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Phytochemical and Microbiological Studies of Some Fijian Plant Extracts

A thesis submitted to the University of the South Pacific in partial fulfilment of the requirements for the degree of

Master of Science in Chemistry

by

Kirti Patel (BSc)
University of the South Pacific
Suva,
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October, 2005

Declaration

I, Kirti Patel, declare that this submission is a result of my own investigation and that to the best of my knowledge, the material contained in this thesis has not been published elsewhere, except where due reference is made and that the thesis has not been used for the award of any degree or diploma of any university or other institute of higher learning.

Candidate: Kirti Patel

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Date Submitted: _____

Dedication

I would like to dedicate this thesis to my parents **Mr Natwarlal Patel and Mrs Pushpa Ben Patel**, and to my brother **Rajeev Patel**, for their guidance and support through the course of this research and write up.

Acknowledgments

Firstly, I would like to take this opportunity to thank my supervisors Professor Subramanium Sotheeswaran (University of the South Pacific) and Associate Professor Sadaquat Ali - **my mentor** (University of the South Pacific), for their advice, encouragement and criticism during the course of this study.

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I would like to give my sincere thanks to my parents (Mr & Mrs Natwarlal Patel) and brother (Rajeev Patel), for their patience, encouragement and contribution to my education.

Sachin Singh (MSc student, Chemistry Department, USP) is duly thanked for his assistance with formatting and layout of the final draft of this thesis.

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Abbreviation

^{o}C	Degree Celsius
A. zerumbet	*Alpinia zerumbet*
%	Percentages
amu	Atomic mass unit
ATR-IR	Attenuated total reflection infrared
ATP^{33}	Adenosine triphosphate (radioactive)
C. albicans	*Candida albicans*
C. coloratus	*Cymbopogon coloratus*
C. sativum	*Coriandrum sativum*
C. verum	*Cinnamomum verum*
cm^3	Cubic centimetre
cm/sec	Centimetre per second
CMC	Carboxy methyl cellose
DCM	Dichloromethane
DMSO	Dimethylsulphoxide
E. coli	*Escherichia coli*
E. foetidum	*Eryngium foetidum*
EDTA	Ethylelediamine tetra acetic acid
EtOAC	Ethyl acetate
ev	Electron volt
FBIT	Ferriprotoporphyrin IX Biomineralization Inhibition test
FID	Flame ionisation detector
FP	Ferriprotoporphyrin
g	Gram
g/cm^3	Grams per cubic centimetre
GC	Gas chromatography
GC-FID	Gas chromatography with flame ionisation detector
GC-MS	Gas chromatography with mass spectrometry
HMQC	Hetero nuclear multiple quantum coherence
HRMS	High resolution mass spectrometry
Hz	Hertz
i.d	Internal diameter
kg	Kilogram
kPa	Kilo Pascal
L15	Culture medium L15
Ltd	Limited
M	Molar
M. quinquenervia	*Melaleuca quinquenervia*
mg/cm^3	Milligram per cubic centimetre
mg/10ml	Milligram per 10 micro litre

mg/ml	Milligram per millilitre
$MgCl_2$	Magnesium chloride
min	Minute(s)
ml	Millilitre
ml/min	Millilitre per minute
μm	Micrometer
mm	Millimetre
NaF	Sodium fluoride
NaOH	Sodium hydroxide
NIR	Near infrared
NIR-FT	Near infrared Fourier transform
nm	Nanometre
^{o}C/min	Degree celsius per minute
P. racemos	*Pimenta racemose*
PBS	Phosphate buffer saline
PfNEK	Protein kinase
ppm	Parts per million
psi	Pound per square inch
R_f	Retention factor
rpm	Revolutions per minute
S. aureus	*Staphylococcus aureus*
S. dulcis	*Spondias dulcis*
scan/sec	Scans per second
SVF	Serum of calf fetal
TLC	Thin Layer Chromatography
™	Trade mark
TP	Control paper
tris HCL	2-amino-2-(hydroxymethyl)-1,3-propanediol, hydrochloride
turns/min	Turns per minute
V	Volts
v/v	Volume by volume

Abstract

This thesis is organised into 2 parts, **A** and **B**. In part **A**, the results of some GC-MS analysis and microbiological studies on essential oils from Fijian Plant species are reported. Part **B** reports the isolation and structure elucidation of desmethoxyyangonin from *A.zerumbet* mixture.

Part A

The essential oils components of the following Fijian plant species *Eryngium foetidum, Coriandrum sativum, Cinnamomum verum, Alpinia zerumbet* and *Spondias dulcis* were analysed using GC and GC-MS.

E. foetidum leaf essential oils had 25 components present with (E)-2-dodecenal (70.68 %) as the major component. *C. sativum* leaf essential oils had 43 components with 2-decen-1-ol (24.25 %) as the major component. *C. verum* leaf essential oils had 31 components with eugenol (86.02 %) as the major component. *S. dulcis* leaf essential oils had 22 components with α-pinene (48.11 %) as the major component In *A. zerumbet* 47 components were identified in the rhizome essential oils and 41 components were identified for the leaf and flower essential oils. 4-Terpineol was the major component in the leaves (25.31 %), rhizomes (19.25 %) and flowers (20.29 %).

Furthermore, the essential oils of the following Fijian plant species: *Melaleuca quinquenervia, C. verum, A. zerumbet, Pimenta racemose, Coleus amboinicus, Cymbopogon coloratus* were tested for antibacterial, antifungal, antimalarial and antidengue activities.

In antibacterial and antifungal activity studies, essential oils from *A. zerumbet* rhizomes showed good inhibition for *Escherichia coli, Staphylococcus aureus* and *Candida albicans*.

Furthermore all essential oils were tested for antimalarial activity in the Ferriprotoporphyrin IX Biomineralization Inhibition test (FBIT) and PfNEK-protein kinase test. Only the essential oils from the leaves of *C. coloratus* and rhizomes of *A. zerumbet* showed antimalarial activity in the FBIT.

Antidengue tests showed negative results, that's no antidengue activity for all the essential oils samples investigated.

Part B

Part B reports the isolation and structure elucidation of desmethoxyyangonin (a kavalactone known to be found in only *Piper methysticum)* from *A. zerumbet.* Non-volatile fractions of the leaves and flowers of *A. zerumbet* were also investigated. Desmethoxyyangonin was isolated from the non-polar extracts of leaves and flowers of *A. zerumbet*. The structure of desmethoxyyangonin was elucidated using MS, ^{1}H NMR, ^{13}C NMR and HMQC spectral data.

1.0 Introduction

This thesis reports the essential oils components of the selected Fijian plant species (*Eryngium foetidum*, *Coriandrum sativum*, *Cinnamomum verum, Alpinia zerumbet, Spondias dulcis*). In addition this thesis reports the microbiological activities of the essential oils of the following Fijian plant species (*Melaleuca quinquenervia, C. verum, A. zerumbet*, *Pimenta racemosa, Coleus amboinicus, and Cymbopogon coloratus*). The microbiological studies were antibacterial, antifungal, antimalarial and antidengue tests. Furthermore, this thesis also reports the isolation of desmethoxyyangonin from the Fijian plant *A. zerumbet*.

1.1 What are essential oils?

Essential oils are strong smelling ingredients found in various parts of some plants (Cook and Pickering, 1979). Essential oils are also known as "essence" or volatile oils and are highly odoriferous liquid components of aromatic plants, trees and grasses. Essential oils evaporate slowly when exposed to air at room temperature and because of this they are sometimes referred to as volatile oils (Cook and Pickering, 1979). Essential oils are usually obtained by steam distillation, a process whose origin can be traced to ancient Mesopotamia (Guenther, 1972). Essential oils accumulate in specific plant tissue (s) harbouring oil glands (Raven *et al*., 1999). Essential oils are highly concentrated substances and therefore rarely used neat, though certain essences such as lavender and "tea-tree" essential oils are sometimes used undiluted as an antiseptic (Balz *et al*., 1999).

1.2 Where are essential oils found?

Most plants contain essential oils, often in such minimal volumes that their extraction would not be worth the effort. Due to low composition of essential oils in plants, hundreds of kilograms (kg) of plant material is required to recover a single kilogram of oil. For example when 100 kg of fresh plant materials are distilled, they yield the following amounts of essential oils; for rose and violet, 3 to 8 g and for lavender 1 to 1.2 kg (Balz *et al*., 1999).

Essential oils are produced by some 2000 plant species scattered throughout about 60 families of higher plants (Guenther, 1972). Important families for essential oils are:

1. Lamiaceae e.g. lavender, thyme savory, sage, mint
2. Myrtaceae e.g. eucalyptus, cajeput, niaouli
3. Apiaceae e.g. caraway, anise, fennel
4. Cupressaceae e.g. pine, cedarwood, cypress, juniper
5. Rutaceae e.g. lime, lemon, orange, bergamot
6. Asteraceae e.g. chamomile, yarrow
7. Burseraceae e.g. myrrh, frankincense
8. Lauraceae e.g. bay, rosewood
9. Poaceae e.g. lemon grass, vetiver
10. Geraniaceae e.g. geranium

In addition, essential oils are also known to occur in gymnosperms such as sandalwood, cardamom and ginger (Guenther, 1972).

Essential oils are ordinarily formed in special cells or group of cells, which may be present as scattered individual glandular cells or as glandular hairs, which are found in many leaves and stems. In these cases essential oils are contained as a globule or globules in cells. Locations of the essential oils are unique to each plant. Essential oils are found in different parts of the plant and also the composition differ with stage of plant development. Essential oils are found in special cells within the petals e.g. rose (Belaventseva, 1953), leaves e.g. eucalyptus (Panda and Panda, 1987), roots of grass e.g. vetiver (Nikiforov *et al*., 1992), bark e.g. cinnamon (Strauss, 1977), heartwood e.g. sandalwood (Brophy *et al*., 1991), citrus rind e.g. lemon (Boelens and Jimenez, 1989), seeds e.g. caraway (Gorunovic *et al*., 1991), rhizomes e.g. valerian (Hikino *et al*., 1969), bulbs e.g. garlic (Khoshoo *et al*., 1960) and the aerial or top parts of the plant e.g. marjoram (Brieskorn *et al*. 1952). Furthermore the compositions of essential oils vary depending on the part of the plant. Lavender for instance yields essential oils from both the flowers (Sharma *et al*., 1983) and the leaves (Guenther, 1972). The orange tree produces three different smelling essences with varying medicinal properties: the heady bittersweet neroli oil from flowers (Kekelidze *et al*., 1977); the similar though less refined scent of petitgrain from leaves (Kamiyama and Amaha, 1972) and the cherry orange from the rind of the fruit (Perse *et al*., 1977).

1.3 The chemistry of essential oils

1.3.1 Physical properties

Essential oils are volatile fragrant substances with an oily consistency, which plants produce. Even though they are technically classified as oil, plant essences are quite different from 'fixed' or fatty acids such as sunflower seed, corn or sweet almond oils. Essential oils are volatile (Guenther, 1972) which means they evaporate when left in open air and they do not leave a permanent mark on paper (Cook and Pickering, 1979). Essential oils can be more-or-less fluid and sometimes resinous (Guenther, 1972). Some essential oils are coloured and some are not. The colour of essential oils may vary from colourless e.g. peppermint essential oils (Guenther, 1979), yellowish e.g. lavender essential oils (Sharma *et al*., 1983), greenish e.g. bergamot essential oils (Guenther, 1979), amber e.g. palchouli essential oils (Balz *et al*., 1999) or dark brown e.g. vetiver essential oils (Nikiforov *et al*., 1992). With a few exceptions, essential oils are lighter than water and have a density between 0.75 - 0.98 g/cm^3 (Guenther, 1972).

1.3.2 Chemical properties

The chemical constituents of plants can be either due to primary metabolism or secondary metabolism. Primary metabolites are chiefly carbohydrates, amino acids and fixed oils produced by photosynthesis. Secondary metabolites arise from the primary metabolites such as glucosides, terpenoids, alkaloids and essential oils (Raven *et al*., 1999). It is the nature of these natural products and the proportion in which they are present that gives each essential oils its distinctive character (Guenther, 1972).

1.3.3 Main Components of Essential oils

The chemical components of essential oils can belong to 1 or more of the following classes of organic compounds (see Table 1). The structures of these are in section 1.3.3.1

1. Monoterpene hydrocarbons
2. Sesquiterpenes
3. Aromatic phenols
4. Alcohols
5. Epoxides and ethers
6. Esters
7. Aldehydes
8. Ketones
9. Aromatic and aliphatic acids
10. Lactones and coumarins
11. Miscellaneous.

Table 1 Main Components of Essential Oils.

Main component	General properties	Examples	Plants that are rich in these components
Monoterpene hydrocarbons	• quite prevalent in a large number of essential oils • builds the framework of essential oils • are general strengthening agents, stimulant and antiseptic. (Balz *et al*., 1999)	α-pinene (**1**), camphene (**2**), β-phellandrene (**3**), *d*-limonene (**4**), myrcene (**5**) and others	conifers (Hui *et al*., 1993), cypress (Chanegriha *et al*., 1993), eucalyptus (Panda and Panda, 1987), coriander Bhattacharya *et al*., 1998), lemon (Boelens and Jimenez, 1989) and others.
Sesquiterpenes	• rare but still found • anti inflammatory properties (Schnaubelt, 1999)	δ-cadinene (**6**), β-caryophyllene (**7**), β-selinene (**8**), germacrene (**9**) and others	cloves (Muchalal and Crouzet, 1983), cedar (Guenther, 1976) and others.
Aromatic phenols	• most important group • pharmaceutical value (i.e. antiseptic and germicidal properties due to their phenolic content) • are most popular flavouring ingredients	thymol (**10**), eugenol (**11**), carvacrol (**12**), chavicol (**13**) and others	origanum (Mockute *et al*., 2001), cloves (Muchalal and Crouzet, 1983), cinnamon (Rao *et al*., 1988) and others
Alcohols	• monoterpene alcohols . • diterpene alcohols • sesquiterpene alcohols • contain only a few saturated monohydroxy alcohols, lots of them being esterified with fatty acids • stimulate immune system • bacteriostatic and germicidal	menthol (**14**), nerol (**15**), phytol (**16**), sclareol (**17**), carotol (**18**), (E)-farnesol (**19**), citronellol (**20**), linalool (**21**), etc	lemongrass (Nigam *et al*., 1987), lemon (Boelens and Jimenez, 1989), lavender (Sharma *et al*., 1983), sandalwood (Brophy et. al., 1991), *Melaleuca* (Jreland *et al*., 2002) and others

Table 1: continued Main Components of Essential Oils

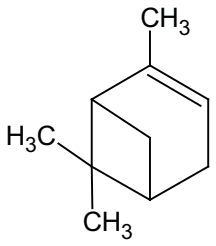
Main component	General properties	Examples	Plants that are rich in these components
Epoxides and ethers	• originate from phenol-methyl ether compounds • 1,8-cineol (**22**) is the most frequently occurring oxide • most important constituent of volatile oils	linalool oxide (**23**) methyl chavicol (**24**) and methyl eugenol (**25**) and others	eucalyptus (Panda and Panda, 1993), mint (Sievers *et al.*, 1945), basil (Maheshwari *et al.*, 1988), cloves (Muchalal and Crouzet, 1983) and bay laurel (Diaz-Maroto *et al.*, 2002) and others.
Esters	• widely distributed in essential oils • important constituents and contributing greatly to the character of their odour and flavour • and oil of sweet birch contain up to 99% of methyl salicylate. (**26**) (Proctor, 1944)	linalyl acetate (**27**), neryl acetate (**28**) and others	lavender (Sharma *et al.*, 1983), bergamot (Guenther, 1972), rosemary (Tucker and Maciarello, 1986) and others
Aldehydes	• with the exception of citral and citronella, aliphatic aldehydes do not play an important role in essential oils	benzaldehyde (**29**), cinnamaldehyde (**30**), vanillin (**31**), citral (**32**), citronellal (**33**) and others	cumin (Jiao *et al.*, 1990), anise (Tkachenko *et al.*, 1999), cinnamon (Rao *et al.*, 1988), bergamot (Guenther, 1979), eucalyptus (Panda and Panda, 1987) and others
Ketones	• several types of ketones. Monotones, diketones, terpene ketones and cyclic and acrylic ketones	carvone (**34**), thujone (**35**), menthone (**36**) and others	caraway (Gorunnovic *et al.*, 1991), mint (Sievers *et al.*, 1945), rosemary (Tucker and Maciarello, 1986) and others

Table 1: continued Main Components of Essential Oils

Main component	General properties	Examples	Plants that are rich in these components
Aromatic, aliphatic acids containing terpene	• are acid strongly oxidised substances • relatively water-soluble and react with alcohol with the formation of esters • are generally present in small amounts	benzoic acid (**37**), cumic acid (**38**), geraniol acid (**39**) and others	ylang-ylang (Duve *et al*., 1975), cumin (Jiao *et al*., 1990), juniper (Bonaga and Galletti, 1985) and others
Lactones and coumarins	• are quite widely distributed in nature • the most important members of this class occurring in essential oils are the coumarins	coumarin (**40**), umbelliferone (**41**) and others	bergamot (Guenther, 1979), citrus rind peel oil (Perse *et al*., 1977), lavender (Sharma *et al*., 1983) and others
Miscellaneous	• are incidental • specific to few species	allyl isothiocyanate (**42**), allyl sulfide (**43**), indole (**44**) and others.	mustard oil (Yu *et al*., 2003), garlic oil (Khoshoo *et al*., 1960) and jasmine (Ramachandriah and Narasimha, 1984)

1.3.3.1 Structures of the various components present in essential oils mentioned in Table 1

(a) Monoterpene hydrocarbons



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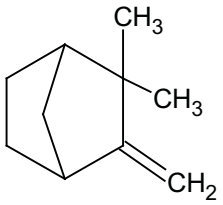

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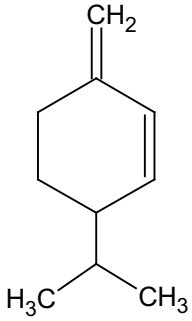

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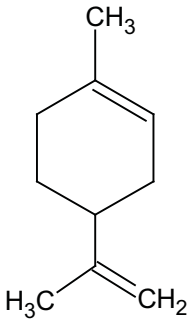

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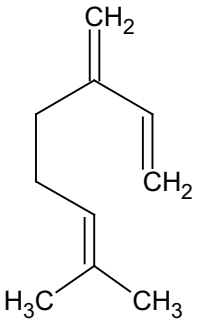

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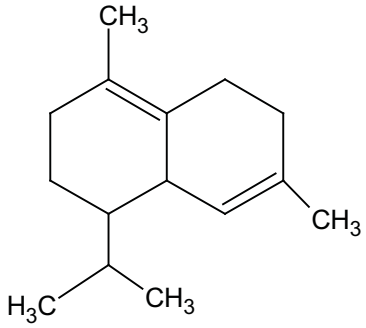
(b) Sesquiterpenes



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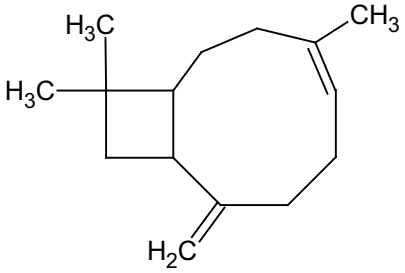

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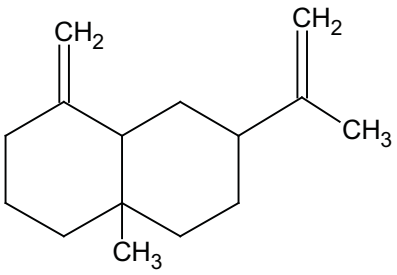

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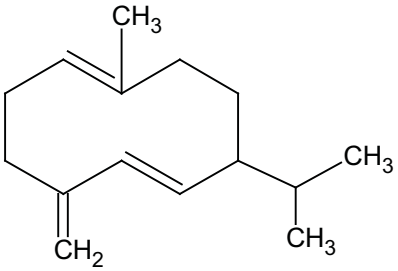

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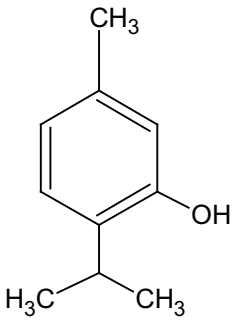
(c) Aromatic phenols



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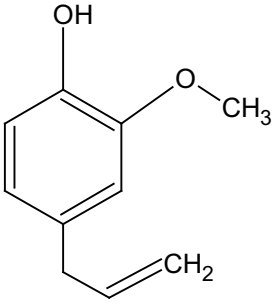

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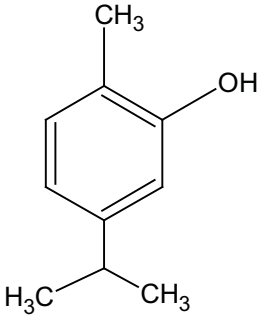

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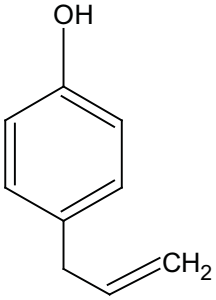

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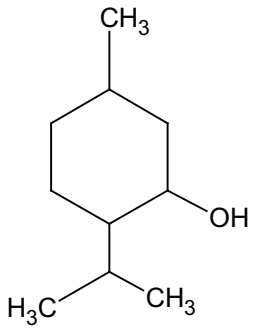
(d) Alcohols



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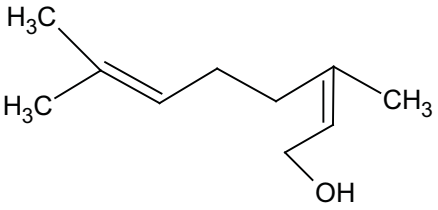

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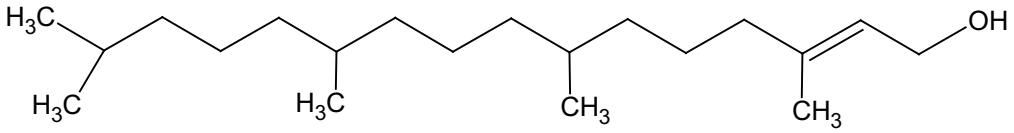

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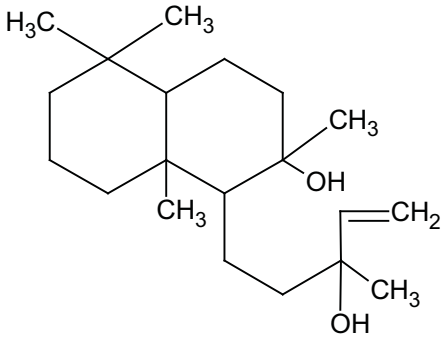

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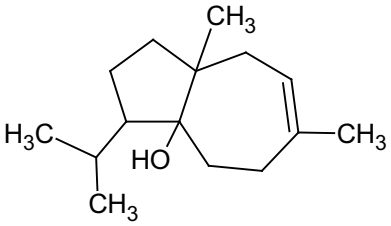

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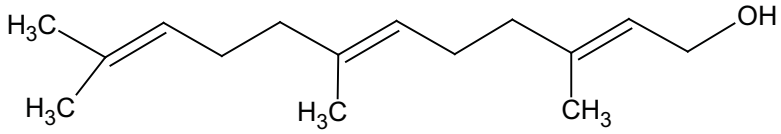

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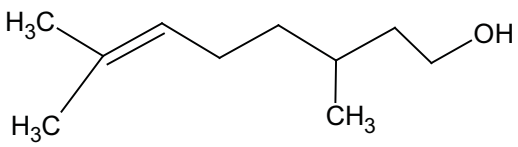

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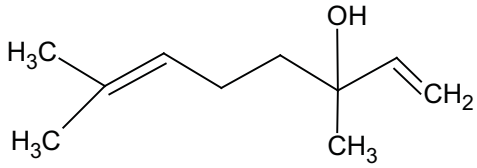

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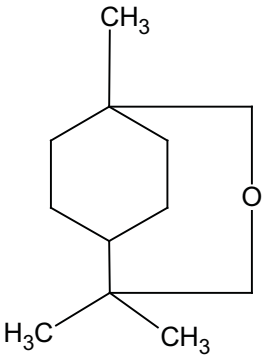

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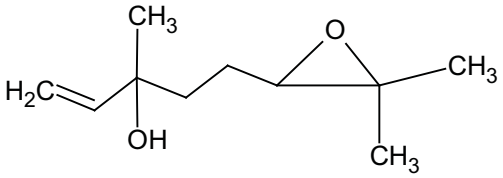

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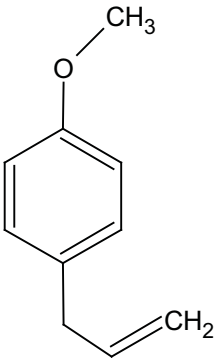

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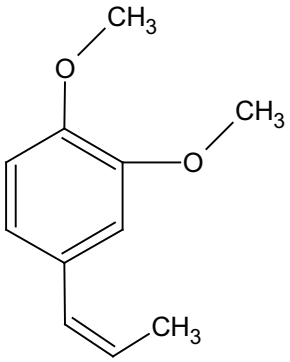

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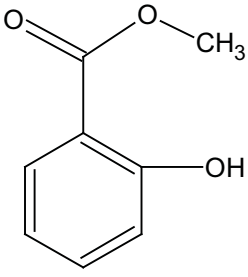

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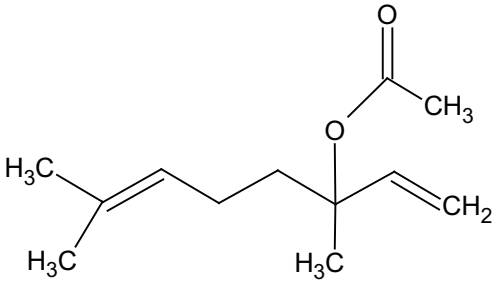

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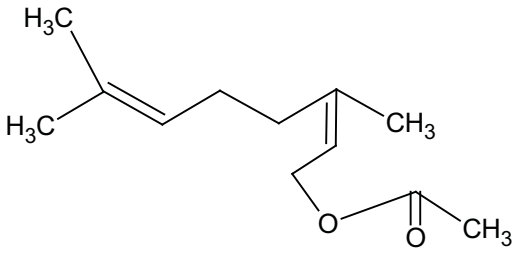

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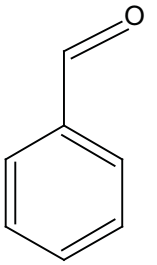

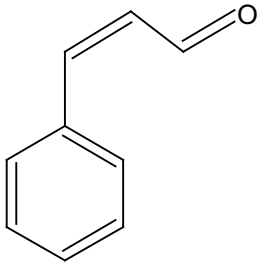

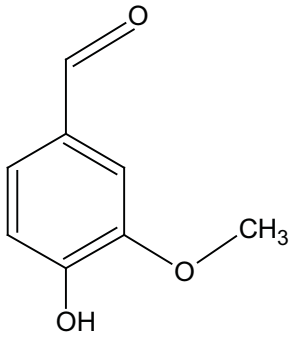

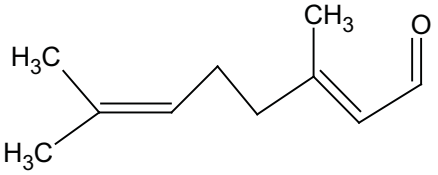

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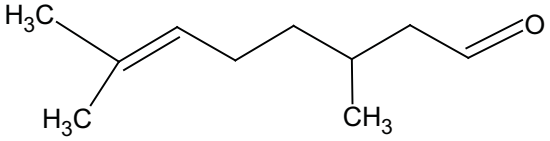

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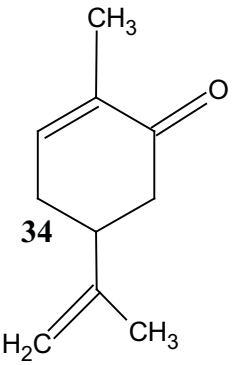

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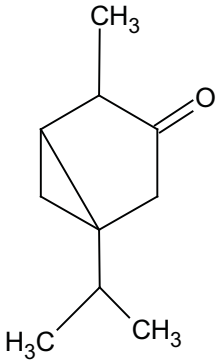

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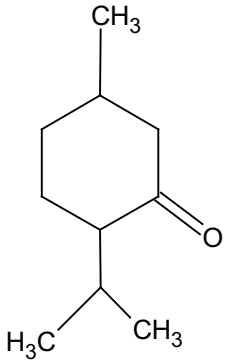

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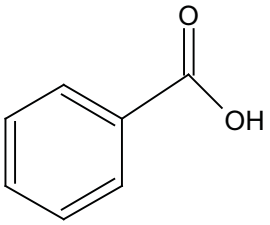
(i) Aromatic and aliphatic acids



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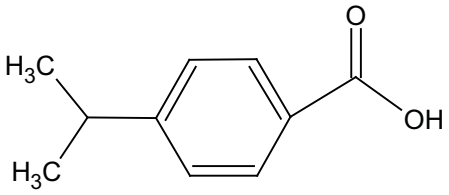

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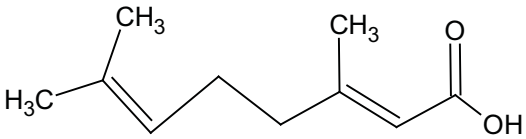

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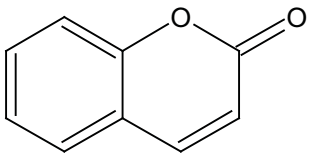
(j) Lactones and coumarins



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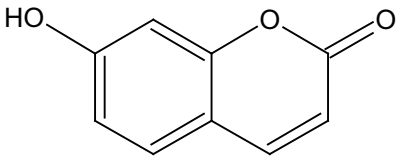

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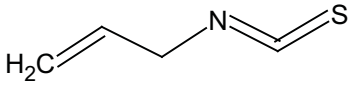
(a) Miscellaneous



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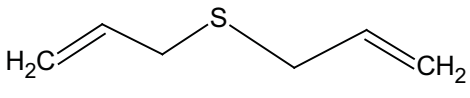

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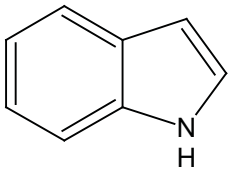

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1.4 Extraction

Our ancestors recognised that many of the pleasing fragrances and flowers associated with plants were due to the essential oils. It was discovered that by squeezing or gently heating certain plant parts, the essential oils could be isolated and these were used as perfumes (Balz *et al.*, 1999).

While most essential oils are distilled from plant material, the type of plant, the unique makeup of its essential oils and the location within the plant determine the extraction process. There are 3 main process namely expression, steam distillation and solvent extraction. Expression is the best process for the isolation of citrus essential oils because the essential oils are found in tiny sacs just inside the fruit skin. Steam distillation is the most common method and most plant essential oils yield to this process, especially herbaceous plants (rosemary and cinnamon). Most flower essences such as jasmine and carnation are too delicate for distillation and solvent extraction is the only method that allows the recovery of these essential oils.

1.4.1 Steam distillation

Steam distillation is normally the main method used for extracting essential oils from plants. It would be more correct to call this common procedure "steam passage" rather than steam distillation (Bruneton, 1999). Steam distillation is based on the principle that when plant material is placed in boiling water, the essential oils in the plant will evaporate with steam (Bruneton, 1999). Once the steam and essential oils have condensed, the essential oil will separate from the water and can be collected. Plants are crushed to encourage the release of their essential oils (Balz *et al*., 1999). When plants are boiled in water, essential oils vaporise and rise up with the steam. The vapours are allowed to condense. Current methods also involve placing the plants on a screen and steam passed through them (Guenther, 1972).

1.4.2 Solvent extraction

This method is used for delicate flowers whose odours are damaged by high heat that is needed to boil water for steam distillation. Essential oils are extracted using solvents, which have lower boiling points than water, various substances such as ether or high-grade petroleum, which evaporate rapidly are used in modern perfumery to dissolve essential oils from fragrant plant materials (Balz *et al*., 1999). The method involves placing the fragrant plant materials on perforated metal plates in a container, the solvent is then passed over the fragrant plant materials and led into a still where the solvent is evaporated leaving a semi-solid mass containing the essential oils together with stearoptene (Balz *et al*., 1999). Essential oils can be then separated from the stearoptene

by extraction with alcohol on producing the substance called “absolute”, the purest and most concentrated form of essential oils known (Guenther, 1972).

Another method is where the fragrant plant materials are placed in chemical solvents in order to obtain the volatile substances (Balz *et al*., 1999). Essential oil extracted in this manner are mixed with solvents from which they must be separated by fractional distillation (Balz *et al*., 1999). However, this method never shows satisfactory results as trace solvents (hexane, acetone, methanol or iso-propanol) always remain in the essential oils (Balz *et al*., 1999). Essential oils extracted with the help of solvents cannot be used for therapeutic or hygienic purpose (Balz *et al*., 1999).

1.4.3 Enfleunage

The “enfleunage” method is where fatty substances are used to absorb the essential oils, which is then separated for cosmetic products since the fat loaded with essential oils can be processed into ointments and creams (Guenther, 1972). This technique is very costly and is rarely used today. It is a labour-intensive process that yields the highest quality of “absolute” because it does not involve heat (Guenther, 1972). Heat always alters the fragrance. This method is used on delicate flowers that cannot stand up to the high heat and on flowers that continue to release essential oils long after they have been picked (Balz *et al*., 1999). Enfleunage works on the principal that fat absorbs odour. Petals or other fragrant parts of the plant are steeped in fat or non-evaporating oil, which will absorb their fragrance. This process is repeated several times with fresh flowers until the fat is totally absorbed with essential oils. The essential oils are then retrieved from the fat by dissolving in an alcoholic solvent (Balz *et al*., 1999). This fat with essential oils is

mechanically mixed with alcohol for up to one week and is chilled to 68 ^{o}F (Guenther, 1972). The essential oils dissolve in the alcohol and the fat does not. The mixture is then chilled and filtered several times to remove all the fat. The alcohol is then evaporated to leave the pure absolute (Guenther, 1972).

1.4.4 Expression

This is a simple technique where the rinds of citrus fruits are cold pressed to extract their essential oils using rollers and sponges (Balz *et al*., 1999). There is no heat involved, thus leaving the essential oils with an odour very close to the original fruit (Guenther, 1972).

1.4.5 Supercritical Fluid Extraction

When carbon dioxide (CO_2) is subjected to high pressure, the CO_2 gas turns into liquid. The liquid CO_2 can be used as a very inert safe "liquid solvent" which will extract the essential oils in a process which is similar to that used to extract "absolutes" (Reverchon, 1997). The advantage of this method is that no solvent residue remains (Reverchon, 1997). Since at normal pressure and temperature, CO_2 simply reverts to gas and evaporates. Essential oils extracted by supercritical fluid extraction have a richer and more intense odour than steam distilled essential oils (Reverchon, 1997).

1.4.6 Phytonic Extraction

This method uses non-CFC (chlorofluro carbon) gaseous solvents and refrigerants hydro flurocarbon 134a now called florasols. The benign gaseous solvents are used for extraction at or below room temperature (Extraction technology, 2005). The advantage is that no heat is used so no thermal degradation of essential oils occurs.

1.4.7 Microwave Assisted Hydro-Distillation

In this method the plant material is placed in a microwave reactor, heat is applied in the form of microwave energy. The internal heating of the *in situ* water within the plant material ruptures the plant cells containing the essential oils, this process frees the essential oils, which evaporates with the *in situ* water (Presti *et al*., 2004). Condensation occurs outside the microwave in the condenser and essential oils are separated from water. Microwave extraction has advantages over other methods such as low costs, the greatly reduced extraction time (18 - 30 minutes against 4 - 6 hours for steam distillation) and the attainment of high quality essential oils as thermal degradation of essential oils are avoided due to less time of extraction (Presti *et al*., 2004).

1.5 Methods used to analyse essential oils

Essential oils may contain as many as 300 different components. Majority of these components do not contribute to the odour and those that do may be present in very low concentrations. Modern analytical techniques enable chemist to separate individual components and identify the key odorous chemicals of essential oils.

Chromatographic techniques are used to separate components while spectroscopic techniques give information about the molecular structure of components present in essential oils.

1.5.1. Gas Chromatography (GC)

This method is commonly employed for routine quality checks of essential oils. Mixtures are separated while they are carried through capillary columns by a carrier gas (Schomburg, 1990).

1.5.2 High Performance liquid Chromatography (HPLC)

This is a very powerful method, which is complementary to GC method and is very useful for essential oil analysis (Hanai, 1999).

1.5.3 Mass Spectrometry (MS)

"Molecules are subjected to high-energy electrons, which cause the molecular ions to fragment to ions. Mass spectrometry records the masses of all different fragments into which the molecular ion fragments. The exact path of fragmentation allows almost unambiguous identification of chemical structures. Consequently competent

interpretation on unknown mass spectra are highly involved and time consuming. In combination with GC (GC-MS), it is very powerful, complex and expensive tool for essential oil research" (Constantin and Schnell, 1990).

1.5.4 Enantioselective gas chromatography (ESGC) and isotope ratio mass spectrometry (IRMS)

"These two analytical methods allow for some of the best assessment of authenticity of essential oils. Many of the most common molecules occurring in essential oils are "chiral" meaning they exist in two enantiomeric forms. Natural essential oil is characterised by distinctive patterns. In the case of monoterpene hydrocarbons this is seen in the distribution of their plus (+) or minus (-) enantiomeric forms. The enantiomeric ratio of the constituents of essential oils of same species but of different origins is similar but not identical. If the specific enantiomeric ratio for a plant essential oil is known, adulteration of essential oil with compounds emanating from the laboratory can be detected. IRMS utilises another endogenous parameter of biosynthesis to ascertain authenticity of essential oils or an essential oil component. When plants photosynthesise they distinguish CO_2 from the common carbon isotope and CO_2 with a heavier isotopes. Organically bound carbon is deficient in heavier isotopes compared to the CO_2 in the air. This deficit is not identical for all plants but is dependent on its type of photosynthesis. The ratio of carbon isotopes in essential oils or their components is often specific for a given plant and these different ratios can be utilized to distinguish between substances or synthetic and natural origin as well as between substances of different natural origins" (Hoffmann *et al*., 1996).

1.5.5 Headspace analysis

There are 2 reasons for the use of headspace analysis and they are insufficient quantities of plant material and essential oils extraction process may give a essential oil whose fragrance may not reflect the fragrance of original plant (Ioffe *et al*., 1984).

In headspace analysis (Figure 1) plant material is enclosed in a container connected to a filtered air inlet and an outlet. The outlet has a "trap" to collect the odorous, volatile molecule given off by the plant material and a pump to draw air and odorous molecules through. When enough odour molecules have been collected, the "trap" is removed and the molecules are set free by heating or using a solvent. The analytical chemist and perfumer then analyse using GC-MS and GC-sniffing (Ioffe *et al*., 1984). The advantage of headspace analysis over conventional extraction is that the plant remains intact in headspace analysis (Ioffe *et al*., 1984).

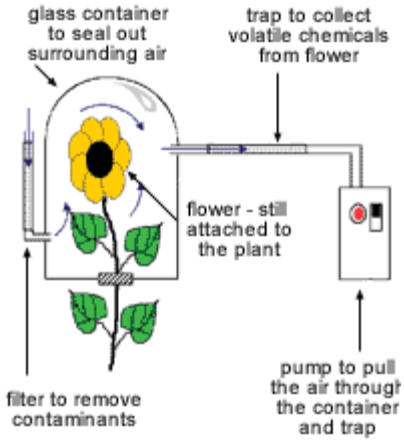


Figure 1:Headspace apparatus

1.5.6 Solid Phase Micro-extraction (SPME)

SPME is a simple effective adsorption technique that eliminates the need for solvents and complicated apparatus for concentrating volatile compounds in liquid samples or headspace (Pawliszyn, 1994). SPME is compatible with gas chromatography. In SPME process there is a length of silica fibre coated with a polymer and a plunger installed in a holder and looks like a micro-litre syringe (Pawliszyn, 1994). The fibre is exposed to a sample in a vial or the headspace above the sample where essential oils are adsorbed onto

the polymer coating on the fibre. After adsorption equilibrium is attained, the fibre is removed and injected into the gas chromatograph injector for analysis (Pawliszyn, 1994).

1.5.7 GC and GC-MS

Either gas chromatography (GC) or a combination of gas chromatography and mass spectroscopy (GC-MS) are commonly used for analysis of essential oils. GC and GC-MS are powerful methods for routine analysis, quality control and research. Due to varying parameters such as different machines, column flow rates, temperature programming and other different column parameters such as column type, column length, column internal diameter; chromatograms of a particular essential oil can only be compared under identical conditions of experiments (Middleitch, 1979). Gas chromatography itself does not identify the substances that cause a given peak. Identity can be confirmed with authentic samples (Middleitch, 1979). Given the complexity of essential oils compositions such identifications (identity with authentic samples) are often ambiguous and mass spectroscopy (MS) methods must be used to identify a component with precision. First the essential oils are separated by gas chromatography, and then the separated components are identified from their mass spectral fragmentations (Rouessac and Rouessac, 2000). Interpretations of mass spectra are tedious and hence they are done by computerised reference libraries. The identification is usually based in retention time and retention indices. Retention time is dependent on temperature and retention indices have an advantage over retention time in that retention indices are in a standard numerical form and relatively invariable over a moderate range of temperature (Rouessac and Rouessac, 2000).

1.5.8 Raman Spectroscopy

"GC coupled with FID or MS methods of analysis for essential oils are time consuming and expensive so vibrational spectroscopy methods [e.g. NIR (near infrared) and ATR-IR (attenuated total reflection infrared)] have been introduced. Raman spectra in most analysis of essential oils present characteristic key bands of individual volatile fraction and therefore in theory allow easy identification of different plant species or chemotypes. Advantages of Raman spectroscopy are the minimal time required for sample preparation and small amounts of essential oil for analysis. Also Raman spectroscopy also allows non-destructive identification of various components in fresh plant materials. NIR FT (near infrared Fourier Transform) is valuable tool for *in vivo* experiments because fluorescence and thermal decomposition of the plant material is reduced to a minimum" (Schulz and Baranska, 2004).

1.6 Uses of essential oils

1.6.1 The function of essential oils in plants

Although sometime essential oils are designated as "waste products" of plant metabolism, studies have shown that plants utilise essential oils for many purposes. Essential oils are secreted in various organs such as closed cells and intercellular spaces or oil glands. Essential oils may either remain there or pass out from the cells into other organs or diffuse into the atmosphere. Essential oils, mainly terpenoids function as attractants for insect pollinators (James, 2003) or to repel insects and other herbivorous animals to prevent the eating of leaves, barks or flowers (Peterson *et al*., 2002). Some essential oils protect plants against microbial infection or growth of other plants, which may be competing for the same habitat. For example α- and β- pinene, limonene and citronellol inhibit the growth of *Amaranthus retrofleuxs* near the orange tree, *Citrus aurantium* (Steele, 1949).

Essential oils from barks may protect plants from insect attacks, as some of the essential oils are deterrents and even insecticides. Timbers of certain species of *Callitris* are immune to the attack of termites due to the presence of a peculiar phenol to which the odour of wood is due to (Steele, 1949). In conifers, terpenoids are stored in resin ducts can have devastating impacts on small herbivores (Gershenzon and Croteau, 1991). Also in conifers, oleoresin, a complex mixture of terpenoids is an important defence against bark beetles and their associated fungal pathogens (Langenheim, 1994). In angiosperms, glandular trichomes that contain terpenes often serve as a first line of defence against would-be herbivores.

A number of herbivores recycle terpenoids, using them for protection without sequestering them, they secret the terpenes and this secretion is used to ward off predators. Some species of the plant genus *Bursera* produce a resin after insect damage; specialist herbivores in the genus *Blepharida* utilise this resin by adoring their backs with a regurgitant and an anal secretion that match the host's defence chemistry (Evans *et al*., 2000).

One of the most exciting recent discoveries involving volatile terpenes and signalling to predators and parasitoids involves the apparently ubiquitous induction of homoterpenes in response to herbivore damage (Degenhardt and Gershenzon, 2000).

The herbivorous mites (*Tetranychus urticae*) induce the release of linalool in *Phaseolus lunatus* (lima beans) following infestation. The herbivore is repelled by this compound but no so by the predatory mite, *Phytoseiulus persimilis* (Dicke, 1986). This is an example of a plant defending itself from predators.

The wood of western red cedar (*Thuja plicata*) is renowned for its ability to withstand insect attack both in timber stands and as saw mill products. This resistance has been due to the presence of monoterpenes including β-thujaplicin, which was found to be highly insecticidal in tests against larvae of the old house borer *Hylotrupes bajulus* (Cerambycidae), a common pest of structural timber in Europe (Becker, 1965).

1.6.2 Uses of essential oils by humans

Schnaubelt (1999) stated that essential oil extracts have traditionally been used to maintain or restore the beauty of healthy skin. Indeed, modern scientific investigations have shown that many of the claimed beneficial effects of such essential oils are with some foundation.

Many of the healing promoting qualities of essential oils are based upon their ability to cleanse the skin. In addition to their cleansing qualities many essential oils have antibacterial properties. These properties are often due to the presence of compounds called phenols, which combine with skin protein to create antiseptic conditions. Essential oils of thyme are especially effective in this regard. Wounds and even small scratches often leave scars and essential oils of lavender are often used for this purpose. Essential oils such as menthol are effective in stimulating flow of blood in the skin and producing a warm glowing sensation. Citronella oil is valued as an insect repellent and for the treatment of insect bites. Menthol, ethers of thymol and eucalyptus oil for example are often found as ingredients in perfumed sprays used to ease breathing complains such as bronchitis and whooping cough. Most essential oils consist of hundred of components such as terpenes, alcohols, aldehydes and esters. For this reason, a single essential oil can help in a wide variety of disorders. Lavender essential oil for instance is endowed with antiseptic, antibacterial, antibiotic, antidepressant, analgesic, decongestant and sedative properties. Essential oils have a pronounced effect on the brain limbic system that affects our moods. For this reason, essential oils have historically seen heaviest use in the soap and perfume industry. Essential oils have pharmacological properties and the fundamental properties that stand out are antiseptic activity, spasmolytic and sedative

properties and also irritating properties. Today there are many ointments, creams or gels based on essential oils and designed to relieve sprains, soreness, strains and muscular pains. When administered internally, essential oils are thought to trigger "irritation" process at different levels

Some examples of the usage of essential oils by humans are as follows:

Calcabrini *et al*. (2004) reported that "tea tree" oil (*Melaleuca alternifolia*) and terpinen-4-ol (main active component) were able to impair the growth of human M14 melanoma cells and appeared to be more effective on their resistant variants. Farag *et al*. (2004) reported the chemical and biological evaluation of the essential oils of different *Melaleuca* species and reported that *M. ericifloia* exhibited highest inhibitory affects against *Bacillus subtilis* and *Aspergillus niger*. Essential oils of *M. armillaris* were effective as a virucidal for Herpers Simples virus type 1 (HSV-1). It was also reported that essential oils of *M. armillaris* exhibited a marked antioxidant effect and it improved vitamin E, vitamin C and super-oxide dismutase parameters so it can be used as a free radical suppressor.

Benoit-Vical *et al*. (1999) reported that *Cochlospermum planchonni* leaf essential oils yielded the best antimalarial affect. While Lopes *et al*. (1999) reported that essential oils from leaves of *Virola surinamensis* caused 100 % of growth inhibition after 48 hours in the development of the young trophozoite to schizont stage and the sesquiterpene nerolidol was identified as one of the active constituents.

Asekum and Adeniyi (2004) reported that fruit essential oils of *Xylopia aethiopica* showed inhibition activity against 4 micro-organisms and cytotoxicity to carcinoma cells (Hep-2 cell line).

Essential oils have also shown antibacterial activities. This can be seen from work done by Nevas *et al*. (2004), which showed antibacterial properties of 13 essential oils derived from spices grown in Finland for both spoilage and pathogenic bacteria. Skaltsa *et al*. (2003) reported that essential oils from 8 *Stachys* species from Greece showed inhibition activity against bacterial species than against fungal species. Ngassoum *et al*. (2003) reported that essential oils from dried fruits of *Zanthoxylum xanthoxyloids* showed extensive inhibition zones. Araujo *et al*. (2003) reported that essential oils from aerial parts of *Melissa officinalis* showed marked fungicidal effect against 5-food spoilage yeast. Sokemn *et al*. (2004) reported that essential oils obtained from *Origanum acutidens* exhibited strong anti microbial activity with a significant inhibitory affect against 27 of the 35 bacteria, 12 of the 18 fungi and a yeast that were tested.

1.6.3 Essential oil products

The 3 main uses of essential oils are:

(a) Pharmacy: The vast majority of drugs are crude, especially for the preparations of infusions. Essential oils are also used for therapeutic interest but the major part of essential oils is the aromatisation of medications for the oral route (Bruneton, 1999).

(b) Perfumery: This is the main outlet for essential oils, concrete, absolutes and other resinoid. Cosmetology and hygiene product industry are also consumers, even though the cost of natural products is often high and leads to the selection for mass production of synthetic alternatives (Bruneton, 1999).

(c) Food technology: The primary benefit of essential oils in food technology is the uniform quality and lack of colour making essential oils popular in food preparations. Some plants are used raw (herbs and spices), others are used as essential oils, resinoids or oleoresins. Although in the past few decades' refrigeration has replaced spices to ensure the conservation of foods. The development of new culinary practices, a taste for exotic, the flavour (or the lack of flavour) of the products from intensive agriculture and other factors have led to a rapid increase in the consumption of natural flavours. All sectors of food technology use essential oils: alcoholic and non-alcoholic beverages, confectionery, dairy products, meat products, sauces, soups, snacks, bakery products and animal foods (Bruneton, 1999).

Balz *et al*. (1999) mentioned that essential oils find amazingly wide and varied applications in many industries. The following list shows some of the various industries

or manufactures that employ essential oils, aromatic isolates or combinations; adhesives, automobile industries, canning industries, chewing gum industries, condiment industries, dental preparations, exterminators and insecticide suppliers, extract industries, household products, ice-cream industries, janitors suppliers, plant industries, paper and printing industries, soap industries and many more.

Most of the industries mentioned above use essential oils in addition to synthetic products, for example turpentine from pines, sclareol and citral of *Litsea cubeba* from China, geraniol of palmarosa (*Cymbopogon martinii*), (+)- and (-)-linalool, (+)-citronella, eugenol and safrole (Bruneton, 1999).. These are starting materials for the synthesis of active principles of medicine, vitamins and fragrances. One example is the use of safrole (extracted from Brasillia *Ocotea* or from *Cinnamomun* species from China) to synthesize the heliotropin used in perfumery or piperonyl butoxide, a pyrethrinoid synergist (Bruneton, 1999).

1.6.4 Toxicity of essential oils

Essential oils are natural products but it is still necessary to follow certain precautions when using essential oils. Essential oils are highly concentrated substances thus their safe use is vital. The dangers of essential oils depend on the dosage, frequency of use, properties of the essential oil and the method of application (Balz *et al*., 1999). The application of undiluted essential oils directly to the skin can cause burning of the skin, photosensitivity and skin irritation (Bruneton, 1999). Acute toxicity is rare with essential oils unless essential oils are ingested in large quantities (Balz *et al*., 1999). In skin toxicity, essential oils can cause irritation, sensitising and photosensitisation.

Photosensitisation occurs when essential oils are applied on to the skin and then the essential oils react with ultra violet light (Bruneton, 1999). Essential oils are also known to have carcinogenic effects. Several allyl- and propenyl- phenols are capable of inducing cancer in rodents e.g. safrole induces the formation of hepatic tumours in rats (Tisserand and Balacs, 1995).

1.7 Aim

This study is in two parts, A and B

The first part (Part A) of the thesis:

- To analyse and report the essential oils compositions of Fijian Plants (*Eryngium foetidum, Coriandrum sativum, Cinnamomum verum, Alpinia zerumbet* and *Spondias dulcis)* using GC and GC-MS.
- To report the results of microbiological investigations of the essential oils from the following Fijian plant species (*Melaleuca quinquenervia, C. verum, A. zerumbet*, *Pimenta racemosa, Coleus amboinicus, and Cymbopogon coloratus*).

The second part (Part B) of this thesis:

- To isolate pure compounds from the Fijian plant species *Alpinia zerumbet*. This particular species was chosen for a detailed phytochemical study since essential oils from this Fijian plant species was also being investigated in Part A.

2.0 Background of the plant species investigated

2.1 *Eryngium foetidum*

E. foetidum (Figure 2) is from the family Umbelliferae. It is a coarse biannual herb, with leaves at the basal and the leaves have sharp pointed spines (Parham, 1972). *E. foetidum* is abundant near sea level as a naturalised weed in waste places, cultivated areas and along roadsides (Smith, 1985). *E. foetidum* has a strong aroma, very similar to fresh coriander leaves and the taste is similar though slightly stronger. The leaves are used to flavour curries and can be used as a substitute for coriander due to the same aroma. The common names are wild coriander, thorny coriander, false coriander or long coriander.

Figure 2: *E. foetidum*

2.1.1 Essential oil

Leaf essential oils of *E. foetidum* have been investigated previously. Leclercq *et al*. (1992) analysed the essential oils of the aerial parts of *E. foetidum* from Vietnam and identified 19 constituents mostly aldehydes and carboxylic acids, of which major components were (E)-2-dodecenal (45.0 %), 2-dodecenoic acid (15.5 %), dodecenoic acid (8.6 %) and (E)-2-tetradecenal (5.3 %). The leaf essential oils of Cuban *E. foetidum* showed the presence of 46 components, major components were 2,4,5-trimethylbenzaldehyde (20.53 %), hexadecanoic acid (12.05 %) and caratol (9.44 %) (Pino *et al*., 1997). Wong *et al*. (1994) analysed essential oils isolated by steam distillation from the leaves of *E. foetidum* and alkanals and alkenals constituted the main chemical composition with (E)-2-dodecenal was the most abundant component (59.72 %). It appears from the above literature that the leaf essential oils composition of *E. foetidum* varies with the origin of the plant.

2.2 *Coriandrum sativum*

C. sativum (Figure 3) is also from the family Umbelliferae like *E. foetidum*. It is an annual herbaceous plant and a coarse herb with basal trifoliate leaves (Parham, 1972). *C. sativum* is native to Southern Europe and Western Mediterranean region but this herb is now cultivated widely in the world. *C. sativum* is an introduced species in Fiji, cultivated in small scale and is a weed in agricultural lands. *C. sativum* is a highly aromatic herb with the leaves and seeds both used in cooking. The leaves are used in chutneys and sauces. The whole herb is also used for medicinal purpose as it helps sooth indigestion and essential oils from this pant is a muscle relaxant and an antiseptic (Schnaubelt, 1999). The common names for this species are coriander or *dhania* (Indian).

Figure 3: *C. sativum*

2.2.1 Essential oil

Analysis of *C. sativum* leaf essential oils has been reported less when compared to reported work on *C. sativum* seed essential oil. Potter and Fagerson (1990) reported that *C. sativum* leaf essential oils had 41 components and composed mainly of (E)-2-decenal (46.6 %). Other principal components reported by Potter and Fagerson (1990) were (E)-2-tetradecanal, 1-decanol, decanal, (E)-2-undecanal, (E)-2-dodecanal and 2-decen-1-ol. MacLeod and Islam (1976) reported that the leaf essential oil had a series of unsaturated aldehydes in the C_8-C_{15} range. Carlbolm (1936) reported that decanal, 2-decenal and 8-methyl-2-noneal were the major components of *C. sativum* leaf essential oils.

2.3 *Cinnamomum verum*

Figure 4: *C. verum*

C. verum (Figure 4) is from the family Lauraceae and is a small evergreen aromatic tree growing wild in Fiji (Smith, 1981). This species was introduced in Fiji for commercial purpose. This species is used as a spice, condiment for flavouring of foods, in incense and perfumes. The essential oils from barks and leaves are also used for the same purpose (Parham, 1972). The essential oils are used as an anti microbial agent, due to high eugenol content (Mallavarup *et al*., 1995). The common name for this species is *macovu* (Fijian).

2.3.1 Essential oil

Reported work in *C. verum* leaf essential oils is limited, though lots of work has been done on other *Cinnamomum* species. Nath *et al*. (1997) identified 12 components in *C. verum* leaf essential oil with eugenol as the major component (90.0 %), other components were linalool (2.4 %) and cinnamaldehyde (1.1 %).

2.3.2 Microbiological Activity

Antibacterial and antifungal activities of *C. verum* essential oils have been demonstrated *in-vitro* experiments. Essential oils of *C. verum* were active *in-vitro* against the following bacteria; *Bacillus subtilis, Escherichia coli, Staphylococcus aureus* (Janssen *et al*., 1986) and against the following fungi; *Candida albicans* (Conner and Beuchat, 1984). The antibacterial and fungicidal effects have been attributed to o-methoxycinnamaldehyde (Bisset, 1994).

2.4 *Spondias dulcis*

S. dulcis (Figure 5) is a member of the family Sapindaceae, a tree that grows up to 25 m with whitish bark and has edible fruits that are similar in appearance to unripe mango (Parham, 1972). Fruits are used to make jellies and jams, while leaves are used to flavour meat (Smith, 1985). The local name for this species is *wi* (Fijian) or *amra* (Indian).

Figure 5: *S. dulcis*

2.4.1 Essential oil

There was no reported work on the analysis of *S. dulcis* leaf essential oils.

2.5 *Alpinia zerumbet*

A. zerumbet (Figure 6) is from the family Zingiberaceae and ginger is another member of this family (Parham, 1972). *A. zerumbet* is a well-known plant in Fiji. The flowers are shelled shaped with pink tips (Smith, 1988) and used for decorations and ornaments. The local name is shell ginger. There is no traditional use of *Alpinia* species in Fijian traditional medicine.

Figure 6: *A. zerumbet*

2.5.1 Essential oil

There is only 1 reported work on essential oils from Fijian *A. zerumbet.* The paper reported 10 components from the rhizome and leaf essential oils. The main components were β-pinene, 1,8-cineol and terpinene-4-ol (Ali *et al*., 2002).

2.5.2 Microbiological Activity

There was no reported work on the antibacterial, antifungal, antimalarial and antidengue activity of the *A. zerumbet* leaf, rhizome and flower essential oils.

2.6 *Melaleuca quinquenervia*

M. quinquenervia (Figure 7) is from the family Myrtaceae and is an introduced species in Fiji Islands (Smith, 1985). *M. quinquenervia* tree can grow up to 25 meters and the trunk is characterised by exfoliating layers of whitish or brownish bark. Leaves of this plant are traditionally used to disinfect water in some places, though overall the tree is used for timber (Smith, 1985). The common names are *niaouli* (New Caledonia) and v*uni veva* (Fijian).

Figure 7: *M. quinquenervi*

2.6.1 Essential oil

The *M. quinquenervia* is known to exist in 2 chemotypes. First chemotype contains large amounts of (E)-nerolidol, while the second chemotype contains large amounts of 1,8-cineole. Other components of *M. quinquenervia* leaf essential oils are (E,E)-farnesol, β-farnesene, linalool, β-carophyllene, α-pinene, myrcene and limonene (Brophy and Doran, 1996).

2.6.2 Microbiological Activity

The *M. quinquenervia* essential oils have shown many biological activities. The commercial samples of *M. quinquenervia* essential oils have shown antibacterial activity against *B. subtilis, S. aureus* and as antifungal agent against *C. albicans* (Christoph *et al*., 2000). *M. quinquenervia* essential oils have also shown anti pyretic activity in rats (Aboutable *et al*., 1996).

2.7 *Pimenta racemosa*

P. racemosa (Figure 8) is also from the family Myrtaceae like *M. quinquenervia*, it is an introduced and now naturalised species in Fiji. The tree can grow up to 12 meters high. Essential oils distilled from leaves and twigs are used in perfumery and in preparation of bay rum, a fragrant cosmetic and medicinal liquid. The tree is also considered useful for ornaments and shade (Smith, 1985). The local name for this species is *cinimoni* (Fijian).

Figure 8: *P. racemosa*

2.7.1 Essential oil

Bello *et al*. (1995) analysed essential oils from the leaves of *P. racemosa* growing in western Cuba and identified 26 components with 1,8-cineol and terpinene-4-ol as the major components. On the other hand Garci'a *et al*. (2004) identified 23 components of which α-terpineol acetate, α-terpineol and 4-methoxy eugenol were found to be the major components from the leaves of the same plant species.

2.7.2 Microbiological Activity

Essential oils from *P. racemosa* showed antifungal activity against the *Microsporum canis* (Chaumot and Bardey, 1989) and *C. albicans* (Hammer *et al*., 1998). *P. racemosa* essential oils have also shown antibacterial activity against *E. coli* and *S. aureus* (Janssen *et al*., 1986).

2.8 *Coleus amboinicus*

C. amboinicus (Figure 9) is from the family Labiate, it is an introduced and now naturalised species in Fiji (Parham, 1972). This species was previously listed as *Salvia officinalis*. *C. amboinicus* is a useful potherb, semi prostate with flesh pubescent, aromatic leaves and pale mauve flowers. *C. amboinicus* leaves are used as condiments e.g. in soups and salads. The common names for this species are wild oregano and sage.

Figure 9: *C. amboinicus*

2.8.1 Essential oil

Pino *et al.* (1990) investigated the essential oil *C. amboinicus* and identified a total of 20 components, which included 13 terpenes and 7 oxygenated compounds. Pino *et al.* (1990) separated oxygenated and non-oxygenated compounds to find the percentage of the terpene hydrocarbons, oxygenated compounds (exclusive of phenols) and phenols in the essential oils of *C. amboinicus.* The essential oil contained about 64 % carvacrol. In another study Smith (1974) only 2 major components namely camphor and carvacol were identified. Limonene was identified as one of the trace components.

2.8.2 Microbiological Activity

Singh *et al*. (2002) reported that the *C. amboinicus* essential oil was insecticidal to white termites (*Odontotermes obesus*) with 100 % mortality at a concentration of 2.5×10^{-2} mg/cm^3 for a 5-hour exposure.

2.9 *Cymbopogon coloratus*

C. coloratus (Figure 10) is from the family Gramineae. It is a perennial, broad, glabrous, tapering plant (Smith, 1979). The local name for this species is *Fiji grass* (Fijian and Indian) and the leaves are used for making tea.

Figure 10: *C. coloratus*

2.9.1 Essential oil

There was no published work found on the analysis of *C. coloratus* essential oils.

2.9.2 Microbiological Activity

There was no reported work on the antibacterial, antifungal, antimalarial and antidengue activity of the *C. coloratus* leaf essential oils.

3.0 Method

Extraction of essential oils, antibiotic assay, anti malarial assays were done by the candidate, while the anti dengue assay was done by Isabelle Desriaux (a staff) at Institut de Recherche pour le Dévelopment, Noumea, New Caledonia and the GC analysis was done by Professor Jean-Pierre Dufour of University of Otago, New Zealand.

3.1 Extraction of Essential oils

Plant materials were blended in water and the resulting mixtures were steam distilled for 6 hours. Table 2 shows the mass of each plant species used. Essential oils were separated from water using an apparatus with a built-in separating funnel (Diagram: Appendix 1). Collected essential oils were dried with excess anhydrous sodium sulphate and stored at 4 ^{o}C until further analysis.

Table 2: Mass and part of Fijian plant species used for essential oil extraction along with other information.

Plant species	**Location of plant collection**	**Part of plant used**	**Other information**	**Mass (kg)**
E. foetidum	Cane Field (Nadi)	Leaves	Fresh plant parts were collected from a mature plant. Essential oil was extracted from the sample on the day the sample was collected.	0.9
C. sativum	Farm (Navua)	Leaves		0.8
C. verum	Namosi	Leaves		0.8
A. zerumbet	University of the South Pacific Botanical Garden	Leaves		0.6
		Rhizomes *		0.9
		Flowers *		1.3
S. dulcis	Namosi	Leaves		0.6
C. coloratus	Cane Field (Nadi)	Leaves		0.9
C. amboinicus	Farm (Nauva)	Leaves		1.1
P. racemosa	Namosi	Leaves		1.0

Note: * The samples were frozen and essential oil extraction was done later.

3.2 GC-FID Analysis (Composition of the essential oils)

Analyses of essential oils were performed using a HP6890A gas chromatograph (Agilent Technologies, Inc.,Wilmington, DE, USA) equipped with a split/splitless injector (with a 4 mm i.d. FocusLiner^{TM}, SGE Australia Pty Ltd, Ringwood, Vic, Australia) and a fast responding flame ionisation detector (FID) at 250 °C. Separations were carried out on a BPX5 (5 % phenyl-equivalent polysilphenylene phase) column (30 m, 0.25 mm i.d., 0.25 μm film thickness; SGE Australia Pty Ltd) using hydrogen as carrier gas (constant flow mode, 1.17 ml/min, 34.5 cm/sec, 7.5 psi at 60 °C). Injections were done at 220 °C in the split mode ratio (50:1) with a sample volume of 1 μl using a HP7683 autosampler (Agilent Technologies, Inc.). Detector gas flow rates were; air 300 ml/min, hydrogen 30 ml/min and nitrogen (makeup gas) combined flow 10 ml/min. The GC oven temperature was increased from 60 °C to 210 °C at 2 °C/min, held at 210 °C for 5 min, and then increased at 10 °C/min to 260 °C. Samples of essential oils (10 %, v/v) were diluted in cyclohexane. Analysis was done in triplicate. HP Chemstation software (Agilent Technologies, Inc.) was used for the collection of data. In order to obtain retention indices, 1 μl of a series of paraffins from 8 to 26 carbon atoms in cyclohexane was injected under the same GC-FID conditions.

3.3 GC-MS Analysis (Characterization of the essential oils)

GC-MS analysis were carried out using a Fisons 8000 Top GC (Carlo Erba Instruments, Milan, Italy) coupled to a Finnigan MAT MD 1000 mass detector (Finnigan Instruments, Manchester, UK). Separations were achieved using a non-polar BPX5 column (50 m, 0.22 mm i.d., 0.25 μm film thickness; SGE Australia Pty Ltd) and a polar Zebron ZB-

Wax (polyethylene glycol) column (60 m, 0.32 mm i.d., 0.5 μm film thickness; Phenomenex, Torrance, CA, USA). Analytical conditions were as follows:

- non polar BPX5 column: carrier gas was helium at 220 kPa (constant pressure mode). Injections were done at 250 °C in the split mode ratio (15.7:1) with a sample volume of 1 μl. The GC oven temperature was increased from 60 °C to 260 °C at 2 °C/min and held at 260 °C for 5 min.

- polar ZB-Wax column: carrier gas was helium at 1.5 ml/min (31.25 cm/sec at 60 °C, constant flow mode). Injections were done at 250 °C in the split mode ratio (50:1) with a sample volume of 1 μl. The GC oven temperature was increased from 60 °C to 240 °C at 2 °C/min.

The split/splitless injector was equipped with a 4 mm i.d. FocusLiner™ (SGE Australia Pty Ltd). The mass spectrometer was operated in the electron impact ionisation mode (70 eV). Source and interface temperatures were 200 °C and 250 °C, respectively. Detector voltage was 250 V; mass range was from 35 to 400 amu; scan rate was 0.9 scan/sec.

Samples of essential oils (1 %, v/v) were diluted in cyclohexane. A series of paraffins was run for each column to establish the retention indices.

3.4 Compound identification

Compounds were identified by matching mass spectral data with the Wiley and NIST library of standard compounds. Finnigan Masslab software version 1.4 (Finnigan Instruments, Manchester, UK) was used for data analysis. In order to obtain retention indices, 1 μl of a series of paraffins from 8 to 26 carbon atoms in cyclohexane was injected under the same GC-MS conditions. Whenever possible identification of

compounds was further confirmed by injecting 1 µl of authentic compounds at a concentration of 1 mg/ml in cyclohexane on each column.

3.5 Antibiotic Assay

Two bacteria: *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) and a fungus: *Candida albicans* (*C. albicans*) were used to test the antibiotic activities of the essential oils.

A suspension of the bacteria or fungus were prepared in physiological water and then flooded over agar in a Petri dish. Excess bacteria or fungus were withdrawn. The Petri dishes were put in an oven at 37 °C for drying and to start the growth of the bacteria or fungus. Essential oils were dissolved in dichloromethane (DCM) to get a concentration of 1 mg/10 µl. Each dissolved extract (10 µl) were placed on a disc. There was disc of DCM for blank control as well. For the antibiotic control-tests, Gentamicin at a concentration of 10 µg was used, but there was no antifungal control-test for *C .albicans*. Discs were placed for 30 minutes in an oven at 37 °C. Discs were then placed at the marked spot in the Petri dish and it was ensured that the discs were adhered well to the agar. Petri dishes were then placed in an oven at 37 °C and observations (inhibition diameter in mm) were made after 24 hours.

3.6 Antimalarial test

There were two kinds of test done: FBIT (Ferriprotoporphyrin IX Biomineralization Inhibition test) and PfNEK (Protein kinase) test.

3.6.1 FBIT (Ferriprotoporphyrin IX Biomineralization Inhibition test)

FBIT was developed by the members of UMR IRD-UPS 152 (Deharo *et al*., 2002).

The test was performed on 96-well micro plate and required 24 hours. The method for testing consisted of incubation in a normal non-sterile flat bottom 96-well plate at 37 °C for 24 hours a mixture containing 50 µl of drug solution (see below), or 50 µl of solvent (control), 50 µl of 0.5 mg/ml of haemin chloride freshly dissolved in dimethylsulphoxide (DMSO) and 100 µl of 0.5 M sodium acetate buffer pH 4.4. The final pH of the mixture was 5 - 5.2. It was important to adhere to the following order of addition: first the haemin chloride solution, second the buffer and finally the solution of drug of the solvent (DMSO). After incubation the wells containing the mixture was emptied into a stew pan. The wells were washed twice with 200 µL DMSO per well. Between each wash, the plate was centrifuged at 3500 rpm for 8 minutes. The β-haematin adhering to the side of the wells was then dissolved in 200 µl of 0.1 M NaOH per well. The plate was then agitated at 750 rpm for 5 minutes. Finally the absorbency was read at 405 nm with micro ELISA reader (Titertk Multiskan MCC/340). The data were expressed as the percentage of inhibition of FP biomineralization calculated by the following equation:

$$\% \text{ Inhibition} = 100 \times \frac{\text{OD control} - \text{OD Drug}}{\text{OD control}}$$

The controls used in FBIT were DMSO and water. The drug test was chloroquine phosphate at a concentration ranging from 0.5 mg/ml to 0.0625 mg/ml dissolved in water. The extracts were at a concentration of 10 mg/ml and the obtained fractions were at a concentration of 1mg/ml.

3.6.2 PfNEK (Protein kinase)

For essential oils the concentration used was 5 mg/ml and 2.5 mg/ml. This was dissolved in DMSO and then diluted by a factor of 10. The diluted essential oils solution (3 µl) put in a 0.5 ml tube

There were 6 controls

2 Enzymes

2 Roscovitine (R) for positive result

1 Control Paper (TP)

1 Control Casein (TK)

Preparation of the buffer

For the preparation of the buffer solution a 1.5ml tube was used. The ATP ^{33} was left at –20 °C. $MgCl_2$ (3.6 mg) was weighed, tris HCL (2-amino-2-(hydroxymethyl)-1,3-propanediol, hydrochloride) (18 µl), NaF (90.1 µl) and ATP ^{33} (9 µl) were transferred into the 1.5 ml tube. The resulting solution (now know as the buffer solution) was homogenized and 17 µl of this buffer solution was put in the test tube labelled control paper (TP). β-casein (62 µl) was added to the remaining buffer solution and homogenized. Then 17 µl of this buffer solution with β-casein were put in all test tubes

including the test tubes containing the diluted essential oils except the test tube with control paper (TP).

Preparation of the Roscovitine

Roscovitine is originally in a powder form and is dissolved in water/DMSO (50/50) to get a concentration of 5 mM. This Roscovitine solution was diluted by a factor of 5. The diluted Roscovitine solution (3 µl) was placed in the roscovitine tube (R).

Preparation of the enzyme

"PfNEK RUN" (50 ml) was taken in a tube, purification made in Toulouse. The enzyme (PfNEK RUN) was diluted by a factor of 10. The diluted enzyme solution (10 µl) were then placed in all test tubes including the test tubes containing the diluted essential oils except in the test tubes labelled TP and TK.

After the addition of the buffer solution, the diluted Roscovitine solution and the diluted enzyme solution all test tubes were centrifuged for 15 seconds at 4000 turns/min, All test tubes with resulting mixtures were placed at 30 ^{o}C for 30 minutes. Block of papers (cut from clean plain paper) were labelled and folded in half. The total content of each test tube was deposited to the corresponding paper and left to dry for 5 minutes. Block papers were then placed in a beaker and covered with 1 % orthophosphoric acid and left for 15 minutes. Block papers were rinsed four times with distilled water and placed on a clean plain paper to dry. During this time test tubes were labelled for scintillation. Block papers were placed in the corresponding test tubes with 4 ml of scintillation liquid and kept for 2 hours before being placed in the Liquid Scintillation Analyzer (1600TR Parker) to

measure the radioactivity. The data obtained after 2 hours were converted to percentage inhibition by the same formula used for FBIT test.

3.7 Anti Dengue Test

There are three stages in the experiment:

Stage 1: Cell culture 2 days

Stage 2: Virus inoculation (sponge extract is inoculated and incubated for 5 days)

Stage 3: Revelation (5^{th} day)

Stage 1

Preparation of the Culture Medium L15 GLU

L15 powder (14.75 g) was placed in a large beaker with 950 ml of distilled water and stirred. The antibiotic (penicillin and streptomycin) (10ml) was added to the L15 solution. The pH was made to 7.2 - 7.3. The solution was transferred to a graduated 1 l beaker and the volume was made to mark The culture medium solution was then filled into 250 ml sterile bottles (4 bottles) and kept at 37 ^{o}C for 24 hours and then at room temperature for another 24 hours.

Trypsination of Cells

The culture medium (L15 GLU), the SVF (serum of calf fetal), the PBS (Phosphate buffer saline) without Ca^{2+} and Mg^{2+}, trypsin and EDTA (ethylenediamine tetra acetic acid) were heated in a water bath at room temperature. With the aid of a syringe 20 ml of culture medium (L15 GLU) and 1 ml of SVF was taken and homogenised and the resulting mixture (10.5 ml) was placed in a large flask. This large flask was shaken well

and the resulting cell carpet was washed to get the medium to all sides of the flask before discarding the excess medium into the stew pan. Water (9 ml) and PBS (1 ml) was taken and homogenized and filtered. This mixture (water and PBS) (5 ml) was placed in the flask with the culture and cell medium and left to incubate for 1 minute. EDTA (4.5 ml) and trypsin (0.5 ml) were homogenized and filtered. This mixture (EDTA and trypsin) (2 ml) was put into the flask, which contained the culture medium. The flask (which contained the culture medium, the SVF, PBS, EDTA and trypsin) was left to incubate for 1 minute before the contents of the flask was emptied into the stew pan, leaving behind a thin film on the inside surface of the flask. This flask was then left at 37 ^{o}C for 2 minutes after which it was transported horizontally and was viewed under a microscope for the SVF cells. Culture medium and SVF mixture (5 ml) was put in the flask and left at 37 ^{o}C.

Stage 2

Inoculation of the virus on to the plate

A plate is filled with culture medium and SVF and left to incubate for 48 hours. Then the different concentrations of the virus are prepared.

Culture medium (19.5 ml) and 3 % SVF (0.6 ml) was taken and filtered into a cold beaker. This mixture (culture medium and 3 % SVF) (1.8 ml) was then placed in 3 ml test tubes (6 test tubes). The first test tube labelled "10^{-1}" and the dengue virus (0.2 ml) was placed. [The test tube was labelled 10^{-1} because the concentration of the virus in the test tube is 0.1] The contents of the test tube labelled "10^{-1}" was filtered into another test tube labelled "10^{-1} filtered". From the "10^{-1} filtered", 200 µl was taken and placed in the tube labelled 10^{-2}, which was already filled with 1.8 ml of culture medium and 3 % SVF mixture. This 10^{-2} test tube was then vortexed and 200 µl was taken and put in the next

tube, this was done until all tubes were filled. The concentrations of the virus were 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6}. For the virus concentration 1/5000, 0.4 µl of virus from the 10^{-4} test tube and 1.6 ml of culture medium and 3 % SVF was added.

The plate with its content (culture medium and SVF) was reversed into a stew pan and then 250 µl of each of the virus solutions prepared were placed in the labelled wells. The mixtures in the wells of the plate were homogenised. The plate was then placed at 37 ^{o}C for 1 hour. During this 1 hour, the CMC (carboxy methyl cellose) and the culture medium (29.5 g/l) were prepared. After 1 hour culture medium (29.5 g/l) with SVF and CMC (500 µl) was added in each well. It was ensured that no bubbles were formed during the process. The plate was left to incubate for 5 days at 37 ^{o}C.

Inoculation of virus with extracts

Final concentrations of essential oils were 2000 ppm. This was prepared by putting DMSO (25 µl) into a test tube containing the essential oil and the rest volume was made up with water to get a concentration of 2000 ppm. It was ensured that the essential oils were completely dissolved in DMSO before the addition of water. From the essential oils solution (2000 ppm), 50 ppm and 10 ppm solutions were made, using the solution of culture medium (29.5 g/l) (14 ml) and SVF (6%) (0.9 ml) that was filtered into a beaker. The test tubes containing the essential oils solutions (50 ppm and 10 ppm) were placed in a water bath (37 ^{o}C) for 1 hour and 30 minutes and then placed in all wells except the control well (medium +SVF). The plate was put in the oven for 1 hour at 37 ^{o}C.

Stage 3

Revelation with purple gentian

Plate contents were turned over into a stew pan. The plate was then rinsed with PBS and care was taken since the carpet of cells is fragile. Rinsing with PBS was done until the pink colour remained. Using a pipette 3 % formol (9.7 ml PBS + 0.3 ml formol) (250 µl) was placed in each well and left for 30 minutes. The contents of the plate were then again emptied into a stew pan. Plate wells were rinsed with PBS solution again. Dilution of the purple gentian was done by adding 1 ml of purple gentian to a solution of distilled water (9 ml) and phenol (200 µl). Volume of this solution (purple gentian, water and phenol) added to the plate wells was based on judgment and left for 15 minutes for revelation to occur. After 15 minutes, the contents of the plate were discarded and the plate was rinsed under tap water. The cells were observed to see if there was any inhibition had occurred.

Note:

All 8 species essential oils were sent for GC-MS analysis and only 5 species results were available when this thesis was written. As for the plant species whose essential oils were tested for microbiological assays was dependant on the volume of essential oils left after sending for GC-MS analysis. Microbiological assays were done in New Caledonia and extraction of essential oils was done in Fiji. Logistics behind the antimalarial and anti dengue tests are that these tests were available to the candidate when the candidate was doing her research in New Caledonia.

Results and Discussions

4.1 Essential oil Analysis

4.1.1 *Eryngium foetidum*

The colour and the yield of essential oils from the leaves of *E. foetidum* are shown in Table 3. The lists of the components with their relative peak areas (%) identified in the essential oils from the leaves of Fijian *E. foetidum* are shown in Table 4. The comparison of Fijian *E. foetidum* leaf essential oils constituents with that of Vietnamese (Leclercq *et al*., 1992) and Cuban (Pino *et al*., 1997) origin are shown in Table 5.

There were 25 components (comprising 98.16 % of the sample) that were identified in the *E. foetidum* leaf essential oils. Major components of the Fijian *E. foetidum* leaf essential oils in this study were (E)-2-dodecenal (70.68 %) (**45**), (E)-2-tetradecenal (11.17 %) (**46**), dodecanal (7.91 %) (**47**), 2,4,5-trimethylbenzaldehyde (2.04 %) (**48**) and (Z)-2-docecanal (1.32 %) (**49**). The absence of sesquiterpenoids [e.g. cadinene (**6**) and selinene(**8**)], presence of small numbers of monoterpene hydrocarbons [e.g. limonene (**4**), sabinene (**50**) and α-pinene (**1**)] and a large number of aldehydes in high percentages [e.g. (E)-2-dodecenal (**45**)] was characterised in the Fijian *E. foetidum* leaf essential oil sample. Aldehydes [e.g. 2,4,5-trimethylbenzaldehyde (**48**)], alkanal [e.g. decanal (**51**) and dodecanal (**47**)] and alkenals [e.g. (E)-2-tetradecenal (**46**)] were dominant and their occurrence could be presumed to be responsible for the intense aroma of *E. foetidum*.

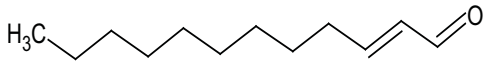
A comparison of the percentage composition of the present Fijian *E. foetidum* leaf essential oils with the leaf essential oils from Vietnamese *E. foetidum* (Leclercq *et al*., 1992) revealed considerable differences, the most significant being the low percentage of (E)-2-dodecenal (**45**) in the Vietnamese sample (major component in the Fijian *E. foetidum*) and the high percentage of the carboxylic acids (2-dodecenoic acid, 15.5 %) (**52**). The high percentage of carboxylic acids reported by Leclercq *et al*. (1992) could not be confirmed in the present Fijian *E. foetidum* leaf essential oils. It was also clear that the Fijian *E. foetidum* leaf essential oils is vastly different from the *E. foetidum* leaf essential oil of Cuban origin (Pino *et al*., 1997), as only 9 components were common in the Cuban and Fijian *E. foetidum* leaf essential oils. The Cuban *E. foetidum* leaf essential oils sample had 46 components with the major being 2,4,5-trimethylbenzaldehyde (20.53 %) (**48**), which was present in small percentages (2.04 %) in the present Fijian *E. foetidum* leaf essential oils sample. The Cuban *E. foetidum* leaf essential oil sample like the Vietnamese samples analysed by Leclercq *et al*. (1992) had high percentage of carboxylic acids. The Cuban *E. foetidum* leaf essential oil sample reports the presences of sesquiterpenoids [carotol, 9.94 % (**18**)], which was absent in the Fijian *E. foetidum* leaf essential oils.

These differences in the present Fijian *E, foetidum* leaf essential oils composition when compared with Cuban and Vietnamese *E. foetidum* leaf essential oils could be due to the location of the plant collected, the time the plant was collected, and the process used for essential oil collection. Another reason for the difference in the number and percentage of components identified could be due to the type of instrument used and the parameters used for analysing the essential oil. Also the difference in composition of components in

the leaf essential oils of *E. foetidum* could be due to the stability of the various components present in essential oils and also which biosynthetic pathway is favoured by the plant species from each region. The difference could also be due to the stress the plant was in the time of collection and also the requirement of each component by the plant.

Table 3: The yield and physical characteristics of the leaf essential oils of *E. foetidum*

Character	Leaf Oil
Oil yield	0.01 - 0.02 %
Colour	Pale clear yellow



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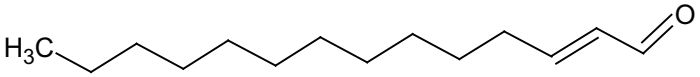

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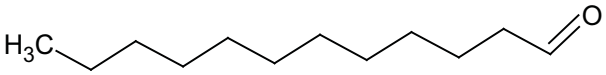

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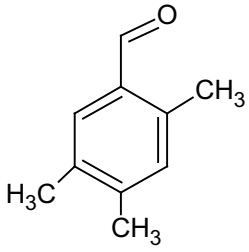

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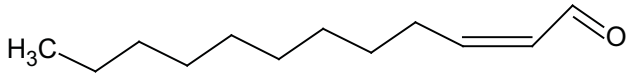

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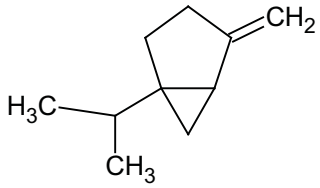

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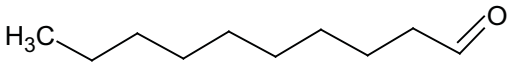

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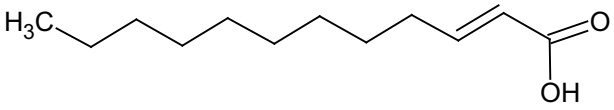

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Table 4: Relative peak areas in % of Fijian *E. foetidum* leaf essential oils components

Components	Average area (%)	RI-EXP BPX5	RI-REF DB5	RI-EXP ZBWax	RI-REF Cwax
α-pinene	0.12	928	934	1033	1007
sabinene	0.04	976	973	1134	1110
β-myrcene	0.07	991	989	1174	1148
1,3,5-trimethylbenzene	0.07	1006	1000	1300	-
p-cymene	0.11	1034	1029	1286	1248
limonene	0.07	1037	1031	1213	1188
eucalyptol	0.06	1042	1033	1221	1213
linalool	0.16	1100	1101	1558	1537
decanal	1.24	1215	1204	1513	1489
undecanal	0.10	1317	1306	1618	1653
2,4,6-trimethylbenzaldehyde	0.14	1337	-	1917	-
eugenol	0.08	1371	1365	-	-
2,4,5-trimethylbenzaldehyde	2.04	1380	-	2006	-
dodecanal	7.91	1420	1412	1724	1756
(E)-β-farnesene	0.09	1457	1455	-	-
(Z)-2-dodecenal	1.32	1463	-	-	-
(E)-2-dodecenal	70.68	1486	1466	1877	-
(E,E)-2,4-dodecadienal	0.13	1541	1514	2045	-
(Z)-nerolidol	0.16	1569	1565	2053	2004
caryophyllene oxide	0.78	1601	1581	1997	1993
tetradecanal	1.24	1622	1618	1934	-
(Z)-2-tetradecenal	0.12	1667	-	-	-
(E)-2-tetradecenal	11.17	1687	1666	2091	-
cyclohexanol	0.07	1066	-	1415	-
neophytadiene	0.18	1837	-	-	-
Unidentified compounds					
unknown1a	0.44	1405	-	1930	-
unknown1b	1.40	1409	-	1936	-
TOTAL	100.00	-	-	-	-

- RI-EXP are the experimental retention indices for BPX5 (non polar) and the ZBWax column (polar).
- RI-REF are the published retention indices values on the DB5 (non polar equivalent to BPX5) and Cwax (polar equivalent to ZBWax)

Table 5: Comparison of % composition of *E. foetidum* leaf essential oils of Fijian, Vietnamese and Cuban origin

Components	**Fijian (%)**	**Vietnamese[Ψ] (%)**	**Cuban [Φ] (%)**
α-pinene*	0.12	trace	-
sabinene*	0.04	-	-
β-myrcene*	0.07	-	-
1,3,5-trimethylbenzene*	0.07	-	-
p-cymene	0.11	0.3	0.34
limonene	0.07	-	0.02
eucalyptol*	0.06	-	-
linalool*	0.16	-	-
decanal	1.24	0.7	4.04
undecanal	0.10	0.5	2.49
2,4,6-trimethylbenzaldehyde	0.14	1.4	-
eugenol*	0.08	-	-
2,4,5-trimethylbenzaldehyde	2.04	1.4	20.53
dodecanal	7.91	1.0	4.03
(E)-β-farnesene*	0.09	-	-
(Z)-2-dodecenal	1.32	0.9	-
(E)-2-dodecenal	70.68	45.5	5.67
(E,E)-2,4-dodecadienal*	0.13	-	-
(Z)-nerolidol	0.16	-	0.30
caryophyllene oxide	0.78	-	0.34
tetradecanal*	1.24	-	-
(Z)-2-tetradecenal	0.12	0.4	-
(E)-2-tetradecenal	11.17	5.3	0.67
cyclohexanol*	0.07	-	-
neophytadiene*	0.18	-	-
2-dodecenoic acid	-	15.5	-
dodecanoic acid	-	8.6	-
decanoic acid	-	3.5	0.35
1-dodecanol	-	0.5	0.14
carotol	-	-	9.94
nonanal	-	0.4	3.43
β-pinene	-	trace	-
octanal	-	trace	0.19
undecanoic acid	-	1.5	-

Table 5 Continued:

Components	Fijian (%)	Vietnamese[Ψ] (%)	Cuban [Φ] (%)
α-phellandrene	-	trace	-
tricyclene	-	-	0.15
cumene	-	-	0.02
(E,Z)-2,4-decadienal	-	-	2.80
γ-terpinene	-	-	0.21
octanol	-	-	0.16
nonanol	-	-	0.08
2-decanone	-	-	0.16
2-undecanone	-	-	0.89
undecanol	-	-	0.54
camphor	-	-	0.03
trans-verbenol	-	-	0.15
trans-α-bergamotene	-	-	0.10
geranyl acetone	-	-	0.35
tridecanal	-	-	1.12
(E)-2-tridecenal	-	-	0.29
tetradecanal	-	-	0.22
(E)-γ-atlantone	-	-	0.08
(Z)-α-atlantone	-	-	1.29
(E)-2-pentadecanal	-	-	0.19
pentadecanol	-	-	0.16
(E)-α-atlantone	-	-	1.03
tetradecanoic acid	-	-	0.39
hexadecanol	-	-	0.20
2-hexadecanone	-	-	0.42
ar-curcumene	-	-	0.18
endo-1-bourbonanol	-	-	0.10
daucene	-	-	0.10
(E)-2-nonenal	-	-	0.16
6-camphenol	-	-	0.07
methyl-(Z,Z)-9,12-hexadecenoate	-	-	4.66
hexadecanoic acid	-	-	12.05

[Ψ] Leclercq *et al*. (1992) [Φ] Pino *et al*. (1997)

*components not previously listed for *E. foetidum* leaf essential oil but identified in the present study.

trace = < 0.1 %

4.1.2 *Coriandrum sativum*

The colour and the yield of the essential oils from the leaves of *C. sativum* are shown in Table 6. The list of the components with their relative peak areas (%) identified in the essential oils from the leaves of Fijian *C. sativum* are shown in Table 7. The comparison of Fijian *C. sativum* leaf essential oil with *C. sativum* leaf essential oils of two different samples from the United States of America (USA) (Potter, 1996 and Potter and Fagerson, 1990) are shown in Table 8.

There were 43 components (comprising 98.69 % of the sample) that were identified in the Fijian *C. sativum* leaf essential oil. The major components of the present Fijian *C. sativum* leaf essential oils were 2-decen-1-ol (24.25 %) (**53**), decanol (18.15 %) (**54**), (E)-2-tetradecenal (10.84 %) (**46**), (E)-2-decenal (9.31 %) (**55**) and decanal (7.69 %) (**56**). Fijian *C. sativum* leaf essential oil was characterised by relatively high percentages of volatile aldehydes [e.g. (E)-2-tetradecenal (**46**) and (E)-2-decenal (**55**)] and alcohols [e.g. 2-decen-1-ol (**53)** and decanol (**54**)], which could be presumed responsible for the flavour that is characteristic of this herb.

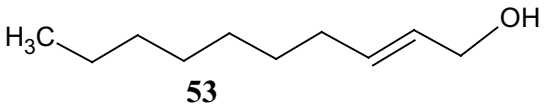
It was of interest to note that the Fijian *C. sativum* leaf essential oils also had 2-undecenol (1.97 %) (**57**), which along with 2-decenal showed antifungal activity in another study (Iriye *et al.* 1988).

A comparison of the composition of the Fijian *C. sativum* leaf essential oils with that of the USA sample investigated by Potter and Fagerson (1990) revealed considerable differences. The most significant being low percentages of (E)-2-decenal (9.31 %) (**55**)

in the Fijian *C. sativum* leaf essential oil sample whereas the USA sample had 46.1 % of (E)-2-decenal (**55**).

A second study of the USA leaf essential oils of *C. sativum* done by Potter (1996) showed (Z)-2-dodecenal (21.60 %) (**49**) as the major component which was absent in the previous USA *C. sativum* leaf essential oil study by Potter and Fagerson (1990). However, the Fijian *C. sativum* leaf essential oil sample investigated had only 0.08 % of (Z)-2-dodecenal (**49**).

These differences in the present Fijian *C. sativum* leaf essential oils composition when compared with USA *C. sativum* leaf essential oils could be due to the location of the plant collected, the time the plant was collected, and the process used for essential oil collection. Another reason for the difference in the number and percentage of components identified could be due to the type of instrument used and the parameters used for analysing the essential oil. Also the difference in composition of components in the leaf essential oils of *C. sativum* could be due to the stability of the various components present in essential oils and also which biosynthetic pathway is favoured by the plant species from each region. The difference could also be due to the stress the plant was in the time of collection and also the requirement of each component by the plant.



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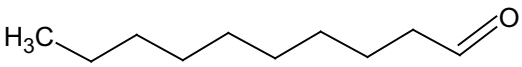

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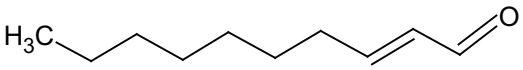

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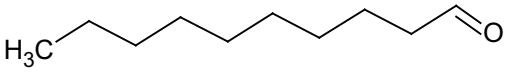

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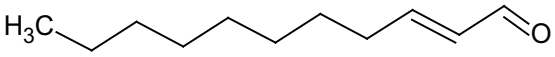

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Table 6: The yield and physical characteristics of the leaf essential oils of *C. sativum*.

Character	Leaf Oil
Oil yield	0.03 %
Colour	Pale yellow

Table 7: Relative peak area in % of Fijian *C. sativum* leaf essential oils components

Components	**Average area (%)**	**RI-EXP BPX5**	**RI-REF DB5**	**RI-EXP ZBWax**	**RI-REF Cwax**
(E or Z)-3-hexenol	1.32	850	-	1395	-
(E)-2-hexenol	0.48	858	887	1417	1409
nonane	1.44	882	900	900	900
α-thujene	0.07	917	925	1030	-
α-pinene	0.06	928	934	1033	1007
sabinene	0.20	976	973	1131	1110
β-myrcene	0.10	985	989	1171	1148
decane	0.09	1000	1000	1000	1000
octanal	0.55	1012	1006	1303	1297
p-cymene	0.26	1034	1029	1284	1248
limonene	0.06	1037	1031	1210	1188
eucalyptol	0.50	1042	1033	1219	1213
γ-terpinene	0.25	1066	1061	1256	1223
undecane	0.07	1100	1100	1098	1100
linalool	0.19	1108	1101	1558	1537
nonanal	0.15	1114	1104	1407	1385
nonanol	0.22	1180	1171	1670	-
terpinene-4-ol	0.09	1195	1182	1614	1591
decanal	7.69	1216	1204	1512	1489
(E)-2-decenal	9.31	1275	1264	1657	1636
2-decen-1-ol	24.25	1281	-	1829	-
decanol	18.15	1284	1272	1773	1765
undecanal	0.66	1317	1306	1617	1653
(E)-2-undecenal	1.08	1377	1366	1764	1720
2-undecen-1-ol	1.97	1379	-	1932	-
undecanol	0.69	1382	1371	1877	1827
(E)-2-decenyl acetate	0.13	1415	-	-	-
dodecanal	2.50	1419	1412	1724	1756
(Z)-2-dodecenal	0.08	1463	-	-	-
(E)-2-dodecenal	6.30	1481	-	1874	-
(E)-2-dodecen-1-ol	3.70	1481	-	2034	-
dodecanol	0.36	1483	-	1978	1972
tridecanal	0.30	1521	1518	-	-

Table 7 Continued:

Components	Average area (%)	RI-EXP BPX5	RI-REF DB5	RI-EXP ZBWax	RI-REF Cwax
(E)-2-tridecenal	0.80	1582	-	1981	-
tetradecanal	1.13	1623	1616	1934	-
(Z)-2-tetradecenal	0.06	1674	-	-	-
(E)-2-tetradecenal	10.84	1686	-	2089	-
pentadecanal	0.13	1724	1711	2039	2059
(E)-2-pentadecenal	1.30	1787	-	2196	-
hexadecanal	0.08	1827	-	2137	-
(E)-2-hexadecenal	0.80	1892	-	2305	-
Unidentified compounds					
????	0.18	1207	-	-	-
????	0.22	1259	-	-	-
????	0.24	1395	-	-	-
unknown1b	0.08	1408	-	1934	-
????	0.14	1611	-	-	-
??	0.16	1752	-	-	-
??	0.14	1837	-	-	-
????	0.12	2183	-	-	-
????	0.28	2213	-	-	-
????	0.05	2230	-	-	-
TOTAL	100.00	-	-	-	-

- RI-EXP are the experimental retention indices for BPX5 (non polar) and the ZBWax column (polar).
- RI-REF are the published retention indices values on the DB5 (non polar equivalent to BPX5) and Cwax (polar equivalent to ZBWax)

Table 8: Comparison of % composition of *C. sativum* leaf essential oils of Fijian and United states origin

Components	**Fijian**	**US$^{\Psi}$**	**US$^{\Phi}$**
(E or Z)-3-hexenol *	1.32	-	-
(E)-2-hexenol*	0.48	-	-
nonane	1.44	0.2	1.28
α-thujene*	0.07	-	-
α-pinene*	0.06	-	-
sabinene*	0.20	-	-
β-myrcene*	0.10	-	-
decane*	0.09	-	-
octanal	0.55	0.5	<0.03
p-cymene*	0.26	-	-
limonene	0.06	-	0.19
eucalyptol*	0.50	-	-
γ-terpinene *	0.25	-	-
undecane	0.07	-	0.06
linalool*	0.19	-	-
nonanal	0.15	0.2	0.07
nonanol*	0.22	-	-
terpinene-4-ol*	0.09	-	-
decanal	7.69	4.4	9.45
(E)-2-decenal	9.31	46.1	0.87
2-decen-1-ol	24.25	9.2	<0.03
decanol	18.15	4.3	0.89
undecanal	0.66	0.5	2.14
(E)-2-undecenal	1.08	5.6	1.18
2-undecen-1-ol	1.97	0.9	<0.03
undecanol	0.69	0.2	<0.03
(E)-2-decenyl acetate*	0.13	-	-
dodecanal	2.50	-	10.30
(Z)-2-dodecenal	0.08	-	21.60
(E)-2-dodecenal	6.30	10.3	-
(E)-2-dodecen-1-ol *	3.70	-	-
dodecanol	0.36	1.6	0.18
tridecanal	0.30	0.1	1.43
(E)-2-tridecenal	0.80	0.7	1.83
tetradecanal	1.13	0.7	2.22

Table 7 Continued:

Components	**Fijian**	**US[Ψ]**	**US[Φ]**
(Z)-2-tetradecenal*	0.06	-	-
(E)-2-tetradecenal	10.84	5.8	20.20
pentadecanal	0.13	0.1	0.61
(E)-2-pentadecenal	1.30	0.7	5.12
hexadecanal	0.08	-	0.10
(E)-2-hexadecenal	0.80	0.4	1.58
(E)-2-nonenal	-	0.2	-
(Z)-4-decenal	-	0.3	-
9-decenal	-	0.8	-
8-methyl-2-nonenal	-	1.3	-
10-undecenal	-	0.1	-
9-methyl-2-decenal	-	0.1	-
4-dodecenal	-	0.1	-
11-dodecenal	-	0.2	-
10-methyl-2-undecenal	-	0.3	-
2-dodecen-1-ol	-	1.4	0.82
2-dodecen-1-ol	-	1.4	-
10-methyl-2-undecenal	-	0.3	-
13-tetradecenal	-	0.1	-
12-methyl-2-tridecenal	-	0.2	-
2-tetradecen-1-ol	-	0.6	-
heptadecenal	-	0.3	-
5-methyltetrahydrofuryl alcohol	-	-	0.06
2,4,6-trimethylheptane	-	-	0.12
phenylacetaldehyde	-	-	0.17
(E)-4-decenal	-	-	<0.03
(E)-9-decenal	-	-	<0.03
germacrene B	-	-	0.18
2-tridecen-1-ol	-	-	<0.03
diallylfumerate	-	-	0.25
phytol	-	-	3.46
methyl sterate	-	-	0.13
1-eicosanol	-	-	0.40
1-docosanol	-	-	2.41
1-tetracosanol	-	-	0.35

[Ψ] Potter and Fagerson (1990) [Φ] Potter (1996)

*components not previously listed for *C. sativum* leaf essential oil but identified in the present study.

Table 9 shows the comparison of present Fijian *E. foetidum* and Fijian *C. sativum* leaf essential oils. It can be seen that 14 components are present in both Fijian *E. foetidum* and Fijian *C. sativum* leaf essential oils. These 14 components could be presumed to be responsible for the similar odour of *E. foetidum* and *C. sativum.*

Table 9: Comparison of % composition of present Fijian *E. foetidum* and *C. sativum* leaf essential oil

Components	***E. foetidum***	***C. sativum***
(E or Z)-3-hexenol	-	1.32
(E)-2-hexenol	-	0.48
nonane	-	1.44
α-thujene	-	0.07
α-pinene	0.12	0.06
sabinene	0.04	0.20
β-myrcene	0.07	0.10
decane	-	0.09
octanal	-	0.55
γ-terpinene	-	0.25
undecane	-	0.07
1,3,5-trimethylbenzene	0.07	-
p-cymene	0.11	0.26
limonene	0.07	0.06
eucalyptol	0.06	0.05
linalool	0.16	0.19
decanal	1.24	7.69
undecanal	0.10	-
2,4,6-trimethylbenzaldehyde	0.14	-
eugenol	0.08	-
2,4,5-trimethylbenzaldehyde	2.04	-
dodecanal	7.91	2.50
(E)-beta-farnesene	0.09	-

Table 9 continued

Components	***E. foetidum***	***C. sativum***
(Z)-2-dodecenal	1.32	0.08
(E)-2-dodecenal	70.68	6.30
(E,E)-2,4-dodecadienal	0.13	-
(Z)-nerolidol	0.16	-
caryophyllene oxide	0.78	-
tetradecanal	1.24	1.13
(Z)-2-tetradecenal	0.12	0.06
(E)-2-tetradecenal	11.17	10.84
pentadecanal	-	0.13
(E)-2-pentadecenal	-	1.30
hexadecanal	-	0.08
(E)-2-hexadecenal	-	0.80
(E)-2-undecenal	-	1.08
2-undecen-1-ol	-	1.97
undecanol	-	0.69
(E)-2-decenyl acetate	-	0.13
dodecanal	-	2.50
dodecanol	-	0.36
tridecanal	-	0.30
(E)-2-tridecenal	-	0.80
(E)-2-decenal	-	9.31
2-decen-1-ol	-	24.25
decanol	-	18.15
undecanal	-	0.66
nonanal	-	0.15
nonanol	-	0.22
terpinene-4-ol	-	0.09
Unidentified compounds	2.02	1.25
TOTAL	100.00	100.00

4.1.3 *Cinnamomum verum*

The colour and the yield of the essential oils from the leaves of *C. verum* are shown in Table 10. The list of components with their relative peak areas (%) identified in the essential oils from the leaves of Fijian *C. verum* are shown in Table 11. The comparison of the present Fijian *C. verum* leaf essential oils with *C. verum* leaf essential oils from India (Nath *et al*., 1997) is shown in Table 12.

There were 31 components (comprising 100% of the sample) that were identified in the Fijian *C. verum* leaf essential oils. The Fijian *C. verum* leaf essential oils were characterised by the presence of eugenol, monoterpenes and sesquiterpenes in high percentages. Major components present in the Fijian *C. verum* leaf essential oils were eugenol (86.02 %) (**11**), (E)-caryophyllene (5.70 %) (**58**) and linalool (2.30 %) (**21**).

Comparison of the composition of the Fijian *C. verum* leaf essential oils with that investigated by Nath *et al*., (1997) revealed similarities such as eugenol (**11**) as the major component in both studies, 86.02 % in Fijian sample and 79.3 % in Indian sample was the major component present in both studies. The Fijian *C. verum* leaf essential oil showed components, which were not identified by Nath *et al*. (1997) such as sabinene (**50**), limonene (**4**) and many more as shown in table 12.

These differences in the present Fijian *C. verum* leaf essential oils composition when compared with Indian *C. verum* leaf essential oils could be due to the location of the plant collected, the time the plant was collected, and the process used for essential oil collection. Another reason for the difference in the number and percentage of

components identified could be due to the type of instrument used and the parameters used for analysing the essential oil. Also the difference in composition of components in the leaf essential oils of *C. verum* could be due to the stability of the various components present in essential oils and also which biosynthetic pathway is favoured by the plant species from each region. The difference could also be due to the stress the plant was in the time of collection and also the requirement of each component by the plant.

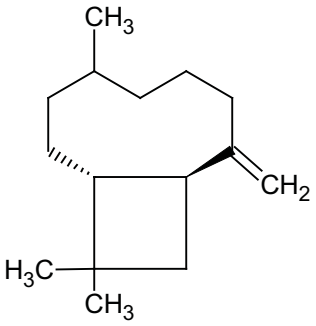

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Table 10: The yield and physical characteristics of the leaf essential oils of *C .verum*

Character	Leaf Oil
Oil yield	0.03 %
Colour	golden yellow

Table 11 Relative peak areas in % of Fijian *C. verum* leaf essential oils components

Components	**Average area (%)**	**RI-EXP BPX5**	**RI-REF DB5**	**RI-EXP ZBWax**	**RI-REF Cwax**
hexanal	0.30	806	801	1098	1024
(Z)-3-hexenal	0.31	848	854	1234	1220
α-thujene	0.07	913	925	1034	1003
α-pinene	0.52	924	937	1031	1032
camphene	0.22	946	946	1078	1050
sabinene	0.04	972	973	1132	1123
benzaldehyde	0.03	972	968	1542	1532
β-pinene	0.23	981	1002	1120	1116
β-myrcene	0.03	990	989	1172	1148
α-terpinene	0.11	1022	1018	1192	1152
p-cymene	0.11	1031	1029	1284	1248
limonene	0.21	1034	1031	1211	1188
β-phellandrene	0.45	1037	1034	1221	1212
eucalyptol	0.22	1039	1033	1220	1213
γ-terpinene	0.09	1063	1061	1257	1223
trans-linalool oxide	0.05	1094	1088	1482	1454
linalool	2.30	1105	1101	1559	1537
terpinene-4-ol	0.09	1191	1182	1615	1591
α-terpineol	0.05	1207	1195	1710	1688
(E)-cinnamic aldehyde	0.32	1292	1266	2060	2015
eugenol	86.02	1369	1365	2185	2122
α-copaene	0.36	1382	1376	1500	1472
(E)-caryophyllene	5.70	1434	1437	1607	1594
(allo)aromadendrene	0.12	1456	1461	1616	1627
α-humulene	0.92	1477	1470	1680	1663
valencene	0.17	1513	1491	1705	1700
bicyclogermacrene	0.19	1518	1515	1744	1714
δ-cadinene	0.05	1537	1538	1767	1749
nerolidol	0.09	1571	1565	2052	2004
spathulenol	0.14	1592	1601	2136	2035
caryophyllene oxide	0.48	1597	1581	1995	1993
TOTAL	100	-	-	-	-

- RI-EXP are the experimental retention indices for BPX5 (non polar) and the ZBWax column (polar).
- RI-REF are the published retention indices values on the DB5 (non polar equivalent to BPX5) and Cwax (polar equivalent to ZBWax)

Table 12: Comparison of % composition of *C. verum* leaf essential oils of Fijian and Indian origin

Components	Fijian	India [Ψ]
hexanal*	0.30	-
(Z)-3-hexenal *	0.31	-
α-thujene	0.07	-
α-pinene	0.52	0.2
camphene	0.22	0.2
sabinene *	0.04	-
benzaldehyde*	0.03	-
β-pinene	0.23	0.1
β-myrcene*	0.03	-
α-terpinene	0.11	-
p-cymene	0.11	0.2
limonene	0.21	-
β-phellandrene	0.45	-
eucalyptol*	0.22	-
γ-terpinene*	0.09	-
trans-linalool oxide*	0.05	-
linalool	2.30	0.2
terpinene-4-ol	0.09	-
α-terpineol	0.05	trace
(E)-cinnamic aldehyde	0.32	1.6
eugenol	86.02	79.3
α-copaene	0.36	-
(E)-caryophyllene	5.70	2.0
(allo)aromadendrene*	0.12	-
α-humulene	0.92	-
valencene*	0.17	-
bicyclogermacrene*	0.19	-
(δ)cadinene*	0.05	-
nerolidol*	0.09	-
spathulenol*	0.14	-
caryophyllene oxide*	0.48	-
α-terpineol	-	trace

*components not previously listed for *C. verum* leaf essential oil but identified in the present study.
[Ψ] Nath *et al*. (1997)
trace - = < 0.05 %

4.1.4 *Alpinia zerumbet*

The colour and the yield of the essential oils of Fijian *A. zerumbet* leaves, rhizomes and flowers are shown in Table 13.

The list of components with their relative peak areas (%) identified in the essential oils from the leaves, rhizomes and flowers of Fijian *A. zerumbet* are shown in Table 14. The comparison with of present Fijian *A. zerumbet* rhizomes and leaf essential oils (respectively) with that of *A. zerumbet* rhizome and leaf essential oils from a separate study in Fiji (Ali *et al*., 2002) are shown in Table 15 and 16 respectively.

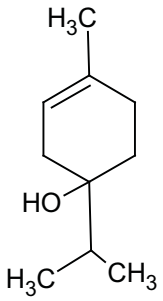
There were 47 components (comprising 96.28 % of the sample) that were identified in the present Fijian *A. zerumbet* rhizome essential oils with 4-terpineol (19.25 %) (**59**), 1,8-cineole (16.13 %) (**22**) and *p*-cymene (14.50 %) (**60**) as the major components. There were 41 components (comprising 87.13 % and 97.57 % of the sample) in the present Fijian *A. zerumbet* leaf and flower essential oils respectively with 4-terpineol (25.31 %) (**59**), 1,8-cineole (15.35 %) (**22**) and γ-terpene (12.72 %) (**61**) as major components in *A. zerumbet* leaf essential oils with 4-terpineol (20.29 %) (**59**), 1,8-cineole (19.36 %) (**22**) and sabinene (16.39 %) (**50**) as major components in *A. zerumbet* flower essential oils. 4-Terpineol and 1,8-cineole are common to the leaf, rhizome and flower essential oils of *A. zerumbet* and this is due to the fact that the plant parts were collected from the same tree. Essential oils components remain same for the plant, but the composition vary with plant parts (see page 3).

A comparison of the present Fijian *A. zerumbet* leaf essential oil with that investigated by Ali *et al*. (2002) revealed that the major component in both studies was 4-terpineol but the relative percentage present is different. Ali *et al.* (2002) reported 40.9 % as the major component [4-terpineol (**59**)] while this study reported 25.31 % of 4-terpineol (**59**). The same was also observed when the present Fijian *A. zerumbet* rhizome essential oils were compared with *A. zerumbet* rhizome essential oils analysed by Ali *et al*. (2002).

These differences in the present Fijian *A. zerumbet* rhizome essential oils composition when compared with Fijian *A. zerumbet* rhizome essential oils investigated by Ali *et al*. (2002) could be due to the location of the plant collected, the time the plant was collected, and the process used for essential oil collection. Another reason for the difference in the number and percentage of components identified could be due to the type of instrument used and the parameters used for analysing the essential oils. Also the difference in composition of components in the *A. zerumbet* rhizome essential oils could be due to the stability of the various components present in essential oils and also which biosynthetic pathway is favoured by the plant species from each region. The difference could also be due to the stress the plant was in the time of collection and also the requirement of each component by the plant. The components of essential oils remain same in plant but the composition may vary with plant parts (refer page 4 of the thesis).

Fresh Fijian *A. zerumbet* rhizome has an odour, which is similar to fresh commercial ginger (*Z. officinale*). A comparison of the present Fijian *A. zerumbet* rhizome essential oils components with ginger essential oils components (Nigam *et al*., 1964) (Table 15) it was seen there were 13 components that were same for both species. These 13

components could be presumed to be responsible for the similar odour of *A. zerumbet* and commercial ginger.



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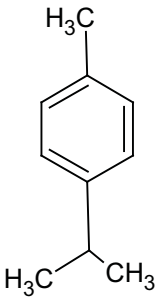

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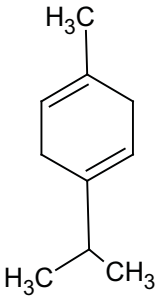

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Table 13: The yield and physical characteristics of the leaf, rhizome and flower essential oils of *A. zerumbet*

Character	Leaf	Rhizome	Flower
Oil yield	0.29 %	0.10 %	0.01 %
Colour	golden yellow	golden yellow	golden yellow

Table 14: Relative peak areas in % of Fijian *A. zerumbet rhizome,* leaf and flower essential oils components

	Average Area (%)			RI-HP	RI-MS
Components	**rhizome**	**leaf**	**flower**	**BPX5**	**ZBWax**
unknown	-	1.54	-	850	-
Unknown	-	0.16	-	860	-
Unknown	0.04	0.13	0.04	891	-
Unknown	-	-	0.03	895	-
α-thujene	1.59	2.17	1.24	917	1036
α-pinene	2.76	1.03	1.58	928	1033
camphene	2.56	0.11	0.54	950	1080
sabinene	0.52	5.66	16.39	976	1132
β-pinene	5.05	2.43	0.97	985	1121
β-myrcene	1.01	0.85	1.50	991	1172
α-phellandrene	0.20	0.20	0.90	1015	1177
unknown	0.09	-	-	1020	-
α-terpinene	1.37	2.44	4.83	1025	1193
p-cymene	14.50	5.06	3.38	1034	1300
limonene	2.88	1.57	1.92	1037	1212
1,8-cineole	16.13	15.35	19.36	1043	1220
unknown	-	-	0.10	1050	-
γ-terpinene	5.60	12.72	9.03	1066	1257
unknown	-	0.66	0.11	1081	-
terpinolene	1.46	1.62	2.11	1094	1296
fenchone coeluting (BPX5) with	0.19	0.05	-	1103	1411
p-cymenene/para-cymenyl	-	-	-	-	1452
linalool	0.46	1.12	1.64	1108	1558
unknown	-	1.03	0.11	1114	-
unknown	-	0.07	-	1125	-
fenchylalcohol	0.40	-	-	1134	1596
sabinene hydrate (Z) and/or (E)	0.40	0.97	0.73	1138	1574
1-terpineol (terpinene-1-ol)	-	-	-	-	1640
unknown	-	0.05	-	1147	-
coelution of epoxyterpinolene with	0.48	0.66	0.45	1156	-
menth-2-en-1-ol, (E)-para	-	-	-	-	-
camphor	0.27	0.08	0.19	1166	1530

Table 14 Continued:

Components	Average Area (%)			RI-HP	RI-MS
	rhizome	leaf	flower	BPX5	ZBWax
exo-methyl-camphenilol	0.09	-	-	1173	-
unknown	-	0.10	-	1177	-
δ-terpineol	0.08	0.17	0.13	1186	-
iso-/borneol	2.11	0.13	0.10	1190	1714
terpinene-4-ol (4-terpineol)	19.25	25.31	20.29	1198	1615
p-cymen-8-ol	0.16	0.29	-	1205	1865
L-α-terpineol	1.62	1.80	1.97	1211	1709
unknown	-	0.36	0.24	1224	-
fenchylacetate	9.49	-	-	1228	1481
unknown	0.20	0.09	-	1240	-
unknown	-	0.22	-	1262	-
unknown	0.40	0.10	-	1272	-
unknown	-	0.13	-	1279	-
iso-/bornyl acetate	0.76	0.07	0.38	1296	1592
unknown	-	0.07	-	1312	-
unknown	-	0.09	-	1317	-
unknown	-	0.09	-	1332	-
unknown	-	0.10	-	1339	-
unknown	0.08	-	-	1343	-
unknown	-	0.21	-	1347	-
isoledene	0.05	-	0.09	1381	-
daucene	0.27	0.12	0.47	1387	1500
unknown	-	0.05	-	1392	-
unknown	-	0.65	-	1398	-
unknown	-	-	0.22	1407	-
unknown	-	0.16	-	1422	-
santalene	0.15	0.29	0.21	1427	-
β-caryophyllene	1.31	3.37	3.20	1432	1607
bergamotene	0.06	0.19	0.09	1440	-
unknown	-	0.05	-	1444	-
3,7-guaiadiene	0.13	0.27	0.12	1451	1503
farnesene	0.08	0.09	0.12	1457	-
selin-4,7(11)-diene	0.06	0.11	0.06	1461	-
α-humulene	0.25	0.47	0.50	1469	1680
unknown	-	0.10	0.11	1485	-

Table 14 Continued:

Components	Average Area (%)			RI-HP	RI-MS
	rhizome	leaf	flower	BPX5	ZBWax
α/β-selinene	0.19	0.15	0.12	1497	1700
unknown	-	0.19	0.14	1504	-
unknown	-	0.15	0.12	1510	-
unknown	0.20	-	-	1513	-
bisabolene	0.09	0.18	0.22	1516	-
epi-ligulyl oxide	0.15	0.13	0.06	1522	-
δ-cadinene	0.22	0.55	0.19	1526	-
unknown	0.19	-	0.15	1530	-
unknown	0.14	-	-	1535	-
unknown	0.15	0.42	0.24	1538	-
unknown	0.08	-	-	1558	-
elemol	0.09	-	0.12	1563	-
nerolidol	0.21	0.51	0.81	1569	2052
unknown	-	-	0.06	1575	-
caryophyllene oxide	0.76	1.89	0.41	1601	1995
guaiol	0.11	-	-	1612	2100
carotol	0.39	0.13	0.45	1622	2030
unknown	0.15	0.28	-	1631	-
unknown	-	0.12	-	1644	-
γ-eudesmol	0.22	0.17	0.54	1651	2180
unknown	0.10	0.19	-	1654	-
unknown	0.16	0.24	-	1659	-
α-eudesmol	0.49	0.65	0.69	1677	2233
unknown	0.09	-	-	1684	-
unknown	0.43	-	-	1689	-
unknown	-	0.21	-	1691	-
unknown	0.10	-	0.15	1697	
unknown	0.08	-	-	1715	-
unknown	0.46	-	-	1830	-
unknown	-	0.12	-	2026	-
unknown	-	-	0.10	2099	-
unknown	0.11	-	-	2111	-
unknown	-	0.64	-	2121	-
unknown	0.05	-	-	2175	-
unknown	0.02	-	0.02	2199	-
TOTAL	100.00	100.00	100.00		

- RI-HP BPX5- are the retention indices for BPX5 (non polar) for the composition of the essential oils
- RI-MS ZBWax - are the retention indices for ZBWax column (polar) for the characterisation of the essential oils
- Note: the published retention indices values are not given in this table as the published RI values are the same for all experiments and components were identified when compared to the published RI values that are given in previous tables.

Table 15: Comparison of % composition of *A. zerumbet* **rhizome** essential oils.

Components	**Present Study**	**Fijian** $^{\Psi}$	**Ginger** $^{\Phi}$
α-thujene*	1.59	-	-
α-pinene	2.76	1.1	-
camphene*	2.56	-	1.1
sabinene*	0.52	-	-
β-pinene	5.05	4.0	0.4
β-myrcene*	1.01	-	0.1
α-phellandrene*	0.20	-	1.3
α-terpinene*	1.37	-	-
p-cymene	14.50	7.2	0.1
limonene*	2.88	-	1.2
1,8-cineole	16.13	28.1	1.3
γ-terpinene*	5.60	-	-
terpinolene*	1.46	-	-
fenchone coeluting (BPX5) with	0.19	-	-
p-cymenene/para-cymenyl*	-		-
linalool*	0.46	-	-
fenchylalcohol*	0.40	-	-
sabinene hydrate (Z) and/or (E) *	0.40	-	-
1-terpineol (terpinene-1-ol)	-	-	-
coelution of epoxyterpinolene with	0.48	-	-
menth-2-en-1-ol, (E)-para*	-	-	-
camphor*	0.27	-	-
exo-methyl-camphenilol*	0.09	-	-
δ-terpineol*	0.08	-	-
iso-/borneol*	2.11	-	0.1
terpinene-4-ol (4-terpineol)	19.25	41.9	-
p-cymen-8-ol*	0.16	-	-
L-α-terpineol	1.62	7.9	-
fenchylacetate*	9.49	-	-
iso-/bornyl acetate*	0.76	-	-
isoledene*	0.05	-	-
daucene*	0.27	-	-
santalene*	0.15	-	-

Table 15: continued

Components	Present Study	Fijian [Ψ]	Ginger [Φ]
β-caryophyllene	1.31	2.9	-
bergamotene*	0.06	-	-
3,7-guaiadiene*	0.13	-	-
farnesene*	0.08	-	9.8
selin-4,7(11)-diene*	0.06	-	-
α-humulene*	0.25	-	-
α/β-selinene*	0.19	-	1.4
bisabolene*	0.09	-	0.2
epi-ligulyl oxide	0.15	1.1	-
δ-cadinene*	0.22	-	-
elemol*	0.09	-	-
nerolidol*	0.21	-	-
α-carene	-	1.8	-
caryophyllene oxide*	0.76	-	-
guaiol*	0.11	-	-
carotol*	0.39	-	-
γ-eudesmol*	0.22	-	-
α-eudesmol*	0.49	-	-

[Ψ] Ali *et al*., 2002 [Φ] Nigam *et al*. (1964)

*components not previously listed for *A. zerumbet* rhizome essential oil but identified in the present study

Table 16: Comparison of % composition of *A. zerumbet* **leaf** essential oils of Present Fijian and Fijian samples.

Components	**Present Study**	**Fijian**[ψ]
α-thujene	2.17	2.0
α-pinene	1.03	1.1
camphene*	0.11	-
sabinene*	5.66	-
β-pinene	2.43	10.0
β-myrcene*	0.85	-
α-phellandrene*	0.20	-
α-terpinene*	2.44	-
p-cymene	5.06	7.1
limonene*	1.57	-
1,8-cineole	15.35	13.2
γ-terpinene*	12.72	-
terpinolene*	1.62	-
fenchone coeluting (BPX5) with	0.05	-
p-cymenene/para-cymenyl*	-	-
linalool*	1.12	-
sabinene hydrate (Z) and/or (E) *	0.97	-
coelution of epoxyterpinolene with	0.66	-
menth-2-en-1-ol, (E)-para*	-	-
camphor*	0.08	-
δ-terpineol*	0.17	-
iso-/borneol*	0.13	-
terpinene-4-ol (4-terpineol)	25.31	40.9
p-cymen-8-ol*	0.29	-
L-α-terpineol	1.80	8.4
iso-/bornyl acetate*	0.07	-
daucene*	0.12	-
santalene*	0.29	-
β-caryophyllene	3.37	7.0
bergamotene*	0.19	-
3,7-guaiadiene*	0.27	-
farnesene*	0.09	-
selin-4,7(11)-diene*	0.11	-

Table 16: continued

Components	**Present Study**	**Fijian[Ψ]**
α-humulene*	0.47	-
α/β-selinene*	0.15	-
bisabolene*	0.18	-
epi-ligulyl oxide*	0.13	-
δ-cadinene*	0.55	-
nerolidol*	0.51	-
α-carene	-	1.9
caryophyllene oxide	1.89	5.5
carotol*	0.13	-
γ-eudesmol*	0.17	-
α-eudesmol*	0.65	-

[Ψ] Ali *et al*., 2002

*components not previously listed for *A. zerumbet* leaf essential oil but identified in the present study.

4.1.5 *Spondias dulcis*

The colour and the yield of the essential oil from the leaves of Fijian *S. dulcis* leaf are shown in Table 17. The list of components with their relative peak areas (percentages) identified in the essential oils from the leaves of Fijian *S. dulcis* are shown in Table 18.

There were 22 components (comprising 100 % of the sample) that were identified in the present Fijian *S. dulcis* leaf essential oils with the major component as α-pinene (48.11 %) (**1**), other components present were limonene (13.94 %) (**4**), β-pinene (13.10 %) (**62**) and (E)-carophyllene (12.42 %) (**58**). α-Pinene is of great interest because it is used principally in the manufacture of camphone and terpineol. α-Pinene also serves as an important constituent in many artificial oils (Guenther and Althausen, 1975). The fact that *S. dulcis* leaf essential oils yields such high percentages of α-pinene (48.11 %), it could be ideal raw material for the manufacture of camphor and terpineol.

There was no other reported data on *S. dulcis* available for comparison. However looking at the trend seen in previous species it can be said that the composition of the Fijian *S. dulcis* will be different from *S. dulcis* from other locations of the world. The reason for the difference could be due to the location of the plant collected, the time the plant was collected, and the process used for essential oil collection. Another reason for the difference in the number and percentage of components identified could be due to the type of instrument used and the parameters used for analysing the essential oils. Also the difference in composition of components in the *S. dulcis* essential oils could be due to the stability of the various components present in essential oils and also which biosynthetic

pathway is favoured by the plant species from each region. The difference could also be due to the stress the plant was in the time of collection and also the requirement of each component by the plant.

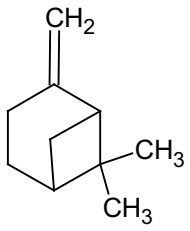

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Table 17: The yield and physical characteristics of the leaf essential oils of *S. dulcis*

Character	Leaf Oil
Oil yield	0.01 %
Colour	pale clear yellow

Table 18: Relative peak areas in % of Fijian *S. dulcis* leaf essential oils components

Components (ref RI)	**Average area (%)**	**RI MS**	**Ref RI**
α-thujene	0.65	917	925
α-pinene	48.11	928	934
fenchene	0.10	947	951
camphene	0.62	950	953
β-pinene	13.10	984	982
myrcene	2.92	991	989
α-phellandrene	0.48	1014	1005
α-terpinene	0.56	1024	1018
p-cymene	0.41	1033	1029
limonene	13.94	1037	1031
β-phellandrene	1.25	1040	1031
ocimene	0.12	1051	(Z)-1040,(E)-1050
γ-terpipene	1.22	1065	1062
terpinolene	2.05	1093	1091
cis-*p*-mentha-2,8-dienol	0.03	1133	1111
terpinene-4-ol	0.08	1193	1182
α-terpineol	0.31	1209	1198
caryophyllene	12.42	1432	1437
α-humulene	0.72	1469	1470
β-Selinene	0.26	1499	1485
α-selinene	0.39	1510	1494
caryophyllene oxide	0.25	1601	1609
TOTAL	100.00	-	

- RI-MS are the experimental retention indices for BPX5 column.
- Ref RI are the published retention indices values on the DB5 (equivalent to BPX5) column.
- All components have not been reported previously for *S. dulcis*

4.2 Microbiological studies of essential oils of the plant species investigated

The bioassays that were conducted for each essential oil were, antibacterial (*E. coli* and *S. aureus*) and antifungal (*C. albicans*) tests. The essential oils were also tested for antimalarial activity using the FBIT (Ferriprotoporphyrin IX Biomineralization Inhibition test) and the PfNEK (Protein kinase) tests. Antidengue test was also conducted on the various essential oil samples.

4.2.1 Antibacterial and antifungal test

The results obtained from the bioassays conducted on the various essential oils are shown in Table 19. From Table 19 it can be seen that Fijian *A. zerumbet* rhizome essential oils had antibiotic activity very similar to that of the commercially available antibiotic (Gentamycin). Leaf essential oils of *C. amboinicus* also had antibiotic activity against the 2 bacteria and the fungus. The *C. verum* leaf essential oil had high activity against the bacteria *S. aureus* and the fungus *C. albicans.* Leaf essential oils of *P. racemosa* and *C. coloratus* showed some antifungal activity, but the inhibition was not as intense as that of *A. zerumbet* rhizome essential oils. The concentration of the essential oil used for the antibacterial test was 1 mg/10 µl (see Methodology 3.1.5, page 39). Gentamycin was used as a standard antibacterial whilst no standard was used for antifungal bioassay because none was available during the time of the experiment.

Table 19. Antibacterial and antifungal activities of the essential oils

	distance inhibited (mm)		
Substance	*E. coli*	*S. aureus*	*C. albicans*
M. quinquenervia (DCM)	-	-	-
C. verum (DCM)	-	10	7
A. zerumbet- leaves (DCM)	-	-	-
A. zerumbet –rhizomes(DCM)	19	16	11
A. zerumbet – flower(DCM)	-	-	-
P. racemosa (DCM)	-	-	7
C. amboinicus (DCM)	8	8	7
C. coloratus (DCM)	-	-	7
Solvent (DCM)	-	-	-
Anti biotic	22	17	-

E. coli – Escherichia coli
S. aureus – Staphylococcus aureus
C. albicans – Candida albicans
Anti Biotic – Gentamycin (10µg)

4.2.2 Antidengue assay.

For the antidengue test, all essential oil samples showed negative results which indicate no anti dengue activity. To identify if any essential oil had any antidengue activity, the wells need to look like TC (normal T cells). However if essential oils did not exhibit any antidengue activity then the wells look like TV (t cells with virus). But if the wells are clear then the extract is cytotoxic (Figure 11).

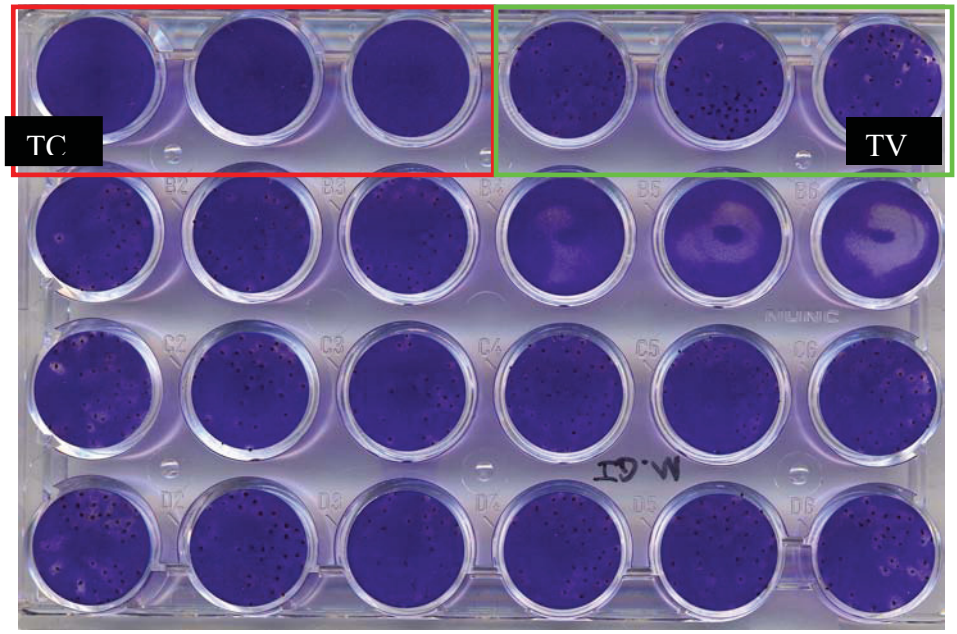


Figure 11: Viewing of cells after stage 3 of antidengue assay

4.2.3 Antimalarial assay

The antimalarial test was done using two methods. The first method was using FBIT (Ferriprotoporphyrin IX Biomineralization Inhibition test). The results are presented in table 20. From Table 20 it can be seen that *A. zerumbet* rhizome essential oils and *C.* coloratus leaf essential oils showed good antimalarial activities. For the second antimalarial test [Test PfNEK (Protein kinase)], all essential oil samples showed negative results, Roscovitine had 69.8 % inhibition.

Table 20: The percentage inhibition of the essential oils tested for antimalarial activity

Haemin 0.5mg/ml	Acetate Buffer pH 3.8
Extract : 10mg/ml	
Control water-1.981	SD-0.02
Control DMSO-2.02:	SD-0.26

Experiments	Average	SD	% INH
Chloroq 0.5 mg/ml	0.69	0.08	65.3
Chloroq 0.25 mg/ml	0.54	0.08	72.5
Chloroq 0.125 mg/ml	0.54	0.02	72.7
Chloroq 0.0625 mg/ml	1.32	0.11	33.6
M. quinquenervia	2.33	0.11	-15.4
C. amboinicus	1.44	0.08	28.7
P. racemosa	2.41	0.23	-19.4
A. zerumbet- rhizomes	0.72	0.06	64.4
C. verum	1.98	0.25	1.9
C. coloratus	0.48	0.09	76.1
A. zerumbet- leaves	1.65	0.1	18.5
A. zerumbet- flowers	1.57	0.26	22.3

- SD- standard deviation
- Chloroq. – chloroquine
- INH- inhibition

Part B

5.0 Chemical Investigation of *Alpinia zerumbet*

5.1 Background of the plant species investigated

A. zerumbet is one of the species that was included for essential oils analysis as reported in Part A (Pages 69-77). In this section (Part B of this thesis), the isolation and structure elucidation of desmethoxyyangonin (**63**) is being reported. This compound is found in *Piper methysticum* [kava] (Smith, 1983). The earlier investigation of the leaves of *A. zerumbet* (Kuster *et al*., 1999) reported the presence of desmethoxyyangonin using GC-MS. In this study, the isolation and detailed characterization and structure elucidation of desmethoxyyangonin from *A. zerumbet* using ^{1}H, ^{13}C, HMQC NMR and MS has been investigated. This is the first report of 2D NMR data on desmethoxyxyangonin. Xu *et al*. (1996) reported the isolation and structural determination of labdane type diterpene; zerumin A (**64**) and zerumin B (**65**) from the seeds of *A. zerumbet.*

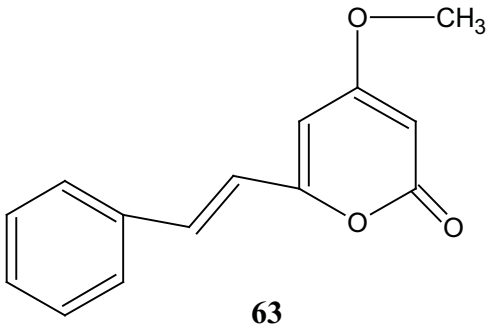
5.2 Method

5.2.1 Solvent Extraction

Essential oil was removed from *A. zerumbet* leaves and flowers (see 3.1 Extraction of Essential oils, page 42). The aqueous layer after steam distillation was first partitioned with n-hexane followed by DCM (the flower aqueous layer was portioned separately and so was the leaf aqueous layer). The resulting 4 organic solvent extracts were passed through excess anhydrous sodium sulphate and concentrated *in vacuo.* The concentrated crude n-hexane and DCM extracts were stored at 4 ^{o}C for future further analysis.

Note:

Even though desmethoxyyangonin is a non-polar compound it has been extracted from aqueous kava extracts. This is because desmethoxyyangonin is in colloidal suspension form in the aqueous extract. If non-polar solvent extractions are done of this aqueous extract, desmethoxyyangonin can be isolated. It is a miss-understood concept that since desmethoxyyangonin is a non-polar compound and can not be extracted from the aqueous medium.



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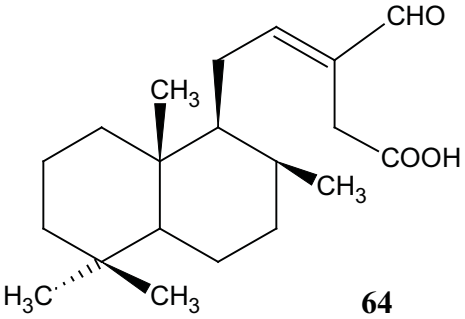

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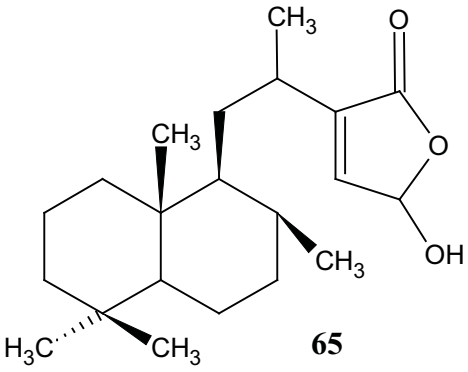

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5.2.2 Isolation of desmethoxyyangonin

Thin Layer Chromatography (TLC) analysis indicated the presence of 1 major compound in all 4 extracts of leaf and flower samples. TLC was performed using glass plates coated with silica gel 60 F_{254} (Merck). Mobile phase used for TLC were n-hexane and ethyl acetate (EtOAc). The 4 extracts were combined and separation was done using column chromatography (column length, 70.0 cm, i.d. 7.0 cm). The stationary phase was silica gel 60 (35 – 70 μm) and mobile phase (n-hexane and EtOAc) in increasing polarity. A total of 30 fractions were collected in test-tubes (10 cm length and i.d. 2 cm). Each fraction collected was about 25ml and of the fractions collected, fractions 13, 14, 15 and 16 were found to contain 1 compound (R_f = 0.25; 20 % EtOAc- 80 % n-hexane). These 4 fractions were combined, separated and purified using a chromatotron (centrifugal thin layer chromatograph [model 8924 and 7924]). The silica layer was F_{254} and 4 mm thick on a glass plate and the mobile phase was n-hexane and EtOAc in increasing polarity. A total of 250 fractions were collected in test-tubes (7 cm length and i.d. 1 cm). Each fraction collected was about 5ml and of the factions collected, fractions 156 to 200 were found to contain 1 compound (R_f value 0.25; 20 % EtOAc- 80 % n-hexane). Fractions 156 to 200 were combined and concentrated *in vacuo,* upon which the residue (white crystals) started to appear. The white crystals were recrystalised with methanol to give desmethoxyyangonin (72.87 mg), melting point of 138–140 ^{o}C (literature value is 138-139 ^{o}C, Klohs *et al.,* 1959). The UV, IR, melting point, MS and NMR data are given in page 94.

5.2.3 Instrumentation

- Melting point was determined using a Jepson Bolton Melting Point apparatus Electrothermal (Model IA9100)
- UV was measured using a Perkin Elmer Spectrophotometer with the computer program Lambda 16
- IR was measured on a Perkin Elmer FTIR spectrometer (Model 1000). Measurements were taken in the range of 4000 – 400 cm^{-1} with a resolution of 2 cm^{-1}. Spectra was obtained using KBr.
- EIMS and HRMS was obtained using a Bruker APEXII FTICR instrument at the University Analytical Laboratory at University of the New South Wales, Australia. EIMS was obtained at 50 – 70 eV
- ^{1}H and ^{13}C NMR and HMQC spectra were recorded on a Bruker DMX500 spectrometer. The internal reference for all NMR experiments were TMS measured at 0 ppm. ^{1}H chemical shifts were reported relative to the solvent at 7.25 ppm and ^{13}C chemical shifts relative to the solvent at 77.0 ppm. All NMR experiments were carried out in $CDCl_3$ and performed at room temperature.

5.3 Results

Desmethoxyyangonin ($C_{14}H_{12}O_3$); white crystals

Melting point: 138–140 ^{o}C (literature value is 138-139 ^{o}C, Klohs *et al.,* 1959)

UV (MeOH) : λ_{max} (log ε)/nm = 210, 243, 341 (4.35) (literature value is 4.37, Klohs *et al.,* 1959

IR (KBr**)**: ν_{max} = 3035, 2830 1725, 1630 960 cm^{-1}

EIMS and **HRMS** : m/z (relative intensity) = 228 [M^{+}] (75 %), 200 (36 %), 157 (78 %), 77 (85 %) 69 (100 %); $C_{14}H_{12}O_3$, calculated 228.0927, found 228.0924

^{1}H-NMR- ($CDCl_3$): δ = 3.82 (3H, s, CH_3-15); 5.49 (1H, d, H-3); 5.94 (1H, d, H-5); 6.5-6.6 (1H, d, H-7); 7.32-7.39 (3H, m, 11, 12, 13-H; 7.47-7.48 (1H, d, H-8); 7.50-7.52 (2H, m, 10, 14-H).

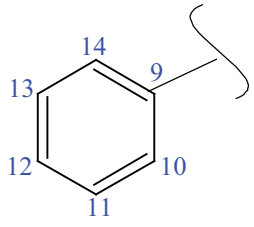
^{13}C-NMR- ($CDCl_3$) δ = 55.8 (C-15); 88.7 (C-3); 101.3 (C-5); 118.5 (C-7); 127.2 (C-14); 127.8 (13, 10-C); 129.3 (11, 12-C); 135.1 (C-9); 153.7 (C-8); 164.0 (C-2).

5.4 Discussion

Desmethoxyyangonin was isolated from the leaves and flowers of *A. zerumbet*. It was isolated as white crystals and had a melting point of 138-140 ^{o}C (literature value 138-140 ^{o}C, Klohs *et al.,* 1959)

The HRMS gave the molecular formula to be $C_{14}H_{12}O_3$. There were 9 degrees of unsaturation as double bond equivalents of which 2 were accounted for 2 cyclic skeletons and the remaining 7 degrees of unsaturation were 7 double bonds.

The ^{1}H-NMR of desmethoxyyangonin was consistent with the presence of mono substituted benzene ring (a total of 5 aromatic protons) assigned as 10, 11, 12, 13 and 14 (δ 7.50 – 7.52, m, 10, 14-H and 7.32 - 7.39, m, 11, 12 13-H). The ^{13}C-NMR was also consistent with the presence of a mono substituted benzene ring. Signals at 127.8, 128.8 and 129.3 ppm (10, 11, 12 13, 14-C) were sp^2 methine carbons of a mono substituted benzene ring. The ^{13}C-NMR signal at 135.1 ppm (C-9) was an sp^2 quaternary carbon of a mono substituted benzene ring, (partial structure I). HMQC-NMR spectrum further identified the aromatic protons with their directly bound carbons (Table 21).



Partial Structure I

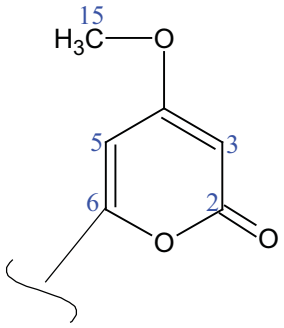
The partial structure I (mono substituted benzene ring) was supported by the IR spectrum that contained absorption bands at 3035 and 1630-1580 cm^{-1} (characteristic peaks of

aromatic ring). The partial structure I was further supported by a fragment ion in EIMS at m/z 77 (characteristic fragment for aromatic ring). UV also confirmed the partial structure I (λ_{max} 210, 243 nm- due to the presence of conjugated system).

The ^{1}H-NMR of desmethoxyyangonin exhibited a sharp 3H singlet at δ 3.82, which was assigned to a methoxy group. HMQC-NMR identified the carbon that was directly bound to the methoxy proton at δ 55.8 (C-15). There was evidence in the IR spectrum for the presence of a methoxy group (ν_{max} 2230 cm^{-1}).

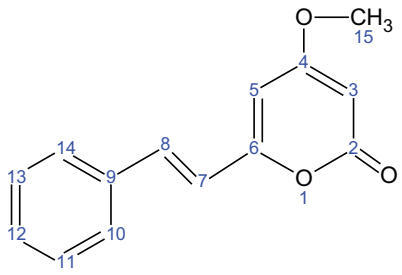
IR spectrum showed significant absorption peak at 1725 cm^{-1} (carbonyl functionality). Since 2 of the 3 oxygen atoms were accounted for by the methoxy group and carbonyl functionality, the remaining oxygen atom was concluded to be present as part of a heterocyclic ring.

The heterocyclic ring had 2 olefinic protons assigned 3 and 5 (δ 5.94, s, 5-H and 5.49, s, 3-H). HMQC and ^{13}C-NMR identified the sp^2 methine carbons that were directly bound these olefinic protons hydrogens (δ 88.7, C-3 and 101.3, C-5). The ^{13}C-NMR signals at δ 164.0 (C-2), an sp2 quaternary carbon was a carboxyl carbon, consistent with the IR spectrum absorption reading (ν_{max} 1725 cm^{-1}). The ^{13}C-NMR signal at δ 171.0 (sp^2 quaternary carbon) was assigned as C-4 due to the signal being more downfield since the carbon is attached to a methoxy group. The ^{13}C-NMR signal at δ 158.5 (C-6) was an sp^2 quaternary carbon. This information gave a partial structure II.



Partial Structure II

The remaining features of this compound, assigned from ^{1}H and ^{13}C-NMR spectra was a alkene (ethene) group, with 1 carbon adjacent to a mono substituted benzene group [δ_H 7.47-7.48 (1H, d, 8-H), δ_C 135.7 (C-8)] and the other carbon adjacent to the hetero cyclic ring [δ_H 6.5-6.6 (1H, d, 7-H), δ_C 118.5 (signals at δ 118.5 and 135.7 ppm (C-7)]. These data led to the conclusion that the two cyclic structures were linked together by an ethene chain and the compound was assigned the structure (**63**).



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Comparisons of the physical and spectroscopic data showed that (**63**) to be identical to desmethoxyyangonin, previously isolated from *Piper methysticum* (kava) (Smith, 1983).

Table 21: ^{1}H and ^{13}C NMR chemical shifts for desmethoxyyangonin in $CDCl_3$

Position	^{13}C (ppm)	HMQC ^{1}H — ^{13}C (δ-multiplet)
2	164.0	-
3	88.7	5.49 (d)
4	171.0	-
5	101.3	5.94 (d)
6	158.5	-
7	118.5	6.5 – 6.6 (d)
8	135.7	7.47 – 7.48 (d)
9	135.1	-
10	128.8	7.50 – 7.52 (m)
11	129.3	7.32 – 7.39 (m)
12	129.3	7.32 – 7.39 (m)
13	128.8	7.32 – 7.39 (m)
14	127.2	7.50 – 7.52 (m)
15	55.8	3.82 (s)

Spectra are in appendix 2

The ^{1}H and ^{13}C NMR data of the present study was compared to the NMR data of Dharamaratne *et al*. (2002) (Table 22 and Table 23). Dharamaratne *et al*. (2002) isolated desmethoxyyangonin from kava (*Piper methysticum*) and elucidated the structure using NMR spectroscopic methods. It was seen that there was no significant difference between the present NMR data and the published NMR data.

Table 22: ^{1}H NMR spectroscopic data of Desmethoxyyangonin

	δ (ppm)	
Hydrogen	Reported [Ψ]	Present study
I0-H	7.48 (m)	7.50 - 7.52 (m)
14-H	7.48 (m)	7.50 - 7.52 (m)
8-H	7.44 (d, J = 15.9)	7.47 - 7.48 (d)
11-H	7.35 - 7.31 (m)	7.32 - 7.39 (m)
12-H	7.35 - 7.31 (m)	7.32 - 7.39 (m)
13-H	7.35 - 7.31 (m)	7.32 - 7.39 (m)
7-H	6.54 (d, J = 16.0)	6.5 - 6.6 (d)
5-H	5.90 (s)	5.94 (s)
3-H	5.44 (d, J = 1.4)	5.49 (d)
15-H	3.76 (s)	3.82 (s)

[Ψ] Dharamaratne *et al*., (2002)

Table 23: ^{13}C NMR spectroscopic data of Desmethoxyyangonin.

	δ (ppm)	
Carbon	Reported[Ψ]	Present Study
2	164.2	164.0
3	89.2	88.7
4	171.4	171.0
5	101.8	101.3
6	158.9	158.5
7	119.1	118.5
8	135.8	135.7
9	135.6	135.1
10	127.8	128.8
11	129.2	129.3
12	129.8	129.3
13	129.2	128.8
14	127.8	127.2
15 – OCH_3	56.3	55.8

[Ψ] Dharamaratne *et al*., (2002)

6.0 Conclusion

In the present study, essential oils from 5 Fijian plants (*E. foetidum, C. sativum, C. verum, S. dulcis* and *A. zerumbet)* were investigated using GC-MS. Some of the major components in the essential oils such as eugenol (from *C. verum*), α-pinene (from *S. dulcis*) can form alternative sources of industrial raw materials.

The uses of techniques such as GC-MS have revealed components hitherto not reported from the same plant species examined previously by other investigators. The list of theses natural products adds to the database of volatile bio-molecules from the 5 plant species mentioned above.

New plant sources for (E)-2-dodecenal, (from *E. foetidum*), 2-decen-1-ol (from *C. sativum*) and 4-terpineol (from *A. zerumbet*) have emerged from this study.

The various reasons mentioned in discussion could affect the percentages of components in the essential oils. These various reasons could affect the mevalonic acid pathway synthesis. Mevalonic pathway is the common pathway followed for the biosynthesis of many components present in essential oils.

7.0 Future work

As new analytical methods are developed, it may be prudent to re-examine previous studies to reveal new chemistry as shown in this present investigation. The fast developing GC-olfactometry and comprehensive two-dimensional gas chromatographic analysis of the essential oils reported in this study may reveal very useful information such as aroma profiles and chemistry of the important odorants present in the essential oils reported in this thesis.

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9.0 Appendix

9.1 Appendix 1

Diagram for the quantitation of essential oils in plant drugs. (Dimensions in millimetres)

Bruneton (1999)

9.2 Appendix 2

Copies of MS, ^{1}H, ^{13}C and HMQC NMR spectra