



UNIVERSITI PUTRA MALAYSIA

**PHYTOCHEMICALS AND ANTI-INFLAMMATORY ACTIVITY OF
MELICOPE PTELEFOLIA CHAMP EX BENTH**

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**PYHTOCHEMICALS AND ANTI-INFLAMMATORY ACTIVITY OF
MELICOPE PTELEFOLIA CHAMP EX BENTH**

**By
SURYATI**

**Thesis Submitted to School of Graduate Studies, Universiti Putra Malaysia in
Fulfilment of the Requirements for the Degree of Master of Science**

May 2005



DEDICATION

My beloved parents (Mama & Papa)

My sisters & brother -in-law

My family in Padang

Thanks to your praying, support, motivation and sacrifice.....

Abstract of thesis presented to the senate of Universiti Putra Malaysia in fulfilment of requirement for the degree of Master of Science

**PHYTOCHEMICALS AND ANTI-INFLAMMATORY ACTIVITY OF
MELICOPE PTELEFOLIA CHAMP EX BENTH**

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Chairman: Associate Professor Khozirah Shaari, PhD

Institute : Bioscience

Leaves of *Melicope ptelefolia* Champ ex Benth were examined for phytochemicals. Using various chromatography techniques such as normal column chromatography, gel filtration on sephadex LH-20 and radial chromatography seven compounds were isolated namely, kokusaginine (**94**), β -sitosterol (**110**), p-*O*-geranylcoumaric acid (**102**), 3-geranyl-2,4,6-trihydroxyacetophenone (**111**), the tentatively assigned compound benzopyranone (**112**), 4',5-dihydroxy-3,3',7-trimethoxyflavone (**113**) and scoparone (**86**). 3-Geranyl-2,4,6-trihydroxyacetophenone was identified as a new natural product, which was previously reported as a synthetic compound.

Nitric oxide (NO) inhibitory assay using RAW 246.7 murine monocytic macrophage and soybean lipoxygenase inhibitory assay were carried out in the primary screening of

the crude methanolic extract, the hexane, dichloromethane and ethyl acetate fractions. Both the hexane and dichloromethane fractions were shown strongly to inhibit nitric oxide production with an IC_{50} of 27.81 $\mu\text{g/ml}$ and 34.13 $\mu\text{g/ml}$ respectively. Meanwhile, inhibition of soybean lipoxygenase activity was shown only by the dichloromethane fraction at IC_{50} 48.01 $\mu\text{g/ml}$

Further anti-inflammatory investigation on the isolated compounds showed that kokusaginine significantly inhibited NO production with an IC_{50} of 12.09 $\mu\text{g/ml}$ (46.69 μM), while 3-geranyl-2,4,6-trihydroxyacetophenone inhibited formation of (9*Z*, 11*E*)-(13*S*)-13-hydroxyoctadeca-9,11-dienoate with an IC_{50} of 7.35 $\mu\text{g/ml}$ (24.17 μM).

3-Geranyl-2,4,6-trihydroxyacetophenone was further evaluated towards inhibition of cysteinyl leukotriene (CysLTs) production. The result showed that this compound significantly inhibited the production of CysLTs with an IC_{50} of 12.13 μM . This was slightly lower than the IC_{50} of nordihydroguaretic acid (8.13 μM) which was used as the reference drug.

Abstrak tesis dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

**KAJIAN FITOKIMIA DAN AKTIVITI ANTI-INLAMASI DARIPADA
MELICOPE PTELEFOILA CHAMP EX BENTH**

Oleh

SURYATI

May 2005

Pengerusi : Profesor Madya Dr. Khozirah Shaari, PhD

Institut : Institut Biosains

Kajian fitokimia telah dilakukan terhadap daun *Melicope ptelefolia* Champ ex Benth. Melalui penggunaan berbagai teknik kromatografi seperti kromatografi turus biasa, filtrasi gel sephadex LH-20 dan kromatografi radial. Tujuh sebatian telah berjaya dipencilkan iaitu, kokusaginin (**94**), p-*O*-geranilkumarik asid (**102**), 3-geranil-2,4,6-trihidroksiasetofenon (**111**), sebatian yang sementara dikenal sebagai benzopiranon (**112**), 4',5-dihidroksi-3,3',7-trimetoksiflavon (**113**) dan skoparon (**86**). Sebatian 3-geranil-2,4,6-trihidroksi asetofenon (**111**) merupakan sebatian baru yang diperolehi dari alam semula jadi yang sebelum ini telah pun dilaporkan sebagai suatu sebatian hasil sintetik.

Untuk menilai sifat anti-inflamasi tanaman ini telah diuji dengan model inflamasi seluler dan asai kinetik enzimatik. Asai perencatan nitrik oksida (NO) menggunakan makrofag

murin monositik (RAW 246.7) dan asai lipoksigenase kacang soya telah digunakan sebagai penabiran awal bagi ekstrak kasar methanol, fraksi heksana, fraksi diklorometana dan fraksi etil asetat. Hasilnya, fraksi heksana dan fraksi diklorometana menunjukkan aktiviti yang kuat menekan penghasilan nitrik oksida, dengan nilai 50% perencatan 27.81 $\mu\text{g/ml}$ dan 34.13 $\mu\text{g/ml}$ setiap satu. Manakala penekanan lipoksigenase kacang soya hanya diperlihatkan oleh fraksi diklorometana dengan nilai 50% perencatan 48.01 $\mu\text{g/ml}$.

Penyelidikan anti-inflamasi keatas sebatian hasil penulinan menunjukkan kokusaginin secara signifikan menekan pembebasan nitrik oksida dengan nilai 50% perencatan 12.09 $\mu\text{g/ml}$ (46.69 μM) manakala sebatian 3-geranil-2,4,6-trihidroksiasetofenon mempunyai aktiviti yang kuat pada asai penekanan lipoksigenase kacang soya dengan nilai 50 % perencatan 7.35 $\mu\text{g/ml}$ (24.27 μM). 3-Geranil-2,4,6-trihidroksiasetofenon dikaji selanjutnya terhadap perencatan sisteinil leukotriena (CysLTs) daripada makrofag mencit putih jantan. Hasilnya menunjukkan bahawa sebatian ini secara signifikan menekan pembebasan CysLTs dengan nilai 50 % perencatan 12.13 μM iaitu lebih rendah sedikit berbanding dengan sebatian asid nordihidroguaretik yang digunakan sebagai sebatian piawai dengan nilai 50% perencatan 8.13 μM .

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TABLE OF CONTENTS

DEDICATION	Page ii
ABSTRACT	iii
ABSTRAK	v
ACKNOWLEDGEMENTS	vii
APPROVAL	viii
DECLARATION	x
LIST OF TABLES	xiii
LIST OF FIGURES	xv
GLOSSARY OF ABBREVIATIONS	xixi
CHAPTER	
1. INTRODUCTION	1
2. LITERATURE REVIEW	5
2.1. Botany, Distribution and Ethnobotany of <i>Melicope</i> species	5
2.1.1. The genus <i>Melicope</i>	5
2.1.2. <i>Melicope ptelefolia</i> Champ ex Benth	6
2.2. A review of Previous Investigation on the <i>Melicope</i> species	9
2.2.1. Chemical constituents from <i>Melicope</i> species	9
2.2.2. Chemical constituents from <i>Melicope ptelefolia</i> Champ ex Benth	16 23
2.3. Biologically active constituents from the <i>Melicope</i> species	26
2.4. Anti-inflammatory activity of <i>Melicope</i> species	28
2.5. Inflammation	30
2.5.1. Mediators of Inflammation	32
2.6. Lipxygenase Enzyme	33
2.6.1. Classification of Lipxygenase	36
2.6.2. Distribution of Lipxygenase	36
2.6.3. Structure of Lipxygenase	37
2.6.4. Substrate Specificity	39
2.6.5. Stimulation of Lipxygenase Activity	40
2.6.6. Products of Lipxygenase	43
2.6.7. Inhibition of Leukotriene Biosynthesis	44
2.7. Involvement of Nitric Oxide (NO) in Inflammatory Process	45
2.7.1. Inhibition of Nitric Oxide Production	46
3. MATERIALS AND METHODS	46
3.1. General Instrumentation	46
3.2. Plant Material	46
3.3. Sample Preparation and Extraction	48
3.4. Chromatography	49
3.5. Isolation Compounds from the Hexane Fraction	52
3.6. Isolation Compounds from the Dichloromethane Fraction	55



3.6.	Isolation Compounds from the Dichloromethane Fraction	55
3.7.	Anti-inflammatory Assay	57
3.7.1.	Principle of Assay	58
3.7.2.	Chemicals and Reagents	59
3.7.3.	Preparation of Test Sample	59
3.7.4.	Methodology	61
3.7.4.1.	In vitro Enzymatic Lipooxygenase Inhibition Assay	63
3.7.4.2.	In-vitro Inhibition Cysteinyl Leukotriene (CysLTs) Product Assay	63
3.7.4.3.	In-vitro Inhibition of Nitric Oxide Production Assay	65
3.7.4.3.1.	Cell Culture	65
3.7.4.3.2.	Griess Assay	66
3.7.4.4.	Cell viability	66
3.7.4.5.	Data Analysis	66
4.	RESULT AND DISCUSSION	74
4.1.	Characterization of Isolated Compounds	79
4.1.1.	Kokusaginine (94)	90
4.1.2.	β -sitosterol (110)	100
4.1.3.	p-O-geranylcoumaric acid (110)	109
4.1.4.	3-geranyl -2,4,6-trihydroxyacetophenone (111)	121
4.1.5.	Tentative Structure of Benzopyranone (112)	129
4.1.6.	4',5-dihydroxy-3,3',7-trimethoxyflavone (113)	129
4.1.7.	Scoparone (86)	
4.2.	Nitric Oxide and Soybean Lipooxygenase Inhibition of <i>Melicope Ptelefolia</i>	131
4.2.1.	Inhibition of Soybean LO by the Crude and Fraction of <i>Melicope ptelefolia</i>	133
4.2.2.	Inhibition of Nitric Oxide Production by the Crude and Fraction of <i>Melicope ptelefolia</i>	135
4.2.3.	Inhibition of Soybean Lipooxygenase by Isolated Compounds	136
4.2.4.	Inhibition of Nitric Oxide Production by Isolated Compound	140
4.2.5.	Inhibition of Cysteinyl Leukotriene by 3-geranyl-2,4,6-trihydroxyacetophenone.	141

5. CONCLUSION

REFERENCE

BIODATA OF THE AUTHOR

LIST OF TABLES

Table		Page
1	4-Geranyloxyacetophenone isolated from <i>Evodia merilii</i> (Chou & Lin., 1992 and Lin, 1993)	13
2	Quinolone alkaloid isolated from fruits of <i>Evodia rutaecarpa</i> (Tang <i>et al.</i> , 1996)	14
3	Benzopyrans isolated from <i>Melicope ptelefolia</i> (Kamperdick, <i>et al.</i> , 1997; 1998, Van <i>et al.</i> , 1999, Li <i>et al.</i> , 1997, Li & Zhu, 1998, Manandar <i>et.al</i> , 1985)	17
4	The assignment of proton and carbon of kokusaginine (94)	74
5	The assignment of proton and carbon of β -sitosterol (110)	75
6	The assignment of proton and carbon of p-O-geranylcoumaric acid (102)	80
7	^1H (500 MHz) and ^{13}C (125 MHz) in CD_3OD NMR data and their short (1J) and long range (2J & 3J) connectivities obtained from gHSQC and gHMBC experiments, respectively, for compound (111)	92
8	^1H (500 MHz) and ^{13}C (125 MHz) in CDCl_3 NMR data and their short (1J) and long range (2J & 3J) connectivities obtained from gHSQC and gHMBC experiments, respectively, for compound (112)	102
9	The assignment of proton and carbon of 4',5-dihydroxy-3,3',7'-trimethoxyflavone	112
10	The assignment of proton and carbon of scoparone (86)	123
11	Inhibition of lipoxygenase by crude and various solvent fraction of <i>Melicope ptelefolia</i>	130
12	Inhibition of NO-released from RAW 264.7 murine macrophage stimulated with <i>E. coli</i> LPS (10 $\mu\text{g/ml}$) and IFN- γ (200 U/ml) by crude and various solvent of <i>Melicope ptelefolia</i> .	133
13	Inhibition of lipoxygenase by isolated compounds of <i>Melicope ptelefolia</i>	134
14	Inhibition of NO-released from RAW 264.7 murine macrophage stimulated with <i>E. coli</i> LPS (10 $\mu\text{g/ml}$) and IFN- γ (200 U/ml) by isolated compounds of <i>Melicope ptelefolia</i> .	136

15	Inhibition of CysLTs-released from male mice macrophage stimulated with 1 μ M of Ca ionophore by 3-geranyl-2,4,6-trihydroxyacetophenone (83)	138
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LIST OF FIGURES

Figure		Page
1	Method for obtaining active substance from plants	2
2	<i>Melicope ptelefolia</i> (Champ. ex Benth)	8
3	4-Geranyloxyacetophenone skeleton	12
4	Quinolone alkaloid skeleton	14
5	Benzopyran skeleton	17
6	The inflammatory mediators derived from phospholipids with an outline of their actions. (Rang <i>et al.</i> , 2003, Gaddi <i>et al.</i> , 2004)	32
7	Formation of unsaturated fatty acid by various lipoxygenases (Yamamoto, 1992).	35
8	Unsaturated fatty acids as substrates for LO enzyme (Rowley, et al., 1998)	38
9	The biosynthesis and chemical structure of leukotriene (Charlier & Michaux, 2003)	42
10	Flowchart of extraction and fractionation of <i>Melicope ptelefolia</i> Champ ex Benth.	47
11	Color reaction involved in NO assay	56
12	Metabolization of MTT to a formazan salt by viable cell	57
13	Flowchart for the enzymatic lipoxygenase inhibition assay	60
14	Flowchart for the inhibition of cysteinyl leukotriene production assay	62
15	Flowchart of nitric oxide assay	64
16	The EI-Mass spectrum of kokusaginine (94)	68
17	IR spectrum of kokusaginine(94)	69
18	¹ H NMR Spectrum of kokusaginine (94) in CDCl ₃	70



19	COSY spectrum of kokusaginine (94) in CDCl ₃	71
20	Proposed mass fragmentation of kokusaginine (94)	72
21	¹³ C NMR Spectrum of kokusaginine (94) in CDCl ₃	73
22	The EI-Mass spectrum of β-sitosterol (110)	76
23	IR spectrum of β-sitosterol (110)	77
24	¹ H NMR Spectrum of β-sitosterol (110)	78
25	IR spectrum of <i>p</i> - <i>O</i> -geranyl coumaric acid (102)	82
26	The EI-Mass spectrum of <i>p</i> - <i>O</i> -geranycoumaric acid (102)	83
27	Mass fragmentation of <i>p</i> - <i>O</i> -geranycoumaric acid (102)	79
28	¹ H NMR Spectrum of <i>p</i> - <i>O</i> -geranycoumaric acid (102) in CDCl ₃	84
29	COSY spectrum of <i>p</i> - <i>O</i> -geranycoumaric acid (102) in CDCl ₃	85
30	¹³ C NMR Spectrum of <i>p</i> - <i>O</i> -geranycoumaric acid (102) in CDCl ₃	86
31	HSQC Spectrum of <i>p</i> - <i>O</i> -geranycoumaric acid (102) in CDCl ₃	87
31a	HSQC Spectrum of <i>p</i> - <i>O</i> -geranycoumaric acid (102) in CDCl ₃	88
32	HMBC spectrum of <i>p</i> - <i>O</i> -geranycoumaric acid (102) in CDCl ₃	89
33	The ESI-Mass spectrum of 3-geranyl-2,4,6-trihydroxyacetophenone (111)	94
34	IR spectrum of 3-geranyl-2,4,6-trihydroxyacetophenone (111)	95
35	¹ H NMR Spectrum of 3-geranyl-2,4,6-trihydroxyacetophenone (111) in CD ₃ OD	96
36	¹³ C NMR Spectrum of 3-geranyl-2,4,6-trihydroxyacetophenone(111) in CD ₃ OD	97
37	COSY spectrum of 3-geranyl-2,4,6-trihydroxyacetophenone (111) in CD ₃ OD	98
38	Mass fragmentation of 3-geranyl-2,4,6-trihydroxyacetophenone (111)	93

39	gHMBC spectrum of 3-geranyl-2,4,6-trihydroxyacetophenone (111) in CD ₃ OD	99
40	The EI-Mass spectrum of benzopyranone (112)	103
41	IR spectrum of benzopyranone (112)	104
42	¹ H NMR Spectrum of in CDCl ₃ benzopyranone (112)	105
43	¹³ C NMR Spectrum of in CDCl ₃ benzopyranone (112)	106
44	gCOSY spectrum of benzopyranone in CDCl ₃	107
45	gHMBC spectrum of benzopyranon in CDCl ₃	108
46	The EI-Mass spectrum of 4',5-dihydroxy-3,5',7-trimethoxyflavone (113)	113
47	IR spectrum of 4',5-dihydroxy-3,5',7-trimethoxyflavone (113)	114
48	¹ H NMR spectrum of 4',5-dihydroxy-3,5',7-trimethoxyflavone (113)	115
49	COSY spectrum of 4',5-dihydroxy-3,5',7-trimethoxyflavone (113)	116
50	Mass fragmentation of 4',5-dihydroxy-3,5',7-trimethoxyflavone (113)	111
51	¹³ C NMR Spectrum of 4',5-dihydroxy-3,5',7-trimethoxyflavone (113)	117
52	HMBC spectrum of 4',5-dihydroxy-3,5',7-trimethoxyflavone (113)	118
52a	HMBC spectrum of 4',5-dihydroxy-3,5',7-trimethoxyflavone (113)	119
52b	HMBC spectrum of 4',5-dihydroxy-3,5',7-trimethoxyflavone (113)	120
53	The EI-Mass spectrum of scoparone (86)	124
54	IR spectrum of scoparone (86)	125
55	¹ H NMR Spectrum of scoparone (86)	126
56	COSY spectrum of scoparone (86)	127
57	Mass fragmentation of Scoparone (86)	122
58	¹³ C NMR Spectrum of scoparone (86) in CDCl ₃	128

59	Inhibition (%) of lipoxygenase by crude and various solvent fraction of <i>Melicope ptelefolia</i>	130
60	Inhibition of NO released from RAW 264.7 murine macrophage cell line by crude and various solvent fraction of <i>Melicope ptelefolia</i>	132
61	Inhibition of lipoxygenase by 3-geranyl-2,4,6-trihydroxyacetophenone (111) in comparison with NDGA	135
62	Inhibition of cysteinyl leukotriene (CysLTs) production by 3-geranyl-2,4,6-trihydroxyacetophenone (111)	137

GLOSSARY OF ABBREVIATIONS

5-HETE	(6 <i>E</i> ,8 <i>Z</i> ,11 <i>Z</i> ,14 <i>Z</i>)-(5 <i>S</i>)-Hydroxyeicosa-6,8,11,14,-tetraenoic acid
8-HETE	(5 <i>Z</i> ,9 <i>E</i> ,11 <i>Z</i> ,14 <i>Z</i>)-(8 <i>S</i>)-Hydroxyeicosa-5,9,11,14,-tetraenoic acid
12-HETE	(5 <i>Z</i> ,8 <i>Z</i> ,10 <i>E</i> ,14 <i>Z</i>)-(12 <i>S</i>)-Hydroxyeicosa-5,8,10,14,-tetraenoic acid
15-HETE	(5 <i>Z</i> ,8 <i>Z</i> ,11 <i>Z</i> ,13 <i>E</i>)-(15 <i>S</i>)-Hydroxyeicosa-5,8,11,13,-tetraenoic acid
5-(S)-HPETE	(6 <i>E</i> ,8 <i>Z</i> ,11 <i>Z</i> ,14 <i>Z</i>)-(5 <i>S</i>)-Hydroperoxyeicosa-6,8,11,14,-tetraenoic acid
8-(S)-HPETE	(5 <i>Z</i> ,9 <i>E</i> ,11 <i>Z</i> ,14 <i>Z</i>)-(8 <i>S</i>)-Hydroperoxyeicosa-5,9,11,14,-tetraenoic acid
12-(S)-HPETE	(5 <i>Z</i> ,8 <i>Z</i> ,10 <i>E</i> ,14 <i>Z</i>)-(12 <i>S</i>)-Hydroperoxyeicosa-5,8,10,14,-tetraenoic acid
15-(S)-HPETE	(5 <i>Z</i> ,8 <i>Z</i> ,11 <i>Z</i> ,13 <i>E</i>)-(15 <i>S</i>)-Hydroperoxyeicosa-5,8,11,13,-tetraenoic acid
LT	Leukotriene
LO	Lipoxygenase
PG	Prostaglandin
TXA ₂	Tromboxane A ₂
PAF	Platelet-activating factor
FLAP	5-lipoxygenase-activating protein
AA	Arachidonic acid
ESIMS	Electron Spray Impact Mass Spectrometry
EIMS	Electron Impact Mass Spectrometry
LCMS	Liquid Chromatography Mass Spectrometry
GC-MS	Gas Chromatography Mass Spectrometry
FT-IR	Fourier Transform Infra Red
NMR	Nuclear Magnetic Resonance
COSY	Correlation Spectroscopy
HMBC	Heteronuclear Multiple Bond Correlation
HSQC	Heteronuclear Single Quantum Coherence
NOESY	Nuclear Overhauser Enhancement Spectroscopy
UV	Ultraviolet
TLC	Thin Layer Chromatography
MTT	3-(4,5)-dimethyl-thiazol-2-yl)2,5-diphenyltetrazolium bromide



CHAPTER 1

INTRODUCTION

Nature has been a rich source of valuable drugs. There are about 35,000 plant species are used for medicinal purposes all over the world. According to WHO, 80 % of the world population is dependent on health care provided by medicinal plants. Herbs and other plants have been used as medicinal agents from ancient to modern times. Many researches have proven this statement. At first, on a folkloric basis and later developed on a scientific basis into single agent drugs such as ephedrine from *Ephedra sinica* (Lee, 2004). For the past few years, the biggest field research that have been conducted shows bioactive plant-derived compounds are anti-tumor drugs, antibiotics, drug active against tropical diseases, contraceptive drugs, anti-inflammatory drugs, immunomodulators, kidney protectors and drug for psychiatric use (Hamburger and Hostettman, 1991).

There are three main research approaches : (Lee, 2004)

- i. Bioactivity or mechanism of action directed isolation and characterization of active compounds.
- ii. Rational drug design based modification and analogue synthesis.
- iii. Mechanism of action studies.

The chart below shows how to get bioactive constituents. It is also shows method of obtaining active substances from plants (Rates, 2001).

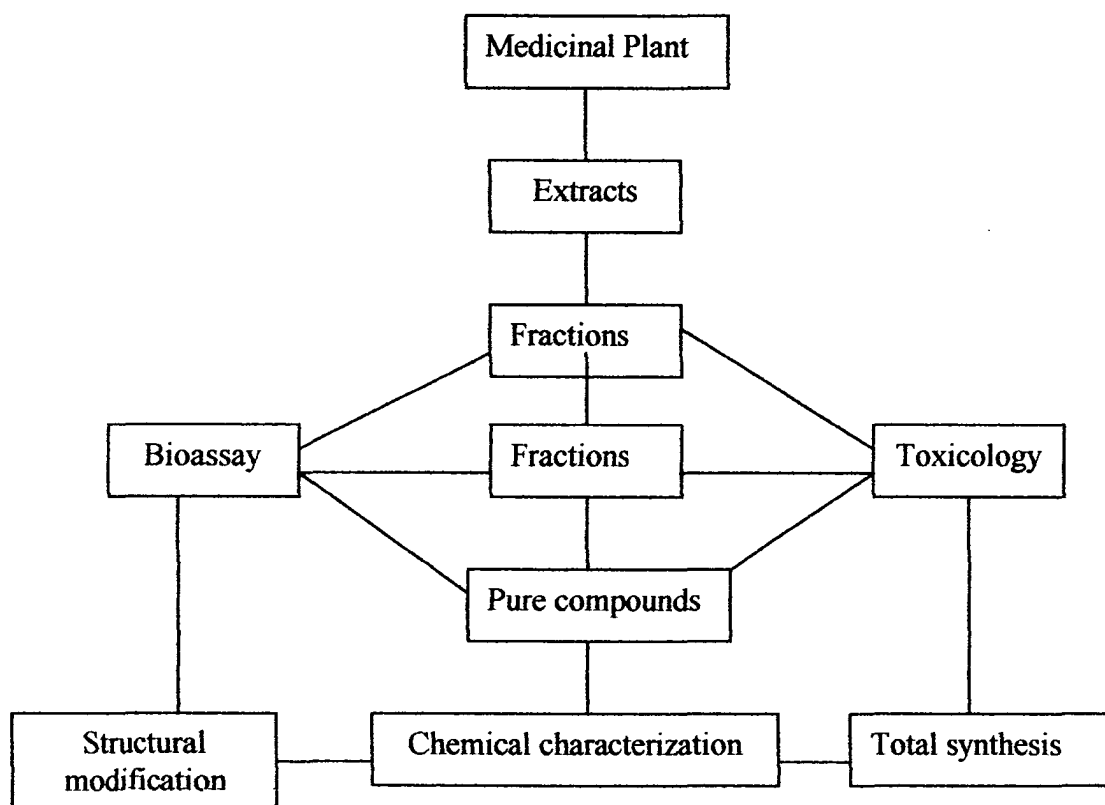


Figure 1. Methods for obtaining active substances from plants

However, the potential use of higher plants as a source of new drugs is still poorly explored. It is estimated about 250,000 – 500, 000 plant species. Only a small percentage has been investigated phytochemically and even a smaller percentage has been properly studied (Payne *et al.*, 1997).

Most cases only pharmacological screening on preliminary studies has been carried out. It is estimated that 5000 species have been studied for medical uses. Between the years 1957 and 1981, the NCI screened around 20,000 plant species from Latin America and Asia for tumor activity, but even these were not screened for other pharmacological activities (Hamburger and Hostettman, 1991).

The plants produce various defense compounds known as secondary metabolites. The secondary metabolites are constitutively express while others are activated upon cell damage. The bioactive nature of many plant secondary metabolites makes them interesting in pharmacy industry. However, the selection of suitable plant for pharmacological study is a very important and decisive step. There are several ways to select a plant for study; based on their traditional uses, chemotaxonomy, toxicity, randomized selection or combination of several criteria (Soejarto, 1996).

It is strongly believed that several *Melicope* species have been prove to grow in Malaysia. There are about eight species can be found in Peninsular Malaysia and fourteen species in Sabah and Sarawak (Soepadmo & Wong, 1995). *Melicope ptelefolia* Champ ex Benth is commonly known as Tenggek Burung in Peninsular Malaysia.

There are other research have been conducted on *Melicope ptelefolia* before. The researcher has found these species on east coast of Malaysia. Furthermore, the study is more toward on anti-insect against *Aedes aegypti* (Ramli *et al.*, 2004),. While my case study are been conducted in west coast of Malaysia. And this research is focused on anti-inflammatory activity. These grounds show the difference of this case study and the case study that have been conducted by other researchers several years ago.

In fact, the *Melicope ptelefolia* species have been collected several years ago may vary from which I have collected recently. This is because the weather and the condition of a soil have a greater impact or influence on the chemical constituent of *Melicope ptelefolia*. This where, it is known as “chemical ecology” (Houghton and Raman, 1998).

Inflammation is the reaction of vascularized tissue upon local injury. Inflammation can be promoted by a variety of mediators including nitric oxide and eicosanoids derived from the lipoxygenase pathway. These mediators are present in white blood cells, macrophages and mast cell. Products of the lipoxygenases (LO) pathway contribute to inflammatory diseases including bronchial asthma, allergic diseases, rheumatoid arthritis and inflammatory bowel disease (Yokomizo, 2000). On the other hand, nitric oxide also contributes to vasodilatation, increasing vascular permeability and increasing the production of prostaglandin. It was also generated at high levels during human inflammatory reaction such as in asthma diseases.

The objectives of this study are:

1. Evaluation of the anti-inflammatory effects of the methanol extract, various fractionated extracts and isolates of the leaves of *Melicope ptelefolia* Champ ex Benth by two *in-vitro* models viz. inhibition of 5-lipoxygenase activity and nitric oxide production,
2. Identification of compounds isolated from the active extracts of the plant by spectroscopic techniques,
3. Characterization of anti-inflammatory activity of the most active compound by inhibition of cysteinyl leukotriene production.

CHAPTER 2

LITERATURE REVIEW

2.1. Botany, Distribution and Ethnobotany of *Melicope* species

2.1.1. The Genus *Melicope*

Melicope is a genus of shrubs, or small trees of the Rutaceae family. There are about 230 species have been recorded and distributed in the world ranging from Madagascar to India, South Cina, throughout Malesia, Polynesia, the Hawaiian Islands, Australia and New Zealand (Soepadmo & Wong, 1995). There are eight species in the Malay Peninsular (Burkill, 1966), and about fourteen species are found in Sabah and Sarawak, i.e; *Melicope triphylla*, *M. jugosa*, *M. sororia*, *M. bonwickii*, *M. denhamii*, *M. latifolia*, *M. clamensiae*, *M. subunifoliolata*, *M. confuse*, *M. glabra*, *M. lunu-ankeda*, *M. accedens*, *M. hookeri* and *M. incana* (Soepadmo & Wong, 1995).

The Malays often call these *Melicope* species collectively as “setenggek burong”. They are also referred as “pauh-pauh” and sometimes are regarded as a form of *Vitex* by calling them “leban” (Burkill, 1966). Hartley (1994) transferred some *Euodia* species into the genus *Melicope*. His revision was based on detailed studies on morphological characteristics. This was supported by chemosystematic data of Rutales family which was published by Waterman in 1983.