalool	10.63	5.42
10	(exo)-fenchol	11.40	0.56
12	$\beta$ -pinene oxide	12.76	0.57
13	trans-verbenol	13.06	0.36
14	cis-chrysanthenol	13.33	0.60
15	$\alpha$ -terpineol	14.21	0.66
17	linalool acetate	15.88	2.98
18	cis-carveol	16.48	1.70
19	geranial	17.81	22.36
22	geranyl acetate	21.15	13.10
	Sesquiterpene		
23	$\alpha$ -copaene	22.51	0.30
24	(E)-caryophyllene	24.36	9.55
25	α-trans-bergamotene	25.00	2.26
26	$(Z)$ - $\beta$ -farnesene	25.34	0.17
27	lpha-humulene	25.89	4.81
28	<i>y</i> -muurolene	26.43	1.88

Table 8 Essential oil composition of Ocimum camum leaves (continued)

Number	Compound	Retention time	% Area
of peak		(min)	
29	$\beta$ -selinene	26.80	0.32
30	$\alpha$ -selinene	27.18	0.10
31	$\beta$ -bisabolene	27.78	0.20
32	$\delta$ -cadinene	28.56	0.14
33	germacrene B	29.51	6,57
	Oxygenated sesquiterpene		
34	caryophyllene oxide	31.09	0.70
35	humulene epoxide II	32.23	0.22
	Phenyl propane		
16	methyl chavicol	14.73	0.38
	Long chain hydrocarbon		
2	6-methyl-5-hepten-2-one	6.53	1.14
	Miscellaneous		
11	unknown	12.05	1.13
20	unknown	18.45	5.57
21	unknown	19.96	12.98



- monoterpene
- oxygenated monoterpene
- **S** sesquiterpene
- a oxygenated sesquiterpene
- 🗵 long chain hydrocarbon
- unknown

Figure 13 The percentage of various terpenoid groups found in the essential oil of *Ocimum canum* leaves

## 4.1.7 Essential Oil Composition of Ocimum gratissimum L.

The yield of essential oil isolated from *Ocimum gratissimum* leaves was found to be 0.3 % (v/w) of fresh weight. Analysis of the essential oil by GC/MS showed 18 peaks (Fig.14). These peaks were identified as 4 monoterpenes, 1 oxygenated monoterpene, 12 sesquiterpenes and 1 non-terpenoid compound (Table 9). Among these, (*E*)-isoeugenol (58.92 %) was found to be the major component, followed by (*Z*)- $\beta$ -ocimene (20.35 %) and germacrene D (7.66 %).

In terms of relative amount, the phenylpropanoid appeared to be the major group, accounting for 59 % of the essential oil (Fig. 15). Monoterpenoids, sesquiterpenoids and oxygenated monoterpenoids were present in lesser amount, with 22 %, 18 % and 1 % respectively (Fig. 15).

Structurally, (E)-isoeugenol is a phenylpropanoid compound. (Z)- $\beta$ -ocimene belongs to the group of acyclic monoterpenoid, while germacrene D belongs to the group of sesquiterpenoid of simple germacrane respectively.

O-Me
HQ (E)-isoeugenol (Z)-
$$\beta$$
-ocimene (phenylpropane) (acyclic monoterpenoid)

germacrene D (simple germacrane )

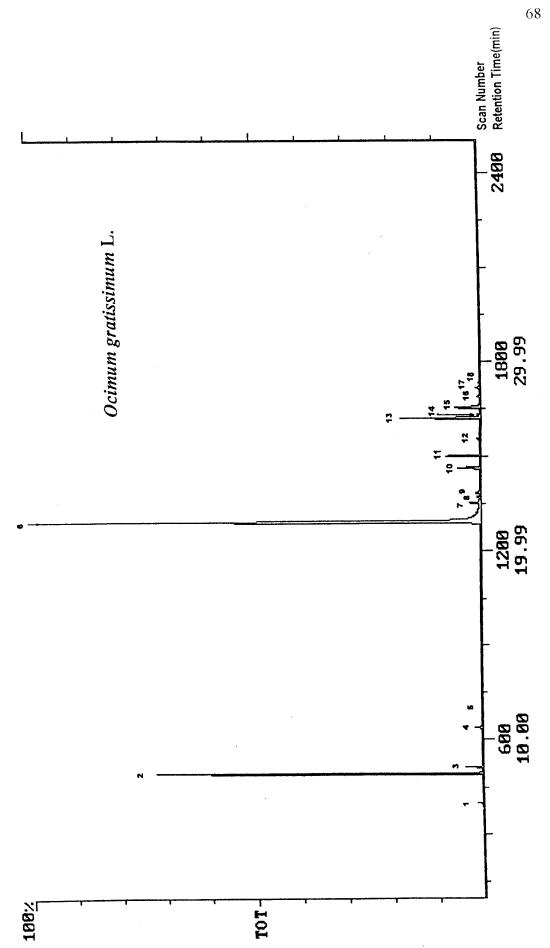


Figure 14 GC chromatogram of the essential oil from Ocimum gratissmum leaves

Table 9 Essential oil composition of Ocimum gratissimum leaves

Number of peak	Compound	Retention time ( min )	% Area
	Monoterpene		
]	myrcene	6.90	0.29
2	$(Z)$ - $\beta$ -ocimene	8.23	20.35
3	(E)-β-ocimene	8.59	1.13
5	neo-allo-ocimene	11.76	0.07
	Oxygenated monoterpene		
4	linalool	10.63	0.64
	Sesquiterpene		
7	lpha-copaene	22.51	0.83
8	$\beta$ -bourbonene	22.60	0.15
9	$\beta$ -cubebene	23.06	0.11
10	(E)-caryophyllene	24.38	2.19
11	α-trans-bergamotene	25.03	3.28
12	$\alpha$ -humulene	25.91	0.24
13	germacrene D	26.56	7.65
14	$\beta$ -selinene	26.80	0.35
15	$(E,E)$ - $\alpha$ -farnesene	27.73	2.94
16	germacrene A	27.90	0.26
17	$\delta$ -cadinene	28.58	0.41
18	$\beta$ -sesquiphellandrene	28.84	0.18
	Phenyl propane		
6	(E)-isoeugenol	21.53	58.92

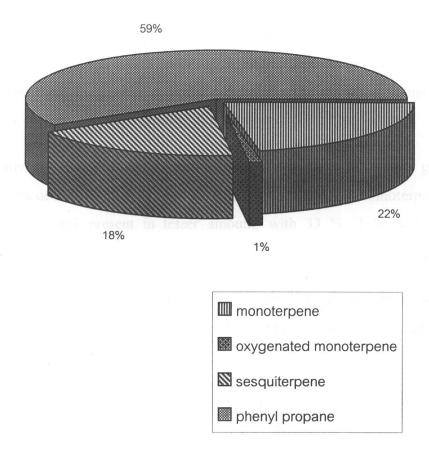


Figure 15 The percentage of various terpenoid groups found in the essential oil of *Ocimum gratissimum* leaves

## 4.1.8 Esssential Oil Composition of Ocimum sanctum L.

The yield of essential oil hydrodistilled from *Ocimum sanctum* L. leaves was found to be 0.2 % (v/w) of fresh weight. Analysis of the essential oil by GC/MS showed 18 peaks (Fig. 16). These peaks were identified as 5 monoterpenes, 3 oxygenated monoterpenes, 8 sesquiterpenes and 2 non-terpenoid components (Table 10). Among these, methyl eugenol (46.08 %) was found to be the major component, followed by 9-*epi* (E)-caryophyllene (23.69 %) and eugenol (19.18 %).

Quantitatively, the phenylpropanoid appeared to be the major group, accounting for 65% of the essential oil. Sesquiterpenoids, oxygenated monoterpenoids and monoterpenoids were present in lesser amount, with 33 %, 1 % and 1 %, respectively (Fig. 17).

Structurally, the major components, methyl eugenol and eugenol belong to the phenylpropanoid group, whereas 9-epi (E)-caryophyllene belongs to the group of sesquiterpenoid of caryophilane.

$$O - Mc$$

$$O - Mc$$

$$O - Mc$$

methyl eugenol

(phenyl propane)

eugenol
(phenyl propane)

9-epi (E)-caryophyllene (caryophilane)

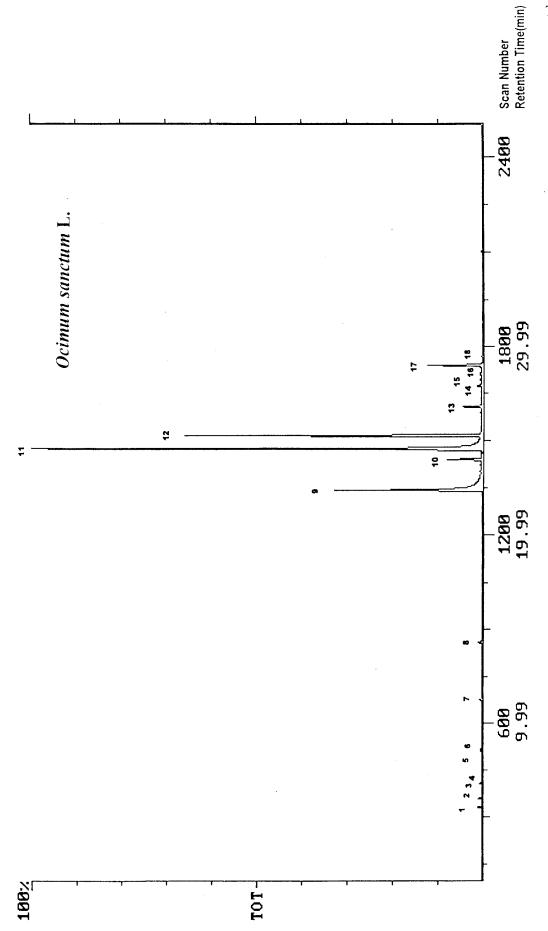
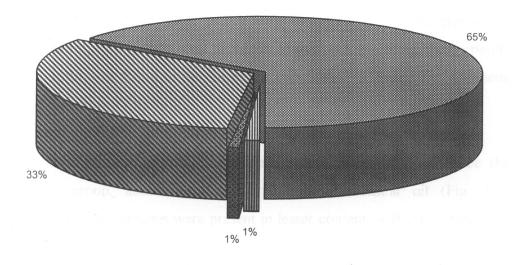


Figure 16 GC chromatogram of the essential oil from Ocimum sanctum leaves

 Table 10
 Esssential oil composition of Ocimum sanctum leaves

Number of peak	Compound	Retention time ( min )	% Area
	Monoterpene		
I	$\alpha$ -thujene	5.50	0.20
2	camphene	5.66	0.21
3	sabinene	6.35	t
4	eta-pinene	6.41	0.14
5	limonene	8.01	0.07
	Oxygenated monoterpene		
6	1,8-cineole	8.13	0.07
7	linalool	10.63	0.32
8	borneol	13.61	0.55
	Sesquiterpene		
10	eta-elemene	23.09	2.65
12	9- <i>epi</i> (E)-caryophyllene	25.43	23.69
13	lpha-humulene	25.91	1.53
14	$\gamma$ -muurolene	26.43	0.39
15	$\beta$ -selinene	26.80	0.12
16	lpha-selinene	27.18	0.14
17	lpha-bulnesene	28.00	4.51
18	$\delta$ -cadinene	28.56	0.11
	Phenyl propane		
9	eugenol	21.31	19.18
11	methyl eugenol	23.66	46.08

t = trace



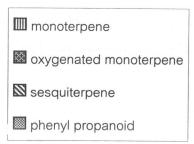


Figure 17 The percentage of various terpenoid groups found in the essential oil of *Ocimum sanctum* leaves

#### 4.1.9 Essential Oil Composition of Perilla frutescens Britt.

By hydrodistillation, the yield of the essential oil from *Perilla frutescens* leaves was found to be 0.1 % (v / w) of fresh weight. GC/MS analysis of the essential oil showed 19 peaks (Fig. 18). These peaks were identified as 4 monoterpenes, 7 oxygenated monoterpenes and 5 sesquiterpenes (Table 11). Among these, perilla aldehyde (57.31 %) was found to be the major component, followed by limonene (20.22 %) and piperitone (7.85 %).

In terms of relative amount, oxygenated monoterpenes appeared to be the major terpenoid group, accounting for 67 % of the essential oil (Fig. 19). Monoterpenes and sesquiterpenes were present in lesser content, with 21 % and 9 %, respectively.

In terms of structure, the major components, perilla aldehyde, piperitone and limonene, belong to the oxygenated monoterpenoid and monoterpenoid groups of menthane.

(menthane)

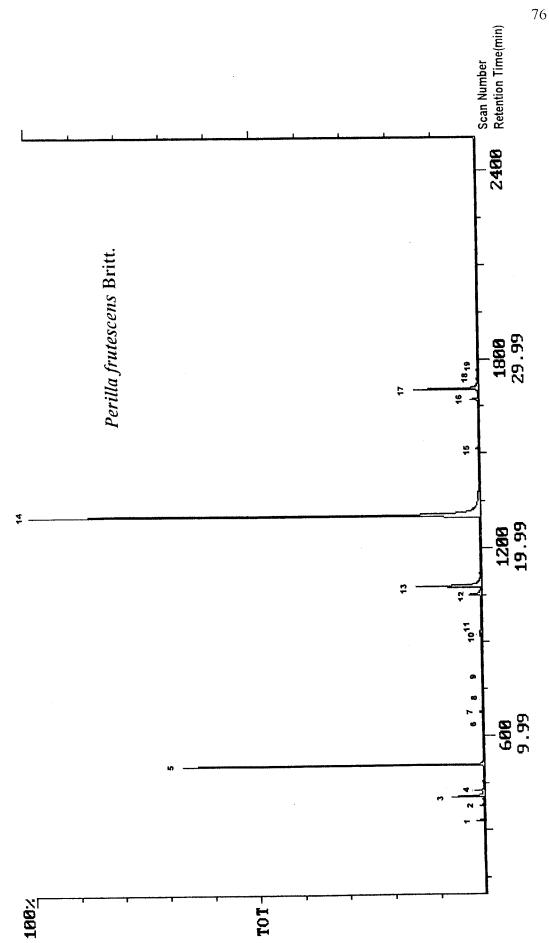
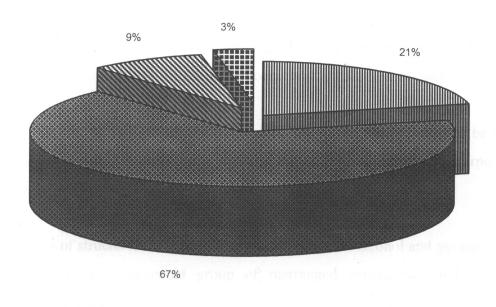


Figure 18 GC chromatogram of the essential oil from Perilla frutescens leaves

 Table 11 Essential oil composition of Perilla frutescens leaves

Number of peak	Compound	Retention time ( min )	% Area
	Monoterpene		
1 .	$\alpha$ -thujene	5.50	0.41
4	myrcene	6.90	0.50
5	limonene	8.01	20.22
6	terpinolene	10.13	0.08
	Oxygenated monoterpene		
8	linalool	10.63	0.39
9	camphor	12.55	t
10	$\alpha$ -terpineol	14.25	0.12
11	dihydro carveol	15.73	0.08
12	carvone	17.86	1.66
13	piperitone	17.93	7.85
14	perilla aldehyde	21.69	57.31
	Sesquiterpene		
15	9-epi-(E)-caryophyllene	25.39	0.23
16	germacrene D	26.56	0.78
17	$(E,E)$ - $\alpha$ -farnesene	27.73	7.29
18	germacrene A	27.89	0.18
19	$\delta$ -cadinene	28.56	0.09
	Miscellaneous		
2	benzaldehyde	6.31	0.35
3	unknown	6.79	2.38
7	dimethyl styrene isomer # 1	10.83	t



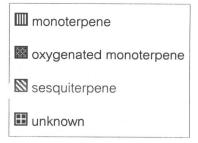


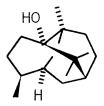
Figure 19 The percentage of various terpenoid groups found in the essential oil of *Perilla frutescens* leaves

#### 4.1.10 Essential Oil Composition of Pogostemon cablin Benth.

The essential oil from the leaves of *Pogostemon cablin* Benth, was isolated by hydrodistillation. The oil yield was found to be 0.30 % (v/w) of fresh weight. Analysis of the essential oil by GC/MS showed 23 peaks (Fig. 20). These peaks were identified as 18 sesquiterpenes. 3 oxygenated sesquiterpenes (Table 12). Among these, patchouli alcohol (60.30 %) appeared to be the major component, followed by germacrene A (11.73 %).

In terms of relative amount, the oxygenated sesquiterpenes appeared to be the major terpenoid group, accounting for 65 % of the essential oil and sesquiterpenes were present in a lesser content 35 %. (Fig.21)

In terms of structure, the major components, patchouli alcohol and germacrene A, belong to the sesquiterpenoid group of rearranged patchoulane and simple germacrane, respectively.



patchouli alcohol (rearranged patchoulane)

germacrene A (simple germacrane)

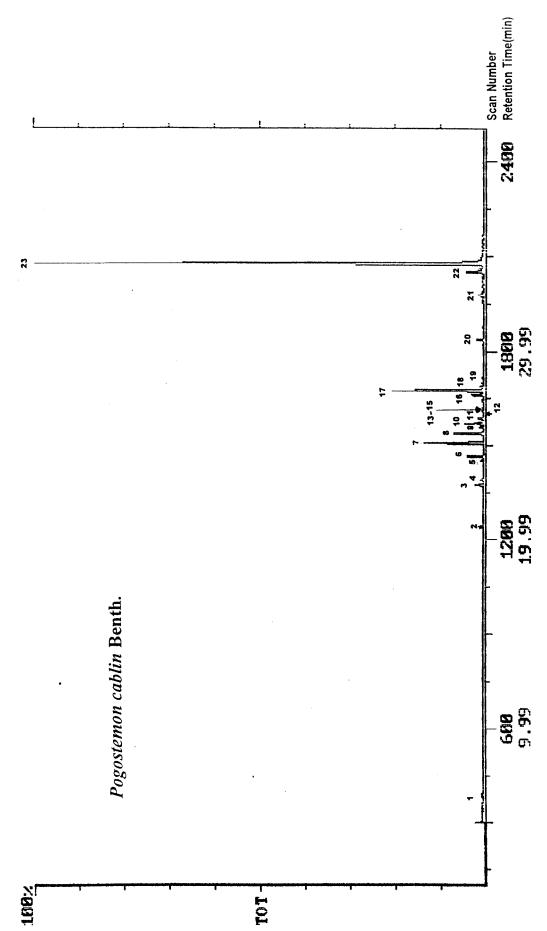
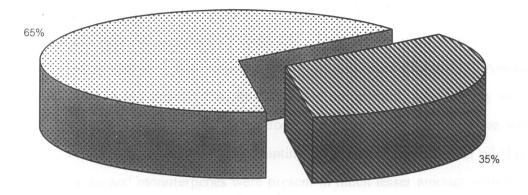


Figure 20 GC chromatogram of the essential oil from Pogostemon cablin leaves

 Table 12 Essential oil composition of Pogostemon cablin leaves

Number of peak	Compound	Retention time (min)	% Area
	Sesquiterpene		
2	$\delta$ -elemene	20.79	t
3	$\beta$ -patchoulene	22.90	t
4	eta–elemene	23.09	0.33
5	cis-thujopsene	24.20	0.25
6	(E)-caryophyllene	24.38	2.24
7	$\alpha$ guaiene	24.85	7.22
8	γ-patchoulene	25.63	3.89
9	lpha-humulene	25.91	0.48
10	lpha-patchoulene	26.15	2.27
11	seychellene	26.28	0.98
12	valencene	26.45	0.85
13	germacrene D	26.56	0.15
14	$\beta$ -selinene	26.80	t
15	$\alpha$ –selinene	27.18	0.23
16	viridiflorene	27.68	1.91
17	germacrene A	27.89	11.73
18	$\alpha$ -bulnesene	28.00	0.86
19	7-epi- $\alpha$ -selinene	28.59	0.17
	Oxygenated sesquiterpene		
21	longipinanol	33.01	t
22	globulol	34.18	4.62
23	patchouli alcohol	34.64	60.30
	Long chain hydrocarbon		
1	I-octen-3-ol	6.79	0.20
	Miscellaneous		
20	unknown	30.12	1.19

t=trace



- sesquiterpene
- ☑ oxygenated sesquiterpene

Figure 21 The percentage of various terpenoid groups found in the essential oil of *Pogostemon cablin* leaves

# 4.2 Chemical Composition of Essential Oil from Western Country

## 4.2.1 Essential Oil Composition of Melissa officinalis L.

The yield of essential oil hydrodistilled from *Melissa officinalis* leaves was found to be 0.1 % (v/w) of fresh weight. Analysis of this essential oil by GC/MS showed that there were 16 components (Fig. 22). These peaks were identified as 3 monoterpenes, 10 oxygenated monoterpenes, and 1 sesquiterpene (Table 13). Among these, geranial (40.75 %) appeared to be the major component, followed by neral (28.85 %) and terpin-4-ol (7.95 %). Therefore, the oxygenated monoterpene was found to be the major terpenoid group, accounting for 90 % of the essential oil (Fig. 23). Sesquiterpenes and monoterpenes were present in much lesser amount with 5 % and 3 %, respectively.

Structurally, geranial and neral belong to the oxygenated monoterpenoid group of acyclic monoterpenoid whereas terpin-4-ol belongs to the group of menthane.

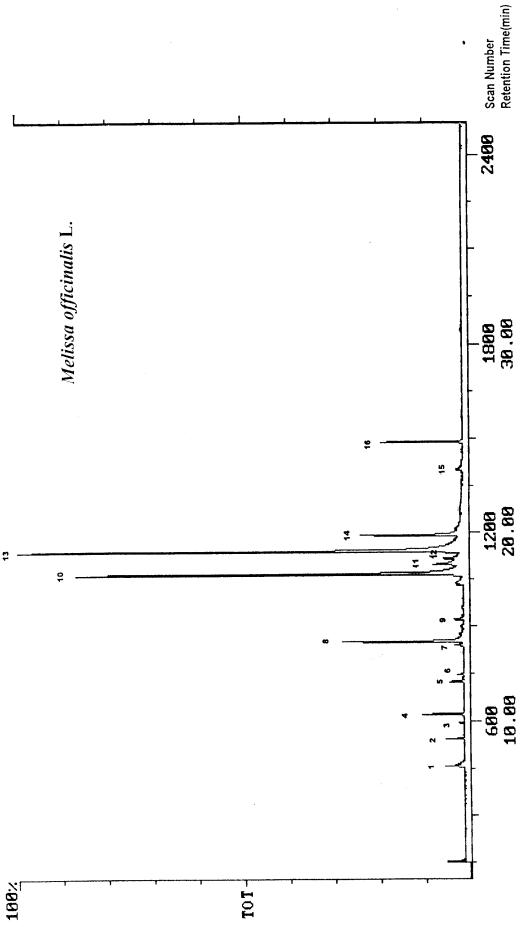


Figure 22 GC chromatogram of the essential oil from Melissa officialis leaves

 Table 13
 Essential oil composition of Melissa officinalis
 leaves

Number of peak	Compound	Retention time ( min )	% Area
	Monoterpene		
2	o-cymene	7.75	0.73
3	γ-terpinene	9.08	0.26
4	terpinolene	10.14	2.06
	Oxygenated monoterpene		
5	isopulegol	12.13	1.04
6	citronellal	12.88	0.34
7	neo-iso-isopulegol	13.30	0.56
8	terpin-4-ol	13.94	7.95
10	neral	16.60	28.85
11	geraniol	17.01	2.77
12	methyl citronellate	17.53	0.78
13	geranial	17.81	40.75
14	thymol	20.01	6.64
15	geranyl acetate	21.15	0.64
	Sesquiterpene		
16	9-epi- <i>(E)</i> -caryophyllene	25.43	4,55
. •		20.10	.,, -
	Long chain hydrocarbon		
1.	<i>6-methyl-5</i> -hepten-2-one	6.53	2.06
	Miscellaneous		
9	unknown	15.33	0.67

t = trace

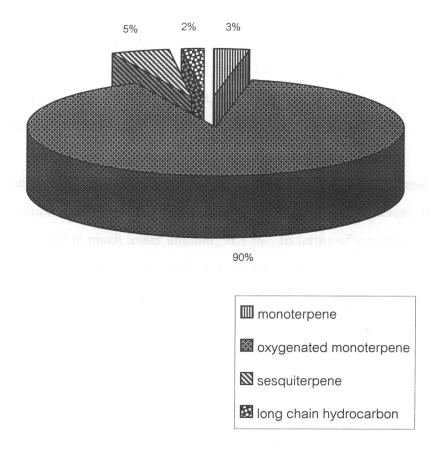


Figure 23 The percentage of various terpenoid groups found in the essential oil of *Melissa officinalis* leaves

### 4.2.2 Essential oil composition of Mentha piperita L.

By hydrodistillation, the yield of the essential oil from *Mentha piperita* leaves was found to be 0.2 % (v/w) of fresh weight. GC/MS analysis of the essential oil showed 16 peaks (Fig. 24). These peaks were identified as 3 monoterpenes, 11 oxygenated monoterpenes and 2 sesquiterpenes (Table 14). Among these, menthol (30.37 %) was found to be the major component, followed by menthone (24.63 %) and isomenthyl acetate (20.46 %).

As for the component, the oxygenated monoterpene appeared to be the major terpenoid group, accounting for 99% of the essential oil (Fig. 25). Whereas the sesquiterpenoid was present in much lesser amount, at 1 %. In terms of structure, the major components, menthol, menthone and isomenthyl acetate, belong to the oxygenated monoterpenoid group of menthane.

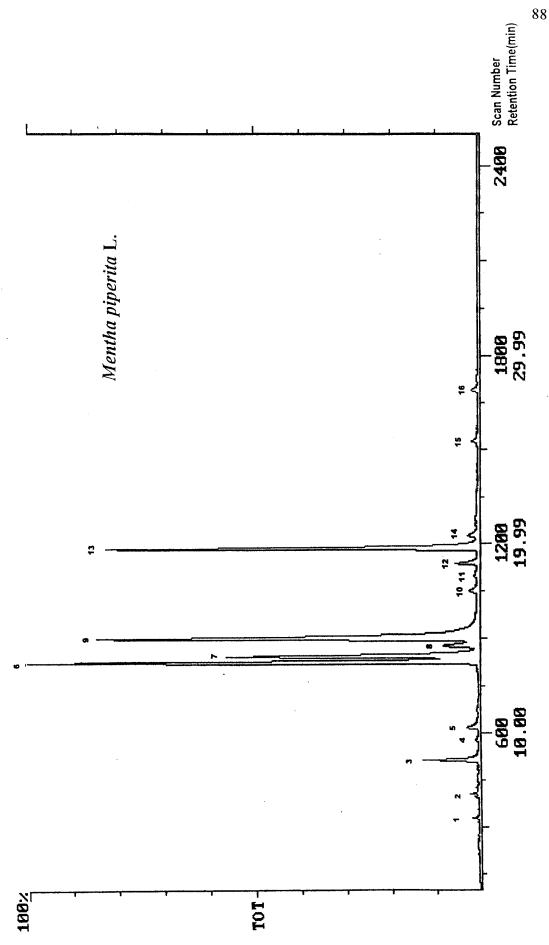
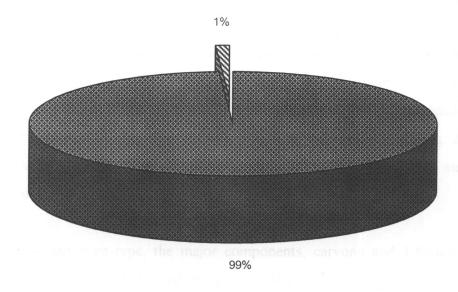


Figure 24 GC chromatogram of the essential oil from Mentha piperita leaves

 Table 14 Essential oil composition of Mentha piperita leaves

Number of peak	Compound	Retention time ( min )	% Area
	Monoterpene		
1	lpha-thujene	5.50	0.12
2	sabinene	6,35	0.22
4	γ-terpinene	9.08	0.11
	Oxygenated monoterpene		
3	1,8-cineole	8.14	2.41
5	trans-sabinene hydrate	10.48	0.71
6	menthone	13.62	24.63
7	menthofuran	14.10	15.32
8	neo-menthol	14.23	2.25
9	menthol	14.64	30.37
10	pulegone	17.23	0.61
11	piperitone	17.95	0.17
12	menthyl acetate	18.73	1.30
13	isomenthyl acetate	19.68	20.46
14	neo-iso-carvomenthyl acetate	20.38	0.58
	Sesquiterpene		
15	9-epi-(E)-caryophyllene	25.43	0.35
16	$\gamma$ -cadinene	28.04	0.39



oxygenated monoterpenesesquiterpene

Figure 25 The percentage of various terpenoid groups found in the essential oil of *Mentha piperita* leaves

### 4.2.3 Essential oil Composition of Mentha spicata L.

The yield of the essential oil isolated from *Mentha spicata* leaves was found to be 0.3 % (v/w) of fresh weight. GC analysis of the essential oil showed that there were at least 25 components present in the oil (Fig. 26). These components were identified as 8 monoterpenes, 9 oxygenated monoterpenes and 5 sesquiterpenes (Table 15). Among these, carvone (57.68 %) was found to be the major components contributed greatly to the overall oxygenated monoterpenoids content which appeared to be the major terpenoid group, accounting for 91 % of the essential oil (Fig. 27). This was followed by the sesquiterpenes and monoterpenes were present in much lesser amount, with 4 % and 4 %.

In terms of structure type, the major components, carvone and 1,8-cineole, belong to the oxygenated monoterpenoid group of menthane.



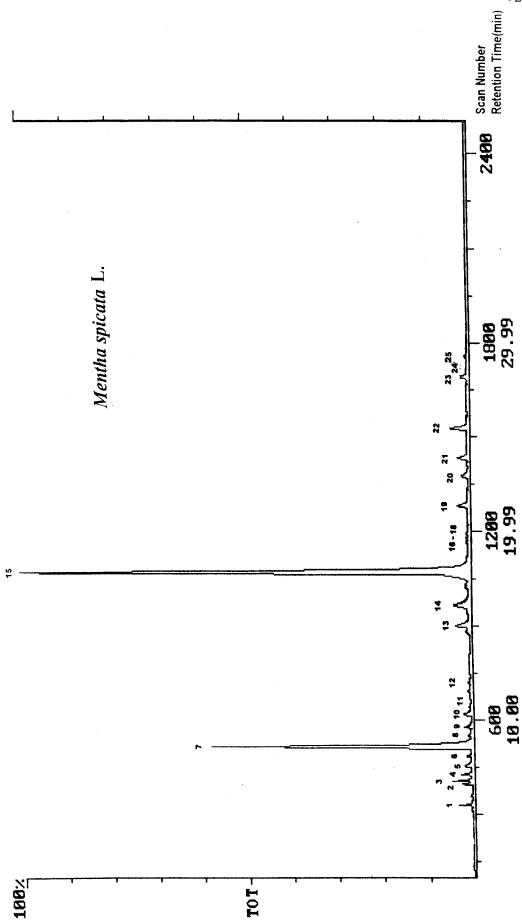


Figure 26 GC chromatogram of the essential oil from Mentha spicata leaves

 Table 15
 Essential oil composition of Mentha spicata leaves

Number of peak	Compound	Retention time ( min )	% Area
or peak	Monoterpene	(11111)	
I	$\alpha$ -thujene	5.50	0.52
2	sabinene	6.35	0.50
3	eta-pinene	6.41	1.08
. 4	myrcene	6.90	0.61
6	lpha-terpinene	7.61	0.21
8	$(E)$ - $\beta$ -ocimene	8.60	t
9	γ-terpinêne	9.08	0.52
11	terpinolene	10.13	0.15
	Oxygenated monoterpene .		
7	1,8-cineole	8.14	25.00
10	cis-sabinene hydrate	10.01	0.80
13	terpin-4-ol	13.94	1.85
14	neo-iso-dihydrocarveol	16.05	2.90
15	carvone	17.86	57.68
16	bornyl acetate	18.50	0.30
19	neo-iso-dihydrocarveol acetate	21.31	1.19
20	cis-carvyl acetate	21.60	0.85
	Sesquiterpene		
21	$\beta$ -bourbonene	22.58	1.24
22	9-epi-(E)-caryophyllene	25.43	2.03
23	germacrene D	26.56	0.74
24	bicycolgermacrene	27.61	0.25
25	germacrene A	27.89	0.21

 Table 15
 Essential oil composition of Mentha spicata leaves (continued)

Number of peak	Compound	Retention time ( min )	% Area
	Long chain hydrocarbon		
5	3-octanol	7.56	0.82
12	3-octanol acetate	11.66	0.16
	Miscellaneous		·
17	unknown	19.59	0.15
18	unknown	19.83	0.24

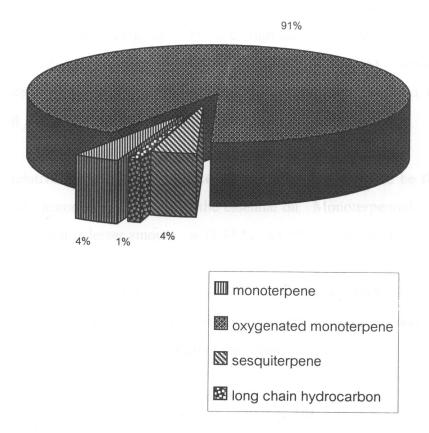


Figure 27 The percentage of various terpenoid groups found in the essential oil of *Mentha spicata* leaves

#### 4.2.4 Essential Oil Composition of Origanum majorana L.

The yield of essential oil from *Origanum majorana* leaves was found to be 0.1 % (v/w) of fresh weight. Analysis of the essential oil by GC/MS showed that there were at least 24 components (Fig. 28). These components were identified as 8 monoterpenes, 5 oxygenated monoterpenes and 3 sesquiterpenes (Table 16). Among these, terpin-4-ol (28.37 %) was found to be the major component, followed by linalool (20.7 %) and  $\beta$ -phellandrene (10.75 %).

In terms of relative amount, the oxygenated monoterpene appeared to be the major terpenoid group, accounting for 54 % of the essential oil. Monoterpenoid and sesquiterpenoid were present in lesser amount, with 33 % and 5 %, respectively (Fig. 29).

Structurally, the major components, terpin-4-ol and linalool, belong to the oxygenated monoterpenoid group of menthane and acyclic monoterpenoid whereas  $\beta$ -phellandrene belongs to the monoterpenoid group of menthane.

(menthane)

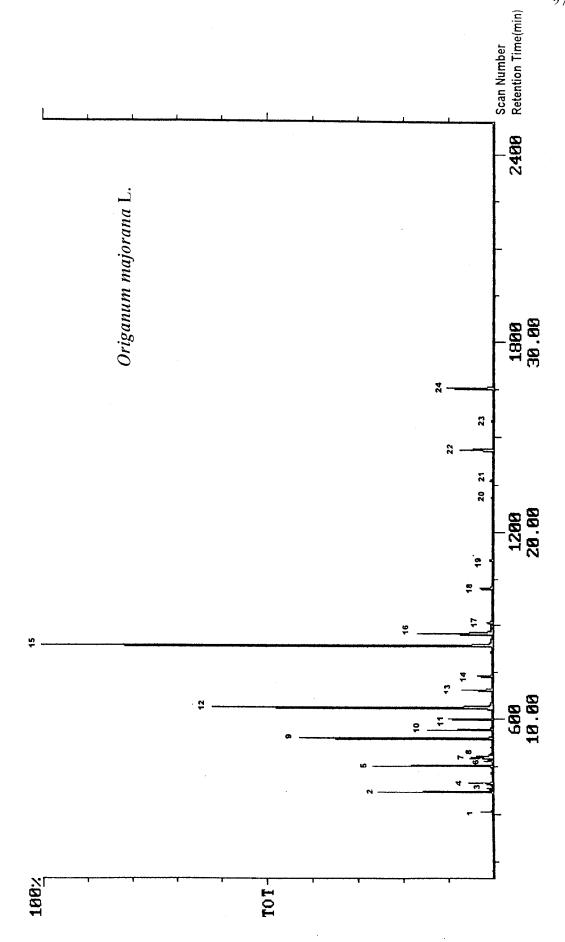


Figure 28 GC chromatogram of the essential oil from Origanum majorana leaves

 Table 16
 Essential oil composition of Origanum majorana leaves

Number of peak	Compound	Retention time ( min )	% Area
ОТРОИК	Monoterpene	()	
1	tricyclene	5.28	0.41
2	sabinene	6.35	3.57
5	$\delta$ -2-carene	7.12	5.12
6	o-cymene	7.75	0.40
7	limonene	8.01	1.26
8	$\beta$ -phellandrene	8.03	10.75
9	γ-terpinene	9.08	8.68
11	terpinolene	10.13	2.30
	Oxygenated monoterpene		
12	linalool	10.64	20.74
15	terpin-4-ol	13.94	28.37
16	lpha-terpineol	14.21	4.97
17	trans-dihydro carvone	14.97	t
19	bornyl acetate	18.49	t
21	geranyl acetate	21.15	0.21
	Sesquiterpene		
22	(E)-caryophyllene	24.33	2.19
23	lpha-humulene	25.91	0.10
24	bicyclogermacrene	27.61	2.99
	Miscellaneous		:
3	unknown	6.36	0.23
4	unknown	6.63	0.96
10	นแห่nown	9.43	3.37
13	unknown	11.57	1.55
14	unknown	12.30	0.88
18	unknown	16.97	0.83
20	unknown	20.90	0.12

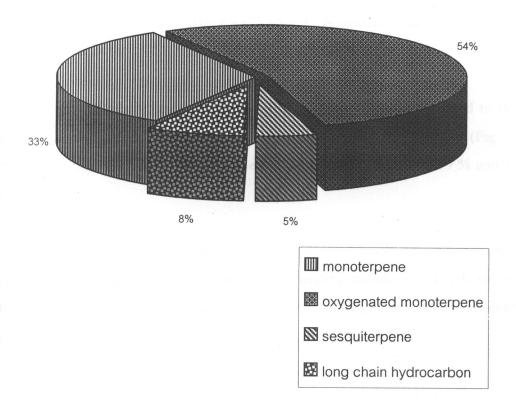


Figure 29 The percentage of various terpenoid groups found in the essential oil of *Origanum majorana* leaves

#### 4.2.5 Essential oil Composition of Origanum vulgare L.

The leaves of *Origanum vulgare* were found to contain essential oil with the content of 0.2 % (v/w) of fresh weight. GC/MS analysis showed at least 26 peaks in its GC chromatogram (Fig. 30). These peaks were identified as 13 monoterpenes, 6 oxygenated monterpenes and 5 sesquiterpenes (Table 17). Among these, the major components were found to be carvacrol (75.63 %), p-mentha-2,4(8)-diene (10.11 %) and o-cymene 2.34 %.

In terms of relative amount, the oxygenated monoterpenes appeared to be the major terpenoid group, accounting for 77 % of the essential oil (Fig. 31). Monoterpenes and sesquiterpenes were present in lesser amount, with 19 % and 4 %, respectively.

In terms of structure, the major components, carvacrol, belongs to the oxygenated monoterpenoid group of menthane whereas p-mentha -2,4 (8) diene and o-cymene belong to the monoterpenoid groups of menthane and o-menthane respectively.

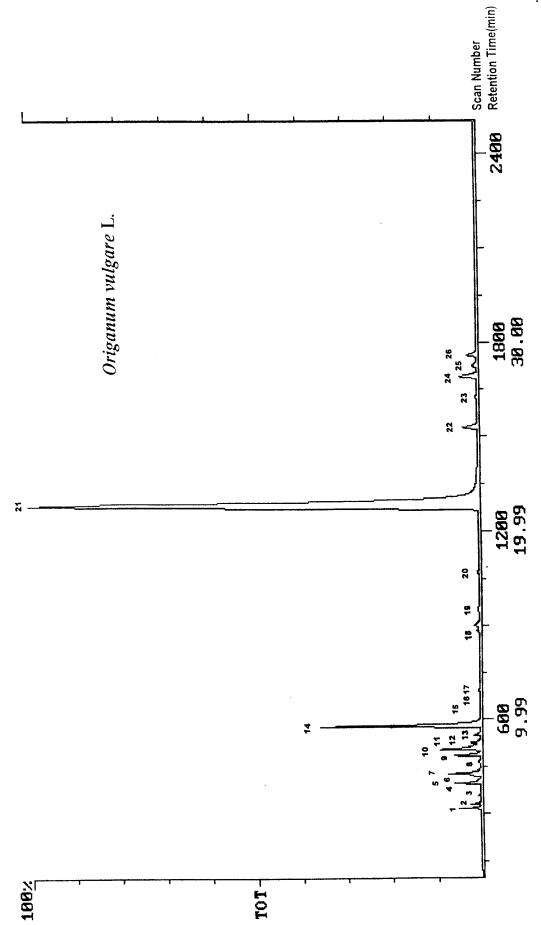


Figure 30 GC chromatogram of the essential oil from Origanum vulgare leaves

 Table 17
 Essential oil composition of Origanum vulgare leaves

Number of peak	Compound	Retention time (min)	% Area
	Monoterpene		
1	tricyclene	5.28	0.71
2	$\alpha$ -thujene	5.50	0.29
3	camphene	5.66	0.11
4	sabinene	6.35	0.98
7	myrcene	6.92	1.65
8	$\delta$ -3-carene	7.41	0.17
9	lpha-terpinene	7.62	1.28
10	o-cymene	7.76	2.34
11	$(Z)$ - $\beta$ -ocimene	8.23	0.51
12	$(I^r)$ - $\beta$ -ocimene	8.60	0.56
13	$\gamma$ -terpinene	9.08	0.36
14	<i>p</i> -mentha-2,4(8)-diene	9.63	10.11
16	terpinolene	10.13	0.06
	Oxygenated monoterpene		
15	<i>trans</i> - sabinene hydrate	10.48	0.07
17	trans-para-menth-2-en-1-ol	11.59	0.19
18	borneol	13.62	0.13
19	α-terpineol	14.20	0.26
20	carvone	17.86	0.18
21	carvacrol	20.33	75.63
	Sesquiterpene		
22	9-epi-E-caryophyllene	25.43	1.19
23	lpha-humulene	25.91	0.14
24	germacrene D	26.56	1.49
25	bicyclogermacrene	27.61	0.38

Table 17 Essential oil composition of Origanum vulgare leaves (continued)

Number of peak	Compound	Retention time (min)	% Area
26	$\beta$ -bisabolene	27.78	0.78
	Long chain hydrocarbon		
6	1-octen-3-ol	6.79	0.25
	Miscellaneous		
5	unknown	6.78	0.18

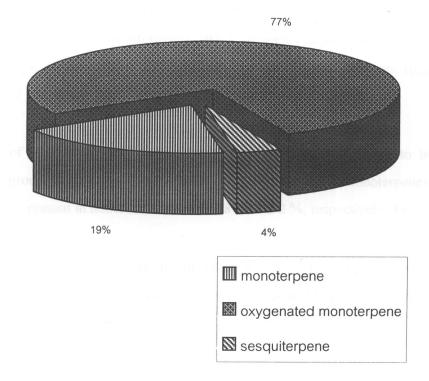


Figure 31 The percentage of various terpenoid found in the essential oil of *Origanum vulgare* leaves

#### 4.2.6 Esssential Oii Composition of Rosmarinus officinalis L.

The yield of essential oil hydrodistilled from *Rosmarinus officinalis* leaves was found to be 0.9 % (v/w) of fresh weight. Analysis of the essential oil by GC/MS showed 40 peaks (Fig.32). These peaks were identified as 14 monoterpenes, 19 oxygenated monoterpenes, 3 sesquiterpenes and 1 oxygenated sesquiterpene (Table 18). Among these,  $\alpha$ -pinene (22.48 %) appeared to be the major component followed by camphor (20.07 %).

In terms of relative amount, the oxygenated monoterpene was found to be the major terpenoid group, accounting for 59 % of the essential oil. Monoterpenes and sesquiterpenes were present in lesser amount, with 38 % and 2 %, respectively (Fig. 33).

In terms of structure type, the major components,  $\alpha$ -pinene, belongs to the monoterpenoid group of pinane, while camphor belongs to the oxygenated monoterpenoid group of camphane.



 $\alpha$ -pinene

(pinane)

camphor

(camphane)

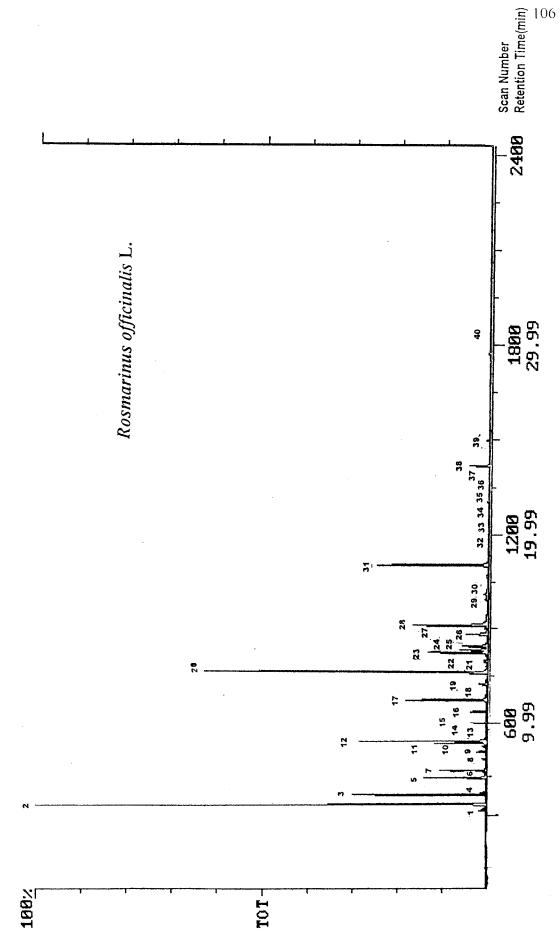


Figure 32 GC chromatogram of the essential oil from Rosmarinus officinalis leaves

 Table 18 Esssential oil composition of Rosmarinus officinalis leaves

Number of peak	Compound	Retention time ( min )	% Area
or peak	Monoterpene	( mm )	
1	tricyclene	5.30	0.26
2	$\alpha$ -pinene	5.33	22.48
3	camphene	5.66	2.96
4	thuja 2,4(10)-diene	5.78	0.32
5	$\beta$ -pinene	6.43	3.41
7	myrcene	6.90	2.42
8	lpha-phellandrene	7.26	t
9	$\delta$ -3-carene	7.40	0.54
10	o-cymene	7.75	t
11	sylvestrene	7.90	3.26
13	(Z)-β-ocimene	8.23	t
14	γ-terpinene	9.08	1.00
16	terpinolene	10.14	0.99
	Oxygenated monoterpene		
12	1,8-cineole	8.13	7.82
17	linalool	10.63	5.63
19	chrysanthenone	11.43	0.50
20	camphor	12.56	20.07
21	trans-pinocamphone	13.09	t
22	pinocarvone	13.34	t
23	borneol	13.61	4.63
24	cis-pinocamphone	13.76	1.95
25	terpin-4-ol	13.94	1.81

 Table 18 Esssential cil composition of Rosma inus officinalis leaves (continued)

Number of peak	Compound	Retention time ( min )	% Area
26	$\alpha$ -terpineol	14.21	1.65
27	isobornyl formate	15.35	t
28	(E)-ocimenone	16.30	6.26
31	bornyl acetate	18.49	8.67
32	cis-pinocarvyl acetate	21.13	0.08
34	neo-iso-dihydro carveol acetate	21.31	0.08
35	trans-myrtanol acetate	22.93	0.21
	Sesquiterpene		
36	γ-elemene	24.60	t
38	9-epi-(E)-caryophyllene	25.43	1.48
39	$(Z)$ - $\alpha$ -bisabolene	27.59	0.23
	Oxygenated sesquiterpene		
40	caryophyllene oxide	31.09	0.29
	Phenpyl propane		
37	(Z)-methyl isoeugenol	25.23	0.12
	Long chain hydrocarbon		
6	3-octanone	6.67	t
	Miscellaneous		
15	unknown	10.00	0.08
18	unknown	11.36	0.09
29	unknown	17.80	0.23
30	unknown	18.06	0.48
33	unknown	21.53	t

t=trace

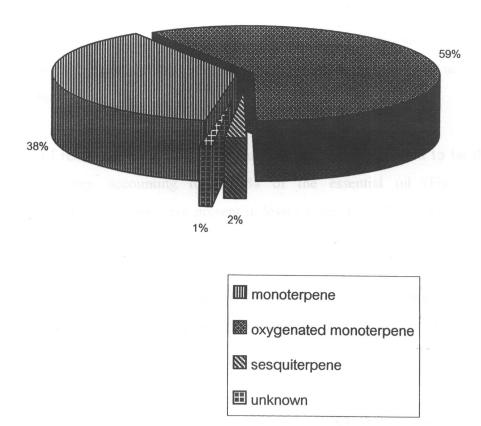


Figure 33 The percentage of terpenoid groups found in the essential oil of *Rosmarinus officinalis* leaves

### 4.2.7 Esssential Oil Composition of Salvia officinalis L.

The yield of essential oil hydrodistilled from *Salvia officinalis* leaves was found to be 0.3 % (v/w) of fresh weight. Analysis of the essential oil by GC/MS showed 26 peaks (Fig. 34). These peaks were identified as 7 monoterpenes, 11 oxygenated monoterpenes and 4 sesquiterpenes (Table 19). Among these, *cis*-thujone (37.49 %) was found to be the major component followed by camphor (13.79 %) and  $\alpha$ -humulene (9.46 %).

In terms of relative amount, the oxygenated monoterpene appeared to be the major terpenoid group, accounting for 71 % of the essential oil (Fig. 35). Sesquiterpenes and monoterpenes were present in lesser amount, at 20 % and 4 %, respectively.

In terms of structure, the major components, *cis*-thujone and camphor, belong to the oxygenated monoterpenoid groups of thujane and camphane, respectively.

α-humulene (humulane)

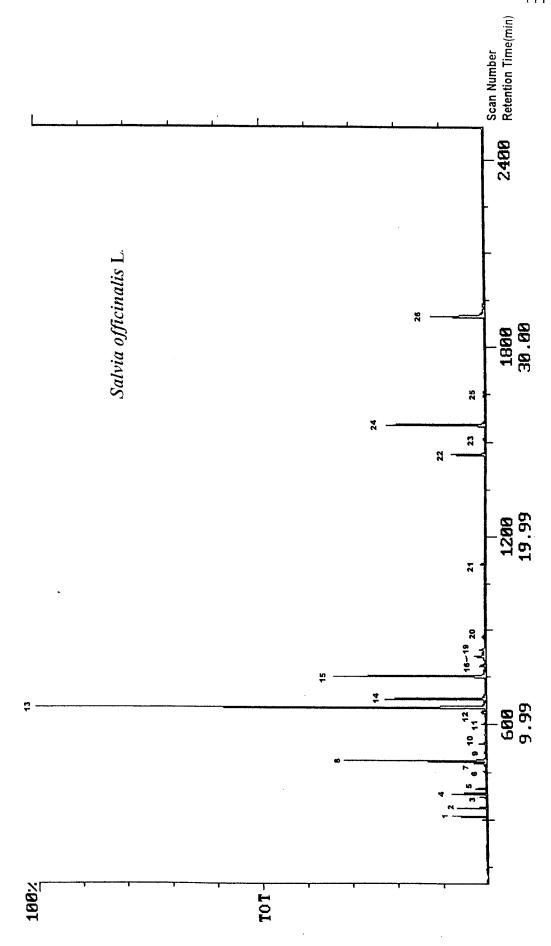


Figure 34 GC chromatogram of the essential oil from Salvia officinalis leaves

 Table 19
 Esssential oil composition of Salvia officinalis
 leaves

Number of peak	Compound	Retention time ( min )	% Area
	Monoterpene		
] 1	tricyclene	5.28	1.80
3	sabinene	6.35	0.35
6	$\delta$ -2-carene	7.12	0.22
7	limonene	8.01	0.81
9	(Z) β-ocimene	8.23	0.44
10	<i>γ</i> -terpinene	9.08	0.44
11	terpinolene	10.13	t
	Oxygenated monoterpene		
8	1,8-cineole	8.14	9.38
12	linalool	10.63	t
13	cis-thujone	10.89	37.49
14	trans-thujone	11.33	7.89
15	camphor	12.55	13.79
16	trans-pinocamphone	13.09	0.59
17	borneol	13.59	0.91
18	cis-pinocamphone	13.76	t
19	terpin-4-ol	13.94	0.41
20	$\alpha$ -terpineol	14.20	t
21	bornyl acetate	18.51	0.44
	Sesquiterpene		
22	(E)-caryophyllene	24.36	3.30
24	lpha-humulene	25.88	9.46
25	bicyclogermacrene	27.61	0.16
26	germacrene B	31.60	7.33

 Table 19
 Esssential oil composition of Salvia officinalis leaves (continued)

Number of peak	Compound	Retention time ( min )	% Area
	Miscellaneous		
2	urknown	5.63	1.63
4	unknown	6.38	2.33
5	unknown	6.66	0.65
23	unknown	25.15	0.15

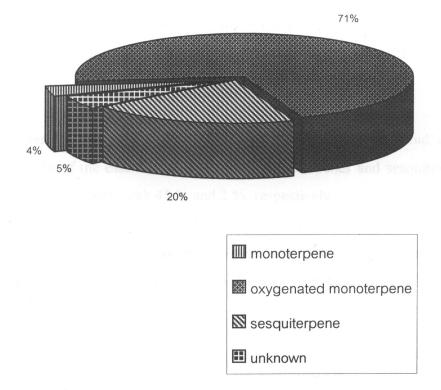


Figure 35 The percentage of various terpenoid groups found in the essential oil of *Salvia officinalis* leaves

### 4.2.8 Esssential Oil Composition of *Thymus* sp. 1 (summer thyme)

The yield of essential oil from *Thymus* sp.1 leaves was found to be 0.9 % (v/w) of fresh weight. Analysis of the essential oil by GC/MS showed that there were at least 18 components (Fig. 36). These components were identified as 8 monoterpenes, 8 oxygenated monoterpenes and 2 sesquiterpenes (Table 20). Among these, thymol (26.43 %) appeared to be the major component followed by  $\gamma$ -terpinene (21.63 %) and  $\sigma$ -cymene (15.87 %).

The oxygenated monoterpene was found to be the major terpenoid group, accounting for 53 % of the essential oil (Fig. 37). Monoterpenes and sesquiterpenes were present in lesser amount, with 45 % and 2 %, respectively.

Structurally, the major components, thymol and  $\gamma$ -terpinene, belong to the monoterpenoid group of menthane, while o-cymene belongs to the group of o-menthane.

*o*-cymene (*o-menthane*)

Figure 36 GC chromatogram of the essential oil from Thymus sp. 1 leaves

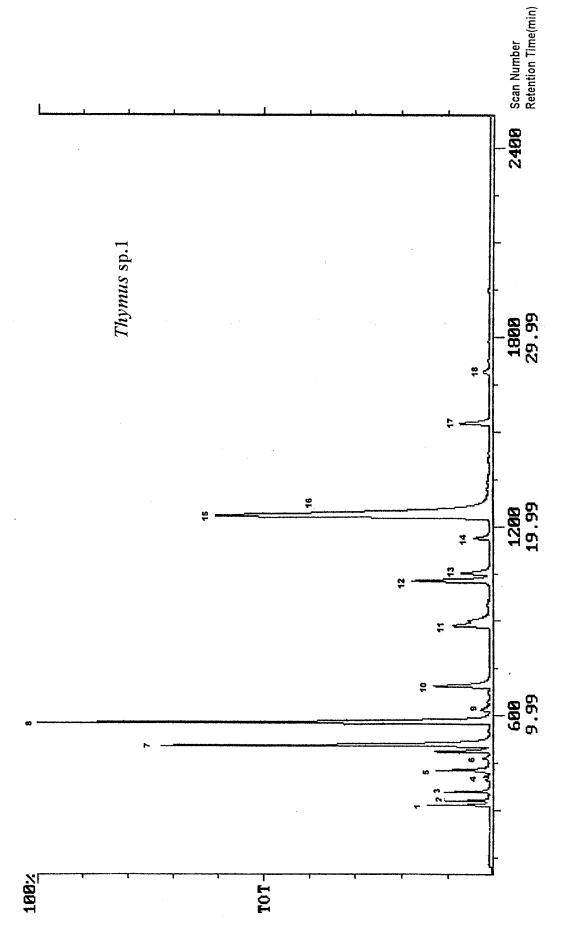


Table 20 Esssential oil composition of Thymus sp. 1 (summer thyme) leaves

Number of peak	Compound	Retention time ( min )	% Area
or peak	Monoterpene	( mir )	
1	tricyclene	5.28	1.44
2	$\alpha$ -thujene	5.49	0.88
3	camphene	5.66	1.30
4	sabinene	6.35	0.17
5	myrcene	6.92	1.51
6	$\alpha$ -terpinene	7.62	2.19
7	o-cymene	7.75	15.87
8	$\gamma$ -terpinene	9.08	21.63
	Oxygenated monoterpene		
9	cis-sabinene hydrate	10.01	t
10	linalool	10.63	4.14
11	borneol	13.61	3.17
12	metnyi ether, thymol	17.06	5.17
13	methyl ether, carvacrol	17.50	2.05
14	bornyl acetate	18.51	0.97
15	thymol	20.04	26.43
16	carvacrol	20.33	11.08
	Sesquiterpene		
17	9-epi-(E)-carylphyllene	25.44	2.00
18	γ-cadinene	28.04	t

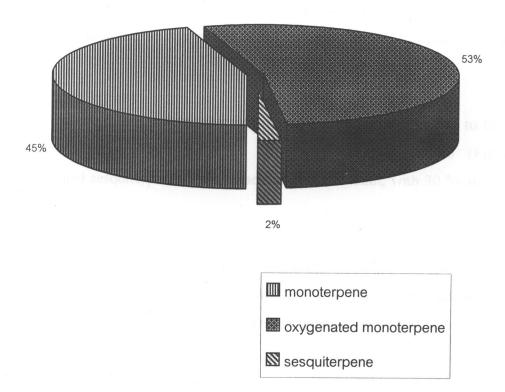


Figure 37 The percentage of various terpenoid groups found in the essential oil of *Thymus* sp.1 leaves

# 4.2.9 Esssential Oil Composition of Thymus sp. 2 (winter thyme)

The leaves of *Thymus* sp.2 was found to contain essential oil with the content of 0.9 % (v/w) of fresh weight. By GC/MS analysis, the oil was shown to have at least 23 components (Fig. 38). These peaks were identified as 9 monoterpenes, 10 oxygenated monoterpenes and 3 sesquiterpenes (Table 21). Among these, the major components were found to be thymol (59.27 %),  $\gamma$ -terpinene (15.20 %) and  $\sigma$ -cymene (9.37 %).

In terms of relative amount, the oxygenated monoterpene appeared to be the major terpenoid group, accounting for 66 % of the essential oil (Fig.39). Monoterpenes and sesquiterpenes were present in lesser amount, with 30 % and 3 %, respectively.

In terms of structure, the major component of thymol belongs to the oxygenated monoterpenoid group of menthane whereas  $\gamma$ -terpinene and o-cymene belong to the monoterpenoid group of menthane and o-menthane, respectively.

*o*-cymene (*o-menthane*)

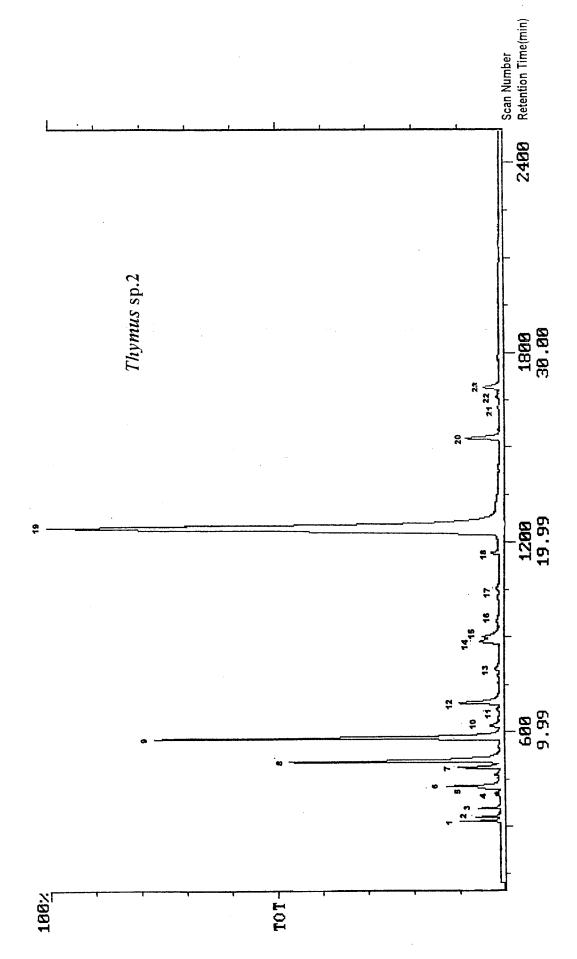


Figure 38 GC chromatogram of the essential oil from Thymus sp. 2 leaves

 Table 21 Esssential oil composition of Thymus sp.2 (winter thyme) leaves

Number of peak	Compound	Retention time ( min )	% Area
	Monoterpene	(1/1111)	
	tricyclene	5.28	0.81
2	$\alpha$ -thujene	5.49	0.53
3	camphene	5.66	0.49
4	sabinene	6.35	0.09
6	myrcene	6.90	1.97
7	lpha-terpinene	7.61	1.52
8	o-cymene	7.75	9.37
9	γ-terpinene	9.08	15.20
11	terpinolene	10.13	0.07
	Oxygenated monoterpene		
10	cis-sabinene hydrate	10.01	0.53
12	linalool	11.54	2.67
13	camphor	12.55	0.19
14	borneol	13.61	1.46
1.5	terpin-4-ol	13.94	1.30
16	lpha-terpineol	14.21	0.10
17	methyl ether, carvacrol	17.49	0.19
18	bornyl acetate	18.50	0.47
19	thymol	20.04	59.27
22	geranyl N propanoate	27.63	0.07
	Sesquiterpene		
20	9-epi-(E)-carylphyllene	25.44	2.11
21	$(Z)$ - $\alpha$ -bisabolene	27.59	0.09
23	γ-cadinene	28.04	0.94
	Long chain hydrocarbon		
5	I-octen-3-ol	6.79	0.55

t = trace

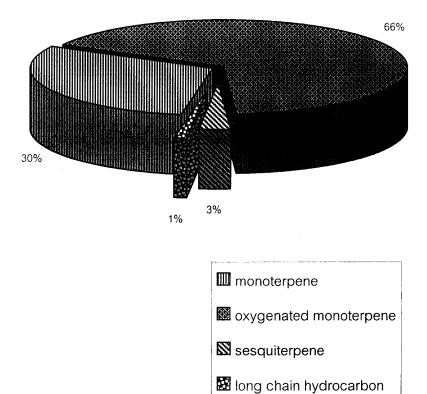


Figure 39 The percentage of various terpenoid groups found in the essential oil of *Thymus* sp.2 leaves

### 4.2.10 Esssential Oil Composition of Thymus vulgaris L.

By hydrodistillation, the yield of the essential oil from *Thymus vulgaris* leaves was found to be 0.2 % (v/w) of fresh weight. GC/MS analysis of the essential oil showed 17 peaks (Fig. 40). These peaks were identified as 8 monoterpene, 7 oxygenated monoterpenes, and 1 sesquiterpene (Table 22). Among these, thymol (47.87%) appeared to be the major component, followed by  $\gamma$ -terpinene (23.35 %) and  $\sigma$ -cymene (9.88 %).

The oxygenated monoterpene appeared to be the major terpenoid group, accounting for 57 % of the essential oil. (Fig. 41). Monoterpenes and sesquiterpenes were present in lesser amount, at 41 %, 1 %, respectively.

Structurally, the major components, thymol, belongs to the oxygenated monoterpenoid group of menthane, while  $\gamma$ -terpinene and o-cymene belong to the monoterpenoid group of menthane and o-menthane, respectively.

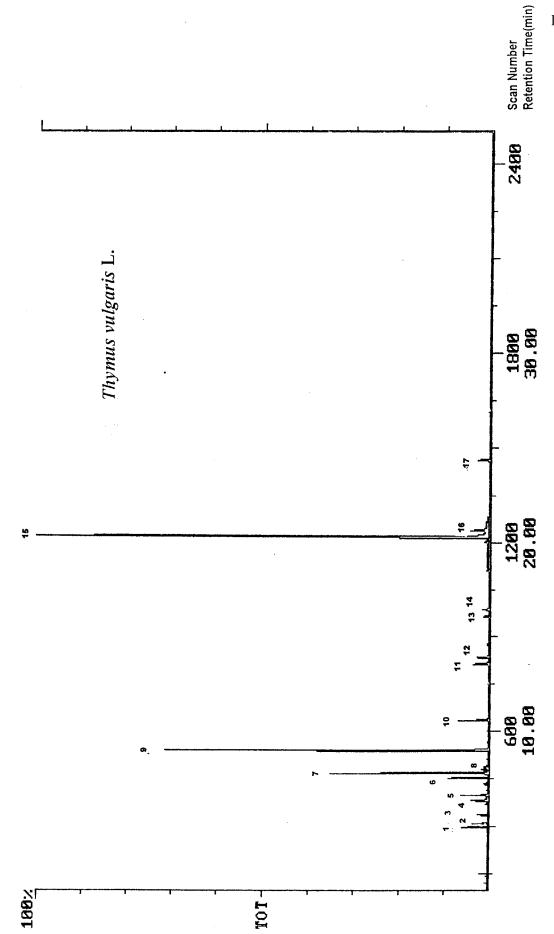


Figure 40 GC chromatogram of the essential oil from Thymus vulgaris leaves

 Table 22
 Esssential oil composition of Thymus vulgaris leaves

Number of peak	Compound	Retention time ( min )	% Area
	Monoterpene		
2	tricyclene	5.28	0.85
3	camphene	5.66	0.66
4	sabinene	6.34	1.47
5	myrcene	6.41	1.60
6	$\alpha$ -terpinene	7.61	2.65
7	o-cymene	7.75	9.88
8	limonene	8.01	0.42
9	γ-terpinene	9.08	23.35
	Oxygenated monoterpene		
10	linalool	10.63	2.86
11	borneol	13.61	1.73
12	terpin-4-ol	13.94	1.14
13	methyl ether, thymol	17.06	0.61
14	methyl ether, carvacrol	17.50	0.66
15	thymol	20.04	47.87
16	carvacrol	20.33	1.65
	Sesquiterpene		
17	(E)-caryophyllene	24.38	1.25
	Miscellaneous		
l	unknown	4.98	1.33

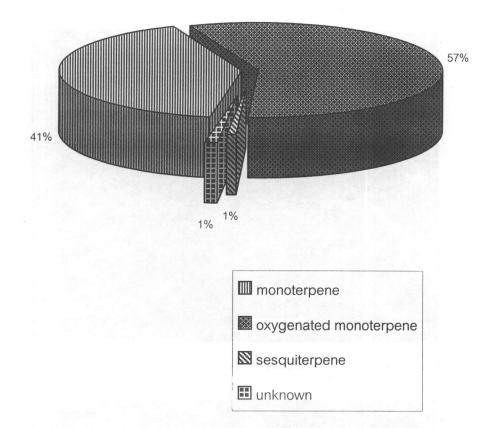


Figure 41 The percentage of various terpenoid groups found in the essential oil of *Thymus vulgaris* leaves

## 4.3 Scanning electron microscopic observation

Leaves of Lamiaceous plants were examined under scanning electron microscope. Their scan electron micrograph revealed for glandular trichomes as shown in figures 42-51.

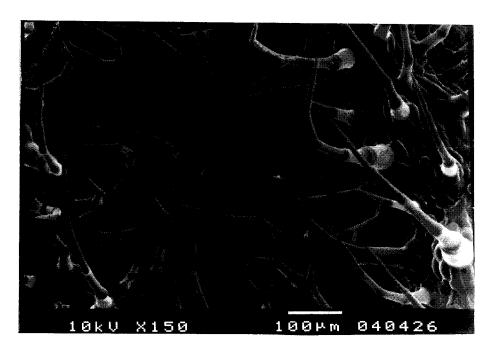


Fig.42 Scanning electron micrograph of glandular trichomes of Coleus amboinicus Lour.

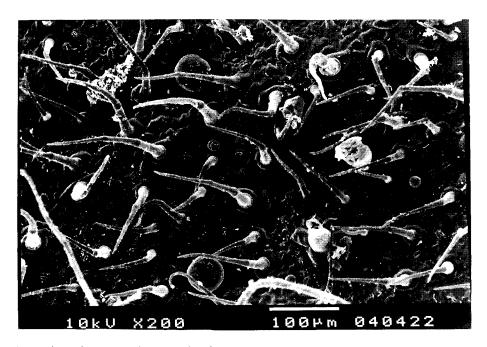
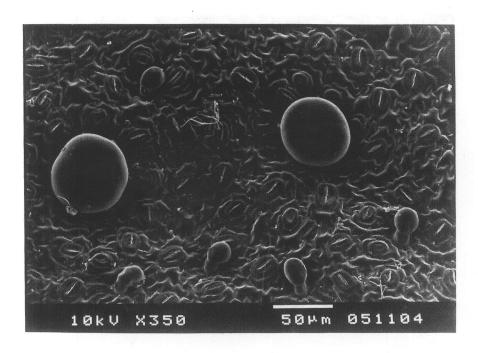


Fig. 43 Scanning electron micrograph of glandular trichomes of Hyptis suaveolens Poit.



**Fig.44** Scanning electron micrograph of glandular trichomes of *Mentha arvensis* L. var *piperascens* Malinvaud

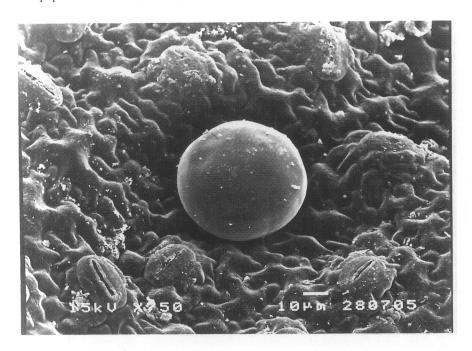


Fig.45 Scanning electron micrograph of glandular trichomes of *Mentha cordifolia*Opiz

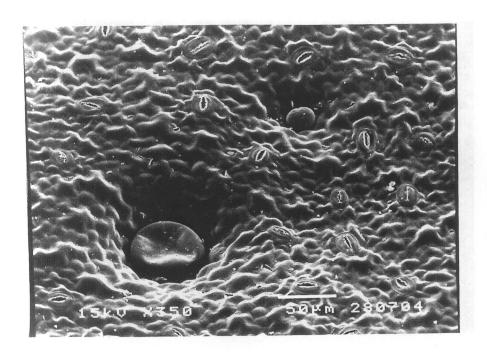


Fig.46 Scanning electron micrograph of glandular trichomes of Ocimum basilicum L.

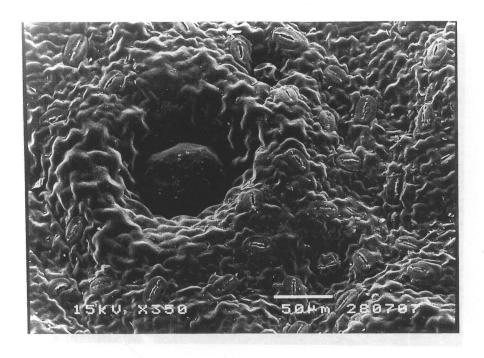


Fig.47 Scanning electron micrograph of glandular trichomes of Ocimum canum Sims.

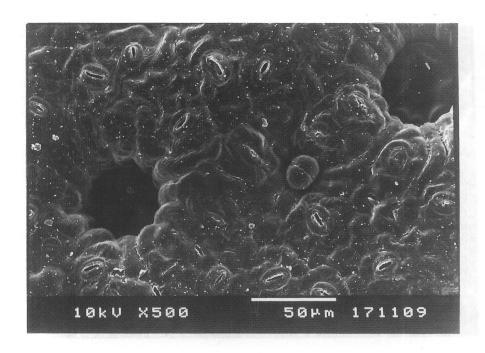


Fig.48 Scanning electron micrograph of glandular trichomes of Ocimum gratissimum L.

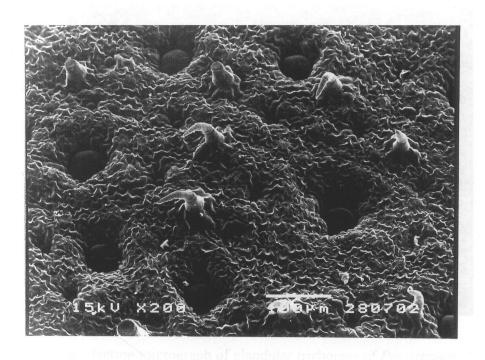


Fig.49 Scanning electron micrograph of glandular trichomes of Ocimum sanctum L.

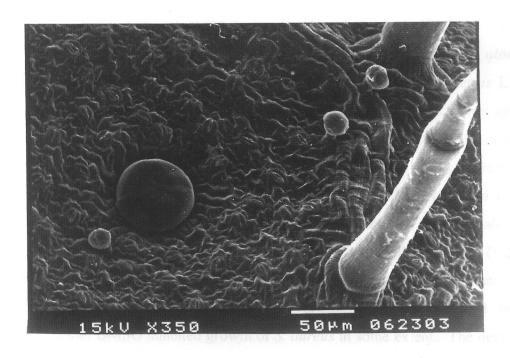


Fig. 50 Scanning electron micrograph of glandular trichomes of Perilla frutescens Britt.

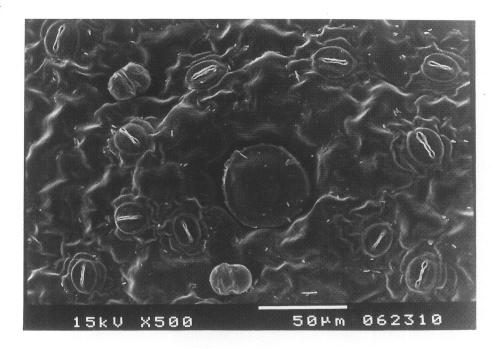


Fig.51 Scanning electron micrograph of glandular trichomes of *Pogostemon cablin*Benth.

### 4.4 Antimicrobial activities of the essential oil from Thai Lamiaceous plants

Antimicrobial activities of the essential oils were shown in Table 23. It was found that the essential oils tested had no activities against *P.aeruginosa*, *C. albicans* and *M. gypseum*. Most of them, except the essential oils from *M. arvensis* L. var *piperascens* Malinvaud and *O. basilicum* L., exhibited antimicrobial activities against *S. aureus* and *B. subtilis*. The essential oils from *O. gratissimum* L. and *O. canum* L. had the lowest MIC against *S. aureus* (0.078%) and *B. subtilis* (0.156%), respectively. Some of them, including the oils from *C. amboinicus* Lour, *O. gratissimum* L. and *P. frutescens* Britt., inhibited *E. coli*. For *E. faecalis*, only the essential oils from *C. amboinicus* Lour. and *O. canum* L. could inhibit this strain and their MICs (1.25%) were the highest comparing to those against other strains. It was found that 0.1% Tween 80 had no activity against the test organisms but 1.25% and higher concentrations of DMSO inhibited growth of *S. aureus* in some extent. The degree of growth inhibition was 91.65%, 58.73% and 29.16% in 5%, 2.5% and 1.25% DMSO, respectively.

 Table 23
 Antimicrobial activities of essential oil

*			-	Day attis 3000000	110001110	Emerococcus Juecuis	is Juecuus
ATCC 29213		ATCC 2592	2	ATCC	633	ATCC 29212	9212
nun ± SD* MIC	$\vdash$	Н	$\vdash$	mm ± SDª	MIC (%)	nun ± SD*	MIC (%)
17.23 ± 0.67				$7.03 \pm 4.07$	ND.	$17.53 \pm 0.35$	1.25
12.50 ± 1.41   0.1			0	9.87 <u>-</u> 0.91	IJ,	0	С
0		<u> </u>	0	0	0	0	©
8.05 ± 0.35			0	0.27 ± 0.75	(J)	0	С
	·# · · ·	-	0	0	е	0	С.
$13.80 \pm 0.36$ 0.1	· · · · · · · · · · · · · · · · · · ·	0	0	17.01 ± 2.1	0.156	10.85 ± 1.20	1.25
$19.87 \pm 0.45$ 0.0		0.21	<u></u>	1.37 - 1.84	(y)	C	<b>=</b>
9.96 ± 1.05 0.3			0	8.75 ± 0.35	IJ,	0	ε
9.87 ± 1.46				12.1 <u>-</u> 1.28	N.	0	С
$8.63 \pm 0.67$ 0.0		0 ——	0	10.4 <u>-</u> 3.14	0.312	0	Э.
	C 25	C 29213  MIC (%)  J.63  0.156  0  0.156  0.078  0.312  1.25	MIC (%) num ± SD*  0.63 11.2 ± 1.06  0.156 0  0  0  0  0  0  0.156 0  0  0.156 0  0  0.156 0  0  0.156 0  0  0.156 0  0  0.156 0  0  0.156 0  0  0.156 0  0  0.156 0  0  0.156 0  0  0  0  0  0  0  0  0  0  0  0  0	MIC (%) mm ± SD* MIC (%)  0.63 11.2 ± 1.06 0.63  0.156 0 0 0  0 0 0  5 0 0 0  0.156 0 0 0  0.025 0 0 0  1.25 8.23 ± 0.21 ≥5  0.312 0 0 0  1.25 8.6 ± 0.69 ND <sup>6</sup>	MIC (%)       mm ± SD*       MIC (%)       mm         0.63       11.2 ± 1.06       0.63       17.03         0.156       0       0       0       9.87         0       0       0       0       10.27         0       0       0       0       17.01         0.078       8.23 ± 0.21       ≥5       11.37         0.312       0       0       8.75         1.25       8.6 ± 0.69       ND <sup>6</sup> 12.1         0.63       0       10.4	MIC (%) $mm \pm SD^*$ MIC (%) $mun \pm SD^*$ MIC (%) $mun \pm SD^*$ MIC (%)           0.63 $11.2 \pm 1.06$ 0.63 $17.03 \pm 4.07$ MIC (%)           0.156         0         0         0 $9.87 \pm 0.91$ 5           0         0         0         0         0         0           5         0         0         0         0         0           0.156         0         0         0         0         0           0.156         0         0         0         0         0         0           0.078         8.23 $\pm 0.21$ $\geq 5$ 11.37 $\pm 1.84$ $\leq$ 5           0.312         0         0         8.75 $\pm 0.35$ $\leq$ ND°           1.25         8.6 $\pm 0.69$ ND°         12.1 $\pm 1.28$ ND°           0.63         0         0         10.4 $\pm 1.14$ 0.312	MIC (%)       num ± SD*       MIC (%)       nm         0.63       11.2 ± 1.06       0.63       17.03 ± 4.07       NI)*       17.         0.156       0       0       0       9.87 ± 0.91       ≤         0       0       0       0       0       0         0.156       0       0       0       0       0       0         0.078       8.23 ± 0.21       ≥5       11.37 ± 1.84       ≤       5         0.312       0       0       8.75 ± 0.35       ≤       10         0.63       0       0       10.4 ± 1.14       0.312

<sup>&</sup>lt;sup>a</sup> inhibition zone diameter resulted from 10 % oils in 0.1% Tween 80

h not determined

#### CHAPTER V

#### DISSCUSSION

Hydrodistillation of leaves from selected Thai Lamiaceous plants yielded various amounts of essential oils ranging from 0.01 % to 0.9 % v/w of fresh weight. It was found that the leaves of *M. cordifolia* and *M. arvensis* var *piperascens* yielded the lowest and the highest amounts, respectively. In terms of the chemical composition analyzed by GC/MS, most of the constituents were quite different. No clear relationship between the distribution of particular components of these plants was observed.

It was found that carvacrol (77.95 %) was the major component of the essential oil from *C. amboinicus*. These was in agreement with the result previously reported (Prudent *et al.*, 1995). The major components of the essential oil from *H. suaveolens* were also in agreement with those reported recently (Ahmed *et al.*, 1995). They were 1,8-cineol (21.70 %), (*E*)-caryophyllene (17.87 %) and sabinene (16.92 %). However, the percentage of 1,8-cineole in our oil were quite different from those of Ahmed *et al.*, 1994 (38.70 % 1,8-cineole).

It was found that major components in the essential oil of *Mentha arvensis* var. *piparascens* and *M. cordifolia* were oxygenated monoterpenes. They were menthol (79.44%) and piperitenone oxide (73.20%), respectively. Most of the constituents of *Mentha arvensis* var. *piparascens* found in this study were similar to those recently reported (Pino *et al.*, 1995). However the percentage of menthol in our oil, were quite different from that report (menthol 51.68%). For *Mentha cordifolia* leaf oil, this study is the first report on its chemical composition.

Comparision of the essential oil composition among *Ocimum* species was shown in Table 24. It was found that  $\alpha$ -humulene and  $\delta$ -cadinene were present in all of four *Ocimun* species including *O. basilicum*, *O. canum*, *O. gratissimun* and *O. sanctum*. Some major components were present specifically in individual species. For example geranial (22.36 %) and geranyl acetate (13.10 %) were present only in

Table 24 Chemical composition of essential oil hydrodistilled from Ocimum leaves

Compound		%	Area	Varus
	O. basilicum	O. canum	O. gratissimum	O. sanctum
Monoterpene				
α-thujene	remaria wa			0.20
camphene	-	-		0.21
sabinene	<u> -</u>	0.26	-	t 65
β-pinene	-	-		0.14
myrcene	0.11	-	0.29	-
δ-3-carene	-	0.14		-
O-cymene	-	0.13	£12,1	- 31
limonene	0.15	1.11		0.07
Z-β-ocimene	-	-	20.35	-
$(E)$ - $\beta$ -ocimene	1.29	0.78	1.13	- 13
neo-allo-ocimene	-		0.07	- 11
Oxygenated monoterpene				
1.8-cineole	0.31	0.63	-	0.07
fenchone	-	0.21	-	-
linalool	-	5.42	0.64	0.32
(exo)-fenchol		0.56		7 11
camphor	0.71	-	-	-
β-pinene oxide	-	0.57		× , , ,
trans-verbenol	-	0.36	-	7
cis-chrysanthenol	-	(),6()	V- 1	-
borneol	0.16	-	-	0.55
α-terpineol	-	0.66	-	-
linalool acetate	-	2.98	-	-
cis-carveol	<b>5</b> (	1.70	-	- ,
geranial	- 1	22.36	-	-
geranyl acetate	· .	13.10		-
Sesquiterpene				
(x-copaene	·	0.30	0.83	-
β-bourbonene		-	0.15	

 Table 24 Chemical composition of essential oil hydrodistilled from Ocimum leaves (continued)

Compound		9/	6 Area	
	O. basilicum	O. canum	O. gratissimum	O. sanctum
β-cubebene	7	~	0.11	-
β-elemene	0.15	-	-	2.65
9-epi-(E)caryophyllene	-	-	-	23.69
(E)-caryophyllene	0.18	9.55	2.19	-
$\alpha$ -transbergamotene	1.98	2.26	3.28	0.00
α-humuene	0.25	4.81	0.24	1.53
γ-muurolene		1.88	- A	0.39
germacrene D	0.38	-	7.65	-
β-selinene	-	0.32	0.35	0.12
α-selinene	- 1	(), [()	· · · · · · · · · · · · · · · · · · ·	0.14
$(Z)$ - $\alpha$ -bisabolene	0.13	-	_	4: 11 -46, 7 1
E.E.α-farnesene	4 40.2000	, · · · · -	2.95	sk million
β-bisabolene	0.35	0.20	-	
germacrene A	0.27	-	0.26	-
\(\alpha\)-bulnesene	0.41	_	, ' . <u>-</u>	4.51
γ-cadinene	0.54	· · · · · · · · · · · · · · · · · ·	-	41112 4
δ-cadinene	0.17	0.14	0.41	0.11
β-sesquiphellandrene			0.18	<b>-</b> . 4
germacrene B	-	6.57		_
Dxygenated sesquiterpene				
caryophyllene oxide	-	().7()	-	-
humulene epoxide II	-	0.22	-	-
1-epi-cubenol	0.24	-	-	-
epi-α-cadinol	2.38	-"		950
$\alpha$ -cadinol	0.41		n julialija je po	and was the
Phenyl propane				
methyl chavicol	88 40	0.38	-	-
eugenol	-	_		19.18
(E)-isoeugenol	·	, in , 200 - 1 ( = -1)	58.92	ms of c4.
methyl eugenol	0.75	-		46.08

(). canum, (Z)- $\beta$ -ocimene (20.35 %) was only in (). gratissimum and 9-epi-(E) caryophyllene (23.69 %) was only in (). sanctum. These components may potentially be used as markers for identification of these individuals.

In terms of the proportion of terpenoid compounds, only O. *camum* leaf oil was found to contain the major component in the oxygenated monoterpene group (50 %). The major components in leaf oil of the other three species were in the phenylpropanoid group, and the non-terpenoid compound. It was found that essential oils in these species were composed of uncommon major constituents. That of O. *basilicum* was composed of methyl chavicol (88.40 %), epi- $\alpha$ -cadinol (2.38 %) and  $\alpha$ -trans-bergamotene (1.98 %). That of O. *camum* contained geranial (22.36 %), geranyl acetate (13.10 %) and (E) caryophyllene (9.55 %). That of O. *gratissimum* contained (E)-isoeugenol (58.92 %), (E)-E-ocimene (20.35 %) and germacrene E0 (7.66 %). Most of the constituents found in this study were different from other reports. E0. *basilicum* leaf oil has been previously reported to contain linalool, (E)-methyl cinnamate and 1,8-cineole (Ozek E1 E1. 1995).

It was found that perilla aldehyde (57.31 %) was the major component of *Perilla frutescens*. This was in agreement with the findings by Kang *et al.*, 1992. In contrast, it was different from the findings by Nguyen *et al.*, 1995 (28.4 % limonene, 25.9 % piperitone). For *Pogostemon cablin* oil, its major component was patchouli alcohol. This agreed with the previous report by Nguyen *et al.*, 1990.

Leaves of selected Lamiaceous plants of western origin contained the essential oil yields comparable to those of indigenous species. They were 0.1-0.9 % v/w of fresh leaves. The plants with the lowest essential oil content included *Melissa officinalis* and *Origanum majorana*. Those containing the highest content were *Rosemarinus officinalis*. *Thymus* sp1. (summer Thyme) and *Thymus* sp.2 (winter Thyme).

In consideration to the chemical composition, the major constituents of oils from two plants of western origin, *Mentha piperita* L. and *M. spicata* L., and two

indigenous spp., *M. arvensis* L. var *piperascens* Malinvaud and *M. cordifolia* Opiz, were different except menthol and menthone in oils of *M. piperita* L. and *M. arvensis* L. var *piparascens* Malinvaud. However, their amounts were different. The latter contained very high amount of menthol (79.44 %) but low amount of menthone (9.44 %) whereas the former contained nearly equal amounts of menthol (30.57 %) and menthone (24.63 %). It was found that most of the constituents of the essential oils in *M. piperita* and *M. spicata* in this study were similar to previous reports of Tisserand and Balacs (1995).

Origanum majorana oil was obtained in 0.1 % v/w. This was different from previous report (3% v/w) (Wren, 1988). The GC/MS analysis indicated terpin 4-ol (28.37 %), linalool (20.70 %) and  $\beta$ -phellandrene (10.75 %). Most of the constituents found in this study were different from previously reported results. Origanum majorana leaf oil had been previously reported to contain carvacrol and thymol (Sarer, E., Scheffer, J.J.C., and Baerheim, S.A., 1982).

Origanum vulgare oil was obtained in 0.2 % v/w. The GC/MS analysis indicated carvacrol (75.63 %) as the major component, together with p-mentha-2,4(8)-diene (10.11 %) and o-cymene (2.34 %). The presence of carvacrol as the major component was in agreement with the results from a recently reported study (Roengsumran  $et\ al.$ , 1997).

Rosemary oil was obtained in 0.9 % v/w. This was quite different from the previous reported 2.35 % v/w by Perez-Alonso *et al.*, 1995. It was found to contain  $\alpha$ -pinene (22.48 %), camphor (20.07 %) and bornyl acetate (8.67 %). The presence of  $\alpha$ -pinene as the major component was in agreement with the results from earlier reports (Tisserand and Balacs, 1995; Perez-Alonso *et al.*, 1995). However, its percentage was quite different.

Salvia officinalis leaf oil was obtain in 0.3 % v/w. This was quite different from the previously reported value (1.4 % v/w) (Tsankova, E.T., Konaktchiev, and Genova, E.M., 1995). The GC/MS analysis indicated that *cis*-thujone (37.49 %) was

the major component, together with camphor 13.79 % and  $\alpha$ -humulene 9.46 %. These were in agreement with those reported recently (Tisserand and Balacs, 1995).

Thymus vulgaris leaves oil was obtained in 0.2 % v/w. The GC/MS analysis indicated that thymol (47.87%) was the major component, This was in agreement with that previously reported (Tisserand and Balacs, 1995). The essential oils obtained from the leaves of *Thymus* sp.1 and *Thymus* sp. 2 were similar in terms of both yields (0.9 % v/w) and major chemical components. However, their percentages were different: thymol (26.43 % and 59.27 %, respectively),  $\gamma$ -terpinene (21.63 % and 15.20 %, respectively) and  $\sigma$ -cymene (15.87% and 9.37%, respectively). This is the first report on their chemical composition.

Essential oils of selected indigenous Lamiaceous plants used in this study showed activity against *S. aureus* and *B. subtilis* except the oil from *Mentha arvensis* L. var *piperascens* and *Ocimum basilicum* L. They showed no activity against *P. aeruginosa*, *C. albicans* and *M.gypseum*. *Coleus amboinicus* Lour. leaf oil also inhibited *E. coli* and *E. faecalis*. This was in agreement with the previous report (Prudent *et al.*, 1995). The antibacterial activities of *C. amboiaincus* Lour. oil might come from its major constituent, carvacrol, a phenolic compound. However, it was previously reported that carvacrol showed activities against *B. cereus* and *E. coli* but it had no activities against *S. aureus* and *P. aeruginosa* (Ross, S.A., El-Keltawi, N.E., and Megalla, S.E., 1980). This suggested that the activity of *C. amboinicus* Lour. oil against *S. aureus* might come from other constituents. On the other hand, the difference of result on *S. aureus* might come from the difference in tested strains.

Hyptis suaveolens leaf oil showed antibacterial effect on gram-positive bacteria (S. aureus and B. subtilis) but it had no activity against gram-negative bacteria. The effect on gram-positive bacteria agreed with a previous report but its effect on gram-negative bacteria was different (Iwu et al., 1990). Its antibacterial effect might be due to 1,8-cineole, its major component. Previously, 1,8-cineole was found to have antibacterial activity (Prudent et al., 1995).

Essential oil from *Mentha arvensis* L.var. *piperascens* Malinvaud showed no activity against both bacteria and fungi despite the presence of menthol as its major

component. Previously, menthol was found to show weak activities against *S. aurens*, *E. coli* and *C. albicans* (Morris, *et al.* 1979; Ross, *et al.*, 1980). The reasons underlying the negative results of this oil might be the followings. First, some constituents in the oil might interfere with menthol activity. Or second, the test organisms used in this study might be different from those used in the previous reports. In contrast to essential oil of *M. arvensis* Linn var *piperascens* Malinvaud, that of *Mentha cordifolia* Opiz showed activities against some bacteria, *S. aureus* and *B. subtilis*.

Essential oil of *Ocimum basilicum* L. leaves showed no activity against test bacteria and fungi. This was different from the finding by Dikshit and Husain (1984). They found that *Ocimum bacillicum* L. oil inhibited mycelial growth of *M. gypseum*. The different results of this study and the previous study might come from the difference in chemical composition of the oils and /or in sensitivity of techniques used in antimicrobial activity screening. In the previous study, they determined the percentage of mycelial inhibition but measurement of clear inhibition zone was used in this study.

In addition to the activities against *S. aureus* and *B. subtilis*, *Ocimum canum* L. oil also showed activity against *E. faecalis*. Its effects might come from its major constituent, geranial, an aldehyde compound. Several aldehydes were shown to possess antimicrobial activity (Russell A.D., Hugo, W,B. and Ayliffe, A. J. 1992). Instead of inhibiting *E. faecalis*, *O. gratissimum* L. oil could inhibit the gram-negative bacterium, *E. coli*. It showed strong activity against *S. aureus* (MIC 0.078 %). Strong antibacterial activities of *O. gratissimum* L. oil might be due to its major component, *(E)*-isoeugenol. Isoeugenol was found to have antibacterial activities against *S. aureus* and *E. coli* (Morris *et al.*, 1979). Essential oil of *Ocimum sanctum* L. leaves showed activities against *S. aureus* and *B. subtilis*. Its activity might be due to its major constituents, methyl eugenol and eugenol. Both major constituents were found to showed activities against some bacteria (Morris, *et.al*, 1979; Ross, *et al.*, 1980).

Besides *S. oureus* and *B. subtilis*, essential oil of *Perilla frutescens* also showed activity against the gram-negative bacterium, *E. coli*. This was in agreement with a previous report (Kang *et al.*, 1992). Perilla aldehyde, its major constituent, seemed to be the active component. It was found to showed activities against several bacteria (Kang *et al.*, 1992).

Essential oil of *Pogostemon cabin* only showed activies against *S. aureus* and *B. subtilis*. Its major constituent was patchouli alcohol. This agreed with the previous report on activies of Patchuli oil (Morris *et al.*, 1979).

#### CHAPTER VI

# **CONCLUSION**

The present investigation deals with the determination of chemical components and antimicrobial activity of essential oils isolated from selected Thai Lamiaceous plants.

A total of 10 species of Thai Lamiaceous plants representing 6 genera have been investigated. Oxygenated monoterpenes and phenylpropanes are commonly found in these particular species. A unique species; *Pogostemon cahlin* was found to contain oxygenated sesquiterpenes as major components. In this investigation, the essential oils among 4 *Ocimum* species were compared. The major constituents were in the phenylpropane group which thereby may be used as markers for the identification of individuals species. Additionally, the ten species of western Lamiaceous plants cultivated in Thailand were also compared with regard to their content and composition, with previously reported data. Each of Thai cultivars was found to produced essential oil with similar constituents but lesser amount, compared with its original counterpart. In addition, it was found that certain essential oils showed antibacterial activity against Gram-positive and Gram-negative bacteria. Although the antibacterial properties of the essential oils in this study were much lower than those of currently used antibiotics, the present study indicates the possibility of using some essential oils as natural antibacterial agents.

#### REFERENCES

#### **Thai**

เต็ม สมิตินันท์. 2523. <mark>ชื่อพรรณไม้แห่งประเทศไทย</mark>. กรุงเทพมหานคร : โรงพิมพ์ฟันนี่พับถิชชิ่ง.

#### **English**

- Adams, R.P. 1995. <u>Identification of Essential oil Components by Gas</u>

  <u>Chromatography / Mass Spectroscopy</u>. Illinois: Allured Publishing.
- Ahmed, M., Scora, R.W., and Ting, I.P. 1994. Composition of Leaf Oil of *Hyptis suaveolens* (L.) Poit. J. Essent. Oil Res. 6: 571-575.
- Ameenah, G.F., Mala, D.S., and Fawsia, N. 1995. Aromatic Plants of Mauritius:

  Volatile Constituents of the Essential Oils of *Coleus aromaticus* Benth., *Triphasia trifolia* (Burm.f.) and *Eucalyptus kirtoniana* F. Muell. J. Essent. Oil

  Res. 7: 215-218.
- Backer, C.A., and Bakhuizen Van Den Brink, R.C.1965 <u>Flora of Java</u> (spermatophytes only). Vol. II. 1st ed.Groningen: N.V.P., p.631.
- Bailey, L.H. 1949. <u>Manual of Cultivated Plants</u>. New York: The macmillan company, p. 847.
- Burkill, I.H. 1935. <u>A Dictionary of the Economic Products of the Malay Peninsula</u>
  Vol. I. London: The Crown Agents for the Colonies., pp. 634-635.
- Burkill, I.H. 1935. <u>A Dictionary of the Economic Products of the Malay Peninsula</u>
  Vol. II. London: The Crown Agents for the Colonies.. pp.1575-1576.
- Council of Scientific and Industrial Research. 1959. The Wealth of India: A

  <u>Dictionary of India Raw Materials and Industrial Products</u>. Vol.V New Delhi:

  Sree Saraswaty Press., p. 159.
- Council of Scientific and Industrial Research. 1966. The Wealth of India: A

  <u>Dictionary of India Raw Materials and Industrial Products.</u> Vol.VII. New Delhi

  Sree Saraswaty Press., pp. 79-81, 84-85.
- Council of Scientific and Industrial Research. 1969. The Wealth of India: A

  <u>Dictionary of India Raw Materials and Industrial Products.</u> Vol.VIII. New

  Delhi: Sree Saraswaty Press., pp. 177-182.

- Dikshit, A., and Husain, A. 1984. Antifungal Action of some essential oils Against Animal Pathogens. <u>Fitoterapia</u> 553: 171-176
- Dro, A.S., and Hefendehl, F.W. 1974. Essential oil of *Ocimum gratissimum* L. <u>Arch. Pharm. Wienham.</u> 307: 168-186.
- Drury, C.H. 1873. <u>The Useful Plants of India</u>. 2 nd ed. Ballimaran, Delhi : Jayyed Press., pp.153-154.
- Edwin, H.L., Albert, B., William. J.Jr and Jean, H.S. 1985. <u>Manual of Clinical Microbiology</u>. 4th ed. American Society For Microbiology, Washington, D.C.
- Haque, I.Ul. 1988. Analysis of volatile Constituents of Pakistani *Coleus aromaticus*Plant Oil by Capillary Gas Chromatography Mass Spectrometry. <u>J.Chem. Soc.</u>

  <u>Pak.</u> 10 (3):369-371.
- Helrich, K. 1990. <u>Association of official Analytical Chemists.</u> Vol. II. 15th ed. USA.: Association of official Analytical Chemists Inc., p. 1001.
- Heywood, V.H. 1978. <u>Flowering Plants of The World.</u> 1st ed. New York: Mayflower Book., p.239.
- Iwu, M.M., Ezeugwu, C.O., and Okunji, C.O. 1990. Antimicrobial Activity and Terpenoids of the Essential Oil of *Hyptis suaveolens*. <u>Int. J. Crude Drug Res</u>. 28 (1): 73-76.
- Kang, R., Helms, R., Stout, M.J., Jaber, H., Chen, Z., and Nakatsu, T. 1992.
   Antimicrobial Activity of the Volatile Constituents of *Perilla frutescens* and Its Synergistic Effects with Polygodial. <u>J. Agric. food Chem.</u> 40: 2328-2330.
- Keng, H. 1978. Flora Malasiana Vol. VIII. pp. 301,307.
- Keys, J.D. 1976. Chinese Herbs. 1st ed. Japan: Charles E. Tuttle., pp.256-257.
- Lorian, V. 1991. <u>Antibiotics in Laboratory Medicine</u>. 3rd ed. Williams & Wikins, Maryland.
- Morris, J.A., Khettry, A., and Weitz, E.W. 1979. Antimicrobial Activity of Aroma Chemical and Essential Oils. J. Am. Oil Chem. Soc. 56: 595-603.
- Morton, J.F. 1992. Country Borage (*Colens amboinicus* Lour.): potent flavoring and medicinal plant. <u>J. Herbs. Spices Med. Plants.1(1-2): 77-90.</u>

- Nguyen, X.D., La, D.M., Lu'u, D.C., and Piet, A.L. 1995. Essential Oil Constituents from the Aerial Parts of *Perilla frutescens* (L.) Britton. <u>J. Essent. Oil Res.</u> 7: 429-432.
- Nguyen, X.D., Leclerck, P.A., Tran, H.T., and La Dinh, M. 1989. Chemical Composition of Patchouli oil of Vietnam. <a href="Proc.Int.Congr.Essent.">Proc.Int.Congr.Essent.</a>
  <a href="Oils,Fragrances Flavours">Oils,Fragrances Flavours</a>. 4:99-102 (CA.117:1472132q)
- Nguyen, X.D., Leclerck, P.A., Tran, H.T., and La Dinh, M. 1990. Results on Study about *Pogostemon cablin* Grown in Vietnam. Proc. Natl. Cent. Sci. Res. Vietnam. 2:118-122 (CA. 116:170210z).
- Ntezurubanza, L., Scheffer, J.J.C., and Looman, A. 1985. Composition of the Essential oi! of Ocimum canum Grown in Rwanda. Pharm Weekbl. 7: 273-276.
- Ntezurubanza, L., Scheffer, J.J.C., and Svendsen, A.B. 1987. Composition of the Essential Oil of *Ocimum gratissimum* Grown in Rwanda. <u>Planta Medica</u> 53: 421-423.
- Ozek, T., Beis, S.H., Demircakmak, B., and Baser, K.H.C. 1995. Composition of the Essential Oil of *Ocimum basilicum* L. Cultivated in Turkey. <u>J.Essent.Oil Res.</u>7: 203-205.
- Perez-Alonso, M.J., Velasco-Negueruela, A., Duru, M.E., Harmandar, M., and Esteban, J.L. 1995, Composition of the Essential Oils of *Ocimum basilicum* var. *glabratum* and *Rosmarinus officinalis* from Turkey. <u>J. Essent.Oil Res</u>. 7:: 73-75
- Perry, L.M. 1980. <u>Medicinal Plants of East and Southeast Asia</u>. Cambridge: The MIT Press., pp.188-189.
- Pino, J.A., Garcia, J., and Martinez, M.A. 1996. Comparative Chemical Composition of the Volatiles of *Coleus aromaticus* Produced by steam Distillation, solvent Extraction and Supercritical Carbon Dioxide Extraction. <u>J. Essent. Oil Res.</u> 8: 373-375.
- Pino, J.A., Roncal, E., Rosado, A., and goire, I. 1994. The Essential Oil of *Ocimum basilicum* L. from Cuba. <u>J. Essent. Oil Res.</u> 6: 89-90.

- Pino, J.A., Rosado, A., and Fuentes, V. 1996. Composition of the Essential Oil from the Leaves and Flowers of *Ocimum gratissimum* L. Grown in Cuba. <u>J. Essent.</u>

  Oil Res. 8: 139-141.
- Pino, J.A., Rosado, A., and Fuentes, V. 1995. Chemical Composition of the Essential Oil of *Mentha arvensis* L. var. *piperascens* Malinv. from Cuba. <u>J. Essent. Oil</u> Res. 7: 685-686.
- Prudent, D., Perineau. F., Bessiere, J.M., Michel, G.M., and Baccou, J.C. 1995.

  Analysis of the Essential Oil of Wild Oregano from Martinique (*Coleus aromaticus* Benth.)-Evaluation of Its Bacteriostatic and Fungistic Properties.

  J. Essent. Oil Res. 7: 165-173.
- Retamar, J., and De-Riscala, E. 1980. Essential oil of *Mentha arvensis*, *piperascens* variety. Rivista Italian Essenze Profumi Piante Officinali Aromi saponi Cosmetici Aerosal. 62: 127-129.
- Roengsumran, S., Petsom, A., Thaniyavarn, S., Pornpakakul, S. and Khantahiran, S. 1997. Antibacterial activity of some essential oils. <u>J Sci. Res. Chula. Univ.</u>, 22 (1): 13-19.
- Ross, S.A., El-keltawi, N.E., and Megalla, S.E. 1980. Antimicrobial Activity of Some Egyptian Aromatic Plants. <u>Fitoterapia</u> 51: 201-205.
- Russel, A. D., Hugo, W.B., and Ayliffe, A. J. 1992. <u>Principle and Practice of Disinifection, Preservative and Sterilization.</u> 2nd ed. London. : Backwell Scientific Rublishing.
- Sainsbury, M., and Sofowora, E.A. 1971. Essential Oil from the Leaves and inflorescence of *Ocimum gratissimum*. Phytochemistry 10:3309-3310.
- Sarer, E., Scheffer, T.T.C., and Baerheim, S.A., 1982. Monoterpenes in the essential oil of *Origanum majorana*. <u>Planta Medica</u> 46(Dec): 236-239.
- Tisserand, R., and Balacs, T. 1995. <u>Essential Oil Safety</u>. New York: Churchill Living stone.
- Tsankova, E.T., Konaktchiev, A.N., and Genova, E. M. 1995. Constituents of Essential Oils from three *Salvia* species. J. Essent. Oil Res. 6: 375-378

- Weakly, B.S. 1972. <u>A Beginner & Handbook, in Biological Electron Microscopy</u>. London: Churchill Livingstone., pp.77-95
- World Health Organization Reginal Officer for the Western Pacific. 1990. <u>Medicinal Plants in Vict Nam.</u> Viet Nam., pp.263, 265, 283.
- Wren, R.C. 1988. <u>Potter's Newcyclopaedia of Botanical Drugs and Preparations</u>. England: The C.W. Daniel Company Limited., p.185.
- Xaasan, C.C., Cabdulraxmaan, A.D., Passananti, S., Piozzi, f., and Schmid, J.P. 1981.
   Constituents of the Essential Oil of *Ocimum canum*. <u>J. Nat. Prod.</u> 44 : 752-753.
- Zamureenko, V.A., Klyuev, N.A., Dmitriev, L.B., and Polykova,S.G. 1986.

  Component Composition of Essential Oils in Eugenol-type. <u>Izv Timiry Azevsk</u>
  S-Kh Akad. 2: 172-175.

# **APPENDICES**

A. The chemical components of essential oil isolated from selected Lamiaceous plants.

Retention time (min)	Compound	Structure
3.86	bergamal	H
5.28	tricyclene	
5.33	$\alpha$ -pinene	
5.50	lpha-thujene	
5.66	camphene	
5.78	thuja 2,4 (10) - diene	
6.31	benzaldehyde	CHO
6.35	sabinene	
6.41	eta-pinene	
6.53	6-methyl-5-hepten-2-one	0
6.67	3-octanone	-CH <sub>3</sub> CH <sub>2</sub> C(=O)(CH <sub>2</sub> ) <sub>4</sub> CH <sub>3</sub>
6.79	1-octen-3-ol	-CH <sub>2</sub> =CHCH(OH)(CH <sub>2</sub> ) <sub>4</sub> CH <sub>3</sub>

Retention time (min)	Compound	Structure
6.90	myrcene	
7.12	δ-2-carene	
7.26	lpha-phellandrene	
7.40	δ-3-carene	
7.56	3-octanol	OH
7.61	lpha-terpinene	
7.75	()-cymene	
7.90	sylvestrene	
8.01	limonene	
8.03	eta-phellandrene	
8.13	1,8-cineole	0

Retention time (min)	Compound	Structure
8.23	(Z)-β-ocimene	
8.60	(E)-β-ocimene	
9.08	γ-terpinene	
9.63	para-mentha-2,4(8)-diene	
10.01	<i>cis</i> -sabinene hydrate	HO 11
10.08	fenchone	o l
10.13	terpinolene	
10.48	<i>trans</i> -sabinene hydrate	HO
10.63	linalool	OH
10.83	dimethyl styrene isomer #	

Retention time (min)	Compound	Structure
10.89	<i>cis</i> -thujone	0
11.33	<i>trans</i> -thujone	0
11.39	(exo)-fenchol	HO, m
11.43	chrysanthenone	ОН
11.59	<i>Irans</i> -para-menth-2-en-1- ol	OH
11.66	octanol acetate	-CH <sub>3</sub> CH <sub>2</sub> CH(O-Ac)(CH <sub>2</sub> ) <sub>4</sub> CH <sub>3</sub>
11.76	neo-allo-ocimene	
12.13	isopulegol	OH
12.55	camphor .	
12.76	β-pinene oxide	, ()
12.88	citronellal	CHO

Retention time (min)	Compound	Structure
13.06	<i>trans</i> - verbenol	OH
13.09	<i>trans</i> -pinocamphone	
13.30	<i>neo-iso-</i> isopulegol	ОН
. 13.33	<i>cis</i> -chrysanthenol	(H)
13.34	pinocarvone	
13.61	borneol	OH OH
13.62	menthone	O
13.76	<i>cis</i> -pinocamphour	tt <sub>o</sub>
13.94	terpin-4-ol	OH
14.10	menthofuran	
14.21	lpha-terpineol	ОН
14.23	neo-menthol	OH

Retention time (min)	Compouna	Structure
14.64	menthol	ОН
14.73	methyl chavicol	O — Me
14.97	<i>trans</i> -dihydro carvone	O
15.07	isomenthol	У ОН
15.35	isobonyl formate	O - CHO
15.73	dihydro carveol	OH
15,88	linalool acetate	<b>0</b> - Ac
16.05	neo-iso-dihydrocarveol	OH
16.30	(E)-ocimenone	
16.32	nerol	OH
16.48	cis-carveol	OH

Retention time (min)	Compound	Structure
16.60	neral	O O
16.86	carvone	o
17.01	geraniol	OH
17.06	methyl ether, thymol	() - Me
17.23	pulegone	
17.50	methyl ether, carvacrol	() · Me
17.53	methyl citronellate	O — Me
17.95	piperitone	
17.81	geranial	H O
18.50	bornyl acetate	OAc
18.73	menthyl acetate	, O-Ac

Retention time (min)	Compound	Structure
19.68	isomethyl acetate	,,, - Ac
20.04	thymol	ОН
20.33	carvacrol	OH
20.38	neo-iso-carvomethyl acetate	O — Ac
20.79	$\delta$ -elemene	
20.86	piperitenone	o de la constant de l
21.13	cis-pinocarvyl acetate	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,
21.15	geranyl acetate	O - Ac
21.28	lpha-cubebene	
21.31	neo-iso-dihydrocarveol acetate	,, O Ac
21.31	eugenol	OH O ·Me

Retention time (min)	Compound	Structure
21.53	(E)-isoeugenol	0 — Me
21.60	<i>cis</i> -carvyl acetate	0 - Ac
21.69	perillaldehyde	CHO
21.83	piperitenone oxide	0
22.50	lpha-copaene	
22.58	eta-bourbonene	H
22.90	eta-patchoulene	
22.93	trans-myrtanol acetate	O· Ac
23.00	(Z)-isoeugenol	O · Me
23.06	eta-cubebene	H H H
23.09	eta-elemene	

Retention time (min)	Compound	Structure
23.48	(Z)-caryophyllene	H//
23.66	methyl eugenol	O - Me O - Me
23.86	α-gurjunene	
24.20	<i>cis</i> -thujopsene	TIME THE THE THE THE THE THE THE THE THE TH
24.38	(E)-caryophyllene	
24.60	γ-elemene	
24.85	α-guaiene	
25.01	<i>α-trans-</i> bergamotene	
25.23	(Z)-methyl isoeugenol	O · Me
25.34	$(Z)$ - $\beta$ -farnesene	
25.43	9 <i>-epi-(E)</i> -caryophyllene	# H

Retention time (min)	Compound	Structure
25.63	γ-patchoulene	H H
25.91	lpha-humulene	
26.08	allo-aromadendrene	
26.10	cis-muurola-4(14), 5-diene	\tag{\tau}
26.15	α-patchoulene	
26.28	seychellene	H
26.43	γ-muurolene	
26.45	valencene	- Hill
26.56	germacrene D	
26.80	eta-selinene	
27.18	lpha-selinene	H
27.59	(Z)- $lpha$ -bisabolene	

Retention time (min)	Compound	Structure
27.61	bicyclogermacrene	
27.63	geranyl N propanoate	
27.68	viridiflorene	H X
27.73	$(E,E)$ - $\alpha$ -farnesene	
27.78	eta-bisabolene	
27.89	germacrene A	
28.00	lpha-bulnesene	H
28.04	γ-cadinene	H H
28.56	$\delta$ -cadinene	N H
28.59	7-epi- $\alpha$ -selinene	H "H
28.61	<i>cis-</i> calamenene	

Retention time (min)	Compound	Structure
28.84	eta-sesquiphellandrene	H.
28.88	lpha-cadinene	H H
29.53	germacrene B	
30.22	geranyl N-butyrate	
30.90	spathulenol	HO H
31.10	caryophyllene oxide	H H
32.23	humulene epoxide II	
32.48	(1-epi)-cubenol	OH OH
32.56	longiborneol acetate	Aco H

Retention time (min)	Compound	Structure
33.01	longipinanol	HO H
33.55	epi- $lpha$ -cadinol	H DIPO
34.04	lpha-cadinol	H H
34.13	lpha-eudesmol acetate	OAc
34.18	globulol	H OH
34.64	patchouli alcohol	HO
35.36	(Z)- $\alpha$ -trans-bergamotol acetate	O -Ac
48.10	abietatriene	
49.13	abietadiene	H H

## **B.** Mass spectra of terpenoid and nonterpenoid compound.

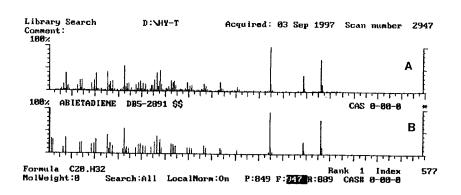


Figure 52 Mass spectra of abietadiene (A) and authentic abietadiene (B) by GC-MS

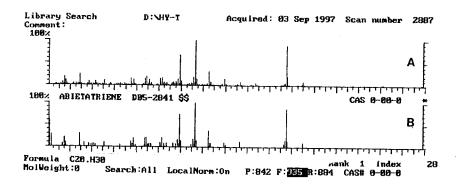


Figure 53 Mass spectra of abietatriene (A) and authentic abietatriene (B) by GC-MS

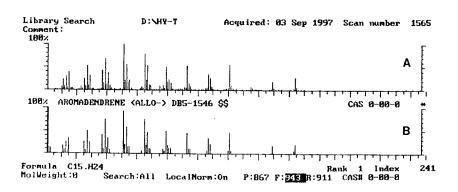


Figure 54 Mass spectra of aromadendrene <allo-> (A) and authentic aromadendrene <allo-> (B) by GC-MS

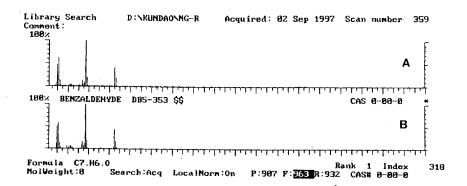


Figure 55 Mass spectra of benzaldehyde (A) and authentic benzaldehyde (B) by GC-MS

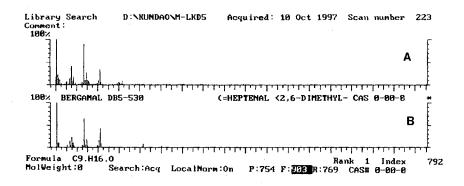


Figure 56 Mass spectra of bergamal (A) and authentic bergamal (B) by GC-MS

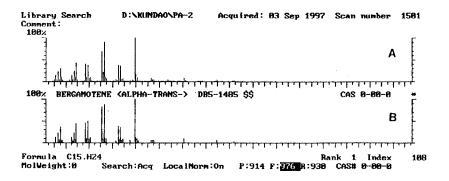
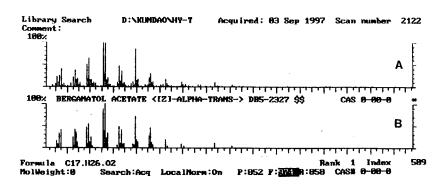


Figure 57 Mass spectra of bergamotene  $<\alpha$ -trans-> (A) and authentic bergamotene  $<\alpha$ -trans-> (B) by GC-MS



**Figure 58** Mass spectra of bergamatol acetate  $\langle (Z)-\alpha - trans - \rangle$  (A) and authentic bergamatol acetate  $\langle (Z)-\alpha - trans - \rangle$  (B) by GC-MS

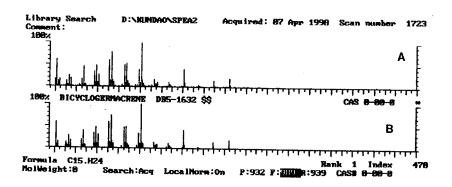
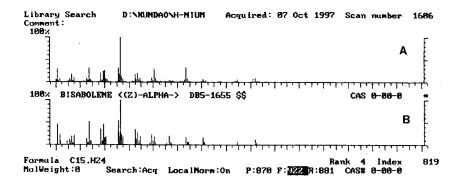
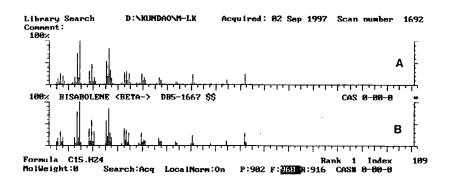


Figure 59 Mass spectra of bicyclogermacrene (A) and authentic bicyclogermacrene (B) by GC-MS



**Figure 60** Mass spectra of bisabolene  $\langle (Z)-\alpha-\rangle$ (A) and authentic bisabolene  $\langle (Z)-\alpha-\rangle$ (B) by GC-MS



**Figure 61** Mass spectra of bisabolene  $<\beta>>$  (A) and of authentic bisabolene  $<\beta>>$  (B) by GC-MS

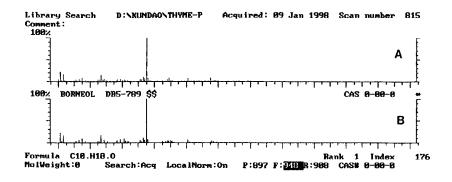


Figure 62 Mass spectra of borneol (A) and authentic borneol (B) by GC-MS

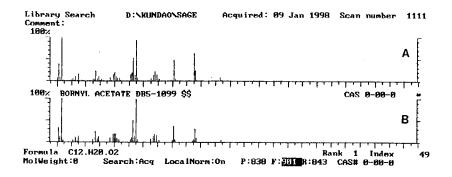
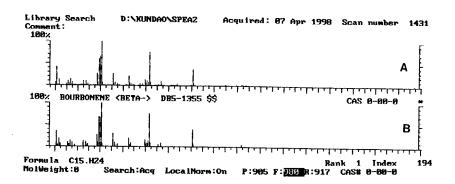


Figure 63 Mass spectra of bornyl acetate (A) and authentic bornyl acetate (B) by GC-MS



**Figure 64** Mass spectra of bourbonene  $<\beta>>$  (A) and authentic bourbonene  $<\beta>>$  (B) by GC-MS

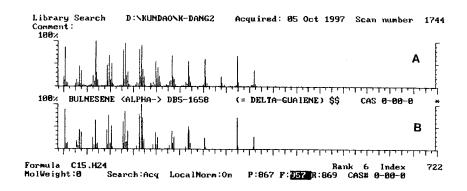
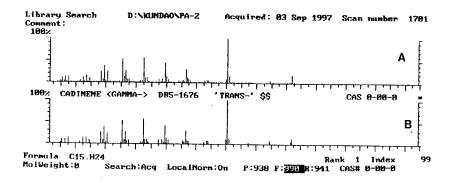
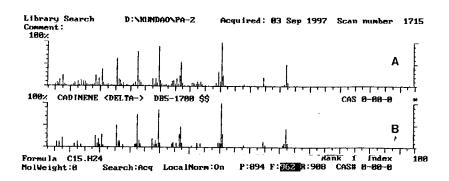


Figure 65 Mass spectra of bulnesene  $<\alpha>$  (A) and authentic bulnesene  $<\alpha>$  (B) by GC-MS



**Figure 66** Mass spectra of cadinene  $\langle \gamma - \rangle$  (A) and authentic cadinene  $\langle \gamma - \rangle$  (B) by GC-MS



**Figure 67** Mass spectra of cadinene  $<\delta->$  (A) and authentic cadinene  $<\delta->$  (B) by GC-MS

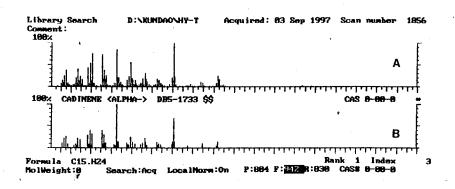
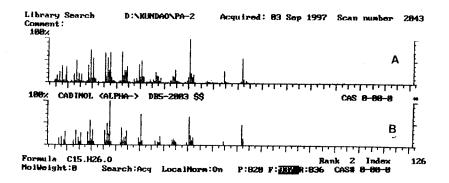
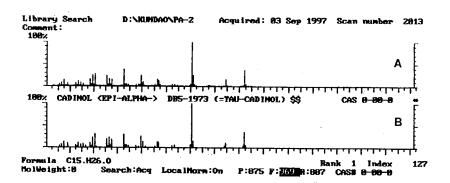


Figure 68 Mass spectra of cadinene  $<\alpha>$  (A) and authentic cadinene  $<\alpha>$  (B) by GC-MS



**Figure 69** Mass spectra of cadinol  $\langle \alpha - \rangle$  (A) and authentic cadinol  $\langle \alpha - \rangle$  (B) by GC-MS



**Figure 70** Mass spectra of cadinol  $\langle epi-\alpha-\rangle$  (A) and authentic cadinol  $\langle epi-\alpha-\rangle$  (B) by GC-MS

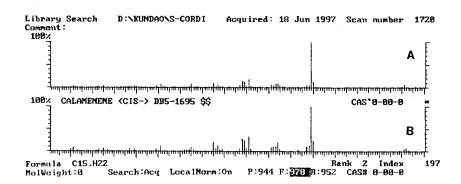


Figure 71 Mass spectra of calamenene  $\langle cis \rangle$  (A) and authentic calamenene  $\langle cis \rangle$  (B) by GC-MS

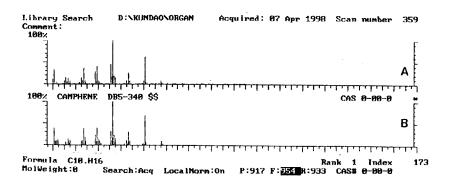


Figure 72 Mass spectra of camphene (A) and authentic camphene (B) by GC-MS

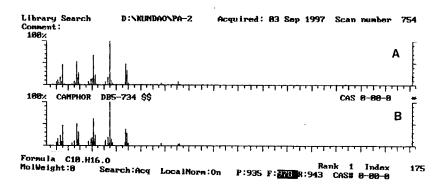


Figure 73 Mass spectra of camphor (A) and authentic camphor (B) by GC-MS

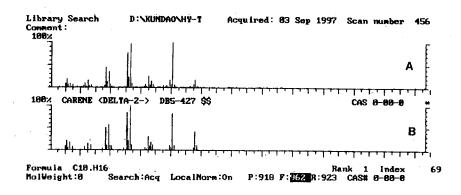


Figure 74 Mass spectra of carene  $<\delta$ -2-> (A) and authentic carene  $<\delta$ -2-> (B) by GC-MS

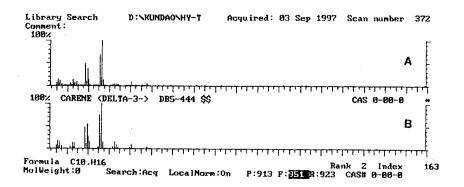


Figure 75 Mass spectra of carene  $<\delta$ -3-> (A) and authentic carene  $<\delta$ -3-> (B) by GC-MS

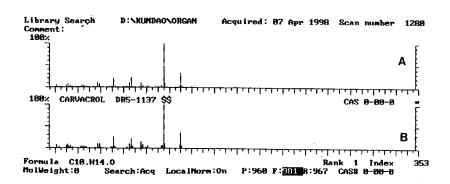


Figure 76 Mass spectra of carvacrol (A) and authentic carvacrol (B) by GC-MS

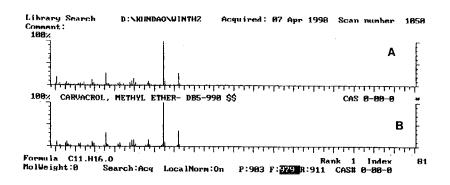


Figure 77 Mass spectra of carvacrol, methyl ether (A) and authentic carvacrol, methyl ether (B) by GC-MS

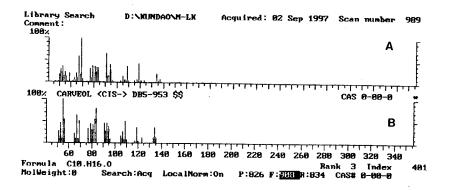


Figure 78 Mass spectra of carveol < cis > (A) and authentic carveol < cis > (B) by GC-MS

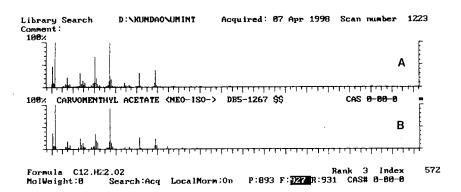


Figure 79 Mass spectra of carvomenthyl acetate <*neo-iso->* (A) and authentic carvomenthyl acetate <*neo-iso->*(B) by GC-MS

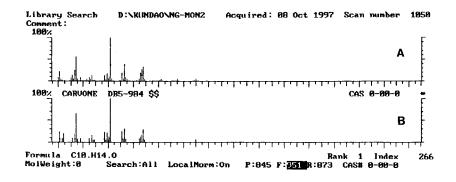


Figure 80 Mass spectra of carvone (A) and authentic carvone (B) by GC-MS

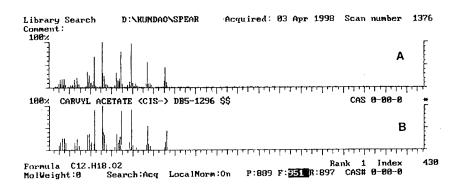
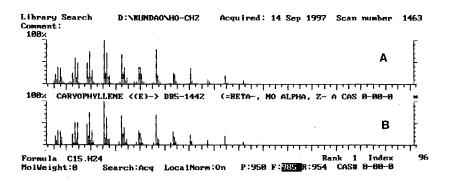
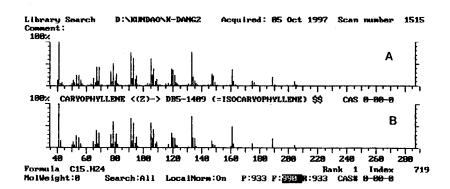


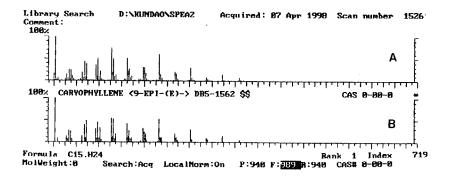
Figure 81 Mass spectra of carvyl acetate <*cis*-> (A) and authentic carvyl acetate <*cis*-> (B) by GC-MS



**Figure 82** Mass spectra of caryophyllene <(E)-> (A) and authentic caryophyllene <(E)-> (B) by GC-MS



**Figure 83** Mass spectra of caryophyllene <(Z)>(A) and authentic caryophyllene <(Z)>(B) by GC-MS



**Figure 84** Mass spectra of caryophyllene <9-epi-(E)-> (A) and authentic caryophyllene <9-epi-(E)-> (B) by GC-MS

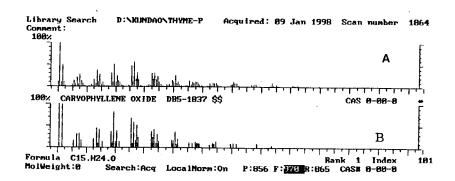


Figure 85 Mass spectra of caryophyllene oxide (A) and authentic caryophyllene oxide (B) by GC-MS

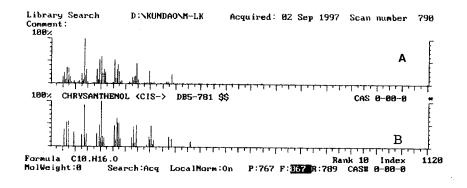


Figure 86 Mass spectra of chrysanthenol <*cis*-> (A) and authentic chrysanthenol <*cis*-> (B) by GC-MS

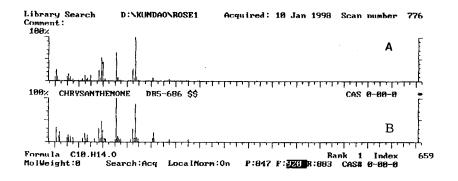


Figure 87 Mass spectra of chrysanthenone (A) and authentic chrysanthenone (B) by GC-MS

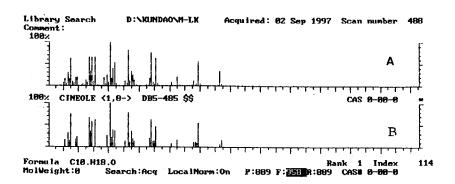


Figure 88 Mass spectra of cineole <1,8-> (A) and authentic cineole <1,8-> (B) by GC-MS

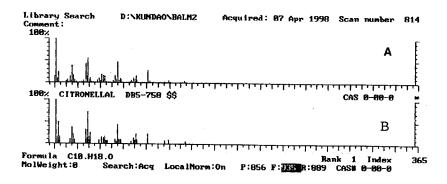
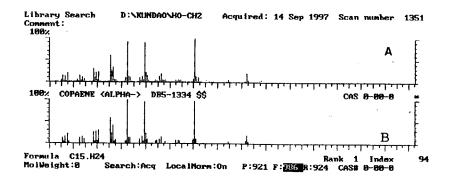
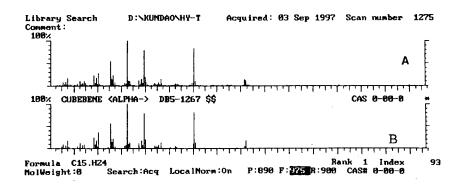


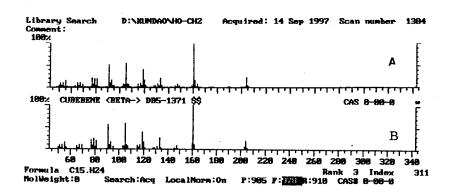
Figure 89 Mass spectra of citronellal (A) and authentic citronellal (B) by GC-MS



**Figure 90** Mass spectra of copaene  $<\alpha>>$  (A) and authentic copaene  $<\alpha>>$  (B) by GC-MS



**Figure 91** Mass spectra of cubebene  $<\alpha>$  (A) and authentic cubebene  $<\alpha>$  (B) by GC-MS



**Figure 92** Mass spectra of cubebene  $<\beta>$  (A) and authentic cubebene  $<\beta>$  (B) by GC-MS

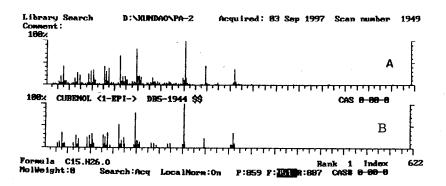


Figure 93 Mass spectra of cubenol < (1-epi)-> (A) and authentic cubenol < (1-epi)-> (B) by GC-MS

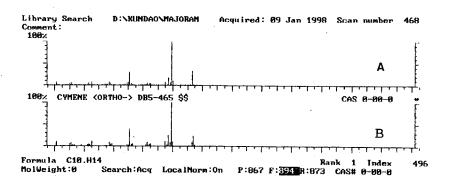


Figure 94 Mass spectra of cymene < ortho-> (A) and authentic cymene < ortho-> (B) by GC-MS

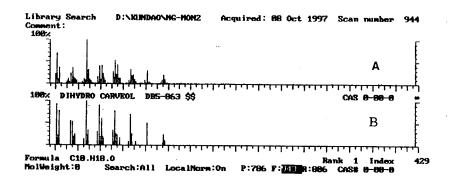


Figure 95 Mass spectra of dihydro carveol (A) and authentic dihydro carveol (B) by GC-MS

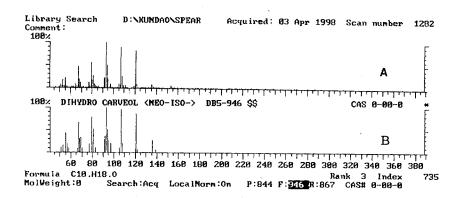


Figure 96 Mass spectra of dihydro carveol < neo-iso-> (A) and authentic dihydro carveol < neo-iso-> (B) by GC-MS

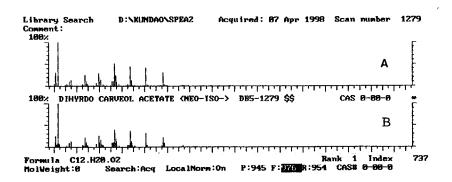


Figure 97 Mass spectra of dihydrocarveol acetate < neo-iso-> (A) and authentic dihydrocarveol acetate < neo-iso-> (B) by GC-MS

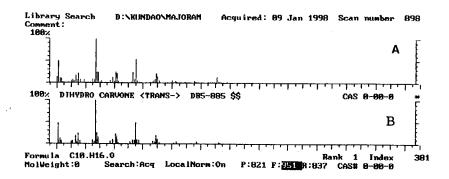


Figure 98 Mass spectra of dihydrocarvone < trans-> (A) and authentic dihydrocarvone < trans-> (B) by GC-MS

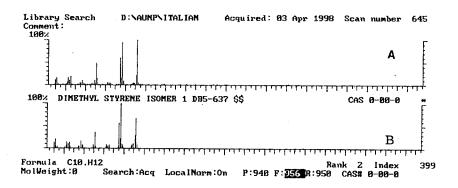
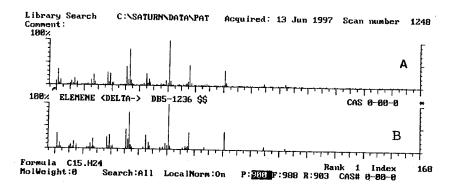
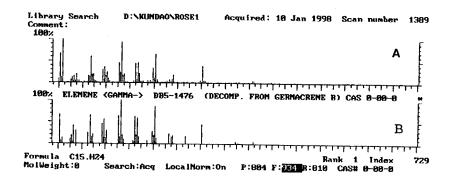


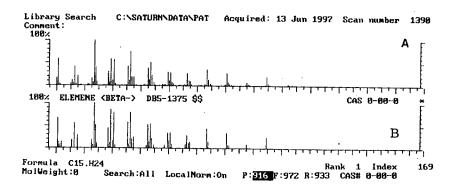
Figure 99 Mass spectra of dimethyl styrene isomer # 1 (A) and authentic dimethyl styrene isomer # 1 (B) by GC-MS



**Figure 100** Mass spectra of elemene  $<\delta>>$  (A) and authentic elemene  $<\delta>>$  (B) by GC-MS



**Figure 101** Mass spectra of elemene  $\langle \gamma - \rangle$  (A) and authentic elemene  $\langle \gamma - \rangle$  (B) by GC-MS



**Figure 102** Mass spectra of elemene  $<\beta>>$  (A) and authentic elemene  $<\beta>>$  (B) by GC-MS

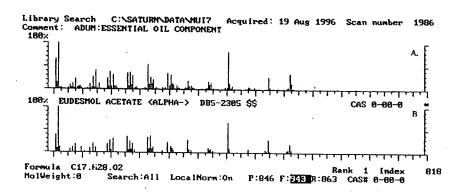


Figure 103 Mass spectra of eudesmol acetate  $\langle \alpha - \rangle$  (A) and authentic eudesmol acetate  $\langle \alpha - \rangle$  (B) by GC-MS

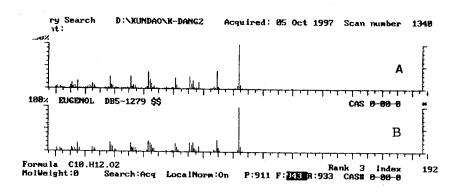


Figure 104 Mass spectra of eugenol (A) and authentic eugenol (B) by GC-MS

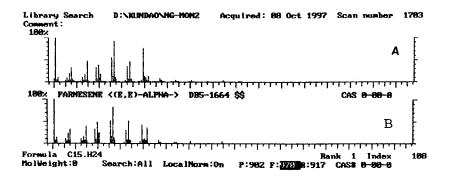
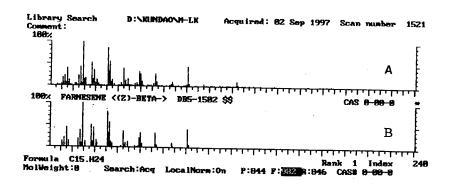


Figure 105 Mass spectra of farnesene  $\langle (E,E)-\alpha-\rangle$  (A) and authentic farnesene  $\langle (E,E)-\alpha-\rangle$  (B) by GC-MS



**Figure 106** Mass spectra of farnesene  $<(Z)-\beta->$  (A) and authentic farnesene  $<(Z)-\beta->$  (B) by GC-MS

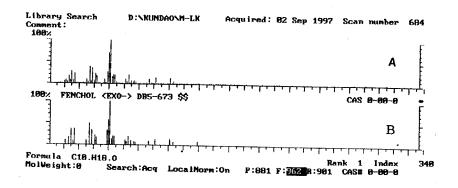


Figure 107 Mass spectra of fenchol <(exo)-> (A) and authentic fenchol <(exo)-> (B) by GC-MS

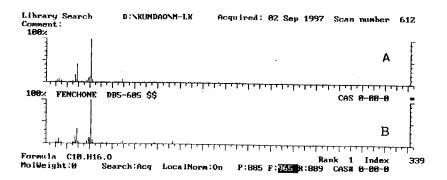


Figure 108 Mass spectra of fenchone (A) and authentic fenchone (B) by GC-MS

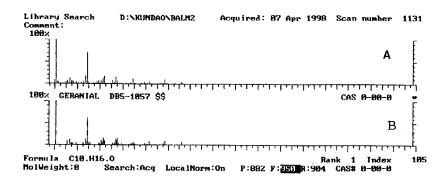


Figure 109 Mass spectra of geranial (A) and authentic geranial (B) by GC-MS

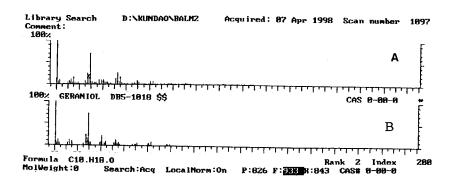


Figure 110 Mass spectra of geraniol (A) and authentic geraniol (B) by GC-MS

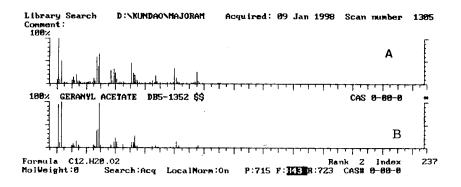


Figure 111 Mass spectra of geranyl acetate (A) and authentic geranyl acetate (B) by GC-MS

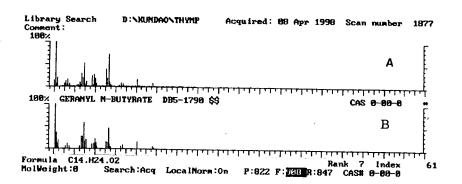


Figure 112 Mass spectra of geranyl N-butyrate (A) and authentic geranyl N-butyrate (B) by GC-MS

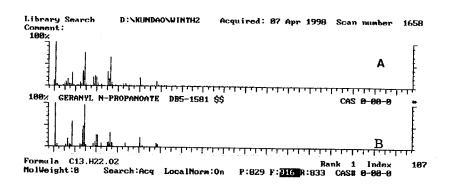


Figure 113 Mass spectra of geranyl N- propanoate (A) and authentic geranyl N- propanoate (B) by GC-MS

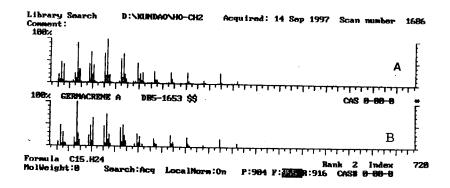


Figure 114 Mass spectra of germacrene A (A) and authentic germacrene A (B) by GC-MS

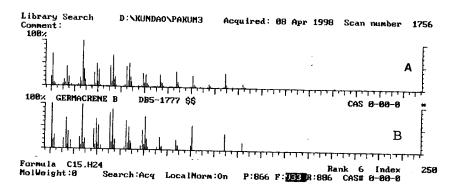


Figure 115 Mass spectra of germacrene B (A) and authentic germacrene B (B) by GC-MS

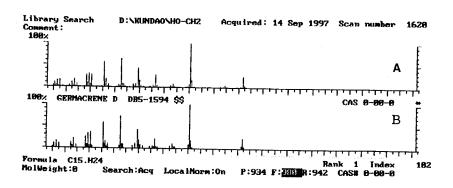


Figure 116 Mass spectra of germacrene D (A) and authentic germacrene D (B) by GC-MS

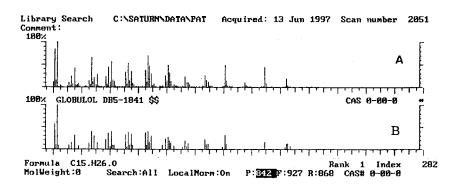
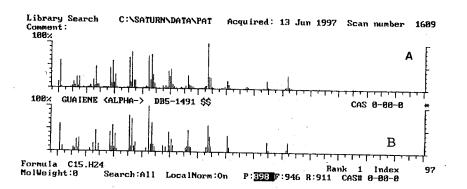


Figure 117 Mass spectra of globulol (A) and authentic globulol (B) by GC-MS



**Figure 118** Mass spectra of guaiene  $\langle \alpha - \rangle$  (A) and authentic guaiene  $\langle \alpha - \rangle$  (B) by GC-MS

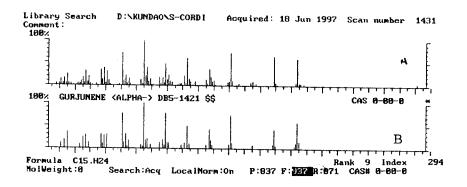


Figure 119 Mass spectra of gurjunene  $<\alpha>$  (A) and authentic gurjunene  $<\alpha>$  (B) by GC-MS

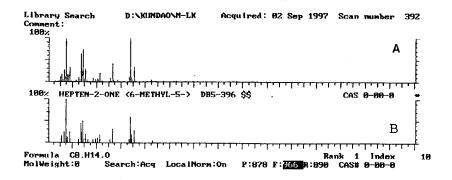
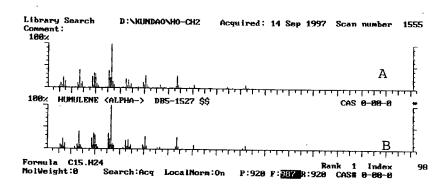


Figure 120 Mass spectra of hepten-2-one -<6-methyl-5-> (A) and authentic hepten-2-one -<6-methyl-5-> (B) by GC-MS



**Figure 121** Mass spectra of humulene  $\langle \alpha - \rangle$  (A) and authentic humulene  $\langle \alpha - \rangle$  (B) by GC-MS

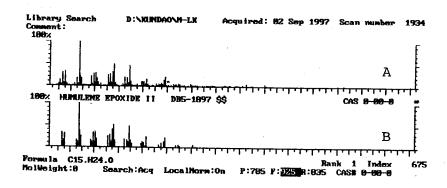
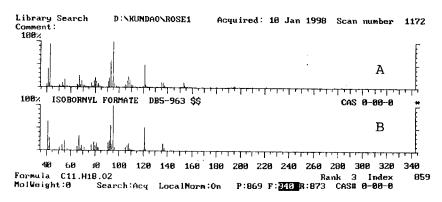


Figure 122 Mass spectra of humulene epoxide II (A) and authentic humulene epoxide II (B) by GC-MS



**Figure 123** Mass spectra of isobonyl formate (A) and authentic isobonyl formate (B) by GC-MS

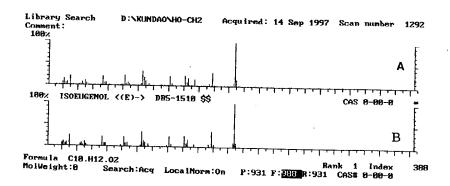
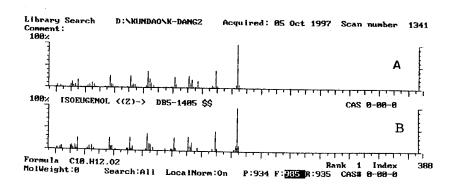


Figure 124 Mass spectra of isoeugenol <(E)->(A) authentic isoeugenol <(E)->(B) by GC-MS



**Figure 125** Mass spectra of isoeugenol  $\leq$ (Z)- $\geq$ (A) and authentic isoeugenol  $\leq$ (Z)- $\geq$ (B) by GC-MS

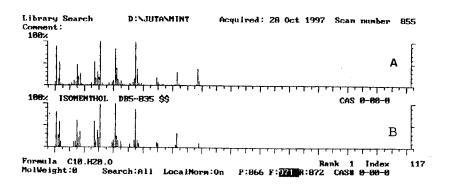


Figure 126 Mass spectra of isomenthol (A) and authentic isomenthol (B) by GC-MS

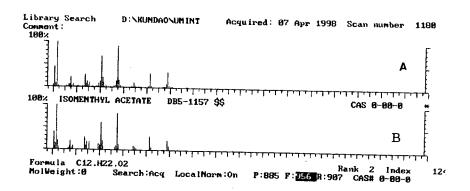


Figure 127 Mass spectra of isomenthyl acetate (A) and authentic isomenthyl acetate (B) by GC-MS

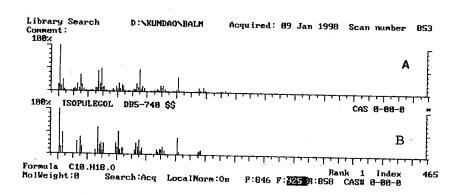


Figure 128 Mass spectra of isopulegol (A) and authentic isopulegol (B) by GC-MS

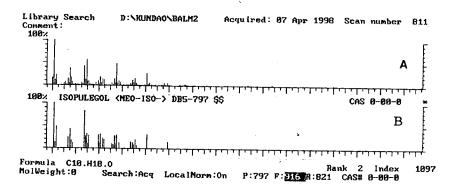


Figure 129 Mass spectra of isopulegol <neo-iso-> (A) and authentic isopulegol <neo-iso-> (B) by GC-MS

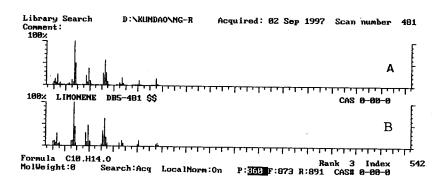


Figure 130 Mass spectra of limonene (A) and authentic limonene (B) by GC-MS

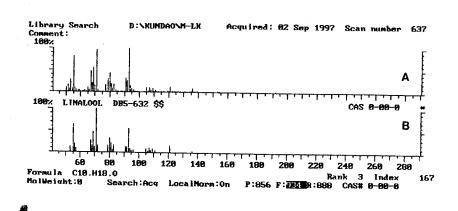


Figure 131 Mass spectra of linalool (A) and authentic linalool (B) by GC-MS

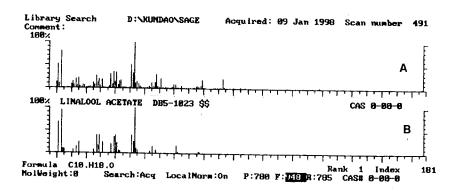


Figure 132 Mass spectra of linalool acetate (A) and authentic linalool acetate (B) by GC-MS

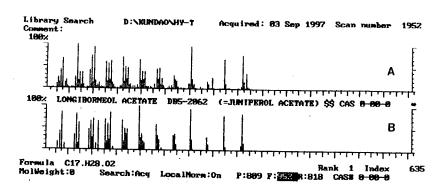


Figure 133 Mass spectra of longiborneol acetate (A) and authentic longiborneol acetate (B) by GC-MS

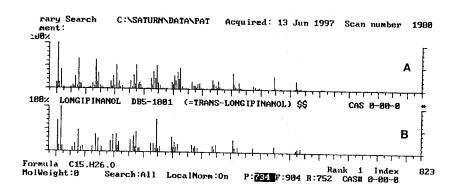


Figure 134 Mass spectra of longipinanol (A) and authentic longipinanol (B) by GC-MS

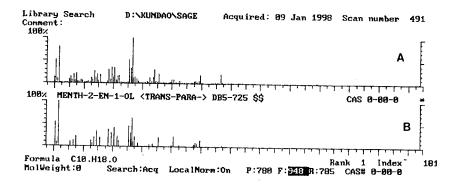


Figure 135 Mass spectra of menth-2-en-1-ol < trans-para-> (A) and authentic menth-2-en-1-ol < trans-para-> (B) by GC-MS

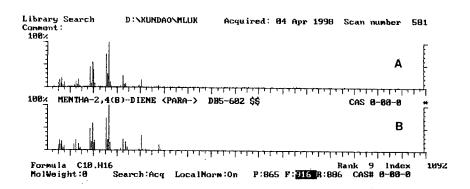


Figure 136 Mass spectra of mentha-2,4(8)-diene <para->(A) and authentic mentha-2,4(8)-diene <para->(B) by GC-MS

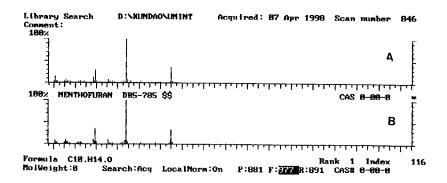


Figure 137 Mass spectra of menthofuran (A) and authentic menthofuran (B) by GC-MS

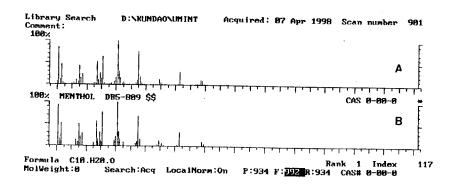


Figure 138 Mass spectra of menthol (A) and authentic menthol (B) by GC-MS

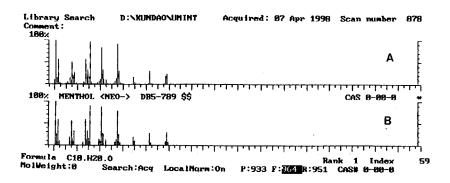


Figure 139 Mass spectra of menthol < neo > (A) and authentic menthol < neo > (B) by GC-MS

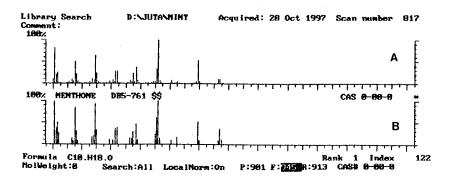


Figure 140 Mass spectrum of menthone (A) and authentic menthone (B) by GC-MS

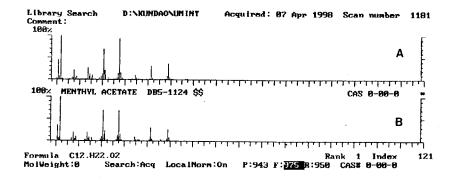


Figure 141 Mass spectra of menthyl acetate (A) and authentic menthyl acetate (B) by GC-MS

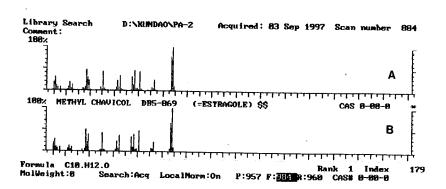


Figure 142 Mass spectra of methyl chavicol (A) and authentic methyl chavicol (B) by GC-MS

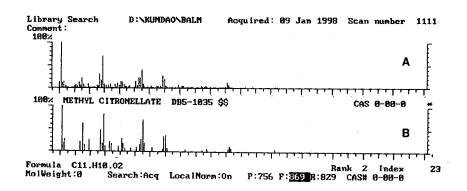


Figure 143 Mass spectra of methyl citronellate (A) and authentic methyl citronellate (B) by GC-MS

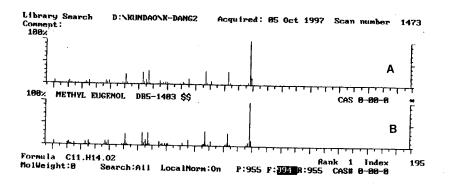


Figure 144 Mass spectra of methyl eugenol (A) and authentic methyl eugenol (B) by GC-MS

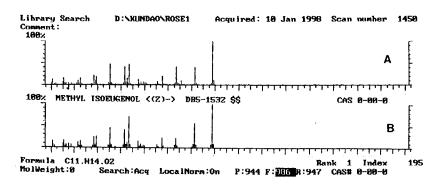


Figure 145 Mass spectra of methyl isoeugenol <(Z)->(A) and authentic methyl isoeugenol <(Z)->(B) by GC-MS

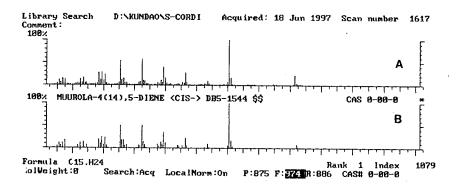


Figure 146 Mass spectra of muurola-4(14), 5-diene <*cis*-> (A) and authentic muurola-4(14), 5-diene <*cis*-> (B) by GC-MS

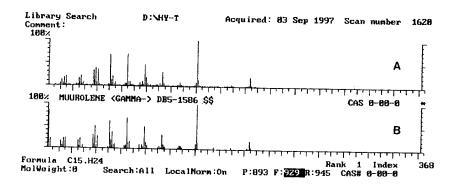


Figure 147 Mass spectra of muurolene  $\langle \gamma - \rangle$  (A) and authentic muurolene  $\langle \gamma - \rangle$  (B) by GC-MS

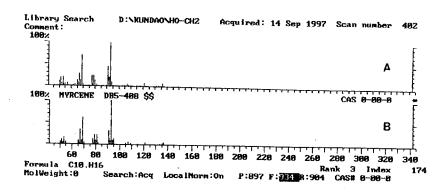


Figure 148 Mass spectra of myrcene (A) and authentic myrcene (B) by GC-MS

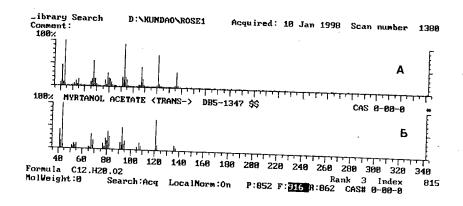


Figure 149 Mass spectra of myrcene (A) and authentic myrcene (B) by GC-MS

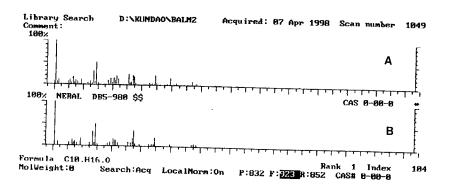


Figure 150 Mass spectra of neral (A) and authentic neral (B) by GC-MS

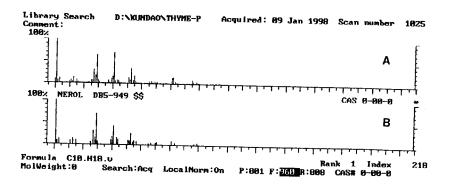
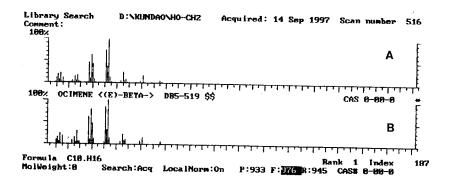


Figure 151 Mass spectra of nerol (A) and authentic nerol (B) by GC-MS



**Figure 152** Mass spectra of ocimene  $\leq$  (*E*)- $\beta$ - $\geq$  (A) and authentic ocimene  $\leq$  (*E*)- $\beta$ - $\geq$  (B) by GC-MS

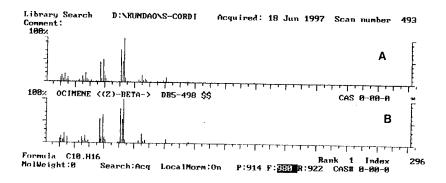


Figure 153 Mass spectra of ocimene  $\langle (Z)-\beta-\rangle$  (A) and authentic ocimene  $\langle (Z)-\beta-\rangle$  (B) by GC-MS

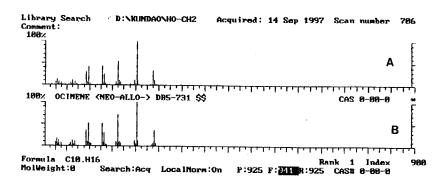


Figure 154 Mass spectra of ocimene < neo-allo-> (A) and authentic ocimene < neo-allo-> (B) by GC-MS

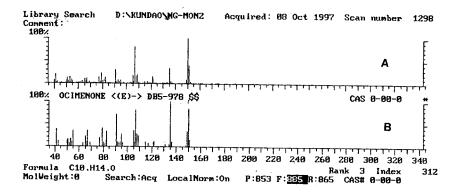


Figure 155 Mass spectra of ocimenone <(E)->(A) and authentic ocimenone <(E)->(B) by GC-MS

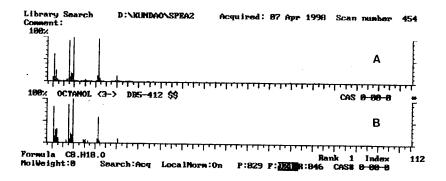


Figure 156 Mass spectra of octanol <3-> (A) and authentic octanol <3-> (B) by GC-MS

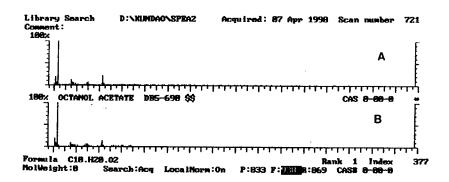


Figure 157 Mass spectra of octanol acetate (A) and authentic octanol acetate (B) by GC-MS

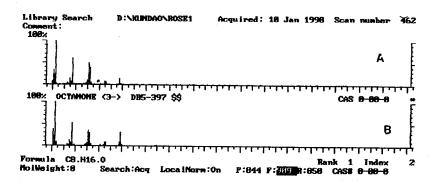


Figure 158 Mass spectra of octanone <3-> (A) and authentic octanone <3-> (B) by GC-MS

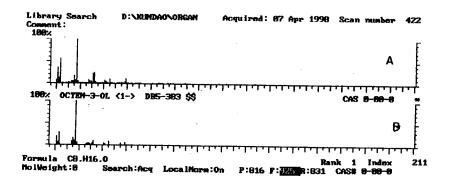


Figure 159 Mass spectra of octen-3-ol <1-> (A) and authentic octen-3-ol <1-> (B) by GC-MS

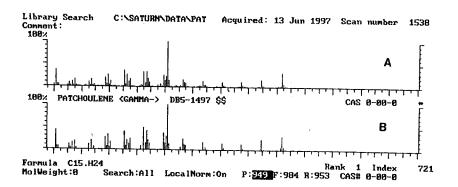


Figure 160 Mass spectra of patchoulene  $\langle \gamma - \rangle$  (A)and authentic patchoulene  $\langle \gamma - \rangle$  (B) by GC-MS

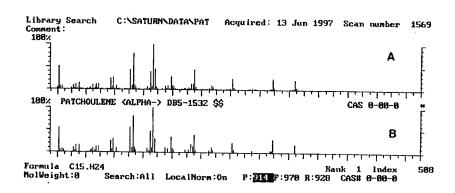


Figure 161 Mass spectra of patchoulene  $<\alpha>$  (A) and authentic patchoulene  $<\alpha>$  (B) by GC-MS

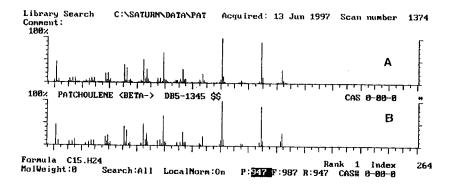


Figure 162 Mass spectra of patchoulene  $<\beta>$  (A) and authentic patchoulene  $<\beta>$  (B) by GC-MS

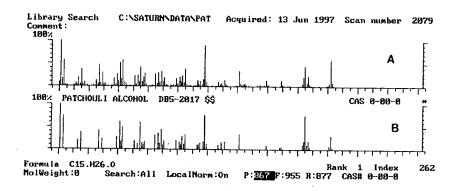


Figure 163 Mass spectra of patchouli alcohol (A) and authentic patchouli alcohol (B) by GC-MS

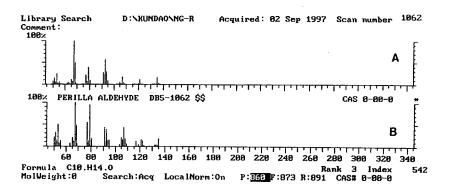


Figure 164 Mass spectra of perilla aldehyde (A) and authentic perilla aldehyde (B) by GC-MS

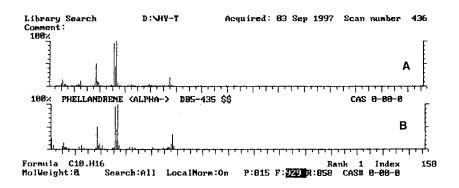
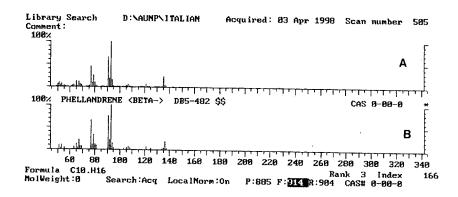
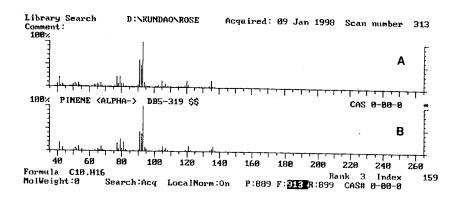


Figure 165 Mass spectra of phellandrene  $<\alpha>$  (A) and authentic phellandrene  $<\alpha>$  (B) by GC-MS



**Figure 166** Mass spectra of phellandrene  $<\beta>>$  (A) and authentic phellandrene  $<\beta>>$  (B) by GC-MS



**Figure 167** Mass spectra of pinene  $<\alpha>$  (A) and authentic pinene  $<\alpha>$  (B) by GC-MS

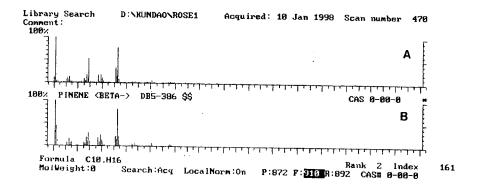
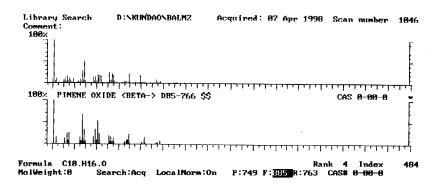


Figure 168 Mass spectra of pinene  $<\beta>$  (A) and authentic pinene  $<\beta>$  (B) by GC-MS



**Figure 169** Mass spectra of pinene oxide  $<\beta>$  (A) and authentic pinene oxide  $<\beta>$  (B) by GC-MS

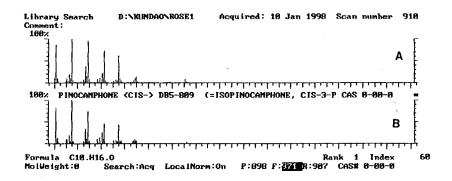


Figure 170 Mass spectra of pinocamphone <*cis*-> (A) and authentic pinocamphone <*cis*-> (B) by GC-MS

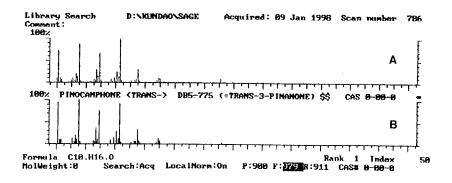


Figure 171 Mass spectra of pinocamphone <trans-> (A) and authentic pinocamphone <trans-> (B) by GC-MS

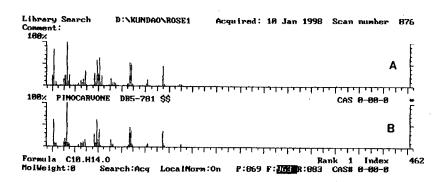


Figure 172 Mass spectra of pinocarvone (A) and authentic pinocarvone (B) by GC-MS

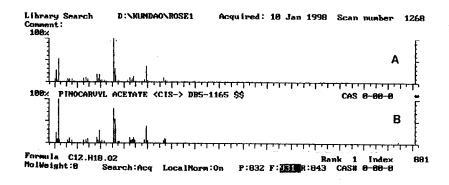


Figure 173 Mass spectra of pinocarvyl acetate  $\langle cis \rangle$  (A) and authentic pinocarvyl acetate  $\langle cis \rangle$  (B) by GC-MS

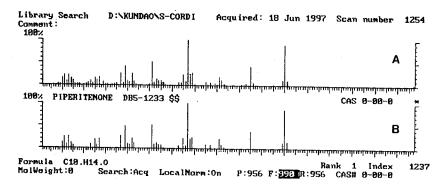


Figure 174 Mass spectra of piperitenone (A) and authentic piperitenone (B) by GC-MS
(B) by GC-MS

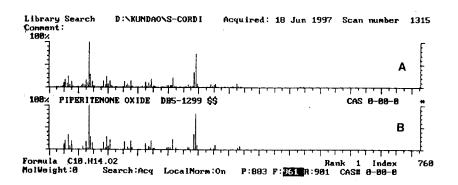


Figure 175 Mass spectra of piperitenone oxide (A) and authentic piperitenone oxide (B) by GC-MS

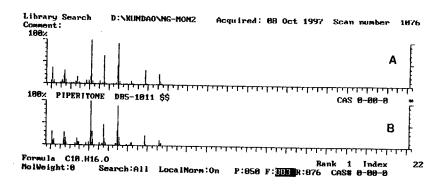


Figure 176 Mass spectra of piperitone (A) and authentic piperitone (B) by GC-MS

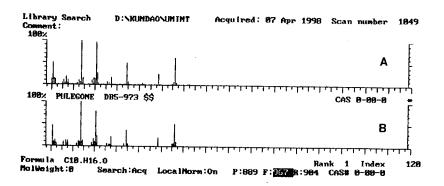


Figure 177 Mass spectra of pulegone (A) and authentic pulegone (B) by GC-MS

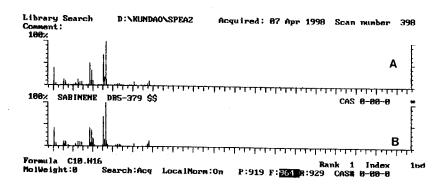


Figure 178 Mass spectra of sabinene (A) and authentic sabinene (B) by GC-MS

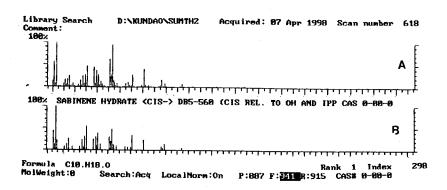
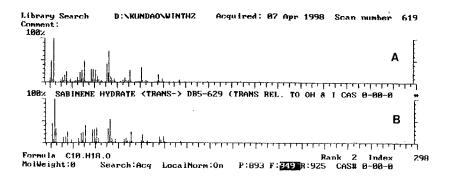
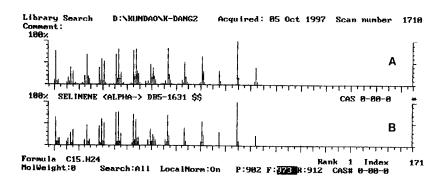


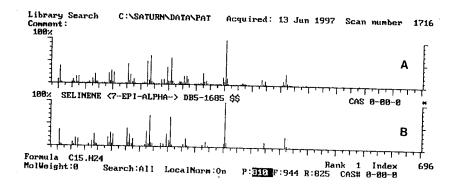
Figure 179 Mass spectra of sabinene hydrate  $\langle cis \rangle$  (A) and authentic sabinene hydrate  $\langle cis \rangle$  (B) by GC-MS



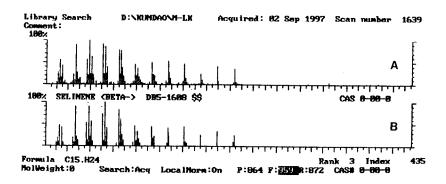
**Figure 180** Mass spectra of sabinene hydrate <*trans->* (A) and authentic sabinene hydrate <*trans->* (B) by GC-MS



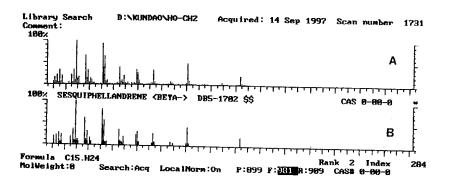
**Figure 181** Mass spectra of selinene  $<\alpha ->$  (A) and authentic selinene  $<\alpha ->$  (B) by GC-MS



**Figure 182** Mass spectra of selinene <7-epi- $\alpha$ -> (A) and authentic selinene <7-epi- $\alpha$ -> (B) by GC-MS



**Figure 183** Mass spectra of selinene  $<\beta>>$  (A) and authentic selinene  $<\beta>>$  (B) by GC-MS



**Figure 184** Mass spectra of sesquiphellandrene  $<\beta>$  (A) and authentic sesquiphellandrene  $<\beta>$  (B) by GC-MS

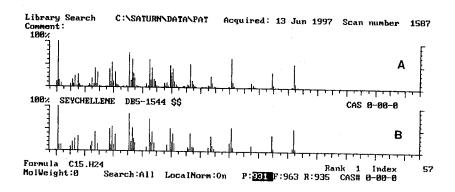


Figure 185 Mass spectra of seychellene (A) and authentic seychellene (B) by GC-MS

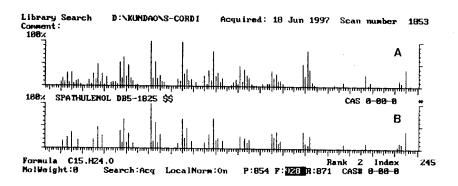


Figure 186 Mass spectra of spathulenol (A) and authentic spathulenol (B) by GC-MS

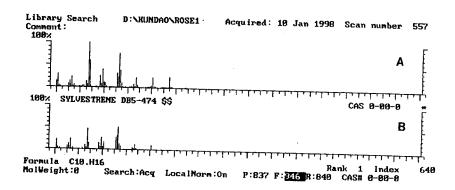


Figure 187 Mass spectra of sylvestrene (A) and authentic sylvestrene (B) by GC-MS

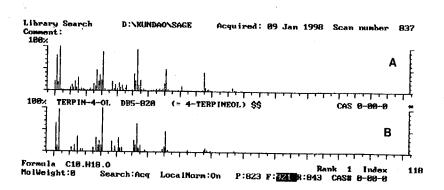


Figure 188 Mass spectra of terpin-4-ol (A) and authentic terpin-4-ol (B) by GC-MS

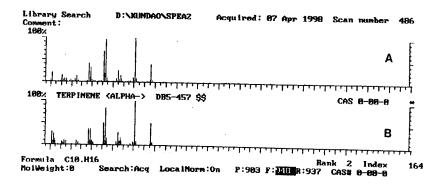
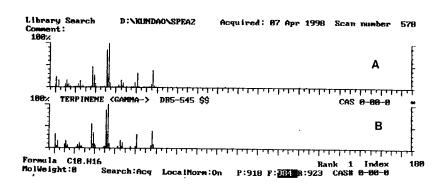
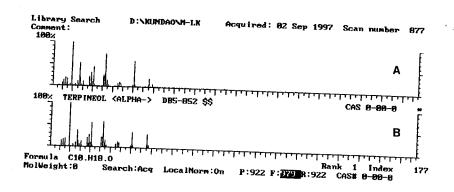


Figure 189 Mass spectra of terpinene  $<\alpha>$  (A) and authentic terpinene  $<\alpha>$  (B) by GC-MS



**Figure 190** Mass spectra of terpinene  $<\gamma>$  (A) and of authentic terpinene  $<\gamma>$  (B) by GC-MS



**Figure 191** Mass spectra of terpineol  $<\alpha>$  (A) and of authentic terpineol  $<\alpha>$  (B) by GC-MS

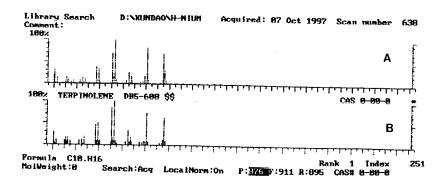


Figure 192 Mass spectra of terpinolene (A) and authentic terpinolene (B) by GC-MS

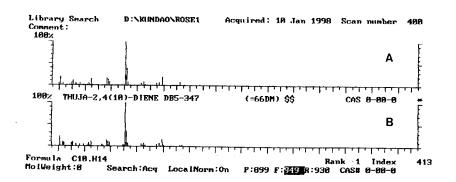
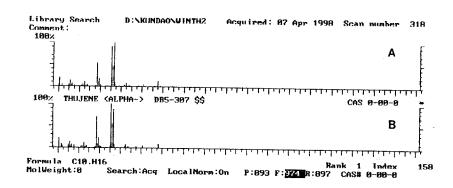


Figure 193 Mass spectra of thuja 2,4 (10) - diene (A) and authentic thuja 2,4 (10)-diene (B) by GC-MS



**Figure 194** Mass spectra of thujene  $<\alpha->$  (A) and authentic thujene  $<\alpha->$  (B) by GC-MS

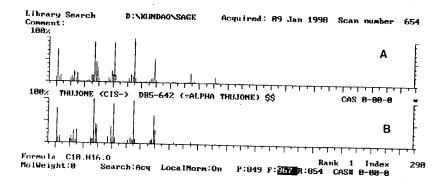


Figure 195 Mass spectra of thujone  $\langle cis \rangle$  (A) and of authentic thujone  $\langle cis \rangle$  (B) by GC-MS

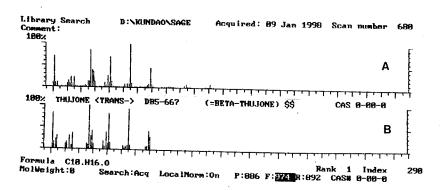


Figure 196 Mass spectra of thujone < trans-> (A) and authentic thujone < trans-> (B) by GC-MS

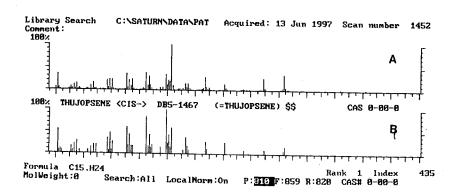


Figure 197 Mass spectra of thujopsene  $\langle cis \rangle$  (A) and authentic thujopsene  $\langle cis \rangle$  (B) by GC-MS

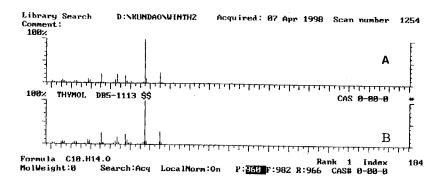


Figure 198 Mass spectra of thymol (A) and authentic thymol (B) by GC-MS

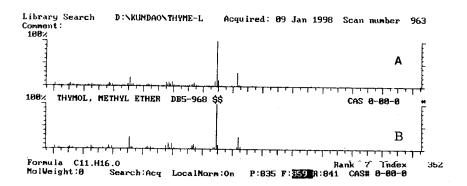


Figure 199 Mass spectra of thymol, methyl ether (A) and authentic thymol, methyl ether (B) by GC-MS

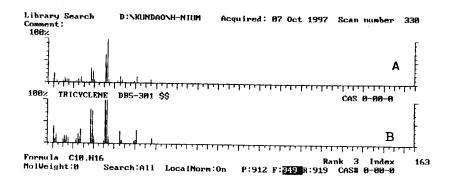


Figure 200 Mass spectra of tricyclene (A) and authentic tricyclene (B) by GC-MS

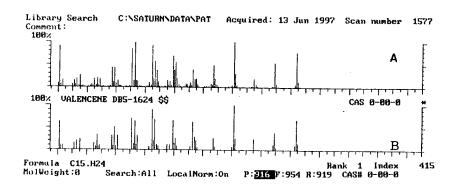


Figure 211 Mass spectra of valencene (A) and authentic valencene (B) by GC-MS

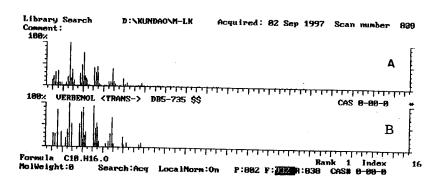


Figure 202 Mass spectra of verbenol < trans-> (A) and authentic verbenol < trans-> (B) by GC-MS

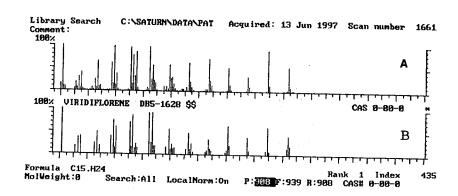


Figure 203 Mass spectra of viridiflorene (A) and authentic viridiflorene (B) by GC-MS

## **VITA**

Miss Daojan Choochoat was born in August 7,1972 in Chanthaburi, Thailand. She received her Bachelor of Science in Pharmacy in 1994 from Rangsit University, Pathum Thani, Thailand. At present, she is a faculty member of the Department of Pharmacognosy, Faculty of Pharmacy, Rangsit University, Pathum Thani, Thailand.