UNIVERSITI PUTRA MALAYSIA

# BIOLOGICAL ACTIVITIES OF *BARRINGTONIA RACEMOSA* (LECYTHIDACEAE) AND THE QUANTITATIVE ANALYSIS OF PHENOLIC ACIDS AND FLAVONOIDS PRESENT IN THE VARIOUS BIOACTIVE EXTRACTS OF THE SPECIES

NURUL MARIAM HUSSIN

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**By**

**NURUL MARIAM HUSSIN**

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

**October 2009**

## DEDICATION

With love to all my family including my mother Ku Nadzrah Ku Aziz, my father Hussin Mohd. Hanif, my sisters (Nurul Huda, Nurul Ashikin, Nurul ‘Ain Sakina, Nurul Suraya, and Nurul Aiman), for enduring, with patience and understanding. Also not forgetting my lovely friends (Nurazah Zain, Dayana Wazir, Rudi Hendra 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

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**October 2009**

**Chairman : Radzali Muse, PhD**

**Faculty : Biotechnology and Biomolecular Sciences**

*Barringtonia racemosa* (Family Lecythidaceae) posses several bioactivities and is used in traditional medicine of Malaysia. However, its antioxidant, antimicrobial, anti-inflammatory and allelopathic activities investigation are still lacking. The aims of this study were therefore to determine those biological activities of methanolic, ethanolic and hot water leaf, stem and bark extracts of *B. racemosa* and quantitative analysis of phenolic acids and flavonoids present in the various bioactive extracts of the species. The methanolic and ethanolic extracts of bark showed strong free radical scavenging activity (89.23 ± 0.40 and 91.35 ± 0.89 %) and this was even higher than butylated hydroxytoluene (BHT), ascorbic acid and α-tocopherol (antioxidant standards). The results also showed that all methanolic extracts (leaf, stem, and bark; 84.47 ± 0.05, 84.41 ± 0.28 and 84.03 ± 0.03 %) and ethanolic extracts of leaf and bark (82.58 ± 0.06 and 88.21 ± 0.06 %), respectively had similarly strong reducing power when compared to the antioxidant standards, tested in this study. The leaf of methanolic and ethanolic

extracts showed strong antioxidant activity (80.03 ± 5.56 and 88.93 ± 8.32 %) in the β-carotene bleaching assay which were higher than the antioxidant standards. Although most of the hot water extracts showed weak to moderately antioxidant activities, the hot water extract of the leaf surprisingly showed strong reducing power potential (83.45 ± 0.07 %). Overall, the methanolic and ethanolic extracts of *B. racemosa* had effective antioxidant activities compared to hot water extracts. In Griess assay, all the *B. racemosa* extracts showed no significant activity on nitric oxide inhibition in RAW264.7 cell line. However, the extracts do not seem to be cytotoxic. In antibacterial assay, *B. racemosa* aerial parts extracts showed weak to moderate inhibitory activities against both Gram-positive and Gram-negative bacteria tested accept for boiling water extracts which was inactive. For antifungal assay, methanolic extracts of *B. racemosa* leaf, stem and bark exhibited the higher inhibitory activity among the four of seven fungi tested. Among different fungi tested, all extracts were found to be more sensitive to *Fusarium* sp. compared to the others. The seed germination assay results showed that *B. racemosa* extracts were able to effect the germination of the *Brassica nigra* L. (allelopathic activity). In the present study, quantitative analysis of phenolic compounds from *B. racemosa* leaf, stem and bark were performed using reversed-phase high performance liquid chromatography (RP-HPLC) technique. The results showed that gallic acid and naringin were the major phenolic acids and flavonoids present in all different aerial parts of *B. racemosa*. Ferulic acid, rutin, kaempferol and luteolin were also detected in several aerial parts of *B. racemosa*. Overall results obtained from the biological assays suggested that *B. racemosa* is a source of bioactive compounds endowed with interesting biological activities, such as strongly antioxidants agents and can be rated as good dietary sources of natural phenolic antioxidants.

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

# AKTIVITI-AKTIVITI BIOLOGI *BARRINGTONIA RACEMOSA* (LECYTHIDACEAE) DAN ANALISA KUANTITATIF ASID FENOL DAN FLAVONOID YANG HADIR DI DALAM PELBAGAI EKSTRAK BIOAKTIF SPESIES


Oleh

**NURUL MARIAM HUSSIN**


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*Barringtonia racemosa* (keluarga Lecythidaceae) memiliki pelagai bioaktiviti dan digunakan di dalam perubatan tradisional di Malaysia, walaubagaimanapun kajian terhadap aktiviti antioksida, antimikrob, anti-radang dan allelopatik masih lagi kurang dan tidak mendalam. Oleh itu, objektif utama kajian ini adalah untuk menentukan aktiviti-aktiviti biologi tersebut dalam ekstrak metanol, etanol dan air panas daun, ranting dan kulit kayu *B. racemosa* dan menjalankan ujian analisa kuantitatif asid fenol dan flavonoid yang hadir di dalam pelbagai ekstrak bioaktif spesies tersebut. Ekstrak metanol dan etanol pada bahagian kulit kayu menunjukkan kekuatan yang tinggi dalam akitiviti pemerangkapan radikal bebas (89.23 ± 0.40 dan 91.35 ± 0.89 %), malah keputusan tersebut adalah lebih tinggi dari hidroksitoluena butilat (BHT), asid askorbik dan α-tokoferol. Keputusan juga menunjukkan kesemua ekstrak metanol (daun, ranting dan kulit kayu; masing-masing 84.47 ± 0.05, 84.41 ± 0.28 and 84.03 ± 0.03 %) dan

ekstrak etanol pada daun dan kulit kayu (masing-masing 82.58 ± 0.06 and 88.21 ± 0.06 %) mempunyai kekuatan aktiviti kuasa penurunan yang hampir sama dengan kesemua antioksidan piawai yang diuji. Dalam uji kaji aktiviti pelunturan β-kerotina, ekstrak metanol dan etanol pada daun menunjukkan aktiviti yang sangat kuat (80.03 ± 5.56 and 88.93 ± 8.32 %) di mana lebih tinggi dari antioksidan piawai. Walaupun kebanyakan ekstrak air panas menunjukkan aktiviti yang sederhana dalam kesemua uji kaji antioksidan, salah satu daripada ekstrak air panas iaitu pada daun telah menunjukkan kekuatan kuasa penurunan yang amat tinggi (83.45 ± 0.07 %). Secara keseluruhannya, ekstrak metanol dan etanol *B. racemosa* mempunyai kesan yang amat efektif terhadap aktiviti antioksidan berbanding ekstrak air panas. Dalam ujian Griess, kesemua ekstrak *B. racemosa* tidak menunjukkan aktiviti yang siknifikan terhadap perencatan nitrik oksida dalam sel RAW 264.7. Walaubagaimanapun, ekstrak tersebut tidak memberi kesan sitotoksik pada sel. Kesemua ekstrak daun, ranting dan kulit kayu *B. racemosa* menunjukkan aktiviti rencatan yang lemah hingga sederhana terhadap bacteria Gram-positif dan Gram-negatif kecuali ekstrak air panas yang tidak menunjukkan sebarang aktiviti rencatan terhadap kedua-dua bakteria tersebut. Dalam ujian antikulat, ekstrak metanol pada daun, ranting dan kulit kayu *B. racemosa* mempamerkan activiti rencatan yang tinggi terhadap empat jenis kulat daripada tujuh jenis kulat yang diuji kaji. Kesemua ekstrak adalah sangat sensitif terhadap *Fusarium* sp. berbanding dengan lain-lain kulat. Keputusan dari ujian germinasi biji benih juga menunjukkan bahawa ekstrak metanol, etanol dan air panas *B. racemosa* berpontensi memberi kesan germinasi biji benih *Brassica nigra* L. (biji benih sawi). Dalam kajian ini, analisa kuantitatif sebatian fenol di dalam daun, ranting dan kulit kayu *B. racemosa* dijalankan dengan menggunakan teknik kromatografi fasa-pembalikan HPLC. Keputusan menunjukkan

asid galik dan naringin merupakan sebatian asid fenol dan flavonoid utama di dalam semua bahagian arial *B. racemosa* (daun, ranting dan kulit kayu). Asid ferulik, rutin, kemferol dan lutiolin juga dapat dikesan di dalam beberapa bahagian arial *B. racemosa*. Secara keseluruhannya, dari keputusan yang diperolehi daripada ujian aktiviti biologi, dapat disimpulkan bahawa *B. racemosa* adalah menjadi sumber sebatian bioaktif yang mengandungi pelbagai aktiviti biologi yang istimewa, misalnya sebagai agen antioksidan yang kuat dan mampu menjadi sumber diet berasaskan antioksidan fenolik semulajadi.

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I certify that an Examination Committee has met on **13th October 2009** to conduct the final examination of Nurul Mariam Hussin on her Master of Science thesis entitled "Biological Activities of *Barringtonia racemosa* (Lecythidaceae) and The Quantitative Analysis of Phenolic Acids and Flavonoids Present In The Various Bioactive Extracts of The Species" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The committee recommends that the student be awarded the relevant degree.

Members of the Examination Committee were as follows:

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This thesis was submitted to 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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Date: 14 January 2010

## DECLARATION

I declare that the thesis is my original work except for the 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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**NURUL MARIAM HUSSIN**

Date:



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| | |
|---|---|
| A | Absorbance |
| ABTS | 2,2'-azino-bis(3-ethylbenzythiazoline-6-sulphonic acid) |
| $AlCl_3$ | Aluminium chloride |
| AP-1 | Activator protein-1 |
| BHA | Butylate hydroxyanisole |
| BHT | Butylated hydroxytoluene |
| HW | Hot water |
| CoA | Coenzyme A |
| $CD_{50}$ | Cluster of differentiation 50 |
| Cu | Cuprum |
| Da | Dalton |
| DMEM | Dulbecco's Modified Eagle's Medium |
| DMSO | Dimethyl sulfoxide |
| DNA | Deoxyribonucleic acid |
| DPPH | 1,1-diphenyl-2-picryl-hydrazyl |
| EtOH | Ethanol |
| FCS | Foetal calf serum |
| FDW | Freezed-dried weight |
| Fe | Ferrum/ion |
| FTC | Ferric thiocyanate |
| FRAP | Ferric reductant antioxidant power |
| HCl | Hydrochloric acid |
| $H_20_2$ | Hydrogen peroxide |
| HOCl | Hypochlorous |
| HOSC | Hydroxyl radical scavenging capacity |
| HPLC | High performace liquid chromatography |
| $IC_{50}$ | Half maximal inhibitory concentration |
| (IFN)-γ | Interferon-gamma |
| -1β | Interleukin -1β |
| iNOS | Nitric oxide synthase |
| $K_3Fe(CN)_6$ | Potassium ferricyanide |
| L-NAME | L-nitro-arginine methyl ester |
| LPS | Lipopolysaccharide |
| MAPK | Mitogen activated protein kinase |
| MAP | Mitogen activated protein |
| MDA | Malaondialdehyde |
| MeOH | Methanol |
| mRNA | Messenger ribonucleic acid |
| MTT | 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide |
| NA | Nutrient agar |
| $NaCO_3$ | Sodium carbonate |

| | |
|---|---|
| $NaNO_2$ | Sodium nitrate |
| $NaNO_3$ | Sodium nitrite |
| NaOH | Sodium hydroxide |
| NB | Nutrient broth |
| NF-κB | Nuclear factor kappa-B |
| nm | Nanometer |
| NMR | Nuclear magnetic resonance |
| NO | Nitric oxide |
| $NO2^-$ | Nitrogen oxide |
| $OONO^-$ | Peroxynitrite |
| $O_2^{\bullet-}$ | Superoxide |
| $^{\bullet}OH$ | Hydroxyl |
| $^1O_2$ | Singlet oxygen |
| $ONOO^-$ | Oxidant peroxynitrite |
| ORAC | Oxygen radical absorbance capacity |
| PDA | Potato dextrose agar |
| PG | Propyl gallate |
| $PGE_2$ | Prostaglandin $E_2$ |
| RAW 264.7 | Murine monocytic macrophage cell line |
| RNS | Reactive nitrogen species |
| RSNO | S-nitrosothiols |
| SOD | Superoxide dismutase |
| TCA | Trichloroacetic acid |
| TFA | Trifluoroacetic acid |
| TNF-α | Tumor necrosis factor-alpha |
| TBA | Thiobarbituric acid |
| UV-B | Ultraviolet-B |

# CHAPTER 1

# INTRODUCTION

Natural phytochemicals derived from plants have been reported to possess a wide range of biological effects, including antioxidant, anti-inflammatory and antimicrobial actions (Ao et al. 2008; Brunet et al. 2009). Of which, phenolics were one of the most notable groups. Among them, phenolic acids and flavonoids have been the object of the great number of studies of their biological activities in traditional medicinal plants. The antioxidative activity may be due to the both phenolic compounds which act as free radical scavengers and/or metal chelators (Ao et al. 2008; Brunet et al. 2009). A positive correlation was observed between the high phenolic content and the strong antioxidant activity (Ao et al. 2008; Barros et al. 2007).

The search for natural antioxidants of plant origin is also being explored as an alternative to the synthetic antioxidants, such as butylated hydroxyanisole (BHA), propyl gallate (PG) and butylated hydroxytoluene (BHT), used in food and pharmaceutical industries (Brunet et al. 2009; Gulcin et al. 2005) as the synthetic antioxidants may posses some side effects and toxic properties such as carcinogenic to human health (Manian et al. 2008). Natural antioxidants can protect the human body from free radicals and retard the progress of many chronic diseases as well as lipid oxidative rancidity in foods (Gulcin et al. 2004). Further more, the area of natural antioxidants developed enormously in the past decade mainly because of the increasing

limitations on the use of synthetic antioxidants and enhanced public consumers because they are considered safe (Madhavi et al. 1996).

Medicinal plants have been used for a large range of purposes, including medicine, nutrition, flavourings, beverages, dyeing, repellents, fragrances, cosmetics, charms, smoking and industrial uses. Nowadays, medicinal plant extracts are very attractive not only in the modern phytotherapy but also for food industry (Brunet et al. 2009).

*Barringtonia racemosa* (Lecythidaceae) is a small tree capable of reaching 20 m with leaves tufted at the ends of stout twigs. In Malaysia, it is commonly grow in the moist low places, especially near the shores of back waters, lakes, rivers and the banks of paddy field. The ethnopharmacological uses of *B. racemosa* indicate it to be rich source of phytomedicine. *B. racemosa* is also widely used in the form of a decoction in traditional medicine in Sri Lanka (Deraniyagala et al. 2003). The bark and leaves are used for rat-snake bites, rat poisoning and on boils. The seeds along with other ingredients are employed in the preparations for the treatment of itch, piles and thypoid fever. The bark is claimed to be specific for gastric ulcers (Deraniyagala et al. 2003; Khan et al. 2001). This species have been shown to contain polyhydroxylated triterpenoids, saponins (Hasan et al. 2000) and phenolic compounds (Sun et al. 2006).

In view of the lack of investigation of antioxidant properties in *B. racemosa*, the present study have investigated the activities of this plant extracts in inhibiting scavenging DPPH radical, bleaching of β-carotene and potential in breaking the free radical chain by donating a hydrogen atom and other biological activities. Antioxidants isolated from

plants also showed antibacterial, anticarcinogenic, anti-inflammatory, antiviral, antiallergic, allelopathic, estrogenic and immune-stimulating effects (Larson, 1988). Even though recent studies conducted internationally have confirmed the efficacy of *B. racemosa* diterpenoids and triterpenoids (Hasan et al. 2000; Khan et al. 2001; Khan and Omoloso, 2002), information on the content of phenolic acids and flavonoids content in the plant is still lacking. These compounds have attracted considerable interest due to their many potential health benefits and the *B. racemosa* possess many biological activities that can be a new source of natural product in the future.

## Objectives of The Study

1. To evaluate the biological activities of hot water, methanolic, and ethanolic extracts of *B. racemosa* leaf, stem and bark using antioxidant, anti-inflammatory, antimicrobial and seed germination assays.

2. To determine and to quantify the major phenolic compounds which are phenolic acids and flavonoids, present in three different aerial parts of *B. racemosa* (leaf, stem and bark) using reversed-phase high performance liquid chromatography (RP-HPLC) technique.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Medicinal Plants

The world is rich with natural and unique medicinal plants. Medicinal plants are now getting more attention than ever because they have offer of many benefits to society or indeed to all mankind, especially in terms of medicinal and pharmacological uses. The medicinal value of these plants lies in the bioactive phytochemical constituents that produce definite physiological action on the human body (Akinmoladun et al. 2007). Medicinal plants and their chemical constituents have been used worldwide in folk medicine as an alternative treatment of various diseases (Castelucci et al. 2007). Some of the most important classes of bioactive phytochemical constituents are alkaloids, essential oils, flavonoids, tannins, terpenoids, saponins, phenolic acids and many more (Edeoga et al. 2005). These natural compounds formed the foundations of modern prescription drugs as we know today (Krishnaiah et al. 2009).

## 2.2 Malaysian Medicinal Plants

Malaysian rainforest store a large number of plant species which are important as a source of traditional medicine. From about 10 000 species of higher plants and 2000 species of lower plants available in Peninsular Malaysia, approximately 16 % are

claimed to be used for medicinal purposes (Lattif et al. 1984; Murakami et al. 2000; Saha et al. 2004; Soepadmo, 1999). It is estimated that about 20% (3,000 species) of angiosperma and gymnosperma plant species found in Malaysia have medicinal properties and have been used in traditional medicine preparation for a considerable time. Those species are from the families of Annonaceae, Apocynaceae, Araceae, Compositae, Dioscoreceae, Ebenaceae, Euphorbiaceae, Flacourtiaceae, Lauraceae, Leguminosae, Menispermaceae, Myrsinaceae, Myrtaceae, Rubiaceae, Rutaceae, Simaroubaceae, Thymelaeaceae, Zingiberaceae and saveral others (Soepadmo, 1999).

Malay traditional vegetables in Malaysia (locally called 'ulam') comprise more than 120 species representing various families. The leaves, shoots or rhizomes of the vegetables are eaten fresh as salad or cooked (Norhanom et al. 1999). They are consumed because of their taste, which adds variety and flavour to the diet, as well as for their health benefits. Nutritional studies have indicated that many of these vegetables are rich in carbohydrates, proteins, minerals and vitamin (Abas et al. 2006). Some of the vegetables are also claimed to have medicinal properties, such as blood cleansing, induction of uterine contractions and prevention or cure of ailments such as diabetes, high blood pressure, cardiovascular disease, arthritis, fever and coughs. In addition, it is also believed that these vegetables play a vital role in lowering the incidence of cancer, as well as the control of ageing and age-related diseases (Abas et al. 2006). Table 1 summarized some common Malaysian medicinal plants and their related traditional uses.