

UNIVERSITI PUTRA MALAYSIA

COMPOSITION AND BIOLOGICAL ACTIVITIES OF SEAWEED IN COASTAL WATERS OF BINTULU, SARAWAK, MALAYSIA

MOHD HAFIZBILLAH BIN ZAWAWI

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By

MOHD HAFIZBILLAH BIN ZAWAWI

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

July 2014

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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

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July 2014

Chair: Mohd Hanafi bin Idris, PhD

Faculty: Agriculture and Food Sciences (BINTULU)

Seaweed communities are considered as important component in the coastal ecosystems and able to produce a great variety of secondary metabolites for biological activities. The coastline of Bintulu geographically surrounded by rocky shore areas and some reef areas provide favorable natural habitat for seaweed communities. Rapid development and anthropogenic disturbance near the shorelines may give negative effect to the organism in the coastal ecology and some seaweed species might have great potential for biological activities. However, no clear information was reported on seaweed distribution and status of seaweed species from the Bintulu coastal waters and none of the study reported on potential of seaweed species for biological activities. Therefore a study was conducted to determine the species composition of seaweed communities from Bintulu coastal waters and to determine the antioxidant and antimicrobial activities of dominant seaweed species. A total of 54 species of seaweeds were identified from six different study areas of Bintulu coast by using NaGISA Protocol; in which 23 species were from Rhodophyta (11 families), 15 species were from Phaeophyta (2 families) and 16 species were from Chlorophyta (10 families). The abundance of seaweeds was high in the rocky substrate, while Rhodophyta (11 families and 23 species) was the common and highest group of seaweeds found in this coastal area. This study recorded 12 species of seaweeds which can be commonly found in Bintulu coastal waters (Chaetomorpha sp., Ulva intestinalis, Padina minor, Padina australis, Gracilaria salicornia, Hydropuntia edulis, Hypnea cervicornis, Hypnea spicifera, Acanthophora spicifera, Laurencia sp., Chondrophycus papillosus and Laurencia similis). Interestingly, the present study recorded high numbers of seaweed species when compared to the previous studies conducted at Bintulu, Sarawak and most of the new species recorded from fringing reef area. The extracts obtained from 20 species of seaweeds were evaluated for their antioxidant and antimicrobial activities. Antioxidant properties from methanolic extracts were evaluated according to the total phenolic contents by using Folin-Ciocalteau method and free radical scavenging activity determined by standard 2, 2-Diphenyl-1-picrylhydrazyl (DPPH). High level of antioxidant properties was recorded from Lobophora variegata, Padina boryana and Acanthophora spicifera. Both Lobophora variegata and Padina boryana showed greater antioxidant properties than the control. There was high correlation between concentration of phenolic contents and antioxidant properties by the DPPH scavenging activity. Antimicrobial activity was examined using disc diffusion method. The samples were subjected to solvent extraction using chloroform, acetone, methanol and ethanol. Maximum inhibition activities were shown from crude extract of Chaetomorpha sp. and Chaetomorpha antennina. Both chloroform crude extract of Chaetomorpha antennina and acetone crude extract of Chaetomorpha sp. showed greater antibacterial activities than the control against Streptococcus gallolyticus. Present study recorded no correlation between antimicrobial and antioxidant activities. Bintulu coastal waters are abounding in seaweed species which provided suitable environment for the marine ecology and present study recorded some seaweed species contain potential active compounds for antimicrobial activities and antioxidant properties. Therefore, study recommended for further monitoring on the seaweed diversity and isolates the active compounds from the high potential seaweed species.

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

KOMPOSISI DAN AKTIVITI BIOLOGI RUMPAI LAUT DI PERAIRAN PANTAI BINTULU, SARAWAK, MALAYSIA

Oleh

MOHD HAFIZBILLAH BIN ZAWAWI

Julai 2014

Pengerusi: Mohd Hanafi bin Idris, PhD

Fakulti: Sains Pertanian dan Makanan (BINTULU)

Komuniti rumpai laut adalah dianggap sebagai komponen yang penting untuk ekosistem persisir laut dan mampu untuk menghasilkan pelbagai metabolit sekunder yang penting untuk aktiviti-aktiviti biologi. Kawasan persisir pantai Bintulu mempunyai geografi yang dikelilingi oleh kawasan pantai berbatu dan beberapa kawasan terumbu karang memberikan habitat semulajadi yang baik untuk komuniti rumpai laut. Pembangunan yang pesat dan gangguan antropogenik berhampiran kawasan pesisir pantai boleh memberikan kesan negatif kepada organisma dalam ekologi pantai dan beberapa spesies rumpai laut mungkin mempunyai potensi yang besar untuk aktiviti biologi. Bagaimanapun, tiada maklumat yang jelas dilaporkan terhadap taburan rumpai laut dan status spesies rumpai laut daripada perairan pantai Bintulu dan tiada kajian melaporkan spesies rumpai laut yang berpotensi dalam aktiviti biologi. Oleh itu, kajian telah dijalankan untuk menentukan komposisi spesies kumpulan rumpai laut dari perairan pantai Bintulu dan untuk menentukan aktiviti antioksidan dan antimikrob daripada spesies rumpai laut dominan. Sejumlah 54 spesies rumpai laut telah dikenalpasti dari enam kawasan kajian yang berbeza di sekitar persisir laut Bintulu dengan menggunakan Protokol NaGISA; di mana 23 spesies daripada Rhodophyta (11 famili), 15 spesies daripada Phaeophyta (2 famili), 16 spesies daripada Chlorophyta (10 famili). Taburan rumpai laut adalah tinggi di substrat berbatu, manakala Rhodophyta (11 famili dan 23 spesies) adalah yang biasa dan kumpulan rumpai laut yang tertinggi dapat dijumpai di kawasan persisir pantai ini. Kajian ini telah merekodkan 12 spesies rumpai laut yang biasanya boleh dijumpai di perairan pantai Bintulu (Chaetomorpha sp., Ulva intestinalis, Padina minor, Padina australis, Gracilaria salicornia, Hydropuntia edulis, Hypnea Acanthophora spicifera, cervicornis, Hypnea spicifera, Laurencia sp., Chondrophycus papillosus dan Laurencia similis). Menariknya, kajian ini mencatatkan bilangan spesies rumpai laut yang tinggi apabila dibandingkan dengan



kajian sebelum ini yang telah dijalankan di Bintulu, Sarawak dan sebahagian besar spesies baru direkodkan di kawasan terumbu karang. Ekstrak yang diperolehi daripada 20 spesies rumpai laut telah dinilai untuk aktiviti antioksidan dan antimikrob mereka. Sifat-sifat antioksidan daripada ekstrak metanol telah dinilai mengikut jumlah kandungan sebatian fenol dengan menggunakan kaedah Folin-Ciocalteau dan aktiviti perencatan radikal bebas ditentukan daripada piawai 2, 2-Diphenyl-1-picrylhydrazyl (DPPH). Tahap antioksidan yang tinggi telah dicatatkan daripada Lobophora variegata, Padina boryana dan Acanthophora spicifera. Keduadua Lobophora variegata dan Padina boryana menunjukkan sifat antioksidan yang lebih baik daripada kawalan. Kajian mencatatkan korelasi yang tinggi di antara kepekatan sebatian fenol dan sifat antioksidan daripada aktiviti perencatan radikal DPPH. Aktiviti antimikrob telah dinilai dengan menggunakan kaedah penyebaran cakera. Sampel telah melalui proses pengekstrakan pelarut dengan menggunakan klorofom, aseton, methanol dan etanol. Aktiviti perencatan yang maksimum telah ditunjukkan daripada ekstrak mentah Chaetomorpha sp. dan Chaetomorpha antennina. Kedua-dua ekstrak mentah Chaetomorpha antennina daripada kloroform dan ekstrak mentah Chaetomorpha sp. daripada aseton menunjukkan aktiviti antibakteria yang baik terhadap Streptococcus gallolyticus berbanding kawalan. Kajian ini mencatatkan tiada korelasi di antara aktiviti antimikrob dan aktiviti antioksidan. Perairan Bintulu mempunyai banyak spesies rumpai laut di mana menyediakan persekitaran yang sesuai untuk ekologi marin dan kajian ini mencatatkan beberapa spesies rumpai laut mengandungi sebatian aktif yang berpotensi terhadap aktiviti antimikrob dan sifat antioksidan. Oleh itu, kajian mencadangkan untuk pemantauan lanjut terhadap kepelbagaian rumpai laut dan membuat pengasingan sebatian aktif daripada spesies rumpai laut yang berpotensi.

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I certify that a Thesis Examination Committee has met on July 23, 2014 to conduct the final examination of Mohd Hafizbillah bin Zawawi on his thesis entitled "Composition and Biological Activities of Seaweed in Coastal Waters of Bintulu, Sarawak, Malaysia" 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:

Osumanu Haruna Ahmed, PhD

Associate Professor Faculty of Agriculture and Food Science (Bintulu Campus) Universiti Putra Malaysia (Chairman)

Joseph Bong Choon Fah, PhD

Associate Professor Faculty of Agriculture and Food Science (Bintulu Campus) Universiti Putra Malaysia (Internal Examiner)

Hishamuddin Omar, PhD

Senior Lecturer Faculty of Science Universiti Putra Malaysia (Internal Examiner)

Siti Aishah Abdullah @Christine A. Orosco, PhD

Associate Professor University Malaysia Terengganu Malaysia (External Examiner)

NORITAH OMAR, PhD

Associate Professor and Deputy Dean School of Graduate Studies Universiti Putra Malaysia

Date: 19 September 2014

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Mohd Hanafi bin Idris, PhD

Senior Lecturer Faculty of Agriculture and Food Science Universiti Putra Malaysia (Chairman)

Wong Sing King, PhD

Senior Lecturer Faculty of Agriculture and Food Science Universiti Putra Malaysia (Member)

Abu Hena Mustafa Kamal, PhD

Senior Lecturer Faculty of Agriculture and Food Science Universiti Putra Malaysia (Member)

BUJANG BIN KIM HUAT, PhD

Professor and Dean School of Graduate Studies Universiti Putra Malaysia

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Signature: Name of Chairman of Supervisory Committee:	Mohd Hanafi bin Idris	_	
Signature: Name of Member of Supervisory Committee:	Wong Sing King	Signature: Name of Member of Supervisory Committee:	Abu Hena Mustafa Kamal

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- 4.32 A: Fresh specimen of *Bostrychia tenella*, B: Closed up view 92 branches of *Bostrychia tenella*, C: Fresh specimen of *Chondrophycus cartilaginous*, D: Close up view branches of *Chondrophycus cartilaginous*, Scale bar specimen equal to 1 cm
- 4.33 A: Fresh specimen of *C. papillosus*, B: Closed up view cross section 94 of branches *C. papillosus*, C: Fresh specimen of *Laurencia similis*, D: Closed up view cross section of *Laurencia similis*, mc: medulla cell, Scale bar specimen equal to 1 cm
- 4.34 A: Fresh specimen of *Laurencia* sp., B: Closed up view cross 96 section of *Laurencia* sp., C: Fresh specimen of *T. glomerulata*, D: Closed up observes branches of *T. glomerulata*, cc: cortical cell, mc: medulla cell, Scale bar specimen equal to 1 cm
- 4.35 A: Fresh specimen of *Scinaia furcellata*, B: Closed up view cross 98 section of *Scinaia furcellata*, C: Fresh specimen of *Solieria* sp., D: Closed up view cross section of *Solieria* sp., cc: cortical cell, mc: medulla cell, Scale bar specimen equal to 1 cm
- 5.1 Negative correlation of total phenolic contents and DPPH radical 107 scavenging from selected methanolic seaweed extracts where r = -0.602 significantly at p<0.005

- 6.1 Antimicrobial activity of Chlorophyta crude extracts on selected pathogenic microbes, Bs: *Bacillus subtilis*, Bc: *Bacillus cereus*, Lm: *Listeria monocytogene*, Sg: *Streptococcus gallolyticus*, MRSA: *Staphylococcus aureus*, VRE: *Enterococcus* sp., Pv: *Proteus vulgaris*, Ec: *Escherichia coli*, Chl: Chloroform, Ace: Acetone, Met: Methanol, Eth: Ethanol, (No activities recorded from Micrococcus luteus, Pseudomonas aeruginosa, Klebsiella pneumonia, Klebsiella oxytoca, Salmonella enteritidis and Candida albicans)
- 6.2 Antimicrobial activity of Phaeophyta crude extracts on selected pathogenic bacteria, Bs: *Bacillus subtilis*, Bc: *Bacillus cereus*, Lm: *Listeria monocytogene*, Sg: *Streptococcus gallolyticus*, MRSA: *Staphylococcus aureus*, VRE: *Enterococcus sp.*, Ko: *Klebsiella oxytoca*, Pv: *Proteus vulgaris*, Ec: *Escherichia coli*, Chl: Chloroform, Ace: Acetone, Met: Methanol, Eth: Ethanol, (No activities recorded from Micrococcus luteus, Pseudomonas aeruginosa, Klebsiella pneumonia, Salmonella enteritidis and Candida albicans)
- 6.3 Antimicrobial activity of Rhodophyta crude extracts on selected 12 pathogenic bacteria. Bs: Bacillus subtilis, Bc: Bacillus cereus, Lm: Listeria monocytogene, Sg: Streptococcus gallolyticus, MRSA: Staphylococcus aureus, VRE: Enterococcus sp., Ko: Klebsiella oxytoca, Pv: Proteus vulgaris, Ec: Escherichia coli, Chl: Chloroform, Ace: Acetone, Met: Methanol, Eth: Ethanol (No activities recorded from Micrococcus luteus, Pseudomonas aeruginosa, Klebsiella pneumonia, Salmonella enteritidis and Candida albicans)

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LIST OF ABBREVIATIONS

BHA	butylated hydroxyaniselo
BHT	butylated hydroxytoluene
PG	propyl gallate
TBHQ	tertiary butylhydroquinone
ТВ	Tanjung Batu
PT	Pantai Telekom
KS	Kuala Similajau
GB	Golden Beach
KN	Kuala Nyalau
BM	Batu Mandi
С	Coverage
Μ	Percentage of mid point
rpm	Revolutions per minute
DMSO	dimethylsulfoxide
ATCC	American Type Culture Collection
MTCC	Microbial Type Culture Collection
ROS	reactive oxygen species
mg GAE/g	milligram gallic acid equivalent per gram
DPPH	2, 2-Diphenyl-1-picrylhydrazyl
EC_{50}	extract required to reduce the DPPH radical at 50%
TPC	Total Phenolic Contents

CHAPTER 1

INTRODUCTION

Seaweeds are macroscopic plants which are divided into three divisions namely Chlorophyta, Phaeophyta and Rhodophyta. Generally, they grow and attach to the solid substrata along the tidal zone worldwide and uptake nutrients from the environment. Seaweeds respond to light which make them vary in color; red, green and brown algae because of different pigments for photosynthesis process (Chapman, 1979). Seaweeds have a multicellular plant-like body structure known as thallus which typically consists of a root-like holdfast for attachment at the substrates and stem-like stipe which support the leaf-like blades (Campbell and Reece, 2005). While seagrasses are considered as vascular plant which posses roots to uptake nutrients from the sediment, produce flowers, seeds and fruits, seaweeds are unique taxonomically group as protists grow attached to the hard substrates and their life forms are categorize into rhizophytic, lithophytic, epiphytic, epizoic and loose-lying or floating near the shorelines (Muta Harah *et al.*, 2006).

Sarawak has a land area which represents 38% of Malaysia's land mass with a coastline length of approximately 1000 km. Tidal range along the Sarawak coast is generally large, up to 6 meters. The coastal waters are enriched with nutrients leached from the land and all these support a highly diverse flora and fauna including the seaweed communities. The coastline of Sarawak is surrounded by rocky shores, sandy areas, coral reefs, mudflats and mangroves with high humidity. Due to this favorable natural habitat and rich of organic matter, seaweeds tend to grow and inhabits on the various substratums along this coastline. Most of the seaweeds tend to grow well at the protected area and some are limited distribute depending on the adaptation ability in different environmental condition like temperature, rainfall, tidal range and wave-exposed (Fisheries Research Centre, 2000).

In Sarawak, marine protected areas covering 206 344 ha and seaweed communities are considered as important component in the coastal ecosystems providing nursery ground to the other marine organisms especially marine fishes, marine molluscs, sea turtles and other marine mammals (Ministry of Natural Resources and Environment, 2009). Seaweeds are important as primary producer and as the base of the food chain in the marine ecosystems, provides protection and shelter to other marine creatures, prevent erosion in the coastal areas and protect marine environment from pollution (Julie, 2013). Seaweed communities have important relationship among species composition, biomass, anthropogenic pressures and physical or biological environmental factors (Juanes *et al.*, 2008). It also can be a good ecological indicator in the coastal environment. Seaweeds diversity in the coastal area provides important information for the coastal management in which different adaptation among seaweed species in the habitat may refer to the different ability to growth against the environmental pressure (Prathep *et al.*, 2011).

Besides ecological importance, biologically seaweeds contain high fiber, minerals, protein, lipids, vitamins and other valuable natural components for human consumption. They are considered as a source of bioactive compounds and able to produce a great variety of secondary metabolites for biological activities. Seaweeds have been screened extensively to isolate life saving drugs or biologically active substances worldwide including pharmacological components (Vallinayagam *et al.*, 2009). These compounds have been purified as antioxidant, antiviral, antifungal and antimicrobial components where in small concentrations they can cause a physiological effect for inhibiting or stimulating specific function against some diseases (Cox *et al.*, 2010; Chew *et al.*, 2008).

Numerous of novel compounds have been isolated from seaweeds and most of these active compounds demonstrated interesting biological activities especially antioxidant and antimicrobial activities. Generally, active compounds which responsible for antioxidant properties are considered as major component in the therapeutic treatments against various diseases and these active substances especially phenol compounds and polysaccharides inhibit activity of many bacterial species (Raja et al., 2013; Chojnacka et al., 2012). Seaweeds are valuable sources produce diverse natural products were broadly effective and safe with less adverse effect for human consumption and most of the synthetic chemicals are toxic to human (Chojnacka et al., 2012). There were highly used of commercialized synthetic components in the market such as butylated hydroxyaniselo (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG) and tertiary butylhydroquinone (TBHQ) where were reported to be toxic for human and suspected to be carcinogenic (Tariq et al., 2011). Some pathogenic microorganisms genetically have an ability to become more resistance to therapeutic agents, therefore isolation of new drugs from natural resources in contrast to using the synthetics components are highly recommended.

Rapid development and anthropogenic disturbance near the Bintulu shorelines may give negative effect to the organism in the coastal ecology. Coastal development and environmental degradation are directly affect on the seaweed communities in terms of species composition, habitat and distribution because of sediment deposition decrease light penetration, disturbance for physiological processes and habitat destruction. However, after a few years the baseline information on seaweed species from Bintulu coastal waters which is remains abundance with some defense mechanisms in any particular situation and tolerant to the environmental condition need to determine, therefore the comparative between previous studies on seaweeds diversity need to be evaluating for better knowledge in the seaweeds biology and ecology. Hence, due to the abounding seaweed communities recorded previously from Bintulu coastal waters and some seaweed species have develop a variety of natural defense mechanism against environmental pressure to remains abundant, is there any species could probably have potential in antioxidant and antimicrobial activities?



1.1 Objectives of the Study

The objectives of the study are:

- 1. To determine the species composition of the seaweed communities in the Bintulu coastal waters.
- 2. To determine the antioxidant properties of the dominant seaweed species from Bintulu coastal waters.
- 3. To determine the antimicrobial activities of the dominant seaweed species from the Bintulu coastal waters.



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BIODATA OF STUDENT

The student, Mohd Hafizbillah Zawawi was born on 19th April 1987, raised in Kemaman, Terengganu. He had his primary education at Sekolah Kebangsaan Kampung Air, Kemaman from 1995 to 1999. His secondary education was at Sekolah Menengah Agama Al-Falah, Kemaman from 2000 until 2003, after PMR he attended Kolej Sains Pendidikan Islam Negeri Terengganu, Kuala Terengganu from 2003 to 2004. After completing SPM, he continues the education at Kedah Matriculation College for a year, his university education at Universiti Putra Malaysia from 2006 to 2010 and graduated with a Bachelor's Degree in Science Bioindustry. He has work experiences in pasture management, fruit crops research and post harvest technology, research and development of hybrid rice varieties, demonstrator and research assistant. In 2010 he continues his Master studies at Department of Animal Science and Fishery, Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia.

LIST OF PUBLICATIONS

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