Chemical investigation of Aromatic and Medicinal plants from the New Guinea highlands and North Queensland

Thesis submitted by

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James Cook University of North Queensland

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Statement of the contribution of others

I, the undersigned author of this thesis, acknowledge the contribution of others to this work. Substantial supervision was provided by A/Prof. Bruce Bowden (School of Pharmacy and Molecular Sciences, JCU; primary supervision) and Prof. Beverley Glass (School of Pharmacy and Molecular Sciences, JCU; co-supervision). Editorial assistance in the preparation of this thesis was provided by A/Prof. Bruce Bowden.

Plant essential oils from PNG were offered by Dr Stewart Wossa (Goroka University, PNG) and assistance in identifying the plant species collected on JCU Douglas campus was provided by A/Prof. Betsy Jackes (School of Marine and Tropical Biology, JCU). Assistance in the antimicrobial assays was provided by Dr Gabriel Padilla, Dr Jeffrey Warner and Ms Kerryn Price-Wilson (School of Veterinary and Biomedical Sciences, JCU). Dr Shane Askew (School of Pharmacy and Molecular Sciences, JCU) performed Gas Chromatography - Mass Spectrometry (GC-MS). Dr Catherine Liptrot at the Australian Institute of Marine Science, Townsville, and Dr Anna-Marie Babey (School of Veterinary and Biomedical Sciences, JCU) performed cytotoxic assays and Dr Cherrie Motti (Australian Institute of Marine Science, Townsville) performed Mass Spectrometry (MS) on samples.

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Finally, I wish to thank my family for supporting me with love during the studies in Australia.

Abstract

Various aromatic and medicinal plant species from the New Guinea highlands and North Queensland were investigated to discover and characterise new molecular entities with useful pharmacological properties, which could potentially become lead compounds for the development of new drug products.

A new optically active diterpene ester was isolated from the plant species *Stylosanthes hamata* and its structure determined using spectroscopic technique (^{1}H, ^{13}C, HSQC, HMBC and COSY). It is the 3-hydroxy-3-methyl glutarate ester of cativol, a diterpene previously reported from *Halimium viscosum*. Some components of crude essential oil samples were identified without the need for separation of their mixture by the use of gradient selective NMR techniques. A total of twenty-six known compounds were identified by either this method or by separating and analysing the essential oils of various species from the New Guinea highlands and North Queensland. They included; monoterpenes, sesquiterpenes, acetate-derived metabolites such as the acetogenins/polyketides methyl salicylate and gibbilimbols, and shikimic acid-derived metabolites such as dillapiole and *trans*-anethole. Some of the minor components detected in one species were the same as major components in other essential oil samples and it is suspected that cross contamination may have occurred during the essential oil distillation in PNG. This problem will make publications of results difficult unless uncontaminated samples are available for comparison.

Cytotoxic and antimicrobial assays were performed on all essential oils and extracts.

Although some essential oil samples displayed cytotoxicity, the levels were considered too low to warrant further investigation. The antimicrobial assays employed included the use of six gram positive and six gram negative bacteria as well as one yeast and five fungi. The major components of the distillates that produced a large zone of inhibition in the initial screening were selected and retested against the microbes. All of the metabolites tested showed lower activity than the crude extracts. This may be due the presence of minor components in the distillates that have much greater activity or synergistic effects from other oil components.

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List of Abbreviations

1D	one dimensional
2D	two dimensional
ATCC	American Type Culture Collection
br	broad
CH_3CN	acetonitrile
CD	Circular Dichroism
$CDCl_3$	deuterated chloroform
COSY	^1H ^1J-Correlated Spectroscopy
d	doublet
dd	double doublet
ddd	doublet of double doublets
ddt	doublet of double triplets
DEPT	Distortionless Enhancement by Polarisation Transfer
dt	double triplet
EtOAc	ethyl acetate
ESI	Electrospray Ionisation Mass Spectrometry
GC	Gas Chromatography
GC-MS	Gas Chromatography - Mass Spectrometry
hept	heptet
HMBC	Heteronuclear Multiple Bond Coherence
HSQC	Heteronuclear Single Quantum Correlation
HPLC	High Performance Liquid Chromatography

IR	Infrared
m	multiplet
MeOH	methanol
mult	multiplicity
MS	Mass Spectrometry
NMR	Nuclear Magnetic Resonance
PDA	photodiode-array
ppm	parts per million
s	singlet
sp.	species (singular)
spp.	species (plural)
t	triplet
td	triple doublet
TLC	Thin Layer Chromatography
TOCSY	Totally Correlated Spectroscopy
UV	ultraviolet
q	quartet

CHAPTER 1 Introduction

1.1 General introduction

Higher plants represent a rich source of new molecules with pharmacological properties that could cause them to be considered as lead compounds for the development of new drug products. During the last few decades, renewed interest in the investigation of natural substances has led to the introduction of several important drugs that are used as chemotherapeutic agents in the fight against cancer. These include some of the early natural anti-cancer substances such as vinblastine, vincristine, and camptothecine derivatives, which are still in use today.[1] In addition, taxol, which was discovered in the 1960's, was approved for clinical use in 2000.[2]

1.2 Medicinal plants

Plants have been an important part of medicinal practice for thousands of years and are still the mainstay of medical practice in many parts of the world including China, Africa, India, South America and other developing parts of our world. Over the centuries, medicinal herbs have become an important part of daily life in the western world despite significant progress in modern medical and pharmaceutical research. According to a report by the World Bank 1997, the use of plant-based medicines have been increasing all over the world. Nearly 50 percent of medicines on the market are made from natural basic materials.[3] Interestingly, the market demand for medicinal herbs is likely to remain high because many of the active ingredients in medicinal plants cannot yet be prepared economically utilising synthetic processes.

Plants have formed the basis of sophisticated traditional medicine systems that extend back thousands of years.[4] The first cuneiform records of medicinal plants are found on hundreds of clay tablets from Mesopotamia dated about 2600 B.C. These records include mention of approximately 1000 plant derived substances all of which are found today in preparations for the treatment of ailments ranging from coughs and colds to parasitic infections and inflammation.[5] Egyptian medicine dates from about 1500 B.C. ('Ebers Papyrus') and documents over 700 drugs. The Chinese *Materia Medica* and documentation of the Indian Ayurvedic system both date from about 1000 B.C.[6, 7] In later years, many other works were formally codified at least Europe in the 1618 publication of the London Pharmacopoeia.

The idea of 'pure' compounds as drugs may be traced back to the 1800s, when the active principles of commonly used plants and herbs such as strychnine, morphine, atropine and colchicine were first isolated. These isolations were then followed by what can be considered the first commercial pure natural product, morphine, by E. Merck in 1826 and the first semi-synthetic pure drug based on a natural product, aspirin, by Bayer in 1899.[8]

Even today, in many countries in Asia, Africa and South America rely on traditional medicines. In China about 40% of the total medicinal consumption is attributed to traditional tribal medicines. In the mid-90s, herbal medicine sales amounted to more than US$2.5 billion and in Japan herbal medicinal preparations are in greater demand than mainstream pharmaceutical products.[9]

1.3 The aim of this study

This project, is part of the wider search for natural drug products, and aimed to investigate medicinal plants for their drug properties. In particular, it aimed to discover and characterise new compounds with pharmacological activity. It focused on cytotoxic activity that might have applications in the development of new anti-cancer agents, and antimicrobial activity that might have some applications in the treatment of those infections that have increasingly developed resistance to conventional antibiotics.

The project investigated aromatic and medicinal highland plants from the Goroka region of PNG through collaboration with Stewart Wossa at the University of Goroka in PNG. In addition, three species were collected on JCU Douglas campus and the essential oil from two, together with a methanol extract of the third, were characterised. Essential oils and plant extracts were screened using *in vitro* cell-based assays to observe cytotoxicity. Culture plates were utilised for antimicrobial activity assays. Many of the essential oils screened were from introduced species that are known to have commercial value, so the project also has potential commercial implications.

1.4 Literature review of the species for which steam distillates have been obtained

1.4.1 *Tithonia diversifolia* (Hemsl.) A. Gray (Asteraceae)

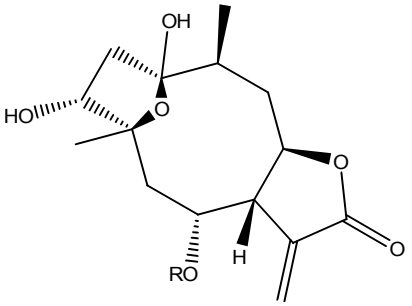
Tithonia diversifolia, commonly known as the Mexican Sunflower, is a 2-5 m tall perennial shrub, that is native to Mexico and also grows in parts of Africa, Australia, Asia, and parts of North America, where it may become locally abundant.[10] Extracts of the aerial parts of *T. diversifolia* have traditionally formed the basis of a decoction for

the treatment of diarrhoea, fever, haematomas, hepatitis, hepatomas, malaria, and wounds.[11-13]

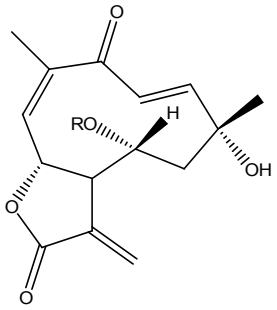
Previous phytochemical investigations of *T. diversifolia* resulted in the isolation of sesquiterpene lactones, cadinanes, chromenes, eudesmanes, flavones, germacranes, and rearranged eudesmane derivatives.[14-21] Sesquiterpene lactones constitute an important group of secondary metabolites which are common constituents of the family Asteraceae. Many secondary metabolites produced by different plants are harmful to other plant species and help to reduce competition in their natural habitats; this phenomenon is called allelopathy.[22] Such plant-derived chemicals are of considerable agronomic interest because of their low level of hazard to nontarget plant species.[23,24] Baruah *et al.,* described the inhibitory effects of two sesquiterpene lactones tagitinin A (**1**), tagitinin C (**2**) and a flavonoid hispidulin (**3**) on the germination and seedling growth of onion, radish and cucumber seeds. The flavonoid hispidulin was more toxic to the crop seeds tested than tagitinin A that was in turn more toxic than tagitinin C.[18] Other tagitinins [B (**4**), D (**5**), E (**6**) and F (**7**)] have been reported but without biological testing data. The main anti-plasmodial activity of this plant has been attributed to the sesquiterpene lactone, tagitinin C, that was originally found in *T. tagitiflora*,[25] and has subsequently been shown to exhibit significant activity against *Plasmodium falciparum* with IC_{50} values of 0.95 μM and 0.72 μM against the chloroquine-sensitive strain FCA and chloroquine-resistant strain FCB1, respectively.[26]

The presence of tagitinin C in *T. diversifolia* varies considerably according to the source of the plant material. Thus, whilst tagitinin C has been found in Indian plants,[17,21] it has

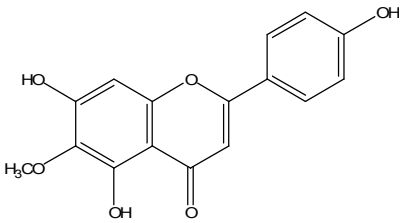
not been described in plants growing in Brazil,[10] Costa Rica[19] or Taiwan.[15]



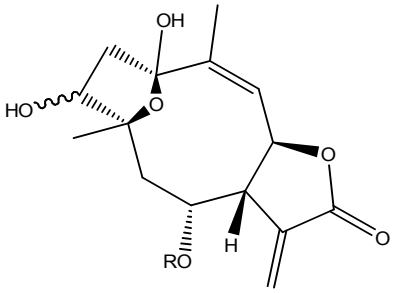
tagitinin A **(1)**
R = $COCH(CH_3)_2$



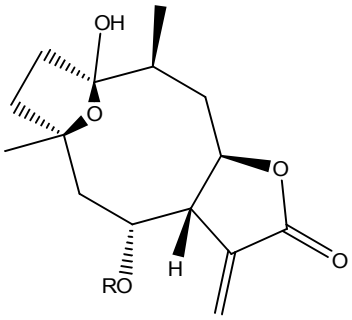
tagitinin C **(2)**
R = $COCH(CH_3)_2$



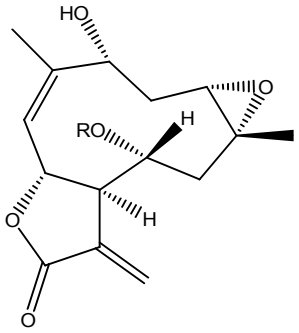
hispidulin **(3)**



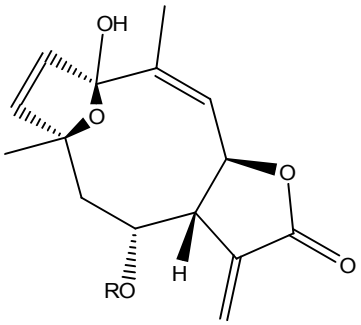
tagitinin B **(4)**
R = $COCH(CH_3)_2$



tagitinin D **(5)**
R = $COCH(CH_3)_2$



tagitinin E **(6)**
R = $COCH(CH_3)_2$



tagitinin F **(7)**
R = $COCH(CH_3)_2$

1.4.2 *Tagetes spp.* (Asteraceae)

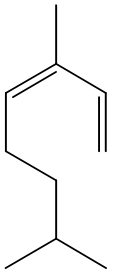
Tagetes is a genus of 52 species of annual and perennial herbaceous plants in the daisy family (Asteraceae or Compositae). They are native to the area stretching from the southwestern United States into Mexico and south throughout South America.

They are known almost universally in North America as Marigold, Mexican marigolds, African marigolds, or French marigolds. At least one species is a naturalized weed in Africa, Hawaii, and Australia. Neither African or French marigolds come from Africa or France but both are native to Mexico. The different species vary in size from 0.05-2.2 m tall.[27]

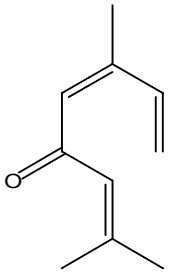
Their foliage has a musky/pungent scent, though some later varieties have been bred to be scentless. This scent is said to deter some common insect pests as well as nematodes. *Tagetes* are hence often used in companion planting. *T. minuta* (Khakibush), originally from South America, has been used as a source of essential oil, known as tagette, for the perfume industry as well as a flavourant in the food and tobacco industries in South Africa, where the species is also a useful pioneer plant in the reclamation of disturbed

land. Some of the perennial species are deer, rabbit, rodent and javalina resistant.[28] The marigold is also widely cultivated in India and Thailand, particularly the species *T. erecta*, *T. patula*, and *T. tenuifolia*. Vast quantities of marigolds are used in garlands and decoration for weddings, festivals, and religious events.[28] In Chinese traditional medicine the flowers of *T. erecta* are used to cure colds, coughs and ailments and the distilled oils are used against warts.[27]

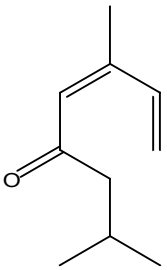
Some species of *Tagetes* have been extensively studied for the composition of their essential oil,[29,30] and for biological activities.[31] Monoterpenes [eg. ocimene (**8**) and the ketones ocimenone (**9**) and *cis*-tagetone (**10**)], thienyl acetylenic compounds[32] and thiophenes [eg. 3'-methoxy-2,2':5',2"-terthiophene, (**11**)][33] have been found as major constituents in species such as *T. terniflora, T. mandonii, T. minuta, T. filifolia, T. lucida, T. patula* and *T. erecta*. In 2002 a new flavonol glycoside [quercetagenin 3,4'-dimethyl ether 7-O-β-D-glucopyranoside (**12**)] and two new phenolic acids [3-(2-O-β-D-glucopyranosyl-4-methoxyphenyl)propanoic acid (**13**) and its methyl ester (**14**)] were isolated from the leaves of *T. lucida*.[34] *T. terniflora* has been found to possess toxicity (*in vitro*) against the brine shrimp *Artemia salina*.[29] Larvicidal activity was also tested in the essential oil of *T. patula* which showed a potent activity against the mosquito species *Aedes aegypti.*[35] The essential oil of *T. terniflora*[36] and *T. lucida* have been reported to possess antibacterial activity against *Staphylococcus aureus*, *Streptococcus pneumoniae*, *S. pyogenes*,[37] *Escherichia coli*, *Salmonella enteritidis*, *S. typhi*, *Shigella dysenteriae* and *S. flexneri*.[38] The antibacterial and antifungal activity of the essential oil of *T. minuta* and *T. tilifolia* has also been reported.[39]



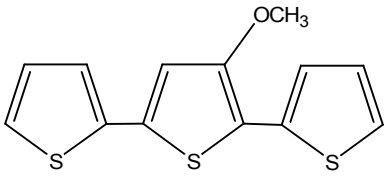
ocimene **(8)**



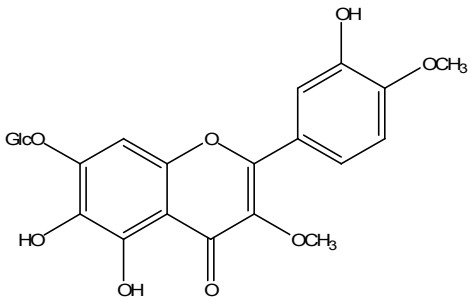
ocimenone **(9)**



cis-tagetone **(10)**

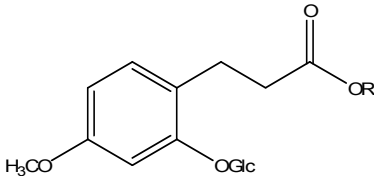


3'-methoxy-2,2':5',2''-terthiophene **(11)**



Glc= β-D-glucopyranosyl

quercetagenin 3,4'-dimethyl ether 7-O-β-D-glucopyranoside **(12)**



Glc= β-D-glucopyranosyl

R = H: 3-(2-O-β-D-glucopyranosyl-4-methoxyphenyl)propanoic acid **(13)**

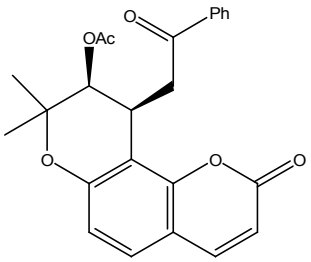
R = CH_3: methyl 3-(2-O-β-D-glucopyranosyl-4-methoxyphenyl)propanoate **(14)**

1.4.3 *Polygala paniculata* L. (Polygalaceae)

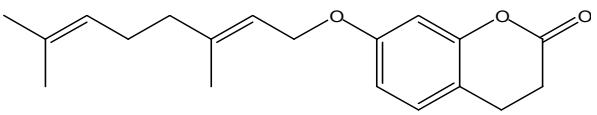
Polygala paniculata, also known by its English name of island snake-root, is an erect

much-branched annual herb, up to 60 cm tall, that belongs to the family Polygalaceae. This plant is native to tropical America from Mexico and the West Indies to Brazil. It was unintentionally introduced into Java in 1845 or 1846 and is now widespread in Malaysia, Micronesia, and eastward.[40]

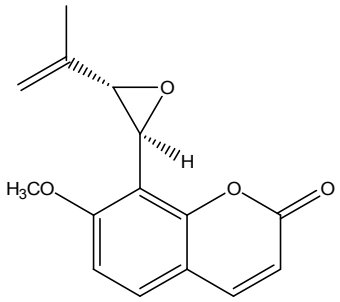
Previous studies on the apolar extracts of *P. paniculata*, possessing both molluscicidal and antifungal properties, led to the isolation of a pyranocoumarin diester[41] 3'-O-acetyl-4'-O-benzoylkhellactone (**15**), and four prenylated coumarins[42]; aurapten (**16**), phebalosin (**17**), murrangatin (**18**), and 7-methoxy-8-(1,4-dihydroxy-3-methyl -2-butenyl)coumarin (**19**). The flavonol rutin and the xanthones 1-hydroxy-5-methoxy-2,3-methylenedioxyxanthone (**20**) and 1,5-dihydroxy-2,3- dimethoxyxanthone (**21**) have also been reported.[43] Xanthones are biologically active natural compounds widely distributed in plants of the Polygalaceae family.[44-47]



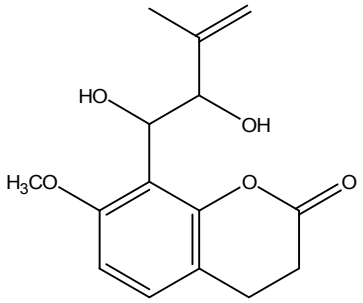
3'-O-acetyl-4'-O-benzoylkhellactone **(15)**



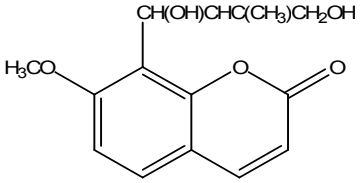
aurapten **(16)**



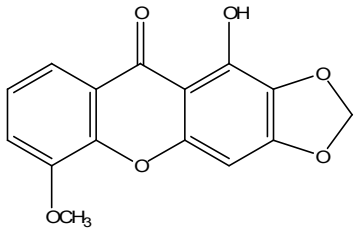
phebalosin **(17)**



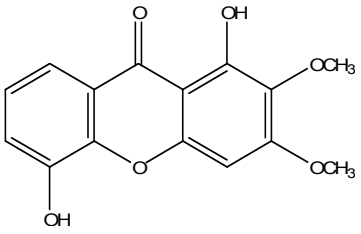
murrangatin **(18)**



7-methoxy-8-(1,4-dihydroxy-3-methyl-2-butenyl)coumarin **(19)**



1-hydroxy-5-methoxy-2,3-methylenedioxyxanthone **(20)**



1,5-dihydroxy-2,3-dimethoxyxanthone **(21)**

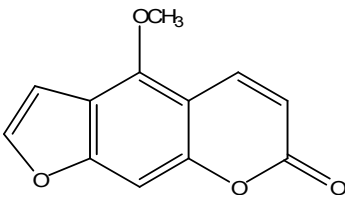
1.4.4 *Euodia sp.* (Rutaceae)

Euodia is a genus belonging to the citrus family Rutaceae with over 50 species originating in Asia, the north-east of Australia, New Guinea and the islands of the

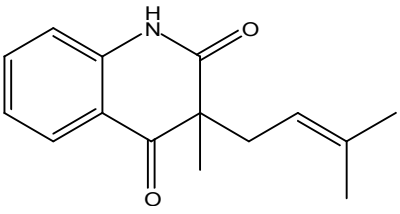
south-west Pacific. Trees, shrubs or herbs grow up to 3-14 m tall and the wood is locally used in construction. The leaves release a translucent aromatic oil with a citrus smell.[48,49]

Previous chemical studies of the genus *Euodia* were prompted by its application in folk medicines by indigenous people from Australia and Asia. Resin from *E. vitiflora* has been applied by Queensland aborigines as an adhesive and for filling cavities in teeth.[50] A decoction of the leaves of *E. latifolia* has been a treatment for fever and cramps.[51] *Euodia borbonica* is a medicinal plant that is used for its aromatic properties and for rheumatism treatment.[52] *E. daniellii* is also a folk medicine for treatment of gastric inflammation, headache and the extermination of noxious insects.[53] The oil from the fruits of this plant has also been applied in various diseases such as dermatitis and scabies.[54,55]

Antifungal[56] and antibacterial[57] activities have been reported for *E. luna-ankenda* extracts. Compounds previously found in *Euodia spp.,* include terpenes,[58] coumarins [eg. bergapten (**22**)],[53,59] and alkaloids [eg. buchapine (**23**)].[50,52,56]



bergapten **(22)**

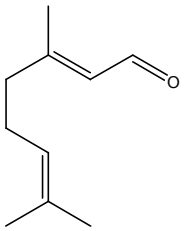


buchapine **(23)**

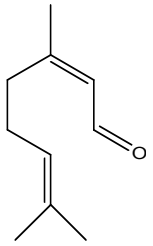
1.4.5 *Cymbopogon citratus* Stapf. (Poaceae)

Cymbopogon citratus commonly known as lemongrass is native to India and Sri Lanka.[60] It belongs to the Family Poaceae, which has about 660 genera and 9000 species.[61] They are important sources of essential oils for the flavour and fragrance industries world wide[62] and are also noted for their medicinal potential.[63] Lemongrass is used as flavouring for food, alcohol and soft drinks and the essential oils in cosmetics.[64] Other uses are to reduce headaches, indigestion, pain, rheumatism and nervousness.[65-67]

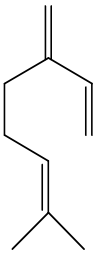
Cymbopogon is widely studied because its oil has widespread application in industry. Geranial (**24**), neral (**25**) and myrcene (**26**) have been identified previously as the major constituents.[68-74] *C. citratus* plants produce up to 75 - 85% citral in their essential oils.[75] Citral is the name given to a natural mixture of two isomeric acyclic monoterpene aldehydes: geranial (*trans*-citral, citral A) and neral (*cis*-citral, citral B). Normally, one isomer does not occur without the other. In addition to citral, the essential oil of *Cymbopogon* species contain small quantities of geraniol, geranyl-acetate and monoterpene olefins, such as limonene (**27**) (in *C. exuosus*) and myrcene (in *C. citratus*).[75,76] Because of its characteristic lemon aroma, citral is of considerable importance in the food and flavour industry. Citral is also an important raw material for the pharmaceutical, perfumery and cosmetics industries, especially for the synthesis of vitamin A and ionones; synthetic citral, derived from conifer turpentine is normally used for those purposes.[77] Citral possesses antifungal activity against plant and human pathogens,[78,79] inhibits seed germination,[80] and has bactericidal[81,82] and insecticidal properties.[83]



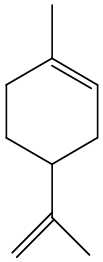
geranial **(24)**



neral **(25)**



myrcene **(26)**



limonene **(27)**

1.4.6 *Ricinus communis* L. (Euphorbiaceae)

Ricinus communis, the castorbean plant, which is native to tropical Africa has been introduced to and is cultivated in many tropical and subtropical areas of the world. It is a perennial shrub growing to 5 m in height.[84]

Castorbean is cultivated for the seeds which yield fast-drying, non-yellowing oil, that is used mainly in industry and medicines. The oil from the seed is a very well-known laxative that has been widely used for over 2,000 years.[85] It is so effective that it is regularly applied to clear the digestive tract in cases of poisoning.[86] It is beneficial in the treatment of non-inflammatory skin diseases, is a good protective in cases of occupational eczema and dermatitis,[87] and also has a remarkable antidandruff effect.[88] A decoction of the leaves and roots has antitussive, discutient and expectorant properties.[89] The leaves can be formed into a poultice to relieve headaches and treat

boils.[87] Not only is castorbean found as a cooking oil, it is also an ingredient of soaps, polishes, flypapers and a transparent component of paints, varnishes, typewriter and printing inks.[88,90-92] It makes an efficient lubricant, can provide lighting in the form of a lamp oil, and is an ingredient in fuels for precision engines.[88,93,94]

The seeds (and to a much lesser extent the leaves) contain ricin, a protein (a toxalbumin)[95] which is highly toxic in small quantities. Ricin is soluble in water and is therefore not present in extracted oil. As a cytotoxic lectin, ricin readily gains access to exposed cells and subsequently inhibits protein synthesis which, in turn, leads cell death.[95] Humans, as well as numerous animal species, have been poisoned after ingesting the seeds. Most reported cases of animal poisoning have occurred overseas where the seed is eaten as food and, if improperly treated, has caused illness and death. The toxin has also been used for suicide and assasination.[96]

1.4.7 *Piper aduncum* L. (Piperaceae)

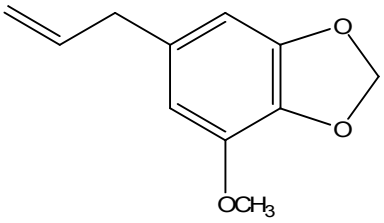
Piper aduncum, an exotic species in Papua New Guinea (PNG), is a multi-stemmed evergreen shrub that can grow up to about 6 m tall. It has alternate leaves with short petioles and elliptic to lanceolate leaf blades that are about 12 - 20 cm long. All plant parts have a characteristic peppery odour.[97] It has also been pointed out that *P. aduncum* has the attributes of an invasive species[98] and is thus now considered a major plant pest in almost all areas of PNG. The dried wood and branches are widely used for firewood, fencing and in temporary constructions, but easily rot away when subjected to long-term exposure to moisture. *P. aduncum* has, however, also been appreciated in many areas

for its medicinal applications.[97] The people of Finschaffen in PNG use the extracts from fresh leaves of this plant as an antiseptic for wound healing,[99-101] while other reports in Peruvian ethnobotany[102] also indicate that the extracts are applied to treat diarrhoea and in Colombian ethnobotany[103] as a treatment for haemostasis and dysentery. In southeast Brazilian folk medicine, *P. aduncum* has been recorded as a tratment for trachoma, vaginitis and stomach aches.[104]

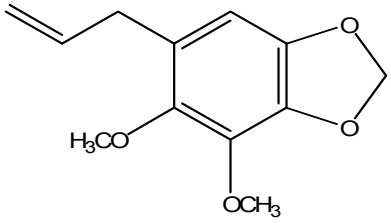
Piperaceae species have been extensively investigated as a source of new natural products with potential antitumoral, antimicrobial, antifungal, and insecticidal activities.[105-108] Phenylpropanoids, such as myristicin (**28**) and dillapiole (**29**), benzoic acid derivatives, chromenes and flavonoids have been reported.[109-112] Dillapiole is the major component in this species and has been described as an insecticide as well as a synergist of several natural insecticides.[113,114] Chromene and benzoic acid derivatives are frequently isolated from *Piper* species and many of them exhibit diverse biological activity such as antimicrobial, molluscicidal,[111] germination inhibition, fungicidal[115] and insecticidal activities.[113]

The crude hexane extract of the leaves from *P. aduncum* yielded aduncamide (**30**) showed significant antibacterial activity against *Bacillus subtilis*, *Micrococcus luteus* and *Escherichia coli* as well as antifungal activity against *Penicillium oxalicum* in *in vitro* biological screening. Molluscicidal potential against *Biomphalaria glabrata* was also detected.[110] The crude petroleum ether extract of the leaves of *P. aduncum* which yielded the sesquiterpenes [viridiflorol (**31**), cubebol (**32**), spathulenol (**33**) and caryophyllenol-II (**34**)] showed strong molluscicidal activity against *Biomphalaria*

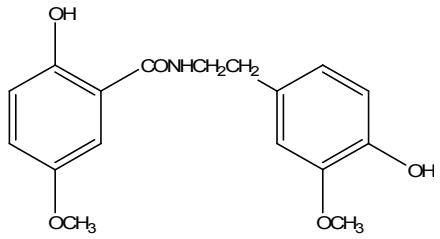
glabrata, known to be a vector of schistosomiasis.[111] The essential oil of *P. aduncum* was also tested against *Crinipellis perniciosa*, the fungus known as `witches' broom' which has been responsible for pathogenic attacks on Cacao. The essential oil at concentrations ranging from 50 to 100 ppm caused 100% inhibition on mycelial growth and germination of *C. perniciosa*.[116-118]



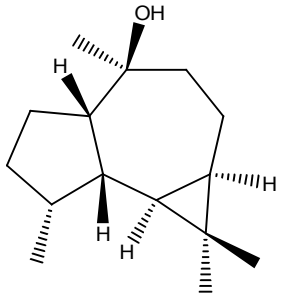
myristicin **(28)**



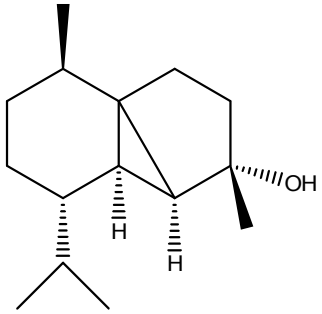
dillapiole **(29)**



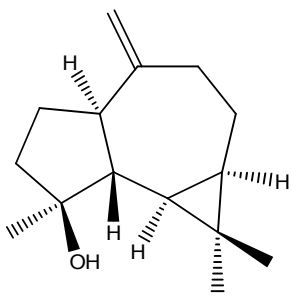
aduncamide **(30)**



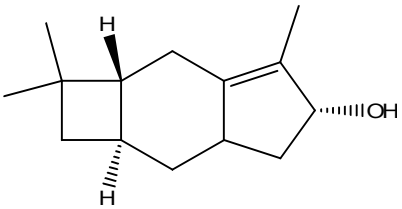
viridiflorol **(31)**



cubebol **(32)**



spathulenol **(33)**



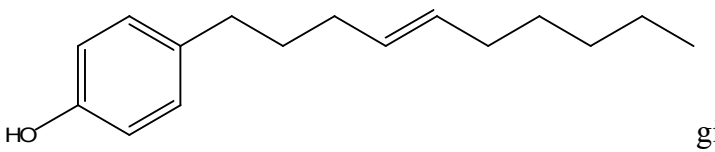
caryophyllenol-II **(34)**

1.4.8 *Piper gibbilimbum* C. DC (Piperaceae)

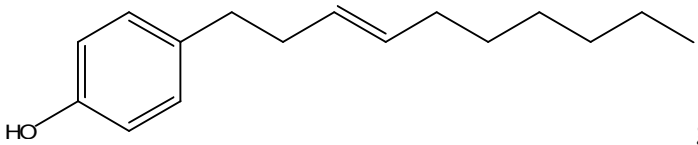
In 1997 *Piper gibbilimbum* was described as a native species of the island of New Guinea.[119] It is a widespread shrub, occasionally reaching a height of about 4 m. The leaves and fruits have an aromatic odour. In PNG, *P. gibbilimbum* is applied as an antiseptic to heal abscesses and skin ulcerations and used to treat fever.[120] The juices extracted from the heated barks are ingested to cure internal sores.[121] The leaves provide wrappers for cooked food and animal protein in the Highlands societies of PNG.[122]

Four new cytotoxic alkenylphenols, designated gibbilimbols A-D (**35-38**) were obtained from *P. gibbilimbum* leaves and their chemical structures were established from spectroscopic evidence. These compounds were found to be toxic to brine shrimps (*Artemia salina*) (LC_{50} of approximately 5μg/mL) and cytotoxic towards KB nasopharyngeal carcinoma cells (ED_{50} 7.8-2.1 μg/mL). All isolates also showed antibacterial activity toward *Staphylococcus epidermidis* and *Bacillus cereus*.[123] Strategies for their synthesis have been recently described.[124,125]

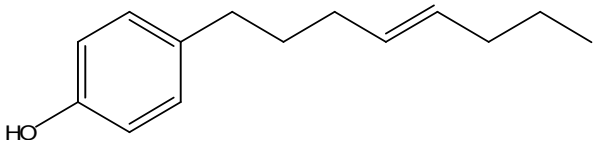
The oil of *P. gibbilimbum* is dominated by the gibbilimbols A-D (74.2 %), positional isomers of *trans*-alkenylphenols where the chain length is either eight or ten carbon atoms. The remaining major constituents are the terpenes camphene (**39**) (13.6 %) and α-pinene (**40**) (6.5 %).[122]



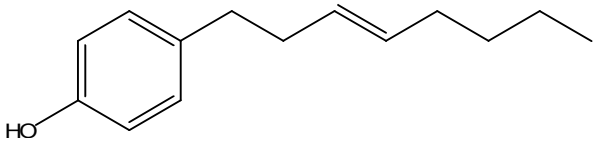
gibbilimbol A **(35)**



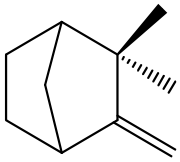
gibbilimbol B **(36)**



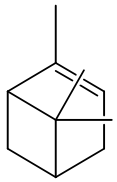
gibbilimbol C **(37)**



gibbilimbol D **(38)**



camphene **(39)**



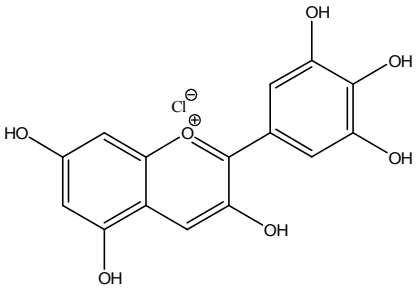
α-pinene **(40)**

1.4.9 unidentified species 'Gorgor' (Zingiberaceae)

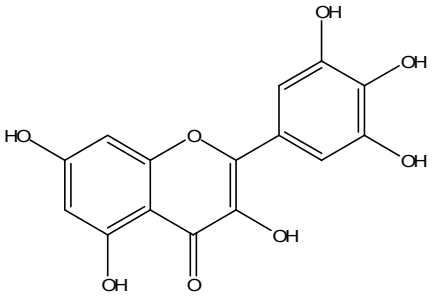
Zingiberaceae, or Ginger, is a family of flowering plants consisting of aromatic perennial herbs with creeping horizontal or tuberous rhizomes, comprising approximately 52 genera and more than 1300 species, distributed throughout tropical Africa, Asia and the Americas.[126] Many species are important ornamental plants, spices, or medicinal plants. Ornamental genera include the shell gingers (*Alpinia*), Siam or summer tulip (*Curcuma alismatifolia*), *Globba*, ginger lily (*Hedychium*), *Kaempferia*, torch-ginger *Nicolaia*, *Renealmia*, and ginger (*Zingiber*). Spices include ginger (*Zingiber*), galangal or Thai ginger (*Alpinia galanga* and others), melegueta pepper (*Aframomum melegueta*), myoga (*Zingiber mioga*), turmeric (*Curcuma*) and cardamom

(*Amomum*, *Elettaria*). Some genera (*Alpinia*, *Hedychium*) yield essential oils for the perfume industry.[127]

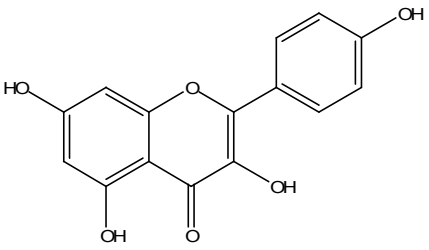
Quite a few constituents have been identified from this family. Anthroquinones were detected in *Aframomum* and *Curcuma*, delphinidin (**41**), a flavylium salt and the flavonol myricetin (**42**) were present in *Brachychilum*. Also flavonols such as kaempferol (**43**) were identified from *Globba* and saponins/sapogenins were present in *Alpinia*.[128]



delphinidin **(41)**



myricetin **(42)**

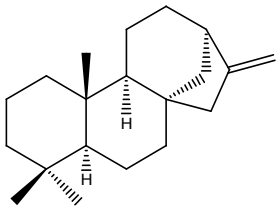


kaempferol **(43)**

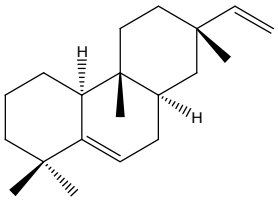
1.4.10 *Dacrydium spp.* (Podocarpaceae)

Dacrydium is a genus of conifers belonging to the family Podocarpaceae, with 25 species native to Southeast Asia and New Zealand. They have scale-like leaves similar

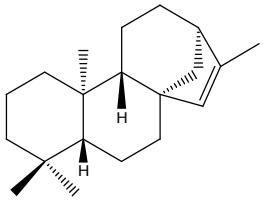
to those of cypress trees. Many are valued for their timber. The huon pine *D. franklinii* of Tasmania resembles a weeping cypress; the mountain pine *D. bidwillii* is a shrub that grows to 3 m in areas of open mountainous scrub in New Zealand.[129] The silver pine *D. colensoi* is a very hardy tree with grey, flaking bark, which grows to 15 m in shady forests in New Zealand; the red pine *D. cupressinum* of New Zealand grows to 40 m and is common in low-lying forest areas. (+)-Phyllocladene (**44**), (+)-rimuene (**45**), (+)-isokaurene (**46**), (-)-sandaracopimaradiene (**47**) and (+)-manoyl oxide (**48**) were identified in addition a new diterpene alcohol, which was shown to be (-)-8β-hydroxysandaracopimar-15-ene (**49**) from the volatile oil of *D. colensoi*[130] and later, a new diterpene laurenene (**50**) was isolated from the latter species[131] by Corbet *et al.* Other than those compounds, some of the major components of the volatile oils identified from the *Dacrydium* include α-pinene (**40**) (up to 58% dry weight), caryophyllene, aromadendrene, viridiflorene, camphene, bicyclogermacrene and β-farnesene.[132]



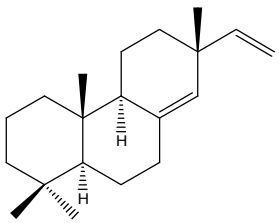
(+)-phyllocladen **(44)**

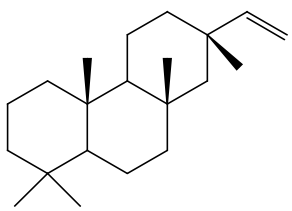


(+)-rimuene **(45)**

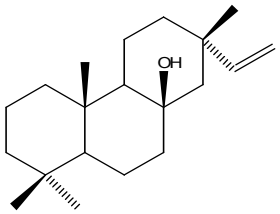


(+)-isokaurene **(46)**

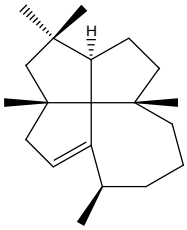




(-)-sandaracopimaradiene **(47)**



(+)-manoyl oxide **(48)**



(-)-8β-hydroxysandaracopimar-15-ene (**49**)

laurenene (**50**)

1.4.11 *Eucalyptus tereticornis* Smith (Myrtaceae)

Eucalyptus tereticornis, commonly known as Forest Red Gum, is a tree which grows up to 50 m tall. The bark is smooth, white or grey and sheds in large plates or flakes.[133] The genera *Eucalyptus*, native to Australia and some islands to the north of it, comprise over 750 tree species. More than 300 species of this genus have been shown to contain volatile oils in their leaves. Only about 20 of these species have ever been exploited commercially for oil production.[134-136]

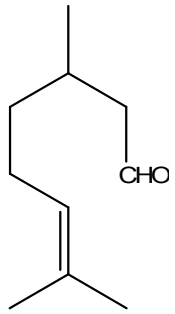
The leaves and bark of *Eucalyptus* species have been widely used for the treatment of many serious diseases and, according to the folk medicine of many countries, it is effective for enhancing physical strength. In particular, the powder of the bark had been extracted for an insecticide in Africa, and the leaves of some species have been used to treat influenza, dysentery, articular pain and tonsillitis in China. Because *Eucalyptus* oil has such an authenticated record of success in treating minor ailments, it has been put in the Pharmacopeias of many countries, such as UK, France, Germany, Belgium, Netherlands, USA and Japan.[137]

Essential oils from *Eucalyptus* species are widely used in modern cosmetics, food, and pharmaceutical industries.[138] In addition, the trees are used in forestry (timber, fuel and paper pulp), environmental planting (water and wind erosion control) and amenity planting.[134] The main uses of *Eucalyptus* oils are found in the pharmaceuticals (those that are rich in 1,8-cineole), perfumery (those that are rich in citronellal) and for industrial use (those that have piperitone and α-phellandrene as their main constituents).[139] About 90% of the oils purchased world-wide are pharmaceutical grade oils, which is a large proportion of industrial and perfumery oil production.[140]

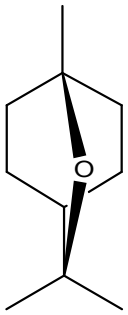
Monoterpenoid components of the aromatic constituents of the oils are commercially available for the treatment of the common cold and other symptoms associated with respiratory infections.[141-143] Phytochemical analysis has shown that the content profile of the monoterpenoids present varies among *Eucalyptus* species, with potential variations in medicinal properties. For example, *E. citriodora* has been shown to contain 60% citronellal (**51**), whereas *E. tereticornis* and *E. globulus* contain 60–90% of eucalyptol (1,8-cineole) (**52**).[143] While citronellal is effective against bacterial and fungal infections,[144] eucalyptol has been reported to inhibit the production/synthesis of tumor necrosis factor-α, interleukin-1β, leukotriene B_4, and thromboxane B_2 in inflammatory cells.[144,145]

Apart from 1,8-cineole, *E. tereticornis* oil from Mali contained α-pinene (**40**) and limonene (**27**) whereas myrcene (**26**), α-pinene and β-pinene (**53**) were reported from an Algerian sample. In addition, *p*-cymene (**54**) was identified from a Cuban sample.[146-148] However, both *E. tereticornis* and *E. grandis* from Ethiopia and Nigeria contain

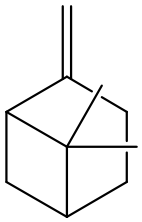
α-pinene and β-pinene as dominant compounds and the absence of 1,8-cineole is noteworthy.[149,150]



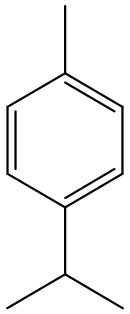
citronellal **(51)**



1,8-cineole **(52)**



β-pinene **(53)**



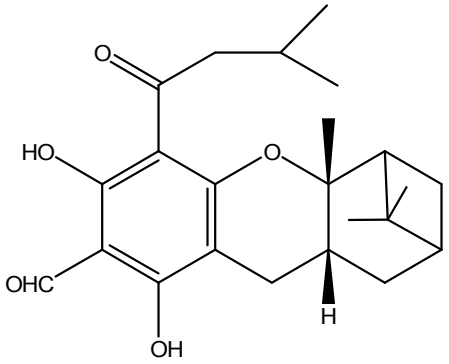
p-cymene **(54)**

1.4.12 *Eucalyptus grandis* W.Hill ex Maiden (Myrtaceae)

Eucalyptus grandis, commonly known as the Flooded Gum or Rose Gum, is a 50 m high tree with smooth grey bark.[151]

It has been reported that *E. grandis* oil has 65 identified components with 1,8-cineole being the main one.[152] Many kinds of phloroglucinol derivatives have also been isolated from *Eucalyptus* plants, and their pharmacological activities are studied.[153-157] Euglobals G1 (**55**) - G5, from the leaves of *E. grandis*, are reported as inhibitors of Epstein Barr Virus (EBV) activation.[158] Furthermore, the phloroglucinol-monoterpene derivative, euglobal-G1 (EG-1), exhibited a remarkable inhibitory effect in the two-stage carcinogenesis test of mouse skin tumors induced by 7,12-dimethylbenz[a]anthracene (DMBA) as an initiator and fumonisin-B1, a mycotoxin produced by *Fusarium monifliforme*, as a tumor promoter. EG-1 also exhibited potent anti-tumor-promoting

activity in a two-stage carcinogenesis test of mouse pulmonary tumor using 4-nitroquinoline-N-oxide (4-NQO) as an initiator and glycerol as a promoter.[159]

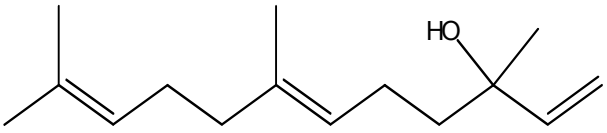


euglobal-G1 (**55**)

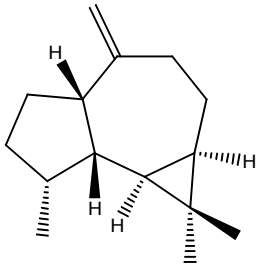
1.4.13 *Melaleuca leucadendron* L. (Myrtaceae)

Melaleuca leucadendron, also known as the Weeping Teatree or Cajeput, is a tall stately tree to 20 m found along natural creek banks. Some forms have weeping foliage but others are more erect in appearance. The most striking feature of *M. leucadendron* is its almost pure white papery bark, which is whiter than other species. Its white bottlebrush flowers bloom in autumn.[160] The genus *Melaleuca* currently contains 236 species, all of which occur in Australia. About 230 species are endemic to Australia and the few remaining species are found as far afield as Indonesia, New Guinea, New Caledonia and even Malaysia. Plants of the genus *Melaleuca* are rich in volatile oils. They are used mainly in the manufacture of cosmetics, germicides and as antiseptic agents. They are also used as caminatives and in the treatment of several ailments.[161]

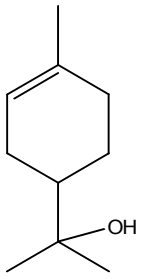
A GC/MS investigation of *M. leucadendron* revealed that 44 constituents were identified from a Venezuelan sample with 1,8-cineole (**52**) (38.4%), nerolidol (**56**) (28.7%), alloaromadendrene (**57**) (14.4%) and α-terpineol (**58**) (12.6%) being the most abundant.[162] In comparison, 26 constituents were obtained from leaves of a north Indian sample with the major constituents being 1,8-cineole (19.9%), α-eudesmol (**59**) (15.8%), β-eudesmol (**60**) (11.3%), viridiflorol (**31**) (8.9%) and guaiol (**61**) (9.0%).[163] Further investigation showed that this essential oil was active against *Bacillus cereus* and *Staphylococcus aureus*, but was inactive against *Escherichia coli* and *Pseudomonas aeruginosa*. It also showed toxicity in the brine shrimp (*Artemia salina*) lethality test (LC_{50} (24 h) = 22.25 μg/mL).[162] Farag *et al.,* described the antiviral activities of the essential oils against *Herpes simplex virus* type 1 (HSV-1) in African green monkey kidney cells (Vero) by a plaque reduction assay. The volatile oil of *M. leucadendron* was up to 92% effective as a virucidal.[164]



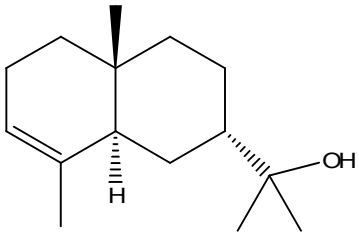
nerolidol **(56)**



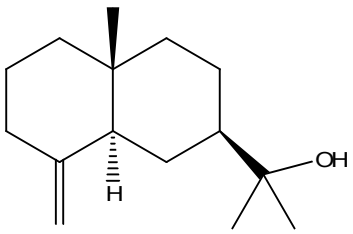
alloaromadendrene **(57)**



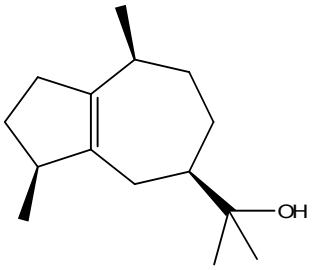
α-terpineol **(58)**



α-eudesmol **(59)**



β-eudesmol **(60)**



guaiol **(61)**

1.4.14 *Euroschinus falcatus* Hook.f. (Anacardiaceae)

The family Anacardiaceae includes 82 genera and 650 species. Plants of their family are probably the most common single cause of contact dermatitis in man. The skin reaction occurs following sensitisation to various alkyl catechols, phenols and quinols. At high concentrations, these compounds are also primary irritants.[165]

Euroschinus falcatus is known as the ribbon wood or maiden's blush and is distributed throughout Oceania and regions of Southeast Asia.[166] It is a small to medium-sized tree and commonly its timber is used to make bearings, bushings, boxes, crates, brush backs, handles, cabinets, coffins, flooring and furniture.[167]

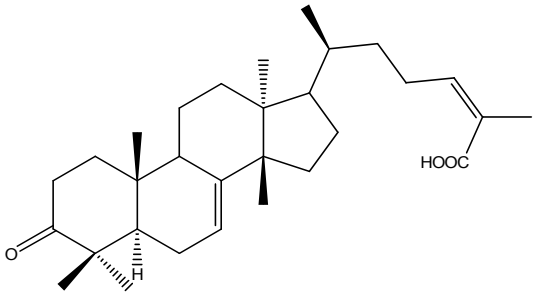
Antibacterial and antifungal activities of *E. papuanus* were reported by Kahn *et al*.[168] However, currently no chemical investigations of *E. falcatus* have been reported.

1.4.15 *Schinus terebinthifolius* Raddi (Anacardiaceae)

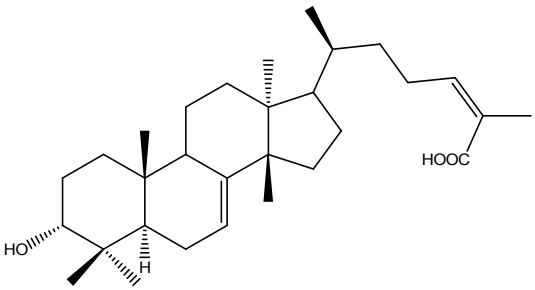
Schinus terebinthifolius, commonly known as the Brazilian pepper tree, belongs to the cashew family. It is a shrub or small tree to 7 m high. This species, which is native to

Central and South America, was introduced to Florida 50 years ago as a shade and ornamental tree and spread rapidly to thousands of acres.[169] In people who are susceptible, the pollen and volatiles, as well as the sap, may cause respiratory problems and dermatitis.[170]

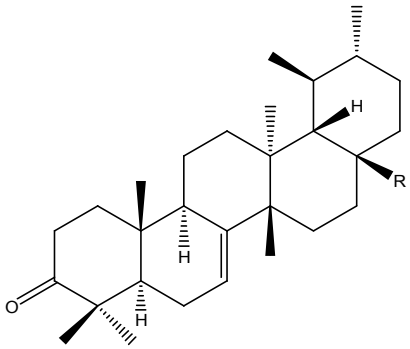
Previous studies of fruits, leaves and bark of this tree have revealed the presence of triterpene alcohols, ketones and acids.[169] Campello *et al.,* extracted a triterpene acid, masticadienonic acid (**62**), 3α-hydroxymasticadienonic acid (**63**), triacontane and sitosterol from the leaves.[171] They also isolated new terpenoid ketones, bauerenone (**64**) and terebenthifolic acid (**65**), from the bark.[172] In 1977, Lloyd *et al*., described the isolation of additional monoterpenes and sesquiterpenes from an extract of its fruit.[171]



masticadienonic acid **(62)**



3α-hydroxymasticadienonic acid **(63)**



R = CH_3 : bauerenone **(64)**

R = COOH : terebenthifolic acid **(65)**

1.4.16 *Stylosanthes hamata* (L.) Taub. (Leguminosae)

S. hamata is a member of the pea family (Leguminosae) and is also known as Caribbean stylo. It is a perennial, broad-leaved, erect or decumbent shrub or herb, up to 0.7 m high. *S. hamata* occurs mainly in the West Indies, in the Caribbean area generally, and in coastal areas of Venezuela and Colombia bordering the Caribbean. It also occurs in coastal areas of southern Florida, the United States.[173]

There have been many reports on chemical composition of this species assessed as forage or pasture, however the constituents of leaf extracts have not been given.

CHAPTER 2 Results and Discussions

2.1 General Discussion

The initial aims of this project were to investigate the components of essential oil and extracts from aromatic and medicinal plants from the highlands of New Guinea. It was thought this would be largely comprised of endemic New Guinea highland plant species, however, a large proportion of the samples were from introduced species, and it became clear that in New Guinea the choice of oil samples by Dr Stewart Wossa was being influenced by potential commercial application.

The methodology that was utilised consisted of initially quantifying individual components in volatile oil samples by gas chromatography (GC), and then trying to identify as many of the components as possible. Two strategies were proposed to that end: The first was to utilise NMR spectroscopy to identify the major components present in each oil sample. Utilising the excellent NMR facilities at the Australian Institute of Marine Sciences (AIMS), the decision was made to try to identify as many components as possible for each oil sample without any prior separation or fractionation. This strategy was possible because of access to a 600 MHz NMR spectrometer fitted with a cryoprobe, and the improvements that have been made in terms of 1D and 2D NMR techniques, particularly through utilisation of gradient selective techniques that might facilitate investigation of resonance signals for one component and avoid overlapping signals from other components: The second strategy was to tentatively identify other (minor) components using gas chromatography-mass spectrometry (GC-MS). GC-MS in some cases indicated the identity of components, because it was

possible to match molecular weights and retention times with potential essential oil components by utilisation of the compound library available on the GC-MS system employed for the analyses. In other cases GC-MS was merely used to match the molecular weight of peaks to components that had been structurally identified by NMR spectroscopy. When NMR spectroscopy was used to identify the structure of components, and assignments of ^{1}H and ^{13}C signals had been obtained, the data was confirmed, when possible, by comparison with published literature values. Where possible, the identity of compounds was also confirmed by comparison with values obtained from a commercially-obtained authentic sample or by isolation of the pure component from the oil sample.

A total of twenty six known and one new compound were identified employing these methods, or by application of these methods to fractions obtained by chromatography of the oils or extracts.

Although it is common for essential oils to be comprised of many components, where some are present in small amounts, as the project progressed, it soon became evident that cross-contamination of some of the oil samples had occurred, as some major components of certain oils were repeatedly observed in small amounts in other oils that would not be expected to contain such components. While monoterpenes such as α and β-pinene, limonene, 1,8-cineole, γ-terpinene, etc., are expected to be components of many essential oils, the presence of the component *trans*-anethole as 74% of the 'Hane grass' oil, as well as 3.7% of the *Dacrydium* sp., oil suggested some contamination of the *Dacrydium* oil had occurred. Similarly, methyl salicylate, which was a major

component of one oil sample, was found in smaller amounts in other oil samples. Some samples appear to have been cross-contaminated in PNG, probably through inadequate cleaning of distilled oil residues from the distillation apparatus between samples. The suspected contaminants are each discussed under the individual oil sample heading. This potential cross contamination only applies to some of the eighteen oil samples that were supplied from PNG, the last three samples investigated were collected from the James Cook University campus, and no cross-contamination occurred.

The twenty one oil samples and extracts were initially screened for antimicrobial activity against a total of eighteen bacteria, yeasts and fungi, as well as for cytotoxic activity. After identification of major oil components, where available, isolated or commercially-obtained samples of components were screened in the same assays to assess their contribution to the overall activity of the oil or extract. These results were compared with literature available on similar screening of essential oils and their components.

2.2 Compounds identified from the collected species

The structures of compounds discussed in the following sections were determined from their NMR spectral data and by comparison with literature spectral values. Proton chemical shifts and couplings in some compounds were determined from a gradient selective TOCSY experiment so that overlapping signals from other components in the oil sample were not observed.

2.2.1 *Tithonia diversifolia* (leaves)

The oil of *Tithonia diversifolia* was found to contain dillapiole (11%), β-caryophyllene (6.1%), carvacrol (5.9%), caryophyllene oxide (3.5%), spathulenol (2.9%), camphor (2.7%), viridiflorol (1.9%) and methyl salicylate (0.6%). It is suspected that methyl salicylate in this sample originated from cross contamination of the oil during steam distillation in PNG as it constituted 74% of the oil from the roots of *Poligala paniculata*. Structure characterisation of carvacrol and camphor have been discussed under 2.2.5 unidentified species (leaves), β-caryophyllene and caryophyllene oxide under 2.2.6 *Euodia sp.* (leaves), viridiflorol under 2.2.7 *Cymbopogon citratus* (leaves) and dillapiole under 2.2.8 *Ricinus communis* (leaves).

Spathulenol

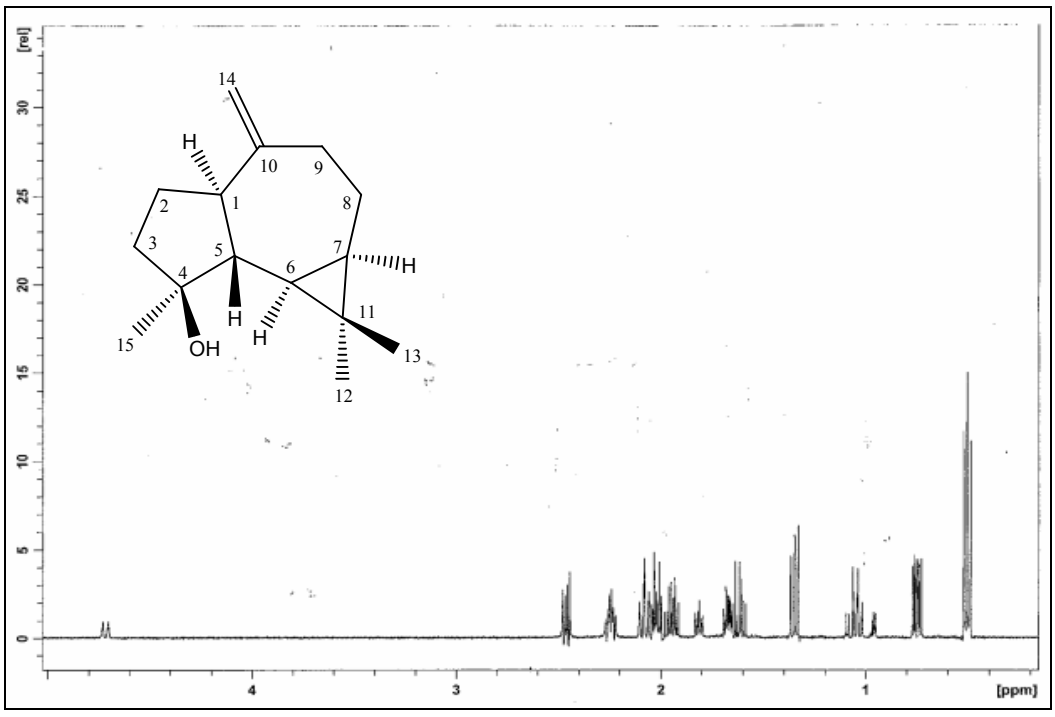


Figure 1. Gradient selective TOCSY (600 MHz) from excitation centered on δ0.47 of spathulenol in $CDCl_3$

The ^{1}H NMR spectrum of spathulenol exhibited the presence of three methyl groups (δ1.04, 1.05, 1.28), two proton signals for a cyclopropane (δ0.47, 0.71), one terminal methylene (δ4.64, 4.69) and resonances from ten other protons giving signals between δ1.0 and 2.5. The ^{13}C NMR spectrum of spathulenol contained resonances for fifteen carbons: three methyl groups (16.4, 26.1, 28.7 ppm), one alkene (106.4, 153.4 ppm) and one oxygenated carbon (81.1 ppm); the remaining nine carbon atoms were assigned as 4x CH_2 groups (24.8, 26.8, 38.8, 42.0 ppm), 4x CH groups (27.5, 29.9, 53.4, 54.4 ppm) and a quaternary carbon (20.4 ppm).

Its HMBC spectrum showed correlations between the resonances for the methyl groups H_3-12 (δ1.05) and H_3-13 (δ1.04) and the resonances for C-6 (29.9 ppm), C-7 (27.5 ppm), C-11 (20.4 ppm) and, CH_3-13 (16.4 ppm) and CH_3-12 (28.7 ppm), respectively; From the resonance of one of the cyclopropane protons H-6 (δ0.47) to C-7, C-11 and CH_3-13 and from the other one H-7 (δ0.71) to CH_3-13. These data indicated that the methyl groups CH_3-12 and CH_3-13 were both attached to C-11, and that C-6, C-7 and C-11 formed a cyclopropane ring. Another methyl group CH_3-15 (26.1 ppm) was suggested to be attached at the C-4 (81.1 ppm) position with C-3 (42.0 ppm) and C-5 (54.4 ppm) connected on either side, as HMBC correlations were seen between H_3-15 (δ1.28) and C-3, C-4 and C-5. Further, correlations between the terminal methylene H-14a (δ4.64) and C-1 (53.4 ppm), C-9 (38.8 ppm) and C-10 (153.4 ppm) and between the 9-CH_2 protons δ2.04 and C-8 (24.8 ppm), C-10 and C-14 (106.4 ppm) and between δ2.42 and C-1, C-7, C-8 and C-10 confirmed the carbon linkages from C-7 to C-10 and C-1, and showed that C-14 was attached to C-10. Additional HMBC correlations between one of the H-2 protons (δ1.91) and C-3, between one of the H-3 protons (δ1.78) and C-1

together with a COSY correlation between H-5 (δ1.31) and H-6 indicated the presence of a cyclopentane ring (C-1 to C-6) and its attachment to C-6. Gradient selective TOCSY experiments centred on the two cyclopropane protons (δ0.47 and 0.71) and two terminal methylene protons (δ4.64 and 4.69) enabled identification of the chemical shifts for the protons of the CH and CH_2 groups. Comparison of the NMR data with literature values[174] confirmed the identity of the sesquiterpene as spathulenol.

		Spathulenol	
C #	δ^{13}C	δ^{1}H mult. *J* (Hz)	HMBC ^{1}H to ^{13}C
1	53.4	2.2 td 11.5, 6.5	H-3b, H-9b, H-14a
2	26.8	1.63 dddd 11.5, 6.5, 6.2, 2.2	
		1.91 qd 11.5, 6.2	
3	42.0	1.57 dt 12.3, 6.2	H-2b, H_3-15
		1.78 m	
4	81.1		H_3-15
5	54.4	1.31 dd 11.7, 9.7	H_3-15
6	29.9	0.47 dd 11.2, 9.5	H_3-12, H_3-13
7	27.5	0.71 ddd 11.2, 9.4, 6.1	H-6, H-9b, H_3-12, H_3-13
8	24.8	1.01 dddd 14.4, 12.7, 11.2, 1.4	H_2-9
		1.98 dtd 14.4, 6.4, 1.6	
9	38.8	2.04 t 13.4, 12.7, 1.6	H-14a
		2.42 ddd 13.4, 6.4, 1.4	
10	153.4		H_2-9, H-14a
11	20.4		H-6, H_3-12, H_3-13
12	28.7	1.05 s	H_3-13
13	16.4	1.04 s	H-6, H-7, H_3-12
14	106.4	4.64 brs	H-9a
		4.69 brs	
15	26.1	1.28 brs	

Table 1. ^{1}H NMR (600 MHz), ^{13}C NMR (150 MHz) and HMBC data of spathulenol in $CDCl_3$

2.2.2 *Tagetes spp.* (leaves)

The oil of *Tagetes spp.* was found to contain piperitone (57%) and methyl salicylate (15%). Structure characterisation of methyl salicylate is discussed under 2.2.3 *Poligala paniculata* (roots).

Piperitone

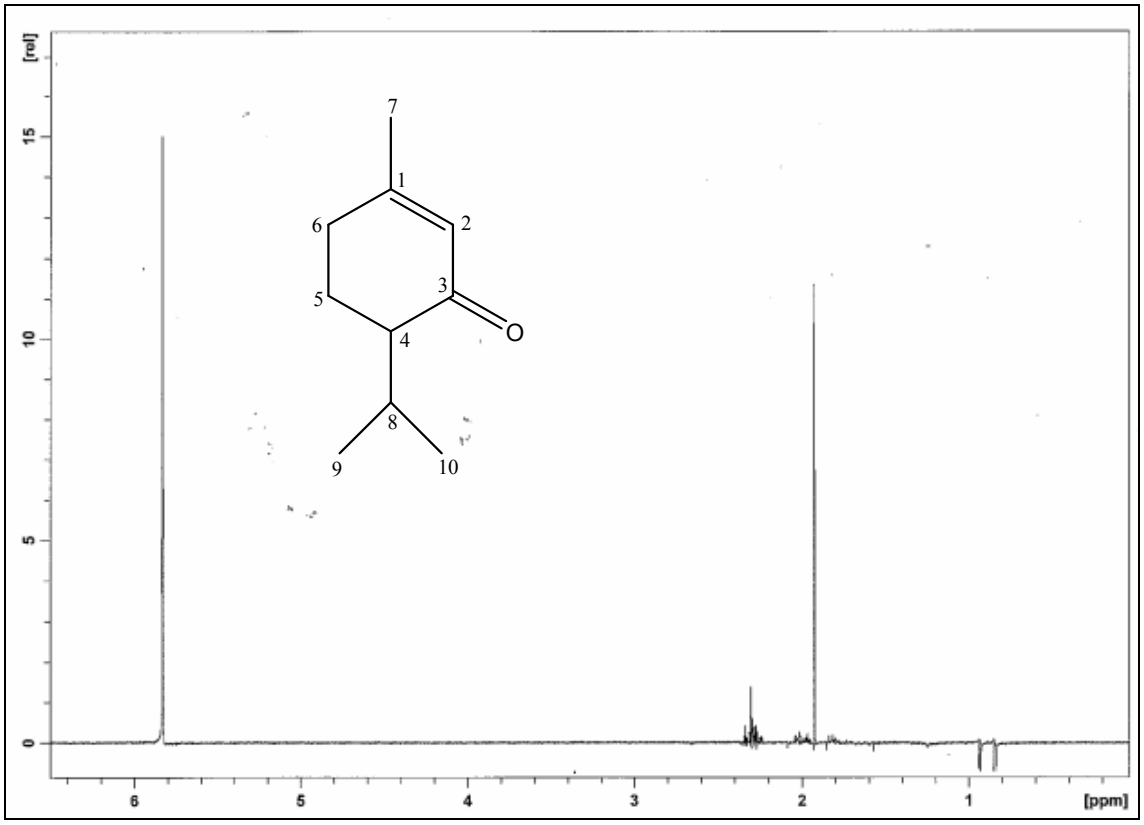


Figure 2. Gradient selective TOCSY (600 MHz) from excitation centered on δ5.81 of piperitone in $CDCl_3$

The ^{1}H NMR spectrum of piperitone exhibited a free methyl singlet at δ1.90, two methyl doublets at δ0.83 and δ0.92, and one olefinic proton signal at δ5.81. It also contained signals for six protons at δ1.78, 1.98, 2.01, 2.24, 2.31 and 2.33. The ^{13}C NMR

spectrum of piperitone revealed the presence of three methyl groups (18.4, 20.5, 24.1 ppm), a conjugated alkene (126.6, 161.1 ppm), a ketone (201.3 ppm) and a methine carbon (51.4 ppm) α to the carbonyl. Two methylene carbon signals were also observed at 22.8 ppm and 30.2 ppm as well as a methine carbon signal at 25.7 ppm.

Piperitone			
C #	$\delta^{13}C$	$\delta^{1}H$ mult. *J* (Hz)	HMBC ^{1}H to ^{13}C
1	161.1		H_3-7, H_2-6
2	126.6	5.81 d 1.8	H_3-7, H_2-6
3	201.3		H_2-5
4	51.4	2.01 m	H-2, H_2-5, H_2-6, H-8, H_3-9, H_3-10
5	22.8	1.78 m	H_2-6, H-8
		1.98 m	
6	30.2	2.24 m	H-2, H_2-5, H_3-7
		2.31 m	
7	24.1	1.90 s	H_2-6
8	25.7	2.33 m	H_2-5, H_3-9, H_3-10
9	18.4	0.83 d 6.9	H-8, H_3-10
10	20.5	0.92 d 6.9	H-8, H_3-9

Table 2. ^{1}H NMR (600 MHz), ^{13}C NMR (150 MHz) and HMBC data of piperitone in $CDCl_3$

HMBC correlations between the methyl group H_3-7 (δ1.90) and C-1 (161.1 ppm), C-2 (126.6 ppm) and C-6 (30.2 ppm) and between H-2 (δ5.81) and C-4 (51.4 ppm) and C-6 indicated that the methyl group was attached to the β-carbon of an α, β unsaturated ketone. Two proton signals at δ2.24 and δ2.31 in the ^{1}H NMR spectrum of piperitone were assigned to C-6 based on HSQC data. These protons (H_2-6) shared HMBC correlations with C-1, C-2, C-4, C-5 (22.8 ppm) and CH_3-7 (24.1 ppm). HMBC

correlations between the two methyl groups (H_3-9 and H_3-10) and C-8 (25.7 ppm), C-4 and, CH_3-10 (20.5 ppm) and CH_3-9 (18.4 ppm) respectively, clearly indicated that C-8, CH_3-9 and CH_3-10 formed an isopropyl group. Correlations from H-8 (δ2.33) to C-4, CH_3-9 and CH_3-10 were also observed to confirm the assignments. Additional HMBC correlations were observed between H_2-5 (δ1.78, 1.98) and C-3, C-4, C-6 and C-8, and COSY correlations were observed between H-4 (δ2.01) and both H-5 protons (δ1.78, 1.98), as well as between H_b-5 (δ1.98) and both H-6 protons (δ2.24, 2.31). These data confirmed that the isopropyl group was attached at C-4, which was next to the carbonyl group, and that C-5 and C-6 were adjacent to each other. The assignments showed that the carbons from C-1 to C-6 formed a six-membered ring, with a conjugated ketone function C-1 and C-3, a methyl group and an isopropyl group separated by two CH_2 groups. Comparison of the NMR data with literature values[175] confirmed the identity of piperitone.

2.2.3 *Poligala paniculata* (roots)

The oil of *Poligala paniculata* was found to contain methyl salicylate (74%) and dillapiole (3.6%). It is suspected that dillapiole in this sample originated from cross contamination during steam distillation.

Methyl salicylate

The ^{1}H NMR spectrum of methyl salicylate revealed one methyl singlet (δ3.95), four protons (two double doublets at δ6.98 and 7.83 and two doublets of double doublet at δ6.88 and 7.45) in the aromatic region and one hydroxyl group (δ10.8). The

arrangement of the four aromatic protons was determined by the *J* values. Vicinal couplings (ortho to each other) were 8.3, 8.1 and 7.2 Hz whereas long-range couplings (meta to each other) were 1.7 and 1.0 Hz and these data confirmed orthodisubstitution on the aromatic ring. The ^{13}C NMR spectrum of methyl salicylate accounted for eight carbon atoms: one methyl group (52.3 ppm) and one carbonyl group (170.6 ppm) both next to an oxygen, and one benzene ring (112.3, 117.6, 119.2, 129.9, 135.7, 161.6 ppm).

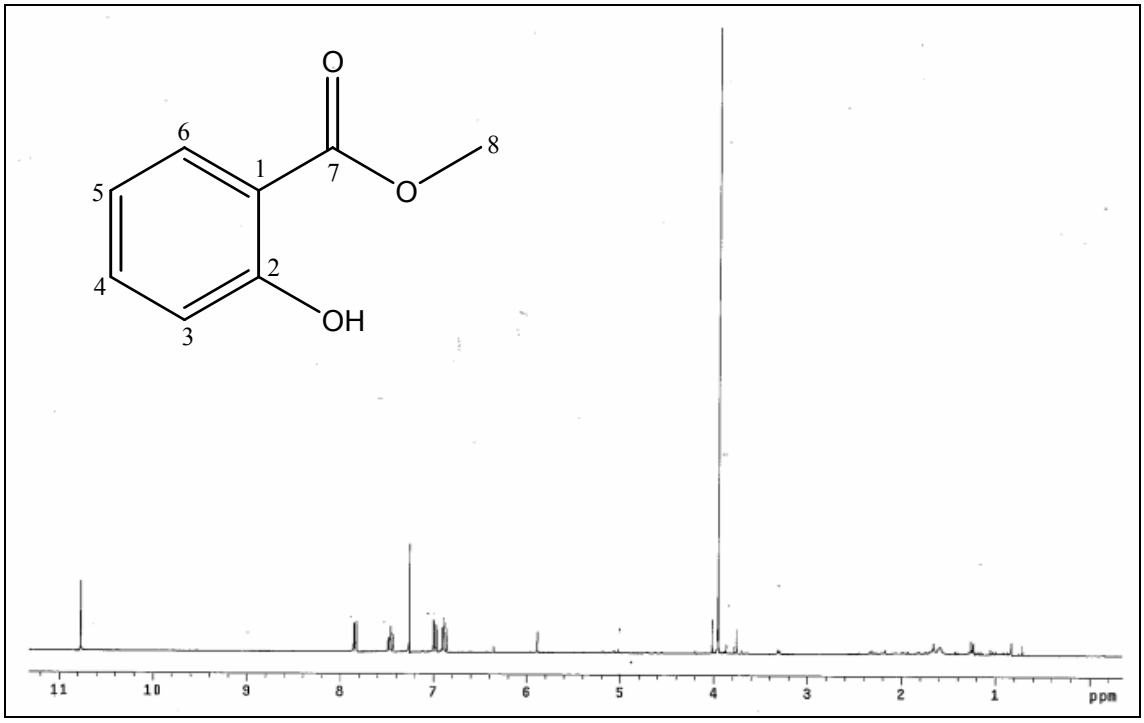


Figure 3. ^{1}H NMR spectrum (300 MHz) of the *Poligala paniculata* root oil in $CDCl_3$ (reference: residual solvent peak at δ7.26)

The methyl group H_3-8 at δ3.95 attached to an oxygen showed a three bond HMBC correlation to the carbonyl group C-7 (170.6 ppm). An HMBC correlation between H-6 (δ7.83) and C-7 together with the fact that C-1 (112.3 ppm) was a quaternary carbon from HSQC data indicated that the carbonyl group (C-7) was attached at the C-1 position on the benzene ring. The hydroxyl group proton showed HMBC correlations to

C-1, another quaternary carbon C-2 (161.6 ppm) and C-3 (117.6 ppm). This confirmed that C-2, bearing the hydroxyl group, was located next to the quaternary carbon C-1 and the protonated aromatic carbon C-3. Further correlations between H-3 (δ6.98) and C-1 and C-5 (119.2 ppm), between H-4 (δ7.45) and C-2 and C-6 (129.9 ppm), between H-5 (δ6.88) and C-1, C-3 and C-6 and, between H-6 and C-2 and C-4 permitted the full ^{1}H and ^{13}C assignments of the benzene ring. Comparison of the NMR data with literature values[176,177] confirmed the identity of methyl salicylate.

Methyl salicylate			
C #	δ^{13}C	δ^{1}H mult. *J* (Hz)	HMBC ^{1}H to ^{13}C
1	112.3		OH, H-3, H-5
2	161.6	10.8 s (OH)	OH, H-4, H-6
3	117.6	6.98 dd 8.3, 1.0	OH, H-5
4	135.7	7.45 ddd 8.3, 7.2, 1.7	H-6
5	119.2	6.88 ddd 8.1, 7.2, 1.0	H-3
6	129.9	7.83 dd 8.1, 1.7	H-4, H-5
7	170.6		H-6, H_3-8
8	52.3	3.95 s	

Table 3. ^{1}H NMR (300 MHz), ^{13}C NMR (75 MHz) and HMBC data of methyl salicylate in $CDCl_3$

2.2.4 'Newton grass' (leaves)

The oil of Newton grass was found to contain β-caryophyllene (17%), dillapiole (3.2%), *p*-cymene (2.3%) and caryophyllene oxide (1.6%). Structure characterisation of *p*-cymene has been discussed under 2.2.5 unidentified species (leaves), β-caryophyllene and caryophyllene oxide under 2.2.6 *Euodia sp.* (leaves) and dillapiole under 2.2.8

Ricinus communis (leaves).

2.2.5 Unidentified species (leaves)

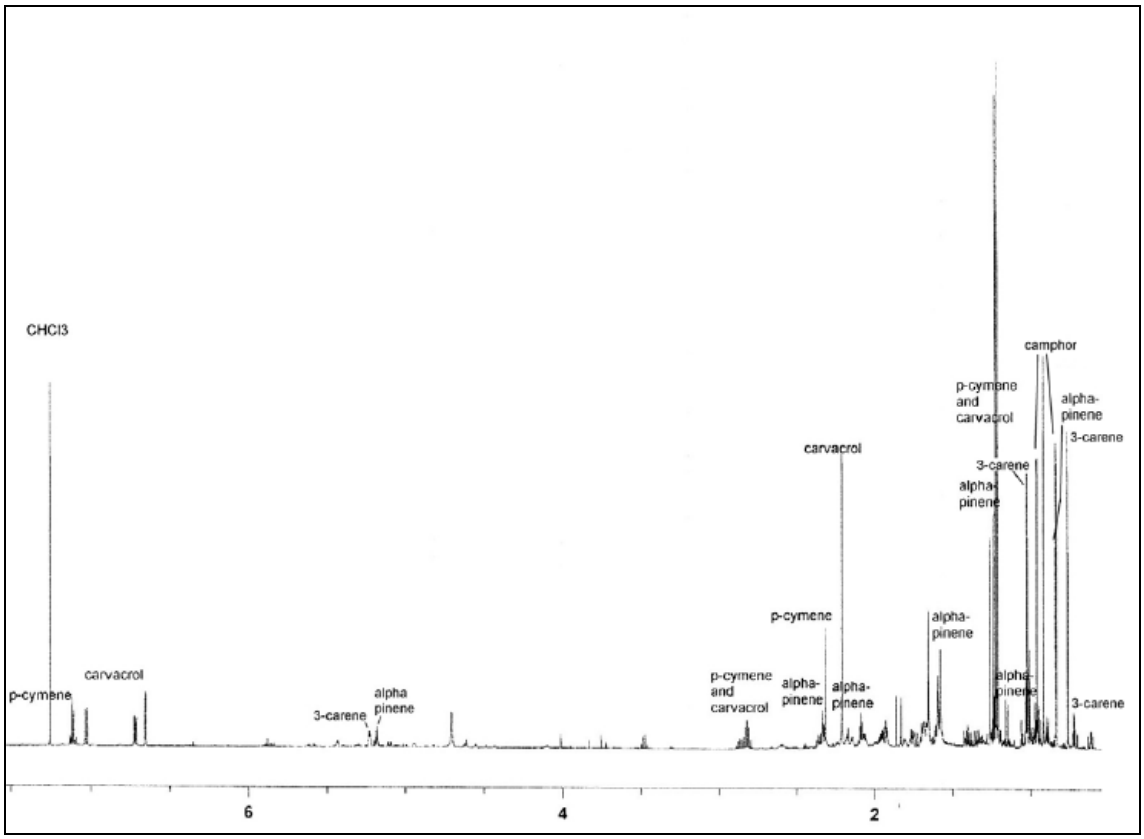


Figure 4. ^{1}H NMR spectrum (600 MHz) of the unidentified species crude essential oil, showing the identified components labelled (reference: residual solvent peak at δ7.26)

The oil was found to contain carvacrol (18%), camphor (15%), α-pinene (13%), 3-carene (11%) and *p*-cymene (8.2%).

Carvacrol

The ^{1}H NMR spectrum of carvacrol exhibited an aromatic methyl singlet at δ2.21, one

large methyl doublet at δ1.24 equivalent for two methyl groups, three proton signals in the aromatic region at δ7.03, 6.71 and 6.66 and a hydroxyl broad singlet at δ5.95. It also contained a heptet at δ2.82. The ^{13}C NMR spectrum of carvacrol disclosed the presence of three methyl groups (15.3, 24.0 x 2 ppm) and resonances for a benzene ring (112.9, 118.6, 120.9, 130.8, 148.3, 153.7 ppm). A methine signal at 33.6 ppm was also observed.

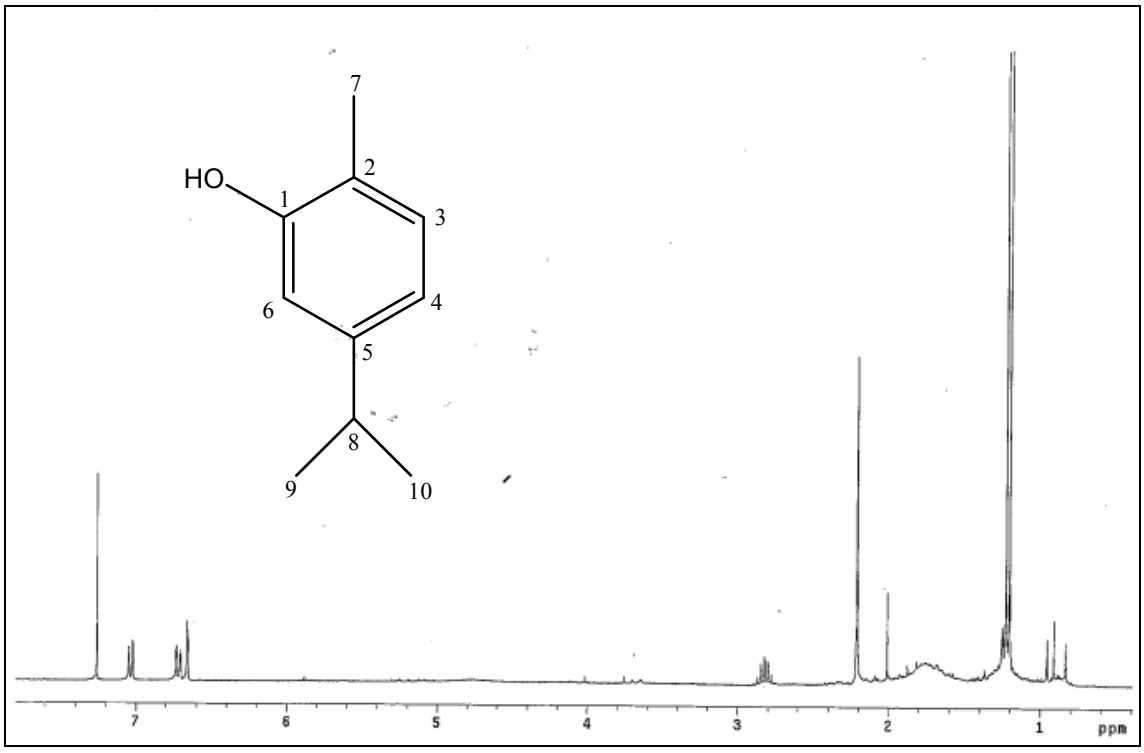


Figure 5. ^{1}H NMR spectrum (300 MHz) of carvacrol (isolated by HPLC) in $CDCl_3$ (reference: residual solvent peak at δ7.26)

HMBC correlations observed between the methyl group H_3-7 (δ2.21) and C-1 (153.7 ppm), C-2 (120.9 ppm) and C-3 (130.8 ppm) as well as between H-3 (δ7.03) and C-1 and CH_3-7 (15.3 ppm) indicated that the methyl group CH_3-7 was attached to a quaternary carbon C-2 which was connected with two carbons, C-1 and C-3, on either

side. Correlations between the two methyl groups H_3-9 and H_3-10 both at δ1.24 and C-8 (33.6 ppm), C-5 (148.3 ppm) and, CH_3-10 and CH_3-9 (24.0 ppm) respectively, clearly indicated that C-8, CH_3-9 and CH_3-10 formed an isopropyl group. It also suggested that the isopropyl group was bonded to C-5, with C-4 (118.6 ppm) and C-6 (112.9 ppm) located on either side. This assignment was confirmed by the HMBC correlation observed between H-4 (δ6.71), H-6 (δ6.66) and C-8 and between H-8 (δ2.82) and C-4, C-5, C-6, CH_3-9 and CH_3-10. Further correlations between H-3 and C-5, between H-4 and C-2 and C-6 and between H-6 and C-1, C-2 and C-4 confirmed that C-4 and C-6 were linked to C-3 and C-1 respectively. As C-2 and C-5 were substituted by the methyl group and the isopropyl group respectively, and C-3, C-4 and C-6 were protonated aromatic carbons as shown in the HSQC spectrum, it was determined that the hydroxyl group was attached at the remaining carbon C-1 position. Comparison of the NMR data with literature values[178] confirmed the identity of carvacrol.

Carvacrol			
C #	δ^{13}C	δ^{1}H mult. *J* (Hz)	HMBC ^{1}H to ^{13}C
1	153.7	5.95 br s (OH)	H-3, H-6, H_3-7
2	120.9		H-4, H-6, H_3-7
3	130.8	7.03 d 8.1	H_3-7
4	118.6	6.71 dd 7.6, 1.7	H-6, H-8
5	148.3		H-3, H-8, H_3-9/H_3-10
6	112.9	6.66 s	H-4, H-8
7	15.3	2.21 s	H-3
8	33.6	2.82 hept 7.0	H-4, H-6, H_3-9/H_3-10
9,10	24.0	1.24 d 7.0	H-8, H_3-10/H_3-9 respectively

Table 4. ^{1}H NMR (300 MHz), ^{13}C NMR (75 MHz) and HMBC data of carvacrol in $CDCl_3$

Camphor

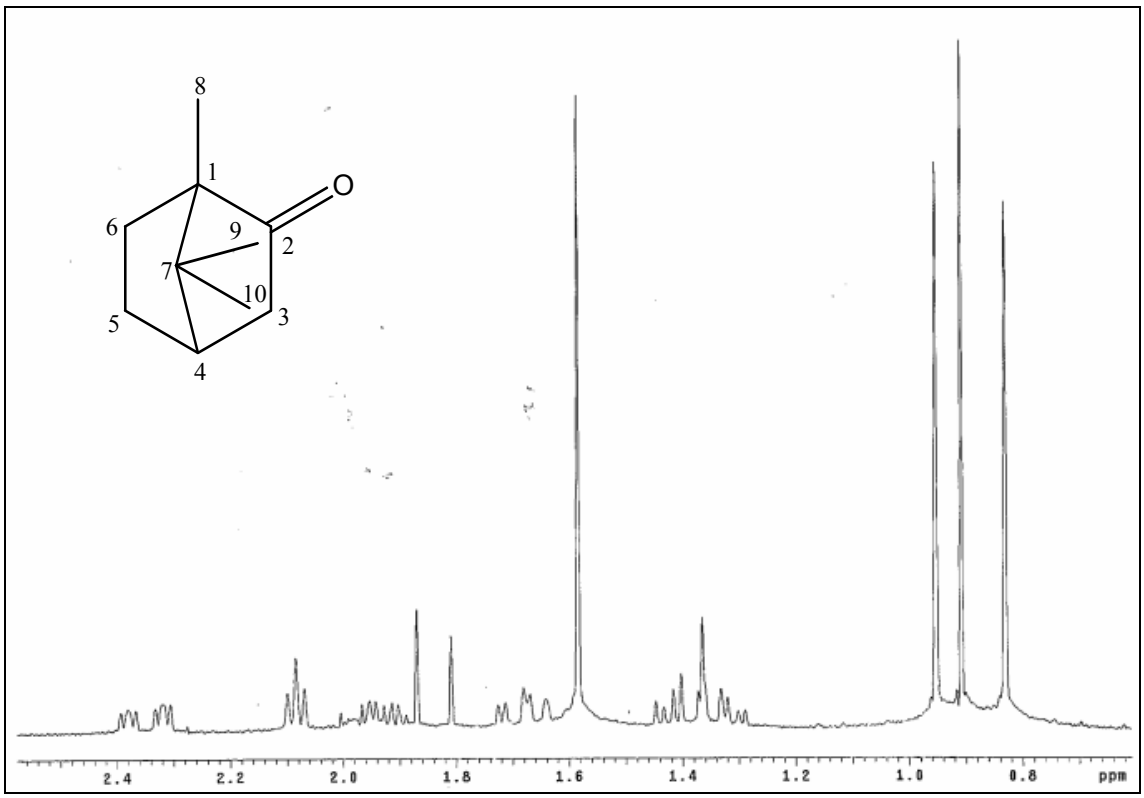


Figure 6. ^{1}H NMR spectrum (300 MHz) of camphor in $CDCl_3$

The ^{1}H NMR spectrum of camphor showed three methyl singlets at δ0.82, 0.90 and 0.94 and signals for seven protons at δ1.32, 1.42, 1.75, 1.80, 1.90, 2.08 and 2.30. The ^{13}C NMR spectrum accounted for ten carbon atoms: three methyl groups (9.2, 19.2, 19.9 ppm), one ketone carbonyl group (220.0 ppm), a quaternary carbon next to the carbonyl group (57.7 ppm) and the remaining five carbons were assigned as three CH_2 groups (27.0, 30.0, 43.1 ppm), one CH group (43.1 ppm) and a quaternary carbon at 47.1 ppm from HSQC data.

Camphor			
C #	δ^{13}C	δ^{1}H mult. *J* (Hz)	HMBC ^{1}H to ^{13}C
1	57.7		H-4, H_3-8, H_3-9, H_3-10
2	220.0		H-3a, H-4, H_2-6, H_3-8
3	43.1	1.80 d 18.3	
		2.30 ddd 18.3, 4.8, 3.0	
4	43.1	2.08 dd 4.8, 4.2	H-6a, H_3-9, H_3-10
5	27.0	1.32 m	H-3a
		1.90 m	
6	30.0	1.42 m	H_3-8
		1.75 m	
7	46.9		H_3-9, H_3-10
8	9.2	0.90 s	
9	19.2	0.94 s	H_3-10
10	19.9	0.82 s	H_3-9

Table 5. ^{1}H NMR (300 MHz), ^{13}C NMR (75 MHz) and HMBC data of camphor in $CDCl_3$

HMBC correlations between the methyl group H_3-8 (δ0.90) and C-1 (57.7 ppm), the carbonyl group C-2 (220.0 ppm) and the CH_2 group C-6 (30.0 ppm) and between H_2-6 (δ1.42, 1.75) and C-2 indicated that CH_3-8 (9.2 ppm) was bonded to C-1 which was connected to C-2 and C-6 on either side. In addition, correlations between both methyl groups H_3-9 (δ0.94) and H_3-10 (δ0.82) and C-1, C-4 (43.1 ppm), C-7 (46.9 ppm) and, CH_3-10 at δ19.9 and CH_3-9 at δ19.2 respectively as well as correlations between H-4 (δ2.08) and C-1 proved that CH_3-9 and CH_3-10 were both attached to C-7 which was linked to C-1 and C-4 on either side. Further correlations were observed between one of the H-3 protons (δ1.80) and C-2 and C-5 (27.0 ppm), between H-4 and C-2 and between one of the H-6 protons (δ1.42) and C-4. As C-4 was a CH group as shown by the HSQC data, the structure was determined to be that of camphor. Comparison of the NMR data with literature values[177] confirmed the identity of camphor.

α-Pinene

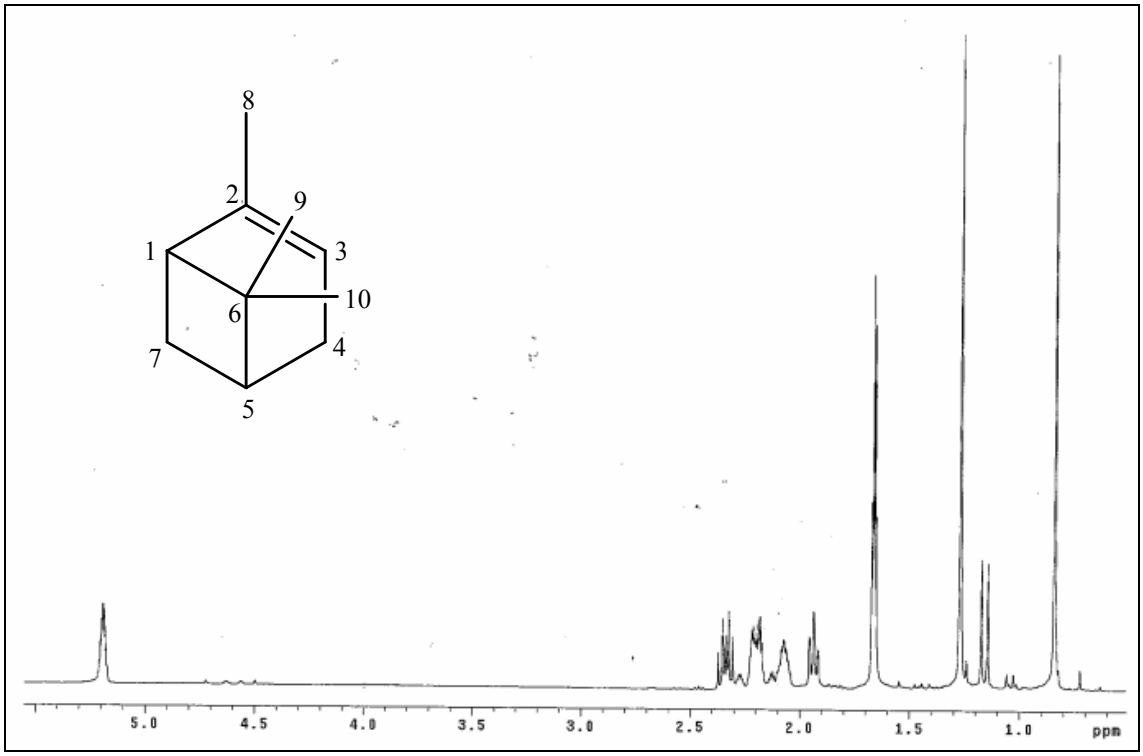


Figure 7. ^{1}H NMR spectrum (300 MHz) of α-pinene in $CDCl_3$

The ^{1}H NMR spectrum of α-pinene exhibited a methyl group signal at δ1.65, two methyl singlets (δ0.83, 1.26) and one olefinic proton at δ5.18. Signals for six protons were also observed at δ1.14, 1.16, 1.94, 2.08, 2.21 and 2.32. The ^{13}C NMR and DEPT experiments confirmed the presence of three methyl groups (20.8, 23.0, 26.3 ppm), one alkene (116.0, 144.5 ppm), two CH_2 groups (31.3, 31.5 ppm), two CH groups (40.7, 47.0 ppm) and a quaternary carbon (38.0 ppm).

α-Pinene			
C #	δ^{13}C	δ^{1}H mult. *J* (Hz)	HMBC ^{1}H to ^{13}C
1	47.0	1.94 td 5.5, 1.3	H-3, H-5, H_2-7, H_3-8, H_3-9, H_3-10
2	144.5		H-1, H-3, H_2-4, H_2-7, H_3-8
3	116.0	5.18 m	H-1, H_3-8
4	31.3	2.21 m	H-3
		2.32 dt 8.6, 5.5	
5	40.7	2.08 m	H-1,H-3, H_2-4, H_2-7, H_3-9, H_3-10
6	38.0		H-1, H_2-7, H_3-9, H_3-10
7	31.5	1.14 m	H_2-4
		1.16 m	
8	23.0	1.65 m	H-1, H-3
9	20.8	0.83 s	H-1, H_3-10
10	26.3	1.26 s	H-1, H_3-9

Table 6. ^{1}H NMR (300 MHz), ^{13}C NMR (75 MHz) and HMBC data of α-pinene in $CDCl_3$

HMBC data showed correlations between the methyl group H_3-8 (δ1.65) and C-1 (47.0 ppm), C-2 (144.5 ppm) and C-3 (116.0 ppm). Correlations were also observed between H-1 (δ1.94) and C-2, C-3 and CH_3-8 (23.0 ppm), between the olefinic proton H-3 (δ5.18) and C-1, C-2 and CH_3-8. Therefore it was confirmed that the methyl group C-8 was attached to one of the alkene carbons, C-2, which was connected with C-1 and C-3 on either side. Correlations between both methyl groups H_3-9 (δ0.83) and H_3-10 (δ1.26) and C-1, C-5 (40.7 ppm), C-6 (38.0 ppm), and CH_3-10 (26.3 ppm) and CH_3-9 (20.8 ppm) respectively together with correlations between H-1 (δ1.94) and C-6 and CH_3-10 proved that CH_3-9 and CH_3-10 were both attached to C-6 which was linked to C-1 and C-5 on either side. Further correlations between H-3 and C-4 (31.3 ppm) and C-5, between H_2-4 (δ2.21 and 2.32) and C-2, C-5 and C-7 (40.7 ppm) together with COSY

correlations between H_2-4 (δ2.21, 2.32) and H-3 and H-5 indicated that C-4 was located in between C-3 and C-5. HMBC correlations between H_2-7 (δ1.14 and 1.16) and C-1, C-2, C-5 and C-6 indicated that C-7 was located in between C-1 and C-5. Additional correlations between H-1 and C-5 and between H-5 (δ2.08) and C-1 were observed, verifying the presence of a cyclobutane ring. The NMR data obtained were consistent with literature values[179,180] for α-pinene except for the assignments for the three methyl signals.

3-Carene

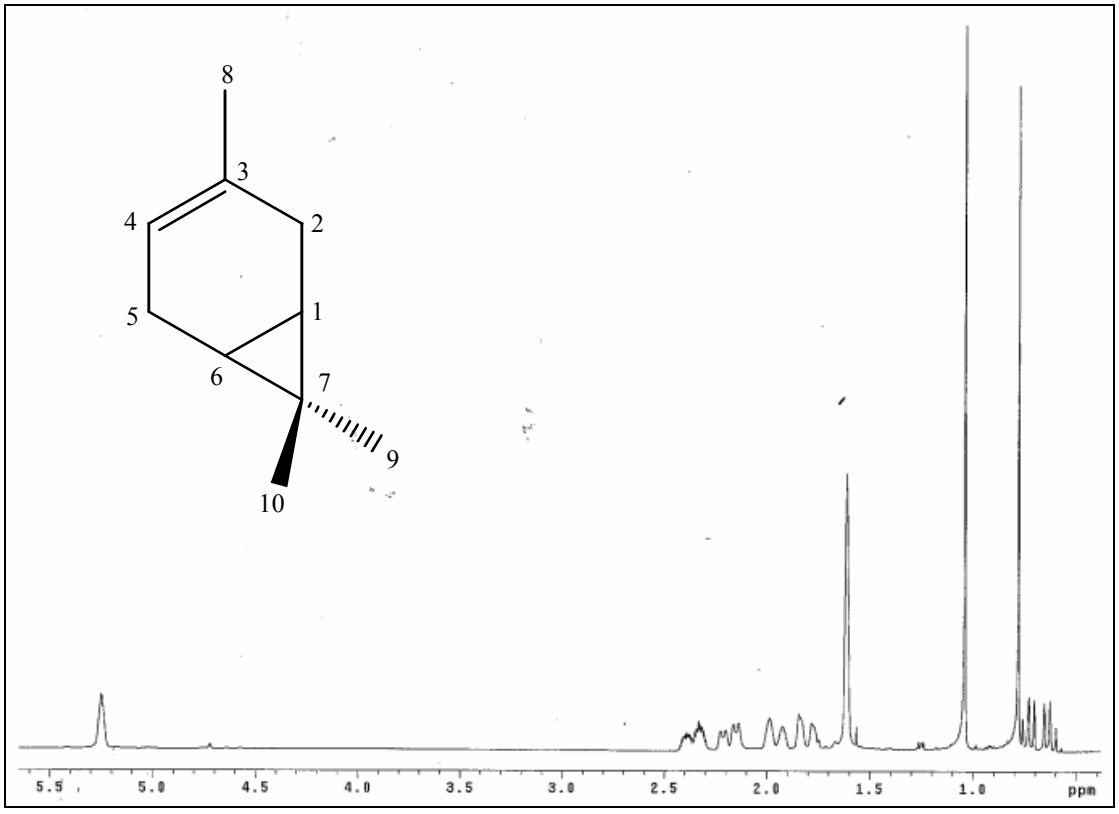


Figure 8. ^{1}H NMR spectrum (300 MHz) of 3-carene in $CDCl_3$

The ^{1}H NMR spectrum of 3-carene exhibited the presence of three methyl group (δ0.76,

1.02, 1.62), an olefinic proton (δ5.24) and two cyclopropane protons (δ0.61, 0.71). Signals for four protons were also observed at δ1.83, 1.94, 2.17 and 2.33. The ^{13}C NMR spectrum of 3-carene accounted for ten carbons: three methyl groups (13.2, 23.5, 28.3 ppm), one trisubstituted alkene (δ119.4, CH; 131.4, C) and a cyclopropane (16.3 ppm, C; 16.6, CH; 18.4, CH). The remaining two carbons at 20.8 ppm and 24.7 ppm were assigned as CH_2 groups by HSQC data.

3-Carene			
C #	δ^{13}C	δ^{1}H mult. *J* (Hz)	HMBC ^{1}H to ^{13}C
1	18.4	0.71 ddd 9.1, 7.6, 0.8	H-6, H_3-9, H_3-10
2	24.7	1.83 d 0.8	H_3-8
		2.17 dd 18.7, 7.6	
3	131.4		H-1, H_3-8
4	119.4	5.24 m	H-6, H_3-8
5	20.8	1.94 dd 19.0, 0.6	
		2.33 dd 19.0, 7.6	
6	16.6	0.61 ddd 9.1, 7.6, 0.6	H-1, H_2-2, H_3-9, H_3-10
7	16.3		H_2-2, H-6, H_3-9, H_3-10
8	23.5	1.60 m	H_2-2
9	13.2	0.76 s	H_3-10
10	28.3	1.02 s	H-1, H_3-9

Table 7. ^{1}H NMR (300 MHz), ^{13}C NMR (75 MHz) and HMBC data of 3-carene in $CDCl_3$

HMBC correlations were observed between the methyl group H_3-8 (δ1.60) and C-2 (24.7 ppm) and the two alkene carbons, C-3 and C-4 (131.4 and 119.4 ppm respectively). Correlations between H_2-2 (δ1.83, 2.17) and CH_3-8 (23.5 ppm) indicated that the methyl group was attached to C-3 which was connected with C-2 and C-4 on

either side. Further correlations between the two methyl groups H_3-9 at δ0.76 and H_3-10 at δ1.02 and C-1 (18.4 ppm), C-6 (16.6 ppm), C-7 (16.3 ppm), and CH_3-10 (28.3 ppm) and CH_3-9 (13.2 ppm) respectively, clearly indicated that C-1, C-6 and C-7 formed a cyclopropane with both CH_3-9 and CH_3-10 attached at the C-7 position. Correlations between H-1 (δ0.71) and C-6 and CH_3-10 and between H-6 (δ0.61) and C-1 and C-7 were also observed. The cyclopropane protons H-1 and H-6 were assigned to C-1 and C-6 respectively by HSQC data. Additional HMBC correlations observed between H-1 and C-3, between H_2-2 and C-6 and C-7 and between H-6 and C-4 enabled identification of the metabolite as 3-carene. Comparison of the NMR data with literature values[177,181] confirmed the identity of 3-carene.

***p*-Cymene**

The ^{1}H NMR spectrum of *p*-cymene exhibited one methyl singlet at δ2.23, two methyl doublets at δ1.21 and δ1.23, and four protons (two doublets, each 2H) in the aromatic region (δ7.10, 7.12). A multiplet proton signal was also observed at δ2.87. The ^{13}C NMR spectrum of *p*-cymene revealed signals for ten carbon atoms, three of which were assigned to methyl groups (20.6, 23.6, 23.6 ppm) and six to a benezene ring, including two sets of equivalent protonated carbon atoms (126.0 x 2, 128.8 x 2, 134.6, 145.2 ppm). A methine was also observed at 33.4 ppm.

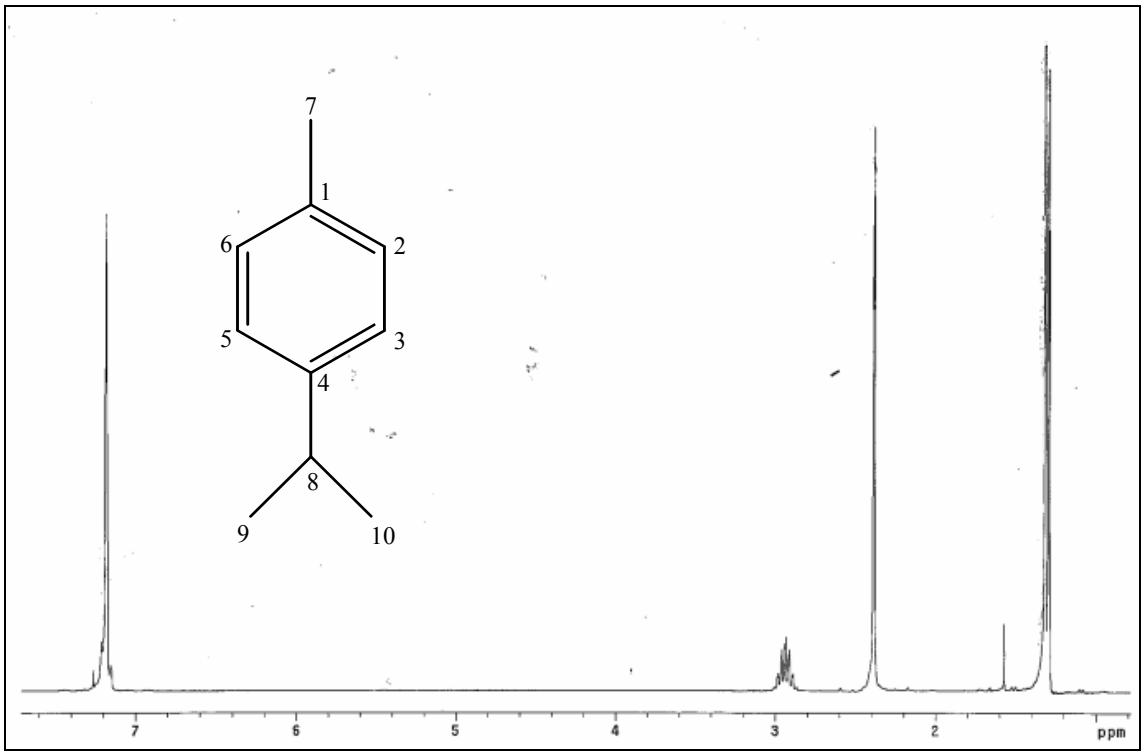


Figure 9. ^{1}H NMR spectrum (300 MHz) of *p*-cymene in $CDCl_3$ (reference: residual solvent peak at δ7.26)

HMBC correlations observed between the methyl group H_3-7 (δ2.23) and C-1 (134.6 ppm) and C-2/C-6 (128.8 ppm) as well as between H-2/H-6 (δ7.10) and C-1 and CH_3-7 (20.6 ppm) indicated that the methyl group CH_3-7 was attached to a quaternary carbon C-1 which was connected with two equivalent carbons, C-2 and C-6, on either side. Further correlations between H-2 and C-3 (126.0 ppm) (and between H-6 and C-5), between H-3 and C-1, C-2 and C-5 (and between H-5 and C-1, C-3 and C-6) and between both H-2 and H-3 and C-4 (145.2 ppm) (and between both H-5 and H-6 and C-4) enabled complete assignments for the aromatic ring. In addition, HMBC correlations between the two methyl groups H_3-9 (δ1.21) and H_3-10 (δ1.23) and C-8 (33.4 ppm), C-4, and CH_3-10 (23.6 ppm) and CH_3-9 (23.6 ppm) respectively, clearly indicated that C-8, CH_3-9 and CH_3-10 formed an isopropyl group that was attached at

the C-4 position. These assignments were confirmed by the HMBC correlations observed between H-3/H-5 and C-8. In addition, COSY correlations between H-8 and H_3-10 and between H-2/H-6 and H_3-7 were observed. Comparison of the NMR data with literature values[175,182] confirmed the identity of *p*-cymene.

p-Cymene			
C #	$\delta^{13}C$	$\delta^{1}H$ mult. *J* (Hz)	HMBC ^{1}H to ^{13}C
1	134.6		H-2/H-6, H-3/H-5, H_3-7
2,6	128.8	7.10 d 8.0	H-3/H-5, H_3-7
3,5	126.0	7.12 d 8.0	H-2/H-6, H-5/H-3
4	145.2		H-2/H-6, H-3/H-5, H_3-9, H_3-10
7	20.6	2.23 s	H-2/H-6
8	33.4	2.87 m	H-3/H-5, H_3-9, H_3-10
9	23.6	1.21 d 6.7	H-8, H_3-10
10	23.6	1.23 d 6.7	H-8, H_3-9

Table 8. ^{1}H NMR (300MHz), ^{13}C NMR (75MHz) and HMBC data of *p*-cymene in $CDCl_3$

2.2.6 *Euodia sp.* (leaves)

The oil of *Euodia sp.* was found to contain β-caryophyllene (25%), dillapiole (8.0%) and caryophyllene oxide (3.4%). Structure characterisation of dillapiole has been discussed under 2.2.8 *Ricinus communis* (leaves).

β-Caryophyllene

The ^{1}H NMR spectrum of β-caryophyllene exhibited three methyl singlets at δ0.98, 1.00 and 1.61, two olefinic doublets at δ4.82 and 4.95, one olefinic proton at δ5.31 and

resonances from twelve other protons giving signals between δ1.4 and 2.4. The ^{13}C NMR spectrum of β-caryophyllene accounted for fifteen carbon atoms: three methyl groups (16.1, 22.6, 30.1 ppm), a terminal alkene (111.5, 154.5 ppm), a trisubstituted alkene (124.3, 135.4 ppm) and the remaining eight carbons were assigned as five CH_2 groups (28.3, 29.0, 34.6, 39.8, 40.2 ppm), two CH groups (48.2 and 53.7 ppm) and a quaternary carbon (33.0 ppm) from HSQC data.

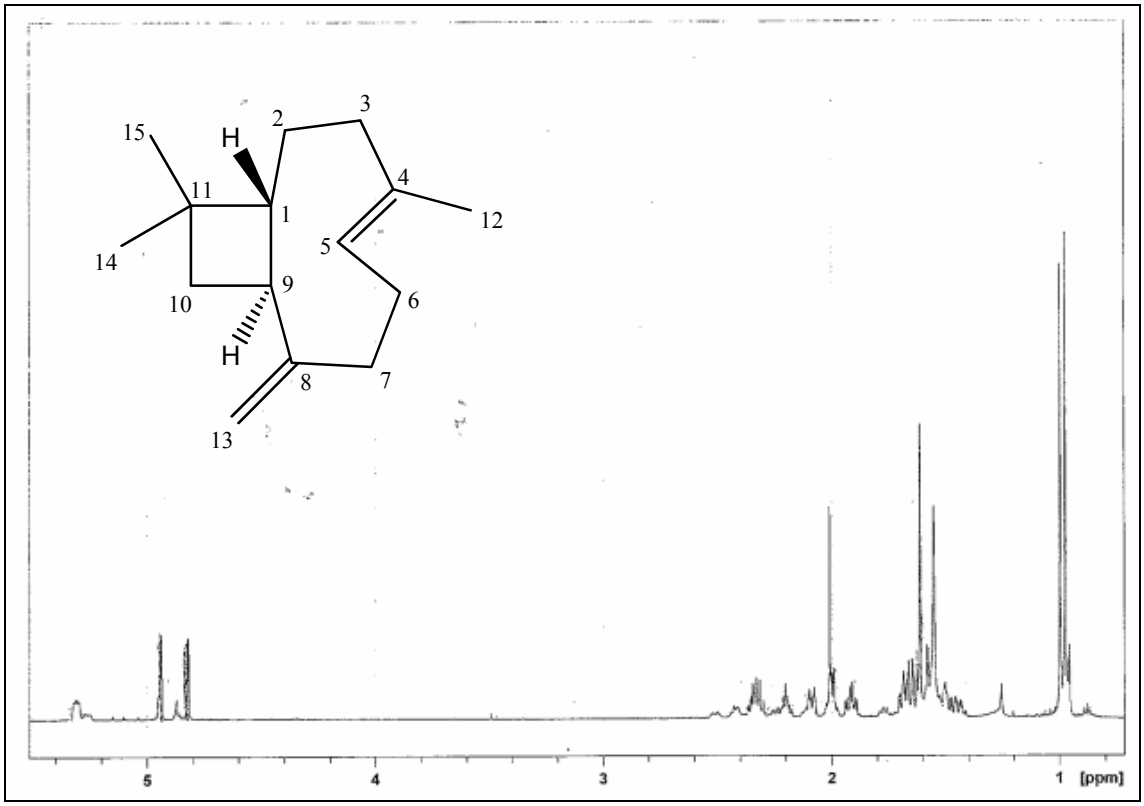


Figure 10. ^{1}H NMR spectrum (600 MHz) of β-caryophyllene (isolated by HPLC) in $CDCl_3$

The two methyl groups at δ1.00 (H_3-14) and 0.98 (H_3-15) showed HMBC correlations to C-1 (53.7 ppm), C-10 (40.2 ppm), the quaternary carbon C-11 (33.0 ppm) and to the carbons CH_3-15 (22.6 ppm) and CH_3-14 (30.1 ppm) respectively. Additional

correlations between H-1 (δ1.69) and C-11 indicated that both methyl groups were attached to C-11 which was further linked to C-1 and C-10 on either side. Further correlations between the terminal methylene H_2-13 (δ4.82, 4.95) and C-7 (34.6 ppm), C-8 (154.5 ppm) and C-9 (48.4 ppm) suggested that the terminal methylene C-13 (111.5 ppm) was attached at the C-8 position which was connected with C-7 and C-9 on either side. This assignment was confirmed by the correlations observed between H_2-7 at δ2.01 and 2.21 and C-8, C-9 and C-13 and between H-9 at δ2.32 and C-7, C-8 and C-13. Next, the methyl group at δ1.61 (H_3-12) showed correlations to C-3 (39.8 ppm), the alkene carbons C-4 (135.4 ppm) and C-5 (124.3 ppm), while one of the H-3 protons (δ1.92) exhibited correlations to C-4, C-5 and CH_3-12 (16.1 ppm) and those data confirmed that the methyl group CH_3-12 was attached at the C-4 position with C-3 and C-5 on either side. The ^{13}C chemical shift for C-5 (124.3 ppm), while one of the H-3 protons (δ1.92) exhibited correlations to C-4, C-5 and C-12 (16.1 ppm) and those data confirmed that the methyl group C-12 was attached at the C-4 position with C-3 and C-5 on either side. The ^{13}C chemical shift for this methyl group (16.1 ppm) indicates *trans* geometry for the double bond (the methyl group is *cis* to the methylene on the adjacent alkene carbon and this causes steric compression that results in a ^{13}C shift of < 20 ppm for the ethyl group, whereas *cis* geometry would produce a shift of > 20 ppm). Further correlations from H-1 to C-9, H-2b (δ1.51) to C-3 and C-9, H-3a to C-1 and C-2 (29.0 ppm) indicated that C-2 was located in between C-1 and C-3 and also that C-1 and C-10 were both linked to C-9. Thus C-1, C-9, C-10 and C-11 were arranged in a cyclobutane ring. Meanwhile, correlations observed between H-7b (δ2.21) and C-6 (28.3 ppm) and C-5 confirmed that C-6 was located in between C-5 and C-7. 1D Gradient selective TOCSY experiments on the olefinic proton (H-5, δ5.31) and H_2-3

(δ2.09 and 1.92) allowed identification of the chemical shifts for the protons of the CH_2 groups C-2, C-6 and C-7. The NMR data obtained were consistent with literature values[183] for β-caryophyllene therefore the relative ring junction geometry must be *trans*.

β-Caryophyllene			
C #	δ^{13}C	δ^{1}H mult. *J* (Hz)	HMBC ^{1}H to ^{13}C
1	53.7	1.69 m	H-3a, H-9, H_3-14, H_3-15
2	29.0	1.45 m	H-3a
		1.51 m	
3	39.8	1.92 m	H-2b, H_3-12
		2.09 m	
4	135.4		H-3b, H_3-12
5	124.3	5.31 dd 10.3, 4.5	H-3b, H-7b, H_3-12
6	28.3	2.00 m	H-7b
		2.35 m	
7	34.6	2.01 m	H-9, H_2-13
		2.21 m	
8	154.5		H_2-7, H-9, H_2-13
9	48.4	2.32 m	H-1, H-2b, H_2-7, H_2-13
10	40.2	1.63-1.68 m	H-9, H_3-14, H_3-15
11	33.0		H-1, H_3-14, H_3-15
12	16.1	1.61 s	H-3a
13	111.5	4.82 d 1.7	H_2-7, H-9
		4.95 d 1.7	
14	30.1	1.00 s	H_3-15
15	22.6	0.98 s	H_3-14

Table 9. ^{1}H NMR (600 MHz), ^{13}C NMR (150 MHz) and HMBC data of β-caryophyllene in $CDCl_3$

Caryophyllene oxide

The ^{1}H NMR spectrum of caryophyllene oxide exhibited three methyl singlets at δ0.98, 1.00 and 1.20, two olefinic doublets at δ4.86 and 4.97 and resonances from thirteen other protons giving signals between δ0.9 and 3.0. The ^{13}C NMR spectrum of caryophyllene oxide accounted for fifteen carbon atoms: three methyl groups (16.3, 22.3, 29.3 ppm), one alkene (112.1, 151.0 ppm) and together with the DEPT experiment, it confirmed the presence of five CH_2 groups (26.6, 29.2, 29.3, 38.5, 39.1 ppm) in addition to the terminal methylene carbon at δ112.1, three CH groups (48.1, 50.2, 63.1 ppm) and two quaternary carbons (33.3, 59.3 ppm) in addition to the one already discussed in the alkene group. The signals at 59.3 (a quaternary carbon) and 63.1 ppm (a CH group) suggested the presence of an epoxide between those two carbons.

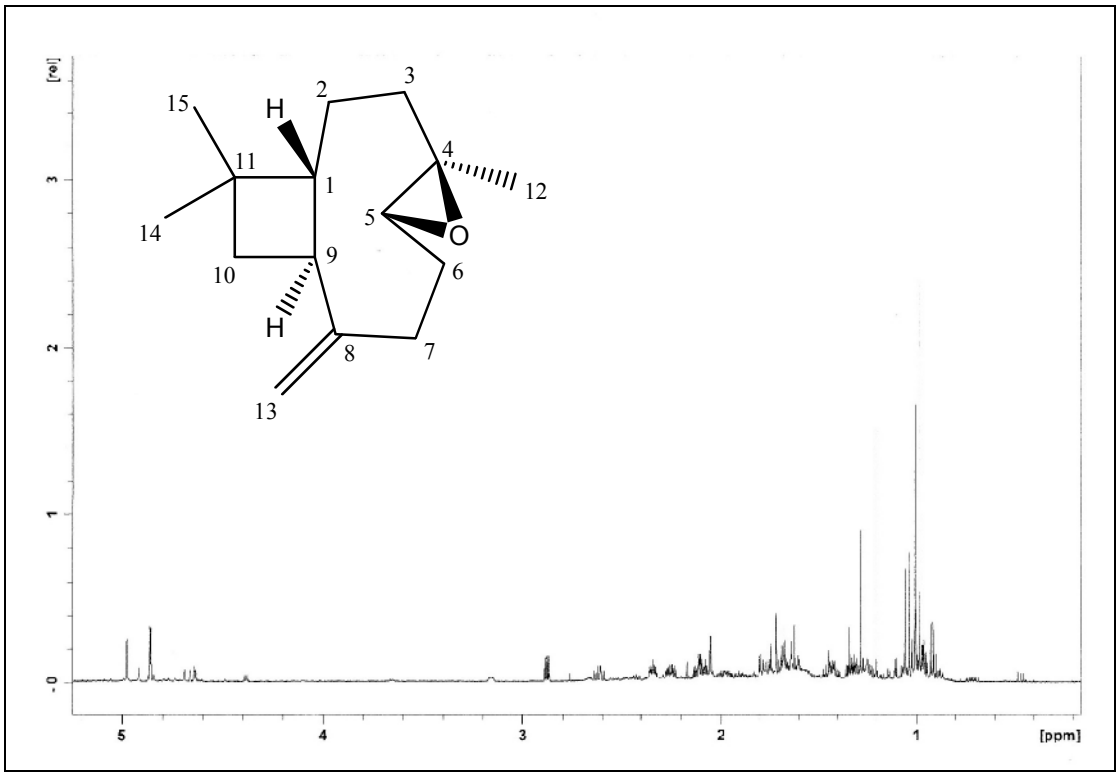


Figure 11. ^{1}H NMR spectrum (600 MHz) of caryophyllene oxide (isolated by HPLC) in $CDCl_3$

The similarity of the spectrum to that of β-caryophyllene with the presence of an epoxide and the absence of the trisubstituted alkene, suggested the metabolite was caryophyllene oxide and HMBC correlations observed were consistent with that structure. The chemical shifts for H-1 and H-9 matched the literature[184] therefore, the relative stereochemistry is the same as that described in the literature.

Caryophyllene oxide			
C #	δ^{13}C	δ^{1}H mult. *J* (Hz)	HMBC ^{1}H to ^{13}C
1	50.2	1.76 t 9.8	H-9, H_3-14, H_3-15
2	26.6	1.45 m	H-9
		1.63 m	
3	38.5	0.96 m	H-1, H_3-12
		2.06 m	
4	59.3		H-3a, H_3-12
5	63.1	2.87 dd 10.6, 4.3	H-6b, H_2-7, H_3-12
6	29.6	1.33 m	H-5, H-7b
		2.23 m	
7	29.2	2.11 m	H-5, H-9, H_2-13
		2.32 m	
8	151.0		H-1, H_2-7, H-9, H-10a, H_2-13
9	48.1	2.62 ddd 10.6, 9.8, 8.3	H-1, H-2b, H-7a, H_2-13
10	39.1	1.62 m	H-9, H_3-14, H_3-15
		1.68 m	
11	33.3		H-1, H_3-14, H_3-15
12	16.3	1.20 s	
13	112.1	4.86 d 1.4	H_2-7, H-9
		4.97 d 1.4	
14	29.3	1.00 s	H-1, H-10a
15	22.0	0.98 s	H-1

Table 10. ^{1}H NMR (600 MHz), ^{13}C NMR (150 MHz) and HMBC data of caryophyllene oxide in $CDCl_3$

2.2.7 *Cymbopogon citratus* (leaves)

The oil of *Cymbopogon citratus* was found to contain geranial (32%), neral (18%), viridiflorol (14%), ledol (6.0%) and geraniol (1.5%).

Geranial (*trans*-citral)

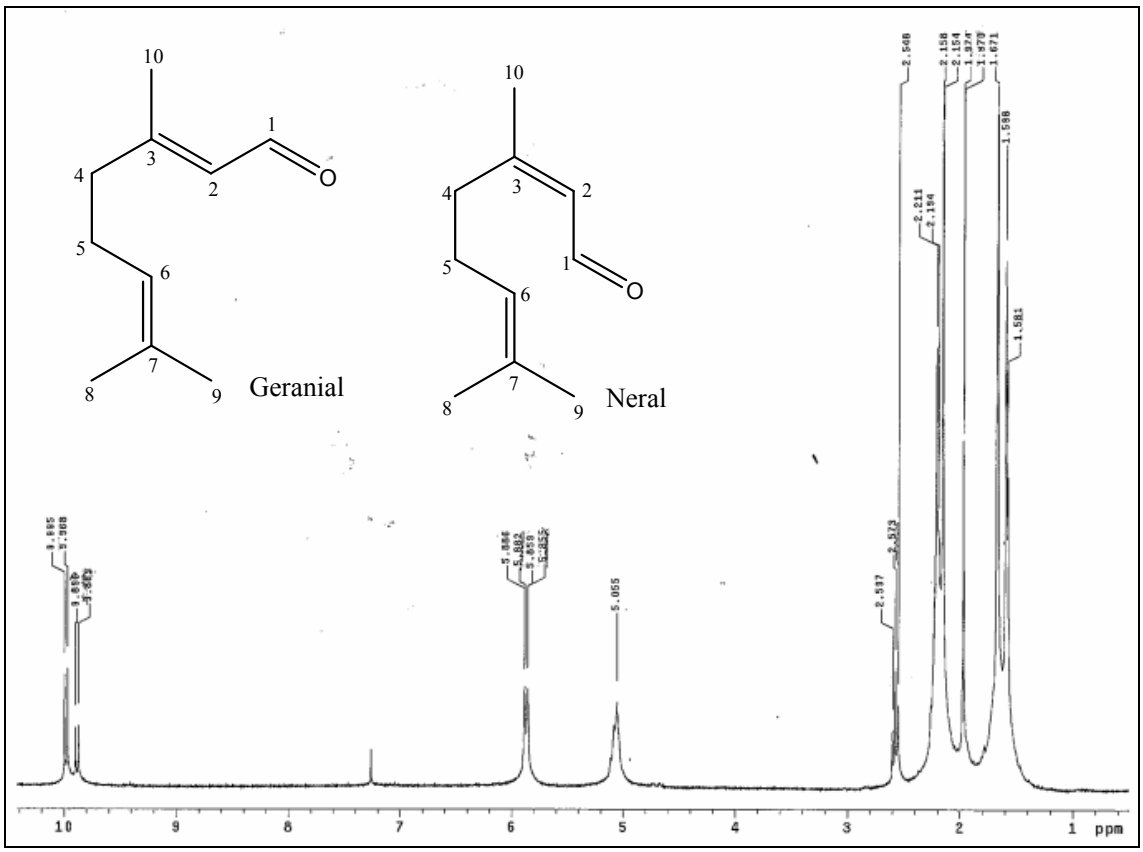


Figure 12. ^{1}H NMR spectrum (300 MHz) of geranial and neral in $CDCl_3$ (reference: residual solvent peak at δ7.26)

The ^{1}H NMR spectrum of geranial revealed the presence of three methyl groups (δ1.61, 1.68, 2.16), two olefinic protons (δ5.07, 5.89) and one aldehyde proton (δ9.99). It also afforded signals for four protons at δ2.20 and 2.21. The ^{13}C NMR spectrum of geranial

accounted for ten carbon atoms: three methyl groups (17.3, 17.4, 25.2 ppm), two alkenes (122.4, 127.2, 132.6, 163.7 ppm) and one aldehyde carbonyl group (191.0 ppm). The remaining two carbons at 25.2 and 40.3 ppm were assigned as CH_2 groups from HSQC data.

Geranial (*trans*-citral)			
C #	$\delta^{13}C$	$\delta^{1}H$ mult. *J* (Hz)	HMBC ^{1}H to ^{13}C
1	191.0	9.99 d 8.1	
2	127.2	5.89 d 8.1	H-1, H_2-4
3	163.7		H_2-4
4	40.3	2.21 m	H-2
5	25.2	2.20 m	H_2-4
6	122.4	5.07 m	H_2-4, H_3-8, H_3-9
7	132.6		H_3-8, H_3-9
8	17.4	1.61 s	H-6, H_3-9
9	25.2	1.68 s	H_3-8
10	17.3	2.16 d 1.1	H-2, H_2-4

Table 11. ^{1}H NMR (300 MHz), ^{13}C NMR (75 MHz) and HMBC data of geranial in $CDCl_3$

HMBC correlations between the two methyl groups H_3-8 (δ1.61) and H_3-9 (δ1.68) and the alkene carbons C-7 (132.6 ppm) and C-6 (122.4 ppm), and the carbons CH_3-9 (25.2 ppm) and CH_3-8 (17.4 ppm) respectively, clearly indicated that CH_3-8 and CH_3-9 were both attached to C-7 which linked to C-6 bearing an olefinic proton at δ5.07 (H-6) which showed a correlation to CH_3-8. Furthermore, the aldehyde proton H-1 (δ9.99) showed a correlation to C-2 (127.2 ppm), an alkene methine with a proton at δ5.89 (H-2). Coupling (8.1 Hz) between H-1 and H-2 was observed in the ^{1}H NMR spectrum. The H-2 proton showed three bond correlation to both C-4 (40.3 ppm) and the methyl group CH_3-10 (17.3 ppm). These data suggested that the carbons from C-1 (191.0 ppm)

to C-4 were linked consecutively with CH_3-10 attached at the C-3 position (163.7 ppm). This assignment was confirmed by the correlations observed from H_2-4 at δ2.21 to C-2, C-3 and CH_3-10. Additional correlations between H_2-4 and C-5 (25.2 ppm) and C-6 indicated that C-5 was located in between C-4 and C-6. The downfield position of the proton signal for the H-10 methyl group, together with the upfield position of the ^{13}C signal for CH_3-10 indicated that the H-10 methyl group was *cis* to the aldehyde function on the C-2/C-3 double bond. Comparison of the NMR data with literature values[184] confirmed the identity of geranial.

Neral (*cis*-citral)

The ^1H NMR spectrum in $CDCl_3$ and structure of neral are shown in the geranial section. The ^1H NMR spectrum revealed the presence of three methyl groups (δ1.59, 1.68, 1.98), two olefinic protons (δ5.09, 5.88) and one aldehyde proton (δ9.89). It also afforded signals for four protons at δ2.24 and 2.58. The ^{13}C NMR spectrum of neral accounted for ten carbon atoms: three methyl groups (17.7, 24.8, 25.2 ppm), two alkenes (123.6, 128.3, 133.8, 163.6 ppm) and one aldehyde carbonyl group (190.4 ppm). The remaining two carbons at 26.7 and 32.1 ppm were assigned as CH_2 groups from HSQC data.

It would be tempting to simply say here that coupling (8.1 Hz) between the aldehyde proton and one alkene signal together with HMBC correlations, indicated the same linkage patterns as had been assigned for geranial, however the upfield positions of the signal for the H-10 methyl group in the ^1H NMR spectrum, and the downfield shift in the ^{13}C spectrum for CH_3-10 (relative to that for geranial) indicated that the H-10 methyl and aldehyde groups were in a *trans* relationships on the C-2/C-3 double bond.

Comparison of the NMR data with literature values[184] confirmed the identity of neral.

Neral (*cis*-citral)			
C #	δ^{13}C	δ^{1}H mult. *J* (Hz)	HMBC ^{1}H to ^{13}C
1	190.4	9.89 d 8.1	
2	128.3	5.88 d 8.1	H-1, H_2-4
3	163.6		H_2-4
4	32.1	2.58 t 7.3	H-2, H_2-5
5	26.7	2.24 m	H_2-4, H-6
6	123.6	5.09 m	H_2-4
7	133.8		H_3-9
8	17.7	1.59 s	H_3-9
9	25.2	1.68 s	
10	24.8	1.98 d 1.1	H-2, H_2-4

Table 12. ^{1}H NMR (300 MHz), ^{13}C NMR (75 MHz) and HMBC data of neral in $CDCl_3$

Viridiflorol

The ^{1}H NMR spectrum of viridiflorol exhibited the presence of four methyl groups (δ0.93, 1.00, 1.02, 1.15), two signals for cyclopropane protons (δ0.11, 0.61) and resonances from eleven other protons giving signals between δ1.26 and 2.0. The ^{13}C NMR spectrum of viridiflorol accounted for fifteen carbon atoms: four methyl groups (15.9, 16.3, 28.2, 31.7 ppm), one oxygenated carbon (74.7 ppm) and a methine carbon at 58.0 ppm. The remaining nine carbons were assigned as four CH_2 groups (18.4, 25.6, 29.2, 37.5 ppm), three CH groups (21.8, 28.2, 39.6 ppm) and two quaternary carbons (18.5, 38.6 ppm) from the DEPT experiment.

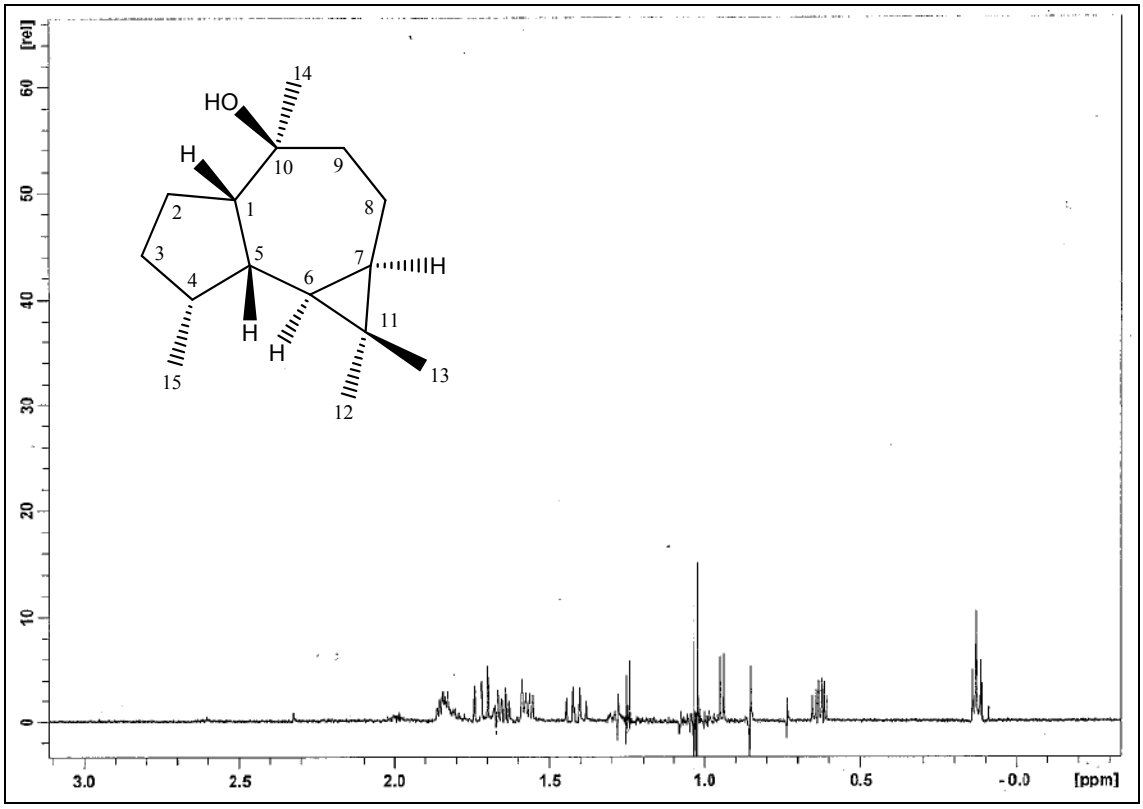


Figure 13. Gradient selective TOCSY (600 MHz) from excitation centered on δ 0.11 of viridiflorol in $CDCl_3$

An HMBC spectrum showed correlations between the methyl group H_3-12 (δ1.02) and C-7 (28.2 ppm), between the other methyl group H_3-13 (δ1.00) and C-6 (21.8 ppm), C-7, C-11 (18.5 ppm) and CH_3-12 (28.2 ppm), from the cyclopropane proton H-6 (δ0.11) and C-7 and C-11 and between the other cyclopropane proton H-7 (δ0.61) and CH_3-12 and CH_3-13 (16.3 ppm). These data indicated that the methyl groups CH_3-12 and CH_3-13 were both attached to C-11 and that C-6, C-7 and C-11 formed a cyclopropane ring. COSY correlations between H-6 and H-7 and between H-7 and H_2-8 (δ1.40 and 1.64) confirmed the carbon linkages from C-6 to C-8 (18.4 ppm). Further HMBC correlations between the methyl group H_3-14 (δ1.15) and C-1 (58.0 ppm), C-9 (37.5 ppm) and C-10 (74.7 ppm) suggested that the methyl group (C-14, 31.7 ppm) was attached at the C-10

position bearing a hydroxyl group, with C-1 and C-9 connected on either side. Correlations were observed between the fourth methyl group H_3-15 (δ0.93) and C-3 (29.2 ppm), C-4 (38.6 ppm) and C-5 (39.6 ppm) and between one of the H-3 protons (δ1.80) and C-2 (25.6 ppm) and CH_3-15. These data together with a COSY correlation between H-4 (δ1.99) and H_3-15 indicated that CH_3-15 (15.9 ppm) was attached at C-4 that linked to C-3 and C-5 on either side. With the correlation between H-6 and C-4, those data confirmed the carbon linkages from C-2 to C-6. COSY data was used to link C-8 to C-9, C-1 to C-2 and C-1 to C-5, completing the planar structure.

Viridiflorol			
C #	δ^{13}C	δ^{1}H mult. *J* (Hz)	HMBC ^{1}H to ^{13}C
1	58.0	1.81 m	H_3-14
2	25.6	1.57 m	H-3b
		1.63 m	
3	29.2	1.26 m	H_3-15
		1.80 m	
4	38.6	1.99 m	H-6, H_3-15
5	39.6	1.86 m	H_3-15
6	21.8	0.11 m	H_3-13
7	28.2	0.61 m	H-6, H_3-12, H_3-13
8	18.4	1.40 m	
		1.64 m	
9	37.5	1.56 m	H_3-14
		1.66 m	
10	74.7		H_3-14
11	18.5		H-6, H_3-13
12	28.2	1.02 s	H-7, H_3-13
13	16.3	1.00 s	H-7
14	31.7	1.15 s	
15	15.9	0.93 d 6.9	H-3b

Table 13. ^{1}H NMR (600 MHz), ^{13}C NMR (150 MHz) and HMBC data of viridiflorol in $CDCl_3$

Two gradient selective TOCSY experiments which utilized the signals at δ0.11 and 0.61, together with HSQC and COSY data allowed identification of chemical shifts for all remaining protons, and the NMR data was consistent with literature values[185] for viridiflorol, thus providing relative stereochemical data.

Ledol

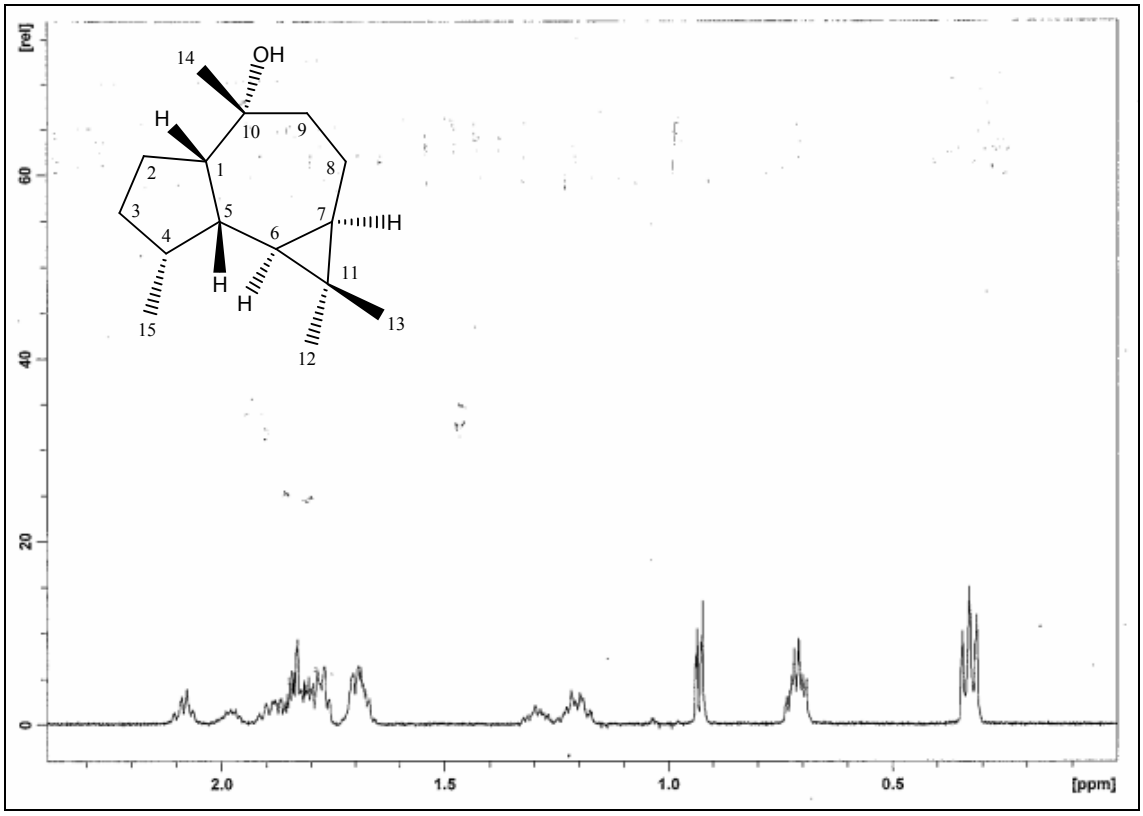


Figure 14. Gradient selective TOCSY (600 MHz) from excitation centered on δ 0.33 of ledol in $CDCl_3$

The ^{1}H NMR spectrum of ledol exhibited the presence of four methyl groups (δ0.94, 0.98, 1.03, 1.14), two signals for cyclopropane protons (δ0.33, 0.72) and resonances from eleven other protons giving signals between δ1.18 and 2.1. The ^{13}C NMR

spectrum of ledol accounted for fifteen carbon atoms: four methyl groups (15.4, 15.9, 28.2, 30.2 ppm), one oxygenated carbon (74.7 ppm) and a methane carbon at 53.7 ppm. Nine other carbons (19.2, 20.3, 23.4, 24.6, 24.8, 30.6, 38.4, 39.2, 40.5 ppm) were present.

HMBC correlations were similar to those of viridiflorol and indicative of an isomer of viridiflorol. The NMR data obtained matched those published for ledol[185], which differs from viridiflorol by its relative stereochemistry at the C-10 centre.

Ledol			
C #	$\delta^{13}C$	$\delta^{1}H$, mult., *J* (Hz)	HMBC ^{1}H to ^{13}C
1	53.7	2.09 ddd 11.0, 10.8, 9.2	H_3-14
2	24.6	1.67 m	
		1.88 m	
3	30.6	1.30 m	H_3-15
		1.70 m	
4	38.4	1.99 m	H_3-15
5	40.5	1.78 m	H-6, H_3-15
6	23.4	0.33 dd 10.4, 9.0	H-8a, H_3-12, H_3-13
7	24.8	0.72 ddd 11.3, 9.0, 6.0	H-6, H_3-12, H_3-13
8	20.3	1.85 m	
		1.18 m	
9	39.2	1.70 m	H_3-14
		1.89 m	
10	74.7		H_3-14
11	19.2		H-6, H_3-12, H_3-13
12	28.2	1.03 s	H_3-13
13	15.4	0.98 s	H-6, H-7, H_3-12
14	30.2	1.14 s	
15	15.9	0.94 d 6.9	

Table 14. ^{1}H NMR (600 MHz), ^{13}C NMR (150 MHz) and HMBC data of ledol in $CDCl_3$

Geraniol

Figure 15. Gradient selective TOCSY (600 MHz) from excitation centered on δ4.16 of geraniol in $CDCl_3$

The ^{1}H NMR spectrum of geraniol revealed the presence of three methyl groups (δ1.61, 1.68, 1.69) and two olefinic protons (δ5.10, 5.43). It also afforded signals for six protons (3 x 2H) at δ2.04, 2.11 and 4.16. The ^{13}C NMR spectrum of geraniol accounted for ten carbons: three methyl groups (16.0, 17.4, 25.3 ppm), two alkenes (122.9, 123.4, 131.9, 139.8 ppm) and one primary oxygenated carbon (58.9 ppm). The remaining two carbons at 26.1 and 39.3 ppm were assigned as CH_2 groups from HSQC data.

Geraniol			
C #	$\delta^{13}C$	$\delta^{1}H$ mult. *J* (Hz)	HMBC ^{1}H to ^{13}C
1	58.9	4.16 d 7.7	
2	122.9	5.43 m	H_2-1, H_2-4
3	139.8		H_2-1, H_2-4, H_3-10
4	39.3	2.04 m	H-2, H_2-5, H_3-10
5	26.1	2.11 m	H_2-4
6	123.4	5.10 m	H_2-4, H_2-5, H_3-8, H_3-9
7	131.9		H_2-5, H_3-8, H_3-9
8	17.4	1.61 br s	H-6, H_3-9
9	25.3	1.69 br s	H-6, H_3-8
10	16.0	1.68 d 2.7	H-2, H_2-4

Table 15. ^{1}H NMR (600 MHz), ^{13}C NMR (150 MHz) and HMBC data of geraniol in $CDCl_3$

HMBC correlations between the two methyl groups H_3-8 (δ1.61) and H_3-9 (δ1.69) and the alkene carbons C-7 (131.9 ppm) and C-6 (123.4 ppm), and the carbons CH_3-9 (25.3 ppm) and CH_3-8 (17.4 ppm) respectively, clearly indicated that CH_3-8 and CH_3-9 were both attached to C-7 which linked to C-6 bearing an olefinic proton (δ5.10, H-6) that showed correlations to CH_3-8 and CH_3-9. Furthermore, protons at δ4.16 (H_2-1) on the oxygenated carbon C-1 (58.9 ppm) showed correlations to C-2 (122.9 ppm) and C-3 (139.8 ppm). The alkene methine proton H-2 (δ5.43) showed HMBC correlations to C-4 (39.3 ppm) and the methyl group CH_3-10 (16.0 ppm). Correlations were also observed between H_3-10 (δ1.68) to C-3 and C-4. These data suggested that the carbons from C-1 to C-4 were linked consecutively with CH_3-10 attached at the C-3 position. This assignment was confirmed by the correlations observed between H_2-4 at δ2.04 and C-2, C-3 and CH_3-10. Additional correlations between H_2-4 and C-5 (25.2 ppm) and

C-6 and between H_2-5 (δ2.11) and C-4, C-6 and C-7 indicated that C-5 was located in between C-4 and C-6, and led to identification as geraniol/nerol. The ^{13}C chemical shift for CH_3-10 was indicative of *cis* orientation with respect to the C-1 group, so the metabolite was identified as geraniol. Comparison of the NMR data with literature values[184] confirmed the identity of geraniol.

2.2.8 *Ricinus communis* (leaves)

The oil of *Ricinus communis* was found to contain dillapiole (38%), piperitone (13%) and β-caryophyllene (12%). Structure characterisation of piperitone has been discussed under 2.2.2 *Tagetes spp.* (leaves) and β-caryophyllene under 2.2.6 *Euodia sp.* (leaves).

Dillapiole

The ^1H NMR spectrum of dillapiole disclosed two methoxyl singlets (δ3.75, 4.01), and a terminal double bond (δ5.04, 5.05 and 5.91). It also afforded an upfield singlet in the aromatic region at δ6.35, another two proton singlet at δ5.88 and a double triplet (*J*=6.5, 1.4 Hz) at δ3.30. The ^{13}C NMR spectrum of dillapiole exhibited signals for twelve carbon atoms, two of which were assigned to methoxyl groups (59.8, 61.2 ppm), one to a methylenedioxy group (100.8 ppm), six to a pentasubstituted benzene ring (102.7, 125.9, 135.8, 137.3, 143.9, 144.1 ppm) and the remaining three to an allyl group (33.7, 115.5, 137.2 ppm).

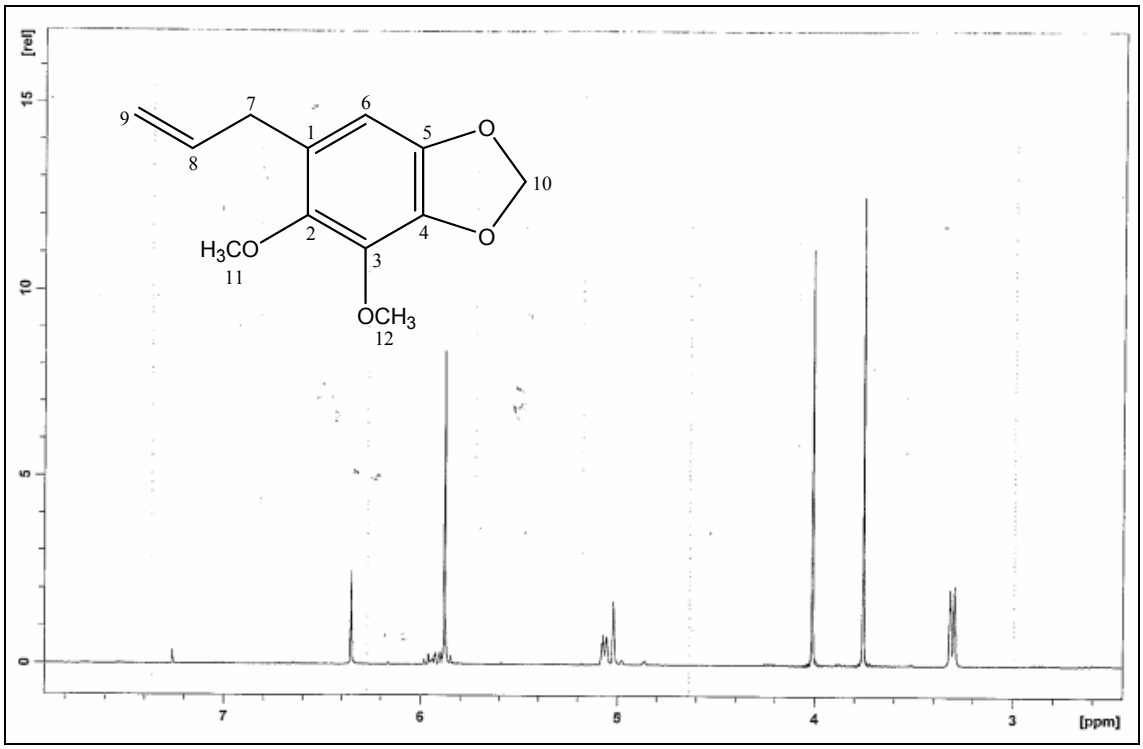


Figure 16. ^{1}H NMR spectrum (600 MHz) of dillapiole (isolated by column chromatography) in $CDCl_3$ (reference: residual peak solvent at δ7.26)

The two methoxyl signals at δ3.75 (H_3-11) and δ4.01 (H_3-12) in the ^{1}H NMR spectrum which corresponded to the ^{13}C NMR signals at 61.2 ppm and 59.8 ppm showed HMBC correlations to the benzene ring carbons C-2 (143.9 ppm) and C-3 (137.3 ppm) respectively and confirmed the attachment positions for the two methoxy groups. Further, HMBC correlations between the methylenedioxy group H_2-10 (δ5.88) and C-4 (135.8 ppm) and C-5 (144.1 ppm) indicated that the two oxygens were attached at C-4 and C-5 positions. The remaining ^{13}C NMR signal at 125.9 ppm (C-1) must have been substituted by the allyl group as the signal at 102.7 ppm (C-6) was a protonated carbon which corresponded to H-6 at δ6.35 by the HSQC data. Additional HMBC correlations between H-6 and C-4 and C-5, between H_2-7 (δ3.30) and C-1, C-2, C-6, C-8 (137.2 ppm) and C-9 (115.5 ppm) permitted unambiguous assignment of substituent positions

on the aromatic ring and confirmed the carbon linkages of the allyl group. The published ^{1}H NMR shifts[186] were inconsistent with our findings, however the ^{13}C NMR data obtained were consistent with literature values for Dillapiole for C-1 to H-11, except for the fact that assignments for C-2 and C-4 were reversed in the literature. The HMBC correlations between H_2-7 and C-1, C-2, C-6 together with HMBC correlations from both H-6 and the methylenedioxy group to C-4 and C-5 unambiguously defined the isolated structure as dillapiole.

Dillapiole			
C #	δ^{13}C	δ^{1}H mult. *J* (Hz)	HMBC ^{1}H to ^{13}C
1	125.9		H_2-7
2	143.9		H_2-7, H_3-11
3	137.3		H_3-12
4	135.8		H-6, H_2-10
5	144.1		H-6, H_2-10
6	102.7	6.35 s	H_2-7
7	33.7	3.30 dt 6.5, 1.4	
8	137.2	5.91 ddt 16.8, 10.3, 6.5	H_2-7
9	115.5	5.04 ddd 10.3, 1.8, 1.4	H_2-7
		5.05 ddd 16.8, 1.8, 1.4	
10	100.8	5.88 s	
11	61.2	3.75 s	
12	59.8	4.01 s	

Table 16. ^{1}H NMR (600 MHz), ^{13}C NMR (150 MHz) and HMBC data of dillapiole in $CDCl_3$

2.2.9 *Piper aduncum* (leaves)

The oil of *Piper aduncum* was found to contain dillapiole (17%), piperitone (10%) and

β-caryophyllene (9.8%). Structure characterisation of piperitone has been discussed under 2.2.2 *Tagetes spp.* (leaves), dillapiole under 2.2.8 *Ricinus communis* (leaves) and β-caryophyllene under 2.2.6 *Euodia sp.* (leaves).

2.2.10 *Piper gibbilimbum* (leaves)

The oil of *Piper gibbilimbum* leaves was found to contain gibbilimbol C, gibbilimbol D (11% in total), dillapiole (2.0%) and 1,8-cineole (0.1%). It is suspected that dillapiole and 1,8-cineole originated from cross contamination of the oil during steam distillation.

Gibbilimbol C and D

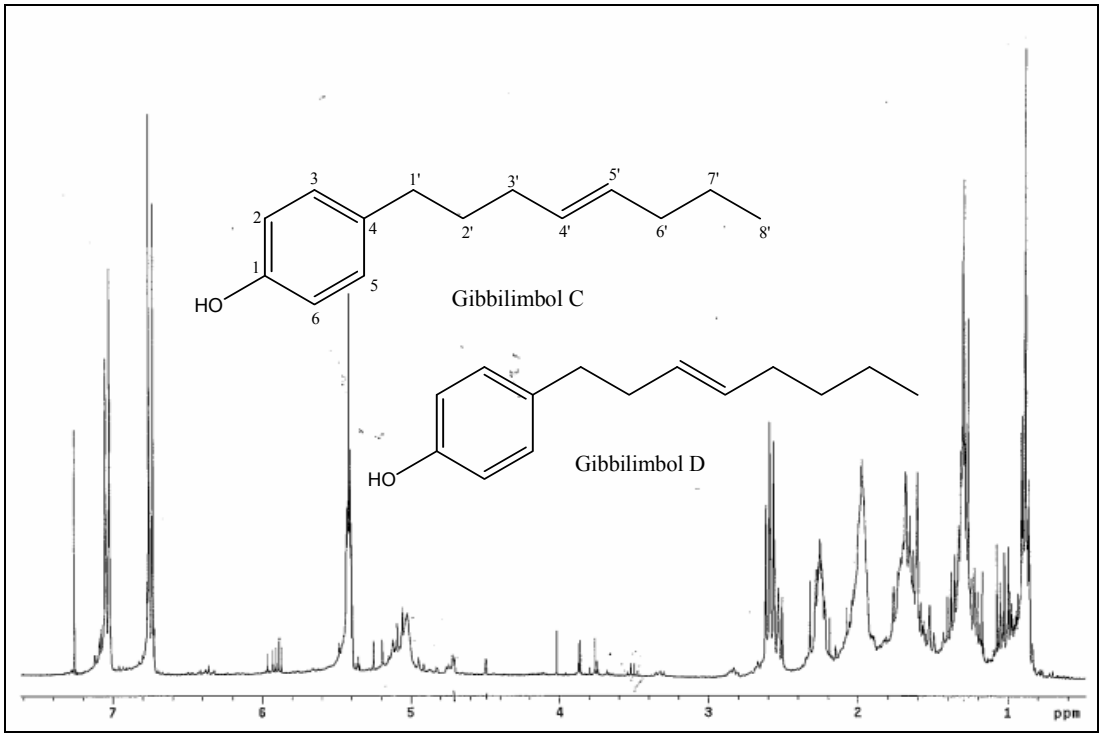


Figure 17. ^{1}H NMR spectrum (300 MHz) of the *Piper gibbilimbum* leaf oil in $CDCl_3$ (reference: residual peak solvent at δ7.26)

Gibbilimbol C			
C #	δ^{13}C	δ^{1}H mult. *J* (Hz)	HMBC ^{1}H to ^{13}C
1	153.5	4.50 br s (OH)	H-2/H-6, H-3/H-5
2,6	115.0	6.73 d 8.5	H-6/H-2
3,5	129.5	7.05 d 8.5	H-5/H-3
4	134.9		H_2-1'
1'	34.4	2.51 t 7.5	
2'	31.6	1.66 m	H_2-1', H_2-3'
3'	32.0	2.04 m	H_2-2', H-4', H-5'
4'	130.0	5.41 m	H_2-3', H_2-6'
5'	130.7	5.41 m	
6'	34.7	1.94 m	H_2-7', H_3-8'
7'	22.7	1.35 m	H_3-8'
8'	13.6	0.90 t 7.4	H_2-7'

Table 17. ^{1}H NMR (300 MHz), ^{13}C NMR (75 MHz) and HMBC data of gibbilimbol C in $CDCl_3$

Gibbilimbol D			
C #	δ^{13}C	δ^{1}H mult. *J* (Hz)	HMBC ^{1}H to ^{13}C
1	153.5	4.72 br s (OH)	H-3/H-5
2,6	115.0	6.76 d 8.5	H-3/H-5
3,5	129.5	7.02 d 8.5	H-5/H-3
4	134.4		H-2/H-6, H_2-1'
1'	35.2	2.56 t 7.5	H-3/H-5
2'	34.7	2.22 m	H_2-1', H-3', H-4'
3'	129.3	5.42 m	H_2-2'
4'	131.1	5.42 m	H_2-2'
5'	32.2	1.98 m	H_2-6'
6'	31.7	1.29 m	H_2-5', H_2-7', H_3-8'
7'	22.1	1.30 m	H_3-8'
8'	13.9	0.88 t 7.1	

Table 18. ^{1}H NMR (300 MHz), ^{13}C NMR (75 MHz) and HMBC data of gibbilimbol D in $CDCl_3$

These compounds have been previously found in *P. gibbilimbum* in PNG and reported.[122] Our data (see Figure 16 and Tables 17 and 18) was in a total agreement with the literature values.

2.2.11 *Piper gibbilimbum* (fruits)

The oil of *Piper gibbilimbum* fruits was similar in composition to that from the leaves, but contained slightly higher yields of gibbilimbols. The oil was found to contain gibbilimbol C, gibbilimbol D (22% in total), dillapiole (0.8%) and 1,8-cineole (0.03%). It is suspected that dillapiole and 1,8-cineole originated from cross contamination of the oil during steam distillation.

2.2.12 'Gorgor' (leaves)

The oil of Gorgor was found to contain α-pinene (37%), β-pinene (22%), β-farnesene (8.8%), caryophyllene oxide (0.9%), dillapiole (1.2%) and methyl salicylate (0.9%). It is suspected that caryophyllene oxide, dillapiole and methyl salicylate originated from cross contamination of the oil during steam distillation. Structure characterisation of α-pinene has been discussed under 2.2.5 unidentified species (leaves) and caryophyllene oxide under 2.2.6 *Euodia sp.* (leaves).

β-Pinene

The ^{1}H NMR spectrum of β-pinene revealed the presence of two methyl groups (δ0.71, 1.23) and a terminal methylene group (δ4.55, 4.62). Signals for eight protons were also

observed at δ1.42, 1.82, 1.83, 2.07, 2.24, 2.44, 2.45 and 2.53. The ^{13}C NMR and DEPT experiments confirmed the presence of two methyl groups (21.8, 25.6 ppm), one terminal alkene (116.0, 144.5 ppm), three CH_2 groups (23.5, 23.5, 26.9 ppm), a saturated quaternary carbon (40.4 ppm) and two CH groups (40.6, 51.4 ppm).

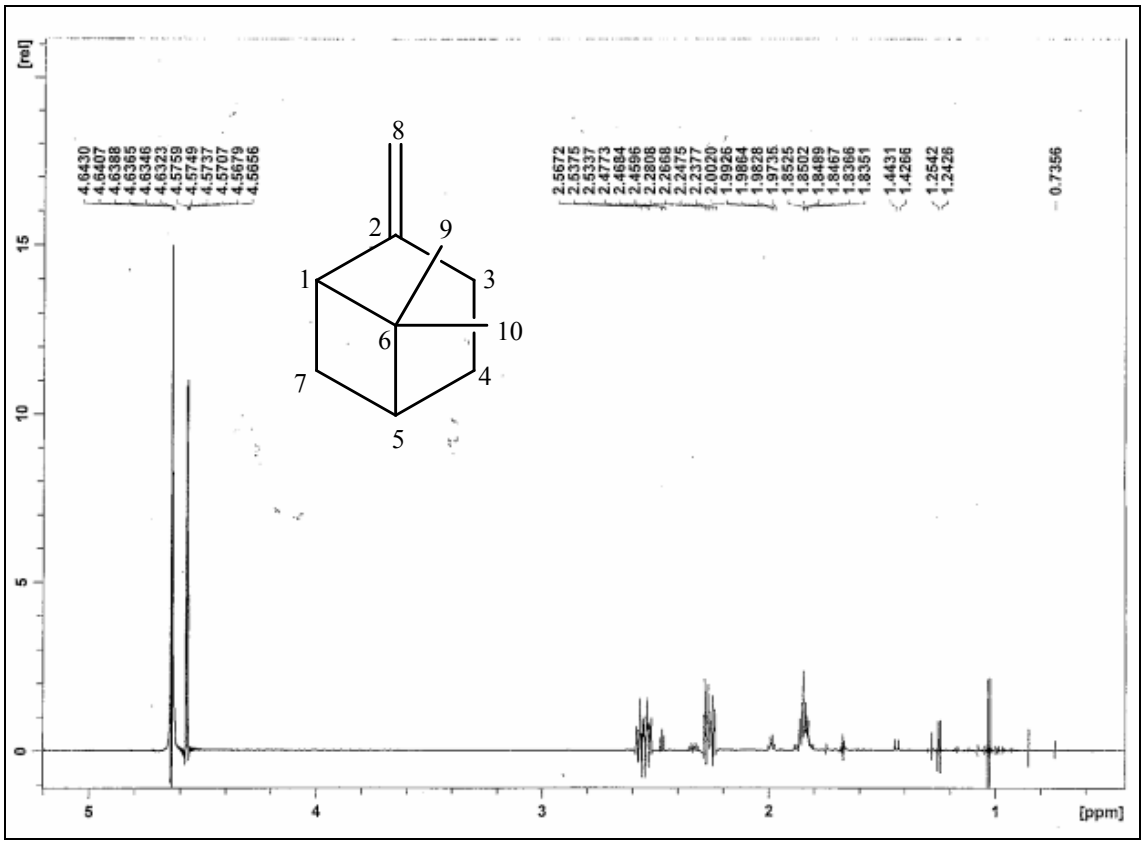


Figure 18. Gradient selective TOCSY (600 MHz) from excitation centered on δ4.62 of β-pinene in $CDCl_3$

HMBC data showed correlations between the terminal methylene group H_2-8 (δ4.55 and 4.62) and C-1 (51.4 ppm), C-2 (152.1 ppm) and C-3 (23.5 ppm). Correlations were also observed between H-1 (δ2.44) and C-2 and C-8 (23.5 ppm) and between H-3b (δ2.53) and C-2. Therefore it was confirmed that the methylene group C-8 was attached to C-2 which was connected with C-1 and C-3 on either side. Correlations between both

methyl groups H_3-9 (δ0.71) and H_3-10 (δ1.23) and C-1, C-5 (40.6 ppm), C-6 (40.4 ppm), and CH_3-10 (25.6 ppm) and CH_3-9 (21.8 ppm) respectively proved that CH_3-9 and CH_3-10 were both attached to C-6 which was linked to C-1 and C-5 on either side. Further correlations between H-3b and C-4 (23.5 ppm), between H_2-4 (δ1.82 and 1.83) and C-2 and C-6 respectively indicated that C-4 was located in between C-3 and C-5. Correlations between H_2-7 (δ1.42 and 2.45) and C-2, C-5 and C-6 and between both H-1 and H-5 (δ2.07) and C-7 indicated that C-7 was located in between C-1 and C-5. The NMR data obtained were consistent with literature values[184,187] for β-pinene.

β-Pinene			
C #	δ^{13}C	δ^{1}H mult. *J* (Hz)	HMBC ^{1}H to ^{13}C
1	51.4	2.44 m	H_2-8, H_3-9, H_3-10
2	152.1		H-1, H-3b, H-4a, H_2-7, H_2-8
3	23.5	2.24 m	H_2-8
		2.53 m	
4	23.5	1.82 m	H-3b
		1.83 m	
5	40.6	2.07 m	H_2-7, H_3-9, H_3-10
6	40.4		H-4b, H_2-7, H_3-9, H_3-10
7	26.9	1.42 m	H-1, H-5
		2.45 m	
8	105.9	4.55 br s	H-1
		4.62 br s	
9	21.8	0.71 s	H_3-10
10	25.6	1.23 s	H_3-9

Table 19. ^{1}H NMR (600 MHz), ^{13}C NMR (150 MHz) and HMBC data of β-pinene in $CDCl_3$

β-Farnesene

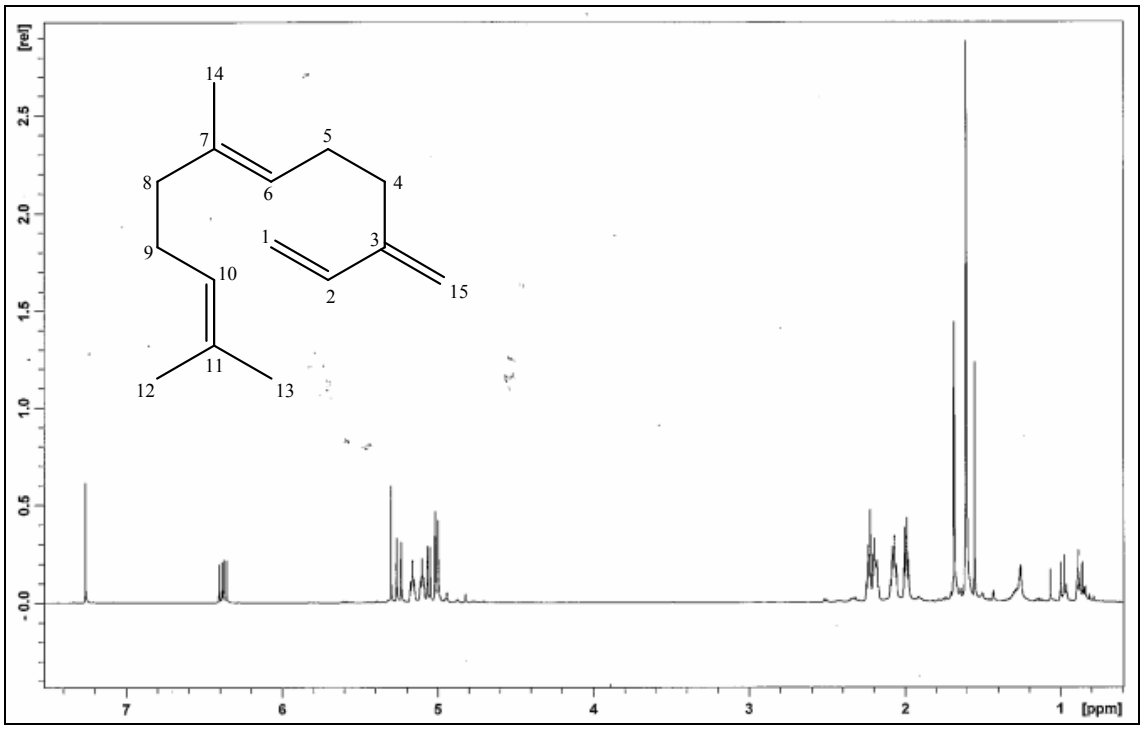


Figure 19. ^{1}H NMR spectrum (600 MHz) of β-farnesene (isolated by column chromatography) in $CDCl_3$ (reference: residual peak solvent at δ7.26)

The ^{1}H NMR spectrum of β-farnesene contained one large singlet at δ1.61 which accounted for two methyl groups and another methyl singlet was observed at δ1.69. A pair of methylene singlets were observed at δ5.00 and 5.02. Two doublets at δ5.06 (*J*=10.8 Hz) and 5.25 (*J*=17.6 Hz), together with the doublet of doublets at δ6.37 (*J*=17.6, 10.8 Hz) accounted for a monosubstituted terminal alkene conjugated with the previously mentioned methylene group. In addition, two olefinic protons at δ5.11 and 5.17 were observed. Resonances were also observed from eight other protons giving signals between δ2.0 and 2.3. The ^{13}C NMR spectrum of β-farnesene accounted for fifteen carbon atoms: three methyl groups (15.9, 17.6, 25.7 ppm), four alkenes (113.0, 115.7, 124.2, 124.3, 131.7, 135.8, 138.8, 146.4 ppm) and the remaining four carbons

(26.5, 26.9, 31.3, 39.6 ppm) were assigned as CH_2 groups by HSQC data.

The terminal alkene carbon signal at 113.8 ppm (C-1) assigned with protons at δ5.06 and 5.25 (H_2-1) were allocated as an alkene together with C-2 (138.8 ppm) through ^1H couplings. The proton H-2 at δ6.37 which was assigned on C-2 showed HMBC correlations to C-3 (146.4 ppm) and C-4 (31.3 ppm). In addition, the terminal methylene protons H_2-15 (δ5.00 and 5.02) which were assigned on C-15 (115.7 ppm) showed correlations to C-2, C-3 and C-4. Correlations were also observed between the CH_2 group protons at δ2.24 (H_2-4) and C-2, C-3, C-5 (26.9 ppm), C-6 (124.2 ppm) and C-15, and between one of the H-5 protons (δ2.19) and C-4, C-6 and C-7 (135.8 ppm). Thus the data indicated that the carbons C-1 to C-7 were connected in a chain. Further correlations between the methyl group H_3-14 (δ1.61) and C-6, C-7 and C-8 (39.6 ppm), between the olefinic proton H-6 (δ5.17) and C-4, C-8 and CH_3-14 and between one of the H-8 protons (δ1.99) and C-6, C-7 and CH_3-14 indicated that the methyl group CH_3-14 was attached to the alkene carbon C-7 with C-8 linked next to it. In addition, correlations between the two methyl groups at δ1.61 (H_3-12) and 1.69 (H_3-13) and C-10 (124.3 ppm) and C-11 (131.7 ppm) suggested that those methyl groups were both attached at the C-11 position. This assignment was confirmed by the correlations observed between the olefinic proton H-10 at δ5.11 and CH_3-12 and CH_3-13 and between one of the H-9 protons at δ2.08 and C-10 and C-11. Additional correlations between H-8a (δ1.99) and C-9 (26.5 ppm) and between H-9a (δ2.08) and C-7 and C-8 indicated that C-9 was located in between C-8 and C-10.

The *cis* and *trans* positions of the protons on C-1 with respect to the proton H-2 were

determined by their coupling constants. The proton at δ5.06 has a coupling constant of 10.8 Hz and is in a *cis* position with respect to H-2 whereas the other proton at δ5.25 shows a coupling constant of 17.6 Hz and is in a *trans* position relative to H-2. Comparison of the NMR data with literature values[188] confirmed the identity of β-farnesene.

β-Farnesene			
C #	δ^{13}C	δ^{1}H mult. *J* (Hz)	HMBC ^{1}H to ^{13}C
1	113.0	5.06 d 10.8	
		5.25 d 17.6	
2	138.8	6.37 dd 17.6, 10.8	H_2-4, H_2-15
3	146.4		H-2, H_2-4, H_2-15
4	31.3	2.24 m	H-2, H-5b, H-6, H_2-15
		2.24 m	
5	26.9	2.00 m	H_2-4
		2.19 m	
6	124.2	5.17 m	H_2-4, H-5b, H-8a, H_3-14
7	135.8		H-5b, H-8a, H-9a, H_3-14
8	39.6	1.99 m	H-6, H-9a, H-10, H_3-14
		2.08 m	
9	26.5	2.08 m	H-8a
		2.23 m	
10	124.3	5.11 m	H-9a, H_3-12, H_3-13
11	131.7		H-9a, H_3-12, H_3-13
12	25.7	1.69 s	H-10, H_3-13
13	17.6	1.61 s	H-10
14	15.9	1.61 s	H-6, H-8a
15	115.7	5.00 s	H_2-4
		5.02 s	

Table 20. ^{1}H NMR (600 MHz), ^{13}C NMR (150 MHz) and HMBC data of β-farnesene in $CDCl_3$

2.2.13 'Hane grass' (leaves)

The main metabolite in 'Hane grass' oil was *trans*-anethole (74%).

trans-Anethole

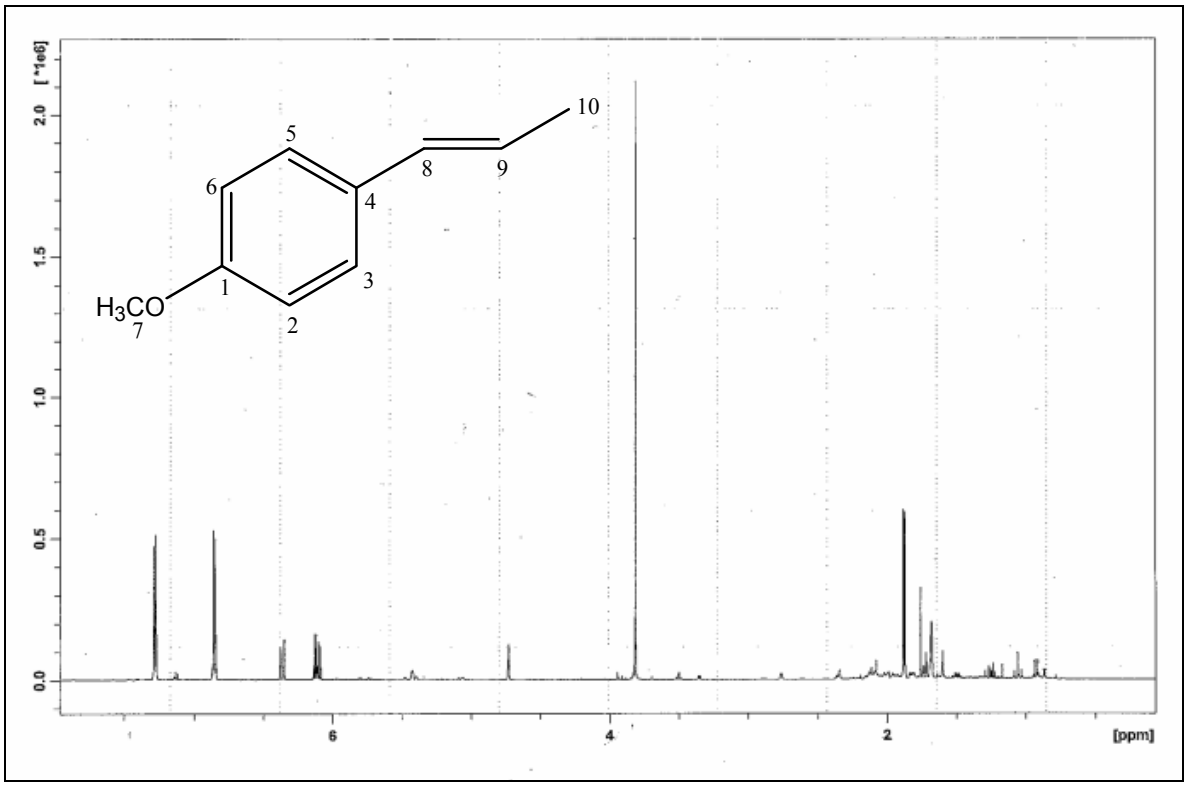


Figure 20. ^{1}H NMR spectrum (600 MHz) of 'Hane grass' leaf oil in $CDCl_3$ (reference: residual peak solvent at δ7.26)

The ^{1}H NMR spectrum of *trans*-anethole exhibited a methyl double doublet at δ1.86 (*J* = 6.7, 1.6 Hz) and a methoxy singlet at δ3.80, two *trans* coupled olefinic protons (δ6.10, dq, *J*=15.7, 6.7 Hz and δ6.36, dq, *J*=15.7, 1.6 Hz) and four protons (two doublets, each 2H) in the aromatic region (δ6.84 and 7.28, *J* = 8.6 Hz). The ^{13}C NMR spectrum of *trans*-anethole revealed signals for ten carbons, one of which was assigned to a methyl

group (18.2 ppm), one to a methoxy group (55.1 ppm), two to an alkene (123.3, 130.1 ppm) and six to a benzene ring that displayed two sets of equivalent protonated carbons (113.7, 126.6, 130.2, 158.1 ppm), and hence was *para*-disubstituted.

trans-Anethole			
C #	$\delta^{13}C$	$\delta^{1}H$ mult. *J* (Hz)	HMBC ^{1}H to ^{13}C
1	158.5		H-3/H-5, H-2/H-6, H_3-7
2, 6	113.9	6.84 d 8.6	H-3/H-5, H-6/H-2
3, 5	126.9	7.28 d 8.6	H-5/H-3, H-8
4	130.8		H-2/H-6
7	55.0	3.80 s	
8	130.3	6.36 dq 15.7, 1.6	H-3/H-5, H-9, H_3-10
9	123.5	6.10 dq 15.7, 6.7	H_3-10
10	18.4	1.86 dd 6.7, 1.6	H-8, H-9

Table 21. ^{1}H NMR (600 MHz), ^{13}C NMR (150 MHz) and HMBC data of *trans*-anethole in $CDCl_3$

An HMBC correlation between the methoxyl group H_3-7 at δ3.80 and C-1 (158.1 ppm) on the benzene ring indicated that the methoxyl group was attached at the C-1 position. Correlations between the methyl group H_3-10 (δ1.86) and the alkene carbons C-8 (130.1 ppm) and C-9 (123.3 ppm), between H-9 (δ6.10) and C-8 and CH_3-10 (18.2 ppm), and between H-8 (δ6.36) and CH_3-10 indicated that the methyl group was attached to one of the alkene carbon atoms while the aromatic ring was attached to the other in a *trans* arrangement. The olefinic side chain was attached at the C-4 position (130.2 ppm) with C-8 since the olefinic proton at δ6.36 (H-8) correlated with C-3/C-5 (126.6 ppm) in the HMBC spectrum. A correlation between H-3/H-5 (δ7.28) and C-8 was also observed. Further correlations between H-2 (δ6.84) and C-1, C-6 (113.7 ppm) and C-4 (and

between H-6 and C-1, C-2 and C-4) and between H-3 (δ7.28) and C-1, C-2 and C-5 (and between H-5 and C-1, C-6 and C-3) allowed full assignment of the benzene ring and also confirmed that the methoxy group and the olefinic side chain were substituted in the *para*-position. The NMR data obtained were consistent with literature values[189] for *trans*-anethole.

2.2.14 *Dacrydium spp.* (leaves)

The oil of *Dacrydium spp.* was found to contain α-pinene (32%) and *trans*-anethole (3.7%). It is suspected that *trans*-anethole originated from cross contamination of the oil during steam distillation. Structure characterisation of α-pinene has been discussed under 2.2.5 unidentified species (leaves).

2.2.15 *Eucalyptus sp.* (leaves)

The main metabolite in *Eucalyptus sp.* oil was α-pinene (45%). Structure characterisation of α-pinene has been discussed under 2.2.5 unidentified species (leaves).

2.2.16 *Eucalyptus tereticornis* (leaves)

The main metabolite in *Eucalyptus tereticornis* oil was α-pinene (53%). Structure characterisation of α-pinene has been discussed under 2.2.5 unidentified species (leaves).

2.2.17 *Eucalyptus grandis* (leaves)

The main metabolite in *Eucalyptus grandis* oil was α-pinene (66%). Structure characterisation of α-pinene has been discussed under 2.2.5 unidentified species (leaves).

2.2.18 *Melaleuca leucadendron* (leaves)

The main metabolite in *Melaleuca leucadendron* oil was 1,8-cineole (60%).

1,8-Cineole

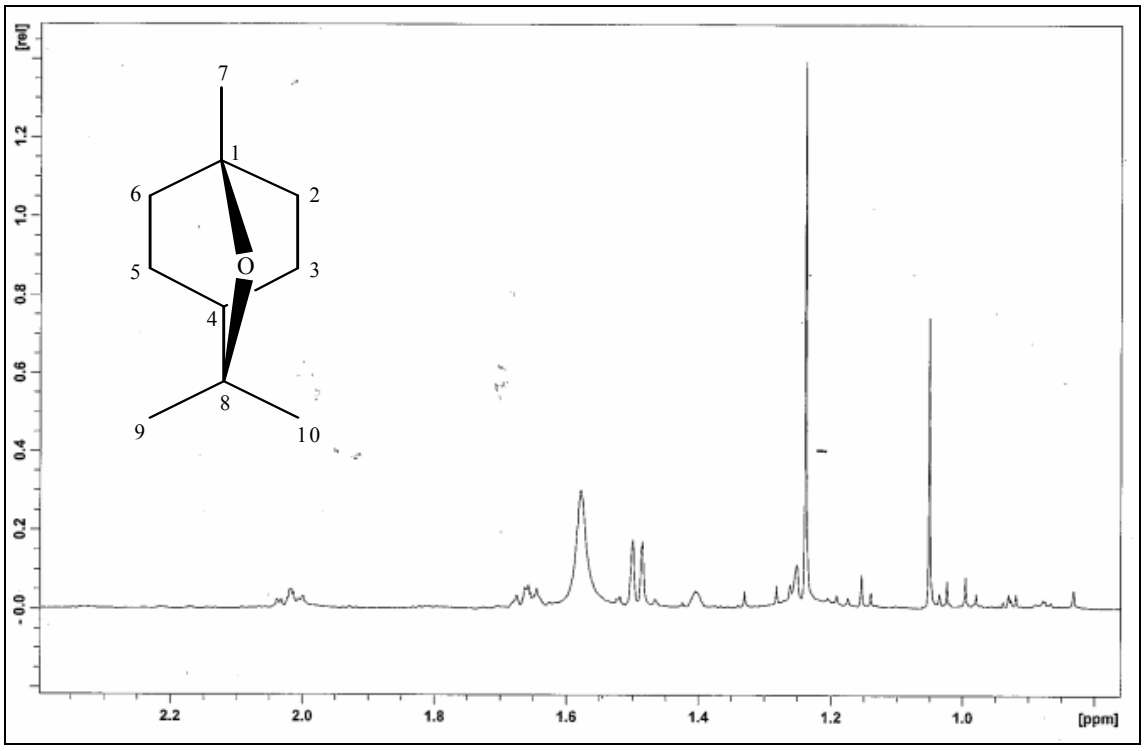


Figure 21. ^{1}H NMR spectrum (600 MHz) of *Melaleuca leucadendron* leaf oil in $CDCl_3$

The ^{1}H NMR spectrum of 1,8-cineole revealed a methyl singlet at δ1.05 and a large methyl singlet at δ1.24 for two equivalent methyl groups. It also afforded proton signals at δ1.40, 1.49, 1.50, 1.66 and 2.02. The ^{13}C NMR spectrum of 1,8-cineole confirmed three methyl groups (27.4 ppm and two for 28.9 ppm) and indicated the presence of two oxygenated carbons (70.0, 73.5 ppm). The presence of four CH_2 groups (22.7 x 2 and 31.3 x 2 ppm) and one CH group (32.8 ppm) and the assignments of protons were confirmed by an HSQC experiment.

1,8-Cineole			
C #	δ^{13}C	δ^{1}H mult. *J* (Hz)	HMBC ^{1}H to ^{13}C
1	70.0		H_3-7, H-2b/H-6b
2, 6	31.3	1.50 t 11.6	H_3-7, H_2-6/H_2-2, H-3a/H-5a
		1.66 m	
3, 5	22.7	1.49 t 11.6	H-5b/H-3b
		2.02 m	
4	32.8	1.40 m	H_3-9/H_3-10
7	27.4	1.05 s	
8	73.5		H-3a/H-5a, H_3-9/H_3-10
9, 10	28.9	1.24 s	H_3-10/H_3-9 respectively

Table 22. ^{1}H NMR(600 MHz), ^{13}C NMR (150 MHz) and HMBC data of 1,8-cineole in $CDCl_3$

HMBC correlations between H_3-9/H_3-10 (δ1.24) and C-4 (32.8 ppm), the oxygenated carbon C-8 (73.5 ppm), and CH_3-10 and CH_3-9 (28.9 ppm) respectively, confirmed that the two methyl groups CH_3-9 and CH_3-10 were both attached to the oxygenated carbon C-8 which was linked to C-4. Correlations between H-3a (δ1.49) and C-2 (31.3 ppm) and C-8, and between H-3b (δ2.02) and C-5 (22.7 ppm) suggested that C-3/C-5 was

linked to the CH group C-4 and the methylene group C-2/C-6. Correlations between H-2b (δ1.66) and the other oxygenated carbon C-1 (70.0 ppm) and C-6, and between H-2a (δ1.50) and C-6, together with correlations between the methyl group H_3-7 (δ1.05) and C-1 and C-2/C-6 suggested that C-3/C-5 were linked to the oxygenated carbon C-1 which was next to C-2/C-6. Those data also confirmed that C-1 and C-8 were linked by an ether bridge. Those data also indicated that carbons from C-1 to C-6 formed a six-membered ring, and that the structure was that of 1,8-cineole. Comparison of the NMR data with literature values[177,190] confirmed the identity of 1,8-cineole.

2.2.19 *Euroschinus falcatus* (leaves)

The oil of *Euroschinus falcatus* was found to contain α-pinene (33%), γ-terpinene (16%), limonene (11%), *p*-cymene (7.9%), terpinolene (4.3%), β-pinene (3.9%), myrcene (3.9%), viridiflorol (1.6%) and spathulenol (1.6%). Structure characterisation of spathulenol has been discussed under 2.2.1 *Tithonia diversifolia* (leaves), α-pinene and *p*-cymene under 2.2.5 unidentified species (leaves), viridiflorol under 2.2.7 *Cymbopogon citratus* (leaves), β-pinene under 2.2.12 'Gorgor' (leaves).

γ-Terpinene

The ^{1}H NMR spectrum of γ-terpinene exhibited one methyl singlet at δ1.68, two equivalent methyl doublets at δ1.03 and two olefinic protons both at δ5.45. The ^{13}C NMR spectrum of γ-terpinene revealed the presence of three methyl groups (21.6 x 2, 23.2 ppm) and two alkenes (116.6, 119.0, 131.3 and 140.9 ppm). Signals for two methylene at 27.5 and 31.8 ppm and a methine at 33.4 ppm were also observed in the

DEPT spectrum.

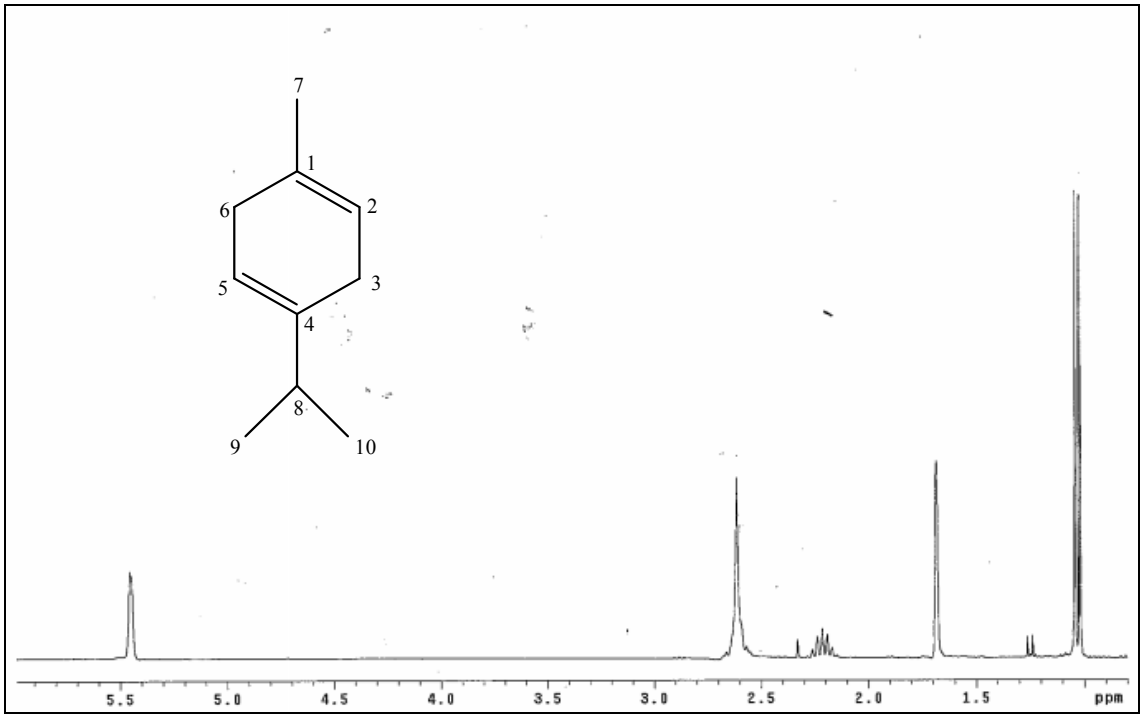


Figure 22. ^{1}H NMR spectrum (300 MHz) of γ-terpinene in $CDCl_3$

HMBC correlations observed between the methyl group H_3-7 (δ1.69) and C-1 (131.3 ppm), C-2 (119.0 ppm) and C-6 (31.8 ppm) as well as between H-2 (δ5.45) and CH_3-7 (23.2 ppm) indicated that the methyl group CH_3-7 was attached to the alkene carbon C-1 which was connected to C-2 and C-6 on either side. In addition, correlations between the two methyl groups H_3-9 and H_3-10 both at δ1.03 and C-8 (34.6 ppm), and CH_3-10 and CH_3-9 (24.0 ppm, ^{3}J couplings H-9 to CH_3-10 and H-10 to CH_3-9) respectively, clearly indicated that C-8, CH_3-9 and CH_3-10 formed an isopropyl group. Correlations between H-8 (δ2.21) and C-4 and C-5 (140.9 and 116.6 ppm respectively) and between H-5 (δ5.45) and C-8 suggested that the isopropyl group was attached to

C-4, which was next to C-5. Further correlations between H_2-3 (δ2.60) and C-1, C-2, C-4 and C-5, between H-2 (δ5.45) and C-4 and between H-5 and C-1, C-3 and C-6 allowed full assignment of the ring formed by C-1 to C-6. COSY data revealed correlations between H-2 and H_2-3, between H_2-6 and H-5, H-8 and between H_3-7 and H-2, H_2-3. The ^{13}C chemical shifts matched the literature[175] and the ^{1}H chemical shifts were consistent with those of authentic γ-terpinene.

γ-Terpinene			
C #	δ^{13}C	δ^{1}H mult. *J* (Hz)	HMBC ^{1}H to ^{13}C
1	131.3		H_2-3, H-5, H_3-7
2	119.0	5.45 br s	H_2-3, H_3-7
3	27.5	2.60 m	H-5
4	140.9		H-2, H_2-3, H-8, H_3-9/H_3-10
5	116.6	5.45 br s	H_2-3, H-8
6	31.8	2.60 m	H-5, H_3-7
7	23.2	1.69 br s	H-2
8	34.6	2.21 m	H-5, H_3-9/H_3-10
9,10	21.6	1.03 d	H-8, H_3-10/H_3-9 respectively

Table 23. ^{1}H NMR (300 MHz), ^{13}C NMR (75 MHz) and HMBC data of γ-terpinene in $CDCl_3$

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Limonene

The ^{1}H NMR spectrum of limonene revealed the presence of two methyl groups on double bonds (δ1.68, 1.75), one terminal methylene group (both protons at δ4.72) and one olefinic proton (δ5.41). The ^{13}C NMR and DEPT experiments showed the presence of two methyl groups (20.8, 23.2 ppm), two alkenes (108.5, 120.6, 133.8, 150.4 ppm), three CH_2 groups (28.0, 30.8, 30.8 ppm) and a methine at 41.4 ppm.

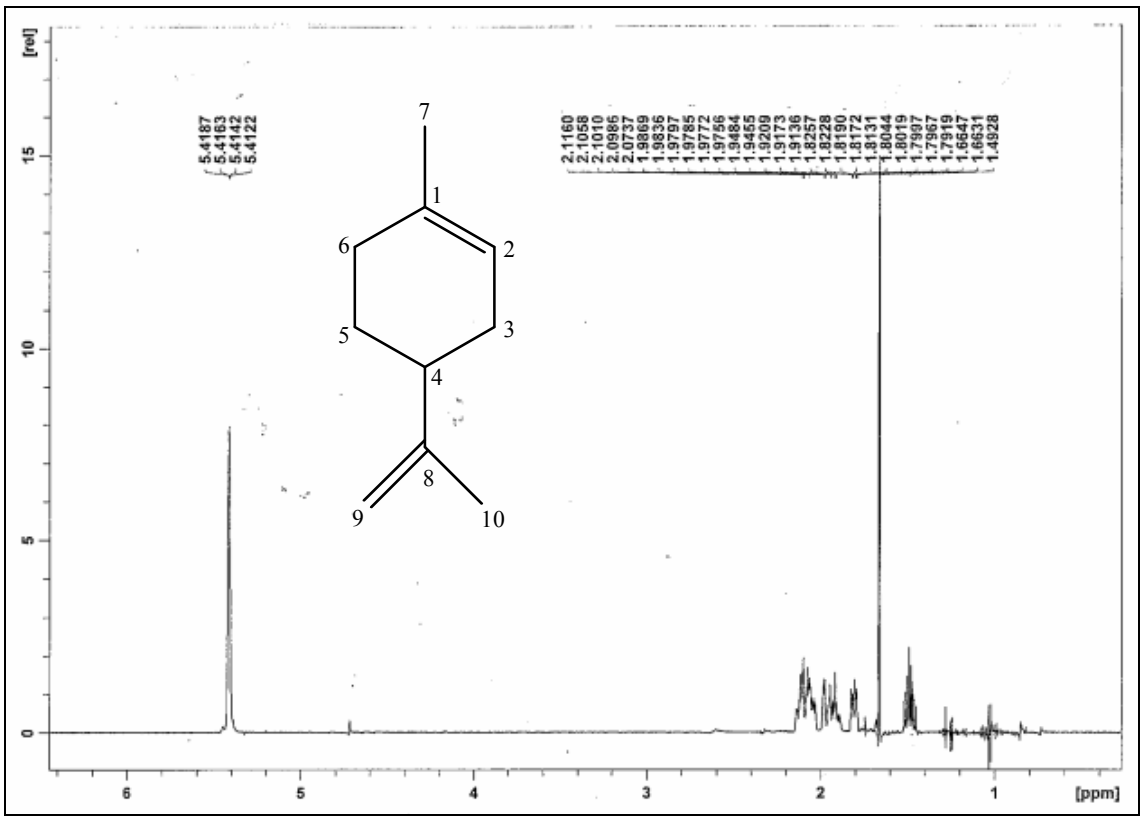

Figure 23. Gradient selective TOCSY (600 MHz) from excitation centered on δ5.41 of limonene in $CDCl_3$

HMBC correlations between H_3-7 (δ1.68) and one of the alkene carbons C-2 (120.6 ppm) and C-6 (30.8 ppm) were observed. Correlations between the methyl group H_3-10 (δ1.75) and the methylene group H_2-9 (δ4.72) and C-8 (150.4 ppm), C-4 (41.4 ppm), and C-9 (108.5 ppm) and CH_3-10 (20.8 ppm) respectively, clearly indicated the presence of an isopropenyl group attached to C-4. The olefinic proton H-2 at δ5.41 showed correlations to C-3 (30.8 ppm), C-4 and CH_3-7 (23.2 ppm). Further correlations were observed from H_2-3 (δ2.07 and 1.95) and C-4 and between H_2-5 (δ1.49 and 1.81) and C-4 and C-6. Together with COSY correlations between H-2 and H_3-7, between H-2 and H_2-3, these data indicated that C-1 to C-6 were cyclised, and that CH_3-7 was attached at C-1 position, and confirmed the structure as limonene. The NMR data

obtained were consistent with literature values[184,191] for limonene.

Limonene			
C #	δ^{13}C	δ^{1}H mult. *J* (Hz)	HMBC ^{1}H to ^{13}C
1	133.8		
2	120.6	5.41 m	H_3-7
3	30.8	1.95 m	H-2
		2.07 m	
4	41.4	2.11 m	H-2, H_2-3, H_2-5, H_2-9, H_3-10
5	28.0	1.49 m	
		1.81 m	
6	30.8	1.95 m	H_2-5, H_3-7
		2.07 m	
7	23.2	1.68 s	H-2
8	150.4		H_2-9, H_3-10
9	108.5	4.72 m	H_3-10
10	20.8	1.75 s	H_2-9

Table 24. ^{1}H NMR (600 MHz), ^{13}C NMR (150 MHz) and HMBC data of limonene in $CDCl_3$

Terpinolene

The ^{1}H NMR spectrum of terpinolene revealed the presence of three allylic methyl groups (δ1.66, 1.67, 1.71) and an olefinic proton (δ5.38). The ^{13}C NMR spectrum of terpinolene accounted for ten carbon atoms: three methyl groups (19.7, 20.3, 23.2 ppm), two alkenes (120.9, 121.9, 127.7, 133.9 ppm) and the three remaining carbons were assigned as CH_2 groups (26.8, 29.5, 31.5 ppm) from DEPT data.

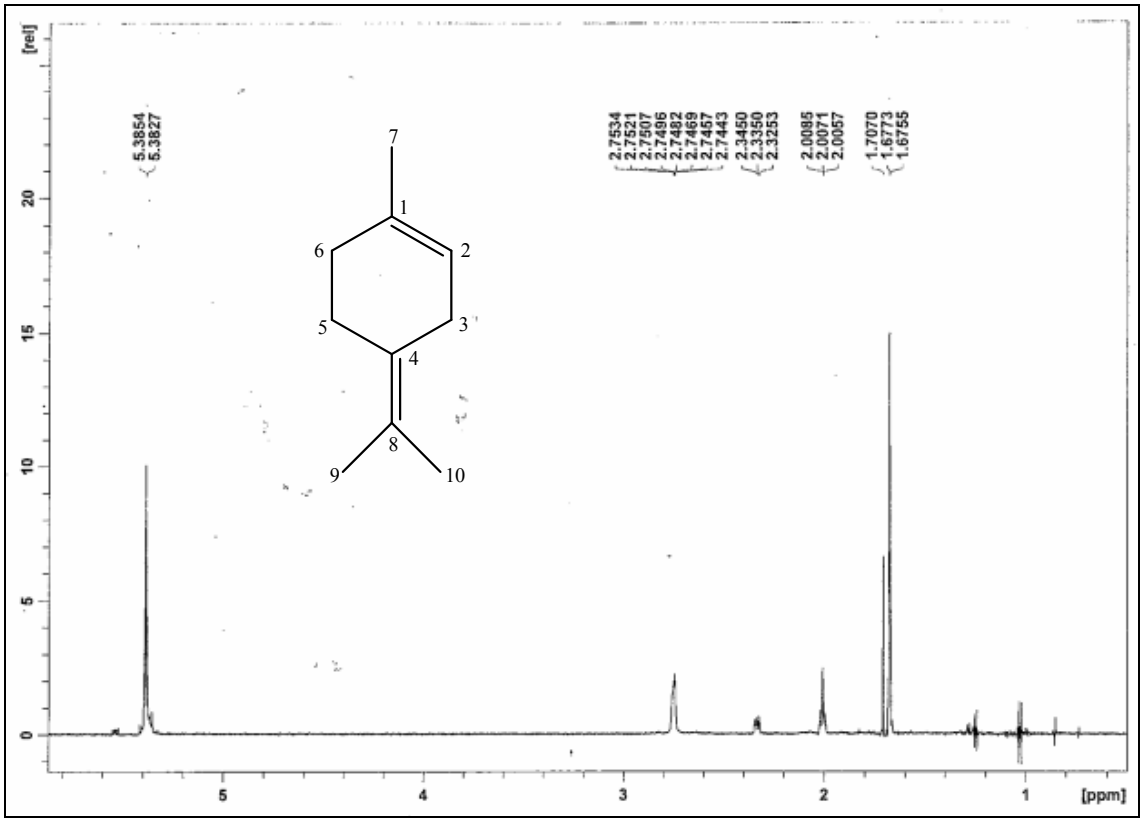

Figure 24. Gradient selective TOCSY (600 MHz) from excitation centered on δ5.38 of terpinolene in $CDCl_3$

An HMBC spectrum showed correlations between H_3-7 (δ1.66) and C-1 (133.9 ppm), C-2 (120.9 ppm) and C-6 (31.5 ppm). As C-1 was a quaternary carbon shown in the DEPT spectrum, it indicated that the methyl group CH_3-7 (23.2 ppm) was attached to C-1 with C-2 and C-6 on either side. This was confirmed by the correlation between the olefinic proton H-2 (δ5.38) and H_2-6 (δ2.00) and CH_3-7. Further HMBC correlations between H-2 and C-4 (127.7 ppm) and C-6, between H_2-3 (δ2.74) and C-2, C-4 and C-5 (26.8 ppm), between H_2-5 (δ2.33) and C-1, C-3 (29.5 ppm), C-4 and C-6 and between H_2-6 and C-1, C-2, C-4 and C-5 confirmed the carbon linkages from C-1 to C-6. Additional correlations between the two methyl groups H_3-9 (δ1.66) and H_3-10 (δ1.71) and C-4, C-8 (121.9 ppm), and CH_3-10 (19.7 ppm) and CH_3-9 (20.3 ppm) respectively

and between H_2-5 and C-8 indicated that the methyl groups were attached to C-8 which was further connected with C-4. Thus, the NMR data established the structure as terpinolene. The NMR data obtained were consistent with literature values[175,192] for terpinolene.

Terpinolene			
C #	δ^{13}C	δ^{1}H mult. *J* (Hz)	HMBC ^{1}H to ^{13}C
1	133.9		H_2-5, H_2-6, H_3-7
2	120.9	5.38 tq 3.8, 1.4	H_2-3, H_2-6, H_3-7
3	29.5	2.74 m	H_2-5
4	127.7		H-2, H_2-3, H_2-5, H_2-6, H_3-9, H_3-10
5	26.8	2.33 t 6.3	H_2-3, H_2-6
6	31.5	2.00 t 6.3	H-2, H_2-5, H_3-7
7	23.2	1.66 s	H-2, H_2-6
8	121.9		H_2-5, H_3-9, H_3-10
9	20.3	1.67 s	H_3-10
10	19.7	1.71 s	H_3-9

Table 25. ^{1}H NMR (600 MHz), ^{13}C NMR (150 MHz) and HMBC data of terpinolene in $CDCl_3$

Myrcene

The ^{1}H NMR spectrum of myrcene revealed the presence of two methyl groups on double bonds (δ1.68, 1.71), two terminal methylene groups (δ5.01, 5.02, 5.06, 5.25) and two olefinic protons (δ5.32, 6.38). It also afforded signals for four protons (2 x 2H) at δ2.23 and 2.33. The ^{13}C NMR spectrum of myrcene accounted for ten carbon atoms: two methyl groups (17.7, 25.8 ppm), three alkenes (113.1, 115.6, 124.5, 132.0, 139.0, 146.3 ppm). The remaining two carbons at 27.2 and 31.7 ppm were assigned as CH_2 groups from DEPT data.

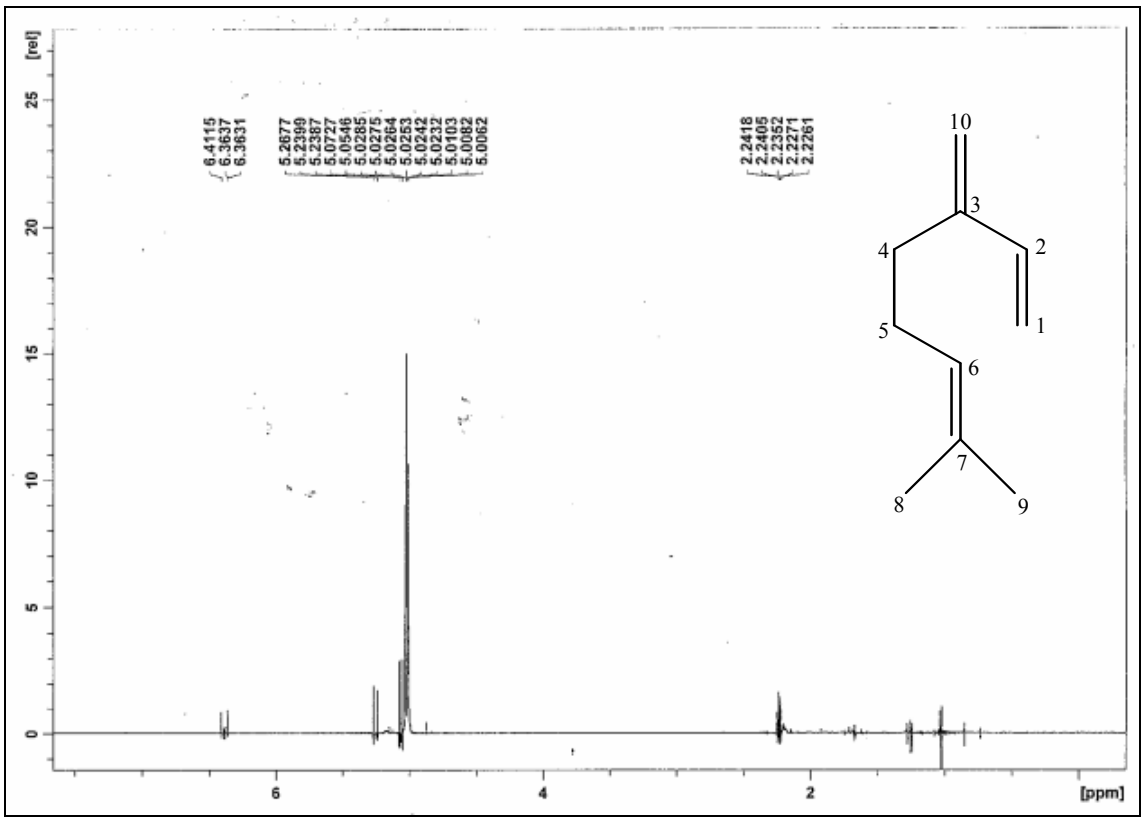

Figure 25. Gradient selective TOCSY (600 MHz) from excitation centered on δ5.02 of myrcene in $CDCl_3$

HMBC correlations between the two methyl groups H_3-8 (δ1.63) and H_3-9 (δ1.71) and the alkene carbons C-7 (132.0 ppm) and C-6 (124.5 ppm), and CH_3-9 (25.8 ppm) and CH_3-8 (17.7 ppm) respectively, clearly indicated that CH_3-8 and CH_3-9 were both attached to C-7 which linked to C-6, which carried an olefinic proton (H-6, δ5.32). In addition, the terminal methylene group protons H_2-1 (δ5.06 and 5.25) showed correlations to C-2 (139.0 ppm) bearing an olefinic proton (H-2, δ6.38) and C-3 (146.3 ppm). The olefinic proton H-2 exhibited correlations to C-4 (31.7 ppm) and the alkene carbons C-3 and C-10 (115.6 ppm). These data suggested that the carbons from C-1 to C-4 were linked consecutively with C-10 attached at the C-3 position. Gradient selective TOCSY experiments on two terminal methylene protons (δ5.02 and 5.06)

allowed identification of the chemical shifts for the protons of the CH_2 groups. These assignments were confirmed by the correlations observed between the terminal methylene group protons H_2-10 at δ5.01 and 5.02 and C-2 and C-4 together with COSY correlations between H-1 and H-2 and between H_2-4 and H_2-10. Further HMBC correlations from H-5 (δ2.33) to C-3 and C-7 and a COSY correlation between H_2-5 and H-6 indicated that C-5 (27.2 ppm) was located in between C-4 and C-6, and confirmed the structure as myrcene. The NMR data obtained were consistent with literature values[180,193] for myrcene.

Myrcene			
C #	δ^{13}C	δ^{1}H mult. *J* (Hz)	HMBC ^{1}H to ^{13}C
1	113.1	5.06 d 10.8	
		5.25 d 17.6	
2	139.0	6.38 dd 17.6, 10.8	H_2-1, H_2-10
3	146.3		H_2-1, H-2, H_2-5
4	31.7	2.23 m	H-2, H_2-10
5	27.2	2.33 m	
6	124.5	5.32 t 6.0	H_3-8, H_3-9
7	132.0		H_2-5, H_3-8, H_3-9
8	17.7	1.63 s	H_3-9
9	25.8	1.71 s	H_3-8
10	115.6	5.01 s	H-2
		5.02 s	

Table 26. ^{1}H NMR (600 MHz), ^{13}C NMR (150 MHz) and HMBC data of myrcene in $CDCl_3$

2.2.20 *Schinus terebinthifolius* (leaves)

The oil of *Schinus terebinthifolius* was found to contain α-pinene (32%), *p*-cymene (9.8%) and limonene (8.5%). Structure characterisation of α-pinene and *p*-cymene has been discussed under 2.2.5 unidentified species (leaves) and limonene under 2.2.19 *Euroschinus falcatus* (leaves).

2.2.21 *Stylosanthes hamata* (leaves)

The methanol extract *of Stylosanthes hamata* was found to contain a new diterpene ester, stylosanthenoic acid.

Stylosanthenoic acid (66)

Stylosanthenoic acid had a molecular formula $C_{26}H_{44}O_5$ at deduced by accurate ESI negative ion mass spectrometry and ^{13}C NMR data. The ^{1}H NMR spectrum of (**66**) contained four methyl singlets (δ0.74, 0.84, 0.87 and 1.39), one methyl doublet (δ0.92), and a broad methyl singlet at δ1.65, one olefinic proton at δ5.38, four one proton doublets at δ2.60, 2.69 (J = 15.8 Hz), δ2.64 and 2.70 (J = 15.5 Hz) and multiplet resonances from nineteen other protons between δ0.9 and 4.2. The ^{13}C NMR spectrum of (**66**) showed the presence of 26 carbons including; two carbonyl groups (172.4, 175.6 ppm), one alkene (122.4, 135.5 ppm) in addition to a primary and a tertiary oxygenated carbons at 63.8 and 69.9 ppm, respectively.

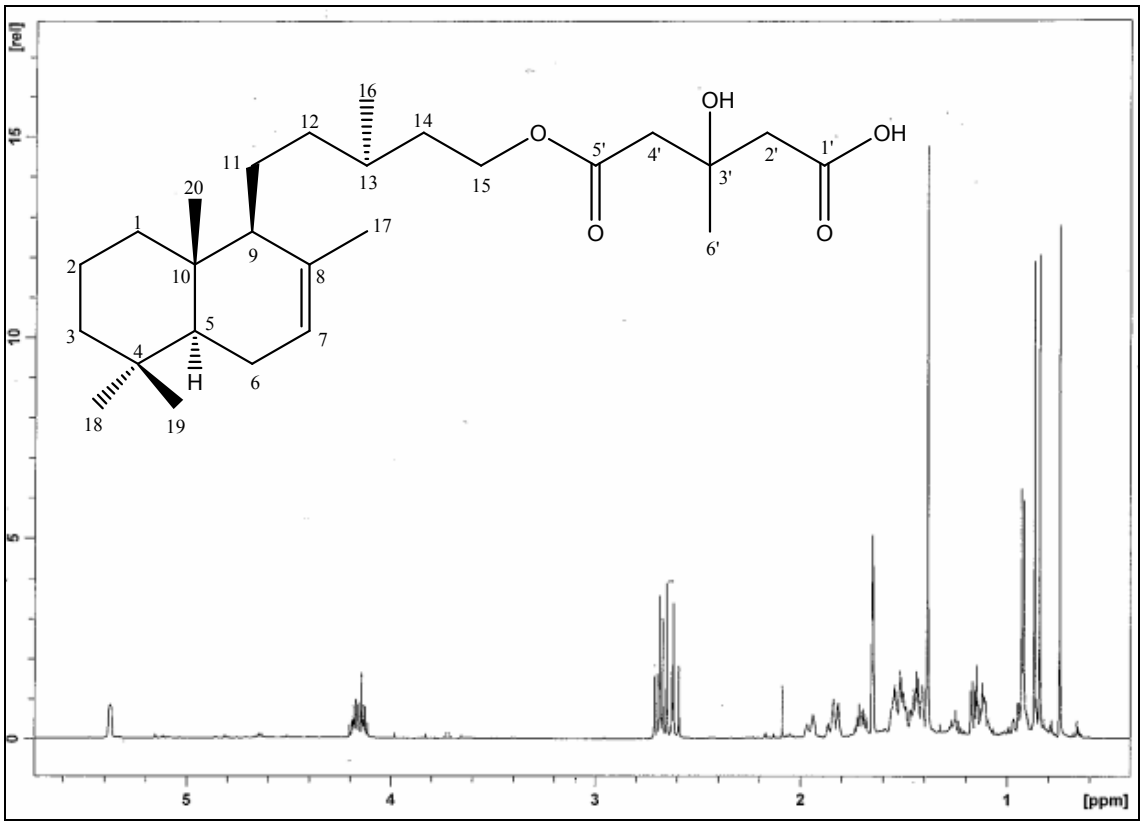

Figure 26. ^{1}H NMR spectrum (600 MHz) of (**66**) in $CDCl_3$

The HSQC spectrum of (**66**) confirmed the presence of six methyl groups (indicated by the ^{1}H NMR spectrum), and identified the presence of ten methylene groups, in addition to four CH groups. Therefore, there were six quaternary carbon atoms in the structure. HMBC correlations were observed between the methyl protons at δ1.39 (H_3-6') and carbons at 45.1 (C-2'), 69.9 (C-3') and 44.6 ppm (C-4'), between the methylene protons at δ2.64 and 2.70 (H_2-2') and C-3', C-4', the methyl group at 27.3 ppm (CH_3-6') and the carbonyl group at 172.4 ppm (C-1') and between the other methylene protons at δ2.61 and 2.69 (H_2-4') and C-2', C-3', CH_3-6' and the carbonyl group at 175.6 ppm (C-5'). These data indicated that C-1' to C-5' composed a 1,5-pentane dicarboxylate moiety with a methyl group and hydroxyl group attached to C-3' (see Figure 28a). HMBC correlations between the methylene protons at δ4.14 and 4.17 (H_2-15) and C-5'

indicated that the 3-hydroxy-3-methyl glutaric acid moiety was mono-esterified and joined to C-15 via oxygen.

Given that the original formula contained twenty-six carbon atoms, and six of these were represented by the 3-hydroxy-3-methyl glutaric ester moiety, a diterpene based structure was most probable for the remaining twenty carbon atoms that included five methyl groups.

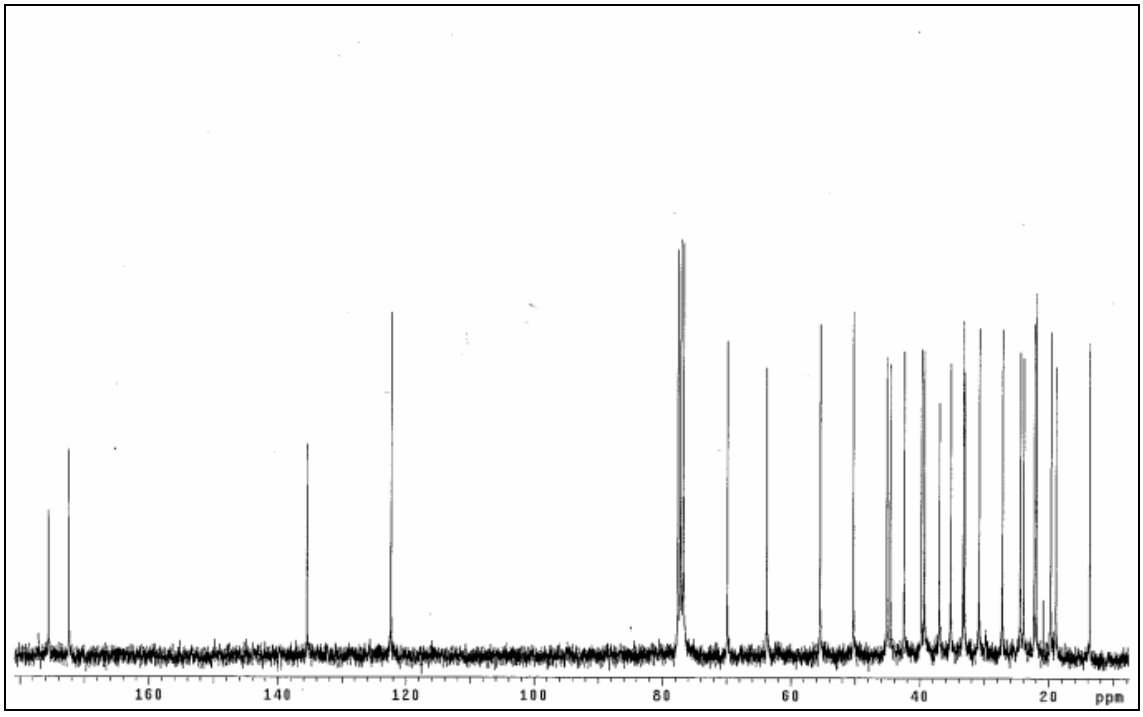

Figure 27. ^{13}C NMR spectrum (75 MHz) of (**66**) in $CDCl_3$ (reference: solvent peak at 77.2 ppm)

The two methyl singlets at δ0.84 (H_3-18) and δ0.87 (H_3-19) showed HMBC correlations to a methylene carbon at 42.5 ppm (C-3), a quaternary carbon at 33.1 ppm (C-4), a methine carbon at 50.3 ppm (C-5) to CH_3-19 (22.0 ppm) and CH_3-18 (33.3 ppm).

Further correlations between H-5 and H_2-3 (δ1.14, 1.39) and C-4, CH_3-18 and CH_3-19 confirmed the two methyl groups CH_3-18 and CH_3-19 were attached to C-4 which was bonded to C-3 and C-5. The methyl singlet at δ0.74 (H_3-20) correlated with the methylene carbon at 39.3 (C-1), C-5, C-9 (55.5 ppm) and the quaternary carbon at 37.0 ppm (C-10), which indicated C-10 was connected with C-1, C-5, C-9 and the methyl group CH_3-20 (13.7 ppm). In addition, correlations between the methyl doublet at δ1.65 (H_3-17) and the alkene carbons at 122.4 (C-7), 135.5 ppm (C-8) and the methine carbon C-9, as well as between δ1.55 (H-9) to the methyl carbon CH_3-17 (22.3 ppm) indicated that the methyl group CH_3-17 was bonded to C-8. Further, HMBC correlations between methylene protons at δ1.84 and δ1.94 (H_2-6) and C-5, C-7, C-8 and C-10, and between the olefinic proton at δ5.38 (H-7) and C-6 (24.0 ppm) and CH_3-17 together with correlations between one of the H-1 protons (δ1.84) and C-2 (18.9 ppm), and between one of the H-3 protons (δ1.39) and C-1 allowed the full assignment of a 6,6-bicyclic ring system to be completed (see Figure 28b). These assignments were supported by COSY correlations observed between H-1a and H_2-2 (δ1.45 and 1.51), H_2-2 and H_2-3, H-5 and H_2-6, H_2-6 and H-7, H-7 and H_3-17 and a long-range "W-coupling" between H-1b and H-9.

Additional HMBC correlations were observed between the methyl doublet at δ0.92 (H_3-16) and C-12 (39.8 ppm), C-13 (30.8 ppm) and C-14 (35.2 ppm) which indicated CH_3-16 (19.8 ppm) was bonded to C-13 where a proton at δ1.51 (H-13) had been assigned. COSY correlations between H-9 and H_2-11 (δ1.11 and 1.47), H_2-11 and H_2-12 (δ1.10 and 1.50), H_2-12 and H-13 (δ1.51), H-13 and H_2-14 (δ1.44 and 1.70) and H_3-16 as well as H_2-14 and H_2-15 were observed, positioning the secondary methyl group with

respect to the esterified primary alcohol. HMBC correlations between H-12a and C-9, between H_2-14 and C-12, C-13 and C-15 (63.8 ppm) and between H_2-15 and C-13 and C-14 confirmed the carbon chain from C-11 (24.5 ppm) to C-15, which was bonded via C-11 to C-9 (see Figure 28c). This led to the total planar structure of (**66**), shown in Figure 29.

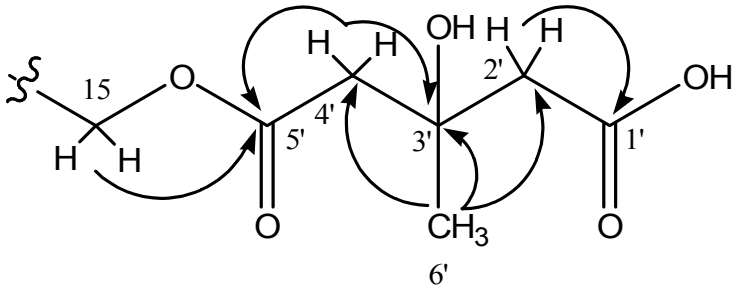

Figure 28a. Selective HMBC correlations for the 3-hydroxy-3-methyl glutarate monoester moiety of (**66**)

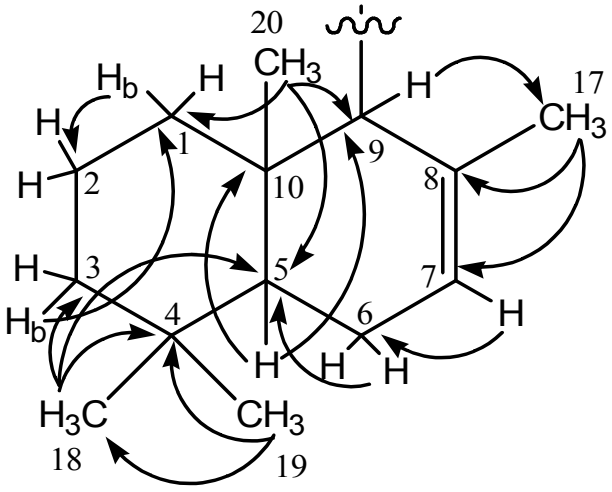

Figure 28b. Selective HMBC correlations for the 6,6-bicyclic ring system of (**66**)

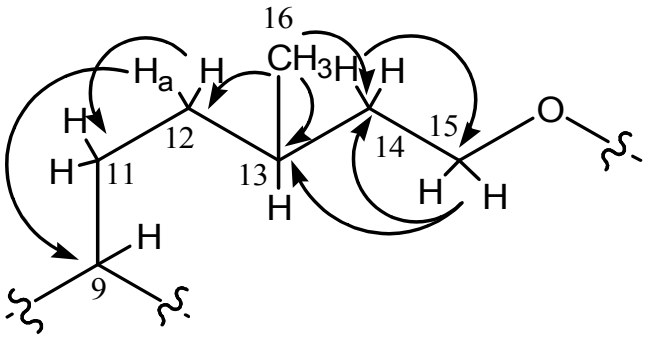

Figure 28c. Selective HMBC correlations for the side chain of (**66**)

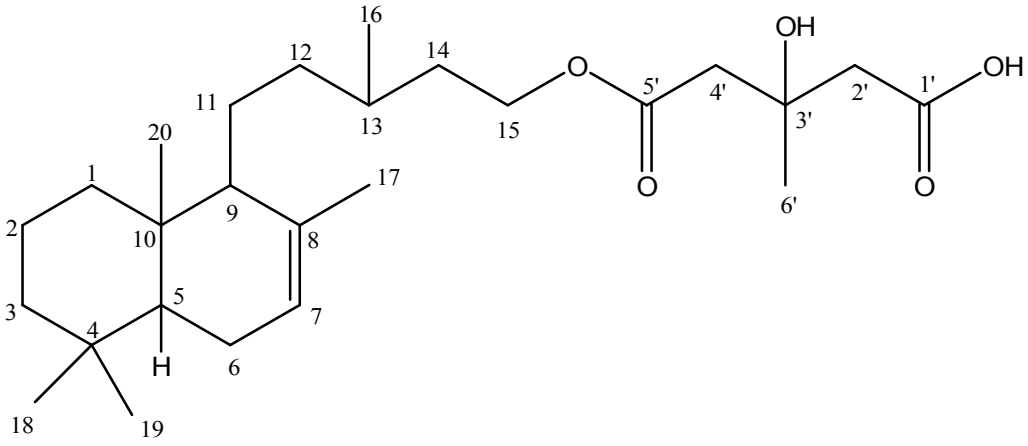

Figure 29. The planar structure of (**66**)

The IR spectrum of (**66**) in $CHCl_3$ solution exhibited a peak at 3504 cm^{-1} (OH group), a broad peak at approximately 3100 cm^{-1} (an acid OH group), and peaks at 1712 and 1208 cm^{-1} (both acid and ester carbonyl groups and C-O bond stretch respectively) confirming the assignments (Figure 30).

A search of the literature revealed this diterpene ester to be a new natural product. The NMR data for the diterpene portion of the ester corresponded well with the published data for cativol, a diterpene alcohol isolated from *Halimium viscosum* (see Table 27)[194].

Based on the similarity of the ^{13}C NMR data, the structure of (**66**) is proposed to have the same configuration at equivalent centre of cativol. The absolute configuration at C-3' centre was not determined, as it is likely that the configuration results from a mono-esterification of an achiral dicarboxylic acid, so potentially may be racemic at the C-3' centre.

C #	Cativol[194]	Stylosanthenoic acid
1	39.8	39.3
2	18.9	18.9
3	42.3	42.5
4	33.0	33.1
5	50.4	50.3
6	23.9	24.0
7	122.2	122.4
8	135.9	135.5
9	55.6	55.5
10	37.0	37.0
11	25.0	24.5
12	39.4	39.8
13	30.6	30.8
14	40.0	35.2
15	61.4	63.8
16	19.8	19.8
17	22.1	22.3
18	33.2	33.3
19	21.9	22.0
20	13.7	13.7

Table 27. Comparison of ^{13}C NMR data of cativol (50.3 MHz, $CDCl_3$) and (**66**) (75 MHz, $CDCl_3$) (C-1 to C-20)

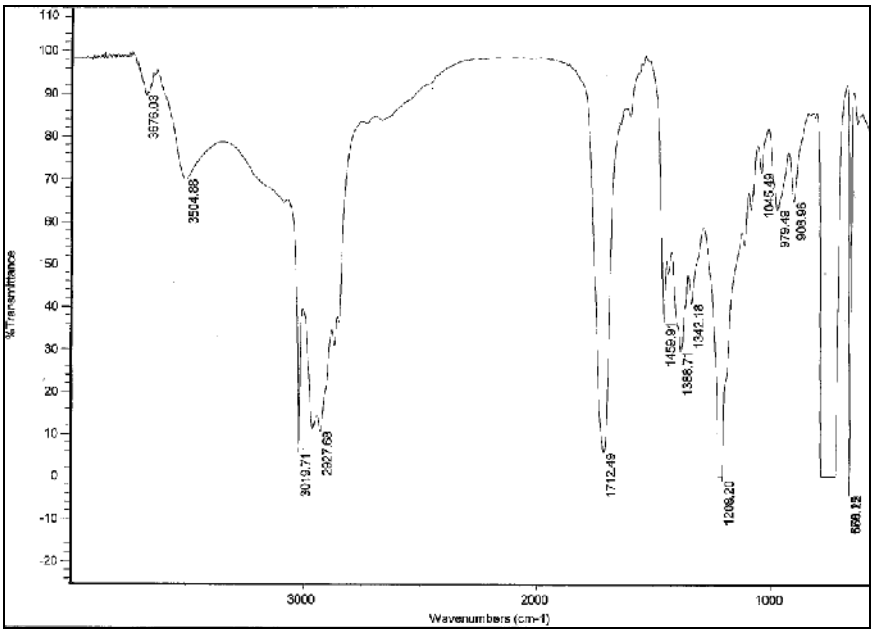

Figure 30. IR spectrum of (**66**)

An $[\alpha]_D$ was measured on the stylosanthenoic acid sample in $CHCl_3$, and found to be -3.9° (c. 0.685 w/v%). Because of the small value of this rotation a Circular Dichroism (CD) spectrum was also run to ensure that the sample was optically active and not racemic (The CD spectrum would show no Cotton effect if the sample was racemic). It was found to have a CD_{max} at 245 nm, confirming its optical activity (Figure 31). An ORD could also be run however lack of access to a suitable instrument prevented us from running the experiment.

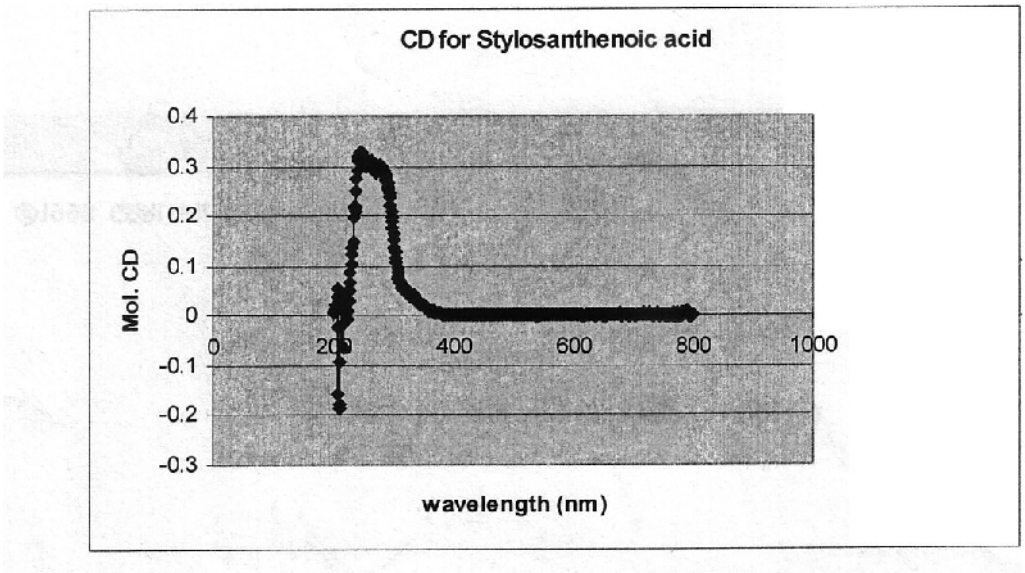

Figure 31. CD spectrum of (**66**) (0.01 M in $CHCl_3$)

stylosanthenoic acid			
C #	δ^{13}C	δ^{1}H, mult., *J* (Hz)	HMBC ^{1}H to ^{13}C
1	39.3	0.93 m	H-3b, H_3-20
		1.84 m	
2	18.9	1.45 m	H-1b
		1.51 m	
3	42.5	1.14 m	H_3-18, H_3-19
		1.39 m	
4	33.1		H_2-3, H-5, H_3-18, H_3-19
5	50.3	1.16 m	H_2-6,H_3-18, H_3-19, H_3-20
6	24.0	1.84 m	H-5, H-7
		1.94 m	
7	122.4	5.38 brs	H_2-6, H_3-17
8	135.5		H_2-6, H_3-17
9	55.5	1.55 m	H-5, H-12a, H_3-17, H_3-20
10	37.0		H-5, H_2-6, H_3-20
11	24.5	1.11 m	H-12
		1.47 m	
12	39.8	1.10 m	H_2-14, H_3-16
		1.50 m	
13	30.8	1.51 m	H_2-14, H_2-15, H_3-16
14	35.2	1.44 m	H_2-15, H_3-16
		1.70 m	
15	63.8	4.14 m	H_2-14
		4.17 m	
16	19.8	0.92 d 6.4	
17	22.3	1.65 br s	H-7, H-9
18	33.3	0.84 s	H_2-3, H-5, H_3-19
19	22.0	0.87 s	H_2-3, H-5, H_3-18
20	13.7	0.74 s	H-5
1'	175.6		H_2-2'
2'	45.1	2.64 d 20.2	H_2-4', H_3-6'
		2.70 d 15.5	
3'	69.9		H_2-2', H_2-4', H_3-6'
4'	44.6	2.60 d 20.5	H_2-2', H_3-6'
		2.69 d 15.8	
5'	172.4		H_2-4', H_2-15
6'	27.3	1.39 s	H_2-2', H_2-4'

Table 28. ^{1}H NMR (600 MHz), ^{13}C NMR (75 MHz) and HMBC data of (**66**) in $CDCl_3$

2.3 Summary

Monoterpenes such as carvacrol, camphor, piperitone, *p*-cymene, 3-carene, α- and β-pinene, geranial, neral, geraniol and 1,8-cineole have been found in the essential oils of species that include *Tithonia Diversifolia*, *Cymbopogon citratus*, *Ricinus communis*, *Piper aduncum*, the family Myrtaceae, the genus *Tagetes* and the family Zingiberaceae. Sesquiterpenes such as β-caryophyllene, caryophyllene oxide, viridiflorol and ledol were present in *Tithonia Diversifolia*, *Cymbopogon citratus*, *Ricinus communis*, the genus *Euodia* and the family Zingiberaceae. Methyl salicylate was detected as a major constituent in *Polygala paniculata* and the family Zingiberaceae. Dillapiole was also found in the family Zingiberaceae as well as *Tithonia Diversifolia*, *Ricinus communis*, *Piper aduncum*. *Trans*-anethole was present in the genus *Dacrydium*. Gibbilimbols C and D were found in the leaves and fruits of *Piper gibbilimbum*. The oil extracted from *Euroschinus falcatus* leaves contained the monoterpenes; *p*-cymene, α- and β-pinene, limonene, γ-terpinene, terpinolene and myrcene and the sesquiterpenes viridiflorol and spathulenol. The oil extracted from *Schinus terebinthifolius* leaves contained the monoterpenes; *p*-cymene, α-pinene and limonene. Stylosanthenoic acid, a new diterpene ester was isolated from the methanol extract of *Stylosanthes hamata* leaves and its structure was determined. Some of the compounds detected in oil samples from New Guinea were present in very small amounts and it is suspected that presence in many samples came from cross contamination during the oil distillation.

This research has demonstrated that it is possible to identify components by NMR that are present in moderate amounts without fractionation of essential oil samples. Even quite minor components (< 1%) can be characterised by NMR provided at least one

signal has a unique chemical shift to enable TOCSY correlations to be generated. Access to a high field NMR, equipped with a cryoprobe and improvements to NMR pulse programs (particularly 1D gradient techniques) have made this possible.

Oil/extract samples	Identified compounds
Tithonia Diversifolia (leaves)	Dillapiole, β-Caryophyllene, Carvacrol, Caryophyllene oxide
	Spathulenol, Camphor, Viridiflorol, Methyl salicylate*
Tagetes spp. (leaves)	Piperitone, Methyl salicylate
Polygala paniculata (roots)	Methyl salicylate, Dillapiole*
'Newton grass' (leaves)	β-Caryophyllene, Dillapiole, *p*-Cymene, Caryophyllene oxide
unidentified (leaves)	Carvacrol, Camphor, α-Pinene, 3-Carene, *p*-Cymene
Euodia sp. (leaves)	β-Caryophyllene, Dillapiole, Caryophyllene oxide
Cymbopogon citratus (leaves)	Geranial, Neral, Viridiflorol, Ledol, Geraniol
Ricinus communis (leaves)	Dillapiole, Piperitone, β-Caryophyllene
Piper aduncum (leaves)	Dillapiole, Piperitone, β-Caryophyllene
Piper gibbilimbum (leaves)	Gibbilimbol C, Gibbilimbol D, Dillapiole*, 1,8-Cineole*
Piper gibbilimbum (fruits)	Gibbilimbol C, Gibbilimbol D, Dillapiole*, 1,8-Cineole*
'Gorgor' (leaves)	α-Pinene, β-Pinene, β-Farnesene, Caryophyllene oxide*
	Dillapiole*, Methyl salicylate*
'Hane grass' (leaves)	*trans*-Anethole
Dacrydium spp. (leaves)	α-Pinene, *trans*-Anethole*
Eucalyptus sp. (leaves)	α-Pinene
Eucalyptus tereticornis (leaves)	α-Pinene
Eucalyptus grandis (leaves)	α-Pinene
Melaleuca leucadendron (leaves)	1,8-Cineole
Euroschinus falcatus (leaves)	α-Pinene, γ-Terpinene, Limonene, *p*-Cymene, β-Pinene
	Myrcene, Terpinolene, Viridiflorol, Spathulenol
Schinus terebinthifolius (leaves)	α-Pinene, *p*-Cymene, Limonene
Stylosanthes hamata (leaves)	Stylosanthenoic acid

Table 29. Compounds identified from the collected species

*Compounds that appear to be contaminants in essential oil samples due to inadequate cleaning of steam distillation apparatus, prior to use.

2.4 Antimicrobial assay

A large zone of inhibition (halo) is not necessarily indicative of good antibacterial/antifungal activity. It may mean the active component diffuses well through the water-based agar medium maintaining a high concentration over a large area. A very good antibacterial/antifungal agent which is relatively insoluble in water might diffuse through the water to a very limited extent, and only produce a very small zone of inhibition.

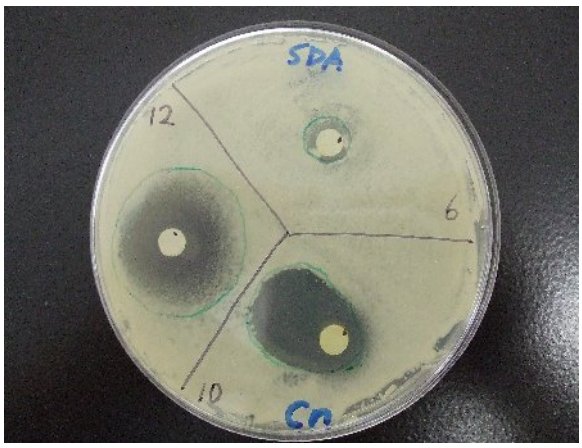

Figure 32. Zones of inhibition obseved on *C. neoformans*

Tables 30a and 30b show the diameter of zones of inhibition observed for various volatile oils/extracts against bacteria, fungi and yeast respectively. Zones of inhibition are expressed as the diameter of the zone in mm. Significant activity (halo size over 30 mm) was shown by distillates of *T. diversifolia*, the unidentified species and *Euodia spp.* against *B. cereus*, *E. grandis* against *S. epidermidis, S. aureus, B. cereus, Neurospora* and *C. neoformans*, 'Gorgor' against *C. neoformans*, 'Hane grass' against *P. aeruginosa*. The crude extract of *P. aduncum* which contained aduncamide has been reported to

exhibit a significant activity against *B. subtilis, E. coli* and *Penicillium*[110] Rather surprisingly in our test, no activity against *B. cereus* was shown but some activity was observed against *E. coli* and *Penicillium*. It should be noted that the *Bacillus* species used in our test differed from that in the literature. The extract of *P. gibbilimbum* has been reported to show antibacterial activity toward *S. epidermidis* and *B. cereus,*[123] however, both the leaf and fruit extracts showed a negative result against *S. epidermidis* and only the leaf extract was negative toward *B. cereus*. In addition, the leaf extract showed a significant activity against *S. aureus* with a halo size of 49.5 mm. The essential oil of *M. leucadendron* was reported to be active against *B. cereus* and *S. aureus* but inactive against *E. coli* and *P. aeruginosa*[162] however results showed positive activity against all those microbes in this test, with an especially large halo observed against *B. cereus* (36.5 mm). Citral in *C. citratus* extract was found to possess antifungal activity against plant and human pathogens[78,79] and indicated positive activity against all of the fungi used in this test.

A further investigation was carried out to see whether the activities were due to specific metabolites. Results are indicated in Table 31. The major components of the distillates that produced a large halo were selected and tested against the microbes. The original crude oils were also tested for comparison. All of the metabolites tested showed reduced activities compared with the oil distillates. This could be indicative of the presence of other minor components in the distillates with much greater activity or of synergistic effects from other oil components.

	gram positive bacteria						gram negative bacteria					
species	*S. e*	*S. a*	*B. c*	*S. p*	*S. v*	*E. f*	*E. c*	*P. v*	*Sal.*	*P. a*	*E. c*	*K. p*
T. d	-	17.0	32.0	16.5	20.0	11.0	10.0	12.0	-	-	8.0	7.5
T. spp.	-	16.5	19.5	15.0	18.0	11.5	-	19.0	11.5	8.0	-	9.0
P. p	-	14.5	16.0	14.5	-	7.0	11.0	10.5	13.5	7.0	-	11.0
Newton	-	-	18.0	11.5	20.0	10.5	8.0	-	-	7.5	8.0	-
unidentified	-	-	36.5	19.5	-	13.5	18.5	-	11.5	9.0	10.0	9.0
E. sp.	-	-	41.0	17.5	15.0	-	9.0	-	-	7.0	7.0	-
C. c	-	18.0	12.0	21.0	20.0	13.5	10.0	9.5	10.0	9.0	9.5	8.0
R. c	10.5	10.5	9.0	7.5	-	10.0	9.0	7.5	-	-	7.5	7.0
P. a	11.0	10.0	-	8.5	14.5	8.5	7.0	-	8.0	-	7.5	7.0
P. g lea	-	49.5	-	23.0	12.0	20.5	-	-	-	-	-	7.0
P. g fru	-	23.0	8.5	16.0	-	11.5	9.5	8.5	8.0	-	7.0	7.0
Gorgor	-	20.0	15.0	17.0	18.0	11.0	10.0	9.0	9.0	-	9.0	7.0
Hane	17.5	14.0	18.0	11.0	11.0	10.5	9.5	10.0	9.5	48.5	7.0	7.5
D. spp.	14.0	8.5	12.5	9.0	13.0	10.0	7.5	-	-	12.5	7.0	-
E. sp.	-	18.0	29.5	18.5	28.0	20.5	8.0	9.0	8.5	7.0	7.5	7.0
E. t	-	21.0	27.5	19.0	14.0	14.5	15.0	11.5	-	7.5	9.0	8.5
E. g	32.0	43.0	36.5	22.0	28.0	12.0	23.5	25.0	22.0	8.5	8.0	16.0
M. l	-	10.5	36.5	15.0	18.0	14.0	11.5	10.0	9.0	21.5	7.0	7.0
E. f	-	10.0	-	-	-	-	-	8.0	-	-	-	-
S. t	-	-	-	-	-	-	-	-	-	-	-	8.0
S. h	-	-	-	-	-	-	-	-	-	-	-	-

Table 30a. Diameter of zone of inhibition (mm); '-' indicates no observed inhibition

	fungi					yeast
species	*Fus.*	*Neu.*	*Rhi.*	*Pen.*	*Hum.*	*C. n*
T.d	-	10.5	8.0	-	7.5	11.0
T. spp.	-	9.0	-	-	-	12.0
P. p	-	7.0	-	7.0	-	11.0
Newton	-	8.5	7.0	-	-	-
unidentified	10.0	-	7.0	16.0	8.0	20.0
E. sp.	-	13.0	9.5	-	7.5	10.0
C. c	15.0	12.0	7.0	20.0	9.5	13.0
R. c	7.0	11.0	9.0	9.0	-	12.5
P. a	-	10.0	7.0	9.0	8.5	8.0
P. g lea	16.0	10.5	7.0	8.5	8.5	13.0
P. g fru	-	9.0	7.0	24.5	8.5	28.0
Gorgor	9.0	-	-	9.5	-	30.5
Hane	8.5	-	-	9.5	-	11.0
D. spp.	-	-	-	8.0	-	10.5
E. sp.	12.5	13.0	7.0	10.0	7.0	15.0
E. t	8.0	10.5	-	12.0	-	3.0
E. g	12.0	31.0	-	17.0	7.0	57.0
M. l	10.0	8.0	-	9.0	-	11.0
E. f	-	-	-	-	-	-
S. t	-	-	-	-	-	-
S. h	-	-	-	-	-	-

Table 30b. Diameter of zone of inhibition (mm); '-' indicates no observed inhibition

components / species	microbes					
	S.epidermidis	*S.aureus*	*B.cereus*	*P.aeruginosa*	*Neurospora*	*C.neoformans*
α-Pinene	11.0	-	13.0	N/A	-	-
E. grandis	33.0	42.0	33.0	N/A	30.0	59.0
1,8-Cineole	N/A	N/A	8.0	N/A	N/A	N/A
M. leucadendron	N/A	N/A	18.0	N/A	N/A	N/A
Dillapiole	N/A	N/A	11.0	N/A	N/A	N/A
T. diversifolia	N/A	N/A	28.0	N/A	N/A	N/A
Carvacrol	N/A	N/A	12.0	N/A	N/A	N/A
unidentified sp.	N/A	N/A	22.0	N/A	N/A	N/A
β-Caryophyllene	N/A	N/A	-	N/A	N/A	N/A
Euodia sp.	N/A	N/A	39.0	N/A	N/A	N/A
trans-Anethole	N/A	N/A	N/A	10.0	N/A	N/A
Hane grass'	N/A	N/A	N/A	12.0	N/A	N/A

Table 31. Diameter of zone of inhibition (mm) for individual components; '-' indicates no observed inhibition

2.5 Cytotoxicity assay

Results obtained are expressed as GI_{50} values in Table 32a and 32b. The GI_{50} value is the concentration of distillate that inhibits the growth of the cancer cells by 50%. Usually for pure compounds, a GI_{50} value of less than 10μg/mL is considered significantly cytotoxic.

None of those steam distillates exhibit high levels of growth inhibition, but distillates of *Piper gibbilimbum*, *Eucalyptus tereticornis*, *Eucalyptus grandis* and *Schinus terebinthifolius* all showed significant activity. *Euodia* and "Gorgor" appear to show some selectivity for the H460 cell line. Although some of the samples displayed cytotoxicity, the levels were not sufficient to warrant further investigation.

	Cell lines and GI_{50} values (µg/mL)		
Species	**H460**	**MCF-7**	**SF268**
Tithonia Diversifolia	34	36	48
Tagetes spp.	56	58	66
Polygala paniculata	82	68	115
"Newton grass"	50	48	52
unidentified	54	48	52
Euodia sp.	22.5	30	40
Cymbopogon citratus	65	48	46
Ricinus communis	170	165	165
Piper aduncum	56	52	52
Piper gibbilimbum	17.5	19	22
Piper gibbilimbum	22	23	25
"Gorgor"	22.5	40	39
"Hane grass"	68	76	90
Dacrydium spp.	58	58	45
Eucalyptus sp.	50	50	45
Eucalyptus tereticornis	19.5	21	21
Eucalyptus grandis	20	20	22
Melaleuca leucadendron	100	125	150
Control	250	250	250

Table 32a. GI_{50} values for different cell lines

Species/compounds	**GI_{50} values (µg/mL)**
Euroschinus falcatus	32
Schinus terebinthifolius	8.1
Stylosanthes hamata	34
Stylosanthenoic acid	39

Table 32b. GI_{50} values for mouse lymphoma cell line (P388D1)

CHAPTER 3 Experimental

3.1 General Experimental Procedures

3.1.1 Solvents

All solvents (technical grade) were distilled prior to use.

3.1.2 Instrumentation

3.1.2.1 NMR Spectrometry

^{1}H and ^{13}C NMR spectra were recorded on a 300 MHz Varian Mercury spectrometer and 600 MHz Bruker Avance spectrometer. Chemical shifts are given relative to tetramethylsilane (TMS) at 0.0 ppm as internal standard in $CDCl_3$. 2D NMR data were also obtained using the same system, and the spectra were measured and reported in ppm. The TOCSY mixing time used for 300 and 600 MHz 1D TOCSY experiments was 0.12 second.

3.1.2.2 Mass Spectrometry (MS)

Electrospray ionisation mass spectrometry (ESI-MS) was performed in HPLC grade methanol with a Bruker BioApex 47e Fourier Transform Ion Cyclotron Resonance Mass Spectrometer at the Australian Institute of Marine Science, Townsville.

3.1.2.3 Infrared Spectroscopy (IR)

Infrared spectra were taken in chloroform using a Nicolet Nexus Fourier Transform IR (FT-IR) spectrometer.

3.1.2.4 Optical Rotations ($[\alpha]_D$)

Optical rotations were recorded in chloroform using a JASCO P-1020 Polarimeter.

3.1.2.5 Circular Dichroism (CD)

CD was observed in chloroform using a Jasco J-715 spectropolarimeter.

3.1.2.6 Column Chromatography

The oils/extracts were separated by column chromatography under reduced pressure. Silica gel 60 G (Merck) was used as the stationary phase, and the elution of the adsorbed mixtures was by step-wise gradient elution (i.e. using mixtures of solvents containing increasing amounts of the more polar solvent). All fractions obtained were examined by thin layer chromatography (TLC) and ^{1}H NMR spectroscopy.

3.1.2.7 Thin Layer Chromatography (TLC)

TLC was carried out on plastic plates precoated with silica gel 60 F (Merck) (0.2 mm, normal phase). After elution, plates were examined under UV light (254 nm) to detect UV absorbing compounds, and then sprayed with vanillin concentrated sulphuric acid in methanol before heating to visualise individual compounds.

3.1.2.8 Gas Chromatography (GC)

The oils were analysed by GC using a Hewlett Packard 5890 system. A Supelcowax 10 column (30m x 0.25 mm inner diameter) was used with helium as carrier gas (1mL/min). The oven temperature was kept at 30 °C for 4 min and programmed to 150 °C at a rate of 4 °C/min, then kept constant at 150 °C for 1.5 min and then programmed to 200 °C at

a rate of 5 °C/min. The injector temperature was set at 150 °C. The percentage compositions were obtained from electronic integration measurements using flame ionization detection (FID, 250 °C). Cyclohexane was used as solvent.

3.1.2.9 Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS analyses were conducted using a 1200 MSMS Gas Chromatograph attached on a Varian CP-3800. A Supelcowax 10 column (30m x 0.25mm inner diameter) was used with helium as carrier gas (1mL/min). Samples of 1 mL were injected into the GC, which was run in splitless mode with a flowrate of 1mL per minute. The GC oven temperature was kept at 30 °C for 4 min and programmed to 150 °C at a rate of 4 °C/min, then kept constant at 150 °C for 1.5 min and then programmed to 200 °C at a rate of 5 °C/min.. The injector temperature was 150 °C. MS were taken at 70 eV. Mass range was from *m*/*z* 50 to 350 and the column pressure was 11.0 psi.

3.1.2.10 High Performance Liquid Chromatography (HPLC)

Preparative HPLC was carried out using a GBC LC 1150 HPLC pump, a Rheodybe 7725i manual injector with a 200 µl sample loop, and various C-18 columns. Samples were observed at multiple UV/Vis wavelengths simultaneously with a GBC LC 5100 Photodiode Array Detector (PDA) and WinChrom Chromatography Data System (Version 1.3.1) software. Flow rates ranged from 1-2 ml/min. Distilled water and LR grade Acetonitrile were filtered through a Millipore 0.45 µm HA (water) or FH (methanol) membrane filter and degassed by sparging with helium for 15 minutes.

3.2 Bioassays

3.2.1 Antimicrobial Assay

Antimicrobial assays were carried out in School of Veterinary and Biomedical Sciences, JCU against a range of gram positive and gram negative bacteria. Yeast and fungi were also included in the assays. Agar plates and filter paper disks were used.

Staphylococcus epidermidis (ATCC 12228), *Staphylococcus aureus* (ATCC 25923), *Bacillus cereus* (ATCC 10870), *Streptococcus pyogenes* (wild*), *Streptococcus viridans* (wild), *Enterococcus faecalis* (wild), *Escherichia coli* (ATCC 25922), *Proteus vulgaris* (wild), *Salmonella serotype* (wild), *Pseudomonas aeruginosa* (ATCC 27853), *Enterobacter cloacae* (wild), *Klebsiella* (wild), *Fusarium* (wild), *Neurospora* (wild), *Rhisopus* (wild), *Penicillium* (wild), *Humicola* (wild) and *Cryptococcus neoformans var gatii* (wild) were used as test microorganisms. The bacteria, fungi and yeast were prepared in Nutrient Broth (Sigma-Aldrich) prior to use. The impregnated broth was poured onto the Nutrient Agar or Sabouraud Dextrose Agar (Sigma-Aldrich). 10µl of each distillates/extracts were added to filter paper disks, and the disk was placed on the surface of the agar. The plates were then incubated at 37 °C or 30 °C for 24-48 hours. A clear zone of inhibition around the disk indicated activity as opposed to the cloudy bacterial/fungal film growing over the rest of the plate. The disk diameter used in this assay was 6.5 mm and results are shown in the Tables in Chapter 2.4.

* wild strain collections from School of Veterinary and Biomedical Sciences, JCU

3.2.2 Cytotoxicity Assay

Cytotoxicity assessment of the eighteen essential oils was conducted at the Australian

Institute of Marine Science, Townsville. Incubated cultures of three different cancer cell lines were used to assess cell growth in the presence of each sample extract. These cell lines covered a range of cancer-types: lung cancer (H460), breast cancer (MCF-7), glioma (SF268). Assays on the three samples (*Euroschinus falcatus, Schinus terebinthifolius* and *Stylosanthes hamata*) which were collected on JCU campus and Stylosanthenoic acid, a purified compound from *Stylosanthes hamata*, were carried out at the School of Veterinary and Biomedical Sciences, JCU using the mouse lymphoma cell line (P388D1). Results obtained were indicated in the Tables in Chapter 2.5.

3.3 Plant selection and extraction

Plants were selected by Dr Stewart Wossa (University of Goroka, Papua New Guinea) in 2006 utilising knowledge of traditional herbal and medicinal use. Plant material was either steam distilled to afford essential oils, or extracted with methanol to afford crude extracts. Eighteen steam-distilled volatile oil fractions were forwarded (see Table 33). Leaf samples of three species (*Euroschinus falcatus, Schinus terebinthifolius* and *Stylosanthes hamata*) were collected on Douglas campus of JCU in 2008. Leaves of *Euroschinus falcatus* (359.28g) and *Schinus terebinthifolius* (431.02g) were each steam distilled and yielded 0.2ml and 0.1ml of oils respectively. Leaves of *Stylosanthes hamata* (258.44g) were extracted with methanol and filtered. The solvent was removed at 50°C under vacuum on a rotary evaporator (Büchi) to give 12.06g of crude extract.

Botanical Name	Family
Tithonia diversifolia (leaves)	Asteraceae
Tagetes spp. (leaves)	Asteraceae
Polygala paniculata (roots)	Polygalaceae
'Newton grass' (leaves)	unidentified
unidentified (leaves)	unidentified
Euodia sp. (leaves)	Rutaceae
Cymbopogon citratus (leaves)	Poaceae
Ricinus communis (leaves)	Euphorbiaceae
Piper aduncum (leaves)	Piperaceae
Piper gibbilimbum (leaves)	Piperaceae
Piper gibbilimbum (fruits)	Piperaceae
'Gorgor' (leaves)	Zingiberaceae
'Hane grass' (leaves)	unidentified
Dacrydium spp. (leaves)	Podocarpaceae
Eucalyptus sp. (leaves)	Myrtaceae
Eucalyptus tereticornis (leaves)	Myrtaceae
Eucalyptus grandis (leaves)	Myrtaceae
Melaleuca leucadendron (leaves)	Myrtaceae
Euroschinus falcatus (leaves)	Anacardiaceae
Schinus terebinthifolius (leaves)	Anacardiaceae
Stylosanthes hamata (leaves)	Leguminosae

Table 33. Species for which steam distillates/extracts were obtained

3.3.1 Identification of components in essential oils

Components in oils from the *Tagetes spp.*, *Polygala paniculata*, *Piper gibbilimbum*, 'Hane grass', *Dacrydium spp.*, *Eucalyptus sp.*, *E. tereticornis*, *E. grandis*, *Melaleuca leucadendron*, *Euroschinus falcatus* and *Schinus terebinthifolius* were identified without separation by the use of NMR spectroscopy.

The distilled oil (50 mg) from *Tithonia diversifolia* was fractionated by reverse phase

HPLC using a C_{18} column (250 x 4.6 mm) eluted with 100% CH_3CN at a flow rate of 1 ml/min (fractions 1-6). The CH_3CN layer was evaporated under reduced pressure. Fraction 1 (5.0-9.2 min) was a mixture of Dillapiole, Carvacrol, Camphor and Methyl salicylate. Fraction 3 (16.2-17.4 min) was a mixture of Caryophyllene oxide and Spathulenol. Fractions 5 (22.6 min) and 6 (36.2 min) contained Viridiflorol and β-Caryophyllene respectively.

For each of the distilled oils from 'Newton grass', *Cymbopogon citratus*, *Ricinus communis* and 'Gorgor', a sample (50 mg) was rapidly chromatographed on silica gel under vacuum eluted with 50 ml each of 33% DCM/Hexane, 50% DCM/Hexane, 66% DCM/Hexane, 100% DCM, 5% EtOAc/DCM and 10% EtOAc/DCM (fractions 1-6). The fractions obtained from these separations contained the components listed below.

'Newton grass'; Fraction 1 (33% DCM/Hexane) was a mixture of *p*-Cymene, β-Caryophyllene and Caryophyllene oxide. Fraction 2 and 3 (50% DCM/Hexane and 66% DCM/Hexane respectively) contained Dillapiole.

Cymbopogon citratus; Fraction 3 (66% DCM/Hexane) was a mixture of Geranial and Neral and fraction 5 (5% EtOAc/DCM) was a mixture of Geraniol, Viridiflorol and Ledol.

Ricinus communis; Fraction 1 (33% DCM/Hexane) was a mixture of Dillapiole and β-Caryophyllene. Fraction 2 (50% DCM/Hexane) contained Dillapiole and fraction 3 (66% DCM/Hexane) was a mixture of Dillapiole and Piperitone.

'Gorgor'; Fraction 1 (33% DCM/Hexane) contained β-Farnesene and fraction 2 (50% DCM/Hexane) was a mixture of Dillapiole and Methyl salicylate. Fraction 3 (66%

DCM/Hexane) was a mixture of Dillapiole and Caryophyllene oxide.

p-Cymene, 3-Carene and α-Pinene were identified without separation from oil of the unknown species by the use of NMR spectroscopy. The distilled oil (50 mg) was fractionated by reverse phase HPLC using a C_{18} column (250 x 4.6 mm) eluted with 92% CH_3CN/H_2O at a flow rate of 0.7 ml/min (fractions 1-5). Fractions were each transferred to a separating funnel, diluted with water and extracted with DCM. The DCM layer was evaporated under reduced pressure. Fraction 2 (3.2 min) and 4 contained (6.8 min) Carvacrol and Camphor respectively.

The distilled oil from the *Euodia sp.* (50 mg) was fractionated by reverse phase HPLC using a C_{18} column (250 x 4.6 mm) eluted with 100% CH_3CN at a flow rate of 1 ml/min (fractions 1-6). The CH_3CN layer was evaporated under reduced pressure. Fraction 2 (6.2 min), 4 (15.0 min) and 6 (33.2min) contained Dillapiole, Caryophyllene oxide and β-Caryophyllene respectively.

The distilled oil from *Piper aduncum* (50 mg) was fractionated by reverse phase HPLC using a C_{18} column (250 x 4.6 mm) eluted with 100% CH_3CN at a flow rate of 1 ml/min (fractions 1-5). The CH_3CN layer was evaporated under reduced pressure. Fractions 1 (6.0 min) and 5 (33.4 min) contained Dillapiole and β-Caryophyllene. Fraction 2 (6.8-7.6 min) was a mixture of Dillapiole and Piperitone.

3.3.2 Isolation and identification of components from crude extracts

The MeOH crude extract from *Stylosanthes hamata* (2g) was rapidly chromatographed

on silica gel under vacuum eluted with 100 ml each of 75% Hexane/DCM, 50% Hexane/DCM, 100% DCM, 75% DCM/EtOAc, 50% DCM/EtOAc/DCM, 100% EtOAc and 75% EtOAc/MeOH (fractions 1-7). Fractions 4 and 5 were combined and the solvent was removed using a rotary evaporator to afford an extract (0.2364g) which was then chromatographed on silica gel under vacuum eluted with 33% Hexane/EtOAc. The solvent of fraction 3 was removed and again was chromatographed on silica gel under vacuum eluted with a step-wise gradient of 50-33% Hexane/EtOAc. The solvent of fraction 2 was removed to afford an extract of 0.0983g which was chromatographed on silica gel under vacuum eluted with 33% Hexane/EtOAc. The solvent of fraction 3 was removed to afford an extract of 0.0690g which was identified as Stylosanthenoic acid by the NMR spectroscopy.

Stylosanthenoic acid

Yellow optically active gum; $[\alpha]_D$ -3.9° (c. 0.685 w/v% in $CHCl_3$); CDmax 245nm (c. 0.01 M in $CHCl_3$); Found: $[M-H]^-$, 435.3119; $C_{26}H_{43}O_5$ requires $[M-H]^-$, 435.3111; IR ν_{max} ($CHCl_3$): 3504, 3100, 1712, 1363, 1208 cm^{-1}.

^{1}H NMR spectrum ($CDCl_3$): Refer to Table 28

^{13}C NMR spectrum ($CDCl_3$): Refer to Table 28

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Appendices

Appendices 1.1 to 1.3 show the GC and GC-MS retention time (minutes) data. The amount of compounds are expressed in percentage (%).

Appendix 1.1

Oil samples	Identified compounds	M.Wt.	Amount	GC	GC-MS
T. diversifolia	Camphor	152	2.7	20.9	22.5
	β-Caryophyllene	204	6.1	23.5	25.0
	Methyl Salicylate*	152	0.6	35.1	30.9
	Caryophyllene oxide	220	3.5	36.4	35.7
	Viridiflorol	222	1.9	38.7	38.6
	Spathulenol	220	2.9	41.1	39.6
	Carvacrol	150	5.9	40.8	41.9
	Dillapiole	222	11	46.9	44.6
Tagetes spp.	Piperitone	152	57	27.6	28.9
	Methyl Salicylate	152	15	34.1	30.5
P. paniculata	Methyl Salicylate	152	74	34.3	30.4
	Dillapiole*	222	3.6	46.8	44.6
Newton grass'	*p*-Cymene	134	2.3	13.6	14.6
	β-Caryophyllene	204	17	23.4	25.1
	Caryophyllene oxide	220	1.6	36.3	35.7
	Dillapiole	222	3.2	46.9	44.6
unidentified	α-Pinene	136	13	5.3	N/A
	3-Carene	136	11	9.5	N/A
	p-Cymene	134	8.2	14.1	N/A
	Camphor	152	15	21.1	N/A
	Carvacrol	150	18	41.0	N/A
Euodia sp.	β-Caryophyllene	204	25	23.4	24.9
	Caryophyllene oxide	220	3.4	36.3	35.7
	Dillapiole	222	8.0	46.8	44.6
C. citratus	Neral	152	18	29.2	27.8
	Geraniol	154	1.5	29.6	28.3
	Geranial	152	32	30.8	29.3
	Ledol	222	6.0	39.9	37.1
	Viridiflorol	222	14	41.6	38.6
R. communis	β-Caryophyllene	204	12	23.4	24.9
	Piperitone	152	13	27.6	28.9
	Dillapiole	222	38	46.9	44.7
P. aduncum	β-Caryophyllene	204	9.8	23.5	25.0
	Piperitone	152	10	27.6	28.9
	Dillapiole	222	17	46.9	44.7

Appendix 1.2

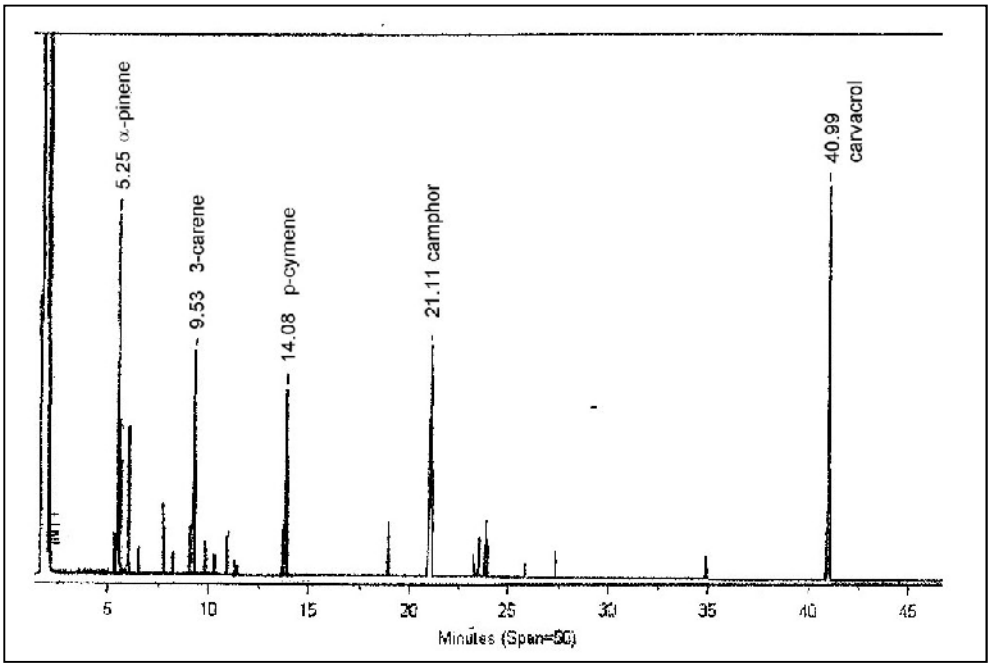
Oil samples	Identified compounds	M.Wt.	Amount	GC	GC-MS
P. gibbilimbum	1,8-Cineole*	154	0.1	12.3	11.7
	Gibbilimbol C/D	204	11	39.8	38.6
	Dillapiole*	222	2.0	46.9	44.6
P. gibbilimbum	1,8-Cineole*	154	0.03	12.3	11.7
	Gibbilimbol C/D	204	22	39.8	38.6
	Dillapiole*	222	0.8	46.9	44.6
Gorgor'	α-Pinene	136	37	5.5	5.1
	β-Pinene	136	22	8.1	7.3
	β-Farnesene	204	8.8	31.7	27.4
	Methyl salicylate*	152	1.0	35.0	30.9
	Caryophyllene oxide*	222	0.9	36.3	35.7
	Dillapiole*	222	1.2	46.8	44.6
Hane grass'	*trans*-Anethole	148	74	30.8	N/A
Dacrydium spp.	α-Pinene	136	32	5.7	5.4
	trans-Anethole*	148	3.7	30.7	32.4
Eucalyptus sp.	α-Pinene	136	45	5.4	5.4
E. tereticornis	α-Pinene	136	53	5.1	N/A
E. grandis	α-Pinene	136	66	4.9	N/A
M. leucadendron	1,8-Cineole	154	60	12.9	N/A
E. falcatus	α-Pinene	136	33	4.2	5.4
	β-Pinene	136	3.9	8.9	6.1
	Myrcene	136	3.9	9.0	8.1
	Limonene	136	11	10.0	9.1
	γ-Terpinene	136	16	12.5	11.0
	p-Cymene	134	7.9	13.7	11.9
	Terpinolene	136	4.3	14.2	12.3
	Viridiflorol	222	1.6	39.4	36.2
	Spathulenol	220	1.6	40.2	37.4
S. terebinthifolius	α-Pinene	136	32	4.3	5.4
	Limonene	136	8.5	10.3	9.3
	p-Cymene	134	9.8	13.7	11.9

Appendix 1.3

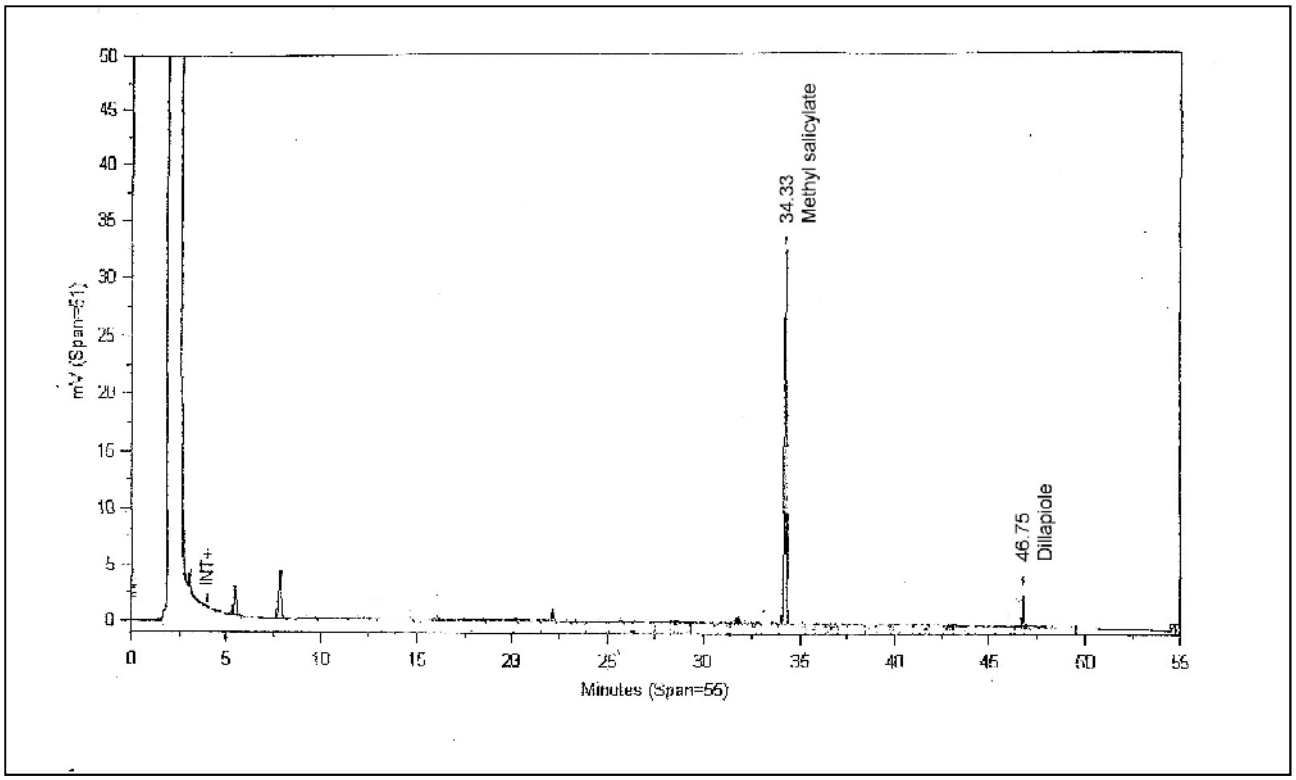
Standard compounds	M.Wt.	GC	GC-MS
α-Pinene	136	5.8	5.0
β-Pinene	136	8.3	7.1
3-Carene	136	9.9	N/A
γ-Terpinene	136	11.8	N/A
Eucalyptus oil (93% 1,8-Cineole)	154	12.7	N/A
p-Cymene	134	14.3	N/A
Camphor	152	21.3	N/A
Citral (Neral)	152	29.3	27.8
Citral (Geranial)	152	30.9	29.3
Aniseed oil (76% trans-Anrthole)	148	30.7	N/A
Methyl salicylate	152	35.3	30.5

Appendices 2.1 to 2.3 show examples of GC and GC-MS spectra from oil samples.

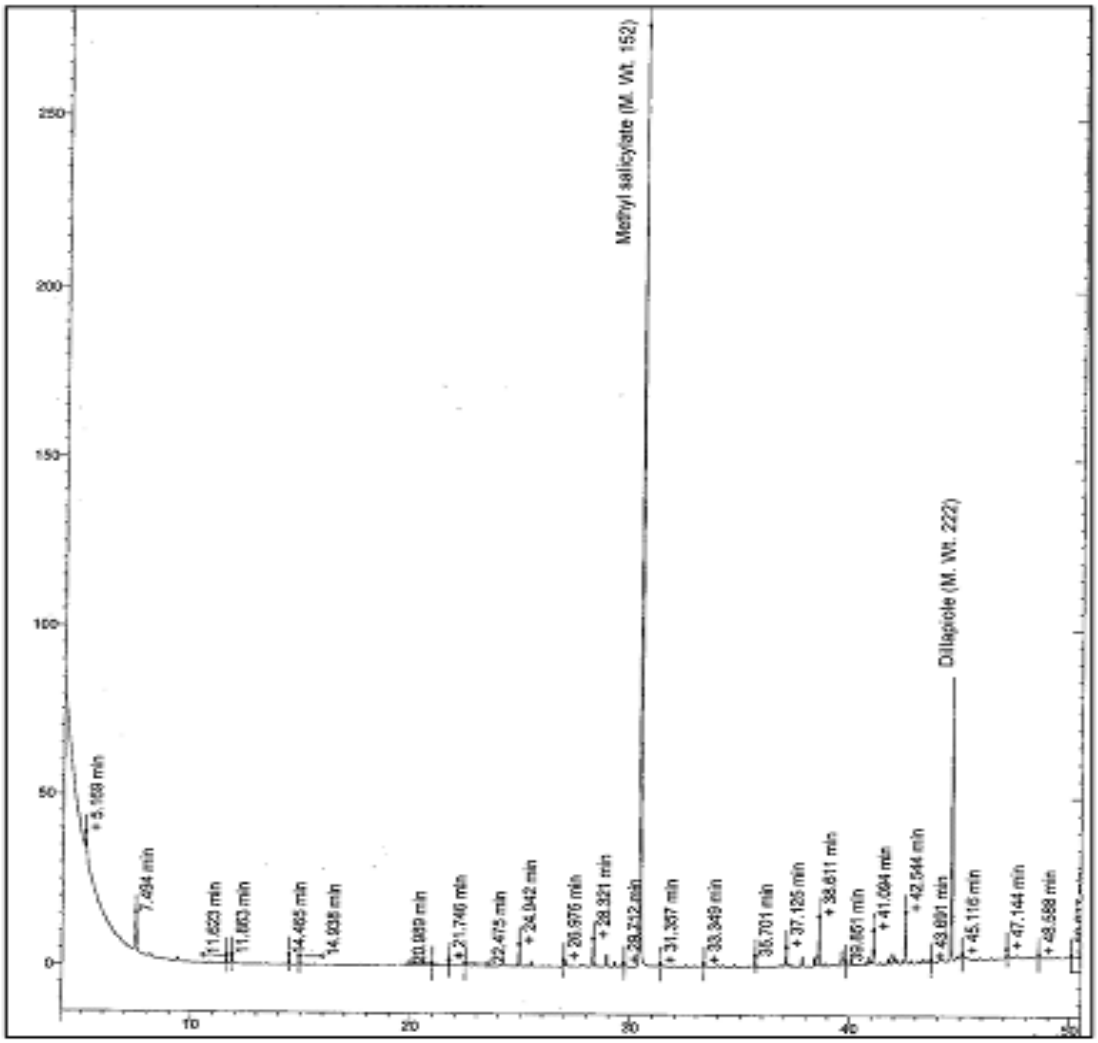
Appendix 2.1. GC spectrum of the essential oil from the unidentified species



Appendix 2.2. GC spectrum of the *Poligala paniculata* root oil



Appendix 2.3. GC-MS spectrum of the *Poligala paniculata* root oil



Appendix 3.1. MS spectrum of Stylosanthenoic acid