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Deep Das , Abimannan Arulkumar , Sadayan Paramasivam , Aroa Lopez-Santamarina , Alicia del Carmen Mondragon , Jose manuel Miranda lopez *

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*Article*

# Phytochemical Constituents, Antimicrobial Properties and Bioactivity of Marine Red Seaweed (*Kappaphycus alvarezii*) and Seagrass (*Cymodocea serrulata*)

**Deep Das [1], Abimannan Arulkumar [2], Sadayan Paramasivam [1] Aroa López-Santamarina [3], Alicia del Carmen Mondragón [3] and Jose Manuel Miranda Lopez [3,*]**

[1] Department of Oceanography and Coastal Area Studies, School of Marine Sciences, Alagappa University, Karaikudi-630003, Tamil Nadu, India; das415269@gmail.com (D.D.); drparamsan@gmail.com (S.P.)
[2] Department of Biotechnology, Achariya Arts and Science College (Affiliated to Pondicherry University), Villiabur, Puducherry -605 110, India; aruul3@gmail.com
[3] Laboratorio de Higiene Inspección y Control de Alimentos, Departamento de Química Analítica, Nutrición y Bromatología, Universidad de Santiago de Compostela, 27002-Lugo, Spain; aroa.lopez.santamarina@usc.es (A.L.-S.); alicia.mondragon@usc.es (A.d.D.C.M.); josemanuel.miranda@usc.es (J.M.M.L.)
* Correspondence: josemanuel.miranda@usc.es

**Abstract:** The present work was performed to evaluate the levels of phytochemical constituents and the antioxidant and antibacterial properties of marine red seaweed (*Kappaphycus alvarezii*) and seagrass (*Cymodocea serrulata*). Quantitative phytochemical analysis, antioxidant activity and antimicrobial activity against 5 potential pathogenic bacteria was investigated. In both cases were found presence of flavonoids, tannins, phenolic compounds, glycosides, steroids, carbohydrates and ashes. Alkaloids were only found in *K. alvarezii*, buT not in *C. serrulata*. The antimicrobial properties of both *K. alvarezii* and *C. serrulata* chloroform extracts were found to be antagonistically effective against the gram-positive bacterium *Bacillus subtilis* and the gram-negative bacterium *Vibrio parahaemolyticus*, *Vibrio alginolyticus, Vibrio harveyi* and *Klebsiella pneumoniae*. GC–MS analysis revealed the presence of 94 bioactive compounds in *K. alvarezii* and 104 *C. serrulata*, including phenol, decane, dodecane, hexadecane, vanillin, heptadecane, diphenylamine, benzophenone, octadecanoic acid, dotriaconate, benzene, phytol, butanoic acid, and 2-hydroxyl-ethyl ether, which played a vital role in antioxidant and antibacterial activities. Thus, in view of the results, both *K. alvarezii* and *C. serrulata* could be considered as sources of ingredients with appreciable nutritional and medicinal value.

**Keywords:** phytochemicals; seaweed; seagrass; *Kappaphycus*; *Cymodocea*; antimicroboal; phenolic; GC–MS; radical scavenging

## 1. Introduction

Seaweeds are a group of autotrophic, halophytic and complex communities that live in marine environments that have potential renewable resources [1,2]. Biologically, they have been classified as Phaeophyta (Brown Algae), Rhodophyta (Red Algae) and Chlorophyta (Green Algae) [3]. Seaweeds grow in salt water, mainly in shallow coastal waters, and can be obtained for human consumption from both wild or cultivated form [4]. Their proximate and nutritional composition varies and is affected by a large variety of factors, including the seaweed species, geographic areas of origin, solar intensity or the seawater temperature [3].

Although in recent years the use of seaweeds as food has been increased in other parts of the world [3], seaweeds are still mostly consumed in Asian countries, such as Japan, China or Korea [3]. Seaweeds consumption has numerous advantages for human health, due to its content in dietary fibers, proteins, essential fatty acids, vitamins or essential minerals [5]. Seaweeds are also between the richest sources of bioactive primary and secondary metabolites, which are characterized by

beneficial biological activities [1]. Between advantages for human health, seaweeds are known for their potential natural antioxidant, antiviral, antiobesity, antitumor and antimicrobial properties [5–7]. Additionally to their use as foods, other uses of seaweeds have been widely increased in recent decades and nowadays, seaweeds are also used as fertilizers, cosmetics, and their extracts were used in pharmaceutical industries as a fresh source of bioactive compounds with a wide range of medicinal properties [7].

Among seaweeds, *Kappaphyus* sp. is a commercially important red seaweed and is cultivated in tropical countries such as the Philippines, Indonesia, and Malaysia, as well as many countries in Eastern Africa [7], because it is relatively easy to cultivate, has short production cycles and has low production costs [7,8]. It is also a common food for the local people and is believed to have various beneficial effects. In India, the southeast coast has a unique marine habitat with a great variety of seaweed species spread around the intertidal zone and shallow- and deep-water regions of the ocean. Specifically, *Kappaphycus alvarezii* (commercially known as "cottoni") grows well in the shores of the Kanyakumari and Ramanthapuram Districts of Tamil Nadu, India [9]. *K. alvarezii* has high economic value since is the principal source of the commercial hydrocolloid κ-carrageenan, and it also contains various inorganic and organic compounds that are beneficial for human health [7,12]. k-carrageenan is used in pharmaceuticals, cosmetics, textiles, organic fertilizers and in the food industry [12].

Other marine source of bioactive compounds with a broad spectrum of beneficial activities for human health are seagrasses [13]. Seagrasses are submerged flowering marine angiosperms living their full lifecycle submerged in marine environments, and they are the primary producers. They are the only flowering plants to recolonize the seaband, are highly productive and play an important ecological role in marine environments (Kim et al., 2021). Seagrasses are found in all coastal areas around the world except in Antarctica [14, 15]. Seagrass biomass are used in some countries as human food, and they are rich sources of secondary metabolites, such as alkaloids, flavonoids, terpenoids, tannins, steroids, and especially phenolic compounds [16]. The phenolic compounds present in seagrasses contribute to pigmentation, growth, reproduction, and resistance against pathogens, and they also act as defensive mechanisms against other aquatic lives as well as protection [17]. They have been used for traditional medicine, such as treating infections caused by pathogenic microbes, fever, inflammation, muscle pain, skin disease, viruses, diarrhea, diabetes, wound healing, sedation, and cancer [15]. Between the different genus forming seagrasses, *Cymodocea*, from Potamogetonaceae family, is represented worldwide by four species: *Cymodocea rotundata*, *Cymodocea serrulata*, *Cymodocea angustata and Cymodocea nodosa* [18]. *C. serrulata* is commonly found in the coastal area of the tropical Indo-West Pacific region. *C. serrulata* can be differentiated from other seagrass species by their shoots with distinctive open leaf scars, triangular, flat leaf sheath fibrous roots on the shoot and serrated leaf tips [14].

Hence, the aim of the present study was to evaluate the phytochemical constituents, antioxidant activity and antibacterial activity present in seaweeds and seagrass. The chemical compounds present in both *K. alvarezii* and *C. serrulata* were also determined.

## 2. Materials and Methods

### *2.1. Collection, identification and processing*

The red seaweed *K. alvarezii* and the seagrass *C. serrulata* were obtained from Thondi coastal waters (Latitude: 9° 44" N and Longitude: 79° 00" E), Palk Bay, southeast coast of India. Freshly collected seaweed (*K. alvarezii*) and seagrass (*C. serrulata*) were cleaned thoroughly in seawater and transported to the laboratory within 1 h after collection. The epiphytes, necrotic parts, muds, dust and other debris were removed by washing thoroughly with fresh water and double distilled water. Then, they were shade dried at 25 ± 2°C for one week, ground into fine powder, and stored at room temperature in an airtight container (Tarsons, Chennai, India) for further analysis. The collected seaweed and seagrass were identified according to those established in the standard manual of Rao [19].

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*2.2. Preparation of extracts*

The seaweed and seagrass extracts were prepared by adding 5 g of dried seaweed or seagrass powder into 50 mL of three different solvents, chloroform, ethanol and distilled water, in a conical flask and placed in a dark bottle in light agitation for 7 days. After that, the extracts were filtered through Whatman No. 1 filter papers and sterile cotton wools, and the supernatants were stored at 4°C for future use [20,21].

*2.3. Determination of alkaloids*

The alkaloid content of *K. alvarezii* and *C. serrulata* was determined by the method proposed by Hikino et al. [22]. One milliliter of test extract phosphate buffer (5 ml, pH 4.7) was added to 5 ml of bromocresol green solution, and the mixture was shaken vigorously added with 4 ml of chloroform. The extracts were collected in a 10 ml volumetric flask. The absorbance of the complex in chloroform was measured at 470 nm in UV–Vis spectrophotometer (Shimadzu, Kyoto, Japan) against a blank prepared as described above but without extract. Atropine (Sigma-Aldrich, St. Louis, MO, USA) was used as a standard material, and the assay was compared with atropine equivalents.

*2.4. Determination of flavonoids*

Total flavonoid content was determined by the aluminum chloride method [23] using catechin (Sigma-Aldrich) as a standard. One milliliter of test sample and 4 ml of water were added to a volumetric flask (10 ml volume). After 5 min, 0.3 ml of 5% sodium nitrite and 0.3 ml of 10% aluminum chloride (Sisco Research Laboratories, Mumbai, India) were added. After 6 min of incubation at room temperature, 2 ml of 1 M sodium hydroxide (Sisco Research Laboratories) was added to the reaction mixture. Immediately, the final volume was brought to 10 ml with distilled water. The absorbance of the reaction mixture was spectrophotometrically measured at 510 nm against a blank in UV–Vis spectrophotometer (Shimadzu). The results were expressed as catechin equivalents (mg catechin/g dried extract).

*2.5. Determination of tannins*

The total tannin content extracts were determined according to the Julkunen-Titto [24] method. 50 µl extracts were mixed with 1.5 ml of 40% vanillin (Sisco Research Laboratories) (prepared with methanol), and then 750 µl of HCl was added. The solution was shaken vigorously and left at room temperature for 20 min in darkness. The absorbance of the mixtures was measured at 500 nm using a spectrophotometer (Shimadzu). Catechin (Sigma-Aldrich) in the range of 20-200 mg/L was used to construct a calibration curve.

*2.6. Determination of phenolic compounds*

The total phenolic content in different solvent extracts was determined with Folin-Ciocalteu's reagent proposed by Sangeeta and Vrunda [25]. In the procedure, different concentrations of the extracts were mixed with 0.4 ml Folin-Ciocalteu's reagent (Sigma-Aldrich) (diluted 1:10 v/v). After 5 min, 4 ml of sodium carbonate solution was added. The final volume of the tubes was brought to 10 ml with distilled water and left for 90 min at room temperature. The absorbance of the sample was measured against the blank at 750 nm using a spectrophotometer (Shimadzu). A calibration curve was constructed using 1,2-dihydroxybenzen (catechol) (Sigma-Aldrich) solutions as standards, and the total phenolic content of the extract was expressed in terms of mg of catechol per g of dry weight.

*2.7. Determination of cardiac glycosides*

The cardiac glycoside content in the samples was evaluated using Buljet's reagent as described by El-Olemy et al. [26]. One gram of the fine powder of *K. alvarezii* and *C. serrulata* was soaked in 10 ml of 70% alcohol for 2 hr. and then filtered. The extract obtained was then purified using lead acetate and Na2HPO4 solution before the addition of freshly prepared Buljet's reagent (containing 95 ml

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aqueous Picric acid + 5 ml 10% aqueous NaOH) (Sigma-Aldrich). The difference between the intensity of colors of the experiment and blank samples gives the absorbance at 217 nm and is proportional to the concentration of the glycosides.

*2.8. Determination of steroids*

The steroid content was determined by Ejikeme et al. [24]. One milliliter of test extract of steroid solution was transferred into 10 ml volumetric flasks. Sulfuric acid) (Sisco Research Laboratories) (4 N, 2 ml and iron (III) chloride (Sisco Research Laboratories) (0.5% w/v, 2 ml) were added, followed by potassium hexacyanoferrate (III) solution) (Sisco Research Laboratories) (0.5% w/v, 0.5 ml). The mixture was heated in a water bath maintained at 70 ± 20°C for 30 minutes with occasional shaking and diluted to the mark with distilled water. The absorbance was measured at 780 nm spectrophotometer (Shimadzu) against the reagent blank.

*2.9. Determination of carbohydrates*

Carbohydrate content was estimated by the phenol–sulfuric acid method [27]. Briefly, 200 mg of powdered sample was hydrolyzed by adding 5 ml of 2.5 N HCl. The sample was kept in a boiling water bath, and after 3 h of incubation, the solution was neutralized with solid Na2CO3 until effervescence ceased. The solution was made up to 50 ml and centrifuged at 8000 rpm for 10 min in a centrifuge (Remi Lab World, Mumbai, India). The supernatant was aliquoted and brought up to 1 ml with deionized water, to which 1 ml of phenol and 5 ml of 96 % sulfuric acid (Sisco Research Laboratories) were added. After mixing the solution, it was kept in a water bath at 25 ± 1°C for 20 min. The absorbance was measured at 490 nm using a UV–Vis spectrophotometer (Shimadzu).

*2.10. Ash content*

The ash content was determined by the method of Yemm and Willis [27]. Two g of each sample was taken and weighed accurately in a clean silica dish. The dish was first heated over a low burner flame. After that, the dish was transferred to a SNOL muffle furnace (Utena, Lithuania) maintained at 3000°C-4500°C for 3-5 h. The ash residue obtained was then cooled in a desiccator and weighed. The percentage of total ash content was calculated by the formula as follows.

Total Ash Percent of plant sample (%) = [Weight of dry ash residue (g) ÷ Weight of plant sample (g)] x 100.

*2.11. Hydrogen peroxide radical scavenging activity*

The antioxidant activity of seaweed and seagrass extracts was evaluated by the hydrogen peroxide radical scavenging activity as described by Ebrahimzadeh et al. [28]. The seaweed and seagrass extracts (100 µg/mL) were reacted with 0.6 mL of 40 mM H2O2 solution prepared in phosphate buffer (pH 7.4) (Sisco Research Laboratories). After incubation at 37°C for 10 min, absorbance was measured at 230 nm using a UV–Vis spectrophotometer (Shimadzu). Phosphate buffer was used as the corresponding blank solution. A similar procedure was repeated with distilled water instead of the extract, which served as a control. Ascorbic acid (Sigma-Aldrich) (20-100 µg/mL) was used as a standard.

*2.10. In vitro antibacterial activity of seaweed and seagrass against human pathogenic bacteria*

The antibacterial activity of seaweed and seagrass extracts was evaluated by the well diffusion method on Muller-Hinton agar (Hi-Media, Mumbai, India). Approximately 100 µl of 105 CFU/ml diluted inoculum of bacterial culture was applied on the surface of Muller-Hinton agar plates. The Muller-Hinton agar well was made with a well borer under aseptic conditions and filled with *K. alvarezii* and *C. serrulata* extracts and methanol served as positive controls. The plates were incubated at 37°C for bacterial growth, and the antibacterial activity of the seaweed and seagrass samples was evaluated by measuring the zone of inhibition (mm) against the tested pathogenic bacteria. All

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experiments were performed in triplicate, and the data are expressed as the mean values of the experiments.

*2.11. Characterization of the active compound by gas chromatography–mass spectrometry (GC–MS)*

The crude extracts of *K. alvarezii* and *C. serrulata* were loaded onto a silica gel (Hi-Media) packed column (20 cm length and 2 cm diameter) and eluted with n-hexane: ethyl acetate (50: 50 v/v) (Sigma-Aldrich). The fractions were characterized by gas chromatography GC-2010 interfaced with a quadrupole mass spectrometer QP-2010 (Shimadzu, Japan) analyzer to determine their chemical constituents using an Rtx-PCB capillary column (60 m x 0.25 mm i.d., 0.25 mm film thickness, Resteck, Bellefonte, PA). Helium with a purity of 99.99% was used as the carrier gas at a flow rate of 1 ml/min. One mil of extract was injected in spilt mode using an autosampler. The injector port, interface and ion source temperature were set at 250, 270 and 230°C, respectively. The GC temperature was programmed as follows: 50°C (1 min), 10°C (1 min) lamp to 320°C (10 min hold). The mass spectrometer was operated in electron ionization (EI) mode at 70 eV and at an emission current of 60 mA. Full scan data were obtained in a mass range of m/z 50-500. Interpretation of mass spectrum analysis was performed using the National Institute Standard and Technology (NIST) database. The spectrum of the unknown components was compared with the spectrum of known components stored in the NIST library.

*2.12. Statistical analysis*

All determinations were given in terms of the mean ± standard deviation (SD). The results obtained were compared by one-way analysis of variance (ANOVA). The significance of the difference between means was determined by Duncan's multiple range test ($P < 0.05$) using SPPS version 14 (Chicago, IL, USA).

## 3. Results and discussion

In the present work, red seaweed *K. alverazii* and seagrass *C. serrulata* were tested for their phytochemical, antioxidant and antibacterial activities. Phytochemical analysis of *K. alvarezii* and *C. serrulata* revealed the presence of alkaloids (only in the case of *K. alverazii*) flavonoids, tannins, phenolic compounds, glycosides, steroids, carbohydrates and ashes. Among the six phytochemicals present in *K. alvarezii*, higher contents were found for phenolic compounds (3.39 ± 0.41 mg/g) and tannins (2.94 ± 0.41 mg/g). Among the five phytochemicals present in *C. serrulata*, the higher contents were found for glycosides (2.47 ± 0.41 mg/g) and flavonoids (2.11 ± 1.40 mg/g) (Table 1). These constituents significantly contributes to the biological activity of seaweeds and seagrass [29]. Similar observations were also made by other works [30–32], in which tannins, flavonoids, phenolic compounds, carotenoids and polysaccharides were found in both seaweed and seagrasses.

**Table 1.** Steroids, tannins, flavonoid, glycosides, alkaloids and phenolic compounds of *Kappaphycus alvarezii* and *Cymodocea serrulata*.

| Parameters | *K. alverazii* | *C. serrulata* |
|---|---|---|
| Alkaloids (ATE/g dry wt) | 1.91±0.58* | - |
| Flavonoids (CAE/g dry wt) | 1.63±2.73 | 2.11±1.40 |
| Tannins (CAE/g dry wt) | 2.94±0.41* | 1.94±0.85 |
| Phenolic Compound (GAE/g dry wt) | 3.39±0.45* | 1.01±0.39 |
| Glycosides (mg/g dry wt) | 1.88±0.11 | 2.47±0.28* |
| Steroids (mg/g dry wt) | 2.51±0.15* | 1.60±0.24 |
| Carbohydrates (% DW) | 2.57±1.89 | 1.44±1.75 |
| Ash (% DW) | 8.5±0.95 | 6.9±0.49 |
| Antioxidant activity | 27.9±0.1 | 22.1±01 |

Values are means of three analyses of the extracts ± standard deviation (n=3); CAE: Catechin equivalent; GAE: Gallic acid equivalents; ATE: Atropine equivalent.

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In the present study, *K. alvarezii* showed a higher tannins content (2.94 ± 0.41 mg Catechin equivalent (CAE)/g) than *C. serrulata* (1.94± 0.85 mg CAE/g). Similarly, Deyad and Ward [33] reported a similar tannins content in the brown seaweed *Dictyota dichotoma* (2.12 ± 0.45 mg CAE/g), whereas Domettila et al. [34] reported a higher presence of tannins in the red seaweed *Sargassum wightii* (27.54 ± 0.54 mg CAE/g). In previous studies [13], it was reported the presence of tannins in *C. serrulata* (264.71 mg/ml tannic acid equivalence). Similarly, another work reported the presence of tannins in seagrass *Syringodium isoetifolium* (80.65 ± 5.64 mg CAE/g [35]. Tannins are polyphenols which have a large influence on the nutritive value of humans and animals, due to its antimicrobial, anti-inflammatory, and astringent activities [13].

Flavonoids content was similar in both *K. alvarezii* and *C. serrulata*, although in global term it was found in lower amounts than in previous work. Vaghela et al. [35] found a much higher content (15.26 ± 0.95 mg CAE 100 $g^{-1}$). Similarly, Smadi et al. [36] reported the flavonoid content of *C. nodosa* as 3.98 ± 0.03 mg CAE/g, which is comparatively much higher than the results of the present study.

*K. alvarezii* showed an alkaloids content of 1.91 ± 0.58 mg CAE/g. Similarly, Domettila et al. [34] showed the alkaloids content of 1.32 ± 0.02 mg CAE/g in *Ulva reticulata*. Previously, Kubbat et al. [38] also reported alkaloids content in two brown algae *Cystoseira compressa* and *Sargassum hornschuchii* of 4125.00 ± 180.28 mg/g DW and 3708.33 ± 152.75 mg/g DW. Alkaloids are proved to have antiplasmodic, antimicrobial, and cytotoxic properties [38].

The phenolic compounds content in seaweeds are in part responsible of their scavenging activity, which protects against lipid oxidation [39]. In this work, *K. alvarezii* showed a higher phenolic content (3.39 ± 0.45 mg gallic acid equivalents (GAE)/g) than *C. serrulata* (1.01 ± 0.39 mg GAE/g). Previously, other authors reported a significantly higher content of phenolic compounds of both *K. alvarezii* (3.14 ± 0.14 mg GAE/g) [40] and *Kappaphycus striatum* (7.24 ± 0.21 mg GAE/g) [41]. Regarding *C. serrulata*, the results obtained in the current work were also significantly lower than those reported by Libin et al. [17] for *C. serrulata* (2.98 ± 0.12 mg GAE/g), and those reported for *Cynodocea rotundata* (2.04 ± 0.1) [42]. The phenolic content of seaweed and seagrass depends on the solvent used for the extraction, environment, habitat and biomass.

The presence of steroids in seaweed *K. alvarezii* (2.51 ± 0.15 mg/g) was higher than in seagrass *C. serrulata* (1.60 ± 0.24 mg/g). Previous, study also showed that the presence of steroids in seaweed *C. elongata* is 2.27 ± 0.26 mg/g [43]. Kumar et al. [44] also reported the presence of steroids in *Champai parvula* (24.30 ± 0.11 mg/g). Previously, it was also reported the presence of steroids *Himanthalia elongata* (2.64 ± 2.21 mg/g) [45]. In previous studies, Kannan et al. [46] reported the presence of steroids in *C. rotundata* (2.37 ± 1.27 mg/g). Similarly, Tango et al. [47] also reported the presence of steroids in seagrass *Haludole pinifolia* (5.62 ± 0.76 mg/g). Steroids isolated from seaweed and seagrass have medicinal values such as antihelmintic, antioxidant, antimicrobial and antiviral [45].

*K. alvarezii* showed a glycosides content of 1.88 ± 011 mg/g and in *C. serrulata* of 2.47 ± 0.28 mg/g. Previously, Kumar et al. [44] also reported the presence of glycosides in seaweed *Cymodocea parvula* (35.33 ± 0.14 mg/g). Similarly, Prabakaran et al. (2018) also reported the presence of glycosides in *Chorella vulgaris* (5.75 ± 0.23 mg/g). Deyad et al. [33] also reported the presence of glycosides in seaweed *D. dichotoma* (2.14 ± 0.15 mg/g). In a previous work done by Regalado et al. [50] showed the presence of glycosides in *Thalassia testudinum* (4.61 ± 1.60 mg/g). Glycosides are well known to lower the blood pressure in humans [44].

With respect to carbohydrates and ash content, the carbohydrate content of *K. alvarezii* was 2.57 ± 1.89 mg/g DW, and that of *C. serrulata* was 1.44 ± 1.75 mg/g DW. The wide variation in the carbohydrate content observed in seaweed and seagrass might be due to the influence of different factors, such as salinity, temperature, and sunlight intensity. Moreover, carbohydrate content is also influenced by biomass, which reveals the link between growth and carbohydrate content. Regarding ashes, *K. alvarezii* had a higher ash content (8.5 ± 0.95 g/100 g) than *C. serrulata* (6.9 ± 0.49 g/100 g). High ash content showed the presence of appreciable amounts of diverse minerals found in both seaweed and seagrass.

Antioxidant effectiveness is measured by monitoring the inhibition of oxidation of a suitable substrate [16]. In biological systems, antioxidant effectiveness is classified into two groups: evaluation

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of lipid peroxidation and measurement of free radical scavenging ability [28]. The *in vitro* antioxidant activity of *K. alvarezii* and *C. serrulata* extracts was evaluated by hydrogen peroxide radical scavenging activity *K. alvarezii* had higher scavenging activity (27.9 ± 0.1%) than *C. serrulata* (22.1 ± 0.1%). Regarding *K. alvarezii*, the results obtained were higher than those previously reported by other authors as Farah et al. [39], or Chew et al. [51], whose reported a lower (18.34 ± 0.57% and 11.8 ± 5.7%, respectively) 2,2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging activity. Regarding *C. serrulata*, the DPPH scavenging activity obtained were lower than those obtained by Kannan et al. [52] (61.85 ± 0.95%) free radical scavenging activity from the same seagrass species, although higher than those reported by Rengasamy et al., 2013 (6.65 ± 0.12%) for other *Cymodocea* species, *C. rotundata.*

The antibacterial activity of both *K. alvarezii* and *C. serrulata* were investigated using chloroform extracts based on those reported by Pusparaj et al. [6], whose reported that best inhibitory effects of *K. alvarezii* was reported using chloroform extracts. The antibacterial activity of both *K. alvarezii* and *C. serrulata* depends on the presence of bioactive compounds, phenolic content and free radical scavenging activity [53]. In all case, it was detected inhibitory activity against the five pathogenic bacteria investigated (Table 2). The higher inhibitory activity was observed in *K. alvarezii* (26 ± 0.03 mm) against *Bacillus subtilis*, as well as in the case of *C. serrulata* exhibited maximum inhibitory activity (26 ± 0.08 mm) against *Vibrio parahaemolyticus.* The chloroform extract of *K. alvarezii* showed maximum activity of 26 ± 0.03 mm against *B. subtilis* at 100 µg/ml, and *C. serrulata* showed maximum activity of 26 ± 0.08 mm against *V. parahaemolyticus* at 100 µg/ml and minimum activity of 22±0.01 mm and 20±0.04 mm against *Vibrio alginolyticus* at 100 µg/ml in both *K. alvarezii* and *C. serrulata,* respectively (Table 2).

**Table 2.** Antibacterial activity of *K. alvarezii* and *C. serrulata* in chloroform extract against human pathogenic bacteria.

| Human Pathogenic Bacteria | Concentration (µg/ml) | Seaweed Extract | Seagrass Extract |
|---|---|---|---|
| | | Zone of inhibition (mm) | |
| *Bacillus subtilis* | 100 | 26±0.03 | 25±0.16 |
| *Klebsiella pneumoniae* | 100 | 23±0.01 | 22±0.20 |
| *Vibrio alginolyticus* | 100 | 22±0.01 | 20±0.04 |
| *Vibrio parahaemolyticus* | 100 | 24±0.02 | 26±0.08 |
| *Vibrio harveyi* | 100 | 24±0.10 | 22±0.01 |

Date were expressed as the mean ± SD values of triplicates (n=3).

Jaswir *et al.* [53] reported the maximum inhibitory activity (12±1.02 mm) against *B. subtilis* using the methanolic extract of *K. alvarezii.* Similarly, Pusparaj *et al.* [6] reported the antibacterial activity of *K. alvarezii* against six human pathogens, *Staphylococcus aureus, B. subtilis, Lactobacillus acidophilus, Pseudomonas aeruginosa, Escherichia coli* and *Proteus mirabillis,* and he reported that the best activity was recorded in chloroform extracts. Kumar *et al.* [54] studied the antibacterial activity of *C. serrulata* against four fish-borne pathogens, namely, *Bacillus cereus, B. subtilis, E. coli* and *Micrococcus luteus,* and reported that *C. serrulata* was effective against several *Bacillus* sp.

The GC–MS running time for the n-hexane:ethyl acetate (50:50 v/v) extracts of *K. alvarezii* and *C. serrulata* was 30 min. The target mass ions *(m/z)* and retention times of all identified compounds in *K. alvarezii* and *C. serrulata* are shown in Tables 3 and 4. The results showed that *K. alvarezii* extracts contained 94 different bioactive compounds, including phenol, decane, dodecane, hexadecane, vanillin, heptadecane, diphenylamine, benzophenone, octadecanoic acid, dotriacontane and benzene (Table 3). In the other hand, *C. serrulata* was found to contain 104 different bioactive compounds, including tetradecane, dodecanal, dodecanal, diphenylamine, heptadecane, phytol, butanoic acid, 2-hydroxy-, ethyl ester, dodecane and benzene (Table 4). These compounds were responsible for the antioxidant and antibacterial activities of both *K. alverazii* and *C. serrulata*.

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**Table 3.** List of compounds identified from the purified extracts of *K. alvarezii* using GC-MS analysis.

| No. | Name | Retention Time | Base m/z |
|---|---|---|---|
| 1 | Phenol | 4.119 | 94.05 |
| 2 | Cyclopropyl methyl carbinol | 4.170 | 58.05 |
| 3 | Decane | 4.347 | 57.05 |
| 4 | Butanoic acid, 2-hydroxy-, ethyl ester | 4.429 | 59.05 |
| 5 | 2-Methylpentyl formate | 4.457 | 56.05 |
| 6 | Benzene, 1,4-dichloro- | 4.530 | 145.95 |
| 7 | Cyclopentane, 1,2-dimethyl-, cis- | 4.568 | 70.10 |
| 8 | Dodecane, 2,6,11-trimethyl- | 5.158 | 57.05 |
| 9 | Undecane, 5-methyl- | 5.237 | 57.10 |
| 10 | Ethane, hexachloro- | 5.395 | 116.90 |
| 11 | Dodecane, 2,6,10-trimethyl- | 5.803 | 57.05 |
| 12 | 3-Ethyl-3-methylheptane | 5.890 | 57.05 |
| 13 | Naphthalene | 6.994 | 128.10 |
| 14 | Dodecane | 7.201 | 57.05 |
| 15 | Benzaldehyde, 2,5-dimethyl- | 7.450 | 133.10 |
| 16 | Octadecanoic acid, phenyl ester | 7.517 | 94.05 |
| 17 | Benzene, 1,3-bis(1,1-dimethylethyl)- | 7.986 | 175.15 |
| 18 | Undecane, 2,4-dimethyl- | 8.093 | 57.10 |
| 19 | Dodecane, 4,6-dimethyl- | 8.317 | 57.05 |
| 20 | Hexadecane | 8.437 | 57.10 |
| 21 | Formamide, N-phenyl- | 8.884 | 121.05 |
| 22 | Dodecane, 2,6,10-trimethyl- | 8.942 | 57.10 |
| 23 | Chloroxylenol | 9.762 | 121.10 |
| 24 | Benzene, 1-cyclobuten-1-yl- | 9.844 | 129.10 |
| 25 | Hexadecane | 9.907 | 57.05 |
| 26 | Vanillin | 9.950 | 151.05 |
| 27 | Heptadecane | 10.186 | 57.05 |
| 28 | Dodecane, 2,6,10-trimethyl- | 10.690 | 57.10 |
| 29 | 1-Dodecanol | 10.864 | 55.05 |
| 30 | Nonadecane | 11.005 | 85.10 |
| 31 | Decane, 1-bromo-2-methyl- | 11.044 | 57.05 |
| 32 | Heneicosane | 11.113 | 71.10 |
| 33 | Nonadecane | 11.159 | 57.05 |
| 34 | 2,4-Di-tert-butylphenol | 11.351 | 191.15 |
| 35 | Hexadecane | 11.655 | 57.05 |
| 36 | Hexadecane | 12.350 | 57.05 |
| 37 | Diphenylamine | 12.664 | 169.15 |
| 38 | Benzophenone | 12.754 | 105.05 |
| 39 | 3-Hydroxydiphenylamine | 13.216 | 185.10 |
| 40 | Hexadecane, 2,6,10,14-tetramethyl- | 13.328 | 57.10 |
| 41 | Heptadecane | 13.476 | 57.05 |
| 42 | Dodecane, 2,6,10-trimethyl- | 13.540 | 71.10 |
| 43 | Heneicosane | 13.590 | 71.10 |
| 44 | Heneicosane | 13.681 | 57.10 |
| 45 | Decane, 1-iodo- | 13.751 | 71.10 |
| 46 | Heptadecane, 8-methyl- | 13.940 | 71.10 |
| 47 | Heneicosane | 14.061 | 71.10 |
| 48 | Tetradecanoic acid | 14.148 | 57.05 |
| 49 | Formamide, N,N-diphenyl- | 14.487 | 168.10 |

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| | | | |
|---|---|---|---|
| 50 | Heneicosane | 14.549 | 57.05 |
| 51 | p-(Benzylideneamino)phenol | 14.651 | 196.10 |
| 52 | Isopropyl myristate | 14.836 | 60.00 |
| 53 | Carbamic chloride, diphenyl- | 14.990 | 196.10 |
| 54 | 2-Pentadecanone, 6,10,14-trimethyl- | 15.036 | 57.05 |
| 55 | Phenoxazine | 15.211 | 183.10 |
| 56 | 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester | 15.305 | 149.05 |
| 57 | Heneicosane | 15.445 | 57.05 |
| 58 | Hexadecane | 15.569 | 57.05 |
| 59 | Tetrapentacontane | 15.735 | 71.10 |
| 60 | 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione | 15.837 | 57.05 |
| 61 | 3-Hydroxydiphenylamine | 15.925 | 185.10 |
| 62 | Benzoic acid, 2-benzoyl-, methyl ester | 16.008 | 163.05 |
| 63 | n-Hexadecanoic acid | 16.227 | 73.05 |
| 64 | 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione | 16.414 | 57.05 |
| 65 | Heneicosane | 16.547 | 57.05 |
| 66 | Cyclic octaatomic sulfur | 16.953 | 63.95 |
| 67 | Palmitic Acid, TMS derivative | 17.018 | 117.05 |
| 68 | Dotriacontane | 17.125 | 57.05 |
| 69 | Tetrapentacontane | 17.472 | 57.05 |
| 70 | Pentatriacontane | 17.560 | 85.10 |
| 71 | Octadecane, 3-ethyl-5-(2-ethylbutyl)- | 17.635 | 71.10 |
| 72 | Dotriacontane | 17.723 | 57.05 |
| 73 | Dotriacontane | 17.820 | 71.10 |
| 74 | Octadecanoic acid | 18.063 | 73.05 |
| 75 | Tetrapentacontane | 18.120 | 71.10 |
| 76 | Tetrapentacontane | 18.193 | 71.10 |
| 77 | Heneicosane | 18.374 | 57.05 |
| 78 | Tetracosane | 19.231 | 57.10 |
| 79 | Tetrapentacontane | 19.605 | 71.10 |
| 80 | 1-Heptadecanamine | 19.985 | 85.10 |
| 81 | Heneicosane | 20.056 | 57.05 |
| 82 | Benzenemethanamine, N-hydroxy-N-(phenylmethyl)- | 20.519 | 91.05 |
| 83 | 9-Octadecenenitrile, (Z)- | 20.833 | 55.05 |
| 84 | Bis(2-ethylhexyl) phthalate | 21.292 | 149.05 |
| 85 | 13-Docosenamide, (Z)- | 21.461 | 59.05 |
| 86 | 9-Octadecenamide, (Z)- | 21.520 | 59.05 |
| 87 | Dotriacontane | 21.670 | 57.05 |
| 88 | Tetracontane | 22.656 | 57.05 |
| 89 | 13-Docosenamide, (Z)- | 23.720 | 59.05 |
| 90 | Squalene | 24.338 | 69.05 |
| 91 | Tetrapentacontane | 25.429 | 57.05 |
| 92 | 13-Docosenamide, (Z)- | 26.983 | 59.00 |
| 93 | Dotriacontane | 27.136 | 57.05 |
| 94 | Cholesterol | 28.552 | 386.35 |

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**Table 4.** List of compounds identified from the purified extracts of *C. serrulata* using GC -MS analysis.

| NO. | Name | R. Time | Base m/z |
|---|---|---|---|
| 1 | 1-Trifluoroacetoxy-2-methylpentane | 3.115 | 71.05 |
| 2 | Propanoic acid, 2-hydroxy-2-methyl- | 3.929 | 59.05 |
| 3 | Cyclopropyl methyl carbinol | 4.168 | 58.05 |
| 4 | Carbamic acid, 2-(dimethylamino)ethyl ester | 4.354 | 58.05 |
| 5 | Butanoic acid, 2-hydroxy-, ethyl ester | 4.427 | 59.05 |
| 6 | 2-Methylpentyl formate | 4.455 | 71.05 |
| 7 | 1-Heptanol | 4.565 | 70.10 |
| 8 | Octane, 3,3-dimethyl- | 4.656 | 71.10 |
| 9 | 3-Heptanol, 4-methyl- | 4.780 | 59.05 |
| 10 | Propane, 1,3-dichloro- | 4.817 | 76.00 |
| 11 | Dodecane, 2,6,10-trimethyl- | 5.156 | 57.05 |
| 12 | Dodecane, 4,6-dimethyl- | 5.236 | 57.10 |
| 13 | Ethane, hexachloro- | 5.392 | 116.90 |
| 14 | Dodecane, 2,6,10-trimethyl- | 5.800 | 57.05 |
| 15 | Naphthalene | 6.993 | 128.10 |
| 16 | Tetradecane | 7.265 | 57.05 |
| 17 | (Z),(Z)-2,4-Hexadiene | 7.332 | 77.00 |
| 18 | Decane, 2-methyl- | 7.399 | 57.05 |
| 19 | Benzaldehyde, 2,4-dimethyl- | 7.455 | 133.10 |
| 20 | Undecane, 4,8-dimethyl- | 7.510 | 71.10 |
| 21 | Tridecane | 7.817 | 57.05 |
| 22 | Tridecane | 7.893 | 57.05 |
| 23 | Benzene, 1,3-bis(1,1-dimethylethyl)- | 7.983 | 175.15 |
| 24 | Nonadecane | 8.092 | 57.05 |
| 25 | Dodecane, 4,6-dimethyl- | 8.316 | 71.10 |
| 26 | Nonadecane | 8.431 | 57.05 |
| 27 | Dodecane, 4,6-dimethyl- | 8.507 | 71.10 |
| 28 | Dodecane, 2,6,10-trimethyl- | 8.615 | 71.10 |
| 29 | Dodecane, 4,6-dimethyl- | 8.940 | 71.10 |
| 30 | Naphthalene, decahydro-1,4a-dimethyl-7-(1-methylethyl)-, [1S-(1.alpha.,4a.alpha.,7.alpha.,8a | 9.200 | 57.05 |
| 31 | Benzene, 1-cyclobuten-1-yl- | 9.841 | 129.10 |
| 32 | Hexadecane | 9.903 | 57.05 |
| 33 | Dodecanal | 10.039 | 57.05 |
| 34 | Heptadecane | 10.184 | 57.05 |
| 35 | Cyclotetrasiloxane, octamethyl- | 10.471 | 281.05 |
| 36 | Hexadecane | 10.570 | 57.05 |
| 37 | Dodecane, 2,6,10-trimethyl- | 10.687 | 71.10 |
| 38 | Heptane, 2,4-dimethyl- | 10.720 | 85.10 |
| 39 | Hexadecane, 1-bromo- | 10.852 | 57.05 |
| 40 | Undecane, 2,4-dimethyl- | 11.005 | 85.10 |
| 41 | Octane, 2-methyl- | 11.039 | 71.10 |
| 42 | Hexadecane | 11.110 | 71.10 |
| 43 | Tetradecane | 11.157 | 57.05 |
| 44 | Octadecane, 1-iodo- | 11.220 | 57.05 |
| 45 | 2,4-Di-tert-butylphenol | 11.349 | 191.15 |
| 46 | Hexadecane | 11.653 | 57.10 |
| 47 | Dodecanoic acid | 11.918 | 73.05 |
| 48 | Octadecane | 12.008 | 57.10 |

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| | | | |
|---|---|---|---|
| 49 | Hexadecane | 12.346 | 57.10 |
| 50 | 1,4-Methanoazulen-9-ol, decahydro-1,5,5,8a-tetramethyl-, [1R-(1.alpha.,3a.beta.,4.alpha.,8a. | 12.425 | 85.10 |
| 51 | Heptadecane | 12.515 | 57.05 |
| 52 | Pentadecane, 4-methyl- | 12.590 | 71.10 |
| 53 | Diphenylamine | 12.653 | 169.10 |
| 54 | Heptadecane | 12.749 | 57.05 |
| 55 | Hexadecane, 2,6,10,14-tetramethyl- | 13.057 | 57.05 |
| 56 | Heneicosane | 13.192 | 57.05 |
| 57 | Heneicosane | 13.249 | 57.05 |
| 58 | Hexadecane | 13.330 | 57.05 |
| 59 | Dodecane, 1-iodo- | 13.385 | 57.05 |
| 60 | Heneicosane | 13.483 | 57.05 |
| 61 | Hexadecane | 13.540 | 71.10 |
| 62 | Heneicosane | 13.588 | 71.10 |
| 63 | Hexadecane | 13.945 | 57.05 |
| 64 | Heneicosane | 14.058 | 71.10 |
| 65 | Pentacosane | 14.156 | 57.05 |
| 66 | 3,5-di-tert-Butyl-4-hydroxybenzaldehyde | 14.250 | 219.15 |
| 67 | Octadecane, 1-iodo- | 14.320 | 57.05 |
| 68 | Heneicosane | 14.546 | 57.05 |
| 69 | Octacosane | 14.645 | 57.10 |
| 70 | 6-Octen-1-ol, 3,7-dimethyl-, acetate | 14.966 | 68.05 |
| 71 | Heneicosane | 15.042 | 57.05 |
| 72 | 1-Tetradecanamine | 15.151 | 59.05 |
| 73 | Phytol | 15.213 | 57.05 |
| 74 | 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester | 15.302 | 149.05 |
| 75 | Phytol | 15.397 | 57.05 |
| 76 | Hexadecane | 15.445 | 57.05 |
| 77 | Hexadecane | 15.489 | 57.05 |
| 78 | Tetracosane | 15.650 | 57.10 |
| 79 | Dotriacontane | 15.695 | 71.10 |
| 80 | Tetracosane | 15.738 | 267.05 |
| 81 | 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione | 15.835 | 57.05 |
| 82 | Benzoic acid, 2-benzoyl-, methyl ester | 16.006 | 163.05 |
| 83 | n-Hexadecanoic acid | 16.202 | 73.05 |
| 84 | Dibutyl phthalate | 16.441 | 149.05 |
| 85 | Heneicosane | 16.544 | 57.05 |
| 86 | Palmitic Acid, TMS derivative | 17.010 | 117.10 |
| 87 | Heneicosane | 17.115 | 57.10 |
| 88 | Dotriacontane | 17.246 | 57.05 |
| 89 | Heneicosane | 17.476 | 57.05 |
| 90 | Tetrapentacontane | 17.564 | 57.05 |
| 91 | Phytol | 17.631 | 71.10 |
| 92 | Tetracosane | 17.725 | 57.05 |
| 93 | Tetrapentacontane | 17.817 | 71.10 |
| 94 | Octadecanoic acid | 18.056 | 57.05 |
| 95 | Tetrapentacontane | 18.116 | 71.10 |
| 96 | Tetrapentacontane | 18.190 | 71.10 |
| 97 | Docosane | 18.371 | 57.05 |
| 98 | 4-Morpholinepropanamine | 18.495 | 100.05 |

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| | | | |
|---|---|---|---|
| 99 | 3-Isopropyl-2,5-piperazine-dione | 18.583 | 114.10 |
| 100 | Heptadecane, 2-methyl- | 18.945 | 57.05 |
| 101 | Tetrapentacontane | 18.997 | 57.10 |
| 102 | Heneicosane | 19.229 | 57.05 |
| 103 | Tetracosane | 19.450 | 57.05 |
| 104 | Tetrapentacontane | 19.529 | 71.10 |

Datchanamurthy et al. [55] reported that red algae (*Acoathophora deilei*) contain major common components, such as hexadecanoic acid methyl ester, dibutyl phthalate, 2-ethyl butyric acid, octadecyl ester, 9-octadecanoic acid, methyl ester, and 1,2-benzendicarboxylic acid. Similarly, Manilal et al. [56] also reported that red algae (*Asparagopsis taxiformis*) contain components such as 4,5-dimethyl-1H-pyrrole-2carboxylic acid ethyl ester, chlorobenzene, 14-methyl-pentadecanoic acid methyl ester, octadec-9-enoic acid, 2,3-dihydroxy-propyl ester, 9-octadecanoic acid, pentadecanoic acid and octadecanoic acid, which might be involved in synergistic bioactivity. Anitha et al. [29] also studied the presence of phenols, hexadecanoic acid, n-hexadecanoic acid, tridecanoic acid, n-nonadecanoic acid, and benzene reported in red algae (*Gracilaria cervicornis*). Pushpabharathi et al. [13] reported 9 bioactive components in seagrass (*C. serrulata*): hexahydofarnesyl acetone, hexadecanoic acid, methyl ester, n-hexadecanoic acid, tetradecanoic acid, pentadecanoic acid, cholestesta 4,6 dien 3-ol and stigmasterol.

## 4. Conclusions

The red seaweed *K. alvarezii* and seagrass *C. serrulata* examined in the present study were found to possess rich sources of phytochemicals. The antioxidant properties of both seaweed and seagrass reveal that they have appreciable levels of protection against free radicals.

GC–MS analysis revealed the presence of large active metabolites (94 in the case of *K. alvarezii* and 104 in the case of *C. serrulate*), such as phenol, decane, dodecane, hexadecane, vanillin, heptadecane, diphenylamine, benzophenone, octadecanoic acid, dotriacontane and benzene, in both red seaweed and seagrass. In view of the results obtained, both *K. alvarezii* and *C. serrulata* could be employed as potential marine drugs and may be used in the pharmaceutical and food processing industries as sources of ingredients with appreciable medicinal value. Since both red seaweed and seagrass were found to be good sources of essential phytochemicals, their commercial value can be enhanced by marketing them as value-added products.

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