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(Research Article)

Phytochemical composition and antioxidant potential of chloroform extract from red seaweed: A GC-MS and DPPH Assay-Based Study

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Abstract

This report investigated the phytochemical constituents and antioxidant capability of the chloroform extract derived from red sea algae. Gas chromatography-mass spectrometry (GC-MS) study showed the existence of 19 metabolites, with citronellal (64.37%), gamma-terpinene (11.42%), (+)-2-bornanone (8.02%), linalool (3.04%), humulene (2.16%), and caryophyllene (2.08%) being the prominent components. These metabolites have been reported to possess various pharmacological functions, comprising antimicrobial, anti-inflammatory, analgesic, and antioxidant properties. The antioxidant properties of the extract were examined utilizing the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. The extract demonstrated dose-dependent antioxidant activity, with 63.21% inhibition at 1000 µg concentration, proportionate to the standard ascorbic acid. The antioxidant activity observed in this study may be attributed to secondary metabolites, such as bromophenols and chlorotannins, which are known to possess significant antioxidant properties. The results emphasize the ability of red sea algae as an effective fount of bioactive compounds with diverse therapeutic applications. The identified compounds, particularly citronellal, terpinen-4-ol, gamma-terpinene, linalool, humulene, and caryophyllene, have the potential to be utilized in different industries, including pharmaceuticals, nutraceuticals, cosmetics, and food preservation. More research is needed to understand the structure-activity correlations of these molecules, examine their synergistic effects, and create innovative drug delivery domains to improve accessibility and specific administration.

Keywords: DPPH; GCMS; Red Seaweed; Red algae

1. Introduction

Red algae (Rhodophyta) have attracted significant interest as potential sources of bioactive molecules with antioxidant, antimicrobial, and other beneficial potentials (Haq et al., 2019). They have been shown to be a prospective provider of bioactive compounds such as proteins, lipids, and polyphenols, which have powerful antibacterial, anticancer, antioxidant, antifungal, and antiviral potentials (Ashwini et al., 2016). These marine organisms produce diverse secondary metabolites as adaptations to their unique aquatic environments. In particular, many red algal species have demonstrated potent antioxidant activity, which may have applications in preventing oxidative stress-related diseases and in food preservation. Recent years have shown an increasing focus on investigating the bioactive substances generated by marine creatures, as they signify a prospective source of novel natural molecules with therapeutic uses.

The assessment of antioxidant phytochemical elements in macro-algae extracts has garnered concern due to its significant importance in disease control in humans. Antioxidant compounds including alkaloids, flavonoids, phenols, tannins, phlorotannins, terpenoids, pigments, glycosides, and steroids in algae are considered to serve as a defence system against reactive oxygen species (ROS) generated by extreme ecological constraints (Senguttuvan et al., 2014).

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Antioxidants in macro-algae safeguard the species' internal structures against oxidative destruction caused by external factors (Karimzadeh et al., 2020).

The present investigation intended to examine the chemical constitution and antioxidant capacity of an extract from *Ceramium diaphanum*, a red alga sourced from the Kunkeshwar coastline in Maharashtra, India. Gas chromatography-mass spectrometry (GC-MS) was utilised to determine the primary components in the algal extract. Additionally, the antioxidant activity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay, a widely used method for assessing antioxidant capacity (Jegan and Manjusha, 2003). GC-MS is an advanced analytical method that facilitates the separation and determination of intricate mixtures of volatile and semi-volatile chemicals (Ouahabi et al., 2023). Application to algal extracts can uncover the existence of bioactive constituents, including phenolic compounds, fatty acids, sterols, and terpenes, which may enhance antioxidant properties (Gunathilaka et al., 2019). The DPPH assay provides a measure of the extract's ability to neutralize free radicals, which is a vital mechanism of antioxidant action (Murugan and Iyer, 2014).

The Red Sea, with its high salinity, elevated water temperatures, and intense solar radiation, presents a unique environment that has shaped the evolution of its resident organisms, including red algae. The adaptive strategies employed by these algae to cope with the harsh conditions of the Red Sea may have led to the production of distinct bioactive molecules with increased antimicrobial and antioxidant potential. Therefore, exploring the natural products derived from Red Sea algae could yield novel and potent compounds with promising applications in the pharmaceutical and biomedical fields. This research seeks to elucidate the bioactive compounds and possible uses for red algal extract by integrating chemical profiling using GC-MS with the evaluation of antioxidant activity. The findings may contribute to the growing body of knowledge on red algae as promising sources of natural antioxidants.

2. Materials and methods

2.1. Red algae extract preparation

A specimen of the red seaweed *Ceramium diaphanum* was collected from the coastline of Kunkeshwar, Maharashtra, India. Ten grams of the crushed plant material were placed into a thimble, which was then positioned inside a Soxhlet extractor. The thimble was filled with 250 mL of an ethanol/chloroform mixture, serving as the organic solvent for extraction. To prevent heat loss, the side arm of the extractor was insulated with glass wool.

The extraction procedure commenced by heating the solvent with a heating mantle, resulting in its evaporation and subsequent movement through the equipment to the condenser. The concentrated solvent subsequently poured into the reservoir carrying the thimble. Upon reaching the syphon, the solvent level was automatically discharged back into the flask, facilitating the resumption of the cycle. This extraction process was carried out continuously for a total of 8 hours, completing 7 extraction cycles. Upon completion, the extracted plant samples were air-dried and collected in the extraction collector for further analysis.

2.2. GCMS Analysis

GC-MS analysis was conducted on a chloroform extract of *Ceramium diaphanum* utilising a GC-MS QP 2010 Plus system (Shimadzu, Kyoto, Japan). The gas chromatograph was connected to a mass spectrometer, including an ion generator temperature of 200°C and an input temperature of 270°C. The column oven temperature was established at 50°C, while the injection temperature remained at 250°C at a pressure of 54.4 kPa. Helium served as the medium for the gas at a steady flow rate, with a total flow of 54.6 mL/min and a column flow of 1.01 mL/min. A 1 µL sample was introduced and analyzed in mass spectrometry full scan mode, spanning a mass range of 45 to 500 m/z. The GC-MS program operated for 50 minutes. Peak determination was conducted by a computer comparing the acquired mass spectra with the NIST 14 collection.

2.3. Antioxidant Assay by DPPH Method

The antioxidant potential of the chloroform extract derived from red sea algae was evaluated by assessing its free radical scavenging capacity using the DPPH (1, 1-Diphenyl-2, Picryl-Hydrazyl) assay. Various concentrations of the extract (200, 400, 600, 800, and 1000 µl/ml) were prepared in test tubes, and 1.5 ml of 0.1% methanolic DPPH solution was introduced to each sample. The mixtures were then incubated for 30 minutes under dark conditions to allow the reaction to proceed. During this period, the samples were monitored for any discoloration, which would indicate a change from the initial purple color of the DPPH solution to yellow. After the incubation process, the absorbance of each sample was quantified using a colorimeter at a wavelength of 510 nm to assess the degree of free radical scavenging activity. The subsequent equation computes the radical scavenging activity:

DPPH radical scavenging activity (%) = [Absorbance of control - Absorbance of the test sample) / (Absorbance of control)] × 100

3. Result and discussion

3.1. GCMS Profile

Red sea algae are known to contain a wealth of bioactive substances that could find use in the pharmaceutical, nutraceutical, and cosmetic industries, among other sectors. GC-MS is a robust analytical method that facilitates the determination and measurement of volatile and semi-volatile chemicals inside intricate mixtures (Priya et al., 2020). The current investigation aimed to investigate the composition of the chloroform extract of red sea algae using GC-MS analysis.

Table 1 Metabolites profile of red sea algae chloroform extract using GCMS analysis

Sr. No.	Name	Formula	Molecular Wt. g/mol	R. Time	Area%
1	Propane, 1,1-diethoxy-2-methyl-	$C_8H_{18}O_2$	146.23	5.042	0.63
2	Gamma-Terpinene	$C_{10}H_{16}$	136.23	6.576	11.42
3	Butane, 1,1-diethoxy-3-methyl-	$C_9H_{20}O_2$	160.25	7.543	0.55
4	Ethane, 1,1,1-triethoxy	$C_8H_{18}O_3$	162.23	7.830	0.25
5	(+)-2-Bornanone	$C_{10}H_{16}O$	152.23	8.266	8.02
6	(1R,2R,5S)-5-Methyl-2-(prop-1-en-2 yl) cyclohexane	$C_{10}H_{18}O$	154.25	9.371	0.85
7	Linalool	$C_{10}H_{18}O$	154.25	9.797	3.04
8	(+)-2-Bornanone	$C_{10}H_{16}O$	152.23	11.555	1.41
9	Citronellal	$C_{10}H_{18}O$	154.25	12.165	64.37
10	Terpinen-4-ol	$C_{10}H_{18}O$	154.25	13.326	1.06
11	Dodecane	$C_{12}H_{26}$	170.33	14.927	0.50
12	Benzene, 1-methoxy-4-methyl-2-(1-methylethyl)	$C_{11}H_{16}O$	164.24	15.407	1.00
13	alpha-Cubebene	$C_{15}H_{24}$	204.35	19.281	0.57
14	Copaene	$C_{15}H_{24}$	204.35	19.532	0.53
15	Caryophyllene	$C_{15}H_{24}$	204.35	20.771	2.08
16	cis-alpha-Bergamotene	$C_{15}H_{24}$	204.35	21.137	0.73
17	Humulene	$C_{15}H_{24}$	204.35	21.750	2.16
18	Naphthalene, decahydro-4a-methyl-1-methylene	$C_{15}H_{24}$	204.35	22.649	0.49
19	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)	$C_{15}H_{24}$	204.35	23.384	0.34

The growing number of drug-resistant infections presents a significant risk to the proper management of microbiological illnesses. Essential oils and plant extracts have historically attracted attention as reservoirs of natural antibacterial agents. The World Health Organization reports that a significant portion of the global population depends on conventional medicine for basic healthcare, with medicinal and aromatic plants extensively utilized as a principal supplier of natural chemical substances (Prabuseenivasan et al., 2006). The GC-MS study of the red sea algae chloroform extract revealed the presence of 19 compounds (Table 1). The major metabolites identified were citronellal (64.37%), gamma-terpinene (11.42%), (+)-2-bornanone (8.02%), linalool (3.04%), humulene (2.16%), and caryophyllene (2.08%). Other compounds, such as terpinen-4-ol, alpha-cubebene, copaene, and cis-alpha-bergamotene, were also detected in lower concentrations.

Citronellal, a monoterpenoid found in various essential oils, exhibits significant antimicrobial and antioxidant properties. Studies have demonstrated their strong antibacterial, antifungal, antiviral, and insecticidal activities (Burt, 2004; Nakahara et al., 2003; Wei and Wee, 2013). These properties make citronellal a promising candidate for utilization as a natural preservative in food, an eco-friendly pesticide in agriculture, and an antimicrobial agent in personal care products (Kordali et al., 2005). While its antioxidant potential is not as potent as some other compounds, citronellal can still contribute to food preservation by inhibiting lipid oxidation and protecting against oxidative stress in cosmetic formulations.

Kamal et al. reported that *C. nardus* essential oil showed an inhibitory effect contrary to *C. albicans* at a dosage of 6.25%, whereas inhibition of *S. mutans* and *S. sobrinus* commenced at a level of 25% (Ahmad Kamal et al., 2020). In another report, a screening for 21 different essential oils was performed. The study revealed that out of 21 essential oils tested, 19 oils showed antibacterial activity against one or more strains. Cinnamon, clove, geranium, lemon, lime, orange, and rosemary oils demonstrated substantial inhibiting properties. Cinnamon oil demonstrated significant suppressive efficacy at low doses, while aniseed, eucalyptus, and camphor oils showed minimal effectiveness against the examined pathogens. *B. subtilis* exhibited the highest susceptibility. *K. pneumoniae* had minimal susceptibility (Prabuseenivasan et al., 2006).

Citronellal's antifungal activity has also been reported, with minimum inhibitory dose (MID) values ranging from 14 to 56 ppm (Nakahara et al., 2003). The higher percentage of citronellal in red algae chloroform extract suggests its potential as a renewable source for natural fungicides. Compounds like citronellal, with lower MID values against various fungal genera, could serve as natural alternatives to synthetic fumigants to protect stored foods.

Terpinen-4-ol (an isomer of terpineol), a monoterpenoid found in various essential oils, has been revealed to have significant anti-inflammatory and antioxidant potential. In a study by Brand et al., terpinene-4-ol was reported to diminish the synthesis of pro-inflammatory cytokines, such as TNF-α, IL-8, IL-1β, and IL-10, as well as prostaglandin E2 by lipopolysaccharide-activated monocytes. Additionally, terpinen-4-ol and α-terpineol were reported to down-regulate superoxide synthesis by agonist-stimulated monocytes (Brand et al., 2001). The antifungal potential of terpinen-4-ol has been evaluated against *Botrytis cinerea*, a common plant pathogen. Yu et al. demonstrated that terpinen-4-ol attacks primarily on the organelles and cell membranes of *B. cinerea*, suggesting its property as an antifungal compound (Yu et al., 2015). Furthermore, Garozzo et al. reported promising antiviral ability of terpinen-4-ol against Influenza A in MDCK cells. Terpinen-4-ol has also been found to enhance the effects of various biological and chemotherapeutic agents (Garozzo et al., 2011). Shapira et al. found that terpinen-4-ol substantially suppresses the proliferation of pancreatic, colorectal, gastric, and prostate cancer cells in a dose-dependent manner, with reduction levels between 10-90% at concentrations of 0.005-0.1% (Shapira et al., 2016). Gamma-terpinene ($C_{10}H_{16}$), another monoterpene, exhibits notable antioxidant properties and some antimicrobial activity. Guo et al. reported that gamma-terpinene displays strong synergistic antioxidant activity when combined with phenolic compounds. This property can be exploited in various applications, such as food preservation to extend shelf life, nutraceutical formulations as a natural antioxidant, and cosmetic products to protect against free radical damage. Although gamma-terpinene's antimicrobial activity is not as potent as some other compounds, it can still be utilized in natural food preservatives and aromatherapy products with mild antimicrobial properties (F. Guo et al., 2021). The extensively reported antioxidant characteristics of gamma-terpinene, especially its synergistic effects with phenols and polyphenols, render it an attractive candidate for additional study and application across many industries.

The strong antimicrobial potential of terpinen-4-ol makes it a promising candidate for various applications. Its remarkable antibacterial and antibiofilm properties, particularly against *S. aureus*, suggest its potential use in the production of novel antibacterial medicines, formulation of natural antiseptics and disinfectants, and creation of antimicrobial coatings for medical devices. The well-documented antimicrobial activity of terpinen-4-ol, especially against *S. aureus* and its biofilms, underscores the need for further research to fully exploit its therapeutic potential (Cordeiro et al., 2020).

Antioxidants are essential for blocking oxidative reactions in food, pharmaceuticals, and cosmetics, and for avoiding disorders associated with oxidative stress in the human body. However, the use of synthetic antioxidants has been associated with notable side effects, leading to a growing concern for natural antioxidants. Microbial contamination presents a substantial concern in the food, cosmetics, and pharmaceutical sectors. The persistent use of synthetic preservatives may lead to the emergence of fungus resistance and residual harm (Y. Guo et al., 2021). In response to these issues, effective and organic antimicrobials have subsequently garnered interest as supplements for diverse products.

Linalool (3,7-dimethyl-1,6-octadien-3-ol), a monoterpene acyclic tertiary alcohol, has a significant worldwide market worth of USD 9,980 million in 2019, with projections indicating an increase to USD 12,300 million by 2024 (Ferraz et al., 2021). It is predominantly acquired via chemical synthesis and has widespread uses in the manufacture of perfumes, lotions, soaps, shampoos, and non-cosmetic everyday products like detergents and cleansers (Caputi and Aprea, 2011). In recent years, linalool has been investigated as an effective packaging component by infusing it into the headspace of food packaging or integrating it into food coatings and films (Liu et al., 2012).

Linalool and its ester, linalyl acetate, are monoterpene chemical substances noted as significant volatile constituents in the essential oils of various aromatic species. Numerous species that produce linalool and linalyl acetate are utilized in traditional medicine to mitigate symptoms and address different acute and chronic conditions. The pharmacological properties of these kinds of substances are ascribed to the existence of alcohols such as linalool and linalyl acetate (Peana and Moretti, 2002). Recent researches have assessed the psychopharmacological properties of linalool in mice, demonstrating significant dose-dependent sedative impacts on the central nervous system (CNS) (Jirovetz et al., 1991).

In addition to its fragrance properties, linalool has been shown to enhance the preservative effect of cosmetic formulations and exhibit anti-inflammatory activity on mild skin lesions (Peana et al., 2002). Numerous studies have highlighted the wide range of biological characteristics possessed by linalool, comprising antioxidant, anti-inflammatory, anticancer, cardioprotective, and antimicrobial properties (Liu et al., 2012a). Linalool's anti-inflammatory properties are believed to contribute to its neuroprotective properties in rats with hemiparkinsonism, since it markedly inhibited the downregulation of tyrosine hydroxylase and dopamine transporter in a Parkinson's disease rat model (de Lucena et al., 2020).

Linalool exhibits antimicrobial potential against bacteria and fungi, with a particularly strong effect against Gram-negative bacteria among opportunistic hospital strains. The mode of action of linalool against microorganisms remains the subject of rigorous research, with biotransformation being a key aspect in determining its susceptibility to detoxification processes (Mączka et al., 2022). Multiple studies in the literature have evidenced the antibacterial efficacy of linalool, particularly against critical pathogens, particularly multi-drug-resistant variants such as *E. coli* O157:H7 (Zengin and Baysal, 2014) and *Pseudomonas aeruginosa* (Liu et al., 2020).

The antimicrobial activity of linalool is primarily associated with gastrointestinal infections caused by pathogens such as *Salmonella enterica* subsp. enterica serovar Typhi, *E. coli*, *C. jejuni, V. fluvial*, and *S. aureus* (Duarte et al., 2016; Ghosh et al., 2019; Prakash and Vadivel, 2020). Additionally, linalool has been shown to be effective against bacteria involved in periodontal diseases, such as *P. gingivalis*, *P. intermedia*, *P. nigrescens*, *F. nucleatum*, *A. actonomycetemcomitans*, *S. mutans*, and *S. sobrinus* (Park et al., 2012). Opportunistic bacteria like *P. aeruginosa* and *S. epidermidis* are also susceptible to the antimicrobial effects of linalool (Liu et al., 2012b; Sokovicx́ et al., 2010).

The addition of linalool into food items could have broad positive effects, as it has demonstrated activity against food-contaminating microbes such as *Campylobacter spp.* (found in undercooked poultry, raw milk, and polluted water), *Salmonella spp.* (found in eggs, meat, poultry, milk, and unwashed green fruit and vegetables), *Listeria monocytogenes* (found in soft and mature cheeses, raw milk, cream, eggs, ice cream, meat, smoked fish, pork, sausages, raw vegetables and fruits, and refrigerated foods, and *E. coli* (found in unwashed vegetables and fruits, raw meat, and water)) (Prakash and Vadivel, 2020).

As a terpene alcohol, linalool possesses significant antimicrobial and antioxidant properties. Its potent antimicrobial efficacy against many pathogens, particularly bacteria and fungi, positions it as a viable option for the formulation of natural food preservatives, environmentally sustainable insecticides and fungicides, as well as antimicrobial personal care and household goods. Furthermore, the potent antioxidant activity of linalool can be utilized in nutraceutical formulations to combat oxidative stress, food preservation to prevent lipid oxidation, and cosmetic products to protect against free radical damage. Research has confirmed the antimicrobial activity of linalool against various microorganisms, including *Aspergillus niger* and *Candida albicans* (Mączka et al., 2022).

Table 2 Prominent metabolites present in the Red Sea algae with their biological activity

Chemical Compound	**Molecular Formula**	**Biological activity**	**References**
Citronellal	$C_{10}H_{18}O$	Antibacterial, antifungal, antiviral, insecticidal, and antioxidant properties	Burt, 2004; Nakahara et al., 2003; Kordali et al., 2005

		As a food preservative	
Gamma-Terpinene	$C_{10}H_{16}$	Antifungal and antiviral properties Good anti-cancer potential As a natural food preservative	Yu et al., 2015 and Garozzo et al., 2011
(+)-2-Bornanone	$C_{10}H_{16}O$	In topical antiseptic formulations Natural preservatives for cosmetics and personal care products	Lee et al., 2022; Dalavaye et al., 2023; Rogerio et al., 2009
Linalool	$C_{10}H_{18}O$	Antioxidant, anti-inflammatory, anticancer, cardioprotective and antimicrobial characteristics As a natural food preservative, As an active ingredient in pharmaceutical and cosmetic items (Perfumes, lotions, soaps, and shampoos) In food packaging material such as food coatings and films.	Liu et al., 2012; Caputi and Aprea, 2011

(+)-2-Bornanone, also referred to as camphor ($C_{10}H_{16}O$), is a bicyclic monoterpene ketone that has been utilized for generations in conventional medicine and has garnered considerable interest in contemporary life sciences studies owing to its varied medicinal characteristics and prospective uses. Camphor has a significant history of application in traditional medicinal practices, especially in Asian nations. It has been utilized to address a range of diseases, notably respiratory disorders, muscle pain, and dermatological concerns (Lee et al., 2022). The ethnopharmacological knowledge surrounding camphor has inspired modern research into its therapeutic potential.

Camphor has been shown to reduce inflammation and relieve pain when applied topically, likely through its interaction with transient receptor potential (TRP) channels, such as TRPV1 and TRPV3. Research has shown that camphor can impede the spread of diverse bacteria and fungi, indicating its efficacy as a natural antibacterial medicine. Camphor exhibits antioxidant activity, potentially aiding in its preventive properties against disorders associated with oxidative stress (Lee et al., 2022). Camphor can relieve itching and minor skin irritations by desensitizing sensory nerve endings when applied topically.

Camphor is a common ingredient in over-the-counter balms and liniments used for the temporary relief of minor muscle and joint pain. Its analgesic properties are attributed to its ability to activate and desensitize TRP channels. Camphor has been used in nasal decongestants and cough suppressants due to its ability to relieve congestion and suppress the cough reflex. Some studies have investigated the anticancer potential of camphor derivatives. Novel camphor-derived pyrimidine derivatives have been demonstrated to trigger apoptosis in cancer cells via a ROS-mediated mitochondrial mechanism. Camphor and its derivatives have been explored as potential organocatalysts in various chemical reactions, such as the enantioselective Henry reaction.

Humulene, or α-humulene/α-caryophyllene, is a monocyclic sesquiterpene present in the essential oils of numerous plants, including hops (*Humulus lupulus*), sage (*Salvia officinalis*), and ginseng (*Panax ginseng*). This compound has gained significant attraction in the area of life sciences due to its diverse therapeutic uses and potential applications in pharmaceuticals, biotechnology, and traditional medicine.

Humulene has demonstrated significant anti-inflammatory effects across various research paradigms. It can diminish the synthesis of pro-inflammatory cytokines and impede the activation of NF-κB and AP-1 transcription factors, which have a role in the inflammatory reaction (Dalavaye et al., 2023; Rogerio et al., 2009). Research has shown the significant anticancer properties of humulene against multiple cancer cell types. It can trigger apoptosis, suppress cell proliferation, and regulate the expression of genes associated with cancer progression (Mendes de Lacerda Leite et al., 2021). Humulene has demonstrated antibacterial, antifungal, and antiparasitic properties, indicating its promise as a natural antimicrobial compound (Dalavaye et al., 2024). Humulene has shown promise as a natural analgesic, with the ability to lower pain and inflammation in animal models (Rogerio et al., 2009).

Humulene has been studied for the ability in treating asthma and other allergy conditions. In a mouse model of airway allergic inflammation, humulene demonstrated significant anti-inflammatory activities when delivered orally or via aerosol, diminishing eosinophil recruitment and inflammatory mediators (Rogerio et al., 2009). The antibacterial characteristics of humulene render it a promising candidate for the creation of new antibiotics and antifungal medicines. Its ability to disrupt biofilm formation and target antibiotic-resistant strains is particularly promising (Dalavaye et al., 2024).

Caryophyllene, a bicyclic sesquiterpene found in various essential oils, has gained significant interest in the area of life sciences due to its diverse therapeutic potential and applications in pharmaceuticals, biotechnology, and traditional medicine.

Caryophyllene exhibits significant anti-inflammatory capabilities by diminishing the synthesis of pro-inflammatory cytokines and obstructing the activation of NF-κB and AP-1 transcription factors (Scandiffio et al., 2020). Research has shown caryophyllene's ability to reduce pain and inflammation, likely through its interaction with the CB2 cannabinoid receptor (Jha et al., 2021). Research suggests that caryophyllene can suppress the multiplication of various bacteria and fungi, making it a potential natural antimicrobial agent. Caryophyllene has been found to possess antioxidant properties, which may enhance its preventive benefits against illnesses associated with oxidative stress (Francomano et al., 2019). The antitumor properties of caryophyllene have sparked interest in its potential use as an adjuvant in cancer therapy. Studies have shown that caryophyllene can enhance the efficacy of chemotherapeutic agents like paclitaxel (Hashiesh et al., 2020). Caryophyllene's anti-inflammatory and neuroprotective effects suggest its potential in treating neurodegenerative diseases like Alzheimer's and multiple sclerosis (The ability of caryophyllene to reduce gut inflammation and protect against gastric issues has led to its exploration as a potential treatment for gastrointestinal disorders. Investigations intimate that caryophyllene may be a therapeutic agent for preventing and treating osteoporosis by promoting bone formation and reducing bone resorption (Francomano et al., 2019).

3.2. Antioxidant Assay (DPPH Method)

Table 3 Antioxidant assessment of red algae chloroform extract using DPPH assay

Sr. No.	Sample	Concentration (mg L^{-1})	% Inhibition	IC_{50} (µg/ml)
1	Control	-	-	-
2	Standard (Ascorbic Acid)	200	24.87%	598.16
		400	37.30%	
		600	52.84%	
		800	56.99%	
		1000	66.83%	
3	Chloroform extract	200	21.76%	729.97
		400	28.49%	
		600	44.55%	
		800	58.03%	
		1000	63.21%	

Antioxidants have gained importance in the fight against free radical-related disorders in the last ten years. The tissue and cellular impairment caused by reactive oxygen species occurs when these molecules assault biological molecules such proteins, lipids, enzymes, and nucleic acids. Because of this, oxidative stress and a host of illnesses can develop when cellular antioxidant equilibrium is disturbed (Tziveleka et al., 2021). Many disorders including cancer, cardiovascular disease, diabetes, and high blood pressure are thought to be caused by free radicals, which have a substantial influence on human health (Farasat et al., 2014). The immune system's defence mechanism against free radicals is comprised of both enzymatic and non-enzymatic antioxidants. Some enzymes, such as glutathione peroxidases, catalases, superoxide dismutases, and lactoperoxidases, directly neutralise free radicals, while others, such as glutathione reductase and glucose6-phosphate dehydrogenase, indirectly neutralise free radicals (Tziveleka et al., 2021). In contrast, when an organism is under stress, it can create flavonoids, polyphenols, β-carotene, vitamin E, vitamin C, and other non-enzymatic antioxidants through diet (Young and Woodside, 2001). The intake of exogenous antioxidants is the most effective and widely utilized technique to reduce oxidative stress.

The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging was utilized to examine the antioxidant potential of red sea algae. It determines if a substance can function as a hydrogen supplier or a free-radical scavenger by using the compounds themselves as free radicals (Baliyan et al., 2022). Here, in this particular study, it was noted that there was dose-dependent increase in scavenging activity. Scavenging activity was noted in % inhibition. At 200 µg concentration of standard ascorbic acid, 24.87% inhibition was noted. Whereas red sea algae chloroform extract possessed 21.76% inhibition at 200 µg concentration. Also, at 1000 µg concentration of standard ascorbic acid 66.83% inhibition was illustrated. In comparison with standard ascorbic acid, red sea algae chloroform extracted at 1000 µg concentration executed 63.21% inhibition. Here, the results obtained for red sea algae chloroform extract were highly comparable with standard ascorbic acid. At 1000 µg concentration, there was a noticeable peak in scavenging activity. For conventional ascorbic acid, the minimal inhibitory dose needed to scavenge the free radicals was 598.16 µg/ml. Similarly, for red sea algae chloroform extract it was noted to be 729.16 µg/ml. With this respective study, several other microalgae including *S. latiuscula*, *G. furcate*, *R. confervoides*, *R. confervoides*, *S. latiuscula*, *R. confervoides*, *S. latiuscula*, *S. latiuscula* also possessed promising antioxidant activity by the assay performed through DPPH (Tziveleka et al., 2021).

The activity found in macroalgae is due to the secondary metabolites present in it. A bromophenol is a secondary metabolite found in marine organisms that consists of phenol and one or more bromine atoms. Marine organisms such as sponges, ascidians, and several types of algae (green, brown, and red) have yielded numerous bromophenol's. Even though they are exclusive to marine algae, chlorotannins are an essential and varied class of polyphenolic secondary metabolites found throughout nature (Liu et al., 2011). These compounds show significant antioxidant activity. In this study, the promising activity observed here may be due to the secondary metabolites present in the red sea algae and thus executing antioxidant properties. Therefore, red sea algae can be used to extract secondary metabolites possessing antioxidant activity to treat several ailments.

4. Conclusion

This study has shown the substantial phytochemical content and considerable antioxidant capacity of the chloroform extract of red sea algae. GC-MS analysis identified 19 substances, including citronellal (64.37%), gamma-terpinene (11.42%), (+)-2-bornanone (8.02%), linalool (3.04%), humulene (2.16%), and caryophyllene (2.08%) as the predominant components. These substances are shown to exhibit a broad spectrum of medicinal effects, encompassing antibacterial, anti-inflammatory, analgesic, and antioxidant actions. Citronellal demonstrates potent antibacterial, antifungal, antiviral, and insecticidal characteristics, positioning it as a viable option for natural preservation, environmentally sustainable pest control, and antimicrobial applications. Terpinen-4-ol and gamma-terpinene exhibit significant antioxidant and antibacterial properties, with prospective uses in food preservation, nutraceuticals, and cosmetics. The DPPH experiment further validated the dose-dependent antioxidant properties of the red sea algal extract, yielding findings comparable to conventional ascorbic acid. At a dose of 1000 µg, the extract demonstrated 63.21% inhibition, underscoring its efficacy as a natural antioxidant source. This research highlights the significance of red sea algae as a crucial source of bioactive substances with various medicinal uses. The detected chemicals, including citronellal, terpinen-4-ol, gamma-terpinene, linalool, humulene, and caryophyllene, has the potential for application in numerous industries, including medicines, nutraceuticals, cosmetics, and food preservation. Additional study is necessary to examine the structure-activity correlations of these substances, assess their synergistic impacts, and create innovative drug delivery vehicles to improve their bioavailability and targeted delivery. Moreover, in vivo investigations and clinical trials are essential to comprehensively assess the therapeutic efficacy and safety profile of red sea algae extract and its components. This study elucidates the phytochemical composition and antioxidant capacity of red sea algae extract, facilitating future studies and the creation of natural, environmentally friendly, and efficacious remedies for many health and industrial uses.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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