



UNIVERSITI PUTRA MALAYSIA

***CHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITIES OF
CALOPHYLLUM VENULOSUM ZOLL. AND CALOPHYLLUM
INOPHYLLUM L.***

AHMAD AZRI FITRI BIN ISMAIL

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CALOPHYLLUM VENULOSUM ZOLL. AND *CALOPHYLLUM*
INOPHYLLUM L.**

By

AHMAD AZRI FITRI BIN ISMAIL

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

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

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August 2016

Chairman : Prof. Gwendoline Ee Cheng Lian, PhD
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A phytochemical investigation on the stem bark of two species from the Clusiaceae family, *Calophyllum venulosum* and *Calophyllum inophyllum* was carried out using various chromatographic techniques and xanthenes, coumarin, terpenes and sterol were successfully isolated and identified. All of the secondary metabolites isolated were structurally characterized on the basis of modern spectroscopic evidence, such as 1D and 2D NMR, MS, IR and UV.

Extensive chromatographic separations applied to the chloroform extract of the stem bark of *Calophyllum venulosum* resulted in one new xanthone; venuloxanthone, along with two other xanthenes, caloxanthone I and tovoxyrifolin C. The hexane extract afforded two pentacycliterpenes; friedelin and lupeol.

Meanwhile, purifications of the chloroform extract revealed the presence of a coumarin, benjaminin together with a xanthone, macluraxanthone. Two sterols, stigmasterol and β -sitosterol were also discovered to be present in the hexane extract.

The crude extracts of both plants were screened for cytotoxic and antimicrobial activities. The cell lines used in the cytotoxic assays were human promyelocytic leukemia (HL60) and positive breast cancer (MCF7). For *Calophyllum venulosum*, only the methanol extract showed significant activity against MCF-7, meanwhile the hexane and chloroform extracts from *Calophyllum inophyllum* were found to be active against both cell lines. The antimicrobial activities was carried out on *Staphylococcus aureus* S276, *Bacillus subtilis* B145,

Staphylococcus epidermidis S273, *Escherichia coli* E266 and *Serratia marcescens* S381. From the tests, only the hexane and chloroform crude extracts from *Calophyllum inophyllum* showed weak inhibition against *Bacillus subtilis* B145, while no activity were indicated by the other crude extracts.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Sarjana Sains

**SEBATIAN KIMIA DAN AKTIVITI BIOLOGI DARIPADA KULIT
CALOPHYLLUM VENULOSUM ZOLL. DAN *CALOPHYLLUM INOPHYLLUM*
L.**

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Satu kajian fitokimia terhadap kulit dua spesies daripada keluarga Clusiaceae, iaitu *Calophyllum venulosum* dan *Calophyllum inophyllum* telah dijalankan menggunakan pelbagai teknik kromatografi, dan berjaya menemui xanton, kumarin, terpene, dan sterol. Struktur kesemua metabolit sekunder yang diasingkan dikenalpasti dengan analisis spektroskopi moden seperti 1D dan 2D, NMR, MS, IR and UV.

Teknik kromatografi yang dijalankan keatas ekstrak klorofom daripada kulit batang *Calophyllum venulosum* memberikan satu xanton baru, diberi nama venuloxanthone, disamping dua xanton lain iaitu caloxanthone I dan tovyprifolin C, manakala ekstrak hexane memberi dua terpene iaitu fridelin dan lupeol.

Sementara itu, kajian keatas kulit batang *Calophyllum inophyllum* menunjukkan kehadiran kumarin dan satu xanton, iaitu macluraxanthone dalam ekstrak klorofom. Dua sterol, iaitu stigmasterol dan β -sitosterol dijumpai di dalam ekstrak heksana.

Ekstrak dari kedua-dua pokok diuji dengan aktiviti sitotoksik dan antimikrob. Sel-sel yang digunakan dalam aktiviti sitotoksik ialah sel leukemia promeilotik manusia (HL60) dan kanser payudara reseptor positif estrogen manusia (MCF7). Hanya metanol ekstrak daripada *Calophyllum venulosum* menunjukkan adanya aktiviti menentang MCF-7, manakala untuk *Calophyllum inophyllum*, heksana dan klorofom ekstrak didapati aktif menentang kedua-dua sel. Aktiviti antimikrob diuji dengan *Staphylococcus aureus* S276, *Bacillus subtilis* B145, *Staphylococcus epidermidis* S273, *Escherichia coli* E266 dan *Serratia marcescens* S381. Hasilnya, hanya heksana dan kloroform ekstrak daripada

Calophyllum inophyllum menunjukkan sedikit aktiviti menentang *Bacillus subtilis* B145, manakala lain-lain ekstrak tidak menunjukkan sebarang aktiviti.



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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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CHAPTER 1

INTRODUCTION

1.1 General Introduction

Plants which are gifts from God have been the source of many cures for illnesses for over the centuries. Plants also offer essential materials for shelter, furniture, food, clothing, writing and colouring materials and weapons (Balick et al., 1996). In the early years, traditional medical practitioners sold medicinal plants in the crude form based on the knowledge they gain from the old folks. In the 1950's, scientific research were established. Scientists started to carry out research on medicinal plants seriously. The first report on a phytochemical survey of plants in Malaysia was carried out by Arthur in 1954 which led to more investigations until today (Teo et al., 1990).

Malaysia has been known for its richness in flora and fauna. Its position near the equator which gives it a typical tropical climate, characterized by high temperatures, humidity and rainfall throughout the year has resulted in a rich and beautiful flora and fauna. It was estimated that Malaysia is home to some 15 000 species or higher plants. Malaysia is listed as the 12th most diverse nation in the world and is mainly covered by tropical rainforests. About 25% of all modern drugs originate from rainforests even though only less than 1% of the world's tropical rainforest plant species have been evaluated for pharmacological properties. Malaysian forest is a rich source of numerous classes of natural compounds such as alkaloids, anthraquinones and phenolic compounds.

The phytochemical study of several well-known plants in folklore medicine such as *Eurycoma longifolia* Jack (Tongkat Ali), *Labisa pumila* (Kacip Fatimah), and *Morinda citrifolia* (noni) yielded many bioactive phytochemicals with good biological activities. A study in 2000 gave evidence of the folk use of *E.longifolia* as an aphrodisiac (Ang et al., 2000). On the other hand, *Labisa pumila* is effective as a skin collagen synthesis promoting herb (Chua et al., 2012) while *Morinda citrifolia* shows antibacterial activity (Atkinson, 1956).

Natural product chemistry is referred to as the study on secondary metabolites produced by living organisms such as plants. Due to their medicinal properties, natural compounds are usually preferred as alternative drugs rather than synthetic drugs used in pharmaceutical industry nowadays. The ancient cultures are known for their systematic collection of information on herbs and their rich and well-defined herbal pharmacopoeias (Mohagheghzadeh et al., 2006). Before synthetic drugs were found, most medicines were obtained from leaves, roots, bark and seeds of plants. The World Health Organization reported that 80% of the population in developing countries depend on traditional medicine for their primary health care. The dominant role played by medicinal plants contributed approximately 60% of anti-cancer compounds and 75% of drugs for infectious

diseases, either from natural products or natural product derivatives (Newman et al., 2003, Shoeb et al., 2006).

This research was conducted due to the advancement in the field of natural products. *Calophyllum venulosum* and *Calophyllum inophyllum* were chosen in this research in our search for bioactive compounds. Previous studies on *Calophyllum* species have shown anti-microbial (Yimdjo et al., 2004), anti-oxidant (Dharmaratne et al, 2009) and anti-cancer (Itoigawa et al, 2001). There should be at least two subheadings to justify having subheadings.

1.2 Botany of Plants Studied

1.2.1 The Family Clusiaceae

Clusiaceae, previously named as the Guttiferae which means “latex bearing plant” grows widely in the Malaysian forests. *Garcinia*, *Calophyllum*, and *Mesua* are some of the genera discovered under this family. This family of plants usually gives a number of useful timbers, drugs, dyes, gums pigments and resins. The Clusiaceae family consists of about 40 genera and over 1600 species of trees and shrubs and are easily found in Tropical Asia and Africa. In Malaysia, the genera that are mainly found are *Calophyllum*, *Mesua*, *Mammea*, and *Garcinia*.

Members of the Clusiaceae family can be recognised as trees, shrubs, or sometimes herbs containing resin or oil in canals and sometimes black or red glands containing hypericin or pseudohypericin. They also have sticky sap and fruits or capsules used for seeds and flowers are bisexual or unisexual with separate sepals and petals. The flowers also appear in bright colours while the leaves are always simple, oblong in shape with horizontal veins.

1.2.2 The Genus *Calophyllum*

The name *Calophyllum* originated from the Greek language where “kalos” means beautiful and “phullon” means leaf. This genus is composed of a great group of tropical trees. There are approximately 180-200 species restricted to the hot and humid tropics and can be found in Australasia, Madagascar, Eastern Africa, South and Southeast Asia, the Pacific islands, the West Indies and Latin America (Morel et al., 2000). Many of these species are employed in popular medicines to treat injuries such as pain, infection, inflammation and ulcer. (Dharmaratne et al., 1999). The genus *Calophyllum* has around 180-200 species of tropical evergreen trees and are widely distributed in Australasia, Madagascar, Eastern Africa, South and Southeast Asia, the Pacific islands, the West Indies and Latin America (Morel et al., 2000).

Calophyllum is also known as “bintangor” locally. (Corner 1998; Whitmore 1973). It usually has a hard and rough bark while its leaves are shiny and leathery. It can reach up from 30 to 60 m in height. On the other hand, it also has fragrant

flowers to attract insects or birds for pollinating purposes. The flowers also are the main source of seed oil production (Dweck et al., 2002).



Figure 1.1 : *Calophyllum* tree



Figures 1.2 : Stem and bark of *Calophyllum* species



Figures 1.3 : Fruits of *Calophyllum* species



Figures 1.4 : Flowers of *Calophyllum* species

1.2.3 The Species *Calophyllum venulosum*

Calophyllum venulosum is a dicotyledenous plant species discovered by Heinrich Zollinger. This plant can be found in Java, Sumatera, Philippine and Malaysia. However, there is not much research carried out on this species. So far, there are only two investigations on this species (Cao et al., 1997, Cao et al., 2001) which resulted in the isolation of new biflavanoids but there is no report on its biological activities. Thus, this plant has been chosen to discover more new bioactive compounds.

1.2.4 The Species *Calophyllum inophyllum*

Calophyllum inophyllum, is also known as *kamani* in Hawaii and *tamanu* in Tahiti. This plant is usually found in warm tropical and sub-tropical areas. It can reach a height of about 8-30 m and its fragrant flower is 25 mm wide. The diameter of the fruit is about 2 to 5 cm and it is round shaped, while its colour varies from yellow to brownish-red. This plant is usually used for medicinal and ornamental purposes due to its fragrant white flowers and glossy leaves, and provides shelter on streets and along the beaches.

1.3 Problem Statement

Natural products screening from *Calophyllum* species can lead to the discovery of new potential anticancer leads that are of great importance to pharmaceutical industry and public health which are not accessible by other technologies. The bioassay results and the knowledge in bioassay screening, as well as isolation techniques and structural elucidation of new compounds will be useful information for future advanced study by natural products scientists in industries worldwide. The discovery and usage of these possible potential anticancer agents can serve as alternatives to the presently available drugs in the market.

1.4 Objectives of Study

This research was designed to carry out extraction, isolation, characterization, elucidation and evaluation of the secondary metabolites present in the extracts of *Calophyllum venulosum* and *Calophyllum inophyllum*. The discovery of new bioactive compounds and their structures is the ultimate goal for this research.

As such, the following specific objectives are to be met as the goals of the project:

1. To extract and isolate chemical constituents from the stem bark of *Calophyllum venulosum* and *Calophyllum inophyllum*.
2. To characterize and elucidate the structures of the isolated compounds using various modern spectroscopic techniques, such as NMR, MS and IR.
3. To evaluate the biological activities of the crude extracts using bioassays; cytotoxic and antimicrobial.

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