UNIVERSITI PUTRA MALAYSIA

CHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITIES OF ESSENTIAL OIL FROM CHEMPAKA (Michelia alba De Candolle)

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By

NOR'AISHAH BINTI ABU SHAH

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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Dedication

My late beloved mother who had always prayed for my wellbeing.
This writing is specially dedicated to her.

To all my loves ones and those who have sacrificed and supported me throughout my studies.

ABSTRACT

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the Degree of Doctor of Philosophy

CHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITIES OF ESSENTIAL OIL FROM CHEMPAKA (*Michelia alba* De Candolle)

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Chairman: Associate Professor Janna Ong Abdullah, PhD

Faculty: Biotechnology and Biomolecular Sciences

Malaysia is one of the twelfth mega-diversity countries in the world with a lot of colourful and aromatic flowers flowering throughout the year. Unfortunately many plant species are not fully exploited towards perfume production. *Michelia alba,* an aromatic plant with fascinating flower odour, has long been used as fragrance in perfume and cosmetics products. However, there is still shortage of information on the chemical profile their pharmacognosy values. The flower only budding mostly during rainy season, concurrently with shoots formation. Furthermore, this local plant is hardly produce fruit or seeds due to pollen shortage and exhibit low efficiency in conventional propagation. This study was carried out to profile the chemical and biological properties of the essential oils in fresh *M. alba* flowers and leaves and establish essential oil production in its callus cultures. The chemical compositions of the essential oils of *M. alba* flowers (from white bud to full bloom

flower) and the leaves were determined using gas chromatography-mass spectrometry (GC-MS) techniques. Approximately 100 compounds were detected and 30 were chromatographically identified. Linalool was found to be dominant in the leaf essential oils at 76.6% while it constituted 67.1% in the stage 8 flower. Other major chemical constituents detected in the leaf oil were farnesol (5.5%), β-elemen (3.7%) and nerolidol (2.2%). Other abundant chemical components in most of the flower stages were 2-methyl-methyl-ester-butanoic acid, phenol, caryophyllene, phenyl ethyl alcohol, ocimene and germacrene D. The compositions of the essential oils in different parts of the flower revealed that the highest percentage of linalool were from gynoecium, stamen and tepal at 74.5%, 74.4% and 38.8%, respectively. Other major compounds found were 2-methyl-butanoic acid and 2-methyl-methyl-ester-butanoic acid.

The essential oils from the fresh flowers and leaves of *M. alba* were specifically extracted to further test their effects towards growth inhibition of different pathogenic bacteria and fungi using disc diffusion assay. Two kind solvents of flower and leaf extracts were used to examine the effect of extraction solvents with different polarities on biological activities. Most of the extracts from different solvents used inhibit the growth of all the bacteria tested. The extracts showed better antibacterial effects on most of the tested bacteria with lower MIC values ranging from 1.25 to 10.00 µl/ml. The least inhibited bacteria was *Pseudomonas aeroginosa,* effective only with leaf essential oil extracted in dichloromethane (MIC value 10.00 µl/ml). All Gram-negative bacteria tested were more resistant compared to the Gram-positive bacteria. *Candida albicans* was found to be more susceptible to the leaf extract, while *Fusarium oxysporium* was more susceptible to the dichloromethane

flower extract. Overall, the results showed that the leaf gave better inhibitory effect from dichloromethane extract (DL) and the flower from n-pentane extract (PF), consistent with the higher percentage of linalool in the leaf (DL: 76.6%) and the flower (PF: 63.2%).

The antioxidant activity of the fresh flowers and leaves extracted with dichloromethane and n-pentane were also investigated with three different established methods: the conjugate diene method, the 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method and β-carotene bleaching method. Approximately 20 mg/ml (v/v) of the essential oils of *M. alba* was found to exhibit moderate antioxidant property (25% - 50%). Even though the essential oils was slow in its scavenging effects either with dichloromethane or pentane, the scavenging activity was independent of the solvent polarity. The oxidation of linoleic acid in β-carotene bleaching test was best inhibited by the leaf extracted in dichloromethane (93.9%), compared to standard butylated hydroxytoluene (BHT) (83.3%). The antioxidant activity tested using the conjugated diene method showed higher inhibition of lipid peroxidation for both flower (PF: 90.7%) and leaf (PL: 77.1%) extracts in n-pentane, which was equivalent to the standard antioxidant, BHT (91.5%) and α-tocopherol (vitamin E) (77.2%). Overall, the essential oils from the flower showed slightly higher antioxidant activity than the leaf oils, both extracted in n-pentane.

In order to ensure sustainability of the essential oils due to seasonal flowering problem, *M. alba* explants were induced for calli production for the corresponding secondary metabolites. Tepal, gynoecium, stamen and leaf explants were separately

cultured on Murashige and Skoog (MS) and Woody Plant (WP) basal media each supplemented with various concentrations of NAA and kinetin in order to select the best medium for callus proliferation and organogenesis. Both media supplemented with phytohormones produced pale (colour) friable calli. However, the WPM medium produced remarkable callus proliferation compared to the MS medium with around 50% to 100% of the explants producing friable to compact nodular calli. Chemical compositions of the essential oils extracted with dichloromethane using simultaneous distillation extraction (SDE) from the callus cultures of gynoecium and stamen of *M. alba* flowers and analyzed by GC-MS revealed there were 60 peaks detected and approximately 15 compounds were identified in both oils. The compound with the highest composition in both essential oils was linalool with 50.0% in gynoecium oil and 28.8% in stamen oil for 0.1g of dry weight callus. The other abundant chemical components of gynoecium oil were β-elemen (10.0%), caryophyllene oxide (8.4%), caryophyllene (6.4%), germacrene D (4.2%), δ-cadinene (5.6%) and eugenol methyl ether (2.3%). The chemical constituents of stamen oil were α-bergamotene (12.5%), β-elemen (4.9%), germacrene-D (4.0%), δ-cadinene (4.8%), caryophyllene (3.4%) and caryophyllene oxide (2.9%). The major compounds of the essential oils from the callus cultures of gynoecium and stamen were not significantly different ($p>0.05$) from the essential oils of fresh gynoecium and stamen of *M. alba* flower.

This study indicates that *in vitro* production of the oils via callus culture from gynoecium and stamen explants has potential in mass fragrance production from *M. alba* replacing the seasonal fresh flowers. In addition, the *M. alba* essential oils have the potentials as antimicrobial and antioxidant agents useful for the cosmetic industry.

ABSTRAK

Abstrak tesis yang dikemukakan kepada Senat Univesiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

KANDUNGAN KIMIA DAN AKTIVITI BIOLOGI MINYAK PATI DARIPADA CHEMPAKA (*Michelia alba* De Candolle)

Oleh

NOR'AISHAH BINTI ABU SHAH

Januari 2013

Pengerusi: Profesor Madya Janna Ong Abdullah, PhD

Fakulti: Bioteknologi and Sains Biomolekul

Malaysia adalah satu daripada dua belas buah negara di dunia dengan kepelbagaian-mega, mempunyai tumbuhan yang berbunga sepanjang tahun dengan pelbagai warna dan aroma bunga. Malangnya tumbuhan ini tidak dimanafaatkan sepenuhnya dalam penghasilan wangian. *Michelia alba* sejenis tumbuhan beraroma dengan bau yang menarik telah lama digunakan sebagai pewangi dalam pembuatan minyak wangi dan kosmetik. Namun profil kimianya masih lagi terhad dan nilai ubatannya belum ramai yang ketahui. Bunga hanya berputik kebanyakannya ketika musim hujan, serentak dengan pembentukan pucuk. Tambahan pula tumbuhan tempatan ini sukar menghasilkan buah atau biji kerana debunganya yang terhad dan propagasi secara konvensional kurang berkesan. Kajian ini dijalankan untuk menentukan profil kimia dan aktiviti biologi minyak pati bunga dan daun segar *M. alba* dan penghasilan minyak pati dari kultur kalusnya. Komposisi kimia minyak pati *M. alba* dari daun, putik bunga putih hingga bunga mekar telah ditentukan dengan menggunakan kromatografi gas (GC-MS). Lebih kurang 100 sebatian dikesan dan 30 sebatian telah

dikenal pasti melalui proses kromatografi. Linalool merupakan sebatian yang dominan dengan 76.6% dalam ekstrak daun dan 67.1% dalam ekstrak bunga peringkat 6. Komponen kimia utama yang dikesan dalam daun ialah caryophyllene, β-elemen dan 'caryophyllene' oksida. Komponen kimia utama yang dikesan di kebanyakan peringkat bunga ialah 2-metil-metil-ester-butanoik asid, fenol, 'caryophyllene', alkohol fenil etil, 'ocimene' dan 'germacrene' D. Bahagian bunga yang berlainan mengandungi berlainan peratusan komposisi minyak pati dan didapati bahawa peratusan tertinggi komposisi minyak pati dalam ginoesium, stamen dan tepal adalah linalool, masing-masing pada 74.5%, 74.4% dan 38.8%. Sebatian utama lain ialah asid 2-metil-butanoik dan asid 2-metil-metil-ester-butanoik.

Minyak pati daripada bunga dan daun segar *M. alba* telah diekstrak secara khusus untuk menguji kesan mereka terhadap perencatan pertumbuhan beberapa jenis kulat dan bakteria patogenik dengan menggunakan kaedah resapan dari cakera. Dua jenis ekstrak bunga dan daun telah digunakan untuk menguji kesan pengekstrakan pelarut dengan polariti berlainan ke atas aktiviti-aktiviti biologi. Kebanyakan ekstrak dengan pelarut yang berbeza yang digunakan, menyekat pertumbuhan semua bakteria yang diuji. Pengekstrakan tersebut menunjukkan kesan antibakteria yang lebih baik terhadap kebanyakan bakteria yang diuji pada nilai MIC sekitar 1.25 hingga 10.00 µl/ml. *Pseudomonas aeroginosa* merupakan bakteria paling sukar disekat pertumbuhannya kecuali dengan minyak pati dari daun yang diekstrak dengan diklorometana sahaja (nilai MIC 10.00 µl/ml). Semua bakteria gram-negatif yang diuji adalah lebih rentan berbanding dengan bakteria gram-positif. *Candida albicans* didapati boleh direncatkan oleh ekstrak daun dengan diklorometana, manakala *Fusarium oxysporium* adalah lebih mudah direncatkan oleh ekstrak bunga

dari pelarut yang sama. Secara keseluruhannya, keputusan menunjukkan bahawa ekstrak daun dengan diklorometana (DL) memberi kesan perencatan yang lebih baik berbanding dengan pati bunga dari ekstrak n-pentana (PF), selaras dengan peratusan linalool yang lebih tinggi di dalam daun (PL:76.6%) berbanding dengan bunga (PF: 63.2%).

Kajian antioksidan ke atas ekstrak bunga segar dan daun dengan diklorometana dan n-pentana juga telah dijalankan melalui tiga kaedah yang berbeza iaitu kaedah diena terkonjugat, kaedah pemusnahan radikal 2,2-'difenil-1-picrylhydrazyl (DPPH) dan kaedah pelunturan β-karotena. Lebih kurang 20 mg / ml (v / v) minyak pati *M. alba* telah didapati mempamerkan kesan antioksidan di antara sederhana kepada baik. Walaupun minyak pati menunjukkan kesan pemusnah radikal yang perlahan dalam pelarut diklorometana dan n-pentana, aktiviti pemusnah radikal adalah tidak dipengaruhi oleh polariti pelarut. Pengoksidaan terbaik asid linoleik dalam ujian pelunturan β-karotena telah direncat oleh pengekstrakan daun dengan diklomethana (93.9%), berbanding dengan piawai butylated hydroxytoluene (BHT) (83.3%). Aktiviti antioksidan diuji dengan menggunakan kaedah diena terkonjugat telah menunjukkan perencatan pengoksidaan lipid yang lebih tinggi di dalam kedua-dua ekstrak bunga (PF: 90.7%) dan daun (PL: 77.1%), yang hampir sama dengan aktiviti antioksidan piawai, BHT (91.5%) dan α-tokoferol (vitamin E) (77.2%). Secara keseluruhan, minyak pati dari bunga menunjukkan aktiviti antioksidan yang tinggi sedikit daripada minyak pati daun yang diekstrak dengan n-pentana.

Bagi memastikan kelestarian penghasilan minyak pati dan mengatasi masalah pembungaan yang dipengaruhi musim, penghasilan kalus dengan pengeluaran

metabolit sekunder yang sepadan telah dijalankan. Tepal, ginoesium, stamen dan daun telah dikultur secara berasingan di atas media Murashige and Skoog (MS) dan Woody Plant (WP) yang dibekalkan dengan pelbagai kepekatan NAA dan kinetin untuk memilih media yang terbaik untuk percambahan kalus dan organogenesis. Kedua-dua media yang dibekalkan dengan fitohormon telah menghasilkan kalus rapuh dan berwarna pucat. Walau bagaimanapun, media WP menghasilkan percambahan kalus yang lebih baik berbanding dengan media MS dengan pertumbuhan sekitar 50% hingga 100% eksplan dengan penghasilan kalus dari yang rapuh hingga kepada kalus padat nodular. Komposisi kimia minyak pati daripada kultur kalus ginoesium dan stamen *M. alba* telah diekstrak dengan diklorometana menggunakan pengekstrakan penulenan serentak (SDE) dan dianalisa dengan GC-MS telah menunjukkan terdapat 60 sebatian yang dikesan dalam kedua-dua minyak tersebut dan kira-kira 15 sebatian telah dikenalpasti. Komposisi sebatian yang tertinggi dalam kedua-dua minyak pati tersebut adalah linalool dengan 49.98% dalam ekstrak ginoesium dan 28.80% dalam ekstrak stamen untuk 0.1g kalus berat kering. Sebatian kimia utama lain yang terdapat dalam minyak pati ginoesium adalah β-elemen (10.0%), 'caryophyllene' oksida (8.4%), 'caryophyllene' (6.4%), 'germacrene' D (4.2%), δ-cadinena (5.6%) dan eugenol metil eter (2.3 %). Manakala sebatian kimia utama lain minyak pati stamen ialah 'α-bergamotene' (12.5%), β-elemen (4.9%), 'germacrene-D' (4.0%), 'δ-cadinene' (4.8%), 'caryophyllene' (3.4%) dan 'caryophyllene' oksida (2.9 %). Sebatian utama minyak pati daripada kultur kalus ginoesium dan stamen tidak mempunyai perbezaan yang ketara dari ginoesium dan stamen bunga segar *M. alba*.

Kajian ini menunjukkan bahawa pengeluaran *in vitro* minyak pati dari *M. alba* melalui kultur kalus eksplan gynoecia dan stamen mempunyai potensi dalam pengeluaran minyak wangi secara besar-besaran menggantikan pengeluaran bunga segar yang bermusim. Di samping itu, minyak pati *M. alba* mempunyai potensi sebagai agen antimikrob dan antioksidan yang berguna dalam industri kosmetik.

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Thank you Allah for showing me the path of your endless knowledge. And to Allah belongs whatever is in heaven and whatever is on earth.

The earth He laid out for the creatures. Therein is fruit and palm trees having sheaths of dates. And grains having husks and scented plants. Ar Rahman.

Great appreciation goes to Associate Professor Dr Janna for having me as her student. Her willingness, guidance, motivation, suggestion and assistance has helped me towards realizing the completion of this study. Appreciation also goes to Professor Dr Mohd Aspollah Hj Sukari and Associate Professor Dr Maheran Abdul Aziz, who have served as supervisory committee members from the beginning.

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May Allah bless all of you.

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I certify that an Examination Committee has met on 8 January 2013 to conduct the final examination of Nor'aishah binti Abu Shah on her PhD thesis entitiled "Chemical constituents and biological activities of essential oil from Chempaka (*Michelia alba* De Candolle)" in accordance with Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 10] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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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 Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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DECLARATION

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

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NOR'AISHAH BINTI ABU SHAH

8 January 2013

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