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ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF Rhizophora apiculata BLUME AND Rhizophora mucronata Lam

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By

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

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the Degree of Master of Science

ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF *Rhizophora apiculata* BLUME AND *Rhizophora mucronata* Lam

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March 2016

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Mangroves have many important roles from coastal protection, as a medicinal plant, conservation of biodiversity to industrial products; such as firewood, charcoal and pole piling. *Rhizophora apiculata* and *Rhizophora mucronata* are the most common species used in these industries. The aim of this study is to investigate the antioxidant and antimicrobial activity of these two species as a potential non-wood product.

Three locations of mangrove type forest were selected, which included TPF (Timber production forest), VGF (Virgin forest) and SCF (Soil conservation forest). Parts of the plant such as fruit, leaf, root and twig were investigated for alcohol solubility, phenolic and flavonoid contents. Plant parts were extracted using ethanol and distilled water (80/20 by volume). The phenolic content was analyzed by Folin-Ciocalteau reagent using UV spectrophotometric technique while flavonoid content was measured by aluminium chloride colometric assay.

The extracted samples were analyzed through a high performance liquid chromatography (HPLC) to identify the existing of phenolic compounds. Three antioxidant activities were investigated, i.e. ABTS [2,2-Azinobis (3-ethyl-benzothiazoline- 6-sulfonic acid)], DPPH (1,1-diphenly-2-picrylhydrazyl) assay and β-carotene bleaching assays. Antimicrobial activities were investigated by the use of disc diffusion method with *Bacillus subtilis* (B145), *Staphylococcus aureus* (S276), *Salmonella cholerasuis* (ATCC 10708), *Escherichia coli* (E266) and *Candida albican* (C244).

Alcohol solubility is shown to be significantly different based on the part of the plant with the highest seen in descending order from fruit, leaf, twig and root. Based on the

location, TPF showed the highest yield compared to SCF and VGF. *R.mucronata* showed the highest value from fruit (32.05%), while, *R.apiculata* presented the highest value at 35.06 % from leaves.

Phenolic and flavonoid content were significantly different ($p<0.05$) on parts, location and also species. Twigs from *R.mucronata* at SCF presented the highest phenolic and flavonoid content with 41.01 mg GAE/g and 72.18 mg QE/g respectively. HPLC result showed that gallic acid was presented in all parts but not for cinnamic acid, caffiec acid and quercetine. Based on species, there were extra compounds present such as benzoic acid and vanillic acid in *R.apiculata* and salicylic acid in *R.mucronata*.

All parts for the both species showed antioxidant activity by DPPH, ABTS and β-Carotene. However fruit and twig are the most prominent. DPPH radical scavenging discovered that *R.apiculata* and *R.mucronata* inhibit more capacity from fruit at 56.78% and 57.35% respectively. Through IC50 measurement on both species showed that the data from fruits have the lowest value at 101.09 µg/mL (*R.apiculata*) and 174.04 µg/mL (*R. mucronata*). ABTS capacity showed that *R.mucronata* inhibits the highest amount from fruits (70.91%) while *R.apiculata* were from twigs (57.60%). Twigs presented high amounts of β-Carotene for both species which were at 42.79% from *R. apiculata* and 54.62 % from *R. mucronata*.

Antimicrobial activity presented that both species showed an inhibition area against bacterial strains of *B. subtilis* but were resistant to other strains except the twig part of *R. mucronata*. Fruits extrated from *R.apiculata* were inhibited more than others parts (10.00 mm), followed by twig (8.5 mm). Fruit and twig parts of *R.mucronata* showed a greater inhibition area than other parts which corresponded to 8.25 mm and 8.5 mm. Only the twig part of *R.mucronata* presented an inhibition zone 9.5 mm against *Salmonella chlerasuis* (ATTC 10708). From this study, these species presented antimicrobial activity within parts of plant, mainly from fruit and twig.

This study revealed that twig and fruit parts presented high phenol and flavonoid content. These parts also exhibited a greater response to antioxidant and antimicrobial activity compared to other parts. *R. mucronata* is the outstanding species which is inhibited by a greater radical scavenging capacity than *R. apiculata*. This species also showed greater efficacy as an antimicrobial agent.

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

AKTIVITI ANTIOKSIDA DAN ANTIMIKROB BAGI SPESIS *Rhizophora apiculata* BLUME DAN *Rhizophora mucronata* Lam

Oleh

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Bakau memainkan pelbagai peranan yang penting daripada sebagai perlindungan pesisir pantai, tumbuhan ubatan dan pemuliharaan biodiversity hinggalah kepada produk industrI seperti kayu api, arang dan tiang cerucuk. *Rhizophora apiculata* and *Rhizophora mucronata* merupakan dua species bakau yang paing biasa digunakan dalam industri ini. Tujuan kajian ini adalah untuk menyiasat aktiviti antioksida dan anti-mikrob bagi kedua spesis tersebut yang berpotensi untuk penghasilan produk bukan kayu.

Tiga lokasi berdasarkan kategori htan bakau telah dipilih iatu TPF (Hutan produksi balak, VGF (Hutan dara), dan SCF (Hutan konservasi tanah). Bahagian pokok seperti buah, daun, akar dan ranting dikenalpasti hasil extraktif, kandungan fenol dan kandungan flavonoid. Bahagian ini diekstrak dengan etanol dan air suling (80/20 mengikut isipadu). Jumlah fenol dianalisis dengan kaedah Folin-Ciocalteau menggunakan teknik spektrofotometri manakala jumlah flavonoid diukur melalui kaedah aluminium klorida kolometri.

Sampel yang telah diekstrak dianalisis menggunakan kromatografi cecair berprestasi tinggi (HPLC) untuk mengenal pasti sebatian fenolik yang wujud.

Tiga kaedah aktiviti antioksida disiasat iaitu ABTS [2,2-Azinobis (3-ethyl-benzothiazoline- 6-sulfonic acid)], DPPH (1,1-diphenly-2-picrylhydrazyl) dan pelunturan β-carotene. Aktiviti anti-mikrob di siasat dengan menggunakan kaedah cakera penyebaran beberapa bakteria dan kulat iaitu *Bacillus subtilis* (B145), *Staphylococcus aureus* (S276), *cholerasuis Salmonella* (ATCC 10708), *Escherichia coli* (E266) dan *Candida albican* (C244).

Terdapat perbezaan hasil ekstraktif (kelarutan alkohol) yang signifikan terhadap bahagian tumbuhan dengan buah menghasilkan jumlah yang teringgi, diikuti daun, ranting dan akar. Berdasarkan lokasi, TPF memberikan jumlah hasil ektraktif yang banyak berbanding SCF dan VGF. *R. mucronata* menunjukkan hasil ekstraktif yang tinggi daripada buah (32.05%), manakala *R. apiculata* daripada daun (30.30%).

Kandungan fenolik dan flavonoid mempunyai perbezaan yang signifikan ($p < 0.05$) terhadap bahagian pokok, lokasi dan juga spesis. Ranting daripada *R.mucronata* di SCF memberikan kandungan fenolik dan flavonoid yang tertinggi iaitu 41.01 mg GAE/g dan 72.18 mg QE/g masing-masing. Cerapan HPLC menunjukkan gallic acid wujud dalam semua bahagian pokok tetapi tidak bagi cinnamic acid, caffiec acid dan quercetine. Berdasarkan spesis, terdapat kompound lain yang wujud seperti benzoic acid dan vanillic acid dalam *R. apiculata* dan salicylic acid dalam *R.mucronata*.

Semua bahagian pokok pada kedua-dua spesis menunjukkan aktiviti antioksida dengan kaedah DPPH, ABTS dan β-carotene. Walaubagaimanapun, buah dan ranting adalah paling optimum. Perencatan DPPH menunjukkan *R. apiculata* dan *R.mucronata* memberikan kapasiti rencatan yang tinggi daripada buah iaitu 56.78% dan 57.35% masing-masing. Melalui pengukuran IC_{50} terhadap kedua-dua spesis, buah memberikan nilai yang terendah iaitu 101.09 µg/mL (*R.apiculata*) dan 174.04 µg/mL (*R.mucronata*). Kapasiti ABTS menunjukkan *R.mucronata* merencat kapasiti yang tertinggi daripada buah (70.91%), sementara *R.apiculata* daripada ranting (57.60%).

Aktiviti anti-mikrob menunjukkan kedua-dua spesis bertindak merencat terhadap bakteria *B. subtilis* tetapi rentan kepada microorganism yang lain kecuali bahagian ranting pada *R. mucronata*. Ekstrak buah daripada *R.apiculata* merencat lebih baik berbanding bahagian pokok yang lain (10mm), diikuti ranting (8.55mm). Bahagian buah dan ranting pada R.mucronata menunjukkan rencatan yang baik berbanding bahagian lain iaitu 8.25mm dan 8.5mm. Hanya bahagian ranting pada R.mucronata menunjukkan aktiviti rencatan 9.5 mm melawan *Salmonella chlerasuis* (ATTC 10708). Kajian ini menunjukkan terdapat aktiviti anti-mikrob dalam bahagian pokok terutama buah dan ranting.

Kajian ini membuktikan bahawa bahagian ranting dan buah mengadungi fenolik dan flavonoid yang tinggi. Bahagian ini juga mempamerkan tindakbalas yang baik terhadap aktiviti antioksida dan anti-mikrob bebanding bahagian pokok lain. *R. mucronata* merupakan spesis yang terbaik terhadap tindakannya sebagai antioksida berbanding *R. apiculata*. Spesis ini juga menunjukkan keberkesanannya sebagai ejen anti-mikrob.

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I certify that a Thesis Examination Committee has met on 20 March 2017 to conduct the final examination of Tuhaila binti Tukimin on her thesis entitled "Antioxidant and Antimicrobial Activity of *Rhizophora apiculata* Blume and *Rhizophora mucronata* Lam." in accordance with the 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 Master of Science.

Members of the Thesis Examination Committee were as follows:

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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 Master of Science.The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

Abs	Absorbance
ABTS	2,2'-azino-bis(3-ethylbenzythiazoline-6-sulphonic acid)
Anova	Analysis of variance
AlCl3	Aluminium chloride
cm	centimetre
DPPH	1,1-diphenly-2-picrylhydrazyl
DW	Dry weight
etc.	*et cetra*, and the rest
et al.,	*at alli* and other people
EtOH	Ethanol
FAO	Food and Agriculture Organization of the United Nation
HPLC	High Performance Liquid Chromatography
$K_2S_2O_8$	Potasium persulfate
m	metre
mg	miligram
MeOH	Methanol
Min	Minute
$NaCO_3$	Sodium carbonate
$NaNO_3$	Sodium nitrate
NaOH	Natrium Hidroksida
UPM	Universiti Putra Malaysia
UV	Ultra violet
spp.	Species
w/v	weight per volume

CHAPTER 1

INTRODUCTION

A long time ago, plants were used for medicinal purposes in the community. Most of the time, the knowledge was passed through word of mouth from one generation to the next generation without documentation. More than 35000 species were reported to be used in various cultures from around the world for medical purposes (Seters, 2003). In Malaysia, many races and cultures have inherited the various roles of plants as medicine for generations and more than 1200 plants has been reported with medicinal properties (Hunter, 2011). Burkill (1996), in his extensive compilation of the economic products of the Malay Peninsula, recorded not less than 1300 plants used in traditional medicine. This value might have increased sources and knowledge of the medicinal plants that could be implemented.

Bioactive compounds in plant extract were essential to pharmaceutical value. These compounds have essential biological and pharmacological activities such as anti-allergic, antioxidant, hypoglycaemic, anti inflammatory and anti carcinogenic (Katalinic et al., 2005; Borneo et al., 2008).Nearly one-fourth of the medicines prescribed by plant extracts contain active ingredients. For example, aspirin, a painkiller made from willow bark. In fact to about 25% of the drugs used in modern medicine owe their origins to plants from tropical rainforests (Elliot, 1986).

Nowadays, the role of phenolic compounds has become an increasingly important area of human nutrition research (Fraga, 2009). It has been a growing interest as an antioxidant and antimicrobial, which are obtained from plants; and has been strongly recommended. Phenolic or phenol is a class of chemical compounds consisting of a hydroxyl group (—OH) bonded directly to an aromatic hydrocarbon group. Phenolics can be classified into 10 subclasses based upon their chemical structure including the simple phenolics, phenolic acids, hydroxycinnamic acids, and flavonoids among others (Lepiniec, 2006). Phenolic compounds consist of secondary metabolites which synthesize a vast range of plants (Crozier et al., 2006) and are involved in a wide range of specialized physiological functions. For example, polyphenols which are widely distributed in plants, contribute to fruit organoleptic and nutritive quality in terms of color, taste, smell and flavor (Serrano, 2010). They are very important for the normal growth, development and defensive mechanism of plants (Maisathisakul et al., 2007).

Flavonoids are one of the types of phenolic compounds of particular interest because of its multiple functions in plants and its impact on human health (Harborne and Williams, 2000). The Flavonoid group such as flavonols are the most widespread in plant which consist mainly of quercetin, kaempferol, myricetin and isorhamnetin (Fraga, 2009). Quercertine in group flavonols is among the most widely distributed in plants (Chen et. al., 2008). Huang and Ferraro, (1992) also reported that almost all plant tissues are able to synthesize flavonoids, which flavonols quercertin and rutin

are the most widely found. These compounds are important to our body and act as antioxidants to prevent diseases such as cancer and heart disease. In plants, phenolic compounds play a role in survival and adaptive strategies.Flavonols, as well are involved in plant protection from ultraviolet (UV) light while promoting the growth of pollen tubes down the style to facilitate fertilization (Fraga, 2009).

The mangrove plant was used as traditional medicine in South Asian long ago, which has produced a lot of natures significant structure (Govindasamy and Kannan, 2012). Mangrove plants such as *Rhizophora* spp., *Avicenia* sp. , *Herotia* sp., and others, also important plants commonly used as medicine, which were inherited from the old generation and passed down to the new generation. Many authors noticed that mangroves species should be considered as a valuable source for chemical constituents with potential medicinal and agricultural values (Miles and Kokpol, et al., 1999; Bunyapraphatsara et al., 2003; Ravikumar, et al., 2010). According to Nebula et al., (2013), *R.apiculata* contains triterpenes, diterpenoids, lupeol, taraverol, steroids, aliphatic alcohol, phenolic compounds and others chemical components. The ethnomedicinal value of this plant has been numerous since a long time ago. Previous studies reported that *Rhizophora apiculata* was used traditionally to treat angina, boils and fungal infections. Beside that, it was also used as an antiseptic and to treat diarrhea, dysentery, fever, malaria and leprosy (Bandaranayake, 2002). A polysaccharide extracted from the leaf of *Rhizophora apiculata* inhibited HIV (Premanathan et al., 1999). *R.mucronata* was used traditionally to treat hepatitis, ulcers, elephantiasis, haematoma and febrifuge (Bandaranayake, 2002). This species obtain alkaloid, tannin, saponin, phenolic, flavonoid, terponoid and steroids (Nurdiani et al., 2012).

The trees have specific characteristics such as tough root systems, special bark and leaf structures, and other unique adaptations that enable them to survive in their habitat's harsh conditions. Mangrove forests play a vital role in coastal ecosystem while the trees have been utilized for many functions including wood production, pole, firewood and charcoal. In order to fulfil wood production in the industries mentioned previously, whole wood products are mainly produced and this involves a clear cutting practice. *Rhizophora* species are common mangrove species that produces a lot of high log value in the wood industry such as pole and charcoal. Different mangrove species have different wood and bark properties, making some more suitable than others for specific uses (FAO, 2013). For example, genera such as *Rhizophora*, *Bruguiera* and *Ceriops* are widely valued for construction, fuel wood and tannin extraction which are their heavy hardwood and tannin-rich bark (Ewel et al., 1998). There is limited research reported on phytochemical properties in terms of other values of *Rhizophora* species.

1.1 Justification

Mangrove plants are used economically as pole piling, lumber, firewood and charcoal. The manufacture of charcoal from *Rhizophora* wood remains the most important forest industry (MTC, 2009; Amir, 2005; Azahar and Nik Mohd Shah, 2003). Annually, Selangor produces 2750,000 pieces of poles with an average 500 ha (Zakariah, 2005). *R. apiculata* and *R. mucronata* are the two species used for commercial charcoal production and pole products as well as the most productive species in terms of timber production. Thus, these species were chosen in this study due to the high quality and dominant population.

With clear felling practice in timber production, mangrove plants provide a vast amount of mass material such as twig, fruit, leaf and root. Various parts of this plant have been used commonly in folk medicine. However, many previous studies reported about bark phytochemical, and limited information to others part. Thus, investigations of chemical constituents for various parts are important in order to provide more information about the specific use of each part. These mass materials might exhibit pharmaceutical value and potential in drug development. Natural sources of plants provided 25% of the drugs for Western medicine (Zhou and Wu, 2006).

Mangrove plants are used traditionally for medicinal purpose as well and its full range of medicinal capabilities has yet to be explored. Ethnomedicinal records regarding the medical use of mangrove plants are very limited (Govindasamy and Kannan, 2012). Therefore, the investigation of mangrove species about its bioactive compounds and their potential against humans, animals and plant pathogens are very narrow (Roome et al., 2008). Previous studies reported that antioxidant activities are shown in *R.apiculata* and *R. mucronata* (Gao and Xiao, 2012; Rahim et al., 2008; Yin, 2008). However, the compounds responsible for the antioxidant ability in crude plants have not been previously investigated. Thus, this study provides more information about the bioactive compounds that contributes to the antioxidant and antimicrobial properties of these species.

Toxicity is a problem to the body which is the cause of many diseases such as cancers, coronary heart disease and stroke. Therefore, phenolic compounds such as phenols, flavanoid, caffeic acid, frolic acid and others play a role as an antioxidant and an antimicrobial against said diseases. Bunyapraphatsara et al. (2007), reported that the plants in the mangrove forest showed potential as a source of antioxidant and cancer chemoprevention agents and further studies may contribute to drug development. From their study, eighteen plants in the mangrove forest showed antioxidant activity with ED50 (effective dose) less than 10 µ/mL such as *Avicennia alba*, *Bruguiera parviflora*, *Rhizophora mucronata, Sonneratia caseolaris*, etc.

Phenolic compounds are most popular antioxidant property nowadays, which exists in all plants. Compared with synthetic phenolics such as BHT (butylated hydroxytoluene), BHA (butylated hydroxyanisole), and propyl gallate, natural

phenolic antioxidant from plants were able to obtain strong activity and low toxicity trait which is more desirable today (Cailet et al., 2006). Rohman and Riyanto (2010) also mentioned that antioxidants from plants are much better than synthetic due to their natural origin. Previous studies also revealed that natural antioxidants were used commonly in food due to their anticarcinogenic activity and potential to prevent heart disease (Siddhuraju and Becker, 2003); thus were strongly recommended (Lindsay and Astley, 2002).

Recently, phenolics have been considered powerful antioxidants in vitro which are classified as simple phenols or polyphenols based on the number of phenol units in the molecule (Robbins and Rebecca, 2003) with hydroxyl group (—OH) bonded directly to an aromatic hydrocarbon group. These compounds are capable of inhibiting free radicals and hence can retard the aging process (Vioux, 2006). According to Navarro (2006), these compound retard lipid auto oxidation by acting as radical scavengers and, consequently, its essential anti oxidation that protects against the propagation of the oxidative chain.

In addition, phenolic constituent has potential as an antimicrobial which are related with antioxidant activities. Due to the pathogen resistance, many researchers have tried to discover a new source, especially a natural resource. Due to the side effects and resistance of synthetic sources, it has become necessary to discover natural sources such as plants, fungi or other microorganism (Nurdiani et al., 2012).

1.2 Objective of study

1.2.1 General Objective

To investigate the antioxidant and antimicrobial activity of *Rhizophora apiculata* and *Rhizophora mucronata.*

1.2.2 Specific Objective

1.2.2.1 To determine the total phenolic and flavonoid contents of two *Rhizophora* species.

1.2.2.2 To identify the flavonoid and phenolic compounds from different parts (fruit, twig, leaf and root) of two *Rhizophora* species using high performance liquid chromatography (HPLC) technique.

1.2.2.3 To evaluate antioxidant properties of different parts of two *Rhizophora* species using DPPH, ABTS radical scavenging method and β-carotene bleaching assays.

1.2.2.4 To investigated antimicrobial activity of different parts of two *Rhizophora* species on several microbial strains using disc diffusions method.

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