UNIVERSITI PUTRA MALAYSIA

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

SURYATI.

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

By

SURYATI

May 2005

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 μg/ml and 34.13 μg/ml respectively. Meanwhile, inhibition of soybean lipoxygenase activity was shown only by the dichloromethane fraction at IC_{50} 48.01μg/ml

Further anti-inflammatory investigation on the isolated compounds showed that kokusaginine significantly inhibited NO production with an IC_{50} of 12.09 μg/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 μg/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 PTELEFOIIA* 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 menunjukan aktiviti yang kuat menekan penghasilan nitrik oksida, dengan nilai 50% perencatan 27.81 μg/ml dan 34.13 μg/ml setiap satu. Manakala penekanan lipoksigenase kacang soya hanya diperlihatkan oleh fraksi diklorometana dengan nilai 50% perencatan 48.01μg/ml.

Penyelidikan anti-inflamasi keatas sebatian hasil penulinan menunjukkan kokusaginin secara signifikan menekan pembebasan nitrik oksida dengan nilai 50% perencatan 12.09 μg/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 μg/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.

ACKNOWLEDGEMENTS

In the name of Allah, most Gracious and most Merciful.

All the great Merciful and Forgiveness of Allah who has bless me with the completion of this thesis even though with many barriers and obstacles I managed to complete this testament.

I would like to express my sincere acknowledgment and deepest appreciation to my supervisor, Assoc. Prof. Dr. Khozirah Shaari for her guidance, constructive criticisms and discussion from the beginning of this research till the final manuscript.

I am also very grateful to members of my supervisory committee, Prof. Dr. Nordin Hj. Lajis and Assoc. Prof. Dr. Daud Ahmad Israf for their discussion and professional assistance in my research.

Much appreciation goes to research officers; Mr. Salahudin, Puan Zurina, Puan Mazina and Puan Normayati for obtaining spectroscopy data. Thanks to all my labmates, especially for kak Habibah, kak Norma, Uwi, Uni Ayu, Khalid and Sharin. Thanks for the laughing, joking and cheerful day. And also I wish to express my special thanks to Pak Deri for encouragement and hard effort in my study. I wish specially thanks to my beloved parents (mama & papa) for constant love, praying and sacrifice and to my sisters and brother in law for all motivation, support and encouragement. Last, but not least, my deepest thank and love to Shahrul Nizam for his support and encouragement.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

GLOSSARY OF ABBREVIATIONS

5-HETE	(6*E*,8*Z*,11*Z*,14*Z*)-(5*S*)-Hydroxyeicosa-6,8,11,14,-tetraenoic acid
8-HETE	(5*Z*,9*E*,11*Z*,14*Z*)-(8*S*)-Hydroxyeicosa-5,9,11,14,-tetraenoic acid
12-HETE	(5*Z*,8Z,10*E*,14*Z*)-(12*S*)-Hydroxyeicosa-5,8,10,14,-tetraenoic acid
15-HETE	(5*Z*,8Z,11*Z*,13E)-(15*S*)-Hydroxyeicosa-5,8,11,13,-tetraenoic acid
5-(S)-HPETE	(6*E*,8*Z*,11*Z*,14*Z*)-(5*S*)-Hydroperoxyeicosa-6,8,11,14,-tetraenoic acid
8-(S)-HPETE	(5*Z*,9*E*,11*Z*,14*Z*)-(8*S*)-Hydroperoxyeicosa-5,9,11,14,-tetraenoic acid
12-(S)-HPETE	(5*Z*,8Z,10*E*,14*Z*)-(12*S*)-Hydroperoxyeicosa-5,8,10,14,-tetraenoic acid
15-(S)-HPETE	(5*Z*,8Z,11*Z*,13*E*)-(15*S*)-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 Magentic 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).

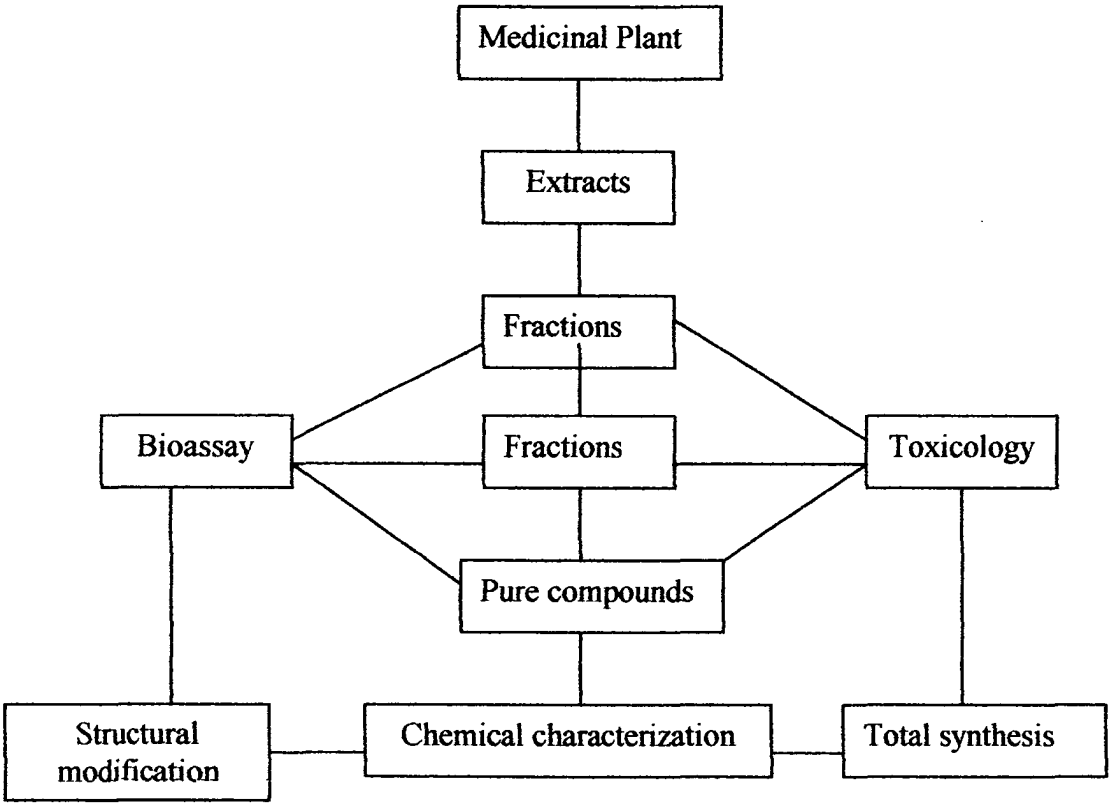

Figure 1. Methods for obtaining active substances from plants

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.