UNIVERSITI PUTRA MALAYSIA

PHYSICO-CHEMICAL PROPERTIES OF WATER, PROXIMATE COMPOSITION, MINERALS AND ANTIOXIDANT OF SIX SPECIES OF SEAGRASSES AT MERAMBONG SHOAL, JOHORE, MALAYSIA

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By

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Chairman : Muta Harah binti Zakaria @ Ya, PhD
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Seagrass has not yet been claimed as a dietary stuff and source of medicine for human consumption. Since several seagrass species are edible, investigation of nutrient, proximate composition and antioxidant properties in marine macrophytes are very important. Thus, the objectives of this study were to assess the physico-chemical and nutrient status of seawater in the seagrass habitat, determine the proximate composition, mineral contents and screening the antioxidant properties of six seagrass species; *Enhalus acoroides*, *Thalassia hemprichii*, *Cymodocea serrulata*, *Halodule uninervis*, *Halophila ovalis* (small and big-leaved) and *Halophila spinulosa* at Merambong shoal, Johore during the low tide, from March to August 2013. Nutritive proximate compositions were estimated following analysis methods described by the Association of Official Analytical Chemists. Results showed that seagrasses were high in carbohydrate (48.99-83.03%), fiber (10.02-27.48%) and ash (11.46-41.69%), and low in lipid content (0.02-1.22%). The protein content showed variation with species ranged 5.50% to 16.04%. The moisture content range from 6.68-21.49%. Macro-(Nitrogen-N, Phosphorus-P, Potassium-K, Calcium-Ca, Magnesium-Mg) and micro-(Zinc-Zn, Iron-Fe, Manganese-Mn, Copper-Cu) minerals measured using Atomic Absorption Spectrophotometer (AAS) and their concentrations varied with species and time. Minerals content; N, P, K, Ca, Mg, Zn, Fe, Mn, and Cu ranged 3308.00-36826.67 mg kg^{-1}, 429.33-3400.00 mg kg^{-1}, 2025.33-29520.00 mg kg^{-1}, 4680.00-25280.00 mg kg^{-1}, 3244.00-13040.00 mg kg^{-1}, 85.33-180.00 mg kg^{-1}, 922.67-5657.33 mg kg^{-1}, 13.33-129.33 mg kg^{-1} and 3.20-29.20 mg kg^{-1} respectively. Trend in micro-nutrient contents categorically followed a simple pattern, Fe>Zn>Mn>Cu. Methanol extracts of seagrasses were used to examine antioxidant activity by DPPH radical scavenging activity, total phenolic content (TPC) content and total flavonoid content (TFC). DPPH scavenging activity, TPC and TFC significantly different ($p<0.05$) between seagrass species with month. Rhizomes of *E. acoroides* and *H. uninervis* had relatively higher antioxidant activity (0.34 mg ml^{-1} in June and 0.26 mg ml^{-1} in July and August of EC_{50} value respectively). Total phenolic content of seagrasses at Merambong shoal ranged 1.02 to 91.77 mg GAE kg^{-1} with lower content in March and higher in August. The values of TFC were also varied among species and months (ranged from 43.25 to

361.46 mg QE kg^{-1}). Based on biplots generated by Principle component analysis (PCA), there were clear separation of proximate composition and mineral contents of seagrass species with locations from Malaysia and other regions. In conclusion, seagrasses possessed essential nutrient content and good antioxidant properties that could be utilized for pharmaceutical and nutraceutical uses.

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

SIFAT FIZIKO-KIMIA AIR, KOMPOSISI PROKSIMAT, MINERAL DAN ANTIOKSIDA PADA ENAM SPESIES RUMPUT LAUT DI BETING MERAMBONG, JOHOR, MALAYSIA

Oleh

WAN HAZMA BINTI WAN NAWI

Mac 2017

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Rumput laut belum diiktiraf sebagai barangan makanan dan sumber perubatan untuk kegunaan manusia. Memandangkan beberapa spesies rumput laut boleh dimakan, kajian nutrien, komposisi proksimat dan antioksida dalam makrofit marin adalah sangat penting. Oleh itu, objektif kajian ini adalah untuk menilai fiziko-kimia dan status nutrien air laut dalam habitat rumput laut, mengkaji komposisi proksimat, kandungan mineral dan pemeriksaan sifat antioksidan enam spesies rumput laut; *Enhalus acoroides*, *Thalassia hemprichii*, *Cymodocea serrulata*, *Halodule uninervis*, *Halophila ovalis* (small and big-leaved) and *Halophila spinulosa* di Beting Merambong, Johor semasa air surut, dari bulan Mac hingga Ogos 2013. Nutrisi komposisi proksimat dianggarkan mengikut kaedah analisis diterangkan oleh Persatuan Official Analytical Chemists. Keputusan menunjukkan karbohidrat dalam rumput laut adalah tinggi (48.99% hingga 83,03%), serat (10.02 % hingga 27.48%) dan abu (11.46% hingga 41.69%), dan rendah kandungan lipid (0.02% hingga 1.22%). Kandungan protein berbeza mengikut spesies dari 5.50% hingga 16.04%. Kandungan kelembapan adalah di antara 6.68% hingga 21.49%. Makro-(Nitrogen-N, Fosforus-P, Kalium-K, Kalsium-Ca, Magnesium-Mg) dan mikro-(Zink-Zn, Ferum-Fe, Mangan-Mn, Tembaga-Cu) mineral diukur menggunakan Spektrofotometer Penyerapan Atom (AAS) kepekatannya adalah berbeza mengikut spesies dan masa. Kandungan mineral; N, P, K, Ca, Mg, Zn, Fe, Mn, dan Cu adalah di antara 3308.00-36826.67 mg kg^{-1}, 429.33-3400.00 mg kg^{-1}, 2025.33-29520.00 mg kg^{-1}, 4680.00-25280.00 mg kg^{-1}, 3244.00-13040.00 mg kg^{-1}, 85.33-180.00 mg kg^{-1}, 922.67-5657.33 mg kg^{-1}, 13.33-129.33 mg kg^{-1} dan 3.20-29.20 mg kg^{-1} masing-masing. Kecenderungan dalam kandungan mikro-nutrien dikategorikan mengikut corak yang mudah, Fe>Zn>Mn>Cu. Ekstrak metanol rumput laut telah digunakan untuk menentukan paras antioksidan dengan aktiviti memerangkap radikal DPPH, jumlah kandungan fenolik (TPC) dan jumlah kandungan flavonoid (TFC). Aktiviti menguraikan DPPH, TPC dan TFC berbeza dengan ketara ($p<0.05$) antara spesies rumput laut dengan bulan. Rizom *E. acoroides* dan *H. uninervis* mempunyai aktiviti antioksidan yang lebih tinggi (nilai EC_{50} masing-masing 0.34 mg ml^{-1} dalam bulan Jun dan 0.26 mg ml^{-1} dalam bulan Julai dan Ogos). Jumlah kandungan fenolik rumput laut di Beting Merambong adalah 1.02-91.77 mg GAE kg^{-1}

dengan kandungan rendah dalam bulan Mac dan tinggi dalam bulan Ogos. Nilai TFC berubah antara spesies dan bulan (adalah di antara 43.25 hingga 361.46 mg QE kg^{-1}). Berdasarkan biplots dihasilkan oleh analisis komponen Prinsip (PCA), terdapat pemisahan yang jelas komposisi proksimat dan kandungan mineral spesies rumput laut dengan lokasi dari Malaysia dan negara lain. Kesimpulannya, rumput laut memiliki kandungan nutrien penting dan ciri antioksida baik yang boleh digunakan untuk industri farmaseutikal dan nutraseutikal.

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For any errors or inadequacies that may remain in this work, of course, the responsibility is entirely my own.

I certify that a Thesis Examination Committee has met on 31 March 2017 to conduct the final examination of Wan Hazma binti Wan Nawi on her thesis entitled "Physico-Chemical Properties of Water, Proximate Composition, Minerals and Antioxidant of Six Species of Seagrasses at Merambong Shoal, Johore, 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.

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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

AAS	Absorption atomic spectrometer
ABTS	2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)
ACA	Aldehyde/carboxylic acid
AI	Adequate intake
$AlCl_3$	Aluminium chloride
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
B	Boron
Ca	Calcium
Ca^{2+}	Calcium ion
Cl^-	Chloride ion
CO_2	Carbon dioxide
Cu	Copper
DNA	Deoxyribonucleic acid
DNMRT	Duncan's New Multiple Range Test
DO	Dissolved oxygen
DPPH	2,2-diphenyl-1-picrylhydrazyl
DW	Dry weight
EC_{50}	Effective concentration at 50%
FAO	Food and Agriculture Organization
Fe	Iron
FNB	Food and Nutrition Board
FOX	Ferrous oxidation-xylenol orange
FRAP	Ferric reducing/antioxidant power
FTC	Ferric thiocyanate
GAE	Gallic acid equivalents
GC	Gas chromatography
GE	Gross energy
H^+	Hydrogen ion
H_2O	Water
HCl	Hydrochloric acid
HCO_3^-	Bicarbonate ion
HPLC	High-performance liquid chromatography
H_2O	Water
H_2O_2	Hydrogen peroxide
H_2SO_4	Sulphuric acid
IOM	Institute of Medicine
K	Potassium
MA	Malonaldehyde
Mg	Magnesium
Mn	Manganese
Mo	Molybdenum
MSL	Mean sea level
N	Nitrogen
Na^+	Sodium ion
Na_2CO_3	Sodium carbonate
$NaNO_2$	Sodium nitrite

NaOH	Sodium hydroxide
NAP	National Academies Press
NCCFN	National Coordinating Committee on Food and Nutrition
NFE	Nitrogen-free extractives
NO^3-N	Nitrate-nitrogen
NO_2^--N	Nitrite-nitrogen
O_2	Oxygen
ORAC	Oxygen radical absorption capacity
P	Phosphorus
PCA	Principle component analysis
PO_4^{3-}	Phosphate ion
QE	Quercetin equivalents
RDA	Recommended Dietary Allowance
SCF	Scientific Committee on Food
TAA	Total antioxidant activity
TBA	Thiobarbituric acid assay
TDS	Total dissolved solids
TFC	Total flavonoid content
TPC	Total phenolic content
Trolox	(S)-(-)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid
UNEP	United Nations Environment Programme
USEPA	United States Environmental Protection Agency
UV-VIS	Ultraviolet–visible
WHO	World Health Organization
Zn	Zinc

CHAPTER 1

INTRODUCTION

Seagrasses are the only flowering plants that have adapted themselves to living in marine and estuarine habitats, and are fully submerged most of the time (Japar Sidik *et al*., 2006a). Malaysian seagrass can be found patchy in shallow intertidal and subtidal habitat, mangrove area, semi-enclosed lagoons, coral reef, rocky shores and shoals in subtidal zones (Japar Sidik and Muta Harah, 2003; Annaletchumy *et al*., 2005; Japar Sidik *et al*., 2006a; Japar Sidik *et al*., 2012). There are 16 seagrass species recorded in Malaysia along its 4800 km coastal area (Japar Sidik and Muta Harah, 2011; Nguyen *et al*., 2014; Japar Sidik *et al*., 2016). Seagrasses are keystone species in many estuaries and shallow lagoons as excellent indicator providing unique ecosystems that act as primary production for economically and ecologically important higher consumers (UNEP, 2004; Japar Sidik *et al*., 2006a; Short *et al*., 2011).

Inhabit the area within the intertidal zone as Merambong shoal whose located near the estuary of Pulai river influenced by tidal much affect the growth of seagrasses. A good environment and suitable condition of water qualities are very important for optimum growth and survival of seagrasses. Therefore, it is significant to investigate the relationship between physico-chemical parameters of the environment with biochemical processes in seagrasses. Seagrass meadows are the most productive plant communities that providing important grazing habitat and food for a variety of marine organisms (Short and Wyllie-Echeverria, 1996). In seagrasses, metals sequestered may be passed through trophic links to higher level consumers such as fish (dengkis, *Siganus* sp.; sea urchin, *Echinoidea* spp.) including dugongs (*Dugong dugon*) and green turtles (*Chelonia mydas*) in tropical ecosystems (Brand-Gardner *et al*., 1999; Japar Sidik *et al*., 2006a).

Besides consumed by animals, there are reports by Green and Short (2003) and Kannan *et al*. (2011) where seagrasses are used as food sources for human in Southeast Asia regions such as Philippines, Indonesia, Thailand and Malaysia especially by the coastal populations. Compared to seaweed, the chemical composition of seagrass has been poorly investigated and most of the available information only deal with the seed of *Enhalus acoroides* (Montano *et al*., 1999). The nutrients in these seagrasses have potential as new material sources for aquatic animal feed development.

There are abundance information regarding variation between the population of a given seagrass species for the chemical components of temperate, subtropical or tropical seagrasses. However, information on the nutritional value of seagrasses, especially in seagrasses of Malaysia are still lacking. Each plant species has its own nutrient composition that is important to both animals and human needs. Since a few of seagrasses was edible, investigation nutrient, mineral composition and antioxidant properties in marine aquatic macrophytes are very important. Although there are a few studies have been conducted around the world the nutritional properties of seagrass are not completely known.

According to the previous study by Kannan *et al*. (2013b) seagrasses such as *Halodule pinifolia* and *Cymodocea rotundata* contain high level of antioxidant acting as major signaling pathways of cells and protector from oxidative damage. Seagrasses also have a good reputation in the medical field especially as remedy. Torre-Castroa and Ronnback (2004) reported that fishermen and seaweed farmers in East Coast of Zanzibar are using the roots of *E. acoroides* as a remedy against stings of different kinds of rays, scorpion fish (Scorpaenidae) as well as for diverse rabbit fish (Siganidae). It is also used for easing wounds, muscle painsand stomach problems. Another seagrass, *Cymodocea* spp. is good remedy against cough, malaria and used as a tranquillizer for babies. *Thalassia hemprichii* is being use as a treatment for malaria and relieving fever. and *Thalassodendron ciliatum* is used to relieve smallpox disease. A mixture of *Cymodocea* and *Thalassia* is good to treat skin diseases and fever. Inspite of the above information very little are known on the usage and utilization of seagrasses.

Seagrasses are threatened by many factors especially by human activities. Rapid development is one of the predominant contributed of their disappearance around the world. This study focuses on a seagrass bed of Merambong shoal, Johor which is undergoing rapid development called Forest City. Thus, the objectives of this study were to:

1. assess the physico-chemical and nutrient status of seawater in the seagrass habitat at Merambong shoal.
2. determine the proximate composition and mineral contents of seagrasses in different sampling month inhabiting the Merambong shoal.
3. screening the antioxidant properties of different seagrasses in different sampling month harvested at Merambong shoal.

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