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CHARACTERIZATION OF BIOACTIVE METABOLITES FROM MARINE MACROALGAE COLLECTED FROM VERAVAL COAST OF GUJARAT

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Keywords:

Seaweeds; Bioactive metabolites; *Ulva lactuca, Ulva faciata, Acanthophora dendroides, Gracilaria corticata, Padina tetrastromatica, Cystoseira indica*

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ABSTRACT: Seaweeds are potential sources of bioactive molecules. They are known to be rich sources of proteins, carbohydrates, lipids, vitamins, minerals, and other secondary metabolites. To evaluate the presence of these compounds in marine macroalgae collected from Veraval coast of Gujarat, present studies was carried out. The methanol extract of six macroalgae species (*Ulva lactuca*, *Ulva faciata*, *Acanthophora dendroides*, *Gracilaria corticata*, *Padina tetrastromatica*, and *Cystoseira indica*) was subjected to LCMS/MS analysis which suggested presence of amino acids, sugar, vitamins and phytohormones in algae extracts. The aminoacids and vitamins present in *Ulva lactuca* (betaine, L-methionine, L-asparagine), *Ulva faciata* (L-arginine, L-proline, L-histidine, L-valine, L-Threonine, Dihydrofolic acid), *Acanthophora dendroides* (L-glutamic acid, L-alanine, L-serine, L-phenylalanine, L-tyrosine, nicotinic acid), *Gracilaria coeticata* (L-canavanine, L-isoleucine, and L-cysteine) and *Padina tetrastromatica* (L-Aspartic acid, L-leucine) can be utilized for the development of dietary supplements and proteinaceous food for domestic animals. The sugar present in *Ulva faciata* (glucosamine), *Acanthophora dedroides* (D-xylose, D-mannose, D-fructose), *Gracilaria corticata* (D-Rhamnose, fucose, D-galactose), *Padina tetrastromatica* (D-glucuronic acid, D-glucose, D-mannuronic acid), and *Cystosera indica* (arabinose, maltose) can be utilized in food products, cosmetic, gelling and thickening agents. The phytohormones present in *Ulva lactuca* (6-Benzylaminopurine, jasmonate), *Gracilaria corticata* (salicylic acid, ethylene), *Padina tetrastromatica* (Abscisic acid), and *Cystosera indica* (Indole-3 acetic acid) can be utilized in agriculture as plant growth regulators. Hence, these algae species are essential raw materials for the production of various pharmaceutical and biotechnological products.

INTRODUCTION: Principally, it was observed that the drugs developed from natural sources mainly come from microorganisms and terrestrial plants. However, marine organisms are the alternative sources to discover novel bioactive compounds [1].

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Secondary metabolites are organic compounds synthesized in living organisms using primary metabolites. These compounds are specific for each and every organism species within a phylogenetic group.

These secondary compounds are economically important for different pharmaceutical and biotechnological industries for synthesizing medicines, flavouring agents, thickening agents, and recreational drugs [2]. Marine macroalgae are natural sources of biologically active therapeutic and bioactive molecules for treating multiple diseases [3, 4].

Macroalgae are the potential sources of primary metabolites and secondary metabolites which are extensively evaluated using bioassays and pharmacological studies [5, 6, 7]. Many secondary metabolites are produced from intermediates and end products of secondary metabolism which are grouped into terpenes, phenolics, and alkaloids [8, 9]. Seaweed resources such as polysaccharides, lipids, proteins, carotenoids, vitamins, sterols, enzymes, antibiotics and many other fine chemicals are used as major metabolites for human benefits [10, 11, 12]. *Ulva fasciata* contains sphingosine derivatives that have *in-vivo* antiviral activity [13]. Extract of Ulva lactuca has anti-inflammatory and antitumour compounds [14]. The secondary metabolites from *Ulva fasciata* and *Hypnea musciformis* enhance the bacterial clearance capability of the hemolymph of shrimp and *Penaeus monodon* [15].

Portieria hornemannii contains halogenated monoterpene called halomon, which is cytotoxic in nature [16]. The *Codium iyengarii* is a potential source of steroidal glycosides known as iyengarosides A and B [17]. *Sargassum carpophyllum* is the source of two bioactive sterols. Which induces morphological abnormality in the plant pathogenic fungus (*Pyricularia oryzae*) and exhibits cytotoxic activity against several cultured cancer cell lines [18]. Laminaria and Sargassum species are used in China for the treatment of cancer. Undaria contains antiviral compounds which inhibit the herpes simplex virus. Corralina species are rich sources of calcium carbonate and hence are used in bone replacement therapies [19]. Brown seaweeds produce terpenoids, acetogenins, and other aromatic compounds [20, 21, 22, 23, 24].

Macroalgae from temperate and polar regions produce high concentrations of polyphenols called phlorotannins [25, 26]. Isoprenoid and acetogenin derivatives recognized from red seaweeds are useful as defensive metabolites [27, 28]. Bryopsidales are probable sources of sesquiterpenoid and diterpenoid compounds [24]. Several marine secondary metabolites have antifouling strategies due to their capability to inhibit the growth, attachment, and settlement of other marine organisms [29, 30, 31]. Seaweed extracts are being used for the treatment of asthma, thyroid, goiter, urinary infections, stomach ulcers, and even tumors. Sulfated polysaccharides such as carrageenan, agar, agarose, and furcellaran have wide applications in medicines. Carrageenan obtained from Chondrus, Eucheuma, Gigartina, and Iridea has an effective therapy for gastric and duodenal ulcers [32, 33]. Fucoidan obtained from *Gracilaria corticata*, is used for colorectal and breast cancer treatments [34]. L-kainic acid obtained from Digenea simplex is used in treatments of cancer, inflammation, and infectious diseases [35]. Phenolic compounds produced by *Rhodomela confervoides*, *Symphyocladia latiuscula* and *Polysiphonia urceolata* have antidiabetic activity. They inhibit the activity of protein tyrosine phosphatase (PTPase), which is a responsive factor for insulin. Polyphenolic compounds obtained from Laurencia undulate used for the treatment of asthma [36].

Fucoxanthin obtained from macroalgae (*Undaria pinnatifida*, *Laminaria japonica*, *Sargassum fulvellum*, and *Hijikia fusiformis*) has antioxidant, anticancer, antiobesity, antidiabetic, and antiphotoaging activities [37]. Anticancer activity against several cell lines was observed from extracts of two Sargassum species (*Sargassum wightii* and *Sargassum ilicifolium*) [38]. Laminarin has strong heparin-like activity which is useful as an anticoagulant, antilipemic, antiviral, and anti-inflammatory agent [39]. Park *et al.* (2011) [40] suggested that fucoidan can be used in obesity therapies as it reduces lipid accumulation by stimulating lipolysis. Spavieri (2010) [41] observed cytotoxic activity of twenty-one brown algae extract on mycobacteria and protozoan species. Fucoidans obtained from brown seaweed species have immune-modulating activity, which increases macrophage-mediated responses [42]. Salgado suggested that the interaction between polyphenolic compounds and cell wall alginates leads to the absorption of ultraviolet radiation [43]. diekol obtained from Ecklonia cava has antifungal, anti-inflammatory, and antitype II diabetes activities [44]. Green seaweeds are rich sources of antioxidants, vitamins, and bioactive peptides [45, 46]. Extracts from *Caulerpa taxifolia*, *Caulerpa racemose*, and *Cladophora pinnulata* have hypotensive activities [47]. Sulfated polysaccharides from green seaweeds have anticoagulant, antioxidant, anticancer, anti-hyperlipidemic, and immune modulation activity [48, 49]. Sulfated polysaccharide obtained from *Ulva pertusa* is used for the treatment of ischemic, cerebrovascular, and cardiovascular diseases [50].

Sulfated polysaccharides from *Ulva pertusa*, *Capsosiphon fulvescens*, and Codium fragile have immune-modulating activity for stimulating macrophages [51]. The ethanolic extracts of *Codium tomentosum* have antigenotoxic and antioxidant activity [52]. Ethanolic extract from *Codium decorticatum* has antibacterial activity [53]. The methanolic extract of *Ulva linza* has high inhibitory activity against inflammatory response due to the presence of high polyunsaturated fatty acids (PUFA) [54].

MATERIALS AND METHODS:

Sample Collection: The six different macroalgae species (*Ulva lactuca*, *Ulva faciata*, *Acanthophora dendroides*, *Gracilaria corticata*, *Padina tetrastromatica*, and *Cystoseira indica*) were collected from the coastal region of Veraval behind College of fisheries (GPS Location: 20’54’41N 70’21’01’E).

Extraction of Secondary Metabolites: Seaweeds were collected and washed thoroughly with distilled water to remove unwanted impurities and other debris. They were allowed to dry for few days under sunlight. After drying, each macroalgae species was cut into small pieces and made into a coarse powder with the help of a mechanical grinder. Dry powder from each algae (*Ulva lactuca*, *Ulva faciata*, *Acanthophora dendroides*, *Padina tetrastromatica*, *Cystoseira indica*, and *Gracilaria corticata*) was filled in a thimble and was subjected for extraction in a soxhlet extractor. Extraction was carried out in different solvents starting from nonpolar solvents (petroleum ether and n-hexane) to semi-polar solvents (chloroform and acetone) and finally polar solvents (methanol and water) at 70 °C using a soxhlet extractor. After extraction, samples of methanol extract were concentrated using a rota evaporator. Then, concentrated samples were subjected to LC-MS analysis for the characterization of secondary metabolites.

LC-MS/MS analysis of samples: Each Sample (10 µl) was run on a Shimadzu UHPLC system composed of two LC-20AD XR UHPLC pumps, a Shimadzu DGU-20A 5R degassing unit, a Shimadzu SIL-20A XR auto sampling unit, a Shimadzu SPD-N20A UV DAD detector equipped with a UHPLC cell, and a Shimadzu IT-TOF detector mounting an Electrospray source. A Reprosil-Pur Basic C18 (1.9 μm^{-100} × 2.0 mm) column was mounted in the UHPLC system and fed at 0.25 ml min^{-1} with solution made of 0.05 M NH4CH-3COO in LC–MS grade water (A) and in LC–MS grade acetonitrile (B) by the following gradient: 95:5 A:B for 0.1 min; linear gradient to 80:20 in 10 min, then to 50:50 in 20 min and finally to 10:90 in 20 min. The system was kept at 10:90 for 5 min and then re-equilibrated to 95:5 in 10 min for a total of 65 min run cycle. The stationary phase was stabilized at 30 °C.

Data Acquisition and Processing: All LC-MS data were converted to the mzdata format. Data preprocessing was performed using MZmine 2.23. Fragmentations were gathered with the corresponding precursor ions. Precursor ions of the metabolites were identified by using the MET-LIN metabolite database (http://metlin.scripps.edu/).

RESULTS: In present studies, extraction and characterization of metabolites from six different macroalgae species were done to identify the different type of bioactive metabolites present in them. From, methanolic extract of *Ulva lactuca* totally five peaks were identified, which included amino acids (betaine, L-Methionine and L-Asparagine) and phytohormones (6-Benzylaminopurine and jasmonate) **Fig. 1**. From, methanolic extract of *Ulva faciata* totally seven peaks were identified, which included amino acids (L-arginin, L-Proline, L-histidine, L-valine, and L-threonine), vitamin (Dihydrofolic acid), and sugar (glucosamine) **Fig. 2**.

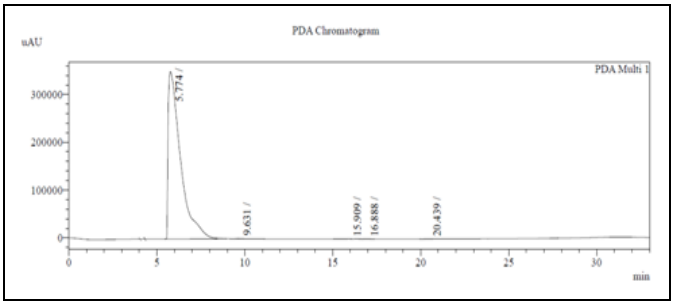

FIG. 1: CHROMATOGRAM OF *ULVA LACTUCA* METHANOL EXTRACT

Peak	Compound name	Ret. Time	Type of Compound
1	Betaine	5.774	aminoacid
2	L-Methionine	9.631	aminoacid
3	6-Benzylaminopurine	15.909	Phytohormone
4	jasmonate	16.888	Phytohormone
5	L-Asparagine	20.439	aminoacid

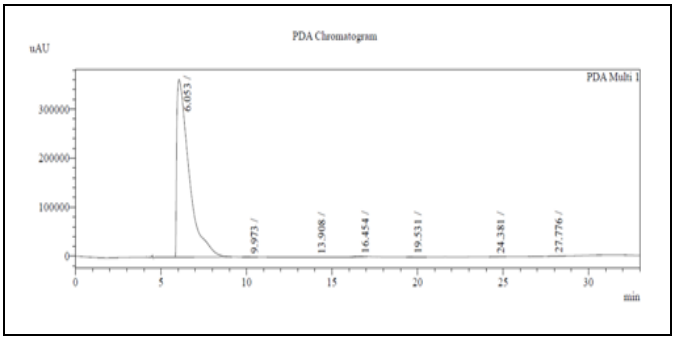

FIG. 2: CHROMATOGRAM OF *ULVA FACIATA* METHANOL EXTRACT

Peak	Compound name	Ret. Time	Type of compound
1	L-Arginine	6.053	aminoacid
2	L-Proline	9.973	aminoacid
3	Dihydrofolic acid	13.908	Vitamin B9
4	L-Histidine	16.454	aminoacid
5	glucosamine	19.531	sugar
6	L-Valine	24.381	aminoacid
7	L-Threonine	27.776	aminoacid

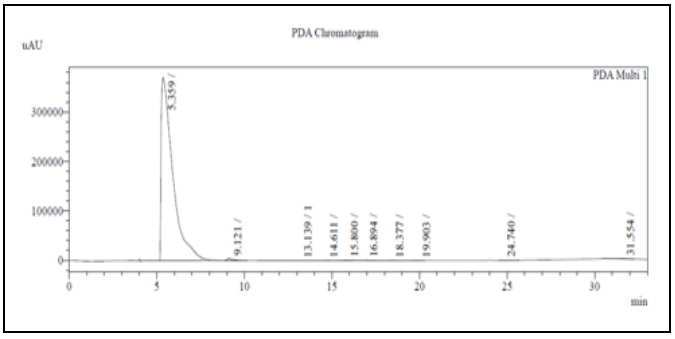

FIG. 3: CHROMATOGRAM *OF ACANTHOPHORA DENDROIDES* METHANOL EXTRACT

Peak	Compound name	Ret. Time	Type of compound
1	D-Xylose	5.359	Sugar
2	Nicotinic acid	9.121	vitamin B3
3	L-glutamic acid	13.139	Amino acid
4	D-mannose	14.611	Sugar
5	L-Alanine	15.800	Amino acid
6	L-Serine	16.894	Amino acid
7	L-phenylalanine	18.377	Amino acid
8	L-Tyrosine	19.903	Amino acid
9	D-Fructose	24.740	Sugar
10	L-Glycine	31.554	Amino acid

From, methanol extract of *Acanthophora dendroides* ten peaks were identified, which included six amino acids (L-glutamic acid, L-alanine, L-serine, L-phenylalanine, L-Tyrosine, and L-glycine), three sugars (D-xylose, mannose and D-fructose) and one vitamin (Nicotinic acid) **Fig. 3**. From methanol extract of *Gracilaria corticata* eight peaks were identified, which included three aminoacids (L-Canavanine, L-isoleucine and L-Cysteine), three sugars (D-Rhamnose, fucose and D-galactose) and two phytohormones (Salicylic acid and ethylene) **Fig. 4**.

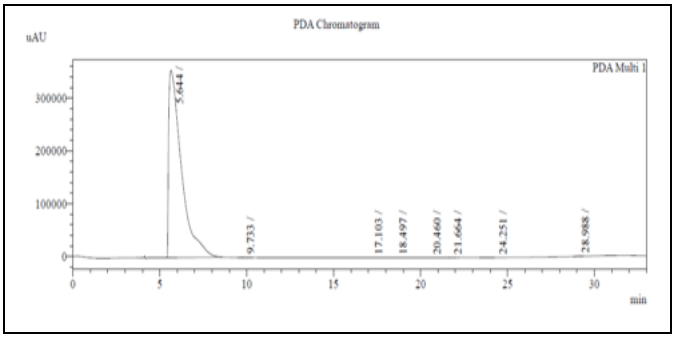

FIG. 4: CHROMATOGRAM OF *GRACILARIA CORTICATA* METHANOL EXTRACT

Peak	Compound name	Ret. Time	Type of compound
1	D-Rhamnose	5.644	sugar
2	L-Canavanine	9.733	aminoacid
3	fucose	17.103	Sugar
4	Salicylic acid	18.497	Phytohormone
5	ethylene	20.460	Phytohormone
6	L-isoleucine	21.664	aminoacid
7	D-galactose	24.251	sugar
8	L-Cysteine	28.988	aminoacid

From methanol extract of *Padiana tetrastromatica* seven peaks were identified which contained compounds such as three aminoacids (L-Aspartic acid, L-Tyrosine, and L-Leucine), one vitamin (Pantothenic acid), two sugars (Glucuronic acid and D-Glucose), and one phytohormones (abscissic acid). Aminoacids identified were L-aspartic acid, L-Tyrosine and L-leucine. Sugars identified were glucuronic acid and D-Glucose. Phytohormone identified was abscisic acid, and vitamin identified was Pantothenic acid **Fig. 5**. From methanol extract of *Cystosera indica* only four peaks were identified, which contained vitamin (pyridoxamine-5-phosphate), sugars (arabinose and maltose) and phytohormones (Indole 3-acetic acid) **Fig. 6**.

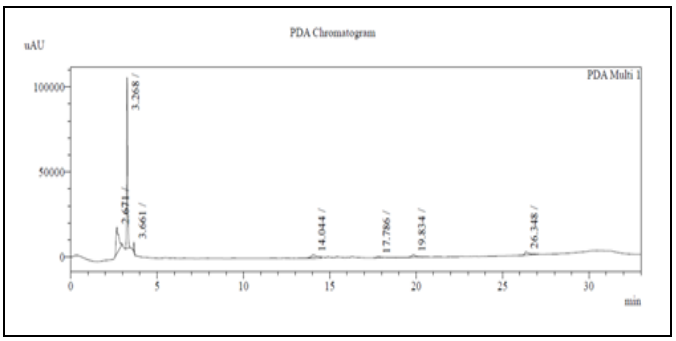

FIG. 5: CHROMATOGRAM OF *PADIANA TETRASTROMATICA* METHANOL EXTRACT

Peak	Compound name	Ret. Time	Type of compound
1	Pantothenic acid	2.671	Vitamin B5
2	D-Glucuronic acid	3.268	Sugar
3	D-Glucose	3.661	Sugar
4	Abscisic acid	14.044	Phytohormone
5	L-Aspartic acid	17.786	aminoacid
6	D-mannuronic acid	19.834	Sugar
7	L-Leucine	26.348	Amino acid

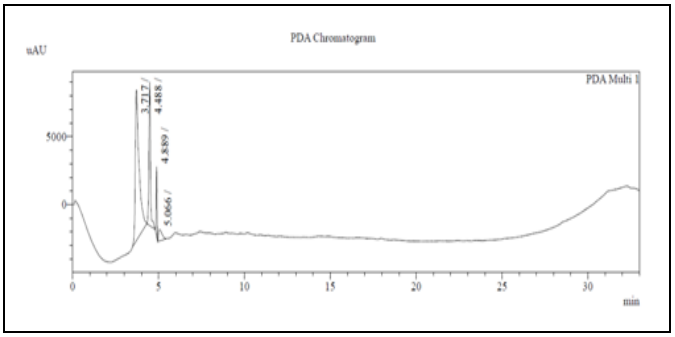

FIG. 6: CHROMATOGRAM OF *CYSTOSERA INDICA* METHANOL EXTRACT

Peak	Compound name	Ret. Time	Type of compound
1	Pyridoxamine-5-phosphate	3.717	Vitamine B6
2	arabinose	4.488	sugar
3	maltose	4.889	sugar
4	Indole-3 acetic acid	5.066	Phytohormone

DISCUSSION: From the present studies, it was observed that all algae species contained aminoacids, vitamins, sugars, and phytohormones. From methanolic extract of all six marine macroalgae amino acids such as betaine, L-methionine, L-asparagine, L-arginine, L-proline, L-histidine, L-valine, L-threonine, L-glutamic acid, L-alanine, L-serine, L-phenylalanine, L-Tyrosine and L-Glycine, L-Canavanine, L-isoleucine, L-Cysteine, L-Aspartic acid, and L-Leucine were identified. Similarly, in previous studies, Ortiz *et al.* 2006 [55] had reported the presence of seventeen aminoacids (asparagine, glutamate, serine, histidine, glycine, therionine, arginine, alanine, proline, tyrosine, valenine, methionine, cysteine, isoleucine, leucine, phenylalanine, and lysine) from Ulva lactuca collected from the coastal area of Northern Chile.

Garcia *et al.* 2016 [56] reported the presence of fifteen amino acids (aspartic acid, threonine, serine, glutamic acid, lysine, arginine, glycine, phenylalanine, typtophan, methionine, cysteine, isoleucine, leucine, histidine, and valine) in four macroalgae (Ulva, Codium, *Halymenia floresia*, and Saccorhiza polyschides) collected from Barbate estuary, Gulf of Cadiz, Spain. Kumar and Kaladharan (2007) [57] reported the presence of eighteen amino acids (asparagine, glutamate, serine, histidine, glycine, therionine, arginine, alanine, proline, tyrosine, valenine, methionine, cysteine, isoleucine, leucine, phenylalanine, tryptophan, and lysine) from *G. corticata*, *H. musciformis*, *A. spicifera*, *S. wightii*, *U. lactuca* and *K. alvarezii* collected from Thikkodi Thangasseri and Quilon of Kerala coast. Recently Kazir *et al.* 2019 [58] reported the presence of seventeen amino acids (alanine, arginine, aspartic acid, cysteine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, therionine, tyrosine, and valenine) in Ulva sp. and Gracilaria sp. cultivated at the seaweed unit of Israel Oceanographic and Limnological Research, Haifa, Israel.

Sugars and sugar acids identified from six marine algal species were glucosamine, D-xylose, D-mannose, D-fructose, D-rhamnose, fucose, D-galactose, D-glucose, D-glucuronic acid, D-mannuronic acid, arabinose, and maltose. Similarly, in previous studies, Aguilar-Briseño *et al.* 2015 [59] suggested the presence of rhamnose, glucuronic acid, xylose, glucose and galactose in the dry powder of *Ulva clathrata*. Robin *et al.* (2017) [60] suggested the presence of glucose, rhamnose, galactose, xylose arabinose, mannitol, fucose, and glucuronic acid in *Ulva* sp., *Gracilaria* sp., *Padina pavonica*, *Cladophora pellucid*, *Galacxaura rugosa*, and *Nemalion helminthoides*. Agili and Mohamed 2012 [61] reported the presence of six sugars (rhamnose, fucose, xylose, mannose, glucose, and galactose) in the polysaccharide of *Padina pavonia*.

Vitamins identified from methanol extract of six marine macroalgae were Pyridoxamine-5-phosphate, Dihydrofolic acid, Pantothenic acid, and Nicotinic acid. Similarly, previous studies by Jesmi *et al.* 2018 [62] suggested the presence of folic acid, riboflavin, pantothanic acid, niacin, vitamin A, vitamin B1, vitamin B12, vitamin C, vitamin D, and vitamin E in seaweeds. Pandithurai and Murugesan (2014) [63] suggested the presence of these vitamins in brown marine algae Spatoglossum asperum. Rodrıguez Bernaldo de Quirós *et al.*, 2004 [64] suggested the presence of folic acid in Himanthalia elongata, *Laminaria ochroleuca*, Palmaria spp., *Undaria pinnatifida*,

Porphyra spp. and Saccorhiza polychides. Rosemary *et al.* 2019 [65] reported the presence of vitamin B1, vitamin B2, vitamin B3, vitamin B6, vitamin B9, and vitamin C, vitamin A, and vitamin E in *G. corticata* and *G. edulis*.

Phytohormones identified from methanol extract of six marine macroalgae were 6-benzylaminopurine, jasmonate, indole butyric acid, salicylic acid, ethylene, abscisic acid, and indole-3 acetic acid. Recently, Yalcın *et al.* 2019 [66] suggested the presence of zeatin, benzylaminopurine, gibberellic acid, kinetin, indole-3 acetic acid, and abscissic acid in nine seaweeds (*Cladostephus spongiosum* f. verticillatum, *Colpomenia sinuosa*, *Cystoseira barbata*, *Halopithys incurva*, *Gracilaria bursapastoris*, *Ellisolandia elongata*, Polysiphonia scopulorum, *Penicillus capitatus*, and *Flabellia petiolata).* Prasad *et al.*, 2010 [67] reported the presence of indole-3 acetic acid (IAA), zeatin, and Giberellic acid in *Kappaphycus alvarezii* and *Sargassum tenerrimum*. He also reported the presence of indole-3-pyruvic acid (IPA), zeatin, and gibberellic acid in *Gracilaria edulis*. Gupta *et al.*, 2011 [68] reported the presence of IAA, indole-3-butyric acid (IBA), salicylic acid (SA), and abscissic acid (ABA) in four wild Ulva species and in laboratory cultured Monostroma oxyspermum. Yokoya *et al.*, 2010 [69] reported presence of indole acetic acid (IAA), Indole-Acetamide (IAM) and ABA in elevan Rhodophyceae species of Bangiales, Gelidiales, Gracilariales and Gigartinales. Duan *et al.*, 1995 [70] reported the presence of zeatin in *L. japonica*. Stirk *et al.*, 2009 [71] reported presence of ABA in *Ulva fasciata* and *D. humifusa*. Plettner et al., 2005 72 reported ethylene production by *Ulva intestinalis*. García-Jiménez *et al.*, 2013 [73] also reported ethylene production by *Gelidium arbuscula*.

CONCLUSION: From the above analysis, it was concluded that the bioactive compounds identified in the methanolic extract of six algae extracts were aminoacids, vitamins, sugars, and phytohormones. The aminoacids and vitamins present in *Ulva lactuca*, *Ulva faciata*, *Acanthophora dendroides*, *Gracilaria coeticata*, and *Padina tetrastromatica* can be utilized for the development of dietary supplements and proteinaceous food for domestic animals. The sugar present in *Ulva faciata*, *Acanthophora dedroides*, *Gracilaria corticata*, *Padina tetrastromatica* and *Cystosera indica* can be utilized in food products, cosmetics, gelling and thickening agents. The phytohormones present in *Ulva lactuca*, *Gracilaria corticata*, *Padina tetrastromatica*, and *Cystosera indica* can be utilized in agriculture as plant growth regulators. Hence, these algae species are essential raw materials for the production of various pharmaceutical and biotechnological products.

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CONFLICTS OF INTEREST: Authors declare that there is no conflict of interest regarding the publication of this research article in the Journal

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