



Phycochemical Characterization of Marine macroalgae, *Sargassum tenerrimum* Collected from Beyt Dwarka, Western Coast of Gujarat, India

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ABSTRACT

Industries are moving towards finding a natural source of functionally active constituents which is better and safer materials to fulfill customers' demand. Marine algae contain a huge variety of biologically active compounds and express a promising role in different applications. Hence, the present study was carried out to characterize different biochemicals from brown alga *Sargassum tenerrimum* by FTIR, GCMS, HRLCMS Q-TOF, and ICP AES technique. First, the macroalga was collected from the Beyt Dwarka sea site, the Western coast of Gujarat, India. In FTIR, different types of bioactive functional groups were characterized as accountable for different beneficial components whereas ethanolic and methanolic extracts of *S. tenerrimum* reported fourteen and nineteen different beneficial phyco compounds in GCMS analysis, respectively. In HRLCMS Q-TOF analysis, two compounds were found carbohydrate derivatives and fifty-nine different compounds were determined to be different types of phycocompounds. Additionally, in the ICP AES study, Silicon was found to be high up in amount whereas Copper remained the minimum among studied elements. Moreover, the HRLCMS-QTOF study of amino acids reported that Glutamic acid (132.13 nmol/mL) was found to be the highest whereas Isoleucine (0.70 nmol/mL) was found to be the least amidst measured twenty-one amino acids. Bioactive potentials of these compounds have been reported in many previous studies. The inclusion of macroalgae-derived constituents in different applications has been broadly rising due to their bioactive potentials.

Keywords: *Sargassum tenerrimum*, Macroalga, Biochemical, FTIR, GCMS, HRLCMS.

INTRODUCTION

High energy requirements, natural, nutritious, harmless, and nontoxic compounds in different types of products such as food, cosmetics, dairy, pharma as well as aquaculture supplements promote the search for alternative natural renewable

and sustainable materials¹. These compounds are eco-friendly, sustainable, and economically cheaper. Mainly, great attention towards using biomass for overcoming the demerits of harmful and synthetic components. There are different types of natural resources utilized for different applications but among them, marine macroalgae are well known



due to having high nutritional compositions as well as the presence of bioactive compounds than terrestrial plants and animals². Marine macroalgae are similarly known as seaweeds, which are macroscopic, multicellular, eukaryotic, marine photosynthetic microorganisms. It is highly diversified in length and morphology³. They are mainly growing in brackish water as well as coastal zone (water close to the sea coast). It is found attached to rocky sea fronts, sand gravels, or floating freely⁴. Some species of macroalgae can reach up to 65 meters in length. Seaweed species occupy several ecological areas. Some algal species are found wet in seafoam whereas other algae can attach to substratum several meter deeps. Its growth expands for miles to the deeper sea⁵. Moreover, other species have become to live in tide pools and must tolerate hasty changes in environments such as temperature, drying, and salinity⁶. According to pigment composition, it can be divided into three types: red algae, brown algae, and green algae belong to Rhodophyta, Phaeophyta, and Chlorophyta, respectively. Among these three types, the Plantae kingdom comprises Chlorophyta and Rhodophyta whereas the Chromista kingdom comprises Phaeophyta⁷⁻¹⁰. Red algae mainly contain phycocyanin, phycoerythrin, chlorophyll a, and carotenoids such as lutein, zeaxanthin, and beta carotene. Brown algae are well known for chlorophyll (a & c), and carotenoids such as fucoxanthin whereas chlorophyll a, b, and carotenoids are found in green algae¹¹. As a result of the diversified components, macroalgae are extensively useful in many regions of the world as an ingredient of human food and animal feed, pharmaceutical, cosmeceutical, agriculture, and many more¹². Marine algae produce proteins, amino acids, carbohydrates, fatty acids (primary metabolites) which play their role in reproduction and normal growth as well as phenols, sterols, minerals, vitamins, and some other active constituents (secondary metabolites) found under stressful environmental factors such as Ultraviolet exposure, temperature, high salinity or pollutants¹³⁻¹⁶.

Many marine algae have been completely practiced by humans for different uses as food for humans, animal feed, fertilizers, dairy and food industries, etc. China and Europe have had old practices to collect marine algae for nutritional food purposes as long as 500 B.C.¹⁷ Macroalgae can be used as an alternative to today's foods that we

consume. It gives us high nutritional content, high caloric, and lesser fat. Macroalgae provide a good amount of carbohydrates, vitamins, minerals, and fibers that improve intestinal transition and reduce the cholesterol amount in the blood¹⁸. They also have an old use as an animal food but it depends on the algal species, variable composition, time of collection, and habitat as well as some other atmospheric factors such as light intensity, temperature, and nutrient availability¹⁹. Macroalgae have wide application in the agriculture sector to improve crop productivity. It contributes certain elements such as nitrogen, phosphorus, potassium, magnesium, iron, and calcium. Besides, it is utilized to regulate the pH of the soils and possesses plant growth-promoting components such as auxins, gibberellin, cytokinin, and others²⁰. It is also utilized to combat different plant infections caused by different insects and fungal pathogens²¹. Marine algae-derived bioactive molecules are widely used in various industrial applications like, shows anticoagulant, antitumor, anti-inflammatory, antimicrobial, antithrombotic, and immunomodulatory^{22,23}. Seaweed based bioactive compounds are useful in making soft drinks, glass, soaps, shampoos, dyes as well as biopolymers²⁴⁻²⁶. The phycocolloids exhibit an important action in pharmaceutical applications as a stabilizer, a thickener, as well as an, impart antimicrobial component²⁷. Macroalgae are widely advantageous in the cure and hair washing due to their binding ability with hair proteins²⁸. Previous studies reported that it can be used to treat warm infection, wound healing, treatment of gastritis, diarrhea, menstrual disorders, hypertension, skin disorders, ulcers, and syphilis²⁹⁻³¹. Moreover, it is also useful in the skin cosmeceutical applications such as antiwrinkle, photoprotection, skin whitening, anti-aging, moisturizer, antiacne, and other skin benefits^{32,33}.

Brown macroalga *Sargassum tenerrimum* is very commonly found on the western coast of Gujarat, India. The thallus of *S. tenerrimum* is soft, slender, light brown to greening brown in the color of about 30-40 cm in height. It has a very short main axis, terete, and alternatively arranged primary cylindrical branches with branchlets. Vesicles of *S. tenerrimum* are stalked, spherical, pointed, and ovate about 3-4 mm broad. It is fusiform, slightly compressive, edged, and possess toothed at the margin. Its holdfast discoid and grow on rocks in subtidal regions^{34,35}. The present study aims

to carry out the characterization of brown alga *S. tenerrimum* derived bioactive components such as fatty acids, amino acids, carbohydrates derivatives, as well as mineral content analysis by using different characterization techniques to show its phytochemical profile.

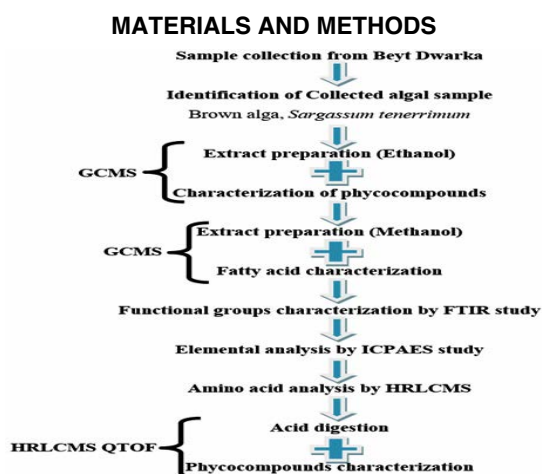


Fig. 1. Flow chart of the present study

Sample collection

The sample of marine macroalga *Sargassum tenerrimum* was done in sterile plastic bags from Beyt Dwarka sea site (22°28'37.7"N 69°07'48.2"E), the West coast of Gujarat, India. (Fig. 2-a) After collection, the sample was transferred (10°C) to the laboratory. This sample collection was carried out by handpicking from the low tide zone and environmental surroundings as follows: 0% Precipitation, 55% Humidity, 12 km/h Wind, and 27°C temperature. Then, this sample was cleaned with deionized water to separate extraneous materials and debris and kept shed dry at room temperature for six to seven days. This dried material was reduced to a fine powder by a mechanical grinder and preserved at freezing temperature until the next analysis.

Identification of *Sargassum tenerrimum*

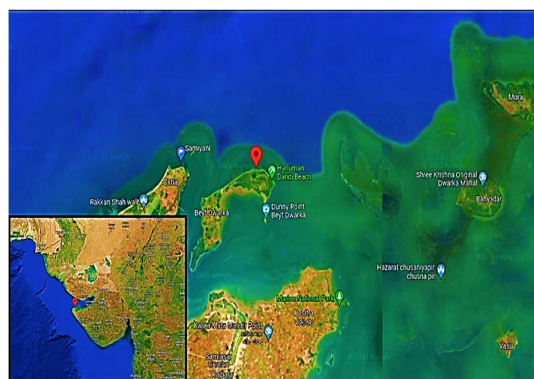
The sample identification was accomplished by taking the help of Dr. N. Joshi, at the Aquaculture department, Veraval, Gujarat-India. The authenticated image is illustrated in Figure. 2-b.

Phytochemicals characterization study by GCMS technique

Extract Preparation

0.5 kg of dry *S. tenerrimum*'s dried powder was added in 80% ethanol for 24 h by the continuous

hot percolation in the Soxhlet procedure. Afterward, the extract was then filtered and dried at 40°C for 24 h in a Hot air oven (REMI, India) to remove the excessive ethanol solvent (Sigma-Aldrich, India). This obtained filtrate was fixated to dryness at 20°C in reduced pressure (150 mbar) by using a vacuum evaporator (Sigma scientific, India). The above-concentrated extract was isolated in the air-free vessel and kept at freezing temperature (-20°C, Esquire Biotech, India).



(a)



(b)

Fig. 2(a). The geographical location of the sample collection site (in respect to Gujarat state, India); (b) Isolated sample of *Sargassum tenerrimum* (Chlorophyta)

GCMS Characterization study

For the characterization study, the T100GCV GC model and EB 5 column were used for chromatographic separation. GC specifications such as Helium (He) used as a Carrier gas; 1 mL/min flow rate; 200°C Injector operation; 50–250°C Column oven temp.; Injection mode: 10 °C/minute.

AccuTof Mass from the jeol model was used in mass spectrometry analysis. MS specifications

are as follows: 70 eV Ionization voltage; 250°C ion source temperature; 250°C interface temperature; 50–600 mass units mass range. The obtained gas chromatogram and mass spectra of the screened compounds were put in comparison with the available data of known compounds in the National Institute of Standard and Technology (NIST) library ver. 2005.

Characterization of Fatty Acids

The methanol solvent (99.8%) was used to prepare an extract from the shed dried fine powder of *S. tenerrimum*. This extract was prepared 10% (ratio 1:10) in a flask for three days. The above mixture was filtered out in another sterile vessel. After filtrates collection, the surplus solvent was eliminated by vacuum evaporator using fresh methanol solvent. This process was carried out twice with the same sample residues using fresh methanol. And this extract was used for further study. The GCMS characterization study was carried out by fixing the HP-5 column. Other specifications of GC and MS are used the same as above. The obtained gas chromatogram and mass spectra of each phycocompounds were put in comparison with known data in the NIST library (ver. 2005). The chemical identity, mol. weight and chemical framework of all obtained phycocompounds were regained and the percentage peak area was also calculated.

Functional Groups characterization study by FTIR

Total 5 mg of shed dried *S. tenerrimum's* a fine powder added with FTIR grade KBr (Potassium bromide) and evenly mixed to get a homogenized texture. This mixture was then placed in the mechanical mold using a sterile spatula and pressed by mechanical support for 30 seconds. This pellet of the mixture was conditioned on the pan proceeded for IR study. In this FTIR study, 3000 Hyperion Microscope with Vertex 80 FTIR model (Bruker, Germany) and 400–4000 cm^{-1} scanning range were used. The peak ratio (the peak value in the IR spectrum) was used to separate the functional groups of components.

Elemental analysis by ICP AES procedure Digestion and extraction procedure

All standard grade reagents (purchased from Sigma-Aldrich, India) were used in this analysis. About 50 milligrams of *S. tenerrimum's* powder was added into the TFM (modify PTFE-PolyTetraFluoroEthylene-) vessels. Following this,

the mixture of reagents (3 mL HCl + 1 mL HNO_3 + 1 mL HF + 1 mL H_2O_2) was added and the vessel was closed immediately. The digestion of this mixture was carried out in a microwave digester (Titan Microwave system, India) based on the below specifications: 15 min hold time 10 min with 130 degrees, and following 190 degrees ramp time. After cooling this hot vessel at 70°C, the sample mixture was vented and opened. By using Milli-Q water, made total volume up to 25 mL and then shaken completely to dilute the rest of the particles adhered to the vessel's wall. Blank was also kept for hydrolysis without adding the sample. The ICP-AES (Inductive Couple Plasma-Atomic Absorption Emission Spectrometer) instrument was considered to measure the amount of different elements. Model of the instrument: ARCOS, Simultaneous ICP Spectrometer.

Amino acid analysis

Acid digestion and detection

0.1 gram of *S. tenerrimum's* fine powder was weighed and added into the 12 mL of 6N hydrochloric acid in it and the mixture was tightly packed after adding pure N_2 gas. Put this test tube at 120°C temperature for 16 h in a hot air oven (REMI, India) for digestion. After hydrolysis, filtration was carried out and flash evaporation was done to eliminate excess hydrochloric acid. 0.05 N HCl was used to make a definite amount. Its filtration was carried out by a Whatman filter (0.45 μ size). The filtrate was used as a sample for analysis. Moreover, standard amino acids were also run to get a standard chromatogram. Specifications such as, model of the instrument: 6550 iFunnel QTOFs (Quadrupole Time of Flights), Agilent Technologies, USA; Column details: Poroshell HPH C18 (4.6 \times 100 mm), 2.7 μ ; 60°C temp. in the oven; analysis mode: non-switch flow.

Phyco compounds analysis by HRLCMS-QTOF Acid Hydrolysis and detection

The uniform fine powder was obtained by drying the algal sample at 40°C for 48 hours. A total of 0.1 g of the selected macroalgal sample was placed in a sterile airtight tube and add 10 mL of 2M HCl containing 1% phenol. Then, the tube was tightly closed in presence of N_2 gas and kept at 80°C for 3 h in a Hot air oven, allowing it to cool and Whatman no. 41 paper was used to carry out vacuum filtration. The obtained filtrate was diluted to make a final volume of 25 mL with ultrapure water which was again filtered to get the hydrolysate. Different phycocompounds were analyzed by the HRLCMS QTOF technique. Specifications include.

Model of the instrument: 6550 iFunnel Q-TOFs, Agilent Technologies, USA; Column detail: Hypersil GOLD C18 (100 mm × 2.1 mm × 3 μm); 250°C Gas Temp.; 3 L/min Flow of gas; 35 psig Nebulizer; 300°C Sheath Gas Temperature.

RESULTS

Characterization of phyco compounds

Total fourteen different phyco compounds were identified in *S. tenerrimum's* ethanolic extract based on retention time (RT) in minutes, percentage peak area (PA), mol. formula, and mol. weight. A gas chromatogram of *S. tenerrimum's*

ethanolic extract is illustrated in Fig. 3. PubChem ID, mol. formula, Kovats Index, mol. weight, %peak area, RT (min), and SMILE structure for each compound are reported in Table 1. Among identified phyco compounds, 1-O-butyl 2-O-(6-ethyloctan-3-yl) benzene-1,2-dicarboxylate is showed the highest percentage peak area (23.33%) with 23.23 min retention time whereas Propanoic acid, 2-[(1-cyclohexylethyl)carbamonyl]-methyl ester compound reported the lowest percentage peak area (0.47%) with 21.19 minutes retention time. The chemical framework of each compound is illustrated in supplementary data Table S1.

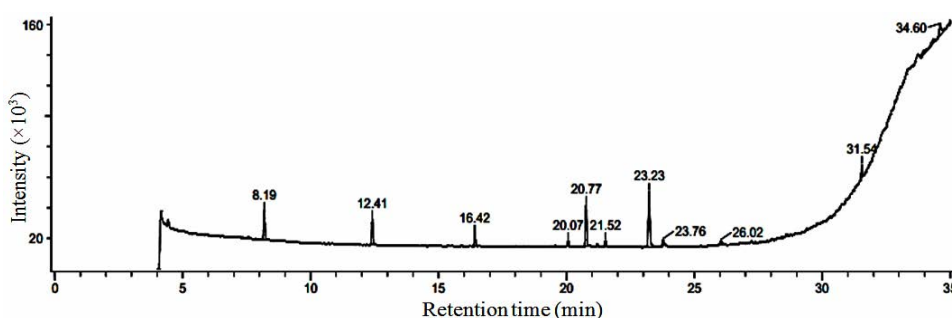


Fig. 3. The Gas chromatogram was obtained from the ethanolic extract of *S. tenerrimum*

Table 1: Chemical information of phycochemicals found in ethanolic extract of *S. tenerrimum*

No.	Name	Pub Chem Id	Mol. Formula	Mol. Wt. (g/mol)	Retention Time (Min)	KovatsIndex (Iu)	Peak Area%	SMILE
1	1-O-tetradecyl 4-O-(2,3,6-trichlorophenyl) butanedioate	91701556	C ₂₄ H ₃₅ Cl ₃ O ₄	493.9	8.19	3358	9.75	CCCCCCCCCCCCC COC(=O)CCC(=O)OC1 =C(C=CC(=C1Cl)Cl)Cl
2	3-Hexanone, 2,5-dimethyl-	15901	C ₈ H ₁₆ O	128.21	12.41	8241	8.22	CC(C)CC(=O)C(C)C
3	Pentanal	8063	C ₅ H ₁₀ O	86.13	16.42	707	4.25	CCCC=O
4	1,3-Propanediamine, N-methyl-	80511	C ₄ H ₁₂ N ₂	88.15	20.7	860	1.72	CNCCCN
5	Pentadecanal-	17697	C ₁₅ H ₃₀ O	226.4	20.77	1701	13.56	CCCCCCCCCCCC CCC=O
6	Propanoic acid, 2-[(1-cyclohexylethyl)carbamonyl]-methyl ester	541974	C ₁₃ H ₂₃ NO ₃	241	21.19	1816	0.47	CC(C1CCCCC1)NC (=O)C(C)C(=O)OC
7	[s]-(+)-1-cyclohexylethylamine	5325951	C ₈ H ₁₇ N	127.23	21.52	1059	1.91	CC(C1CCCCC1)N
8	1-O-butyl 2-O-(6-ethyloctan-3-yl) benzene-1,2-dicarboxylate	6423866	C ₂₂ H ₃₄ O ₄	362.5	23.23	2505	23.33	CCCCOC(=O)C1=CC= CC=C1C(=O)OC(CC) CC(C)CC
9	t- Boc-sarcosine	83692	C ₈ H ₁₅ NO ₄	189.21	23.77	1305	1.95	CC(C)C(OC(=O)N(C) CC(=O)O
10	2-Pyrrolidinone,1-phenyl-4-[1-(phenylmethyl)-1H-1,3-benzimidazol-2-yl]-	2947212	C ₂₄ H ₂₁ N ₃ O	367.4	26.02	3277	1.95	C1C(CN(C1=O)C2=CC =CC=C2)C3=NC4=CC= CC=C4N3CC5=CC= CC=C5
11	Benzyl icosanoate	562252	C ₂₇ H ₄₆ O ₂	402.7	31.54	2949	4.24	CCCCCCCCCCCCCC CCCCC(=O)OCC1= CC=CC=C1
12	Benzyl oleate	5368218	C ₂₅ H ₄₀ O ₂	372.6	34.09	2758	0.56	CCCCCCCCC=CCCCC CCCC(=O)OCC1=CC= CC=C1
13	1,1,3,3,5,5,7,7,9,9,11,11-Dodecamethylhexasiloxane	6329090	C ₁₂ H ₃₆ O ₅ Si ₆	428.92	34.33	1341	3.99	C[Si](C)O[Si](C)C)O [Si](C)C)O[Si](C)C)O [Si](C)C)O[Si](C)C
14	Propane, 1,3-bis(octadecyloxy)-	624534	C ₃₉ H ₈₀ O ₂	581.1	34.60	4050	6.22	CCCCCCCCCCCCCCC CCCCOCCOCCCCC CCCCCCCCCCCCC

1-*O*-tetradecyl 4-*O*-(2,3,6-trichlorophenyl) butanedioate and 1-*O*-butyl 2-*O*-(6-ethyl octan-3-yl) benzene-1,2-dicarboxylate are found to be carboxylic acid derivatives. 1,3-Propanediamine, *N*-methyl- α -1-undecyl ester are amine derivatives and primary aliphatic amine, respectively. Moreover, *t*-Boc-sarcosine and 2-Pyrrolidinone, 1-phenyl-4-[1-(phenylmethyl)-1*H*-1,3-benzimidazol-2-yl]- are *N*-methylglycine and 2-Pyrrolidone derivative, respectively. Hydrocarbon derivative Propane, 1,3-bis(octadecyloxy)- was found whereas Pentanal; Benzyl icosanoate; Benzyl oleate; Propanoic acid, 2-[(1-cyclohexylethyl) carbamonyl]- methyl ester; Pentadecanal-; and 3-Hexanone, 2,5-dimethyl- were also detected that belong to class fatty acid and fatty acid like molecule. Lastly, 1,1,3,3,7,7,9,9,11,11-

Dodecamethylhexasiloxane; is found to be Polymers (Siloxanes).

Characterization of fatty acids and derivatives

Different fatty acids and derivatives are identified in this characterization depending on RT (in min), % peak area, mol. formula, and mol. weight. The gas chromatogram for the methanolic extract is illustrated in Fig. 4 whereas nineteen different phytochemicals were identified which are tabulated in Table 2 with its chemical information. Among identified phytochemicals, *cis*-Vaccenic acid is found to be the considerable compound that had the large value of % peak area (52.96%) with 19.68 min retention time whereas 9-Octadecenamide compound reported the lowest percentage peak area (0.29%) with 27.41 min retention time.

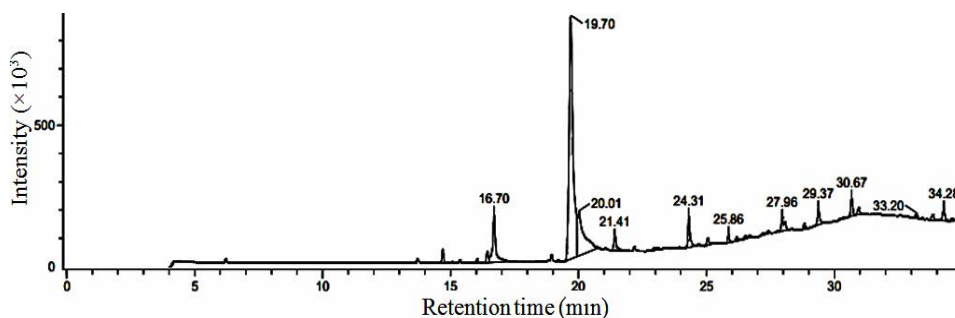


Fig. 4. The Gas chromatogram of *S. tenerrimum*'s methanolic extract

2-Monolinolenin, 2TMS derivative; is a lipid-like compound whereas *cis*-Vaccenic acid; Erucic acid; 4-Tridecene, (*Z*)-; 10-Undecenyl pentanoate; Methyl dihydrohydnicarbate; 9-Octadecenal; 9 Octadecenamide; 17-Octadecynoic acid; Linoleic acid, phenylmethyl ester; Oleic acid benzyl ester and Oleic acid are fatty acyl compounds which belong to class fatty acids or fatty acid derivatives. In addition, *n*-Hexadecanoic acid; is a saturated fatty acid. Some other compounds such as 9,17-Octadecadienal, [*Z*]- and 1-(4-Bromobutyl)-2-piperidinone are aldehyde derivative and organonitrogen heterocyclic compounds, respectively. In addition, carboxylic acid derivative cyclooctane acetic acid, 2-oxo- was found. Lastly, Phytol and Patchouli alcohol is found that belong to terpenes (Diterpenes) whereas hydrocarbon derivative methyl di-*t*-butyl hydroxyhydro cinnamate was also detected. The chemical framework of each compound is revealed in supplementary data Table S2.

FTIR characterization study

Characterization of the functional groups of an active constituent was done by FTIR depending on the value of peaks in the IR spectrum. The FTIR spectrum of *S. tenerrimum* is depicted in Fig. 5. Peak values, functional group, and compound class are revealed in Table 3. The bands found at different intensities such as 669.92 cm^{-1} , 820.73 cm^{-1} , 1064.33 cm^{-1} , 1091.40 cm^{-1} , 1338.88 cm^{-1} , 1420.08 cm^{-1} , 1509.02 cm^{-1} , 1543.82 cm^{-1} , 1655.96 cm^{-1} , 1702.36 cm^{-1} , 1733.29 cm^{-1} , 1771.96 cm^{-1} , 1872.50 cm^{-1} , 1992.37 cm^{-1} , 2928.14 cm^{-1} , 3450.16 cm^{-1} , and 3651.23 cm^{-1} corresponding to C-Br/C-Cl/C=C/C-H/C-F/S=O/C-O/C-N/O-H/N-O/C=N/C=C=N/N=C=S/N-H. These functional groups revealed the presence of halo, alkene, hydrocarbon, fluoro, sulfoxide, amine, Imino/oxime, alkene, isothiocyanate, amine salt, alcohol, and substituted constituents in the extracts of *S. tenerrimum*.

Table 2: Chemical information of phytochemicals found in *S. tenerrimum*'s methanolic extract

No.	Name	Pub Chem ID	Mol. Formula	Mol. Wt. (g/mol)	RT (Min)	KovatsIndex (lu)	Peak Area%	SMILE
1	Phytol	5366244	C ₂₀ H ₄₀ O	296.5	14.69	2045	1.36	CC(C)CCCC(C)CCC C(C)CCCC(=CO)C
2	Methyl 3,5-bis (tert-butyl)-4-hydroxy- hydrocinnamate	62603	C ₁₈ H ₂₈ O ₃	292.4	16.43	2134	1.45	CC(C)(C)C1=CC(=CC (=C1O)C(C)(C)O)CCC (=O)OC
3	n-Hexadecanoic acid	985	C ₁₆ H ₃₂ O ₂	256.42	16.70	1968	6.69	CCCCCCCCCCCC CCC(=O)O
4	cis-Vaccenic acid	5282761	C ₁₈ H ₃₄ O ₂	282.5	19.68	2175	52.96	CCCCC/C=C\CCCCC CCCCC(=O)O
5	Erucic acid	5281116	C ₂₂ H ₄₂ O ₂	338.6	20.02	2572	16.67	CCCCCCCC=CCCC CCCCCCCC(=O)O
6	4-Tridecene, (Z)-	5362712	C ₁₃ H ₂₆	182.35	21.41	1321	1.93	CCCCCCCC=CCCC
7	9,17-Octadecadienal, [Z]-	5365667	C ₁₈ H ₃₂ O	264.4	24.31	1997	3.62	C=CCCCCCCC=CCC CCCCC=O
8	Pentanoic acid,10- undecenyl ester	543363	C ₁₆ H ₃₀ O ₂	254.41	25.04	1769	0.92	CCCC(=O)OCCCC CCCC=C
9	Cyclopentaneundecanoic acid, methyl ester	535041	C ₁₇ H ₃₂ O ₂	268.4	25.85	1921	0.83	CO(=O)CCCCCCCC CC1CCCC1
10	9-Octadecenal	5283381	C ₁₈ H ₃₄ O	266.5	26.17	2007	0.42	CCCCCCCC=CCCC CCCC=O
11	Cyclooctane acetic acid, 2-oxo-	536995	C ₁₀ H ₁₆ O ₃	184.23	26.68	1647	0.38	C1CCCC(=O)C(CC1) CC(=O)O
12	9-Octadecenamide	5353370	C ₁₈ H ₃₅ NO	281.5	27.41	2228	0.29	CCCCCCCC=CCCC CCCC(=O)N
13	17-Octadecynoic acid	1449	C ₁₈ H ₃₂ O ₂	280.4	27.95	2165	1.84	C#CCCCCCCCCCCC CCCC(=O)O
14	Oleic acid	445639	C ₁₈ H ₃₄ O ₂	282.5	28.83	2140	0.62	CCCCCCCC=CCCC CCCC(=O)O
15	1-(4-bromobutyl)-2-piperidone	536377	C ₉ H ₁₆ BrNO	234.13	30.67	1635	2.08	C1CCN(C(=O)C1) CCCCBr
16	2-Monolinolenin, 2TMS derivative	5362857	C ₂₇ H ₅₂ O ₄ Si ₂	496.9	30.94	2804	0.83	CCC=CCC=CCC=CC CCCCC(=O)OC (CO[Si](C)(C)C)CO [Si](C)(C)C
17	Linoleic acid, phenylmethyl ester	5368290	C ₂₅ H ₃₈ O ₂	370.6	33.20	2760	0.69	CCCCC=CCC=CCC CCCCC(=O)OCC1= CC=CC=C1
18	Patchouli alcohol	10955174	C ₁₅ H ₂₆ O	222.37	34.27	1420	1.72	CC1CC2(C(C3CCC2 (C1C3)C)(C)C)O
19	9-Octadecadienoic acid[Z]-, phenylmethyl ester	5368218	C ₂₅ H ₄₀ O ₂	372.6	34.60	2758	0.47	CCCCCCCC=CCCC CCCC(=O)OCC1= CC=CC=C1

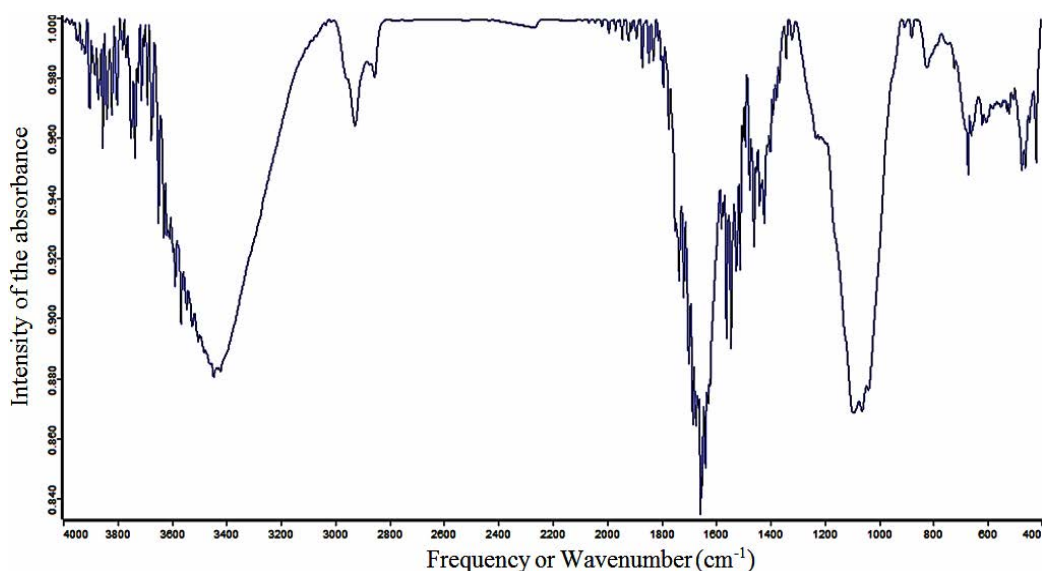
**Fig. 5. FTIR spectrum of *Sargassum tenerrimum***

Table 3: Characterization of functional groups of *S. tenerrimum*

Absorption	Appearance	Group	Compound Class
669.92	Strong	C-Br (S)	halo compound
	Strong	C-Cl (S)	halo compound
	Strong	C=C (B)	alkene(disubstituted)
820.73	Strong	C-Cl (S)	halo compound
	Medium	C=C (B)	alkene(trisubstituted)
	Strong	C-H (B)	1,4 disubstituted or 1,2,3,4 tetra substituted
1064.33	Strong	C-F (S)	Fluoro compound
	Strong	S=O (S)	Sulfoxide
	Strong	C-O (S)	Primary alcohol
1091.4	Medium	C-N (S)	Amine
	Strong	C-F (S)	Fluoro compound
	Medium	C-N (S)	amine
1338.88	Strong	C-O (S)	Secondary alcohol
	Strong	C-O (S)	aliphatic ether
	Strong	C-F (S)	Fluoro compound
1420.08	Strong	S=O (S)	Sulfone
	strong	C-N (S)	aromatic amine
	Medium	O-H (B)	Phenol
	Strong	S=O (S)	Sulfonamide
	Strong	S=O (S)	Sulfonate
1509.02	Medium	O-H (B)	alcohol
	medium	O-H (B)	Carboxylic acid
1543.82	Strong	N-O (S)	nitro compound
1655.96	strong	N-O (S)	nitro compound
	Weak	C-H (B)	aromatic
1702.36	Medium	C=N (S)	imine/oxime
	Medium	C=C (S)	alkene(vinylidene)
	medium	C=C (S)	alkene(distributed)
	Weak	C-H (B)	aromatic
	Strong	C=O (S)	Conjugated aldehyde
1733.29	strong	C=O (S)	Conjugated acid
	Weak	C-H (B)	aromatic
1771.96	Strong	C=O (S)	aldehyde
	Weak	C-H (B)	aromatic
1872.5	Strong	C=O (S)	Conjugated acid halide
	Strong	C=O (S)	Vinyl/ phenol ester
1992.37	Weak	C-H (B)	aromatic
	Medium	C=C=N (S)	alkene
2928.14	Strong	N=C=S (S)	isothiocyanate
	Weak	C-H (B)	aromatic
	Strong broad	O-H (S)	Carboxylic acid
3450.16	Weak broad	O-H (S)	alcohol
	Strong broad	N-H (S)	amine salt
	medium	C-H (S)	alkene
3651.23	Strong broad	O-H (S)	alcohol
	Medium sharp	O-H (S)	alcohol

Bond type: (S) Stretching; (B) bending;

Determination of Elements

Among different mineral elements, Silicon, Potassium, Calcium, and Magnesium were found in large proportion as compared to Sodium, Iron, Boron, and Copper in water extract of *S. tenerrimum* in ICP AES analysis. The result reported that Silicon (13.67%) was found in the highest amount whereas Boron (0.01%) remained at the lowest. The mineral % follows Silicon > Potassium > Calcium > Magnesium > Sodium > Iron > Boron order. The amount of each element (%) is tabulated in Table 4.

Determination of Amino Acids

In this analysis, a total of twenty-one

different types of amino acids were measured in *S. tenerrimum* by the HRLCMS-QTOF technique. A gas chromatogram containing different peaks for amino acids is illustrated in Fig. 6. Among 21 amino acids, Glutamic acid, Alanine, Glycine, and Aspartic acid were determined to be higher than 100 nmol/mL whereas Leucine, Serine, Arginine, Threonine, Tyrosine, Lysine, Phenylalanine, valine, and Isoleucine were found to be lower than 100 nmol/ml. Content (nmol/mL) of these amino acids were detected in the below order: Glutamic acid > Alanine > Glycine > Aspartic acid > Leucine > Serine > Arginine > Threonine > Tyrosine > Lysine > Phenylalanine > Valine > Isoleucine. Concentrations of detected amino acids (in nmol/mL) are presented in Table 5.

Table 4: Element contents of *S. tenerrimum*

Minerals	Amount in %
(Mean± S.E.M.)	
Boron (B)	0.01±0.01
Calcium (C)	1.36±0.09
Copper (Cu)	NO
Iron (Fe)	0.10±0.02
Potassium (K)	10.31±0.13
Magnesium (Mg)	0.66±0.06
Zinc (Zn)	NO
Sodium (Na)	0.12±0.03
Silicon (Si)	13.67±0.28
Selenium (Se)	NO

"NO" means less than 0.01 PPM;

Table 5: The total amino acid content of *S. tenerrimum*

Sr. No	Amino Acids	Amount in (nmol/ml)
1	Aspartic acid	106.13
2	Glutamic Acid	132.13
3	Methionine	ND
4	Asparagine	ND
5	Serine	55.45
6	Valine	9.37
7	Glutamine	ND
8	Histidine	ND
9	Isoleucine	0.70
10	Glycine	110.18
11	Threonine	28.35
12	Norvaline	ND
13	Arginine	34.67
14	Alanine	129.05
15	Tryptophan	ND
16	Tyrosine	25.46
17	Cystine	ND
18	leucine	63.82
19	Lysine	17.16
20	Hydroxproline	ND
21	phenylalanine	11.90

ND: Not detectable

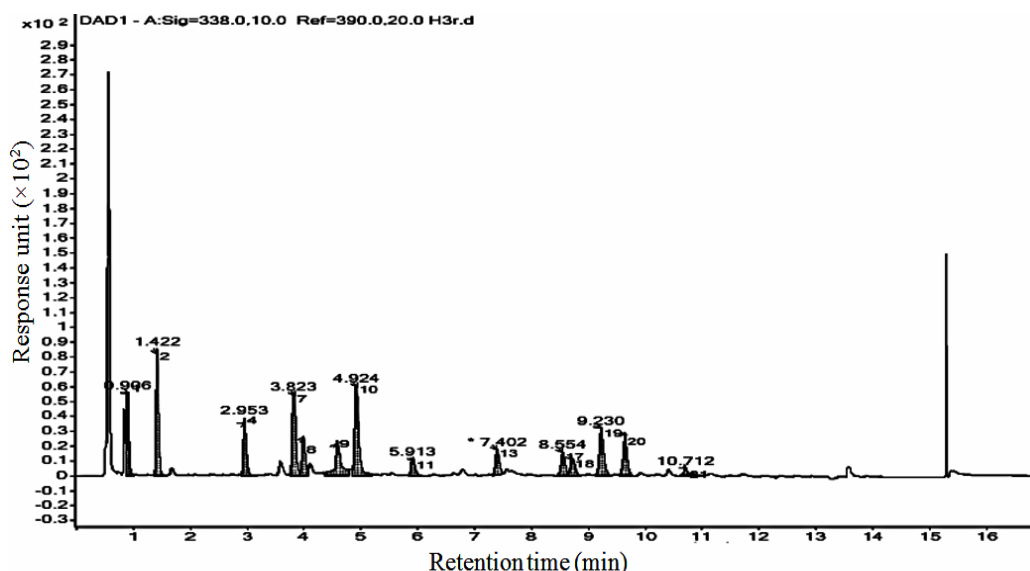


Fig. 6. Chromatogram for different amino acids by HRLCMSQTOF

Characterization of phycocompounds and derivatives

Total sixty-one compounds were found in methanolic extract of *Sargassum tenerrimum* by HR-LCMS Q-TOF analysis at different RT. In HRLCMS-QTOF analysis, obtained liquid chromatogram for this analysis is illustrated in Fig. 7. The different types of phycocompounds and their chemical information were revealed in Table 6. By comparing obtained data with the main library, all these compounds were characterized and identified. The chemical frameworks of compounds are illustrated in supplementary table S3. In this analysis, 9Z,11E-Hexadecadienal; 9,12,13-trihydroxy-10,15octadecadienoic acid; formyl 2E,4E,6Z-decatrienoate; 12-deoxy-J2-IsoP; 2(R)-HPOT; 5,8,11-Octadecatriynoic acid and 5-deoxy-J2-IsoP were belong to Fatty Acyls, Fatty acids and derivatives. Likewise, C16 Sphinganine; Phytosphingosine and 17-phenyl trinor Prostaglandin E2 serinol amide are amino alcohol that belongs to lipid and lipid-like molecule. In addition, organic heterocyclic compounds such as Isopiperolein B; Diversifolide; 2-Morpholinomethylestrone; Hydroxyatrazine; 2,4,6-Trimethyl-4-phenyl-1,3dioxane; Phendimetrazine; Imiquimod and 2-Phenylethyl propanoate were found whereas two carbohydrate derivatives 25-O-(2''-beta-Dglucopyranosyl-beta-Dglucopyranosyl)-25-hydroxy11E-eicosenoic acid and (1RS,2RS)-

Guaiacylglycerol 1 glucoside were found. Benzyl alcohol, Benzenemethanol, 2-(2hydroxypropoxy)-3-methyl-; secondary alcohol, Convallasaponin A; carboxylic ester, Heptyl p-hydroxybenzoate; carboxylic acid derivative, Procaine; aldehyde derivative, 2,3-Butanedione trimer; amino acid derivative, Alpha,beta-Didehydrotryptophan; Amine, Benzonatate, and Nylidrin; alkaloid, Codonocarpine; Narwedine; Isocorydine (+); 3-Ethylethcathinone and Salsolidine; organophosphorus compound, 13-Octadecene-9,11-diynoic acid, (Z)- and Triphenylphosphine oxide; terpenes (Diterpenes) like compound, Cyathin A3 and 18-Nor-4(19),8,11,13 abietatetraene were identified. Vitamin B complex, (nicotinic acid), Beta-Butoxyethyl nicotinate was also identified in *S. tenerrimum*. An antibiotic N2'-Acetylgentamicin C1a (gentamycin) and peptide Tumonoic Acid I were also detected. Moreover, hydrocarbon derivatives, 1,4-Diethylbenzene and Benzo[a]fluorene; an aromatic ether, Juvocimene 2; organic amino compound, Pirimicarb; organic compounds, Buspirone; Dehydrozyleuton; (E)-1-Cinnamoylpyrrolidine and Ecklonialactone A; organonitrogen compounds, PAC-1; Distichonic acid A; 5-(alpha-Phenylethyl)semioxamide and Istamycin C1; as well as organosulfur compounds, Sulfabenzamide; 3-(2-Methyl-3-furylthio)-2-butanone and Dicyclohexyl disulfide were identified in *S. tenerrimum*.

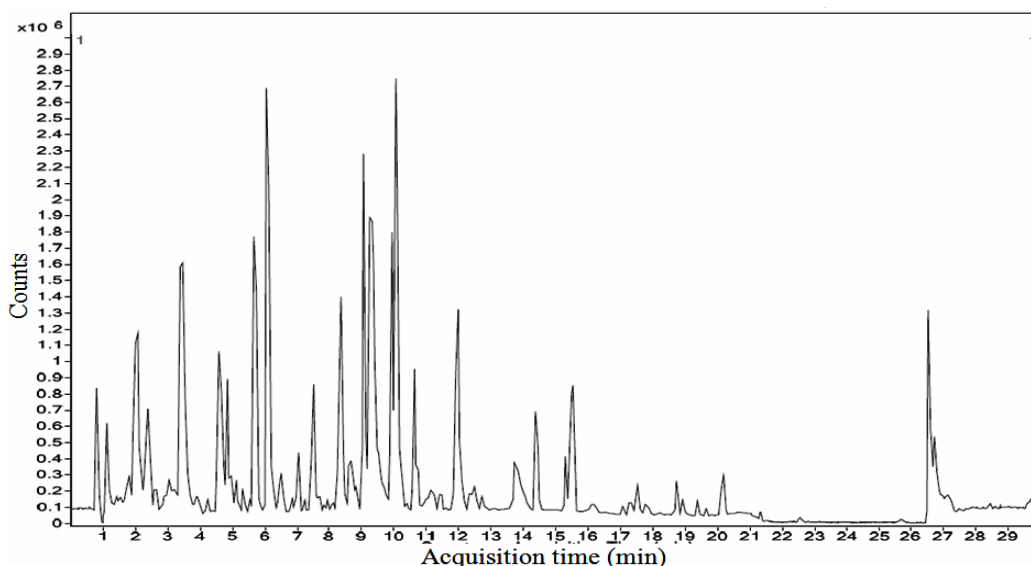
Fig. 7. Chromatogram for phycocompounds' of *S. tenerrimum* by HRLCMSQTOF

Table 6: Chemical information of identified phycocompounds and derivatives by HRLCMS-QTOF

No.	Name	PubChem ID	Molecular Formula	RT (min)	Mass (Da)	Hits (DB)
1	3-(2-Methyl-3-furylthio)-2-butanone	12980878	C ₉ H ₁₂ O ₂ S	0.79	184.0576	3
2	Sulfabenzamide	5319	C ₁₃ H ₁₂ N ₂ O ₃ S	0.795	276.0554	3
3	9Z,11E-Hexadecadienal	11172431	C ₁₆ H ₂₈ O	1.945	236.2118	10
4	1,4-Diethylbenzene	7734	C ₁₀ H ₁₄	1.995	134.1074	10
5	Benzo[a]fluorene	9195	C ₁₇ H ₁₂	2.235	216.0966	5
6	5-(alpha-Phenylethyl)semioxamazide	249877	C ₁₀ H ₁₃ N ₃ O ₂	2.3	207.1003	6
7	Hydroxyatrazine	135398733	C ₈ H ₁₅ N ₅ O	2.384	197.1273	9
8	Salsolidine	10302	C ₁₂ H ₇ NO ₂	2.618	207.125	10
9	2,3-Butanedione trimer	193527	C ₁₂ H ₁₈ O ₆	3.203	258.1092	9
10	Procaine	4914	C ₁₃ H ₂₀ N ₂ O ₂	3.487	236.1518	3
11	Nylidrin	4567	C ₁₉ H ₂₅ NO ₂	3.842	299.1929	2
12	Distichonic acid A	85405750	C ₁₀ H ₁₈ N ₂ O ₈	3.897	294.1069	10
13	Isopiperolein B	16041826	C ₂₁ H ₂₉ NO ₃	4.264	343.2197	5
14	Diversifolide	73232471	C ₁₃ H ₂₀ O ₃	4.535	224.1406	10
15	Isocorydine (+)	10143	C ₂₀ H ₂₃ NO ₄	4.637	341.1619	10
16	(1RS,2RS)-Guaicylglycerol 1glucoside	131751407	C ₁₆ H ₂₄ O ₁₀	4.671	376.1377	10
17	2-Morpholinomethylestrone	235463	C ₂₃ H ₃₁ NO ₃	4.675	369.2368	10
18	Istamycin C1	46174030	C ₁₉ H ₃₁ N ₅ O ₆	4.87	431.2721	6
19	Dehydroxzyuleton	10104880	C ₁₁ H ₁₂ N ₂ OS	5.186	220.0666	10
20	17-phenyl trinor ProstaglandinE2 serinol amide	35026314	C ₂₆ H ₃₇ NO ₆	5.199	459.2662	10
21	Tumonoic Acid I	2505812	C ₂₇ H ₄₇ NO ₇	5.344	497.3423	1
22	Benzenemethanol, 2-(2hydroxypropoxy)-3-methyl-	85909061	C ₁₁ H ₁₆ O ₃	5.623	196.109	10
23	Imiquimod	57469	C ₁₄ H ₁₆ N ₄	5.821	240.1355	4
24	beta-Butoxyethyl nicotinate	14866	C ₁₂ H ₁₇ NO ₃	5.984	223.1212	7
25	Benzenemethanol, 2-(2hydroxypropoxy)-3-methyl-	85909061	C ₁₁ H ₁₆ O ₃	6.089	196.1092	10
26	2-Phenylethyl propanoate	31225	C ₁₁ H ₁₄ O ₂	6.092	178.0989	10
27	2,4,6-Trimethyl-4-phenyl-1,3dioxane	107381	C ₁₃ H ₁₈ O ₂	6.466	206.1301	10
28	Convallasaponin A	441883	C ₃₂ H ₅₂ O ₉	6.854	580.3744	2
29	N2'-Acetylgentamicin C1a	16069998	C ₂₁ H ₄₁ N ₅ O ₈	6.901	491.2955	4
30	(E)-1-Cinnamoylpyrrolidine	2056198	C ₁₃ H ₁₅ NO	7.597	201.115	6
31	Heptyl p-hydroxybenzoate	14138	C ₁₄ H ₂₀ O ₃	7.639	236.1408	10
32	13-Octadecene-9,11-diyonic acid, (Z)-	5312685	C ₁₈ H ₂₆ O ₂	8.297	274.1925	10
33	Phendimetrazine	30487	C ₁₂ H ₁₇ NO	8.355	191.1307	10
34	9,12,13-trihydroxy-10,15octadecadienoic acid	5312876	C ₁₈ H ₃₂ O ₅	8.419	328.2252	10

35	13-Octadecene-9,11-diyonic acid, (Z)-	5312685	$C_{18}H_{26}O_2$	8.655	274.193	10
36	Benzonate	7699	$C_{30}H_{53}NO_{11}$	15.582	603.3757	2
37	alpha,beta-Didehydrotryptophan	5280990	$C_{11}H_{10}N_2O_2$	8.71	202.076	5
38	Codonocarpine	5281820	$C_{26}H_{31}N_3O_5$	8.856	465.22	3
39	beta-Butoxyethyl nicotinate	14866	$C_{12}H_{17}NO_3$	8.878	223.1213	7
40	13-Octadecene-9,11-diyonic acid, (Z)-	5312685	$C_{18}H_{26}O_2$	8.995	274.1926	10
41	9,12,13-trihydroxy-10,15octadecadienoic acid	5312876	$C_{18}H_{32}O_5$	9.118	328.2252	10
42	3-Ethylethcathinone	91696115	$C_{13}H_{19}NO$	9.291	205.1462	8
43	formyl 2E,4E,6Z-decatrienoate	11052245	$C_{11}H_{16}O_2$	9.468	180.114	10
44	12-deoxy-J2-IsoP	52921986	$C_{20}H_{28}O_3$	9.519	316.2034	10
45	2(R)-HPOT	23724727	$C_{18}H_{30}O_4$	9.522	310.2137	10
46	5,8,11-Octadecatriynoic acid	5312681	$C_{18}H_{24}O_2$	9.581	272.1769	10
47	Ecklonialactone A	23428255	$C_{18}H_{26}O_3$	9.719	290.1876	10
48	C16 Sphinganine	656816	$C_{16}H_{35}NO_2$	9.989	273.2665	1
49	Phytosphingosine	122121	$C_{18}H_{39}NO_3$	10.013	317.2925	1
50	Triphenylphosphine oxide	13097	$C_{18}H_{15}OP$	10.096	278.0855	10
51	17beta-Hydroxy-2-oxa-5alphaandrostan-3-one	252289	$C_{18}H_{28}O_3$	10.155	292.2033	10
52	Cyathin A3	442017	$C_{20}H_{30}O_3$	10.22	318.2185	10
53	18-Nor-4(19),8,11,13abietatetraene	5320205	$C_{19}H_{26}$	10.593	254.2084	7
54	Buspirone	2477	$C_{21}H_{31}N_5O_2$	10.667	385.2457	3
55	Juvocimene 2	131752882	$C_{20}H_{26}O_2$	10.913	298.1926	10
56	5-deoxy-J2-IsoP	52921971	$C_{20}H_{28}O_3$	10.977	316.2031	10
57	Narwedine	10356588	$C_{17}H_{19}NO_3$	11.148	285.136	10
58	PAC-1	135421197	$C_{23}H_{28}N_4O_2$	12.134	392.2216	10
59	25-O-(2'-beta-Dglucopyranosyl-beta-Dglucopyranosyl)-25-hydroxy11E-eicosenoic acid	42607366	$C_{32}H_{58}O_{13}$	14.394	650.3867	9
60	Pirimicarb	31645	$C_{11}H_{18}N_4O_2$	26.713	238.1402	4
61	Dicyclohexyl disulfide	17356	$C_{12}H_{22}S_2$	26.721	230.1177	1

Many seaweeds derived bioactive compounds have been applicable in skin cosmetic applications for varieties of skin benefits. Many previous studies reported that Dicarboxylic acids and derivatives reported benefits as a pH adjuster, fragrance enhancer as well as emollient³⁶. Phthalic acid ester showed antibacterial activities against different bacterial species such as *Streptococcus epidermidis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Bacillus subtilis*³⁷. Different commercially available products were reported containing Hexadecane. The former commercially available products can be used as a softener, emollients, stabilizers, and thickeners. Likewise, Fatty acids and derivatives have an important role in regulation and healthy benefits for the skin. First, fatty acids are important useful in normal skin barrier functions. Long-chain fatty acids have become helpful in skin hydration, protective barrier, and metabolic regulators. Medium-chain fatty acids show antiinflammation as well as antitumor activity. In addition, short-chain fatty acids promote the immune system and anti-inflammatory benefits³⁸. Moreover, fatty acids have antibacterial properties which can be used as preservatives and lubricants^{39,40}. Ahmad *et al.*,⁴¹ revealed the presence

of different bioactive constituents such as alcohol, carboxylic acids, ethers, esters, ketones, amides, alkanes, and aldehydes by FTIR characterization study. These types of compounds are beneficial for various applications mainly antimicrobial, photoprotection, food flavoring, color enhancer, preservatives as well as anticancer activity⁴²⁻⁴⁵. Halogenated compounds are reported to use in face care products, antiseptic and antiacne, deodorants, antiperspirant, as well as antimicrobial benefits^{46,47}. Many previously existing studies demonstrated the benefits of minerals and amino acids for skin health benefits. Mainly, it plays its role in the regulation of epithelial layers, skin nourishment, skin repair, topical formulations as well as skin protectants⁴⁸⁻⁵⁰. Except for skin benefits, it is helpful in nutraceutical and pharmaceutical formulation, in food and dairy applications, as well as in animal feed supplements.

CONCLUSION

Oceans contain a huge diversified marine organism, comprise the majority area of the earth. Among them, marine macroalgae offer a wide variety of bioactive ingredients such as polysaccharides, amino acids, proteins, vitamins, fatty acids,

bioactive peptides, etc. It confers a broader range of beneficial actions such as antimicrobial, anticancer, antioxidant, anti-inflammation, antiaging, anticancer as well as other benefits in food and dairy sectors, medicinal and pharmaceutical sectors, fuel and remediation, agricultural benefits, etc. Researchers from around the world have demonstrated the biological potential of different macroalgae and derived compounds. The present findings showed that the Phaeophyta *S. tenerrimum* is a valuable source of bioactive ingredients. In the finding, 1-O-butyl 2-O-(6-ethyloctan-3-yl) benzene-1,2-dicarboxylate; Pentadecanal-; 1-O-tetradecyl 4-O-(2,3,6-trichlorophenyl) butanedioate; 3-Hexanone, 2,5-dimethyl-; Propane, 1,3-bis(octadecyloxy)-; Pentanal; Benzyl icosanoate, 1,1,3,3,5,5,7,7,9,9,11,11-Dodecamethylhexasiloxane, and t- Boc-sarcosine, etc. compounds in ethanolic extracts whereas cis-Vaccenic acid, Erucic acid, n-Hexadecanoic acid, 9,17-Octadecadienal, [Z]-, 1-(4-bromobutyl)-2-piperidone, 4-Tridecene, (Z)-, 17-Octadecynoic acid and Oleic acid were majorly found in methanolic extract of *S. tenerrimum*. Likewise, Lipid and lipid-like molecules, Carboxylic acid derivatives, Carbohydrate derivatives, Terpenes like compounds, alkaloids as well as some other organic compounds were screened in the HRLCMS characterization study. In addition, Glutamic Acid, Alanine, Glycine, Aspartic acid, Leucine, Serine, Arginine, Threonine, Tyrosine, and Lysine amino acids were obtained in larger amounts whereas Silicon, Potassium, Calcium, Magnesium, and Sodium elements were measured higher in amount. This finding revealed a very good phytochemical profile and

that compounds can be applied in different types of applications after successful experimentation further. These compounds are natural, less toxic, economical, almost inexhaustible, and safer than synthetic ingredients. By different characterization studies, selected marine alga reported a good type of potentially active components. *S. tenerrimum* or derived bioactive constituents can be utilized whole or part in various applications such as food, beauty enhancer, medicinal and pharmacological properties, antioxidant activities, antimicrobial, anti-inflammatory, anticancer, antidiabetic activity, antiviral activity, cellular growth, plant growth promotion, vermifuge activity, antitumor activity, antiulcer, wound healing treatment, Goitre treatment, industrial for fuel production, renewable energy suppliers, animal feed preparation, organic manure preparation, domestic sewage treatment, wastewater treatment, etc. Along with its large availability of biomass (particularly on the Beyt Dwarka sea site), no need to worry about its cultivation, it could be utilized in various applications or product preparations after successful *In vitro* and *in vivo* evaluation as well as clinical assessment.

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Conflict of Interest: "No"

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