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Marine seaweeds with economic importance in the philippines: Valuation of six specie *Caulerpa racemosa* (Forsskål), *Ulva fasciata* (Delile), *Sargassum polycystum* (C. Agardh), *Sargassum ilicifolium* (Turner) C. Agardh, *Halymenia durvillei* (Bory de Saint-Vincent), and *Halymenia dilatata* (Zanardini) from the Philippines

Rexie Magdugo

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THÈSE DE DOCTORAT DE

L'UNIVERSITE BRETAGNE SUD

ECOLE DOCTORALE N° 598

Sciences de la Mer et du littoral

Spécialité : « (Biotechnologie marines) »

Par

Rexie MAGDUGO

**ALGUES MARINES D'IMPORTANCE ÉCONOMIQUE AUX PHILIPPINES
: ÉVALUATION DE SIX ESPÈCES *Caulerpa racemosa* (Forsskål), *Ulva lactuca* (Linnaeus), *Sargassum polycystum* (C. Agardh), *Sargassum ilicifolium* (Turner) C. Agardh, *Halymenia durvillei* (Bory de Saint-Vincent) et *Halymenia dilatata* (Zanardini) des Philippines**

« Wild seaweed species from the Philippines »

Thèse présentée et soutenue à « UBS, Vannes », le 4 décembre 2020

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**Dedicated to the one who broke my heart,
-especially to the one who healed it-**

**“Life is short and funny”
RPMagdugo**

**“Simplicity is the ultimate sophistication”
Leonardo da Vinci**

**“It is true intelligence for a man to take a subject that is
mysterious and great in itself and to unfold and simplify it so that
a child can understand it”
John Taylor**

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**ALGUES MARINES D'IMPORTANCE ÉCONOMIQUE AUX
PHILIPPINES : ÉVALUATION DE SIX ESPÈCES *Caulerpa racemosa*
(Forsskål), *Ulva lactuca* (Linnaeus), *Sargassum polycystum* (C. Agardh),
Sargassum ilicifolium (Turner) C. Agardh, *Halymenia durvillei* (Bory de
Saint-Vincent) et *Halymenia dilatata* (Zanardini) des Philippines**

Résumé :

Les Philippines sont connues pour sa riche flore, et ses 1291 taxons d'algues macrobenthiques marines qui représentent une production végétale naturelle importante et diversifiée. Sur 350 espèces considérées comme d'importance économique, seulement 5% d'entre elles sont utilisées, la plupart doivent encore être développées.

Les macroalgues vertes (*Caulerpa* et *Ulva*), brunes (*Sargassum* spp.) et rouges (*Halymenia* spp.) présentent de nombreuses application dans l'alimentation humaine ou animale, source de vitamines et de minéraux. Cependant, les propriétés nutritionnelles et les activités biologiques des espèces *Caulerpa*, *Ulva*, *Sargassum* et *Halymenia* aux Philippines sont peu connues. Par conséquent, il est intéressant de valoriser davantage ces algues pour diverses applications industrielles, telles que l'alimentaire, la pharmacologie et la cosmétique. Cette étude a été menée afin de fournir des informations supplémentaires sur le potentiel des espèces sauvages de *Caulerpa*, *Ulva*, *Sargassum* et *Halymenia* des Philippines. La présente étude traite (1) de la composition biochimique, des lipides, des protéines, des acides aminés, de la composition minérale comme valeurs nutritionnelles, et pour déterminer sa composition en acides gras à partir des lipides totaux; (2) caractérisation des polysaccharides de la paroi cellulaire, nature des sucres, spectres infrarouges, compositions pigmentaires; et (3) de l'évaluation des activités antivirales et antioxydantes des polysaccharides pariétaux dans un contexte de santé.

Mots-clés : *Caulerpa*; *Ulva*; *Sargassum*; *Halymenia*; **nutrition**; **activités biologiques**

MARINE SEAWEEDS WITH ECONOMIC IMPORTANCE IN THE PHILIPPINES: VALUATION OF SIX SPECIES *Caulerpa racemosa* (Forsskål), *Ulva lactuca* (Linnaeus), *Sargassum polycystum* (C. Agardh), *Sargassum ilicifolium* (Turner) C. Agardh, *Halymenia durvillei* (Bory de Saint-Vincent), and *Halymenia dilatata* (Zanardini) from the Philippines

Abstract:

Philippines is known for its rich flora, and its 1291 taxa of marine macrobenthic algae are significant and diversified natural vegetable production. Of 350 species considered as economic importance, only 5% of these are being used, most have still to be developed.

These species of green (*Caulerpa* and *Ulva*), brown (*Sargassum* spp.), and red (*Halymenia* spp.) seaweeds present many uses as human or animal food, source of vitamins and minerals. However, little is known for its nutritional properties, and biological activities of *Caulerpa*, *Ulva*, *Sargassum*, and *Halymenia* species in the Philippines. Therefore, it is interesting to further valorize these algae for various industrial applications, such as food, pharmasology and cosmetic. This study was conducted to give additional information on the potential of wild *Caulerpa*, *Ulva*, *Sargassum*, and *Halymenia* species from the Philippines. The present study deals with (1) the biochemical composition, lipids, proteins, amino acids, mineral composition as nutritional values, and to clarify its fatty acids composition from total lipids; (2) characterization of cell wall polysaccharides, nature of sugars, infrared spectra, pigment compositions; and (3) evaluation of the antiviral and antioxidant (DPPH radical scavenging) activities of parietal polysaccharides in health context.

Keywords: *Caulerpa*; *Ulva*; *Sargassum*; *Halymenia*; nutrition; biological activities

Scientific contribution

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Poster Presentation

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List of abbreviations

AA: Amino Acids

AA: Arachidonic Acid

AAS: Atomic Absorption Spectrophotometry

AGPs: ArabinoGalactan Proteins

AI: Atherogenicity Index

ALA: α -Linolenic Acid

BHA: Butylated Hydroxyanisole

BHT: Butylated Hydroxytoluene

BSA: Bovine Serum Albumin

CC₅₀: Cytotoxic Concentration 50%

CEVA: Centre d'Études et de Valorisation des Algues

CVD: Cardiovascular Disease

CPE: CytoPathic Effect

DAP: Depigmented Algal Powder

DHA: Docosahexaenoic Acid

DPPH: 2,2-DiPhenyl-1-PicrylHydrazyl

DW: Dry Weight

EAA: Essential Amino Acids

EPA: Eicosapentaenoic Acid

FA: Fatty Acid

FAME: Fatty Acid Methyl Ester

FAO: Food and Agriculture Organization

FCS: Fetal Calf Serum

FCSPs: Fucose Containing Sulfated Polysaccharides

FT-IR: Fourier Transform InfraRed

FW: Fresh Weight

GC-FID: Gas Chromatography - Flame Ionization Detector

HPAEC-PAD: High-Performance Anion Exchange Chromatography – Pulse
Amperometric Detection

HPLC: High Performance Liquid Chromatography

HSV: Herpes Simplex Virus

HWE: Hot Water Extraction

MAA: Mycosporine-like Amino Acids

MEM: Minimal Essential Medium

MHDP: Meta-HydroxyDiPhenyl

MOI: Multiplicity Of Infection

MUFA: Monounsaturated Fatty Acids

NEAA: Nonessential Amino Acids

NMF: Natural Moisturizing Factor

OD: Optical Density

-OH: hydroxyl group

PSB: Phosphate-Buffered Saline

PUFA: Polyunsaturated Fatty Acids

ROS: Reactive Oxygen Species

SAR: Stramenopiles, Alveolates, and Rhizaria

SFA: Saturated Fatty Acids

SIAP: Seaweed Industry Association of the Philippines

Sp: *Sargassum polycystum*

Si: *Sargassum ilicifolium*

TAA: Total Amino Acids

TI: Thrombogenicity Index

TL: Total Lipid

UBS: Université Bretagne Sud

GENERAL

INTRODUCTION

General introduction

Marine has a lot to offer to humanity, some of which are yet to be explored. The Philippines, made up of 7,641 groups of Islands, which are mostly made up of coastal areas, estuaries, ponds lakes, mountainous areas, are areas of growth where alga thrives (Rao and Mantri 2006). Research on seaweeds opened the door to the right knowledge and use (Trono and Largo, 2019). It was also found out that there are over 350 species of seaweeds that are valued in the market (Trono 1999). A significant business venture to individuals who reside in the Philippines is one of the significant producers, along with China and Indonesia as the leading one (FAO, 2018; Trono and Largo, 2019). The strategic geographical location of the Philippines in the heart of the Coral Triangle as it is considered as the global epicenter of marine biodiversity (Hehre, 2015), produces a large number of seaweeds and has proven to be favorable for the commercial farming of eucheumatoids (Trono and Largo, 2019).

The Philippines is known for its rich flora, and its marine algae are significant and diversified natural vegetable production. There are 1,291 taxa of marine macrobenthic algae with 306 published names of taxa (including species, varieties, and forms) of greens, 234 names of browns, and 751 names reds currently listed respectively on the Philippines littoral zones (Ang *et al.* 2013). Many of these species (350 species) are of economic importance as food, sources of industrial products such as polysaccharides, bioactive and nutritional natural products, and growth-promoting substances. However, about 5% of these are economically important; most have still to be developed (Trono 1999).

Out from almost 30 genera of macroalgae that are cultivated worldwide, species from 17 genera are commercially cultivated which includes; *Agardhiella*, *Euचेuma*, *Gelidium*, *Gigartina*, *Gracilaria*, *Hydropuntia*, *Hypnea*, *Kappaphycus*, *Meristotheca*, *Porphyra/Pyropia* (Rhodophyta), *Saccharina*, *Laminaria*, *Undaria*, *Cladosiphon* (Phaeophyceae), *Monostroma*, *Ulva*, and *Caulerpa* (Chlorophyta). *Agardhiella*, *Gelidium*, *Gigartina*, *Porphyra/Pyropia*, *Saccharina*, *Laminaria*,

Undaria, *Monostroma*, and *Ulva* are cultivated in the temperate zone, and *Eucheuma*, *Gracilaria*, *Hydropuntia*, *Hypnea*, *Kappaphycus*, *Cladosiphon*, and *Caulerpa* are mainly grown in the tropics and subtropics (de Gaillande *et al.* 2017). In the Philippines, most species have been harvested in the Pacific coast since the early 60's when American Seaweed Company was forced to seek an alternative source of dried seaweeds due to Indonesia's (primary source) political problems. Since then, farming technology was developed, and it is a sustainable harvest up to today for just a few species such as *Kappaphycus alvarezii*, and *Eucheuma denticulatum*, and non-traditional seaweed resources such as *Caulerpa lentillifera* and *Caulerpa racemosa* as an export product, and some species of *Gracilaria*, while all others are gathered from wild stocks (Trono, 1999; Trono and Largo, 2019). The Philippines used to be a world leader for the production of eucheumatoids but, due to ease of technology as well as other factors affecting production such as the frequent occurrence of typhoons, the Philippines slid down to second place next to Indonesia with more than 80% of the world's *Eucheuma* and *Kappaphycus* (Trono and Largo, 2019). Other seaweed resources are likewise explored for their high-value products, such as the brown seaweed *Sargassum*, and red seaweed *Halymenia* are sources of natural extracts in the form of biostimulants, hydrocolloids (alginate, sulfated polysaccharides rich in fucose or carrageenan) and pigments (fucoxanthine or phycoerythrin). *Halymenia* for example have essential, value-added industrial applications that could boost the local carrageenan industry for its lambda-type colloid, but also find commercial applications for its natural pigment phycoerythrin especially when a viable and cost-effective culture technique can be developed (Trono and Largo, 2019).

1. Contribution to Philippine economy

Asia as a prime producer of seaweed farming, large groups of islands came to the point of doing lucrative ways to get onto the top market of producers. The Philippines' total export of seaweeds and seaweed products has grown tremendously, which amounted to US\$198 million in 2016. Recently, most seaweed products are exported in processed forms representing 65% of the total seaweed exports (Trono and Largo, 2019). This has sustained fishers as an alternative income other than fishing, which gathered up considerable evidence that the activity is a profitable business venture amongst them, thus boosted the economy of the regions (Crawford, 2002; Sievanen *et al.* 2005). People from the Asian regions, especially the Philippines, have depended on their needs on the sea. Due to the fact, the coastal areas have a massive seaweed supply; thus, seaweed farming is one of the livelihoods a fisher can have for sustainable economic development in the countryside (Trono and Largo, 2019). Unless alternative methods are used, subsistence fishing will decrease. In the Indo-Pacific region, seaweed farming consists mostly of small subsistence farms ($\sim 1 \text{ ha}^2$) (Hehre and Meeuwig, 2015).

In the country, 'hard data' were difficult to obtain from official sources since seaweed shipping channels were complex. However, the Seaweed Industry Association of the Philippines (SIAP) has pooled its commercial data to support common causes (SIAP, 2018). SIAP members reported total production of all types of dried seaweeds in the Philippines, with an average of almost 88,000 MT per annum, from 2011-2015. The Philippine government reported for 2015 that live seaweed production was in the order of 1,566,161 MT; this was a year when SIAP reported 101,900 MT dw production. This indicates a wet-to-dry ratio of approximately 15.4 to 1.0, which is more than 1.5 times the level of wet: dry ratio experienced by the industry. Despite unavailable precise numbers, general trends are still evident from consensus estimates production volumes and price trends. Industry estimates of global *Kappaphycus* production, over the past decade, hovered in the range of $180,000 \pm 20,000$ MT per year. This is roughly equivalent to 1.3-1.7 million MT fresh weight if one assumes a wet-to-dry ratio of 8.5:1. These numbers are consistent with industry production estimates; however, they are not consistent with FAO (2014) estimates supplied by government sources (Hurtado *et*

al. 2017). A study about seaweed farming as an alternative occupation for fishers (Hill *et al.* 2012) perceived a decrease of fishing due to the availability of seaweed farming in some villages of the Philippines. Access to this has dramatically boosted the Philippines' economy and fed the poverty line. Thus, Seaweed farming is beneficial in the Philippines, but due to declines, it may be hard to maintain as seaweed farming emphasizes an opportunistic nature.

2. Seaweed farming

In the early 1950s, fishers began farming these green algae *C. racemosa* and *C. lentillifera* in fishponds initially used for the aquaculture of milkfish (*Chanos chanos*) cleared mangrove areas in the island of Mactan, Cebu, Philippines. The ponds were flooded to a depth of 0.5 to 0.6 m depending on the tidal range, and sluice gates-controlled water level and renewal. Pond's dams were constructed with dried black mud and clusters of coral stone. The area of the ponds ranges from 1000 m² to about 1 ha. Planting was done by burying (2 cm) or by merely spreading rhizoidal thalli or young plants in the mud or on the bottom, then harvesting after around 45 days by uprooting the plants from the mud leaving several plants to propagate new growth—each harvest lasting several days. The freshly collected seaweeds could be stored in baskets for up to 48 h (de Gaillande *et al.*, 2017). About 30 million T (wet weight) of red, brown, and green seaweeds were harvested in 2016, mainly for human food, animal feed, and hydrocolloids production. The most collected macroalgae from the wild and sold on the markets are *Caulerpa* species.

In contrast, the most widely cultivated species include Japanese kelp (*Laminaria japonica*), *Eucheuma* seaweeds, *Kappaphycus alvarezii*, and *Undaria pinnatifida* (FAO, 2018). However, farms can use a range of culture techniques. Because of the increasing demand for food, mainly aquatic food in most regions of the world, the aquaculture industry became popular since the 1970s. It continued to

have a significant contribution, especially for *Caulerpa* with total annual crop harvested in Fiji, Samoa, and Tonga Islands combined represented 123 T of wet material with a value of US\$266,492. This suggests that, for every edible and potentially cultivatable species of interest, it should be genetically characterized by rapid molecular traceability methods (de Gaillande *et al.*, 2017). However, in *Ulva* species, the cultivation method is similar to *Monostroma*, a non-clonal marine plant that does not have fragmentation ability. In the natural seeding method, rope nets are submerged in places where this alga grows naturally, and once germination starts, the nets are taken and brought to the farm. In the artificial seeding method, gamete release and collection are induced from matured thalli with a combination of brief drying and floating in seawater with intense illumination. Gametes from thalli fragmentation used for its artificial seeding are allowed to settle on culture nets in dark conditions, and these nets are taken to the farm once germinated. These germinated algae are then cultivated on poles fixed in shallow, calm areas of the sea or in estuaries, where the nets are periodically exposed to air during low-tide. Tank cultivation or also known as the "germling cluster method," has also been developed. Information on phylogenetics and phylogeography is known, making it one of the well-studied green algal lineages (Bast, 2014).

Due to the high potential yields of seaweeds' biomass, its utilization as raw materials in a biorefinery is a promising alternative to fossil resources to produce energy and chemicals. Seaweeds as an alternative source of energy could help reduce or mitigate greenhouse gas (GHG) emissions (by 42-48%) compared to the use of natural gas (Pechsiri *et al.* 2016) and thus enhance energy security. Every year, about 1 million T of algal phycocolloids are extracted in these industries worldwide (Bixler and Porse, 2011), which nearly equal the number of industrial wastes disposed of in landfills and open sea, causing fouling smell and water pollution. Thus, the waste from these industries, which contains high amounts of carbohydrates, maybe a source of raw material for bioethanol or biogas production (Teh *et al.* 2017) . So technically, in this scenario, bioethanol production from seaweeds and industrial seaweed waste will reduce the usage of freshwater, synthetic fertilizers, and that agricultural land and will produce high-value

commodities in an environmentally friendly biorefinery manner (Baweja *et al.* 2016; Maneein *et al.* 2018).

There have been many other reports in the recent years of ethanol production from various seaweeds such as *Ulva pertusa*, *U. lactuca*, *Gelidium amansii*, *G. elegans*, *Sargassum fulvellum*, *S. sagamianum*, *Chigaso crassifolia*, *Saccharina japonica*, *S. latissimi*, *Laminaria japonica*, *L. digitata*, *L. hyperborean*, *Nizimuddinia zanardini*, *Euchema* sp., *Gracilaria verrucosa*, and *G. Salicornia* (Baweja *et al.* 2016). Bioconversion of algal material into bioethanol and other valuable co-products depends on the technologies available for converting biomass to fuels and has been applied in limited ways. For example, the high-water content of seaweed (ca. 80–90%) will harm the energy balance of applications dependent on dry biomass. This makes seaweed (wet biomass) undesirable for processes like; direct combustion, pyrolysis, and gasification, but suitable for processes that depend on the use of wet biomass. These methods include hydrothermal liquefaction for bio-oil production, fermentation for ethanol production, and anaerobic digestion (AD) for biogas production. All three methods are still under development to produce biofuel from seaweed (Maneein *et al.* 2018).

In the past years, there have been continuing efforts to optimize pre-treatments to achieve better yields and lower costs, whether using microalgae or seaweed. Pre-treatments of biomass that modify the bioavailability of polysaccharides for their hydrolysis to sugars. Different pre-treatment methods (mechanical, thermal, enzymatic, chemical, biological) to improve cellular access to polysaccharide-hydrolysis agents were tested and improved (Barbot *et al.* 2015; Maneein *et al.* 2018). Other factors to consider during different pre-treatment methods are the season of collection and location of seaweed biomass, salt content, heavy metal content, polyphenols, structural make-up, and the relative content of the different polymeric carbohydrates. The biogas production, sugars from hydrolyzed polysaccharides were converted to acetate, CO₂, and H₂ by acidogenesis and acetogenesis, and proceed to methane and CO₂ by methanogenesis. While for ethanol production, glucose and galactose from the hydrolysis of the major polymers in red seaweed (e.g., carrageenan and agar), in brown seaweed (e.g.,

laminarin and fucoidan along with glucuronic and mannitol), and in green seaweed (e.g., cellulose, starch, and ulvan) were converted by the natural microbial process to pyruvate by glycolysis in anaerobic respiration, and then proceed to ethanol and CO₂ by alcoholic fermentation (Maneein *et al.* 2018).

Currently, seaweed biomass is harvested for high-value natural products and food only. A biorefinery approach can sustainably produce and maximize biofuel and process co-products on a condition to meet the required and appropriate pre-treatments for different seaweeds. The future of biofuel production from seaweeds depends on developing new technologies, with an appropriate and market-friendly regulatory environment (Baweja *et al.* 2016). Therefore, it is possible to design suitable seaweed pre-treatments that will maximize the sustainability and energy return of investments (EROI) for biofuel production at the lowest cost (Maneein *et al.* 2018).

3. Ecological impact of seaweed farming

Problems on aquaculture worldwide have been afloat for some time as sectors failed to regulate or due to poor management of the areas. These problems are caused by the expansion of the cultured regions, spike of aquaculture installations, and the increased feed resources produced away from the immediate area (Soto *et al.* 2008). Human activities such as aquaculture will lead to some loss of biodiversity; this may be a minimal change or crucial to an extent, is a concept by Odum, who pioneered the relationship between human activity and the natural process of biodiversity (Odum and Barrett, 1972). Ecological issues are the focal point of interest rather than the changed ecosystem to an agro-ecosystem.

Seaweed aquaculture is a complex sector conditioned by environmental, biological, and external factors, relying on cultivation techniques and sites which may face environmental issues, and subject to legislation and market demand

(Barbier *et al.* 2019). As seaweeds are common in Asia, factors such as nutrient levels, phytoplankton blooms, temperature, turbidity, and fluctuations are the significant ones that may affect the yield of the product (Beveridge *et al.* 1997). Continuing to the effects on the study on impacts of effluents of aquaculture, it has been sought out that it contains negative impacts on the particular area where it has been done, this is due to particulate matters which are desecrated by the types of machinery; this may be inorganic or organic components that may harm the existing living organisms (Piedrahita, 2003). Due to aquaculture installations, the place's ecosystem might be at risk due to alteration of ecosystem structure and function, as natural bioturbation may change due to ecological disturbances that may cause greater damage to the locality (Piedrahita, 2003; Barbier *et al.* 2019).

Because of increasing aquatic food demand in the early 1960s, aquaculture farms were booming in the Philippines, and many of these farms were in mangrove areas. At present, the conversion of mangroves into ponds has been banned because of its environmental impact, and therefore less *Caulerpa* production (Tanduyan *et al.* 2013). Also, harvesting of the brown seaweed *Sargassum* from the wild has been banned since 2014 due to its ecological importance (Trono and Largo, 2019). Because of this prohibition, cultivation of *Caulerpa* was tried in open and natural waters at different culture media, at different water levels, at different sites using: 1. plastic screen cage, 2. tubular plastic screen, 3. nylon screen cage, and 4. tubular nylon screen at the surface, middle, and bottom water at muddy, sandy, or rocky sites (Tanduyan *et al.* 2013). At the same time, it hurt the livelihood opportunities of some sectors and the young *Sargassum* industry (Trono and Largo, 2019). In the study of Tanduyan *et al.* the plastic screen cage is the best culture method, the middle layer is the best water level, and there is no significant difference in the culture sites (Tanduyan *et al.* 2013). The need to protect natural habitats while providing sufficient raw materials for an industry that is looking for opportunities to utilize local resources to produce value-added products must be balanced and based on scientifically proven evidence (Barbier *et al.* 2019; Trono and Largo, 2019). This can only be addressed by developing appropriate sustainable culture technologies that are on-going (Trono and Largo, 2019).

Seaweeds, unlike terrestrial plants, do not compete for arable land, do not require the use of freshwater, fertilizers, or pesticides to grow much faster than land plants. The seaweeds' cell wall consists of alginates, carrageenan, agar, cellulose, hemicellulose, fucoidan, ulvan, and protein (Trono, 1999; Stiger-Pouvreau *et al.* 2016). Aquaculture is one of the main livelihoods and fastest-growing producers of the world. It is more than ever, harder to prevent environmental degradation due to aquaculture intensification. This would most likely affect a large area to the places in Asia, where it is more common (Ahmed and Thompson, 2017). Sites such as the eastern countries may have a hard time doing this since they are new to the aquaculture world and barely have traditions in using the pollutants as products. A new ecological concept in agriculture addresses these problems, which are the Ecological intensification concepts. It is made to address the challenges of producing the human population's sufficient needs and the thought of conserving the environment at the same time. The goal of this concept is to protect and preserve the remaining resources and the quality of living of humans (Aubin *et al.* 2019). Harvesting due to intensification has provided with the right produce provided but soon may change since the population is continuously rising; thus, demands are higher. The concept was later adopted in the eastern regions of the globe, Asia. China, the leading seafood producer, treats effluents as products rather than pollutants, commonly used as enhancements of natural stocks. The two primary forms of an Integrated multi-trophic Aquaculture or IMTA are sea-ranching and suspended aquaculture. This tradition opened a path to intensive aquaculture on the scale and intensity of Chinese waters (Leung and Bates, 2013). However, this does not apply to the western world as they are recent to aquaculture and have no traditions on the usage of effluents as inputs, which it is difficult for them in following IMTA.

Nonetheless, seaweed farming may impact fishing behavior due to pieces of machinery, expansion of areas, overproduction of goods, and disturbance in the ecosystem. Seaweed farming often leads to immigration, which attracted outsiders in Tawi-Tawi and Sibu, Philippines (Sievanen *et al.* 2005). To prevent overexploitation of the natural beds, most seaweed industries sourced research to

improve knowledge and understanding of the seaweed diversity, sustainable harvesting techniques, and the development of efficient culture systems to cope up with increasing global demands. As this became a thing, Sulu archipelago begins advertising and attracting people to participate in a new way of earning money (Trono and Largo, 2019).

4. Seaweed species selected in this study

The selected species of green (*C. racemosa* and *U. lactuca*), brown (*S. polycystum* and *S. ilicifolium*), and red (*H. durvillei* and *H. dilatata*) seaweeds collected from different parts of the country were based on their popularity or abundance, economic, and biochemical potential. The *C. racemosa* was one of the most popular seaweed salads in the Philippines. It was cultured in the Philippines since the 1960s together with *C. lentillifera* and is still sustainable upto today (Trono and Largo 2019). *Caulerpa* is the most collected seaweed species from the wild and directly sold to the market (de Gaillande *et al.* 2017). The genus *Ulva* was seasonally abundant and considered as nuisance in the beach shores of the Philippines. This genus was encouraged by the Philippine government to be developed as human and animal feed, and as potential source of biogas feedstock (SIAP 2018; <http://business.inquirer.net/227401/ph-seaweed-gears-resurgence>). The genus *Sargassum* is the most dominant genus in the Philippines. The reason for selecting *S. polycystum* as specimen was because of its easier identification than other *Sargassum* species, while *S. ilicifolium* was utilized as food. Although exploitation of this genus *Sargassum* began in the 1970s for the production of organic liquid fertilizer, it was just recently tested for possible cultivation (Trono and Largo 2019). Both *H. durvillei* and *H. dilatata* were utilized as food in the Philippines (Trono and Largo 2019). The *H. durvillei* is one of the seven emerging red seaweeds from the Philippines as a potential alternative source of lambda-

carrageenan, and r-phycoerythrin (Trono and Largo 2019; Hurtado *et al.* 2020). Less research on *Halymenia* species in the Philippines (Trono and Largo 2019).

4.1 Green seaweeds (*C. racemosa* and *U. lactuca*)

The thallus of *Caulerpa* is formed by a giant multinucleated cell (coenocytic organization), which develops into a complex branching siphon. *Caulerpa* is characterized by this siphon (called trabeculae) in an inward projection of the wall. The thallus is composed of upright fronts arising from creeping stolons anchored by branched rhizoids. These vertical branches bear distinctive branchlets (called metamers) and are used for morphological species identification. (de Gaillande, 2017). Specimen of *C. racemosa* shown in Fig. 1.

Classification of green algae *Caulerpa* according to Guiry and Guiry, (2020) is:

Eukaryota

Kingdom: Plantae

Phylum: Chlorophyta

Class: *Ulvophyceae*

Order: Bryopsidales

Family: *Caulerpaceae*

Genus: *Caulerpa*



Figure 1. Plant specimen of *C. racemosa* (Forsskål) displayed at the local public market in Langihan, Butuan City, Philippines (personal documentation).

The thalli of *Ulva* are composed of two layers of cells in which each cell contains a cup-shaped chloroplast (Baweja *et al.* 2016). The plant is greenish to yellowish, often twisted and crumpled in appearance, and with irregular perforations (Hurtado-Ponce *et al.* 1992). This macroalga of the genus *Ulva* is also known as Sea lettuce (Bast 2014). Macroalgae having a resemblance to lettuce are, in fact, *U. fasciata*, *U. lactuca*, or that *Ulva* genus that has distromatic blades. At the same time, commercially cultivated species include *U. prolifera* (= *Enteromorpha prolifera*) and *U. intestinalis* tubular walls with walls of the tube one cell thick. Thalli are coarsely filamentous or foliaceous at 5-12 cm diameter (Hurtado-Ponce *et al.* 1992) that can be branched or unbranched and have disc-like holdfasts developed from basal cells that attach firmly on suitable substrata like pebbles or rocks. Plant specimen of *Ulva lactuca* shown in Fig. 2.

Classification of green algae *Ulva* according to Guiry and Guiry, (2020) is:
Eukaryota

Kingdom: Plantae

Phylum: Chlorophyta

Class: *Ulvophyceae*

Order: Ulvales

Family: *Ulvaceae*

Genus: *Ulva*



Figure 2. Plant specimen of *Ulva lactuca* (Linnaeus) collected at Lawigan, Bislig City, Philippines (personal documentation).

4.2 Brown seaweeds (*S. polycystum* and *S. ilicifolium*)

In the Philippines, the genus *Sargassum* is commonly known as Samo (Cebuano), while *S. polycystum* is called Aragan (Iloku), and Kulapu (Hiligaynon) (Hurtado-Ponce *et al.* 1992). The *S. polycystum* has a cylindrical, small spiny thallus with a disc-shaped holdfast and air bladders in every corner. Typical characteristics of *S. polycystum* are their small, pointed and jagged blades. The lower part of the main axis giving rise to stolon-like horizontal branches, often bearing secondary haptera from which a new plantlet may arise (Mattoo and Payri 2011). Consequently, they inhabit mostly along the coast with 0.5-5 m. Plant specimen of *S. polycystum* shown in Fig. 3.

According to Guiry and Guiry (2020), the classification of brown algae *Sargassum* is:

Eukaryota

Kingdom: Chromista

Phylum: Ochrophyta

Class: *Phaeophyceae*

Order: Fucales

Family: *Sargassaceae*

Genus: *Sargassum*



Figure 3. Plant specimen of *S. polycystum* (C. Agardh) collected at Lamanok island, Anda, Bohol, Philippines (personal documentation).

Another species, *S. ilicifolium*, is generally brown or dark green, having a taxonomic synonym as *S. duplicatum*. Oogonia and antheridia occur in conceptacles embedded in receptacles on special branches. Their branchlets are modified pedicles with berry-like bladders. Linear-lanceolate fronds possess a dentate sinuate margin, distinct midrib, and small, numerous irregularly placed cryptostomata. The shapes of their vesicles are globular, often without prolongation at the top. Then shapes of their receptacles are cylindrical, filiform, and irregularly

ramified (Mattio and Payri, 2011; Sumithra and Arunachalam, 2014). Plant specimen of *S. ilicifolium* shown in Fig. 4.



Figure 4. Plant specimen of *S. ilicifolium* (Turner) C. Agardh, collected at Currimao, Ilocos Norte, Philippines (personal documentation).

4.3 Red seaweeds (*H. durvillei* and *H. dilatata*)

The *H. durvillei* is one of the new species developed for cultivation in the Philippines. It is characterized by large, bushy thalli that may grow up to 35 cm. It is soft, cartilaginous, and slimy when fresh and found in upper subtidal attached to the rocky substrate by its discoid holdfast (Trono and Largo, 2019). It is abundant in a vast area of the Indian Ocean (Filaire *et al.* 2019). Plant specimen of *H. durvillei* shown in Fig. 12.

Classification of red algae *Halymenia*, according to Guiry and Guiry (2020)

is:

Eukaryota

Kingdom: Plantae

Phylum: Rhodophyta

Class: *Florideophyceae*

Order: Halymeniales

Family: *Halymeniaceae*

Genus: *Halymenia*



Figure 5. Plant specimen of *H. durvillei* (Bory de Saint-Vincent) collected at Currimaos, Ilocos Norte, Philippines (personal documentation).

The *H. dilatata* is characterized by the discoidal holdfast bearing a relatively small stipe, the brownish spots on the brightly colored thallus (Irregular sheet-like thalli) surface, and the minute teeth which often form a fringed margin. The thallus of *H. dilatata* has thinner cortex (up to about 50 μm thick), the minutely dentate

margins, and the relatively smooth surface with only small proliferations. The cross-section of the mid-region of a thallus had a 4-cell-layered outer cortex and a 2-cell-layered inner cortex (De Smedt *et al.* 2001). Plant specimen of *H. dilatata* shown in Fig. 6.



Figure 6. Plant specimen of *H. dilatata* (Zanardini) collected at Lawigan, Bislig City, Philippines (personal documentation).

5. Objectives of the study

Philippines is a source of enormous number of rich natural resource, especially the marine environment. And yet, little is known about the nutritional properties, health values and biological activities of the wild green (*C. racemosa* and *U. fasciata*), brown (*S. polycystum* and *S. ilicifolium*), and red (*H. durvillei* and *H. dilatata*) economically important seaweeds species from the Philippines.

Therefore, this study was conducted to give additional information on the potential of these wild seaweed species for various industrial applications, and it is divided into two parts.

Part I: The first part focuses on “Nutritional values” by:

1. determining the biochemical composition, especially proteins and its amino acids, ash and its mineral composition; and

Part II: The second part focuses on the “Health value and biological activities” by:

2. extracting polysaccharides fractions using appropriate methods for each seaweed;
3. determining each of its polysaccharides fraction yields;
4. characterization of cell wall polysaccharides fractions;
5. determining its polysaccharides sugar compositions, infrared spectra;
6. evaluation of the antiviral and antioxidant (DPPH radical scavenging) activities of polysaccharides; and
7. analyses of lipids and its fatty acids, and pigments composition.

Chapter 1 Review of Literature

1. Definition

Macroalgae (seaweeds) represents a group of aquatics, oxygen-evolving, photosynthetic autotrophs eukaryotic organisms ((Tirichine and Bowler 2011; Leliaert et al. 2012; Adl et al. 2019). They are a varied group, with sizes ranging from a few centimeters to 100 m in length. Their habitat is mostly limited to a certain depth of 60 m (Gomez-Zavaglia *et al.* 2019) or up to maximum depths of 100 to 236 m in tropical kelp taxa such as *Eisenia galapagensis*, and *Laminaria philippinensis* (Graham *et al.* 2007) and growth is usually vertical, looking for sunlight (Gomez-Zavaglia *et al.* 2019). Some algae are microscopic such as the phytoplankton that provides the base for most marine food chains. Some are enormous like the giant kelp, and most are medium-sized that come in colors of red, green, and brown that randomly wash up on beaches and shorelines everywhere (Guiry 2012). These primary producers (protista microorganisms) are responsible for 30-50% of the oxygen in the atmosphere (Gomez-Zavaglia *et al.* 2019). Aside from its function as ecosystem engineers in reef-building communities, macroalgae also serve as a refuge for fish and invertebrates, serve as a food source for human consumption, and are harvested to extract useful natural products such as hydrocolloids and pigments. The multitude of seaweeds applications in various fields and industries led to a global increase in demand for the biomass of a variety of seaweed species (Trono and Largo, 2019). All lineages of these aquatic groups have primary plastids, organelles that originated directly from cyanobacteria (Burki *et al.* 2020).

2. Taxonomy and classification

More than 30,000 species of algae have been described (Guiry 2020; Gomez-Zavaglia *et al.* 2019). In the biological sense of the word, algae are used to designate a vast, varied, and heterogeneous group of organisms, at present, classification is not well defined, and taxonomic status may vary according to the authors, under constant and rapid revision at all levels (Gomez-Zavaglia *et al.* 2019). The groupings of the macroalgae: green (Chlorophyta), the red (Rhodophyta), both belonged to kingdom Plantae; and brown (Ochrophyta) of kingdom Protista, as well as microalgae kingdom, were grouped according to their pigment composition, and through their photosynthetic activity to carry out autotrophic feeding. Other authors include some green macroalgae in another differentiated group and at a taxonomic level equivalent to Chlorophyta, which are responsible for life expanding beyond the oceans, and are precursor of terrestrial plants. Table I showed the summary of the phylogeny of these four large groups of macroalga. The taxonomic classification of algae classes is very complicated due to the number of existing varieties and many applicable classification criteria (Gomez-Zavaglia *et al.* 2019), such as the nature and locations of the reserve glucans, the nature of their cell walls, the number of periplastidial membranes, the shape of the mitochondrial crystal, the presence and ultra-structure of flagella, the type of mitosis, the mode of nutrition, reproduction, and habitat (de Reviere, 2002). Seaweed is the common name for approximately 15,000 species of marine plants and algae that grow in the ocean and rivers, lakes, and other water bodies (Chandler *et al.* 2017; Gomez-Zavaglia *et al.* 2019).

Table I. Phylogenetic summary of the most common macroalgae (Gomez-Zavaglia *et al.* 2019).

Kingdom	Phylum/Division	Classes	Orders
Chromista	Ochrophyta	Phaeophyceae	Ascoseirales; Desmarestiales; Discosporangiales; Dictyotales Ectocarpales; Fucales; Laminariales; Nemodermatales Ralfsiales
	Charophyta	Charophyceae; Chlorokybophyceae; Coleochaetophyceae; Klebsormidiophyceae; Mesostigmatophyceae; Zygnematophyceae.	Bryopsidales; Cladophorales; Dasycladales;
Plantae	Chlorophyta	Ulvophyceae	Oltmannsiellopsidaes; Trentepohliales;
	Rhodophyta	Bangiophyceae; Compsopogonophyceae; Florideophyceae; Porphyridiophyceae; Rhodellophyceae; Stylonematophyceae.	Ulotrichales; Ulvales

By the early 2000s, a model of the tree emerged that divided almost all known eukaryote diversity among five to eight significant taxa, usually referred to as 'supergroups' (Burki *et al.* 2020). The category of the supergroup was a purely informal one, denoting extensive assemblages that contain, for example, the traditional 'kingdoms' like Metazoa and Fungi as subclades. A typical list of these groups included Archaeplastida that is also known as Plantae. Archaeplastida is distinguished by the presence of primary plastids, also known as the photosynthetic organelles deriving directly from cyanobacteria by primary endosymbiosis around 1500 Mya (Chandler *et al.* 2017; Burki *et al.* 2020). In comparison, the divergence

of the brown algae (Phaeophyceae), as a consequence of secondary endosymbiosis, or the occurrence of a separate distinct iridescent bodies (with structural coloration) suggest that it may have evolved independently in brown algae occurred relatively recently, at around 200 Mya (Fig. 7) (Chandler *et al.* 2017). The three main groups with primary plastids are the green algae and land plants, the red algae, and glaucophyte algae (Burki *et al.* 2020).

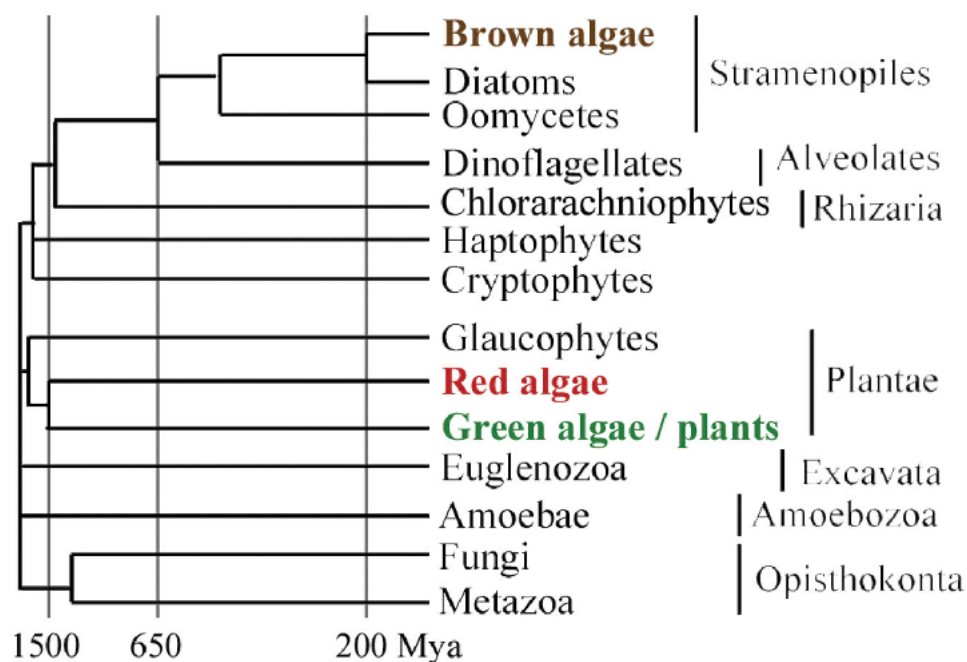
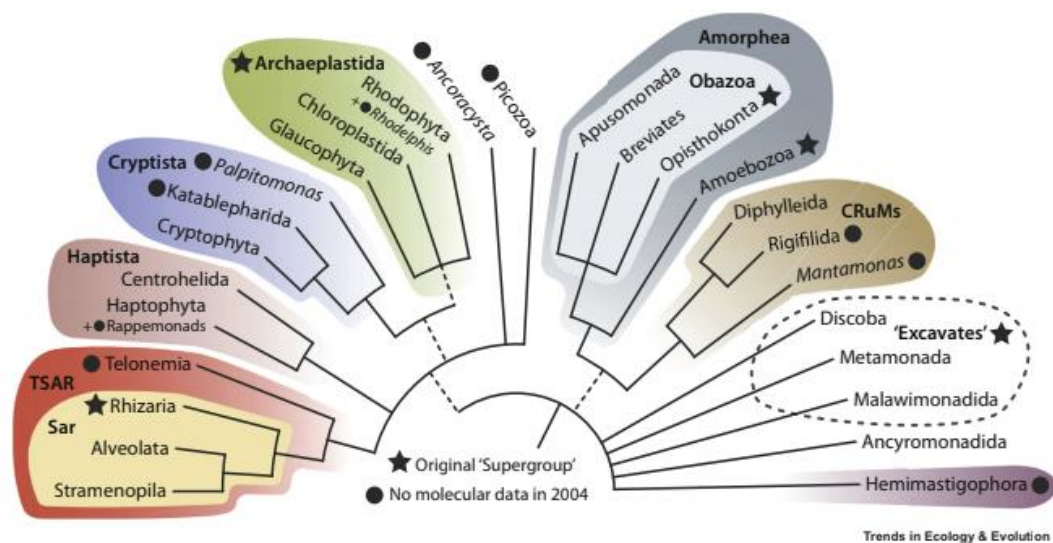


Figure 7. Evolutionary tree of the eukaryotes shows the position of red, green, and brown algae and the divergence of red algae, dinoflagellates, and brown algae (cited by Chandler *et al.* 2017, and taken from the paper of Cock *et al.* 2010).

Today, Archaeplastida is generally considered a supergroup, although most phylogenomic analyses do not actively support its monophyly (Fig. 8). Hence, the three taxa that comprise Archaeplastida are the Chloroplastida (green algae and land plants), Rhodophyta (red algae), and Glaucophyta. All three lineages have primary plastids, which are photosynthetic organelles that originated directly from cyanobacteria (Burki *et al.* 2020).



This summary is based on a consensus of recent phylogenomic studies. The colored groupings correspond to the current 'supergroups.' Unresolved branching orders among lineages are shown as multifurcations. Broken lines reflect lesser uncertainties about the monophyly of certain groups. Star symbols denote taxa that were considered supergroups in early versions of the supergroup model; thus, all original supergroups except Archaeplastida have either disappeared or been subsumed into new taxa. The circles showed major lineages with no molecular data when the supergroup model emerged, most often because they had not yet been discovered.

Figure 8. The new tree of eukaryotes showing Archaeplastida, and Stramenopila – the lineage of marine algae (Burki *et al.* 2020).

The clade 'SAR' (Stramenopiles, Alveolates, and Rhizaria) has been estimated to comprise up to half of all eukaryote species (Burki *et al.* 2020). Most macroalgae come from either brown alga (class Phaeophyceae) or red algae (phylum Rhodophyta). A few marine macroalgae occur in the phylum Charophyta since about 600 large green algal taxa are in the phylum Chlorophyta (C. Dawes, 2016). Macroalgae in these four groups have more fundamental differences (cytological, chemical, and life histories) between them (C. J. Dawes *et al.* 1998). Since then, advancements in phycology have resulted in significant changes in the classification of algae (Wynne and Schneider, 2010), and the number of seaweed species has increased. There are 3330 species of green algae, 2060 brown algae, and 7102 red algae worldwide (Stiger-Pouvreau *et al.* 2016).

3. Biochemical composition

Among marine macroorganisms, seaweed gained considerable significance due to its utilization as a precious raw material for the extraction of various valuable materials, such as polyphenols, organic pigments, proteins, unsaturated fatty acids, and polysaccharides, and other biochemical compounds (Al-Nahdi *et al.* 2019). Seaweeds are among the wealthiest sources of known and novel bioactive compounds, and the biological activity of compounds derived from this group has been extensively reviewed (Bedoux *et al.* 2016; Hurtado *et al.* 2020; Theophilus *et al.* 2020). However, the biochemical composition of algae varies according to species, location and season of collection, growth conditions or sensitivity of methods used were seen in lipids and fatty acids (Kelman *et al.* 2012; Kok and Wong, 2018; Artemisia *et al.* 2019; Hurtado *et al.* 2020), in polysaccharide yields (Chattopadhyay *et al.* 2007; Ji *et al.* 2008; Kok and Wong, 2018; Artemisia *et al.* 2019), in minerals and ash contents (Kelman *et al.* 2012; Alam Bhuiyan and Qureshi, 2016; Garcia *et al.* 2016; de Gaillande *et al.* 2017; Wanyonyi *et al.* 2017; Wells *et al.* 2017; Kok and Wong, 2018; Artemisia *et al.* 2019), in β -carotene and other pigments (Paul *et al.* 2013), and proteins and amino acids (Kelman *et al.* 2012; Anh *et al.* 2013; Hardouin *et al.* 2016; Garcia *et al.* 2016; de Gaillande *et al.* 2017; Kok and Wong, 2018; Artemisia *et al.* 2019). Macroalgae have many uses; food for human consumption, ingredients in cosmetics and fertilizers, treated products for extracting gelling agents, or animal feed additives (de Gaillande *et al.* 2017). They are mainly used as food in the form of fresh vegetables. Their popularity tends to be on the increase due to the general awareness of health-conscious consumers of the advantages of its natural products.

3.1 Minerals

Seaweeds are absorbed from the sea, an incomparable wealth of mineral elements and are known as excellent sources of vitamins and minerals, especially potassium and iodine (Leandro *et al.* 2020). However, nutritional generalization about algal mineral contents is difficult because of sometimes significant seasonal, geographic, and taxonomic variations in mineral contents of marine algae (Wells *et al.* 2017). Green algae *Caulerpa* and *Ulva* present many uses as human or animal food, source of minerals like (Ca, K, Mg, Na, Cu, Fe, Zn), or vitamins E. Brown algae, particularly from the genera *Laminaria*, are a great source of iodine. The highest concentration of iodine is in *L. japonica*, 5.6, and 3.0 mg kg⁻¹ from all investigated algae (Mišurcová 2011). The high ash content can reach up to 50.2% of dw in *H. floresii* and represent a high content in minerals (Garcia *et al.* 2016). Other red seaweed species such as *Gracilaria* spp., *Gelidium* sp., and *Porphyra* sp. reported varying ash contents from 7.72% to 29.06% (Alam Bhuiyan and Qureshi, 2016), and 58% from *K. alvarezii* (Wanyonyi *et al.* 2017). Table II showed mineral composition results from different groups of seaweeds. The percentages of the recommended daily intake covered by an intake of 8g of dry seaweed are also presented as well as the daily recommended intake allowance (MacArtain *et al.* 2007).

Table II. Composition of minerals (% dw), recommended daily intakes and recommended daily allowances from different seaweed groups

Species	Macroelements					Microelements			
	Na	K	Ca	Mg	Na/K	Fe	Zn	Mn	Cu
<i>C. racemosa</i> ^a	0.02-9.0	0.7-1.1	0.6-1.9	0.6-1.6	7.8≥22.0	0.02-81.3	0-2.5	0-5.8	0-0.8
<i>C. lentillifera</i> ^a	0.02-7.0	0.3-3.9	0.6-5.9	0.4-4.1	0.3≥100	0.02-21.4	0.002-3.5	0.001-7.9	0-0.1
<i>U. reticulata</i> ^b	-	1.54	0.14	0.14	-	0.174	0.0033	0.481	0.0006
<i>S. polycystum</i> ^c	1.36	8.37	3.79	0.48	0.162	0.068	0.0021	-	0.00003
<i>S. ilicifolium</i> ^d	-	4.64	4.32	3.34	-	0.018	0.00264	0.0096	0.0012
<i>Sargassum spp.</i> ^e	2.71	7.80	1.39	1.57	0.35	0.006	0.003	0.002	0.0005
<i>H. floresii</i> ^f	5.54	5.96	0.73	0.9	0.93	0.0143	0.0021	-	0.0006
<i>K. alvarezii</i> ^g	3.7	20.0	2.89	0.57	0.19	tr	-	tr	-
<i>P. umbelicalis</i> ^h	11.97	30.22	3.42	10.83	0.25	0.52	0.07	-	0.01
RDI (g day ⁻¹)*	2.0	3.8	0.84	0.35		0.006	0.12	0.0055	0.0017
ULI (g day ⁻¹)**	2.3	-	2.5	0.35		0.045	0.040	-	0.01

a= de Gaillande *et al.* 2017; b= Arporn and Chirapart, 2014; c= Matanjun *et al.* 2009; d= Pise and Sabale, 2010; e= Thadlani *et al.* 2019; f= Garcia *et al.* 2016; g= Wanyonyi *et al.* 2017; h= MacArtain *et al.* 2007; tr= trace amount. *= Recommended daily intake values for macroelements and microelements for an adult male with a body weight of 70 kg according to the World Health Organization (WHO 2002); **= Upper limit intake according to National Health and Medical Research council (2006).

3.2 Proteins

Seaweed and microalgae are considered a viable source of protein. Some seaweed and microalgae species are known to contain protein levels similar to those of traditional protein sources, such as meat, egg, soybean, and milk, as cited by Bleakley and Hayes, 2017. Algae use for protein production has several benefits over traditional high-protein crop use in terms of productivity and nutritional value (Bleakley and Hayes, 2017). Proteins of marine algae have attracted less attention, and their structure and biological properties are not as widely documented as their polysaccharides (Chojnacka, 2012). However, recently, they have gained a great deal of interest due to their functional properties and bioactivity potential for further application (Michalak and Chojnacka, 2015; Xu *et al.* 2017).

Proteins are shown in Table III. Other nutritional values differ from phylum to phylum and from species to species in seaweeds (Table III) with all its vitamins and essential amino acids in reasonable proportions (de Gaillande *et al.* 2017).

Table III showed that *C. racemosa* contains a higher amount of proteins (19.72±0.77%), compared to other green, brown, and red algae (Alam Bhuiyan and Qureshi 2016). The protein content of brown algae is generally low, usually ranging between 2.4-16.8% of dry weight compared to the green (3.2-35.2%) and red algae (6.4-37.6%) (Øverland *et al.* 2019). However, their protein content in different species is dependent on the season. It is linked to their surrounding variables, including nutrient supply (Gaillard *et al.* 2018), or even the sensitivity of methods used (Gaillard *et al.* 2018; Øverland *et al.* 2019). Gaillard *et al.* (2018) reported that *Porphyra* sp. had an average crude protein (CP) content (33.4% dw), which is comparable to high protein seeds such as soybeans (35.0% dw) indicates a suitable substitute for soybeans in terms of CP content. Furthermore, the brown seaweeds (*Alaria* sp., *Laminaria* sp., and *Saccharina* sp.) had CP content that ranges from 6.8 to 16.4% dw and further explained that the higher CP content might be influenced by the amount of sunlight and nutrients during spring season compared to autumn season (Gaillard *et al.* 2018). Crude protein content is measured based on the assessment of crude protein from overall nitrogen content using the traditional nitrogen-to-protein (N-Prot) conversion factor of 6.25, while protein (true protein) is quantified using the sum of amino acid residues (Biancarosa, *et al.* 2017).

Table III. Proximate protein results (% dw) of different species of green, brown, and red seaweeds.

Green seaweeds	Protein	Brown seaweeds	Protein	Red seaweeds	Protein	Reference
<i>C. lentillifera</i>	12.49	<i>S. polycystum</i>	5.4	<i>H. durvillei</i>	13	a, c, d
<i>C. racemosa</i>	0.6–18.3	<i>S. pallidum</i>	0.1-1.2	<i>H. dilatata</i>	10	b, d, g
<i>C. racemosa</i>	19.72	<i>S. filipendula</i>	8.72	<i>H. maculata</i>	11	a, d
<i>U. rigida</i>	6.40	<i>S. vulgare</i>	15.76	<i>G. cornea</i>	5.47	a
<i>U. lactuca</i>	7.06	<i>S. polycystum</i>	4.7	<i>G. changii</i>	6.9	a, e
<i>U. pertusa</i>	15.4	<i>S. fusiforme</i>	1.01	<i>G. changii</i>	12.57	a, f, h
<i>U. intestinalis</i>	17.9					a

a= Alam Bhuiyan and Qureshi, 2016; b= de Gaillande *et al.* 2017; c= Matanjun *et al.* 2009; d= Kho *et al.* 2016; e= Palanisamy *et al.* 2018 (crude fucoidan); f= Ye *et al.* 2018 (polysaccharide); g= Li *et al.* 2018 (purified polysaccharides by DEAE-sepharose fast flow); h= Chan and Matanjun, 2018.

The total amino acid composition of several green, brown, and red seaweed species is shown in Table IV. Table IV showed a higher concentration of aspartic acid (9.2-13.68% dw), and glutamic acid (10.7-31.19% dw) was observed in *C. racemosa*, *C. fastigiata*, *U. fasciata*, *Alaria* sp., *Laminaria* sp., *Saccharina* sp., and *Porphyra* sp. Alanine (12.18-17.58% dw) was also dominant in *Alaria* sp., *Laminaria* sp., *Saccharina* sp., *Porphyra* sp., and Arginine (35.57% dw) *H. floresii*. While histidine (0.11-4.11% dw), methionine (0.17-5.12% dw), and tyrosine (0.44-3.9% dw) were the limiting amino acids in all seaweeds except *H. porphyroides*. The abundance of glutamic and aspartic acids is responsible for the characteristic flavor and taste of seaweeds (Gaillard *et al.* 2018). These results suggest that the seaweed species mentioned above could be potentially used as a nutritious and functional food item for human consumption (Alam Bhuiyan and Qureshi 2016).

Table IV. Total amino acid composition of several species of green, brown, and red seaweeds.

	Essential amino acids (EAA)							Nonessential amino acids (NEAA)								
	THR	VAL	LYS	ILE	LEU	PHE	HIS	MET	ASP	SER	GLU	GLY	ARG	ALA	TYR	PRO
<i>C. racemosa</i> ^a	0.51	0.57	0.33	0.43	0.69	0.64	0.11	0.17	0.94	0.45	1.13	0.61	0.49	0.49	0.44	0.57
<i>C. racemosa</i> ^b	6.2	5.1	6.6	5.8	6.9	5.0	2.6	1.4	9.2	5.8	12.7	6.5	6.4	7.6	3.8	5.1
<i>C. fastigiata</i> ^c	4.8	6.1	7.1	4.0	8.7	6.6	2.2	1.5	10.1	6.2	10.7	7.1	5.5	6.2	3.9	7.7
<i>Ulva fasciata</i> ^c	5.1	5.7	5.1	3.9	7.6	5.1	2.4	0.9	13.0	5.8	12.6	6.5	5.6	8.5	3.3	4.6
<i>Alaria</i> sp. ^d	4.88	5.59	5.44	3.75	6.18	4.22	1.42	1.95	11.32	5.14	31.19	5.25	4.25	17.58	-	3.62
<i>Laminaria</i> sp. ^d	5.69	6.35	5.65	4.48	7.55	5.03	1.75	2.0	13.68	5.55	15.31	6.13	4.81	12.23	-	4.95
<i>Saccharina</i> sp. ^d	5.92	6.54	5.49	4.4	7.56	4.94	1.53	2.11	13.23	5.51	22.33	6.17	4.75	12.18	-	4.9
<i>H. floresii</i> ^e	6.96	0.08	8.47	2.85	4.45	4.11	4.11	5.12	-	-	6.80	1.17	35.57	-	-	-
<i>H. porphyroides</i> ^f	1.94	3.35	9.33	3.4	3.54	3.04	9.93	8.81	3.35	3.94	3.04	6.2	7.71	6.75	8.93	1.14
<i>Porphyra</i> sp. ^d	6.92	8.06	6.38	4.89	8.83	4.97	1.6	2.56	12.01	6.24	14.39	7.9	7.63	14.79	-	5.31
RDI (mg/kg/day)	15	26	30	20	39	25	10	10	-	-	-	-	-	-	-	-
% RDI	4.6	3.0	2.2	2.9	2.5	2.8	2.3	2.1	-	-	-	-	-	-	-	-

Values=mean percent 100mg⁻¹ dw of algal protein; a=de Gaillande *et al.* 2017; b=Alam Bhuiyan and Qureshi, 2016; c=Lourenço *et al.* 2002; d=Gaillard *et al.* 2018; e=Garcia *et al.* 2016; f= Manam and Subbiah, 2020. RDI=recommended daily intake values for EAAs according to the World Health Organization (Joint WHO 2007); %RDI= is based on a daily intake of 8g dw of *Caulerpa racemosa* var *turbinata* for an adult human with a bodyweight of 70kg (de Gaillande *et al.* 2017).

In the paper of Hamed *et al.* (2015), the green alga *Ulva rigida* contains Leu, Phe, and Val as primary essential amino acids. Hamed *et al.* also recorded that His

content in *U. rigida*, an essential amino acid for infants, is at levels close to those in legumes and eggs. Algal polysaccharides have numerous human health benefits that can be easily isolated and are ingredients in functional foods. Gaillard *et al.* (2018) reported a rich concentration of aspartic and glutamic acids, and low methionine and histidine in all seaweeds tested. Compared with soybean, most of the seaweed species were deficient with EAA except for sulfur amino acids (Met, and Cys). Proline contents were low (1.68-4.78 g/16gN), and is known to accumulate in large quantities in response to environmental stressors, as a nontoxic and protective osmolyte under saline conditions (Gaillard *et al.* 2018). Gaillard *et al.* (2018) also reported EAA in different proportions (24-42% TAA) with EAA/NEAA ratio for red and green seaweeds at around 0.7 and lower ratio for brown seaweeds.

3.3 Sugars

In general, total sugars were abundant components compared to proteins, phenols, and flavonoids (Hao *et al.* 2019). In the last decades, sulfated polysaccharides from algae have attracted much attention as functional additives in the pharmaceutical field, food, and cosmetic industries. The major sulfated polysaccharides found in marine algae are ulvan (green algae), sulfated polysaccharide rich in fucose – fucan and fucoidan- (brown algae), and carrageenan (red algae) (Cunha and Grenha 2016).

3.3.1 Cell wall

Seaweed cell walls represent at least 50% of its dry weight and typically consist of a fibrillar skeleton and an amorphous embedding matrix (Stiger-Pouvreau *et al.* 2016). Marine algae are well-known by their chemically complex and heterogeneous structure, i.e., sulfated and branched polysaccharides associated with proteins and various bound ions, including calcium and potassium. The number and chemical structure of these polymers vary according to the specific algal species (Cunha and Grenha, 2016).

3.3.1.1 Green seaweeds

In green algae, it has polymer assemblage cell walls similar to the cellulose, pectins, hemicelluloses, and exceptionally arabinogalactan proteins (AGPs). Ulvophyceae seaweeds have abundant fibrillar constituents' cell wall components. Most may change from cellulose to β -mannans to β -xylans during different life cycle phases; these also produce complex sulfated polysaccharides, AGPs, and extensin. Chlorophyceae green algae had a wide array of walls ranging from cellulose–pectin complexes to ones made of hydroxyproline-rich glycoproteins (Domozych *et al.* 2012). The schematic diagram of the green seaweed (Lahaye and Robic, 2007) is shown in Fig. 9. Cell wall polymers common to green seaweed, particularly for *Caulerpa* and *Ulva* species is shown in Table V (Holdt and Kraan, 2011).

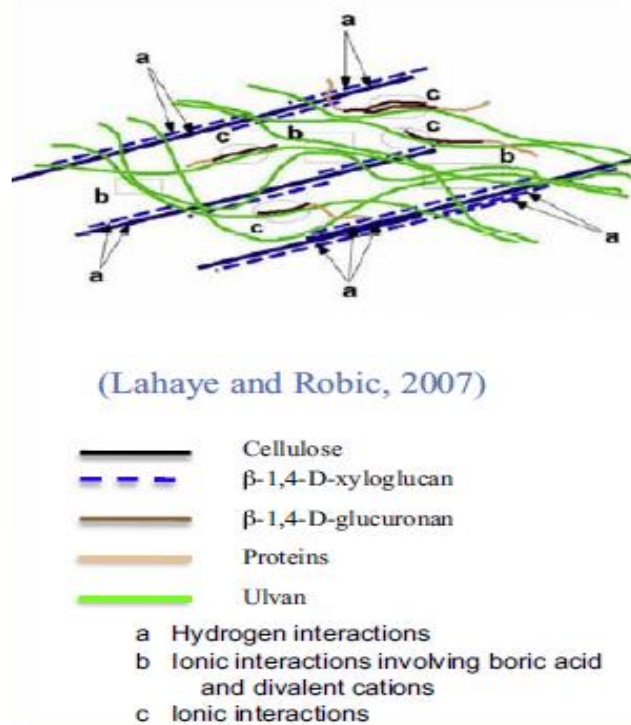


Figure 9. Schematic cell wall present in green seaweeds, with natural polysaccharides (Lahaye and Robic, 2007).

Polysaccharides, especially extracellular polysaccharides derived from *Caulerpa*, are complex in composition and structurally diverse. It has water-soluble polysaccharides (mostly glucans and sulfated polysaccharides). These sulfated polysaccharides are mainly of sulfated galactose (commonly of xylose, glucose, and mannose). The cell wall of *C. racemosa* is described as a branched polymer containing (3-linked galactose, terminal- and 4-linked xylose, as well as 4- and 3,4-linked arabinose residues). When sulfate groups are present, are linked to (C-3 of 4-linked arabinose and C-6 of 3-linked galactose) units (Chattopadhyay *et al.* 2007; Arata *et al.* 2015).

Table V. Major storage carbohydrate and cell wall polymers present in *C. racemosa* and *U. fasciata* (Lahaye and Robic 2007; Stengel *et al.* 2011).

<i>Polysaccharide</i>	<i>C. racemosa</i>	<i>U. fasciata</i>
<i>Fibrillar polysaccharides of the crystalline phase</i>	β -(1,4)-D-mannans β -(1,3)-D-xylans	<i>Cellulose</i>
<i>Hemicelluloses</i>	<i>Xyloglucan, Mannans, Glucuronan, β-(1,3)-glucans</i>	
<i>Carboxylic polysaccharides of the matrix phase</i>	<i>Sulfated glucuronoxylorhamnans</i>	<i>Ulvans</i>
<i>Sulfated polysaccharides of the matrix phase</i>	<i>Sulfated xyloarabinogalactans</i>	<i>Ulvans, Sulfated xylorhamnoglucuronans</i>
<i>Storage carbohydrates</i>	<i>Starch</i>	

The seaweed *Ulva* (Ulvales) also has a complex composition and structure sulfated polysaccharide ulvan, a potential source of new functional biopolymer. The name ulvan is derived from the original terms ulvin and ulvacin introduced by Kylin, 1946 as cited (Lahaye and Robic 2007) about different fractions of *U. lactuca* water-soluble sulfated polysaccharides from members of the Ulvales. The pioneering work of Brading *et al.* 1954 and McKinnel and Percival 1962, as cited by (Lahaye and Robic, 2007), established that sulfate, rhamnose, xylose, and glucuronic acid are the main constituents of ulvan. Thus, its main composition is made of disaccharide (sulfated rhamnose and glucuronic acid, iduronic acid, or xylose) repeating sequences. The two major repeating disaccharides are aldobiuronic acids as type A: ulvanobiuronic acid 3-sulfate (A3s) and type B: ulvanobiuronic acid 3-sulfate (B3s) is shown in Fig. 10 (Lahaye and Robic, 2007; Cunha and Grenha 2016). In surface view, the cells are usually hexagonal or pentagonal, which remain loosely arranged (Baweja *et al.* 2016).

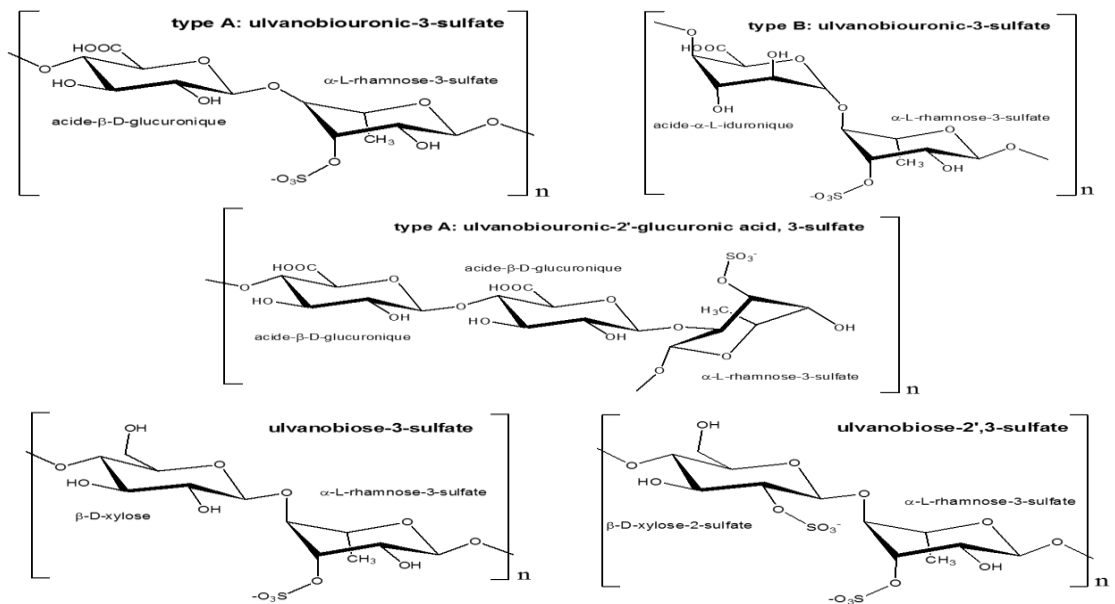


Figure 10. The main repeating disaccharide units of ulvan. A. $[\rightarrow 4)\text{-}\alpha\text{-D-Glcp-(1}\rightarrow 4)\text{-}\beta\text{-L-Rhap3S-(1}\rightarrow]_n$; B. $[\rightarrow 4)\text{-}\alpha\text{-L-Idop-(1}\rightarrow 4)\text{-}\alpha\text{-L-Rhap3S-(1}\rightarrow]_n$.

3.3.1.2 Brown seaweeds

This cell wall structure in brown algae is even more complicated as they consist of an amorphous matrix of acid polysaccharides linked to each other by proteins (Fig. 11). These acid polysaccharides are mainly composed of sulfated polysaccharides rich in fucose – fucan and fucoidan- and alginic acid, which confer structural toughness and flexibility to seaweed (Cunha and Grenha 2016). This physical barrier has limited the efficiency of the solid-liquid extraction technique of biologically active compounds from the marine algae (Wijesinghe and Jeon, 2011). Sulfated polysaccharides rich in fucose usually constitutes about 5-10% of dry algal biomass, depending on the species, the part of the thallus being used, and the harvesting period (Cunha and Grenha 2016). Alginate generally makes up to 40% of the algae dry weight (Dobrinčić *et al.* 2020). Fucoidan can differ in structure

among and within algal species, and extraction conditions may alter the natural structure, and produce distinct fucoidan forms that may affect bioactivity and physicochemical properties (Cunha and Grenha 2016).

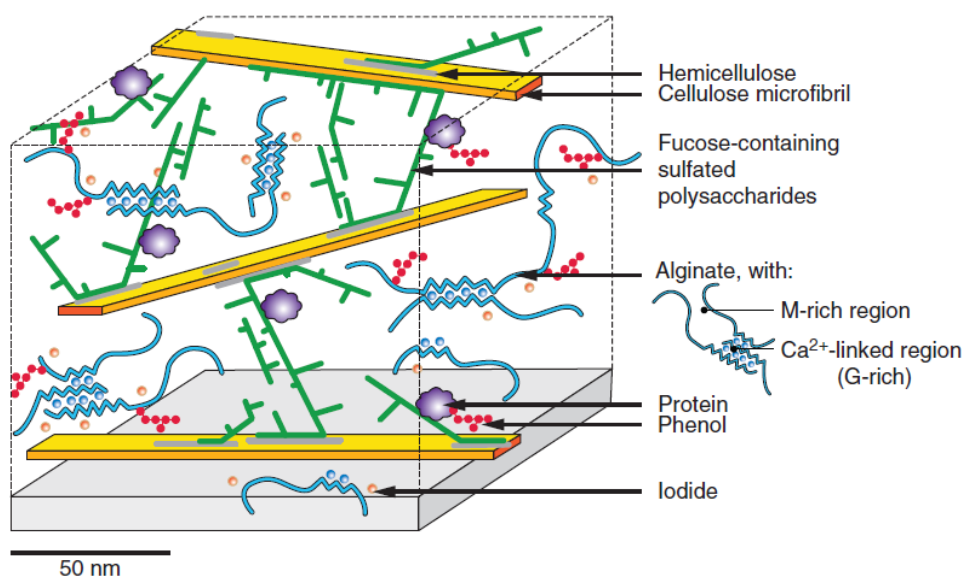
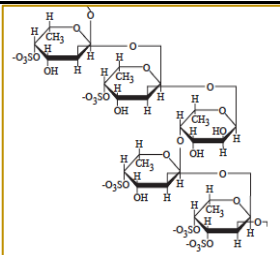
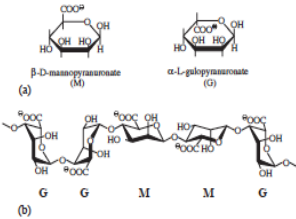
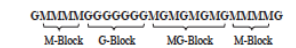


Figure 11. Cell wall model of brown algae from the order Fucales (Deniaud-Bouët *et al.* 2014).

Table VI showed the amorphous component of the brown algal cell wall is made up of alginic acid and sulfated polysaccharides rich in fucose. In contrast, their mucilage and cuticle polysaccharides are mainly sulfated polysaccharides rich in fucose, alginate, and laminarin (Cunha and Grenha 2016). In general, the cell wall and storage carbohydrate in brown algae varied strongly with the season, maturity, environmental factors and are species-specific, which increases the difficulty for industrial processing. For example, the Phaeophyceae *Ascophyllum nodosum* and *Laminaria digitata* have a composition of alginic acid, sulfated polysaccharides rich in fucose, laminarin equivalent to the ratio of 6:3:1 and 6:1:3, respectively (Ravanel *et al.* 2019). Besides structural polysaccharides, algae also contain storage polysaccharides, notably laminarin and mannitol, shown in Table VI (Stiger-Pouvreau *et al.* 2016). Sulfated polysaccharides rich in fucose primarily consists of α -L-fucose units (usually referred to as α -L-fucopyranose). The sulfation

pattern of the sugar backbone is species-related, and sulfation of α -L-fucose may occur at positions C-2 and C-4 and, at the rare position, C-3 structure (Cunha and Grenha 2016). Besides fucose and sulfate, fucoidan may also contain additional sugar constituents (mannose, galactose, glucose, xylose, uronic acids, and acetyl groups). Alginic acid consists of (1-4) linked β -D-mannuronic acid (M) and α -L-guluronic acid (G) residues. In the mixed salts form known as alginates- they contain several cations such as Na^+ , K^+ , Mg^{2+} , and Ca^{2+} (Puspita *et al.* 2020). Alginic acids may differ in the M/G ratio depending on the species, the part of the thallus being used, and the harvesting period (Artemisia *et al.* 2019; Puspita *et al.* 2020). Also, the molecules may differ in block composition, since uronic acid residues are usually arranged in two homopolymeric blocks (poly-M and poly-G), interspersed with the alternating structure of MG-blocks. Alginates have been used as thickeners, stabilizers, and gelling agents in food and pharmaceutical industries due to their hydrophilic and viscous properties (Puspita *et al.* 2020).

Table VI. Primary storage carbohydrate and cell wall polymers present in *Sargassum* species (Deniaud-Bouet *et al.* 2014).

<i>Polysaccharide</i>	<i>S. polycystum</i>	<i>S. ilicifolium</i>
<i>Fibrillar polysaccharides of the crystalline phase</i>	Cellulose (mostly xylose) Presence of hlorotannins	
<i>Hemicelluloses polysaccharides of the matrix phase</i>	Hexose, pentose, and uronic acids Sulfated rich fucose	
<i>Polysaccharides of the matrix phase</i>	rich alginate (~40%) mostly associated to phenols	 

Storage carbohydrates Laminarin (~34%), mannitol (~30%),

Several species with their total polysaccharides content (Table VII) from order Chordariales and Laminariales contain sulfated polysaccharides rich in fucose with a linear backbone composed of (1-3)-linked α -L-fucose residues. In contrast, order Fucales mostly display a backbone composed of alternating (1-3)- and (1-4)-linked α -L-fucose residues (Cunha and Grenha 2016). Seaweed from order Fucales have been reported to have abundant amounts of fucose and galactose, called galactofucans, and mainly composed of (1-6)- β -D-galactose and/or (1-2)- β -D-mannose units. In these cases, sulfated polysaccharides rich in fucose molecules differ in composition and terms of glycosidic bond positions (Cunha and Grenha 2016). In general, fucoidan polysaccharides may be branched, presenting a variety of substituting groups and side-chain compositions. The typical positions referred to link sulfate groups (C-2, C-3 or C-4) may also be occupied by neutral, and partially sulphated residues of glucuronic acid, mannose, galactose, and xylose have been further reported as side chain (e.g., *Sargassum* sp., *Fucus serratus*, *Punctaria plantaginea*, *Ascophyllum nodosum*) (Cunha and Grenha 2016). Polysaccharides contents (% of dry weight) of different species of *Sargassum* is shown in Table VII.

Table VII. Content of total polysaccharides in some *Sargassum* spp. (% dw)

Genera	Total polysaccharides (% dw)	Extraction	References
<i>S. pallidum</i>	21.3, 32.4, 5.3, 8.9	DEAE-sepharose	A
<i>Sargassum</i> sp.	8.5	Aquadest	B
<i>S. polycystum</i>	8.3	Methanolic	C
<i>S. ilicifolium</i>	3.1	Methanolic	C
<i>S. aquifolium</i>	5.7	Methanolic	C
<i>S. fusiforme</i>	6.59	Hot water	D
<i>S. tenerimum</i>	33.0	0.1 M HCl; K ₂ CO ₃	E
<i>S. polycystum</i>	22.3, 16.9, 19.4, 5.3	DEAE-cellulose	F
<i>S. ilicifolium</i>	2.89-3.48	Hot water	G

a= Li *et al.* 2017; b= Hidayati *et al.* 2019; c= Puspita *et al.* 2017; d= Ye *et al.* 2018; and e= Sinha *et al.* 2010; f= Palanisamy *et al.* 2018; and g= Artemisia *et al.* 2019.

3.3.1.3 Red seaweeds

Carrageenans is a generic name for a family of galactans (Cunha and Grenha 2016), a family of linear sulfated polysaccharides. They are found and extracted from the cell wall of certain species of red seaweeds (Al Nahdi *et al.* 2019). Rhodophyta is characterized by their content on non-fibrillar and sulfated polysaccharides such as carrageenan which is up to 20-38% (Lalegerie *et al.* 2020), agars and complex sulfated galactans, which are the main components of their cell walls (Fenoredosa *et al.* 2009), shown in Fig. 12 and Table VIII. These polysaccharides, well known for their gelling, stabilizing, and thickening properties in food, pharmaceutical, and cosmetic industry (Cunha and Grenha 2016), are made up of linear chains of alternating 3-linked-galactopyranosyl and 4-linked galactopyranosyl residues. The 3-linked units always belong to series, whereas the 4-linked ones may have the D or L configurations, often occurring as a 3,6-anhydrogalactopyranosyl moiety after enzymatic or alkaline treatment. Many rhodophycean galactans have been described depending on their structural features. Generally, the large carrageenan family is obtained from different species of Rhodophyta, including *Gigartina*, *Chondrus crispus*, *Euclidean*, and *Hypnea* (Fenoredosa *et al.* 2009). The source of carrageenan was the red seaweed *Chondrus crispus* which contains a mixture of *kappa* and *lambda* carrageenan that cannot be separated during commercial extraction. Today, most of the carrageenan is extracted from *K. alvarezii* and *E. denticulatum*, a predominant source of *kappa*- and *iota*-carrageenan, respectively (Cunha and Grenha 2016).

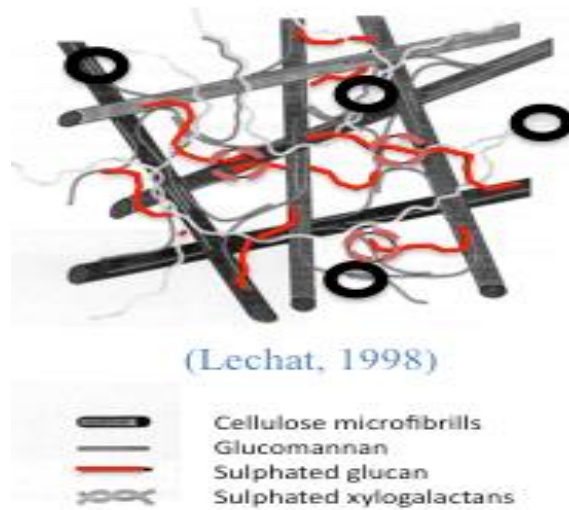
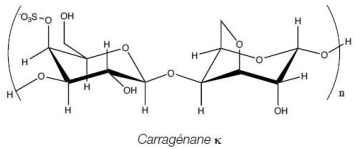
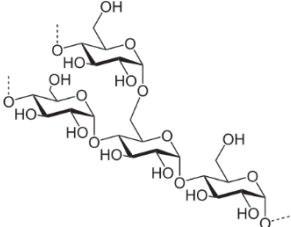


Figure 12. Cell wall model of red algae from the order Halymeniales (Stiger-Pouvreau *et al.* 2016).

They are variably sulfated on C-2 and C-4 of the 3-linked-D-galactopyranosyl unit and have a part of 4-linked-D-galactopyranoses in 3,6-anhydrogalactopyranosyl configuration. Cell wall polymers common to red seaweed, particularly for *Halymenia* species (Rhodophyta) is shown in Table VIII (Fenoradosoa *et al.* 2009). The principal feature of the highly sulfated carrageenan is D-galactose and anhydro-D-galactose, while the less sulfated agars the D-galactose, L-galactose, or anhydro-L-galactose (Stiger-Pouvreau *et al.* 2016).

Table VIII. Primary storage carbohydrate and cell wall polymers present in *H. durvillei* and *H. dilatata* (Santibañez *et al.* 2016; Fenoradosoa *et al.* 2009).

<i>Polysaccharide</i>	<i>H. durvillei</i>	<i>H. dilatata</i>
<i>Fibrillar polysaccharides</i>	Cellulose, β -(1,4)-D-mannans, β -(1,3)-D-xylans	
<i>Hemicelluloses</i>	Glucomannan, Mixed Linkage Glucan sulfates; β -(1,3), (1,4)-D-xylans	
<i>polysaccharides of the matrix phase</i>	Kappa-carrageenan, Lambda-carrageenan	 <p style="text-align: center;">Carragénane κ</p>
<i>Storage carbohydrates</i>	Floridean starch	
		

The repeating units may be modified in several ways; for example, the repeating carrabiose structure of carrageenans may be altered by replacing the 3,6-anhydro-D-galactose with D-galactopyranose residues. The glycosyl units may be substituted by sulfate hemiesters in various patterns, or more rarely by methyl ethers, or by the 4,6-O-carboxyethylidene group of pyruvic acid. Similarly, the agars' agarobiose repeating structure may be masked by replacing 3,6-anhydro-L-galactose with L-galactose or adding methyl ethers, sulfate hemiesters, and, exceptionally, pyruvic acid ketal at specific sites on either glycosyl unit (Stiger-Pouvreau *et al.* 2016).

4. Lipids content and fatty acids

Generally, seaweeds are characterized by a relatively low content of saturated fatty acids (SFA) and substantial amounts of polyunsaturated fatty acids (PUFAs), which are considered beneficial for human health as long as they are not oxidized or converted into saturated lipids (Hamed *et al.* 2015).

Brown algae contain relatively lower contents of lipids having less than 5% of dry weight. Quantitative analysis revealed that the total lipid content of a major brown algae family, Sargassaceae, was higher in subarctic zones (approximately 5% of dry weight) than tropical zones (0.9-1.8% of dry weight). Despite their low total lipid content, brown algae are a rich source of polyunsaturated fatty acids (PUFAs) (van Ginneken *et al.* 2011). In red seaweed, lipids and fatty acids are present in low amounts, generally 1–5% of the dry weight (Torres *et al.* 2019). The mean total lipid content of *Halymeniales* species can vary (0.1-3.9% dw). Kumari *et al.* 2013 compiled the total lipid content and fatty acid distribution of different seaweeds and suggested that the variations observed between different species of the same genus were more likely to be due to the inter-specific/intra-generic variations rather than to geographical and environmental conditions as apparent from the minor variations found with the environmental parameters for the studied collection sites.

Table X shows the total lipid content in several species of brown and red seaweeds, including *Sargassum* and *Halymenia* species. Macroalgae also contain various other lipids and lipids like compounds such as sterols, phospholipids, and glycolipids, but red seaweeds have a high ω -3 fatty acids content, being a rich source of α -linolenic acid (ALA) [18:3(n-3)], AA, eicosapentaenoic acid (EPA) [20:5(n-3)], and docosahexaenoic acid (DHA) [22:6(n-3)], and most species showed a nutritionally beneficial ω 6/ ω 3 ratio (Torres *et al.* 2019) (Table IX).

Table IX. Total lipid (TL) content, polyunsaturated and saturated fatty acids (PUFA/SFA) ratio, and distribution in some brown and red seaweed extracts, including *Sargassum* sp. and *Halymenia* sp.

Algae	TL (% of dw)	PUFA/SFA	$\omega 6/\omega 3$	References
<i>U. pinnatifida</i>	5	-	-	a
<i>S. horneri</i>	0.82-0.96	-	-	b
<i>Alaria esculenta</i>	1.3-1.5	-	-	c
<i>L. digitata</i>	0.8-1.1	-	-	c
<i>L. hyperbora</i>	1.1-1.4	-	-	c
<i>F. vesiculosus</i>	2.6-3.5	-	-	c
<i>Pelvetia canaliculata</i>	3.7-5.8	-	-	c
<i>A. nodosum</i>	4.5	-	-	c
<i>F. serratus</i>	3.7	-	-	d
<i>S. natans</i>	1.0	-	-	d
<i>Acanthophora</i>	6.8-10.4 mg g ⁻¹ fw	0.79-0.94	0.9-1.8	e
<i>Bortryocladia</i>	2.3-5.2 mg g ⁻¹ fw	0.49-0.54	1.7-3.6	e
<i>Gracilaria</i>	2.9-9.7 mg g ⁻¹ fw	0.15-2.13	0.6-1.9	e
<i>Grateloupia</i>	5.0-6.4, 13.6 dw	0.74-1.4	0.5-1.9	e; f
<i>Halymenia</i>	10-18.8 dw	1.37-1.8	1.7-5	e; f
<i>Porphyra</i>	11.2-12.4 dw	2.4-2.5	1.2-9.1	f; g
<i>Palmaria</i>	14-46 dw	0.49-1.1	0.21-0.41	h; i

a= Billakanti *et al.* 2013; b= Hossain *et al.* 2003; c= Maehre *et al.* 2014; d= van Ginneken *et al.* 2011; e= Kumari *et al.* 2013; f= Chen and Chou, 2002; g= Patra *et al.* 2017; h= Kumari *et al.* 2010; i= Maehre *et al.* 2014.

Total fatty acid concentrations vary among species, accounting for 1–8 in % of dry weight, showing significant differences in the fatty acid profiles, which can also be depending on the storage conditions (time and temperature) and the solvent also influences the yields and composition of the lipid extracts (Torres *et al.* 2019). *Sargassum* is rich in fatty acids with 20 carbon atoms, such as eicosapentaenoic acid (EPA, C20:5n-3), and arachidonic acid (AA, C20:4n-6). Certain species show different content of these fatty acids. As an example, *S. kjellmanianum* is a rich source of $\omega 6$, whereas *S. thunbergii* has a high content of $\omega 3$ (Burtin, 2003).

PUFAs generally contain high levels of omega-3 ($\omega 3$) and omega-6 ($\omega 6$) (Holdt and Kraan 2011). Seafood is the primary sources of long-chain PUFAs (Lordan *et al.* 2011), and humans should get them from food since humans are

incapable of synthesizing PUFAs with more than 18 carbons, e.g., long-chain fatty acids such as eicosapentaenoic acid (EPA, C20:5n-3) and arachidonic acid (AA, C20:4n-6). EPA and AA are mostly found in seafood and have the following health benefits which include the regulation of blood clotting, blood pressure, and develop the function of the brain and nervous systems, decrease the risk of many chronic diseases (Hamed *et al.* 2015), and regulate inflammatory responses by producing inflammation mediators called eicosanoids (Lordan *et al.* 2011). Studies indicate that inflammation is decreased by increasing the ratio of omega-3 to omega-6 fatty acids in the diet, Eicosanoids from omega-6 PUFAs (such as AA) have pro-inflammatory and immunoactive functions. In contrast, eicosanoids derived from omega-3 PUFAs (EPA and docosahexaenoic acid or DHA) have anti-inflammatory properties. A much bigger ratio of omega-6 PUFAs compared with omega-3 found in western food. Preclinical and clinical research on PUFAs has shown that fish oils (with EPA and DHA) consumption, affect inflammation. Prostaglandins and leukotrienes from EPA by cyclooxygenases (COXs) and lipoxygenases (LOXs) are less pro-inflammatory compared to AA (omega-6) thus, the increase of (DHA EPA)/AA ratio decreases the inflammatory mediators. EPA and AA are present in green seaweeds including *Caulerpa lentillifera* (0.83-1.60%, 0.84-6.70%), *Caulerpa racemosa* (1.70-9.54%, 2.40-6.70%) (de Gaillande *et al.* 2017), *Enteromorpha intestinalis* (5.43±0.45%, 1.51±0.12%), *Ulva rigida* (1.64±0.15%, 0.39±0.0%) (Ragonese *et al.* 2014), *Ulva pertusa* (2.0%, 0.6%) (Li *et al.* 2002), and docosahexaenoic acid (DHA, C22:6n-3) from *Caulerpa lentillifera* (0.83-3.64%), *Caulerpa racemosa* (0.81-3.39%) (de Gaillande *et al.* 2017), *Ulva linza*, *Ulva fasciata*, and *Ulva rigida* by which DHA levels is reported to be between 4-5% (Kumari *et al.* 2010) and *Ulva pertusa* as anti-allergic (Hamed *et al.* 2015).

However, the *Halymeniales* species contain significantly higher levels of polyunsaturated fatty acids than vegetables (Torres *et al.* 2019; Hurtado *et al.* 2020), considered essential nutritional components in humans and animals (Hurtado *et al.* 2020) and have been proposed as a chemotaxonomic tool to differentiate macroalgae (Torres *et al.* 2019). Fatty acid data revealed chemotaxonomic characteristics with C18 PUFAs (LA, ALA, and stearidonic acid or STA) being

higher in Chlorophyta, C20 PUFAs (AA and EPA) in Rhodophyta, while depicted even distribution of C18 and C20 PUFAs in Phaeophyta. Moreover, the green, red, and brown macroalgae acquired their ability of producing long-chain PUFAs from the coupling of the chloroplastic desaturase enzymatic systems of their respective endosymbionts and FA desaturase and elongase system of the eukaryotic host (Kumari *et al.* 2013). Some macroalgae present a low $\omega 6/\omega 3$ ratio; the $\omega 3$ polyunsaturated fatty acids (PUFAs) cannot be synthesized by humans and are thus obtained only through dietary sources (Torres *et al.* 2019). Their therapeutic role, especially EPA and other unsaturated fatty acids during pregnancy, has been crucial for transport across the placenta for the production of prostaglandins (Ramu Ganesan *et al.* 2020). EPA is also shown to reduce blood cholesterol and protect against cardiovascular and coronary heart diseases, anti-inflammatory, anti-thrombotic and anti-arrhythmic properties (Kumari *et al.* 2010; Torres *et al.* 2019). High DHA and unsaturated fatty acids will improve the fetus's visual acuity, mental development, and psychomotor skills (Ramu Ganesan *et al.* 2020).

5. Applications

For centuries, marine macroalgae within the entire algal group have been harvested in a wide variety along with coastal communities. Macroalgae were frequently used for domestic human consumption and are harvested to extract useful natural products such as hydrocolloids and pigments (Delaney *et al.* 2016; Trono and Largo, 2019). Macroalgae is a lucrative business, driven by economic rather than environmental conditions (Delaney *et al.* 2016). As the human population increases, so are the need to find alternative natural resources that could replace the demand for consumptive forms of needs (Henchion *et al.* 2017). It has compelled humanity to search more into the depth of the ocean (Murty and Banerjee, 2011). Aside from consumptive forms, macroalgae include non-consumptive forms such as medicine, fertilizer and animal feed, inputs into the

industrial process, and other purposes such as building materials (Delaney *et al.* 2016). The multitude of applications of seaweeds in various fields and industries led to a global increase in demand for the biomass of a variety of seaweed species. (Trono and Largo, 2019).

Algae have been subjected to many researches these past years, and some of them have been introduced and used in biotechnological, nutraceutical, and pharmaceutical applications. Biochemical constitutions of algae, especially the valorization of bioactives, is emphasized (Torres *et al.* 2019). These algae constituents, including proteins (with EAA) and minerals (iron and calcium), lipids, vitamins, and fibers, provide high nutritional values that are beneficial for humans. Because of their high nutritional and nutraceutical value, algae are consumed traditionally as food, as herbal medicine (Okolie *et al.* 2018), as an alternative to agricultural products to address the nutritional deficiencies of the current food (Leandro *et al.* 2020). Furthermore, they are also used as fodder, fertilizer (Okolie *et al.* 2018), fungicides, herbicides, dietary supplements, and as a resource of phycocolloids such as agar, alginate, and carrageenan for various industrial applications (Peng *et al.* 2015). It must be remembered that seaweeds and their extracts are very complex and, as such, there is no one compound which conveys the many beneficial properties, instead of that various seaweeds and their extracts contain many compounds which may work synergistically and antagonistically with biological processes (Hurtado *et al.* 2020).

5.1 Algae as vegetable in human consumption

Of the 221-seaweed species worldwide that have commercial value, only about ten are intensely cultivated, and use also as food such as brown seaweed (Phaeophyceae—*Saccharina japonica*, *Undaria pinnatifida*, and *Sargassum fusiforme*); red seaweed (Rhodophyta—*Porphyra* spp., *Euclima* ssp., *Kappaphycus alvarezii*, and *Gracilaria* spp.); and green seaweed (Chlorophyta—

Ulva clathrata, *Monostroma nitidum*, and *Caulerpa* spp.) (Pereira 2018a). Seaweeds in East Asia have been documented to have been harvested as consumptive and non-consumptive for thousands of years (6000 BCE–300 BCE) in Japan, (600 BC) in China, (AD 918–1392) in Korea (Dicks and Doll, 1983; Delaney *et al.* 2016), and the rest of the Pacific islands. The introduction of seaweeds in Europe as human food began in the 15th century, especially by littoral populations suffering from famine. Now, seaweeds as human food is widely distributed throughout the world, especially as a resource for the production of food, food additives, and nutritional supplements in Asia, Europe, North and South America, Africa, and Pacific Islands nations (Fleurence 2016).

Although nutritional values can vary and are widely affected by physical and environmental factors, edible macroalgae are mostly rich in bioactive antioxidants, soluble dietary fibers, proteins, minerals, vitamins, phytochemicals, and polyunsaturated fatty acids (Hamed *et al.* 2015; de Gaillande *et al.* 2017). Seaweeds are also very rich in essential minerals and trace elements required for the human diet compared to terrestrial foods due to their ability to maintain inorganic atoms from seawater (Hamed *et al.* 2015). Macroalgae have many uses; food for human consumption, ingredients in the manufacture of cosmetics and fertilizers, treated products for extracting gelling agents, or animal feed additives (de Gaillande *et al.* 2017). In brown algae, *Sargassum fusiforme*, also known as Hijiki is served as food, named as “vegetable for longevity” in food industries of Japan. It is a side dish prepared by soaking and boiling the dried algae with water and served with soy sauce (Liu *et al.* 2012). *S. polycystum* has been eaten as sea vegetables in Asian countries such as Malaysia and Vietnam (Hong *et al.* 2007; Matanjun *et al.* 2009). Macroalgae are mainly used as food in the form of fresh vegetables, and their popularity tends to be on the increase due to the general awareness of health-conscious consumers of the advantages of its natural products. Annual production of seaweed for direct (sea vegetables) and indirect (phycocolloids) human consumption is estimated at 2 million tonnes of dry matter, and 90% of this production is from China, Korea, Japan, Vietnam, Chile, the Philippines, Norway, France, Spain, and the United Kingdom (Fleurence, 2016).

The Philippines is the third-largest producer of seaweed in the world following China and Indonesia, where *Eucheuma* species is the most commercially significant. In 2015, 90% of the Philippines' farmed output comprised *Eucheuma* species, such as *E. cottonii* (*Kappaphycus*) and *E. denticulatum*. Also, about 80% of the *E. cottonii* (*Kappaphycus*) production comes from the Philippines (777 963 tons), and about 30% traded in dry matter (FAO, 2018). Several edible species of the green, brown, and red seaweeds' nutrient contents are shown in Table X.

Table X. Nutrient composition of different edible green, brown, and red seaweed species (% dw).

Species	Protein	Lipid	Carbohydrate	Ash	Moisture	Reference
<i>Caulerpa racemosa</i>	19.72	7.65	48.97	12.15	15.37	a
<i>C. lentillifera</i>	12.49	0.86	59.27	24.21	25.31	a
<i>C. cupressoides</i>	20.8	3.8	47.4	11.3	nd	b
<i>C. racemosa</i>	0.6–18.3	0.1–3.8	3.6–83.2	14.5–55.1	88.8–91.5	b
<i>C. sertularioides</i>	3.1; 11.96	2.3; 0.92	nd	2.9; 35.54	82.4, 88.2 ww	b, d
<i>Codium fragili</i>	8-11	0.5-1.5	39-67	21-39	91.4	c
<i>Ulva rigida</i>	6.40	0.30	18.10	52.00	-	a
<i>U. reticulata</i>	21.06	0.75	55.77	17.58	22.51	a
<i>U. lactuca</i>	7.06	1.64	14.60	55.40	10.60	a
<i>U. pertusa</i>	15.4	4.8	-	27.2	6.0	a
<i>U. intestinalis</i>	17.9	8.0	-	27.6	6.3	a
<i>U. expansa</i>	4.12	0.65	-	35.66	84.59 ww	d
<i>Fucus vesiculosus</i>	3-14	1.9	46.8	14-30	-	c
<i>L. digitata</i>	5-9	0.9-2.1	42-62	19-36	-	e
<i>Saccharina japonica</i>	7.5	1.0	51.9	26.63	-	c
<i>Sargassum fusiforme</i>	11.6	1.4	30.6	19.77	-	c
<i>Pyropia tenera</i>	33-47	0.7	44.3	20.5	-	c
<i>P. umbilicalis</i>	29-39	0.3	43	12	-	c
<i>P. yezoensis</i>	31-44	2.1	44.4	7.8	-	c
<i>H. durvillei</i>	13.02	1.29	53.65	39.54	12.4	f
<i>H. dilatata</i>	9.99	0.29	40.53	21.92	8.85	f
<i>K. alvarezii</i>	1.34	0.62	38.3	58	-	g

dw= dry weight; ww= wet weight of seaweeds; - = no data; nd= not determined. a= Alam Bhuiyan and Qureshi 2016; b= de Gaillande *et al.* 2017; c= Pereira *et al.* 2016; d= Osuna-Ruiz *et al.* 2019; e= Fleurence, 2016; f= Kho *et al.* 2016; g= Wanyonyi *et al.* 2017.

5.2 Algae as a source of phycocolloid

The brown algae processing industry in Europe is divided into two main categories, first is the alginic acid production, and second is the production for agriculture such as fertilizer and animal feeds. Colloids have been applied to multiple industrial applications, such as food processing, cosmetics, and pharmaceuticals. Alginate, carrageenan, and agar are widely known for their gelling, stabilizing, and thickening properties. These products have been commercialized as additives, with an average of 1 million tons of *wet algae* that are used every year to extract these three compounds, as shown in Fig. 13 (Frangoudes *et al.* 2012).

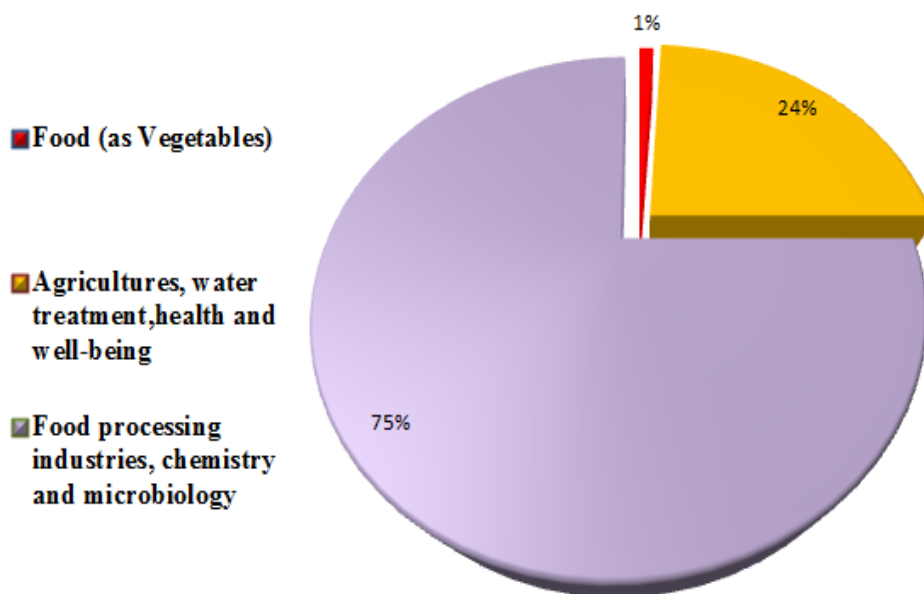


Figure 13. Utilization of algae in France (production and imports) according to CEVA 2009 (Mesnildrey *et al.* 2012).

Valuable products such as agar and carrageenan extracts are not immediately recognizable as human food since it is used in particular as thickening agents in beverages (de Gaillande *et al.* 2017). For more than four decades. There has been a lot of published works presented on economically important red

seaweeds, including the carrageenophytes – especially the genera *Kappaphycus* and *Eucheuma* (Hurtado *et al.* 2017). The amounts of total protein and profiles of amino acids in red seaweeds make them interesting sources for industrial food and even aquaculture feed sectors (Hamed *et al.* 2015; Baweja *et al.* 2016). Commercial carrageenans (used at the food level) have a high molecular weight, usually ranging from 400 to 600 kDa, with a minimum of 100 kDa (Baweja *et al.* 2016). Molecular forms of carrageenan (kappa, lambda, and iota) can be tailored for a wide variety of uses, as additives in the manufacture of dessert gels, especially for dairy products, fish gels, pizzas, barbecues, sauces, and medical and industrial applications (Dawes, 2016; Fleurence, 2016). Agar consists of two polymers (agarose and agaropectin) used in food processing such as baked goods, ice cream, candies, juices, wine, and vinegar (Dawes, 2016; Fleurence, 2016). Carrageenan contributes 15–20% in the use of food hydrocolloids globally; the most important algal sources are *C. crispus* harvested in the North Atlantic, and species of *Eucheuma* and *Kappaphycus*, farmed in the Philippines (C. Dawes, 2016; FAO, 2018). These species, *C. crispus* (in Ireland and the British Isles) and *Eucheuma*, and *Kappaphycus* (in the Philippines) are used directly as sea vegetables, in stews, or salads. The *K. alvarezii* (Doty) contains kappa-carrageenan, while *E. isiforme* (C. Agardh) contains iota-carrageenan (Dawes, 2016).

5.3 Active ingredients (cosmetics and health)

Viral infections, as this present pandemic that the world is facing for COVID-19, count as one of the most predominant cause of death in human life and the economy worldwide. So far this year, COVID-19 has killed more than 1.5 million people and forecasters predict that by the end of the year 2020, the pandemic death toll could rise to 1.9 million. If that happens, COVID-19 would rank as the 6th-deadliest disease in the world (WHO, 2019). Seaweeds are known rich source

for active antiviral metabolites. Extracted polysaccharides exhibit antiviral activity against a broad spectrum of viruses including; Human Immunodeficiency Virus (HIV), Herpes Simplex Virus (HSV), Vesicular stomatitis virus (VSV), Cytomegalovirus (CMV) (Bouhlal *et al.* 2011; Wang *et al.* 2014; Mahomoodally *et al.* 2019).

Algae proteins and their derivatives have anti-inflammatory, antioxidant, antitumor, anti-aging, and protective activity. They are also applied as a moisturizer to hair and skin. Amino acids are generally used as skin moisturizers since many are constituents of the natural moisturizing factor (NMF) in human skin (Pereira, 2018b). Xanthophylls (astaxanthin, fucoxanthin, loraxanthin, lutein, violaxanthin), and carotene (α -carotene, β -carotene, lycopene) are widely used as natural dyes and vigorous antioxidants with antitumor, anti-inflammatory and radical sequestering properties (Stengel *et al.* 2011; Pereira 2018a). Pro-vitamin A activity can also be found in carotenoids where it can remain active when standard vitamin C cannot, especially under low oxygen condition; β -carotene, protect the skin and eyes from photo-oxidation against UV radiation and prevent eye disease such as cataracts; Astaxanthin performs a variety of activities, e.g., UV-mediated photo-oxidation, tumors, and inflammatory processes; and fucoxanthin, a typical xanthophyll of brown algae with extraordinary antioxidant activity. Its main bioactivities are antimalarial, antitumor, anti-obesity, anti-inflammatory, antidiabetic, and antiangiogenic activities. It also has protective effects on the brain, skin, liver, blood vessels, bones, and eyes (Joshi *et al.* 2018; Pereira 2018a).

The fatty acid alpha-linolenic acid (ALA), the precursor to EPA and DHA were reported to inhibit the production of eicosanoids, lowering blood pressure, and blood triglyceride levels (Oomah and Mazza, 1999) while docosapentaenoic acid (DPA) was shown to inhibit platelet aggregation and has strong endothelial cell migration abilities (Kaur *et al.* 2011). Commercial ALA or hybrid liposomes of ALA ethyl ester (HL-ALAE) seemed to provide benefits against several health problems, e.g., decrease in blood cholesterol levels, reduction in the risk of heart attacks, and a growth reduction in breast, colon, and prostate cancer (Tanaka *et al.* 2008). Researchers have suggested that elevated blood pressure in adulthood may

be associated with peri-natal omega-3 fatty acid deficiency and that early exposure to dietary, long-chain omega-3 played a critical role in supporting heart health and reducing CVD risk in later life (Papanikolaou *et al.* 2014). Given the situation above, then it is safe to assume that consumption of recommended amounts of omega-3s as ALA in food, or in a supplement, would be generally beneficial to consumers.

5.3.1 *Caulerpa* and *Ulva* species

Antioxidant compounds from macroalgae (*Caulerpa* and *Ulva* spp.) have earned a reputation for protecting healthy cells against various diseases, aging processes, and considerable commercial potential in medicine, food production, and cosmetics industry (de Gaillande *et al.* 2017; Olasehinde *et al.* 2019). Several studies isolating *C. racemosa* as cited by de Gaillande *et al.* (2017) in her paper that Liu *et al.* (2013) found caulerprenylols A and B (new prenylated para-xylenes) which have antifungal and antitumoral activities, and the β -sitosterol which is a plant stanol known to reduce blood levels of cholesterol as it inhibits cholesterol absorption in the intestine was also isolated from *C. racemosa* (Ragasa *et al.* 2015). A toxic constituent called caulerpicin (from *C. racemosa*) has anesthetizing sensation and causes numbness of the tongue, lips and cold sensation in the fingers and the feet (Kumar and Sharma, 2020). Another compound Caulerpin has painkilling and anti-inflammatory properties (*C. racemosa*, *C. serrulata* and *C. sertularioides*) (de Gaillande *et al.* 2017), however, it also produces mild anesthetic action, difficulty in breathing, sedation and loss of balance (Kumar and Sharma, 2020). Simultaneously, Caulerpenyne is also known as a repellent against other organisms (grazers, epiphytes, competitors) and exhibiting anti-neoplastic, antibacterial, and anti-proliferative activities (de Gaillande *et al.* 2017). Tropical species of *Caulerpa* are highly variable in their production of caulerpenyne; for

example, in Guam are higher than those from the Mediterranean alga *C. sertularioides*. However, caulerpenyne also represents the most abundant cytotoxic sesquiterpenoid in *Caulerpa taxifolia*, showed ichthyotoxic activity, which blocked cleavage of developing sea urchin eggs and can induce apoptosis in mammalian (neuroblastoma) cells. Caulerpenyne also shows neurological activity in invertebrate model organisms (leeches) by modifying the electrophysiological properties of touch mechanosensory cells. These electrophysiological effects on neurons might explain human poisoning incidents after the fish *Sarpa salpa*, which eats *C. taxifolia*, was consumed as having neurological symptoms of amnesia, vertigo, and hallucinations. These findings may have implications about the neuroecological effects of caulerpenyne on other marine consumers. Caulerpenyne's cytotoxic activity is at the concentration of 60-90 μM leading to the destruction of human hematopoietic progenitors (melanocytes, keratinocytes and fibroblasts) but not toxic to normal melanocytes at $<10.5 \mu\text{M}$ and normal keratinocytes at $12.6 \mu\text{M}$ (Kumar and Sharma, 2020). It is important to remember the importance of monitoring *Caulerpa* species' toxicity before consumption (de Gaillande *et al.* 2017), as they exhibited significant increase in their toxic metabolites when eaten by predators, are then transferred to the marine food chain, resulting into toxicity to its predators (Kumar and Sharma, 2020).

5.3.2 *Sargassum* species

In brown algae, recent pharmacological research discovered that alginates isolated from *Sargassum* demonstrated anticancer (*in vivo*), antiviral (*in vitro*), and hypolipidemic (*in vivo*) activity (Mao *et al.* 2004; de Sousa *et al.* 2007; Sinha *et al.* 2010). Pharmacological properties of *Sargassum* crude extracts or isolated pure compounds have been widely recognized, including antibacterial, antifungal, antiviral, anticoagulant, antioxidant, antimelanogenic, anti-inflammatory,

hypoglycemic, hepatoprotective and neuroprotective activities (Puspita *et al.* 2017; Dobrinčić *et al.* 2020). This suggests that *Sargassum* is a rich source of health and promoting agents. Before these exceptional findings, *Sargassum* has been traditionally used as medicine in ancient times in China to treat goiter. *Sargassum* has been mentioned as "Hai Zao," It is believed that *Sargassum* could soften hard lumps, dispel nodes, eliminate phlegm, and induce urination in humans (Liu *et al.* 2012). In traditional medicine of China, *Sargassum* has been formulated in more than 226 prescriptions to treat various diseases. *S. pallidum* (Turner) C. Agardh and *S. fusiforme* (Harvey) Setchell have been listed as treatments for arteriosclerosis, skin diseases, high blood pressure, hepatolienomegaly, neurosis, angina pectoris, chronic bronchitis, and esophagitis. *S. fulvellum* (Turner), *S. henslowianum* C. Agardh, *S. thunbergii* (Mertens ex Roth) Kuntze, *S. horneri* (Turner) C. Agardh, commonly named as "Hai Qian" are another *Sargassum* species that are popular in traditional medicines and food ingredients in the southeast region of China. *Sargassum* was also used exclusively for medicinal purposes in ancient times in Monte Verde, South America (Dillehay *et al.* 2008). *Sargassum vulgare* C. Agardh and *Sargassum thunbergii* Yendo from Japan have been used as anthelmintics (Chapman, 1980; Kang *et al.* 2008). Records about the traditional use of *Sargassum* in medicine have made this genus interesting to explore. Thus, *Sargassum* has been the most extensively as alternative natural resources with economic value. Identified bioactive meroterpenoids such as 2-methyl-6-(3-methyl-7oxo-2,5-octadienyl)-1,4-benzoquinone and sargafuran (Kamei *et al.* 2009) with significant antibacterial activity ($2 \mu\text{g mL}^{-1} < \text{MIC} < 15 \mu\text{g mL}^{-1}$) could provide scientific evidence for the traditional therapeutic claims of *Sargassum*. The pharmacological activity of *Sargassum* extracts and research of its bioactive constituents provide scientific evidence that underpins the traditional therapeutical claims made for *Sargassum* such as furuncle and abscesses (Liu *et al.* 2012). The iodine in *Sargassum* was believed to play an important role in therapeutical function. However, the role of iodine may have been overestimated, and other bioactive metabolites may have made more significant contributions toward *Sargassum*'s therapeutic claims (Liu *et al.* 2012). Research suggests that *Sargassum* may play a

role as an immunomodulator (Yende *et al.* 2014). The antimalarial activity has been found in *Sargassum natans* (Linnaeus) Gaillon (Orhan *et al.* 2006). *S. hemiphyllum* lipophilic fraction of the dichloromethane/methanol extract exerted potent in vitro antiplasmodial activity ($IC_{50} = 2.8 \mu\text{g mL}^{-1}$; Lategan *et al.* 2009). Samee *et al.* (2009) showed that semipurified hydroethanolic extracts from *Sargassum* growing in Pakistan or China had an antihyaluronidase activity proportional to the contents of the phlorotannin contents the ethyl acetate fractions. Also, the IC_{50} of the extract from *Sargassum tennerimum* was similar to that of catechin. *Sargassum* in India is mostly exploited for its alginate production in hydrocolloid industries either for local or export (Subba Rao and Mantri 2006).

5.3.3 *Halymenia* species

Some particular red seaweed is reported to contain antihypertensive, anticoagulant, and antihemorrhagic compounds. Several scientific studies on the collective biomasses of carrageenophytes demonstrate their potential as sources of structurally diverse, biologically active compounds with potentially multiple pharmaceutical and biomedical applications (Pereira, 2018a). They also contain specific lectins, enzymes, and derivatives, such as peptides, which could present bioactive potential. Peptides from *Pyropia columbina* were reported as immunosuppressive and antihypertensive (Cian *et al.* 2012). Lectins from five species of red seaweeds of *Eucheuma* were reported to have strong hemagglutination activity. Lectins, in general, have also been reported to present many advantages, such as detection of disease-related to glycan synthesis, detection of infectious agents (viruses, bacteria, fungi, parasites), an antibiotic, mitogenic, and cytotoxic activities (Dumay and Morançais, 2016).

Recent studies on human gut microbes or host/microbe inter-dependence (dysbiosis) were reported to be associated with various pathologies, e.g., inflammation, obesity, hypertension, endothelial dysfunction, diabetes,

cardiovascular disease (CVD), colitis, and neurological disorders (Cornish *et al.* 2019). Since CVD is highly influenced by human food digestion, carbohydrate fermentation, energy harvesting and storage, gene expression, the secondary generation of beneficial compounds (cross-feeding), and the facilitation of metabolic functions, makes applications and utilization of therapeutic dietary impacts of seaweeds to be viable and essential for intervention strategies related to food manufacturing and consumption (Cornish *et al.* 2015, 2019).

A study of the human intestine (Caco-2) and hepatic (HepG2) cell lines showed apoptotic induction using *k*-carrageenan [from *Kappaphycus alvarezii* (cottonii)]. At the same time, no toxicity was observed in corresponding normal and cancerous intestinal and liver cell-lines, using non-degraded carrageenan (Ariffin *et al.* 2014). According to (Murad *et al.* 2015), a pro-apoptotic activity of ι -carrageenan [from *Eucheuma denticulatum* (spinosum)] was shown to be mediated by caspase-3, caspase-9, p53, Bax, and Bcl-2 genes within an MDA-MB-231 metastatic, human, breast cancer cell-line. Generally, the reported antiviral compounds from seaweeds include (Hurtado *et al.*, 2019):

- (a) carrageenans (such as λ -carrageenan and partially cyclicized μ/ι -carrageenan) against HSV-1 and 2 from *Gigartina skottsbergii* (Carlucci *et al.* 1997), from *Gymnogongrus griffithsiae* and *Cryptonemia crenulata* (Talarico *et al.* 2005),
- (b) DL-galactan hybrids, against HSV-1 and 2 from *G. griffithsiae* and *C. crenulata* (Talarico *et al.* 2005), and HSV-2 and Dengue virus type 2 (DEN-2) from *G. torulosus* (Pujol *et al.* 2002),
- (c) kainic and domoic acids, as anthelmintic agents from *Digenea simplex* and *Chondria armata* (Freile-Pelegrín and Tasdemir 2019),
- (d) major glycolipids: MGDG (from *Hydrolithon reinboldii*), DGDG (from *Sargassum horneri*), and SQDG (from *Gigartina tenella*, *Palmaria palmata*) as antibacterial, antitumor, antiviral and anti-protozoal agents (Plouguerné *et al.* 2014),
- (e) xylomannan sulfate, against HSV-1 from *Sebdenia polydactyla* (Ghosh *et al.* 2009), and

(f) galactan sulfate, and sulfated glucurono-galactan (from *Schizymenia binderi*), against HSV-1 and HSV-2, HIV, RSV, HCMV, Dengue virus, Pseudorabies virus, and Influenza A and B virii (Pujol *et al.* 2002; Talarico *et al.* 2005).

Studies revealed that the above compounds, with particular emphasis on carrageenans, inhibited the cytopathic effect of HIV. They prevented HIV-induced syncytium (giant cell) formation, as well as other sexually transmitted viruses, such as genital warts, human papillomavirus (HPV), and different strains of *Herpes simplex* virus Type 1 and 2 (HSV-1 and 2) (Mendis and Kim 2011). Reportedly, κ , and ι -carrageenan inhibited the human papillomavirus (HPV) infection process (Buck *et al.* 2006), and are potent inhibitors of the multiplication of the Dengue virii, Type-2 and Type-3 (DENV-2 and 3) (Talarico *et al.* 2005).

However, it must be remembered that seaweeds and their extracts are very complex. Although, even one compound conveys many beneficial properties; more so of that, various seaweeds and their extracts contain many compounds that may work synergistically or antagonistically with biological processes (Morán-Santibañez *et al.* 2016). Some of the bioactivity of extracts is a direct result of the chemical interactions between raw materials and the extraction processes. Several examples of health benefits with its functional ingredients taken from different species of green, brown, and red seaweeds that could at least represent the present study seaweed samples are shown in Table XI.

Table XI. Health benefits summary of green, brown, and red seaweeds (Hurtado *et al.*, 2019).

Species	Fraction	Health benefits	Category	Reference
<i>Codium fragile</i>	Galactan	Antiviral, Immunostimulant	Polysaccharides	a
<i>Chaetomorpha crassa</i> <i>Ulva pertusa</i>	Ca	Strengthen teeth and bone, Anti-osteoporosis	Minerals	a
<i>Enteromorpha compressa</i>	Zn	Antidepressant, Reduce miscarriage, Malformations	Minerals	a
<i>Ulva pertusa</i>	DHA, EPA	Antiallergic	Lipids	a
<i>Ulva reticulata</i>	Vit. C	Antioxidant, Improve immune system	Vitamins	a
<i>Ascophyllum nodosum</i> <i>Laminaria digitata</i>	I	Antigoiter, Reduce miscarriage, stillbirth	Minerals	a
<i>Ascophyllum nodosum</i> <i>Laminaria digitata</i>	Mg	Neuroprotective, Antidepressant, Antiasthmatic	Minerals	a
<i>Ascophyllum nodosum</i> <i>Laminaria digitata</i> <i>Undaria pinnatifida</i>	Vit. E	Antioxidant, Prevention of CVD	Vitamins	a
<i>Laminaria digitata</i> <i>Padina pavonica</i>	Vit. C	Antioxidant, Improve immune system	Vitamins	a
<i>Sargassum hornerii</i>	Protein extract	Antioxidant, Anticoagulant	Proteins	a
<i>S. ringgoldianum</i>	Zn	Antidepressant, Reduce miscarriage, Malformations	Minerals	a
<i>S. fulvellum</i>	B ₁₂	Anti-aging, Antianemia	Vitamins	a
<i>Laminaria japonica</i>	Fucoidan	Antioxidant, Anticoagulant	Polysaccharides	a
<i>Undaria pinnatifida</i>	DHA, EPA	Antiallergic	Lipids	a
<i>All classes of algae</i>	Chlorophylls	Antioxidant, Anticancer	Pigments	a
<i>Asparagopsis taxiformis</i>	Sulfated polysaccharide	Anticoagulant	Polysaccharides	b
<i>Gigartina skottsbergii</i>	Carrageenans, (1C ₃ , 1T ₁ , λ)	Anticoagulant, Antiviral (HSV-1 and 2)	Polysaccharides	c
<i>Gymnogongrus torulosus</i>	DL-galactan	Anticoagulant, HSV-2 and DENV-2	Polysaccharides	d
<i>Halymenia floresii</i>	polysaccharide	Anticoagulant	Polysaccharides	b
<i>Porphyra umbilicalis</i> <i>Palmaria palmata</i>	Vit. C	Antioxidant, Improve immune system	Vitamins	a
<i>Porphyra umbilicalis</i> <i>Palmaria palmata</i>	Vit. E	Antioxidant Prevention of CVD	Vitamins	a
<i>Porphyra umbilicalis</i> <i>Palmaria palmata</i>	I	Antigoiter, Reduce risk of abortion, stillbirth	Minerals	a

CVD, cardio vascular disease; 1C₃, cystocarpic fraction; 1T₁, tetrasporic fraction; HSV, herpes simplex virus; DENV, dengue virus; a= Hamed *et al.* 2015; b= Pereira *et al.* 2018a; c= Carlucci *et al.* 1997; d= Pujol *et al.* 2002.

Chapter 2 Materials and Methods

2.1 Sampling areas and abiotic factors

In this work, sampling areas were chosen in order to determine and compare the biochemical composition of different species of seaweed and their extracts along with their biological activities. The Philippines is divided into three (3) main group of islands: Luzon islands, the largest island located on the northern part of the archipelago; Visayas islands, are the scattered islands in the central Philippines; and the Mindanao islands, the second largest group located in the southern part of the archipelago. Illustration of the sampling location is described in Fig. 14 showing the selected seaweed species.

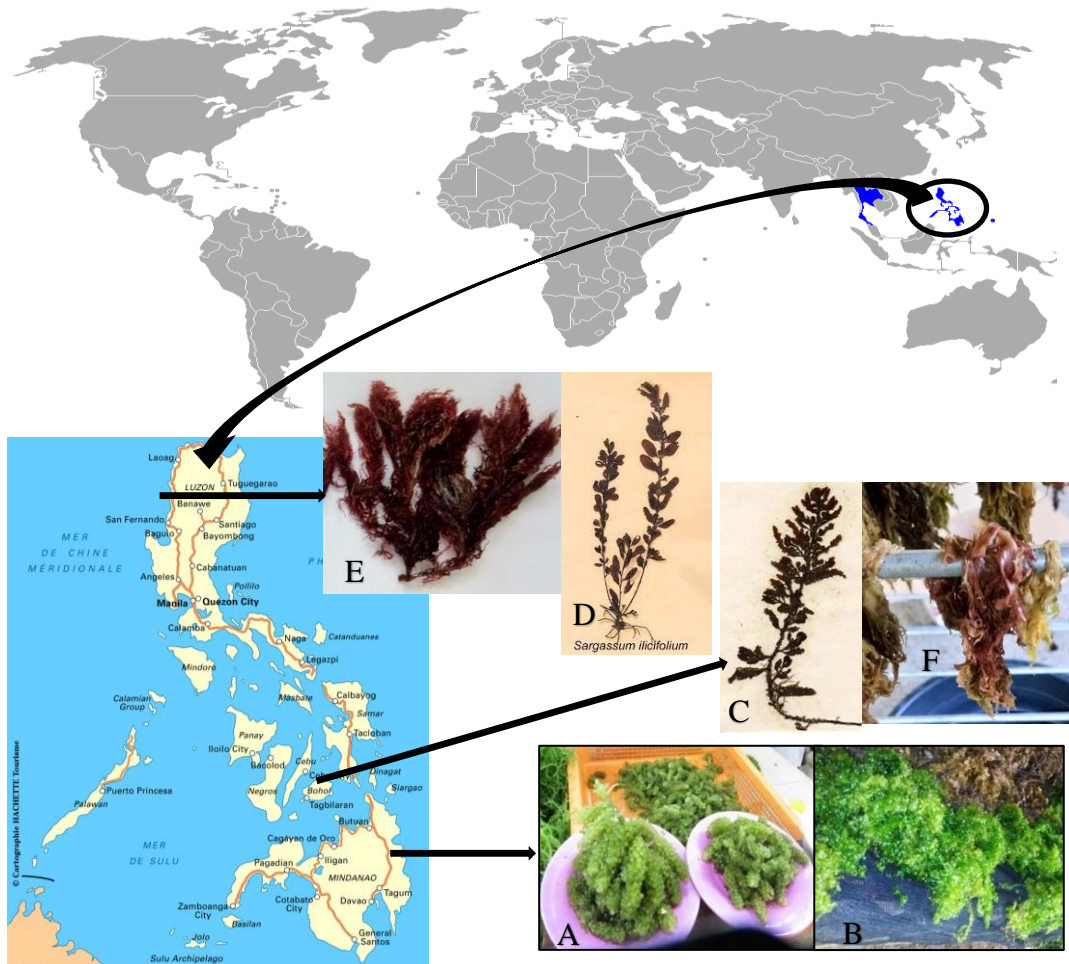


Figure 14. Perspective map of the world and sampling locations in the Philippines showing the site collection of selected species of green seaweeds (A) *Caulerpa racemosa* var. Forsskal, and (B) *Ulva lactuca* Linnaeus; brown seaweeds (C) *Sargassum polycystum* and (D) *Sargassum ilicifolium*; and red seaweeds (E) *Halymenia durvillei* and (F) *Halymenia dilatata*. It was collected from June 25 to August 16, 2017.

2.1.1 Overview of the studied location

The Philippines is a tropical archipelagic country with more than 7,000 islands located in the Indo-Pacific region at 12.8797° N and 121.7740° E. It has a tropical climate is, for the most part, hot and humid all year round. There are two seasons, the wet season (from June to November) and the dry season (from December to May). Philippines marine water covers an area of 220 million hectares, a shelf area of 18.6 million hectares, with a coastline length of 36,289 kilometers (<http://nast.ph/index.php/downloads/>).

It has an average temperature between 25.5 and 28.3 °C, with mean annual precipitation that varies (from 965 to 4,064 mm) occurred highest during the rainy season between June and November. Baguio City, eastern Samar, and eastern Surigao receive the highest amount of rainfall, while the southern part of Cotabato receives the least amount of rain. The combination of warm temperature and high relative, absolute humidity gives rise to sensible high temperatures throughout the archipelago. Maximum levels of temperature and humidity are mostly felt during March to May (<https://www1.pagasa.dost.gov.ph/index.php/climate-of-the-philippines>).

There were different sampling sites chosen to represent the main group of islands of Luzon, the Visayas, Mindanao (Fig. 15), and primary groups of green, brown, and red seaweed. From the **Luzon**, in Currimao coast, Ilocos Norte (18.021 N and 120.483 E), collected *Sargassum ilicifolium* (brown), and *Halymenia durvillei* (red). From the **Visayas**, in Lamanok island, Anda, Bohol (9.804° N and 124.596° E) collected *Sargassum polycystum* (brown). From the **Mindanao**, in the coastal area of Lawigan, Bislig City, Surigao del Sur (8.226° N and 126.431° E) collected *Caulerpa racemosa* var. Forsskal (green), *Ulva lactuca* Linnaeus (green), and *Halymenia dilatata* Delile (red).

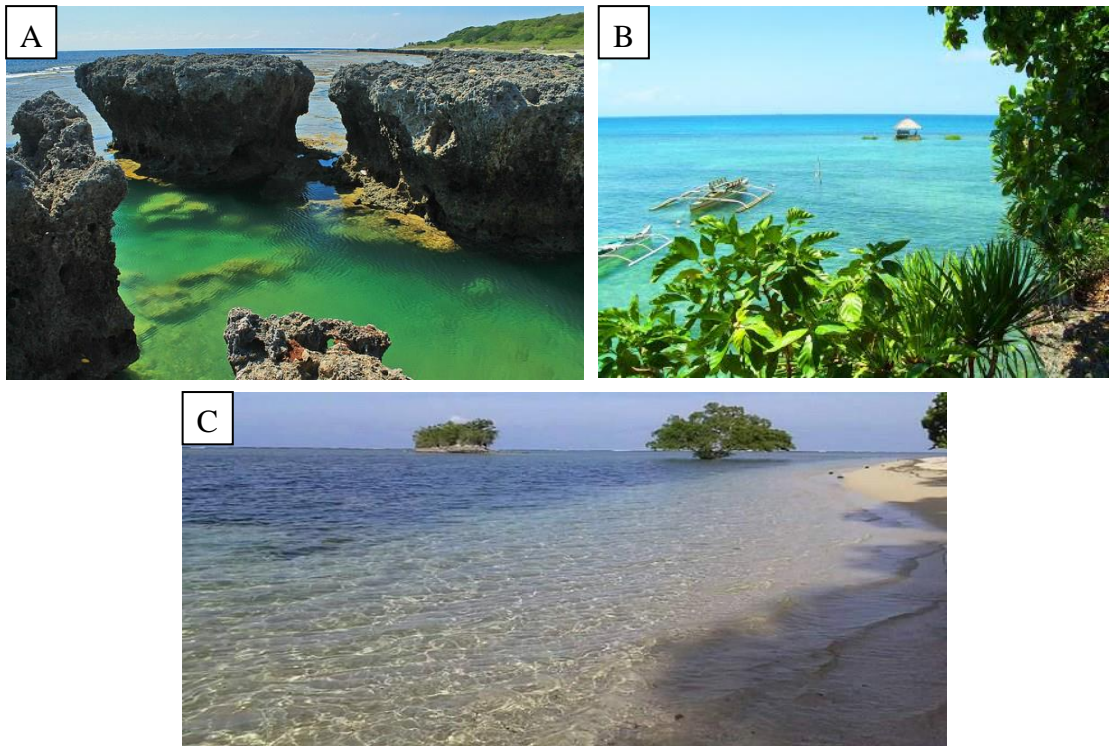


Figure 15. Sampling areas in the different parts of the Philippines (Above) during low tide at; Currimaos coast, Ilocos Norte in Luzon (A), Lamanok Island, Anda, Bohol in Visayas (B), and Lawigan coast, Bislig City in Mindanao (C). The collection is from June 25 to August 16, 2017.

2.1.2 *Samples preparation, drying, and grinding*

All seaweed samples were handpicked by cutting or pulling off the basal part of the thalli near the holdfast. Each sample of seaweed species collected from different patches or stations in each chosen site were pooled according to its kind. Samples were immediately washed with seawater after sampling and were put in a covered styroboxes to keep away from direct sunlight.

Algae *Sargassum ilicifolium* and *Halymenia durvillei* were collected last July 2017 in the subtidal area of Currimao coast, Province of Ilocos Norte, from 6:00 in the morning up to 3:00 p.m. (Fig. 16).



Figure 16. Sampling and collection of *S. ilicifolium* and *H. durvillei* during low tide in the coastal area of Currimao, Province of Ilocos Norte in Luzon. The collection was in July 2017.

The algae *Sargassum polycystum* was collected last August 22, 2017, in the subtidal area of Lamanok Island, Anda, in the Island Province of Bohol from 6:00 a.m. up to 3:00 p.m. (Fig. 17).



Figure 17. Sampling and collection of *Sargassum polycystum* during low tide in the coastal area of Lamanok Island, Anda, Island Province of Bohol in the Visayas. The collection was on August 22, 2017.

Furthermore, algae *Caulerpa racemosa*, *Ulva fasciata*, and *Halymenia dilatata* were collected last August 15-16, 2017, at the subtidal areas of the coast of barangay Lawigan, Bislig City, Province of Surigao Del Sur from 6:00 in the morning up to 3:00 p.m. (Fig. 18).

Collected algae from all selected sites were rinsed thoroughly with running freshwater from the remaining sand and epiphytes and left to dry under the shade avoiding sunlight for 1 to 2-weeks. The dried samples were then put in ziplock and was then transported to the Laboratoire de Biotechnologie et Chimie Marines (LBCM), in Université Bretagne Sud (UBS), Campus de Tohannic, Vannes, France.

Transported materials were then ground or cut into small pieces with either scissors or blender and were then freeze-dried in LBCM, and were stored in the dark for further analyses.



Figure 18. Sampling and collection of *Caulerpa racemosa*, *Ulva lactuca*, and *Halymenia dilatata* during low tide in the coastal area of barangay Lawigan, Bislig, Province of Surigao del Sur in Mindanao. The collection was 15-16 August 2017.

The water quality in all areas was taken *in-situ* for its salinity, temperature, pH, and dissolved oxygen. The summary of sampling and collection were shown in Table XII, and summary of processed samples and its respective analyses were shown in Table XIII.

Table XII. List of seaweed samples, location, date of collection, and mean water quality of the sampling area based on *in-situ* measurements.

Water Quality	Place Collected		
	Currimaos (Luzon)	Anda, Bohol (Visayas)	Lawigan, Bislig (Mindanao)
Salinity (‰)	34	36	34
Temperature (°C)	29	28	27
pH	7.0	8.0	8.16
Dissolved oxygen (DO/ mg L ⁻¹)	8.5	8.0	6.5
	Date and Species Collected		
	July 2017	22 August 2017	15-16 August 2017

<i>S. ilicifolium</i>	<i>S. polycystum</i>	<i>C. recemosa</i>
<i>H. durvillei</i>		<i>U. fasciata</i>
		<i>H. dilatata</i>

Table XIII. Summary table of samples processed for each analysis in the present study.

Species	Extract	Biochemical composition		Sugar units (HPAEC)		FTIR	Pigments HPLC	Anti-viral HSV-1	Anti-oxidant DPPH	Lipid	Fatty acids GC-FID	Minerals AAS	Amino Acid GC-FID	Location and Date collected
		raw	extract	raw	extract									
<i>C. recemosa</i>	HWE	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		Lawigan, Bislig, Mindanao (August 15, 2017)
	1OH	.	✓	.	✓	✓		✓	✓	
	4OH	.	✓	.	✓	✓		✓	✓	
	INS	.	✓	.	✓	✓		✓	✓	
<i>U. lactuca</i> (Linn.)	DS	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	Lawigan, Bislig, Mindanao (August 15, 2017)
	DA	.	✓	.	✓	✓		✓	✓	
	PP1	.	✓	.	✓	✓		✓	✓	
	PP2	.	✓	.	✓	✓		✓	✓	
	PP3	.	✓	.	✓	✓		✓	✓	
<i>S. polycystum</i>	Sp CaCl ₂	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	.	Anda, Bohol, Visayas (August 22, 2017)
	Sp HCl	.	✓	.	✓	✓		✓	✓	
	Sp Na ₂ CO ₃	.	✓	.	✓	✓		✓	✓	
	Sp KOH	.	✓	.	✓	✓		✓	✓	
<i>S. ilicifolium</i>	Si CaCl ₂	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	.	Currimao, I.N., Luzon (July 2017)
	Si HCl	.	✓	.	✓	✓		✓	✓	
	Si Na ₂ CO ₃	.	✓	.	✓	✓		✓	✓	
	Si KOH	.	✓	.	✓	✓		✓	✓	
<i>H. durvillei</i>	Hdur	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	Anda, Bohol, Visayas (August 22, 2017)
<i>H. dilatata</i>	Hdil	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		

2.2 Biochemical analyses

2.2.1 Acid and water extraction for the characterization of the raw material

Acid hydrolysis: to characterize the raw matter, 10 mg of freeze-dried matter were mixed with 5 mL of HCl 1 M in a sealed vial at 100 °C for 2 h, after which 5 mL NaOH 1 M were added. The final solution was to measure the protein, neutral sugar, uronic acid, and polyphenol contents. This solution was kept at 4 °C and should be used within a week for further analysis. The biochemical composition of *C. racemosa* (Cr), *U. fasciata* (Uf), *S. polycystum* (Sp), *S. ilicifolium* (Si), *H. durvillei* (Hdur), and *H. dilatata* (Hdil) was defined as percentage proportions of each chemical compound found in the total dry weight of each raw material. All analyses were done in triplicate.

Aqueous hydrolysis: Ten mg of freeze-dried, finely ground material in a mortar and pestle were introduced to 5mL of ultrapure H₂O in different vials. All prepared samples were then put in the heating block of 100 °C for 2 h with agitation every 30 min to optimize the process. Samples were left to cool for 5 min. In order to complete the concentration of the samples into 1 mg mL⁻¹, 5 mL of ultrapure H₂O was then added to each different vial. This solution was kept at 4 °C and should be used within a week for further analysis. The designated analysis for this solution is for the determination of sulfate groups (Burlot, 2016). Sulfated groups' content was determined by the Azure A method that reacts specifically with sulfates linked to the polysaccharides (Jaques *et al.* 1968). For this estimation, sulfated dextran (17%) (0–100 µg mL⁻¹) was used as the standard.

The final solution was used to measure the protein, uronic acid, neutral sugar, and polyphenol contents (Burlot, 2016).

2.2.2 Proteins and amino-acids content

The principle of this assay, according to the method of Smith *et al.* (1985), is detailed in Fig. 19

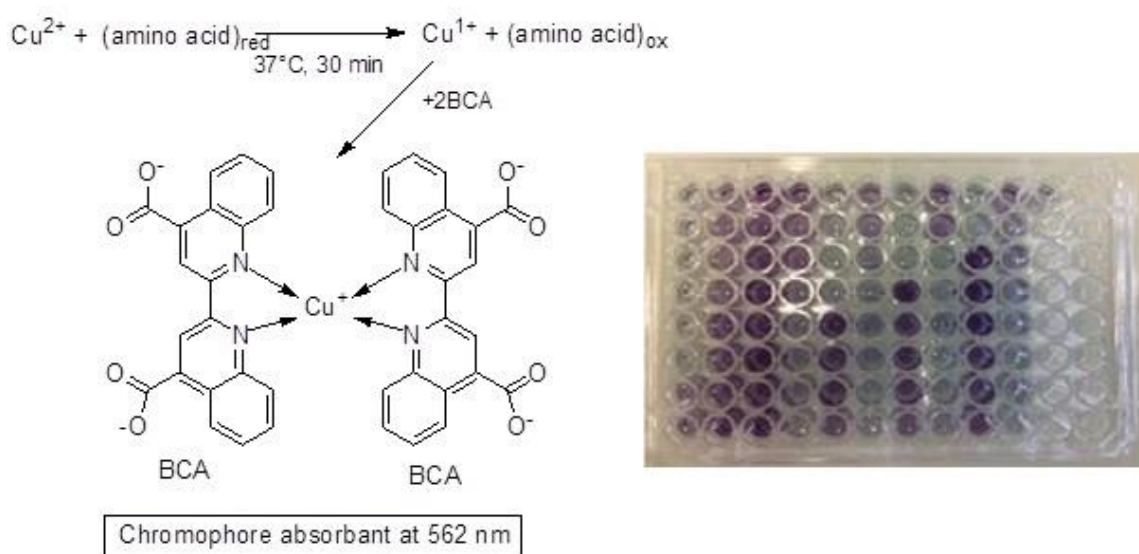


Figure 19. Principle and reaction of the protein assay by the method of Smith *et al.* (1985) (Burlot, 2016).

Principle: Dosage of total protein uses the BCA (BicinChoninic Acid) method based on the reaction of Biuret reagent, which results in the reduction of Cu^{2+} ion to Cu^+ by the protein in the alkaline medium. The cuprous ions will then be chelated by 4,4'-dicarboxy-2,2'-biquinoline (bicinchoninic acid, BCA) to form an absorbent chromophore $\lambda = 562 \text{ nm}$.

Standards, reagents and samples:

- BSA solution (bovine serum albumin) (2 mg mL^{-1} , Pierce BCA Protein Assay Kit, Thermo Scientific, 23227) to be diluted in ultra-pure water (Purelab Ultra, ELGA, France) at 1 mg mL^{-1} .
- Pierce BCA Protein Assay Kit, (Thermo Scientific, 23228)

- Sample to be analyzed at 1 mg mL⁻¹, the dilution factor to be determined so that the ODs measured are among those in the standard range.

Procedure: Twenty-five µL of standards (Bovine Serum Albumin) or samples (diluted if necessary) was introduced into the microplate 96 wells then added with 200 µL of reactive A + B (Pierce BCA protein assay kit, Thermo Scientific, 23228; Interchim, Montluçon, France). The microplate was then incubated for 30 min at 37 °C. Optical density was then measured at 562 nm. Bovine Serum Albumin was prepared in different concentrations ranged before each run (050, 100, 150, 200, 250 µg mL⁻¹). All samples were made in triplicate. Content of protein was calculated using the following formula:

$$\% = \left[\frac{(OD - b)}{a} \right] \times D \times \frac{1}{1000} \times 100$$

where:

- OD is the optical density of the designated analysis,
- b is the intercept of the calibration curve,
- a is the slope of the calibration curve,
- D is 1/factor of dilution,
- 1/1000 is the conversion of mg from µg, 100 is from 1 mg samples and conversion to %

(Smith *et al.* 1985; Kessler and Fanestil, 1986; Wiechelman *et al.* 1988; Brown and Jeffrey, 1992).

2.2.2.1 Amino-acids content

The amino acid composition of the raw seaweed materials (5-25 nM of protein) and or its purified fractions were determined by gas chromatography (GC) after acid hydrolysis of the samples with 6 M HCl 110 °C for 24 h, for microcharacterization of proteins. While this method gives good results for a majority of amino acids, there are several amino acids that are either partially or completely destroyed by such method. While 6 M HCl acid hydrolysis is the most common procedure, there are several limitations to this method. ASN and GLN are deamidated to ASP and GLU, and thus are quantitated as a mixture. Peptide bonds of hydrophobic amino acids (VAL, ILE, LEU) may be difficult to break and require additional hydrolysis time (upto 72 hours). Residual oxygen in the hydrolysis vessel can increase the thermal breakdown of hydrogen and sulfur containing amino acids (typical recoveries for SER, THR, HYP, and TYR range between 50-90%, MET ranges from 25-75%). Reducing hydrolysis time improves recoveries but reduces other amino acid yields (see above). GLY yields tend to exceed 100% (especially for low level samples) due to background protein contamination. Finally, both TRP and CYS are completely destroyed by acid hydrolysis and must be analyzed by alternate methods. The above listed limitations are based on hydrolysis chemistry and are not related at all to the EZ: faast process. The Amino acid samples were prepared according to the procedure recommended by the kit of EZ™ faast assay (Phenomenex, Torrance, California, USA). The preparation consisted of a solid-phase extraction step followed by derivatization and, finally, a liquid/liquid extraction. Amino acid samples were analyzed on gas chromatography coupled to a flame ionization detector (GC-FID, TRACE™ 1300, Thermo-Fisher Scientific™, Milan, Italy). The system consisted of a Zebron ZB-AAA-GC column (10 m x 0.25 mm, Agilent, CA, USA). Two µL of samples were introduced into the injector (250 °C.) with a split ration of 1:10 and separated into using the following program: 110 °C to 320°C (increase 32 °C min⁻¹), held for 2 minutes. The temperature detector was set at 320°C and the gas pressure at 1.7bar.

Nitrogen gas was used as the carrier gas. The signals were recorded using Chromeleon™ 7.2 software (DIONEX™, Thermo-Fisher Scientific™, Sunnyvale, CA., USA). Amino acids have been identified by their time of retention and quantified by their response factor relative to Norvaline internal standard added at a concentration of 200 μmol L⁻¹.

2.2.3 Total sugars content analysis

The principle of the determination of total sugars, according to the method of Dubois *et al.* 1956, is shown in Fig. 20 below.

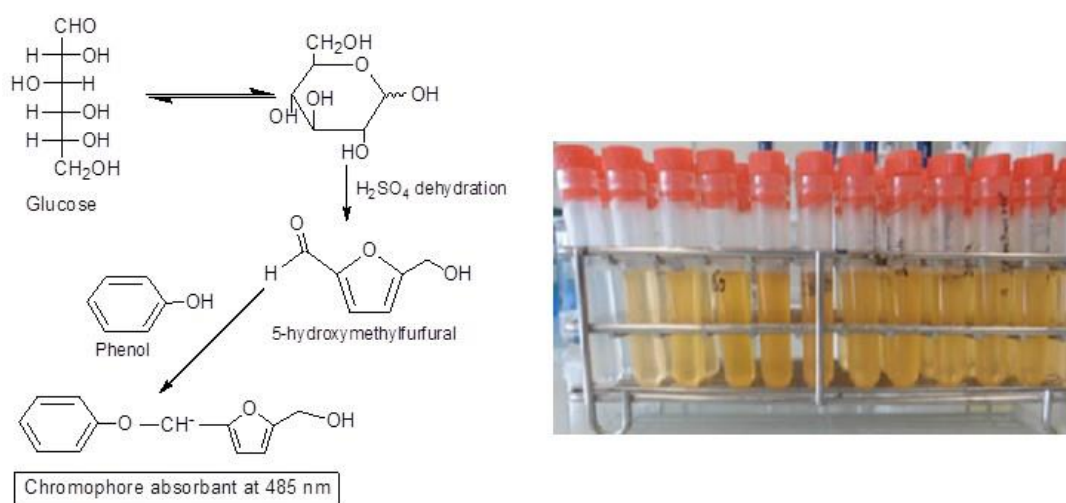


Figure 20. Principle and reaction of the determination of total sugars by the method of Dubois *et al.* (1956) (Burlot, 2016).

Principle: The principle of this analysis is based on dehydration of hexoses and pentoses (in Fig. 10, glucose is exemplified as hexose) under the action of hot and concentrated mineral acid cyclization forming derivatives of furfural like 5-hydroxymethylfurfural, which reacts with phenol. So, a yellow complex is formed if there is a presence of sugars. This complex concentration is measured by

spectrophotometer (UV-1800 Spectrophotometer, Shimadzu Europe GmbH, Germany).

Standards, reagents, and samples:

- D-Glucose monohydrate (Fisher Scientific) in ultra-pure water (Purelab Ultra, ELGA, France) at 1mg mL⁻¹.
- 75% phenol solution (Fisher Scientific), 96% sulfuric acid (Across organics, 302070010)
- Sample to be analyzed at 1 mg mL⁻¹ from acid extraction, the dilution factor of which is determined so that the measured ODs are among those in the standard range.

Procedure: One mL of samples or standard was put in the tubes, then added with 50 µL of phenol 75% and followed by agitation by using the vortex. 2.5 mL of sulfuric acid was added, and the mixture was then cooled (exothermic reaction) in the cold water of 20 °C for 10 min. After 10 min, all samples were agitated and placed in the water bath of 30°C for 10 min. Optical density is read against the blank at 490 nm. A standard range of glucose is performed before each series of analyzes (0, 20, 40, 60, 80, and 100 µg mL⁻¹. All samples were made in triplicate (Dubois *et al.* 1956).

The concentration and total sugar content are calculated with the rate of dry matter of the sample, according to the following equation:

$$\% = \left[\frac{(OD - b)}{a} \right] \times D \times \frac{1}{1000} \times 100$$

where:

- OD is the optical density of the designated analysis,
- b is the intercept of the calibration curve,
- a is the slope of the calibration curve,
- D is 1/factor of dilution,

- 1/1000 is the conversion of mg from μg , 100 is from 1 mg samples and conversion to %

(Smith *et al.* 1985; Kessler and Fanestil, 1986; Wiechelman *et al.* 1988; Brown and Jeffrey, 1992).

2.2.4 Uronic acid content analysis

The dosage of uronic acids, inspired by the method of Blumenkrantz and Asboe-Hansen (1973) modified by Filisetti-Cozzy *et al.* (1991) and cited by Burlot (2016) is shown in Fig. 21 below.

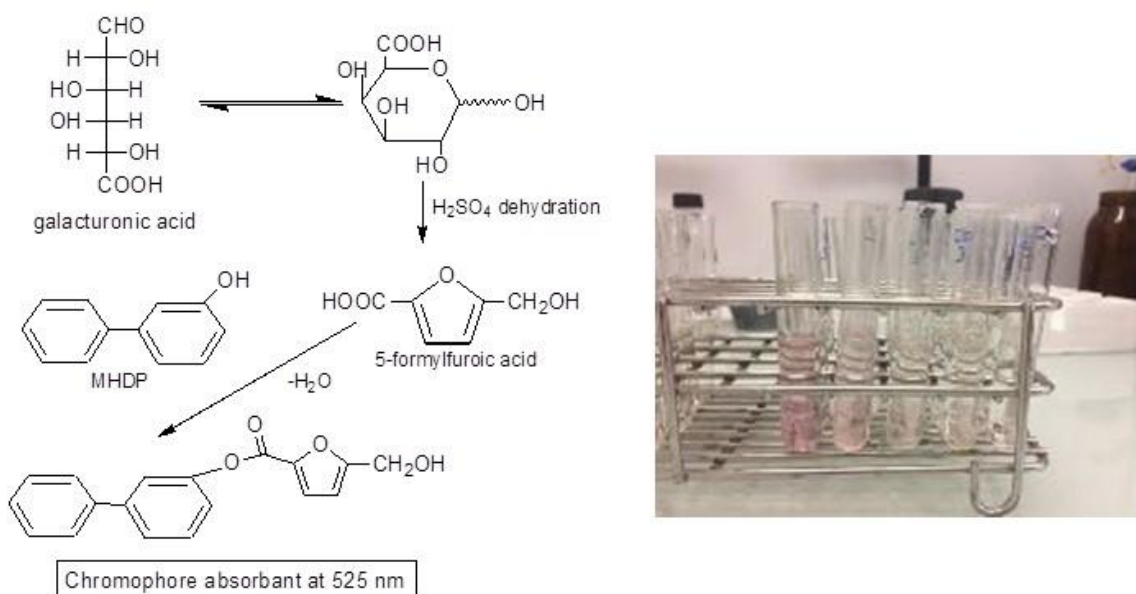


Figure 21. Principle and reaction of the dosage of uronic acids by the method of Blumenkrantz and Asboe-Hansen (1973) modified by Filisetti-Cozzy *et al.* (1991) (Burlot, 2016).

Principle: Under the action of a hot concentrated strong acid, the uronic acids undergo dehydration than a cyclization. 5-formylfuroic acid derivatives are formed. These derivatives in contact with meta-hydroxydiphenyl (MHDP) form a

chromophore of color pink absorbent at $\lambda = 525$ nm. The coloring is more intense in the presence of borate but remains very sensitive to interferences due to neutral sugars, which are stained in the presence of concentrated sulfuric acid. This is why potassium sulfamate is added to limit these interferences.

Uronic acid could react to the presence of meta-hydroxydiphenyl (MHDP), and this reaction creates a pink coloration. To enhance the coloration, tetraborate is added, and the addition of sulfamic acid minimizes the interferences of neutral sugars detection in the presence of sulfuric acid (Blumenkrantz and Asboe-Hansen, 1973).

Standards, reagents, and samples:

- Glucuronic acid (Sigma-Aldrich G5269) in ultrapure water (Purelab Ultra, ELGA, France) at 1 mg mL^{-1} .
- The solution of potassium sulfamate 98% at 4 M (Alfa Aesar, B24447), solution of tetraborate 75 M sodium (Merk, 524 A12408) diluted in 96% sulfuric acid, solution of 0.15% MHDP in 0.5% sodium hydroxide (Fisher Scientific 11983223)
- Sample to be analyzed at 1 mg mL^{-1} from acid extraction, the dilution factor of is determined so that the measured ODs are among those in the standard range.

Procedure: Diluted samples solution or standard of 200 μL are put in vials and added with 20 μL of 98% sulfamic acid 4 M. Before introducing 1.2 mL of sodium tetraborate 75 mM, the solution was agitated and re-agitated afterward. All samples were capped and incubated for 20 min at 80 °C then put in the ice for 5 min. Then, 40 μL of MHDP 0.15% was then added. Leave the solution at ambient temperature for 10 min for the reaction to develop before reading the optical density (OD). The optical density was read at 525 nm. Glucuronic acid with concentrations ranged from 0-200 $\mu\text{g mL}^{-1}$ served as standards for the calibration curve. A standard range of glucuronic acid is carried out before each analysis (0, 20, 40, 60, 80, and 100 $\mu\text{g mL}^{-1}$) (Guillaume, 2010).

The concentration and total sugar content are calculated with the rate of dry matter of the sample, according to the following equation:

$$\% = \left[\frac{(OD - b)}{a} \right] \times D \times \frac{1}{1000} \times 100$$

where:

- OD is the optical density of the designated analysis,
- b is the intercept of the calibration curve,
- a is the slope of the calibration curve,
- D is 1/factor of dilution,
- 1/1000 is the conversion of mg from μg , 100 is from 1 mg samples and conversion to %

(Smith *et al.* 1985; Kessler and Fanestil, 1986; Wiechelmann *et al.* 1988; Brown and Jeffrey, 1992).

2.2.5 Polyphenol content analysis

The principle of the determination of polyphenols, according to the method of Turkmen *et al.* 2005, is shown in Fig. 22 below.

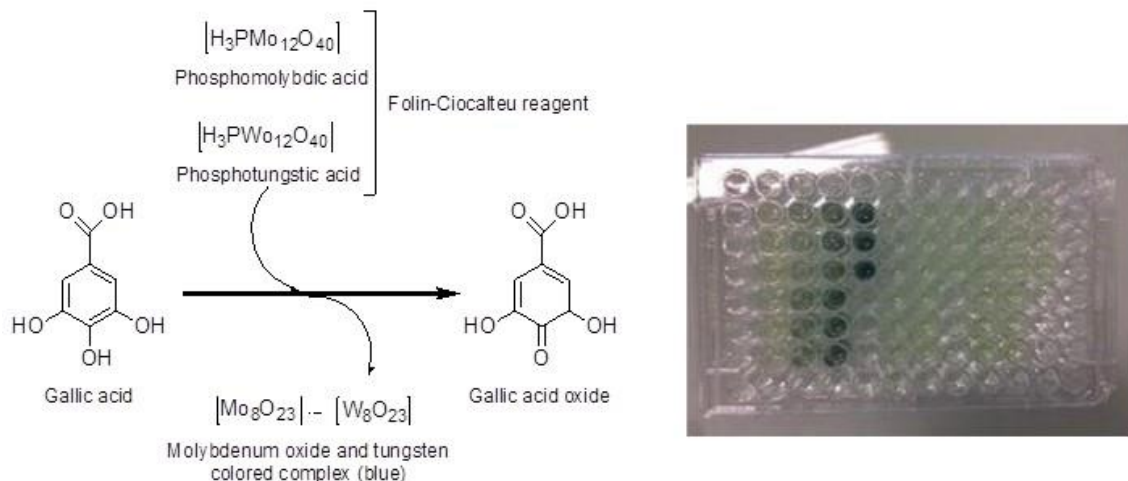


Figure 22. Colorimetric determination of total phenols by the Folin-Ciocalteu (Hardouin, 2015; Burlot, 2016).

Principle: The samples' polyphenol content was determined by a method adapted from Folin-Ciocalteu (Turkmen *et al.* 2005). This assay method is based on the oxidation of the phenolic compounds by the Folin-Ciocalteu reagent. The reagent is composed of a mixture of phosphotungstic acid and phosphomolybdic acid. The reduction of these acids by phenolic compounds causes the appearance of a mixture of oxides of tungsten and molybdenum, blue. Gallic acid is used as a standard for calibration, and the results are expressed in gallic acid equivalents (EAG) per gram of extract (Wang *et al.* 2010).

Folin-Ciocalteu is a commonly used method for measuring the polyphenol content. This assay is based on the reduction-oxidation (redox) reactions, which are usually considered to be relatively stoichiometric and on the redox potential of the polyphenol hydroxyl group (Hagerman and Butler, 1989).

Standards, reagents, and samples:

- Sodium carbonate 5%: 200 g of Na₂CO₃ dissolved in 1L ultrapure water (Purelab Ultra, ELGA, France).
- Gallic acid solution at 1mg mL⁻¹: 10 mg Gallic acid in 50% methanol to make 10 mL.
- 50% Folin-Ciocalteu reagent solution in distilled water: 10 mL reagent with 10 mL distilled water. Note! 100% Folin-Ciocalteu reagent is used for the microplate method.
- Sample to be analyzed at 1 mg mL⁻¹, the dilution factor to be determined so that the ODs measured are among those in the standard range.

The procedure of 20 µL of samples (0.25 mg mL⁻¹) or standard was added with 130 µL of ultrapure water. Followed by 40 µL of Folin-Ciocalteu was then introduced to the samples or standard solution. In the end, 40 µL of 200g Na₂CO₃ solution was added. The reaction was then incubated at 70 °C for 10 min and put

in the box of ice to stop the reaction for 5 min. Then the optical density was read against the blank prepared at 620 nm. A standard range of gallic acid is carried out before each series of analyses (0, 20, 40, 60, 80, and 100 $\mu\text{g mL}^{-1}$). All samples were made in triplicate.

The contents of the phenolic groups are calculated according to the following equation:

$$\% = \left[\frac{(OD - b)}{a} \right] \times D \times \frac{1}{1000} \times 100$$

where:

- OD is the optical density of the designated analysis,
- b is the intercept of the calibration curve,
- a is the slope of the calibration curve,
- D is 1/factor of dilution,
- 1/1000 is the conversion of mg from μg , 100 is from 1 mg samples and conversion to %

(Smith *et al.* 1985; Kessler and Fanestil, 1986; Wiechelman *et al.* 1988; Brown and Jeffrey, 1992).

2.2.6 Sulfates group content analysis

The amount of sulfates bound to the polysaccharides was determined by the use of the colorimetric assay method developed by Jaques *et al.* (1968). The principle and Chemical reactions are shown in Fig. 23 below.

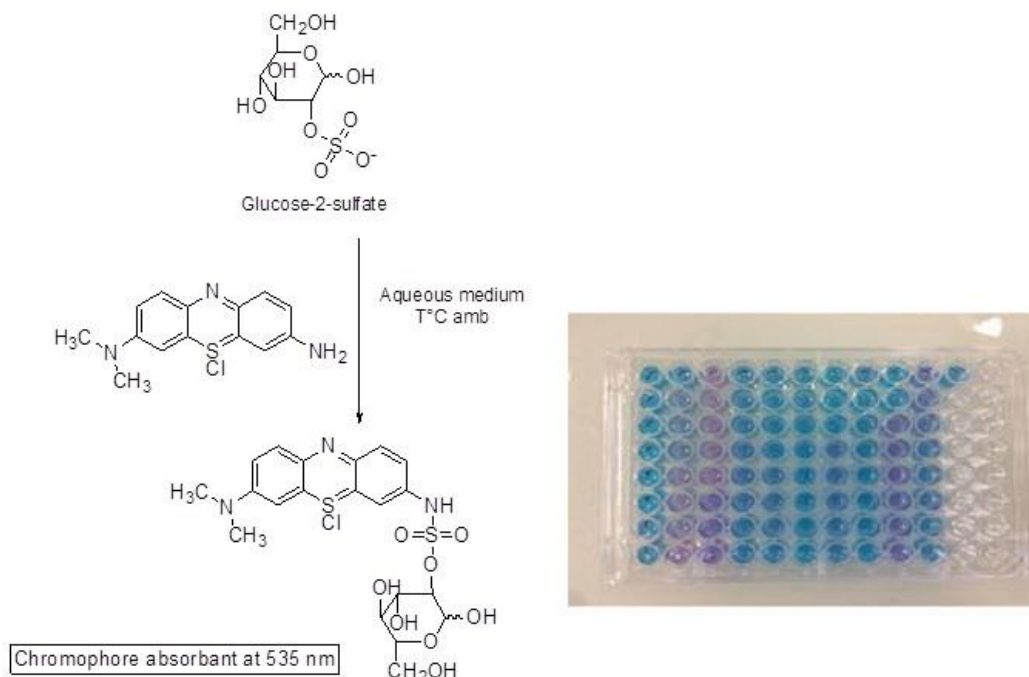


Figure 23. Principle and reaction of the determination of polysaccharide-linked sulfate groups by the method of Jaques *et al.* (1968) (Burlot, 2016).

Principle: The reaction, shown in Fig. 2-10, shows that in an aqueous medium, 3-amino-7-(dimethylamino) phenothiazin-5-ium chloride (Azure A) will complex the sulfate groups. The chromophore complex emits a violet color measurable at a spectrophotometer at $\lambda = 535 \text{ nm}$.

Standards, reagents and samples:

- A solution of 17% dextran sulfate (Sigma-Aldrich D6049) in ultra-pure water (Purelab Ultra, ELGA, France) at 1 mg mL^{-1} .
- Azure A solution (Sigma-Aldrich A6270) at 10 mg L^{-1} is prepared in ultrapure water.
- Sample to be analyzed at 1 mg mL^{-1} from extraction with ultrapure water, including the dilution is to be determined so that the measured ODs are among those in the range standard.

Procedure: In a 96-well microplate, 20 μL of the sample (diluted if necessary) and the standards are mixed with 200 μL of Azure A aqueous solution at 10 mg L^{-1} . The concentration of sulfate groups is determined from the linear line of the range standard made with 17% sulfate dextran (0, 10, 20, 30, 40, 50 $\mu\text{g mL}^{-1}$). Optical density is read against the blank at 535 nm. All samples were made in triplicate.

The contents of the sulfate groups linked to the polysaccharides are calculated according to the following equation:

$$\% = \left[\frac{(OD - b)}{(a \times D \times 0.17)} \right] / [32 \times 96 \times 0.1]$$

where:

- The average OD is calculated by subtracting the OD from the blank (0 $\mu\text{g mL}^{-1}$ of sulfated dextran,
- b is the intercept of the calibration curve,
- a is the slope of the calibration curve,
- D is 1/factor of dilution, and the dilution factor is taken into account with a sample solution at 1 g L^{-1} ,
- The multiplying factor 0.17 is used to obtain the sulfur concentration in the sample, knowing that dextran is sulfated to 17%,
- 32 is the molar mass in g mol^{-1} of sulfur,
- In this reaction, it is considered that one mole of sulfur equals one mole of sulfate. Multiply by 96 to change to the mass concentration of sulfate,
- The multiplicative factor 0.1 is a unit conversion factor.

2.2.7 Ash content analysis

Ash is the general term used to describe the inorganic matter in fuel. In biomass fuels, the ash content may originate from the biomass itself, *e.g.*, materials that the plant absorbed from the water during its growth. In food analysis, ash content represents the total mineral content. Ashing is the first step in preparing a food sample for specific elemental analysis. Since certain foods are high in particular minerals, ash becomes important (Marshall, 2010).

The principle is to incinerate materials at high temperatures (585 °C or higher). A hundred mg of dry material was put in the oven of 100 °C for 30 min then burnt on the Bunsen flame for 10 min. Later, the samples were kept in the incinerator (Carbolites FurnacesCSF, UK) at 585 °C for 3 h (Fig. 24A). Before measure their final weight, all samples were put in the desiccators for 1 h (Fig. 24B).

$$\% = \frac{M_f}{M_i} \times 100$$

where: M_f = final mass of algae (g);
 M_i = initial mass of algae (g)

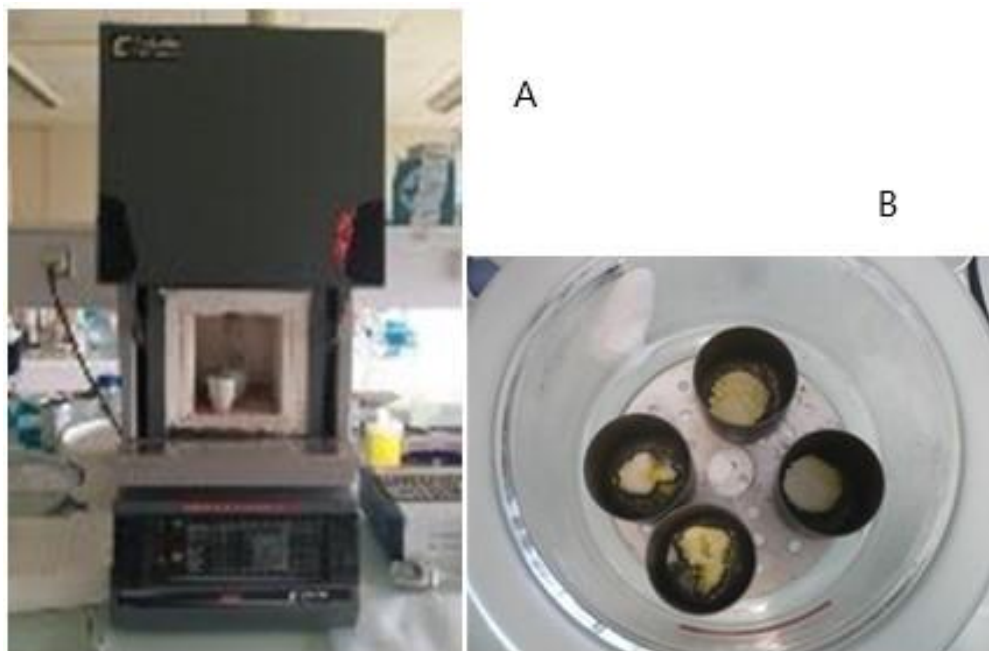


Figure 24. Incineration of the organic material of the samples in a muffle furnace at 585 °C (A), and obtaining the mineral material (B).

2.2.8 *Lipid and fatty acid methyl ester (FAME) analysis*

The total lipids extraction protocol was based following the method of Bligh and Dyer (1959) and Kendel *et al.* (2015) with modifications (Fig. 25). Lipids were extracted from 10 grams of freeze-dried algae (crushed) with a 30-mL mixture of chloroform/methanol (1:1, v/v) and were stirred for over 12 hours at room temperature under agitation. The process was repeated three times. The extract was filtered using a Büchner funnel and washed with saturated brine (100 mL), dried over magnesium sulfate, and evaporated under reduced pressure. Lipid content was determined gravimetrically as a percentage of the seaweed dry weight before it was saponified, and esterified according to the method of Terme *et al.* (2017).

The proportion of lipids relative to the dry mass corresponds to the weight ratio of the total lipid extract and biomass. The percentage (%) of lipid was calculated according to the following equation:

$$Fat(\%) = \frac{total\ lipid}{(total\ lipid + dry\ mass\ without\ lipid)} \times 100$$

where;

for example, the mass of dry *C. racemosa* Forsskal and *U. lactuca* Linnaeus = 10.0 g each; Mass of the lipid content of *C. racemosa* Forsskal = 448.5 mg; Mass of the lipid content of *U. lactuca* Linnaeus = 408.6 mg.

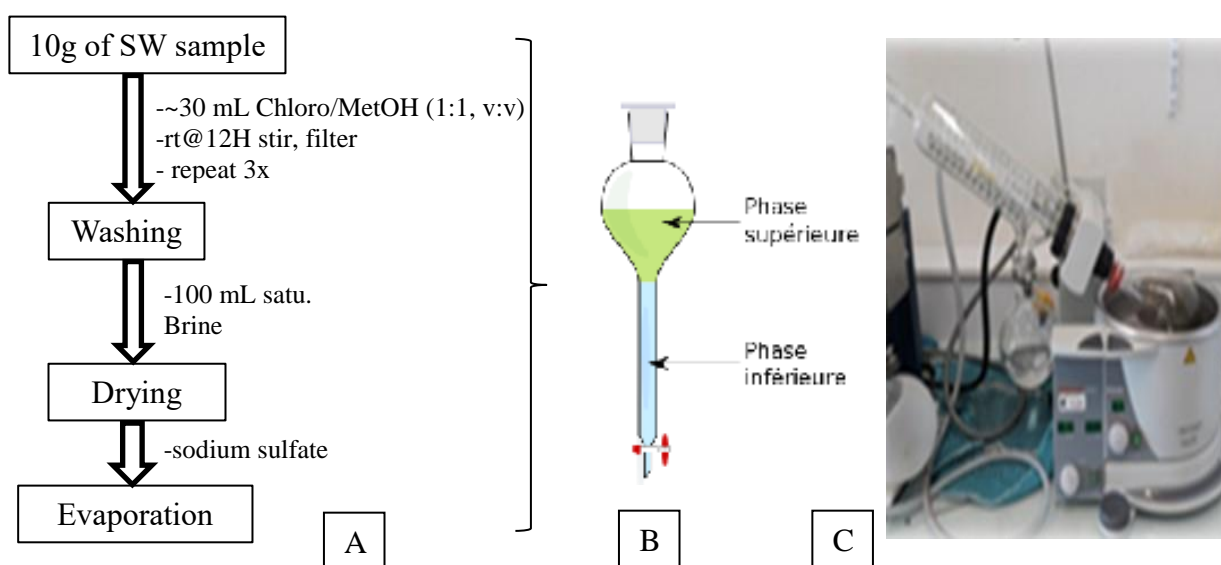


Figure 25. The first treatment of total or partition lipid extraction common to all groups of algae (A), washing and drying (B), and evaporation (C).

Saponification: A part of lipids (30 mg) from each sample was saponified with 2 M ethanolic potassium hydroxide mixture into the round flask with a magnetic stirrer submerged in a metal basin with water (Fig. 26A). Set into maximum heat under a heat plate until it reached the boiling point of the solvent ethanol (78°C). Time was

recorded, and the temperature was lowered just enough for condensation. The heat plate was turned off after one and a half-hour condensation and cool the sample for thirty minutes at room temperature. Water was then added for layer separation, and hexane/diethyl ether mixture (1:1, v/v) was then added in the round flask for the extraction of aqueous and organic phases. Extraction was done four times. Each volume added is twice the amount of the initial ethanol volume. The aqueous phase containing potassium salts of fatty acids (FAs) was acidified by 6 M HCl (pH = 4–5), and FA was extracted by hexane/diethyl ether mixture (1:1, v/v), washed with water, dried over magnesium sulfate, and evaporated under reduced pressure.

Esterification: FAs were then esterified with methanolic hydrogen chloride mixture into the round flask with a magnetic stirrer (Fig. 26B). Five percent sodium chloride with water was then added for washing and was extracted by hexane. Extraction was done four times. Each volume added is thrice the amount of the initial methanolic hydrogen chloride mixture. The organic phase was added with 2% sodium bicarbonate in water, dried over magnesium sulfate, and evaporated under reduced pressure. Finally, the obtained fatty acids and its FAME were then diluted to hexane at a ratio of 50 mg mL⁻¹ and were analyzed by gas chromatography coupled with flame ionization detection (GC-FID, TRACE™ 1300, ThermoFischer Scientific™, Milan, Italy), equipped with a capillary column CP-Sil 5 CB (60 m × 0.25 mm × 0.25 μm), the carrier gas was nitrogen (0.5 mL·min⁻¹). The injector and detector were set at 250 °C, and a temperature gradient was used for FAME analysis: the temperature was held at 170 °C for 4 min and programmed to 300 °C at 4 °C min⁻¹. The FAs were then identified by comparing their equivalent chain lengths (ECLs) values and their retention time with commercial mixtures (Supelco 37 Component FAME Mix, ThermoScientific, Illkirch, France). The ECLs of FAs were determined by expressing their elution positions relative to known straight-chain saturated FAs.

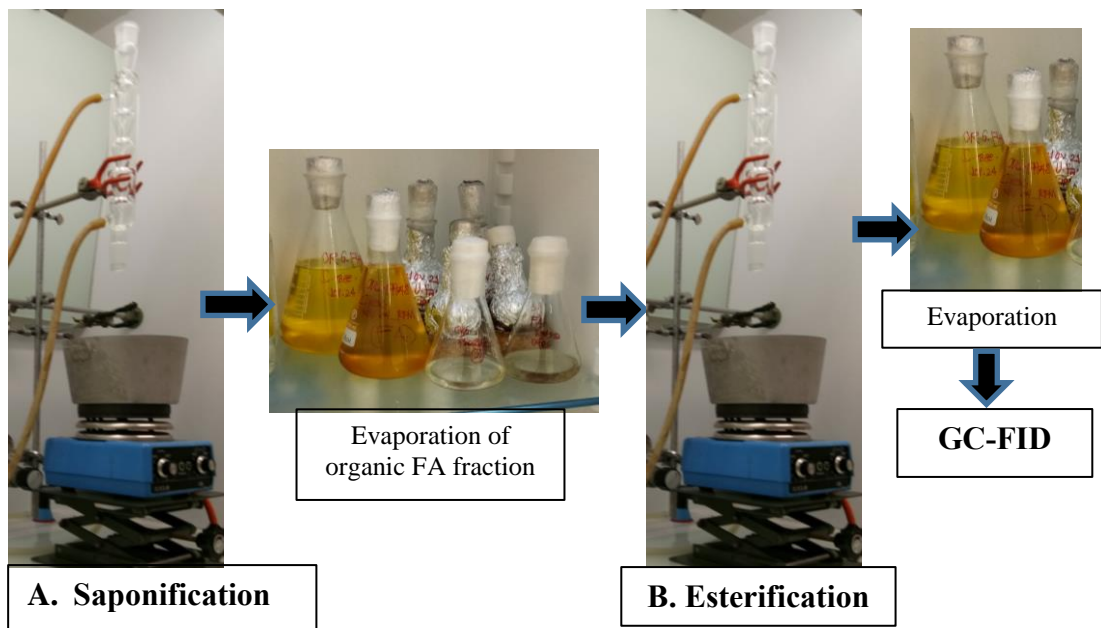


Figure 26. The second treatment of total lipid extraction common to all groups of algae: (A) *Saponification* and (B) *Esterification* (adapted from Terme *et al.* 2017).

2.2.8.1 Nutritional quality indexes

The nutritional quality indexes were assessed according to different parameters calculated with the concentration of fatty acids.

The equations of the indexes are the following:

- (1) C18/C20 PUFA ratio,

$$\frac{C18}{C20} = \frac{\sum C18PUFA}{\sum C20PUFA}$$

- (2) $\omega 6/\omega 3$ ratio,

$$\omega 6/\omega 3 = \frac{\sum PUFA\omega 6}{\sum PUFA\omega 3}$$

- (3) Atherogenicity Index (AI) (Matos *et al.* 2016),

$$AI = \frac{C12:0 + 4 \times C14:0 + C16:0}{\sum MUFA + \sum PUFA\omega6 + \sum PUFA\omega3}$$

- (4) Thrombogenicity Index (TI),

$$TI = \frac{C14:0 + C16:0 + C18:0}{0.5 \times \sum MUFA + 0.5 \times \sum PUFA\omega6 + 3 \times \sum PUFA\omega3 + \frac{\sum PUFA\omega3}{\sum PUFA\omega6}}$$

- (5) Fatty acids hypocholesterolemic/hypercholesterolemic (h/H), and

$$h/H = \frac{C18:1\omega9 + C18:2\omega6 + C20:4\omega6 + C18:3\omega3 + C20:5\omega3}{C14:0 + C16:0}$$

- (6) PUFA/SFA

$$PUFA/SFA = \frac{\sum PUFA}{\sum SFA}$$

where:

PUFA = Polyunsaturated Fatty Acids;

MUFA = Monounsaturated Fatty Acids, and;

SFA = Saturated Fatty Acids.

Unsaturation Index (UI) was calculated by multiplying the number of double bonds by the percentage of each fatty acid, followed by summing up their contributions (Kumari *et al.* 2013).

2.3 Characterization of polysaccharides

Ground freeze-dried seaweed samples, e.g., 15 g (Fig. 27), are inserted into a Soxhlet cartridge and were extracted sequentially inside the Soxhlet extractor. An extraction for 1 h or until the cartridge turns off-white or white, performed with 96% ethanol, of which 300 mL were **first** introduced into the flask with pumice. Switch on the ballon heater just enough for the solvent to condense). **Second** extraction with a Soxhlet extractor is carried out for 1h (same above) with 300 mL chloroform/methanol mixture (1:1, v/v). The **third** extraction with a Soxhlet extractor was carried out for 1 h with 300 mL 100% acetone. Let the cartridge dry (air, hair drier, or oven-dry) in between each extraction. Solvents from each extraction were filtered and evaporated under reduced pressure. Record each initial and final weight before and after extraction and evaporation. Degraded residues of pigments, lipids and proteins are obtained from these three sequential extractions. These extracts are then used for polysaccharides extraction in each samples.

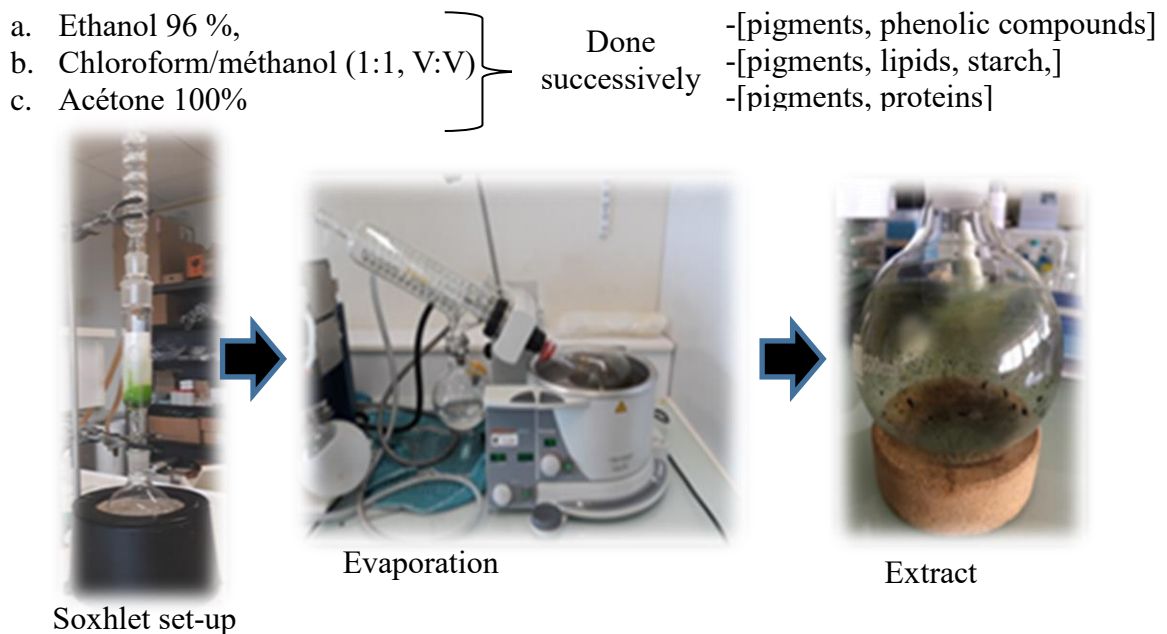


Figure 27. Extraction and isolation of polysaccharides common to all groups of algae.

2.3.1 *Caulerpa racemosa* and *Ulva lactuca* cell wall polysaccharides

Extraction was performed, following a simplified and adapted protocol for *C. racemosa* (Ghosh *et al.* 2004) and *U. fasciata* (Lahaye and Robic, 2007). The extraction of *Caulerpa* polysaccharides was done in several stages. Each stage was separated by filtration, where its residue was precipitated in ethanol and dialyzed with a 12-14 kDa Spectra/Por membrane. A first enriched extract was obtained from 0.5 kg of freeze-dried algae (at, i.e., 10 g dw), by depigmentation.

2.3.1.1 *C. racemosa* hot water extraction (HWE)

The depigmented algal powder (DAP) (e.g., 4 g) from *C. racemosa* (Fig. 28) is first mixed with 200 mL distilled water at 80 °C, pH 6.5 for 30 minutes, and separation of the insoluble particles and the aqueous fraction was obtained by filtration on Buchner cloth. This process was repeated three times (3x), giving the hot water extracted (HWE) fraction (0.52g). The hot water insoluble residue is then washed twice or successively extracted in a strong alkali (1- and 4M-KOH/NaBH₄) to isolate hemicellulosic polysaccharides. This mixture is initially at a concentration 1 M or 500 mL of (1M KOH + 0.4% NaBH₄ at 30-35 °C for 4h, and 4-6 °C for 16 h) giving rise to fraction 1OH, and then at 4M concentration or 500 mL of (4M KOH + 0.4% NaBH₄ at 30-35 °C for 4 h, and 4-6 °C for 16 h) resulting to 4OH fraction. Alkaline extracts were acidified with HCl to pH 6, and all extracts dialyzed extensively against water using a membrane having a molecular weight cut-off of 12 kDa. Soluble materials were recovered by diluting the retentate with water and ethanol at (1:5, v/v). The final residue was suspended in water, acidified to pH 5.2, dialyzed, and lyophilized to produce the insoluble residue (INS).

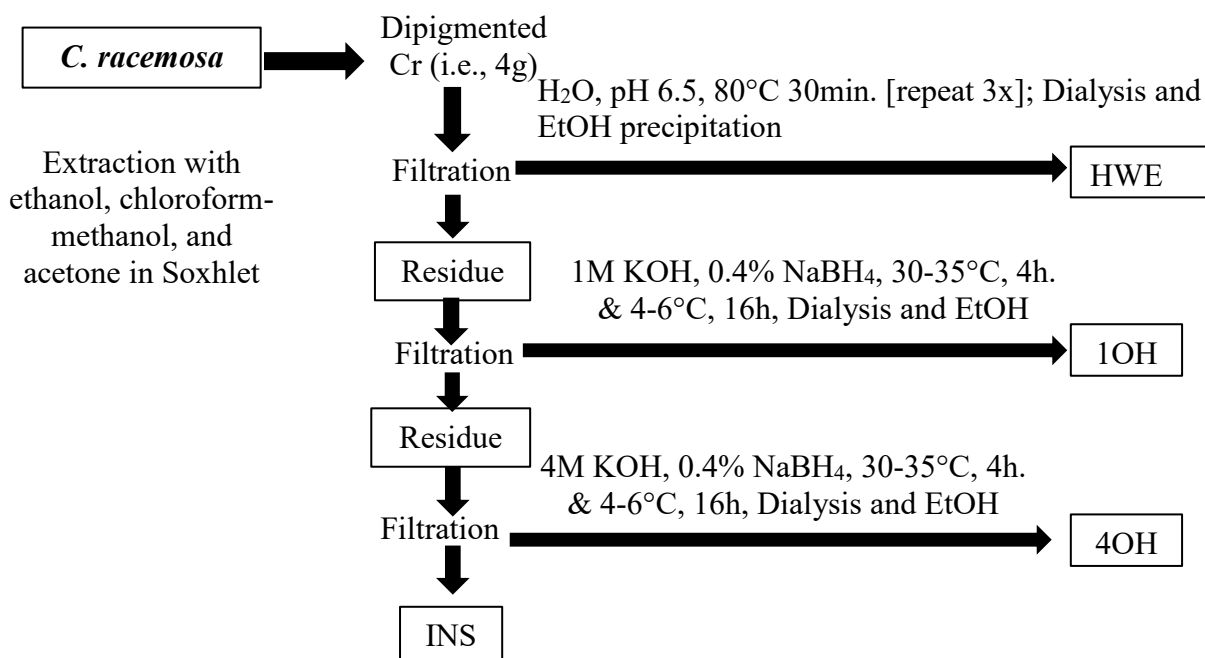


Figure 28. Schematic diagram of sequential extraction of polysaccharides of *Caulerpa racemosa* (Forsskål) with inorganic solvents.

2.3.1.2 *U. lactuca* Ulvan extraction

The depigmented algal powder (e.g., 10 g) for *U. lactuca* (Fig. 29) is first mixed with 200 mL water by maceration at 90 °C, with stirring for 2 hours. The separation of the insoluble particles and the aqueous fraction was obtained by filtration on Buchner cloth. The crude extract of ulvans was concentrated by ethanol precipitation (1:5, v:v), during 24h at +4 °C. The precipitate obtained, representing the crude extract of ulvans, was stored at -20 °C. This dry raw ulvan was weighed (2.37 g) and divided into two parts and were then tested for two purification methods, (route 1) successive precipitation purification and (route 2) dialysis.

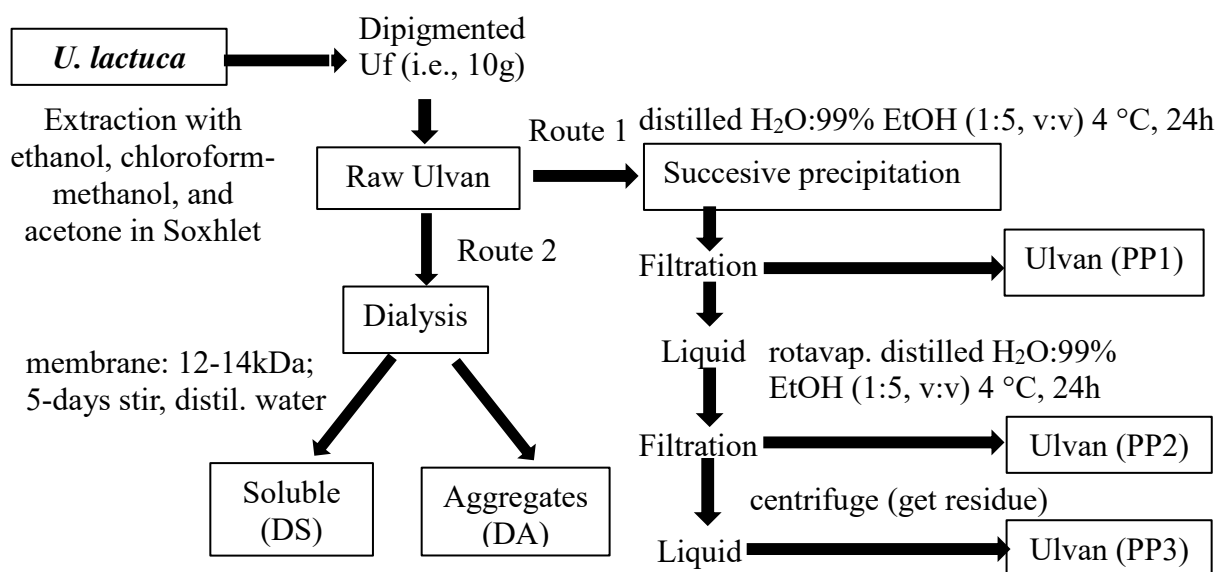


Figure 29. Routes of purification of sulfated polysaccharides, by successive precipitations and by dialysis of *Ulva lactuca* (Linnaeus).

Route 1: Successive precipitation was done with water and 99% ethanol (1: 5. v: v) starting from 1.37 g dw of crude ulvans for 24 hours at +4 °C. The precipitates were separated by filtration to obtain Ulvan PP1 fraction, and the liquid fraction was then concentrated by rotary evaporation and was then dissolved under the same previous condition. Second, precipitation was done to recover Ulvan PP2 fraction, and its liquid fraction was then concentrated by rotary evaporation and was named Ulvan PP3 fraction. These three precipitates or isolated polysaccharides were then dried and characterized.

Route 2: Dialysis was performed on a diluted crude extract 1.0 g Ulvan on a membrane 12-14 kDa for five days' agitation in 4.5 L of distilled water. To ensure maximum efficiency of dialysis, distilled water was renewed every 6 hours during the first two days, then every 12 hours for 3-5 days. At the end of the dialysis, the purified extract was heterogeneous, revealing a soluble fraction (Ulvan DS) and aggregates fraction (Ulvan DA) inside the membrane. Both fractions were separated by decantation, then freeze-dried and analyzed separately.

2.3.2 *Sargassum polycystum* and *Sargassum ilicifolium* polysaccharides

The extraction of polysaccharides from brown algae was performed following and adapted the protocol from Deniaud-Bouët *et al.* (2017). The depigmented algal powder was purified sequentially for the extraction of polysaccharides. The extraction of *Sargassum* polysaccharides was done in several stages, and each stage was separated by filtration where its residue was precipitated in four (4) different solutions and its liquid filtrate were dialyzed with a 12-14 kDa Spectra/Por membrane (Fig. 30).

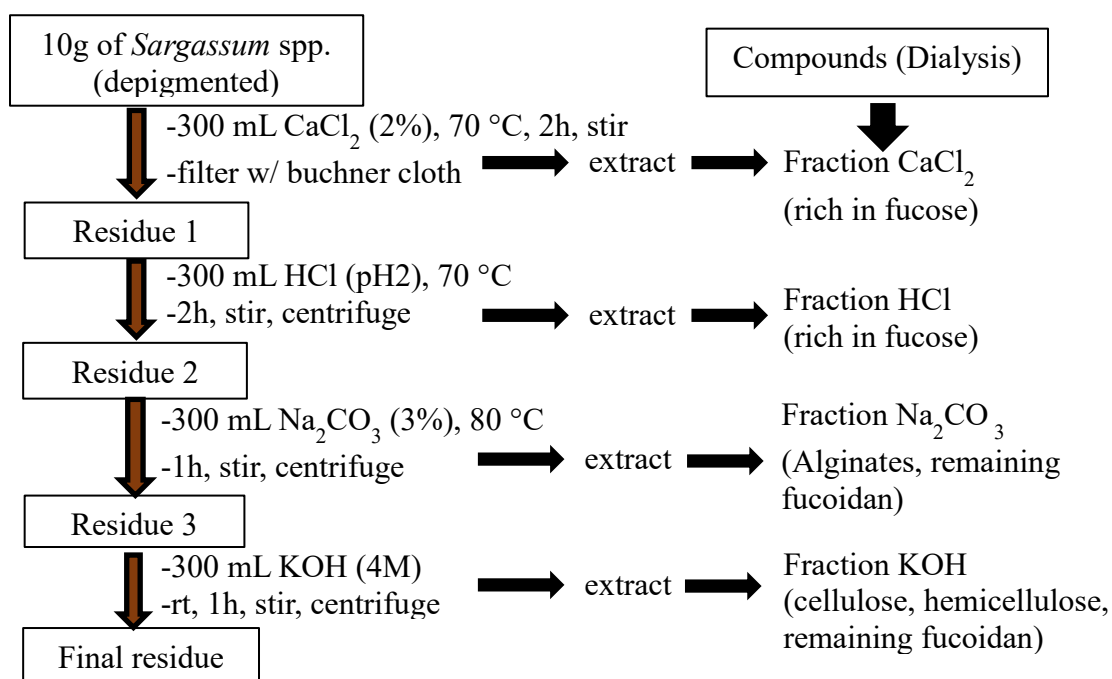


Figure 30. Schematic diagram of sequential extraction of sulfated polysaccharides from *S. polycystum* and *S. ilicifolium* with inorganic solvents.

The depigmented algal powder (DAP) (e.g., 10 g) from *S. polycystum* or *S. ilicifolium* is first mixed with 300 mL CaCl_2 (2%) solution at 70 °C, for 2 h with agitation, and filtered on Buchner cloth to separate the insoluble and aqueous

fraction. The insoluble residue is kept, while the liquid filtrate was then dialyzed and freeze-dried, giving the CaCl₂ fraction (rich in fucose). Second, the residue is then washed in a 300 mL HCl (pH 2) solution at 70 °C for 2 h with agitation. Separation of the insoluble and aqueous fraction was obtained by centrifugation and filtration on Buchner cloth. The insoluble residue is kept, while the liquid filtrate was then dialyzed and freeze-dried, giving the HCL fraction (rich in fucose). Third, the residue is then washed in a 300 mL Na₂CO₃ (3%) solution at 80 °C for 1 h with agitation. Separation of the insoluble and aqueous fraction was obtained by centrifugation and filtration on Buchner cloth. The insoluble residue is kept, while the liquid filtrate was then dialyzed and freeze-dried, giving the Na₂CO₃ fraction (rich in alginates with remaining fucoidans). Lastly, the residue is then washed in a 300 mL KOH (4 M) solution at room temperature for 1 h with agitation. Separation of the insoluble and aqueous fraction was obtained by centrifugation and filtration on Buchner cloth. The insoluble residue is thrown, while the liquid filtrate was then dialyzed and freeze-dried, giving the KOH fraction (compose of cellulose, hemicellulose, with remaining fucoidans).

2.3.3 *Halymenia durvillei* and *Halymenia dilatata* polysaccharides

Extraction was performed, following a simplified and adapted protocol for red algae *Solieria chordalis* by Burlot *et al.* (2016). The extraction of *Halymenia* polysaccharides was done in a simple linear sequence by filtration, agitation, and ethanol precipitation. A first enriched extract was obtained from 0.5 kg of freeze-dried algae (at, i.e., 10 g dw), by depigmentation (Fig. 31).

The depigmented algal powder (DAP) (e.g., 5 g) from *H. durvillei* or *H. dilatata* are immersed with 125 mL distilled water and extracted at 80 °C, for 2 hours with agitation. Separation of the insoluble particles and the aqueous fraction was obtained by filtration on Buchner cloth. The filtrate containing the

polysaccharides were kept and let cool for about 5-10 minutes, and the volume of recovered filtrate is measured. Absolute ethanol equal to twice the volume of the recovered filtrate was then poured gradually to the filtrate to make a ratio of (1:2, v:v) solution. This solution will then be vigorously agitated manually, the polysaccharides precipitate and form a ball around the glass stirrer. This precipitate is disintegrated or removed and crushed manually and washed with ethanol. The fibers obtained are placed in an oven at 60 °C for one day. These are then crushed finely in a mortar or a blender and stored in an Eppendorf tube in a dark and dry place at room temperature (18-22 °C) for further use. For the same sample, extractions of the polysaccharides were carried out in duplicate, and the mass yields expressed as a percentage of the dry matter of the seaweed were measured in triplicate.

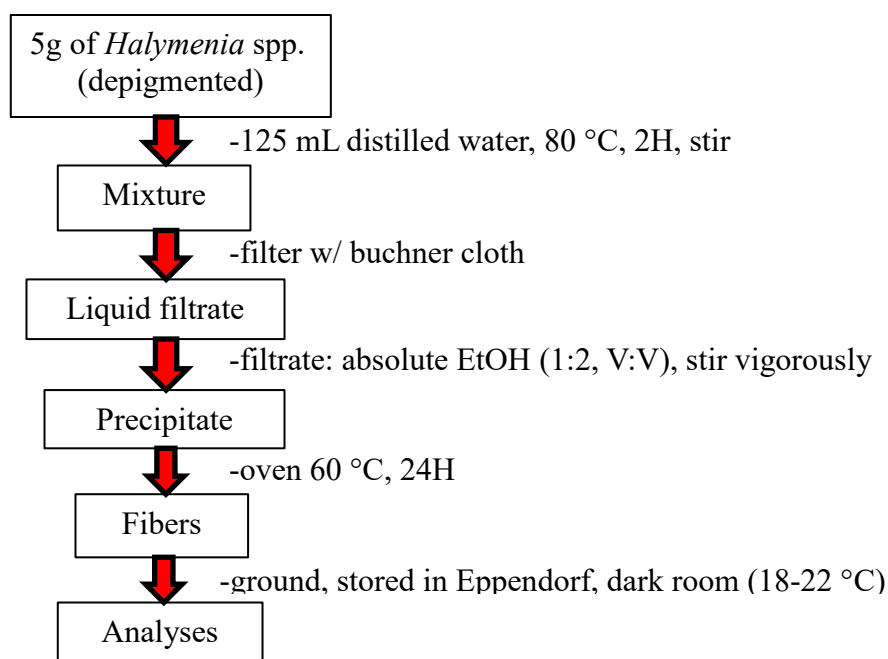


Figure 31. Schematic diagram of extraction and purification of sulfated polysaccharides from *H. durvillei* and *H. dilatata* with inorganic solvents (adapted from AS. Burlot, 2016).

2.3.4 Monosaccharides profile by HPAEC-PAD

The simple sugar composition of the samples was determined by High-Performance Anion Exchange Chromatography (HPAEC) with Pulsed Amperometric Detection (PAD) (Thermo Dionex, Sunnyvale, CA., USA) based on Pliego-Cortés *et al.* (2019) with brief modifications. Monosaccharides were identified and quantified based on their standard curves at different concentrations (1.95–125 ppm). Results were expressed as micrograms of monosaccharides per milligram of dry weight ($\mu\text{g mg}^{-1}$ dw).

Principle: HPAEC is based on sugars' property, which becomes weakly acidic in a very alkaline medium ($\text{pH} > 12$). This type of chromatography allows a selective separation of the monosaccharides on a stationary anion exchange phase, stable at high pH. The column CarboPac PA1 (Dionex, Thermo Scientific) is suitable for this type of analysis. It constitutes the stationary phase and is composed of a $10\ \mu\text{m}$ polystyrene/divinylbenzene substrate of a diameter that agglomerated 350 nm latex microbeads, having quaternary amines functions, which bind to SO_3 groups - the surface of the substrate, such as shown in Fig. 32.

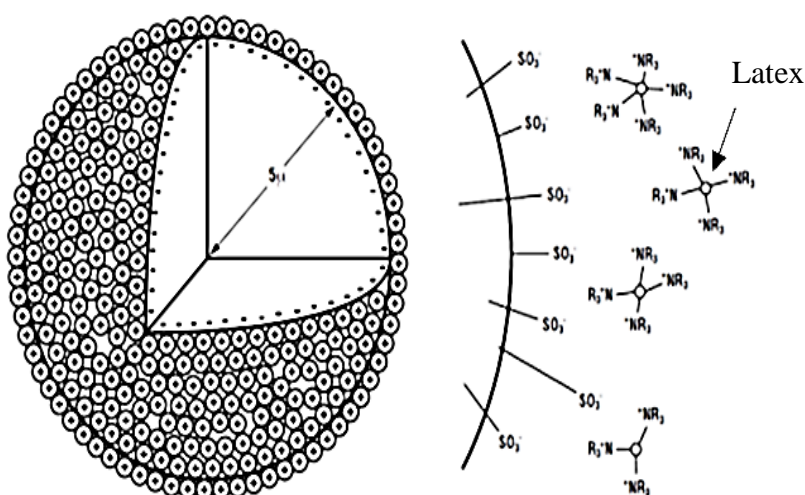


Figure 32. CarboPac PA1 column. The quaternary amine functions of latex microbeads bind to SO_3 groupings - the substrate of the column (Rohrer, 2013).

The column separates the sugars according to their pKa. The latter depends on hydroxyl groups of sugars, their number, and their position. Microbeads quaternary amines, which are positively charged, will attract the -OH groups of sugars. By introducing the eluent, here NaOH, there will be an exchange of anions between the soda and the hydroxyl groups of the sugars. Thus, monosaccharides, with the lowest pKa, interacting most strongly with the stationary phase, will be eluted last.

Pulsed Amperometric Detection (PAD) allows quantifying the monosaccharides after their passage in the column. Carbohydrates are detected by measuring the electric current generated by the oxidation of their hydroxyl groups, which are 0.10 volts on the surface of a gold electrode. Then, to clean the gold electrode and to continue the analysis of other sugars, a variation cycle of voltaic potentials is applied, as shown in Fig. 33.

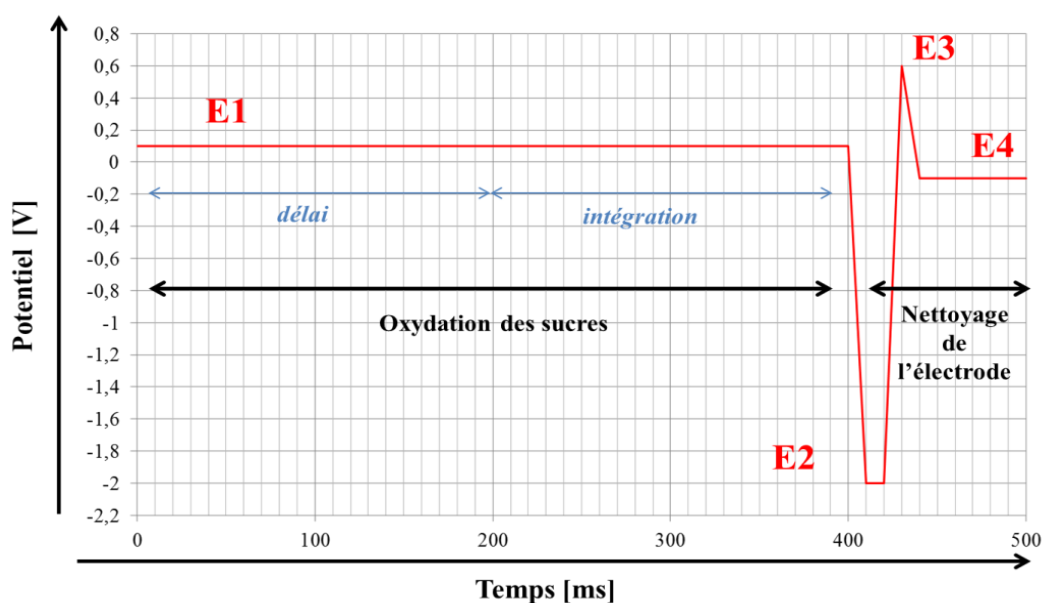


Figure 33. Operation of the PAD detector. Potential E1 is applied for 400 ms. During this time, the intensity of the current related to sugars oxidation (integration) is measured. Potential E2, at -2.00V, cleans the gold electrode by decomposing the oxidation products of sugars. Once the electrode is clean, the potential E3 goes up to 0.60 V to "reactivate" it. Finally, immediately after, a potential E4 is applied for 60 ms to reduce the native gold surface of the electrode and to allow better oxidation of sugars. This wave of potential is repeated throughout the chromatography.

The HPAEC-PAD consists of several modules shown in Fig. 34.

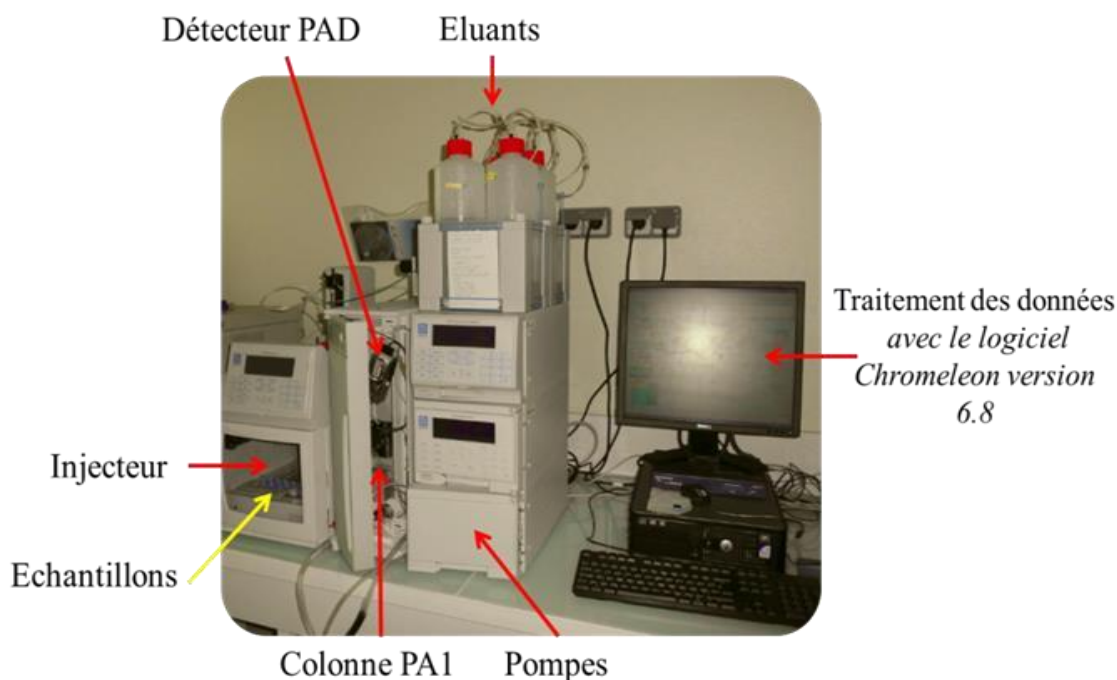


Figure 34. HPAEC-PAD system. The samples are in a sampler where they are injected independently of one after the other. They injected the eluent, which, beforehand, propelled the system pumps—the set between the column where the sugars are separated. At the column outlet, the sugars are quantified by the PAD. The quantifications of the sugars present in the samples are calculated by integrations and using standards (fucose, rhamnose, arabinose, glucosamine, galactose, glucose, mannose, xylose, fructose, ribose, glucoheptose, glucuronic acid, and (standard internal) deoxyribose).

Standards: Preparation of standards were done by preparing a mother solution of each sugar composed of glucoheptose (Sigma- Aldrich), rhamnose (R3875, Sigma- Aldrich), glucosamine (G4875, Sigma- Aldrich), galactose (G0750, Sigma- Aldrich), mannose (63580, Sigma- Aldrich), fructose (F0127, Sigma- Aldrich), deoxyribose (533-67-5, ACROS Organics), arabinose (A3256, Sigma- Aldrich), glucose (G5767, Sigma- Aldrich), xylose (X1075, Sigma- Aldrich), ribose (R7500, Sigma- Aldrich), and glucuronic acid (G5269, Sigma- Aldrich) at 1000 ppm or 50 mg 50mL⁻¹ of pure water. Two (2) mixtures of these sugars were made to make a

calibration curve (Table XIV). Calibration of standards has been performed over a concentration range of 1.95, 3.90, 7.80, 15.6, 31.25, 62.5, and 125 ppm. It is important to note that each of these sugars will have a final concentration (C_f) of 125 ppm except for deoxyribose, which has a final concentration (C_f) of 50 ppm. The concentration of 125 ppm was based on the maximum concentration of sugars that the HPAEC can detect, or its sensitivity limit. The two mixtures created were based on its retention time (rt), and the peak of detection to separate each peak or avoid overlapping of peaks. Mixture one (1) were; deoxyribose, glucoheptose, rhamnose, glucosamine, galactose, mannose, and fructose. Mixture two (2) were; deoxyribose, arabinose, glucose, xylose, ribose, and glucuronic acid. The solution of deoxyribose for 50 ppm, was done by taking 1 mL of deoxyribose from 1000 ppm mother solution and was added with 19 mL pure water to make 20 mL solution. To determine how much volume must be taken from each prepared mother solution to make the mixture, we must decide how much our final volume (V_f) is, and in this case, $V_f = 1.5$ mL. Using the formula $(C_i)(V_i) = (C_f)(V_f)$ and its given values. Where;

C_i = initial concentration or 1000 ppm,

V_i = initial volume or unknown,

C_f = final concentraion or 125 ppm for all except for deoxyribose, and

V_f = final volume or 1.5 mL.

Table XIV. Standard sugar mixtures used for calibration curves.

	Mixture 1	(μL)	Mixture 2	(μL)
1	Deoxyribose	75	Deoxyribose	75
2	Glucoheptose	187.5	Arabinose	187.5
3	Rhamnose	187.5	Glucose	187.5
4	Glucosamine	187.5	Xylose	187.5
5	Galactose	187.5	Ribose	187.5
6	Mannose	187.5	Glucuronic acid	187.5
7	Fructose	187.5	Pure water	<u>487.5</u>
+	Pure water	300		
Total		1500		1500

Serial dilution was then made according to the determined concentration ranged. Monosaccharides have been identified and quantified using calibrations of standards. The standard solutions were prepared in ultrapure water and filtered at 0.22 µm. Fig. 35 shows an example of a chromatogram of Mixture 2 standards, used at a given concentration (125 ppm), and its peak summary for each standard monosaccharide.

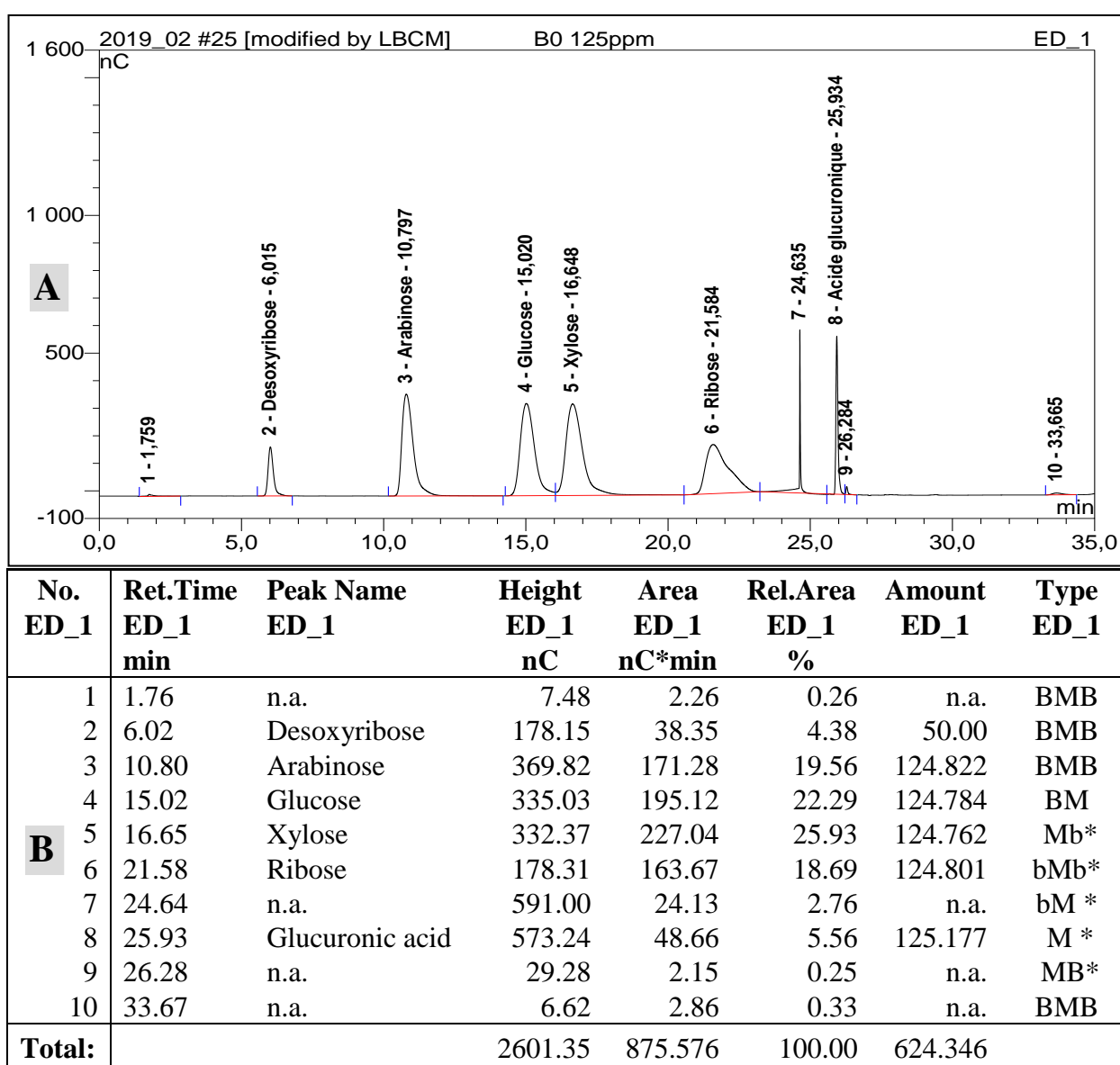


Figure 35. Chromatogram showing the retention time of the different standards of mixture 2 (A) and its peak summary Table (B).

Procedure: Before analysis, each dry matter samples (4 mg dw) undergo complete acid hydrolysis for 48 hours at 100 °C using 110 µL of 1 M hydrochloric acid (Carlo Erba, 7647-01-0) and 1 mL of ultrapure water in a sealed glass ampoule. The samples were then neutralized with 110 µL of 1M sodium hydroxide to each sample, and 780 µL of ultrapure water containing deoxyribose (internal standard) to a final concentration of 50ppm. The 2 g L⁻¹ neutralized samples were then filtered (0.22 µm) and are inserted into specific vials for HPAEC-PAD analysis.

The separation of the sugars present in the samples is carried out on the Carbo column Pac PA1 at a flow rate of 1 mL min⁻¹ and a pressure of 110 bar. The simple sugars are identified by comparing injected standards (50 µM) and eluted under the same conditions (glucoheptose, rhamnose, glucosamine, galactose, mannose, fructose, arabinose, glucose, xylose, ribose, and glucuronic acid). Twenty-five (25) µL of sample or standard are injected. The elution program (Fig. 36) requires three eluents: Solution A, 0.1M NaOH; Solution B, 1M NaOAc in 0.1M NaOH; and Solution C, ultrapure water. The program elution started with 30 min of isocratic elution (Sol A: Sol C 20:80) followed by a gradient of sodium acetate (Sol B) from 0 to 100 % in 5 min supplemented by 5 min of isocratic elution to 100% of solution B and finally a return to initial conditions for 20 min (Sol A: Sol C, 20:80). The retention times obtained for the standards and the height of the peaks obtained for known concentrations allow the identification and quantification of sugars present in the samples.

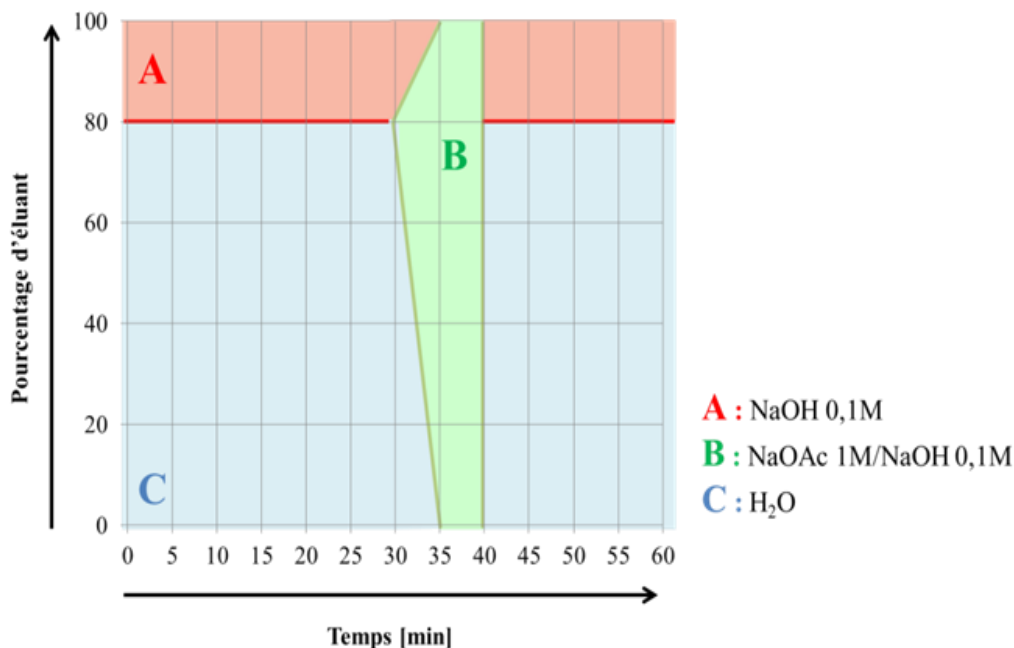


Figure 36. HPAEC-PAD elution program. The elution of monosaccharides was mainly in the isocratic mode. Sodium acetate is added after 30 minutes to elute the compounds in the column. Indeed, the acetate ions, more electronegative, will be exchanged by binding to quaternary amine microbeads.

2.3.5 *Fourier transform infrared (FT-IR) spectroscopy*

FT-IR is a vibrational spectroscopic technique and is based on the principle that most molecules absorb light in the infrared wavelength region of the electromagnetic spectrum. This absorption corresponds precisely to the chemical bonds present in molecules. FT-IR can facilitate to confirm the identity of a pure compound by using the unique collection of absorption bands (Fernando *et al.* 2017). Spectra of polysaccharides extracted by conventional technique from green seaweeds *C. racemosa* (Cr crude, 1OH, 4OH, and INS), and *U. lactuca* (PP1, PP2, PP3, DS, and DA); from brown seaweeds *S. polycystum* (Sp CaCl₂, Sp HCl, Sp Na₂CO₃, Sp KOH) and *S. ilicifolium* fractions (Si CaCl₂, Si HCl, Si Na₂CO₃, Si

KOH); and from red seaweeds, *H. durvillei* (Hdur) and *H. dilatata* (Hdil) were determined using a Nicolet™ iS™ 5 FT-IR spectrometer (Thermo Scientific™, Madison, WI, USA) with diamond crystal plate. The spectra were recorded in reflexive mode ranging from 4000–500 cm⁻¹ wavenumber as the percentage transmittance was collected with 16 subsequent scanings of samples. About 2-mg of dry extracts were introduced onto the crystal plate and were then acquired as the percentage transmittance. A background scan with the diamond plate in place was run before each analysis. The detected peaks were then compared with algal polysaccharide standards with their respective structural characteristics.

2.3.6 Minerals by flame atomic absorption spectrophotometry (AAS)

Major mineral elements (Ca, Mg, Na, and K) and trace elements (Fe, Zn, Cu, and Mn) were analyzed using a Perkin Elmer *AAnalyst 200* atomic absorption spectrophotometer (AAS) with a single hollow cathode lamp for each element and an air-acetylene burner. The 200-mg raw samples of dried seaweed were placed into digestion vessels with 1.3 mL of 1 M HCl was added 19.7 mL of ultrapure water (18.2MΩ) for final sample concentration at 10 mg mL⁻¹ and incubated 48 hours at 118 °C. Samples were filtered (0.22 µm, Minisart High Flow; Sartorius Stedim Biotech, Göttingen, Germany). Filtered samples were diluted in a 100-mL flask with distilled water at 1-mL sample added 99-mL distilled water to obtain a 1 mg mL⁻¹ concentration.

Preparation of standards was done by diluting 20 mL of each element solution into 200ml of distilled water; then, a standard range has been established depending on the suggested wavelengths with its respective standard range (i.e., for Zn, 213.86nm at 0.15- 0.75 mg L⁻¹). Quantification was performed using standard ranges depending on the element.

2.4 Analysis of pigments

Pigment analysis was based on the method used by Silkina *et al.* (2009) with some modifications. The analysis was performed by high-performance liquid chromatography (HPLC) on a Nucleodur C18 column (EC 250/4.6 100-5 C18ec Macherey-Nagel). A two-solvent elution (solvent A 100 %, 0–27 min; solvent B 100 %, 27–42 min) was used at a flow rate of 1.4 mL min⁻¹ to separate the pigments. Pigment extraction from raw material (20 mg) was diluted with methanol (2 mL, at 4 °C) and was sonicated (pulse 2, amplitude 100) for 2 minutes in test tubes (inserted in water and ice). After sonication, samples were kept in the dark cold room (4 °C) 24 hours before the injection of the sample (100 µL). After 24 hours, the diluted methanol extracts were centrifuged (36,000 x g, 4 min.), and then filtered (0.22 µm, Minisart High Flow; Sartorius Stedim Biotech, Göttingen, Germany) before analysis. Eluent preparation of Solvent A consisted of methanol (510 mL), acetonitrile (360 mL), and ammonium acetate (23.145 g) dissolved in water (130 mL); and solvent B was acetonitrile (30%), and ethyl acetate (70%). Pigments were detected and characterized by diode array measurements of their absorption spectra recorded between 300 and 600 nm. Chromatograms were recorded at a wavelength of 440 nm, and standards of chlorophyll *a*, *b*, and β- carotene (Sigma–Aldrich) were used for pigment identification and quantification. Pigment concentrations were expressed as mg g⁻¹.

2.5 Screening of biological activities

2.5.1 Evaluation of cytotoxicity and antiviral activity by cell viability

Each sample's antiviral activity is evaluated in parallel with the cytotoxicity (Burlot *et al.* 2016; Hardouin *et al.* 2016). These tests are performed on previously

extracted and freeze-dried polysaccharides and carried out in vitro using the Vero cells, a green monkey kidney fibroblast cell line (line n° ATCC CCL81). All of the manipulations are performed in Eagle's Minimal Essential Medium (MEM) culture (Appendix 1). The medium was renewed daily; cells need to be serviced twice a week (Appendix 2) and enumerated before each test to have an equivalent number of cells for each sample (Appendix 3).

The cell viability assessment method's principle is based on the staining of cells by the neutral red dye that only enters living cells by integrating, especially lysosomes (Langlois *et al.* 1986). So, cell viability is quantifiable in spectrophotometer by reading an optical density at a wavelength fixed at $\lambda = 540$ nm.

The freeze-dried extracts were homogenized in MEM medium enriched with 10% of DMSO, reaching a final concentration of 1 mg mL^{-1} . Five concentrations of one same extract are evaluated: $200 \text{ }\mu\text{g mL}^{-1}$; $100 \text{ }\mu\text{g mL}^{-1}$; $50 \text{ }\mu\text{g mL}^{-1}$; $10 \text{ }\mu\text{g mL}^{-1}$ and $1 \text{ }\mu\text{g mL}^{-1}$. The dilutions are carried out in cascade in enriched MEM. Each sample is made in quadruplets.

One hundred μL of cell suspension at $350,000$ Vero mammalian cells mL^{-1} in enriched Eagle's MEM are inserted into all wells (Appendix 4). In all the wells of the 12th column (Cell Control), $100 \text{ }\mu\text{L}$ of enriched MEM are added. In all the wells of the 11th column (Virus control), $50 \text{ }\mu\text{L}$ enriched MEM are added. The evaluation of cytotoxicity is carried out in the wells in columns 1 to 10 and lines A to D, which are added $50 \text{ }\mu\text{L}$ of enriched MEM. In all the wells of an entire column from A to H, $50 \text{ }\mu\text{L}$ of the same sample at a given concentration, previously prepared, are added. In all the wells of column 11 and the wells of columns 1 to 10 and lines from E to H, were infected by adding $50 \text{ }\mu\text{L}$ of HSV-1 viral suspension, diluted to obtain an MOI (multiplicity of infection) equal to 0.001 ID_{50} per cell. In this way, each sample is made in quintuplet for each concentration and vero cells were optimally protected when polysaccharides are deposited at the same with the viruses. The microplate is then placed in an oven at $37 \text{ }^{\circ}\text{C}$ under $5\% \text{ CO}_2$ atmosphere and saturated with water vapor for 72 hours.

The neutral red revelation of cell viability: Fifty μL of the neutral red solution is introduced into all wells. The microplate is again placed in an oven at $37\text{ }^{\circ}\text{C}$ for 45 minutes. A step of washing is then performed and repeated twice successively with a solution PSB (Phosphate-buffered saline) at 450 mOsm at pH 7.4 and a citrate-ethanol buffer. The bottom of the wells is scraped to homogenize the neutral red released in cells by the citrate-ethanol buffer. The ODs of all the wells are then read at 540 nm spectrophotometer.

Cells and Virus: Vero cell lines (line n° ATCC CCL81) were cultivated in Eagle's MEM supplemented with 8% fetal calf serum (FCS, Eurobio, France), 1% L-glutamine (200 mM), and 1% PCS (penicillin (10,000 U), colimycin (25,000 U) and streptomycin (10 mg). The culture was performed at $37\text{ }^{\circ}\text{C}$ under a 5% CO_2 atmosphere, and the medium was renewed daily. Pr. Agut provided herpes simplex virus type 1 (HSV-1; family Herpesviridae) (Laboratoire de Dynamique, épidémiologie et traitement des infections virales de la Pitié Salpêtrière Paris, France). The virus collection was obtained by infection of Vero cells monolayers (75 cm^2 culture flasks, $350,000\text{ cells mL}^{-1}$) incubated at 95/5 air/ CO_2 , $37\text{ }^{\circ}\text{C}$ for three days. After incubation, the suspension was frozen and thawed two times before centrifugation at low velocity ($10,000 \times g$, 10 min) to collect the supernatant. The liquid phase containing the virus suspension was stored at $-80\text{ }^{\circ}\text{C}$ before use.

Cytotoxic and antiherpetic activity evaluation by cell viability: The fractions were prepared in MEM supplemented medium (8% FCS), and $50\text{ }\mu\text{L}$ was distributed into 96 wells microplate (micro-test III tissue culture plate, Nuclon Intermed, France). A dilution series included five assays (quadruplicate) ranging from 1 to $200\text{ }\mu\text{g mL}^{-1}$ extract concentrations. A $100\text{ }\mu\text{L}$ of cellular suspension diluted to a concentration at 3.5×10^5 Vero cells mL^{-1} in Eagle's MEM (8% FCS) is added to the wells. The cell suspension was infected by the addition of two different viral suspensions at a multiplicity of infection (MOI) of 0.001 ID_{50} per cell. The microplates were incubated at $37\text{ }^{\circ}\text{C}$, 5% of CO_2 for three days. The cells were examined daily under an inverted microscope to determine the minimum concentration of extracts that induced alterations in cell morphology, including swelling, shrinkage, granularity,

and detachment. Cytotoxicity by cell viability was tested using the neutral red dye method (Langlois *et al.* 1986). Optical density (OD) was measured at 540 nm using a spectrophotometer (SpectraCount™, Packard). The 50% cytotoxic concentration (CC₅₀) was defined as the concentration that reduced the OD of treated cells to 50% of that of untreated cells. CC₅₀ values were expressed as the percentage of destruction (%D):

$$CC_{50} = \left(\frac{OD_{control\ cells} - OD_{cells+sample}}{OD_{control\ cells}} \right) \times 100$$

where:

OD_{control cells}= were the OD values of the untreated cells, and

OD_{cells+sample}= were the OD values of the treated cells (Langlois *et al.* 1986).

After incubation, antiviral activity was evaluated by the neutral red dye method. The antiherpetic compound acyclovir [9-(2-hydroxyethoxymethyl) guanine] was used as a reference inhibitor. The 50% effective antiviral concentration (EC₅₀) was expressed as the concentration achieved by 50% protection of virus-infected cells from virus-induced destruction. The OD was related directly to the percentage of viable cells, which was inversely related to the cytopathic effect (CPE). The linear regression was determined for each assay based on cell controls (0% CPE) and virus controls (100% CPE). Data were expressed as a percentage of protection (%P):

$$EC_{50} = \left(\frac{OD_{control\ cells+virus+drug} - OD_{control\ virus}}{OD_{control\ cells+drug} - OD_{control\ virus}} \right) \times 100$$

where:

OD_{control cells+virus+drug}= were the OD values of the test sample,

OD_{control virus}= were the OD values of the virus control, and

OD_{control cells+drug}= were the OD of the infected cells control (Langlois *et al.* 1986).

2.5.2 Antioxidant activity

DPPH radical scavenging assay

Antioxidant 2,2-diphenyl-1-picrylhydrazyl or DPPH radical scavenging activity was determined according to Terme *et al.* (2018). DPPH assay is a stable free radical and is based on reduction reaction or the theory that a hydrogen donor is an antioxidant (Škrovánková *et al.* 2012). It measures compounds that are radical scavengers or reacts with compounds that can donate hydrogen atoms. DPPH shows a strong absorption maximum at 517 nm. There are two general types of assays widely used for different antioxidant studies. To the first group belong assays that are associated with electron or radical scavenging, including the DPPH assay, Trolox equivalent antioxidant capacity (TEAC) assay, and FRAP assay, while the other types are assays associated with lipid peroxidations, including the thiobarbituric acid assay and [β-carotene](#) bleaching assay (Škrovánková *et al.* 2012). The DPPH method is widely used due to relatively short time required for the analysis (Škrovánková *et al.* 2012). A series of butylated hydroxyl-anisole (BHA), butylated hydroxyl-toluene (BHT) (respectively 2(3)-*t*-Butyl-4-hydroxyanisole, and 2,6-Di-*tert*-butyl-4- methylphenol, Sigma, Saint Quentin Fallavier, France) solutions – serves as affirmative standard – is prepared in different concentrations (2-50 µg mL⁻¹) in methanol. DPPH solution of 0.25 mM is introduced to 100 µL samples solution. Samples solution of hydrolysates from *C. racemosa*, *U. lactuca*,

S. polycystum, *S. ilicifolium*, *H. durvillei*, and *H. dilatata* were made in different concentration by diluting the solution stock in water (0–5000 µg mL⁻¹). Before reading the optical density at 517 nm, all samples are incubated at 40 °C for 30 min. Then, the percentage of inhibition is calculated by using the following formula:

$$I (\%) = \left[\frac{(A_{control} - A_{sample})}{A_{control}} \right] \times 100$$

where:

$A_{control}$ = absorbance of control, and

A_{sample} = absorbance of samples

IC₅₀ of samples is determined based on the regression obtained from dose response curve. IC is defined as the concentration sufficient to obtain 50% of a maximum scavenging capacity.

2.6 Statistical Analyses

Statistical analysis used: All measurements were done in triplicate for each alga (n=3), except for GC-FID (fatty acids, amino-acids) and HPAEC (monosaccharides) analyses were done only in duplicate. All values were reported as mean±standard deviation (SD). Student t-test was used to compare the degree of effectivity of EC₅₀ or IC₅₀ between different doses and control for each fraction of *C. racemosa*, *U. lactuca*, *S. polycystum*, *S. ilicifolium*, *H. durvillei*, and *H. dilatata* species.

Chapter 3 Results and Discussion

Green Seaweed (*Caulerpa racemosa* and *Ulva lactuca*)

3. Results and Discussion

The purpose of this part is to identify and compare the composition of the polysaccharides extracted from *C. racemosa* and *U. lactuca*, and also their nutritional and biological properties.

3.1 Cell wall polysaccharides purification

3.1.1 First treatment

The objective of this first treatment was to remove salts, small proteins, phlorotannins, and pigments. The yields were calculated by the ratio between the initial mass and the final mass of dry algae. Extraction yield in percent after three solvents (ethanol, chloroform/methanol, and acetone) are shown in Table XV. We obtain 89.92% and 89.65 % for *C. racemosa* and *U. lactuca*, respectively. Initial and final weights of raw *C. racemosa* and *U. lactuca* used to calculate extraction yield in percent after three solvents were shown in appendix 5.

Table XV. Percent extraction efficiency of Philippine green seaweeds *Caulerpa racemosa* and *Ulva lactuca*.

Species	Solvents	Yields (%)
<i>C. racemosa</i>	Ethanol	89.92
	Chloroform/Methanol	
	Acetone	
<i>U. lactuca</i>	Ethanol	89.65
	Chloroform/Methanol	
	Acetone	

3.1.2 Polysaccharide extraction

The cell wall polysaccharide was extracted from Depigmented Algal Powder (DAP). In *C. racemosa*, the soluble Hot Water Extracted fraction (designated HWE) and then successively fractions (1-and 4M-KOH) extracted with strong alkali to isolate hemicellulosic polysaccharides, and finally, the insoluble fraction (INS) were prepared. In *U. lactuca*, crude ulvan was extracted separately in two groups: successive precipitation fractions (PP1, PP2, and PP3), and by dialysis fractions (DS and DA). The yields of the different extracted fractions are given in Table XVI. Initial and final weights of raw *C. racemosa* and *U. lactuca* that were used to calculate extraction yield in percent at each purification step were shown in appendix 6.

Table XVI. Extraction yield and purification of polysaccharides from Philippine green seaweeds *Caulerpa racemosa* and *Ulva lactuca*.

EXTRACTION	Yields (% dw)
<i>C. racemosa</i>	
Depigmented	89.92
HWE	13.0
1OH	6.5
4OH	8.0
INS	28.0
<i>U. lactuca</i>	
Depigmented	89.65
Successive Precipitation	
Ulvan PP1	63.41
Ulvan PP2	28.51
Ulvan PP3	4.55
Dialysis	
Ulvan DS	91.22
Ulvan DA	8.35

Overall yield= expressed as a percentage of total dry algae at the start.

Purification yield= expressed as a percentage of the dry mass used at each stage.

From HWE (see Magdugo *et al.* 2020), we had obtained three more sequential fractions; (1OH=6.5%, 4OH=8.0%, and INS=28% dw) (Table IX).

In comparison with the study of Ghosh *et al.* (2004) on *C. racemosa* polysaccharide extraction, the authors reported higher values with (HWE= 20%, 1OH=12%, 4OH=16%, and INS= 40% dw) in similar extraction conditions. In the study of Chattopadhyay *et al.* (2007), the HWE fraction was purified from *C. racemosa* by anion exchange chromatography to obtain three sub-fractions with its corresponding values (F1=20%, F2=11%, and F3=69%) of total material recovered. Another study reported a 7 to 8% yield of crude polysaccharide from *C. racemosa* after ethanol precipitation by hot water extraction (Ji *et al.* 2008).

The differences between Ghosh *et al.* (2004) results can be explained by geographic location. The specimens collected by Ghosh *et al.* came from the Gujarat coast of Western India. Seasonal variations and other factors, like extraction and characterization processes, may be responsible for the variation of yield and biochemical composition (Ji *et al.* 2008; Chattopadhyay *et al.* 2007).

Ulvan polysaccharide was extracted from DAP *U. lactuca*. The extraction yield of the crude Ulvan extract from DAP *U. lactuca* was 23.71%. According to Lahaye and Robic, (2007), the initial contents of Ulvan is between (8 and 29%) of the algal dry matter. Also, the yield obtained by Hardouin *et al.* (2016) Ulvan extraction from *U. armoricana* was 24.5% of the algal dry matter. In the study of Yaich *et al.* (2013) on the yields and quality of Ulvans from *Ulva lactuca* by acid extraction conditions, followed by ethanol precipitation, reported that specific yields of Ulvans are between (21.7 and 32.7%) depending on pH, temperature, and extraction time. Other studies obtained (24.3%) Ulvans from *Ulva rigida* by hot water extraction, after dialysis, then with ethanol precipitation (Toskas *et al.* 2011), and from 9 to 36% Ulvan from several ulvales species and using different methods of extraction (Kidgell *et al.* 2019). These results confirmed data from previous papers and the effectiveness of the extraction method.

From crude Ulvan, we had obtained five Ulvan fractions: three from successive precipitation (PP1=63.41%, PP2=28.51%, and PP3=4.55%), and two from dialysis (DS=91.22%, and DA=8.35%). In the thesis manuscript of Hardouin in 2015 (<http://www.theses.fr/2015LORIS373#>), the author recorded for successive precipitation (PP1=33%, PP2=57%, and PP3=6%) and dialyzed extracts (DS=76.70%, and DA=23.30%). Despite the similar method used, the present study differs in the amounts of PP1, and PP2, while PP3 is somewhat similar. DS fraction is 15% higher, and DA is 15% lower than that of Hardouin (2015, <http://www.theses.fr/2015LORIS373#>). These different results may be due to the amount of the crude Ulvan that is being used, beginning with the extraction and purification (from 13 g: 10, 3 g (Hardouin, 2015), and from 2.37 g: 1.37, 1 g (present study) used for successive precipitation and dialysis, respectively). Different factors, such as species, season, and place of the collection again influence the yields (Ji *et al.* 2008; Chattopadhyay *et al.* 2007).

3.1.3 Biochemical composition analysis of extracts

The biochemical composition of *C. racemosa* (HWE, 1OH, 4OH, and INS) and *U. lactuca* (PP1, PP2, PP3, DS, and DA) extracts are shown in Table XVII.

The composition of isolated fractions (1OH, 4OH, and INS) revealed that 1OH fraction is rich in neutral sugars (16.2%) followed by sulfates (10.58%), and proteins (7.8%); with small amounts of polyphenols and uronic acids; 4OH fraction is composed of proteins (11.0%) and sulfates (8.19%), with small amounts of polyphenols, neutral sugars, and uronic acids. Insoluble polysaccharide (INS) is abundant in sulfates (10.21%), followed by small amounts of neutral sugars, proteins, polyphenols, and uronic acids.

Another study from *C. racemosa* var *peltata* fraction WEC (Water Extracted *Caulerpa*) extracted by hot water at 85 °C for 3 h reported 0.67% protein, 0.19% polyphenols, and 3.4% total sugar (Hao *et al.* 2019), which are quite small compared to the present study. In general, total sugars were abundant components compared to proteins, phenols, and flavonoids (Hao *et al.* 2019).

Table XVII. Biochemical composition of polysaccharide extracts from Philippine green seaweeds *Caulerpa racemosa* and *Ulva lactuca*.

ALGAE	Content (% dry algal material)					
	Yield (%)	Proteins	Neutral sugars	Sulfates group	Uronic acids	Polyphenol
<i>C. racemosa</i>						
HWE	13.0	19.9±0.5	31.2±4.9	13.0±1.5	3.6±0.4	4.9±1.6
1OH	6.5	7.8±1.7	16.2±1.4	10.58±0.7	0.5±0.2	3.0±0.7
4OH	8.0	11.0±0.4	1.2±0.1	8.19±0.1	0.1±0.0	3.8±0.0
INS	28.0	3.7±0.5	4.0±0.1	10.21±0.1	0.9±0.4	2.1±0.9
<i>U. lactuca</i>						
Ulvan PP1	63.41	13.5±2.4	5.0±0.2	15.79±0.3	6.2±0.3	2.3±0.8
Ulvan PP2	28.5	19.6±1.5	12.8±0.8	16.45±0.4	7.0±1.4	2.5±0.9
Ulvan PP3	4.5	7.1±1.0	7.2±0.9	20.03±0.4	2.2±0.1	2.8±0.5
Ulvan DS	91.2	18.3±2.6	6.7±0.7	16.0±0.2	9.5±0.2	3.2±1.4
Ulvan DA	8.4	18.9±0.8	6.1±0.2	5.44±0.7	7.0±0.3	1.7±0.9

The purification of *U. lactuca* raw Ulvan, had five Ulvan fractions mainly composed of sulfates and proteins in successive precipitation fractions (PP1, PP2, and PP3), while proteins, sulfates, and uronic acids in dialyzed fractions (aggregates DA, and soluble DS). Fraction PP1 (yield, 63.41%) had sulfates (15.79%), proteins (13.5%) contents, followed by small amounts of uronic acids, neutral sugars, and polyphenols. Fraction PP2 (yield, 28.51%) had proteins (19.6%), followed by sulfates (16.45%), neutral sugars (12.8%), small amounts of uronic acids, and polyphenols. Fraction PP3 (yield, 4.55%) had sulfate (20.03%) followed by neutral sugars (7.2%), proteins (7.1%), and small amounts of polyphenols and uronic acids—Dialyzed fractions with yield values of 91.22 and 8.35% for Ulvan DS and Ulvan DA, respectively. Fraction DS is rich in proteins (18.3%) and sulfates (16.0%) followed by uronic acids (9.5%), neutral sugars, and a small amount of polyphenols. Fraction DA is rich in proteins (18.9%) followed by uronic acids, neutral sugars, sulfates, and a small amount of polyphenols. The amount of proteins (7.11-19.57%), neutral sugars (5.0-12.8%), sulfates (5.44-20.03%), and uronic acids (2.2-9.5%) from the present study are quite small or different from the thesis results of Hardouin, (2015, <http://www.theses.fr/2015LORIS373#>) using similar methods of extraction especially fractions PP1 (22.3%), and DA (41.7%) in proteins; all fractions in neutral sugars; fraction DS (31.8%) in sulfates; and fractions PP2 (18.1%), PP3 (13.9%), and DS (25.3%) in uronic acids. He stated in his results that EC₅₀ activity is not only dictated on Ulvans or polysaccharides alone but also of its protein contents, which he also observed at fractions PP1 (EC₅₀<400.0 ug mL⁻¹), and DA (EC₅₀=68.6 ug mL⁻¹). Sulfates content in the present study is within the values published in the literature (Costa *et al.* 2012; Hernández-Garibay *et al.* 2011) and somewhat similar to the results of Hardouin *et al.* (2016) except for fraction DA. Proteins content in the present study are way higher (up to 20 times) than Costa *et al.* (2012), up to 2 times than Hernández-Garibay *et al.* (2011), and up to 2 to 3 times higher than Hardouin *et al.* (2016) except for fraction PP3 which is almost similar to his results. The uronic acid content in the present study except for fraction PP3 are slightly lower or just comparable to Hernández-Garibay *et al.* (2011), and up to 2 to 3 times lower compared to Hardouin *et al.* (2016). Neutral

sugars content in the present study are mostly 2 to 3 times lower than Hardouin *et al.* (2016). The present study's total phenolic contents are 3 to 6 times higher than Hardouin *et al.* (2016).

3.1.4 Monosaccharide composition analysis

The unit sugar composition of crude and purified fractions of *C. racemosa* and Ulvan of *U. lactuca* fractions were determined by High-Performance Anion Exchange Chromatography (HPAEC). The results are given in Table XVIII and XIX and expressed as a percentage of sugars relative to the total sugars detected.

Eleven monosaccharides have been identified present in raw *C. racemosa*, 9 in Cr crude, 11 in 1OH, 10 in 4OH, and 11 in INS. Main contents of monosaccharides in raw *C. racemosa* were xylose with 10.03%, and glucose with 7.88%, small amounts (<10%) of glucose, galactose, mannose, fucose, and ribose; and trace amount (<1.0%) of fructose, glucosamine, rhamnose, glucuronic acid, and arabinose. The Cr crude fraction had glucose (54.8%), and galactose (18.4%) as major monosaccharides, with small amounts (<10%) of glucuronic acid, xylose, fucose, glucosamine, mannose, and rhamnose; and a trace amount of arabinose. Identified monosaccharides from isolated fractions (1OH=11, 4OH=10, and INS=11) revealed that 1OH is rich in glucose (50.41%); followed by small amounts (<10%) of galactose, mannose, xylose, fructose, and fucose; and trace amounts (<1.0%) of glucuronic acid, arabinose, and rhamnose. Fraction 4OH only had small amounts (<10%) of glucose, galactose, and mannose; and trace amounts (<1.0%) of fructose, xylose, mannitol, fucose, rhamnose, arabinose, and glucosamine, that varies (from 0.09 to 0.9%). Insoluble polysaccharide (INS) had small amounts (<10%) of glucose, and rhamnose; and trace amounts (<1.0%) of galactose, mannose, glucuronic acid, fructose, mannitol, xylose, fucose, arabinose, and glucosamine that ranged from 0.12 to 0.99%. The total monosaccharides contents

for its raw materials and each fraction were; raw=87.55±7.47, Cr crude=11.68±0.06, 1OH=201.50±0.0, 4OH=33.93±0.0, and INS=67.49±0.0 (Table XVIII).

Table XVIII. Unit sugar composition of raw and extracted fractions from the Philippines green seaweed *C. racemosa*.

Monosaccharide	<i>C. racemosa</i>				
	Raw	Cr crude	1OH	4OH	INS
	(% of total sugars)				
Mannitol	-	-	0.12±0.00	0.24±0.00	0.50±0.00
Fucose	1.49±0.06	2.12±0.00	1.09±0.00	0.17±0.01	0.12±0.08
Rhamnose	0.37±0.01	1.07±0.00	0.43±0.00	0.09±0.00	2.19±0.01
Arabinose	0.22±0.03	0.68±0.00	0.62±0.00	0.09±0.00	0.09±0.02
Glucosamine	0.46±0.04	2.08±0.00	0.36±0.00	0.09±0.00	0.09±0.03
Galactose	4.98±0.34	18.40±0.11	8.16±0.21	1.39±0.00	0.99±0.00
Glucose	7.88±0.52	54.80±0.15	50.41±0.81	6.89±0.21	6.01±0.14
Mannose	2.41±0.32	1.88±0.01	7.73±0.21	1.15±0.04	0.68±0.08
Xylose	10.03±0.94	3.38±0.01	4.47±0.16	0.56±0.02	0.44±0.00
Fructose	0.83±0.20	-	2.66±0.00	0.90±0.34	0.56±0.09
Ribose	1.41±0.02	-	-	-	-
Glucuronic acid	0.23±0.00	3.90±0.00	0.87±0.05	-	0.65±0.04
Others	4.81±0.40	-	3.66±0.12	1.99±0.08	14.65±0.10
Total (µg mg⁻¹) dw	87.55±7.47	11.68±0.06	201.50±0.12	33.93±0.05	67.49±0.05

- = not detected; The values are expressed in g 100g⁻¹ of total sugars in the fractions.

The present study sugar composition is somewhat similar to the results of Ghosh *et al.* (2004) using the same method of extraction from *C. racemosa*, where it showed the presence of glucose, xylose, galactose, smaller amounts of mannose, and traces of rhamnose, arabinose, and fucose. We obtain high percentages of glucose and galactose in fractions Cr crude, and 1OH which is in line with Ghosh *et al.* (2004), and other dominant sugars such as mannose, and xylose are also present in all fractions. However, although dominant, these sugars are just in small proportions, especially fractions 4OH and INS compared to Ghosh *et al.* (2004). In Ghosh *et al.* (2004), the insoluble residue's primary sugar is xylose, indicating the presence of a xylan. In the present study, we observe xylose in all fractions, and it

is even the primary sugar in the raw *C. racemosa* sample. Another study of Shevchenko *et al.* (2009) using the same extraction (except INS) from *C. lentillifera* and *C. sertularioides* showed sugar composition of glucose, galactose, mannose, and xylose. Glucose dominates among these sugars in all fractions except in fraction 4OH *C. sertularioides*, which is dominated by galactose and a lesser portion of glucose. These results of Shevchenko *et al.* (2009) supported the present study in which all of its sugar composition was also found in different proportions, and glucose was also the dominant sugar in all fractions.

Table XIX. Unit sugar composition of raw and extracted fractions from the Philippines green seaweed *U. lactuca*.

Monosaccharide	<i>U. lactuca</i>					
	Raw	DS	DA	PP1	PP2	PP3
	(% of total sugars)					
Mannitol	-	-	-	-	-	0.63±0.05
Fucose	-	0.23±0.09	0.29±0.13	0.20±0.09	0.20±0.00	-
Rhamnose	5.02±0.20	5.13±3.11	3.56±2.03	5.87±1.28	6.44±0.75	0.54±0.08
Arabinose	-	0.01±0.00	0.01±0.00	0.01±0.00	0.03±0.00	0.02±0.00
Glucosamine	-	0.01±0.01	0.07±0.04	0.02±0.01	0.02±0.00	0.04±0.01
Galactose	0.29±0.01	0.43±0.16	0.49±0.21	0.18±0.00	0.29±0.02	0.64±0.08
Glucose	6.52±0.16	0.99±0.27	6.40±2.21	3.73±0.96	12.42±1.02	2.55±0.32
Mannose	0.13±0.01	0.15±0.07	0.40±0.19	0.15±0.06	0.19±0.00	0.19±0.02
Xylose	1.06±0.02	0.15±0.13	0.18±0.16	0.20±0.16	0.34±0.00	0.06±0.02
Fructose	0.03±0.00	0.27±0.00	0.51±0.01	0.15±0.00	-	0.29±0.19
Ribose	0.04±0.00	0.16±0.00	-	-	-	-
Glucuronic acid	1.26±0.05	0.17±0.08	0.32±0.12	0.43±0.00	0.43±0.06	0.08±0.03
Others	2.42±0.10	3.27±0.44	2.94±0.66	3.16±0.63	4.10±0.92	13.02±0.62
Total (µg mg⁻¹) dw	41.83±1.45	26.91±11.43	37.94±14.40	35.03±8.21	60.62±7.46	45.19±3.59

- = not detected; The values are expressed in g 100g⁻¹ of total sugars in the fractions.

Eight monosaccharides have been identified in raw *U. lactuca*, 11 in DS, 10 in DA, 10 in PP1, 9 in PP2, and 10 in PP3. Primary sugar units of raw *U. lactuca*, were smaller amounts (<10%) of glucose, rhamnose, glucuronic acid, and xylose; and a trace amount (<1.0%) of galactose, mannose, and fructose. Fraction PP1 had rhamnose (5.87%) and glucose (3.73%) as main sugars; and trace values that ranged

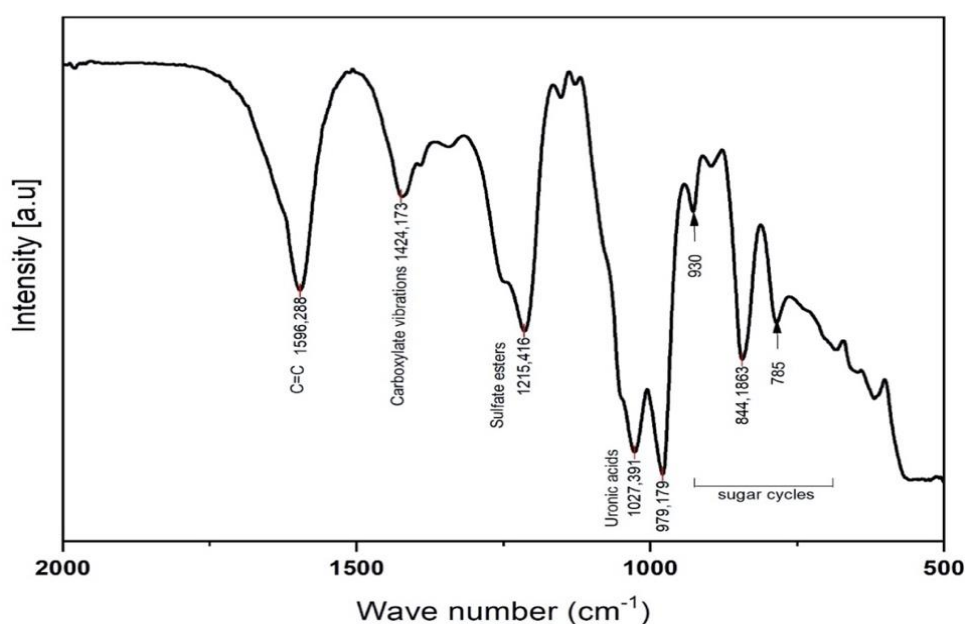
(from 0.02 to 0.43%) of glucuronic acid, fucose, xylose, galactose, mannose, and fructose. Fraction PP2 had glucose (12.42%) as major sugar, with a smaller amount (<10%) of rhamnose, and trace values that ranged (from 0.02 to 0.43%) of glucuronic acid, xylose, fucose, mannose, and galactose. Fraction PP3 had glucose (2.55%), and trace values ranged (from 0.04 to 0.64%) of galactose, rhamnose, fructose, mannose, glucuronic acid, and xylose. Ulvan DS primary sugar was rhamnose (5.13%), and trace sugar values (0.01-0.99%) of glucose, galactose, fructose, fucose, glucuronic acid, xylose, and mannose. Residual sugars of Ulvan DA with ten monosaccharides composed of glucose (6.40%) and rhamnose (3.56%) as main sugars and trace amounts (0.07-0.51%) of fructose, galactose, mannose, glucuronic acid, fucose, and xylose. The total monosaccharides contents for its raw materials and each fraction were; raw=41.83±1.45, DS=26.91±11.43, DA=37.94±14.403, PP1=35.03±8.21, PP2=60.62±7.46, and PP3=45.19±3.59 (Table XIX). Ulvan backbone is most commonly made-up of α - and β -(1,4)-linked monosaccharides (rhamnose, xylose, glucuronic acids, and iduronic acids) with characteristic repeating disaccharide units (Kidgell *et al.* 2019).

Ulvans from *U. armoricana* (Lahaye and Robic, 2007) and *U. rotundata* (Robic *et al.* 2009) reported that it consists of rhamnose, glucose, xylose, glucuronic acids, and iduronic acids. Their results showed that rhamnose was the dominant sugar, followed by sulfates and glucose. These sugars also indicate the presence of Ulvans in the extracts. Similar results were also found in the present study where sulfates, uronic acids, glucose, and rhamnose were the main sugars but in smaller proportions in all *U. lactuca* fractions. According to Hardouin *et al.* (2016) and Lahaye and Robic, (2007) that alcohol extraction decreased the content in minor sugars and glucose in the material. These small proportions of all sugar composition in all fractions in the present study could be attributed to the extraction method. The presence of glucose and other sugars in the present study indicates the presence of other types of saccharides aside from Ulvans. According to Lahaye and Robic (2007), these other sugars are probably not in the form of free oligosaccharides since they have not been removed during dialysis. Their presence in the aggregated fraction or fraction DA in the present study indicates the presence of carbohydrates

of a different nature aside from Ulvans (Lahaye and Robic, 2007). Both Lahaye and Robic, (2007) and Hardouin *et al.* (2016) reported the presence of β -glucans, polymers of glucose in their samples. This result could also explain the dominant levels of glucose in all fractions in the present study.

3.1.5 Fourier Transform Infra-Red (FT-IR) spectroscopy

Each type of polysaccharide having different spectral number characteristics, the wavenumbers of the spectral bands are therefore specific to each polymer. The values indicated by lines or arrows are the spectral bands' characteristics of Ulvans shown in Fig 37. The polysaccharide used as the standard for the *U. lactuca* fractions measurements was the Ulvan DS produced and characterized (Fig. 37).



The value specified on the spectrum indicates the specific wavenumbers for the identification of Ulvans and is expressed in cm⁻¹.

Figure 37. Infra-red spectrum of the Ulvan DS sample on the 2000-500 cm^{-1} zone. Ulvan DS was also used as a standard for polysaccharide differentiation of *U. lactuca* in the present study.

Infrared spectroscopy provides useful information on the position of sulfate groups of polysaccharides (Ghosh *et al.* 2004). The calculated FTIR spectra of green seaweeds *C. racemosa* (Cr crude, 1OH, 4OH, and INS) and *U. lactuca* (PP1, PP2, PP3, DS, and DA), spectral bands of interest for polysaccharide extracts were shown in Fig. 38 and 39.

The IR spectrum of *C. racemosa* extracts (Fig. 38) within 2000-500 cm^{-1} zone or the fingerprint region for polysaccharides were used for data analysis. Extracts showed spectral bands of high intensity between 1025 and 1000 cm^{-1} , while bands of medium or low intensity at [1641-1630 cm^{-1}], [1561-1546 cm^{-1}], and [1245-1240 cm^{-1}], in all extracts. Moreover, low-intensity peak at 871 cm^{-1} was shown in fraction Cr crude, and at 793 cm^{-1} in fraction INS. Table XX summarizes some of the major IR vibrational modes of *C. racemosa* polysaccharides (Ghosh *et al.* 2004; Marungrueng and Pavasant, 2007; Robic *et al.* 2009; Gómez-Ordóñez and Rupérez, 2011; Sarada *et al.* 2014; Fernando *et al.* 2017, Barbosa *et al.* 2019; Figueira *et al.* 2020).

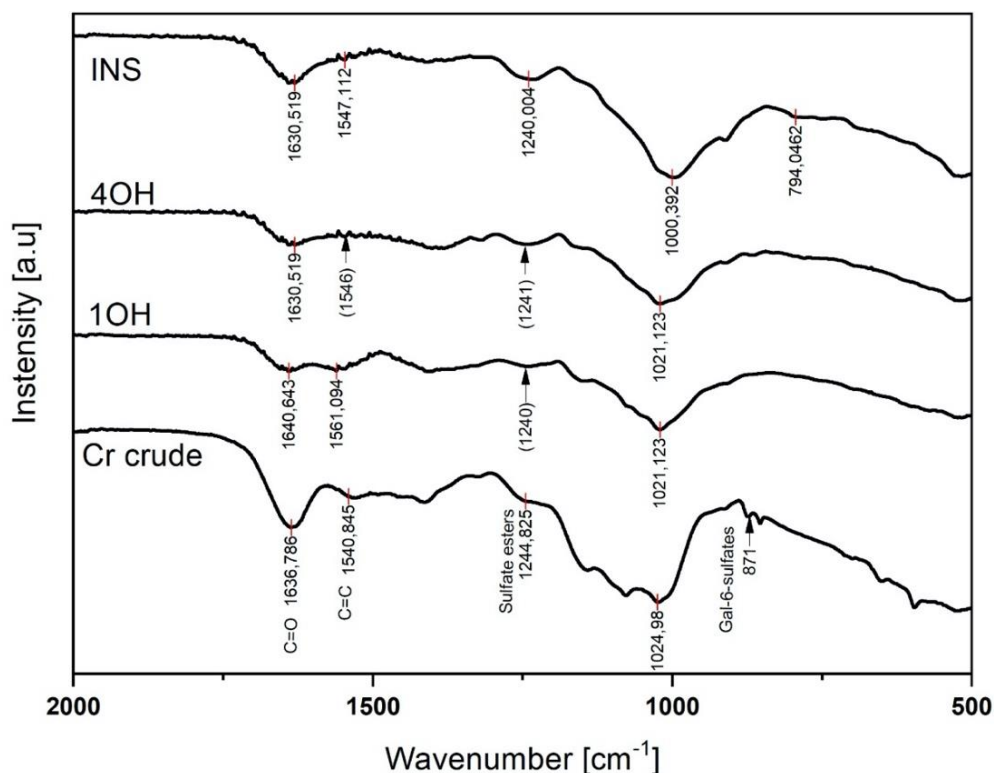


Figure 38. FT-IR spectra of *C. racemosa* extracts (Cr crude, 1OH, 4OH, and INS).

The FTIR intensity bands in the ranges of [1260-930 cm^{-1}] and [850-800 cm^{-1}] are distinguishing characteristics between different types of main galactans as these regions are known as stretching vibrations of the sulfate esters and the 3,6-anhydrogalactose ring. All spectral bands between [1260 and 1220 cm^{-1}] are related to the sulfation level in general (Rochas *et al.* 2009; Barbosa *et al.* 2019). The spectral bands at [1245-1240 cm^{-1}] and at [1025-1000 cm^{-1}] in all *C. racemosa* extracts were due to stretching of $-\text{SO}_3$ and C-O in ring groups (Gómez-Ordóñez and Rupérez 2011; Sarada *et al.* 2014). The medium and weak intensity bands at [1640-1630 cm^{-1}] in all extracts were due to carboxylic acid vibrations. The peaks at [1561-1540 cm^{-1}] bands in all extracts indicate amide N-H bending vibrations, where the previous *C. racemosa* extracts reported the presence of N-H involving in metal adsorption binding process (Javaid *et al.* 2011; Sarada *et al.* 2014). The bands at 871 and 793 cm^{-1} found in Cr crude and INS extracts are characteristics of the presence of sulfate groups on the molecule. At 871 cm^{-1} , this indicates the presence of S=O and C-S-O bands from ester sulfonate (Ghosh *et al.* 2004; Sarada *et al.* 2014), and associated with 3,6-anhydrogalactose and the sulfation of C-4, C-6 of

galactose units and C-2 of 3,6-anhydrogalactose (Fernando *et al.* 2017). The band at 793 cm^{-1} is of Si-C group, and the bands present below 800 cm^{-1} are fingerprint zone of phosphate and sulfur functional groups and N containing bioligands (Sarada *et al.* 2014). This silica particle was observed attached to the thallus during collection, and even after cleaning, and drying of samples. A similar peak was reported in Sarada *et al.* (2014) for *Caulerpa fastigiata*, where the presence of siliceous from diatomaceous earth in algal waste and composite material.

Table XX. List of InfraRed (IR) vibrational modes characteristic to polysaccharides used in determining spectral bands of interest for the identification and discrimination of green algal polysaccharides from *C. racemosa* (adapted from Ghosh *et al.* 2004; Marungrueng and Pavasant, 2007; Robic *et al.* 2009; Gómez-Ordóñez and Rupérez, 2011; Sarada *et al.* 2014; Fernando *et al.* 2017; Barbosa *et al.* 2019; Figueira *et al.* 2020)

Region/Band (cm^{-1})	Type of bond	Identification
Common to <i>Caulerpa</i> species		
1780-1670	C=O	Carboxyl group
1650, 1400	C=O-OH	Carbonyl of a carboxylic acid group
1549	-NH	Amide N-H bending vibrations
1420	C-N	C-N stretching
1248	-SO ₃	-SO ₃ stretching
1200-1040	S=O	Sulfonyl
1033	-C-O	-C-O benzene ring stretching
854	S=O; C-O-S	S=O; C-O-S bands from ester sulfonate, 3,6-anhydrogalactose and the sulfation of C-4, C-6 of galactose units and C-2 of 3,6-anhydrogalactose
791.72	Si-C	Presence of siliceous group

The spectrum of *U. lactuca* Ulvans (Fig. 37 and 39) within 2000-500 cm^{-1} zone or the fingerprint region for polysaccharides were used for data analysis. Extracts from Ulvan DS used as the standard, have specific spectral bands of high intensity at [1596, 1215, 1027 and 979 cm^{-1}], and bands of medium or low intensity at [1424, 930, 844, and 785 cm^{-1}]. Table XXI summarizes some of the significant IR vibrational modes of *U. lactuca* (Robic *et al.* 2009; Suresh *et al.* 2012; Pezoa-Conte *et al.* 2015; Fernando *et al.* 2017; Prabhu *et al.* 2019; Olasehinde *et al.* 2019; Figueira *et al.* 2020).

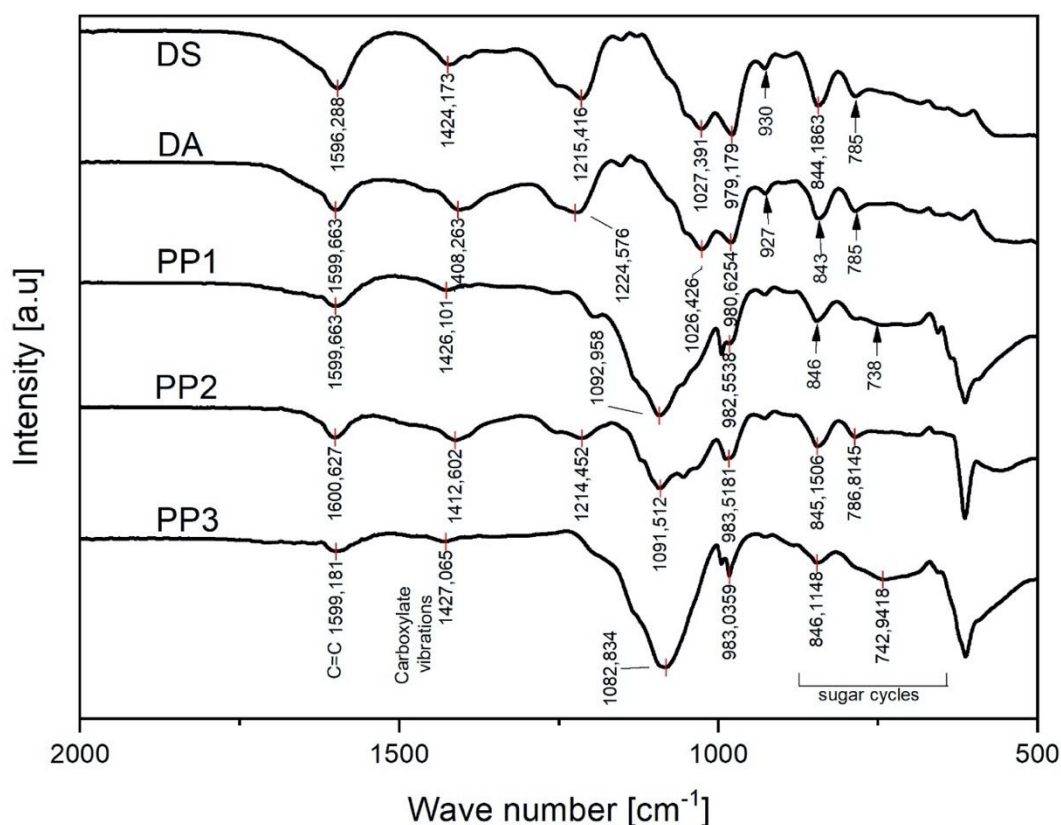


Figure 39. FT-IR spectra of *U. lactuca* extracts (DS, DA, PP1, PP2, and PP3).

The strong and weak intensities of carboxylic vibrations were found around 1600 and 1420 cm^{-1} for all Ulvan fractions. The strong intensity at around 1600 cm^{-1} corresponded to an asymmetrical stretching band attributed to the presence of OH groups within the uronic acids' spectral bands. This similar peak was reported in the previous study for *Ulva lactuca* (Olasehinde *et al.* 2019) and *U. fasciata* (Figueira

et al. 2020) polysaccharide fractions. While the weaker intensity band, at around 1420 cm^{-1} was symmetric stretching vibration of carboxylic groups (Robic *et al.* 2009; Hernández-Garibay *et al.* 2011; Figueira *et al.* 2020). The intensity range at $[1200-1000\text{ cm}^{-1}]$ was dominated by sugar ring vibrations that overlapped stretching vibrations of C-OH side groups and the C-O-C glycosidic bond vibrations. The bands at $1092-1082\text{ cm}^{-1}$ (fractions PP1, PP2, and PP3) and about 1026 cm^{-1} (fractions DS and DA) with the highest intensities were likely due to C-O stretching from the two main sugars rhamnose and glucuronic acid. These similar intensity bands were also found in the previous study for *Ulva armoricana* and *Ulva rotundata* fractions (Robic *et al.* 2009). The double spectral band at $[1224-1214\text{ cm}^{-1}]$ in the Ulvan fraction DS, DA, and PP2 indicates the presence of sulfate groups that belongs to a band of S=O elongation bond. At intensity bands $[983-979\text{ cm}^{-1}]$ in all fractions, corresponds to glycosidic linkages, which have also been reported in previous studies (Robic *et al.* 2009; Hardouin, 2015; Figueira *et al.* 2020). The bands around 845 cm^{-1} (in all Ulvan fractions), around 785 cm^{-1} (fractions DS, DA, and PP2) and probably a shift of intensity bands at around 740 cm^{-1} (fractions PP1 and PP3) are characteristics of the presence of sulfate groups on the molecule. At 845 cm^{-1} , this is of a C-O-S elongation band in the axial position, and at 785 cm^{-1} is of C-O-S vibration deformation in the equatorial position. These bands are due to these sugar cycles, according to Robic *et al.* (2009), and are a typical signal for Ulvans (Robic *et al.* 2009; Hernández-Garibay *et al.* 2011; Olasehinde *et al.* 2019; Figueira *et al.* 2020). The lower intensity band at about 930 cm^{-1} for fractions DS and DA indicates the bonds' deformation vibrations. This peak also indicates the presence of 3,6-anhydro-D-galactose from which was reported in *Ulva rigida* extract extracted by distillable ionic liquid (DIL) (Pezoa-Conte *et al.* 2015). According to Olasehinde *et al.* (2019), the presence of sulfate groups in algal polysaccharides is the origin of their biological activities.

Table XXI. List of InfraReds (IR) vibrational modes characteristic to polysaccharides used in determining spectral bands of interest for the identification and discrimination of green algal polysaccharides from *U. lactuca* (adapted from Robic *et al.* 2009; Suresh *et al.* 2012; Pezoa-Conte *et al.* 2015; Fernando *et al.* 2017; Prabhu *et al.* 2019; Olasehinde *et al.* 2019; Figueira *et al.* 2020)

Region/Band (cm ⁻¹)	Type of bond	Identification
Common to <i>Ulva</i> species		
1650	COOH	The asymmetrical stretch of a carboxyl group
1618, 1608	O-H	Presence of OH groups within uronic acid bands
1400	COOH	The symmetrical stretch of a carboxyl group
1235, 1225, 1260	S-O	Elongation of S=O (sulfate esters)
1200-1000		Sugar ring vibrations overlap with stretch vibrations of (C-OH) side groups and (C-O-C) glycosidic bonds
1055	C-O	Likely due to C-O stretching from two main sugars (rhamnose and glucuronic acid)
975 (980)		Glycosidic linkages
933-928		3,6-anhydro- D-galactose
847-853 (845)	C-O-S	C-O-S elongation in axial position (sugar cycles)
(789-770), 785, 748	C-O-S	C-O-S deformation in equatorial position (sugar cycles)

3.2 Biological activity

3.2.1 Cytotoxicity and antiviral activity evaluation

As shown in the previous part of this chapter (3.1.3. Biochemical composition of extracts), the extracts obtained have biochemical contents that differ with its raw material (see Magdugo *et al.* 2020). Aside from sulfates, monosaccharides composition (Wang *et al.* 2014), the amount of proteins (Hardouin *et al.* 2016), and its AA could impact the antiviral activity reported in the extracts of *C. racemosa* and *U. lactuca*.

After three days of treatment, microscopically visible alteration of normal cell morphology was observed, and viability assay showed destruction of the cell layer. No cytotoxic effect of polysaccharides (CC_{50} greater than $200 \mu\text{g mL}^{-1}$) on the Vero cells was observed for both *C. racemosa*, and *U. lactuca* extracts in ranged of concentrations assayed. All fractions had low EC_{50} values except fractions PP1 and PP3 in *U. lactuca*. Fraction 4OH from *C. racemosa* was insoluble and thus, cannot be tested (Table XXII). Efficacy or the cellular protection of *C. racemosa* fractions showed that fraction Cr crude had maximum efficacy of 88.20%, is higher than 1OH (82.36%), and is higher than INS (63.85%) all at $200 \mu\text{g mL}^{-1}$ after 72 h infection. The potency or the effectivity of the Cr crude at 50% concentration, only needs $25.33 \mu\text{g mL}^{-1}$ of sample (EC_{50} : $25.33 \mu\text{g mL}^{-1}$), which is slightly higher than 1OH (EC_{50} : $32.15 \mu\text{g mL}^{-1}$), and four times potent than INS (EC_{50} : $112.26 \mu\text{g mL}^{-1}$). Efficacy and potency evaluation are shown in appendix 7 and 8, while its statistical analysis is shown in appendix 17.1 and 17.2 for *C. racemosa* and *U. lactuca* fractions, respectively. These antiviral activities mentioned here in the present study for *C. racemosa* extracts, although active, yet appear to be less potent compared to Ghosh *et al.* (2004) on HWE extract from *C. racemosa* against HSV-1 strains (EC_{50} =2.2-4.2 $\mu\text{g mL}^{-1}$). The HWE fraction from Ghosh *et al.* (2004) was considered a selective inhibitor of thymidine kinase (TK) acyclovir-resistant HSV-1 strains in Vero cells. Wang *et al.* (2014) reported antiviral activities against HSV-1 of sulfated polysaccharides from *Caulerpa brachypus* (EC_{50} =9.6 $\mu\text{g mL}^{-1}$) having a direct correlation with the degree of sulfation (0.5), and sugar units found (rhamnose, xylose, and glucose). In the present study, major sugar units (glucose, galactose, and mannose) were found highest to lowest in this trend: fraction Cr crude > 1OH > INS. Also, FTIR confirms the presence of sulfate groups and the 3,6-

anhydrogalactose. Moreover, no direct correlation found in the small amounts of rhamnose and xylose with antiviral activities in each *C. racemosa* fraction. Several factors could influence the variation of antiherpetic activity results between the present and previous studies such as; monosaccharide composition, sulfate content, polysaccharides chemical structure and its molecular weight, location, and period of collection, and the sensitiveness of methods used. The *Caulerpa racemosa* extracts results showed great potential in developing as an antiviral drug. However, these various factors mentioned must be understood better, and therefore extracts from *Caulerpa racemosa* must be studied further.

Table XXII. Evaluation of cytotoxicity and antiviral activity by cellular viability of Philippine green seaweeds *Caulerpa racemosa* and *Ulva lactuca*.

SPECIES	FRACTION	CC ₅₀ (µg mL ⁻¹)	EC ₅₀ (µg mL ⁻¹)
		Destruction	Protection
<i>C. racemosa</i>	Cr crude	>200	25.33±5.13
	1OH	>200	32.15±20.26
	INS	>200	112.26±43.45
Zovirax		>200	0.47±0.07
<i>U. lactuca</i>	DS	>200	136.82±15.54
	DA	>200	88.32±12.36
	PP1	>200	>200
	PP2	>200	122.13±14.9
	PP3	>200	>200
Zovirax		>200	0.37±0.01

CC₅₀: The 50% Cytotoxic Concentration reduced the absorbance of mock-infected cells to 50% of that of controls. EC₅₀: The 50% antiviral Effective Concentration: concentration that achieved 50% protection of virus-infected cells from the HSV-induced destruction. Fraction Cr crude, 1OH, and INS are polysaccharides that were successively extracted from HWE insoluble residue. Fraction DS and DA are dialyzed polysaccharides, while fraction PP1, PP2, and PP3 are polysaccharides that were successively extracted from crude Ulvan of *U. lactuca*.

In *U. lactuca*, the maximum efficacy or the cellular protection of fraction DA (75.24%) is slightly higher than DS (71.25%) and PP2 (70.34%), while much lesser found in PP1 (30.81%) and PP3 (14.17%). In terms of potency or the effectivity, fraction DA achieved 50% at 88.32 µg mL⁻¹ sample (EC₅₀: 88.32 µg

mL⁻¹) is more potent than PP2 (EC₅₀: 122.13 µg mL⁻¹) and DS (EC₅₀: 136.82 µg mL⁻¹). Kidgell *et al.* (2019) reported that the antiviral activity of Ulvan extends to different enveloped viruses of HPV, Newcastle disease, Japanese encephalitis, West Nile, dengue, yellow fever, influenza, and Measles Virus. In the present study, the antiviral activities for *U. lactuca* extracts had range values of EC₅₀ from 88.32 to 136.82 µg mL⁻¹) According to the methodology used to evaluate the antiviral effects of ulvans against viral targets, the concentration of ulvan required to inhibit viral yield by 50% are mixed ranging from weak (EC₅₀ >150 µg mL⁻¹) (Jiao *et al.* 2012; Lopes *et al.* 2017) to significant (EC₅₀ =0.1-30 µg mL⁻¹) (Chiu *et al.* 2012; Aguilar-Briseño *et al.* 2015; Morán-Santibañez *et al.* 2016). The present study of *U. lactuca* fractions had EC₅₀ values range (88.32-136.82 µg mL⁻¹) that appeared to be more potent and effective compared to Hardouin *et al.* (2016). Glucose (0.99-12.42%) and rhamnose (0.54-6.44%) content, although in a smaller proportion, were the major sugar constituents in the present study for *U. lactuca* fractions, which is also in accordance to Hardouin *et al.* (2016). From the thesis results of Hardouin (2015) using similar extraction methods, EC₅₀ activity is not only dictated on Ulvans or polysaccharides alone but also of its protein contents. Hardouin, (2015) reported (EC₅₀) values in fraction PP1 (EC₅₀ <400.0 ug mL⁻¹), and DA (EC₅₀=68.6 ug mL⁻¹) with proteins content of 22.3%, and 41.7%, respectively. In the present study of *U. lactuca* extracts, no pattern of sulfate content found to relate it to EC₅₀ values. However, the protein content of the fractions with antiviral activities against HSV-1 was high in the present study. Variations in the antiviral activity of ulvan from different sources indicate a significant effect of the structure. However, only a few studies probe the antiviral activity of ulvan, and consequently, the understanding of its structure-activity relationships is restricted (Kidgell *et al.* 2019).

3.2.2 Antioxidant activity, DPPH radical scavenging activity

The free radical scavenging of the blank and the fractions of *C. racemosa* and *U. lactuca* polysaccharide extracts were estimated by the decrease in absorbance due to the reduction of the 2,2-DiPhenyl-1-PicrylHydrazyl (DPPH) radical by the extracts. DPPH is a stable synthetic free radical chemical compound used for measuring the free radical scavenging activities of compounds or extracts. DPPH is scavenged by antioxidant molecules through the donation of hydrogen, forming the reduced diphenyl picrylhydrazine (DPPH-H), thus neutralizing the reactive chain and reducing the risk of cellular oxidative damage (Terme *et al.* 2018; Vinosha *et al.* 2019). The Butylated Hydroxyanisole (BHA) and Butylated Hydroxytoluene (BHT) standards presented an inhibiting concentration (IC₅₀) of 7.05±0.59 and 5.32±0.59 µg ml⁻¹, respectively. Percent maximum scavenging ability and inhibition activity of *C. racemosa* and *U. lactuca* polysaccharide extracts at (5 mg mL⁻¹) were shown in Table XXIII.

Antioxidant activity at 50% (IC₅₀) for *C. racemosa* polysaccharides fractions was found highest to lowest in this trend: fraction 4OH>1OH>INS>Cr crude with (203.07, 268, 548.7, and 5253.47 µg mL⁻¹), respectively. While antioxidant activity at 50% (IC₅₀) for *U. lactuca* fractions were found highest to lowest in this trend: fraction DA>PP3>DS>PP1>PP2 with (805.6, 970.9, 2072.2, 2883.2, and 2918.2 µg mL⁻¹), respectively. All polysaccharides extract from *C. racemosa*, and *U. lactuca* had <50% scavenging ability except fractions 4OH and PP1, which are slightly over 50%. All *C. racemosa* fractions except fraction Cr crude had lower (IC₅₀) values compared to *U. lactuca* fractions. The low IC₅₀ value indicates that the sample has a strong antiradical activity. Free radical scavenging activity evaluation was shown in appendix 9 and 10, while its statistical analysis was shown in appendix 17.6 and 17.7 for *C. racemosa* and *U. lactuca* polysaccharide fractions with its BHA and BHT standards.

Table XXIII. Summary of antioxidant activity, scavenging assay (DPPH) of Philippine green seaweeds *Caulerpa racemosa* and *Ulva lactuca*.

	IC ₅₀ (µg mL ⁻¹)
BHA (0-50 conc)	7.05±0.27
BHT (0-50 conc)	5.32±0.18
<i>C. racemosa</i>	
Cr crude	5253.47±0.55
1OH	267.99±0.24
4OH	203.07±0.25
INS	548.69±0.15
<i>U. lactuca</i>	
Uf DS	2072.16±0.57
Uf DA	805.59±0.28
Uf PP1	2883.26±10.19
Uf PP2	2918.20±10.52
Uf PP3	970.90±0.31

IC₅₀: corresponding to the concentration sufficient to obtain 50% of a maximum scavenging capacity. Cr crude: crude polysaccharide extract; fraction 1OH, 4OH, and INS: are polysaccharides extracted from HWE insoluble residue; fraction DS, and DA: are dialyzed polysaccharides; fraction PP1, PP2, and PP3: are successively extracted polysaccharides from crude Ulvan of *U. lactuca*. BHA: Butylated HydroxyAnisole; BHT: Butylated Hydroxytoluene.

Viral infections are often promoted by oxidative processes, favoring replication in infected cells, induction, and inhibition of cell proliferation (Sansone *et al.* 2020). In patients affected by herpes simplex, it was observed that an increase of Reactive Oxygen Species ROS-induced membrane phospholipids peroxidation caused dysfunction of vital cellular processes such as membrane transport and mitochondrial respiration. Seaweeds and their compounds are known to possess high antioxidant properties and are effective in protecting living organisms against oxidative damages caused by ROS (Vinosha *et al.* 2019). Discovery and screening of antioxidant compounds with antiviral properties are promising since the treatment of viral diseases requires the suppression of viral replication and cell survival promotion. Therefore, commercial antioxidants have been in high demand, and most of them are synthesized, including BHA and BHT. However, these

synthetic antioxidants have been reported to be toxic and carcinogenic in animal models (Sanjeeva *et al.* 2018). Fernando *et al.* (2017) reported DPPH activity with IC₅₀: >2 mg mL⁻¹ from crude polysaccharide of *C. racemosa*, and that sulfates (10.51% dw), or total polyphenols (4.38% dw) content, influenced scavenging activity. This polysaccharide from *C. racemosa* had galactans and mannans from its major sugars (15.36-32.71% dw), glucose, galactose, mannose, and xylose. However, the dose concentration in the present study for *C. racemosa* fractions was all twice as low (0.25-5 mg mL⁻¹) except for fraction crude. Thus, fraction 4OH, 1OH, and INS have better antioxidant activity than *C. lentillifera* polysaccharides of Tian *et al.* (2019) and *C. racemosa* crude polysaccharides of Fernando *et al.* (2017). Galactans and mannans were also present in *C. racemosa* fractions in the present study, which is in agreement with Fernando *et al.* (2017). Another study of crude polysaccharide (methanol extraction) from *C. racemosa* reported a 2-times higher DPPH scavenging activity (10.40-88.87%) at a lesser dose concentration of 31.25-2000 µg mL⁻¹ (Lavanya *et al.* 2015) than *C. racemosa* fractions in the present study. Another study correlated an increase in antioxidant activity (DPPH scavenge: 84.18, 84.75, 87.79, and 88.60%) with the increase in total polyphenols content (0, 1, 5, and 10% levels) in biscuits supplemented with *C. racemosa* powder (Kumar *et al.* 2018). In the present study of *C. racemosa* fractions, however, no pattern of relationships found between antioxidant activities and its sulfates (2.46-10.58% dw), uronic acids (0.1-1.5% dw), or total polyphenols (2.1-6.2% dw) contents.

The DPPH scavenging activity in the present study for all *U. lactuca* extracts except fraction PP1 is in accordance to (<50%) in sulfated polysaccharides from *Ulva lactuca* against DPPH (Olasehinde *et al.* 2019), and ulvan extracts from *U. pertusa* against superoxide radical (Qi *et al.* 2005). However, the concentration used for polysaccharides from *Ulva lactuca* against DPPH (333.33 µg mL⁻¹) was 15 times lower (Olasehinde *et al.* 2019), and therefore, had stronger antiradical activity compared to the present study *U. lactuca* extracts. Qi *et al.* (2005) also reported stronger antiradical activity of ulvan extracts from *U. pertusa* with different Mw (157.7-28.2 kDa); against superoxide (<50%, IC₅₀=22.1 µg mL⁻¹), against hydroxyl (5-60%, IC₅₀=1-2.8 mg mL⁻¹), and against Fe²⁺-chelating (23.9-

94.3%) activities all at 0.6-3.4 mg mL⁻¹. Shao *et al.* (2013) reported ulvan extracts from *U. lactuca* with different Mw (264-2 kDa), showed highest scavenged against superoxide (66.17%), and hydroxyl (82.56%, IC₅₀=6.03.8 mg mL⁻¹) radicals at 2-10 mg mL⁻¹ concentration were at lowest sulfate content (11.67% dw). Also, the strongest inhibition (IC₅₀: 805.59 µg mL⁻¹) in the present study had the lowest sulfate content (5.44% dw), which is somewhat in accordance to Shao *et al.* (2013) but is contrary to Qi *et al.* (2005). These activities mentioned, were said to be influenced: by protein and sulfate contents, or conjugated proteins to polysaccharides in *Ulva lactuca* polysaccharides (Olasehinde *et al.* 2019); by low molecular weights in *U. pertusa* polysaccharides (Qi *et al.* (2005); by combined factors of Mw, sulfates content, and proteins content in *U. lactuca* extracts with different Mw (Shao *et al.* 2013). No pattern of relationships found between antioxidant activities and proteins, sulfates, uronic acids, or polyphenols content of *U. lactuca* extracts in the present study. These activities would need to be studied. At the opposite, *Caulerpa cupressoides* var. *flabellata* has also been reported to contain sulfated polysaccharides with total antioxidant capacity and ferrous chelating activity (Costa *et al.* 2012) but showed no activity in superoxide and hydroxyl radical scavenging. Possibly, the antiradical activity could be related to phenolic compounds.

The *C. racemosa* and *U. lactuca* extracts from the present study are considered significant since they are derived from natural resources; therefore, they can be utilized to develop various functional and health foods. There are seven species of *Caulerpa* utilized as food in the Philippines, four of which are used for medical applications (Trono and Largo, 2019), and *Ulva* sp. was one of the main species farmed and sold on European market (Barbier *et al.* 2019). However, various factors mentioned must be understood better, and therefore extracts from *C. racemosa* must be studied further with a priority of investigating the structure of sulfated polysaccharide and antiviral and antioxidant mechanisms of action.

Conclusion and perspectives

In conclusion, the extraction yield results of *C. racemosa* are affected by geographic location, and even by the sensitiveness of the methods used. Biochemical composition of *C. racemosa* extracts is mainly composed of sulfates group (range 8.18-10.58%) and proteins (range 3.7-11.0%). Previous data of this species reported rich in lipids at 4.5% dw and showed real nutritional value for consumption requirements (see Magdugo *et al.* 2020). FTIR spectrum of *C. racemosa* extracts confirmed the presence of sulfate ester, sugars, and proteins, where, *C. racemosa* polysaccharide is mainly composed of sulfated heteropolysaccharides with arabinose, glucose, and xylose sugar units. The use of strong alkali (1-and 4M-KOH) to isolate hemicellulosic polysaccharides from *C. racemosa* HWE fraction, for the purpose of biochemical characterization is not necessary since all the information found in these fractions are already found in HWE fraction (see Magdugo *et al.* 2020). The mineral contents in the previous study for *C. racemosa* and *U. lactuca* are within safe levels and are potential functional foods for humans. Also, *C. racemosa* and *U. lactuca* had high pigments and carotene contents that can be used in the food industry and human health development. The *C. racemosa* fractions showed in vitro antiherpetic activity without cytotoxicity.

While, the extraction yield results of *U. lactuca* in the present study confirmed the effectiveness of the extraction method used based on the previous papers. Despite similar extraction methods used, different yield was observed and is due to the amount of crude ulvan used at the beginning of extraction and purification (successive precipitation and dialysis). Other contributing factors are geographic location, and even the sensitiveness of the methods used. Biochemical composition of *U. lactuca* extracts is mainly composed of sulfates group (range 15.79-20.03%) and proteins (range 7.1-19.6%) for successive precipitation

fractions; and sulfates group (range 5.44-16.0%), proteins (range 18.3-18.9%), and uronic acids (range 7.0-9.5%) for dialyzed fractions. The FTIR spectrum of *U. lactuca* extracts confirmed the presence of sulfate groups and sugars, where *U. lactuca* polysaccharides is mainly composed of Ulvan with rhamnose and glucose as the dominant sugar units. All *U. lactuca* fractions showed in vitro antiherpetic activity without cytotoxicity.

The *C. racemosa* and *U. lactuca* antioxidant results suggest that fresh seaweed should be preferred for human consumption. It is also important to evaluate the heavy metal and microbiological contents of this seaweeds in order to established reputation as food. The extracts from both species are considered significant since they are derived from natural resources; therefore, they can be utilized to develop various functional and health foods. The presence, degree, and distribution of the sulfate groups are important in determining the biological activity and to analyze its anti-viral potency in relation to structure and position of sulfate groups of polysaccharides (Figueira *et al.* 2020), and therefore must be studied further for these extracts in the future.

Chapter 4 Brown Seaweed **(*Sargassum polycystum* and *Sargassum ilicifolium*)**

4.1 Biochemical composition analysis of raw material

The biochemical composition of raw material from brown seaweed *Sargassum polycystum* and *Sargassum ilicifolium* for the present study were given in Table XXIV. The raw *S. polycystum* biochemical composition showed highest to lowest contents in this trend: neutral sugars>ash>proteins>uronic acids>sulfates>polyphenols>lipids. While the biochemical composition of raw *S. ilicifolium* showed highest to lowest contents in this trend: neutral sugars>proteins>ash>polyphenols>uronic acids>sulfates>lipids. Carbohydrate contents, including sulfated polysaccharides (corresponding to uronic acids, sulfate groups, and neutral sugars) in raw *S. polycystum* and raw *S. ilicifolium*, were about 62.0% and 42.4%, respectively.

Table XXIV. Biochemical composition of Philippine brown seaweeds *Sargassum polycystum* and *Sargassum ilicifolium*.

ALGAE	Content (% dry algal material)						
	Proteins	Neutral sugars	Sulfate groups	Uronic acids	Polyphenols	Ash	Lipids
<i>S. polycystum</i>	24.2±7.4	35.4±2.5	10.0±0.3	16.6±5.1	5.6±1.5	24.9±8.41	2.86
<i>S. ilicifolium</i>	17.8±2.9	24.2±1.0	8.88±0.2	9.4±1.4	11.1±1.1	17.0±2.96	3.66

Values are means ± SD (n=9) except for lipids.

Neutral sugars content for raw *S. polycystum* (35.4%) and raw *S. ilicifolium* (24.2%) are the most abundant component in the present study, as was also the case in most of the seaweeds in general (Hao *et al.* 2019). A lower amount of neutral sugars is reported (23.2%) from *Sargassum fluitans* (Bedoux *et al.* 2017), and (4.9-13.6%) from *Sargassum muticum* (Vandanjon *et al.* 2017) than the present study. Higher ash content (42.4%) was reported from *S. polycystum* (Matanjun *et al.* 2009) of the same species, and (37%) from *Sacchoriza polyschides* (Garcia *et al.* 2016)

than the raw *S. polycystum* (24.9%), and twice as high than the raw *S. ilicifolium* (17.0%) of the present study. However, the ash content in the present study was about and within the concentration range of edible brown seaweeds *Laminaria digitata* (19-36%) (Fleurence, 2016), and from *Sargassum muticum* (20.3-41.2%) (Vandanjon *et al.* 2017). The protein contents from raw *S. polycystum* (24.2%) and raw *S. ilicifolium* (17.8%) in the present study was within the range (17.6-34.6%) for *S. muticum* (Vandanjon *et al.* 2017), and higher than the range (5-9%) for edible brown seaweed *Laminaria digitata* (Fleurence, 2016). It is also found to be 4-times higher than (5.40%) *S. polycystum* (Matanjon *et al.* 2009) of the same species and 3-times lower than raw *S. ilicifolium* in the present study. About 2-times lower protein content (12.3%) from *Sargassum fluitans* (Bedoux *et al.* 2017), and about 3-times lower (7.3%) from *Sacchoriza polyschides* (Garcia *et al.* 2016) compared to the present study. Uronic acids content (1.1-4.1%) from *S. muticum* (Vandanjon *et al.* 2017) was quite low compared to the raw *S. polycystum* (16.6%) and raw *S. ilicifolium* (9.4%) in the present study. However, a similar result was found (9.8%) from *S. fluitans* (Bedoux *et al.* 2017) with raw *S. ilicifolium*, but it is lower than raw *S. polycystum* in the present study. Sulfates contents for raw *S. polycystum* (10.0%) and raw *S. ilicifolium* (8.88%) in the present study was within the range (6.5-10.3%) from *S. muticum* (Vandanjon *et al.* 2017), and higher than (1.5%) from *S. fluitans* (Bedoux *et al.* 2017). Carbohydrates contents for raw *S. polycystum* (62.0%) and raw *S. ilicifolium* (42.4%) in the present study were within the concentration range (42-62%) from edible brown seaweeds *Laminaria digitata* (Fleurence, 2016). Matanjon *et al.* (2009) reported a lower carbohydrates content (33.49%) from *S. polycystum* which is about half the amount of the same species, and a little lower compared to raw *S. ilicifolium* in the present study. Twice as low carbohydrates content (34.5%) reported from *Sargassum fluitans* (Bedoux *et al.* 2017), up to 4-times lower (4.2-15.2%) from *Sargassum muticum* (Vandanjon *et al.* 2017), and up to 9-times lower (6.3%) reported from *Sacchoriza polyschides* (Garcia *et al.* 2016), compared to the present study. The polyphenols contents (1.9-4.6%) from *S. muticum* (Vandanjon *et al.* 2017) was reported comparable to raw *S. polycystum* (5.6%), and quite lower than (11.1%) from raw *S. ilicifolium* in the present study.

Lipid contents for raw *S. polycystum* (2.86%) and raw *S. ilicifolium* (3.66%) in the present study were a little over the concentration range value (0.9-2.1% dw) for edible brown seaweeds *L. digitata* (Fleurence, 2016). Matanjun *et al.* (2009) reported about 8-times lower lipid content (0.29%) from *S. polycystum* compared to the same species in the present study. Lipid contents from *S. muticum* (3.15, 3.0, and 2.84% dw) with corresponding extraction procedures (chloroform/methanol, chloroform, and chloroform/methanol with centrifugation, respectively) (Terme *et al.* 2017), and (3.24% dw) from *Colpomenia sinuosa* (Ragonese *et al.* 2014) were found similar to the present study. Also, higher lipid contents found in (6.27%) *Dictyota dichotoma* (Ragonese *et al.* 2014), and (8.2%) *Sacchoriza polyschides* (Garcia *et al.* 2016) compared to the present study. These variations of results of carbohydrates, ash, proteins, uronic acids, polyphenols, and lipids from both present and previous studies in brown seaweeds, could be affected by geography, season, or growth conditions as stated in previous papers (Kok and Wong, 2018; Artemisia *et al.* 2019).

Conclusion and perspectives

In conclusion of this first part of the study for the biochemical analyses, the raw materials from *S. polycystum* and *S. ilicifolium* are mainly composed of carbohydrates (62.0%, 42.4%), and significant presence of ash (24.9%, 17.0%) and proteins (24.2%, 17.8%), respectively. Both species are rich in lipids (2.86%, 3.66%) and are higher than the concentration range value for edible brown seaweeds *L. digitata*. We acknowledge the intra-species variation of biochemical results. However, the biochemical composition of raw *S. polycystum* and raw *S. ilicifolium* were within the values specified for brown seaweeds. Thus, there is a potential for these species to be used as a raw material in food industry development or human health. Although *S. polycystum* is not traditionally utilized as food unlike

the *S. ilicifolium*, it is also important to evaluate the heavy metal and microbiological contents of this seaweeds in order to established reputation as food.

4.2 Cell wall polysaccharides purification

4.2.1 First treatment

Extraction yield in percent after three solvents (ethanol, chloroform/methanol, and acetone) are shown in Table XXV. We obtain respectively 71.57% and 79.94% for *S. polycystum* and *S. ilicifolium*. Initial and final weights of raw *S. polycystum* and *S. ilicifolium* that were used to calculate extraction yield in percent after three solvents were shown in appendix 5.

Table XXV. Percent extraction efficiency of Philippine brown seaweeds *Sargassum polycystum* and *Sargassum ilicifolium*.

Species	Solvents	Yields (%)
<i>S. polycystum</i>	Ethanol	71.57
	Chloroform/Methanol	
	Acetone	
<i>S. ilicifolium</i>	Ethanol	79.94
	Chloroform/Methanol	
	Acetone	

4.2.2 Polysaccharide extraction

The cell wall polysaccharides for *S. polycystum* and *S. ilicifolium* were extracted from Depigmented Algal Powder (DAP) with an initial weight of 10 g. From that 10 g, we extracted sequentially using four different solvents and obtained four different fractions (CaCl₂, HCl, Na₂CO₃, and KOH). The yields of the different extracted fractions for *S. polycystum* and *S. ilicifolium* are given in Table XXVI. Initial and final weights of raw *S. polycystum* and *S. ilicifolium* that were used to calculate extraction yield in percent at each purification step were shown in appendix 6.

The extraction yield of polysaccharides from *S. polycystum* fractions was found highest to lowest in this trend: Sp CaCl₂>Sp Na₂CO₃>Sp KOH>Sp HCl. While the extraction yield of polysaccharides from *S. ilicifolium* fractions was found highest to lowest in this trend: Si Na₂CO₃>Si CaCl₂>Si KOH>Sp HCl, respectively (Table XXVII). The fractions CaCl₂ and HCL are Fucoses Containing Sulfated Polysaccharides (FCSPs), while Na₂CO₃ is composed of alginates extract, and KOH is of cellulose and hemicellulose extracts (Deniaud-Bouët *et al.* 2017).

Table XXVI. Extraction yield and purification of polysaccharides from Philippine brown seaweeds *Sargassum polycystum* and *Sargassum ilicifolium*.

EXTRACTION	Yield (% dw)
<i>S. polycystum</i> (Sp)	
Depigmented	71.57
Sp CaCl ₂	13.83
Sp HCl	1.61
Sp Na ₂ CO ₃	10.77
Sp KOH	6.23
<i>S. ilicifolium</i> (Si)	
Depigmented	79.94
Si CaCl ₂	6.00
Si HCl	2.55
Si Na ₂ CO ₃	29.62
Si KOH	5.50

Overall yield= expressed as a percentage of total dry algae at the start.

Purification yield= expressed as a percentage of the dry mass used at each stage.

The highest yields obtained (>10% dw) from the present study were: Si Na₂CO₃ (29.6%), Sp CaCl₂ (13.8%), and Sp Na₂CO₃ (10.08%), and the rest are small (1.6-6.23%). The amount of FCSPs (CaCl₂ and HCl fractions) in *S.*

polycystum (15.44%) is almost twice as high than in *S. ilicifolium* (8.55%). However, the amount of fractions Na₂CO₃ (alginates) is in contrast and is about 3-times lower in Sp Na₂CO₃ than in Si Na₂CO₃. Fraction KOH (cellulose and hemicellulose) with remaining FCSPs were approximately the same (6.23, and 5.50%) in both species, respectively (Table XXVI).

Fucoidan extracts by DEAE-cellulose from *Sargassum polycystum* (Palanisamy *et al.* 2018) reported higher yields (22.3, 16.9, and 19.4%) and also lower yield (5.3%) compared to (15.4%) FCSPs from Sp CaCl₂ + Sp HCl of the same species, and (8.6%) FCSPs from Si CaCl₂ + Si HCl of the present study. Another study of crude fucoidan extracts subjected to different temperatures (60-90 °C), reported lower yields (6.0-7.36%) from *S. turbinarioides*, and (2.89-3.49%) from *S. ilicifolium* (Artemisia *et al.* 2019) compared to (15.4%) FCSPs from Sp CaCl₂ + Sp HCl, and (8.6%) FCSPs from Si CaCl₂ + Si HCl of the same *S. ilicifolium* species in the present study. Fucoidan extracts usually consisted of about 5-10% from dry brown seaweed (Artemisia *et al.* 2019). However, Usoltseva *et al.* (2017) reported a meager yield of 0.28% laminarin, and 0.15% fucoidan from *Sargassum duplicatum*. In contrast, Puspita *et al.* (2017) produced better yield (22.8%) from the water extract from *S. polycystum* than the present study Sp CaCl₂ (13.8%) and Sp Na₂CO₃ (10.08%) of the same species. However, lower yields of water extracts from *S. ilicifolium* (14.7%), and from *S. aquifolium* (17.3 %) (Puspita *et al.* 2017) than (29.6%) from Si Na₂CO₃ in the present study.

The alginate yield (15.85%) from *S. polycystum* (Kok and Wong, 2018) was about 50% higher than alginate rich fraction Sp Na₂CO₃ (10.08%) of the same species and was 2-times lower than fraction Si Na₂CO₃ (29.6%) in the present study. Sinha *et al.* (2010) reported impure alginate yield (33%) from *Sargassum tenerrimum*, which is similar to fraction Si Na₂CO₃ but is 3-times higher than Sp Na₂CO₃ in the present study. Another study of crude alginates yield (23%) from *Sargassum natans*, and (17%) from *Sargassum vulgare* (Rhein-Knudsen *et al.* 2017) were reported lower than fraction Si Na₂CO₃, and higher than fraction Sp Na₂CO₃ in the present study. However, alginate yield varies with species, seasonal growth, pretreatment, and extraction methods (Kok and Wong, 2018).

Moreover, the present study fractions Si Na₂CO₃, Sp CaCl₂, and Sp Na₂CO₃ produced better yields than the following: Aquadest extract from Indonesian *Sargassum* (8.5%) (Hidayati *et al.* 2019), methanolic extract (8.3, 3.1, and 5.7%) from *S. polycystum*, *S. ilicifolium*, and *S. aquifolium*, respectively (Puspita *et al.* 2017), and hot water extract (6.59%) from *S. fusiforme* (Ye *et al.* 2018). While, fucoidan fraction from *S. tenerrimum* produced better yield (31%) (Sinha *et al.* 2010) than FCSPs fractions Sp CaCl₂ + Sp HCl, or Si CaCl₂ + Si HCl in the present study.

4.2.3 Biochemical composition analysis of extracts

Biochemical composition of *S. polycystum* (Sp) and *S. ilicifolium* (Si) extracts (CaCl₂, HCl, Na₂CO₃, and KOH) were shown in Table XXVII.

The *S. polycystum* fraction Sp CaCl₂ (yield, 13.8%) showed biochemical composition from highest to lowest in this trend: proteins>neutral sugars>uronic acids>sulfates>polyphenols with corresponding range values (11.8-3.2%). Fraction Sp Na₂CO₃ (yield, 10.77%) had highest to lowest composition trend: neutral sugars>sulfates>proteins>uronic acids>polyphenols with range values (22.1-5.4%). Fraction Sp KOH (yield, 6.23%) and composition trend: neutral sugars>sulfates>uronic acids>proteins>polyphenols with range values (23.1-3.2%); and fraction Sp HCl (yield, 1.6%) and composition trend: neutral sugars>sulfates>proteins>uronic acids>polyphenols with range values (17.0-6.4% dw). While the *S. ilicifolium* fraction Si Na₂CO₃ (yield, 29.6%) showed biochemical composition from highest to lowest in this trend: neutral sugars> sulfates>uronic acids>proteins>polyphenols with range values (33.2-1.8%). Fraction Si CaCl₂ (yield, 6.0%) and composition trend: neutral sugars>sulfates>proteins>uronic acids>polyphenols with range values (22.2-2.0%). Fraction Si KOH (yield, 5.5%) and composition trend: neutral sugars>uronic acids>sulfates>proteins>polyphenols

with range values (24.5-2.5%); and fraction Si HCl (yield, 2.55%) and composition trend: proteins>neutral sugars>uronic acids>polyphenols>sulfates with range values (25.5-6.98% dw).

Table XXVII. Biochemical composition of polysaccharide extracts from Philippine brown seaweeds *Sargassum polycystum* and *Sargassum ilicifolium*.

ALGAE	Content (% dry algal material)					
	Yield (%)	Proteins	Neutral sugars	Sulfates group	Uronic acids	Polyphenol
<i>S. polycystum</i>						
Sp CaCl ₂	13.83	11.8±0.7	8.8±0.8	6.36±0.3	7.1±0.7	3.2±1.6
Sp HCL	1.61	10.0±0.2	17.0±0.7	11.62± 0.6	7.3±0.6	6.4±1.9
Sp Na ₂ CO ₃	10.77	9.6±0.3	22.1±0.2	12.0±0.2	8.5±0.4	5.4±1.2
Sp KOH	6.23	8.9±0.4	23.1±1.1	12.68±0.5	9.4±0.2	3.2±0.0
<i>S. ilicifolium</i>						
Si CaCl ₂	6.0	9.9±0.4	22.2±1.9	15.21±0.2	6.7±0.4	2.0±1.2
Si HCL	2.55	25.5±2.5	18.5±0.5	6.98±0.4	8.4±0.3	7.2±0.4
Si Na ₂ CO ₃	29.62	8.7±0.6	33.2±0.8	10.37±0.4	8.9±0.6	1.8±1.5
Si KOH	5.5	9.6±0.5	24.5±0.7	12.58±0.9	13.0±0.7	2.5±2.4

All fractions in the present study have high protein contents than 1.01% from *S. fusiforme* polysaccharides (Ye *et al.* 2018), and 0.1-1.2% from *S. pallidum* purified polysaccharide fractions by DEAE-sepharose fast flow (Li *et al.* 2017). The highest protein contents were fractions Si HCl (25.5%), Sp CaCl₂ (11.8%), and Sp HCl (10.0%), the rest are <10% or (8.7-9.9%). The protein content in crude fucoidan from *S. polycystum* reported 2-times lower (4.7%) (Palanisamy *et al.* 2018), than all *S. polycystum* and *S. ilicifolium* fractions in the present study except fucoidan fraction Si HCl (25.5%), which is 5-times higher.

The highest neutral sugars contents were fractions Si Na₂CO₃ (33.2%), Si KOH (24.5%), Sp KOH (23.1%), Si CaCl₂ (22.2%), and Sp Na₂CO₃ (22.1%), the rest are <20% or (8.8-18.5%). In the present study, neutral sugars were also found abundant components than proteins, and phenols, as was stated in the literature (Hao

et al. 2019). The present study neutral sugar contents FCSPs fraction Sp HCl (17.0%), and Sp CaCl₂ (8.8%) was somewhat in accordance to F3=19.71%, and F4=11.53% from purified crude fucoidan fractions of *S. polycystum* (Palanisamy *et al.* 2018). However, F1=23.51% and F2=51.12% of Palanisamy *et al.* (2018) were almost 3-times higher than FCSPs fraction Sp CaCl₂, and fraction Sp HCl in the present study of the same species. Also, from Palanisamy *et al.* (2018), reported similar sugar contents from two fractions (F1=23.51% and F3=19.71%), but reported 2-times higher in F2=51.12%, and 2-times lower in F4=11.53% compared with the present study FCSPs fractions [Si CaCl₂ (22.2%) and Si HCl (18.5%)], cellulose/hemicellulose rich fractions [Sp KOH (23.1%), and Si KOH (24.5%)]. Another study reported up to 3-times higher total sugar contents from (*fucoidan*=59%, and *sulfated fucoidan*=38%) *S. tenerrimum* (Sinha *et al.* 2010) compared to all FCSPs fractions from Sp and Si, and to cellulose/hemicellulose rich fraction Sp KOH (23.1%) and Si KOH (24.5%) in the present study. While Sinha *et al.* (2010) reported about 2-times higher total sugar contents (*impure alg*=51%, *sodium alginate* or *NaAlg*=64%, and *sulfated NaAlg*=42%) from *S. tenerrimum* than alginate rich fractions [Sp Na₂CO₃ (22.1%), and Si Na₂CO₃ (33.2%)] in the present study.

The highest sulfates contents in the present study were fractions Si CaCl₂ (15.21%), Sp KOH (12.68%), Si KOH (12.58%), and Sp Na₂CO₃ (12.0%), the rest are <12% or (6.36-11.62%). Polysaccharides extracts reported comparable sulfates contents (17.5%) from *S. fluitans* (Bedoux *et al.* 2017), and (13.18%) from *S. fusiforme* (Ye *et al.* 2018) compared to all fractions in the present study except FCSPs fraction Sp CaCl₂ (6.36) and Si HCl (6.98%) which was about 2-times lower. Similar sulfates result in purified polysaccharides (F1=7.2% and F3=6.5%) by DEAE-sepharose fast flow from *Sargassum pallidum* (Li *et al.* 2017) was reported with Sp CaCl₂ and Si HCl in the present study. While fraction F4=16.2% was similar to the rest of the fractions, and fraction F2=24.6% was up to 4-times higher than all fractions in the present study (Li *et al.* 2017). Palanisamy *et al.* (2018) reported similar sulfates contents of purified crude fucoidan (F1=14.21, F3=11.36, and F4=6.19%) from *S. polycystum* with the present study FCSPs fractions [Sp HCl

(11.62%), Sp CaCl₂ (6.36%), Si CaCl₂ (15.21%), and Si HCl (6.98%)] . In contrast, sulfates from crude fucoïdan (22.35%) and purified crude fucoïdan F2=20.41% (Palanisamy *et al.* 2018) reported 2-times higher than FCSPs (Sp HCl, Sp CaCl₂, Si CaCl₂, and Si HCl) in the present study. The cellulose-rich fractions [Sp KOH (12.68%), and Si KOH (12.58%)] in the present study have similar sulfates results to fraction F1=14.21%, and F3=11.36%, in Palanisamy *et al.* (2018). Sinha *et al.* (2010) reported up to 7-times lower sulfates content from *S. tenerrimum* fractions [fucoïdan (2%), and sulfated fucoïdan (6%)], and 2-times lower sulfates (7.66%) in crude fucoïdan from *S. binderi* (Lim *et al.* 2016), compared to all FCSPs fractions (Sp and Si) in the present study. Another fucoïdan extract reported up to 5-times higher (31.7%) sulfates content from *Sargassum duplicatum* (Usoltseva *et al.* 2017), than FCSPs fractions in Sp and Si and about 3-times higher than the rest of the fractions in the present study. While, about 6-times lower sulfates content in sulfated NaAlg (2%) from *S. tenerrimum* (Sinha *et al.* 2010), than alginate rich fractions [Sp Na₂CO₃ (12.0%), and Si Na₂CO₃ (10.37%)] in the present study.

All Sp and Si fractions in the present study have similar uronic acids contents <10% or (6.7-9.4%) except fraction Si KOH (13.0%). The uronic acids content (3.9%) of crude fucoïdan from *S. polycystum* (Palanisamy *et al.* 2018) was at least 2-times lower than all the Sp and Si fractions in the present study. Another study reported uronic acids contents of fucoïdan (9.0%) and sulfated fucoïdan (5.0%) from *S. tenerrimum* (Sinha *et al.* 2010) were similar compared to all FCSPs fractions from Sp and Si in the present study. While impure alginate reported similar uronic acids result (7%), the NaAlg (62%) and sulfated NaAlg (41%) fractions from *S. tenerrimum* were up to 7-times higher (Sinha *et al.* 2010) than alginate rich fractions [Sp Na₂CO₃ (8.5%), and Si Na₂CO₃ (8.9%)] in the present study.

The highest polyphenols contents were fractions Si HCl (7.2%), Sp HCl (6.4%), and Sp Na₂CO₃ (5.4%), the rest are <5% or (1.8-3.2%). The polyphenols content (1.2%) from *S. fusiforme* (Ye *et al.* 2018) was reported similar to the present study fractions Si Na₂CO₃ (1.8%), Si CaCl₂ (2.0%), and Si KOH (2.5%), while the rest of the fractions in the present study were up to 6 times higher than 1.2%. Puspita

et al. (2017) reported similar polyphenols contents of hot water extract (2.6%) from *S. muticum* compared to all fractions of Sp and Si in the present study.

These variations of biochemical results of from both present and previous studies in brown seaweeds extracts, could be affected by geography, season, or growth conditions as stated in previous papers (Artemisia *et al.* 2019; Kok and Wong, 2018). However, the biochemical composition of *S. polycystum* and raw *S. ilicifolium* extracts in the present study were within the values specified for brown seaweeds. Thus, there is a potential for these species to be used as a raw material in food industry development or human health, especially as there is an on-going development of appropriate sustainable culture technologies for *Sargassum* species in the Philippines (Trono and Largo, 2019).

4.2.4 Monosaccharide composition analysis

The unit sugar composition of raw and purified fractions (CaCl₂, HCl, Na₂CO₃, and KOH) of *S. polycystum* (Sp) and *S. ilicifolium* (Si) were determined by High-Performance Anion Exchange Chromatography (HPAEC). The results were given in Table XXVIII and XXIX and expressed as a percentage of sugars relative to the total sugars detected.

Eleven monosaccharides have been identified present in raw *S. polycystum*, 11 in Sp CaCl₂, 11 in Sp HCl, 9 in Sp Na₂CO₃, and 9 in Sp KOH. Main contents of monosaccharides in raw *S. polycystum* were small amounts (<10%; (5.0-1.98%)) of fucose, glucose, and galactose; and trace amount (<1.0%) of mannose, glucuronic acid, mannitol, glucosamine, xylose, fructose, rhamnose, and arabinose. The fraction Sp CaCl₂ only had small amounts (<10%; (4.90-1.84%)) of fucose, galactose, glucose, fructose, and mannose; and trace amount (<1.0%) of glucosamine, xylose, glucuronic acid, rhamnose, mannitol, and arabinose. Fraction Sp HCl had small amounts (<10%; (5.50-1.00%)) of fucose, galactose, glucose,

mannose, fructose, and glucosamine; and trace amount (<1.0%) of xylose, glucuronic acid, rhamnose, mannitol, and arabinose. Fraction Sp Na₂CO₃ had glucuronic acid (17.11%) as main sugar, small amounts (<10%; (6.87-1.58%)) of fucose, mannose, galactose, xylose; and trace amount (<1.0%) of glucose, mannitol, and glucosamine. Fraction Sp KOH had main sugar glucose (22.86%), small amounts (<10%; (7.04-1.74%)) of glucuronic acid, fucose, mannose, galactose, xylose; and trace amount (<1.0%) of fructose, mannitol, and glucosamine. The total monosaccharides contents for its raw materials, and each fraction were; Sp raw=42.23±3.97, Sp CaCl₂=53.80±1.77, Sp HCl=60.89±2.71, Sp Na₂CO₃=108.82±47.47, and Sp KOH=121.74±4.78 (Table XXVIII).

Table XXVIII. Unit sugar composition of raw and extracted fractions from Philippines brown seaweed *S. polycystum*.

Monosaccharide	<i>S. polycystum</i>				
	Raw	Sp CaCl ₂	Sp HCl	Sp Na ₂ CO ₃	Sp KOH
	(% of total sugars)				
Mannitol	0.75±0.03	0.16±0.00	0.25±0.01	0.28±0.03	0.27±0.01
Fucose	5.00±0.04	4.90±0.08	5.50±0.11	6.87±0.09	5.62±0.34
Rhamnose	0.32±0.01	0.34±0.01	0.38±0.02	0.14±0.01	-
Arabinose	0.28±0.01	0.11±0.02	0.16±0.01	-	-
Glucosamine	0.66±0.04	0.93±0.03	1.00±0.03	0.10±0.00	0.09±0.00
Galactose	1.98±0.08	3.75±0.10	4.55±0.20	4.15±0.26	2.69±0.12
Glucose	2.50±0.17	3.49±0.14	4.29±0.21	0.72±0.05	22.86±0.81
Mannose	0.95±0.00	1.84±0.08	2.06±0.04	4.32±0.27	4.65±0.21
Xylose	0.50±0.00	0.62±0.02	0.79±0.02	1.58±0.05	1.74±0.16
Fructose	0.40±0.10	2.20±0.09	1.46±0.34	-	0.31±0.00
Glucuronic acid	0.95±0.00	0.37±0.04	0.70±0.02	17.11±15.63	7.04±0.21
Others	3.33±0.39	2.82±0.10	3.22±0.08	8.25±2.58	3.42±0.05
Total (µg mg⁻¹) dw	42.23±3.97	53.80±1.77	60.89±2.71	108.82±47.47	121.74±4.78

- = not detected; The values are expressed in g/100g of total sugars in the fractions.

All Sp fractions in the present study had sugar units that are somehow similar or slightly lower than the reported mean range values for fucose (8.2-13%), galactose (5.4-11.5%), mannose (3.7-4.7%), and xylose (1.3-3.9%); while reported

atleast 5-times higher in glucose except fraction Sp KOH than (25.5-33.5%) by Thadhani *et al.* (2019) using almost similar condition of extraction from 3 *Sargassum* spp. (*S. wightii*, *S. crassifolium*, *S. polycystum*). Another similar sugar unit results (<6%) with the present study Sp fractions were; glucose (3%) from crude fucoidan and (5%) from sulfated fucoidan from *S. tenerrimum* (Sinha *et al.* 2010); glucose (1.13%) from crude fucoidan from *S. binderi* and galactose from purified fucoidan from *S. polycystum* (Palanisamy *et al.* 2018); mannose (4.16%), and xylose (2.9%) from crude fucoidan from *S. binderi* (Lim *et al.* 2016).

Several *Sargassum* studies reported higher monosaccharide contents than the present study Sp fractions: crude fucoidan fraction from *S. polycystum* had 8-times higher fucose content (46.8%), 3-times higher galactose (14.3%) 2-times higher glucose (11.5%) (Palanisamy *et al.* 2017). Sinha *et al.* (2010) reported crude fucoidan, sulfated fucoidan, and impure alginate samples from *S. tenerrimum* had 10-times higher contents in fucose (73, 70, and 68%), 20-times higher xylose (15, 16, and 17%), and slightly higher galactose (9, 9, and 11%), respectively. Also reported from *S. duplicatum* 10-times higher of fucose (51.3%), and galactose (48.6%) contents (Usoltseva *et al.* 2017); crude fucoidan from *S. binderi* had 6-times higher fucose (34.5%), and 2-times higher galactose (12.14%) (Lim *et al.* 2016); as well as hot water extracted polysaccharides from *S. fusiforme* had 6-times higher fucose (34.95%), galactose (35.09%), and mannose (12.41%), while 2-times higher glucose (13.41%) (Wu *et al.* 2014). Other sugar units such as mannitol, arabinose, rhamnose, and xylose were in trace amounts in all Sp fractions in the present study as was also reported in the literatures (Ale *et al.* 2011; Li *et al.* 2017; Ye *et al.* 2018).

While, there are ten monosaccharides identified present in raw *S. ilicifolium*, 11 in Si CaCl₂, 11 in Si HCl, 9 in Si Na₂CO₃, and 10 in Si KOH. Main contents of monosaccharides in raw *S. ilicifolium* were glucose (18.64%), mannitol (18.09%), fucose (17.44%), and galactose (10.73%); small amounts (<10%; (6.57-3.88%)) of mannose, xylose, glucuronic acid; and trace amounts (<1.0%) of arabinose, rhamnose and glucosamine. Fraction Si CaCl₂ had glucose (33.69%), fucose (15.95%), and galactose (14.64%) as main sugars, with small amounts (<10%;

(6.88-1.0%)) of mannose, xylose, glucuronic acid, fructose, and mannitol; and trace amounts (<1.0%) of glucosamine, rhamnose, and arabinose. Fraction Si HCl had glucuronic acid (14.94%), fucose (13.52%), and galactose (11.49%), with smaller amount (<10%; (4.70-1.52%)) of glucose, mannose, xylose; and trace values (<1.0%) of mannitol, rhamnose, glucosamine, fructose, and arabinose. Fraction Si Na₂CO₃ had smaller amounts (<10%; (5.42-1.18%)) of fucose, mannose, galactose, glucuronic acid, xylose, glucose; and trace values (<1.0%) of mannitol, fructose, and glucosamine. Fraction Si KOH had main sugar glucose (15.55%), and smaller amounts (<10%; (7.07-1.90%)) of fucose, mannose, galactose, glucuronic acid, xylose; and trace amounts (<1.0%) of mannitol, fructose, glucosamine, and rhamnose. The total monosaccharides contents for its raw materials and each fraction were; Si raw=299.67±8.48, Si CaCl₂=212.41±23.86, Si HCl=145.14±27.87, Si Na₂CO₃=80.69±3.97, and Si KOH=114.51±21.64 (Table XXIX).

Table XXIX. Unit sugar composition of raw and extracted fractions from the Philippines brown seaweed *S. ilicifolium*.

Monosaccharide	<i>S. ilicifolium</i>				
	Raw	Si CaCl ₂	Si HCl	Si Na ₂ CO ₃	Si KOH
	(% of total sugars)				
Mannitol	18.09±0.38	1.00±0.04	0.92±0.02	0.23±0.00	0.36±0.01
Fucose	17.44±0.52	15.95±1.64	13.52±0.31	5.42±0.12	7.07±0.45
Rhamnose	0.32±0.02	0.32±0.03	0.27±0.01	-	0.06±0.06
Arabinose	0.42±0.03	0.24±0.02	0.21±0.01	-	-
Glucosamine	0.29±0.01	0.71±0.08	0.23±0.01	0.07±0.00	0.10±0.01
Galactose	10.73±0.75	14.64±1.74	11.49±0.10	3.77±0.09	3.93±0.64
Glucose	18.64±0.08	33.69±4.17	4.70±0.10	1.18±0.34	15.55±5.60
Mannose	6.57±0.05	6.88±0.94	4.68±0.00	4.32±0.11	5.95±0.37
Xylose	4.06±0.02	2.08±0.39	1.52±0.11	1.12±0.04	1.90±0.04
Fructose	-	1.47±0.07	0.22±0.22	0.12±0.12	0.11±0.11
Glucuronic acid	3.88±0.07	1.81±0.17	14.94±9.59	1.64±0.19	2.00±0.01
Others	39.43±1.48	6.17±0.25	5.36±0.68	14.42±0.59	8.76±1.34
Total (µg mg⁻¹) dw	299.67±8.48	212.41±23.86	145.14±27.87	80.69±3.97	114.51±21.64

- = not detected; The values are expressed in g/100g of total sugars in the fractions.

All Si fractions in the present study had fucose, galactose, glucose, and mannose as major sugar units. These major sugars in all Si fractions are similar or slightly higher than the reported mean range values for fucose (8.2-13%), galactose

(5.4-11.5%), and mannose (3.7-4.7%) by Thadhani *et al.* (2019). However, Thadhani *et al.* (2019) had 5-times higher glucose content (25.5-33.5%) than fraction Si HCl (4.7%), 2-times higher than fraction Si KOH, similar glucose content with fraction Si CaCl₂ (33.69%), and more than 20-times higher than fraction Si Na₂CO₃ (1.18%) in the present study. Palanisamy *et al.* (2017) reported similar mannose content (5.6%) from crude fucoidan fraction of *S. polycystum* to all Si fractions, and 3-times higher fucose (46.8%) than FCSPs fraction (Si CaCl₂ and Si HCl), and upto 9-times higher fucose than fraction Si Na₂CO₃ and Si KOH, in the present study. In contrast, Palanisamy *et al.* (2017) reported 3-times lower glucose (11.5%) than fraction (Si CaCl₂), 2-times higher than (Si HCl), upto 8-times higher than Si Na₂CO₃, and similar content to Si KOH.

Several *Sargassum* species reported mostly higher monosaccharide contents. Crude fucoidan and sulfated fucoidan samples: from *S. tenerrimum* with fucose (73, and 70%) respectively, had 4-times higher than FCSPs fraction (Si CaCl₂ and Si HCl) and 10-times higher than fractions Si Na₂CO₃ and Si KOH. While slightly lower galactose (9, and 9%) than FCSPs fraction (Si CaCl₂ and Si HCl) and 2-times higher than fractions Si Na₂CO₃ and Si KOH, respectively (Sinha *et al.* 2010). Usoltseva *et al.* (2017) also reported 3-times higher fucose (51.3%) and galactose (48.6%) from fucoidan extract in *S. duplicatum*; also, crude fucoidan from *S. binderi* reported 2-times higher fucose (34.5%), and a similar result of galactose (12.14%) (Lim *et al.* 2016). Also, hot water extracts from *S. fusiforme* reported 2 to 7-times higher fucose (34.95%), galactose (35.09%), and mannose (12.41%) (Wu *et al.* 2014) than all Si fractions in the present study. Although this hot water extracted polysaccharides from *S. fusiforme* reported almost 3-times lower glucose (13.41%) (Wu *et al.* 2014) than fraction Si CaCl₂, it is also 3-times higher than fraction Si HCl, similar with fraction Si KOH, and way higher than fraction Si Na₂CO₃ in the present study.

All Si fractions in the present study had very low glucuronic acid (about 2.0%), except for fraction Si HCl (14.94%) than the range value of 13.7-24.2% (Thadhani *et al.* 2019). Other sugar units such as mannitol, arabinose, rhamnose,

and xylose were in trace amounts in all Sp fractions in the present study as was also reported in the literatures (Ale *et al.* 2011; Li *et al.* 2017; Ye *et al.* 2018).

The sugar profiles found that all of the fractions from *S. polycystum* and *S. ilicifolium* in the present study contained mostly the same types of sugars in different proportions. Several factors affect fucoïdan, and alginate contents, which include; the type of samples used, whether between or across the same species, life cycle, period, and location of the collection. These factors were also observed in the literature (Thadhani *et al.* 2019).

4.2.5 Fourier Transform Infra-Red (FT-IR) spectroscopy

The calculated FTIR spectra of brown seaweeds *S. polycystum* (Sp CaCl₂, Sp HCl, Sp Na₂CO₃, Sp KOH) and *S. ilicifolium* (Si CaCl₂, Si HCl, Si Na₂CO₃, Si KOH) spectral bands of interest were shown in Fig. 40 and 41, respectively. The IR spectrum of *S. polycystum* extracts (Fig. 40) within 2000-500 cm⁻¹ zone or the fingerprint region for polysaccharides were used for data analysis. Extracts showed spectral bands of high intensity at [1412-1408 cm⁻¹] for Sp CaCl₂, and Sp Na₂CO₃; at [1026-1025 cm⁻¹] for Sp CaCl₂, Sp HCl, and Sp Na₂CO₃; at 1596 cm⁻¹ for Sp Na₂CO₃; and at 1484 cm⁻¹ for Sp KOH. While bands of medium or low intensity between [1641-1637 cm⁻¹ and 535-526 cm⁻¹] for Sp CaCl₂, and Sp HCl; at [1325-1321 cm⁻¹ and 883-873 cm⁻¹] for Sp CaCl₂, Sp HCl, Sp KOH; at 1407 cm⁻¹ for Sp HCl; at [1063, and 815 cm⁻¹] for Sp Na₂CO₃. Table XXX summarizes some of the major IR vibrational modes of *S. polycystum* polysaccharides (Marudhupandi *et al.* 2015; Vandanjon *et al.* 2017; Palanisamy *et al.* 2018; Kok and Wong, 2018; Artemisia *et al.* 2019).

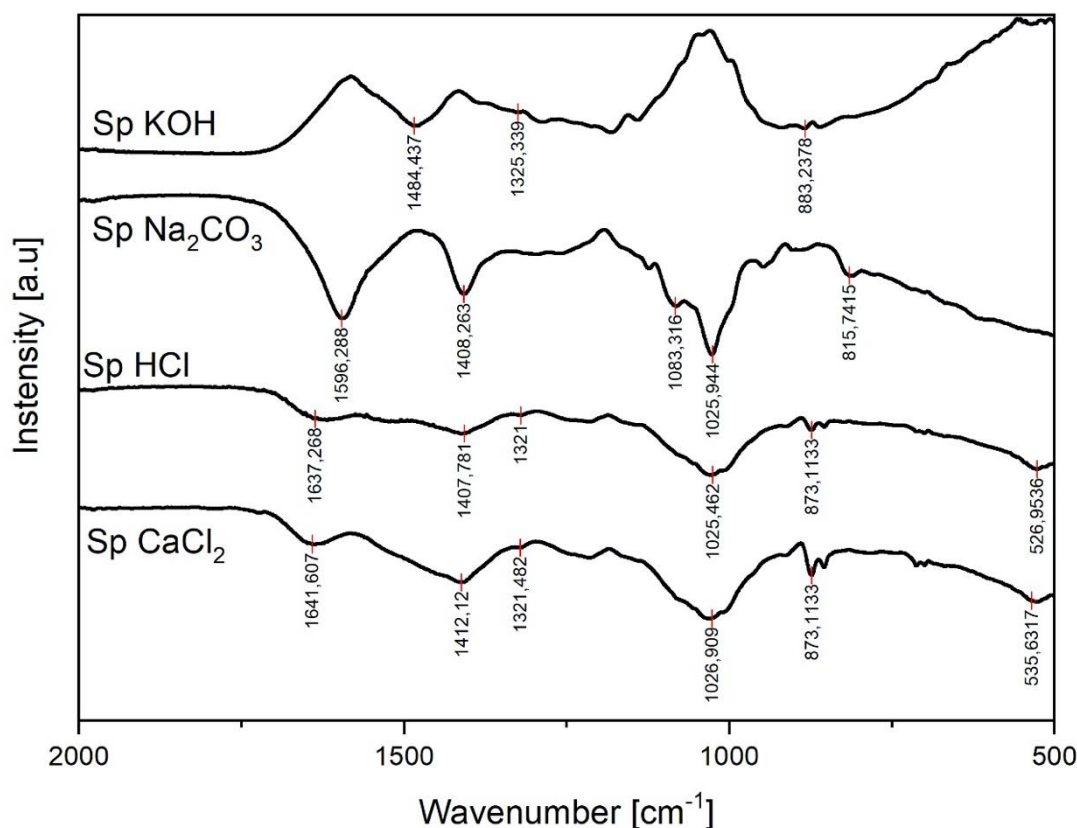


Figure 40. FT-IR spectra of *S. polycystum* extracts (Sp CaCl₂, Sp HCl, Sp Na₂CO₃, and Sp KOH).

The spectral bands at [1641-1637 cm⁻¹] in the fractions Sp CaCl₂ and Sp HCl indicates the presence of CH stretching vibration of the polysaccharides. These bands have been found in the study of Palanisamy *et al.* (2018) in the fucoidan fraction of *S. polycystum*, which has the following composition; fucose, D-glucose, D-mannose, D-xylose, and galacturonic acid. At peak [1596 cm⁻¹], fraction Sp Na₂CO₃ indicates the presence of C-C stretching vibration, as reported by Vandanson *et al.* (2017) for *S. muticum*. At spectral bands of [1412-1408 cm⁻¹] and [1325-1321 cm⁻¹] in all *S. polycystum* fractions were due to the symmetrical and asymmetrical stretching of carboxylate groups or the C-H scissoring and bending. This similar peak at 1409.70 cm⁻¹ was also recorded in the study of Kok and Wong, (2018) for the sodium alginate extract from *S. polycystum*. Moreover, Kok and Wong added that the absorption bands showed a decrease in intensity when the alginate was cross-linked with calcium. This decrease in intensity for this peak was

observed in the present study, decreasing from fraction Sp Na₂CO₃ (an alginate rich) to fucose rich fractions Sp CaCl₂ and Sp HCl. The bands at [1026-1025 cm⁻¹] in all extracts except Sp KOH were due to stretching vibrations of pyranoid rings with C-O-C stretches of the glycosidic bonds, was also present in the study of Marudhupandi *et al.* (2015) for fucoidan fraction of *T. conoides* and Kok and Wong, (2018) for sodium alginate fraction of *S. polycystum*. The band at [815 cm⁻¹] at fraction Sp Na₂CO₃ is due to the presence of sulfates group at the equatorial C-2 or C-3 positions, which has been reported in the isolated fucoidan of *Sargassum henslowianum* at 817 cm⁻¹ (Ale *et al.* 2011). The peaks at [535-526 cm⁻¹] bands in fractions Sp CaCl₂ and Sp HCl indicates the presence of the C-O-S secondary axial sulfate group at C-4 of fructopyranose residue, where the similar peak was also found in Palanisamy *et al.* (2018).

Table XXX. List of InfraRed (IR) vibrational modes characteristic to polysaccharides used in determining spectral bands of interest for the identification and discrimination of brown algal polysaccharides from *Sargassum polycystum* (adapted from

Vandanjon *et al.* 2017; Sun *et al.* 2018; Palanisamy *et al.* 2018; Kok and Wong, 2018; Artemisia *et al.* 2019).

Region/Band (cm ⁻¹)	Type of bond	Identification
<i>Common to Sargassum polycystum</i>		
3425 (3451-3374.12)	O-H	OH stretching vibrations
2960	C-H	aliphatic C-H stretching
2944.05, 2923-2839	C-H	Stretching vibrations of C-H
2927	C=O	C=O stretching vibration of <i>O</i> -acetyl group
1705-1595	C-C	C-C stretching vibrations in the aromatic ring
1654.2, 1605.3		The symmetric and asymmetric stretch of carboxylate
1641	CH	CH vibration of the polysaccharides (fucose, D-glucose, D-mannose, D -xylose and galacturonic acid)
1409.70 (1452-1339)	C-H	The symmetric and asymmetric stretch of carboxylate; C-H scissoring and bending, typical for carotenoids
1261	CH ₃	Fucose, <i>O</i> -acetyl
1230	S=O	Stretching vibration of esterified sulfate
1090.1, 1030.72, 1091.8, 1025.5		Stretching vibrations of pyranose rings
1056	S=O	Stretching of alkyl sulfoxide
945.5	C-O	Stretching vibrations of uronic acid
840.36, 810.67		Characteristic peak of mannuronic acid
802		Anomeric region of carbohydrates.
543	C-O-S	Secondary axial sulfate group at C-4 of fructopyranose residue

The IR spectrum of *S. ilicifolium* extracts (Fig. 41) within 2000-500 cm⁻¹ zone or the fingerprint region for polysaccharides were used for data analysis. Extracts showed spectral bands of high intensity at [1032-1025 cm⁻¹] in all fractions; at 1597 cm⁻¹ for Sp HCl, Si Na₂CO₃, and Si KOH; and at 1408 cm⁻¹ for Si Na₂CO₃, and Si KOH. While bands of medium or low intensity at [1612, and 1546 cm⁻¹] for Si CaCl₂; at [1419-1414 cm⁻¹ and 1255-1249 cm⁻¹] for Si CaCl₂, and Si HCl; at [831-816 cm⁻¹] in all fractions; and [962-943 cm⁻¹] for Si CaCl₂ and Sp Na₂CO₃, and Si KOH. Table XXXI summarizes some of the major IR vibrational

modes of *S. polycystum* polysaccharides (Ale *et al.* 2011; Marudhupandi *et al.* 2015; Vandanjon *et al.* 2017; Sun *et al.* 2018; Palanisamy *et al.* 2018; Kok and Wong, 2018; Artemisia *et al.* 2019).

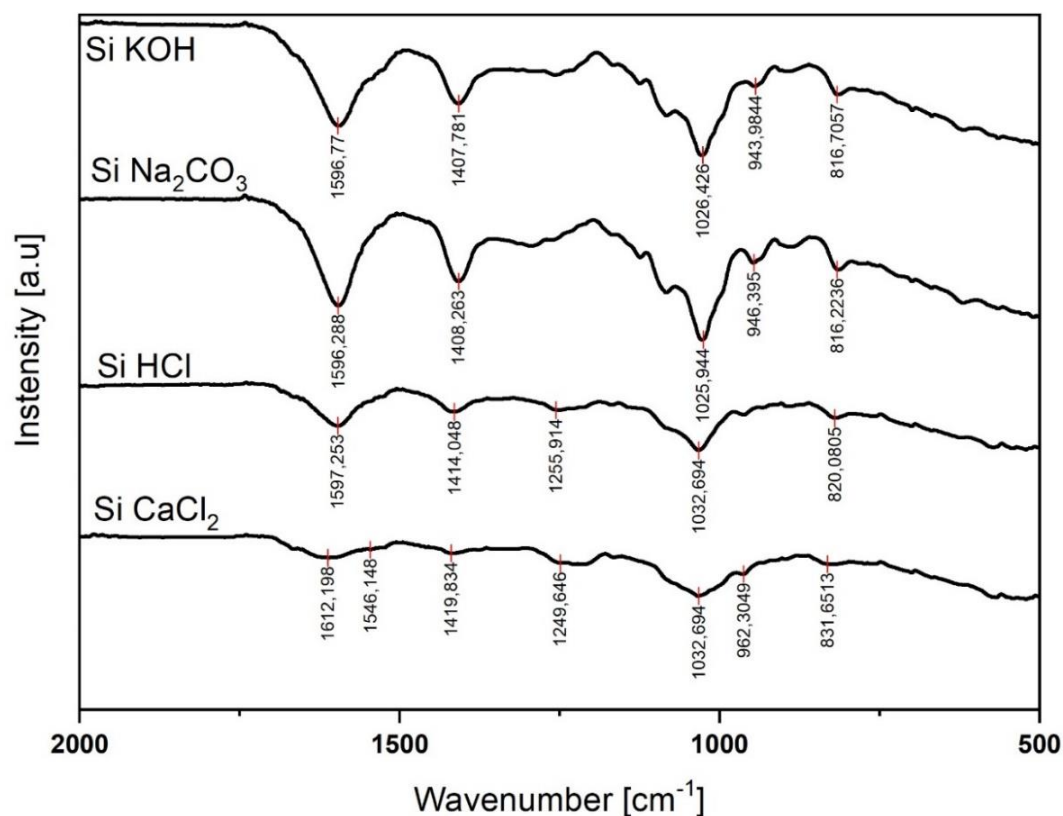


Figure 41. FT-IR spectra of *S. ilicifolium* extracts (Si CaCl₂, Si HCl, Si Na₂CO₃, and Si KOH).

The spectral bands at [1612-1596 cm⁻¹] in all fractions indicate the presence of the C-C stretching vibration of the polysaccharides, which similar peak has been reported by Vandanjon *et al.* (2017) for *S. muticum*. These bands have been found in the study of Palanisamy *et al.* (2018) in the fucoidan fraction of *S. polycystum*, which has the following sugar composition: fucose D-glucose, D-mannose, D-xylose, and galacturonic acid. At spectral bands of [1419-1407 cm⁻¹] in all *S. ilicifolium* fractions were due to the symmetrical and asymmetrical stretching of carboxylate groups, or the C-H scissoring and bending. This similar peak was also

recorded in the study of Kok and Wong, (2018) for the sodium alginate extract from *S. polycystum*, and peaks at [1452-1339 cm^{-1}] were typical for carotenoids (fucoxanthin) extracted from *Undaria pinnatifida* (Sun *et al.* 2018). Decrease of intensity for this particular peak was also observed in the present study from fraction Si Na_2CO_3 (an alginate rich) decreasing in fraction Si KOH (composed of cellulose, hemicellulose, and little fucose), and followed by fucose rich fractions Si HCl and Si CaCl_2 . As mentioned earlier in *S. polycystum* fractions, Kok and Wong, (2018) record the same observation decrease in intensity of absorption bands when the alginate was cross-linked with calcium. The bands at [1255-1249 cm^{-1}] in fucose rich fractions Si HCl and Si CaCl_2 were due to S=O stretching, which was also reported in the study of Artemisia *et al.* (2019) from *Sargassum ilicifolium* fractions extracted at different temperature (60-90 °C) conditions. The fractions Si HCl and Si CaCl_2 in the present study were extracted at 70 °C. At [1032-1025 cm^{-1}] in all extracts were due to stretching vibrations of pyranose rings, which was also present in the study of Kok and Wong, (2018) for sodium alginate fraction of *S. polycystum* and Marudhupandi *et al.* (2015) for fucoidan fraction of *T. conoides*. The band at 962 cm^{-1} of fraction Si CaCl_2 is attributed to the bending vibration of =CH, which was also reported in Artemisia *et al.* (2019) for all fucoidan fractions of *Sargassum ilicifolium*. The band at [946-943 cm^{-1}] fractions Si Na_2CO_3 and Si KOH is attributed to the stretching vibration of uronic acid, which was also found in Kok and Wong (2018). The band at [831-815 cm^{-1}] in all fractions is due to the complex pattern of substitution at the C-S-O. This similar peak has been reported in the isolated fucoidan of *S. ilicifolium* and *Sargassum turbinarioides* (Artemisia *et al.* 2019) and *Sargassum henslowianum* in the study of Ale *et al.* (2011).

The purified extracts from *S. polycystum* (Sp) and *S. ilicifolium* (Si) in the present study contained ester sulfates (at 831-815 cm^{-1} , 535-526 cm^{-1}) and demonstrated antioxidant and antiviral properties against HSV-1.

Table XXXI. List of InfraRed (IR) vibrational modes characteristic to polysaccharides used in determining spectral bands of interest for the identification and discrimination of brown algal polysaccharides from *Sargassum ilicifolium* (adapted from Ale *et al.* 2011; Marudhupandi *et al.* 2015; Vandanjon *et al.* 2017; Sun

et al. 2018; Palanisamy *et al.* 2018; Kok and Wong, 2018; Artemisia *et al.* 2019).

Region/Band (cm ⁻¹)	Type of bond	Identification
<i>Common to Sargassum ilicifolium</i>		
1705-1595	C-C	C-C stretching vibrations in the aromatic ring
1635	C=O	Stretching vibration
1409.7 (1452-1339)	C-H	Symmetric and asymmetric stretch of carboxylate; C-H scissoring and bending, typical for carotenoids
1323	C-H	Bending
1250 (1255-1240)	S=O, C-O	Asymmetric S=O stretching vibration and symmetric C-O vibration associated with the CO-SO ₃ group
1230	S=O	Stretching vibration
1200-900		Carbohydrates
1137	C-O	Stretching
1027		Carbohydrate
963, 833	=CH, =CH ₂	Bending vibration of =CH and =CH ₂
<i>Other Sargassum species</i>		
1615.6	O-C-O	Asymmetric stretching of carboxylate O-C-O vibration
1593	C-H	CH bending
1250	O=S=O; S=O	Asymmetric O=S=O stretching vibration of sulfate group; Stretching vibration
1028 (1030-1025)	C-C; C-O	C-C and C-O stretching vibrations of pyranose ring with C-O-C stretches of the glycosidic bonds
961		Carbohydrate
817 (840-815)	C-S-O	Sulfates group at the equatorial C-2 or C-3 positions; complex pattern of substitution of C-S-O

4.3 Minerals analysis

The mineral contents expressed as content of each mineral relative to the seaweed dry weight (% of dw) of *S. polycystum* and *S. ilicifolium* are given in Table XXXII. The percentages of the recommended daily intake covered by intake of 8 g of dry seaweed are also presented, as is the daily recommended intake allowance (Mac Artain, 2007). The *S. polycystum* had a high total macroelement content (29.42% dw), potassium as the highest content (17.13%), followed by sodium (10.0%). Its microelement total content (1.41% dw), had iron (1.17%) as the highest. While, *S. ilicifolium* macroelement total content (5.43% dw) are rich in potassium (2.10%), followed by calcium (1.54%). Its microelement total content (2.06% dw), had high iron (1.63%). Eating 8 g of dry *S. polycystum* represents between 6.3% and 1553.33% of the recommended daily intake (RDI) for minerals. These percentages were high, especially for the microelements. For *S. ilicifolium*, 8 g of dry seaweed represents 2175.56% of the RDI of iron. The Na/K ratios in *S. polycystum* and *S. ilicifolium* were 0.58 and 0.56, respectively.

Table XXXII. Composition of macroelements and microelements (% dw), recommended daily intakes, and recommended daily allowances of Philippine brown seaweeds *Sargassum polycystum* and *Sargassum ilicifolium*.

Species	Macroelements					Microelements			
	Na	K	Ca	Mg	Na/K	Fe	Zn	Mn	Cu
RDI ^a (g day ⁻¹)	2.0	3.8	0.84	0.350		0.006	0.012	0.0055	0.0017
Upper limit intake ^b (g day ⁻¹)	2.3	-	2.5	0.35		0.045	0.040	-	0.01
<i>S. polycystum</i>	10.0±0	17.13±0	0.94±0	1.36±0	0.58	1.17±0.02	0.13±0	0.004±0	0.11±0
% RDI	40.0	36.06	8.93	31.01		1553.33	83.33	6.30	533.33
RDA (g day ⁻¹)	23.0	-	266.52	25.80		3.86	32.00	-	8.82
<i>S. ilicifolium</i>	1.17±0	2.10±0	1.54±0	0.62±0	0.56	1.63±0.03	0.37±0	0.002±0	0.06±0
% RDI	4.68	4.42	14.64	14.23		2175.56	246.67	2.91	266.67
RDA (g day ⁻¹)	196.58	-	162.65	56.22		2.76	10.81	-	17.65

^aRDI = Recommended daily intake and upper limit intake values for an adult male with a bodyweight of 70 kg according to the World Health Organization (2002); ^bUpper limit intake according to National Health and Medical Research Council (2006); % RDI is based on a daily intake of 8 g dry weight of seaweed; - not detected; RDA: recommended daily allowance. Data are mean ± SD (n=2). SD = standard deviation.

Mineral content is one of the main nutritional characteristics of seaweeds taken up directly from their environment. Seaweeds absorb an incomparable wealth of mineral elements from the sea and are known as excellent sources of vitamins

and minerals, especially potassium and iodine (Leandro *et al.* 2020). However, nutritional generalization about algal mineral content is difficult because of large seasonality, geographic location, taxonomic variations, and laboratory manipulations (Wells *et al.* 2017). The high ash content can reach up to 42% of dw in *S. polycystum* and represent a high content in minerals (Matanjan *et al.* 2009). The present study showed that *S. polycystum* ash content (24.9%) was in accordance with (25%) from three *Sargassum* spp. (*S. polycystum*, *S. wightii*, and *S. crassifolium*) (Thadhani *et al.* 2019), and *S. ilicifolium* ash content (17%) was in accordance with (15.9%) from *S. ilicifolium* (Pise and Sabale, 2010). Other *Sargassum* species reported varying ash contents such as 25% from *S. wightii*, 14.2% from *S. vulgare*, 44.29% from *S. filipendula* (Alam Bhuiyan and Qureshi, 2016), and 30.7% from *Saccorhiza polyschides* (Garcia *et al.* 2016).

Macroelements of *S. polycystum* in the present study showed that Na, K, and Mg had higher values than previous results of 1.36, 8.37, and 0.48%, respectively, while Ca was 4-times lower than 3.79% from previous results of the same species (Matanjan *et al.* 2009). In contrast, macroelements of *S. ilicifolium* in the present study showed that K, Mg, and Ca had lower values than 4.64, 4.32, and 3.34% respectively, while Na was not determined in previous results of the same species (Pise and Sabale, 2010). Also, the present study macroelements of *S. polycystum* had higher values than *S. ilicifolium* except for Ca. Macroelements contents from *S. wightii* was considered small (Syad *et al.* 2013) compared to *S. polycystum* and *S. ilicifolium* in the present study. However, *S. polyschides* reported comparable contents of Na (2.65%), K (10.17%), Ca (1.12%), and Mg (0.56%) (Garcia *et al.* 2016) with the present study *S. polycystum* except for the obvious difference in Na content (2.65%); and it is also somewhat similar with the present study *S. ilicifolium* except for K content (10.17%). The ratio of Na/K in *S. polycystum* and *S. ilicifolium* in the present study were similar and were considered small (<1.5) based on the previous report. It is also found higher than 0.16 of the same species *S. polycystum* (Matanjan *et al.* 2009), or higher than 0.34 from different *Sargassum* spp. (Thadhani *et al.* 2019), and 0.26 from *S. polyschides* (Garcia *et al.* 2016). However, a higher Na/K ratio (1.45) was reported from *S. wightii* (Syad *et al.* 2013) than both

species in the present study. Dietary salt intake was seen to be related to the increase in blood pressure with age, and Na/K ratio was a risk factor for mortality from CVDs (Seko *et al.* 2018). The Na/K ratio in the *S. polycystum* and *S. ilicifolium* in the present study were considered normal or low (<4.0) based on the urinary Na/K ratio and different food items consumed (Seko *et al.* 2018). Thus, consumption of *S. polycystum* and *S. ilicifolium* seaweeds or its nutritional derivatives can help balance high Na/K ratio diets common today.

The microelements (Fe, Zn, Mn, and Cu) were all in higher amounts from *S. polycystum* in the present study compared with previous results of the same species (Matanjun *et al.* 2009). While the microelements in *S. ilicifolium* of the present study also showed higher values except for Mn (0.002%), which was lower than (0.009%) of the same species (Pise and Sabale, 2010). Microelements of both species in the present study were also found higher than the different *Sargassum* spp., except for Mn (0.002%), which had a similar amount (Thadhani *et al.* 2019), and also higher than *S. polyschides* (Garcia *et al.* 2016). Iron is essential for cell functions (oxygen and electron transport, and DNA synthesis), while, manganese helps in protein, lipid, and carbohydrate metabolism (Mišurcová, 2011). According to Garcia *et al.* (2016), microelement concentrations vary with species, which could be due to morphological and physiological affinities for different metals. As the average daily consumption of dry seaweeds in Asia is 8 g, and based on the results in the present study, mineral contents are within safe levels and are potential functional foods for humans. For example, an 8 g portion of *S. polycystum* or *S. ilicifolium* represents 9% and 15%, respectively of the RDI of calcium as reported in other seaweeds, while the same quantity of cheddar cheese provides only 5% of the RDI (Mc Artain, 2007; Finglas *et al.* 2015). Results of daily recommended intake allowance showed that the use of not more than 3.86 g of *S. polycystum* or 2.76 g of *S. ilicifolium* would not compromise health due to the iron content of this seaweed. The accumulation of excess iron may generate oxidative stress and can promote and increase cardiovascular risk (Pietrangelo, 2002; Martínez-Navarrete *et al.* 2002; Puntarulo, 2005). However, the biological availability of minerals is influenced by diet composition by synergistic and antagonistic interactions

(Watanabe *et al.* 1997). Moreover, only between 1 and 53% of iron was absorbed by the human body from food (Skikne *et al.* 1983).

4.4 Pigments

Total pigment content of *S. polycystum* (197.43 ± 28.32 mg g⁻¹ dw) is seventeen times higher than *S. ilicifolium* (11.49 ± 2.28 mg g⁻¹ dw) shown in Table XXXIII. Chlorophyll *a*, fucoxanthin, and β -carotene were detected in both seaweeds. Chlorophyll *a*, and β -carotene were much higher in *S. polycystum* (6.25 ± 1.31 , 188.89 ± 26.70 mg g⁻¹ dw) than *S. ilicifolium* (1.04 ± 0.18 , 7.84 ± 1.67 mg g⁻¹ dw) respectively. Total chlorophyll in *S. polycystum* (8.54 ± 1.62 mg g⁻¹ dw) is more than twice the amount in *S. ilicifolium* (3.65 ± 0.60 mg g⁻¹ dw).

Table XXXIII. Pigment content (mg g⁻¹ dw) of Philippine brown seaweeds *Sargassum polycystum* and *Sargassum ilicifolium*.

Seaweeds	Chlorophyll <i>a</i>	Fucoxanthin	β -Carotene	Total chlorophyll	Total pigments
<i>S. polycystum</i>	6.25 ± 1.31	2.28 ± 0.30	188.89 ± 26.7	6.25 ± 1.31	197.43 ± 28.3
<i>S. ilicifolium</i>	1.04 ± 0.18	2.60 ± 0.41	7.84 ± 1.67	1.04 ± 0.18	11.49 ± 2.28

Data are mean \pm SD (n=2). SD = standard deviation

Of all the pigments, β -carotene dominates in both studied species. The amount of β -carotene in *S. polycystum* is thirty times higher than of the chlorophyll *a*, and almost eighty-three times higher than of fucoxanthin. In contrast, β -carotene in *S. ilicifolium* is seven times higher than the amount of the chlorophyll *a*, and three times higher than of fucoxanthin. Hidayati *et al.* (2019) reported higher contents of chlorophyll *a* (18.23 mg g⁻¹) and carotenoid (32.56 mg g⁻¹) from ethyl acetate extract *Sargassum* sp. sample compared to the present study *S. ilicifolium*.

However, its chlorophyll a content was about 3-times higher, and carotenoid content was about 6-times lower than the present study, *S. polycystum*. Also, very low contents of chlorophyll a (0.0065 mg g⁻¹ fw), and chlorophyll b (0.0037 mg g⁻¹ fw) was reported from fresh weight sample *S. wightii* (Syad *et al.* 2013), and comparable content of chlorophyll a [(3.13 mg g⁻¹) after a 4-weeks photon flux treatment], and [(3.86 mg g⁻¹) after a 4-weeks at 8 h, 6 °C condition] compared to *S. polycystum* and *S. ilicifolium* of the present study. Several *Sargassum* species reported varying results of carotenoids: the fucoxanthin contents (27.4 mg g⁻¹) from *Sargassum polycystum* was 10 times higher than fucoxanthin (2.2, 2.6 mg g⁻¹) from the present study *S. polycystum* and *S. ilicifolium*, respectively (Balasubramaniam *et al.* 2020), fucoxanthin (7.4 mg g⁻¹) from *Sargassum binderi* (Wu *et al.* 2014) was 3 times higher than fucoxanthin (2.6 mg g⁻¹) and similar with β-carotene content from the present study *S. ilicifolium*; and the very low contents from *S. siliquosum* (1.41 mg g⁻¹), *S. polycystum* (0.31 mg g⁻¹) (Nagappan *et al.* 2017); the trans-fucoxanthin (0.107 mg g⁻¹) and minor pigments (Chlorophyll a and β-carotene) from *Sargassum* sp. (Indrawati *et al.* 2015) compared to the present study *S. polycystum* and *S. ilicifolium*.

The β-carotene contents from *Sargassum obtusifolium* (0.0359 mg g⁻¹), and *Sargassum echinocarpum* (0.0581 mg g⁻¹) are similar to β-carotene content found in spinach (0.040 mg g⁻¹) (McDermid, 2003). Another study reported lower β-carotene contents from *S. polycystum* (0.0004 mg g⁻¹) (Balasubramaniam *et al.* 2020), from *Ascophyllum nodosum* (0.003 mg 8g⁻¹), *Laminaria digitata* (0.782 mg 8g⁻¹), and (0.988 mg 8g⁻¹) *Undaria pinnatifida* (MacArtain *et al.* 2007). The β-carotene contents in the present study *S. polycystum* and *S. ilicifolium* were at least 100 times higher than found in spinach and to the brown seaweeds reported in the literature (McDermid, 2003 ; MacArtain *et al.* 2007). The pigment β-carotene is said to modulate UVA-induced gene expression, protect the skin and eyes from photo-oxidation against UV light, and prevent eye disease in humans, e.g., cataracts (Pereira, 2018). β-carotene, which, besides its anticancer activity, has been reported to be absorbed 10-times more easily by the body than the synthetic one (Christaki

et al. 2013). Pigments from macroalgae are also important in this industrial range (Leandro *et al.* 2020).

4.5 Biological activity

4.5.1 Cytotoxicity and antiviral activity evaluation

No cytotoxic effect of polysaccharides (CC_{50} greater than $200 \mu\text{g mL}^{-1}$) on the Vero cells was observed for both *S. polycystum* and *S. ilicifolium* extracts in ranged of concentrations assayed. All fractions had low EC_{50} values in *S. polycystum* and *S. ilicifolium* (Table XXXIV). Efficacy or the cellular protection of *S. polycystum* fractions showed that fraction Sp Na_2CO_3 had maximum efficacy of (94.0%), is slightly higher than Sp CaCl_2 (91.78%), followed by Sp HCl (90.55%), and the least was Sp KOH (73.21%), all at $200 \mu\text{g mL}^{-1}$ after 72 h infection. The potency or the effectivity of the Sp HCl at 50% concentration, only needs $30.08 \mu\text{g mL}^{-1}$ of sample (EC_{50} : $30.08 \mu\text{g mL}^{-1}$), which is slightly higher than Sp CaCl_2 (EC_{50} : $31.63 \mu\text{g mL}^{-1}$), followed by Sp Na_2CO_3 (EC_{50} : $33.25 \mu\text{g mL}^{-1}$) and almost two times potent than Sp KOH (EC_{50} : $67.02 \mu\text{g mL}^{-1}$). The maximum efficacy of Zovirax control is 87.62% at $5 \mu\text{g mL}^{-1}$ and potency of $0.49 \mu\text{g mL}^{-1}$. Efficacy and potency evaluation were shown in appendix 11 and 12, while its statistical analysis was shown in appendix 17.3 and 17.4 for *S. polycystum* and *S. ilicifolium* fractions, respectively.

Fucoidans or fucan sulfates, and alginates (main acidic polysaccharides) are predominantly found in the cell wall of brown algae showed antiviral activity against HSV (Mahomoodally *et al.* 2019). A previous study reported antiviral activities of anionic polysaccharides (NaAlg, and fucoidan) and their derivatives (sulfated NaAlg, and sulfated fucoidan) from *Sargassum tenerrimum* had EC_{50} value that ranges from 0.5 to $15 \mu\text{g mL}^{-1}$ against HSV-1 (Sinha *et al.* 2010).

Fucoidans extracted from three different brown seaweeds (*Sargassum polycystum*, *Sargassum meclurei*, and *Turbinaria ornata*) reported antiviral activity against HSV with EC₅₀ value that ranges from 0.33 to 0.7 µg mL⁻¹ (Thuy *et al.* 2015)(Thuy *et al.* 2015). All *S. polycystum* extracts in the present study possess antiviral activities against HSV-1 with values range (EC₅₀=30.08-67.02 µg mL⁻¹) that are higher and are less potent compared to the results (EC₅₀=0.5-15 µg mL⁻¹) of Sinha *et al.* (2010), and (EC₅₀=0.33-0.7 µg mL⁻¹) Thuy *et al.* (2015). According to Puspita *et al.* (2017), the antiviral activity of brown algae is usually shown by polysaccharides, and some by phlorotannins. The total polyphenols content of *S. polycystum* extracts in the present study had a range value of (3.2-6.4 % dw), which is comparable to its raw material value of 5.6% dw. No pattern or relationship was found between polyphenols contents and EC₅₀ values in *S. polycystum* extract and is in accordance with Puspita *et al.* (2017). Thuy *et al.* (2015) had sulfates contents range value of (23.4-30.5 % dw), and sugar constituents with large portions of fucose (20.3-40% dw), galactose (13.7-25.6% dw), and small portions of mannose, xylose, and glucose (trace amounts-2.6% dw). Thuy *et al.* reported that antiviral activity was not significantly different, and the amount of 2- and 4-sulfated fucose in fucoidans were all different, suggest no relationship in antiviral activity against HIV.

In terms of sulfates content, *S. polycystum* extracts in the present study had a range value of (6.36-12.68 % dw), which is comparable to its raw material value of 10% dw. Sugar constituents (fucose, galactose, glucose, mannose, and xylose) in *S. polycystum* extracts are in very small proportions that range from 5.62 to 6.87%. No pattern or relationship was found between sulfates contents and EC₅₀ values in *S. polycystum* extracts and is in accordance with Thuy *et al.* (2015) and Puspita *et al.* (2017). Sugars constituents in *S. polycystum* extracts in the present study had very small amounts compared to Thuy *et al.* (2015). In the present study of *S. polycystum* extracts, no pattern of sulfate content found to relate it to EC₅₀ values. Protein's content of the fractions with antiviral activities against HSV-1 was in a sufficient amount in the present study.

Table XXXIV. Evaluation of cytotoxicity and antiviral activity by cellular viability of Philippine brown seaweeds *Sargassum polycystum* and *Sargassum ilicifolium*.

SPECIES	FRACTION	CC ₅₀ (µg mL ⁻¹)	EC ₅₀ (µg mL ⁻¹)
		Destruction	Protection
<i>S. polycystum</i>	Sp CaCl ₂	>200	31.63±0.61
	Sp HCl	>200	30.08±0.83
	Sp Na ₂ CO ₃	>200	33.25±2.3
	Sp KOH	>200	67.02±0.36
Zovirax		>200	0.49±1.2
<i>S. ilicifolium</i>	Si CaCl ₂	>200	6.63±1.64
	Si HCl	>200	17.29±0.64
	Si Na ₂ CO ₃	>200	19.707±0.49
	Si KOH	>200	17.44±0.58
Zovirax		>200	0.32±0.25

CC₅₀: The 50% Cytotoxic Concentration. The concentration that reduced the absorbance of mock-infected cells to 50% of that of controls. EC₅₀: The 50% antiviral Effective Concentration: concentration that achieved 50% protection of virus-infected cells from the HSV-induced destruction. CaCl₂ and HCl= are sequential sulfated polysaccharide extracts rich in fucose; Na₂CO₃= extracts containing alginates with remaining fucoidan; KOH= are cellulose, hemicellulosic residues with remaining fucoidan.

In *S. ilicifolium*, the maximum efficacy or the cellular protection of fraction Si KOH (118.91%) is higher than Si Na₂CO₃ (105.59%), followed by Si HCl (97.86%), and Si CaCl₂ (83.0%). In terms of potency or the effectivity, fraction Si CaCl₂ achieved 50% at 6.63 µg mL⁻¹ sample (EC₅₀: 6.63 µg mL⁻¹) is two to three times more potent than Si HCL (17.29 µg ml⁻¹), Si KOH (17.44 µg mL⁻¹), and Si Na₂CO₃ (19.70 µg mL⁻¹). The maximum efficacy of Zovirax control is 102.64% at 5 µg mL⁻¹ and potency of 0.32 µg ml⁻¹. All the *S. ilicifolium* extracts in the present study possess antiviral activities against HSV-1 with values range of (EC₅₀=6.63-19.7 µg mL⁻¹). These values are 2 to 5-times lesser and more potent than *S. polycystum* extracts in the present study, but a little higher and are less potent compared to the results of Sinha *et al.* (2010), and Thuy *et al.* (2015). The total polyphenols content of *S. ilicifolium* extracts in the present study had a range value of (1.8-7.2 % dw), which is comparably less to its raw material value of 11.1% dw.

No pattern or relationship was found between polyphenols contents and EC₅₀ values in *S. ilicifolium* extracts and is in accordance with Puspita *et al.* (2017). In terms of sulfates content, *S. ilicifolium* extracts in the present study had a range value of (6.96-15.21 % dw), which is comparable to its raw material value of 8.8% dw. Also, no pattern or relationship was found between sulfates contents and EC₅₀ values in *S. ilicifolium* extracts and is in accordance to Thuy *et al.* (2015), and Puspita *et al.* (2017). Sugar constituents (fucose, glucose, galactose, and mannose) in *S. ilicifolium* extracts are in different proportions that range from 3.93 to 33.69% dw. Sugars constituents in *S. ilicifolium* extracts of the present study had minimal amounts compared to Thuy *et al.* (2015). In the present study of *S. ilicifolium* extracts, no pattern of sulfate content found to relate it to EC₅₀ values. Protein's content of the *S. ilicifolium* fractions with antiviral activities against HSV-1 was in sufficient amount, as was for *S. polycystum* in the present study.

As shown in the previous part of this chapter (4.2.3. Biochemical composition of extracts), the extracts obtained have biochemical contents that differ with their raw material. Aside from sulfates, monosaccharides composition (Mahomoodally *et al.* 2019), protein, and other polysaccharides (Hardouin *et al.* 2016; Puspita *et al.* 2017) could have an impact on the antiviral activity reported in the extracts of *S. polycystum* and *S. ilicifolium*. The variation of antiherpetic activity results of fucoidans, alginates, and their derivatives between the present and previous studies could be influenced by several factors such as monosaccharide composition, sulfate content, polysaccharides chemical structure and its molecular weight, location and period of collection, and sensitiveness of methods used (Kidgell *et al.* 2019). These activities may be due to other compounds such as proteins and polysaccharides, as stated by Puspita *et al.* (2017). The *Sargassum polycystum* and *Sargassum ilicifolium* extracts results showed great potential in developing as an antiviral drug. However, these various factors mentioned must be understood better and therefore extracts from *Sargassum polycystum*, and *Sargassum ilicifolium* must be studied further.

4.5.2 Antioxidant activity, DPPH radical scavenging activity

The free radical scavenging of the blank and the fractions of *S. polycystum* and *S. ilicifolium* polysaccharide extracts were estimated by the decrease in absorbance due to the reduction of the 2,2-diphenyl-1-picrylhydrazyl radical by the extracts. Percent maximum scavenging ability and inhibition activity of *S. polycystum* and *S. ilicifolium* polysaccharide extracts at (5 mg mL⁻¹) were shown in Table XXXV.

Its antioxidant activity at 50% (IC₅₀) for *S. polycystum* polysaccharides fractions was found highest to lowest in this trend: fraction Sp HCl>Sp KOH>Sp Na₂CO₃>Sp CaCl₂ with (105.49, 242.35, 277.74, and 384.27 µg mL⁻¹), respectively. While *S. ilicifolium* antioxidant activity at 50% (IC₅₀) for polysaccharides fractions were found highest to lowest in this trend: fraction Si KOH>Si CaCl₂>Si Na₂CO₃>Si HCl with (364.74, 595.27, 708.92, and 1134.83 µg mL⁻¹), respectively. Polysaccharides extracts from *S. polycystum* had higher scavenging ability at fractions CaCl₂ and KOH, and lower (IC₅₀) values in all extracts compared to *S. ilicifolium* fractions. The low IC₅₀ value indicates that the sample has a strong antiradical activity. Free radical scavenging activity evaluation was shown in appendix 13 and 14, while its statistical analysis was shown in appendix 17.8 and 17.9 for *S. polycystum* and *S. ilicifolium* fractions, respectively, with its BHA and BHT standards.

Table XXXV. Summary of Antioxidant activity, scavenging assay (DPPH) of Philippine brown seaweeds *Sargassum polycystum*, and *Sargassum ilicifolium*.

IC ₅₀ (µg mL ⁻¹)

BHA (0-50 conc)	7.05±0.27
BHT (0-50 conc)	5.32±0.18
<i>S. polycystum</i>	
Sp CaCl ₂	384.27±0.18
Sp HCl	105.49±0.17
Sp Na ₂ CO ₃	277.74±0.21
Sp KOH	242.35±0.63
<i>S. ilicifolium</i>	
Si CaCl ₂	595.27±0.27
Si HCl	1134.83±0.48
Si Na ₂ CO ₃	708.92±0.29
Si KOH	364.74±0.28

IC₅₀: corresponding to the concentration sufficient to obtain 50% of a maximum scavenging capacity. CaCl₂ and HCl= are sequential sulfated polysaccharide extracts rich in fucose; Na₂CO₃= extracts containing alginates with remaining fucoidan; KOH= are cellulose, hemicellulosic residues with remaining fucoidan; BHA = Butylated HydroxyAnisole; BHT = Butylated Hydroxytoluene

Antioxidant activity varied significantly among 30 species of Hawaiian algae from 27 genera, and the brown algae as a group had the highest mean antioxidant activity, followed by green algae, and red algae with FRAP mean values of 3.55±3.16, 2.29±2.34, and 1.59±1.17 µM µg⁻¹ extract, respectively (Kelman *et al.* 2012). This present study antioxidant activity for brown algae *S. polycystum* and *S. ilicifolium* is somewhat in accordance to what is reported by Kelman *et al.* (2012) where its IC₅₀ mean value is low (476.71 µg mL⁻¹ at 5 mg mL⁻¹). Note that the dose concentration used for red algae is twice as high as brown and green algae; that is why the IC₅₀ mean value appears small. Thus, antioxidant activity in the present study is most effective in brown algae, followed by green algae and red algae. This result is in accordance to Kelman *et al.* (2012), where antioxidant activities of algae collected from shallow waters (0-3m) were more active than from those in the deep waters, where brown algae were also collected in shallow waters (0-3 m) than green algae (0-5 m), and red algae (5-10 m) in the present study.

In the present study, fraction Sp HCl (IC₅₀=105 µg mL⁻¹) had the best antiradical activity, which is comparable to ethyl acetate extract (IC₅₀=102.4 µg mL⁻¹) and is higher to Aquadest extract (at IC₅₀=72.95 µg mL⁻¹), which had the best antiradical activity, according to Hidayati *et al.* (2019). Other fractions with its IC₅₀

values from Sp KOH>Sp Na₂CO₃>Sp CaCl₂ (IC₅₀=242.35-384.27 µg mL⁻¹) and fractions Si KOH (IC₅₀=364.74 µg mL⁻¹) were comparable to methanol extract (IC₅₀=270 µg mL⁻¹), and n-hexane extract (IC₅₀=320 µg mL⁻¹) that was considered weak antioxidants by Hidayati *et al.* (2019). The remaining fractions from the present study, Si Na₂CO₃ (IC₅₀=708.92 µg mL⁻¹), and Si HCl (IC₅₀=1134.83 µg mL⁻¹) were considered moderate antioxidants (IC₅₀=700-1000 µg mL⁻¹) according to Puspita *et al.* (2017).

In terms of percent scavenged against DPPH, fraction Sp CaCl₂>Sp KOH (>50% at 5 mg mL⁻¹) were in accordance with (55.94% at 1000 µg mL⁻¹) of purified fucoidan extracts by DEAE-cellulose from *Sargassum polycystum* (Palanisamy *et al.* 2018), and all samples in the Sp and Si fractions of the present study were also dose-dependent as in the study of Palanisamy *et al.* (2018). All the rest of the Sp and Si fractions in the present study had a scavenging percentage of less than <50% at 5 mg mL⁻¹ concentration, which is lower and 5-times weaker in dose concentrations used, therefore, less effective compared to Palanisamy *et al.* (2018). All samples from the present study Sp and Si fractions have IC₅₀ values of at least 4-times lower than IC₅₀=4.3-5.13 mg mL⁻¹ from *S. glaucescens* (Sanjeewa *et al.* 2018) and are therefore more effective antioxidant. While *S. horneri* had better DPPH scavenging activity (42.6-86.74% at 2.5 mg mL⁻¹) at lower dose concentration than those in the present study, and therefore, were better antioxidant; and the *S. plagiophyllum* extract DPPH scavenged (36% at 5 mg mL⁻¹) (Sanjeewa *et al.* 2018) is in accordance to all Sp and Si fractions except Sp CaCl₂ and Sp KOH in the present study.

All the Sp and Si fractions in the present study are also in accordance with Pádua *et al.* (2015) that seaweeds containing fucoidan are dominant in L-fucose. Pádua *et al.* (2015) added that fucoxanthin, an abundant carotenoid in brown seaweeds have much higher antioxidant activities than any other antioxidant, including tocopherols, and content varies during season and life cycle of seaweed. FTIR analyses at [1641-1637 cm⁻¹] for Sp CaCl₂, and Sp HCl fractions and at [1612-1596 cm⁻¹] for all Si fractions in the present study confirms the presence of

fucoidan, in which according to Palanisamy *et al.* (2018) that fucoidan fraction of *S. polycystum* contains fucose.

The variation of antioxidant activity results between the present study (Sp and Si extracts) and previous studies from *Sargassum* species could be influenced by several factors such as season and location of collection, total polyphenols, low Mw polysaccharides, proteins or peptides, sulfate esters linked with sugar constituents, or sensitiveness of methods used. The DPPH scavenging activity of Sp and Si extracts is considered significant since these extracts are derived from natural resources; therefore, they can be utilized to develop various functional and healthy foods.

Conclusion and perspectives

In conclusion, the extraction yield results of *S. polycystum* and *S. ilicifolium* are affected by species used, geographic location, seasonal growth, and even by the sensitiveness of the methods used. Biochemical composition for both *S. polycystum* and *S. ilicifolium* extracts is mainly composed of neutral sugars, sulfates group, and proteins, where *S. polycystum* had range values of (8.8-23.1%), (6.36-12.68%), and (8.9-11.8%) dw of alga, respectively. While, *S. ilicifolium* extracts had range values of (18.5-33.2%), (6.98-15.21%), and (8.7-25.5%) dw of alga, respectively.

FTIR spectrum of *S. polycystum* and *S. ilicifolium* extracts confirmed the presence of ester sulfates, and sugars, where, *S. polycystum* polysaccharides is mainly composed of fucoidan, alginate, and cellulose/hemicellulose at a ratio of 3:2:1 with fucose, glucose, galactose, mannose, and xylose as the dominant sugar units. While, *S. ilicifolium* cell wall polysaccharides are composed of alginate, fucoidan, and cellulose/hemicellulose at a ratio of 6:2:1 with fucose, glucose, galactose, mannose, and xylose as the dominant sugar units. The *S. polycystum* and *S. ilicifolium* mineral contents are within safe levels and are potential functional foods for humans. Also, *S. polycystum* and *S. ilicifolium* had β -carotene contents of

at least 100 times higher than found in spinach. Besides its anticancer activity, β -carotene has been reported to be absorbed 10-times more easily by the body than the synthetic one (Christaki *et al.* 2013). Thus, *S. polycystum* and *S. ilicifolium* are excellent sources of pigments that can be used in the food industry and human health development.

The *S. polycystum* and *S. ilicifolium* fractions showed in vitro antiherpetic activity without cytotoxicity. Polysaccharides from *S. ilicifolium* fractions are more potent than *S. polycystum* fractions. The *S. polycystum* and *S. ilicifolium* antioxidant results (DPPH scavenging assay) had better activity than the green (*C. racemosa* and *U. lactuca*), and the red (*H. durvillei* and *H. dilatata*) seaweeds in the present study, suggest that the seaweeds are safe for human consumption.

The presence, degree, and distribution of the sulfate groups are important in determining the biological activity and to analyze its anti-viral potency in relation to structure and position of sulfate groups of polysaccharides, and therefore must be studied further for these extracts in the future. The extracts from both species are considered significant since they are derived from natural resources; therefore, they can be utilized to develop various functional and health foods.

Chapter 5 Red Seaweed **(*Halymenia durvillei* and *Halymenia dilatata*)**

5.1 Biochemical composition analysis of raw material

Determining the biochemical composition and carrageenan content from seaweed *Halymenia durvillei* and *Halymenia dilatata* for the present study may help in providing data for its use as potential natural source raw material for carrageenan industry. The biochemical composition of raw material from red seaweed *Halymenia durvillei* and *Halymenia dilatata* for the present study were given in Table XXXVI.

Halymenia durvillei composition showed a high amount of ash (41.0%), followed by neutral sugars (21.2%), and smaller amounts (<10%; (7.2-2.08%)) of sulfate groups, lipids, proteins, polyphenols, and uronic acids content of the dry weight, respectively. While *Halymenia dilatata* composition was rich in ash (46.3%), followed by neutral sugars (16.2%), and smaller amounts (<10%; (6.7-2.32%)) of sulfate groups, proteins, polyphenols, and uronic acids of the dry weight of alga, respectively. Carbohydrates including sulfated polysaccharides (corresponding to uronic acids, sulfate groups, and neutral sugars) in *H. durvillei* and *H. dilatata* are about 30.59% and 25.2%, respectively.

Table XXXVI. Biochemical composition of Philippine red seaweeds *Halymenia durvillei* and *Halymenia dilatata*.

ALGAE	Content (% dry algal material)						
	Proteins	Neutral sugars	Sulfate groups	Uronic acids	Polyphenols	Ash	Lipid
<i>H. durvillei</i>	5.3±0.29	21.2±0.05	7.2 ±0.08	2.08±0.04	5.06±0.64	41.0±1.43	7.1
<i>H. dilatata</i>	5.5±0.29	16.2±0.12	6.7 ±0.12	2.32±0.04	3.17±0.23	46.3±0.61	0.6

Values are means ± SD (n=9) except for lipids.

Ash content for raw *H. durvillei* (41%) and raw *H. dilatata* (46.3%) are the most abundant component in the present study. Kho *et al.* (2016) reported a similar ash content (39.54%) from *H. durvillei* of the same species, a lower ash content (21.92%) from *H. dilatata* of the same species, and also a lower ash content

(23.61%) from *H. maculata* than raw *H. durvillei* and raw *H. dilatata* in the present study. In contrast, higher ash content was reported (58% dw) from *K. alvarezii* (Wanyonyi *et al.* 2017), and (50.2% dw) from *Halymenia floresii* (Garcia *et al.* 2016) than raw *H. durvillei* and raw *H. dilatata* in the present study. The protein contents from raw *H. durvillei* (5.3%) and *H. dilatata* (5.5%) were below the range value (9.4-47% dw) for selected red seaweeds from the Philippines (Hurtado *et al.* 2020). It is also found to be 2-times lower than Kho *et al.* (2016) from *H. durvillei* (13.02%), and *H. dilatata* (9.99%), and *H. maculata* (11.07%). However, the protein content in the present study was 3-times higher compared to *K. alvarezii* (1.34%) (Wanyonyi *et al.* 2017), while about 3-times lower than 15.6% from *Solieria filiformis* (Bedoux *et al.* 2017), and lower than (9.4%) from *Halymenia floresii* (Garcia *et al.* 2016). Lipid contents for raw *H. durvillei* (7.1%) and *H. dilatata* (0.6%) were within the range value (0.1-12% dw) from selected red seaweeds of the Philippines (Hurtado *et al.* 2020). It is also found to be higher than Kho *et al.* (2016) for *H. durvillei* (1.29%), and *H. dilatata* (0.29%) of the same species, while *H. maculata* (1.09%) was reported higher than *H. dilatata*. Wanyonyi *et al.* (2017) reported lipid content (0.62% dw) from *K. alvarezii*, which is similar to raw *H. dilatata*, but 10-times lower than raw *H. durvillei* in the present study. However, the present study lipid content was lower than (12.3%) from *Halymenia floresii* (Garcia *et al.* 2016). Neutral sugars contents for raw *H. durvillei* (21.2%) and *H. dilatata* (16.2%) in the present study was slightly lower than (25.4%) from *Solieria filiformis*, and (24.1%) from *Rhodomenia pseudopalmata* (Bedoux *et al.* 2017). Sulfates contents for raw *H. durvillei* (7.2%) and *H. dilatata* (6.7%) in the present study was similar to (7.5%) from *Solieria filiformis* and about 3-times higher than (2.3%) from *R. pseudopalmata* (Bedoux *et al.* 2017). Uronic acids contents for raw *H. durvillei* (2.08%) and *H. dilatata* (2.32%) in the present study showed similar results to 1.6% from *Solieria filiformis*, and to 1.9% from *R. pseudopalmata* (Bedoux *et al.* 2017). The polyphenols contents from raw *H. durvillei* (5.06%) and *H. dilatata* (3.17%) in the present study were lower than (9.02%) from *Halymenia porphyroides* (Manam and Subbiah, 2020). Carbohydrates contents for raw *H. durvillei* (30.59%) and *H. dilatata* (25.2%) in

the present study were within the range value (13.2-56% dw) for selected red seaweeds from the Philippines (Hurtado *et al.* 2020). However, it is found to be lower than reported in the literature (Kho *et al.* 2016) for *H. durvillei* (53.65%), *H. dilatata* (40.53%) of the same species, and *H. maculata* (51.18%). Slightly higher carbohydrates contents reported (38.3%) from *K. alvarezii* (Wanyonyi *et al.* 2017), and (34.5%) from *Solieria filiformis* (Bedoux *et al.* 2017), while reported lower (15.5%) from *Halymenia floresii* (Garcia *et al.* 2016), than the raw *H. durvillei* and raw *H. dilatata* in the present study respectively.

These variations of results from carbohydrates, ash proteins, and lipids from both present and previous studies in red seaweeds could be affected by geography, season, or growth conditions (Kelman *et al.* 2012). However, the biochemical composition of raw *H. durvillei* and raw *H. dilatata* in the present study were within the values specified for red seaweeds. Thus, there is a potential for these species to be used as a raw material in food industry development or human health.

5.2 Amino acids composition of raw material

The protein contents in red macroalgae, in general, are much higher compared to brown and green groups, accounting to 10-50% dw, and also higher than known high-protein vegetables such as soybean (40.3% dw) (Hurtado *et al.* 2020). The amino acid composition was investigated to understand the nutritional value of the raw seaweeds *Halymenia durvillei* and *Halymenia dilatata* from the Philippines. The sulfur amino acids (methionine, cysteine), when synthesized, are closely related to taurine and its derivatives, which have active biological activities against heart attack, and ameliorating diabetic complications (Okolie *et al.* 2018; Hao *et al.* 2019). Gas chromatography-flame ionization detection (GC-FID) was used to identify and quantify 13, and 14 amino acids (AA) for *H. durvillei* and *H. dilatata*, respectively and is presented in Table XXXVII. The amino acid

composition of ovalbumin, a reference protein from the egg white, is also shown in Table XXXVII for comparison. Amino acids have been classified according to their quality as essential (EAAs) or non-essential (NEAAs) amino acids. Results provided information on the protein composition of the seaweeds and were expressed as a percentage of the total AA detected. The content of AA in the samples is divided into three categories: high (>10%), medium (5-10%), and low (<5%) (Lourenço *et al.* 2002).

In the present study, five dominant AA that constitutes more than or around 10% of the extracted proteins were found for the raw *H. durvillei* and *H. dilatata*. Lysine (11.03%), and leucine (9.81%) are the most represented EAAs in *H. durvillei*, while, *H. dilatata* is rich in the EAAs lysine (9.16%), leucine (9.08%), and methionine (15.55%). High aspartic acid and glutamic acid (NEAAs) contents have been observed in both seaweeds (13.23% and 20.56%) for *H. durvillei*, respectively; and (11.84% and 20.11%) for *H. dilatata*, respectively. Both seaweeds in the present study are deficient in the EAAs threonine, isoleucine, and phenylalanine (0.46-4.69% in *H. durvillei* and 0.18-3.13% in *H. dilatata*) compared to (4-6%) from ovalbumin (Kazir *et al.* 2019) or (25-30%) from soymeal (Makkar *et al.* 2016). The EAA/NEAA ratio has been calculated for both algae. This value, which indicates the quality of proteins, must tend towards or be greater than 1 to contribute to an optimal EAA intake. For the present study, the amino acid contents for *H. durvillei* and *H. dilatata* ranges from 0.18 to 20.56% (Table XXXVII). Observed EAA/NEAA ratios were 0.62 in *H. durvillei*, and 0.87 in *H. dilatata*.

Table XXXVII. Amino acid composition of *Halymenia durvillei* and *Halymenia dilatata* expressed in percentage (%) of the total amino acids detected by gas chromatography.

		<i>H. durvillei</i> (% total AA)	<i>H. dilatata</i> (% total AA)	Ovalbumin ^a (% total AA)
Essential Amino Acids (EAA)	Threonine	0.46	0.18	3.95
	Valine	7.96	6.40	7.10
	Lysine	11.03	9.16	10.14
	Isoleucine	4.69	3.13	6.32

	Leucine	9.81	9.08	8.16
	Phenylalanine	4.13	3.03	5.39
	Histidine	-	-	5.39
	Methionine	-	15.55	4.08
	Total EAA	38.08	46.51	50.52
Non-Essential Amino Acids (NEAA)	Aspartic acid	13.23	11.84	8.16
	Serine	3.51	2.32	8.95
	Glutamic acid	20.56	20.11	13.03
	Glycine	7.30	4.98	4.47
	Alanine	6.25	5.44	8.82
	Tyrosine	6.72	4.90	2.37
	Proline	4.34	3.89	3.69
	Hydroxyproline			-
	Total NEAA	61.92	53.49	49.48
EAA/NEAA ratio	0.62	0.87	1.02	

- = not detected; ^aValues for the ovalbumin composition in amino acids were adapted from (Kazir *et al.* 2019).

The adequate intake of the EAAs/NEAAs ratio is crucial to preserve cell integrity and whole-body metabolism (Romano *et al.* 2019). According to Romano *et al.* (2019) that the percentage survival of mice fed with different diets was negatively affected when EAA was absent (0%/NEAAs) or deficient (30%/NEAAs). The present study *H. durvillei* and *H. dilatata* had EAAs values of 38.08 and 46.51%, respectively, that are within the reported range of EAAs content among red algae (25-50% of the total AA) and are said similar to other protein sources such as casein, ovalbumin, and leguminous plants (Torres *et al.* 2019). The EAAs/NEAAs ratio in the present study for *H. durvillei* (0.62) is within the reported range ratio (0.49-0.68) from 9 different seaweeds, while *H. dilatata* (0.87) is even more significant and better than the reported range ratio (Gaillard *et al.* 2018). Moreover, EAAs/NEAAs ratio is in accordance with the reference value of 0.6 recommended by FAO/WHO (Hao *et al.* 2019). It is also considered favorable or nutritive compared to reference data ovalbumin and in its EAAs/NEAAs values. In the present study, four dominant AA (lysine, leucine, aspartic acid, and glutamic acid) that were found for *H. durvillei* and five dominant AA (lysine, leucine, methionine, aspartic acid, and glutamic acid) were found for *H. dilatata* were also

identified as dominant AA from different red seaweeds in the literature (Hamed *et al.* 2015; Garcia *et al.* 2016; Okolie *et al.* 2018). Aspartic and glutamic acids (up to 15% of the total AA) from *Pyropia tenera* (Hamed *et al.* 2015; Okolie *et al.* 2018) was reported 2-times lower than the present study aspartic and glutamic acids for *H. durvillei* (33.79%) and *H. dilatata* (31.97%). However, no aspartic acid in *H. floresii* (Garcia *et al.* 2016) and its glutamic content (6.80%) is contrary to both species in the present study. Respective leucine and lysine contents (8.7, 6.0%) from *Pyropia tenera* (Hamed *et al.* 2015), and (5.0, 8.47%) from *Halymenia floresii* (Garcia *et al.* 2016) was slightly lower than the present study leucine and lysine contents (9.81, 11.03%) from *H. durvillei*, and (9.08, 9.16%) *H. dilatata*. Methionine content, the highest EAAs, and only found in the present study raw *H. dilatata* (15.55%) is in accordance reported in *Palmaria palmata* (Hamed *et al.* 2015), but in contrast as reported (4.0%) in *H. floresii* (Garcia *et al.* 2016). In terms of NEAAs, aspartic and glutamic acids are the major components of both *H. durvillei* and *H. dilatata*. Aspartic and glutamic acids are responsible for distinctive flavor and taste for seaweeds (de Gaillande *et al.* 2017; Hao *et al.* 2019), suggesting that both *H. durvillei* and *H. dilatata* are potential source flavor enhancers of food products. Also, glutamic acid plays a vital role in maintaining brain function and mental activity, while aspartic acid helps to initiate the Krebs cycle and urea cycle (Okolie *et al.* 2018). However, recent study dares to recognize the fact that glutamate/MSG passed to fetuses and neonates causes brain damage and disrupts the endocrine system will provide the researchers with new knowledge to apply to their studies of glutamate-related abnormalities – including obesity and reproductive disorders (Samuels, 2020). The present study had poor content (<5%) in the EAAs threonine (<0.5%), isoleucine (<4.0%), and phenylalanine (<4.0%) are in accordance with the range results (0.08-4.11%) from *Pyropia tenera* and *H. floresii* (Hamed *et al.* 2015; Garcia *et al.* 2016; Okolie *et al.* 2018). However, methionine content was reported poor from *P. tenera*, which is in high amount (15.55%) from the present study *H. dilatata*.

The presence of methionine in *H. dilatata*, therefore, indicates that it could be a promising natural source of a taurine-like compound and its derivatives with

human health benefits. Variation of results between past and present study on respective amino acid content could be due to the type of species used, season of collection, and seaweed life cycle, which produce different types of proteins during the year, or it could be the extraction method used (Anh *et al.* 2013; Hardouin *et al.* 2016; Garcia *et al.* 2016; de Gaillande *et al.* 2017).

5.3 Lipid composition

The Fatty Acid (FA) composition of *H. durvillei* and *H. dilatata* in the present study are shown in Table XXXVIII. Saturated fatty acids (SFAs) in *H. durvillei* are the primary fatty acids with a value of 51.15% of the total fatty acids (TFAs), while its unsaturated fatty acids such as; polyunsaturated fatty acids (PUFAs), and monounsaturated fatty acids (MUFAs) have (9.81, and 15.75%), respectively. In contrast, unsaturated fatty acids in *H. dilatata* constitute about 35%; PUFAs (12.0%), and MUFAs (23.28%), while its SFAs is (32.19%). Unidentified FAs were (23.29%) in *H. durvillei* and (32.53%) in *H. dilatata*. In *H. durvillei*, palmitic acid (C16:0) was the most abundant FA with a 30.34% TFA value. There was less diversity in unsaturated FA with six different MUFAs with content values that ranged (from <0.3 to 11.68%), and four different PUFAs with content values that ranged (from <0.6 to 4.9%). Unsaturated fatty acids from *H. dilatata* represented a variety of structures with 14 different FA identified by GC ranging from 0.16 to 14.51% of total FA. The main FA detected was oleic acid (C18:1n-9, 14.51% TFA).

Table XXXVIII. Fatty acid composition (% of total fatty acids) of Philippine red seaweed *Halymenia durvillei* and *Halymenia dilatata*.

Fatty Acid (FAs)	ECL	<i>H. durvillei</i> (%)	<i>H. dilatata</i> (%)
Saturated fatty acids (SFAs)			
C6:0 (Caproic)	6.00	0.41±0.02	0.62±0.00
C8:0 (Caprylic)	8.00	0.16±0.00	-
C10:0 (Capric)	10.00	-	1.97±0.00
C11:0 (Undecanoic)	11.00	-	2.68±0.01

C12:0 (Lauric)	12.00	9.99±0.62	2.57±0.01
C13:0 (Tridecanoic)	13.00	0.32±0.00	0.40±0.00
C14:0 (Myristic)	14.00	2.45±0.16	0.55±0.00
C15:0 (Pentadecanoic)	15.00	3.48±0.26	0.40±0.00
C16:0 (Palmitic)	16.00	30.34±0.96	13.89±0.47
C17:0 (Heptadecanoic)	17.00	0.37±0.01	0.47±0.00
C18:0 (Stearic)	18.00	0.73±0.00	4.69±0.03
C20:0 (arachidic)	20.00	2.43±0.02	nd
C22:0 (Behenic)	22.00	0.23±0.00	1.12±0.00
C23:0 (Tricosanoic)	23.00	0.23±0.00	-
C24:0 (Lignoceric)	24.00	-	2.82±0.02
Total SAFs		51.15±1.23	32.19±0.55
Monounsaturated fatty acids (MUFAs)			
C14:1 (Myristoleic)	14.11	11.68±0.60	3.01±0.02
C15:1 (Cis-10-pentadecanoic)	15.00	-	0.38±0.00
C16:1 (Palmitoleic)	16.00	1.36±0.01	2.62±0.00
C17:1 (Cis-10-Heptadecenoic)	17.29	0.16±0.00	1.68±0.00
C18:1n-9c (Oleic)	18.00	1.62±0.06	14.51±0.39
C20:1n-9 (cis-11-Eicosenoic)	20.00	-	0.54±0.00
C22:1n-9 (Erucic)	21.72	0.65±0.01	0.16±0.00
C24:1n-9 (Nervonic)	23.52	0.28±0.00	0.38±0.00
Total MUFAs		15.75±0.64	23.28±0.47
Polyunsaturated fatty acids (PUFAs)			
C18:2n-6c (Linoleic)	18.00	0.59±0.09	0.85±0.00
C18:2n-6t (Linolelaidic)	18.00	0.83±0.03	nd
C18:3n-6 (γ-Linolenic)	18.03	4.90±0.46	0.78±0.00
C18:3n-3 (α-Linolenic)	18.05	3.50±0.12	5.32±0.03
C20:2 (cis-11, 14-Eicosadienoic)	20.00	-	1.00±0.00
C20:3n-6 (Eicosatrienoic)	20.00	-	1.81±0.00
C22:6n-3 (Docosahexaenoic)	22.00	-	2.24±0.01
Total PUFAs		9.81±0.51	12.00±0.22
Σ PUFAs ω6		6.32±0.49	3.44±0.05
Σ PUFAs ω3		3.50±0.25	7.56±0.14
Σ Ratio ω6/ω3		1.81±0.08	0.46±0.00
Σ ω3/Σ ω6		0.55±0.00	2.20±0.00
Unidentified fatty acids		23.29±0.75	32.53±0.39

FA = Total Fatty Acid; - = not detected; SFA = Saturated Fatty Acid; MUFA = Monounsaturated Fatty Acid; PUFA = Polyunsaturated Fatty Acid.

The nutritional quality indexes calculated with the fatty acid profiles of *H. durvillei* and *H. dilatata* are presented in Table XXXIX. The ratio of C18/C20 PUFA was only found in *H. dilatata* species with a value 2.47, which indicates a lesser amount of C20 PUFAs (AA and EPA). The PUFA/SFA ratio of *H. durvillei* was 0.19, whereas it was 0.37 for *H. dilatata*. Ratios based on the functional effects of FA had also been calculated. Atherogenic index (AI), and thrombogenic index

(TI) are related to the protection against coronary artery diseases were found to have higher values in *H. durvillei* than *H. dilatata*. While its high fatty acid hypocholesterolemic/Hypercholesterolemic ratio (h/H) are considered more beneficial as these are directly related to high PUFA content (Matos *et al.* 2016), *H. durvillei* had smaller value (0.2) compared to *H. dilatata* (1.43). The higher the value, the higher the protection properties (Turan, 2007).

Table XXXIX. Nutritional quality indexes of fatty acids from *Halymenia durvillei* and *Halymenia dilatata*.

Seaweed	C18/C20	$\omega 6/\omega 3$	AI	TI	h/H	PUFA/SFA	UI
<i>H. durvillei</i>	-	1.81	1.96	1.52	0.2	0.19	44.60
<i>H. dilatata</i>	2.47	0.46	0.54	0.5	1.43	0.37	64.14

AI: Atherogenic Index; TI: Thrombogenic Index; h/H: fatty acid hypocholesterolemic/Hypercholesterolemic ratio; PUFA: Polyunsaturated Fatty Acid; SFA: Saturated Fatty Acid; UI: Unsaturation Index; - = not detected

A comparison of total average fatty acids (%) in C₁₈, C₂₀, and C₂₂ PUFAs between *H. durvillei* and *H. dilatata* is shown in Fig. 42. Total average fatty acids (%) in C₁₈ PUFAs were found higher in *H. durvillei* (9.82%) than in *H. dilatata* (6.95%). There was no C₂₀, and C₂₂ PUFAs detected in *H. durvillei* while in *H. dilatata* was found to be about 2-3%.

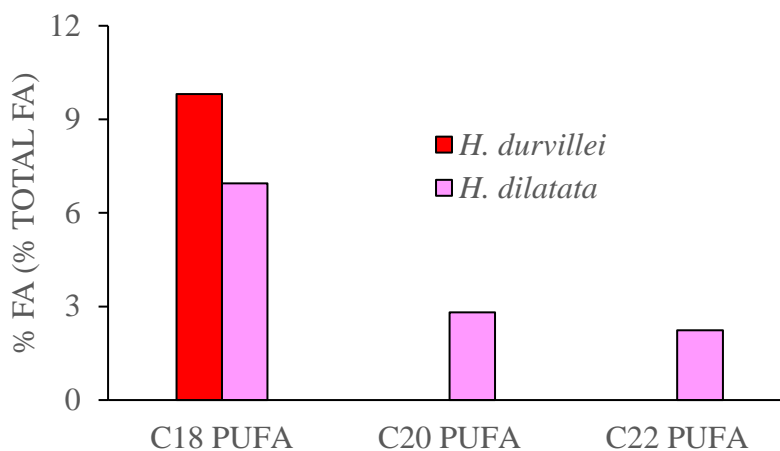


Figure 42. Mean % values for C₁₈, C₂₀, and C₂₂ PUFA red seaweed *H. durvillei* and *H. dilatata* in the present study; TFA= total fatty acid.

The mean total lipid content of *Halymeniales* species can vary (0.1-3.9% dw) with a high content of PUFA considered essential nutritional components in humans and animals. Several red seaweed species are reported to have mean total lipid for *A. taxiformis* (4-12%), *G. filicina* (22%), *H. durvillei* (1.3%), *H. dilatata* (0.3%), and *H. maculata* (1.1%) (Hurtado *et al.* 2020). In the present study, we observed higher lipid contents (7.1 and 0.6% dw) for *H. durvillei* and *H. dilatata* of the same species. The highest PUFA content was measured in *H. porphyroides* at 51.47% (Manam Subbiah *et al.* 2020), *H. sinensis* at 42.2% (Hurtado *et al.* 2020), *H. floresii* at 32.6% (Garcia *et al.* 2016), and *A. taxiformis* at 33.47% (Ragonese *et al.* 2014) of total fatty acids. We observed lower PUFA contents (9.81 and 12.0%) in the present study for *H. durvillei* and *H. dilatata*, respectively, compared to the previous results. The total lipid and fatty acids composition may vary during the life cycles of various seaweeds, which may be seasonal, geographical, or growth conditions (Hurtado *et al.* 2020). The most important of PUFA are the essential fatty acids eicosapentaenoic acid (EPA; C_{20:5n-3}) and docosahexaenoic acid (DHA; C_{22:6n-3}) along with their precursors α -linolenic (ALA; C_{18:3n-3}) and linoleic acids (LA; C_{18:2n-6}). γ and α -Linolenic (C_{18:3n-6} and n-3) acids are the most abundant PUFA in *H. durvillei* while ALA (C_{18:3n-3}) and DHA (C_{22:6n-3})

are the most abundant PUFA in *H. dilatata* of the present study. LA (C18:2n-6) was detected in *H. durvillei* and *H. dilatata* for the present study but only in trace amounts. EPA was not detected in both *H. durvillei* and *H. dilatata*, and DHA was not detected in *H. durvillei* in the present study. Although in different proportions, the present study *H. durvillei* is in accordance with *H. porphyroides*, where ALA (31.21%) and LA (16.14%) was its main PUFAs (Manam Subbiah *et al.* 2020). LA (28.6%) was the major PUFA in *H. floresii*, which was too high compared to both species in the present study, while EPA and DHA were not detected (Garcia *et al.* 2016) as well as in *H. durvillei* in the present study. ALA (2.29%) and DHA (0.93%) PUFAs from *A. taxiformis* (Ragonese *et al.* 2014) were lower than *H. dilatata* (5.32% and 2.24%) in the present study, respectively. Polyunsaturated fatty acids with more than 18 carbons, e.g., EPA, DHA, and arachidonic acid (AA; C20:4n-6), which cannot be synthesized naturally are present in red seaweeds such as *Pyropia dioica*, *Gracilaria gracilis*, and *Palmaria palmata* (Hurtado *et al.* 2020). Besides, PUFAs and carotenoids are most noteworthy as functional foods (McCauley *et al.* 2018). They have been reported to cause the production of different eicosanoids, and are known to regulate cellular processes. EPA, AA, and DHA have several health benefits such as effect on immune-regulation, cancer prevention, pre- and post-natal development of the brain and retina, and regulate inflammation and immune reaction on essential functions in skin growth and protection (Hurtado *et al.* 2020). Manam Subbiah *et al.* (2020) reported that the highest SFA content in *H. porphyroides* was Stearic acid (C18:0) at 29.12% and was way too high than the present study (*H. durvillei* (0.73%), and *H. dilatata* (4.69%)), while highest MUFA, the Oleic acid (C18:1n9c) at 31.21% was way too high than *H. durvillei* (1.62%), but in accordance to *H. dilatata* (14.51%) as the main MUFA in the present study. The main SFA in *H. floresii* at 5.2-5.6% for heptadecanoic (C17:0), stearic, and arachidic (C20:0) acids, while palmitic acid (C16:0) at 1.2% (Garcia *et al.* 2016) were way too small compared to the main SFA (C16:0) for *H. durvillei* (30.34%) and *H. dilatata* (13.89%) in the present study. On the other hand, the main MUFA of *H. floresii* was Myristoleic (C14:1) at 32.6% (Garcia *et al.* 2016) which was in accordance to present study *H. durvillei* as main

MUFA (11.68%) but only in different proportions, while *H. dilatata* main MUFA, the Oleic acid (C18:1n9c) was not detected in *H. floresii*. The main SFA from *A. taxiformis* at 38.05%, the palmitic acid (Ragonese *et al.* 2014) was in accordance with the main SFA in *H. durvillei* and at lower proportion in *H. dilatata* for the present study. The highest MUFA from *A. taxiformis*, palmitoleic acid (C16:1) at 5.6%, was slightly higher than 1.36%, and 2.62% in *H. durvillei* and *H. dilatata*, respectively in the present study. However, the major MUFA in the present study *H. durvillei* (C14:1) was not detected in *A. taxiformis*, and the major MUFA in present study *H. dilatata* (C18:1n9c) was 3-times higher than in *A. taxiformis*.

The nutritional quality indexes (Table XXXIX) can be assessed according to parameters calculated with the concentration of fatty acids. The FAO/WHO considered that a PUFA/SFA ratio below 0.45 is undesirable, as the SFA potentially increases the blood cholesterol level (FAO/WHO, 2008). The present study *H. durvillei* and *H. dilatata* with 0.19 and 0.37 ratios, respectively, do not potentially conform for consumption in terms of PUFA/SFA ratio. For health, high h/H ratios are considered more beneficial, as they are directly related to high PUFA content (Matos *et al.* 2016). The results (1.43) for *H. dilatata* agree with h/H values for marine fishes like sardine or mackerel that are recognized for beneficial health consumption, while (0.2) for *H. durvillei* does not conform with the report (Fernandes *et al.* 2014). AI and TI are related to the protection against coronary artery diseases. Lower AI and TI values indicate more excellent protection (Turan, 2007). The value of AI and TI for *H. dilatata* (0.54, and 0.5), respectively in the present study are in accordance to palatable *C. racemosa* species from the Philippines (Magdugo *et al.* 2020), or lower than those of meat (1.08-1.58) and milk (2.1) (Kumar, 2011), while *H. durvillei* (1.96, and 1.52) was somewhat similar to meat and milk. Moreover, $\omega 6/\omega 3$ ratio (<10) with 1.81 and 0.46, respectively, confirms the potential health benefits of the consumption of *H. durvillei* and *H. dilatata* from the Philippines regarding the lipid distribution (Chan and Matanjun, 2017). Although PUFA/SFA ratio for both studied species was <0.45 , this values was also attributed to other SFAs with health benefits (i.e., lauric, pentadecanoic,

and heptadecanoic acids) which prompts apoptosis in cancer cells (Lappano *et al.* 2017), or promotes against cardiometabolic diseases (Venn-Watson *et al.* 2020). Also, *H. durvillei* and *H. dilatata* from the Philippines should be considered a good-health promoter because of the presence of C22:1n-9 (erucic) and C24:1n-9 (nervonic) acids, compounds which are said to prevent demyelinating disease (Sargent *et al.* 1994).

Conclusion and perspectives

In conclusion of this first part of the study for the biochemical analyses, the raw materials from *H. durvillei* and *H. dilatata* are mainly composed of ash or minerals (41.0%, 46.3%), followed by carbohydrates (30.59%, 25.2%), and proteins (5.3%, 5.5%), respectively. Both species have lipids content that are within the range value from the selected red seaweeds of the Philippines. *H. durvillei* and *H. dilatata* had $\omega 6/\omega 3$ ratio (<10) of 1.81 and 0.46, respectively, confirms the potential health benefits of the consumption requirements of both species regarding the lipid distribution. Although PUFA/SFA ratio for both *H. durvillei* and *H. dilatata* was <0.45 and do not conform to FAO/WHO requirement, these values were also attributed to other SFAs with health benefits (i.e., lauric, pentadecanoic, and heptadecanoic acids) which prompts apoptosis in cancer cells, or promotes against cardiometabolic diseases. Also, *H. durvillei* and *H. dilatata* from the Philippines should be considered a good-health promoter because of the presence of C22:1n-9 (erucic) and C24:1n-9 (nervonic) acids, compounds which are said to prevent demyelinating disease.

We acknowledge the intra-species variation of biochemical results. However, the biochemical composition of raw *H. durvillei* and raw *H. dilatata* in the present study were within the values specified for red seaweeds. Thus, there is a potential for these species to be used as a raw material in food industry development or human health.

5.4 Cell wall polysaccharides purification

5.4.1 First treatment

Extraction yield in percent after three solvents (ethanol, chloroform/methanol, and acetone) are shown in Table XL. In *H. durvillei*, we obtain 95.95%, and in *H. dilatata*, we obtain 97.42%. Initial and final weights of raw *H. durvillei* and *H. dilatata* that were used to calculate extraction yield in percent after three solvents were shown in appendix 5.

Table XL. Percent extraction efficiency of Philippine red seaweeds *Halymenia durvillei* and *Halymenia dilatata*.

Species	Solvents	Yields (%)
<i>H. durvillei</i>	Ethanol	95.95
	Chloroform/Methanol	
	Acetone	
<i>H. dilatata</i>	Ethanol	97.42
	Chloroform/Methanol	
	Acetone	

5.4.2 Polysaccharide extraction

The cell wall polysaccharide for *H. durvillei* and *H. dilatata* were extracted from Depigmented algal powder with the initial weights of 24.02 g and 30 g, respectively. From that 24.02 g and 30 g, after a series of hot water extraction and ethanol precipitation, we obtained the final fraction yield of polysaccharides (Hdur extract, and Hdil extract). The yields of the extracted fraction for *H. durvillei* and *H. dilatata* are given in Table XLI. The initial and final weights of raw *H. durvillei*

and *H. dilatata* that were used to calculate the extraction yield in percent at each purification step were shown in appendix 6.

The extraction yield of polysaccharides from *H. durvillei* and *H. dilatata* fractions was 5.41% and 26.33% dw, respectively (Table XLI).

Table XLI. Extraction yield and purification of polysaccharides from Philippine red seaweeds *Halymenia durvillei* and *Halymenia dilatata*.

EXTRACTION	Yield (% dw)
<i>H. durvillei</i>	
Hdur extract	5.41
<i>H. dilatata</i>	
Hdil extract	26.33

In the present study, the amount of polysaccharides yield in *H. durvillei* (5.41%) was almost 5-times lower than polysaccharides yield in *H. dilatata* (26.33), and comprised of about 15% and 76%, respectively of the carrageenan content found in *Kappaphycus alvarezii* (Wanyonyi *et al.* 2017). The *H. durvillei*, *H. dilatata*, and *H. maculata* from Kho *et al.* (2016) reported a 28.41% yield for *H. durvillei*, which is 5-times higher than Hdur extract (5.41%) of the same species in the present study. While *H. dilatata* yield (9.74%) is about 3-times lower compared to Hdil extract (26.33%) of the same species in the present study, and *H. maculata* reported (11.50%) which is 2-times higher and 2-times lower than Hdur extract, and Hdil extract, respectively. Moreover, the *Halymenia* species from Kho *et al.* (2016) were collected from Iligan Bay, Philippines, and extracted by hydration, followed by hot water extraction and precipitation, and oven-dried at 60 °C, 24 h and 105 °C, 2 h. Another study by Fenoradosoa *et al.* (2009) reported that alkali-treated carrageenan from *H. durvillei* had a 15% yield or 2-times higher than Hdur extract of the same species in the present study. While Hdil extract in the present study was within the range yield reported by Trono and Largo, (2019) that a total of 21-47% g⁻¹ of the air-dry weight of crude λ-like carrageenan can be extracted from

Halymenia as grown in culture. This study suggests that *H. durvillei* and *H. dilatata* could potentially be used as raw material for carrageenan production in the country. Extraction yields between the present and previous studies from seaweeds *Halymenia* vary significantly for different reasons: may be due to the amount of the crude extract that is being used, beginning with the extraction and purifications, or other factors such as species used, season and place of collection, as stated in previous papers (Kok and Wong, 2018; Artemisia *et al.* 2019).

5.4.3 Biochemical composition analysis of extracts

The biochemical composition of *H. durvillei* (Hdur extract) and *H. dilatata* (Hdil extract) carrageenan extracts were shown in Table XLII.

The *H. durvillei* carrageenan fraction (Hdur extract) is rich in neutral sugars (17.6%), followed by sulfates (15.73%), and with small amounts of proteins (7%), polyphenols (4%), and uronic acids (0.7%). While the *H. dilatata* carrageenan fraction (Hdil extract) is rich in sulfates (15.22%), followed by neutral sugars (12.2%), and with small amounts of proteins (8.7%), polyphenols (3.4%), and uronic acids (0.5%). Sulfated polysaccharides or carbohydrates (corresponding to uronic acids, sulfate groups, and neutral sugars) in *H. durvillei* (Hdur extract) and *H. dilatata* (Hdil extract) are of about 34.07% and 27.92%, respectively.

Table XLII. Biochemical composition of polysaccharide extracts from Philippine red seaweeds *Halymenia durvillei* and *Halymenia dilatata*.

ALGAE	Content (% dry algal material)				
	Proteins	Neutral sugars	Sulfates group	Uronic acids	Polyphenol
<i>H. durvillei</i>					
Hdur extract	7.0±1.3	17.6±0.5	15.73±0.4	0.7±0.0	4.0±0.5
<i>H. dilatata</i>					
Hdil extract	8.7±0.9	12.2±1.5	15.22±0.2	0.5±0.2	3.4±0.3

The protein contents from Hdur extract (7.0%) and Hdil extract (8.7%) in the present study were below the range value (9.4-47% dw) for selected red seaweeds from the Philippines (Hurtado *et al.* 2020). Lower protein contents were also reported (1.45%) from crude polysaccharide *Grateloupia filicina*, and (0.18-0.96%) from purified fractions *G. filicina* (Sun *et al.* 2017) than the present study. However, Burlot *et al.* (2016) reported somewhat similar protein content (4.5%) from hot water fraction from *Solieria chordalis* compared to the present study.

Sulfates contents for Hdur extract (15.73%) and Hdil extract (15.22%) in the present study were within the range value (13.2-56% dw) for selected red seaweeds from the Philippines (Hurtado *et al.* 2020). Also, similar sulfates content was reported (15.3%) from crude polysaccharide *Solieria filiformis*, and (16.5%) from *Rhodomenia pseudopalmata* (Bedoux *et al.* 2017), while slightly higher sulfates (21.52%) from crude polysaccharide, and (16.86-20.99%) purified fractions from *G. filicina* (Sun *et al.* 2017). Talarico *et al.* (2004) reported 2-times higher sulfates contents of 33.1%, and 28% from crude extracts from *G. griffithsiae* and *C. crenulata*. Also, according to Talarico *et al.* (2004), that purified fractions from *G. griffithsiae* and *C. crenulata*, produced sulfates contents (16.1-29.4%) and (17.5-28.3%), respectively that were similar to the present study. Conversely, soluble fractions from *G. griffithsiae* and *C. crenulata* (Talarico *et al.* 2004), reported lower sulfates (9.1%), and higher sulfates (28.7%) than Hdur and Hdil extracts in the present study. Burlot *et al.* (2016) reported lower sulfates contents (1.6%) from hot water fraction from *Solieria chordalis* compared to (Hdur and Hdil extracts) in the present study.

Burlot *et al.* (2016) reported a similar neutral sugar content (12.8%) from hot-water fraction from *S. chordalis* compared to Hdur and Hdil extracts in the present study. However, total sugars reported higher contents (55.22%) from crude polysaccharides, and (41-49.74%) from purified fractions from *G. filicina* (Sun *et al.* 2017), than (17.6%, and 12.2%) from Hdur extract and Hdil extract in the present study, respectively.

Uronic acids contents for Hdur extract (0.7%) and Hdil extract (0.5%) in the present study showed similar results to Burlot *et al.* (2016) from *S. chordalis*

extracts from hot water fraction (1%). The polyphenols contents from Hdur extract (4%) and Hdil extract (3.4%) in the present study was lower than (9.02%) from *Halymenia porphyroides* (Manam and Subbiah, 2020).

Carbohydrates contents for Hdur extract (34.07%) and Hdil extract (27.92%) in the present study were within the range value (13.2-56% dw) for selected red seaweeds from the Philippines (Hurtado *et al.* 2020). However, the present study carbohydrates were found to be lower than the carbohydrates reported in crude extract *Gymnogongrus griffithsiae* (55.2%) and *Cryptonemia crenulata* (59.3%); for purified fractions *G. griffithsiae* (39.2-54%) and *C. crenulata* (61-61.3%), and for soluble fraction *G. griffithsiae* (73.5%) and *C. crenulata* (63%) (Talarico *et al.* 2004). In contrast, Burlot *et al.* (2016) reported lower carbohydrates contents of hot water fraction (15.4%) from *Solieria chordalis* extracts than the present study.

These variations of results of carbohydrates, proteins, and lipids from both present and previous studies in red seaweeds could be affected by geography, season, or growth conditions (Kelman *et al.* 2012; Kok and Wong, 2018; Artemisia *et al.* 2019). However, the biochemical composition of Hdur extract and Hdil extract in the present study were within the values specified for red seaweeds. Thus, there is a potential for these species to be used as a raw material in food industry development or human health.

5.4.4 Monosaccharide composition analysis

The unit sugar composition of raw and purified fractions of *H. durvillei* (Hdur extract) and *H. dilatata* (Hdil extract) was determined by High-Performance Anion Exchange Chromatography (HPAEC). The results were given in Table XLIII and expressed as a percentage of sugars relative to the total sugars detected.

There are only four monosaccharides identified present in raw *H. durvillei*, 6 in Hdur extract, 7 in raw *H. dilatata*, and 5 in Hdil extract. Main contents of monosaccharides in raw *H. durvillei* were: small amounts (<10%) of xylose, fucose, trace amounts (<1.0%) of glucoheptose, glucuronic acid, and others (94.24%). The Hdur extract monosaccharides contents were, small amounts (<10%) of xylose, fucose, fructose, glucoheptose, trace amounts (<1.0%) of glucuronic acid, glucosamine, and others (93.17%). While raw *H. dilatata* monosaccharides were, in small amounts (<10%) of xylose, fucose, fructose, trace amounts (<1.0%) of glucoheptose, glucuronic acid, glucosamine, rhamnose, and others (93.57%). The Hdil extract monosaccharides were, small amounts (<10%) of fucose, fructose, glucoheptose; trace amounts (<1.0%) of glucuronic acid, glucosamine; and others (94.88%). The total monosaccharides contents for its raw materials, and each fraction were; raw *H. durvillei*=11.22±0.86, Hdur=13.57±0.47, raw *H. dilatata* =9.79±1.85, and Hdil=15.35±0.53 (Table XLIII).

Table XLIII. Unit sugar composition of raw and extracted fractions from Philippines red seaweed *Halymenia durvillei* and *Halymenia dilatata*.

Monosaccharide	<i>H. durvillei</i>		<i>H. dilatata</i>	
	Raw	Hdur extract	Raw	Hdil extract
	(% of total sugars)			
Fucose	1.84±0.07	1.45±0.18	1.43±0.88	1.66±0.11
Galactose		77.0±0.2		76.0±0.2
Arabinose		8.0±0.2		8.0±0.0
Rhamnose	-	7.0±0.0	0.11±0.02	7.0±0.0
Glucosamine	-	0.23±0.01	0.34±0.05	0.36±0.00
Xylose	2.34±0.24	2.25±0.05	2.41±0.49	-
Fructose	-	1.30±0.09	1.01±0.20	1.26±0.15
Glucoheptose	0.68±0.03	1.04±0.11	0.76±0.21	1.16±0.05
Acide glucuronique	0.44±0.01	0.56±0.02	0.38±0.05	0.64±0.02
Total (µg mg⁻¹) dw	11.22±0.86	13.57±0.47	9.79±1.85	15.35±0.53

- = not detected; The values are expressed in g/100g of total sugars in the fractions.

Fenoradosoa *et al.* (2009) reported similar monosaccharide contents of xylose (2.8, and 1.82%), and fucose (2.1, and 1.6%), from native polysaccharide fractions (water fraction and sodium hydroxide fraction, respectively) from *H. durvillei*, with xylose (2.34-241%), and fucose (1.43-1.84%) contents for all fractions in the present study. Similar results of xylose (2.7, and 2.3%), and fucose (2.0, and 1.7%) was also reported from desulfated polysaccharide fractions (Fenoradosoa *et al.* 2009) compared to the present study Hdur extract. Xylose was not detected in the Hdil extract of the present study.

Galactose was the primary sugar (57.3-65%) in all samples from *H. durvillei* as reported by Fenoradosoa *et al.* (2009), followed by glucose (14.75-16%), then arabinose (15.3-16%), and anhydrogalactose (6.2-9.8%) by which all of these significant sugars were not detected in the present study. However, the unknown major sugars in the present study for raw *H. durvillei* and polysaccharide fraction Hdur extract were about 77%, 8%, and 5% at (13.79, 10.27, and 6.66 min.) retention times (rt), respectively. Also, the unknown major sugars in raw *H. dilatata* and Hdil extract were found to be about 76%, 8%, and 7% at (13.84, 10.27, and 6.66 min.) retention times, respectively. The retention times for standard sugars (galactose, glucose, arabinose, and rhamnose) in the present study were recorded at 14.54, 14.82, 10.6, and 8.64 min. respectively. Given this information above, it might be possible that; (77%, at rt=13.79 min.), (8% at rt=10.27 min.), and (5% at rt=6.66 min.) are representing galactose, arabinose, and rhamnose sugars and their retention times shifted for these sugar components. The same also follows with raw *H. dilatata* and Hdil extract in the present study. The spectral bands at [940-935 cm⁻¹] for both Hdur and Hdil fractions confirm the presence of 3,6-anhydro-D-galactose residues.

Another study, hot water extracted fractions at different temperatures (25, 80, and 80 °C) extracted from *H. floresii* reported sugar contents of xylose (2.7%), and glucose (5.1%) which are only detected on the first fraction (25 °C), and galactose content range (66.9-80.5%) in all fractions (Amorim *et al.* 2011). This reported xylose (2.7%) is also similar to the present study except for Hdil extract which it is not detected, and the galactose contents (about 70%) of all samples in

the present study was within the range value (66.9-80.5%) reported by Amorim *et al.* (2011). Wang *et al.* (2013) reported fucose (76.34%) as the main sugar unit from purified polysaccharides of *Pyropia haitanensis*, followed by galactose (12.98%), xylose (7.63%), and 1.53% each for glucose and mannose. Fucose content (76.34%) reported by Wang *et al.* (2013) is way too big compared to fucose content (1.43-1.84%) found in the present study. Its xylose content (7.63%) was 3-times higher than the present study. The sugar profiles found that all of the samples and its extracts from *H. durvillei* (Hdur extract) and *H. dilatata* (Hdil extract) contained mainly the same types of sugars and are therefore sulfated galactans with a slight difference in proportions. Several factors affect monosaccharide contents, including the type of samples used, whether between or across the same species, life cycle, period and location of collection, and methods of extraction used. These factors were also observed in the literature (Fidelis *et al.* 2014).

5.4.5 *Fourier Transform Infra-Red (FT-IR) spectroscopy*

The calculated FTIR spectra of red seaweeds *H. durvillei* fraction (Hdur) and *H. dilatata* fraction (Hdil) spectral bands of interest were shown in Fig. 43. The IR spectrum of Hdur, and Hdil extracts within 2000-500 cm^{-1} zone or the fingerprint region for polysaccharides were used for data analysis.

The IR spectrum of *H. durvillei* extract (Hdur) and *H. dilatata* extract (Hdil) showed spectral bands of high intensity at [1224-1221 cm^{-1}], while bands of medium or low intensity at [1640-1636 cm^{-1}], [1007, 987 cm^{-1}], [940-935 cm^{-1}], [814-812 cm^{-1}], [769-766 cm^{-1}], and 576 cm^{-1} . Table XLIV summarizes some of the major IR vibrational modes of *H. durvillei* fraction (Hdur) and *H. dilatata* fraction (Hdil) polysaccharides (Fenoradosoa *et al.* 2009; Kim *et al.* 2016; Fernando *et al.* 2017; Al-Nahdi *et al.* 2019).

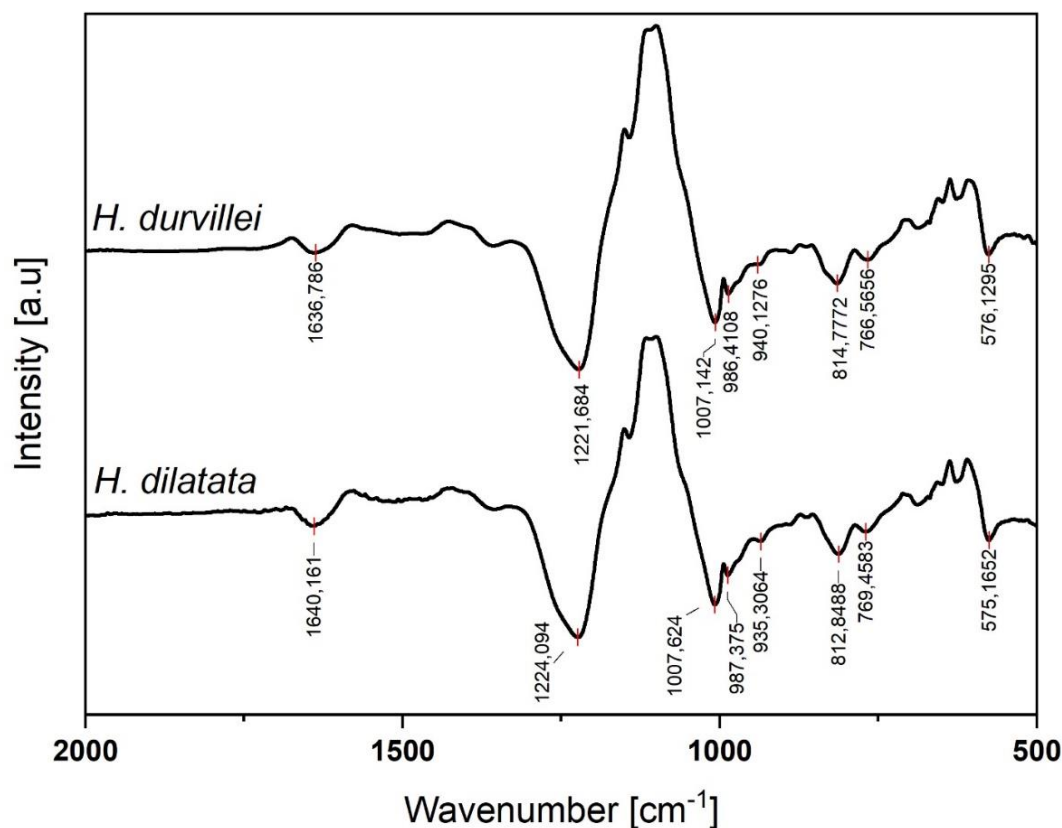


Figure 43. FT-IR spectra of *H. durvillei* and *H. dilatata* polysaccharide extracts.

The high-intensity spectral bands at [1224-1221 cm^{-1}] from both Hdur and Hdil fractions were attributed to O=S=O stretching vibration of sulfate esters with some contribution from the vibration of COH, CC, and CO. This similar peaks also of high intensity were reported in the previous study for *Iridaea cordata* fractions (gelling, and soluble) (Kim *et al.* 2016), also in native and alkali-extracted polysaccharides from *H. durvillei* (Fenoradosoa *et al.* 2009), and in carrageenan extract from *Hypnea bryoides* (Al-Nahdi *et al.* 2019). Accordingly, Kim *et al.* (2016) reported that the intensity of these bands is proportional to the degree of sulfation. Moreover, sulfate contents account for 15% for each Hdur and Hdil fractions in the present study. The spectral bands at [940-935 cm^{-1}] from both Hdur and Hdil fractions indicates the presence of 3,6-anhydro-D-galactose residues, has also been found in all galactan fractions (desulfated, native, and alkali-modified) from *H. durvillei* (Fenoradosoa *et al.* 2009), and in carrageenan extract from

Hypnea bryoides (Al-Nahdi *et al.* 2019). Also, this peak has been detected in very low intensities, which also has been confirmed even in the alkali-modified fraction from Fenoradosoa *et al.* (2009). The medium intensity bands at [814-812 cm^{-1}] for both Hdur and Hdil fractions in the present study indicate the presence of the C-O-SO₄ group on C6 of galactose reported in the study of Al-Nahdi *et al.* (2019) for *Hypnea bryoides* extracts. Al-Nahdi adds that this peak implies the existence of μ -carrageenan in the raw seaweed, which is a natural precursor of K-carrageenan that is present in the seaweed. Therefore, this implies that the red seaweeds *H. durvillei* and *H. dilatata* are natural sources of K-carrageenan. The small spectral bands at [769-766 cm^{-1}] in the present study fractions, indicates the presence of skeletal bending of galactose rings. These similar peaks were also observed and reported in small intensity, for several polysaccharide extracts of red seaweeds (such as; *Gracilaria corticata* var *ramalinoides*, *Gracilaria foliifera*, *Ahnfeltiopsis pygmaea*, *Gracilaria corticata*, *Jania adhaerens*, and *Gracilaria edulis*) in the study of Fernando *et al.* (2017). Small spectral bands at [576-575 cm^{-1}] in the present study fractions were due to asymmetric and symmetric O=S=O deformation of sulfates, was also found in Kim *et al.* (2016). High, medium and small intensity bands between 1200 and 970 cm^{-1} are mainly caused by CC and CO stretching in the pyranose ring and COC stretching of the glycosidic bonds. Intense absorption in this region is common to all types of polysaccharides (Kim *et al.* 2016).

Table XLIV. List of InfraRed (IR) vibrational modes characteristic to polysaccharides used in determining spectral bands of interest for the identification and discrimination of red algal polysaccharides from *Halymenia durvillei* and *Halymenia dilatata* (adapted from Fenoradosoa *et al.* 2009; Kim *et al.* 2016; Fernando *et al.* 2017; Al-Nahdi *et al.* 2019).

Region/Band (cm ⁻¹)	Type of bond	Identification
<i>Common to Halymenia durvillei</i>		
1253	S=O	Stretching vibration of sulfate groups
1375		Sulfate ester substitution
933	C-O-C	3,6-anhydro-D-galactose residues
820		λ-carrageenans; sulfate ester substitution at O-6 at 4- and 3-linked galactose residues
835, 823	O-2 and O-6 gal residues	Equatorial sulfate esters at O-2 and O-6 gal respectively
<i>Other Halymenia and related red species</i>		
1651, 1643	O-H	Indicates the presence of water
(1270-1210), 1219, 1211	O=S=O	Asymmetric stretching vibration of sulfate esters with some contribution from the vibration of COH, CC, and CO
1200-970	CC and CO; COC stretch	CC and CO stretch in pyranoid ring, and COC stretching in glycosidic bonds
1035 (1010-1090)	C-O-C	Stretching vibrations of glycoside bridge
820-810, 867 (shoulder)		D-galactose-6-sulfate (D6S) or the presence of C-O-SO ₄ group on C6 of galactose
770		Skeletal bending of galactose rings
586-578	O=S=O	Asymmetric and symmetric O=S=O deformation of sulfates

5.5 Mineral analysis

The mineral contents expressed as content of each mineral relative to the seaweed dry weight (% of dw) of *H. durvillei* and *H. dilatata* are given in Table XLV. The percentages of the recommended daily intake covered by intake of 8 g of dry seaweed are also presented as well as the daily recommended intake

allowance (Mac Artain, 2007). The species *H. durvillei* had a high total macroelement content (16.20% dw) with sodium (9.96%) as the highest, followed by potassium, calcium, and magnesium. Its microelements total content (0.80% dw), had zinc, iron, copper, and manganese. While *H. dilatata* macroelements total content (31.70% dw) are rich in sodium (19.64%), followed by potassium, calcium, and magnesium. Its microelement total content (1.64% dw), had high iron (1.24%), followed by zinc, copper, and manganese. Eating 8 g of dry *H. durvillei* represents between 1.45% and 360.0% of the recommended daily intake (RDI) for minerals. These percentages were high, especially for the microelements. For *H. dilatata*, 8 g of dry seaweed represents 1646.67% of the RDI in iron. The Na/K ratios in *H. durvillei* and *H. dilatata* were 2.06 and 2.53, respectively.

Table XLV. Composition of macroelements and microelements (% dw), recommended daily intakes, and recommended daily allowances of Philippine red seaweeds *Halymenia durvillei* and *Halymenia dilatata*.

Species	Macroelements					Microelements			
	Na	K	Ca	Mg	Na/K	Fe	Zn	Mn	Cu
RDI ^a (g day ⁻¹)	2.0	3.8	0.84	0.350		0.006	0.012	0.0055	0.0017
Upper limit intake ^b (g day ⁻¹)	2.3	/	2.5	0.35		0.045	0.040	/	0.01
<i>H. durvillei</i>	9.96±0	4.84±0	1.74±0.01	0.42±0	2.06	0.27±0	0.47±0.03	0.001±0	0.06±0
% RDI	39.84	10.19	9.34	9.67		360.00	310.00	1.45	282.35
RDA (g day ⁻¹)	23.09	/	254.84	82.74		16.67	8.60	/	16.67
<i>H. dilatata</i>	19.64±0	7.76±0	2.77±0.02	1.53±0	2.53	1.24±0.03	0.33±0.02	0.002±0	0.07±0
% RDI	78.56	16.34	26.41	34.97		1646.67	218.33	2.91	337.25
RDA (g day ⁻¹)	11.71	/	90.16	22.88		3.64	12.21	/	13.95

^a RDI = Recommended daily intake and upper limit intake values for a male adult with a bodyweight of 70 kg according to the World Health Organization (2002); ^bUpper limit intake according to National Health and Medical Research Council (2006); % RDI is based on a daily intake of 8 g dry weight of seaweed; RDA: recommended daily allowance; Data are mean ± SD (n=2). SD = standard deviation

The high ash content can reach up to 50.2% of dw in *H. floresii* and represent a high content in minerals (Garcia *et al.* 2016). The present study showed that *H.*

durvillei ash content (41%), and *H. dilatata* ash content (46.3%) was a little lower than 50.2% from *H. floresii* (Garcia *et al.* 2016), but is in accordance with 39.54% from *H. durvillei*, and was twice as high than 21.92% from *H. dilatata* of the same species from Iligan bay, Philippines (Kho *et al.* 2016). Other red seaweed species such as *Gracilaria* spp., *Gelidium* sp., and *Pyropia* sp. reported varying ash contents from 7.72% to 29.06% (Alam Bhuiyan and Qureshi, 2016), and 58% from *K. alvarezii* (Wanyonyi *et al.* 2017).

In the present study, *H. dilatata* had higher macroelements (Na, K, Ca, and Mg) than *H. durvillei*. In the study of Garcia *et al.* (2016), macroelements in *H. floresii* reported almost 2-times lower Na (5.54%), slightly higher K (5.96%), 2-times lower Ca (0.73%), and 2-times higher Mg (0.9%) than of *H. durvillei* in the present study. While, macroelements in *H. floresii* reported 3-times lower Na, slightly lower K, about 4-times lower Ca, and about 2-times lower Mg (Garcia *et al.* 2016) than (19.64, 7.76, 2.77, and 1.53%) from *H. dilatata* in the present study, respectively. Several red seaweeds reported higher macroelements contents: of K (13.15%) from *E. cottonii* (Matanjan *et al.* 2009), of Ca (4.3%), Mg (3.3%) from *G. corticata* (Pise and Sabale, 2010), and K (20%), and Ca (2.89%) from *K. alvarezii* (Wanyonyi *et al.* 2017) than the respective macroelements for both *H. durvillei* and *H. dilatata* in the present study.

The ratio of Na/K in *H. durvillei* (2.06) and *H. dilatata* (2.53) in the present study are considered big (>1.5) based on the previous report, and was 2-times higher than 0.93 from *H. floresii* (Garcia *et al.* 2016), way higher than 0.14 from *E. cottonii* (Matanjan *et al.* 2009), 0.25 from *G. acerosa* (Syad *et al.* 2013), higher than 0.4 from *P. umbilicalis*, and 0.22 from *P. palmata* (MacArtain *et al.* 2007), and 0.19 from popular edible red seaweed *K. alvarezii* (Wanyonyi *et al.* 2017). However, a similar Na/K ratio with 1.90 was reported from *C. crispus* (MacArtain *et al.* 2007). Dietary salt intake was seen to be related to the increase in blood pressure with age, and Na/K ratio was a risk mortality factor for CVDs or cardiovascular diseases. However, *H. durvillei* and *H. dilatata* Na/K ratio in the present study were still considered normal or low (<4.0) based on the urinary Na/K ratio and different food items consumed (Seko *et al.* 2018). Thus, consumption of *H. durvillei* and *H.*

dilatata seaweeds or its nutritional derivatives can help balance high Na/K ratio diets common today. The microelements (Fe, Zn, Mn, and Cu) were all in higher amounts in *H. durvillei* and *H. dilatata* in the present study, than the trace amounts reported from *H. floresii* (Garcia *et al.* 2016), from *E. cottonii* (Matanjan *et al.* 2009), from *G. corticata* [except that Mn (0.0036%) was found similar] (Pise and Sabale, 2010), from *G. acerosa* (Syad *et al.* 2013), and *K. alvarezii* (Wanyonyi *et al.* 2017).

Iron is essential for cell functions (oxygen and electron transport, and DNA synthesis), manganese helps in protein, lipid, and carbohydrate metabolism (Mišurcová, 2011). According to Garcia *et al.* (2016), microelement concentrations vary with species, which could be due to morphological and physiological affinities for different metals. As the average daily consumption of dry seaweeds in Asia is 8 g, based on the results in the present study, mineral contents are within safe levels and are potential functional foods for humans. For example, an 8 g portion of *H. durvillei* and *H. dilatata* represented around 9% and 26%, respectively of the RDI of calcium as in other seaweeds, while the same quantity of cheddar cheese provides only 5% of the RDI (Finglas *et al.* 2015; Mc Artain, 2007). Results of daily recommended intake allowance showed that eating of not more than 16.67 g of *H. durvillei* or 3.64 g of *H. dilatata* would not compromise health, because of the iron content of this seaweed. Accumulation of excess iron may generate oxidative stress, can promote and increase cardiovascular risk (Pietrangelo, 2002; Martínez-Navarrete *et al.* 2002; Puntarulo, 2005). However, the biological availability of minerals is influenced by the diet composition by synergistic and antagonistic interactions (Watanabe *et al.* 1997). Moreover, only between 1 and 53% of iron was absorbed by the human body from food (Skikne *et al.* 1983).

5.6 Pigments

Total pigment contents of *H. durvillei* (60.41 ± 5.0 mg g⁻¹ dw) is eight times higher than *H. dilatata* (7.21 ± 0.02 mg g⁻¹ dw) shown in Table XLVI. Chlorophyll *a*, and β -carotene were detected in *H. durvillei*, while chlorophyll *a*, and chlorophyll *b* were detected in *H. dilatata*. The amount of chlorophyll *a* in *H. durvillei* (5.93 ± 5.0 mg g⁻¹ dw), is more than eight times higher than *H. dilatata* (0.68 ± 0.023 mg g⁻¹ dw). Total chlorophyll in *H. durvillei* (5.94 ± 5.0 mg g⁻¹ dw) is slightly lower than in *H. dilatata* (7.21 ± 0.02 mg g⁻¹ dw). The amount of β -carotene (54.47 ± 0.0 mg g⁻¹ dw) or the primary pigment in *H. durvillei* is nine times higher than of the chlorophyll *a* (5.93 ± 5.0 mg g⁻¹ dw), while chlorophyll *d* was not detected. The amount of chlorophyll *d* (6.53 ± 0.0 mg g⁻¹ dw) or the primary pigment in *H. dilatata* is nine times higher than of the chlorophyll *a* (0.68 ± 0.02 mg g⁻¹ dw), while β -carotene in *H. dilatata* was not detected.

Table XLVI. Pigment content (mg g⁻¹ dw) of Philippine red seaweeds *Halymenia durvillei* and *Halymenia dilatata*.

Seaweeds	Chlorophyll			Total chlorophyll	Total pigments
	Chlorophyll <i>a</i>	unidentified	β -Carotene		
<i>H. durvillei</i>	5.93 ± 4.99	-	54.47 ± 0.00	5.94 ± 5.00	60.41 ± 5.00
<i>H. dilatata</i>	0.68 ± 0.02	6.53 ± 0.00	-	7.21 ± 0.02	7.21 ± 0.02

Data are mean \pm SD (n=2). SD = standard deviation. - = not detected.

H. durvillei chlorophyll *a* content was higher than *H. dilatata* (0.68 mg g⁻¹) in the present study. Several red seaweed species reported low chlorophyll content; (0.25 - 0.35 mg g⁻¹ fw) from fresh weight sample *Pyropia haitanensis* after using different light qualities (H. Wu, 2016). Also, low chlorophyll content (0.150 mg g⁻¹ fw) from *H. floresii* (Freile-Peligrin and Robledo, 2009), low (0.288 mg g⁻¹) from *A. taxiformis*, low (0.625 mg g⁻¹) from *C. crispus*, and low (1.84 mg g⁻¹) from

Grateloupia lanceola (Nunes *et al.* 2017). While, meager contents of chlorophyll a and b (0.00158, and 0.00189 mg g⁻¹ fw) from *Gelidiella acerosa* (Syad *et al.* 2013); and (0.5778, and 0.0011 mg g⁻¹ fw) from *Gracilaria changii*, respectively (Chan and Matanjun, 2017) compared with chlorophyll a from *H. durvillei* (5.93, and 0 mg g⁻¹), but are somewhat similar compared with chlorophyll a from *H. dilatata* (0.68, and 6.58 mg g⁻¹) in the present study.

Several varying results of carotenoids contents from different red seaweeds (e.g., *H. floresii*, *G. changii*, *A. taxiformis*, *C. crispus*, *G. lanceola*, *Pyropia dioica*, *H. porphyroides*, *P. umbilicalis*, *P. palmata*, *Gracilaria* spp., *P. vietnamensis*, *E. denticulatum*, and *Gracilaria Salicornia*) were all very low (0.05-1.31 mg g⁻¹) compared to the β -carotene content (54.47 mg g⁻¹) from *H. durvillei* in the present study (McDermid, 2003; Godinez-Ortega *et al.* 2008; Freile-Peligrin and Robledo, 2009; Chan and Matanjun, 2017; MacArtain *et al.* 2007; Nunes *et al.* 2017; Lalegarie *et al.* 2019; Manam Subbiah *et al.* 2020). Chlorophylls and carotenoids had the potential effects of reducing DPPH radicals as antioxidants or in food development. Phycoerythrobilin extracted from *Pyropia* sp. reported antioxidant activity by scavenging peroxy radicals in vitro (Okolie *et al.* 2018). However, the IC₅₀ value of *H. durvillei* and *H. dilatata* in the present study, although active, but are considered weak. The β -carotene content from *H. durvillei* (54.47 mg g⁻¹) was way too high than found in spinach (0.040 mg g⁻¹) and other vegetables (McDermid, 2003; MacArtain *et al.* 2007), while no β -carotene detected in *H. dilatata* in the present study. The pigment β -carotene is said to modulate UVA-induced gene expression, protect the skin and eyes from photo-oxidation against UV light, and prevent eye disease in humans, e.g., cataracts (Pereira, 2018). β -carotene, which, besides its anticancer activity, has been reported to be absorbed 10-times more easily by the body than the synthetic one (Christaki *et al.* 2013). Pigments from macroalgae are also crucial in this industrial range (Leandro *et al.* 2020).

5.7 Biological activity

5.7.1 Cytotoxicity and antiviral activity evaluation

No cytotoxic effect of polysaccharides (CC_{50} greater than $200 \mu\text{g mL}^{-1}$) on the Vero cells was observed for both *H. durvillei* and *H. dilatata* extracts in the range of the concentrations tested. Both *H. durvillei* and *H. dilatata* extracts had low EC_{50} values or lower than those of CC_{50} , signifies antiherpetic activity (Table XLVII). Efficacy or the cellular protection of *H. durvillei* and *H. dilatata* extracts showed that *H. dilatata* extract had maximum efficacy of 136.24% is higher than *H. durvillei* extract 114.0% at $200 \mu\text{g mL}^{-1}$ after 72 h infection. The potency or the effectivity of *H. dilatata* extract at 50% concentration, only needs $1.21 \mu\text{g mL}^{-1}$ of sample (EC_{50} : $1.21 \mu\text{g mL}^{-1}$), which is almost five times more potent than *H. durvillei* extract (EC_{50} : $5.89 \mu\text{g mL}^{-1}$). Efficacy and potency evaluation were shown in appendix 15, while its statistical analysis was shown in appendix 17.5 for both *H. durvillei* and *H. dilatata* extracts.

Table XLVII. Evaluation of cytotoxicity and antiviral activity by cellular viability of Philippine red seaweeds *Halymenia durvillei* and *Halymenia dilatata*.

SPECIES	FRACTION	CC_{50} ($\mu\text{g mL}^{-1}$)	
		Destruction	Protection
<i>H. durvillei</i>	Hdur	>200	5.89 ± 1.13
<i>H. dilatata</i>	Hdil	>200	1.21 ± 0.97
Zovirax		>200	0.37 ± 0.01

CC_{50} : The 50% Cytotoxic Concentration. The concentration that reduced the absorbance of mock-infected cells to 50% of that of controls. EC_{50} : The 50% antiviral Effective Concentration: concentration that achieved 50% protection of virus-infected cells from the HSV-induced destruction.

Red algae as a potential source of antiviral agents against HSV and other viruses were already established by many authors (Mahomoodally *et al.* 2019).

Linear chains of galactopyranosyl residues (λ - and ι - carrageenans) are potent inhibitors against dengue viruses DENV-2 and 3 (Bedoux *et al.* 2017). The water extract from red marine alga *Polysiphonia denudata* selectively inhibits HSV-1 and 2 reproduction with EC₅₀ value range from 8.7 to 47.7 mg mL⁻¹ (Mahomoodally *et al.* 2019). Another study using a similar extraction and analysis condition with the present study reported extract from *Solieria filiformis* had antiviral activity (EC₅₀=136 μ g mL⁻¹) against HSV-1 (Bedoux *et al.* 2017). In the present study, *H. durvillei* and *H. dilatata* extracts possess antiviral activities against HSV-1 with EC₅₀ values (5.89, and 1.21 μ g mL⁻¹) that are way lesser and are more potent compared to the literature (Bedoux *et al.* 2017; Mahomoodally *et al.* 2019). Also, according to Bedoux *et al.* that *Solieria filiformis* extract with 15.6% protein, 7.5% sulfates, and 13.3% polysaccharides sulfate content in dw suggest that aside from degree of sulfation, other factors such as molecular weight, protein content, and position of sulfate groups along the chain may be responsible for this antiviral activity (Bedoux *et al.* 2017). In terms of sulfates content, *H. durvillei* and *H. dilatata* extract in the present study had comparable sulfate content that was also found in the literature (Bedoux *et al.* 2017). Galactopyranose residues as potent antiviral inhibitors from carrageenans (Bedoux *et al.* 2017; Mahomoodally *et al.* 2019) are also found in the present study. The variation of antiherpetic activity results of carrageenans and their derivatives between the present and previous studies could be influenced by monosaccharide composition, sulfate content, polysaccharides chemical structure, molecular weight, location and period of collection, and sensitiveness of methods used. These activities may be due to the presence of other compounds such as proteins and polysaccharides (Bedoux *et al.* 2017; Maya Puspita *et al.* 2017; Sun *et al.* 2017; Kidgell *et al.* 2019; Mahomoodally *et al.* 2019). The *H. durvillei* and *H. dilatata* extracts results showed great potential in developing as an anti-HSV, or anti-DENV drug. However, these various factors mentioned must be understood better, and therefore extracts from *H. durvillei* and *H. dilatata* must be studied further.

5.7.2 Antioxidant activity, DPPH radical scavenging activity

Percent maximum scavenging ability and inhibition activity of *H. durvillei* and *H. dilatata* polysaccharide extracts at (10 mg mL⁻¹) were shown in Table XLVIII.

Scavenging ability for *H. durvillei* polysaccharide extract (Hdur extract) showed that it is higher than *H. dilatata* polysaccharide extract (Hdil extract) with values of 33.9%, and 24.13% at 10 mg mL⁻¹, respectively. Antioxidant activity at 50% (IC₅₀) for *H. dilatata* polysaccharide extract (Hdil extract) had a value of 305.0 µg mL⁻¹ is found lesser than *H. durvillei* polysaccharide extract (Hdur extract) with 449.85 µg mL⁻¹. The low IC₅₀ value indicates that the sample has a strong antiradical activity. Free radical scavenging activity evaluation was shown in appendix 16. In contrast, its statistical analysis was shown in appendix 17.10 for *H. durvillei* and *H. dilatata* polysaccharide fractions with BHA and BHT standards.

Table XLVIII. Summary of antioxidant activity, scavenging assay (DPPH) of Philippine red seaweeds *Halymenia durvillei* and *Halymenia dilatata*.

	IC ₅₀ (µg mL ⁻¹)
BHA (0-50 conc)	7.05±0.27
BHT (0-50 conc)	5.32±0.18
<i>H. durvillei</i>	
Hdur extract	449.85±0.15
<i>H. dilatata</i>	
Hdil extract	305.008±0.32

IC₅₀: corresponding to the concentration sufficient to obtain 50% of a maximum scavenging capacity. *H. durvillei* extracts= sulfated polysaccharide fraction from *H. durvillei*; and *H. dilatata* extracts= sulfated polysaccharide fraction from *H. dilatata*.; BHA = Butylated HydroxyAnisole; BHT = Butylated Hydroxytoluene.

Antioxidant activities of aqueous extract from *Halymenia dilatata* (Hd-extract) had a DPPH assay value range of 3.7-25% at 200-1000 µg mL⁻¹ (Vinosha *et al.* 2019). In the present study, DPPH assay for *H. durvillei* extract (Hdur extract)

with range values of 18.8-33.9%, and *H. dilatata* extract (Hdil extract) with range values of 14.49-24.13% both at 500-10000 $\mu\text{g mL}^{-1}$ are at least 10-times weaker in terms of doses used compared to Vinosha *et al.* (2019). However, if we just consider the dose of up to 1000 $\mu\text{g mL}^{-1}$ in the present study which is the maximum dose used by Vinosha *et al.* (2019), then our antioxidant activities for (Hdur extract) and (Hdil extract) were just somewhat similar with Hd-extract which is also weak from Vinosha *et al.* (2019).

Another study of antioxidant activity of ethanol extract and ethyl acetate extract from *Eucheuma cottonii* and *Eucheuma spinosum* from Indonesia had scavenging activity against DPPH and IC_{50} value (Putri *et al.* 2019). Where, reported for *E. cottonii* ethanol extract (72.41% at 50 $\mu\text{g mL}^{-1}$, $\text{IC}_{50}=35.5 \mu\text{g mL}^{-1}$); *E. cottonii* ethyl acetate extract (1.29% at 50 $\mu\text{g mL}^{-1}$, $\text{IC}_{50}=435.36 \mu\text{g mL}^{-1}$); while *E. spinosum* ethanol extract (5.3% at 500 $\mu\text{g mL}^{-1}$, $\text{IC}_{50}=1386.79 \mu\text{g mL}^{-1}$); and *E. spinosum* ethyl acetate extract (50.4% at 500 $\mu\text{g mL}^{-1}$, $\text{IC}_{50}=430.5 \mu\text{g mL}^{-1}$). Among different extracts from *E. cottonii* and *E. spinosum*, ethanol extract from *E. cottonii* had the strongest extract against DPPH (Putri *et al.* 2019). Despite using 20 to 100 times higher in dose concentrations in comparison with the study of Putri *et al.* (2019), the present study ethanol extracts (Hdur extract and Hdil extract) still had <50% scavenged against DPPH which is lower than *E. cottonii* ethanol extract (72.41% at 50 $\mu\text{g mL}^{-1}$) and *E. spinosum* ethyl acetate extract (50.4% at 500 $\mu\text{g mL}^{-1}$). However, the present study (Hdur extract and Hdil extract) scavenged against DPPH are up to 20 times higher compared to *E. cottonii* ethyl acetate extract (1.29% at 50 $\mu\text{g mL}^{-1}$), and *E. spinosum* ethanol extract (5.3% at 500 $\mu\text{g mL}^{-1}$) with comparable IC_{50} values. Souza *et al.* (2011) Souza *et al.* (2011) reported ethanolic extracts of *gracilaria birdiae* (Gb) exhibit better scavenging activity (approx. 60%, at 5mg mL^{-1}). However, a weaker antioxidant activity ($\text{IC}_{50}=0.76 \text{ mg mL}^{-1}$) compared to the ethanolic extracts of (Hdur extract), and (Hdil extract) with ($\text{IC}_{50}<0.5 \text{ mg mL}^{-1}$) in the present study. These results suggest that antioxidant activity is affected by methods of extraction used, among other factors, even among similar test species. The weak values of antioxidant activity for (Hdur extract) and (Hdil extract) in the present study is probably due to its group, as Red algae group

reported to have the smallest FRAP mean value of $1.59 \pm 1.17 \mu\text{M } \mu\text{g}^{-1}$ extract compared to brown and green algae (3.55 ± 3.16 , and 2.29 ± 2.34) $\mu\text{M } \mu\text{g}^{-1}$ extract, respectively (Kelman *et al.* 2012). Another reason of weak values of antioxidant activity is due to its location and depth of the collection, where Kelman *et al.* (2012) reported antioxidant activities of algae collected from shallow waters (0-3m) were more active than those algae collected in the deep with mean FRAP values of 2.26 ± 2.29 , and $0.21 \pm 0.13 \mu\text{M } \mu\text{g}^{-1}$ extract, respectively. The *H. durvillei* and *H. dilatata* in the present study were collected in the subtidal zones at depths of approximately 5 to 10 meters. The variation of antioxidant activity, DPPH radical scavenging activity results of carrageenans, between the present and previous studies, could be influenced by several factors. Previous papers reported a high correlation of DPPH radical scavenging activity versus total polyphenols, low Mw polysaccharides, pigments, proteins, or peptides (Souza *et al.* 2011) or sensitiveness of methods used, or period, location, and depth of collection (Kelman *et al.* 2012). The present study of Hdil extract is better than Hdur extract. Although both extracts are weak antioxidants, they still are considered significant since these extracts are derived from natural resources.

Conclusion and perspectives

In conclusion, the extraction yield results of *H. durvillei* and *H. dilatata* are affected by species used, geographic location, seasonal growth, and even by the sensitiveness of the methods used. Biochemical composition for both *H. durvillei* and *H. dilatata* extracts is mainly composed of neutral sugars (17.6, 12.2%), sulfates group (15.73, 15.22%), and proteins (7.0, 8.7%) dw of alga, respectively.

FTIR spectrum of *H. durvillei* and *H. dilatata* extracts confirmed the presence of ester sulfates, and sugars, where, *H. durvillei* and *H. dilatata* polysaccharides is mainly composed of carrageenan with galactose, arabinose, and rhamnose as the dominant sugar units. The *H. durvillei* and *H. dilatata*

polysaccharides implies the presence of K-carrageenan, due to the medium intensity bands at [814-812 cm^{-1}] that indicates the presence of its natural precursor, μ -carrageenan. Therefore, this implies that the red seaweeds *H. durvillei* and *H. dilatata* are natural sources of K-carrageenan. Based on the results, the *H. durvillei* and *H. dilatata* mineral contents are within safe levels and are potential functional foods for humans. Also, *H. durvillei* and *H. dilatata* are excellent sources of pigments that can be used in the food industry and human health development. The *H. durvillei* and *H. dilatata* fractions showed in vitro antiherpetic activity without cytotoxicity. Polysaccharides from *H. dilatata* fraction is five times more potent (EC_{50} : 1.21 $\mu\text{g mL}^{-1}$) than *H. durvillei* fraction (EC_{50} : 5.89 $\mu\text{g mL}^{-1}$). Although both *H. durvillei* and *H. dilatata* extracts are weak antioxidants (DPPH scavenging assay), they still are considered significant since these extracts are derived from natural resources and therefore can be cultivated, extracted, and can be a renewable source of bioactive metabolites.

The presence, degree, and distribution of the sulfate groups are important in determining the biological activity of polysaccharides, and therefore must be studied further for these extracts in the future.

Chapter 6 Conclusion

The Philippines is a source of an enormous number of rich natural resources, especially the marine environment. Nevertheless, little is known about the nutritional properties or biological activities of the wild green (*C. racemosa* and *U. lactuca*), brown (*S. polycystum* and *S. ilicifolium*), and red (*H. durvillei* and *H. dilatata*) seaweed species from the Philippines. Thus, we initially characterized these macroalgae to understand their biochemical composition better. Table XLIX showed the summary of results in the present study.

The *C. racemosa* and *U. lactuca* species reported have high amounts of protein. The *C. racemosa* offers real nutritional value, while *U. lactuca* could also be considered good for consumption. The total EAAs of *C. racemosa* and *U. lactuca* species were comparable to FAO/WHO requirement values. The fresh *C. racemosa* and *U. lactuca* seaweed species should be preferred for human consumption. The brown algae, *S. polycystum*, and *S. ilicifolium* are mainly composed of carbohydrates (62.0%, 42.4%), significant presence of ash (24.9%, 17.0%), and proteins (24.2%, 17.8%), respectively. Moreover, the red algae, *H. durvillei* and *H. dilatata* are mainly composed of ash or minerals (41.0%, 46.3%), followed by carbohydrates (30.59%, 25.2%), respectively. The total EAAs of *H. durvillei* and *H. dilatata* were similar to casein, ovalbumin, and leguminous plants. The mineral contents of these seaweeds (green, brown, and red) in the present study are within safe levels and are potential functional foods for humans. The author acknowledged the intra-species variation of their biochemical composition for the green, brown, and red algae of this present work where they were within the values specified.

Thus, it is a potential for these wild seaweeds species to be used as a raw material in the food industry, however, microbial and ecotoxicological analyses are needed to complete the study.

Table XLIX. Summary results of the major analysis of all seaweed samples in the present study.

	GREEN		BROWN		RED	
	<i>C. racemosa</i>	<i>U. lactuca</i>	<i>S. polycystum</i>	<i>S. ilicifolium</i>	<i>H. durvillei</i>	<i>H. dilatata</i>
Proteins (% dw)	19.9	11.1	24.2	17.8	5.3	5.5
Total EAAs (%)	45.2	42.1	n.d.	n.d.	38.0	46.5
Total NEAAs	54.7	57.8	n.d.	n.d.	61.9	53.4
EAA/NEAA	0.8	0.7	n.d.	n.d.	0.6	0.8
Ash (% dw)	29.4	23.3	24.9	17.0	41.0	46.3
Na (%)	7.5	4.7	-	-	9.9	19.6
K (%)	-	-	17.1	2.1	-	-
Fe (%)	0.5 or (9.2 g/day RDA)	1.8 or (2.5 g/day RDA)	1.2 or (3.8 g/day RDA)	1.6 or (2.7 g/day RDA)	0.3 or (16.6 g/day RDA)	1.2 or (3.6 g/day RDA)
Extraction Yields (% dw)	HWE (13.0), 1OH (6.5), 4OH (8.0), INS (28.0)	DS (63.41), DA (28.5), PP1 (4.5), PP2 (91.2), PP3 (8.4)	Sp CaCl ₂ (13.8), Sp HCl (1.6), Sp Na ₂ CO ₃ (10.7), Sp KOH (6.2)	Si CaCl ₂ (6.0), Si HCl (2.5), Si Na ₂ CO ₃ (29.6), Si KOH (5.5)	H. dur. Extract (5.4)	H. dil. Extract (26.3)
Polysaccharides biochemical composition	mainly of sulfates group, and proteins	mainly of sulfates group, proteins, and uronic acids	mainly of neutral sugars, sulfates group, and proteins		mainly of neutral sugars, sulfates group, and proteins	
Major Sugar units (% dw)	Glc (6.0- 54.8), Xyl (0.4- 4.4), Gal (0.9- 18.4)	Glc (0.9- 12.4), Rham (0.5- 6.4), GlcA (0.08- 0.4)	Fuc (4.9- 6.8), Glc (0.7- 22.8), Gal (2.7- 4.5), Man (1.8- 4.6), Xyl (0.6- 1.7)	Fuc (5.4- 15.9), Glc (1.2- 33.7), Gal (3.7- 14.6), Man (4.3- 6.8), Xyl (1.1- 2.08)	Gal (77.0), Ara (8.0), Rham (7.0)	Gal (76.0), Ara (8.0), Rham (7.0)
FTIR analysis	sulfated heteropolysaccharides	Ulvan	fucoidan, alginate, and cellulose/hemicellulose at a ratio of 3:2:1	alginate, fucoidan, and cellulose/hemicellulose at a ratio of 6:2:1	sulfated lambda carrageenan	sulfated lambda carrageenan

Antiviral: EC50 ($\mu\text{g mL}^{-1}$)	fraction Cr crude (25.3 \pm 5.1)	fraction Ulvan DA (88.3 \pm 12.3)	all fractions (30.0- 67.0)	all fractions (6.6- 19.7)	5.9 \pm 1.1	1.2 \pm 0.9
DPPH: IC50 ($\mu\text{g mL}^{-1}$)	fraction 4OH (203.0 \pm 0.2)	fraction Ulvan DA (805.6 \pm 0.3)	fraction Sp CaCl2 (384.3 \pm 0.2)	fraction Si KOH (364.7 \pm 0.3)	449.8 \pm 0.1	305.0 \pm 0.3
Lipids. (% dw)	4.5	4.1	2.86	3.86	7.1	0.6
	α -LA, and Linoleic acids (prevention of skin scaling, hair loss, cardiovascular diseases, and cancer ^a)		-	-	Lauric, Pentadecanoic, Heptadecanoic (prompts apoptosis ^b ; anti- cardiometabolic disease ^c).	
	EPA, DHA (prevention of.. ^a)	n.d.	-	-	(Erucic, Nervonic) Prevent demyelinating disease ^{d, e}	
Fatty acids (health benefits)	Erucic, and Nervonic (Prevent demyelinating disease ^{d, e})		-	-	-	-
	high h/H ratios (directly related to high PUFA content, agree with h/H values for marine fish like sardine or mackerel ^f)		-	-	n.d.	n.d.
	low AI and TI (low values had higher protection against coronary artery diseases ^g)		-	-	n.d.	n.d.
Total PUFAs	21.6 \pm 0.2	10.5 \pm 0.0	-	-	9.81 \pm 0.51	12.00 \pm 0.22
Pigments analysis (mg g⁻¹ dw)	had high pigments and carotene contents that can be used in the food industry and human health development.		β -carotene contents were at least 100 times higher than found in spinach and to the brown seaweeds ^{h, i}		had higher Chl a than <i>Pyropia haitanensis</i> , <i>Halymenia floresii</i> , <i>Asparagopsis taxiformis</i> , <i>Chondrus crispus</i> , <i>Grateloupia lanceola</i> ^{j, k, l} .	β -carotene contents were at least 100 times higher than found in spinach ^{h, i}

Values are means \pm SD (n=9) except for lipids; a= Hamed *et al.* 2015; b= Lappano *et al.* 2017; c= Venn-Watson *et al.* 2020; d= Sargent *et al.* 1994; e= Kumar *et al.* 2010; f= Matos *et al.* 2016; g= Turan 2007; h= McDermid, 2003; i= MacArtain *et al.* 2007; j= Freile-Peligrin and Robledo, 2009; k= Wu, 2016; l= Nunes *et al.* 2017; n.d., not detected; -, not determined; RDA, recommended daily allowance; EAAs, essential amino acids; NEAAs, non-essential amino acids.

With this preliminary biochemical characterization of the raw material, we proceeded to extract these seaweed samples' polysaccharides. The extraction yields of the green, brown, and red seaweeds of this study are affected by the species used, geographic location, and seasonal growth and even by the sensitiveness of the methods used. Biochemical composition of *C. racemosa* and *U. lactuca* (successive precipitation) extracts is mainly composed of sulfates group (8.18-10.58%, 15.79-20.03%) and proteins (3.7-11.0%, 7.1-19.6%), respectively; and sulfates group (5.44-16.0%), proteins (18.3-18.9%), and uronic acids (7.0-9.5%), respectively for dialyzed fractions of *U. lactuca*. The biochemical composition of *S. polycystum* and *S. ilicifolium* extracts is mainly composed of neutral sugars (8.8-23.1%, 18.5-33.2%), sulfates group (6.36-12.68%, 6.98-15.21%), and proteins (8.9-11.8%, 8.7-25.5%) dw of alga, respectively. Furthermore, the red algae *H. durvillei* and *H. dilatata* extracts are mainly composed of neutral sugars (17.6, 12.2%), sulfates group (15.73, 15.22%), and proteins (7.0, 8.7%) dw of alga, respectively. FTIR spectrum of these macroalgal extracts confirmed the presence of sulfate ester, sugars, and proteins. The *C. racemosa* polysaccharide is mainly composed of sulfated galactan with arabinose, glucose, and xylose sugar units. The use of strong alkali (1- and 4M-KOH) to isolate hemicellulosic polysaccharides from *C. racemosa* HWE fraction for biochemical characterization is not necessary since all the information found in these fractions are already found in HWE fraction (see Magdugo *et al.* 2020). The FTIR spectrum of *U. lactuca* extracts confirmed the presence of sulfate groups and sugars, where *U. fasciata* polysaccharide is mainly composed of Ulvan with rhamnose and glucose as the dominant sugar units. *S. polycystum* polysaccharide is mainly composed of fucoidan, alginate, and cellulose/hemicellulose at a ratio of 3:2:1 with fucose, glucose, galactose, mannose, and xylose as the dominant sugar units. *S. ilicifolium* cell wall polysaccharides are composed of alginate, fucoidan, and cellulose/hemicellulose at a ratio of 6:2:1 with fucose, glucose, galactose, mannose, and xylose as the dominant sugar units. *H. durvillei* and *H. dilatata* polysaccharides are mainly composed of sulfated Lambda-carrageenan with galactose, arabinose, and rhamnose as the dominant sugar units. Therefore, this implies that the red seaweeds *H. durvillei* and *H. dilatata* are natural

sources of Lambda-carrageenan. The *C. racemosa*, *U. lactuca*, *S. polycystum*, *S. ilicifolium*, *H. durvillei*, and *H. dilatata* fractions showed in vitro antiherpetic activity without cytotoxicity. Polysaccharides from *S. ilicifolium* fractions are more potent than *S. polycystum* fractions. Polysaccharides from *H. dilatata* fraction is five times more potent (EC₅₀: 1.21 µg mL⁻¹) than *H. durvillei* fraction (EC₅₀: 5.89 µg mL⁻¹). The *C. racemosa* and *U. lactuca* antioxidant results (DPPH scavenging assay) suggest that fresh seaweed should be preferred for human consumption. The *S. polycystum* and *S. ilicifolium* antioxidant results had better activity than the green (*C. racemosa* and *U. lactuca*) and the red (*H. durvillei* and *H. dilatata*) seaweeds in the present study. Suggest that the seaweeds are safe for human consumption. Although both *H. durvillei* and *H. dilatata* extracts are weak antioxidants, they still are considered significant since these extracts are derived from natural resources.

The presence, degree, and distribution of sulfate groups are important in determining the biological activity of polysaccharides, and to analyze its antiviral potency in relation to structure and position of sulfate groups, therefore, must be studied further for these extracts in the future.

The *C. racemosa* and *U. lactuca* are rich in lipids at 4.5% dw and 4.1% dw, respectively. At the same time, *U. lactuca* was evident by its high amount of palmitic acid, presence of PUFAs (C20:4n-6 and C20:5n-3), and a considerable amount of C22:1n-9 (erucic) and C24:1n-9 (nervonic) acids (Magdugo *et al.* 2020). The red algae, *H. durvillei* and *H. dilatata* lipids are within the range value of selected red seaweeds from the Philippines. Also, both *H. durvillei* and *H. dilatata* had ω6/ω3 ratio (<10) of 1.81, 0.46), respectively confirms the potential health benefits of the consumption requirements. Although PUFA/SFA ratio for both *H. durvillei* and *H. dilatata* was <0.45, they shall be considered a good-health promoter because of the presence of C22:1n-9 (erucic) and C24:1n-9 (nervonic) acids. These compounds are said to prevent demyelinating disease. The *C. racemosa* and *U. lactuca* had high pigments and carotene contents that can be used in the food industry and human health development. Also, *S. polycystum* and *S. ilicifolium* had β-carotene contents of at least 100 times higher than found in spinach. Besides its

anticancer activity, β -carotene has been reported to be absorbed 10-times more easily by the body than the synthetic one. Thus, *S. polycystum* and *S. ilicifolium* are excellent sources of pigments used in the food industry and human health development. Also, *H. durvillei* and *H. dilatata* are excellent sources of pigments.

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APPENDICES

Appendix 1. MEM culture medium

The culture medium used for this part is the Eagle's Minimal Essential Medium (MEM) with Earle salts (Eurobio). This medium is enriched with 8% Sterile fetal Calf Serum (FCS, Eurobio, France). They also add 1% L-glutamine to 200 mM (Eurobio) and 1% PCS: a solution of antibiotics and antifungals to avoid any contamination by bacteria and fungi. This solution is prepared previously in 200 mL of phosphate solution. PCS: penicillin (10,000 U, Sigma-Aldrich), colimycin (25,000 U, SigmaAldrich) and streptomycin (10 mg, Sigma-Aldrich).

Appendix 2. Cell maintenance

The cells are cultured in 30 mL of enriched MEM medium in cellular culture flasks (Falcon, Corning). They are incubated at 37°C in an oven containing 5% of CO₂ atmosphere and saturated with water vapor. The medium was renewed daily. The cells are maintained by multiplying them when the cellular carpet is confluent, ie 2 times a week. The viability of the cells is verified under the inverted microscope (G x 100, Olympus CK2). If healthy, the 30 mL of MEM culture medium of the vial is removed in bleach. 20 mL of a phosphate solution are introduced to rinse the cell carpet from the vial. This rinse is performed twice. Then 2 mL of trypsin (Eurobio, Trypsin/EDTA 0.05/0.02%) are then added and contacted with the cell mat for about 1 minute. The excess of the trypsin is removed and the flask is placed back into the oven at 37°C for 2 minutes. By tapping the vial, dissociation of the cell layer should be noticeable. Then 6 mL of enriched medium are inserted and the cells separated from each other are homogenised. They are then seeded with 2 mL of cell suspension in 3 new cell culture flasks in which were deposited at previously 30 mL of enriched MEM. These 3 flasks are put in the oven at 37 °C under 5% of CO₂ atmosphere and saturated with water vapor for 2-3 days. About 25 subcultures like this can be done before starting a new cell culture from cells stock.

Appendix 3. Cell enumeration

The cell count allows to have the same number of cells whatever the sample analyzed. For the cytotoxicity and antiviral activity evaluated in the context of this study Vero cell concentration is set at 350,000 cells mL⁻¹. The Cell count is performed by counting under an inverted microscope using a Cell of Malassez. 1 µL of cell suspension after trypsin deposited between a blade and a lamella constituting the Malassez cell. The quantity of cells is counted on 5 rectangles. The dilution factor (DF) to obtain 350,000 cells mL⁻¹ (in enriched MEM) is determined by multiplying by 2 the amount of cells previously found on 5 rectangles, then divide by 35.

Appendix 4. Organization diagram of a microplate for cytotoxicity and viral tests

	Sample 1 (µg/mL)					Sample 2 or Zovirax (µg mL ⁻¹)					Control virus	Control cell	
Cytotoxicity	200	50	10	5	1	5	1	0.5	0.1	0.005			A
													B
													C
													D
Virus													E
													F
													G
													H
	1	2	3	4	5	6	7	8	9	10	11	12	

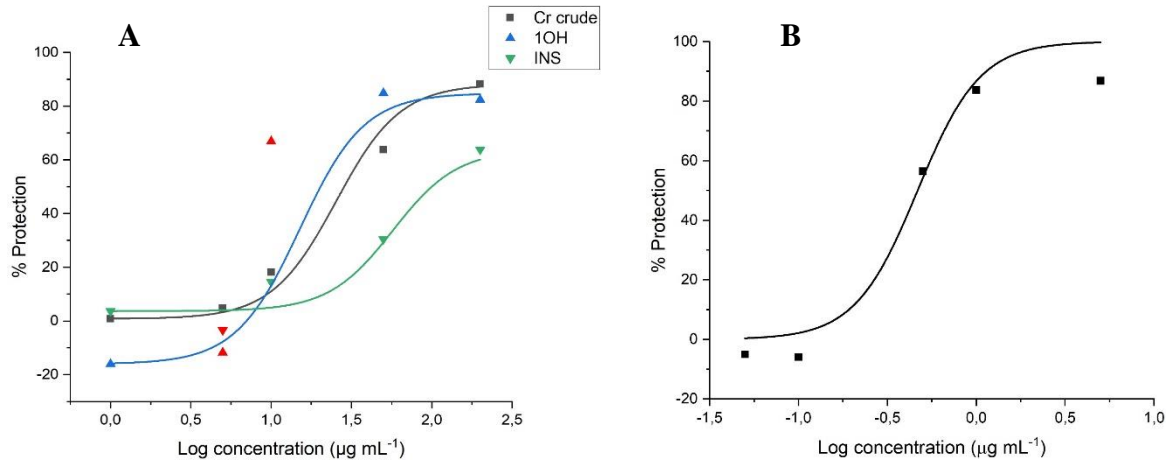
Appendix 5. The initial and final dry weights of green (*C. racemosa* and *U. lactuca*), brown (*S. polycystum* and *S. ilicifolium*), and red (*H. durvillei* and *H. dilatata*) seaweeds used to calculate percent extraction efficiency yield after three solvents.

SPECIES	SOLVENTS	Initial weight (g)	Final weight (g)
<i>C. racemosa</i>	Ethanol	15.00	14.06
	Chloro/MeOH	14.06	13.80
	Acetone	13.80	13.49
<i>U. lactuca</i>	Ethanol	15.01	13.74
	Chloro/MeOH	13.74	13.51
	Acetone	13.51	13.46
<i>S. polycystum</i>	Ethanol	21.10	18.30
	Chloro/MeOH	18.30	16.67
	Acetone	16.67	15.10
<i>S. ilicifolium</i>	Ethanol	21.64	19,63
	Chloro/MeOH	19.63	17.96
	Acetone	17.96	17.30
<i>H. durvillei</i>	Ethanol	26.57	25.92
	Chloro/MeOH	25.92	25.64
	Acetone	25.64	25.49
<i>H. dilatata</i>	Ethanol	58.42	57.41
	Chloro/MeOH	57.41	57.06
	Acetone	57.06	56.91

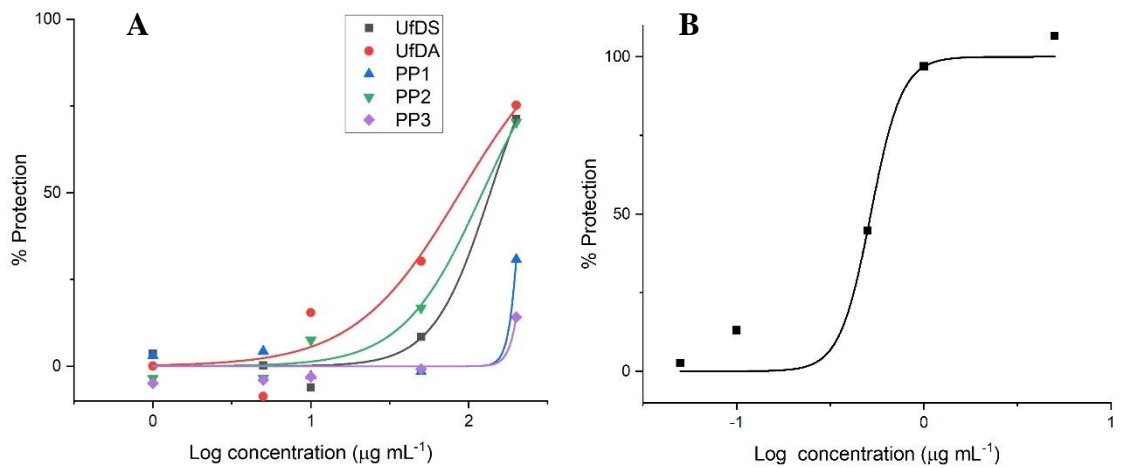
Appendix 6. The initial and final dry weights of green (*C. racemosa* and *U. lactuca*), brown (*S. polycystum* and *S. ilicifolium*), and red (*H. durvillei* and *H. dilatata*) seaweeds used to calculate the extract yield at each purification step.

EXTRACTION	Initial weight (g)	Final weight (g)
<i>C. racemosa</i>		
Depigmented	4	
HWE	4	0.52
1OH	1.80	0.12
4OH	1.20	0.10
INS	0.50	0.014
<i>U. lactuca</i>		
	10	
Raw ulvan	2.37	
SUCC. PRECIPITATION		
Ulvan extract	1.37	
Ulvan PP1	1.37	0.87
Ulvan PP2	0.87	0.39
Ulvan PP3	0.39	0.06
DIALYSIS		
Ulvan extract	1.00	
Ulvan DS	1.0	0.91
Ulvan DA	1.0	0.08
<i>S. polycystum</i>		
	10	
<i>Spoly</i> CaCl ₂	10	8.62
<i>Spoly</i> HCL	8.62	8.46
<i>Spoly</i> Na ₂ CO ₃	8.46	7.38
<i>Spoly</i> KOH	7.38	6.76
<i>S. ilicifolium</i>		
	10	
<i>Spoly</i> CaCl ₂	10	9.40
<i>Spoly</i> HCL	9.40	9.15
<i>Spoly</i> Na ₂ CO ₃	9.15	6.18
<i>Spoly</i> KOH	6.18	5.63
<i>H. durvillei extract</i>	24.02	1.3
<i>H. dilatate extract</i>	30.00	7.9

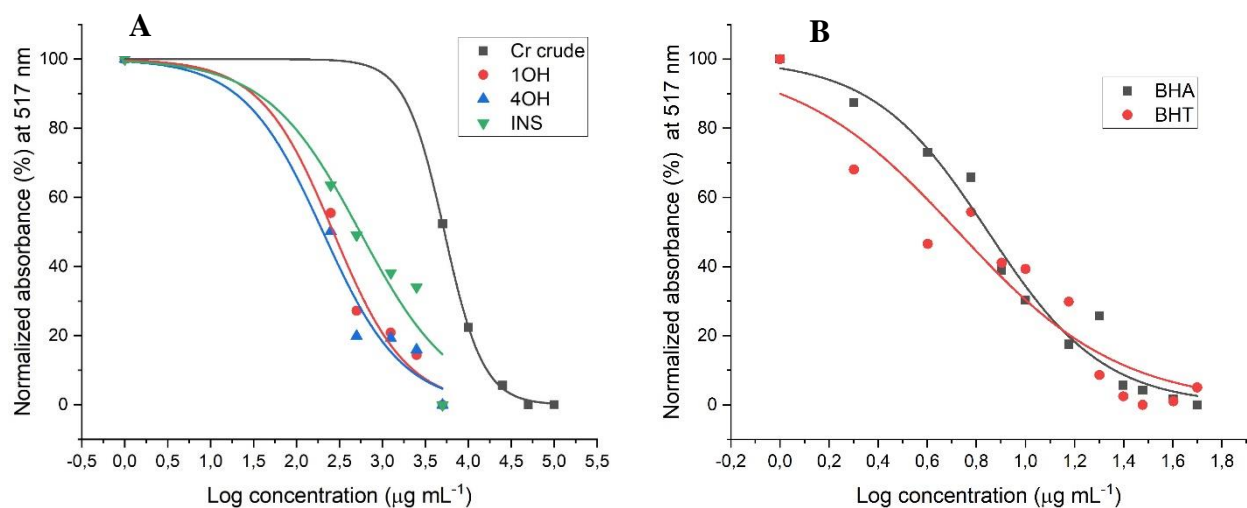
Appendix 7. Efficacy and potency evaluation of (A) *C. racemosa* extracts (Cr crude, 1OH, and INS) compared with (B) zovirax standard.



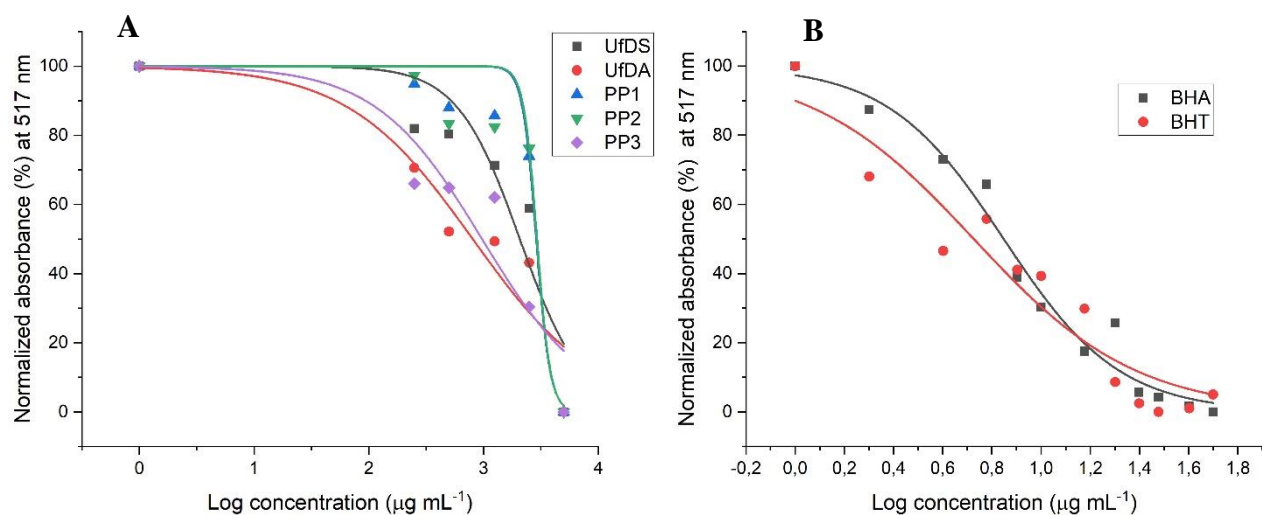
Appendix 8. Efficacy and potency evaluation of (A) *U. lactuca* extracts (Cr crude, 1OH, and INS) compared with (B) zovirax standard.



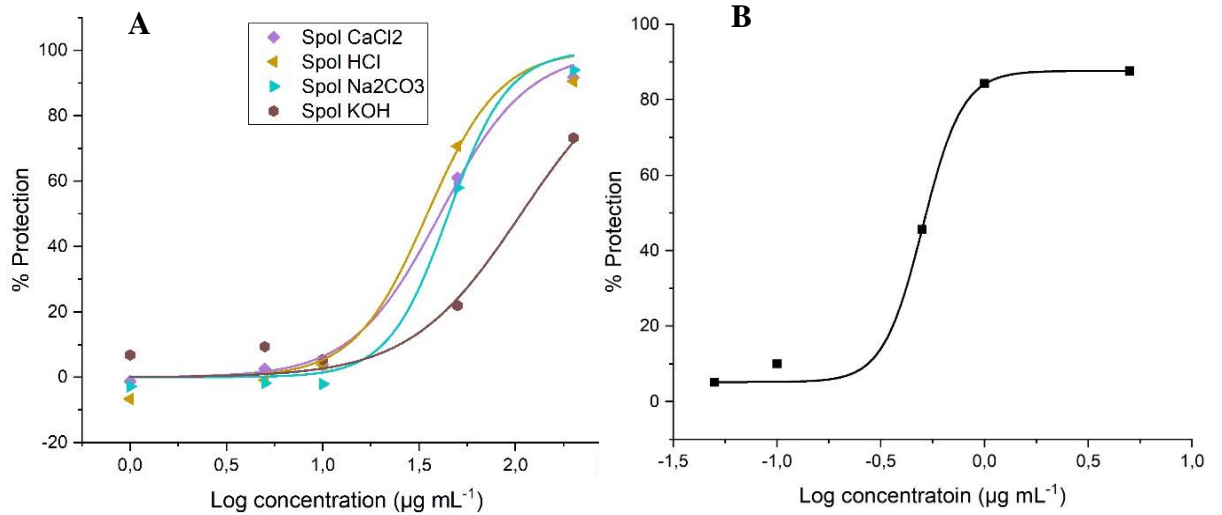
Appendix 9. Free radical scavenging activity of (A) *C. racemosa* extracts (Cr crude, 1OH, 4OH, and INS) compared with (B) BHA and BHT standards.



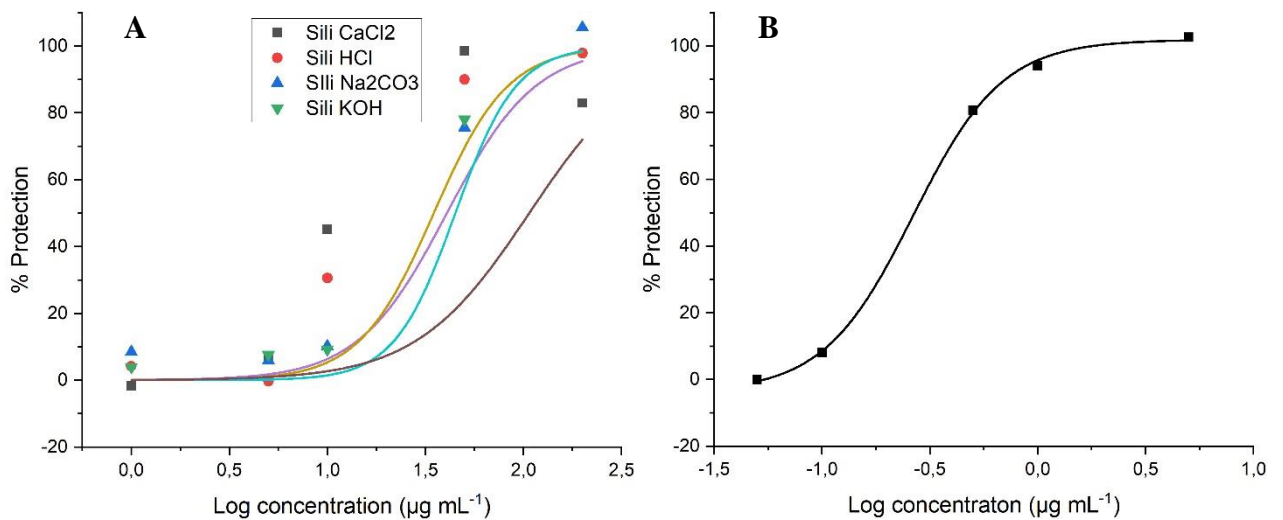
Appendix 10. Free radical scavenging activity of (A) *U. lactuca* extracts (DS, DA, PP1, PP2, and PP3) compared with (B) BHA and BHT standards.



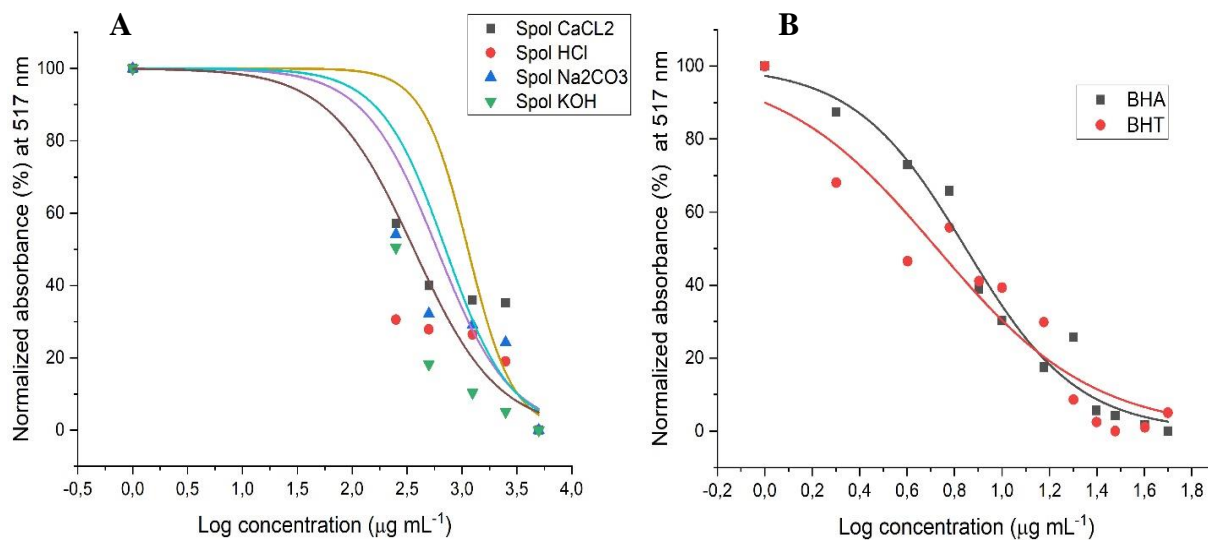
Appendix 11. Efficacy and potency evaluation of (A) *S. polycystum* extracts (CaCl₂, HCl, Na₂CO₃, and KOH) compared with (B) zovirax standard.



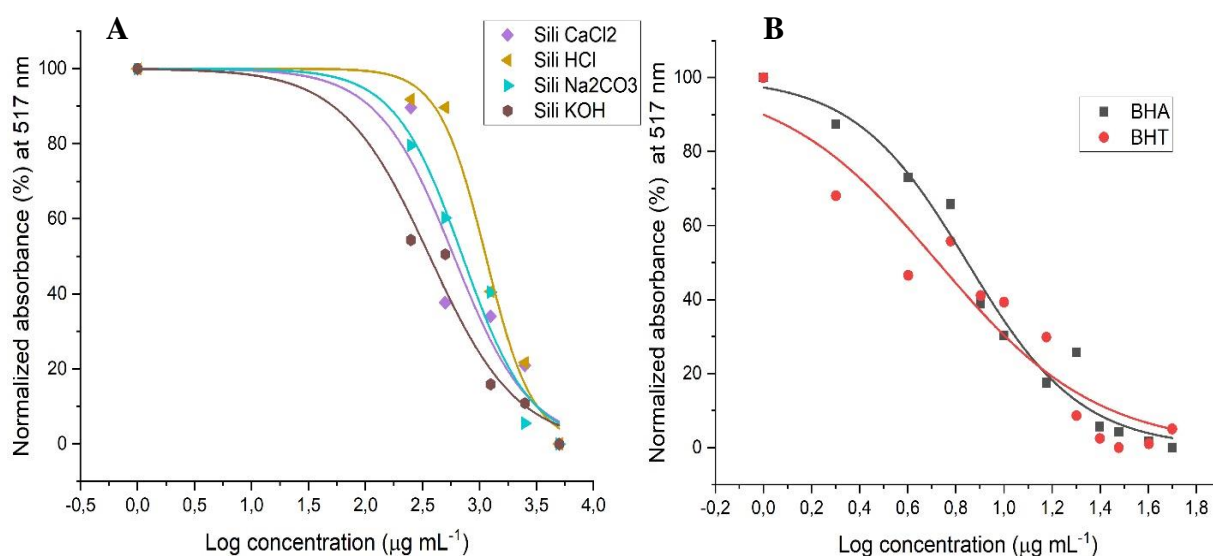
Appendix 12. Efficacy and potency evaluation of (A) *S. ilicifolium* extracts (CaCl₂, HCl, Na₂CO₃, and KOH) compared with (B) zovirax standard.



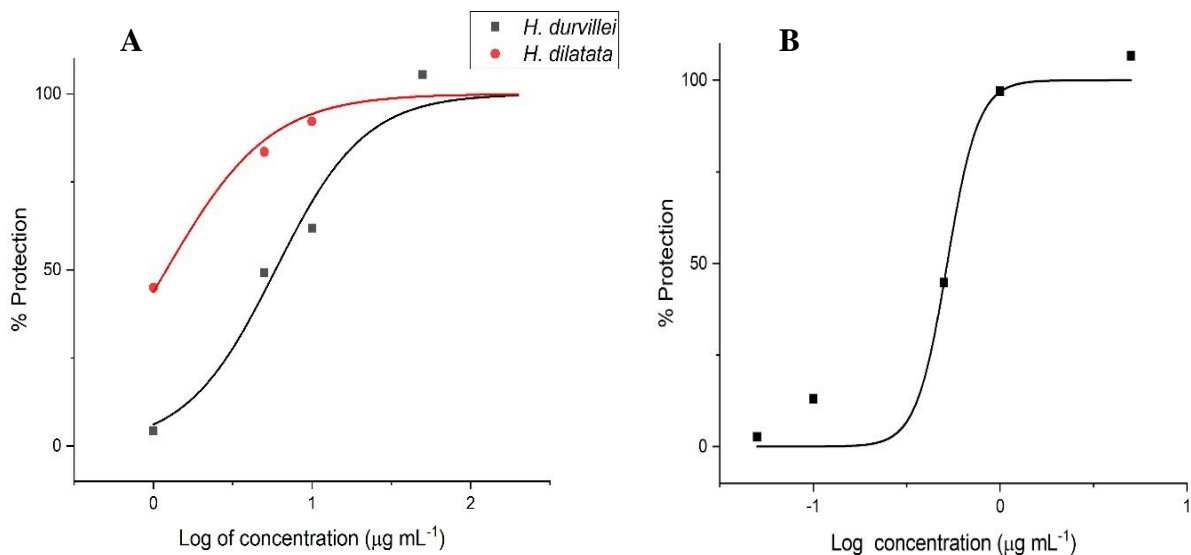
Appendix 13. Free radical scavenging activity of (A) *S. polycystum* extracts (CaCl₂, HCl, Na₂CO₃, and KOH) compared with (B) BHA and BHT standards.



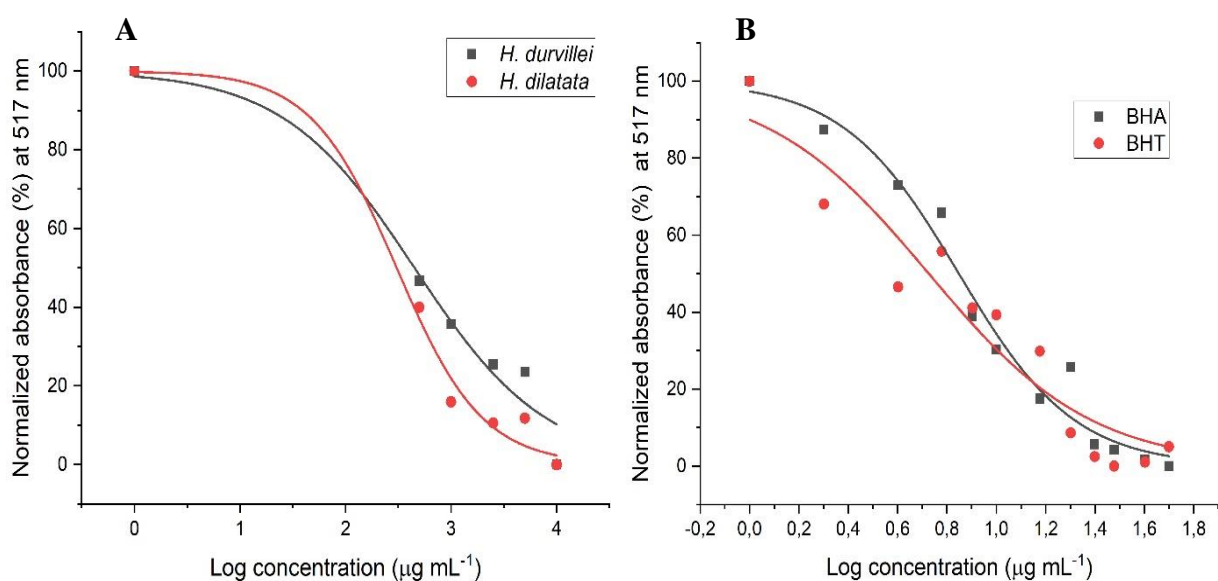
Appendix 14. Free radical scavenging activity of (A) *S. ilicifolium* extracts (CaCl₂, HCl, Na₂CO₃, and KOH) compared with (B) BHA and BHT standards.



Appendix 15. Efficacy and potency evaluation of (A) *H. durvillei* and *H. dilatata* extracts (Hdur and Hdil) compared with (B) zovirax standard.



Appendix 16. Free radical scavenging activity of (A) *H. durvillei* (Hdur), and *H. dilatata* (Hdil) extracts compared with (B) BHA and BHT standards.



Appendix 17. Statistical Analysis

Appendix 17.1. CC₅₀ and EC₅₀ of *C. racemosa* polysaccharide fractions and its zovirax control.

STATISTICS				
PROTECTION	Cr crude	1OH	INS	Global
Number of Points				14
Degrees of Freedom				8
Reduced Chi-Sqr				142.87271
Residual Sum of Squares	121.53394	708.70146	105.43518	1142.9817
R-Square (COD)	0.97983	0.92562	0.9631	0.93673
Adj. R-Square				0.89718
Fit Status	Succeeded(100)	Succeeded(100)	Succeeded(100)	Succeeded(100)

SUMMARY	LOGx0	LOGx0	p	p	span	EC20	EC50	EC80
PROTECTION	Value	Standard Error	Value	Standard Error	Value	Value	Value	Value
Cr crude	1.40364	0	2.17294	0	87.41488	13.38342	25.33022	47.94138
1OH	1.50715	2.82705	3.86363	56.90514	100	22.45525	32.14738	46.02283
INS	2.05023	0.1681	0.97439	0.38158	100	27.06123	112.26173	465.71034

STATISTICS		
Zovirax (Cr)	Destruction	Protection
Number of Points		5
Degrees of Freedom		1
Reduced Chi-Sqr	10.99313	91.21741
Residual Sum of Squares	10.99313	273.65223
R-Square (COD)	0.95972	0.96761
Adj. R-Square	0.8389	0.95681
Fit Status	Succeeded(100)	Succeeded(100)

SUMMARY										
Zovirax (Cr)	LOGx0	LOGx0	p	p	span	EC20	EC50	EC80	Statistics	Statistics
	Value	Standard Error	Value	Standard Error	Value	Value	Value	Value	Reduced Chi-Sqr	Adj. R-Square
Destruction	0.2835	0.1269	3.11487	3.57715	17.1821	0.3335	0.52054	0.8123	10.9931	0.838
Protection	0.3304	0.07145	2.46799	1.16513	100	0.2664	0.46722	0.8193	91.2174	0.956

Appendix 17.2. CC₅₀ and EC₅₀ of *U. lactuca* polysaccharide fractions and its zovirax control.

STATISTICS						
PROTECTION	UIDS	UIDA	PP1	PP2	PP3	Global
Number of Points						25
Degrees of Freedom						15
Reduced Chi-Sqr						28.78454
Residual Sum of Squares	52.49079	224.99955	37.93909	66.98446	49.35427	431.76816
R-Square (COD)	0.98685	0.94847	0.94985	0.98224	0.80232	0.97066
Adj. R-Square						0.95305

SUMMARY

PROTECTION	LOGx0 Value	LOGx0 Standard Error	p Value	p Standard Error	span Value	EC20 Value	EC50 Value	EC80 Value
UIDS	2.13614	0.04933	2.39935	0.5469	100	76.7743	136.81753	243.81905
UIDA	1.94605	0.06079	1.29761	0.22406	100	30.3443	88.31848	257.055
PP1	2.3308	2799.16945	11.80322	1.11E+06	100	190.45299	214.18838	240.88182
PP2	2.08682	0.05301	1.72016	0.31598	100	54.5525	122.12848	273.41306
PP3	2.3788	2061.89545	10.05872	266673.322	100	208.42446	239.22315	274.57292

STATISTICS

Zovirax (Uf)	Destruction	Protection
Number of Points	5	5
Degrees of Freedom	1	3
Reduced Chi-Sqr	0.02194	73.79185
Residual Sum of Squares	0.02194	221.37555
R-Square (COD)	0.99889	0.97545
Adj. R-Square	0.99555	0.96726
Fit Status	Succeeded(100)	Succeeded(100)

SUMMARY	LOGx0 Value	LOGx0 Standard Error	p Value	p Standard Error	span Value	EC20 Value	EC50 Value	EC80 Value	Statistics Reduced Chi-Sqr	Statistics Adj. R-Square
Destruction	-0.5994	0.07917	1.4377	0.25678	5.485	0.095	0.2515	0.6596	0.02194	0.995
Protection	-0.2838	0.03021	5.2451	4.10329	100	0.3993	0.3701	0.6774	73.79185	0.917

Appendix 17.3. CC₅₀ and EC₅₀ of *S. polycystum* polysaccharide fractions and its zovirax control.

STATISTICS					
PROTECTION	Spol CaCl ₂	Spol HCl	Spol Na ₂ CO ₃	Spol KOH	Global
Number of Points					40
Degrees of Freedom					24
Reduced Chi-Sqr					53.62737
Residual Sum of Squares	24.1977	111.11604	44.38859	130.49891	1287.05695
R-Square (COD)	0.997	0.987	0.994	0.960	0.980
Adj. R-Square					0.968

SUMMARY	LOGx0	LOGx0	p	p	span	EC20	EC50	EC80
PROTECTION	Value	Standard Error	Value	Standard Error	Value	Value	Value	Value
Spol CaCl ₂	1.60914	0.06897	1.91347	0.61313	100	19.70151	31.63	83.90431
Spol HCl	1.54238	0.077	2.29603	0.83812	100	19.0618	30.08	63.7675
Spol Na ₂ CO ₃	1.65242	0.06004	2.78997	2.29562	100	27.3294	33.25	73.82768
Spol KOH	2.02891	0.07703	1.5106	0.36625	100	42.69256	67.02	267.58857

STATISTICS		
Zovirax	Destruction	Protection
Number of Points		3
Degrees of Freedom		1
Reduced Chi-Sqr		0.03908
Residual Sum of Squares		0.03908
R-Square (COD)		0.96138
Adj. R-Square		0.92276
Fit Status	Succeeded(100)	Succeeded(100)

SUMMARY										
Zovirax	LOGx0	LOGx0	p	p	span	EC20	EC50	EC80	Statistics	Statistics
	Value	Standard Error	Value	Standard Error	Value	Value	Value	Value	Reduced Chi-Sqr	Adj. R-Square
Destruction	4.2814	1.0978	0.37818	0.092	100	489.177	19118.89	747237.46	0.03908	0.92276
Protection	0.2979	0.0127	4.541	1.209	82.457	0.3711	0.503	0.683	7.705	0.995

Appendix 17.4. CC₅₀ and EC₅₀ of *S. ilicifolium* polysaccharide fractions and its zovirax control.

STATISTICS						
PROTECTION	Sili CaCl ₂	Sili HCl	Sili Na ₂ CO ₃	Sili KOH	Global	
Number of Points						40
Degrees of Freedom						24
Reduced Chi-Sqr						53.62737
Residual Sum of Squares	292.3302	98.95465	136.08886	449.48201		1287.05695
R-Square (COD)	0.963	0.989	0.984	0.959		0.980
Adj. R-Square						0.968

SUMMARY	LOGx0	LOGx0	p	p	span	EC20	EC50	EC80
PROTECTION	Value	Standard Error	Value	Standard Error	Value	Value	Value	Value
Sili CaCl ₂	1.02508	0.04196	3.43406	1.64036	100	7.07554	6.63	15.86354
Sili HCl	1.19375	0.08025	2.22928	0.64433	100	8.38847	17.29	29.0951
Sili Na ₂ CO ₃	1.44917	0.07809	2.06401	0.49716	100	14.37069	19.70	55.06381
Sili KOH	1.4347	0.08126	2.29146	0.58076	100	14.85801	17.44	49.82436

STATISTICS		
Zovirax	Destruction	Protection
Number of Points		3
Degrees of Freedom		1
Reduced Chi-Sqr		4.08407
Residual Sum of Squares		4.83876
R-Square (COD)		0.88033
Adj. R-Square		0.761
Fit Status	Succeeded(100)	Succeeded(100)

SUMMARY										
Zovirax	LOGx0	LOGx0	p	p	span	EC20	EC50	EC80	Statistics	Statistics
	Value	Standard Error	Value	Standard Error	Value	Value	Value	Value	Reduced Chi-Sqr	Adj. R-Square
Destruction	1.718	0.935	0.429	0.172	100.000	2.060	52.264	1326.274	4.084	0.761
Protection	-0.579	0.042	2.094	0.255	105.509	0.136	0.324	0.512	4.839	0.998

Appendix 17.5. CC₅₀ and EC₅₀ of *H. durvillei* and *H. dilatata* polysaccharide fractions and its zovirax control.

STATISTICS			
PROTECTION	Hdur	Hdil	Global
Number of Points			10
Degrees of Freedom			6
Reduced Chi-Sqr			378.40572
Residual Sum of Squares	383.9632	1886.4711	2270.43429
R-Square (COD)	0.95192	0.63081	0.85055
Adj. R-Square			0.77582
Fit Status	Succeeded(100)	Succeeded(100)	Succeeded(100)

SUMMARY								
PROTECTION	LOGx0	LOGx0	p	p	span	EC20	EC50	EC80
	Value	Standard Error	Value	Standard Error	Value	Value	Value	Value
Hdur	0.7699	0.16799	1.54224	1.13904	100	2.39617	5.88706	14.46369
Hdil	0.08181	0.237	1.32554	0.97299	100	0.42424	1.20728	3.43565

STATISTICS		
Zovirax (Hdur and Hdil)	Destruction	Protection
Number of Points		5
Degrees of Freedom		1
Reduced Chi-Sqr		0.02194
Residual Sum of Squares		221.37555
R-Square (COD)		0.99889
Adj. R-Square		0.99555
Fit Status	Succeeded(100)	Succeeded(100)

SUMMARY	LOGx0	LOGx0	p	p	span	EC20	EC50	EC80	Statistics	Statistics
	Value	Standard Error	Value	Standard Error	Value	Value	Value	Value	Reduced Chi-Sqr	Adj. R-Square
Destruction	-0.5994	0.07917	1.4377	0.25678	5.485	0.095	0.2515	0.6596	0.02194	0.995
Protection	-0.2838	0.03021	5.2451	0.01329	100	0.3993	0.3701	0.6774	73.79185	0.917

Appendix 17.6. Antiradical scavenging activity (IC₅₀) for *C. racemosa* polysaccharide fractions (Cr crude, 1OH, 4OH, and INS) with its BHA and BHT standards.

STATISTICS	Cr crude	1OH	4OH	INS	Global
Number of Points					24
Degrees of Freedom					16
Reduced Chi-Sqr					46.86491
Residual Sum of Squares	2.64301	131.63904	220.23129	364.76339	749.8385
R-Square (COD)	0.99966	0.97987	0.9662	0.93417	0.97134
Adj. R-Square					0.9588

SUMMARY	LOGx0	LOGx0	p	p	span	EC20	EC50	EC80
	Value	Standard Error	Value	Standard Error	Value	Value	Value	Value
Cr crude	3.72045	0.05621	-1.91368	0.55561	100	10840.6268	5253.47379	2545.8847
1OH	2.42812	0.09762	-1.01715	0.24673	100	1047.21081	267.99289	68.58236
4OH	2.30764	0.12977	-0.93766	0.25641	100	890.69493	203.06825	46.29724
INS	2.73933	0.08692	-0.79753	0.15766	100	3120.574	548.693	96.47712

STATISTICS	BHA	BHT	Global
Number of Points			24
Degrees of Freedom			20
Reduced Chi-Sqr			61.24191
Residual Sum of Squares	337.46559	887.37269	1224.83828
R-Square (COD)	0.97566	0.91903	0.95089
Adj. R-Square			0.94352
Fit Status	Succeeded(100)	Succeeded(100)	Succeeded(100)

SUMMARY	LOGx0	LOGx0	p	p	span	EC20	EC50	EC80
	Value	Standard Error	Value	Standard Error	Value	Value	Value	Value
BHA	0.84819	0.03664	-1.84925	0.27441	100	14.91964	7.04998	3.33133
BHT	0.7266	0.04844	-1.31583	0.18087	100	15.2809	5.32841	1.858

Appendix 17.7. Antiradical scavenging activity (IC₅₀) for *U. lactuca* polysaccharide fractions ((DS, DA, PP1, PP2, and PP3) with its BHA and BHT standards.

STATISTICS	UfDS	UfDA	PP1	PP2	PP3	Global
Number of Points						30
Degrees of Freedom						20
Reduced Chi-Sqr						143.57539
Residual Sum of Squares	68.99238	2713.58155	69.22419	11.3433	8.36638	2871.50781
R-Square (COD)	0.92614	-2.48589	0.96516	0.9475	0.94798	0.39619
Adj. R-Square						0.12447

SUMMARY	LOGx0	LOGx0	p	p	span	EC20	EC50	EC80
	Value	Standard Error	Value	Standard Error	Value	Value	Value	Value
UfDS	3.92269	0.30032	1.07741	0.67998	100	2311.4528	8369.31925	30303.67083
UfDA	93500.7094	0	11.66543	0	100	0	0	0
PP1	3.67957	0.07871	2.49522	1.28754	100	2743.34929	4781.52504	8333.96676
PP2	4.071	0.68688	1.81483	2.8894	100	5485.99406	11776.0617	25278.1224
PP3	5.10564	2.51455	0.51668	0.74853	100	8717.49798	127537.518	1.87E+06

STATISTICS	BHA	BHT	Global
Number of Points			24
Degrees of Freedom			20
Reduced Chi-Sqr			61.24191
Residual Sum of Squares	337.46559	887.37269	1224.83828
R-Square (COD)	0.97566	0.91903	0.95089
Adj. R-Square			0.94352
Fit Status	Succeeded(100)	Succeeded(100)	Succeeded(100)

SUMMARY	LOGx0	LOGx0	p	p	span	EC20	EC50	EC80
	Value	Standard Error	Value	Standard Error	Value	Value	Value	Value
BHA	0.84819	0.03664	-1.84925	0.27441	100	14.91964	7.04998	3.33133
BHT	0.7266	0.04844	-1.31583	0.18087	100	15.2809	5.32841	1.858

Appendix 17.8. Antiradical scavenging activity (IC₅₀) for *S. polycystum* polysaccharide fractions (CaCl₂, HCl, Na₂CO₃, and KOH) with its BHA and BHT standards.

STATISTICS	Spol CaCL2	Spol HCl	Spol Na2CO3	Spo KOH
Number of Points				
Degrees of Freedom				
Reduced Chi-Sqr				
Residual Sum of Squares	455.92148	260.03328	248.58834	54.01744
R-Square (COD)	0.91558	0.95549	0.95734	0.99266
Adj. R-Square				
Fit Status	Succeeded(100)	Succeeded(100)	Succeeded(100)	Succeeded(100)

SUMMARY	LOGx0	LOGx0	p	p	span	EC20	EC50	EC80
	Value	Standard Error	Value	Standard Error	Value	Value	Value	Value
Spol CaCL2	2.58464	0.13706	-0.67559	0.17972	100	2990.8297	384.26968	49.37198
Spol HCl	2.02325	0.25152	-0.6108	0.1778	100	1020.80162	105.49852	10.90313
Spol Na2CO3	2.44364	0.14662	-0.75611	0.2125	100	1737.40909	277.74172	44.39971
Spol KOH	2.38446	0.08396	-1.70103	0.63069	100	547.51389	242.35755	107.2798

STATISTICS	BHA	BHT	Global
Number of Points			24
Degrees of Freedom			20
Reduced Chi-Sqr			61.24191
Residual Sum of Squares	337.46559	887.37269	1224.83828
R-Square (COD)	0.97566	0.91903	0.95089
Adj. R-Square			0.94352
Fit Status	Succeeded(100)	Succeeded(100)	Succeeded(100)

SUMMARY	LOGx0	LOGx0	p	p	span	EC20	EC50	EC80
	Value	Standard Error	Value	Standard Error	Value	Value	Value	Value
BHA	0.84819	0.03664	-1.84925	0.27441	100	14.91964	7.04998	3.33133
BHT	0.7266	0.04844	-1.31583	0.18087	100	15.2809	5.32841	1.858

Appendix 17.9. Antiradical scavenging activity (IC₅₀) for *S. ilicifolium* polysaccharide fractions (CaCl₂, HCl, Na₂CO₃, and KOH) with its BHA and BHT standards.

STATISTICS	Sili CaCl₂	Sili HCl	Sili Na₂CO₃	Sili KOH
Number of Points				
Degrees of Freedom				
Reduced Chi-Sqr				
Residual Sum of Squares	655.09196	109.91598	208.36247	166.31843
R-Square (COD)	0.91571	0.98764	0.97399	0.97601
Adj. R-Square				
Fit Status	Succeeded(100)	Succeeded(100)	Succeeded(100)	Succeeded(100)

SUMMARY	LOGx0	LOGx0	p	p	span	EC20	EC50	EC80
	Value	Standard Error	Value	Standard Error	Value	Value	Value	Value
Sili CaCl ₂	2.77471	0.07114	-1.29825	0.27376	100	1731.65162	595.26996	204.6291
Sili HCl	3.05493	0.05431	-2.10851	0.48106	100	2190.13315	1134.83543	588.02427
Sili Na ₂ CO ₃	2.8506	0.06591	-1.45905	0.29637	100	1833.3261	708.92825	274.13523
Sili KOH	2.56199	0.08882	-1.12986	0.28638	100	1244.09156	364.74389	106.93594

STATISTICS	BHA	BHT	Global
Number of Points			24
Degrees of Freedom			20
Reduced Chi-Sqr			61.24191
Residual Sum of Squares	337.46559	887.37269	1224.83828
R-Square (COD)	0.97566	0.91903	0.95089
Adj. R-Square			0.94352
Fit Status	Succeeded(100)	Succeeded(100)	Succeeded(100)

SUMMARY	LOGx0	LOGx0	p	p	span	EC20	EC50	EC80
	Value	Standard Error	Value	Standard Error	Value	Value	Value	Value
BHA	0.84819	0.03664	-1.84925	0.27441	100	14.91964	7.04998	3.33133
BHT	0.7266	0.04844	-1.31583	0.18087	100	15.2809	5.32841	1.858

Appendix 17.10. Antiradical scavenging activity (IC₅₀) for *H. durvillei* (Hdur), and *H. dilatata* (Hdil) polysaccharide fractions with its BHA and BHT standards.

STATISTICS	Hdur	Hdil	Global
Number of Points			12
Degrees of Freedom			8
Reduced Chi-Sqr			34.60327
Residual Sum of Squares	177.10521	99.72098	276.82619
R-Square (COD)	0.96911	0.98535	0.97833
Adj. R-Square			0.97021
Fit Status	Succeeded(100)	Succeeded(100)	Succeeded(100)

SUMMARY	LOGx0 Value	LOGx0 Standard Error	p Value	p Standard Error	span Value	EC20 Value	EC50 Value	EC80 Value
Hdur	2.65307	0.1278	0.69908	0.1521	100	3268.02914	449.85092	61.9229
Hdil	2.48431	0.14187	1.06993	0.32244	100	1114.34685	305.00775	83.48363

STATISTICS	BHA	BHT	Global
Number of Points			24
Degrees of Freedom			20
Reduced Chi-Sqr			61.24191
Residual Sum of Squares	337.46559	887.37269	1224.83828
R-Square (COD)	0.97566	0.91903	0.95089
Adj. R-Square			0.94352
Fit Status	Succeeded(100)	Succeeded(100)	Succeeded(100)

SUMMARY	LOGx0 Value	LOGx0 Standard Error	p Value	p Standard Error	span Value	EC20 Value	EC50 Value	EC80 Value
BHA	0.84819	0.03664	-1.84925	0.27441	100	14.91964	7.04998	3.33133
BHT	0.7266	0.04844	-1.31583	0.18087	100	15.2809	5.32841	1.858