



UNIVERSITI PUTRA MALAYSIA

**ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITIES OF LEAVES,
CALLI AND CELL SUSPENSION OF PUTAT (*BARRINGTONIA
RACEMOSA*)**

MANDANA BEHBAHANI

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(*BARRINGTONIA RACEMOSA*)**

By

MANDANA BEHBAHANI

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

September 2007



Dedicated

To my dearly beloved family for all their love, supports, understanding and patient.



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

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Chairman: Abdul Manaf Ali, PhD

Faculty: Biotechnology

The medicinal plant of *Barringtonia racemosa* (Lecythidaceae family) has been used widely in traditional medicine for anti-inflammation and anticancer in Malaysia. The present investigation was carried out to study anti-oxidant and anti-inflammatory effects of leaves, callus, cell suspension and *in vitro* regenerated shoots and roots of *B. racemosa*.

The results showed that different crude extracts of fully expanded leaf extracts of *B. racemosa* have a very strong nitric oxide (NO) inhibitory and antioxidant activities. In the Griess assay, non polar extracts such as chloroform and hexane extracts were found to be strong inhibitors of NO at different concentrations (25, 50, 100 and 200 µg/ml) in comparison with polar extract (ethanol extract).

Calli were aseptically obtained by placing surface sterilized leaf explants on Woody Plant Medium (WPM) supplemented with different concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D). On the shoot induction medium, the callus induced on the WPM medium containing 2 mg/L (w/v) KIN+0.2 mg/l (w/v) IBA and 2 mg/L (w/v) of KIN + 0.4 mg/L(w/v) of NAA was the most effective, providing high shoot regeneration frequency of 85.6 and 76.5 %, respectively. In addition, the highest number of shoots produced was 8.2 and 6.3 shoots per explant respectively in the medium containing the mentioned plant growth regulators. The rooting percentage and number of roots per shoot which achieved on WPM medium supplemented with 3g/L (w/v) of activated charcoal and 0.8 mg/L (w/v) of IBA were 62 and 5.6 %, respectively. 96 % of the *in vitro* rooted plantlets with well developed shoots and roots were survived when transferred to soil.

Results obtained from this study revealed that *B.racemosa* is one of the important sources of lycopene. Lycopene has long been recognized as important antioxidants both *in vivo* and *in vitro*. Lycopene level was detected at a range of 0.02 to 4.14 mg/g dry weight in *in vitro* regenerated shoots and roots respectively. Lycopene level was also successfully detected in the callus (0.34 to 2.12 mg/g dry weight) and cell suspension cultures (0.18 to 0.68 mg/g dry weight) under dark and light conditions and the amount was lower than that produced in the intact plant tissues. However, manipulating the physical conditions, feeding of precursor and elicitation managed to increase the lycopene content in cultured tissues. Studies on the effects of the medium composition show that fully strength of the basal Woody Plant Medium and B5 containing 3% (w/v) of sucrose increased the lycopene content in both callus and cell suspension cultures. The precursor-feeding studies revealed

that concentrations of 3 mg/L (w/v) of isopentenyl pyrophosphate and 2 to 4 mg/L (w/v) of Mevalonate were preferred for lycopene production. The elicitor studies exhibited that the different elicitors showed distinctive effects on lycopene production. Nevertheless, casein hydrolysate at 10 and 15 mg/l (w/v) was found to be the best in increasing the lycopene production in callus and cell suspension cultures. The study further concluded that there was correlation between anti-oxidant and anti-inflammatory activities and lycopene content in callus, cell suspension and *in vitro* regenerated organs of *B.racemosa*.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia bagi memenuhi keperluan untuk ijazah Doktor Falsafah

**ANTI-OXIDADANT DAN ANTI-PEMBEKAKAN AKTIVITI DARI DAUN,
KALUS DAN SEL AMPAIAN DARI PUTAT (*BARRINGTONIA RACEMOSA*)**

Oleh

MANDANA BEHBAHANI

September 2007

Pengerusi : Abdul Manaf Ali, PhD

Fakulti : Bioteknologi

Barringtonia racemosa (Lecythidaceae family) adalah tumbuhan perubatan yang biasa digunakan dalam perubatan traditional untuk anti-inflamasi dan anti-kanser di Malaysia. Kajian ini dijalankan untuk mengesan aktiviti anti-oksidan dan anti-inflamasi dari daun, kalus, sel ampaiian dan *in-vitro* regenerasi organ dari akar *B.racemosa*.

Keputusan telah menunjukkan pelbagai ekstrak kasar dari daun *B. racemosa* mempunyai kesan anti-oxidan and perencatan "nitric oxide" (NO). Dalam kajian esai "Griess", ekstrak yang tidak berpolar seperti ekstrak dari kloroform dan heksan telah menunjukkan kesan perencatan terhadap NO atas kepekatan yang berlainan (25, 50, 100, 200 µg/ml) jika dibandingkan dengan ekstrak dari bahagian berpolar (ekstrak dari etanol).

Kalus diperoleh setelah daun eksplan yang sterile diletak di atas "Woody Plant Medium" (WPM) yang mengandung pelbagai kepekatan asid dichlorophrnoxyacetic (2,4-D). Dalam medium perangsangan pucuk, kalus didapati tumbuh pada medium WPM yang mengandung 2 mg/l (w/v) KIN + 0.2 mg/l (w/v) IBA dan 2 mg/l (w/v) KIN + 0.4 mg/l (w/v) NAA menunjukkan kesan paling baik, dalam regenerasi pucuk iaitu dengan frekuensi 85.6 dan 76.5%. Tambahan pula, jumlah bilangan pucuk yang dihasilkan di dalam media yang mengandung fito-hormon adalah 8.2 dan 6.3 per eksplan. Peratusan pembentuk akar dan nombor bagi bilangan akar terhadap pucuk yang diperolehi melalui medium WPM yang mengandung 3g/L (w/v) arang aktif dan 0.8 mg/L (w/v) IBA adalah 62% and 5.6. 96% plantlet yang membentuk akar dengan perkembangan pucuk telah berjaya hidup apabila dipindahkan ke tanah.

Keputusan yang diperolehi daripada kajian menunjukkan bahawa likopin adalah bahan komponen aktif di dalam *B.racemosa*. Lipokin telah lama dikenai sebagai bahan anti-oxidan secara *in-vitro* dan *in-vivo*. Tahap likopin dikesan pada kadar 0.02-4.14 mg/g berat kering bagi regenerasi organ secara in vitro dari pucuk dan akar. Tahap likopin juga berjaya dikesan di dalam kalus (0.43-2.12 mg/g berat kering), kultur ampai sel (0.18-0.68 mg/g berat kering) dan jumlah ini adalah rendah berbanding jumlah yang dihasilkan di dalam "intact" tisu tumbuhan. Walau bagaimanapun, manipulasi keadaan fizikal, prekursor penyusunan dan elisitasi berjaya meningkatkan kandungan likopin di dalam tisu kultur. Kajian terhadap kesan komposisi media menunjukkan bahawa media asas iaitu basal WPM dan B5 yang mengandung 3% (w/v) sukrosa menunjukkan kesan yang

ketara dan dapat meningkatkan kandungan likopin di dalam kedua-dua kalus dan sel ampaiian kultur. Kajian penyusunan prekursor menunjukkan bahawa kepekatan 3 mg/l (w/v) bagi isopentenyl pyrophosphate dan 2-4 mg/l (w/v) mevalonate adalah sesuai untuk penghasilan likopin. Kajian elisitor dengan pelbagai elisitor menunjukkan bahawa kesan nyata keatas penghasilan likopin. Walau bagaimanapun, casein hydrolysate pada 10-15 mg/L (w/v) didapati adalah terbaik untuk meningkatkan penghasilan likopin di dalam kalus atau sel ampaiian kultur. Kesimpulannya, anti-oxidan and anti-inflamasi aktiviti dalam *B. racemosa* adalah berkaitan dengan kandungan likopen di dalam kalus, ampaiian sel and *in-vitro* regenerasi organ dari *B. racemosa*.

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I certify that an Examination Committee met on 18th Oct 2007 to conduct the final examination of Mandana Behbahani on her Doctor of Philosophy thesis entitled Antioxidant and Anti-inflammatory Activities Of Leaves, Calli And Cell Suspension Of Putat (*Barringtonia Racemosa*) in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

Janna Ong Abdullah, PhD

Lecturer
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Parameswari a/p Namasivayam, PhD

Lecturer
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

Azizah Abd. Hamid, PhD

Associate Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Internal Examiner)

Chong Ving Ching, PhD

Professor
Institute of Biological Science
University of Malaya
(External Examiner)

GULAM RUSUL RAHMAT ALI, PhD

Professor / Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia
Date:



This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirement for the Degree of Doctor of Philosophy. The members of the Supervisory Committee are as follows:

Abdul Manaf Ali , PhD

Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Intan Safinar, PhD

Lecturer
Faculty of Sciences
Universiti Putra Malaysia
(Member)

Noorjahan Banu Mohd, PhD

Lecturer
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

Radzali Muse, PhD

Lecturer
Faculty of Chemistry and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

AINI IDERIS, PhD

Professor / Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:



DECLARATION

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

MANDANA BEHBAHANI

Date:

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LIST OF ABBREVIATIONS

mg	Milligram
g	Gram
mL	Millilitre
L	Litre
μ M	Micromolar
%	Percentage
$^{\circ}$ C	Degree Celsius
v/v	Volume per volume
w/v	Weight per volume
FW	Fresh weight
DW	Dry weight
PGR(s)	Plant Growth regulator(s)
NAA	Naphthalene acetic acid
2,4-D	2,4-Dichlorophenoxy acetic acid
IBA	Indol-3-butyric acid
IAA	Indol-3-butyric acid
BAP	6-benzyl aminopurine
IPP	Isopentenyl pyrophosphate
MVA	Mevalonate acid
CH	Casein hydrolysate
v/v	Volume per volume
w/v	Weight per volume

CHAPTER 1

INTRODUCTION

Antioxidant is a chemical substance extremely useful to humans. It helps us ward off many kinds of disease related to lungs, kidneys, heart, cardiovascular system, muscle and brain, and it helps to retard the aging process. Antioxidant has the ability to prevent or delay the formation of free radicals and lipid peroxidation in the human bodies, two main causes of human disease and aging (Galati and Brien, 2004).

Antioxidant can be obtained from synthesis and natural sources. Antioxidant from natural sources include a wide variety of compounds from a wide range of classes, including plant-based antioxidants, amino acids, peptides and protein hydrolyzates, phytates, phospholipids, vitamins and enzymes (Halliwell *et al.*, 1995). In the recent year, there has been an increase in the use of plants as sources of natural antioxidants for scavenging of free radicals (Galati and Brien, 2004).

Among the plants that have been known to provide antioxidant compounds are those belonging to Lecythidaceae. Lecythidaceae is a large, mainly nut-bearing family of trees which includes well known nuts such as *Barringtonia* (Berkov and Tavakilian, 1998). *Barringtonia* comprises some 39 species of trees and shrubs. These are scattered over a wide area extending from east Africa and India, over the islands of the East Indian Archipelago and of Oceania, even to Australia (Edwin, 1977).



The medicinal plant of *B. racemosa* has been used widely in traditional medicine for anti-inflammation and anticancer in Malaysia. Previous studies on some *Barringtonia* species including *B. asiatica*, *B. acutangul* and *B. lanceolata* have showed that most of the species possess medicinal properties (Grosvenor *et al.*, 1995; Khan and Omoloso, 2002). The literature survey revealed that there are no scientific studies carried out regarding antioxidant and anti-inflammatory activity of the leaves of *B. racemosa*. In this study, we attempted to study the production of antioxidant and anti-inflammatory compound in *B. racemosa* using plant cell culture. In relation to the study and production of plant secondary metabolites, application of plant tissue culture technique offers many advantages compared to the intact plant (Ushiyama, 1991), such as: relatively easy to grow and can be maintained under strictly controlled nutritional and environmental conditions, simple and more convenient in experimental system than intact plant, very effective way of incorporating precursor materials which are often difficult to administer to the entire plant, the uncertainties of climate and soils can be avoided.

Several products were found to be accumulated in cultured cells at a higher level than those in intact plants through optimization of cultural conditions. For example, ginsenosides by *Panax ginseng* (Palazón *et al.*, 2003), rosmarinic acid by *Lavandula vera* (Pavlov and Ilieva, 2006), shikonin by *Lithospermum erythrorhizon* (Chung *et al.*, 2006), diosgenin by *Dioscorea* spp (Katarina *et al.*, 1998), ubiquinone-10 by *Nicotiana tabacum* (Hartmann and Bach, 2001) were accumulated in much higher levels in cultured cells than in the intact plants.

