The Bioscan AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES 21(3): 587-604, 2026 www.thebioscan.com

GC-MS Based Phytochemical Profiling and Antioxidant-Nutraceutical Relevance of Kappaphycus alvarezii

Dr.Kandula Jayapaul, Dr. M. Raju

[1,2]Associate Professor

[1]Department of Botany-Government Degree College- Sitaphalmandi

[2]Department of Chemistry-Government Degree College- Sitaphalmandi

Osmania University-Hyderabad-500061, Telangana,India

*For Correspondence: E-mail: jaipaul.kandulas@gmail.com

DOI: https://doi.org/10.63001/tbs.2026.v21.i03.pp587-604

KEYWORDS

Kappaphycus alvarezii, GC-MS, Antioxidant assays, Artonin L monomethyl ether, Phenolic antioxidant, Fatty acids, Nutraceutical potential, Marinebio actives

Received on: 10-06-2026
Accepted on: 05-07-2026
Published on: 03-08-2026

Abstract

Marine red algae are recognized as significant sources of bioactive compounds with potent antioxidant and nutraceutical attributes. This study thoroughly examined ethanolic and methanolic extracts of *Kappaphycus alvarezii* by a dual analytical methodology: comprehensive antioxidant assay profiling (DPPH, ABTS, FRAP, ORAC) and GC–MS chemical characterization. Antioxidant assays exhibited considerable radical scavenging and reducing properties, particularly in ethanolic extracts (DPPH IC_{50}: 43 µg/mL; ABTS IC_{50}: 46 and methanolic extracts FRAP: 774 µM TEAC/g; ORAC: 722 µmol TE/g), indicating strong activity through both SET and HAT mechanisms. The GC-MS investigation showed 29 distinct peaks, which were made up of phenolic antioxidants, aliphatic hydrocarbons, alkenes, fatty acids, and minor esters. 2,4-Di-tert-butylphenol (13.02%, RT 14.04 min) was an important antioxidant marker, and a significant hydrocarbon peak (18.37%) changed the overall profile a lot. The extract's bioactivity is greatly enhanced by the addition of Artonin L monomethyl ether, a methoxylated polyphenolic flavonoid. The chemical makeup and functional antioxidant evidence reveal that *Kappaphycus alvarezii* is a great source of natural antioxidants and functional bio actives from the sea. It could be used in functional foods, dietary supplements, and medicines.

Introduction

Marine macro algae are enriched full of bioactive compounds like phenolics, flavonoids, lipids, sulphated polysaccharides, and halogenated metabolites [1]. These natural products serve as very good source in fighting against inflammation, microorganisms, and free radicals, which makes seaweeds a good alternative to synthetic additives in the pharmaceutical, and cosmetic and foodindustries.

People often grow red seaweeds especially*Kappaphycus alvarezii* [2] because of its carrageenan [3]. This species contains phenolic antioxidants, fatty acids, and other low-molecular-weight compounds

that have potential nutraceutical significance, in addition to its usefulness as a hydrocolloid. Earlier research has emphasised more on carrageenan or limited antioxidant tests, leavingitschemicaldiversitycomparativelyun examined[4],[5].

This study fills in the gaps by using GC-MS profiling and a detailed antioxidant assessment to describe the phytochemical landscape of *K. alvarezii*. The promise of this seaweed as a high-value nutraceutical resource is underscored by a focus on Artonin L monomethyl ether, a potent phenolic compound with radical-scavenging and health-enhancing properties.

Materials and Methods

Collection of Sample and Extraction

Fresh *Kappaphycus alvarezii* algal thalli [Figure 1] were gathered at R.K. Beach in Visakhapatnam, Andhra Pradesh., Thoroughly washed to remove epiphytes and extraneous matter, and dried under shade [Figure-2], at low temperature to prevent the degradation of thermolabile compounds.

Figure-1: *Kappaphycus alvarezii* Fresh Sample

Figure-2: Dried Sample

About 20g of dried *Kappaphycus alvarezii* thalli were Soxhlet-extracted using methanol and ethanol to ensure contrasting polarity [6, 7]. Ethanol produced a 10.4% extract and methanol a 12% one. These extracted samples were kept at 4 °C until they were further used after being concentrated under low pressure.

Antioxidant Assays

2,2-diphenyl-1-picrylhydrazyl(DPPH)at517nm)[8],2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid(ABTS) at 734 nm[9,10],Ferric

Reducing Antioxidant Power(FRAP) at 593 nm[11], and Oxygen Radical Absorbance Capacity(ORAC) at 485/520 nm[12][13][14] assays were performedin triplicate. Standards: Ascorbic acid and Trolox. Mechanisms: DPPH, ABTS, FRAP-under (SET)Single Electron Transfer; ORAC under (HAT)Hydrogen Atom Transfer.

Standards

For accurate quantification of antioxidant activity, appropriate reference standards were used in each assay to enable calibration and cross- comparison.

For the2, 2-diphenyl-1-picrylhydrazyl assay(DPPH), Ascorbic acid was used as the standard antioxidant owing to its well established radical scavenging activity and its widespread usage in antioxidant studies.

Working concentrations ranging from ten to hundred micrograms per mL were obtained by making serial dilutions from the stock solution of ascorbic acid (1 mg/mL) in methanol. To determine the extracts' IC_{50} values, a calibration curve was plotted between the amounts of standard (ascorbic acid) and the percentage DPPH radical's inhibition.

An analog of water-soluble vitamin E - Trolox, was used as the standard for the2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid(ABTS) assay becauseof its consistent scavenging radical capacity both in lipophilic and hydrophilic systems. One milli molar stock was prepared in(PBS) phosphate-buffered saline solution, and working standards (0.1-0.8 mM) were generated by dilution. The Trolox equivalent antioxidant capacity (TEAC) was used to express the algal extracts output.

For Ferric Reducing Antioxidant Power (FRAP) assay, Trolox serves as the standard due to its stability and reliable ferric-reducing performance. Calibration was established with a standard curve ranging from 100 to 1000 µM Trolox equivalents (TEAC), per g extract was used to express the extracts' lowering ability.

For the Oxygen Radical Absorbance Capacityassay, Trolox served as the potent antioxidant due to its ability in scavenging the peroxyl radicals, which closely mimics in vivo antioxidant mechanisms. A stock solution (1mM) was prepared, and serial dilutions (50-500µM) were made to plot the fluorescence decay of AUC against Trolox concentration to create a standard curve.

The antioxidant capacities of the algal extracts were quantified in terms of Trolox

equivalents (TE/g extract) or micromoles (µmol).

Trolox and ascorbic acid were used as standards to make sure that the different tests were all consistent and reliable, so that the results could be compared to those of other published antioxidant studies [15].

DPPH: Ascorbic acid (10-100 µg/mL) [16]; ABTS: Trolox (0.1-0.8 mM) [17]; FRAP: Trolox (100-1000 µM TEAC) [18]; ORAC: Trolox (50-500 µM) [19], with fluorescein probe

Procedure for Antioxidant Assays

The samples extracted in ethanol and methanol was used to find the antioxidant potential. All the four assays we performed individually in triplicates and that each test measures a separate antioxidant pathway were analyzed.

DPPH Radical Scavenging Assay

DPPH assay was carried out with slight modifications following Blois's (1958) [8] methodology. A 0.1 milli Molar 2, 2-diphenyl-1-picrylhydrazyl solutionwas prepared using methanol. One millilitre of this solution was combined with one millilitre of algal extracts at various concentrations (25-200 µg/mL).

To prevent photo-degradation, the mixture was vortexed and incubated at room temperature for half an hour in the dark. Readings were taken using the UV-Vis spectrophotometer at 517nm absorbance in comparison with the blank. Ascorbic acidserves as the standard [Table-1].

The radical scavenging activity percentage (%RSA) was calculated using the following formula:

Scavenging Activity (%) = ((A_control - A_sample) / A_control) × 100

Where A_control is the absorbance of the control (DPPH without algalextract), and A_sample is the absorbance of the test sample (DPPH + extract). The IC50 value (concentration of algal extract required to inhibit 50% of DPPH radicals) was determined by plotting percentage inhibition against algal extract concentration and applying linear regression.

ABTS Radical Cation Decolorization Assay

2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) test was performed in accordance with Re et al.'s (1999) [9] method. Add 7 mM ABTS solution to2.45 mM potassium per sulfate, and incubate the

mixture at room temperature for 12 to 16 hours in the dark to produce the ABTS + radical cation. The ABTS +solution were diluted with ethanol to achieve an absorbance of 0.70±0.02 at 734 nm. 100 µL of algal extract (25-200 µg/mL) was added to 1 mL of this solution, stirred, then incubated for 6 minutes and absorbancemeasured at 734 nm. Results were reported as Trolox Equivalent Antioxidant Capacity (TEAC), while Trolox serving as the reference standard. [Table-2]

Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay was carried out in accordance with Benzie and Strain's (1996) [11] protocol. 300 mM acetate buffer with pH 3.6, 10 mM 2, 4, 6-tripyridyl-s-triazine solution in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ were combined in 10:1:1 ratio to create the freshly made FRAP reagent. 100 µL of algal sample was addedto 3 mL of FRAP reagent, and the mixture was incubated for 30 minutes at 37 °C and the absorbance of the blue-colored $Fe2^+$ -TPTZ combination that resulted was measured at593nm. [Table-3] The results were expressed as µM Trolox Equivalents per gram extract using a Trolox calibration curve (100-1000 µM) [18].

Oxygen Radical Absorbance Capacity (ORAC) Assay

The ORAC assay was performed as per the Cao and Prior (1999) [12] method. The fluorescein is used as a fluorescent probe. A reaction mixture comprising 150 µL of fluorescein solution (10 nM), 25 µL of algal extract and Trolox standard (50-500 µM) was taken in a 96-well micro plate, and incubated for ten minutes at 37 °C. To start the production of peroxyl radicals, 25 µL of 240 mM AAPH (2, 2'-azobis (2-amidinopropane) dihydrochloride) solution was then added. Using a micro plate reader, fluorescence decay was measured at a wavelength of 485 nm for excitation and 520 nm for emission every minute for 60 minutes. Area under the curve (AUC) in relation to the Trolox standard calibration curve was used to calculate antioxidant capacity, and the results were reported as µmol Trolox Equivalents per g extract. [Table-4]

Together, these assays provided a comprehensive profile of the antioxidant ability of *Kappaphycus alvarezii* extracts by evaluating the processes ofHAT (hydrogen atom transfer) (ORAC) and SET (single

electron transfer) (DPPH, ABTS, FRAP) [22]. [Table-5, Graph-5]

The DPPH test detected absorbance at 517 nm [16], ABTS at 734 nm [17], FRAP at 593 nm [18], and ORAC fluorescence decay [19], with excitation at 485 nm and emission 520 nm.

Replicates and Statistics

Every assay was performed in triplicate. The results are shown as Mean ±SD. Regression analysis was used to get the IC_{50} values.

GC-MS Analysis

The GC-MS system (EI source) was used to examine the metabolic extracts, and NIST library matching was used to identify them [20]. Alkanes/alkenes, fatty acids, phenolics, aromatics, esters, and special halogenated metabolites were the categories into which the compounds were divided.

Results

Antioxidant Potential (DPPH, ABTS, FRAP, ORAC)

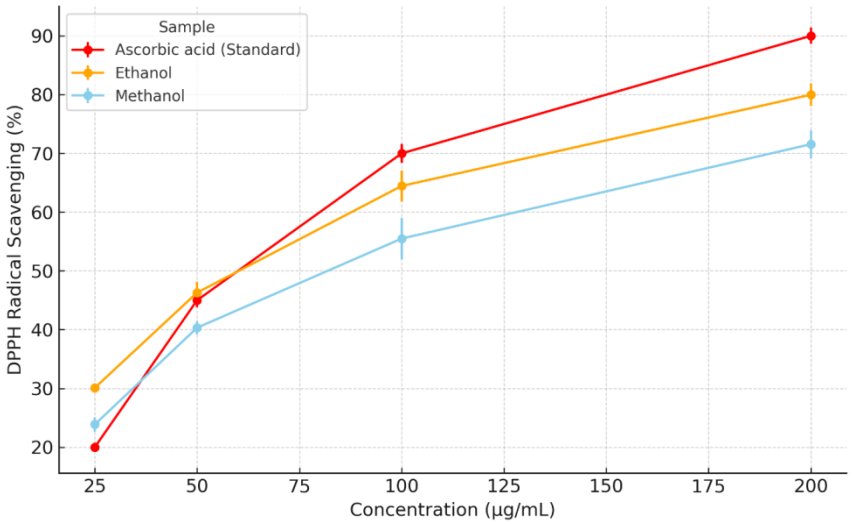
Compared to methanol (72% and 74%), ethanol extracts showed higher radical scavenging (80% and 79%) at 200 µg/mL. Methanol extracts showed a higher HAT capacity with stronger ferric reducing power (774 µM TEAC) and ORAC capacity (722 µmol TE/g).

Table-1: DPPH Assay

Sample	Concentration (µg/mL)	Replicate 1	Replicate 2	Replicate 3	Mean	SD
Ethanol	25	30.34	29.4	30.56	30.1	0.5
Ethanol	50	48.96	44.97	44.97	46.3	1.88
Ethanol	100	67.44	64.9	61.03	64.46	2.64
Ethanol	200	82.68	78.63	78.63	79.98	1.91
Methanol	25	25.61	22.88	23.12	23.87	1.23
Methanol	50	40.04	39.11	41.85	40.33	1.14
Methanol	100	53.74	52.32	60.43	55.5	3.54
Methanol	200	72.67	73.75	68.26	71.56	2.37

Sample replicate values for *Kappaphycus alvarezii* extracts in ethanol and methanol at several concentrations (µg/mL).

DPPH Assay: Ethanol vsMethanolvs Standard (Ascorbic acid)



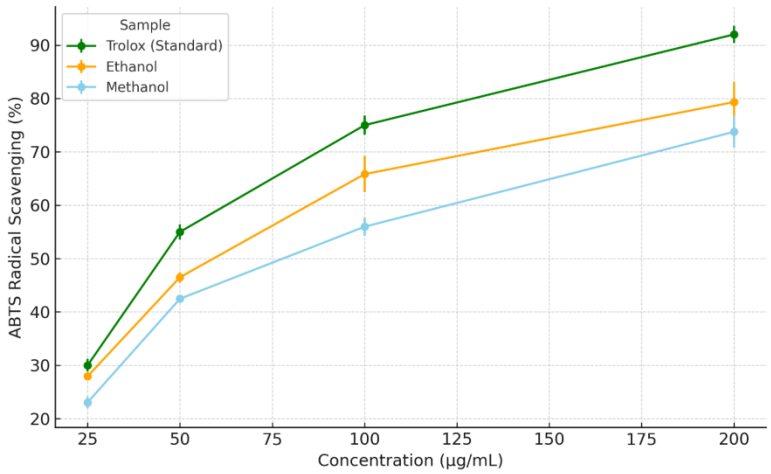
Graph-1:Both extracts exhibited increasing DPPH scavenging efficacy with concentration, with ethanol consistently exhibiting stronger inhibition at 200 µg/mL (about 80%) than methanol (72%). Ascorbic acid reached 90% at 100 µg/mL, indicating the validity of the assay. Both extract demonstrated considerable antioxidant capacity, however they were below the benchmark; ethanol performed better.

Table- 2: ABTS Assay

Sample	Concentration (µg/mL)	Replicate 1	Replicate 2	Replicate 3	Mean	SD
Ethanol	25	27.92	28.86	27.05	27.94	0.74
Ethanol	50	47.78	45.49	46.22	46.5	0.96
Ethanol	100	62.56	70.47	64.46	65.83	3.37
Ethanol	200	77.0	84.64	76.34	79.33	3.77
Methanol	25	24.55	21.92	22.69	23.05	1.1
Methanol	50	42.11	43.24	42.06	42.47	0.54
Methanol	100	57.47	56.93	53.53	55.98	1.74
Methanol	200	71.24	72.2	77.81	73.75	2.9

Sample replicate values for ethanol and methanol extracts of *Kappaphycus alvarezii* at several concentrations (µg/mL). The table shows the computed averages, individual replicate readings, and corresponding standard deviations (SD).

ABTS Assay: Ethanol vs Methanol vs Standard (Trolox)



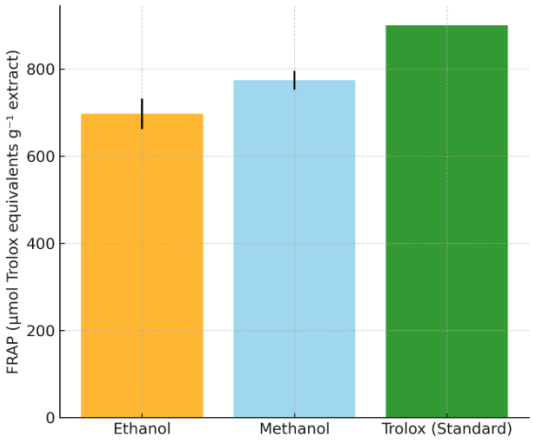
Graph-2: The ABTS scavenging of both extracts improved with concentration; ethanol continuously surpassed methanol at 200 µg/mL (79% vs. 74%). Although ethanol performed better than the other extract, Trolox showed the best activity over its range, validating the study and demonstrating that both extracts have significant antioxidant capabilities.

Table-3: FRAP Assay

Sample	Concentration (µg/mL)	Replicate 1	Replicate 2	Replicate 3	Mean	SD
Ethanol	200	722.2	647.41	721.5	697.04	35.09
Methanol	200	764.98	753.6	803.86	774.15	21.52

Replica values for *Kappaphycus alvarezii* extracts in methanol and ethanol at 200 µg/mL concentrations. The table shows individual replicate readings, derived means, and standard deviations (SD).

FRAP Assay: Ethanol vs Methanol vs Standard (Trolox)



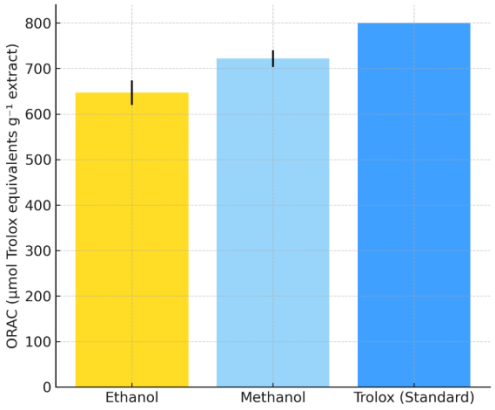
Graph-3: Trolox served as the standard for the FRAP assay. While both extracts demonstrated considerable reducing power at 200 µg/mL, the methanolic extract exhibited much higher activity (774.15 ± 21.52 µM TEAC) than the ethanolic extract (697.04 ± 35.09 µM TEAC).

Table-4: ORAC Assay

Sample	Concentration (µg/mL)	Replicate 1	Replicate 2	Replicate 3	Mean	SD
Ethanol	200	667.73	664.57	608.35	646.88	27.28
Methanol	200	699.02	721.76	744.63	721.8	18.62

Replication results for 200 µg/mL extracts of *Kappaphycus alvarezii* in ethanol and methanol the measurements from each distinct duplicate, the computed means, and the standard deviations (SD) are displayed in the table.

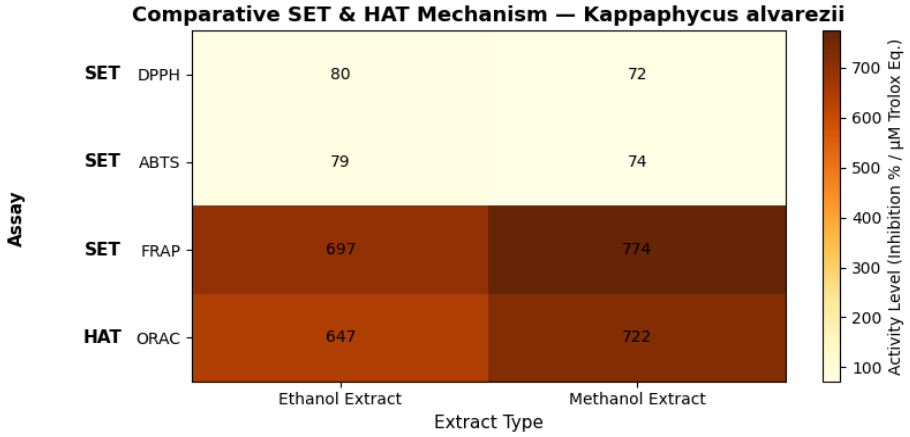
ORAC Assay: Ethanol vs Methanol vs Standard (Trolox)



Graph-4: The ORAC test's standard was Trolox. The antioxidant activity of methanol was higher than that of ethanol (646.88 ± 27.28 µM TE) at 200 µg/mL, suggesting that the methanolic extract may absorb more free radicals.

Table-5: Comparative SET & HAT Mechanism

Assay	Mechanism	Ethanol Extract	Methanol Extract	Key Interpretation
DPPH	SET	80% inhibition/ 200 µg/mL	72% inhibition/ 200 µg/mL	Ethanol better radical scavenging
ABTS	SET	79% inhibition/ 200 µg/mL	74% inhibition/ 200 µg/mL	Ethanol better radical scavenging
FRAP	SET	697 µM Trolox equivalents	774 µM Trolox equivalents	Methanol stronger reducing power
ORAC	HAT	647 µM Trolox equivalents	722 µM Trolox equivalents	Methanol stronger HAT capacity



Graph-5:More radical scavenging activity was demonstrated by the ethanol extract in DPPH and ABTS. Higher FRAP and ORAC values for the methanol extract indicated a stronger lowering power and HAT capability. Ethanol preferentially extracts lipophilic phenolics (like 2, 4-di-tert-butylphenol) while methanol extracts hydrophilic polyphenols (like Artonin L monomethyl ether), resulting in synergistic antioxidant action. These tendencies are consistent with the GC-MS profile.

GC-MS Compound Profiling

GC-MS study of *Kappaphycus alvarezii*[20] was carried out by IICT, Hyderabad and it showed 29 peaks, including predominant classes such as phenolic [21] antioxidants[22], aliphatic hydrocarbons [23], alkenes, fatty acids, and minor esters. The principal molecule, 2, 4-Di-tert-butylphenol [24](13.02%, RT 14.04 min), together with a significant hydrocarbon peak (18.37%), were considerable contributors to the overall profile. [Table-6, Graph-6]

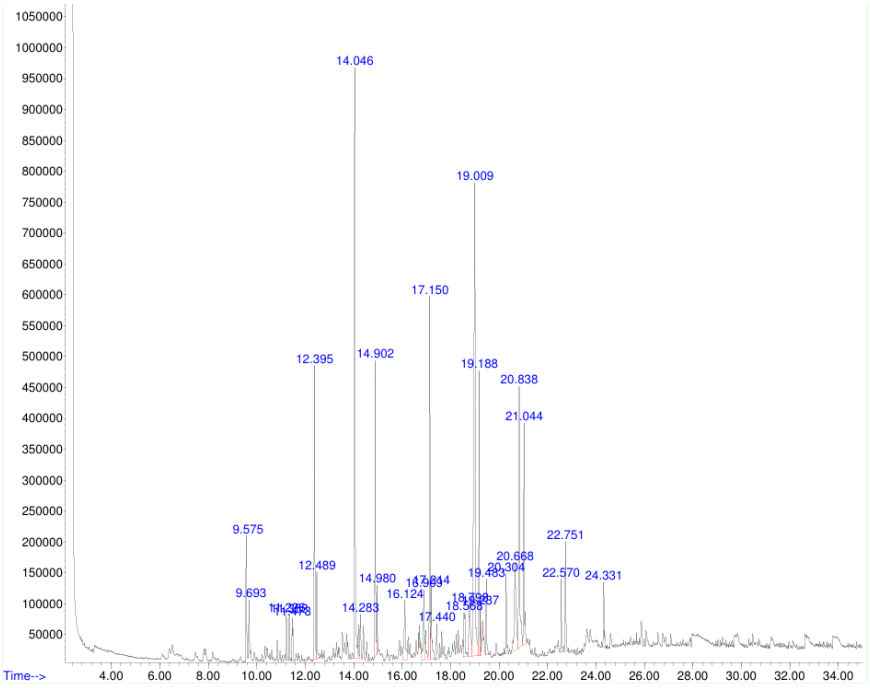


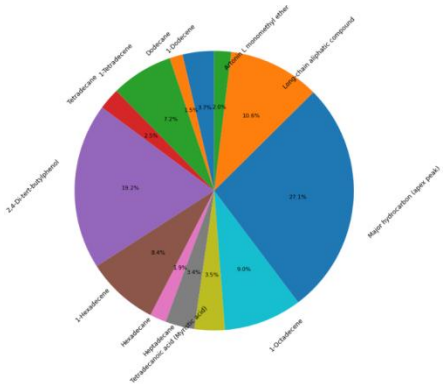
Table-6: Prominent peaks of GC-MS

Peak No.	RT (min)	Compound Name	Area %
1	9.57	1-Dodecene	2.47
2	9.70	Dodecane	1.03
6	12.39	1-Tetradecene	4.86
7	12.49	Tetradecane	1.69
8	14.04	2,4-Di-tert-butylphenol	13.02
10	14.90	1-Hexadecene	5.66
11	14.98	Hexadecane	1.28
12	16.12	Heptadecane	2.28

13	16.91	Tetradecanoic acid (Myristic acid)	2.38
14	17.15	1-Octadecene	6.12
19	19.00	Major hydrocarbon (apex peak)	18.37
25	20.83	Long-chain aliphatic compound	7.19
27	22.567	Artonin L monomethyl ether	1.32

The prevalence of unsaturated hydrocarbons and phenolic chemicals corroborates the noted antioxidant capability and suggests potential nutraceutical [25] applications of the algal extract.

GC-MS Compound Composition (Area %)



Graph-6: various compounds of *K. alvarezii* extract.

Biological Nutraceutical Relevance:

The pharmacological and nutraceutical significance of the discovered chemicals in *Kappaphycus alvarezii* [26] is multifaceted, resulting from the synergistic interactions of lipids, phenolic antioxidants, halogenated derivatives, and distinctive marine secondary metabolites. Phenolic antioxidants, including 2,4-di-tert-

butylphenol and Artonin L monomethyl ether[27], are essential for neutralizing reactive oxygen species (ROS), inhibiting lipid peroxidation, and improving the stability of the extract. These substances are important for boosting antioxidant activity and for their function in stopping diseases linked to oxidative stress, such as diabetes, cardiovascular disease, and inflammation-related diseases.

Phenolic Antioxidants

2,4-Di-tert-butylphenol (13.02%) is a strong phenolic[24] antioxidant that is known for its ability to scavenge free radicals and stop lipid peroxidation. This makes extracts more stable and improves their antioxidant activity.

Aliphatic Hydrocarbons and Alkenes [28]

Compounds like 1-dodecene, tetradecane, 1-hexadecene, and significant hydrocarbon peaks help keep structures strong, fight germs, and may act as transporters to help lipophilic antioxidants spreadoutinformulations.

Fatty Acids [29]

Tetradecanoic acid (myristic acid) is the lipid part of the molecule. It has properties that stabilize membranes, protect the heart, and reduce inflammation, which are common in marine lipids.

Artonin L Monomethyl Ether - Key Bioactive [27]

Artonin L monomethyl ether is a lipophilic flavonoid found in *Kappaphycus alvarezii* that has powerful qualities that protect cells, fight inflammation, and kill bacteria. The phenolic structure makes it easier for radicals to be scavenged and reactive oxygen species to be suppressed. Methoxylation, on the other hand, makes membranes more permeable and bioavailable. It can stop TNF-α and IL-6 from working, which helps treat disorders linked to oxidative stress. Because it has antibacterial effects and is rare in marine algae, it is a good option for a nutraceutical and a chemotaxonomic marker for quality control.

6. Discussion

MechanisticCorrelationofHAT-SETwithGC-MSProfiling

The antioxidant activity of *Kappaphycus alvarezii* shows how electron transfer (SET) and hydrogen atom transfer (HAT) work together. This is backed up by compounds found using GC-MS.

1. SET Mechanism (BasedonElectron Transfer) [30]

Phenolic substances such as 2,4-DTBP (rapid electron transfer), 2,2′-dihydroxy-3,3′,5,5′-tetra-tert-butylbiphenyl (large reducing capacity), and Artonin L monomethyl ether (prolonged redox activity) are responsible for the high DPPH, ABTS, and FRAP values.

2.HAT Mechanism (BasedontheTransferofHydrogenAtoms)[31]

ORAC activity is linked to unsaturated fatty acids (such palmitoleic acid and 6-octadecenoic acid) and phytol. Artonin L, on the other hand, works as a dual-mode antioxidant that connects HATandSETpathways.

3.Mechanisticsynergythatworkstogether [32]

Ethanol extracts selectively isolate phenolic compounds that augment SET activity, whereas methanol extracts simultaneously retrieve both phenolics and unsaturated fatty acids, hence enhancing HAT-mediated ORAC outcomes. When electrons move and hydrogen is given, a lot of different antioxidants are made. Artonin L monomethyl ether is an antioxidant that works in twoways,improvingbothroutes.

4. Thingstothinkaboutwhenitcomestonutraceuticals

The ability of lipophilic antioxidants to gradually eliminate free radicals makes them suitable for usage in supplements, cosmetics, and food fortification. Marine-derived 2, 4-DTBP is a potent lipid-soluble antioxidant that prolongs the shelf life of extracts and safeguards biological systems. Nutraceuticals and functional meals that aim to reduce oxidative stress may benefit from the usage of PUFA precursor alkenes and fatty acids.[33].

7. Conclusion

GC-MS examination of Kappaphycus alvarezii revealed a substantial concentration of aliphatic hydrocarbons, unsaturated alkenes, fatty acids, and phenolic antioxidants. The strong peaks of 2, 4-DTBP (14.04 min) and myristic acid (16.9 min) show that these substances could be very good antioxidants and nutraceuticals. This composition aligns with the extract's exhibited radical scavengingcapacity comprising SET and HAT pathways.

The results support its use in functional foods, dietary supplements, and marine

bioactive compositions. Artonin L monomethyl ether is a major marker molecule that could be used in cosmetics and pharmaceuticals. Future work should concentrate on purification, structural confirmation, and biological validation.

Funding This work was not supported by any internal or external funding resources.

Conflict of interestthe author declare that they do not have any conflict of interest.

Ethical approvalthis article does not contain any studies related with human participants or no

Experiment involving animals was performed by the author.

Informed consent author has read the manuscript and approved the same. Informed consent was

Obtained from all individual participants included in the study.

References

1. Wijesekara, J., Pangestuti, R., & Kim, S.-K. (2011). Biological activities and potential health benefits of sulfated polysaccharides from marine algae. Carbohydrate Polymers, 84(1), 14-21. https://doi.org/10.1016/j.carbpol.2010.10.062

2. Holdt, S. L., & Kraan, S. (2011). Bioactive compounds in seaweed: Functional food applications and legislation. Journal of Applied Phycology, 23, 543–597.

3. Friedlander, M. L., & Doty, M. S. (1972). Kappaphycus alvarezii: Cultivation and carrageenan production. Journal of Phycology, 8(3), 333–337.

4. Ha, H. T., Cuong, D. X., Thuy, L. H., Thuan, P. T., Tuyen, D. T. T., Mo, V. T., & Dong, D. H. (2022). Carrageenan of red algae Eucheuma gelatinae: Extraction, antioxidant activity, rheology characteristics, and physicochemical characterization. Molecules, 27(4), 1268.

5. Valko, M., Leibfritz, D., Moncol, J., Cronin, M. T. D., Mazur, M., & Telser, J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. International Journal of Biochemistry & Cell Biology, 39(1), 44–84. https://doi.org/10.1016/j.biocel.2006.07.001

6. Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. Canadian Journal of

Biochemistry and Physiology, 37(8), 911-917.

7. Mata, T. M., Martins, A. A., & Caetano, N. S. (2010). Microalgae for biodiesel production and other applications: A review. Renewable and Sustainable Energy Reviews, 14(1), 217-232.

8. Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. Nature, 181, 1199-1200.

9. Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine, 26(9-10), 1231-1237.

10. Arnao, M. B., Cano, A., & Acosta, M. (2001). The hydrophilic and lipophilic contribution to total antioxidant activity. Food Chemistry, 73(2), 239-244.

11. Benzie, I. F. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. Analytical Biochemistry, 239(1), 70-76.

12. Cao, G., & Prior, R. L. (1999). Measurement of oxygen radical absorbance capacity in biological samples. Methods in Enzymology, 299, 50–62. https://doi.org/10.1016/S0076-6879(99)99008-0

13. Ou, B., Hampsch-Woodill, M., & Prior, R. L. (2001). Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. Journal of Agricultural and Food Chemistry, 49(10), 4619–4626. https://doi.org/10.1021/jf010586o

14. Kadam, S. U., Tiwari, B. K., & O'Donnell, C. P. (2013). Extraction, structure and biofunctional activities of laminarin from brown algae. International Journal of Food Science & Technology, 48, 1-8.

15. Olszowy-Tomczyk, M. (2021). How to express the antioxidant properties of substances. Food Science and Technology, 41(1), 1-10.

16. Gulcin, I. (2025). Antioxidants: A comprehensive review. Archives of Toxicology.

17. Dechayont, B. D., Phuaklee, P., et al. (2019). ABTS radical cation decolorization assay -in vitro antioxidant capacity. protocols.io. https://doi.org/10.17504/protocols.io.42xgyfn

18. Touhamia, Y., et al. (2025). In vitro and in silico assessment of antioxidant potential of marine algal extracts- FRAP, DPPH, ABTS. Journal of Marine Science and Engineering.

19. Thangsiri, S., et al. (2024). Bioactive compounds and in vitro biological properties of Arthrospira platensis and Arthrospira maxima: A comparative study. Scientific Reports, 14, 23786.

20. Ganesan, P., Kumar, C. S., & Bhaskar, N. (2008). Proximate composition and bioactive compounds of five species of tropical seaweeds. Food Chemistry, 107(3), 1023–1032. https://doi.org/10.1016/j.foodchem.2007.09.055

21. Prior, R. L., Wu, X., & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. Journal of Agricultural and Food Chemistry, 53(10), 4290-4302. https://doi.org/10.1021/jf0502698

22. Rice-Evans, C., Miller, N., & Paganga, G. (1996). Structure–antioxidant activity relationships of flavonoids and phenolic acids. Free Radical Biology and Medicine, 20(7), 933-956. https://doi.org/10.1016/0891-5849(95)02227-9

23. Shakeel, F., Saleem, M., Malik, A., & Khan, T. (2018). Chemical profiling and antioxidant potential of marine red algae (Hypnea musciformis) methanolic extract. Journal of Food Biochemistry, 42(5), e12528. https://doi.org/10.1111/jfbc.12528

24. Zhao, F., Wang, P., Lucardi, R. D., Su, Z., & Li, S. (2020). Natural sources and bioactivities of 2,4-di-tert-butylphenol and its analogs. Toxins, 12(1), 1–13. https://doi.org/10.3390/toxins12010001

25. Calder, P. C. (2012). Mechanisms of action of (n-3) fatty acids. The Journal of Nutrition, 142(3), 592S-599S. https://doi.org/10.3945/jn.111.155259

26. Pereira, L., & Neto, A. I. (2013). Seaweeds as a source of bioactive compounds for functional foods and nutraceuticals. Marine Drugs, 11(12), 4836–4863. https://doi.org/10.3390/md11124836

27. Suciati, S., et al. (2024). Phytoconstituents, antioxidant, and cholinesterase inhibitory activities of Artocarpus sericicarpus. Pharmacia, 71(1), 1-12. https://doi.org/10.3897/pharmacia.71.e112499

28. Rajauria, G., Jaiswal, A. K., Abu-Ghannam, N., & Gupta, S. (2016). Bioactive seaweed phenolics: Extraction, characterization, and applications. Journal of Functional Foods, 20, 112-135. https://doi.org/10.1016/j.jff.2015.10.005

29. Fawzy, M. A., El-Said, A. M. A., & Issa, A. A. (2017). GC–MS determination of bioactive constituents of the red alga Laurencia obtusa. Arabian Journal of Chemistry, 10(S2), S2295-S2301. https://doi.org/10.1016/j.arabjc.2013.07.005

30. Benzie, I. F. F., & Strain, J. J. (1999).Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods in Enzymology, 299*, 15-27. https://doi.org/10.1016/S0076-6879(99)99005-5

31. Wu, X., Beecher, G. R., Holden, J. M., Haytowitz, D. B., Gebhardt, S. E., & Prior, R. L. (2004). Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. Journal of Agricultural and Food Chemistry, 52, 4026-4037.

32. Halliwell, B., & Gutteridge, J. M. C. (2015). Free radicals in biology and medicine (5th ed.). Oxford University Press.

33. Zakaria, M. F., et al. (2025). PUFA from microalgae: Challenges, factors affecting high-yield production, and applications. Microorganisms, 11(1), 48.