UNIVERSITI PUTRA MALAYSIA

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MASTER OF SCIENCE
UNIVERSITI PUTRA MALAYSIA
2012

CHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITIES OF *CALOPHYLLUM NODUSUM* VESQUE AND *CALOPHYLLUM GRACILIPES* MERR

By

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Thesis Submitted to the School of Graduate Studied, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Master of Science

April 2012

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

CHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITIES OF *CALOPHYLLUM NODUSUM* VESQUE AND *CALOPHYLLUM GRACILIPES* MERR

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NADIAH BINTI MAD NASIR

April 2012

Chairman: Professor Mawardi bin Rahmani, PhD

Faculty: Science

The family Guttiferae includes some of the well known and important trees in Malaysia. The *Calophyllum* belongs to the family Guttiferae is the largest genus and locally known as 'bitangor'. In this investigation the dried stem bark of *Calophyllum nodusum* and dried stem bark together with dry leaf *Calophyllum gracilipes* were phytochemically studied and screened for their biological activities. The stem bark of *Calophyllum nodusum,* stem bark and dried leaf of *Calophyllum gracilipes* were collected form Sabah, East Malaysia and identified by the Department of Forestry in Sandakan.

The extracts were separated by various chromatographic techniques including column chromatography, chromatotron and preparative thin layer chromatography. The compounds were analysed by using MS, NMR, IR and UV techniques. Based on interpretation of these spectral data and comparison with literature reports, the structures of the new and known compounds were established. The crude extracts and pure isolated compounds from all plants were screened for free radical

scavenging activity by using 1, 2-Diphenyl-2-picrylhydrayl (DPPH) assay, cytotoxic activity by tetrazolium salt (MTT) assay and antibacterial activity using disc diffusion assay.

Seven compounds were isolated from the *Calophyllum nodusum*. From the hexane extract four known triterpenes were isolated and identified as friedelin **(95)**, lupeol **(96)**, stigmasterol **(99)** and betulinic acid **(100)**. A new xanthone was isolated from the chloroform extract and identified as nodusuxanthone **(110)**. Another new xanthone, trapezifolixanthone A **(112)** was also obtained from the methanol extract together with a known compound 4, 5-dihydroxy-2,3-dimethoxyxanthone **(111)**. Chromatogarphic separation of the extracts of stem bark and dried leaf of *Calophyllum gracilipes* afforded three compounds. From the hexane stem bark and dried leaf extract, similar compounds as in the hexane extract of *Calophyllum nodusum* were isolated. From the chloroform stem bark extract, two known compounds were isolated and identified as zeyloxanthanone **(93)** and trapezifolixanthone **(79)**. Similar chromatographic separation procedure for the methanol extract of dried leaf led to a new xanthone, gracixanthone **(113)**.

The free radical scavenging activity of the plant extracts and pure isolated compounds were carried out using 1, 2-diphenyl-2-picrylhydrayl (DPPH). However, none of the crude extracts of both plant species gave positive test results while the methanol extracts of *Calophyllum nodusum* showed moderate activity (IC_{50} < 182.86 µg/mL). Similarly, all the compounds also displayed negative scavenging activity on DPPH assay. One of the isolated compounds, zeyloxanthanone **(93)** **e**xhibited excellent cytotoxic activity against four cell lines, human prostate (PC-3), colon

(HCT-116), breast (MCF-7) and mouse Macrophages (RAW 264.7) cells with IC_{50} values ranging from 3.6-4.5 µM. The results for the antimicrobial tests using disc diffusion assay indicated that the methanol extract of *Calophyllum nodusum* showed 3 mm inhbition zone against *Salmonella typhimurium* bacteria and the chloroform extract of *Calophyllum gracilipes* showed 2 mm and 2 mm inihibition zone towards *Salmonella typhimurium* and *Escherichia coli* bacteria respectively. The disc diffusion assay was further tested on isolated compounds. Trapezifolixanthone A **(112)** showed moderate inhibition activity towards *Staphylococcus* bacteria with 4 mm inhibition zone and moderate activity against *Salmonella typhimurium* with 5 mm. Both trapezifolixanthone **(79)** and zeyloxanthonone **(93)** exhibited medium inhibition activity against some of the microbes tested.

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

KANDUNGAN KIMIA DAN AKTIVITI BIOLOGI DARIPADA *CALOPHYLLUM NODUSUM* VESQUE DAN *CALOPHYLLUM GRACILIPES* MERR

Oleh

NADIAH BINTI MAD NASIR

April 2012

Pengerusi: Profesor Mawardi bin Rahmani, PhD

Fakulti: Sains

Famili Guttiferae mengadungi pokok yang terkenal dan penting di Malaysia. *Calophyllum* adalah genus yang terbesar dalam family ini dan di tempatan yang dikenali sebagai 'bitangor'. Dalam penyiasatan ini, kulit batang *Calophyllum nodusum* dan kulit batang bersama daun kering *Calophyllum gracilipes* telah dikaji fitokimianya dan disaring bagi aktiviti biologi. Kedua-dua *Calophyllum nodusum*, daun *Calophyllum gracilipes* dikumpul dari Sabah, Malaysia dan dikenalpasti oleh Jabatan Perhutanan di Sandakan. Kajian fitokimia yang terlibat adalah pengekstrakan bahan tumbuhan dengan pelarut organik seperti heksana, kloroform dan metanol.

Ekstrak telah dipisahkan dengan pelbagai teknik kromatografi termasuk kromatografi turus, kromatotron dan kromatografi lapisan nipis penyediaan. Sebatian dianalisis menggunakan teknik MS, NMR, IR dan UV. Struktur sebatian dikenalpasti berdasarkan data spektroskopi dan perbandingan data spektrokopi diperolehi dengan rujukan. Ekstrak mentah dan tulen sebatian daripada semua sampel telah disaring untuk aktiviti radikal bebas memerangkap dengan

menggunakan 1,2-diphenil-2-pikrilhidral (DPPH) asai, aktiviti sitotoksik dengan tetrazolium salt (MTT) asai dan aktiviti antibakteria menggunakan cakera reasapan asai.

Tujuh sebatian tulen telah diekstrak dari *Calophyllum nodusum*. Dari ekstrak heksana empat triterpena telah berjaya dipercilkan dan dikenalpasti sebagai friedelin **(95)**, lupeol **(96)**, stigmasterol **(99)** dan asid betulinik **(100)**. Satu zanthon yang baru telah diasingkan daripada ekstrak kloroform dan dikenalpasti sebagai nodusuxanthone **(110)**. Satu lagi zanthon baru, trapezifolixanthone A **(112)** juga diperolehi dari ekstrak metanol bersama-sama dengan sebatian yang dikenali 4, 5-dihidroxil-2, 3-dimethoxyxanthon **(111)**. Pemisahan kromatografi ekstrak heksana kulit kayu batang dan daun kering *Calophyllum gracilipes* menghasilkan tiga sterol yang sama dengan hasil ekstrak heksana *Calophyllum nodusum*. Ekstrak kloroform telah menghasilkan dua zanthon yang dikenalpasti sebagai zeyloxanthanon **(93)** dan trapezifolixanthon **(79)**. Prosedur pemisahan kromotografi ekstrak methanol telah membawa kepada xanthon baru, gracixanthon **(113).**

Aktiviti radikal bebas telah dijalankan keatas ekstrak tumbuhan dan sebatian terasing tulen dengan menggunakan 1,2-diphenil-2-pikrilhidral (DPPH). Walau bagaimanapun, tiada satu pun daripada ekstrak mentah kedua-dua spesies tumbuhan memberikan keputusan ujian yang positif tetapi ekstrak metanol *Calophyllum nodusum* menunjukkan aktiviti sederhana (IC_{50} <182.86 μg/mL). Begitu juga semua sebatian tulen juga memberi aktiviti negatif pemerangkapan terhadap DPPH asai. Salah satu daripada sebatian yang dipencil zeyloxanthanon **(93)** mempamerkan

aktiviti sitotoksik tinggi terhadap empat jenis sel prostat manusia (PC-3), kolon (HCT-116), payudara (MCF-7) dan makrofasa tikus (RAW 264,7) sel dengan IC_{50} dari 3.6-4.5μM. Keputusan untuk ujian antimikrob menggunakan asai resapan cakera menunjukkan bahawa semua ekstrak *Calophyllum nodusum* menunjukkan aktiviti yang lemah. Cakera resapan asai terus diuji ke atas sebatian tulen dan Cuma zeyloxanthonon **(93)** menunjukkan aktiviti perencatan sederhana terhadap bakteria *Bacillus cereus.*

ACKNOWLEDGEMENTS

The successful of my study has come through from the support and help that come from many people. I would like to express my sincere and appreciation to my supervisor, Prof Dr Mawardi Rahmani for his guidance, advised, suggestion on this research. My sincere gratitude is extended to my co-supervisor, Prof Dr Khozirah for her supportive and suggestion and to my second co-supervisor, Associate Prof Dr Muhajir for his kindness and permission to work in his lab.

I also wish to thanks my labmate, Dr Najihah, Mrs Kartinee, Mrs Winda, Maizatulakmal, Kamilah, Aizat and my two junior, Nazil and Faiqah for their help and supportive throughout my research. I am also grateful to En Zainal for GC-MS, En Johadi, En Fadli and Miss Rina for the guided and advised on handling NMR machines, Mrs Ros for IR and En Shaharudin for UV machine. Special thanks to Associate Prof Dr Johnson and Miss Ethel for helping me in toxicity bioactivity.

Last but not least, I also thank and grateful to my parents, Prof Dr Mad Nasir, Mrs Norsiah and my brothers, Dr Nasirudin and Dr Nizam for the encouragement and support toward my successful research.

Finally, my regard and thanks to all those who have supported and encouraged me during my entire masters programs.

I certify that a Thesis Examination Committee has met on 27 April 2012 to conduct the final examination of Nadiah binti Mad Nasir on her thesis entitled "Chemical Constituents And Biological Activities Of *Calophyllum Nodusum* Vesque and *Calophyllum Gracilipes* Merr" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

Members of the Thesis Examination Committee were as follows:

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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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DECLARATION

I declare that this thesis is my original work except for quotations and citations, which have been duly acknowledge. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or other Institution.

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NADIAH MAD NASIR

Date: 27 April 2012.

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