UNIVERSITI PUTRA MALAYSIA

PRODUCTION AND BIOLOGICAL ACTIVITY OF MONOTERPENES FROM FLOWERS AND CALLUS CULTURES OF MICHELIA ALBA DC.

NOR AZIZUN RUSDI

FBSB 2005 7

PRODUCTION AND BIOLOGICAL ACTIVITY OF MONOTERPENES FROM FLOWERS AND CALLUS CULTURES OF *MICHELIA ALBA* DC.

By

NOR AZIZUN RUSDI

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

November 2005

DEDICATION

With love to all my family including my mum Khatijah Said, my dad Rusdi Mat Din, my loving husband (Che Hasin Che Lah), my sisters (Nor Hakimah, Nor Nasrina, Nor Akmal, Nor Mukminah) and my brothers (Mohd Hakimi, Nasron, Muhammad and Abdul Hafiz), for enduring, with patience and understanding. Also not forgetting my lovely friends (Siti Sarah, Siti Aishah, Rozaina, Azlena, Rafidah and to my entire lab mates in lab 230) for their patience and guidance.

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

PRODUCTION AND BIOLOGICAL ACTIVITY OF MONOTERPENES FROM FLOWERS AND CALLUS CULTURES OF *MICHELIA ALBA* DC.

By

NOR AZIZUN BT RUSDI

NOVEMBER 2005

Chairman : Associate Professor Radzali Muse, PhD

Faculty : Biotechnology and Biomolecular Sciences

Analysis of volatile compounds from the selected flower buds, grown *in -vitro* and callus cultures of *Michelia alba* D.C were performed using the gas chromatography technique with mass spectrometry detector (GC-MS). The results showed that the major volatile compound obtained from the three-selected flower bud was dihydrocarveol. Petal grown *in-vitro* on the Murashige and Skoog (MS) basal medium with different concentrations of plant growth regulators did not produce any dihydrocarveol. But callus grown in the culture medium containing 1.25 mg/L (w/v) of NAA plus 1.35 mg/L (w/v) of BAP (T20) and 0.8 mg/L (w/v) of NAA plus 1.85 mg/L of BAP (T23) produced linalool as a major compounds. On the other hand, callus treated with 1.25 mg/L (w/v) of NAA plus 0.45 mg/L (w/v) of BAP (T10) and T20 (1.25 mg/L (w/v) of NAA plus 1.35 mg/L (w/v) of BAP were able to synthesis cyclohexane (32.96±1.94%) compared to T23 (17.015±1.06%). Callus derived from flower petal of *M. alba* treated with 1.25 mg/L (w/v) of NAA plus 0.45 mg/L (w/v) of

BAP (T10) gave the higher growth (23.83±1.44 g F.wt/ culture; 0.23±0.09 g D.wt/culture) respectively at pH 5.7, 25±2°C and in the complete dark condition after 5 weeks of culture. Treatment of *M. alba* flower petal derived callus with 1.0 mg/L (w/v) NAA plus 1.0 mg/L (w/v) BAP supplied with 150 mg/L (w/v) of casein hydrolysate showed higher growth (0.211±0.02 g D.wt/ culture) than all other concentrations of casein hydrolysate. Most of the new volatile compounds in the callus cultures were found after treatment with 150 mg/L (w/v) of casein hydrolysate. Addition of various concentrations of bioelicitors, jasmonic acid (0 to 0.25 mg/mL (w/v) yeast extract (0-0.3% (w/v)) and pectinase (0-0.25 mg/mL (w/v)) into the culture medium, decreased the cell growth. However, treatment of flower petal derived callus with (0.25 mg/mL (w/v)) of pectinase showed the highest growth (0.118±0.002 g D.wt/culture) on day 18th compared to other treatment. On the other hand, petal derived callus treated with jasmonic acid and L-phenylalanine decreased the callus growth but significantly increased the production of new volatile compounds i.e. cinnamaldehyde, caryophyllene, nerolidol, cinnamic acid, ocimene, farnasene, undecene, germacrene D and also linalool. The essential oils obtained from flower bud showed the insecticidal activities, against *Tribolium castaneum* adults. The insecticidal property of essential oil obtained from flower and petal-derived callus was probably associated with presence of linalool, eugenol and geraniol. Result from seed germination assay also showed that pentane extract of *Michelia alba* flowers was able to effect the germination of the *Brassica nigra* L (mustard seeds) tested. The phytotoxicity effect and cytotoxicity action may also be possibly associated with the presence of cineol, limonene and linalool though they are minor components.

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

PENGHASILAN DAN AKTIVITI BIOLOGIKAL SEBATIAN MONOTERPENO DARIPADA BUNGA DAN KULTUR KALUS *MICHELIA ALBA* DC.

Oleh

NOR AZIZUN BT RUSDI

November 2005

Pengerusi : **Profesor Madya Radzali Muse, PhD**

Fakulti : **Bioteknologi dan Sains Biomolekul**

Kaedah kromatografi gas spektrometri jisim (GC-MS) telah digunakan untuk menganalisis kandungan sebatian meruap daripada sampel bunga cempaka putih yang dipilih. Keputusan yang diperolehi menunjukkan bahawa kandungan sebatian meruap terbanyak dari pengestrakan 3 peringkat bunga cempaka putih adalah dihidrokarveol. Kelopak bunga cempaka putih yang dikulturkan secara *in-vitro* pada medium asas pertumbuhan Murashige dan Skoog yang mengandungi pengawalatur pertumbuhan tumbuhan pada kepekatan yang berbeza gagal menghasilkan sebatian dihidrokarveol. Tetapi kultur kalus tisu yang telah ditumbuhkan pada medium yang mengandungi 1.25 mg/L (b/i) (NAA) ditambah dengan 1.35 mg/L (b/i) (BAP) (T20) dan 0.8 mg/L (b/i) (NAA) ditambah dengan 1.85 mg/L (b/i) (BAP) (T23) telah berjaya menghasilkan linalool. Selain itu, kalus yang ditumbuhakan pada medium yang

mengandungi 1.25 mg/L (b/i) NAA ditambah dengan 0.45 mg/L (b/I) BAP (T10) dan T20 (1.25 mg/L (b/i) of NAA ditambah dengan 1.35 mg/L (b/i) BAP telah berjaya menghasilkan sebatian sikloheksana (32.96±1.94%) berbanding dengan rawatan T23 (17.015±1.06%). Kalus yang terhasil dari kelopak bunga cempaka putih (*M. alba*) yang diuji pada media mengandungi 1.25mg/L (b/i) (NAA) ditambah dengan 0.45mg/L (b/i) (BAP) telah memberikan pertumbuhan yang tertinggi (23.83±14.44.g berat basah/kultur; 0.23±0.09g berat kering/kultur) selepas 5 minggu dieramkan pada pH 5.7, suhu 25±2°C dan dalam keadaan gelap sepenuhnya. Rawatan kalus dari kelopak bunga cempaka putih pada kepekatan 1.0 mg/L (b/i) (NAA) ditambah dengan 1.0 mg/L (b/i) (BAP) ditambah dengan 150 mg/L (b/i) kasein hidrolisat telah menunjukkan peningkatan pertumbuhan kalus (0.211±0.02g.berat kering/kultur) berbanding dengan kepekatan kasein hidrolisat yang lain. Banyak sebatian meruap yang baru telah ditemui pada kalus yang diberi rawatan dengan 150 mg/mL (b/i) kasein hidrolisat.Penambahan beberapa siri kepekatan bioelisitor, asid jasmonik (0-0.05) mg/mL (b/i), ekstrak yis (0-0.3% (b/i)), dan pektinase (0-0.25 mg/mL (b/i)) ke dalam medium asas telah menurunkan kadar pertumbuhan sel kultur tisu. Tetapi didapati rawatan kelopak bunga kalus dengan pektinase pada kepekatan 0.25 mg/mL (b/i) telah berjaya menunjukkan pertumbuhan tertinggi pada hari 18 pengeraman jika dibandingkan dengan rawatan lain. Di dalam hal lain, kelopak bunga kalus yang dirawat dengan asid jasmonik dan L-fenilalanin telah menurunkan kadar pertumbuhan kalus tetapi telah berjaya mengsintesis beberapa sebatian meruap yang baru seperti sinamaldehida, karyofilin, nerolidol, asid sinamik, osimin, farnasin, undecin, germaserin D dan juga linalol. Minyak perlu yang diperolehi daripada bunga cempaka menunjukkan sifat insektisidal keatas serangga *T. castaneum* dewasa. Ciri - ciri penghindar serangga yang terdapat pada ekstrak minyak bunga cempaka putih dan

kultur kalus mungkin disebabkan oleh kehadiran beberapa sebatian seperti linalol, cineol dan limonen. Keputusan daripada asai percambahan *Brassica nigra* L (biji sawi) juga menunjukkan ekstrak bunga cempaka yang menggunakan pelarut pentana telah mempengaruhi percambahan *Brassica nigra* L (biji sawi) yang telah digunakan. Ciri-ciri fitotoksisiti dan sitotoksisiti yang hadir mungkin disebabkan oleh kehadiran cineol, limonen dan linalol walaupun dalam jumlah yang sedikit.

ACKNOWLEDGEMENTS

I would like to sincerely thank to Assoc. Prof. Dr. Radzali Muse, my main supervisor, for his patience, guidance, and advice during this research. I also wish to thank the other members of my supervisory committee: Assoc. Prof. Dr. Johari Ramli from the Department of Biochemistry, Assoc. Prof. Dr. Md. Aspollah Sukari from the Department of Chemistry, Cik Sanimah Simoh from the Biotechnology Center (MARDI) and Encik Suri Roowi from the Food Technology Center (MARDI), for their valuable review and assistance to this research. I am deeply grateful for the opportunity to learn and work with the staff from UPM and MARDI.

I am sincerely thankful and greatly appreciative for all of the help, encouragement, guidance and friendship that I have gained during working with this project from the staff of the Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia; all staff of tissue culture laboratory, Biotechnology Center, Malaysian Agriculture Research and Development Institute (MARDI) Serdang. I hereby knowledge, with gratitude, Mrs Siti Shaleha, Mrs Habsah, and Ms Nor Afni Zulkifli for their help on the tissue culture techniques. I would like to give special thanks to the Pasca siswazah fellowship, UPM for two years of financial support. Finally, I would like to express my heartfelt gratitude and appreciation to my family for their advice, encouragement, understanding, support, and prayers. May God bless you all.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS AND SYMBOLS

Abs	Absorbance
ADM	Artificial diet media
App	Appendix
Approx.	Approximately or about
Aq	Aqueous
BAP	6-benzylaminopurine
b/i	berat per isipadu
b.p	Boiling point
°C	Degrees Celsius
CF(s)	Culture filtrate (s)
CH	Casein Hydrolysate
Conc. (s)	Concentrations
2, 4-D	2, 4-dichlorophenoxy acetic acid
d	Day
D.wt	Dry weight
e.g.	Example (s)
et al.,	*at alli* and other people
etc.	*et cetra*, and the rest.
EB	Evans blue (stain)
EO	Essential oil
EtOH	Ethanol or ethyl alcohol
FeNaEDTA	Ethylenediaminetetraacetic acid, Ferric Sodium salt trihydrate,
F.wt	Fresh weight
GC	Gas Chromatography
GC-MS	Gas chromatography mass spectrometry
h	Hour
IBA	Indole butyric acid
IAA	Indole acetic acid
JA	Jasmonic acid
KIN	Kinetin
LD_{50}	Lethal dose at 50%
LD_{90}	Lethal dose at 90%
MS	Murashige and Skoog
NAA	Naphthalene Acetic Acid
PVP	Polyvinylpyrrolidone
SD	Standard deviation
SDE	Simultaneous Distillation Extraction
SEM	Standard error of mean
µl	Micro liter
w/v	weight per volume
ZEA	Zeatin

CHAPTER I

INTRODUCTION

For centuries, mankind is totally dependent on plants as sources of carbohydrates, proteins and fats for food shelter. In addition, plants are valuable sources of a wide range of secondary metabolites, which are used as biopharmaceuticals, agrochemicals, flavours, fragrances, colours, biopesticides and food additives as reported by Balandrin and Klocke (1988).

Plants produce a variety of chemical compounds that have large economical value. Harborne (1991) reported that first of all the chemical compounds are connected with important traits of the plant itself, *e.g.*, colour or fragrance of flowers, taste and color of foods, and resistance against pests and diseases and also for the production of fine chemicals such as drugs, antioxidants, flavours, fragrances, dyes, insecticides and pheromones.

Monoterpenes such as linalool, champhene and cineol, are involved in pollinator attraction and allelopathy (Langenheim, 1994; Harborne, 1991). Monoterpenes has also found expensive industrial applications as flavoring agents, topical medicines, perfumes and insecticides (Croteau *et al.*, 2000; Little and Croteau, 1999; Loza-Tavera, 1999; Phillips and Croteau, 1999).

Monoterpenes are widespread in the plant kingdom (Banthorpe and Charlwood, 1980) and are often responsible for the characteristic odours of plants. These substances are believed to function principally in ecological roles, serving as herbivore-feeding deterrents, antifungal defense, and attractants for pollinators (Langenheim, 1994; Romagni *et al.*, 2000). There is also evidence that monoterpenes have potential uses as herbicides, pesticides, antimicrobial agents and dietary anticarcinogens (Crowell, 1999). The monoterpenoids are the major component of many essential oils and as such, have economic importance as flavors and perfumes (Brielmann, 1998).

Michelia alba flowers is a native plant of tropical and subtropical Southeast Asia *Michelia* ('cempaka') species is interestingly known to produce high value essential oil. The popularity of the fragrance has resulted in many species of *Michelia* being grown all over the world. It is reported that the essential oil of *Michelia* has been used as a key ingredient in Joy and J'adore, among the world's most expensive perfumes. In China, it is reported by Yamanishi *et al.* (1988) that the flowers are used to scent Yulan tea. The chemical composition of a few species of *Michelia* spp has been the subject of limited study.

Many plant secondary metabolites produced by higher plants are of economically importante (Stafford *et al.*, 1986; Parr, 1989). Many secondary metabolites are obtained directly from tissues whose availability may be limited. Plant cell culture offer an alternative for the production of these metabolites because the cells can be cultured in large quantities under controlled conditions and harvesting of the desired product can be less problematic (Parr, 1989; Buitelaar and Trampler, 1992).