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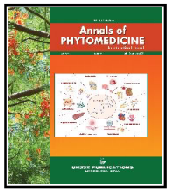

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Antioxidant and antibacterial characteristics of aqueous and alcoholic extracts of *Cystoseira trinodis* (Forsskål) C. Agardh seaweed obtained from a coastal area of Gujarat, India

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Abstract

The brown seaweed contains high amount of polyphenols that have good antioxidant and antibacterial properties. The *Cystoseira trinodis* (Forsskål) C. Agardh seaweed was collected from the Okha and Sikka coast of Gujarat, India. This study examined the antioxidant and antimicrobial properties of aqueous, methanolic and ethanolic extracts of *C. trinodis* seaweed. Significant differences ($p<0.05$) were observed in the antioxidant activities of methanolic, ethanolic, and aqueous extracts. The TPC was found to be highest in methanolic extract, followed by ethanolic and aqueous extracts. The DPPH radical-scavenging activities of ethanolic extract was higher than any other extracts. FRAP activity differed significantly amongst the three extracts. The aqueous extract had much stronger FRAP activity than the other two. The antimicrobial activity of all the extracts were observed against food spoilage microorganisms by agar well diffusion method. All three extracts were found to be more active against Gram-positive bacteria compared to Gram-negative bacteria. Among of all the extracts, the aqueous extract was shown to possess much higher antibacterial properties against Gram-positive bacteria.

1. Introduction

Edible seaweed, also known as marine macroalgae, is one of the most abundant sources of naturally occurring antioxidants and antimicrobials among marine flora that are raised for human consumption (Gupta and Abu-Ghannam, 2011; Pingili *et al*., 2023). Seaweed grows in severe conditions where it is subjected to high oxygen concentrations, tidal forces, temperature and salinity fluctuations, and incessant sun exposure. Red, brown, and green seaweeds have adapted and created a number of secondary metabolites to withstand and flourish in such unfavorable environments (Lomartire *et al*., 2021). From macroalgae, substances were extracted that displayed awide-ranging biological properties, including antimicrobial, antioxidant, antifungal, anti-inflammatory, antiviral, and antitumoral (de Sousa *et al*., 2017; Barzkar*et al*.,2019). Due to their wide range and prospective uses, phenolic compounds of seaweed are grabbing the attention of the scientific community and also several manufacturing industries. Regardless of the recognized variability of phenolic compounds'structures, they have a benzene ring with at least one hydrogen replaced by ahydroxyl group. One of the most notable qualities is their activities as antioxidant, which stop the formation of numerous free radicals because of the capability to chelate metal ions (Pinela *et al*., 2016). The major five groups are used to classify phenolic compounds: a flavonoid (the leading subgroup and related with different bioactivities, *i.e*., antioxidant properties, antimicrobial action (Albuquerque *et al*., 2021; Hemalatha *et al*., 2023), tocopherols, lignans, tannins, and phenolic acids (Thomas and Kim, 2011; Manasaand Shobha, 2022).

Phlorotannins, which are polymers of phloroglucinols (1,3,5-tri hydroxybenzene), are present in some species of brown seaweed and can make up as much as 15% of their dry weight (Wang *et al*., 2012; Sujatha and Meraj, 2023). Additionally, they have antioxidant properties that are much higher than those of many polyphenols obtained from terrestrial plants because they are made up of up to eight rings that are connected. Most polyphenols obtained from terrestrial plants only have three or four rings in their structure (Cox *et al*., 2010; Malik *et al*., 2020). The study meant to assess the

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antioxidant and antibacterial characteristics of *C. trinodis*, one of the brown seaweeds that was obtained from the Okha and Sikka coasts of Gujarat, India.

2. Material s and Methods

The fresh seaweed samples were handpicked from the Okha and Sikka coasts of Gujarat and instantaneously washed by means of seawater to eliminate any foreign particles. The clean samples were placed in buckets and taken to the laboratory for identification. The authentication of the sample was done by Dr. N.H. Joshi, Associate Professor and Seaweed Expert, Department of Aquaculture, Fisheries College, Veraval as per Jha *et al*. (2009).The Voucher Specimen (No. JVC/LPT/SP/2/2022) has been stored in the herbarium of this department.

2.1 Preparation and processing of *C. trinodis* seaweed

The collected seaweed was cleaned by tap water to remove dirt and epiphytes. Seaweed was cut into short fragments (3-4 cm in length) before being dried up in a shelter at room temperature. The drying was done until a constant weight was achieved upon a periodic weighing of the seaweed. The seaweed fragments were crushed into powder with a spice grinder and then passed from a sieve with a netting size of 36. Powder of *C. trinodis* was then placed in individual airtight amber-colored containers and was stored at room temperature up until their use.

2.2 Antioxidant analysis and antibacterial activity assessment of *C. trinodis*

The phenolic content, DPPH activity and ferric reducing antioxidant power (FRAP) as well as antibacterial action of seaweed extracts were measured with the following details:

2.2.1 Preparation of seaweed extracts

Seaweed powder extracts using different solvents were prepared following the method given by Banerjee *et al*. (2020) with slight modifications. In nutshell, a conical flask was precisely filled with 20 g of seaweed powder. 1000 ml of solvent (methanol, ethanol or water) was added to it and held at room temperature (30 ± 1°C) for 10 h in a shaker (Shaker Model No.8406, Equitron, India) at a constant rate (80 oscillation/min), and finally centrifuged (Centrifuge 5430R, Eppendorf AG, Hamburg, Germany) at 3000 g at 5°C for 10 min. The crude extract's supernatant was further clarified via WhatmanNo. 1 filter paper (HiMedia®, Mumbai, India). The filtrates were dried up in an oven at 40°C for 72 h (for ethanolic and methanolic extract) and lyophilized by means of Benchtop Freeze-Dryer (LABCONCO Free-Zone 4.5 Liter (-50°C)) for 36 h (for water extract) (ElNaker*et al*., 2021). These methods are highly reliable, but they take a long time.The dried extracts were kept in a containers and stored at freezing temperature (-20°C) for further studies. The effectiveness of the extracts was considered based on the dry-weight of seaweed extracts.

2.2.2 Assessment of total phenolic content (TPC)

The TPC of seaweed extracts were estimated *via* the Folin-Ciocalteu (F-C) method (Banerjee *et al*., 2020). In a nutshell, a 0.2 ml (water or alcoholic) extract was carefully blended with 1.0 ml of F-C reagent diluted 1/10 in deionized water. After 10 min, 800 µl of a Na_2Co_3 solution (7.5% w/v) was transferred to every test tube. After that, the tubes were left to incubate at room-temperature for 30 min in the dark. A spectrophotometer (WSP-V500, Wensar, India) was used to check the absorbance of materials at 743 nm in comparison to a blank. The TPC was determined as gallic-acid equi. (GAE) in mg/g of dry-weight of seaweed extract. A standard-curve was prepared using gallic-acid concentrations ranging from 10 to 70 mg/l (Figure 1).

2.2.3 Estimation of DPPH radical scavenging activity

The technique finalized by Brand-Williams *et al*. (1995) was utilized for the assessment of DPPH scavenging capacity. The trolox standard curve was used for the estimation of DPPH inhibition activity (Figure 2).

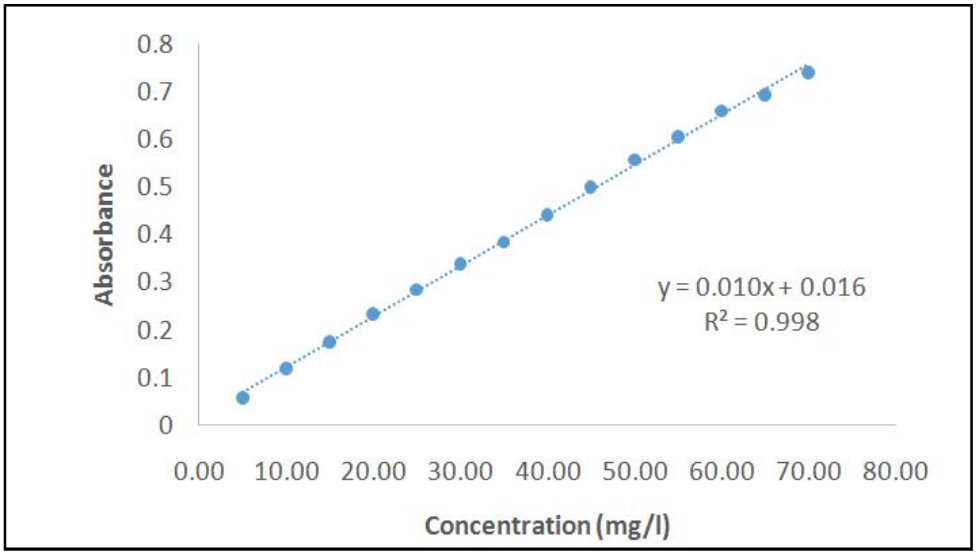

Figure 1: Standard-curve of gallic-acid to check total phenolic content.

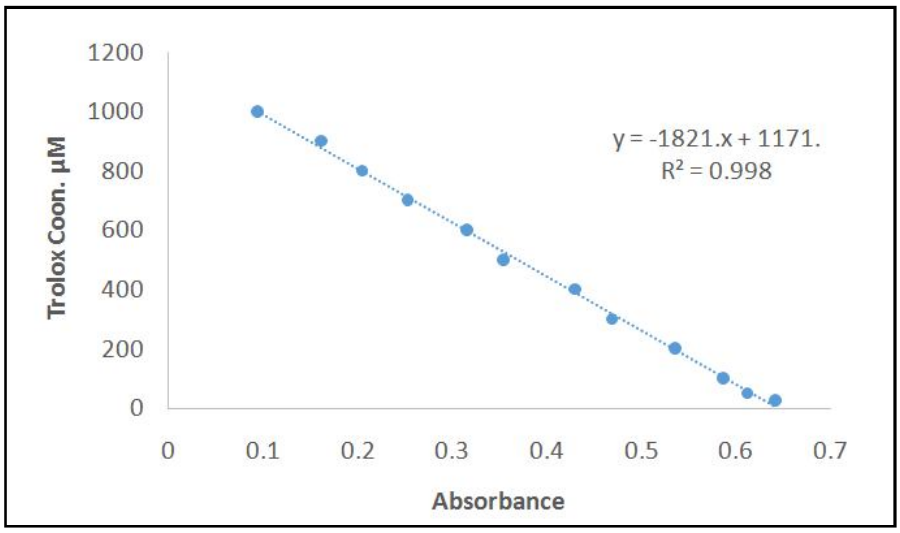

Figure 2:Trolox standard curve for determination of DPPH inhibition activity.

2.2.4 Estimation of ferric-reducing antioxidant power (FRAP)

The method defined by Xiao *et al*. (2020) was used with some modifications to measure the FRAP values of seaweed extracts. The method to measure the reducing activity of samples is adapted for 2 ml microcentrifuge tubes. Mix 250 µl of extract diluted in alcohol or water (or use trolox as a standard), 250 µl phosphate-buffer (pH 6.6, 0.2 M), and 250 µl 1%K*f* [Fe(CN)$^{+}$] (potassium-ferricyanide). Following 20 min of incubation at 50°C, 250 µl of 10% TCA was added to every tube. 250 microliters of the tube's supernatant were removed. After mixing 250 µl of supernatant with 250 µl distilled-water and 50 µl of 0.1% $FeCl_3$, the absorbance was checked at 700 nm. Results were presented as mg trolox equivalent per g of dry-weight extract (mg TE/g). The trolox standard curve was used for the estimation of FRAP activity (Figure 3).

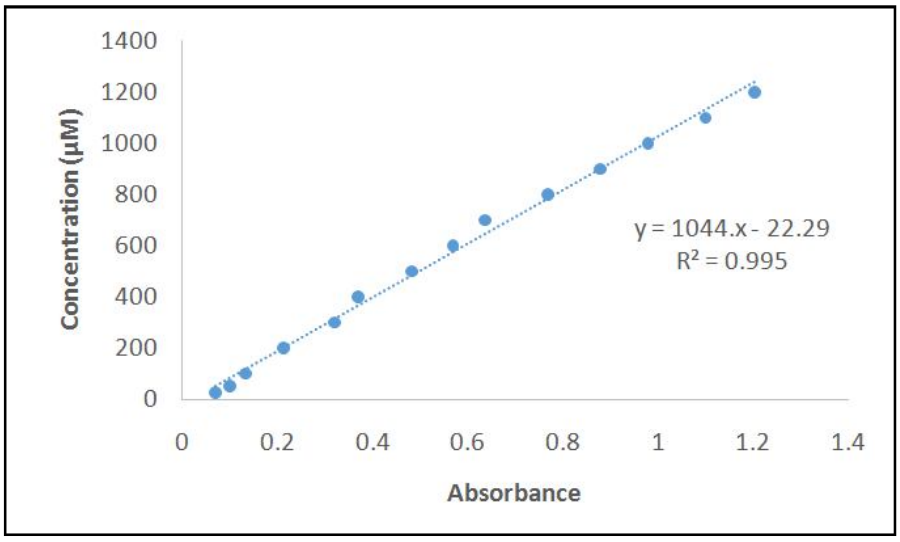

Figure 3: Trolox standard curve for determination of FRAP activity.

2.2.5 Bacterial strains and growth conditions

Identified Gram-positive and Gram-negative microbial cultures (molecular identification) were obtained from the Department of Veterinary Microbiology, CoVSAH, Kamdhenu University, Junagadh for the study. *In vitro*, antimicrobial studies were carried out against four food spoilage bacterial strains. Four different types of microbes-*Staphylococcus aureus, Bacillus cereus, Escherichia coli, and Pseudomonas aeruginosa* were utilized. 20% glycerol stocks were used to keep all of the microbial cultures at -80°C. After being revived, the bacterial cultures were cultured in Mueller Hinton Broth (MHB) at 37°C. A minimum of 18 h was given for the working cultures to mature under ideal circumstances prior to analysis. To get a concentration of 1×10^8 colony forming units (CFU)/ml, bacteriological suspensions were mixed with 0.85% NaCl-saline solution, which is the similarto the McFarland standard of 0.5. An effective concentration of 1×10^6 CFU/ml was achieved by diluting the sample with MHB.

2.2.6 Determination of zone of inhibition

The antimicrobial activity of seaweed was estimated as per the method defined by Martelli *et al*., (2020) with slight modifications. The agar well diffusion technique was used to qualitatively test four different concentrations of alcoholic and aqueous seaweed extracts (1, 2, 3, and 4 mg/ml) for the detection of antibacterial activity. The bacterial lawn on the Mueller Hinton Agar (M173, Hi-Media Laboratories Pvt. Ltd., Mumbai) plates were made from four different bacterial cultures (100 µl), each containing 1×10^6 CFU/ml (as stated above). The agar was then punched with a sterile corkborer to make wells of a 7 mm diameter, and 80 µl of each extract was added. For this test, we employed a positive control consisting of antibiotic discs [Gentamycin (10 µg/disk), Chloramphenicol (30 µg/disk)] and a negative control consisting of solvent without extracts. After 24 h of incubation (37°C) in aerobic environments, the antibacterial power was measured as a diameter of the inhibition-zone (in mm). The measurement of inhibition zone diameter (DIZ) was utilized as an indicator of the antibacterial efficacy. The measurement of the inhibitory zone was recorded in millimeters (mm). The recorded DIZ value was obtained by subtracting 7 mm, from the total DIZ, as it was the diameter of the well. The criterion for defining a no inhibition zone (NIZ) was established as DIZ values less than 7 mm. In this context, a diameter greater than 7 mm but less than 9 mm is considered as indicating trace activity (+). Similarly, a diameter falling between 9 mm and 14 mm is read as moderately active (++), while a diameter above 14 mm is regarded as extremely active (+++) (Afrin *et al*., 2023).

3. Results

An indicator of an extract's or fraction's antioxidant activity is its capacity to neutralize free radicals (DPPH), which can form through electron or hydrogen donation mechanisms. Further, the extracts' electron-transfer-mediated metal-ion reduction capabilities were examined using FRAP tests. Since aqueous and organic solvents are able to extract a higher total phenolic content, the extracts performed satisfactorily in terms of antioxidant activity. Ultrasonic, hot plate and reflux are a few other alternatives to solvent maceration that can be used to extract active compounds. Though, it may extract fewer antioxidant compounds, the maceration approach is the simplest and may be done in larger quantities (Zhang *et al*., 2018). Some of the food supplements and other health-related products can be made from this process, which is still thought to be the most economical way to extract natural antioxidant phenolic compounds with moderately strong antioxidant activity.

3.1 Total phenolic content (TPC) of *C. trinodis*

The phenolic compounds found in macroalgal species may be responsible for their radical-scavenging and antibacterial effects. The aqueous and alcoholic extract of seaweed powder was prepared and the TPC (mg GAE/g dried extract) analyzed was given below in Table 1. There was a substantial difference ($p<0.05$) in the values of the methanolic-extract and ethanolic-extract as well as the aqueous extract. The TPC was observed to be maximum in methanolic-extract followed by ethanolicas well as aqueous extract.

Table 1: Total phenolic content of aqueous, methanolic and ethanoic extract of *C. trinodis* seaweed

	Aqueous extract	Ethanolic extract	Methanolic extract
Total phenolic content (mg gallic-acid equi./gram of dried extract)	12.5 ± 0.12^b	12.7 ± 0.21^b	13.9 ± 0.18^a

Note: Values are in means ± SEM, n = 9 per treatment group. Significant dissimilarities ($p<0.05$) are observed in the mean values when different superscripts are used.

3.2 Antioxidant power of *C. trinodis*

Phenolic compounds, proteins, micro- and macro-elements, precursor contents, pigments, polysaccharides, vitamins, and other chemical components give seaweeds their antioxidant action. The antioxidants found in seaweeds have the potential to scavenge free radicals, reducing the amount of reactive oxygen species (ROS) and other harmful substances. They may also help prevent the formation of hydroxyl radicals by chelating free metal ions or converting hydrogen peroxide to harmless byproducts like water and oxygen. It is commonly recognized that brown algae, out of all the forms of algae, often has the most antioxidant activity (Vladkova *et al*., 2022). The antioxidant power of *C. trinodis* seaweed was analyzed by estimation of DPPH scavenging capacity and FRAP. The results obtained are presented in Table 2.

Table 2: Antioxidant activity of aqueous, methanolic and ethanoic extract of *C. trinodis* seaweed

	Aqueous extract	Ethanolic extract	Methanolic extract
DPPH radical scavenging activity (ì moltrolox equivalent/gram of dried extract)	95.3 ± 8.72^a	96.2 ± 6.53^a	68.9 ± 8.56^b
FRAP activity (ì moltrolox equivalent/gram of dried extract)	385 ± 13.82^a	122 ± 8.33^b	69.7 ± 9.21^c

Note: Values are in means ± SEM, n = 9 per treatment group. Mean value in a row have different superscripts are significantly unlike ($p<0.05$).

There was a considerable dissimilarity ($p<0.05$) in the DPPH scavenging capacity of aqueous extract and methanolic extract. The activity of the ethanolic extract was also considerably higher than the methanolic extract. The activity of the ethanolic-extract was non-significantly higher than the aqueous-extract. The DPPH scavenging power of the methanolic extract of *C. trinodis* was the lowest among all three types of extract.

Among all three extracts, there was a significant difference in the FRAP activity. The FRAP activity of the aqueous extract was quite higher than the other two extracts. The FRAP activity of the aqueous extract is followed by ethanolic extract and methanolic extract. The results suggest that these extracts can effectively neutralize free-radicals by converting them into lesser reactive species, thus ending the radical chain reaction. The reduction of ferric ion (Fe^{3+}) ligand complexes to ferrous (Fe^{2+}) complexes, which are strongly blue, in acidic environments is how the FRAP assay determines antioxidant activity.

3.3 Antimicrobial activity assessment of *C. trinodis*

The antimicrobial action of different seaweed extracts were assessed on food spoilage and pathogenic bacteria, *i.e., S. aureus*, *B. cereus*, *E. coli,* as well as *P. aeruginosa*. Bioactive chemicals found in seaweeds may impede the spread of certain bacteria, both those with Gram-positive as well as Gram-negative strains. Table 3 displays the antibacterial activity of seaweed extracts at different concentrations, including aqueous, ethanolic, and methanolic extracts. The inhibition zone that formed around the well served as an indicator of how effective the seaweed's antibacterial activities were. The activity of different concentrations of extracts was compared with a solvent (vehicle) without extracts, which was used as a negative control, while commercial antibiotic discs containing gentamycin (G_{10}) (10 µg/disc) as well as chloramphenicol (C_{30}) (30 µg/disc) were utilized as positive controls. The antibacterial action of various seaweed extracts was increased in concentration-dependent manner against all the microorganisms. The aqueous extract of *C. trinoidis* is more active against *S.aureus* in comparison to ethanolic or methanolic extract.

The activity of aqueous extract and methanolic extract against *S.aureus* was non-significant with each other; however, it was significantly higher than ethanolic extract. The activity of all the extracts was lower as compared to positive control G_{10} and C_{30}. The activity of the vehicle (solvent) was not reported against *S. aureus*. The antimicrobial activity of *C. trinoidis* against *E. coli* was noted at a higher concentration (4 mg/ml). Among all the extracts, the methanolic-extract was better effective against *E. coli* as compared to other two. The alcoholic-extracts were considerably higher active than the aqueous-extract. The activity of methanolic-extract was non-significantly higher than the ethanolic-extract against *E. coli*. Among the positive controls, gentamycin has shown a positive effect. The effect of various extracts against *B. cereus* was in a concentration-dependent gradient. The outcome of the aqueous-extract was significantly greater compared to alcoholic-extracts. The effect of both positive controls was higher than all the extracts.

Table 3: The antibacterial activities of aqueous, methanolic and ethanoic extract of *C. trinodis* seaweed against various food spoilage causing microorganisms at different concentration

	Zone of inhibition (mm)						
	1 mg/ml	2 mg/ml	3 mg/ml	4 mg/ml	Solvent	G_{10}	C_{30}
Staphylococcus aureus							
Aqueous extract	NIZ	4.78 ± 0.22^{a}	6.11 ± 0.26^{a}	8.00 ± 0.24^{a}	NIZ	35.08 ± 0.22^{d}	13.47 ± 0.29^{d}
Ethanolic extract	NIZ	3.56 ± 0.29^{b}	5.56 ± 0.29^{a}	6.22 ± 0.22^{b}	NIZ		
Methanolic extract	NIZ	5.00 ± 0.24^{a}	6.22 ± 0.22^{a}	7.56 ± 0.24^{a}	NIZ		
Escherichia coli							
Aqueous extract	NIZ	NIZ	NIZ	2.00 ± 0.37^{b}	NIZ	17.24 ± 0.20^{d}	NIZ
Ethanolic extract	NIZ	NIZ	NIZ	4.89 ± 0.31^{a}	NIZ		
Methanolic extract	NIZ	NIZ	NIZ	5.78 ± 0.32^{a}	NIZ		
Bacillus cereus							
Aqueous extract	NIZ	4.33 ± 0.24^{a}	5.56 ± 0.18^{a}	7.33 ± 0.24^{a}	NIZ	22.32 ± 0.28^{d}	17.41 ± 0.24^{d}
Ethanolic extract	NIZ	2.22 ± 0.28^{b}	4.44 ± 0.18^{b}	6.11 ± 0.20^{b}	NIZ		
Methanolic extract	NIZ	1.22 ± 0.28^{c}	5.11 ± 0.26^{a}	6.67 ± 0.24^{b}	NIZ		
Pseudomonas aeruginosa							
Aqueous extract	NIZ	1.00 ± 0.29^{a}	2.33 ± 0.24^{b}	4.44 ± 0.24^{a}	NIZ	26.18 ± 0.37^{d}	NIZ
Ethanolic extract	NIZ	1.22 ± 0.28^{a}	3.11 ± 0.20^{a}	4.33 ± 0.24^{a}	NIZ		
Methanolic extract	NIZ	NIZ	1.00 ± 0.29^{c}	4.00 ± 0.29^{a}	NIZ		

Note: Values are in means ± SEM, n = 9 per treatment group. There is a substantial difference (p<0.05) in the mean value of a column with various superscripts. G_{10}-Gentamycin and C_{30}-Chloramphenicol.

***NIZ- No inhibition zone.** Zone of inhibition inactivity is defined as 7 mm or less, trace activity as >7 mm and less than 9 mm, moderate activity as >9 mm and less than 14 mm, and extremely active as >14 mm.

The antimicrobial action against *Pseudomonas aeruginosa* for all the extracts was non-significantly dissimilar from each other. The inhibition zone (DIZ) of all the extracts with concentration of 4 mg/ml ranged from 4 to 4.44 mm, whereas the DIZ value for the G_{10} was 26.18 ± 0.37 mm and no inhibition zone was reported for C_{30}. Among all the tested organisms, the Gram-positive organisms were more sensitive when being comparable to Gram-negative organisms. The reported activity for all the types of extracts against tested organisms did not show a DIZ value of more than 9 mm diameter at the maximum concentration used 4 mg/ml.

4. Discussion

In estimation of total phenolic content, a similar trend was also conveyed by Khudir *et al*. (2021) who stated that the TPC content of methanolic extract is higher than the aqueous extract. The value observed for methanolic extract of *Cystoseira crinite* was 15.0 ± 0.58 mg GAE/g DW. The reported value of the aqueous extract in this study was greater than the reported value for *Cystoseira crinite* by Khudir *et al*. (2021). Manev and Petkova (2021) determined that *Cystoseira barbata* had a TPC of 0.26 ± 0.05 mg GAE/g dried seaweed in the acetone extract and 0.37 ± 0.05 mg GAE/g dried seaweed in the ethanol extract (95%). They concluded that variations in seaweed species, aquatic ambient factors, and extraction techniques accounted for the observed wide range of total phenol concentrations. In comparison to rhodophytes and chlorophytes, the phenolic levels of phaeophytic macroalgae are higher. This is because brown algae (Phaeophyta) contain physodes within their cells that contain a high concentration of phlorotannins, which are phloroglucinol polymers (Sankar *et al*., 2023). The values stated by Cagalj *et al*. (2022) for the ethanolic extract of *C. compressa* were higher than the reported value in the present study. Season, location, light intensity, photoperiod, temperature, available nutrients, and growth phase are some of the variables that might affect the TPC of algae.

The result of DPPH scavenging capacity in this study was found to be comparable to the work reported by Duan *et al*. (2023). They reported that ethanolic-extract had a higher DPPH-scavenging capacity than extract from other solvents. A high concentration of total phenolics (25.33 ± 1.45 mg GAE/g) as well as effective antioxidant capacity against DPPH-scavenging capacity (33.65 ± 0.03 mg TE/g) were observed in ethanolic-extract of *Sargassum* sp. among all tested seaweed. Similarly, Chakma *et al*. (2023) have also reported higher DPPH inhibition activity in ethanolic extract of Stevia as compared to aqueous extract. Jegan and Manjusha (2023) have also reported that the DPPH inhibition activity of the ethanolic-extract of various seaweed was greater than the methanolic-extract of the same and it was increased in a dose-dependent way. The antioxidant activity of brown seaweed *Padinatetra stromatica* aqueous methanolic extract was analyzed by Lekshmi *et al*., (2021). They reported a value of 30.94 ± 0.20 µmol trolox equivalent/g DPPH-scavenging capacity. The methanolic *P. tetrastromatica* extract has greater DPPH radical-scavenging action than ethanolic-extract, according to Sobuj *et al*. (2021). A possible explanation for this finding: methanolic extracts possess an H-donating ability, which enables them to convert free radicals into stable molecules and thereby halt the oxidation process.

The presence and degree of polyphenol conjugation may explain why all seaweed extracts have different FRAP activities. Boisvert *et al*. (2015) observed that the FRAP activity of ethanolic extract of *Saccharina longicruris*, *Ascophyllum nodosum* and *Ulva lactuca* were 13.0 ± 0.01, 461.5 ± 0.02 and 276.4 ± 0.01 µmol TE/g DW, respectively. The FRAP values of brown seaweeds ranged from 4.87 to 64.34 %, green from 6.45 to 38.6 %, and red from 7.78 to 34.78%, according to Dixit *et al*. (2018). According to the results, the reduction capacity for *D. dichotoma* was highest at a concentration of 200 µg/ml of MeOH extracts, followed in descending order by *C. trinodis*, *U. fasciata*, *A. spicifera*, *C. sertularioides, H. valentiae, L. papillosa*, and *G. corticata*. One of the key components of brown seaweeds, phloroglucinol, is liable of their FRAP activity. Duan *et al*. (2023) also conveyed that the aqueous-extract had the greatest FRAP activity among all the tested solvent extract with the highest reported FRAP activity in the aqueous extract of *Fucus* sp.

The antibacterial action of the various seaweeds was also assessed by researchers. Sellimi *et al*. (2017) reported that *S. aureus* was the most sensitive among all tested organisms against the aqueous extract of *C. barbata* with concentration of 10 mg/ml. Afrin *et al*., (2023) have reported the antibacterial capacity of aqueous, methanolic and ethanolic extracts of different seaweed against *S. aureus* with concentrations of 12.5 mg/ml, 25 mg/ml and 50 mg/ml. The work reported by Dayuti (2018) was also in favor of the current study. He stated that *Gracilaria verrucosa* extract showed a positive effect against *E. coli*as well as *S. typhimurium*. Belattmania *et al*. (2016) stated that the methanolic extract of *C. humilis* at lower concentrations was not effective on Gram-negative organisms like *P. aeruginosa*, *Escherichia coli, Klebsiella pneumonia*, or *Salmonella* sp. Afrin *et al*. (2023) described that the crude extracts of various seaweed have a positive effect against *E. coli*. Parallel outcomes were also conveyed by Belattmania *et al*. (2016). Layana *et al*. (2019) reported that *S. aureus* and *B. subtilis* were more susceptible to the hydroethanolic extract of brown seaweed *Padinatetra stromatica*.

Wanja *et al*. (2020) has also reported that *P. aeruginosa* was sensitive to gentamycin (10 µg/disc) as well as resistant to chloramphenicol C_{30}(30 µg/disc). The results reported by various researchers were also in agreement with the present study (Tajbakhsh *et al*. 2011; Kosanic *et al*. 2015; Belattmania *et al*. 2016). The results reported by Sellimi *et al*. (2017) showed a moderate antibacterial activity of brown seaweed *C. barbata* against Gram-negative bacteria with minimal inhibition concentrations (MIC) of more than 40 mg/ml. Sellimi *et al*. (2017) concluded that the required MIC level for moderate antibacterial activity (DIZ value >9 mm and <14 mm) of *C. barbata* aqueous-extract on Gram-positive as well as Gram-negative microbes was 10 mg/ml and 40 mg/ml, respectively. Dixit *et al*., (2018) reported an inhibition zone (DIZ) of 2 to 3 mm on Gram-positive as well as Gram-negative germs with a concentration of 200 µg/ml and a volume of 30 µl of methanolic extract of brown seaweed *D. dichotoma* and *C. trinodis*. Khudir *et al*. (2021) noticed considerable activity of aqueous and methanolic extract of *Sargassum linearifolium* and *Cystoseira crinite* against Gram-negative microbes such as *E. coli*, *P. aeruginosa*as well as *K. pneumoniae*, whereas moderate to high antibacterial capacityon Gram-positive microbes *S. aureus* and *B. subtilis*. The size of inhibition zone, which indicates the antibacterial action, is predisposed by the concentration of the extract and the volume (µl) used (El Shafay *et al*., 2016; Othman *et al*., 2018; Afrin *et al*., 2023).

The increased resistance observed in Gram-negative microbes can be ascribed to the reduced permeability of the surface membrane, primarily due to the presence of lipid-based compounds (Othman *et al*., 2018). The antibacterial activity can be affected by a number of aspects, including time of year the samples were taken, the solvent used, and the specific extraction procedure. The variation in the outcome can be attributed to these factors. The polarity and solubility of the solvents contribute significantly to the varied extraction capacities of the various phytoconstituents, which in turn are influenced by the various types of solvents used (Zhang *et al*., 2018).

5. Conclusion

The methanolic extract of *C. trinodis* seaweed had a higher total phenolic compared to ethanolic or aqueous-extract. Capacity of ethanolicas well as aqueous extracts to scavenge free radicals is better than methanolic extract. FRAP activity was highest in aqueous extract.The antibacterial activity of *C. trinodis* seaweed extracts with concentration of up to 4 mg/ml was found to be less compared to commercially available gentamycin and chloramphenicol antibiotic discs. The seaweed extracts showed greater antibacterial activity at 4 mg/ml compared to 3 mg/ml, 2 mg/ml, and 1 mg/ml. However, the lower concentrations were also effective against Gram-positive microorganisms, *i.e., S. aureus* and *B. cereus.* This study indicates the use of *C. trinodis* seaweed as a natural antioxidant and antibacterial compound.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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