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Saponins and the *in vitro* bioactivities of different solvent extracts of some tropical green and red seaweeds

Abhilash Parameswaran Kailas*, Muraleedharan Sukumaran Nair

Department of Chemical Oceanography, School of Marine Sciences, Cochin University of Science and Technology, Cochin 682016, India

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ABSTRACT

Objective: To evaluate the antioxidant and antimicrobial activities of the saponins content and the corresponding sequential extracts prepared using petroleum ether, chloroform, ethyl acetate and acetone, of some seaweed species [consisting of red *Gracilaria corticata, Gracilaria corticata* var. *cylindrica* and *Gracilaria foliifera* and green *Enteromorpha prolifera*, *Ulva fasciata* and *Chaetomorpha antennina* (*C. antennina*) seaweeds], obtained from the southwest coast of India.

Methods: The fractions were collected during the extraction of saponins and the saponins were chemically investigated for the saponification and iodine value. The antioxidant activities were estimated using the 2,2-diphenyl-1-picrylhydrazyl, UV-vis ferrous tartrate and $KMnO_4$ titration methods against four standards. The antimicrobial activities were estimated against four pathogenic organisms. Bioactive fractions except saponins, were further investigated through gas chromatography-mass spectrometer. Statistical correlations were done to identify the correlation pattern between the activity and constituents.

Results: The seaweed extracts exhibited appreciable biochemical activities. In general, the Chlorophyta had rich resource of saponins. *C. antennina* showed high content of saponins. Antioxidant activities were seen to be high when the saponins and fatty acid fractions were screened. *C. antennina, Enteromorpha prolifera, Gracilaria corticata* var. *cylindrica* and *Gracilaria foliifera* exhibited commendable antioxidant activities. Antibacterial activity was exhibited the highest in the extracts of *Ulva fasciata*. Selective antimicrobial inhibition was observed throughout. Gas chromatography-mass spectrometer investigation showed appreciable contents of ω-3, ω-5, ω-6, ω-7, ω-8, ω-9 and rare ω-11 fatty acids along with other saturated fatty acids.

Conclusions: The correlation studies underlined the relation between the biochemical compositions and activities. These seaweeds could be considered as a potential source of valuable natural antioxidants, antibiotics and nutraceutical compounds.

1. Introduction

Algae are photosynthetic organisms which exhibit greater diversity and have gained potential interest as a resource of bioactive compounds. Seaweeds were seen to be eaten for centuries by humans living along the coastlines all around the world. Ireland has a rich tradition of using algae in soups. In many other countries, it is also used as an animal fodder. Henceforth, the cultivation was promoted, with special considerations on red and brown species which has a traditional background for the use in medicinal purposes due to their richness in bioactive compounds[1,2]. Seaweeds are not only edible but also tasty and healthy, with large and fairly unexplored potential use in the cuisine. No daily recommendation for intake of seaweeds is available. It's mainly due to the high volatility of its biochemical compositions. In most of the countries, no special regulations have been defined for seaweed consumptions, so that, it has to follow the general safety regulations. The direct human consumption of a broad range of seaweeds as food is increasing over Asia. The western world has shown the increase in terms of seaweed extracts as an application in nutraceuticals[1].

Assessment of the chemical composition of marine organisms is an essential element to evaluate its economical, ecological and biological roles. Seaweeds produce multifaceted compounds

*Corresponding author: Abhilash Parameswaran Kailas, Department of Chemical Oceanography, School of Marine Sciences, Cochin University of Science and Technology, Cochin 682016, India.
Tel: +91 9656 104185
E-mail: abhilashpkailas@gmail.com

like vitamins, proteins, polysaccharides, flavonoids, carotenoids, phenolics and secondary metabolites including terpenoids, saponins, fatty acids and its combinations[3]. These compounds exhibit a wide range of actions on adverse agents that are present in the marine environment[4] and vary with the intrinsic and extrinsic factors such as genetic aspects, age, heat, light, air, mode of extraction, extraction solvent medium, extraction duration, drying procedure and storage[5].

Florae offer a large range of natural compounds that belong to various molecular groups with different bioactivity properties in humans. This versatility in chemical properties led to the rise in several researches on plants to derive on compounds that could be used in advanced medicines. Saponins are considered as a key ingredient in the traditional Indian and Chinese medicine, which are observed for most of the biological effects. This group of compounds are known to produce healing effects on inflammations and so is used up commercially by the nutraceutical industries. They exhibit beneficial effects on lowering the blood cholesterol, acting against cancer along with antiviral and antimicrobial properties[6]. They include the cardiac glycosides which are used in the treatment of cardiac disorders and cardiac arrhythmias[7]. Seaweeds have been already reported with appreciable amounts of saponins content[8,9].

Selection of the extraction solvents plays a great role in the determination of the chemical constituents and their antioxidant and antimicrobial activities. Studies promote the use of methanolic extract for higher antimicrobial activity than the direct *n*-hexane and ethyl acetate extracts[10]. Organic solvents were observed to yield high activities in comparison to water based extractions[11].

Fresh and processed food products are preserved in order to maintain its quality and to extend the shelf life. Chemical preservatives and antioxidants are generally used, but legislations have restricted their elaborate use in foods. Reactive oxygen species and oxidative stress generated in human beings due to the changing living conditions have led to the occurrence of a variety of chronic diseases, including coronary heart disease, cancers, diabetics, *etc*. Reactive oxygen species are highly toxic that result in cell death and degradation of tissues. These highly reactive species have to be destroyed or neutralised by antioxidants supplied through foods which are either artificial or natural[12]. Artificial antioxidants like the butylated hydroxytoluene (BHT), butylated hydroxyanisole, resorcinol, *etc*., were found to be highly effective, but the concerns on its toxicity and carcinogenicity, led to the search for the natural alternatives. Severe light, temperature and oxygen, to which the marine algae are exposed to, lead to the generation of free radicals and other reactive species. But, seaweeds are observed to be resistant to these conditions, and propagating in their growth patterns[12]. Their stability suggested the presence of protective and antioxidative defence systems that include compounds such as phenolics, vitamins (C, D and E), carotenoids, saponins, monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs), *etc*.[12]. The antioxidant assay was seen important in the chemical investigation of seaweeds. Many related works suggested the need to carry out more than one type of antioxidant activity measurement, in order to determine the various mechanisms of antioxidative activities as no particular studies would reflect all the aspects of the antioxidant activities. Methods for the determination of antioxidant capacity differ in terms of their assay principles and experimental conditions.

Current consumer preferences lead to foods that contain minimal chemicals and are microbiologically safe. Many sterilisation methods are adopted to maintain the microbiological load to minimum level, with non-thermal processes gaining higher attractions. These processes include the addition of natural antibacterial extracts[13], high pressure CO_2, high intensity pulsed electric field, irradiation, ultrasound *etc*., which could preserve food without degradation of its organoleptic quality. The use of natural antioxidant and antimicrobial agents that inhibit pathogen growth and deterioration has gained recent attractions. Seaweeds with high bioactive composition have strong bactericidal and antioxidant activity. Phytochemicals with antioxidant and antimicrobial activity were observed in red, green and brown algae[14]. Bacterial diseases are usually arrested using drugs or chemicals, which results in the mutation of bacteria due to their indiscriminate use. It becomes a greater problem of giving treatments to those infected with such mutated microorganisms. Advanced antibiotics are being developed whose cost is high and the efficacy is still interrogative. The decreased efficacy of antibiotics to drug resistance bacteria has made the search for alternatives from natural sources. Marine biota has proved to be worth in this search which consisted of diverse secondary metabolites, especially seaweeds, that exhibit bactericidal and bacteriostatic properties[15]. Brown algae were reported to be active against both Gram-positive and Gram-negative organisms[16]. Since ancient times, the antimicrobial properties of the seaweeds were recognized, even though the in-depth exploration has not been done so far.

Treatment of cut vegetables with edible seaweed juice containing such PUFAs and volatile compounds were worthy for the studies in the prevention of vegetable browning and poisoning[17]. Omega-3 PUFAs including eicosapentaenoic acid and docosahexenoic acid were found to have beneficial clinical and nutraceutical applications[18]. Omega-3 fatty acids are essential unsaturated fatty acids which have to be provided through diet for human and the primary sources are represented by marine fishes and fish oils[19]. As an alternative source, marine macro algae have been studied as potential sources of unsaturated fatty acids which could be cultivated on large scale[20]. Fatty acids like the linolenic acid, palmitoleic acid, hexadecatrienoic acid, eicosapentaenoic acid, other saturated fatty acids (SFAs), ω-3 and ω-6 unsaturated fatty acids were reported with antimicrobial activities which act through their detergent activity by destroying the bacterial membranes through auto-oxidation[21]. Many topical applications containing these fatty acids have seen to be highly effective against bacterial infections[22]. Mixture of dichloromethane, methanol and acetone were reported to extract diterpenes from *Dictyota menstrualis*[5]. Macro algae were reported to have low lipid contents with high PUFA contents in comparison to terrestrial plants[23]. Gas chromatography-mass spectrometer (GC-MS) analysis of the essential oils of brown, red and green seaweeds, extracted using dichloromethane and pentane mixture showed the presence of flavour compounds such as hexenal, nonanal and nonenal with antimicrobial activities against *Escherichia coli* (*E. coli*) and *Erwinia carotovora*[17].

Current study focused on the determination of the bioactive potentials of the six selected seaweeds from the southwest coast of India (Kerala coast). Saponins content, sequential solvent extractions using petroleum ether, chloroform and ethyl acetate and its corresponding saponification and iodine values, antioxidant assay using 3 methods, antimicrobial assay against 2 Gram-positive and 2 Gram-negative bacteria and the GC-MS profiling were determined to substantiate the purpose of this study.

2. Materials and methods

2.1. Chemicals used

All the standards were brought from Sigma (USA) and controls from ChromaDex (USA). Chemicals used were of analytical grade and the solvents were purchased from Merck (Germany) in high performance liquid chromatography grade. Water used was purified on a Milli-Q® system from Millipore (Bedford, Massachusetts, USA). Quantifications were done in triplicates against standards and results were reported with standard deviations. Chloramphenicol and tetracycline were purchased from Sigma (USA). Mannitol salt agar, nutrient agar, Levine eosin methylene blue agar, xylose lysine deoxycholate agar and soyabean casein digest agar were purchased from Difco, USA.

2.2. Pathogen culture used

Lyophilised cultures (live strains) of *E. coli* ATCC 25922 - Gram-negative rod, *Salmonella abony* (*S. abony*) NCTC 6017 - Gram-negative rod, *Bacillus cereus* (*B. cereus*) ATCC 10876 - Gram-positive rod and *Staphylococcus aureus* (*S. aureus*) ATCC 6538 - Gram-positive cocci were used.

2.3. Sampling

Six seaweeds, comprising Chlorophyta and Rhodophyta groups were collected from the Kerala coast, South India (Table 1). *E. prolifera* was obtained from two locations. Samples were cleaned in salt water thrice followed up with fresh water and then dried under shade, ground and sifted through 300 microns nylon sieves. The fine powder was stored at room temperature maintaining the moisture level < 2% and the ground samples were used up in further analysis.

2.4. Estimation of saponins

Saponins contents of the seaweed samples were estimated by the double solvent gravimetric method[24]. 2 g of the ground seaweed sample was mixed with 50 mL of 20% aqueous ethanol solution and heated at 50–60 ˚C in a water bath for 90 min. The supernatant solution was filtered off through a Whatman No. 40 filter paper. The residue was re-extracted twice and the supernatants were combined. The combined supernatant was reduced to 40 mL at 90 ˚C and transferred to a separating funnel. A volume of 40 mL of diethyl ether was added and vigorously shaken. The ether layer was separated off and the aqueous layer was re-extracted twice with 40 mL of diethyl ether. The diethyl ether layer was kept aside. The aqueous layer was extracted further with 60 mL of *n*-butanol thrice. Combined *n*-butanol layer was dried in a pre-weighed evaporating dish at 60 ˚C, and the weight of the residue was estimated, identified as saponins fraction (SF) and reported as the percentage of saponins to dry weight of the seaweeds.

2.5. Preparation of fractions

The residue of the above saponins extractions including the seaweed residue, diethyl ether layer and aqueous layer were combined together desolventized and then repeatedly extracted using 50 mL each of 90% methanol-water until the supernatant was clear. The aqueous methanolic solution was then reduced to half of the extract volume. Later, 100 mL 6 mol/L methanolic KOH was added and refluxed for 3 h. The extract volume was again reduced to one fourth under vacuum at 50 ˚C and transferred to a separating funnel and sequentially extracted using petroleum ether and ethyl acetate. The extracts were collected after desolventization under vacuum at 40 ˚C and named as petroleum ether fraction (PEF) and ethyl acetate fraction (EAF). The left over aqueous phase was acidified to pH 2.0 to 2.5 and extracted using chloroform. The dried layer was named as chloroform fraction (CF). All the above fractions were further subjected to saponification value, iodine value, antioxidant activity, antibacterial activity and GC-MS profiling analysis.

2.6. Determination of saponification and iodine values

Saponification and iodine values provide information regarding the extent of unsaturation and availability of saponifiable matters. Estimations were done based on the analytical procedures evidenced in the Indian Pharmacopoeia[25,26]. The saponification value was reported as the milligram of KOH required per gram of the sample and iodine value was reported as the weight of iodine absorbed per gram of the sample.

2.7. Antioxidant activity

In the present study, antioxidant activities of the extracts of seaweeds were evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method which established the potency of the extracts in free radical quenching, UV-vis ferrous tartrate method which evaluated the potency of the extracts in acting as a reducing agent and $KMnO_4$ titration method which estimated the efficiency of the extracts in undergoing complete oxidation, which in return extended its antioxidant potential. These three analytical methods were used for the *in vitro* antioxidant activity studies and its efficacy was reported as the percentage of equivalence to the corresponding standards - ascorbic acid, α-tocopherol, BHT and resorcinol.

2.7.1. Antioxidant assay: DPPH method

The assay was done as per the method given by Brand-Williams *et al*.[27]. The standard curve was observed to be linear between 403 to 1613 μg/g for ascorbic acid, 409 to 1637 μg/g for α-tocopherol, 406 to 1626 μg/g for BHT and 405 to 1621 μg/g for resorcinol. The r^2 values were > 0.95. Results were expressed as the percentage of

equivalence to the corresponding standard. Additional dilutions were done upon the requirement, when observed to be deviating from linear range.

2.7.2. Antioxidant assay: UV-vis ferrous tartrate method

Antioxidant activity was determined by using the modified UV-vis spectrophotometric methods[28]. The standard curve was observed to be linear between 403 to 1 613 μg/g for ascorbic acid, 406 to 1 622 μg/g for BHT, 409 to 1 637 μg/g for α-tocopherol and 405 to 1 622 μg/g for resorcinol. The r^2 values were > 0.95. Results were expressed as the percentage of equivalence to the corresponding standard. Additional dilutions were done upon the requirement, when observed to be deviating from linear range.

2.7.3. Antioxidant assay: $KMnO_4$ method

Antioxidant assay was estimated by adopting the Ribereau-Gayon-Maurié titrimetric method with $KMnO_4$ and indigo carmine dye as an indicator[29]. Results were calculated with the estimated observations against the standards such as ascorbic acid, α-tocopherol, BHT and resorcinol and were expressed in percentage of equivalence to the corresponding standard.

2.8. Antibacterial study

Positive cultures purchased were initially cultured in the respective selective culture medium and the log phase of the culture was subsequently swabbed into the soyabean casein digest agar plates. pH was maintained at 6.8 to 7.2. Antibacterial activity study was done based on the Kirby-Bauer disc diffusion method[30]. All the four fractions, namely, PEF, EAF, CF and SF were dissolved in methanol at a concentration level of 100 mg/L and were screened with pathogenic bacteria [two Gram-positive (*S. aureus* and *B. cereus*) and two Gram-negative (*E. coli* and *S. abony*)] against 100 mg/L of chloramphenicol and tetracycline as the positive controls. Methanol was used as the analytical blank. Activity was measured in mm of the clearance area around the disc, discarding the diameter of the disc and reported as percentage of inhibition against the positive controls.

2.9. Preparation of fatty acid methyl esters for GC analysis

Fatty acid methyl esters of the algal CF extracts were obtained as follows: 100 mg of the dried chloroform extract was taken in a Pyrex tube and dried under nitrogen. 1 mL of 50% sodium methoxide (NaOH/methanol) in methanol was added and heated in a boiling water bath for 15 min. After cooling, 1 mL of BF_3 was added and the solution was kept in boiling water bath for 15 min. After cooling, 1 mL of high performance liquid chromatography grade *n*-hexane was added followed by 1 mL of saturated NaCl solution and swirled for 10 min. The upper layer was collected, moisture removed by addition of Na_2SO_4 and injected to GC-MS.

2.10. GC-MS analysis

Different fractions were chromatographically analyzed on Perkin Elmer Clarus 680 gas chromatograph equipped with mass spectrometer with electron ionization as mass identification source. A 30 m × 250 μm dimethyl polysiloxane with 5% diphenyl (Elite-5MS) column was used. The extract was diluted with methanol and filtered through 0.2 μm sterile syringe filters and 0.5 μL of each fraction solution injected and analyzed with single quadruple mass spectrometric detector. Structural assignments were based on the analysis of fragmentation pattern of mass spectra in direct comparison with the profiles in the National Institute of Standards and Technology library (version 2.2) at a hit rate > 85%. Results were reported upon the relative concentration of the compounds in the injected volume of sample to the dry weight of the seaweed. Economically important unsaturated fatty acids were reported as the actual concentrations and the relative concentrations of unsaturated fatty acids.

2.11. Statistical correlations

All the values of observation were the means of three replicate determinations (± SD). All the statistical correlation analyses were carried out using SPSS 16.0 for Windows[31]. The Pearson correlation test with bivariate significance was adopted and the positive r^2 values explained the relation between the chemical constituents and activities.

3. Results

3.1. Saponins

Saponins are steroids or similar compounds with glycosidic linkages. Saponins were observed and quantified in all the analyzed samples (Table 1). Chlorophyta exhibited the maximum

Table 1
Taxonomic classification of seaweeds collected from the Kerala coast and the solvent extraction yields. $n = 3$.

Species	Sampling location	Extraction yields (%)			
		PEF	EAF	CF	SF
C. antennina (Bory de Saint-Vincent) Kützing	Njarakkal	0.98 ± 0.04	0.02 ± 0.00	1.98 ± 0.09	1.72 ± 0.16
E. prolifera (O. F. Müller) J. Agardh	Kayamkulam	0.57 ± 0.03	0.13 ± 0.01	0.98 ± 0.05	1.31 ± 0.12
E. prolifera (O. F. Müller) J. Agardh	Njarakkal	1.00 ± 0.05	0.07 ± 0.01	1.18 ± 0.05	1.19 ± 0.11
G. corticata (J. Agardh) J. Agardh	Njarakkal	0.48 ± 0.02	0.16 ± 0.01	1.10 ± 0.05	0.56 ± 0.05
G. corticata var. *cylindrica* Umamaheswara Rao	Njarakkal	0.54 ± 0.02	0.17 ± 0.01	2.46 ± 0.11	0.27 ± 0.02
G. foliifera (Forsskål) Børgesen	Njarakkal	0.43 ± 0.02	0.34 ± 0.02	1.01 ± 0.05	1.21 ± 0.11
U. fasciata Delile	Kayamkulam	1.14 ± 0.05	1.50 ± 0.07	0.63 ± 0.03	1.54 ± 0.14

C. antennina: *Chaetomorpha antennina*; *E. prolifera*: *Enteromorpha prolifera*; *G. corticata*: *Gracilaria corticata*; *G. corticata* var. *cylindrica*: *Gracilaria corticata* var. *cylindrica*; *G. foliifera*: *Gracilaria foliifera*; *U. fasciata*: *Ulva fasciata.*

concentrations (1.19% to 1.72%). *C. antennina* had the highest (1.72%). Among the Rhodophyta, *G. foliifera* alone exhibited similar concentration levels of Chlorophyta (1.21%). The least saponins content was observed in *G. corticata* var. *cylindrica* (0.27%).

3.2. Seaweed fractions

Fractions collected after sequential solvent extractions were dried and the yields were estimated. Values were reported as the percentage dry weight of the sample (Table 1). High concentrations of the non polar fractions (PEF) were obtained from Chlorophyta (0.57% to 1.14%). EAF (slightly polar) was seen the highest in *U. fasciata* (1.50%), but generally in Rhodophyta (0.16% to 0.34%). CF was seen higher in Rhodophyta (1.01% to 2.46%) even though Chlorophyta such as *C. antennina* (1.98%) and *E. prolifera*-Njarakkal (1.18%) also yielded appreciable concentrations.

3.3. Saponification and iodine values of the four fractions of seaweed extractions

Determination of saponification values provided an understanding of the total saponifiable and acidic matters in the sample. Saponification values were seen > 300 in the CF which indicated the contribution of fatty acids in the fraction (Table 2). PEF had low saponification values with an exception by the fractions obtained from *U. fasciata* and *E. prolifera* from both locations. The exceptional saponification values of PEF in *U. fasciata* and *E. prolifera*, remarked the availability of fatty acids which were not saponified during the extraction procedures. SF also exhibited moderate saponification values indicating the presence of acidic functions in the glycosidic linkages. Iodine values which denoted the extent of unsaturation, also exhibited similar patterns with exceptions in SF. SF and EAF had relatively low iodine values. The CF had high iodine values which highlighted the content of unsaturated fatty acids.

Table 2
Saponification and iodine values of the fractions of seaweed extractions. $n = 3$.

Seaweeds	Fractions	Saponification value	Iodine value
C. antennina	PEF	7.558 ± 0.690	7.550 ± 0.690
	EAF	34.360 ± 3.140	13.870 ± 1.270
	CF	335.310 ± 30.610	59.480 ± 5.430
	SF	90.810 ± 8.290	12.640 ± 1.150
E. prolifera - Kayamkulam	PEF	34.330 ± 3.130	97.660 ± 8.920
	EAF	76.430 ± 6.980	79.440 ± 7.250
	CF	356.090 ± 32.510	113.530 ± 10.360
	SF	43.870 ± 4.000	17.650 ± 1.610
E. prolifera - Njarakkal	PEF	23.950 ± 2.190	87.320 ± 7.970
	EAF	10.770 ± 0.980	18.540 ± 1.690
	CF	413.660 ± 37.760	131.660 ± 12.020
	SF	43.300 ± 3.950	11.650 ± 1.060
G. corticata	PEF	5.490 ± 0.500	3.800 ± 0.350
	EAF	7.450 ± 0.680	11.250 ± 1.030
	CF	322.450 ± 29.440	33.640 ± 3.070
	SF	41.440 ± 3.780	10.870 ± 0.990
G. corticata var. *cylindrica*	PEF	1.560 ± 0.140	4.970 ± 0.450
	EAF	22.650 ± 2.070	19.330 ± 1.760
	CF	332.60 ± 30.360	27.540 ± 2.510
	SF	53.980 ± 4.930	13.220 ± 1.210
G. foliifera	PEF	3.130 ± 0.290	102.000 ± 9.310
	EAF	12.330 ± 1.260	12.000 ± 1.090
	CF	315.950 ± 28.840	24.350 ± 2.220
	SF	92.630 ± 8.460	9.650 ± 0.880
U. fasciata	PEF	65.230 ± 5.950	29.430 ± 2.690
	EAF	44.300 ± 4.040	14.220 ± 1.300
	CF	311.070 ± 28.400	67.090 ± 6.120
	SF	95.640 ± 8.730	22.760 ± 2.080

3.4. Antioxidant activity

The antioxidant properties are not purely based on the free radical quenching[32]. Henceforth, the antioxidant activity analysis was done with three methods, namely, the DPPH, the ferrous tartrate and the $KMnO_4$ titration methods. Throughout these methods, the activity showed a concentration dependency which increased with concentrations. The antioxidant activities of the seaweed extracts were estimated in comparison with the respective standards (Tables 3 and 4) and the results were reported in 4 equivalent values (Tables 5–7).

Table 3
Calibration data for the antioxidant activities of standards using DPPH and ferrous tartrate titration methods.

Standard		Calibration equations	r^2	Concentration in mg/kg			
				1	2	3	4
DPPH radical scavenging activity	Ascorbic acid	y = -3.2176e-05 - 0.081	0.9952	403.36	806.72	1210.08	1613.44
	Resorcinol	y = -3.1709e-05 - 0.088	0.9992	406.56	813.12	1219.68	1626.24
	Tocopherol	y = -4.9594e-05 + 0.008	0.9999	409.36	818.72	1228.08	1637.44
	BHT	y = -2.9294e-05 - 0.064	0.9956	405.32	810.64	1215.96	1621.28
Ferrous tartrate oxidising activity	Ascorbic acid	y = 5.6376e-04 - 0.013	0.9783	403.36	806.72	1210.08	1613.44
	Resorcinol	y = 7.2978e-06 - 0.012	0.9924	405.60	811.20	1216.80	1622.40
	Tocopherol	y = 1.808e-03 + 0.556	0.9950	409.36	818.72	1228.08	1637.44
	BHT	y = 2.0351e-03 + 0.421	0.9937	406.56	813.12	1219.68	1626.24

Table 4
Calibration data for the antioxidant activities of standards using $KMnO_4$ titration methods.

Standard		Weight of standards (g/50 mL)	Blank readings	Titre readings			Equivalence of 0.01 mol/L $KMnO_4$ (g)
				1 mL	2 mL	3 mL	
$KMnO_4$ titrimetry	Ascorbic acid	0.5042	0.9	23.0	41.0	61.3	0.00046
	Resorcinol	0.5070	0.9	2.4	4.0	6.0	0.05069
	Tocopherol	0.5069	0.9	1.1	1.3	1.4	0.00664
	BHT	0.5028	0.9	3.1	5.9	6.8	0.00457

Table 5
Antioxidant activity study of the fractions of seaweed extracts based on DPPH method. $n = 3$.

Seaweed	Fraction	DPPH method (% of inhibition)			
		AAE	BHTE	α-TE	RE
C. antennina	PEF	0.018 ± 0.010	0.017 ± 0.010	0.005 ± 0.010	0.044 ± 0.010
	EAF	1.226 ± 0.060	1.372 ± 0.070	2.452 ± 0.120	1.462 ± 0.070
	CF	0.966 ± 0.050	1.069 ± 0.050	1.628 ± 0.080	1.131 ± 0.050
	SF	0.886 ± 0.040	0.978 ± 0.050	1.402 ± 0.070	1.032 ± 0.050
E. prolifera-Njarakkal	PEF	0.097 ± 0.010	0.091 ± 0.010	0.039 ± 0.010	0.248 ± 0.010
	EAF	1.982 ± 0.090	2.162 ± 0.100	2.427 ± 0.120	2.261 ± 0.110
	CF	2.325 ± 0.110	2.548 ± 0.120	3.201 ± 0.150	2.675 ± 0.130
	SF	2.412 ± 0.110	2.651 ± 0.130	3.477 ± 0.170	2.786 ± 0.130
E. prolifera-Kayamkulam	PEF	0.003 ± 0.010	0.002 ± 0.010	0.003 ± 0.010	0.004 ± 0.010
	EAF	0.075 ± 0.010	0.064 ± 0.010	0.387 ± 0.020	0.054 ± 0.010
	CF	0.081 ± 0.010	0.071 ± 0.010	0.355 ± 0.020	0.061 ± 0.010
	SF	0.078 ± 0.010	0.036 ± 0.010	1.242 ± 0.060	0.002 ± 0.010
G. corticata	PEF	0.003 ± 0.010	0.002 ± 0.010	0.001 ± 0.010	0.006 ± 0.010
	EAF	0.075 ± 0.010	0.075 ± 0.010	0.097 ± 0.010	0.073 ± 0.010
	CF	0.143 ± 0.010	0.141 ± 0.010	0.237 ± 0.010	0.135 ± 0.010
	SF	0.075 ± 0.010	0.074 ± 0.010	0.102 ± 0.010	0.072 ± 0.010
G. corticata var. *cylindrica*	PEF	0.096 ± 0.010	0.093 ± 0.010	0.057 ± 0.010	0.261 ± 0.010
	EAF	2.367 ± 0.110	2.699 ± 0.130	6.094 ± 0.290	2.914 ± 0.140
	CF	3.974 ± 0.190	4.626 ± 0.220	12.774 ± 0.610	5.064 ± 0.240
	SF	0.276 ± 0.010	0.291 ± 0.010	0.051 ± 0.010	0.296 ± 0.010
G. foliifera	PEF	0.119 ± 0.010	0.113 ± 0.010	0.055 ± 0.010	0.311 ± 0.010
	EAF	1.137 ± 0.050	1.215 ± 0.060	0.707 ± 0.030	1.251 ± 0.060
	CF	3.252 ± 0.150	3.621 ± 0.170	6.028 ± 0.290	3.845 ± 0.180
	SF	3.871 ± 0.180	4.342 ± 0.210	8.025 ± 0.380	4.635 ± 0.220
U. fasciata	PEF	0.006 ± 0.010	0.003 ± 0.010	0.008 ± 0.010	0.009 ± 0.010
	EAF	0.296 ± 0.010	0.263 ± 0.010	1.276 ± 0.060	0.228 ± 0.010
	CF	0.219 ± 0.010	0.207 ± 0.010	0.613 ± 0.030	0.191 ± 0.010
	SF	0.262 ± 0.010	0.241 ± 0.010	0.916 ± 0.040	0.216 ± 0.010

AAE: Ascorbic acid equivalence; BHTE: Butylated hydroxy toluene equivalence; α-TE: α-Tocopherol equivalence; RE: Resorcinol equivalence.

Table 6
Antioxidant activity study of the fractions of seaweed extracts based on ferrous tartrate method. $n = 3$.

Seaweed	Fraction	Ferrous tartrate method (% of activity)			
		AAE	BHTE	α-TE	RE
C. antennina	PEF	0.036 ± 0.010	0.061 ± 0.010	0.067 ± 0.010	0.117 ± 0.010
	EAF	0.139 ± 0.010	0.756 ± 0.040	0.751 ± 0.040	1.282 ± 0.060
	CF	0.086 ± 0.010	0.637 ± 0.030	0.633 ± 0.030	1.181 ± 0.060
	SF	0.627 ± 0.030	0.592 ± 0.030	0.586 ± 0.030	0.695 ± 0.030
E. prolifera-Njarakkal	PEF	0.039 ± 0.010	0.043 ± 0.010	0.047 ± 0.010	0.049 ±0.010
	EAF	0.479 ± 0.020	0.433 ± 0.020	0.432 ± 0.020	0.573 ± 0.030
	CF	0.117 ± 0.010	0.119 ± 0.010	0.118 ± 0.010	0.112 ± 0.010
	SF	0.522 ± 0.020	0.472 ± 0.020	0.469 ± 0.020	0.625 ± 0.030
E. prolifera-Kayamkulam	PEF	0.034 ± 0.010	0.026 ± 0.010	0.029 ± 0.010	0.062 ± 0.010
	EAF	0.222 ± 0.010	0.117 ± 0.010	0.116 ± 0.010	0.457 ± 0.020
	CF	0.116 ± 0.010	0.119 ± 0.010	0.118 ± 0.010	0.109 ± 0.010
	SF	0.574 ± 0.030	0.232 ± 0.010	0.231 ± 0.010	1.345 ± 0.060
G. corticata	PEF	0.041 ± 0.010	0.044 ± 0.010	0.051 ± 0.010	0.049 ± 0.010
	EAF	0.448 ± 0.020	0.433 ± 0.020	0.431 ± 0.020	0.472 ± 0.020
	CF	0.197 ± 0.010	0.157 ± 0.010	0.156 ± 0.010	0.282 ± 0.010
	SF	0.551 ± 0.030	0.552 ± 0.030	0.548 ± 0.030	0.537 ± 0.030
G. corticata var. *cylindrica*	PEF	0.053 ± 0.010	0.057 ± 0.010	0.063 ± 0.010	0.066 ± 0.010
	EAF	0.472 ± 0.020	0.473 ± 0.020	0.474 ± 0.020	0.453 ± 0.020
	CF	0.395 ± 0.020	0.394 ± 0.020	0.391 ± 0.020	0.389 ± 0.020
	SF	0.684 ± 0.030	0.593 ± 0.030	0.586 ± 0.030	0.884 ± 0.040
G. foliifera	PEF	0.028 ± 0.010	0.025 ± 0.010	0.028 ± 0.010	0.044 ± 0.010
	EAF	0.196 ± 0.010	0.197 ± 0.010	0.196 ± 0.010	0.189 ± 0.010
	CF	0.078 ± 0.010	0.079 ± 0.010	0.079 ± 0.010	0.075 ± 0.010
	SF	0.401 ± 0.020	0.354 ± 0.020	0.352 ± 0.020	0.503 ± 0.020
U. fasciata	PEF	0.029 ± 0.010	0.037 ± 0.010	0.041 ± 0.010	0.028 ± 0.010
	EAF	0.334 ± 0.020	0.435 ± 0.020	0.432 ± 0.020	0.095 ± 0.010
	CF	0.281 ± 0.010	0.276 ± 0.010	0.274 ± 0.010	0.285 ± 0.010
	SF	0.354 ± 0.020	0.355 ± 0.020	0.352 ± 0.020	0.345 ± 0.020

AAE: Ascorbic acid equivalence; BHTE: Butylated hydroxy toluene equivalence; α-TE: α-Tocopherol equivalence; RE: Resorcinol equivalence.

Table 7
Antioxidant activity study of the fractions of seaweed extracts based on $KMnO_4$ method. $n = 3$.

Seaweed	Fraction	$KMnO_4$ method (% of activity)			
		AAE	BHTE	α-TE	RE
C. antennina	PEF	0.015 ± 0.010	0.181 ± 0.010	2.256 ± 0.110	0.214 ± 0.010
	EAF	0.087 ± 0.010	0.871 ± 0.040	9.637 ± 0.460	1.262 ± 0.060
	CF	0.076 ± 0.010	0.764 ± 0.040	8.463 ± 0.400	1.109 ± 0.050
	SF	0.173 ± 0.010	1.727 ± 0.080	19.143 ± 0.910	2.507 ± 0.120
E. prolifera-Njarakkal	PEF	0.018 ± 0.010	0.218 ± 0.010	2.709 ± 0.130	0.258 ± 0.010
	EAF	0.069 ± 0.010	0.687 ± 0.030	7.612 ± 0.360	0.997 ± 0.050
	CF	0.040 ± 0.010	0.403 ± 0.020	4.467 ± 0.210	0.585 ± 0.030
	SF	0.318 ± 0.020	3.186 ± 0.150	35.325 ± 1.680	4.626 ± 0.220
E. prolifera-Kayamkulam	PEF	0.023 ± 0.010	0.281 ± 0.010	3.492 ± 0.170	0.332 ± 0.020
	EAF	0.179 ± 0.010	1.786 ± 0.090	19.801 ± 0.940	2.593 ± 0.120
	CF	0.078 ± 0.010	0.785 ± 0.040	8.703 ± 0.410	1.141 ± 0.050
	SF	0.365 ± 0.020	3.646 ± 0.170	40.431 ± 1.930	5.295 ± 0.250
G. corticata	PEF	0.021 ± 0.010	0.259 ± 0.010	3.219 ± 0.150	0.306 ± 0.010
	EAF	0.095 ± 0.010	0.951 ± 0.050	10.533 ± 0.500	1.383 ± 0.070
	CF	0.043 ± 0.010	0.418 ± 0.020	4.629 ± 0.220	0.607 ± 0.030
	SF	0.339 ± 0.020	3.394 ± 0.160	37.635 ± 1.790	4.929 ± 0.230
G. corticata var. *cylindrica*	PEF	0.021 ± 0.010	0.242 ± 0.010	3.009 ± 0.140	0.286 ± 0.010
	EAF	0.141 ± 0.010	1.401 ± 0.070	15.533 ± 0.740	2.035 ± 0.100
	CF	0.123 ± 0.010	1.233 ± 0.060	13.672 ± 0.650	1.791 ± 0.090
	SF	0.244 ±0.010	2.438 ± 0.120	27.034 ± 1.290	3.541 ± 0.170
G. foliifera	PEF	0.021 ± 0.010	0.261 ± 0.010	3.241 ± 0.150	0.308 ± 0.010
	EAF	0.158 ± 0.010	1.581 ± 0.080	17.525 ± 0.830	2.295 ± 0.110
	CF	0.083 ± 0.010	0.833 ± 0.040	9.228 ± 0.440	1.209 ± 0.060
	SF	0.263 ± 0.010	2.631 ± 0.130	29.171 ± 1.390	3.821 ± 0.180
U. fasciata	PEF	0.013 ± 0.010	0.162 ± 0.010	2.019 ± 0.100	0.192 ± 0.010
	EAF	0.121 ± 0.010	1.198 ± 0.060	13.277 ± 0.630	1.739 ± 0.080
	CF	0.028 ± 0.010	0.282 ± 0.010	3.107 ± 0.150	0.407 ± 0.020
	SF	0.118 ± 0.010	1.181 ± 0.060	13.096 ± 0.620	1.715 ± 0.080

AAE: Ascorbic acid equivalence; BHTE: Butylated hydroxy toluene equivalence; α-TE: α-Tocopherol equivalence; RE: Resorcinol equivalence.

DPPH free radical scavenging was seen the highest in the CF of *G. corticata* var. *cylindrica* with 3.974%, 4.626%, 12.774% and 5.064% of ascorbic acid, BHT, α-tocopherol and resorcinol equivalences respectively. SF of *G. foliifera* also exhibited similar patterns with 3.871%, 4.342%, 8.025% and 4.635% of ascorbic acid, BHT, α-tocopherol and resorcinol equivalences respectively. The highest antioxidant PEF and EAF were exhibited by *G. foliifera* and *G. corticata* var. *cylindrica* respectively. The least antioxidant potential was observed in *G. corticata*.

Ferrous tartrate method indicated the potency of the extracts in acting as a reducing agent. This activity was observed in general to the maximum in the SF fractions. *G. corticata* var. *cylindrica* and *C. antennina* generally exhibited the maximum activity with respect to all the 4 standards. The highest reducing activity of the PEF and EAF was exhibited by *C. antennina*. Reducing activity of the CF was found highest in *C. antennina*. EAF of *C. antennina* exhibited the highest BHT (0.756%), α-tocopherol (0.751%) and resorcinol (1.282%) equivalences. SF of *G. corticata* var. *cylindrica* exhibited the highest ascorbic acid (0.684%) equivalence. In general, the highest total reducing potential was observed for the extracts of *C. antennina*. The least potential was exhibited by the extracts of *G. foliifera*.

$KMnO_4$ titrimetric method indicated the total oxidation potential, which was observed to be the maxima for the SF. SF of *E. prolifera* from the Kayamkulam location and *G. corticata* showed

the highest oxidation potentials. The highest total oxidising activity for the CF was observed in the *G. corticata* var. *cylindrica* with 0.123%, 1.233%, 13.672% and 1.791% of ascorbic acid, BHT, α-tocopherol and resorcinol equivalences. SF of *E. prolifera* from the Kayamkulam location possessed 0.365%, 3.646%, 40.431% and 5.295% of ascorbic acid, BHT, α-tocopherol and resorcinol equivalences. In general, *E. prolifera* and *G. corticata* var. *cylindrica* exhibited the highest total oxidising capabilities.

3.5. Antibacterial activity

Each fraction segregated by the solvent extractions as above, was observed to possess moderate antioxidant activities which could be incorporated into antimicrobial studies too (Figure 1, Tables 8 and 9). Cell walls of microorganisms were made of polysaccharides and lipophilic materials which could be easily oxidized upon with compounds indicating the antioxidant activities. Gram-negative bacteria were more resistant due to the additional presence of a lipid polysaccharide layer on the outer surface which protected it from easy attack of drugs and antibiotics. The solvent impregnated disc (blank) indicated 2.00 mm diameter in the streaked plates of *S. aureus* and *S. abony*, whereas 3.00 mm in *B. cereus* and *E. coli*. Thus, 2.00 mm of inhibitory diameter was reduced as the blank correction upon the observations made on the activity against *S. aureus* and *S. abony* and 3.00 mm deduction against *B. cereus* and *E. coli*. Tetracycline exhibited the inhibitory diameter of 9.00 mm on *S. aureus* and *S. abony* whereas 10.00 mm on *B. cereus* and 8.00 mm on *E. coli*. Chloramphenicol exhibited an inhibitory diameter of 8.00 mm on *S. aureus*, 12.00 mm on *B. cereus*, 10.00 mm on *E. coli* and 12.00 mm on *S. abony*. Both the inhibitory diameters of the positive controls were measured after deducting the solvent inhibition.

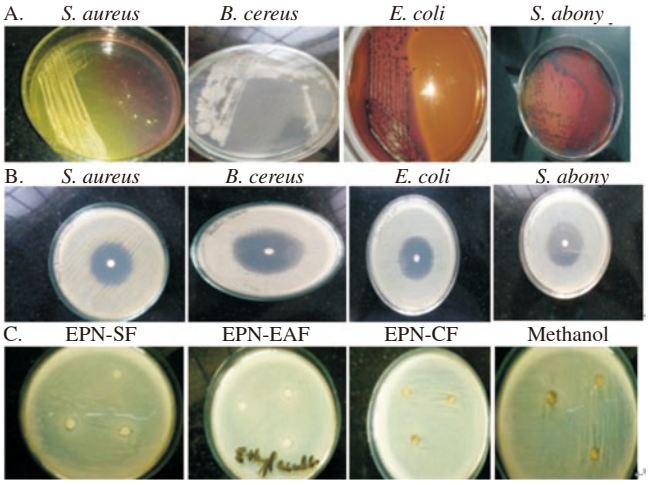

Figure 1. Antimicrobial studies.
A: Selective culture of *S. aureus*, *B. cereus*, *E. coli* and *S. abony*; B: Positive control disc placed on the culture plates; C: Sample discs placed on the culture plates; EPN: *E. prolifera*-Njarakkal.

Table 9
Antimicrobial activity with respect to chloramphenicol and tetracycline as positive control respectively.

Analyte	Fraction	Chloramphenicol equivalents (% of activity)				Tetracycline equivalents (% of activity)			
		S. aureus	*B. cereus*	*E. coli*	*S. abony*	*S. aureus*	*B. cereus*	*E. coli*	*S. abony*
C. antennina	PEF	0.00 ± 0.00	0.00 ± 0.00	10.10 ± 0.43	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	13.00 ± 0.66	0.00 ± 0.00
	EAF	0.00 ± 0.00	8.00 ± 0.41	10.00 ± 0.47	0.00 ± 0.00	0.00 ± 0.00	10.00 ± 0.47	13.10 ± 0.54	0.00 ± 0.00
	CF	0.00 ± 0.00	8.10 ± 0.35	10.10 ± 0.45	0.00 ± 0.00	0.00 ± 0.00	10.00 ± 0.46	13.10 ± 0.58	0.00 ± 0.00
	SF	0.00 ± 0.00	8.20 ± 0.38	10.10 ± 0.41	0.00 ± 0.00	0.00 ± 0.00	10.00 ± 0.40	13.10 ± 0.63	0.00 ± 0.00
E. prolifera-Kayamkulam	PEF	25.30 ± 1.20	0.00 ± 0.00	5.00 ± 0.20	0.00 ± 0.00	22.00 ± 1.06	0.00 ± 0.00	6.00 ± 0.29	0.00 ± 0.00
	EAF	19.00 ± 0.90	0.00 ± 0.00	10.00 ± 0.43	0.00 ± 0.00	17.00 ± 0.82	0.00 ± 0.00	13.00 ± 0.66	0.00 ± 0.00
	CF	13.00 ± 0.64	0.00 ± 0.00	10.10 ± 0.44	0.00 ± 0.00	11.00 ± 0.53	0.00 ± 0.00	13.10 ± 0.59	0.00 ± 0.00
	SF	19.00 ± 0.93	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	17.00 ± 0.62	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
E. prolifera-Njarakkal	PEF	25.20 ± 1.22	0.00 ± 0.00	10.10 ± 0.16	0.00 ± 0.00	22.00 ± 1.01	0.00 ± 0.00	13.00 ± 0.53	0.00 ± 0.00
	EAF	13.00 ± 0.87	0.00 ± 0.00	10.20 ± 0.27	0.00 ± 0.00	11.00 ± 0.38	0.00 ± 0.00	13.00 ± 0.51	0.00 ± 0.00
	CF	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	SF	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	8.00 ± 0.51	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	11.00 ± 0.48
G. corticata	PEF	0.00 ± 0.00	0.00 ± 0.00	5.00 ± 0.21	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	6.00 ± 0.32	0.00 ± 0.00
	EAF	6.00 ± 0.27	0.00 ± 0.00	10.10 ± 0.37	0.00 ± 0.00	6.00 ± 0.29	0.00 ± 0.00	13.00 ± 0.57	0.00 ± 0.00
	CF	13.00 ± 0.60	0.00 ± 0.00	10.00 ± 0.41	0.00 ± 0.00	11.00 ± 0.50	0.00 ± 0.00	13.00 ± 0.71	0.00 ± 0.00
	SF	13.00 ± 0.57	0.00 ± 0.00	10.00 ± 0.47	8.10 ± 0.31	11.00 ± 0.51	0.00 ± 0.00	13.00 ± 0.06	11.00 ± 0.46
G. corticata var. *cylindrica*	PEF	0.00 ± 0.00	17.00 ± 0.75	10.20 ± 0.49	0.00 ± 0.00	0.00 ± 0.00	20.00 ± 0.96	13.00 ± 0.59	0.00 ± 0.00
	EAF	25.10 ± 1.20	0.00 ± 0.00	10.10 ± 0.47	13.00 ± 0.61	22.00 ± 1.04	0.00 ± 0.00	13.00 ± 0.52	17.00 ± 0.77
	CF	0.00 ± 0.00	8.10 ± 0.40	10.10 ± 0.46	0.00 ± 0.00	0.00 ± 0.00	10.00 ± 0.47	13.00 ± 0.55	0.00 ± 0.00
	SF	0.00 ± 0.00	13.00 ± 0.60	15.20 ± 0.73	0.00 ± 0.00	0.00 ± 0.00	15.0 ± 0.72	19.00 ± 0.91	0.00 ± 0.00
G. foliifera	PEF	0.00 ± 0.00	0.00 ± 0.00	10.10 ± 0.15	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	13.00 ± 0.34	0.00 ± 0.00
	EAF	13.00 ± 0.43	8.00 ± 0.42	10.20 ± 0.04	13.00 ± 0.03	11.00 ± 0.50	10.00 ± 0.08	13.00 ± 0.64	17.00 ± 0.84
	CF	13.00 ± 0.38	4.00 ± 0.20	5.10 ± 0.16	8.00 ± 0.14	11.00 ± 0.53	5.00 ± 0.14	6.00 ± 0.57	11.00 ± 0.50
	SF	13.00 ± 0.52	8.00 ± 0.40	10.10 ± 0.14	8.20 ± 0.18	11.00 ± 0.42	10.00 ± 0.44	13.00 ± 0.78	11.00 ± 0.54
U. fasciata	PEF	25.10 ± 1.20	8.10 ± 0.39	5.00 ± 0.23	0.00 ± 0.00	22.00 ± 1.05	10.00 ± 0.49	6.00 ± 0.30	0.00 ± 0.00
	EAF	38.00 ± 1.81	4.00 ± 0.20	5.30 ± 0.26	17.00 ± 0.81	33.00 ± 1.58	5.00 ± 0.22	6.00 ± 0.28	22.10 ± 1.03
	CF	25.10 ± 1.21	4.00 ± 0.23	10.10 ± 0.41	13.00 ± 0.60	22.00 ± 1.07	5.00 ± 0.25	13.00 ± 0.61	17.10 ± 0.81
	SF	19.00 ± 0.91	8.00 ± 0.39	10.20 ± 0.43	4.10 ± 0.22	17.00 ± 0.81	10.00 ± 0.45	13.00 ± 0.63	6.00 ± 0.29

Table 8
Antimicrobial assays of the fractions of seaweed extracts and antimicrobial inhibition. $n = 3$.

Analyte	Fractions (100 mg/kg)	Inhibition level (mm)			
		Gram-positive		Gram-negative	
		S. aureus	*B. cereus*	*E. coli*	*S. abony*
Positive control	Solvent	2.00 ± 0.09	3.00 ± 0.14	3.00 ± 0.15	2.00 ± 0.10
	Tetracycline	9.00 ± 0.43	10.00 ± 0.48	8.00 ± 0.38	9.00 ± 0.43
	CP	8.00 ± 0.38	12.00 ± 0.57	10.00 ± 0.43	12.00 ± 0.37
C. antennina	PEF	2.10 ± 0.10	3.00 ± 0.14	4.00 ± 0.23	2.00 ± 0.09
	EAF	1.50 ± 0.07	4.00 ± 0.19	4.00 ± 0.07	2.00 ± 0.04
	CF	2.00 ± 0.13	4.00 ± 0.20	4.00 ± 0.06	2.00 ± 0.13
	SF	2.10 ± 0.06	4.00 ± 0.09	4.00 ± 0.29	2.00 ± 0.19
E. prolifera-Kayamkulam	PEF	4.00 ± 0.20	2.50 ± 0.12	3.50 ± 0.17	2.00 ± 0.10
	EAF	3.50 ± 0.17	3.00 ± 0.11	4.00 ± 0.21	1.50 ± 0.04
	CF	3.00 ± 0.15	3.00 ± 0.17	4.00 ± 0.02	1.50 ± 0.03
	SF	3.50 ± 0.10	3.00 ± 0.08	3.00 ± 0.17	2.00 ± 0.10
E. prolifera-Njarakkal	PEF	4.00 ± 0.11	3.00 ± 0.03	4.00 ± 0.21	1.50 ± 0.07
	EAF	3.00 ± 0.13	3.00 ± 0.06	4.00 ± 0.27	2.00 ± 0.11
	CF	2.00 ± 0.19	3.00 ± 0.14	3.00 ± 0.08	2.00 ± 0.14
	SF	2.00 ± 0.08	2.50 ± 0.12	3.00 ± 0.05	3.00 ± 0.12
G. corticata	PEF	1.50 ± 0.08	3.00 ± 0.18	3.50 ± 0.17	2.00 ± 0.11
	EAF	2.50 ± 0.12	2.50 ± 0.12	4.00 ± 0.21	2.00 ± 0.13
	CF	3.00 ± 0.10	3.00 ± 0.15	4.00 ± 0.05	2.00 ± 0.06
	SF	3.00 ± 0.11	3.00 ± 0.17	4.00 ± 0.08	3.00 ± 0.07
G. corticata var. *cylindrica*	PEF	2.00 ± 0.18	5.00 ± 0.24	4.00 ± 0.03	2.00 ± 0.16
	EAF	4.00 ± 0.26	3.00 ± 0.05	4.00 ± 0.06	3.50 ± 0.09
	CF	2.00 ± 0.15	4.00 ± 0.01	4.00 ± 0.08	2.00 ± 0.12
	SF	2.00 ± 0.07	4.50 ± 0.22	4.50 ± 0.02	2.00 ± 0.04
G. foliifera	PEF	2.00 ± 0.11	3.00 ± 0.04	4.00 ± 0.11	2.00 ± 0.08
	EAF	3.00 ± 0.08	4.00 ± 0.09	4.00 ± 0.31	3.50 ± 0.17
	CF	3.00 ± 0.13	3.50 ± 0.06	3.50 ± 0.10	3.00 ± 0.12
	SF	3.00 ± 0.11	4.00 ± 0.10	4.00 ± 0.14	3.00 ± 0.14
U. fasciata	PEF	4.00 ± 0.20	4.00 ± 0.21	3.50 ± 0.04	1.50 ± 0.08
	EAF	5.00 ± 0.24	3.50 ± 0.12	3.50 ± 0.08	4.00 ± 0.18
	CF	4.00 ± 0.21	3.50 ± 0.17	4.00 ± 0.14	3.50 ± 0.27
	SF	3.50 ± 0.17	4.00 ± 0.22	4.00 ± 0.25	2.50 ± 0.12

CP: Chloramphenicol.

With respect to the tetracycline as the positive control, highest antibacterial activity with broad spectrum activity on both Gram-positive and Gram-negative bacteria was exhibited by the fractions of *U. fasciata* except PEF. *G. corticata* var. *cylindrica* extracts had high inhibitory activity on *E. coli*, whereas moderate and selective activity on the other strains. *E. prolifera* (collected from Kayamkulam area) showed good inhibitory responses to *S. aureus* and *E. coli*. Similar activity was shown by the extracts of *G. corticata*. Fractions of *G. foliifera*, except PEF exhibited moderate broad spectrum activity.

With respect to chloramphenicol as the positive control, the activity was observed almost in the similar pattern to that of tetracycline. The analytical conclusion thereby stated the extracts of *U. fasciata* to be comprehensively active against a broad spectrum of pathogens. Selective extracts of seaweeds were seen to yield good and appreciable activities. Prominent bactericidal activities against the strains of *S. aureus* by the EAF of *U. fasciata*, *B. cereus* by the PEF of *G. corticata* var. *cylindrica*, *E. coli* by the SF of *G. corticata* var. *cylindrica* and *S. abony* by the EAF of *U. fasciata* were observed.

3.6. GC-MS profiling

The 4 fractions (PEF, EAF, CF and SF) extracted were observed to have good antioxidant and antimicrobial activities. Hence, these bioactive fractions were subjected to GC-MS analysis, which revealed the major chemical compositions of these extracts (Table 10). PEF of Chlorophyta exhibited large combinations of alkanes, alkenes, alkynes and alcohols. High alkane content among the Chlorophyta was seen in PEF of *E. prolifera* collected from both (C-8 to C-43; 0.367% and C-8 to C-44; 0.453%) locations. In Rhodophyta, *G. foliifera* had 0.211% of alkanes (C-11 to C-32). Alkenes were observed in the PEF of *E. prolifera* collected from the Njarakkal location (C-7 to C-17; 0.091%). Alkynes were seen in PEF of *E. prolifera* (C-18, C-19; 0.044%) and *G. foliifera* (C-20; 0.022%). Five alcohols were seen in the PEF of *E. prolifera* (C-10 to C-20; 0.115%). Phytol was the most common alcohol observed in the analysed seaweeds. EAF of the seaweeds exhibited the presence of sterols with cholesterol and its analogues as the common occurrence. Sterols ranged from C-17 to C-30 in the Chlorophyta. *U. fasciata* contained 0.182% of sterols. And 0.196% of C-27 to C-29 sterols was seen in *G. foliifera* belonging to the Rhodophyta family.

Table 10
GC-MS profiling of the fractions of seaweed extracts.

Seaweed	Fraction	Name	Chemical group	Compounds	C (%)
C. antennina	PEF	Alkanes	C-13	2,6,8-Trimethyl-decane	0.038
			C-13	6-Ethyl-2-methyl-decane	0.024
			C-19	2,6-Dimethyl-heptadecane	0.028
			C-19	Nonadecane	0.024
			C-21	2,6,10,14-Tetramethyl-heptadecane	0.045
			C-21	2,6,10,15-Tetramethyl-heptadecane	0.021
		Alcohols	C-15	(Z)6,(Z)9-Pentadecadien-1-ol	0.025
			C-20	Phytol	0.023
	CF	SFAs	C-13	Tridecanoic acid	0.069
			C-15	Pentadecanoic acid	0.066
			C-16	4,8,12-Trimethyl-tridecanoic acid	0.085
			C-16	Hexadecanoic acid	0.071
			C-16	14-Methyl-pentadecanoic acid	0.060
			C-17	Heptadecanoic acid	0.065
			C-18	Octadecanoic acid	0.064
		MUFAs	C-16:1 ω-7	9-Hexadecenoic acid	0.067
			C-18:1 ω-3	15-Octadecenoic acid	0.069
		PUFAs	C-16:4 ω-3	4,7,10,13-Hexadecatetraenoic acid	0.060
			C-18:2 ω-6	(Z,Z)-9,12-octadecadienoic acid	0.071
			C-18:2 ω-7	8,11-Octadecadienoic acid	0.063
			C-18:2 ω-7	9,11-Octadecadienoic acid	0.064
			C-20:4 ω-6	5,8,11,14-Eicosatetraenoic acid	0.069
			C-20:5 ω-3	Cis-5,8,11,14,17-Eicosapentaenoic acid	0.088
E. prolifera-Kayamkulam	PEF	Alkanes	C-08	Octane	0.023
			C-11	4,5-Dimethyl-nonane	0.022
			C-13	2,6,7-Trimethyl-decane	0.022
			C-13	2,5,6-Trimethyl-decane	0.024
			C-13	2,4,6-Trimethyl-decane	0.022
			C-18	7-Methyl-hexadecane	0.022
			C-19	Nonadecane	0.029
			C-21	2,6,10,14-Tetramethyl-heptadecane	0.020
			C-21	2,6,10,15-Tetramethyl-heptadecane	0.028
			C-22	Docosane	0.022
			C-22	2,4-Dimethyl-eicosane	0.021
			C-24	Cyclotetracosane	0.022
			C-26	3-Ethyl-5-(2-ethylbutyl)-octadecane	0.019
			C-26	11-(1-Ethylpropyl)-heneicosane	0.019
			C-28	Octacosane	0.023
			C-43	Tritetracontane	0.029
	EAF	Sterol	C-18	Estra-1,3,5(10)-tri-en-17-β-ol	0.020
			C-24	Scillarenin	0.029
			C-27	Cholesta-3,5-diene	0.030
	CF	SFAs	C-13	Tridecanoic acid	0.060
			C-14	Tetradecanoic acid	0.065
			C-16	Hexadecanoic acid	0.051
			C-17	Heptadecanoic acid	0.049
			C-17	15-Methyl-hexadecanoic acid	0.046
			C-18	Octadecanoic acid	0.046
			C-27	Heptacosanoic acid	0.049
		MUFAs	C-16:1 ω-5	(Z)-11-Hexadecenoic acid	0.049
			C-18:1 ω-9	(Z)-9-octadecenoic acid	0.043
		PUFAs	C-18:2 ω-6	9,12-Octadecadienoic acid	0.052
			C-22:2 ω-5	6Z,8Z-Dodecadienoic acid	0.051

Table 10 (*continued*)

Seaweed	Fraction	Name	Chemical group	Compounds	C (%)
E. prolifera-Njarakkal	PEF	Alkanes	C-08	3-Methyl-heptane	0.027
			C-08	3,4-Dimethyl-hexane	0.030
			C-09	Nonane	0.025
			C-10	2,6-Dimethyl-octane	0.023
			C-12	5,6-Dimethyl-decane	0.032
			C-13	4,7-Dimethyl-undecane	0.041
			C-13	2,10-Dimethyl-undecane	0.020
			C-13	2,6,6-Trimethyl-decane	0.020
			C-15	2,6,11-Trimethyl-dodecane	0.030
			C-18	2-Methyl-heptadecane	0.022
			C-19	2,6-Dimethyl-heptadecane	0.021
			C-21	3-methyl-eicosane	0.041
			C-21	2,6,10,14-Tetramethyl-heptadecane	0.027
			C-24	2-Methyl-tricosane	0.033
			C-24	2,21-Dimethyl-docosane	0.020
			C-26	11-(1-Ethylpropyl)-heneicosane	0.021
			C-44	Tetratetracontane	0.020
		Alkenes	C-07	2,4-Dimethyl-pent-2-ene	0.021
			C-10	Z-(6)-Tridecene	0.024
			C-14	2,6,10-Trimethyl-undeca-1,3-diene	0.024
			C-17	6,9-Heptadecadiene	0.022
		Alkynes	C-18	1-Octadecyne	0.022
			C-19	2,4-Nonadiyne	0.022
		Alcohols	C-10	3,7-Dimethyl-2-octen-1-ol	0.022
			C-10	3,7-Dimethyl-6-octen-1-ol	0.019
			C-14	E-11,13-tetradecadien-1-ol	0.021
			C-16	1-Hexadecanol	0.027
			C-20	Phytol	0.026
	EAF	Sterols	C-30	26,26-Dimethyl-5,23ergostadien-3β-ol	0.030
	CF	SFAs	C-13	Tridecanoic acid	0.022
			C-15	2-Methyl-tetradecanoic acid	0.023
			C-16	Hexadecanoic acid	0.020
			C-17	15-Methyl-hexadecanoic acid	0.021
		MUFAs	C-16:1 ω-7	9-Hexadecenoic acid	0.066
			C-16:1 ω-9	7-Hexadecenoic acid	0.074
			C-18:1 ω-7	11-Octadecenoic acid	0.070
		PUFAs	C-16:4 ω-3	4,7,10,13-Hexadecatetraenoic acid	0.050
			C-18:2 ω-6	10-Trans-12-cis-octadecadienoic acid	0.044
			C-18:3 ω-3	(Z,Z,Z)-9,12,15-octadecatrienoic acid	0.044
			C-18:4 ω-3	Stearidoic acid	0.051
			C-20:3 ω-3	11,14,17-Eicosatrienoic acid	0.060
			C-20:4 ω-6	5,8,11,14-Eicosatetraenoic acid	0.041
G. corticata	PEF	Alkanes	C-13	6-Ethyl-2-methyl-decane	0.024
			C-21	2,6,10,14-Tetramethyl-heptadecane	0.024
			C-26	11-(1-Ethylpropyl)-heneicosane	0.032
		Alcohols	C-20	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	0.025
	EAF	Sterols	C-29	Cholest-5-en-3-ol-3β-acetate	0.054
	CF	SFAs	C-13	Tridecanoic acid	0.067
			C-16	Hexadecanoic acid	0.069
			C-16	14-Methyl-pentadecanoic acid	0.085
		MUFAs	C-17:1 ω-9	7-Hexadecenoic acid	0.066
			C-18:1 ω-8	10-Octadecenoic acid	0.071
G. corticata var. *cylindrica*	PEF	Alkanes	C-19	2-Methyl-octadecane	0.018
			C-21	2,6,10,14-Tetramethyl-heptadecane	0.024
		Alcohols	C-18	E,Z-2,13-octadecadien-1-ol	0.023
			C-20	Phytol	0.026
	CF	SFAs	C-13	Tridecanoic acid	0.030
			C-14	Tetradecanoic acid	0.027
			C-16	14-Methyl-pentadecanoic acid	0.025
			C-16	Hexadecanoic acid	0.032
			C-27	Heptacosanoic acid	0.027
		MUFAs	C-18:1 ω-9	Z-9-octadecenoic acid	0.032
G. foliifera	PEF	Alkanes	C-11	Undecane	0.023
			C-13	6-Ethyl-2-methyl-decane	0.021
			C-13	2,6,6 Trimethyl-decane	0.021
			C-13	2,5,9 Trimethyl-decane	0.020
			C-17	3-Methyl-hexadecane	0.022
			C-18	2-Methyl-heptadecane	0.021
			C-19	2,6 Dimethyl-heptadecane	0.020
			C-21	2,6,10,14 Tetra-methyl-heptadecane	0.022
			C-24	2-Methyl-tricosane	0.020
			C-32	Dotriacontane	0.021
		Alkynes	C-20	1-Eicosyne	0.022
		Alcohols	C-14	1-Tetradecanol	0.020
			C-20	Phytol	0.021
	EAF	Sterols	C-27	Cholesta-3,5-diene	0.065
			C-27	Cholesterol	0.067
			C-29	Cholest-5-en-3-ol-3β-acetate	0.064
	CF	SFAs	C-08	2-Methyl heptanoic acid	0.088
			C-13	Tridecanoic acid	0.069
			C-16	14-Methyl pentadecanoic acid	0.060
			C-17	15-Methyl hexadecanoic acid	0.071
			C-27	Heptacosanoic acid	0.063
		MUFAs	C-14:1 ω-3	Trans-11-tetradecenoic acid	0.074
			C-16:1 ω-7	9-Hexadecenoic acid	0.064
			C-17:1 ω-9	7-Hexadecenoic acid	0.066
			C-18:1 ω-9	9-Octadecenoic acid	0.069
		PUFAs	C-25:2	10,12-Pentacosadiynoic acid	0.070
U. fasciata	PEF	Alkanes	C-13	2,5,6-Trimethyldecane	0.024
			C-13	2,6,7-Trimethyl decane	0.021
			C-13	6-Ethyl-2-methyl-decane	0.030
			C-16	2-Methyl-pentadecane	0.023
			C-19	2,6-Dimethyl-heptadecane	0.021
			C-19	Nonadecane	0.024
			C-20	Eicosane	0.023
			C-21	2,6,10,14-Tetramethyl-heptadecane	0.022
			C-24	Tetracosane	0.020
			C-26	7-Hexyl-eicosane	0.024
			C-30	Squalene	0.022
			C-34	11-Decyl-tetracosane	0.020
			C-34	Tetratriacontane	0.022
			C-44	Tetratetracontane	0.020
		Alkene	C-24	2,6,10,15,19,23-Hexamethyl-2,6,10,14,18,22-tetracosahexaene	0.024
			C-35	17-Pentatriacontene	0.019
		Alcohol	C-14	E-11,13-tetradecadien-1-ol	0.032
			C-20	Phytol	0.022
	EAF	Sterols	C-27	Cholesterol	0.049
			C-29	Gamma-sitosterol	0.047
			C-29	Fucosterol	0.044
			C-30	Cholest-5-en-3-ol,24-propylidene-3β	0.042
	CF	SFAs	C-13	Tridecanoic acid	0.042
			C-16	Hexadecanoic acid	0.061
			C-16	14-Methyl-pentadecanoic acid	0.052
		MUFAs	C-12:1	11-Dodecenoic acid	0.048
			C-16:1 ω-7	9-Hexadecenoic acid	0.045
			C-18:1 ω-9	Z-9-octadecenoic acid	0.049
		PUFAs	C-18:2 ω-3	Z,Z-9,15-octadecadienoic acid	0.045
			C-20:3 ω-3	11,14,17-Eicosatrienoic acid	0.024

C: Concentration.

No specific peaks were observed upon the direct GC-MS injection of CF. Hence, it was subjected to esterification to yield the fatty acid methyl esters. Fatty acid methyl esters were detected in the GC-MS and was found to be possessed with SFA, MUFA and PUFAs. SFAs (C-13 to C-18) were seen the highest in Chlorophyta with 0.480% in *C. antennina*. *E. prolifera* collected from the Njarakkal location exhibited low SFAs (C-13 to C-17) with 0.086%. Among the Rhodophyta, *G. foliifera* had the highest SFAs with 0.351% (C-08 to C-27).

Pharmacologically important unsaturated fatty acids were seen the highest in Chlorophyta. *E. prolifera*-Njarakkal had 0.210% of MUFAs (C-16:1 and C-18:1) and 0.290% of PUFAs (C-16:4, C-18:2, C-18:3, C-18:4, C-20:3 and C-20:4). Omega fatty acids were observed in majority with 41% ω-3, 17% ω-6, 27% ω-7 and 15% ω-9. Similarly, *C. antennina* had 0.136% MUFAs (C-16:1 and C-18:1) and 0.415% PUFAs (C-16:4, C-18:2, C-20:4 and C-20:5). Acids were also belonging to the omega fatty acids with 39% ω-3, 26% ω-6 and 35% ω-7. 0.142% of MUFAs (C-12:1, C-16:1 and C-18:1) and 0.069% of PUFA (C-18:2 and C-20:3) were observed in *U. fasciata* with 23% ω-3, 23% ω-7, 21% ω-9 and 23% ω-11 omega fatty acids. *E. prolifera* collected from the Kayamkulam location was observed to have low contents of MUFAs and PUFAs. Rhodophyta had low variations of MUFAs and

PUFAs. High variations were observed in *G. foliifera* with 0.273% MUFAs (C-14:1, C-16:1, C-17:1 and C-18:1) and 0.070% PUFAs (C-25:2). 0.032% of MUFAs (C-18:1) was observed in *G. corticata* var. *cylindrica* which belonged to ω-9 fatty acids. *G. corticata* was observed with 0.137% of MUFAs with 48% C-17:1 ω-9 and 52% C-18:1 ω-8 fatty acids. CF of *E. prolifera* and *C. antennina* had the ω-6/ω-3 ratio within the World Health Organization (WHO) (< 10) prescribed standards. SF of the analysed seaweeds couldn't be detected in GC-MS. This observation highlighted the presence of non-volatile high molecular glycosidic linkages which required more detailed investigation hereafter.

3.7. Statistical correlation

The biochemical constituents upon correlation analysis with the bioactivities exhibited and indicated appreciable positive correlations (Tables 11 and 12). The amount of total extractives correlated with the antioxidant and antimicrobial activities. The alcohols, alkanes, alkenes and alkynes contents in the PEF correlated positively with the iodine value and microbiological activities. The presence of these indicated a lethal effect on *E. coli*, *S. aureus* and *B. cereus*. The fatty acids such as the SFAs, MUFAs and PUFAs exhibited positive correlations with all the three antioxidant activities, saponification and iodine value. It was also active against *B. cereus* and *E. coli*. Sterol rich EAF correlated with the antimicrobial activities indicated its broad spectrum antimicrobial activity. Antioxidant activities were also seen to be correlated with the antimicrobial activities too. Both the DPPH and ferrous tartrate active fractions were seen to correlate with all the four microbes which indicated the broad spectral activities. The area of interest of the current study was upon the saponins content and it exhibited full segment antioxidant activity. It was positively correlated to the lethality of *B. cereus* and *S. abony*.

Table 12
Pearson's bivariate correlation analysis.

	TCSA	TCBC	TCEC	TCSAB	CPSA	CPBC	CPEC	CPSAB
TCSA	1.00							
TCBC	-0.23	1.00						
TCEC	-0.13	0.36	1.00					
TCSAB	0.50	0.02	-0.08	1.00				
CPSA	1.00	-0.23	-0.13	0.51	1.00			
CPBC	-0.24	1.00	0.36	0.01	-0.24	1.00		
CPEC	-0.12	0.36	1.00	-0.08	-0.12	0.37	1.00	
CPSAB	0.50	0.02	-0.08	1.00	0.51	0.01	-0.08	1.00

TCSA: Tetracycline-*S. aureus*; TCBC: Tetracycline-*B. cereus*; TCEC: Tetracycline-*E. coli*; TCSAB: Tetracycline-*S. abony*; CPSA: Chloramphenicol-*S. aureus*; CPBC: Chloramphenicol-*B. cereus*; CPEC: Chloramphenicol-*E.coli*; CPSAB: Chloramphenicol-*S. abony*.

Table 11
Pearson's bivariate correlation analysis.

	YIELD	ALC	ALKA	ALKE	ALKY	MUFA	PUFA	SFA	STER	SC	SV	IV	DAAE	DBHTE	DTE	DRE	FTAAE	FTBHTE	FTTE	FTRE	KAAE	KBHTE	KTE	KRE
YIELD	1.00																							
ALC	-0.03	1.00																						
ALKA	-0.05	0.78	1.00																					
ALKE	0.07	0.83	0.74	1.00																				
ALKY	-0.03	0.79	0.67	0.80	1.00																			
MUFA	0.24	-0.23	-0.24	-0.13	-0.13	1.00																		
PUFA	0.34	-0.16	-0.17	-0.09	-0.10	0.64	1.00																	
SFA	0.39	-0.22	-0.24	-0.13	-0.13	0.77	0.70	1.00																
STER	-0.14	-0.17	-0.18	-0.10	-0.10	-0.19	-0.14	-0.19	1.00															
SC	0.22	-0.26	-0.28	-0.15	-0.15	-0.29	-0.21	-0.29	-0.22	1.00														
SV	0.51	-0.31	-0.31	-0.15	-0.19	0.85	0.66	0.82	-0.24	-0.21	1.00													
IV	0.02	0.15	0.36	0.22	0.39	0.39	0.47	0.32	-0.15	-0.37	0.47	1.00												
DAAE	0.30	-0.33	-0.35	-0.20	-0.19	0.19	0.01	0.31	-0.04	0.09	0.33	-0.07	1.00											
DBHTE	0.30	-0.33	-0.34	-0.19	-0.19	0.18	0.00	0.31	-0.05	0.08	0.33	-0.07	1.00	1.00										
DTE	0.42	-0.29	-0.31	-0.17	-0.17	0.13	-0.03	0.24	-0.11	0.05	0.34	-0.13	0.93	0.93	1.00									
DRE	0.30	-0.31	-0.32	-0.19	-0.18	0.17	0.00	0.30	-0.06	0.07	0.32	-0.07	1.00	1.00	0.94	1.00								
FTAAE	0.05	-0.51	-0.54	-0.29	-0.30	-0.30	-0.27	-0.29	0.08	0.72	-0.15	-0.39	0.22	0.21	0.23	0.20	1.00							
FTBHTE	0.09	-0.52	-0.55	-0.31	-0.32	-0.17	0.11	0.00	0.08	0.43	-0.02	-0.34	0.25	0.25	0.24	0.24	0.68	1.00						
FTTE	0.09	-0.51	-0.55	-0.31	-0.32	-0.18	0.11	0.00	0.07	0.43	-0.03	-0.34	0.25	0.25	0.24	0.24	0.67	1.00	1.00					
FTRE	0.04	-0.41	-0.44	-0.24	-0.24	-0.14	0.18	0.06	-0.15	0.46	-0.04	-0.3	0.10	0.10	0.11	0.09	0.55	0.75	0.75	1.00				
KAAE	0.08	-0.43	-0.46	-0.25	-0.24	-0.25	-0.17	-0.25	0.04	0.81	-0.21	-0.45	0.22	0.22	0.21	0.20	0.78	0.49	0.49	0.58	1.00			
KBHTE	0.08	-0.42	-0.45	-0.24	-0.23	-0.26	-0.17	-0.25	0.04	0.81	-0.22	-0.44	0.22	0.21	0.21	0.20	0.78	0.48	0.48	0.58	1.00	1.00		
KTE	0.08	-0.41	-0.44	-0.24	-0.23	-0.26	-0.17	-0.26	0.03	0.81	-0.22	-0.45	0.21	0.21	0.20	0.19	0.77	0.48	0.48	0.57	1.00	1.00	1.00	
KRE	0.08	-0.43	-0.46	-0.25	-0.24	-0.25	-0.17	-0.25	0.04	0.81	-0.21	-0.44	0.22	0.22	0.21	0.20	0.78	0.49	0.49	0.58	1.00	1.00	1.00	1.00
TCSA	-0.05	0.06	0.26	0.33	0.13	-0.08	-0.24	-0.12	0.36	-0.12	-0.11	0.12	0.02	0.02	-0.02	0.00	0.01	-0.09	-0.09	-0.37	-0.08	-0.08	-0.08	-0.08
TCBC	0.21	-0.01	-0.19	-0.07	-0.22	-0.09	0.03	0.02	0.05	0.18	0.00	-0.38	0.13	0.14	0.15	0.14	0.06	0.28	0.28	0.18	0.01	0.01	0.01	0.01
TCEC	-0.24	0.07	-0.08	0.01	0.14	-0.23	-0.16	0.05	0.01	-0.05	-0.09	-0.13	0.04	0.05	0.03	0.05	0.11	0.28	0.28	0.07	-0.17	-0.17	-0.17	-0.17
TCSAB	-0.03	-0.29	-0.30	-0.17	-0.17	0.07	-0.12	-0.06	0.51	0.10	-0.05	-0.27	0.15	0.14	0.16	0.13	0.25	0.14	0.14	-0.04	0.31	0.30	0.30	0.31
CPSA	-0.05	0.06	0.26	0.33	0.13	-0.07	-0.24	-0.11	0.36	-0.12	-0.10	0.12	0.03	0.02	-0.02	0.01	0.00	-0.10	-0.10	-0.37	-0.08	-0.08	-0.08	-0.08
CPBC	0.19	0.00	-0.19	-0.07	-0.21	-0.09	0.02	0.01	0.04	0.18	-0.01	-0.37	0.12	0.12	0.14	0.13	0.07	0.28	0.28	0.17	0.01	0.01	0.01	0.01
CPEC	-0.25	0.08	-0.07	0.01	0.13	-0.22	-0.17	0.05	0.01	-0.06	-0.10	-0.14	0.04	0.04	0.03	0.05	0.1	0.27	0.27	0.06	-0.18	-0.18	-0.18	-0.18
CPSAB	-0.03	-0.28	-0.30	-0.16	-0.17	0.06	-0.12	-0.06	0.52	0.08	-0.05	-0.26	0.15	0.14	0.16	0.12	0.25	0.14	0.14	-0.04	0.30	0.29	0.29	0.29

YIELD: Yield of the fractions collected; ALC: Alcohols content; ALKA: Alkanes content; ALKE: Alkenes content; ALKY: Alkynes content; STER: Sterol content; SC: Saponins content; SV: Saponification value; IV: Iodine value; DAAE: DPPH ascorbic acid equivalence; DBHTE: DPPH BHT equivalence; DTE: DPPH α-tocopherol equivalence; DRE: DPPH resorcinol equivalence; FTAAE: Ferrous tartrate ascorbic acid equivalence; FTBHTE: Ferrous tartrate BHT equivalence; FTTE: Ferrous tartrate tocopherol equivalence; FTRE: Ferrous tartrate resorcinol equivalence; KAAE: $KMnO_4$ ascorbic acid equivalence; KBHTE: $KMnO_4$ BHT equivalence; KTE: $KMnO_4$ tocopherol equivalence; KRE: $KMnO_4$ resorcinol equivalence; TCSA: Tetracycline-*S. aureus*; TCBC: Tetracycline-*B. cereus*; TCEC: Tetracycline-*E. coli*; TCSAB: Tetracycline-*S. abony*; CPSA: Chloramphenicol-*S. aureus*; CPBC: Chloramphenicol-*B. cereus*; CPEC: Chloramphenicol-*E.coli*; CPSAB: Chloramphenicol-*S. abony*.

4. Discussion

Most of the *in vitro* bioactivity studies done in seaweed species were concluded the activity in relation with the phytochemicals such as polyphenolic compounds, flavonoids and terpenes. Generation of new degenerative chemicals and mutation of microorganisms has led to the urgent need for alternative compounds with advanced multifaceted activities. Phytocompounds such as saponins, fatty acids, sterols, alkanes, alkenes, alkynes and alcohols that are produced by the marine algae could be extracted and used for fulfilling the expected demands. Marine algae estimated in the present study contain novel antioxidant and antimicrobial compounds which could act against free radical generations, oxidative stress and in eliminating the pathogenic bacteria growths.

The saponins content of seaweed have gained recent scientific attention due to its pharmaceutical importance. They have positive effects on the lowering of heart diseases and are also observed to be the key point in the pharmaceutical properties of traditional herbs. The observations of the current study were aligning with the previous studies done on seaweeds[8,9]. Saponins were observed in extracts of seaweeds collected from the Indian coast[8]. 0.35%–1.08% of saponins were reported in the five Chlorophyta and five Ochrophyta seaweeds collected from the southwest coast of India[9].

Seaweeds are an antioxidant compound resource. Many studies have evidenced the presence of antioxidant activities. Seaweeds such as the *Eucheuma cottonii*, *Eucheuma spinosum*, *Halymenia durvillaea*, *Caulerpa lentillifera*, *Caulerpa racemosa*, *Dicranopteris dichotoma*, *Sargassum polycystum* and *Padina* sp., exhibited good free radical scavenging properties along with reducing powers[12]. *Terrapene ornata* and *Sargassum polycystum* collected from Thailand were reported to have high free radical scavenging properties and reducing properties[33]. The observations of the three methods, as discussed in the current study, showed remarkable antioxidant activities for all the fractions. The activities of free radical quenching were seen in the range of 0.002% to 0.311% in PEF, 0.054% to 6.094% in EAF, 0.061% to 12.774% in CF and 0.002% to 8.025% in SF. The reducing potential was seen in the range of 0.025% to 0.117% in PEF, 0.095% to 1.282% in EAF, 0.075% to 1.181% in CF and 0.231% to 1.345% in SF. The activities of PEF was in the range of 0.013% to 3.492%, EAF was in the range of 0.069% to 19.801%, CF was in the range of 0.028% to 13.672% and SF was in the range of 0.118% to 40.431% with respect to total oxidising potentials. The results therefore concluded that the SF and CF possessed excellent anti-oxidizing capabilities which could be used up in the functional foods and nutraceuticals.

A huge number of studies have been reported with antimicrobial activities in seaweeds. Methanolic water extracts of brown seaweed *Heliamphora elongata* exhibited antimicrobial activity against *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Listeria monocytogenes* and *S. abony*. The ethyl acetate crude extract of *Caulerpa* showed appreciable antibacterial inhibition activity on the strains of *E. coli*, *S. aureus*, *Streptococcus* sp. and *Salmonella* sp.[34]. A total of 14 seaweeds collected from the southwest coast of India, which included *G. corticata*, *C. antennina*, *U. fasciata etc.*, showed the extracts of toluene and methanol (1:3) with high antibacterial activity. The extract of *G. corticata* was highly active against Gram-negative pathogen, *Proteus mirabilis*. The results showed better response to Gram-negative than Gram-positive due to the lipophilic nature of the active components in the crude extract[35]. Methanolic extracts of fifteen red seaweeds samples collected from the southwest coast of India exhibited *in vitro* antimicrobial activity against Gram-negative bacteria, like *E. coli*, and Gram-positive bacteria like *S. aureus*. Zone inhibition technique was used which was directly related to the concentration of extracts[36]. The PEF of *Laminaria japonica* was observed to be active against *Clavibacter michiganensis* subsp. *sepedonicus*[37]. All the solvent extract fractions *viz.*, benzene, acetic acid, hexane, dimethyl sulfoxide, diethyl ether and chloroform of *Sargassum longifolium* exhibited good antibacterial activities against *Streptococcus* sp., *Proteus* sp., *Bacillus subtilis*, *Klebsiella pneumonia* and *Enterococci* sp.[38]. The ethyl acetate crude extract of *Caulerpa* showed appreciable antibacterial inhibition activity on the strains of *E. coli*, *S. aureus*, *Streptococcus* sp. and *Salmonella* sp.[34]. Potent antimicrobial activity was exhibited by the extracts of *Enteromorpha linza* on Gram-negative pathogens, *Prevotella intermedia* and *Porphyromonas gingivalis* which initiated periodontitis, a chronic inflammatory disease, without any side effects[21]. The observations of the present study were comparable with the previous reports. PEF of *G. corticata* var. *cylindrica* exhibited > 17% of activity against *B. cereus*. EAF of *U. fasciata* exhibited > 30% activity against *S. aureus*, > 17% activity against *S. abony* and moderate activity against other strains. SF of *G. corticata* var. *cylindrica* exhibited > 15% activity against *E. coli*. *S. aureus* and *S. abony* were observed to be resistant to all fractions of *C. antennina*. *B. cereus* and *S. abony* were resistant to the fractions of *E. prolifera* collected from the Kayamkulam location. *B. cereus* was resistant against the fractions of *G. corticata* and *E. prolifera* collected from the Njarakkal location. The present study was an in-depth analysis on bactericidal activity after solvent speciation.

Earlier studies have reported the presence of phenolic compounds as the source of antioxidant and antimicrobial activities. The data gathered from this study demonstrated the alkanes, alkenes, alkynes, alcohols, sterols and fatty acids as contributors to the observed bioactivities. The presence of these compounds was already reported in earlier studies in seaweeds. Methanolic extract of *Laurencia brandenii*, collected from the southwest coast of India upon GC-MS analysis exhibited the presence of hexanols, dodecanol, hexadecanoic acid, 9-dodecanoic acid, PUFAs *etc.*[36]. The GC-MS analysis of the methanolic extract of *Solanum marginatum* showed the presence of tetradecanoic

acid, n-hexadecanoic acid and phytols[39]. The highly active methanolic extracts of the 15 red seaweed samples collected from the southwest coast of India, upon GC-MS analysis evidenced the presence of ω-9 PUFAs and some other fatty acids[36]. GC-MS analysis of the fractions of red and brown seaweeds evidenced 3%–5% of ω-9, 3%–32% of ω-6 and 8%–63% of ω-3 PUFAs[40]. *Ulva lactuca* and *Durvillaea antarctica* collected from the coastal area of Chile, had high contents of PUFAs and MUFAs. The saturated acids ranged from C-12 to C-24, MUFAs from C-14 to C-22 (ω-7 and 9) and PUFAs from C-16 to C-22 (ω-3, 6 and 7). Total SFAs content was 33% and 25%, total MUFAs content was 36% and 38% and PUFAs was 18% and 34% respectively[41]. *Porphyra* sp. collected from Japan, Korea and China, *Undaria pinnatifida*, *Laminaria* sp., and *Hizikia fusiforme* collected from China were reported to have the contents of SFAs in the range of C-12 to C-24, MUFAs in the range of C-14 to C-22 (ω-5, 7, 9, 11 and 13) and PUFAs in the range of C-18 to C-22 (ω-3 and 6). The total SFAs content was 35%, 44%, 17%, 41% and 28% respectively. MUFAs content was 18%, 20%, 7%, 17% and 13% respectively and PUFAs content was 44%, 33%, 73%, 39% and 57% respectively[42]. Twenty-two tropical seaweeds collected from the Saurashtra coast, India, showed high level of ω-6 and ω-3 PUFAs with their ratios underlying with the WHO prescription limits[43]. The fatty acid composition was with high palmitic acid (C16:0) content and traces of other SFAs. High contents of PUFA with 11% to 65% in Rhodophyta, 39% to 49% in Phaeophyta and 14% to 43% in Chlorophyta were reported. Omega-3 and ω-6 were seen in all analysed seaweeds. Omega-6/ω-3 ratio was seen to be < 10 which was within the WHO prescribed standards except in *G. corticata*, *Gracilaria dura, Gracilaria debilis* and *Gracilaria fergusonii* where the values exceeded[43]. The total lipid content varied from about 0.4%–1.8% in *Paradoxophyla palmata*[1]. Three edible species of *Caulerpa* genus exhibited high PUFA contents with ω-3 and ω-6 fatty acids[34]. GC-MS analysis of the solvent extracted fractions of red and brown seaweeds also evidenced 3%–5% of ω-9, 3%–32% of ω-6 and 8%–63% of ω-3 PUFA[40].

This study was able to prove that the six selected seaweeds from the southwest coast of India were a significant source of pharmacologically active saponins and fatty acids, which possessed high antioxidant and antimicrobial activities. All the seaweed extracts were studied for the chemical compositional profiling in GC-MS and were observed to have the potentiality to be considered as a potent bioactive resource by meeting the standard requirements. The selective extraction of bioactive compounds from seaweeds could be adopted in the emerging needs for natural bioactive compounds. In all the analysed seaweeds, the extracted fractions were observed to exhibit either antioxidant or antimicrobial activities or both. Saponins/cardiac glycosides which are considered as the pharmacological input of traditional medicines were also observed in the seaweeds. Broad spectrum antimicrobial activity was observed in *U. fasciata*, *G. corticata* and *G. foliifera*. Economically and pharmaceutically important omega fatty acids were seen in all seaweeds with *C. antennina* and *E. prolifera* having the ω-6/ω-3 ratio in line with the WHO prescribed standards (< 10). The statistical correlation also evidenced the constituent activity relations. The study concluded that, green seaweeds especially *U. fasciata*, *E. prolifera* and *C. antennina* representing important sources of bioactive constituents should be used up in cultivation and as an input source of functional food and nutraceuticals. The cultivation of the studied species like the *G. corticata* var. *cylindrica* and *C. antennina* as a resource for food may not be advisable as it may not necessarily be edible. But the cultivation would be viable where the species could be considered as a commercial raw material or as a nutraceutical extractive source.

Conflict of interest statement

We declare that we have no conflict of interest.

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