ANTIOXIDANT AND ANTIMICROBIAL PROPERTIES OF CHEMICAL EXTRACTIVES FROM *Nauclea subdita*

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2013

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THESIS SUBMITTED IN FULFILLMENT FOR THE DEGREE OF MASTER OF SCIENCE

SCHOOL OF INTERNATIONAL TROPICAL FORESTRY
UNIVERSITI MALAYSIA SABAH
2013

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1 February 2013

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CERTIFICATION

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DEGREE	:	**MASTER OF SCIENCE (WOOD FIBER TECHNOLOGY AND INDUSTRY)**
VIVA DATE	:	**1 FEBRUARY 2013**

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ACKNOWLEDGEMENT

Alhamdulillah, my humble thanks to almighty Allah, without His benevolence, spirit and courage, His gave me a mental and physical strength for me to complete this project.

First of all, I am grateful and indebted to my research supervisor Professor Dr. Razak Wahab, Faculty of Earth Sciences, Universiti Malaysia Kelantan, who have constantly given me guidance and support, both mental and physical strength in completing this thesis.

Thanks are also due to all lecturers, academic and non-academic staffs, Mr. Azli and Mr. Rizan, from School of International Tropical Forestry (SITF), Universiti Malaysia Sabah (UMS).

A great thanks is also due to Dean Kuliyyah of Science, academic and non-academic staffs, Mr. Muzammil from International Islamic University of Malaysia (IIUM) and Dr. Ani Sulaiman from Forest Research Institute Malaysia (FRIM) for their cooperation.

I also dedicate my acknowledgement to my parents, Aishah Hashim and Jamaluddin Mohd Daud, who gave me strength and support mentally and physically, and have prayed for me to achieve success in this project and also support my financial which the most important to done well my project. Finally, great thanks to all my siblings and friends; Mr. Jerry J. Kangon, Norizyan Izzati and Maslini Japar Ali for their encouragement.

ABSTRACT

ANTIOXIDANT AND ANTIMICROBIAL PROPERTIES OF CHEMICAL EXTRACTIVES FROM *Nauclea subdita*

Solvent extraction of young and matured woods and barks of *Nauclea subdita* in polar solvent (methanol) and non-polar solvent (hexane) have been carried out in this study. Phytochemical screening tests of the crude extracts of *Nauclea subdita* in methanol indicated that flavanoids, terpenoids, alkaloids, phytosterols, saponins and phenolic compounds were present in the sapwood, heartwood and bark, whereas only phytosterols were present in hexane extracts. Therefore, determination of antioxidant and antimicrobial activities were carried out only for the methanolic crude extracts of wood and bark. The methanolic crude extracts were tested for primary and secondary antioxidant properties by measuring the total phenolic contents (TPC) and the 2, 2, diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity. The extracts demonstrated higher TPCs and free radical scavenging activities against DPPH, especially for the bark and most parts of young wood as compared to matured wood of *N. subdita*. Study was also carried out to test antimicrobial activities of the methanolic crude extracts against four bacteria, i.e. *Bacillus subtilis, Vibrio parahaemoliticus, Vibrio alginolyticus, Bukholderia cepacia,* and two fungi, i.e. *Candida albican* and *Aspergillus niger*. The results indicated that all the samples have moderate to good zones of inhibition on the bacteria growth when compared to the positive control. However, there was no zone of inhibition against the two fungi (*A. niger* and *C. albican*) used. Gas chromatography-mass spectrometry (GC-MS) technique was also employed for the analysis of semi-volatile compounds in six selected crude extracts from young and matured woods of *N. subdita*. The groups of compound were identified are from hydrocarbon, alcohol, ester, phenol, ether, ketone, organofluorine, organoclorine and organic acid groups.

ABSTRAK

SIFAT-SIFAT ANTIOKSIDAN DAN ANTIMIKROBIAL DALAM KIMIA EKSTRAKTIF DARIPADA Nauclea subdita

Ekstraksi pelarut bagi kayu serta kulit kayu Nauclea subdita muda dan matang dalam pelarut berkutub (metanol) dan pelarut tak berkutub (heksana) telah dilakukan dalam kajian ini. Ujian pengenalan fitokimia bagi ekstrak mentah N. subdita dalam methanol menunjukkan terdapat flavanoid, terpenoid, alkaloid, fitosterol, saponin dan sebatian finolik dalam bahagian kayu teras dan kayu gubal (sapwood and heartwood)*, manakala hanya fitosterol terdapat dalam ekstrak heksana. Oleh itu, penentuan aktiviti-aktiviti antioksidaan dan antimikrob hanya dilakukan bagi ekstrak mentah bagi kayu dalam methanol. Ekstrak mentah metanol di periksa aktiviti antioksidaan primer dan sekunder melalui penentuan jumlah kandungan fenolik* (TPC) *dan pengukuran aktiviti pemerangkap radikal bebas 2, 2, difenil-1-pikrillhidrazil* (DPPH)*. Ekstrak tersebut menunjukkan nilai yang tinggi bagi TPC dan aktiviti pemunggut radikal bebas terhadap DPPH, terutama bagi kulit kayu dan kebanyakan bahagian kayu N. subdita yang muda berbanding dengan kayu yang matang. Ekstrak N. subdita telah juga di uji sifat antimikrob dengan menggunakan empat bacteria iaitu Bacillus subtilis, Vibrio parahaemoliticus, Vibrio alginolyticus, Bulkholderia cepacia, dan dua fungus iaitu Candida albican dan Aspergillus niger. Semua sampel menunjukkan zon perencatan bagi pertumbuhan bakteria yang memuaskan ke baik apabila dibandingkan dengan rujukan positif. Walau bagaimanapun, tiada zon perencatan dicatat dalam ujian menggunakan dua fungus (A. niger dan C. albican) tersebut. Analisis kromatografi gas-spektrometer jisim* (GC-MS) *untuk pengenalan sebatian separa-meruap dalam enam sampel pilihan bagi ekstrak mentah N. subdita muda dan matang. Kumpulan sebatian yang di analisis adalah daripada kumpulan hidrokarbon, alkohol, ister, fenol, iter, keton, organoflorin, organoklorin dan asid organik.*

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LIST OF ABBREVIATIONS

NAUCLEA SUBDITA	Bangkal Kuning
ANOVA	Analysis of Variance
TPC	Total Phenolic Content
DPPH	2, 2-dipenyl-1-picryhdrazyl
ROS	Reactive Oxygen Superoxide
FRIM	Forest Research Institute Malaysia
ITIS	Integrated Taxonomy Information System
FC	Folin-Ciocalteu
MeOH	Methanol
GC-MS	Gas Chromatography-Mass Spectrometer
HPLC	High Pressure Liquid Chromatography
TLC	Thin Layer Chromatography
LC-MS	Liquid Chromatography
MBC	Minimal Bactericidal Concentration
MFC	Minimal Fungicidal Concentration
MIC	Minimal Inhibitory Concentration
BHA	Butylated hydroxyanisole
Trolox	6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid
BHT	Butylated hydroxytoluene
GAE	Gallic Acid Equievalent
SITF	School of International Tropical Forestry
IIUM	International Islamic University of Malaysia
UMS	Universiti Malaysia Sabah
IC_{50}	half maximal inhibitory concentration
WHO	World Health Organization
NIST	National Instiute of Standard and Technology
IUPAC	International Union of Pure and Applied Chemistry
NB	Nutrient Broth
DMSO	Dimethyl sulfoxide

LIST OF SYMBOLS

mM	Milimolar
mm	Millimeter
mL	Mililiter
L	Liter
mg	Miligram
g	Gram
m	Meter
cm	Centimeter
Kg m^{-3}	Kilogram meter cubic
µL	Microliter
nm	Nanometer
˚C	Degree Celsius
psi	Per Square Inch
min	Minute
CH_3OH	Methanol
$CH_3 (CH_2)_4CH_3$	n-Hexane
C_6H_5OH	Phenol
H_2SO_4	Sulfuric Acid
FeCl3	Ferric chloride
NaOH	Sodium hydroxide
HCI	Hydrochloric Acid
%	Percent
sec	Second
o	Degree
eV	Electron Volt
µm	Micrometer
ha	Hectare
pK_a	- $\log_{10} K_a$

CHAPTER 1

INTRODUCTION

1.1 Background Research

Plant materials have been used for centuries to improve health by many societies around the world. They use plant materials for their health care as well as trying to find how to apply the products from plants since long time ago (Houghton and Raman, 1998). Some plants were ignored as being useless, and now modern medical science is finding that these compounds from the plants really do have effects on human body.

Antioxidants are vital substances which possess the ability to depend the body from damage caused by free radical induced oxidative stress (ROS). A variety of free radical scavenging antioxidants exist within our body which many of them are derived from dietary sources like stem, barks, leaves, twigs, roots, fruits, and vegetables. There are increasing practical use of medicinal plant preparations possessing antioxidant properties for the regulation of free radical processes in the human organism. Many of the biologically active substances found in plants, including phenolic compounds (flavonoids, phenolic acids), sugars, vitamins, saponins, ethereal oils, polyunsaturated fatty acids, phospholipids, enzymes, amino acids and other compounds, are known to possess antioxidant properties (Brown and Rice-Evans, 1998). The antioxidative and antimicrobial properties in some plant species which contain polyphenols are currently being investigated for therapeutic use topically and orally.

This study focuses on the antioxidant and antimicrobial activities of methanolic extracts of *N. subdita* plant materials which are traditionally used to treat stomach ache by local in Borneo (Beaman and Anderson, 2004). Normally, biological tissues are not similar when considering their structure, texture, sensitivities and lipid contents. The ideal solvent for extraction would completely extract all the components from a sample, while leaving all the other components

behind. The efficiency of solvent extraction depends on the polarity of the lipids present compared to that of the solvent (Christie, 1993). The antioxidants obtained from plants are of greater benefits in comparison to synthetic one (Kappus *et al.,* 1993; Stankovic', 2011). Methanol is the best solvent for extraction of phenolic compounds from *N. subdita* extracts. Methanolic extracts of wood and bark parts of *N. subdita* as the subject of this study, the presence of antioxidant and antimicrobial properties are done by screening process and special tests.

Numerous studies on certain plants have indicated their antioxidant and antimicrobial properties which can protect the human body against both cellular oxidation reactions and pathogens. They also had known to produce certain bioactive molecules which react with other organisms in the environment, inhibiting bacterial or fungal growth (Bruneton *et al.*, 1995). The substances that can inhibit pathogens and have little toxicity to host cells are considered candidates for developing new antimicrobial drugs. Thus, it is important to characterize different types of medicinal plants for their antioxidant and antimicrobial potential (Wojdylo *et al.,* 2007).

An antimicrobial is a substance that kills or inhibits the growth of microorganisms such as bacteria, fungi or protozoan. Antimicrobial drugs are substances that either kill microbes (microbicidal) or prevent the growth of microbes (microbistatic). Generally, prolong use of antimicrobial may cause negative effect on human health because it kill gut flora, thus formidable threat posed by microbial species appear to develop drug resistance (Lamikanra, 1981; Cimanga *et al.,* 1991). Therefore, it becomes an urgent need to develop new types of highly effective and non-toxic antimicrobial from natural sources. Scientific studies have proved that, most of the plant possessed medicinal properties for their biological activities ranging from antimicrobial to antitumor. Many plants have the capacity to produce a large number of organic phytochemicals with complex structural diversity that is known as secondary metabolites. According to Evan *et al.* (1986), some of these secondary metabolites are produced for the plant's self-defense.