UNIVERSITI PUTRA MALAYSIA

VOLATILE COMPOUNDS AND BIOLOGICAL ACTIVITIES OF EXTRACTS OF *Cananga odorata* AND ITS PETAL-DERIVED CALLUS

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By

NURAZAH ZAIN

Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, In Fulfillment of the Requirements for the Degree of Master of Science

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DEDICATION

This thesis is dedicated to my parents, Zain Eusof and Marwiyah Paidi, my sisters, Siti Masnurin Zain and Nurfatin Zain and my brother, Muhammad Asyraf Zain who have been a great source of motivation and supported me since the beginning of my studies.

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

VOLATILE COMPOUNDS AND BIOLOGICAL ACTIVITIES OF EXTRACTS OF *Cananga odorata* AND ITS PETAL-DERIVED CALLUS

By

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November 2009

Chairman : Radzali b. Muse, PhD

Faculty : Biotechnology and Biomolecular Sciences

Ylang-ylang is a flower of *Cananga odorata* (family of Annonaceae) tree or locally known as 'kenanga'. The flowers produce pleasant, sweet-scented and high value essential (volatile) oil that is widely used as aromatherapy and in perfumery industries. The importance of volatile oil in *C. odorata* has led to the alternative production of volatile compounds through plant cell culture such as callus and cell suspension culture. In this study, optimization of pH of the culture medium, different light incubation, plant growth regulators and carbon sources were conducted to develop a suitable growth medium for *C. odorata* petal-derived callus induction. The essential oil from flower, leaf and petal-derived callus of *C. odorata* were extracted through hydro-distillation process using simultaneous distillation extraction (SDE)

and analysis of the volatile compounds were performed by using gas chromatography equipped with flame ionization detector (GC-FID). On the other hand, the biological properties of *C. odorata* flower, leaf and petal-derived callus were evaluated by using antioxidant, antimicrobial and seed germination assays. Results showed that *C. odorata* callus was best induced from petals of the *C. odorata* flowers, which cultured on basal Murashige and Skoog (MS) medium, Gamborg (B5) vitamins containing 30 g/L sucrose and 3 g/L agar supplemented with 3 mg/L NAA plus 0.5 mg/L BAP which gave the highest growth (0.98 ± 0.00 g/culture FW) at pH 5.7, 25 ± 2 ^{o}C and in dark condition after 4 weeks of culture. The volatile compounds detected by GC-FID from *C. odorata* flower essential oils were such as limonene, linalool, benzyl acetate and β-caryophyllene. While, from the leaf essential oils were α-pinene and β -caryophyllene. However, none of the volatile compounds mentioned above was detected from *C. odorata* petal-derived callus. The essential oil obtained from *C. odorata* flower and leaf showed antioxidant activity, especially in inhibiting lipid peroxidation. Result from antibacterial assay showed that flower essential oil was able to inhibit the growth of bacterial strains tested. The flower and leaf essential oil of *C. odorata* also showed antifungal activity against fungal strains tested. In seed germination assay, the germination percentage of *Brassica nigra* seeds was reduced when exposed to *C. odorata* flower and leaf essential oils at concentration of more than 2 mg/ml. The biological properties of *C. odorata* essential oils were possibly due to the presence of volatile compounds such as linalool, eugenol and other volatile compounds that could cause synergistic effects.

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

SEBATIAN MERUAP DAN AKTIVITI BIOLOGI EKSTRAK *Cananga odorata* DAN KALUS DARI KELOPAK BUNGA

Oleh

NURAZAH BINTI ZAIN

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Ylang-ylang merupakan bunga dari pokok *Cananga odorata* (keluarga Annonaceae) atau dikenali dengan nama tempatannya sebagai ‘kenanga’. Bunga kenanga menghasilkan minyak perlu (meruap) berbau harum dan bernilai yang digunakan secara meluas sebagai aromaterapi dan industri minyak wangi. Kepentingan minyak meruap dari *C. odorata* telah menjurus kepada penghasilannya secara alternatif melalui kultur sel tumbuhan; di antaranya kultur kalus dan ampaian sel. Dalam kajian ini, optimisasi pengawalatur pertumbuhan tumbuhan, sumber karbon, pH media kultur dan pengeraman terang yang berlainan telah dijalankan untuk menghasilkan media pertumbuhan yang sesuai bagi kultur kalus dari kelopak bunga *C. odorata* . Minyak perlu dari bunga, daun dan kalus dari kelopak bunga diekstrak melalui proses penyulingan menggunakan pengekstrakan penyulingan serentak (SDE) dan analisa sebatian meruap dilakukan menggunakan kromatografi gas dilengkapi dengan

pengesan pengionan haba (GC-FID). Selain itu, aktiviti biologi bunga, daun dan kalus dari kelopak bunga dinilai menggunakan esei antioksida, antimikrob dan percambahan benih. Keputusan menunjukkan kalus paling sesuai ditumbuhkan dari kelopak bunga pada media Murashige and Skoog (MS), vitamin Gamborg (B5) yang mengandungi 30 g/L sukros dan 3 g/L agar dan ditambahkan dengan 3 mg/L NAA dan 0.5 mg/L BAP yang memberikan pertumbuhan tertinggi (0.98 ± 0.00 g/kultur berat basah) pada pH 5.7, 25 ± 2 ^{o}C dalam keadaan gelap selepas 4 minggu. Sebatian meruap utama yang dikesan oleh GC-FID dari minyak perlu bunga *C. odorata* adalah linalool, benzyl acetate dan β-caryophyllene manakala dari minyak perlu daun adalah α-pinene dan β -caryophyllene. Walau bagaimanapun, tiada sebatian meruap yang diterangkan di atas dapat dikesan dari kalus kelopak bunga. Minyak perlu yang didapati dari bunga dan daun kenanga menunjukkan aktiviti antioksida terutamanya dalam menghalang pengoksidaan lipid. Keputusan dari esei antibakteria menunjukkan minyak perlu bunga mampu menghalang pertumbuhan bakteria yang diuji. Minyak perlu dari bunga dan daun kenanga juga menunjukkan aktiviti antikulat yang menentang kulat yg diuji. Dalam esei percambahan benih, peratus percambahan benih *Brassica nigra* menurun apabila didedahkan kepada minyak perlu bunga dan daun *C. odorata* pada kepekatan lebih daripada 2 mg/ml. Aktiviti biologi dari minyak perlu *C. odorata* kemungkinan disebabkan oleh kehadiran sebatian meruap seperti linalool, eugenol, cineole dan sebatian meruap yang lain yang berkemungkinan menyebabkan kesan sinergisme.

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I certify that a Thesis Examination Committee has met on 4 November 2009 to conduct the final examination of Nurazah Binti Zain on her thesis entitled "Volatile Compounds and Biological Activities of Extracts of *Cananga odorata* and Its Petal-Derived Callus" in accordance with 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 degree of Master of Science.

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DECLARATION

I hereby declare that the thesis is based on my original work except for equations and citation, which have been duly acknowledged. I also declare that it has not been previously of concurrently submitted for any other degree at UPM or other degree at UPM or other institutions.

_____

NURAZAH BINTI ZAIN

Date: 25 February 2010

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LIST OF ABBREVIATIONS

Abs	Absorbance
BAP	6-benzylaminopurine
BHT	Buytlated hydroxytoluene
B5	Gamborg
^{o}C	Degree Celsius
cm	Centimeter
2,4-D	2, 4-dichlorophenoxy acetic acid
DCM	Dichloromethane
DPPH	2, 2-diphenyl-1-picrylhydrazyl
DMSO	Dimethyl sulphoxide
DW	Dry weight
e.g.	Example (s)
etc.	*et cetra*, and the rest
et al.,	*at alli* and other people
FDA	Food and drug administration
$FeCl_{3.}6H_2O$	Ferric chloride
FEMA	Flavour and extract manufacturers association
FEO	Flower essential oil
FRAP	Ferric reducing antioxidant power
FW	Fresh weight

g	Gram
GC-FID	Gas chromatography-flame ionization detector
GRAS	Generally recognized as safe
HPLC	High performance liquid chromatography
IAA	Indole acetic acid
IBA	Indole butyric acid
Kin	Kinetin
LEO	Leaf essential oil
mg	Miligram
mg/L	Milligram per liter
ml	Mililiter
mm	Milimeter
MS	Murashige & Skoog
µg	Microgram
µl	Microliter
nm	Nanometer
NA	Nutrient agar
NAA	1-naphtalene acetic acid
NB	Nutrient broth
No.	Number
NP	*n*-Pentane
PDA	Potato dextrose agar

PGR	Plant growth regulator
SD	Standard deviation
SDE	Simultaneous distillation extraction
SEM	Standard error of mean
SPME	Solid phase micro extraction
TPTZ	Tripyridyltriazine
w/v	weight per volume
v/v	volume per volume

CHAPTER 1

INTRODUCTION

Plant secondary metabolites have been studied over the last 50 years (Bourgaud *et al*., 2001). These secondary metabolites play a major role in the adaptation of plants to their environment, but also represent an important source of active biochemicals or bioactive compounds. Higher plants are one of the valuable sources of wide range of secondary metabolites, which are used as pharmaceuticals, agrochemicals, flavours, fragrances, colours, biopesticides and food additives.

Essential oils are volatile, natural, complex compounds characterized by strong odour and are formed by aromatic plants as secondary metabolites (Bakkali *et al*., 2008). The main group is composed of terpenes (volatile terpenes) and terpenoids and the other of aromatic and aliphatic constituents. They are the most representative molecules constituting 90% of the essential oils (Keeling and Bohlmann, 2008). Volatile compounds in essential oils from medicinal and aromatic plants have been known since ancient times to possess many biological activities especially antibacterial, antifungal and antioxidant properties (Sacchetti *et al*., 2005; Baratta *et al*., 1998).