ANALYSIS OF BIOACTIVE COMPOUNDS IN AVAILABLE SEAWEEDS IN BANGLADESH AND DEVELOPMENT OF CRACKERS BY USING THESE SEAWEEDS

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Roll No.: 0119/03
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A thesis submitted in the partial fulfilment of the requirements for the degree of

Master of Science in Food Chemistry and Quality Assurance

Department of Applied Chemistry and Chemical Technology
Chattogram Veterinary and Animal Sciences University
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June 2022

DEDICATED TO MY RESPECTED AND BELOVED FAMILY AND TEACHERS

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The author

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List of abbreviations

AOA	Antioxidantactivity
BHT	ButylatedHydroxyQuinone
BHA	Butylated hydroxyanisole
DPPH	2,2-Diphenyl-hydrazyl-hydrate
FC	FolinCiocates
GAE	GallicAcidEquivalent
LDL	LowDensityLipoprotein
QE	Quercetin
ROS	ReactiveOxygenSpecies
TA	TotalAnthocyanin
TAC	TotalAnthocyaninContent
TE	TroloxEquivalent
TEAC	TroloxEquivalentAntioxidantCapacity
TFC	TotalFlavonoidContent
TPC	TotalPhenolicContent

Abstract

Seaweed is an unrevealed treasure of the ocean. Due to its high nutrition value such as its bioactive compounds it can be called as a treasure. But most of the people are not concern about it. So the study was conducted to disclose the nutritional value of seaweeds and to incorporate seaweed in a food as we chose crackers. Proximate analysis was done to determine protein, fiber, fat and ash percentage.*Enteromorpha*, *Gracilaria*, *Hypnea*was chosen to conduct the study.Total polyphenol content (TPC), total flavonoid content (TFC), total anthocyanin content (TAC), and antioxidant activity (AOA) of seaweed were all determined as part of the bioactive analysis. Using the DPPH test technique, antioxidant activity was measured. The antioxidant activity was7 mg/100g on average.*Enteromorpha*had the highest antioxidant activityand *Hypnea* was the lowest.Total polyphenol content ranged from 236.667±5.773 to 603.333±5.773 mg GAE/100g.Greater total polyphenol content was in *Gracilaria*(603.333±5.773 mg GAE/100g), whereas*Hypnea* (236.667±5.773 mg GAE/100g) had lowest TPC. TFC content varied from *Hypnea* (18.169±.016 mg QE/100g) to *Gracilaria*(429.506±.079 mg QE/100g). The highest anthocyanin was found in *Enteromorpha*(152.830±.209 mg TA/100 g), while the lowest was found in *Hypnea* (41.137±.180 mg TA/100 g).*Hypnea* had the maximum moister content (16.00 %). Highest protein percentage was found in *Gracilaria*(27.13%), where *Enteromorpha*had (8.93%). *Enteromorpha*had fiber content of (16.45%), which was the highest. *Enteromorpha*had greater ash content (21.39%) and *Gracilaria*had lowest ash content (7.71%). According to the study we can say that seaweed is a great resource of bioactive compounds with high nutritious value and functional food such as seaweed crackers will be acceptable to the consumer.

Keywords: *Antioxidant activity, Total polyphenol content, Total Flavonoid contents, Total Anthocyanin Contents and Functional food.*

CHAPTER I: INTRODUCTION

Seaweeds known as ocean algal biomass, are a type of renewable living element that is used as foodstuff and nourishment in many regions of the world. Seaweeds are well-known for their nutritional value, which includes vitamins, minerals, proteins, polysaccharides, and dietary fibers in addition to being a low-calorie meal (Silva et al., 2013).They are a primitive species of plant that can be extremely little or rather huge, reaching a length of up to 30 meters.The three categories of seaweeds are called Chlorophyta (Green seaweeds), Rhodophyta (Red seaweeds), and Phaeophyceae (Brown seaweeds), based on pigment content and metabolite composition, respectively (Li et al.,2011).Red algae (33%), brown algae (66.5%) and green algae (5%)are consumed more often by humans in Asia, particularly in China, Korea, and Japan. As marine veggies, seaweeds are widely consumed in Asian nations (Marinho-Soriano et al., 2006).

Seaweeds are regarded marine veggies as well as foundation of aquatic life ecosystems.They have been too used as compost, food for humans, and fodder for animals since a long time ago (Gutierrez et al., 2016;Pacheco et al.,2020). Bioactive compounds abound in seaweeds. Polysaccharides, proteins, peptides, amino acids, and secondary metabolites are only a few examples. Polyphenolic chemicals and natural colours are among them. These bioactive materials have been demonstrated to have a variety in terms of genetic action as well as medical and health benefits (Shannon and Abu-Ghannam, 2019).Bioactive substances include molecules developed from synthetic either natural resources for have been physiologically evaluated for action in several important therapeutic zones (Kris-Etherton et al., 2002).

Anti-cancer, anti-inflammatory, anti-microbial, anti-viral, anti-allergic, anti-oxidant, anti-diabetic, hepatoprotective,hypotensive,anti-pruritic, anti-photoaging and antiviral effects are all found inphenolic chemicals that can be found in seaweed (GeneralićMekinić et al., 2019; Gómez-Guzmán et al., 2018; Chen et al., 2015; Murray et al., 2018; Pangestutiet al., 2011; Nwosu et al., 2011; Pereira, 2018).Seaweeds contain phenolic substances. Seaweeds, contain both organic and inorganic substances, similar to other plants compounds that might be beneficial health of people (Kuda et al., 2007).

Antioxidants are chemical substances that can eliminate extra free radicals produced by the body and prevent biological components being harmed by oxidation including lipids, DNA, and protein (Vertuani et al., 2004).To avoid cell damage, our systems should constantly be able to maintain a balance between free radical generation and antioxidant availability.Free radicals have an important function in the human body, boosting oxidation and causing illness. However, the way oxygen is used in the human body varies from person to person; this can lead to a physical imbalance and the reactive oxygen species generation (ROS), which can cause cancer (Gunathilake and Ranaweera, 2016).The virgin antioxidant that entered our bodies by the consumption of various fruits, seaweeds, green vegetables, and other foods might naturalize naturally formed ROS (Bhattacharjee and Islam, 2014).Natural antioxidants include vitamin C, derivatives of chlorophyll, flavonoids, amines, amino acids, and polyphenols may be more effective more than artificial antioxidants.Artificial antioxidants are being used, including butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA)banned because of their carcinogenicity and negative health impacts (Liu et al., 2020).Natural antioxidants generated from seaweed are in great demand due to their health advantages and lack of adverse effects (Gupta &AbuGhannam, 2011). Seaweed has lately been revealed being a plentiful resource of bioactive natural substances with antioxidant potential (Kim et al., 2012; Rafiquzzaman et al., 2013;Rafiquzzaman et al., 2016; Rafiquzzaman et al., 2016).Due to its vast a variety of chemical and biological processes, including as free radical scavenging and antioxidants characteristics, flavonoids are the most important natural phenolic (Kahkonen et al., 1999).Seaweeds are high in secondary metabolites such phenolic compounds and flavonoids (Tanna& Mishra, 2018).The effects of flavonoids have been studied in a number of ways. They discovered incredible advantages, particularly decreases inflammatory reactions regards heart valves, lowering the chances of rheumatic fever and infectious endocarditis, which are two of the most serious cardiovascular hazards in contemporary culture (Rees et al., 2018). These polyphenols, which mostly in the form of flavonoids and ingested, have been shown to reduce prostate cancer danger, which is most likely, the second most common neoplasm in the population of older males (El-Din and El-Ahwany, 2016).Seaweed has significant levels of proteins and bioactive peptides, which are considered primary metabolites, even more than other "protein-rich" foods (Pangestuti& Kim, 2015).

The seaweeds accessible whole of Bangladesh's coastline, with the greatest concentrations on Cox's Bazar, St. Martin Island, and the Sundarbans Mangrove Forest. This region is densely forested with seaweeds. There are a total of 193 seaweed species detected, consisting of 19 commercial significant species from 94 genera. A total of biomass of seaweed is 5,000 metric tons.Seaweeds are normally accessible from October to April owing to the season variations quality of the water characteristics, although from January through March, there is a peak in abundance. The Mog people have been eating seaweed salad and sauce for a long time. At St. Martin's, there are 400 or so people harvest seaweed. In St. Martin Island harvests six to nine tons of moist seaweed (Hypnea Sp.) per year for illegal export to Myanmar (Sarkar et al., 2016).In Bangladesh, seaweed has not been adequately utilized.In Bangladesh, local consumption of seaweeds is negligible and most of them do not know that seaweed can take as a food.The seaweed resource and its use could be a potential source of income for the Bangladeshi economy.

Seaweeds' nutrient-dense composition makes them ideal options for use in functional foods. For better utilization of these huge amount of harvested seaweeds and to make them acceptable to all it is a demand of time to focus on seaweed based food products. Very few researchers focused on it. Moreover the people ofBangladesh are not accustomed to take seaweeds as a food. So we selected a bakery product which is crackers to introduce seaweeds as a functional food to all kinds of peoples.Because dried seaweed is abundant in dietary fiber and a variety of other potentially bioactive components, it has the potential to improve a product's nutritional quality (Chauhan et al., 2016).Crackers, sometimes known as biscuits, are a type of baked confectionary that has been dried to a low moisture level and the name crackers comes from Latin. It's frequently produced with wheat flour that's rich in energy (Chauhan et al., 2016).Bakery items are extensively consumed throughout the world and are the ideal way to incorporate marine beneficial ingredients while also reaching the peoples (Kadam and Prabhasankar, 2010).Mold development is inhibited when seaweeds are used to substitute salt (as sodium chloride) in bakery items (Brownlee et al., 2012).Thus, the aim of the research is to analyze the bioactive substances, antioxidant activity, nutritive values, and development of crackers type biscuits using seaweeds.

1.1**Aims & Objectives**

The aims of this experiment were –

- To analysis bioactive compounds from different types of seaweeds available in Bangladesh.
- To determine nutritional value of different seaweeds and their comparison by proximate analysis.
- Development of functional products which is crackerstypes biscuits by using seaweeds.
- Cost effect analysis between local market products and seaweed products.

1.2Anticipated outcome

The anticipated outcome of this study was

- Identification of morphology of seaweeds
- Estimation of bioactive compounds of three different seaweeds available in coastal area of Bangladesh.
- Formulation of seaweeds functional products as crackers.
- Supply highly nutritious product in a reasonable price.

CHAPTER II: REVIEW OF

LITERATURE

Guiry(2013) stated that seaweeds are photosynthetic marine algae that may be found in every ocean. Seaweed is divided into three phyla: Phaeophyceae (brown algae), Rhodophyta (red algae), and Chlorophyta (green algae) (green algae). Each phylum has thousands of species.

Princely and Dhanaraju(2017) said that seaweeds, like other plants contain a variety of organic and inorganic components that are beneficial to health of people and are secondary metabolite source with a wide range biological nature functions.

Stirk et al.(2003) asserted that seaweeds, also known as marine macroalgae, are a type of renewable living resource that is used widely as food and fertilizer regions of the planet. The seaweeds are well-known for their nutritional value, which includes nutrients, dietary fibers, proteins, polysaccharides, vitamins, and minerals in addition to being a low-calorie meal.

Kim(2011)indicated that seaweeds provide A, D, E, K, C, B1, B2, B9, and B12 are water-soluble vitamins, along with vital elements including phosphorus, potassium, zinc, copper, selenium, fluoride, calcium, iron, iodine, and magnesium..

Chojnacka et al. (2012) found that seaweed and hence their extracts, can be a gold mine of physiologically active chemicals. Their beneficial benefits for people, animals, and plants have long been known, and they continue to be valued in the creation of novel biotechnological products today.

Li and kim(2011)found that polysaccharides are abundance in seaweeds. Many of these are soluble dietary fibers, which have a beneficial effect on animals' digestive systems (i.e. alginic acid). Polysaccharides obtained from seaweed are also powerful and non-toxic antioxidants. They also state that several pigments are present in seaweeds, most of them are strong antioxidants. These pigments have free radicals scavenging properties.

Souza et al.(2012) reported that the DPPH freeradical scavenging effect was used to assess the antioxidant capabilities of polysaccharides present in seaweed, revealing that this polysaccharide has a modest impact in reducing the generation of free radicals.

Keyrouz et al. (2011) showed that seaweeds generate polyphenols. Phenolic acids, flavonoids, isoflavones, cinnamic acid, benzoic acid, quercetin, and lignans are examples of polyphenols.

Tapiero et al. (2002) stated that polyphenols are reducing agents that, when combined with other dietary reducing agents like vitamin C, E, and carotenoids, are known as antioxidants and prevent the body's tissues from oxidative stress and diseases including cancer, coronary heart disease, and inflammation.

Li et al. (2011) reported that the value of food bioactive components as functional components has increasingly been known because of its ability to improve health and reduce ailment threat. Nutraceuticals derived from seaweeds, in particular, have been promoted as a rich source of wellness ingredients.

Furthermore, Ganesan et al.(2008) found a link between antioxidant activity and total phenolic content in extracts extracted from diverse seaweeds.Because of their crude extracts and fractions display antioxidant activity, seaweeds or marine macroalgae can be used as a source of natural antioxidant agents.Bioactive chemicals discovered in seaweeds are ready for a big breakthrough in a number of culinary and medicinal applications, with the potential to be used as natural antioxidants in a variety of food and pharmaceutical products.

According to Shi et al. (2017) there is an inverse relationship between dietary consumption of antioxidant-rich foods and the prevalence of human illnesses. Seaweeds have substantial antioxidant activity due to made from algae chemicals such as sulfated polysaccharides, phenolics, terpenoids, and carotenoids (ulvan, porphyran, alginate) that have significant antioxidant activity.

Furthermore,Chakraborty (2017) found that its polysaccharides have been shown to have immunomodulatory and anticancer properties.

Cunha and Grenha(2016) stated that seaweeds bioactive compounds and secondary metabolites have been shown to have a wide spectrum of antibacterial properties. Derivatives of chlorin and halogenated molecules such as polysaccharides, sulfate polysaccharides, cyclic polysulfides, alcohols, aldehydes, hydroquinones, sterols, ketones, peptides, proteins, vitamins, haloforms, halogenated alkanes and alkenes, and aldehydes and alcohols are some examples of these agents.

Radhika et al. (2014) showed that lots of them might be turned into antiseptics and cleaning treatments, but their antibacterial action is frequently only produced in vivo at hazardous levels.

Mohan et al. (2019) described with fresh discoveries of their potentials and pharmacological actions as anticancer, antimicrobials, antivirals, anti-inflammatories, and other medicinal benefits, the value of these compounds found in seaweed has exploded.

Yuan et al. (2005) stated that due to their robust antioxidative defense systems in their cells, macroalgae are resistant to the oxidative effects of reactive oxygen species (ROS) when exposed to both light and oxygen. Protective enzymes (e.g., superoxide dismutase, peroxidase, glutathione reductase, and catalase) and antioxidative molecules (e.g., carotenoids, ascorbic acid, phlorotannins, tocopherols, phospholipids, chlorophyll-related compounds, bromophenols, catechins, mycosporine-like amino acids, and polysaccharides.

Chauhan and Chauhan (2006)demonstrated that ROS induce oxidative damage, which leads to the development of chronic disease states such coronary heart disease, cancer, diabetes, rheumatoid arthritis, chronic inflammatory illness of the gastrointestinal tract, Alzheimer's disease, and other neurological diseases associated with aging.

2.1 Bioactive Compounds

2.1.1 Fatty Acids

Gill and Valivety (1997) asserted that fatty acids having two or more double methylene bonds help cells function properly and are now employed in biomedical and nutraceutical applications. Understanding how fatty acids work biologically has led to their widespread use in Western society to prevent obesity and heart disease.

Furthermore,Funk (2001) showed that polyunsaturated fatty acids (PUFAs) have an important role in cellular and tissue metabolism, as well as the regulation of membrane fluidity, oxygen and electron transport, and heat tolerance.

Nettleton (1995) showed that the PUFA family, namely EPA, is gaining popularity (5, 8, 11, 14, and 17-eicosapentaenoic acid). EPA is a 20-carbon fatty acid with five double bonds at the carboxy terminus and the the last double bond at methyl terminus' carbon third.

Wen and Chen (2003) demonstrated that EPA is polymerized inside the cell to create an intricate lipid molecule, and it serves as a precursor to eicosanoids, hormone-like compounds including thrombboxanes, leucotrienes, and prostaglandins, which are important in governing both developmental physiological processes in higher plants and animals. Fish oil appears to be the most common source of EPA. Omnivores, carnivores, and humans all transport EPA up the food chain.EPA was discovered in a several types of marine algae groups, yet only a handful having the among them ability to produce commercially. This is due to the fact that when cultivated in an autotrophic environment, the majority of marine algae have modest cell densities and specific growth rates.

2.1.2 Phenols Found in Seaweeds

Hussain et al. (2019) stated that phenolic compounds are distinguished by the presence of an aromatic ring with one or more hydroxyl groups. They can range in structure from simple molecules as hidroxycinnamic acids or flavonoids to more complex polymers with a wide range of molecular weights (126–650 kDa).Resorcinol with cathecol (benzenediols)possess two hydroxyl groups in phenol, while phloroglucinol and Pyrogallol both contain three (benzenetriols).

2.1.3 Simple Phenolic Compounds

Machu et al. (2015) studied that with gallic acid other hydroxybenzoicderivatives were discovered in a variety of macroalgae species that are green, red, and brown. Epicatechin and epigallocatechin are examples of flavan-3-ol derivatives found red, brown, and green macroalgae.Hesperidin, myricetin, morin, kaempferol, rutin, quercitrin, and cirsimaritinare some of the other flavonoids found in species of Chlorophyta, Rhodophyta, and Phaeophyta.

Yoshie-Stark et al. (2003) observed that unique species of macroalgae-specific compounds likewise been discovered. Various isoflavones, including genistein and daidzein, have been found macroalgae that are red, as well as the brown macroalgae.

Hartmann et al. (2018) investigated that simple structures, which including hydroxybenzoic acid brominated derivatives, have been discovered macroalgae that are green, as well as classes that are more complicated, such the two chlorine auronesfound into brown microalgae. Furthermore, a number of species of the Rhodophyta, Chlorophyta, and Phaeophytahave been shown to have a substantial amount of sulfated phenolic substances.

2.1.4 Flavonoids

Mukherjee (2019) reported that flavonoids are phenolic compounds having heterocyclic oxygen connected to two aromatic rings. The structure of flavonoids varies depending on the degree of hydrogenation. In terrestrial plants, there are around 2000 flavones, flavanols, flavanones, flavonols, anthocyanins, and isoflavones that have been classified into important categories like flavones, flavanols, flavanones, flavonols, anthocyanins, and isoflavones.

Yoshie-Stark et al. (2003) overserved that catechins and other flavonoids are abundant in seaweed. Flavonoids like hesperidin, quercetin, and rutinhave been found in various species of Rhodophyta, Chlorophyta, and Phaeophyceae, while chemicals unique to macroalgae including hesperidin, kaempferol, catechin, and quercetin have been discovered.

Santos et al. (2019) also reported that red macroalgae contains isoflavones such as daidzein and genistein. The brown macroalgae has a large quantity of flavonoid glycosides. Green seaweed contains flavonoids called C-glycosides.

According to Yonekura-Sakakibara et al. (2019), there is no flavonoid content in algae (micro and macroalgae)because they nor two major flavonoid biosynthesis enzymes; nonetheless, algae have Enzymes for such shikimate pathway are encoded by genes..The chalcone synthase converts malonyl-CoA from the acetate-malonate (polyketide) pathway, which is comparable to the phlorotannins pathway, and p-coumaroyl-CoA from of the terpenoids into naringeninchalcone. Tyrosine is biosynthesized through the shikimate pathway, which is then catalyzednaringeninwhich is the first step in the synthesis of all flavonoids.. The flavanone 3-hydroxylase then converts naringenin to dihydrokaempferol, and the enzymatic process then converts this molecule into a variety of flavonoid compounds, including anthocyanin, flavones, flavonols, catechins, and others.

Osuna-Ruíz et al. (2019) stated that quantitative assessment flavonoid content is a frequent assay that is typically used in conjunction with phenolic content analysis as component of the biochemical characterisation of seaweed extracts in the bibliography reviewed. However, there are very few articles on flavonoid characterisation, isolation, and specific bioactivity analysis in the examined bibliography. The high quantity flavonoids present in the material under investigation, as well as their bioactivity evaluated are simply hypotheses.

Dixit et al. (2018) showed with different extraction procedures, the findings obtained support the existence of flavonoid components, also demonstrating antioxidant and radical scavenging activities in these extracts that is favorably linked with flavonoid content. Location, date, season, and laboratory information for harvesting and collectionmethods all contribute to the detection of flavonoids and their variations.

2.1.5 Polysaccharide

According to Kumar et al. (2008),polysaccharides, primarily cell wall structure, as well as marine algae have large amounts of storage polysaccharides and mycopolysaccharides.The bulk of the polysaccharide is composed of cellulose, hemicelluloses, and neutral polysaccharides cell wall. The cellulose and hemicellulose content of seaweed species of relevance ranges from 2% to 10% by dry weight. Sulphatedgalactans and sulphuric acid polysaccharide are found in the Chlorophyceae, whereas alginic acid, fucoidan or sulphatedfucose, laminarian or 1–3 glucan are found in the Pheophyceae. Carrageenans, a sugar that resembles amylopectin and is often referred to as floridean starch, and highly soluble in water sulphatedgalactan, and porhyran as a mucopolysaccharide found in intracellular spaces, are all found in Sargassan and red algae, or Rhodophyceae.

Tseng (2001) stated that polysaccharides are polymers composed of simple chain monosaccharides or simple sugars joined together by a glyosidic bond. They are used in a wide range of applications, including food, beverages, stabilizers, emulsifiers, thickeners, feed, and so on.

Holdt and Kraan (2011) found out that green seaweed species with high polysaccharide content, such as Enteromorpha, comprise 65 percent of dry weight. Other seaweeds with a high polysaccharide content include Ascophyllum, Porphyra, and Palmaria.

Mouritsen (2009) described that the majority of seaweeds have polysaccharide concentrations ranging from 4% to 76%. Seaweeds have a low cholesterol and high carbohydrate percentage, the majority of which is dietary fiber, despite the fact that the human body does not absorb them. Dietary fibers, however are beneficial to the human health.

2.1.6 Phenolic Acids

Liwa et al. (2017) asserted that PAs are bioactive molecules that perform a variety of tasks, including nutrition protein production, enzymatic activity, photosynthesis, and allelopathy are all examples of absorption. These are typically joined to other molecules, such as organic acids, simple and/or complex carbohydrates, and other bioactive substances, such as flavonoids or terpenoids. These PAs are frequently categorized according to how many carbons in the chain are connected to the phenolic ring. They are composed of a single phenol ring and at least one functional carboxylic acid group. These phenolic acids are thus divided into three groups: C6-C1 for hydroxybenzoic acid (HBA; with one carbon chain linked to the phenolic ring), C6-C2 for acetophenones and phenylacetic acids (with two carbon chains attached to the phenolic ring), and C6-C3 for hydroxycinnamic acids (with three carbon chains linked to the phenolic ring).

Pietta et al. (2003) stated that gallic acid, p-hydroxybenzoic acid, vanillic acid, syringic acid, and protocathecins are examples of HBAs with modifications in the basic structure, comprising the aromatic ring's hydroxylation and methoxylation. Although they can be discovered as unbound acids, they are most commonly found as conjugates. Gallic acid and ellagic acid, its dimer, for example, may produce hydrolysable tannins when esterified with a sugar (usually glucose). Trans-phenyl-3-propenoic acids (HCA) are hydroxycinnamic acids with different ring structures. Sinapic (3,5-dimethoxy-4-hydroxy), caffeic (3,4-dihydroxycinnamic), ferulic (3-methoxy-4-hydroxy), and p-coumaric (4-hydroxy) acids are among the HCA derivatives, with a broad dispersion of these compounds as esters, principally of quinic acid, conjugates (chlorogenic acids).

Luna-Guevara et al. (2018) reported that these acids can be different depending on the kind, quantity, and location of the acyl residue classified into four groups(1) Caffeic, ferulic, and p-coumaric acid monoesters; (2) Caffeic acid di-, tri-, and tetra-esters; (3) Caffeic acid mixed di-esters of ferulic acid or sinapic acid; and (4) Caffeic acid mixed esters with dibasic acids. Furthermore, cinnamic acids can condense with molecules other than quinic acid, such as rosmaric and malic acid, as well as aromatic amino acids and choline.

Mancini-Filho et al. (2009) studied that show the existence of PAs in seaweed. However, these investigations are few and mostly focus on phenolic characterization, with no bioactivities investigated. Green macroalgae like Cladophorasocialis and green seaweeds like Dasycladusvermicularis have both been reported to contain coumarins and certain vanilic acid derivatives.

Xu et al. (2015) found out that brown seaweeds have been identified as having HBAs, rosmarinic acid, and quinic acid derivatives. As well as benzoic acid, p-hydroxybenzoic acid, salicylic acid, gentisic acid, protocatechuic acid, vanillic acid, gallic acid, and syringic acid, have been identified in the genus Gracilaria.

2.2 Properties of Seaweeds

2.2.1 Antiviral Activity

Damonte et al. (1994) reported that several sulphatedpolysaccharides from red algae have been shown to have antiviral effects against viruses that cause human illness.

De Clercq(2000) showed that seaweeds have antiviral activity against the most infectious viruses, including as the respiratory syncytial virus, genital herpes types 1 and 2, and hiv infection (HIV).When it attaches to the cell surface during the first step of RNA replication, the polysaccharide present in these seaweeds becomes active. The most significant condition for an antiviral polysaccharide is that it has a very low cytotoxic impact on mammalian cells, which most seaweeds meet. Carrageenan has potential antiviral action in vitro. Carrageenan comes in a variety of forms, including -carrageenan, -carrageenan, -carrageenan, and -carrageenan.

Wu et al. (2012) found out that some carrageenans have antiviral action against distinct strains of HSV 1 and 2. Carragaurd is a carrageenan-based microbicide that is currently in phase 3 studies for the prevention of HIV and other sexually transmitted diseases. A sulphated polysaccharide from Schizymeniapacifica inhibits HIV reverse transcriptase in vitro, which is a later step in HIV replication, but has little or little influence on DNA and RNA polymerase activity. Some high molecular weight galactansulphates, commonly known as agaroids from Gracilariacorticata, have antiviral properties against HSV types 1 and 2. This is due to the suppression of early pathogen binding to the host cell.

2.2.2 Antibiotic Activity

Lincoln et al. (1991) demonstrated that many antibiotic-active chemicals are found in macroalgae. Some of the notable components found in macro algae includealcohol, aldehyde, hydroquinone, ketone, halogenated alkanes, haloforms, and alkenes. Antibiotic properties are demonstrated by substances sterols, heterocyclic, and phenolic compounds, for example. Most of the chemicals have antiseptic and cleaning properties, but their antibacterial action in vivo is frequently only at hazardous concentrations.

Høiby (2002) stated that a heterocyclic furanone, commonly possible antibacterial agent is fimbrolide from the Deliseapulchara lactone class. It has been studied for its efficacy in bacterial anti-fouling and as a therapy for Pseudomonas aeruginosa infections that are ongoing. In the lungs of people with cystic fibrosis, the production of mucoid alginate and the development of biofilm are the main causes of Pseudomonas aeruginosa infection..

According to Rasmussen et al. (2000) bacterial prevention is primarily accomplished by blocking the as an intracellular signal antagonist, furanone has an effect on the quorum sensing system, resulting in interruption of intracellular and intercellular communication between cells. The impact is most common in gram-negative bacteria. Chemicals such as phenolic, heterocyclic, and sterol substances have antibacterial properties. These qualities able can be transformed into antiseptics and cleaning agents, therefore the antibiotic quality can only be produced in vivo at hazardous concentrations.

2.2.3Anti-Inflammatory Activity

Imbs et al. (2001) reported that macroalgae, particularly red seaweeds, are high in polyunsaturated fatty acids with 20 carbon atoms, primarily eicosapentaenoic and docosahexanoic, commonly known as PUFAS.Gracilariales and prostaglandin are the two primary metabolites of C20 PUFAS oxidative metabolization in seaweeds. The first technique involves fatty acid cyclooxygenase working the second method includes lipooxygenase reacting on archidonic acid, as in mammalian cells as well. PUFAS metabolized metabolites are known as oxylipins in many red algae and are similar to higher plants produce eicosanpoid hormone and mammals, both of which perform physiological functions.

Lee et al. (1998) observed that the abnormal synthesis of these molecules causes a variety of diseases associated with inflammation, and eicosanoid and its derivatives have attracted considerably greater interest in study due to their anti-inflammatory properties. Eicasanoids, such as leukotriens and hydroxyleicotetraenoic acid, have active physiology qualities such as chemical stimulation of netrophillssuch as smooth muscle cells, and muscular contraction, and are linked to a variety of illnesses in animals. In obstetrics and gynecology, where it is utilized as a cervical dilator, the combined impact of prostaglandins and Laminaria stipe development is healthy documented.

2.2.4 Anti-Thrombic and Anti-Coagulant Activity

Matou et al. (2002) described that fucoidan has in vivo and in vitro antithrombic and anticoagulant activity, which is mediated via anti-thrombin III and heparin cofactor II are examples of blood coagulation inhibitors. Direct interaction between fucan and thrombin results in anti-coagulant activity, which normally increases with the quantity of sulphation.

Trento et al. (2001) asserted that anticoagulant compounds derived from Fucusvesiculosus and Ascophyllumnodosum have been recognized. The necessity to identify a substitute for heparin manufactured from cattle, but also the concern over the spread of BSE through the use of products obtained from cattle drove the research. Sulphatedfucan has many favorable circumstances over heparin, including concentration dependent inhibition of thrombin generated from platelets, Decreased adhering of polymorph nucleated leucocytes of rabbit aorta, concentration dependent suppression of thrombin dependent manner aggregation, absence of the hypotensive effect seen in thrombin, and dosage dependent inhibition of thrombin generated thrombosis.

2.2.5 Hypocholesterolaemic,Antilipemic Activity

Nishide et al. (2003) stated that there are many severe diseases that are detrimental to our community, such as cardiovascular disease, which is mostly caused by excessive plasma cholesterol levels and high blood pressure. Some macroalgal compounds, such as it has been demonstrated that alginate, funoran, fucoidan, laminaran, porphyran, and ulvanto cause hypocholesterolemic and hypolidemic reactions brought on by a reduction in intestinal cholesterol absorption. Both a hypoglycemic response and a rise in fecal lipid profile are to blame for this. Lower levels of total free cholesterol, cholesterol, phospholipids and triglycerides in the hepatic have been seen as well as a drop in systolic blood pressure, commonly known as the hypertensive response.

2.2.6 Enzyme Inhibitors and Stimulants

Angulo and Lomonte (2003) reported that fucoidan is also recognized for numerous actions, it inhibits the myotoxic and cytotoxic and effects of PLA2 myotoxins seen in crotaline snake venom, for example, which cause cellular destruction after a snake bite. In humans, phospholipase A2 (PLA2) is released and is involved in a number of via the production of archidonic acid, a source of prostaglandins and leukotrienes, inflammatory disorders. Phospholipase A2 was therefore employed as a target for the class of anti-inflammatory medications, which is why researchers concentrated on this group of enzymes.

CHAPTER-III: MATERIALS AND METHODS

3.1 Study area

Cox's Bazar, on Bangladesh's eastern coast, was chosen as the sample location. Furthermore, Cox's Bazar has a southern connection to the Bay of Bengal. There are around 193 seaweed species in the region discovered in Bangladesh. However, due to a shortage of funds, this resource cannot be fully used of appropriate initiation. Furthermore, a lack of sufficient knowledge about the nutritional and antioxidant qualities of seaweed must be a reason. The research location has a seaweed farm with two species extracted from it (owner: Falcon International Limited). Another species of seaweed was collected from Zahanara Green Agro. Proximate analysis of the samples was done in PRTC lab at Chattogram veterinary and animal Sciences University. Bioactive compound analysis was done in the Faculty of Food Science and Technology, CVASU.

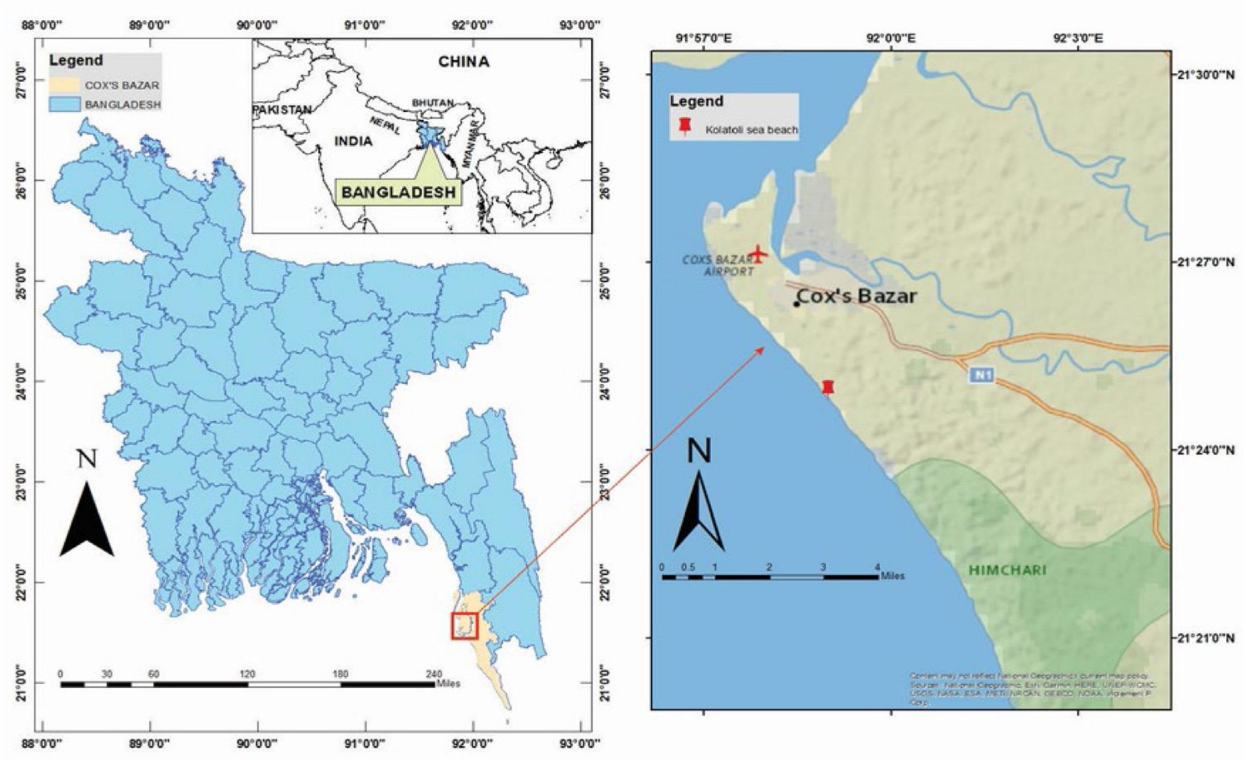


Figure 3.1. The sampling area of Cox's Bazar, Bangladesh

3.2 Sample collection

The seaweed was harvested by hand. Seaweeds were carefully dried for 3-5 days, until the utmost moisture had disappeared. They were subsequently delivered to the Faculty of Food Science and Technology (FFST) at Chattogram Veterinary and Animal

Sciences University (CVASU), in Bangladesh. The samples were washed multiple times with tap water to remove contaminants and sand particles before being gently cleaned and rinsed with de-ionized distilled water. The seaweed was then dried again using a cabin dryer until the moisture was eliminated. The samples were then immediately put in airtight zip-bags and stored for later examination.

3.3 Sample preparation

A blender was used to grind the dried samples. The powdered samples were immediately put in sealed zip-bags. A sample:solvent ratio of 1:10 was employed to prepare the stock solution.The sample was prepared for methanolic extract by combining 10 mL of methanol with 1 g of each powdered component. The sample mixture was then thoroughly shaken. The extract was then kept and stirred for 72 hours. Following that, the crude extracts were frozen at 4°C for future investigation.

3.4 Chemicals and reagents

- 2, 2-Diphenyl -1-picryl hydrazyl (DPPH),
- Gallic acid
- Quercetin,
- The FC Reagent (FolinCiocatea),
- Aluminum chloride,
- distilled water,
- absolute methanol,
- absolute ethanol,
- Potassium acetate,
- sodium carbonate,
- Potassium acetate.

3.5 Equipment

- Conical flasks
- Electric blender.
- Digital analytical balance

- Beakers
- Falcon tubes
- Volumetric flask
- Test tubes
- UV visible spectrophotometer

3.6 Antioxidant activity measurement using the DPPH scavenging technique

The extracts' potential as antioxidants was measured by use of the DPPH test, which was slightly modified from the technique reported by AzlimAlmey et al. (2010). In 100 mL of 100% methanol, 6 mg of DPPH was dissolved to make a DPPH solution with methane. After that, Methanoic extract (1 mL) was combined with DPPH solution (2 ml). Then carefully stirred the mixture mixed and kept in the dark for 30 minutes at room temperature. The absorbance was measured using a UV-VIS spectrophotometer at 517 nm Shimadzu Corporation, UV-2600, USA. Methanol was used as a control, and 1 mL was added to 2 mL of the DPPH solution. By contrasting the absorbance of the samples with that of the DPPH reference solution, the scavenging activity was determined.The antioxidant ability of extracts is determined using the following equation based on their DPPH free radical scavenging activity.

$$\text{Scavengingability}(\%)=\frac{(\text{Absorbance of Sample})\%100}{(\text{Absorbance of the Control})}$$

As the standard Trolox was utilized, and the calibration standard curve was constructed using TEAC compound (Trolox equivalent antioxidant capacity). On a dry weight (DW) basis, the outcome were represented corresponds to Trolox in mg/100 g for each gram of powder.

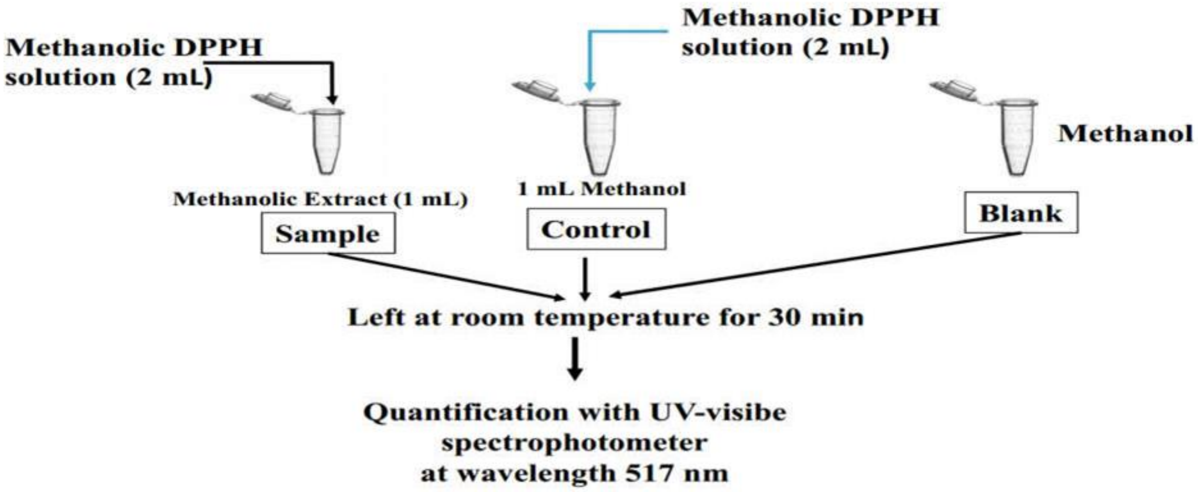


Figure 3.2. An illustration of the process for determining antioxidant activity (AOA)

3.7 Counting the overall amount of phenolics (TPC)

The seaweed's total polyphenol content (TPC) was assessed using a slightly modified Folin-Ciocalteu technique reported by Parthasarathy et al. (2009). In a falcon tube, 1 ml of ethanoic extract was mixed with 1.5 ml of FC reagent was added and kept at room temperature for three minutes.. The mixture was then treated with 1.5 ml Na2CO3 (7.5 percent) for 60 minutes. It was carried out using a UV-VIS Spectrophotometer (UV-2600, Shimadzu Corporation,USA) to measure absorbance at 765 nm, with ethanol serving as the sample of blank. The amount of TPC was calculated and expressed as milligrams of extract equivalents to gallic acid (mg GAE/g).

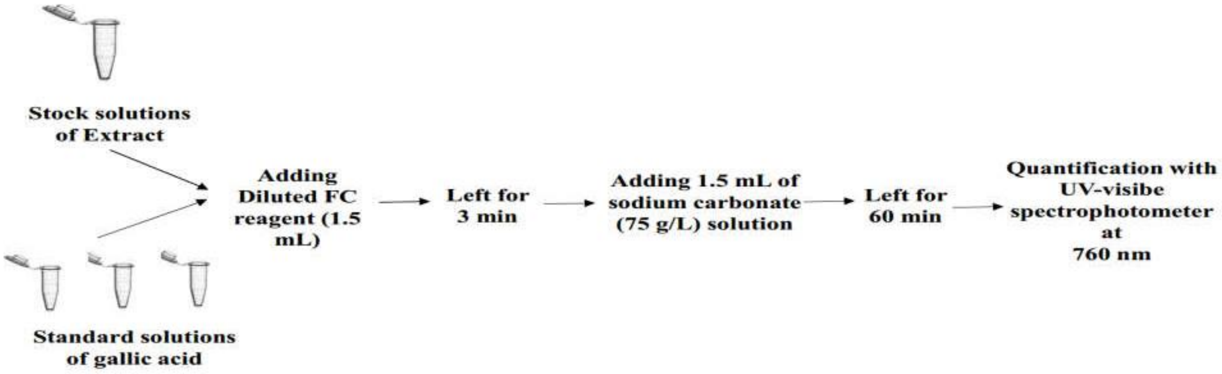


Figure 3.3. Flowchart for the process used to determine the total polyphenol content (TPC)

3.8 Estimating the Total Flavonoid Contents (TFC)

The aluminum chloride colorimetric method described by Chang et al. was slightly adjusted in order to determine the total flavonoid content (TFC) of the seaweed samples (2002). Prepared extract stock solutions (1 mg/mL), aliquots of 0.5 mL diluted extract, and 1.5 mL of 95 percent ethanol were mixed in a cuvette. The liquid in the cuvette was then treated with 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 mol/L potassium acetate, and 2.8 mL of distilled water. For 30 minutes, the mixture was kept at room temperature. The absorbance was measured at 415 nm using aultraviolet-visible spectrophotometer (UV-2600, Shimadzu Corporation, USA), and the blank was 10% aluminum chloride substituted with the same quantity of distilled water.Total flavonoid concentration was calculated by comparing sample extract absorbance to a typical curve for quercetin. TFC is evaluated and measured in milligrams of extract per gram of quercetin equivalents (mg QE/g).

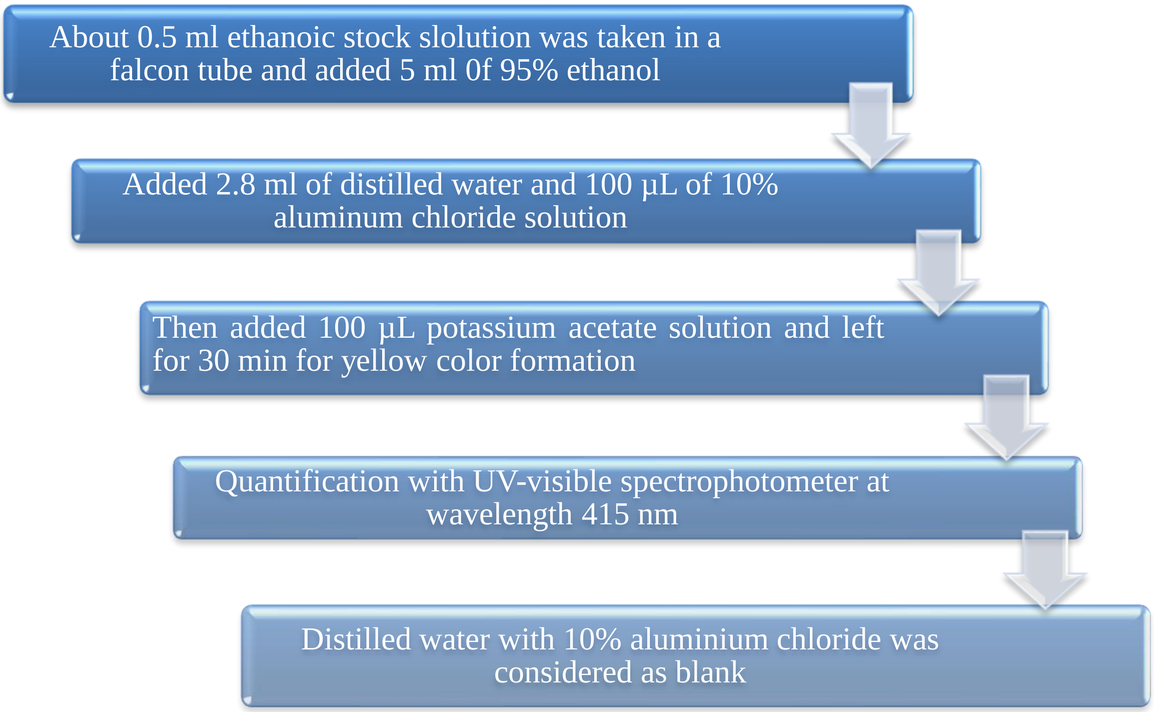


Figure 3.4. Flowchart of the process for determining total flavonoid contents (TFC)

3.9 Estimating of Total Anthocyanin Contents (TAC)

The above-mentioned technique will be used to colorimetrically measure TAC of seaweed extracts with some minor modifications (Selim et al., 2008). A cuvette was filled with 3 mL of ethanoic extract, and the color intensity was measured at using a UV-VIS spectrophotometer, 520 nm (UV-2600, Shimadzu Corporation,USA). Ethanol was utilized as a control. Using the following equation, TAC computed and represented as mg per 100 g (mg/100 g):

$TAC = \text{Sample Absorbance} \times DF \times 100 / E \times m$

Where,

E = refers to extinction coefficient (55.9)

DF = dilution factor

m = means the weight of sample

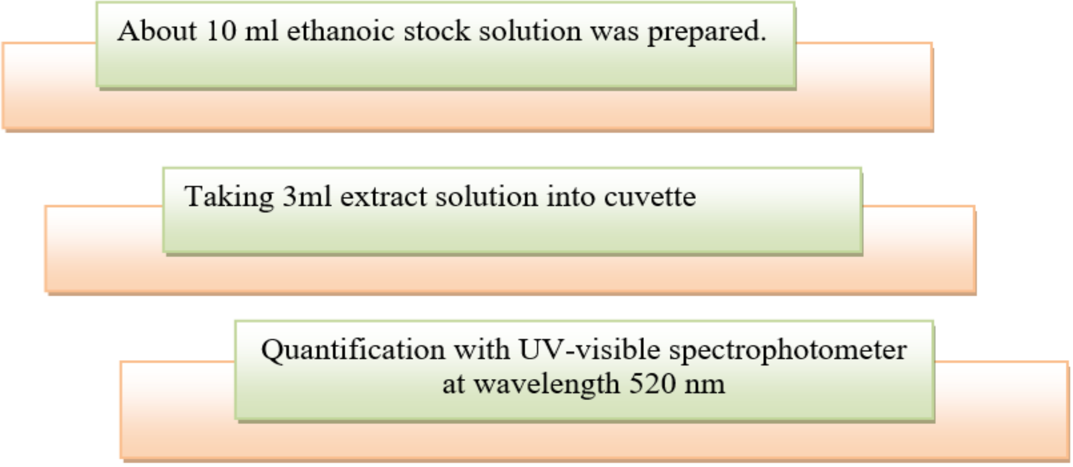


Figure 3.5. Flowchart showing the process for determining the total anthocyanin content (TAC)

3.10 Statistical investigation

For statistical assessment, data were saved in MS Excel 2010. For each variable, Standard deviation, mean, and percentages were used in the descriptive analysis. Finally, SPSS version 22 was used to calculate the correlation coefficient and its significance level. The level of significance is ≤ 0.05 .

3.11 Manufacture of seaweed Crackers

Enteromorpha Sp. was used for the production of crackers.

3.11.1 Ingredients for Crackers

- Regular shortening / all-purpose
- Pasteurized skimmed milk (>90% water)
- Table sugar (sucrose)
- Baking powder
- Pastry flour / all-purpose flour
- Salt
- Table sugar (sucrose)
- Artificial Flavor
- Seaweeds Powder

3.11.2 Manufacturing Process

- Scaling/metering of ingredients.
- Mixing. The versatility of the multi-stage mixing technique to create reliable doughs that weren't completely matured made it the favored approach.
- Blending all of the dry ingredients together with the seaweeds before rubbing or chopping the shortening into the flour until no more pea-sized lumps remain. Depending on the mixing speed (RPM), kind of beater, and load of the mixer, the total mixing time may vary between 2 and 3 minutes.
- Adding liquid components (milk, water, or other liquids) until the dough was homogenous. Depending on the RPM, kind of beater, and mixer load, mixing time might be anywhere between 1 and 2 minutes.
- The ideal dough temperature is between 13 and 16 °C (55 and 60 °F). As a result, the baked good's hardness is decreased, flakiness is enhanced, chemical leavening processes are slowed down, and fat cannot melt.

- Benching or resting the dough for 10 to 15 minutes.
- Dough is extruded, folded, and sheeted to a certain thickness.
- Cutting dough into round pieces.
- Baking for 30 minutes at 165 °C.
- Remove from oven and let cool on racks for 20 minutes.

CHAPTER IV: RESULTS

The study's findings are organized under the following topics. The seaweeds are distinguished by their names. This chapter includes information on the antioxidant properties and bioactive components of certain seaweeds.

4.1 Seaweeds' antioxidant activity using the DPPH scavenging technique

The DPPH test was used to assess the total antioxidant activity of seaweeds. Table 4.1 shows the antioxidant activity of the fruits. By plotting percent inhibition against the mg/100 g trolox test concentration, a calibration curve was created.

Table 4.1: Seaweeds Antioxidant activity (AOA)

Sl No	Seaweed	Antioxidant activity DPPH mg / 100 g (troloxequ).
01.	*Enteromorpha Sp.*	10.067±.100
02.	*Gracilaria Sp.*	8.667±.011
03.	*Hypnea Sp.*	1.204±.001
–	Maximum	10.067±.100
–	Minimum	1.204±.001
–	Average	7

Data are displayed as means with standard deviations, with a sample size of 3.

The DPPH activity of seaweeds spread from 1.2 to 10 mg/100 g, with *Enteromorpha Sp*. having the highest activity and *Hypnea Sp*. having the lowest. Activity of DPPH was 7 mg/100 g on average. Activity of total antioxidant was identified in the following order: Total antioxidant activity was found in the following order: *Enteromorpha Sp. >Gracilaria Sp. >Hypnea Sp.* Maximum absorbance at 515 nm, DPPH is a strong free radical scavenger that becomes yellow after receiving an electron or a proton to stabilize magnetic material molecule.

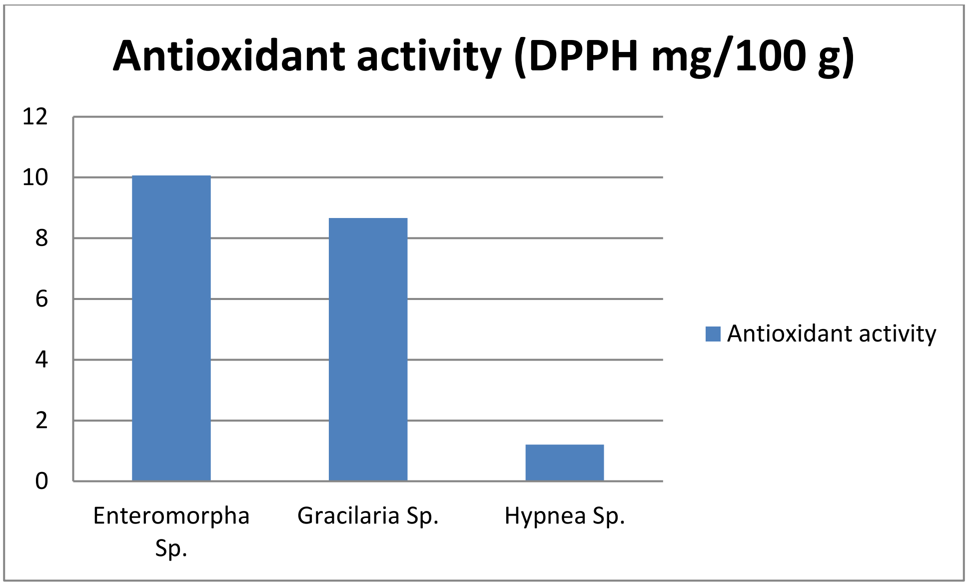


Figure 4.1: Antioxidant activity of *Enteromorpha, Gracilaria, Hypnea.*

4.2 Determination of Bioavtive compounds of available seaweeds of Bangladesh

4.2.1 EstimatingofTotalPolyphenolcontents(TPC)inavailable seaweeds in Bangladesh

The Folin-Ciocalteu technique was used to determine the total polyphenol content (TPC) of seaweed. Table 4.2 displays the total polyphenol content of three seaweeds. In this case, the outcome was represented as milligram GAE/100 g.

Table 4.2: Seaweeds Total polyphenol content (TPC)

Sl No	Seaweed	Totalphenoliccontent(TPC) (milligramGAE/100g)
01.	*Enteromorpha Sp.*	533.333±5.773
02.	*Gracilaria Sp.*	603.333±5.773
03.	*Hypnea Sp.*	236.667±5.773
–	Maximum	603.333±5.773
–	Minimum	236.667±5.773
–	Average	457.77

Data are displayed as means with standard deviations, with a sample size of 3.

The extract's TPC varied from 236.667±5.773 to 603.333±5.773 mg GAE/100 g. (gallic acid equivalent). In this experiment, *Gracilaria Sp.* (603.333±5.773 mg GAE/100g) had considerably greater total polyphenol content than the others, whereas *Hypnea Sp.* (236.667±5.773 mg GAE/100g) had lower TPC and *Enteromorpha Sp.* had (533.333±5.773 mg GAE/100g).

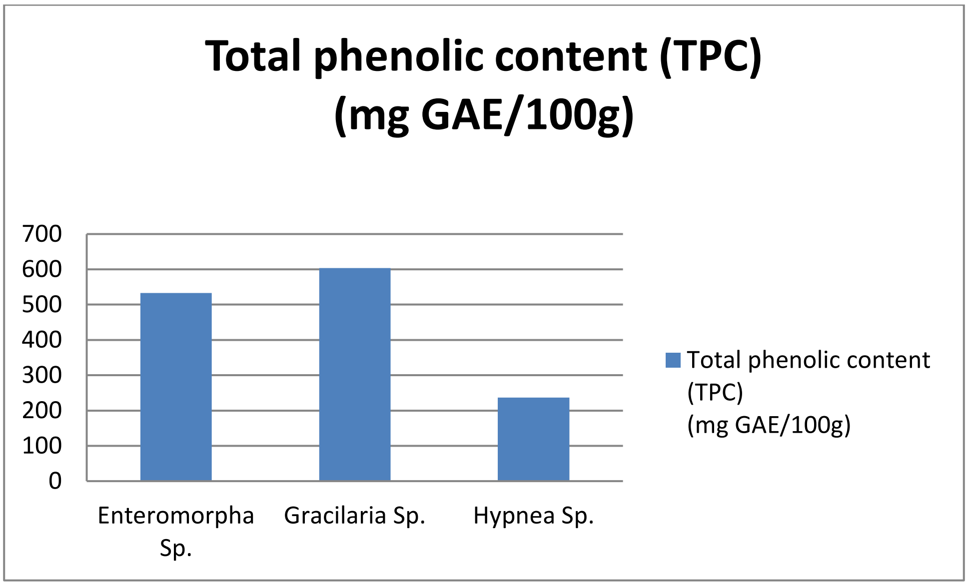


Figure 4.2: Total polyphenol content (TPC) of *Enteromorpha, Gracilaria, Hypnea*

4.2.2 EstimatingofTotalFlavonoidcontents(TFC)of seaweeds

The TFC of the seaweed extract was measured using a slightly modified aluminum chloride colorimetric technique. Table 4.3 displays the total flavonoid result. In this case, the outcome was represented as mg QE/100g extract.

Table 4.3: Total flavonoid content (TFC) of available seaweeds of Bangladesh

Sl No	Seaweed	Totalflavonoidcontent(TFC) (milligramQE/ 100gextract)
01.	*Enteromorpha Sp.*	374.442±.360
02.	*Gracilaria Sp.*	429.506±.079
03.	*Hypnea Sp.*	18.169±.016
–	Maximum	429.506±.079
–	Minimum	18.169±.016
–	Average	274

Dataaredisplayedasmeanvalueswithstandarddeviation,With a sample size of 3

TFC ranged from *Hypnea Sp.* (18.169±.016 mg QE/100g) to *Gracilaria Sp.* (429.506±.079 mg QE/100g), with an average total flavonoid concentration of 274 mg QE/100g. Other seaweeds include *Enteromorpha Sp.* (374.442±.360 mg QE/100g)

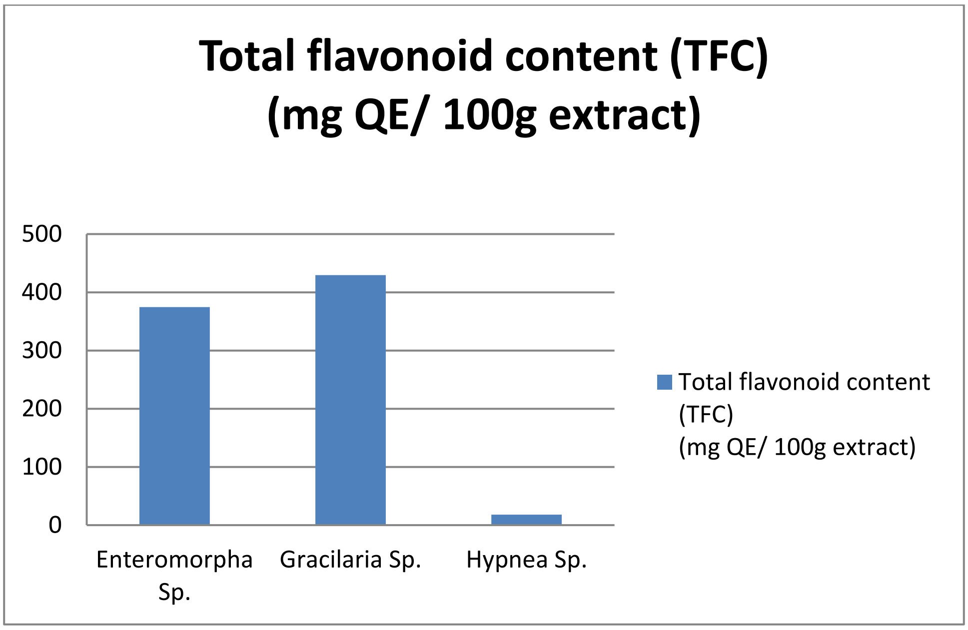


Figure 4.3: Total flavonoid content (TFC) of *Enteromorpha, Gracilaria, Hypnea*

4.2.3 Estimating of Total Anthocyanin contents (TAC) in seaweeds

The majority of the red, blue, and purple coloration in microalgae are due to anthocyanins, water-soluble antioxidant pigments. Table 4.4 shows the total anthocyanin content of the seaweeds.The outcome was represented as mg TA/100 g extract in this study.

Table 4.4: Seaweeds Total anthocyanin content (TAC)

Sl No	Seaweed	Total anthocyanin content (TAC) (milligram TA/100 g)
01.	*Enteromorpha Sp.*	152.830±.209
02.	*Gracilaria Sp.*	59.510±.104
03.	*Hypnea Sp.*	41.137±.180
–	Maximum	152.830±.209
–	Minimum	41.137±.180
–	Average	84.5

Data are displayed as means with standard deviations, with a sample size of 3

The maximal anthocyanin was found in *Enteromorpha Sp.* (152.830±.209 mg TA/100 g), while the least was found in *Hypnea Sp.* (41.137±.180 mg TA/100 g). The seaweeds had an average anthocyanin concentration of 84.5 mg TA/100 g. The following is the sequence in which total anthocyanin content was discovered: *Enteromorpha Sp.>Gracilaria Sp. >Hypnea Sp.*

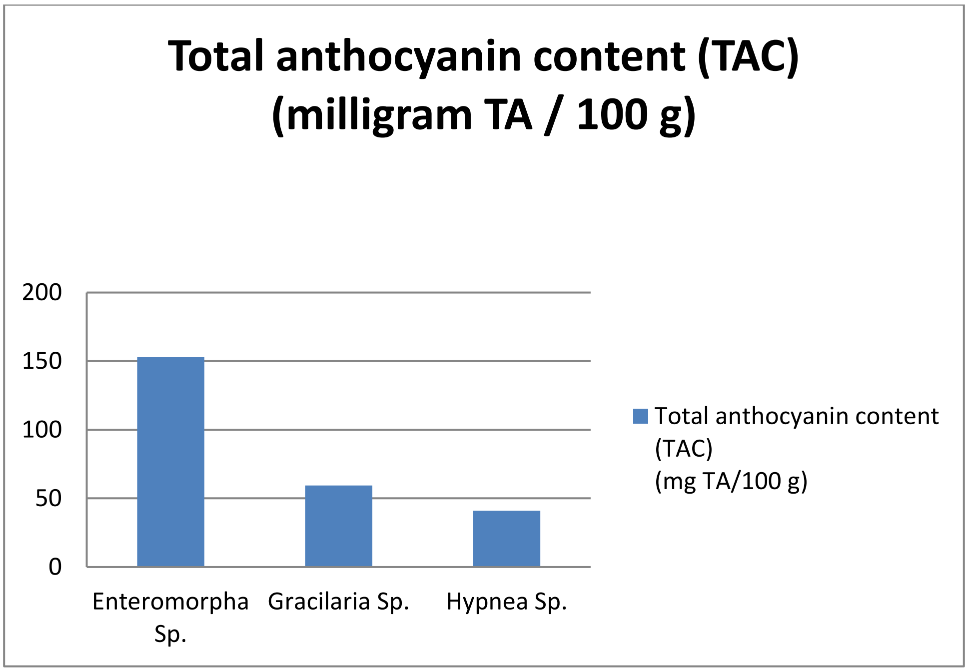


Figure 4.4: Total anthocyanin content (TAC) of *Enteromorpha, Gracilaria, Hypnea*

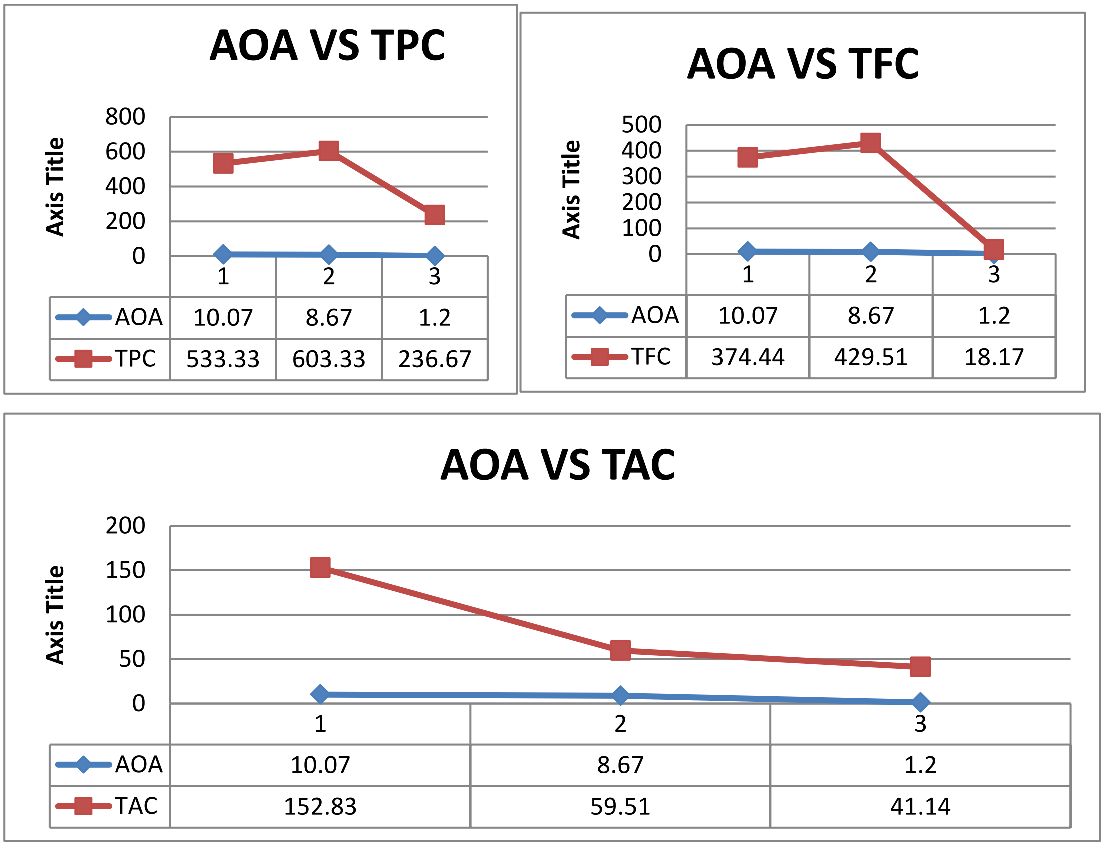
4.3 Relationship between bioactive chemicals and antioxidant activity in available seaweeds in Bangladesh

Table 4.5 shows the correlation coefficient between antioxidant activity and bioactive substances. Correlations between AOA and TPC, TFC and TAC were not significant (P≥0.05).

Table 4.5: Relationships between bioactive chemicals and antioxidant activity of seaweeds available in Bangladesh

Correlations	r	r^2(%)	P
AOAvs.TPC	0.95	90	*P≥0.05*
AOAvs.TFC	0.96	92	*P≥0.05*
AOAvs.TAC	0.74	54	*P≥0.05*

*. Correlation is significant at the 0.05 level (2-tailed).



	1	2	3
AOA	10.07	8.67	1.2
TPC	533.33	603.33	236.67

	1	2	3
AOA	10.07	8.67	1.2
TFC	374.44	429.51	18.17

	1	2	3
AOA	10.07	8.67	1.2
TAC	152.83	59.51	41.14

Figure 4.5: Correlation coefficient between three bioactive chemicals and antioxidant activity (AOA).

Table 4.6 shows three bioactive chemicals correlation coefficient. TPC and TFC had a significant ($P \leq 0.05$) correlation. Total polyphenol concentration (TPC) and total flavonoid content (TFC) of bioactive substances exhibited a moderate positive correlation. TPC vs TFC and TAC versus TAC were not significant ($P \leq 0.05$).

Table 4.6: correlation value between three seaweed bioactive substances

Correlations	**r**	**r^2(%)**	**p**
TPCvs.TFC	0.99	98	*$P \leq 0.05$*
TPCvs.TAC	0.48	23	*$P \geq 0.05$*
TFCvs.TAC	0.57	32	*$P \geq 0.05$*

*. Correlation is significant at the 0.05 level (2-tailed).

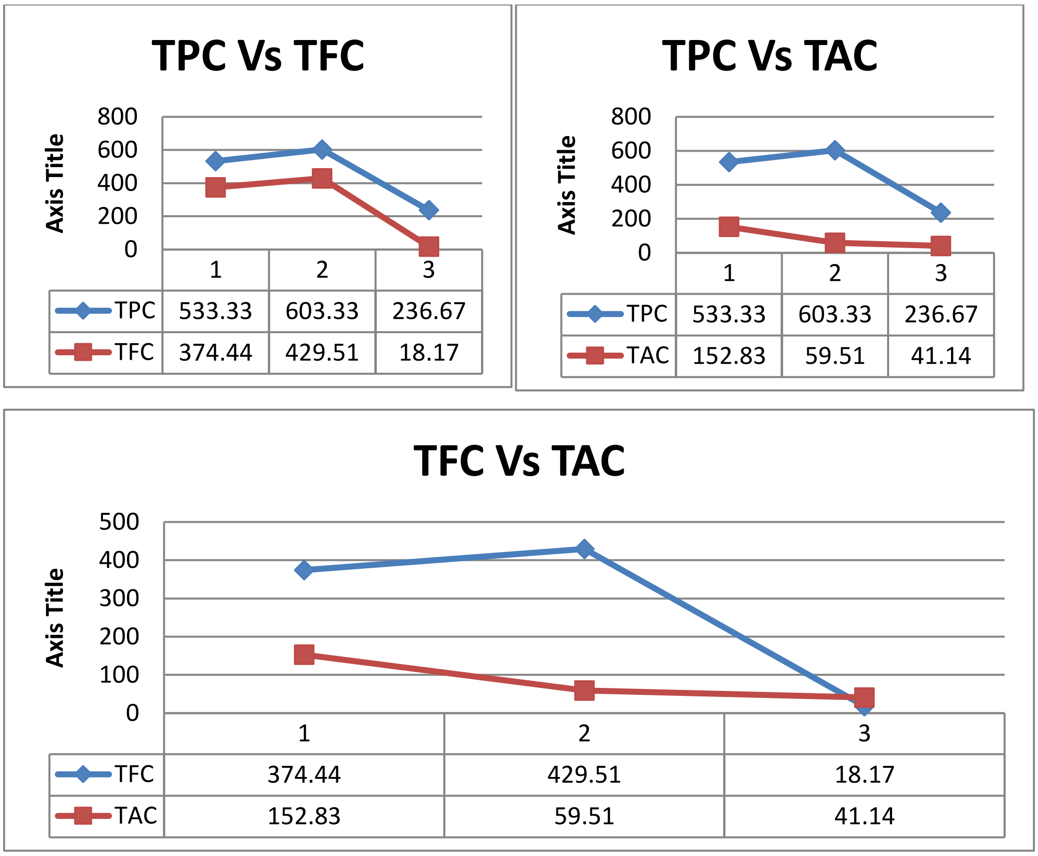


Figure 4.6: Coefficient of correlation between three bioactive substances

4.4 Proximate Analysis of available Seaweeds of Bangladesh

Moisture content, protein percentage, fiber content, fat percentage, ash percentage of the seaweeds are shown in the table 4.7.

Serial No	Name of the Seaweed	Moisture %	Protein %	Crude Fiber %	Crude Fat %	Ash %
01.	*Enteromorpha Sp.*	14.55	8.93	16.45	nil	21.39
02.	*Gracilaria Sp.*	13.00	27.13	7.31	nil	7.71
03.	*Hypnea Sp.*	16.00	13.48	11.06	nil	11.21

Maximum moister content was found in *Hypnea Sp.* (16.00 %)where *Gracilaria Sp.* had lowest moisture with 13%. Protein percentage was found highest in *Gracilaria Sp.*(27.13%), where *Enteromorpha Sp.* And *Hypnea Sp.* had (8.93%) and (13.48%). *Gracilaria Sp.* had the lowest fiber (7.13%), highest fiber content was found in *Enteromorpha Sp.* (16.45%) and *HypneaSp.*had (11.06%). No crude fat was found in any species of seaweed.*Enteromorpha Sp.* had considerably greater ash content

(21.39%) and *Gracilaria Sp*. had lowest ash content (7.71%), where *Hypnea Sp*. had (11.21%) ash content.

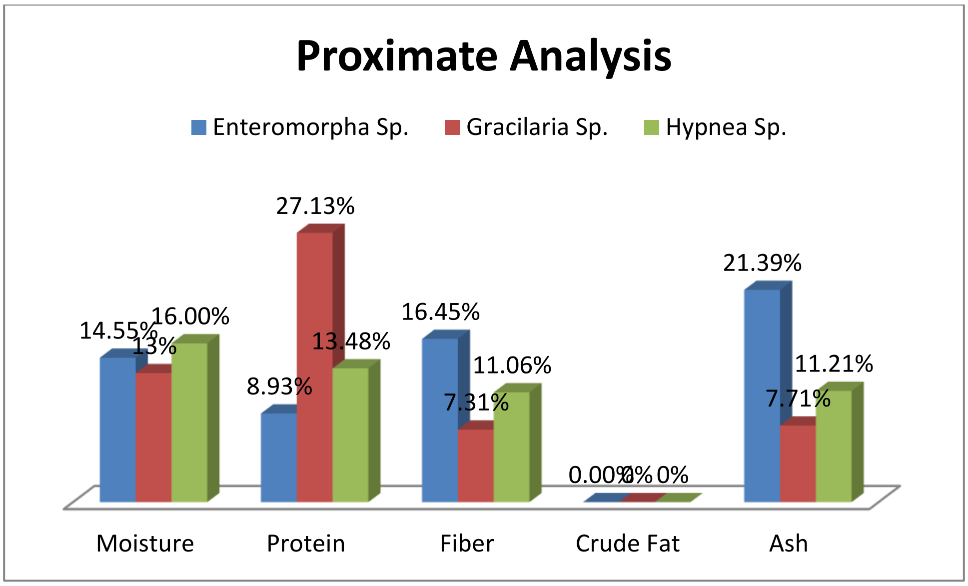


Figure 4.7: Proximate analysis of three seaweeds.

4.5 Consumer's acceptance for manufactured seaweed crackers

Consumer acceptability was determined by sensory assessment. During panel testing, natural light was employed. To avoid odors, the windows and door were kept closed. The door was also kept closed to prevent any potential distractions for precise sensory evaluation.

Figure 4.8: Seaweeds Crackers.

Total 12 individual done the panel test according to 9 point hedonic scale. The result was calculated and evaluated in MS Excel 2010.

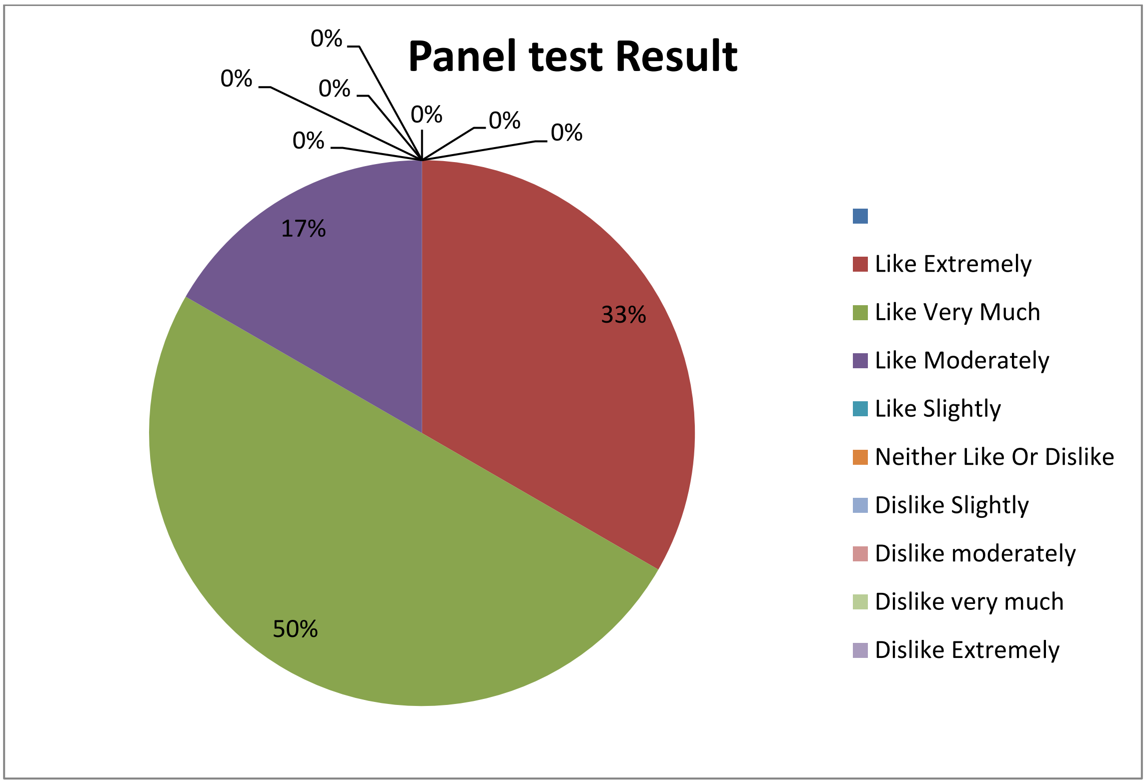


Figure 4.9: Panel test result of Seaweeds Crackers in 9 point hedonic scale.

The result was found for consumer acceptance test of seaweeds crackers.

From the result we found that 50% individual like very much of all quality attributes.

33% like extremely of all quality attributes.

17% like moderately of all quality attributes

CHAPTER V: DISCUSSIONS

5.1 General

Seaweeds are important components of all ecosystems. Seaweed extracts are also an essential component of most bio-stimulate products on the market today, and they are well recognized for their high polysaccharide, mineral, and vitamin content.

Many advantages of seaweed extract have been known for thousands of years, but only recently have their bioactive constituents been discovered.

5.2 Antioxidant activity of seaweeds

Antioxidant activity is responsible for preventing the growth of cancerous cells in the human body. Seaweeds may be an excellent source of antioxidants, and they have a wide range of applications in the food, cosmetics, and pharmaceutical sectors. The antioxidant indices tested, namely antioxidant activity (AOA) and DPPH radical scavenging activity, revealed substantial variance in their findings, indicating good results for the seaweed samples (Table 4.1). DPPH is a nitrogen free radical retaining compound that is easily eliminated by a free radical scavenger. The reduction is carried out using either single electron transfer (SET) or hydrogen atom transfer. All crude extracts' DPPH radical-scavenging capabilities were found to be extract and concentration dependent, and their activity differed considerably amongst extracts. However, there was no significant association between AOA and other bioactive chemicals in this investigation. All of the variables were unconnected.Seaweeds' DPPH activity varied from 1.2 to 10 mg/100 g, with *Enteromorpha Sp.*having the greatest and *Hypnea Sp.* having the lowest. DPPH activity was 7 mg /100 g on average..Antioxidant activity overall was determined as follows: Total antioxidant activity was discovered in the following sequence: *Enteromorpha Sp. >Gracilaria Sp.>Hypnea Sp.*With a maximum absorbance of 515 nm, the stable free radical scavenger DPPH scavenges from purple to yellow after absorbing an electron or a proton to form a sustainable magnetism compound.*Gracilaria Sp.* had an antioxidant capacity of 18.04 mg /100g extract (Ganesan et al., 2008). In this study *Gracilaria Sp.* had lower AOA 8.667 mg /100g. This variation may be due to the variation of solvent, species and origin of the seaweed. 72.86 mg /100g antioxidant capacity also reported for some *Hypnea Sp*. (Hossain et al. 2021). But in some several study lower AOA also reported as some

Hypnea Sp. contain 15.40 mg /100g extract (Chakraborty et al., 2015), which is similar to this study. In this study we found 10.067 mg /100g extract for *Enteromorpha Sp.*

The antioxidant activity of the seaweeds differed as well, which might be due to a range of elements including harvest season, age, weather,variety, geographical location, and so on. However, there is little information available on the chemical composition and antioxidant activity of accessible seaweed resources in Bangladesh. As a result, we discovered numerous bioactive chemical compositions and antioxidant activity of crude extracts from three Bangladeshi seaweeds in this study.

5.3 Bioactive compounds of available seaweeds of Bangladesh

Seaweeds are a source of bioactive substances that may be beneficial to human health. There are a lot of papers on phenolic, flavonoid, and anthocyanin analyses of seaweeds from nations all over the world, but just a few reports on seaweeds from the Bangladesh coast. As a result, we investigated bioactive substances such as phenolic, flavonoid, and anthocyanin analysis in Bangladeshi seaweeds.

5.3.1 Total polyphenol content (TPC)

Table 4.2 displays the total polyphenol content (TPC) of seaweeds. The average is relatively high, at 457.77 mg GAE/100g; nevertheless, there is a wide range of values, with a maximum of 603.33 mg GAE/100 g and a low of 236.66 mg GAE/100 g. The levels are quite high, much exceeding those seen in many fruits and vegetables.The extract's overall polyphenol content varied from 236.667±5.773 mg GAE/100 g to 603.333±5.773 mg GAE/100 g. (gallic acid equivalent). *Gracilaria Sp.* (603.333±5.773 mg GAE/100g) compared to the others in this study, had a considerably greater concentration of total polyphenols, but *Hypnea Sp.* (236.667±5.773 mg GAE/100g) had a lower TPC. There is correlation between total polyphenol content with total flavonoids content. Polyphenol content also correlate with anthocyanin.

Some study found that *Gracilaria sp.* total poly phenol content ranged 1025mg GAE/ 100g (Sanz-Pintos et al., 2017).Some other reports found out that *Gracilaria Sp.* contained 309 mg GAE/ 100g total polyphenol content (Morales et al., 2019). In our study we found 603.33 mg GAE/100g for *Gracilaria Sp. Hypnea Sp*. had TPC 497.7 mg GAE/ 100g (Rafiquzzaman et al., 2016). Some study also found for *Hypnea Sp.* 98.4mg GAE/ 100g (Chakraborty et al., 2015) .Where in this study we found nearly

603.33 mg GAE/ 100g.The result may be differ due to change of species, climate and geographical changes and also due to different solvent using.

5.3.2 Total flavonoid content (TFC)

Due to its vast spectrum of chemical and biological activities, including antioxidant and free radical scavenging characteristics, flavonoids are the most important natural phenolic.With an average total flavonoid concentration of 274 mg QE/100g, TFC content ranged from *Hypnea Sp*. (18.169±.016 mg QE/100g) to *Gracilaria Sp*. (429.506±.079 mg QE/100g). *Enteromorpha Sp*. (374.442±.360 mg QE/100g) is another seaweed. There was correlation found between flavonoids content with polyphenol content. The relation between flavonoids content with polyphenol content was significant.

In a previous study *Enteromorpha Sp*. TFC found 491mg QE/100g (Sameeh et al., 2016). Therefor in this study we found TFC of *Enteromorpha Sp*. was 374.44 mg QE/100g which was supported to the previous findings. TFC of *Hypnea Sp*. reported 660 mg QE/100g in the previous study of (Hossain et al., 2021) ,Which was higher than our present study report 18.16 mg QE/100g . TFC, on the other hand, is prone to seasonal fluctuation, since prior research have demonstrated variation in TFC of the same species collected in various seasons (Manivannan et al., 2008).

5.3.3 Total anthocyanin content (TAC)

The total anthocyanin concentration of the seaweeds is shown in Table 4.4. *Enteromorpha Sp*. has the most anthocyanin (152.830±.209 mg TA/100 g), whereas *Hypnea Sp*. had the least (41.137±.180 mg TA/100 g). The typical amount of anthocyanins in the seaweeds was 84.5 mg TA/100 g. The order in which total anthocyanin content was identified is as follows: *Enteromorpha Sp.>Gracilaria Sp.>Hypnea Sp*.

The methanolic extracts' overall anthocyanin activity differed considerably amongst the seaweeds.The variation in anthocyanin content between this study and others might be attributed to environmental factors, species differences, harvesting methods, and so forth.

5.4 Proximate Analysis of Seaweeds

Table 4.7 shows the moisture content, protein %, fiber percentage, fat percentage, and ash percentage of seaweeds. *Hypnea Sp*. had the highest moisture content (16.00%), whereas *Gracilaria Sp*. had the lowest moisture content (13%). *Gracilaria Sp*. had the greatest protein content (27.13%), followed by *Enteromorpha Sp*. and *Hypnea Sp*. (8.93%) and (13.48 percent). *Gracilaria Sp*. had the lowest fiber content (7.13%), *Enteromorpha Sp*. had the highest fiber content (16.45%), and *Hypnea* had the greatest fiber content (16.45%). (11.06 percent). There was no crude fat identified in any of the seaweed species. *Enteromorpha Sp.*had the highest ash level (21.39%) and *Gracilaria Sp*. had the lowest ash content (7.71%), while *Hypnea Sp*. had the lowest ash content (11.21%).

The protein was discovered in its raw dried form. In the previous survey, *Hypnea Sp.* varied from (15.953 percent) to (1.27 percent) (Hossain et al., 2021).In a previous study, the protein content of seaweeds was found to be between 10% to 47%, which was similar to the average range of 5 to 47% for seaweed (Shannon and Abu-Ghannam, 2019). For *Gracilaria Sp*. protein percentage was found 23% in a previous reseaech (Neto et al., 2018). So we can say that this study support the previous study for proximate analysis of seaweed.Macroalgae ashes have been found to range from 8% to 40% in previous investigations (Mabeau and Fleurence, 1993), which also support the present investigation.

5.5 Limitations of the current study

- In this study, we could collect and select only three species of seaweed, but there are other various types of seaweed which we could not collect due to unavailability.
- We could not examine all types of bioactive compounds due to limitation of resources.
- We measured bioactive chemicals using a UV visible spectrophotometer; however a GC-MS might yield better results.

CHAPTER VI: CONCLUSION

A wide range of edible seaweeds may be found in Bangladesh. The bioactive qualities of three seaweeds, *Enteromorpha Sp.,Gracilaria Sp., and Hypnea Sp*. are shown in this study.Seaweeds and sea grasses are important source of antioxidants and bioactive compounds. Seaweeds are also employed as dietary supplements and for medicinal purposes. The chemical properties of this macro algae can be used to solve many human issues, such as severe illness, and to develop new technologies. Perhaps the key aspects of the significant bioactive metabolites as well as the specific antiviral and anti-cancer capabilities of seaweeds were covered in this review. However, many bioactive metabolites or molecules remain a mystery, and scientists are working to identify every significant component that might improve human welfare. Additionally rich in iron and other elements necessary for proper physiological function are seaweeds.Furthermore the coastal residents of Cox's Bazar, Bangladesh may have new prospects as a result of the promotion of seaweed use as a vegetable. These new varieties of vegetables (seaweeds) can also be processed by the food industry. In essence, these plants have the ability to help some parts of Bangladesh and other nations overcome nutritional deprivation while also improving anti-inflammatory capacity.Seaweeds were discovered to have a greater content of protein, fiber, and minerals. Thus, the current study concludes that seaweeds are potentially beneficial foods in our diets and may be useful to the food industry as a source of high-nutritional-value components. Because of their nutritional worth, seaweeds can provide a dietary alternative, and their commercial value can be increased by enhancing the quality and expanding the variety of seaweed-based value added products.

CHAPTER VII: RECOMMENDATIONS & FUTURE PERSPECTIVES

Bangladesh have a large sea side area. By cultivating seaweeds in this area can help in increasing our blue economy which can help in achieving sustainable goal to reach vision 2041. For achieving the goal firstly, we have made aware our people about the health benefits of seaweeds by making rally, seaweeds fair etc. Marine microalgae, also known as seaweeds, are considered an economically important biological resource because they contain a diverse range of phytonutrients and phytochemicals, including vitamins, proteins, micro and macro elements, poly- and oligosaccharides, polyunsaturated fatty acids, terpenoids, alkaloids, polyphenols, particularly phlorotannins, and polyamines with potential pharmacological properties. Among these beneficial chemicals, research on alkaloids and polyamines is still in its infancy, possibly due to a lack of expertise on isolation and structural chemistry among seaweed biologists.Recent data suggests that dietary polyamines not only have health advantages but also play an important role in plant growth and development; however, there is little information available on seaweed polyamines.The seaweeds investigated here looked to be an attractive prospective source of plant food due to their high protein content and balanced micronutrient profile. The nutritional values of the seaweeds found here were determined only by chemical studies.

To determine the nutritional content of these seaweeds, biological assessment utilizing human and animal feeding experiments would be necessary. More research is needed (e.g., fatty acids, vitamins, hazardous elements, and other bioactive substances) to expand our understanding and encourage the use of these marine algae.

More detailed investigations on the current state of naturally accessible seaweeds and their usage status should be done, and a long-term plan for utilizing these resources should be established. Cookies are a high-energy bakery food, but seaweed cookies are a mineral-rich bakery product. There were several seaweed products including seaweed cookies available across the world. Moreover seaweeds are good source of protein .So seaweeds can be use as a protein supplement in protein based product, which may have numerous health benefits.

However, no Bangladeshi food manufacturer has come up to take such a step to make seaweed-based goods, despite the fact that they are nutritionally appealing and a very successful business.

In the whole world there is high demand of seaweeds products such as seaweeds soup, noodles are popular in Korea and Japan. If any Bangladeshi industry comes forward to make such products they can easily earn high profit by exporting them. This can help in increasing Bangladeshi remittance.

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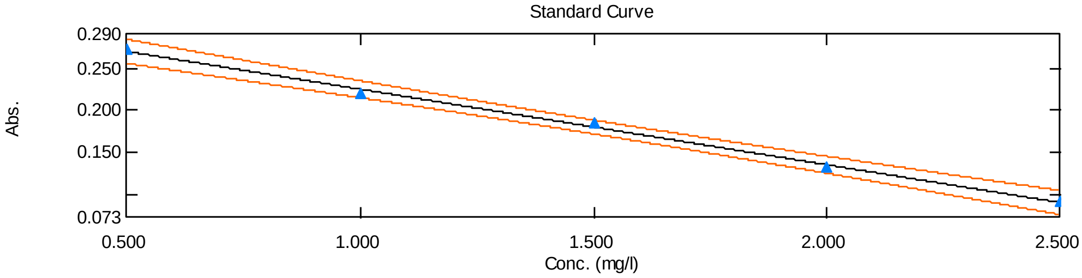
Appendices

AppendixA:StandardCurve&SampleCurve

Table:ConcentrationandAbsorbanceforStandardsolutionforAOA

SampleID	Type	Ex	Conc.(ppm)	WL517. 0	Wgt. Factor	Comments
1	Std2	Standard	1.000	0.221	1.000	
2	Std3	Standard	1.500	0.185	1.000	
3	Std4	Standard	2.000	0.133	1.000	
4	Std5	Standard	2.500	0.092	1.000	

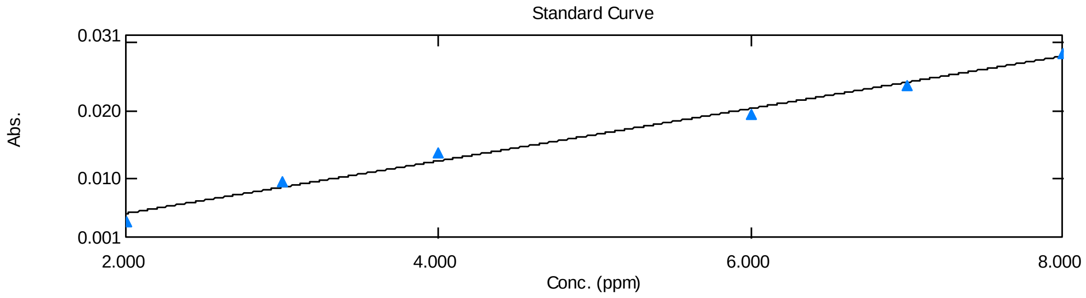


y = - 0.0894539 x + 0.314536
r2 = 0.99735

Figure:Standardcurveforantioxidantcapacity

Table:ConcentrationandAbsorbanceforStandardsolutionforTFC

Sample ID	Type	Ex	Conc	WL517.0	Wgt.Factor
1	Std1	Standard	1.000	0.041	1.000
2	Std2	Standard	3.000	0.088	1.000
3	Std3	Standard	5.000	0.171	1.000
4	Std4	Standard	7.000	0.234	1.000

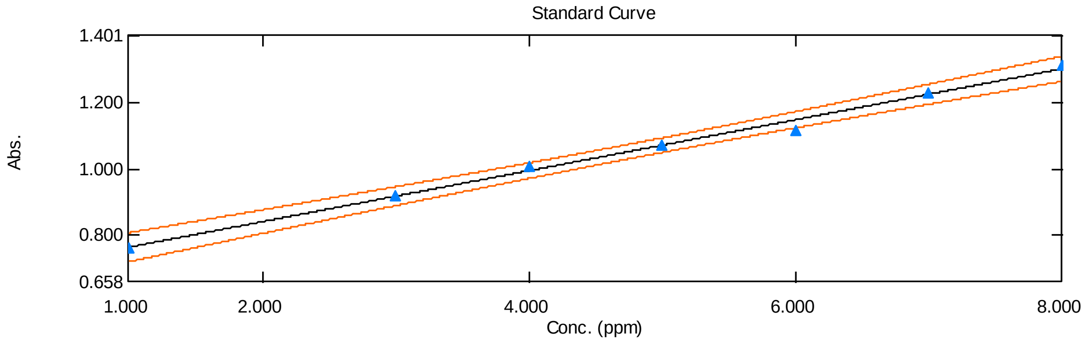


y = 0.00385110 x - 0.00271158
r2 = 0.98868

Figure:StandardcurveforTFCcapacity

Table:ConcentrationandabsorbanceforStandardsolutionforTPC

SampleID	Type	Ex	Conc	WL517.0	Wgt.Factor
1	Std1	Standard	1.000	0.763	1.000
2	Std2	Standard	2.000	0.780	1.000
3	Std3	Standard	3.000	0.920	1.000
4	Std4	Standard	4.000		
5	Std5	Standard	5.000	1.007	1.000
6	Std6	Standard	6.000	1.074	1.000
7	Std7	Standard	7.000	1.115	1.000
8	Std8	Standard	8.000	1.230	1.000



y = 0.0768527 x + 0.687090
r2 = 0.99301

Figure:StandardcurveforTPCcapacity

Figure: Cabinet Drying of Seaweed

Figure: Vaccum Packing Of seaweed

Figure: Sample Preparation

Figure: Stock Solution Preparation

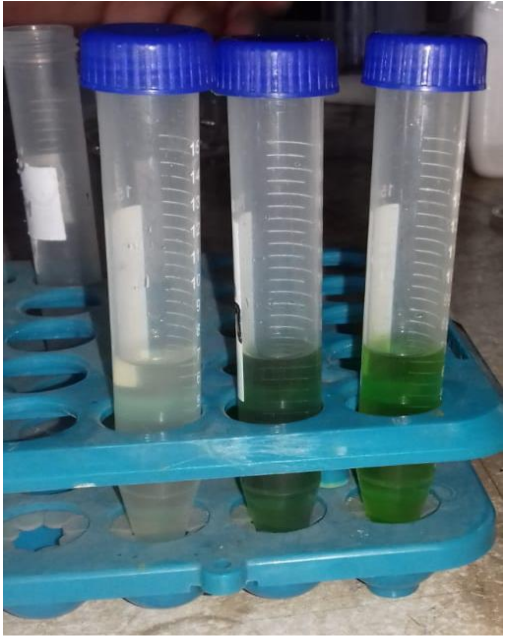


Figure : Sample Preparation

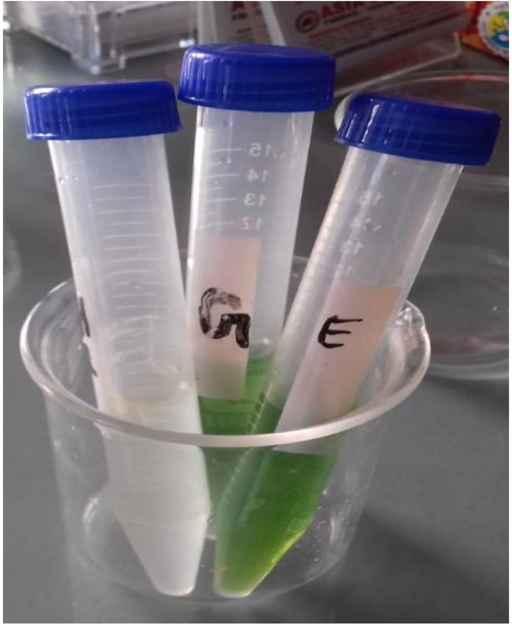


Figure: Crackers Preparation

Figure: Prepared Crackers

Figure: Sensory Evaluation of Prepared Crackers

Brief biography

Md. Mostafizur Rahman Khan passed the Secondary School Certificate Examination in 2010 and then Higher Secondary Certificate Examination in 2012. He obtained his B.Sc. (Hons.) in Food Science & Technology in 2017 from Chattogram Veterinary and Animal Sciences University (CVASU), Bangladesh. Now, he is a candidate for the degree of MS in Dept. of Applied Chemistry and Chemical Technology under the faculty of Food Science & Technology, CVASU. He has immense interest to work in different food issues including nutrition, food safety, food chemistry, quality assurance, food quality control, environmental chemistry, product development and processing, malnutrition, reduction of nutritional changes in food etc.