INVESTIGATION ON PHYSICOCHEMICAL AND NUTRITIONAL PROPERTIES OF *Caulerpa racemosa, Gracilaria manilaensis* and *Padina tetrastromatica*

AROYEHUN ABDULQUDUS BOLA

UNIVERSITI SAINS MALAYSIA

2020

INVESTIGATION ON PHYSICOCHEMICAL AND NUTRITIONAL PROPERTIES OF *Caulerpa racemosa*, *Gracilaria manilaensis* and *Padina tetrastromatica*

by

AROYEHUN ABDULQUDUS BOLA

Thesis submitted in fulfilment of the requirements

for the degree of

Doctor of Philosophy

APRIL 2020

ACKNOWLEDGEMENTS

I would like to express my gratitude to Allah SWT for the mercies and blessings bestowed on me throughout the period of my candidature. Allah alone has been my comforter and source of strength.

Firstly, I would like to deeply express my sincere appreciation and gratitude to my main supervisor Associate Professor Dr. Shariza Abdul Razak. You have provided tremendous mentorship, innovative research direction, encouragement, enthusiastic supervision and the opportunity to conduct postgraduate research study in the School of Health Sciences USM. Secondly, I would like to thank my co-supervisors, Associate Professor Dr. Farid Che Ghazali from Biomedicine Programme, and Dr Kishneth Palaniveloo from Institute of Ocean and Earth Sciences, University of Malaya for their valuable advice, knowledge of marine species and providing the seaweed samples required throughout my course of study. I wish to express my profound appreciation to all students, laboratory and management staff of the School of Health Sciences, and particularly those of the Nutrition Programme for awarding me a research grant for this study.

My appreciation also goes to the management and staff of Standards Organisation of Nigeria (S.O.N) for providing the needed support towards my academic pursuit in this prestigious university. Special thanks to Toyosi Aroyehun and Dr Dauda Gonni for their endless support.

My sincere gratitude also goes to my family for their encouragement and prayers for me. Many thanks to all my friends for brightening up my PhD life! Without you, my PhD life would not have been complete.

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

LIST OF SYMBOLS

mm	Millimetre
µm	Micrometre
nm	Nanometre
>	Greater than
<	Lower than
≥	Greater than or equal to
≤	Lower than or equal to
mL	Millilitre
µL	Microlitre
g	Gram
mg	Milligram
kg	Kilogram
%	Percentage
°C	Degree Celsius
g	Gravity force
Cl	Confidence intervals
P	p-value
Δ	Precision
χ^2	Chi-square

LIST OF ABBREVIATIONS

ABZ	Albendazole
AECUSM	Animal Ethics Committee Universiti Sains Malaysia
AKSB	Air Kelantan Sdn. Bhd.
CVI	Content validity index
CVR	Content validity ratio
ELISA	Enzyme-Linked Immunosorbent Assay
et al.	And others
FBZ	Fenbendazole
IgG	Immunoglobulin G
Inc.	Incorporation
ITS	Internal Transcribed Spacer
JEPEM	Human Ethics Committee
NaCl	Sodium Chloride
OD	Optical density
PCR	Polymerase Chain Reaction
rDNA	Recombinant deoxyribonucleic acid
RFLP	Restriction Fragment Length Polymorphism
spp.	Plural form species
SPSS	Statistical Package for Social Sciences
TCBZ	Triclabendazole
TMB	Tetramethylbenzidine
USA	United States of America
USD	US Dollar
WHO	World Health Organization

KAJIAN TERHADAP GIRI FIZIKOKIMIA DAN PEMAKANAN *CAULERPA RACEMOSA, GRACILARIA MANILAENSIS* DAN *PADINA TETRASTROMATICA*

ABSTRAK

Rumpai laut dikenali sebagai makanan berfungsi dan disyorkan untuk diambil setiap hari kerana komposisinya yang unik dan nilai nutrisinya. Kajian ini bertujuan untuk mengkaji variasi musim (Monsun Timur Laut (NEM)-monsun Barat Daya (SWM) dan geografi (kolam dan kawasan terbiar) terhadap ciri-ciri fizikokimia dan bahan-bahan nutrisi dalam tiga rumpai laut yang boleh dimakan (*Caulerpa racemosa, Glacilaria manilaensis, Padina tetrastromatica*) yang dikumpulkan dari bahagian barat Semenanjung Malaysia. Perubahan geografi luar biasa telah direkodkan dalam komposisi proksimat *C.racemosa* yang dituai dari kolam dan kawasan terbiar. Hasil kajian mendapati rumpai kolam *C. racemosa* mengandungi lebih banyak lipid (4.20 ± 0.32 % berat kering, DW), protein (20.27 ± 0.14 % DW), serabut diet (27.57 ± 0.14 % DW) dan kandungan tenaga (25.45 ± 0.07 kcal kg^{-1}) berbanding *C. racemosa* yang tumbuh liar dengan nilai bacaan abu yang tinggi (44.98 ± 1.60 % DW). *G. manilaensis* yang dituai pada bulan November (NEM) menunjukkan kandungannya yang tinggi dengan protein (19.39 ± 0.12 % DW), karbohidrat (39.58 ± 0.20 % DW), serabut diet (31.07 ± 1.08 % DW) dan nilai tenaga (27.22 ± 0.11 kcal kg^{-1}) jika dibandingkan dengan SWM-*G. manilaensis* yang dituai dalam bulan Ogos di mana hanya kandungan tinggi abu yang ketara (38.48 ± 0.23 % DW). Antara semuanya, rumpai laut *P. tetrastmatica* menunjukkan tahap abu lebih tinggi (64.819 + 0.26 % DW) berbanding *C. rasemosa* dan *G. manilaensis*. Kajian asid lemak menunjukkkan asid palmitik (C16:0) yang paling banyak

diperolehi antara (83.87 - 89.29) dalam *G. manilaensis*, diikuti oleh (51.70 - 63.27%) *C. racemosa* dan (0.83 %) untuk *P.tetrastmotica*. Tambahan pula, asid oleik telah membentuk lemak yang tidak tepu asid lemak tak tepu mono (MUFA) yang dominan iaitu di antara (5.80 - 6.33%) dalam *C. racemosa*, diikuti oleh (1.47- 6.33) in *G manilaensis* dan (0.40 %) pada *P.tetrastromatica*. Jumlah asid amino yang terkandung adalah di antara (64.63 - 159.69 mg/g) dalam *C.racemosa*, (122.43-163.58 mg/g) *G.manilaensis* dan (61.02 mg/g) dalam *P.testromatica*. Berkenaan dengan sifat fizikal, kapasiti pegangan air (WHC) dan kapasiti pegangan minyak (OHC) menunjukkan perbezaan yang ketara mengikut habitat dan turun naik musim. Jumlah kandungan fenol masing-masing ialah (96.91 – 178.78 ± 5.93 mg GAE/g), (74.05 ± 220.36 mg GAE/g) dan (61.20 mg GAE/g) untuk *C. racemosa*, *G. manilaensis* dan *P. tetrastromatica.* Analisis GC-MS mendedahkan kehadiran sebatian fitokimia termasuk eter, aldehid, ester, asid lemak, terpen, sterol, dan asid organik yang bertanggungjawab terhadap aktiviti antioksida. Secara keseluruhan, keputusan yang diperolehi menunjukkan bahawa ciri fizikokimia dan nilai nutrisi rumpai laut dipengaruhi oleh variasi geografi dan musim. Penggunaan rumpai laut secara konsisten boleh memberi kebaikan kepada kesihatan manusi

INVESTIGATION ON PHYSICOCHEMICAL AND NUTRITIONAL PROPERTIES OF *CAULERPA RACEMOSA*, *GRACILARIA MANILAENSIS* AND *PADINA TETRASTROMATICA*

ABSTRACT

Seaweeds are recognised as an important ingredient of functional foods recommended for daily food due to their unique compositions and nutritional value. This study was the first time conducted whereby three tropical edible seaweeds (*Caulerpa* species, *Gracilaria* species, and *Padina* species) were selected from Western Peninsular Malaysia to investigate their proximate, physicochemical and nutritional properties according to change in season and geographical habitat. Remarkable geographical variability was recorded in the proximate composition of *C. racemosa* harvested from the pond and wild. Analysis revealed the farm-*C. racemosa* contain higher concentration of lipids (4.20 ± 0.32 % DW), protein (20.27 ± 0.14 % DW), dietary fiber (27.57 ± 0.14 % DW) and energy values (25.45 ± 0.07 kcal kg^{-1}) than the wild-*C. racemosa* with higher ash level (44.98 ± 1.60 % DW). *G. manilaensis* harvested in November during the Northeast monsoon (NEM) showed a relatively higher level of proteins (19.39 ± 0.12 % DW), carbohydrates (39.58 ± 0.20 % DW), dietary fibers (31.07 ± 1.08 % DW) and energy value (27.22 ± 0.11 kcal kg^{-1}) than the *G. manilaensis* collected in August during Southwest monsoon (SWM) with a significant higher ash content (38.48 ± 0.23 % DW). Amongst the seaweeds *P. tetrastromatica* revealed ash levels of (64.819 ± 0.26 % DW) comparatively higher than both *C. racemosa* and *G. manilaensis*. Investigation of the fatty acids (FAs) profiles showed palmitic acid (C16:0) was the most abundant ranging from (83.87 – 89.29 %) in *G. manilaensis*, followed by (51.70 – 63.27 %) *C.*

racemosa and (0.83 %) *P. tetrastromatica*. Furthermore, oleic acid (C18:1) constituted the dominant monounsaturated fatty acids (MUFA) occurring at (5.80 – 6.33 %) in *C. racemosa*, followed by (1.47- 6.33 %) *G. manilaensis* and (0.40 %) in *P. tetrastromatica*. Total amino acid content occurred between (64.63 – 159.69 mg/g) in *C. racemosa*, (122.43 – 163.58 mg/g) in *G. manilaensis* and (61.02 mg/g) in *P. tetrastromatica*. With respect to physical properties, water holding capacity (WHC) and oil-holding capacity (OHC) showed significant difference according to habitat and seasonal fluctuations. Total phenol content were (96.91 - 178.78 ± 5.93 mg GAE/g), (74.05 – 220. 36 mg GAE/g) and (61.20 mg GAE/g) for *C. racemosa*, *G. manilaensis* and *P. tetrastromatica* respectively. GC-MS analysis revealed the presence of phytochemical compounds including ether, aldehyde, ester, fatty acids, terpenes, sterols, and organic acids responsible for their antioxidant activities. Overall, this thesis revealed that physicochemical and nutritional value of seaweeds have geographical and seasonal variations and that its regular consumption may add benefits to human.

CHAPTER 1

INTRODUCTION

1.1 Background of the study

The role of diet in human health is increasingly gaining attention because nutrition plays a very fundamental role in body metabolism and physiology. Generally, we obtained our diets from different sources, which is from either animals or vegetables throughout history. However, the human race has consistently been confronted with challenges of food production on a large scale because of increasing population growth (Tilman and Clark, 2014). To meet their nutritional demands according to the Food and Agricultural Organisation (FAO, 2016), global food production would require an estimated increase of 70 % of current production (Alexandratos and Bruinsma, 2012 ; Searchinger *et al.*, 2014; Tilman *et al.*, 2011). This makes global food insecurity a growing concern considering lack of variety in agriculture.

In fact, studies show that an estimated 1 billion people are currently suffering from malnutrition and food-related diseases due to insufficient dietary energy accessibility and micronutrient undernourishment (FAO, 2016; Godfray *et al.*, 2010). While nearly two billion people worldwide suffer from specific micronutrient deficiencies, including iron, iodine, vitamin A, and zinc. The most vulnerable groups being pregnant women, lactating women, and young children within low-income countries (WFP, 2012). Consequently, global burden of malnutrition and food insecurity makes it the world's number one health risk as compared to other public health diseases including acquired immunodeficiency syndrome (AIDS), malaria, and tuberculosis combined (Tacon and Metian, 2013; Vermeulen *et al.*, 2012).

According to the World Health Organisation, cereals and legumes represent the most important and largest food source. Moreover, corn, rice, and wheat constitute nearly 60 % of these plant-based calories intake in most diets (WHO, 2013). However, increased commercial cultivation of these cereals requires increased freshwater supply and larger arable land areas both scarcity factors in several countries (Mæhre *et al.,* 2014; Wu *et al.,* 2014). An additional disadvantage using cereals as the main food energy source is the lack of certain essential nutrients (Wijesekara *et al.,* 2011). Their protein content is generally deficient in several essential amino acids, lysine in particular as well as long-chain omega-3 fatty acids (Mæhre *et al.,* 2014). Therefore, enhancing crop production demanded renewed interest in alternatives food supplies such as the ocean because of its colossal chemical and biological diversity for unconventional global food supplies and nutritional value-added nutritional ingredients (Charette and Smith, 2010; Paiva *et al.*, 2014; Plaza *et al.*, 2008).

In the last decade, marine organisms including fishes, sponges, ascidians, bryozoans, corals, mollusks and algae have provided enormous resources for a new natural product (Mahadevan, 2015). Indeed, the increasing awareness of the relationship between diet and health from the consumption of marine bioproducts is increasingly gaining consideration. While marine food products form an essential component of the global food basket (Tacon and Metian, 2013). Seaweeds an essential component of marine resources is considered an excellent option as the plant-based food of the future.

Consequently, seaweed mainly as primary producers in the ocean are responsible for oxygen and some of the organic compounds used as food for other living beings, central to food chains of all marine ecosystems (Israel *et al.,* 2010;

Pereira, 2016). Seaweeds contains a number of unique properties that make them an attractive alternative for food and bioactive compounds (Paiva *et al.*, 2018). Owing to their simple structure they do not compete with agricultural plants for the use of arable land and freshwater and energy resources (Kraan, 2013; Radulovich *et al.*, 2015). Therefore, considering seaweeds as a dietary source can be an important economic option of adequate nutrient supply for the well-being of peoples, be it food-insecure regions of the world or developed countries. Moreover, seaweeds have shown their potential to serve as food ingredients in functional and nutraceutical applications in Malaysia and India (Ahmad et al., 2016; Tanna *et al.*, 2018).

Despite the promising potentials the main difficulty in selecting a species for commercial exploitation process is that the chemical compounds from seaweed show variations in their yield and properties as the result of adaptive responses to the environmental conditions in which they live (Landa-Cansigno *et al.*, 2017; Wells *et al.*, 2017). Therefore, effort are made in research to describe the variation in the chemical constituents of some species of seaweed, particularly those of commercial interest, in order to optimize their use, promote new industrial applications and select the best harvesting period. To the best of our knowledge, there is no reports on seasonal and geographical variability in the physicochemical and nutritional composition of macroalgae species in Malaysia. In view of this, the present study was undertaken to examine the physicochemical properties, nutritional profile, and phytochemical properties of the common tropical macroalgae species in Malaysia representing the red (*Gracilaria manilaensis*), green (*Caulerpa racemosa*) and brown (*Padina tetrastromatica*) classes and based on nutritional requirements, assess their potential as alternatives to cereals in food and feed..

1.2 Problem statement

In the last decade, there was an increasing search for new health-promoting natural compounds of marine biodiversity in order meet dietary nutritional requirements with less strain to the environment. Food and Agriculture Organization (FAO) of the United Nations (UN) estimates that 1 billion people are currently suffering from malnutrition and food-related diseases due to insufficient dietary energy accessibility and micronutrient undernourishment. Seaweeds represent an important components of primary biomass production in coastal maritime ecosystems with a unique and complex physicochemical and nutritional properies that have gained special research attention in different fields of life sciences including agriculture, food, pharmaceuticals, nutraceuticals, and medicine. Despite their promising uses and potential applications, the main difficulty in selecting a species for industrial process is that the physicochemical, nutritional profile as well as extract from seaweeds show variations both within and between species as a result of adaptive responses to the environmental conditions in which they live (Holdt and Kraan, 2011; Kumar *et al.*, 2015; Praiboon *et al.*, 2018). Moreover seasonal and geographical changes in ecological conditions (i.e. water temperature, salinity, nutrient and light), species types, maturity, geography, location, and seasonality can influence the synthesis or inhibit the synthesis of nutrients in seaweeds (Gosch *et al.*, 2015; Paiva *et al.*, 2018). Aquaculture of seaweed by large scale cultivation requires understanding of either the singular or synergistic effect of these factors in order to achieve optimal utilization of seaweed biomass in large-scale commercialization due to interest in the stability of products or production of a standardized product with chemically defined composition. This information is important in order to develop a management plan for these species, including the cultivation practices that will assist in the efficient commercialization of these species as a nutritional and caloric footstock.

1.3 Hypothesis

a) The proximate and nutritional of seaweeds vary significantly over seasonal flunctuations and habiatat.

b) The physicochemical properties of seaweeds vary significantly over seasonal flunctuations and habiatat.

c) The phytochemicals and *invitro* antioxidant activities of seaweeds vary significantly over seasonal flunctuations and habiatat.

1.4 Research objectives

1.4.1 General objectives

The aim of the present study was to investigate the proximate constituents, physicochemical and nutritional properties of three edible seaweeds species that are of commercial interest (*Caulerpa racemosa*, *Gracilaria manilaensis*, and *Padina tetrastromatica*) inhabiting Western Peninsular Malaysia with respect to changes in geographical conditions (i.e. seasonality and habitat) as an approach to understanding the relationships between environmental factors and nutritional value.

1.4.2 Specific objectives

a) To determine the proximate composition (moisture, crude lipids, crude protein, ash, dietary fiber, carbohydrate and energy value) of these three species of seaweed.

b) To determine the nutritional profile (fatty acids, amino acids, vitamins and minerals element) of the three species of seaweeds.

c) To determine the phyicochemical properties (hydration properties, oil-holding capacity) of these three species of seaweeds.

d) To determine total phenolic and total flavonoid contents in the algal extracts of these three species of seaweeds

e) .To determine the phytochemicals and *in vitro* antioxidant activities of these three species of seaweeds using three bioassays techniques (ferric reducing antioxidant power (FRAP), total antioxidant activity (TAC) and Hydrogen peroxide (H_2O_2).

CHAPTER 2

LITERATURE REVIEW

2.1 Seaweeds utilization as food and food ingredients

For centuries, humans all over the world have utilized seaweeds. They provide a sustainable source of essential macronutrients and micronutrients in food. In Oriental communities, seaweed is often consumed mainly as food in the form of marine vegetables (Marinho-Soriano, 2017). Evidence from archaeological investigations in Chile that humans had used seaweeds as food and condiments for about 14,000 years (Dillehay *et al.*, 2008; Erlandson *et al.*, 2015). Currently, seaweeds are not listed in the food balance sheets compiled by the FAO for fish and fishery products owing to the absence of data. Nevertheless, in 2012, an estimated 9 million tonnes of cultivated seaweeds were planned for direct human consumption, mostly in East Asia, and in processes recognized by consumers (FAO 2014). Seaweeds are wholesome, nutritious, and delicious even though global production as food and feed to human and animals are on a modest scale. Their use as human food makes a significant contribution to regular meals where approximately one-fifth of meals contain seaweed (Lange *et al.*, 2015; MacArtain *et al.*, 2007).

Seaweeds are cooked and eaten as local cuisines in several Japanese diets as well as applications in the production of many food products, such as jam, tea, soup, cheese, wine, and noodles (Chapman *et al.*, 2015; Chopin, 2015). However, in western cultures inclusion in the diet is limited traditionally to the artisans and some coastal Atlantic communities such as Ireland, Brittany, Maine and Nova Scotia (Birch *et al.*, 2018; Chapman *et al.*, 2015; Fleurence, 2016). Although this scenario is now changing because seaweed can be further processed for their techno-functional polysaccharides i.e., carrageenans, agars, and alginates mainly as phycocolloids in the food and pharmaceuticals industry (Cao *et al.*, 2016; Song *et al.*, 2011; Venugopal, 2008). More importantly, seaweed consumption is gradually becoming common worldwide, including both whole seaweed and seaweed extracts.

According to human dietary consumption, the brown algae 66.5% is most often eaten in comparison, to the red 33% and the green algae 5% (Radulovich *et al.*, 2015). However, significant variability exists in the chemical composition and nutritional profile of seaweeds due mainly to several factors and influences such as species types, geographical origin or area of cultivation, environmental growth conditions, seasonal, and physiological variations, time of harvest, water temperature, and processing methods (Dawczynski *et al.*, 2007; de Gaillande *et al.*, 2017; Nomura *et al.*, 2013). Some seaweeds may contain natural toxins or environmental toxic compounds which, can be a limiting factor for their application in the food and medical industry (Mozzachiodi *et al.*, 2001). These toxic compounds in addition to other human health effects when consumed have been accountable for several episodic mortalities of marine animals, fishes, and beds that belong to the marine food chain (Van Dolah, 2000).

2.2 Algae

Algae are autotrophic organisms classified into microscopic single-cell 'phytoplankton' or macroscopic multi-cellular macroalgae that either can exist in groups to form colonies or sometimes forming simple tissues (Kim, 2012; Martínez–Hernández *et al.*, 2018). They are ubiquitous, found practically in every known habitat including in the sea, lakes, rivers, soil, walls, animals, and even plants. Microalgae are found growing either in benthic, littoral habitats and also as phytoplankton all through the ocean waters (Kim, 2012). Some examples of microalgae organisms include bluegreen algae (Cyanophyta), diatoms (Bacillariophyta) and dinoflagellates (Dinophyta) (El Gamal, 2010).

Macroalgae referred to as seaweed exist as multi-cellular organisms with a vast diversity occurring predominantly in the marine environment nonetheless also in freshwaters. They are fast-growing plant species that can grow to a considerable height capable of reaching 70m in length (Hillson, 1977) representing one of the most ecologically and economically important resources of the ocean. Additionally, more than 14,000 species of macroalgae have been described to date (Diaz-Pulido and McCook, 2008). They are botanically classified systematically in the order phylum, class, order, family, genus and species (Guiry and Guiry, 2015). They differ from other marine plants such as seagrasses and mangroves in that the macroalgae show strong morphological, taxonomical and phylogenetic differentiation (Baldauf, 2008; Charrier *et al.*, 2012). The taxonomic distribution of some edible macroalgae flora are summarised in (Table 2.1).

Table 2.1 Seaweed species exploited as food or in food products (modified from (MacArtain *et al.*, 2007).

Phylum	Scientific name	Common name	Product/Application
Phaeophyceae (Brown Seaweed)	*Laminaria japonica*	Kombu or Japanese kelp	Dried sea vegetable; pickled; Source of alginates
	Laminaria digitata	Kombu	Sea vegetable
	Laminaria saccharina	kombu	Sea vegetable
	Undaria pinnatifida	Wakame or Sea mustard	Dried seaweed; served in soups and salads; miso soup
	Ascophyllum nodosum	Arame	Source of alginate
	Eisenia bicyclis		Dried seaweed
	Himanthalia elongate	Sea sphagetti	Salad
	Cladosiphon okamuranus	Mozuku	Source of fucoidan
	Sargassum spp.	Gulfweed or Sea holly	Source of alginate
	Alaria esculenta	Winged kelp or Badderlocks	Eat fresh or cooked
	Ecklonia cava		Herbal remedy in form of a Seanol (polyphenolic) and Ventol (Phlorotannin
Chlorophyceae (Green Seaweed)	*Caulerpa lentillifera; Caulerpa racemose;*	Seagrapes or green caviar	Fresh salad
	Monostroma latissimum	Ao-nori or Green laver	Roasted seaweed
	Enteromorpha spp.	Bo-Ao-nori or Gut weed	salad
	Ulva prolifera; Ulva intestinalis;	Sea lettuce	Fresh salad
Rhodophycea (Red Seaweed)	*Chondrus crispus*	Irish moss or carrageenan moss	Salad; Sources of carrageenan
	Palmaria palmata	*Dulse or Dilisk*	Dried sea vegetable
	Porphyra umbilicalis Porphyra yezoensis Hizikia fusiforme	Nori or Purple larver	Nori sheets/Roasted nori; used as wrap for sushi
	Gracilaria verrucosa	Ogo, ogonori or sea moss	(Salad vegetable, Source of thickener agar)
	Gelidium		Salad or processed into Jelly; Sources of carrageenan
	Mastocarpus stellatus	Clúimhín Cait (false Irish moss)	Source of carrageenan

2.2.1 Classification of seaweeds

Accordingly, these divisions include Cyanobacteria, Cyanophyta, Chrysophyta, Phaeophyta, Charophyta, Chlorophyta, Euglenophyta, Pyrrophyta, Prochlorophyta, Cryptophyta, and Rhodophyta (Baweja *et al.*, 2016; Guiry and Guiry, 2015). However, the main classes include Phylum Charophyta with about 4422 species, Phylum Chlorophyta 6055 species, Phylum Phaeophyta 2041 species and Phylum Rhodophyta 7107, species respectively (Guiry *et al.*, 2014). Several key features are used in the classification of seaweeds, and they include the structure and life cycle (presence or absence of flagella), nature of chlorophyll and reserve polymer, biochemical composition, cell wall chemistry (Hurd *et al.*, 2014; Stengel *et al.*, 2011).

2.2.2 Brown seaweed

The brown seaweeds occur as large macroscopic multicellular taxa in distribution consisting of about 2000 species and 265 genera (Guiry and Guiry, 2015). The brown seaweed comprises of 13 orders according to the classification of (Bold and Wynne, 1985). However, only four orders, namely Laminariales, *Ectocarpales,* Fucales, and Dictyotales, were researched broadly for their phytochemicals. Common brown seaweeds that are cultivated commercially for human consumption are *Undaria pinnatifida* (wakame), and *Laminaria japonica* (kombu) and *Hizikia fusiformis* (hijiki), (Fitzgerald *et al.*, 2011). While the types of brown algae used for animal feed include the *Fucus vesiculosus* (bladderwrack), *Laminaria digitata* (oarweed), and *Ascophyllum nodosum* (rockweed) (Kadam *et al.*, 2013).

2.2.3 Red seaweed

The red seaweeds have a more diverse evolution in comparison to the green and the brown (Kılınç *et al.*, 2013). They come from a diverse photosynthetic eukaryotic ancestry that grows predominantly in marine water except for a few (Rindi *et al.*, 2012). They range from microscopic unicellular to large macroscopic multicellular taxa in distribution (Guiry and Guiry, 2014). The red algae contain a substantial amount of red phycobilin pigments responsible for their red color. Other accessory photosynthetic pigment presents in their plastids include chlorophyll-a, phycocyanin, and phycoerythrin (Kadam *et al.*, 2013). The red seaweeds are classified into four subphylum and eight classes. The two most important classes regarding hydrocolloid, production are the Bangiophyceae and the Florideophyceae, consisting of 161 and 6224 species, respectively (Gabrielson *et al.*, 1990; Guiry and Guiry, 2014). The red seaweed species exploited for their carrageenan productions include *Mastocarpus sp.*, *Eucheuma sp*. (Gigartinales), *Gracilaria sp.* (Gracilariales)., *Gelidium sp* and *Chondrus crispus* (Bocanegra *et al.*, 2009; Holdt and Kraan, 2011; Rhein-Knudsen *et al.*, 2017). Other species of red algae, including *Porphyra tenera* (nori) and *Palmaria palmata* (dulse), are among the highest consumed species of seaweed in Asia, as well as Western countries, because of their high protein content and their delicious flavor (Mouritsen *et al.*, 2013).

2.2.4 Green seaweed

The green seaweeds are found growing both on rocky and sandy beaches. Most of them can tolerate low salinity and will inhabit areas where rivers meet the ocean (Falkowski *et al.*, 2004). The green seaweeds are photosynthetic eukaryotes, carrying plastids containing chlorophyll a and b along with starch (Lewis and McCourt, 2004). The phylum comprises nine classes with the common ones including Ulvophyceae (1610 species), Chlorophyceae (3046 species), Chlorodendrophyceae (46 species), and Trebouxiophyceae (672 species) (Guiry and Guiry, 2014). The green algae are the largest species-rich of the algal groups, and they include more than 500 genera comprising an approximate 14,000 species (Guiry, 2012). They contain cell walls composed of fibrous matrix cellulose with specialized organelles such as chloroplasts containing chlorophylls a and b as well as other accessory pigments, including β-carotene and xanthophylls (Kadam *et al.*, 2013).

The most commonly consumed green seaweeds widely exploited are *Enteromorpha* spp., *Monostroma* spp., *Codium* spp., *Caulerpa* spp. (i.e., *C. lentillifera and C. racemosa*) and *Ulva* spp. (i.e. *U. lactuca*, *U. intestinalis*, *U. compressa*). In Asia countries, particularly Japan, dried fronds of edible *Enteromorpha* spp. and *Monostroma spp.*, are branded as ′aonori-green laver-ele ele-lulua-lumi boso′. The green seaweeds are eaten as edible raw, dried, or cooked by humans. They can also be used in the preparation of ′nori-jam′ soup (Bocanegra *et al.*, 2009; Novaczek, 2001).

2.2.5 Global production of edible seaweeds

The marine aquaculture is rich with the potentials of meeting the ever-increasing challenges of food demand. It represents one of the fastest food production sectors and constitutes almost 50 % of aquatic resources globally (FAO, 2016). The global utilization of seaweed comes from the farm and wild, with an annual seaweed harvest of 28.4 million tons in 2014 (FAO, 2014). However, less than 800,000 tonnes harvested is from the wild and an approximate 27.3 million tonnes (96%) from aquaculture. Through human endeavors in aquaculture, the seaweeds have been introduced to several non-native sites around the world (Inderjit, 2005; Schaffelke and Deane, 2005). Moreover, seaweed aquaculture is only from a small group of fewer than 221 species worldwide and only about ten genera are intensively cultivated, such as brown seaweed (i.e., *Laminaria/Saccharina japonica*, *Undaria pinnatifid and Sargassum fusiforme)* red seaweed (*Porphyra spp.*, *Eucheuma spp.*, *Kappaphycus alvarezii* and *Gracilaria spp.*) and green seaweed (*Enteromorpha clathrate*, *Monostroma nitidum*, and *Caulerpa spp*). These species account for about 83% of the world's seaweed production (Pereira, 2011; Pereira, 2016). Seaweeds are currently cultivated in about 35 countries spreading from the Northern and Southern Hemispheres and in waters ranging from cold, through temperate, to tropical (FAO, 2006). While global seaweed aquaculture is dominated principally by Asian countries with China (13.9 million tonnes), followed by Indonesia (11.3 million tonnes), Japan, the North, and South Korea, Malaysia and the Philippines (Admassu *et al.*, 2015; Cian *et al.*, 2015; Stévant *et al.*, 2017). The global status of seaweed production from aquaculture and wild stocks in the year 2014 is shown in (Figure 2.1). China tops the list, trailed by North Korea, South Korea, Japan, Philippines, Chile, Norway, Indonesia, USA, and India (White and Wilson, 2015). These countries also contribute approximately 95% of the world's commercial seaweed utilization (Fleurence, 2016; Zemke-White and Ohno, 1999). Figure 2.2 shows the major seaweeds producing countries and percentage distributions.

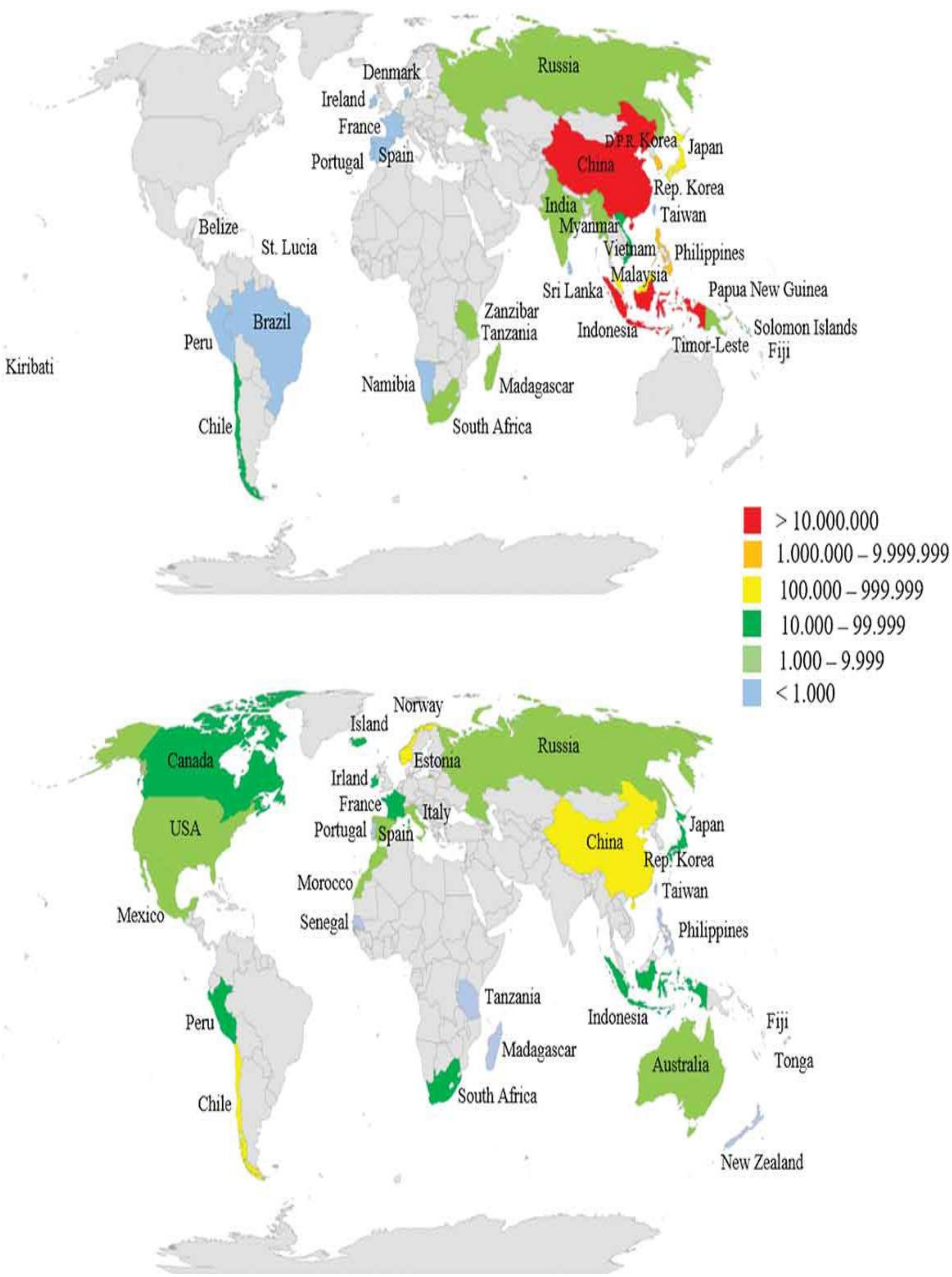

Figure 2.1 Global seaweed production (Source: FAO 2018)

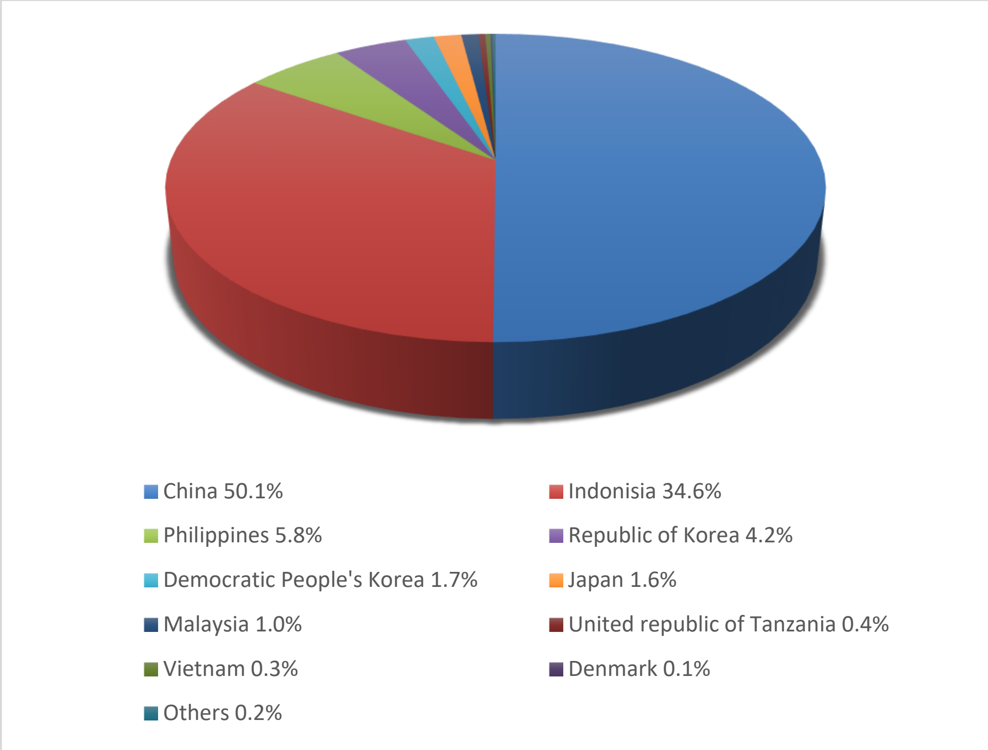

Figure 2.2 Seaweed producing countries (Source: FAO, 2017)

2.2.6 The seaweed biodiversity of the Malaysia geographical habitat

Malaysia is a tropical country located in Southeast Asia extending from 2° 30′ to 112° 50′N with an extensive coastline of approximately 4675 km, and 418 000 km^2 of the continental shelf (Phang *et al.*, 2019). Malaysia's land area is divided into two distinct parts namely the Peninsular Malaysia and East Malaysia separated by the South China Sea besides the numerous clusters of islands (Sabah, Sarawak) (Sidik *et al.*, 2012). The inland aquatic ecosystem of Peninsular Malaysia occupies 2068 km while the East Malaysia coastline inhabits 2,607 km (Phang *et al.*, 2019; Phang, 2006). They share primarily a similar landscape given that they both feature coastal plains rising to hills and mountains. They are characterized into two monsoon wind seasons. The Northeast Monsoon (peak period of rainfall) which originates in China and the north Pacific blowing between November and March, and the Southwest Monsoon (lesser rainfall) from the deserts of Australia between May and September (Phang *et al.*, 2019). Figure 2.3 shows the geographical map of Malaysia including both the coastline and mainland where most of the macroalgae are distributed in the coastline of Malaysia.

The Malaysian aquatic habitats occupy about 39000 km^2, which is more than 10 % of the total land area of 330,000 km^2. They include natural ecosystems such as lakes, rivers, reservoirs, and streams which comprises coral reefs, rocky shores and sandy bays that provide the perfect niches for a diverse seaweed flora (Phang *et al.*, 2006). However, the difference in latitude is responsible for the evolution of variation in genera, species, or strains amongst the algae and seaweed resources (Phang, 2010). Some common tropical taxa in Malaysia waters are green seaweeds (*Ulva* and *Caulerpa*), red seaweed (*Gracilaria*, *Eucheuma,* and *Laurencia*) and brown seaweed (*Sargassum*, *Padina*, *Tubinaria,* and *Dictyota*).

Seaweed mariculture first started in the Philippines, before spreading to Malaysia and then Indonesia, Thailand, Vietnam, Cambodia, and India. The eastern coast of Sabah particularly Kudat and Semporna provides a suitable agro-climatic environment ideal for seaweeds meant for commercial-scale such as *Eucheuma*, *Kappaphycus*, *Caulerpa*, and *Gracilaria* (Phang, 2006). The traditional coastal people have harvested and utilized seaweeds as the main source of livelihood (Hussin and Khoso, 2017). According to the Department of Fisheries, the aquaculture production prediction for the country as of 2017 is estimated to be 1.44 million tonnes, of which around 730000 tonnes was seaweed. In line with that projection, *Gracilaria* cultivation is expanding due to the growing consumer request for the agar. However, supplies are currently met through importation. The demand for carrageenan is also projected to grow because of phycocolloids applications in the food and pharmaceutical sectors.

Figure 2.3 Geographical Map of Malaysia (Source World atlas.com)

2.2.7 Seaweed processing and storage

The processing and storage of seaweeds used as food differ significantly from one nation to another depending on the raw materials available and the peculiarities of each country (Bocanegra *et al.*, 2009; Pak and Araya, 1996). The harvested seaweeds are washed with marine water to get rid of contaminants and parasites, cut and separated from thalli, and then rewashed under tap water. Afterward, subjected to drying to reduce the moisture content and increase shelf life either using a freeze dryer, sun-dried or using forced hot air. However, freeze-drying is most suitable to prevent cell membrane lysis and loss of water-soluble compounds (Boulom *et al.*, 2014; Chapman and Chapman, 1980). Seaweed prepared for human consumption is either cut into strips or powdered under suitable conditions display a nutritional value nearly as rich as the fresh product. The major seaweeds exploited as food products are summarised in (Table 2.8)

Table 2.2 Seaweeds exploit as food or in food products

Phylum	Scientific name	Common name	Product/Application
Phaeophyceae (Brown Seaweed)	*Laminaria japonica*	Kombu or Japanese kelp	Dried sea vegetable; pickled; Source of alginates
	Laminaria digitata	Kombu	Sea vegetable
	Laminaria saccharina	Kombu	Sea vegetable
	Undaria pinnatifida	Wakame or Sea mustard	Dried seaweed; served in soups and salads; miso soup
	Ascophyllum nodosum	Arame	Source of alginate
	Eisenia bicyclis		Dried seaweed
	Himanthalia elongate	Sea sphagetti	Salad
	Cladosiphon okamuranus	Mozuku	Source of fucoidan
	Sargassum spp.	Gulfweed or Sea holly	Source of alginate
	Alaria esculenta	Winged kelp or Badderlocks	Eat fresh or cooked
	Ecklonia cava		Herbal remedy in form of a Seanol (polyphenolic) and Ventol (Phlorotannin
Chlorophyceae (Green Seaweed)	*Caulerpa lentillifera; Caulerpa racemose;*	Seagrapes or green caviar	Fresh salad
	Monostroma latissimum	Ao-nori or Green laver	Roasted seaweed
	Enteromorpha spp.	Bo-Ao-nori or Gut weed	Salad
	Ulva prolifera; Ulva intestinalis;	Sea lettuce	Fresh salad
Rhodophycea (Red Seaweed)	*Chondrus crispus*	Irish moss or carrageenan moss	Salad; Sources of Carrageenan
	Palmaria palmata	*Dulse or Dilisk*	Dried sea vegetable
	Porphyra umbilicalis Porphyra yezoensis Hizikia fusiforme	Nori or Purple larver	Nori sheets/Roasted nori; used as wrap for sushi
	Gracilaria verrucosa	Ogo, ogonori or sea moss	(Salad vegetable, Source of thickener agar)
	Gelidium		Salad or processed into Jelly; Sources of carrageenan
	Mastocarpus stellatus	Clúimhín Cait (false Irish moss)	Source of carrageenan

2.2.8 Geographical variability in the nutritional composition of seaweeds

Like most terrestrial plants, seaweeds are a rich source of carbohydrates, proteins, lipid, ash and dietary fiber (Hamid *et al.*, 2015; Holdt and Kraan, 2011; Wells *et al.*, 2017). However, it has being established that the nutritional values can vary widely within diverse genera of seaweed, depending on the taxon, seasonality, and environmental conditions (Kumar *et al.*, 2015; Paiva *et al.*, 2018). These changes in ecological conditions can influence the synthesis or inhibition of nutrients in seaweeds (Dawczynski *et al.*, 2007; Kumar *et al.*, 2016; Manns *et al.*, 2017). Furthermore, at higher growth densities, competition with respect to irradiation and nutrition is known to influence the chemical composition of seaweeds (Duarte *et al.*, 2017; Kerrison *et al.*, 2015; Kim *et al.*, 2017). This may explain why no less than 145 species of macroalgae are used worldwide for food (Astorga-España *et al.*, 2016; Fleurence *et al.*, 2012). A review of the nutritional values of some edible seaweed species from different geagrahical location as it relates to temporal and spatial variation were undertaken and is summarized in (Table 2.3).

Table 2.3 Geographical variation in the nurtitional composition of seaweeds in % w/w dry weight (DW)

Collection site & Season	Specie	Moisture	Ash	Protein	Crude Lipid	Carbohydrate	Dietary Fiber	References
Brown seaweeds								
Northeastern Mediterranean coast of Turkey spring (April–May), summer (July), autumn (October) and winter (December).	*Padina pavonia*	77.77 ± 0.75	9.71 ± 0.90	1.97 ± 0.46	0.86 ± 0.19	-	-	(Polat and Ozogul, 2013)
	Padina pavonia	77.37 ± 0.26	11.81 ± 1.06	1.81 ± 0.08	0.65 ± 0.13	-	-	
	Padina pavonia	32.25 ± 1.05	18.19 ± 2.66	31.91 ± 4.59	-	14.74 ± 3.08	-	(Ozgun *et al.*, 2015)
	Stypopodium schimperi	82.47 ± 0.52	2.73 ± 0.14	2.68 ± 0.06	2.03 ± 0.21	-	-	
	Styopodium schimperi	78.35 ± 1.24	3.75 ± 0.42	2.37 ± 0.24	2.16 ± 0.52	-	-	
	Styopodium schimperi	30.13 ± 0.87	4.66 ± 1.72	65.19 ± 4.32	-	41.92 ± 2.32	-	
Azores, Portugal (Autumn, winter, and spring)	*Fucus spiralis*	-	22.43 ± 0.45	4.14 ± 0.10	11.54 ± 0.51	17.03 ± 0.59	52.27 ± 1.53	(Paiva *et al.*, 2018)
	Fucus spiralis	-	29.57 ± 0.55	6.85 ± 0.10	4.40 ± 0.10	12.77 ± 0.65	50.24 ± 1.40	
Aveirot, Portugal July, 2016	*Fucus vesiculosus*	-	25.5 ± 0.20	-	-	56.4 ± 0.40	45.0 ± 0.10	(Neto *et al.*, 2018)
Bahía Asunción, Mexico (March and April , 2009)	*Eisenia arborea*	10.56 ± 0.03	29.32 ± 0.01	5.54 ± 0.02	0.64 ± 0.02	47.59 ± 0.05	6.44 ± 0.05	(Hernández-Carmona *et al.*, 2009)
	Eisenia arborea	10.49 ± 0.02	26.15 ± 0.02	9.10 ± 0.08	0.63 ± 0.01	48.86 ± 0.01	4.77 ± 0.01	
Punta Arenas, Mexico (September & November 2014.	*Eisenia arborea*	9.21 ± 0.08	21.72 ± 0.12	12.06 ± 0.06	0.15 ± 0.03	61.43 ± 0.20	4.63 ± 0.11	(Landa-Cansigno *et al.*, 2017)
	Eisenia arborea	9.92 ± 0.05	34.05 ± 0.20	8.69 ± 0.07	0.25 ± 0.04	48.15 ± 0.31	8.85 ± 0.17	
Red seaweeds								
Wando, China	*Porphyra tenera*	3.66	8.78	32.16	1.96	53.44	-	(Hwang *et al.*, 2013)
Wando, china	*Porphyra haitanensis*	6.74	9.07	36.88	2.25	45.06	-	
Central West coast India (Vayangani, Malvan, Redi, Dona Paula and Cola)	*Pyropia acanthophora*	21.54 ± 1.10	6.30 ± 0.10	14.11 ± 1.12	1.70 ± 0.00	41.37 ± 0.35	60.90 ± 10.19	(Kavale *et al.*, 2017)

Geograhical variation in the nutritional composition of seaweed in % w/w dry weight (DW) continuation

Collection site & Season	**Specie**	**Moisture**	**Ash**	**Protein**	**Crude Lipid**	**Carbohydrate**	**Dietary Fiber**	**References**
Korean coast, South Korea (January to April	*Pyropia yezoensis*	-	6.08 ± 0.45	38.16 ± 0.81	2.69 ± 0.24	53.06 ± 1.08	-	(Jung *et al.*, 2016)
	Pyropia yezoensis	-	4.70 ± 0.12	37.59 ± 0.81	2.91 ± 0.15	54.74 ± 0.55	-	
	Pyropia yezoensis	-	3.78 ± 0.09	36.80 ± 0.74	3.05 ± 0.08	56.36 ± 0.74	-	
	Pyropia yezoensis	-	3.76 ± 0.07	36.15 ± 0.45	3.08 ± 0.05	57.93 ± 1.79	-	
Palk Bay, India (Latitude: 9° 44′ N and Longitude: 79° 00′ E)	*Gracilaria corticata*	8.40 ± 0.65	8.30 ± 1.89	22.84 ± 0.87	7.07 ± 0.33	8.30 ± 0.48	49.64 ± 3.89	(Rosemary *et al.*, 2019)
	Gracilaria edulis	10.40 ± 0.69	4.71 ± 0.60	25.29 ± 0.67	4.76 ± 0.60	7.36 ± 0.39	38.02 ± 4.32	
Tamil Nadu, India	*Gracilaria edulis*	87.14 ± 1.10	7.63 ± 0.11	14.26 ± 0.88	0.93 ± 0.00	32.39 ± 1.90	63.18 ± 0.46	(Debbarama *et al.*, 2016)
Green seaweeds								
Pattani Bay, Thailand (Rainy and summer)	*Ulva Pertusa*	6.0 ± 0.3	28.6 ± 1.4	16.1 ± 0.6	7.4 ± 1.0	-	52.2 ± 3.8	(Benjama and Masniyom, 2011)
	Ulva pertusa	5.9 ± 0.3	25.9 ± 0.1	14.6 ± 0.3	2.1 ± 0.0	-	59.0 ± 0.3	
	Ulva intestinalis	5.4 ± 0.9	28.4 ± 0.2	16.4 ± 0.1	8.7 ± 0.6	-	62.2 ± 2.8	
	Ulva intestinalis	7.2 ± 0.2	26.9 ± 0.6	19.5 ± 0.3	7.3 ± 0.3	-	51.3 ± 0.3	
Tamil Nadu, India	*Ulva latuca*	84.81 ± 0.22	12.41 ± 0.32	13.84 ± 3.55	0.86 ± 0.00	43.19 ± 1.75	53.63 ± 0.18	(Debbarama *et al.*, 2016)
Archipelago, Portugal (April 2014).	*Ulva compressa*	90.00 ± 0.91	18.03 ± 0.23	15.66 ± 0.09	1.67 ± 0.16	14.45 ± 0.43	33.67 ± 0.35	(Paiva *et al.*, 2018)
	Ulva rigida	84.50 ± 0.70	20.60 ± 0.27	15.78 ± 0.10	1.02 ± 0.09	16.74 ± 0.17	34.67 ± 0.55	
Aveirot, Portugal July, 2016	*Ulva rigida*	-	31.70 ± 0.60	-	-	58.1 ± 0.70	36.60 ± 1.50	(Neto *et al.*, 2018)
Veraval, India (lat. 200 54' 33.7'' N, long. 700 21' 06.0'' E)	*Caulerpa scalpelliformis*	21.14 ±0.27	23.90 ± 0.81	16.85 ± 0.25	1.83 ± 0.18	36.28 ± 0.98	-	(Manas *et al.*, 2017)
	Caulerpa racemosa	18.11 ± 0.36	30.95 ± 0.37	9.21 ± 0.53	1.29 ± 0.19	40.44 ± 0.90	-	
Naozhou Island, South China	*Caulerpa racemosa var peltate*	-	7.97 ± 0.46	11.39 ± 0.32	1.03 ± 0.01	71.67 ± 0.36	-	(Hao *et al.*, 2019a)
Sepanggar Bay (6°05′0″N 116°09′0″E) Sabah, Malaysia	*Caulerpa racemosa var lv.*	-	26.74 ± 2.17	17.28 ± 0.63	2.11 ± 0.04	53.37 ± 0.00	3.18 ± 0.38	(Nagappan and Vairappan, 2014)