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A comparative analysis of antioxidant activity and total phenolic content of four seaweeds collected from southcoast of India

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Abstract
The intention of this research aimed to investigate the antioxidant capacity of seaweeds collected in Rameswaram along the south coast of India. The antioxidant activity of four seaweeds; Turbinaria conoides, Sargassum squarrossum, Gracilaria foliifera and Dictyota dichotoma were determined in this study.
The highest level of DPPH activity is seen in the DCM fraction, which is as follows: Turbinaria conoides> Sargassum squarrossum> Gracilaria foliifera >Dictyota dichotoma. The DCM fraction of Gracilaria foliifera exhibited the highest total antioxidant activity. (105.44 ± 0.52 mg AA/g). The DCM fraction of Gracilaria foliifera had the strongest ferric reducing antioxidant power (FRAP) among four seaweeds (1.246±0.004 mg AA/g). The ethyl acetate and hexane fraction has less reducing ability as compared with DCM fraction.
The total phenol content of the DCM fraction is greater than that of the ethyl acetate and hexane fractions. The DCM fraction of Sargassum squarrossum has the highest phenol content (3.71 mg/g) and Turbinaria conoides (3.50 mg/g). In comparison to ethyl acetate and hexane fractions, DCM fraction of all seaweeds has strong antioxidant activity and total phenolic content. A search for new natural resources, such as seaweeds used as food and antioxidants, has proven to be nutritious, cost-effective, and environmentally benign.
Due to their antioxidant properties, seaweeds are promising prospects for medicinal, pharmaceutical, and nutritional applications.

Keywords: marine seaweeds, TAC, DPPH, FICA, FRAP, TPC

Introduction
Seaweeds have drawn a lot of interest from scientists in recent years due to their biological properties and a variety of human applications [1]. Phytochemicals by macro algae can have a wide range of biological functions, they are plentiful sources of natural bioactive chemicals. Seaweeds grow in a hostile environment where where they are subjected to a combination of sunlight and higher oxygen levels. Free radicals and other powerful oxidising agents can be produced under these conditions. Seaweed structural components' lack of reactive oxygen species and oxidation resistance over storage, on the other hand, show indicate seaweed cells possess antioxidant protection systems [2].
Furthermore, natural antioxidants are preferable to synthetic antioxidants since they do not contain chemical pollutants and have a wide range of therapeutic properties. As a result, natural antioxidants are deemed safe to use in medicine, dietary supplements, nutraceuticals, and cosmetics for promoting consumer health, minimising the effects of serious diseases, and other aspects of immune system function [3]. Natural antioxidants must be utilised instead of synthetic antioxidants like butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT), which have been shown to be harmful and cancerous in animal studies [4]. These secondary metabolites' high antioxidant capability is primarily responsible for a wide range of biological actions, like anti-inflammatory, antiviral, antiatherogenic, antibacterial, and anticancer properties [5]. Marine macroalgae are a high-nutrient, low-calorie food source that is high in vitamins, minerals, proteins, polysaccharides, steroids, and dietary fibres. In 2004, Bio active substances such as vitamins, minerals, dietary fibre, and polysaccharides have been found in some seaweed, according to Ahn *et al* [6]. As a result, the current research looked into the antioxidant capacity, total phenolics, and flavonoids of four common seaweed species from India's southern coast, Rameshwaram, for future needs in medicine, dietary supplements, cosmetics, and the food industry. Furthermore, new natural-source antioxidant supplies that are both safe and inexpensive should be identified.

Materials and methods
Materials
Hi- Media provided all of the chemicals (Mumbai, India). The solvents and chemicals that were employed were all of the best quality.

Sample preparation
Seaweed was harvested off the coast of India in Mandapam, Rameshwaram. Before being carried to the lab in polybag and preserved aseptically in ice boxes, the obtained sample was thoroughly washed with seawater to remove all foreign materials, such as epithets, sand particles, stones, and shells. After that, the samples were thoroughly rinsed with tap water, followed by distilled water. Seaweeds were washed and wiped clean on blotting paper, then shade dried at room temperature and ground into a fine powder with a blender [7]. The Types of seaweed are depicted in the Table 1.

Table 1: Types of seaweeds

Sr. No	Seaweed	Phylum	Class	Subclass	Order	Family
1.	*Turbinaria conoides* Kuetzing	Ochrophyta	Phaeophyceae	Fucophycidae	Fucales	Sargassaceae
2.	*Sargassum squarrossum* Greville	Ochrophyta	Phaeophyceae	Fucophycidae	Fucales	Sargassaceae
3.	*Gracilaria foliifera* (Forsskal) Boergesen	Rhodophyta	Florideophyceae	Rhodymeniophycidae	Gracilariales	Gracilariaceae
4.	*Dictyota dichotoma* (Hudson) Lamouroux	Ochrophyta	Phaeophyceae	Dictyotophycidae	Dictyotales	Dictyotaceae

Extraction process

The dry macroalgae powder with 90 percent ethanol solvent in a maceration vessel and let to stand for 3x24 hours with occasional stirring before being filtered. The extraction was performed two times, and the extract was then filtered through Whatmann No. 1 filter paper. With use of a rotary evaporator, the filtrate was dried at reduced pressure [8].

In distilled water, the ethanolic extracts were combined and dissolved. Hexane (HEX), dichloromethane (DCM), and ethyl acetate were used to gradually partition the aqueous solution into polar and non-polar components in the crude ethanolic extract (EA). In a rotary vaccum evaporator, the HEX, DCM, and EA fractions were evaporated to dryness. All fractions were kept at 4^0c degrees Celsius until needed [9].

Antioxidant activities estimation

DPPH radical-scavenging activity

DPPH free-radical scavenging assay was done three times. In various fraction dilutions, two millilitres of 0.15 mM DPPH were added (amounting to 1 ml). After 30 minutes of incubation, the absorbance of the reaction mixture was measured at 517 nm.

DPPH scavenging (%) = (Acontrol - Asample) / Acontrol × 100

Where 'A sample' refers to the sample's absorbance after the time required to attain the peak is (30 minutes) and 'A control' refers to the absorbance of DPPH [10].

Total Antioxidant Capacity (TAC) assay

0.3 mL of sample and 3 mL of reagent were mixed together (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 Mm ammonium molybdate). In a water bath, the reaction mixture was heated to 95°C for 90 minutes. The absorbance of all of sample solutions is estimated at 695 nm. In milligrammes of ascorbic acid equivalents per gramme of extract fraction, the total antioxidant capacity was calculated [11].

Determination of total phenolic content (TPC)

Total phenolic content was determined using the Folin–Ciocalteu reagentas gallic acid equivalents (GAE) (Tiitto, 1985). Deionized water was used to dilute macroalgae fraction (0.1 mL) (7.9 mL). The contents were thoroughly mixed after adding the Folin–Ciocalteu phenol reagent (0.5 mL). 1.5 mL of 20 percent sodium carbonate solution mixed after 1 minute and the mixture was vigorously stirred once more. After 1 hour of incubation at 37°C, the absorbance was taken at 750 nm. On a dry weight basis, phenolic content was calculated using a gallic acid (GA) [12].

Ferric- reducing antioxidant power (FRAP) assay

1.25 mL sodium phosphate buffer (0.2 M, pH 6.6) and 1.25 mL potassium ferricyanide were added to aliquots (0.5 mL) of the fraction.

The mixture was then placed in a water bath for 20 minutes at 50°C. After cooling the mixture, 1.25 mL of 10% trichloroacetic acid and 1.25 mL of the mixture were added to 0.25 mL ferric chloride solution (0.1 percent) to 1.25 mL distilled water. The absorbance was compared to a blank. Higher absorbance was linked to higher reducing power. FRAP (ferric-reducing antioxidant power) was calculated as mg of ascorbic acid equivalents per gramme of crude extract fraction [4].

Ferrous ion chelating (FICA) assay

Equal quantities of 0.25mM ferrozine, 0.1mM FeSO4, 0.1mM FeSO4 was mixed with extract fractions from various dilutions (conc range of 1.0–7.0 mg/ml). The reaction mixtures were incubated in triplicates over 10 minutes before being measured at 562 nm (Chew *et al.*, 2008) [10].

Using the following equation, As a percentage of chelating capacity, the result was calculated (percent chelating ability):

Ferrous ion chelating ability (%) = 1-Asample /Acontrol × 100

Results and Discussion

To antioxidant potential of DCM, Ethyl acetate, and Hexane fraction of ethanolic extract of seaweed were determined by various method and presented in Table 2, 3 and 4.

Table 2: Anti-oxidant capacity of seaweeds in dichloromethane fraction

Sr. No	Seaweeds	DPPH (% inhibition)	FRAP (mg AA/g)	Reducing Power (mg AA/g)	Ferrous ion chelating capacity (%)	Total anti-oxidant capacity (mg AA/g)	Phenolic content (mg CE/g)
1.	*Turbinariaconoides*(g)	85.51± 0.15	0.967± 0.005	1.021 ± 0.005	81.01 ± 0.15	103.13 ± 0.21	3.50 ± 0.005
2.	*Sargassum squarrossum*(g)	83.80± 0.15	0.867± 0.002	0.966 ± 0.002	77.30 ± 0.15	102.78 ± 0.032	3.71 ± 0.02
3.	*Gracilaria foliifera*(g)	72.41± 0.13	1.246±0.004	0.868 ± 0.002	69.20 ± 0.15	105.44 ± 0.52	2.97 ± 0.005
4.	*Dictyota dichotoma*(g)	64.46± 0.63	0.971± 0.002	0.79 ± 0.004	72.20 ± 0.20	98.81 ± 0.38	3.21 ± 0.01

The values are the average of three observations with the standard deviation (SD) calculated.

Table 3: Anti-oxidant capacity of seaweeds in ethyl acetate fraction

Sr. No	Seaweeds	DPPH (% inhibition)	FRAP (mg AA/g)	Reducing Power (mg AA/g)	Ferrous ion chelating capacity (%)	Total anti-oxidant capacity (mg AA/g)	Phenolic content (mg CE/g)
1	*Turbinariaconoides*(g)	80.18 ± 0.15	0.907± 0.005	0.67 ± 0.005	62.96 ± 0.20	95.85± 0.20	3.35 ± 0.005
2	*Sargassum squarrossum*(g)	65.09 ± 0.15	0.761± 0.001	0.51 ± 0.001	61.86 ± 0.30	94.18 ± 0.50	3.05 ± 0.001
3	*Gracilaria foliifera*	70.07 ± 0.15	0.833±0.0010	0.45 ± 0.001	57.13 ± 0.30	87.23 ± 0.30	2.55 ± 0.001
4	*Dictyota dichotoma*	59.15 ± 0.10	0.705±0.005	0.49 ± 0.005	66.20 ± 0.50	75.01 ± 0.35	2.89 ± 0.001

The values are the average of three observations with the standard deviation (SD) calculated.

Table 4: Anti-oxidant capacity of seaweeds in hexane fraction

Sr. no	Seaweeds	DPPH (% inhibition)	FRAP (mg AA/g)	Reducing Power (mg AA/g)	Ferrous ion chelating capacity (%)	Total anti-oxidant capacity (mg AA/g)	Phenolic content (mg CE/g)
1	*Turbinariaconoides*(g)	56.66 ± 0.10	0.503± 0.002	0.456± 0.001	32.70± 0.15	55.10 ± 0.001	1.95 ± 0.005
2	*Sargassum squarrossum*(g)	46.17 ± 0.15	0.731 ± 0.001	0.426 ± 0.001	42.53 ± 0.25	49.23 ± 0.005	1.54 ± 0.005
3	*Gracilaria foliifera*	47.54 ± 0.10	0.618 ± 0.005	0.208 ± 0.005	43.10 ± 0.35	45.97 ± 0.02	1.23 ± 0.001
4	*Dictyota dichotoma*	41.39 ± 0.10	0.525 ± 0.001	0.421 ± 0.001	44.51 ± 0.38	48.17± 0.76	1.14 ± 0.001

The values are the average of three observations with the standard deviation (SD) calculated.

DPPH Radical Scavenging Activity

The capacity of anti-oxidant compounds found in the seaweed extract fraction to serve as radical proton scavengers or hydrogen donors is determined using the DPPH assay. This study looked at the anti-oxidant characteristics of four different seaweed extract fractions. DPPH activity in DCM fraction is as *Turbinaria conoides*> *Sargassum squarrossum*> *Gracilaria foliifera*>*Dictyota dichotoma* and in the case of ethyl aetate fraction it as *Turbinaria conoides*> *Gracilaria foliifera* > *Sargassum squarrossum* >*Dictyota dichotoma* and in the hexane fraction, there was a slight decline in activity.

The maximum DPPH activity was found in the DCM fraction in all four seaweeds. The anti-oxidant activity of the ethyl acetate fractions is greater than 50%. According to the findings, all of the seaweeds examined in this study had anti-oxidant properties, as shown in Fig. 1.

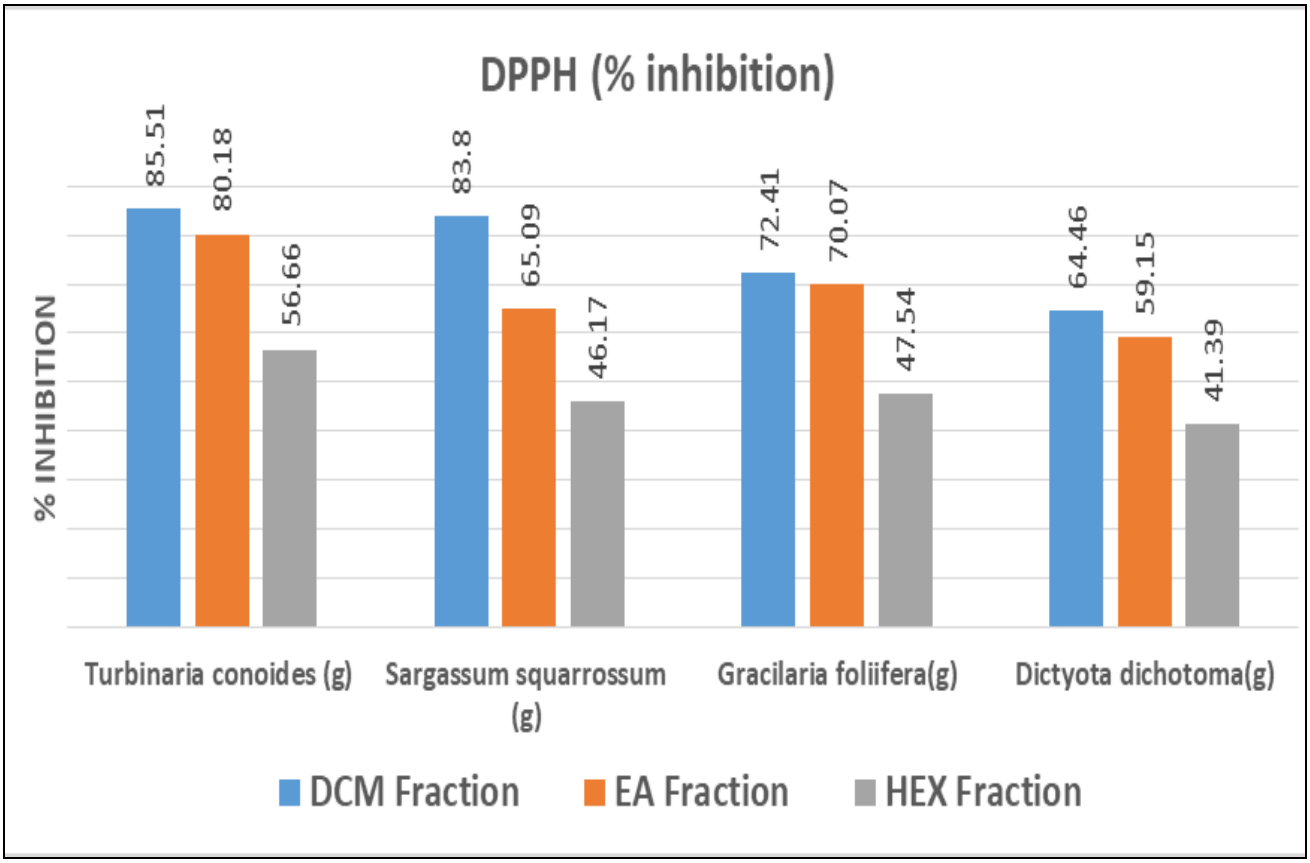

Fig 1: DPPH Radical Scavenging Activity (% Inhibition)

Ferric Reducing Antioxidant Property (FRAP)

The FRAP assay revealed that all four seaweed DCM fractions had the highest anti-oxidant activity, and it's especially significant in *Gracilaria foliifera*seaweeds (1.246 mg/g). Figure 2 depicts the results of the FRAP assay.

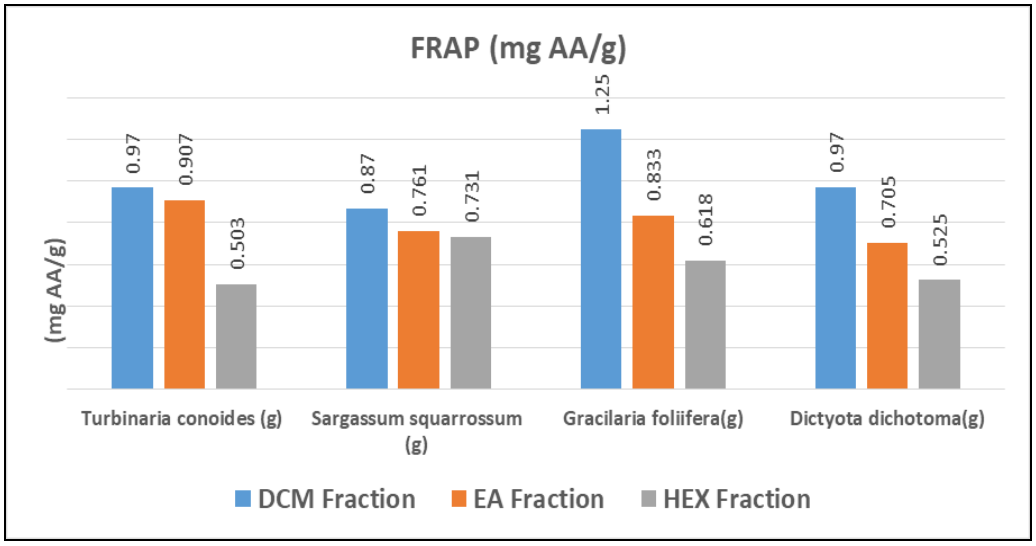

Fig 2: Ferric Reducing Antioxidant Property (FRAP)

Reducing Power

In the DCM fraction, the reducing power was high. The reducing power of various seaweed DCM fraction is as follows: *Turbinaria conoides*>*Sargassum squarrossum*> *Gracilaria foliifera* >*Dictyota dichotoma.* In comparison to the DCM fraction, the ethyl acetate and hexane fractions have less reducing power. The reducing property of anti-oxidant substances suggested that they are electron donors capable of reducing oxidised intermediates in the lipid peroxidation process. As a result, they have the potential to be utilized as primary and secondary antioxidants. The reducing power of DCM, ethyl acetate, and hexane fraction (s) from various seaweeds is shown in Figure3.

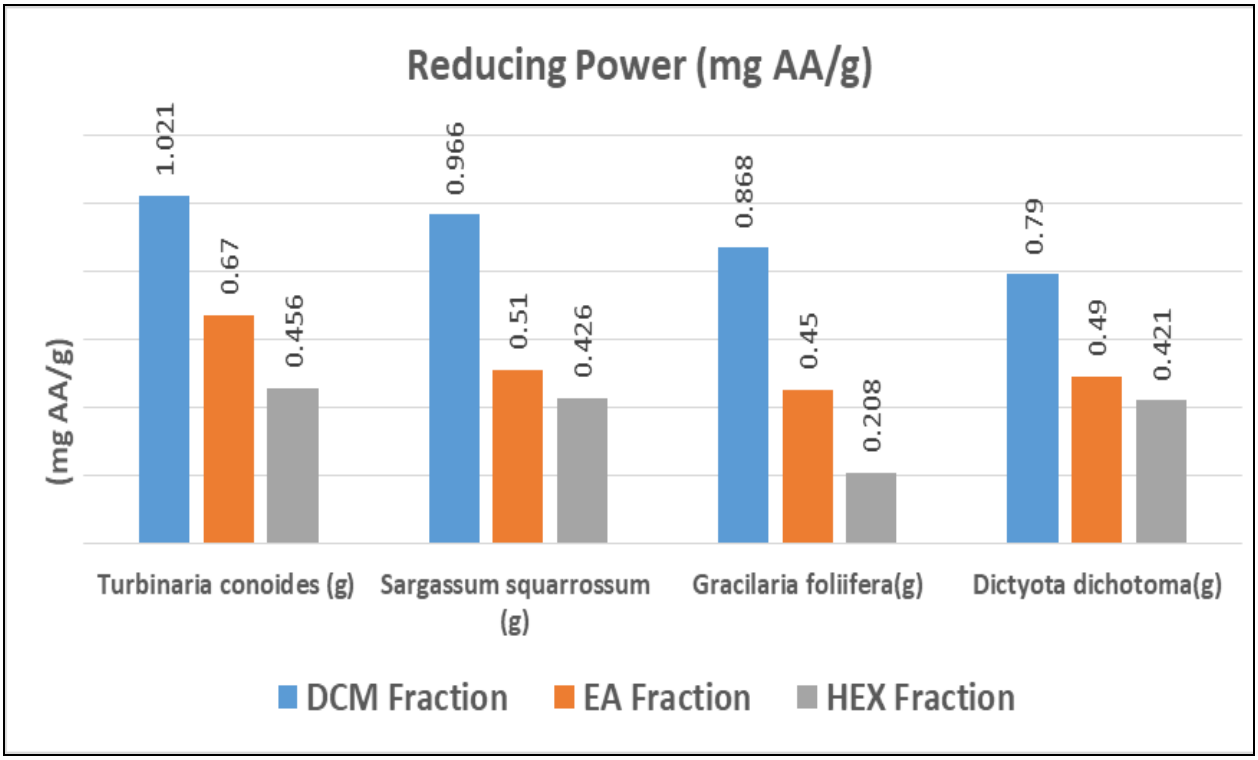

Fig 3: Reducing Power of Different Extract Fraction

Ferrous Ion Chelating Ability (FICA)

A seaweed extract fraction's potential to chelate metals was investigated. FICA percent is (69.20 to 81.01%) in DCM extract fraction, (57.13 to 66.20%) in ethyl acetate fraction, and in hexane fraction (32.70 to 44.51 percent). For all seaweeds, the FICA was the greatest in DCM fraction and was all together as *Turbinaria conoides*>*Sargassum squarrossum*>*Dictyota dichotoma*> *Gracilaria foliifera.*

The DCM fraction of seaweed chelated ferrous ions more effectively than the ethyl acetate and hexane fractions. The Fenton type reaction, which produces dangerous hydroxyl radicals, would be limited or suppressed by a higher binding ability extract. Figure 4 depicts the results of ferrous ion chelating activity of various fractions of collected seaweed(s).

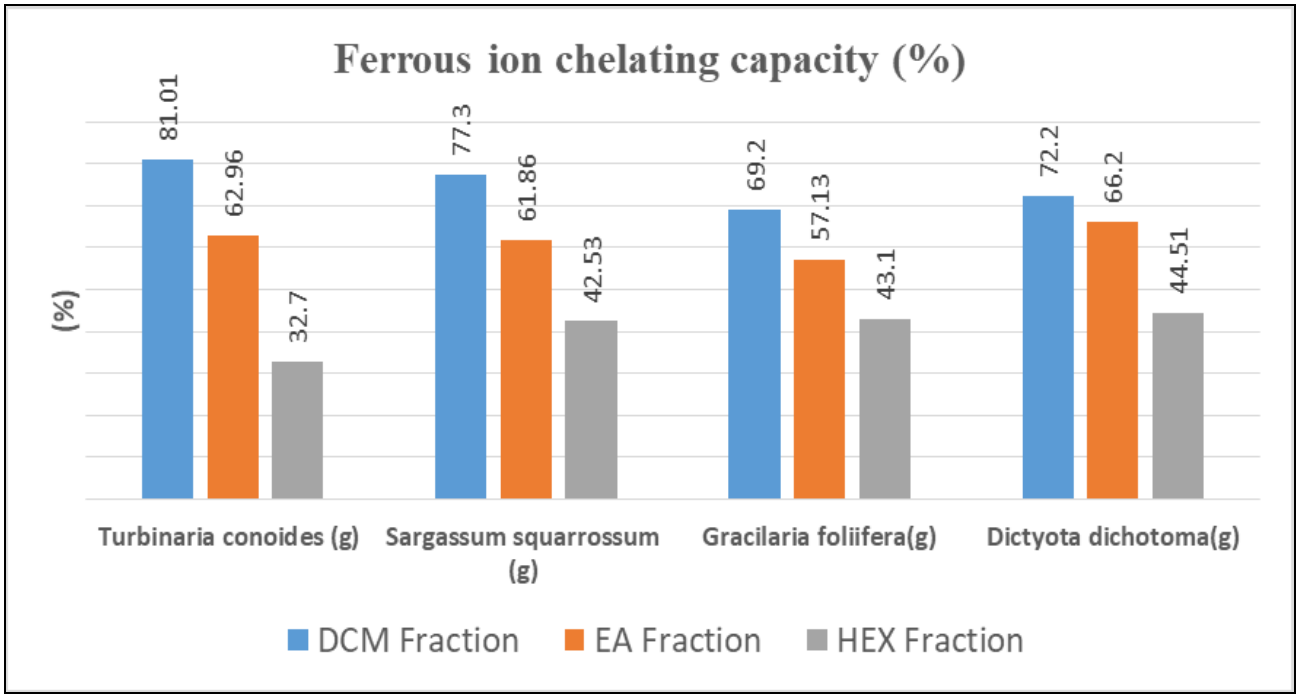

Fig 4: Ferrous Ion Chelating Ability (FICA) of Different Extract Fraction

Total Anti-oxidant Activity (TAC)

The DCM fraction had a high total anti-oxidant capacity (98.81 to 105.44 mg/g), whereas the anti-oxidant activity of the ethyl acetate fraction was lower (75.01 to 95.85 mg/g) than the DCM fraction. Gracilaria foliifera and Turbinaria conoides scored the highest in the DCM fraction (105.44 mg/g and 103.13 mg/g, resp). Figure 5 shows the total anti-oxidant activity results.

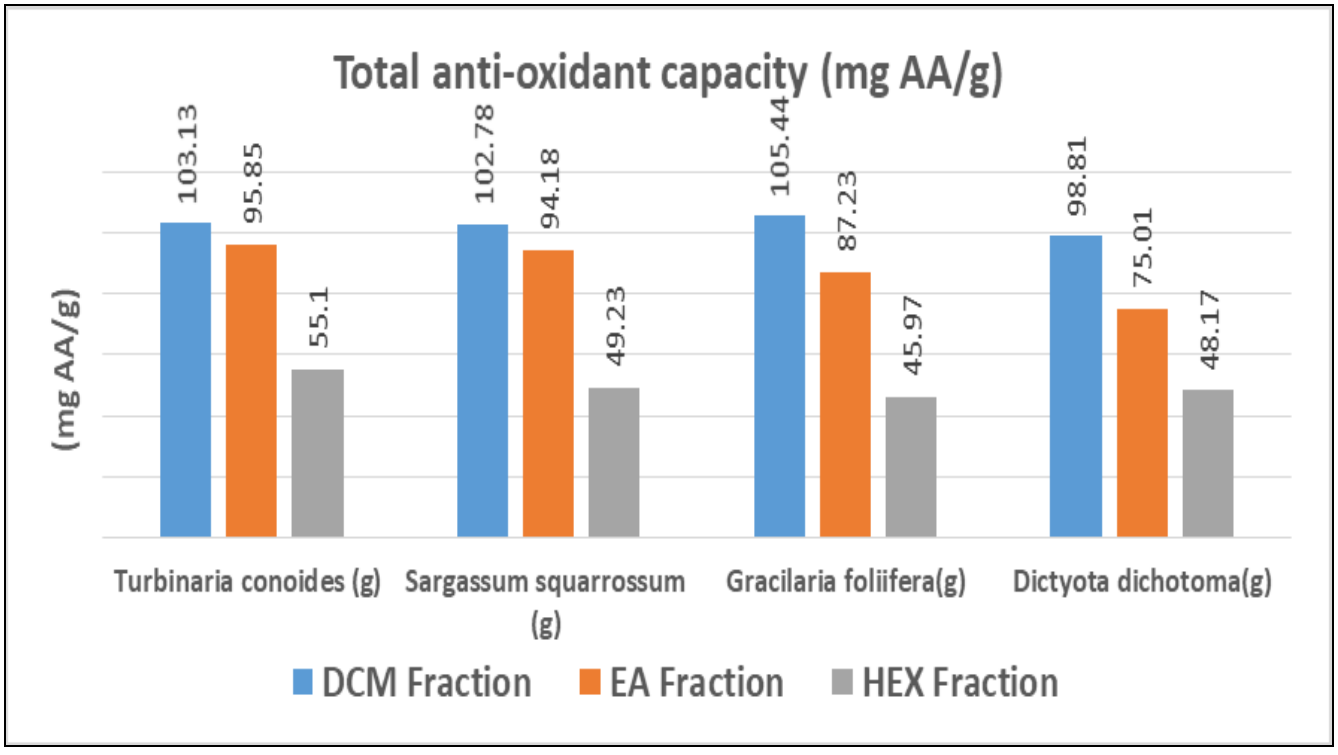

Fig 5: Total Anti-Oxidant Capacity of Different Extract Fraction

Determination of Total Phenolic Content

The DCM fraction had a higher overall phenolic content than the ethyl acetate and hexane fractions in this investigation. The DCM fractions of Sargassum squarrossum (3.71 mg/g) and Turbinaria conoides (3.50 mg/g) had the most phenols. Figure 6 Illustrates the total phenolic content of Turbinaria conoides, Sargassum squarrossum, Gracilaria foliifera, and Dictyota dichotoma in various solvent fractions.

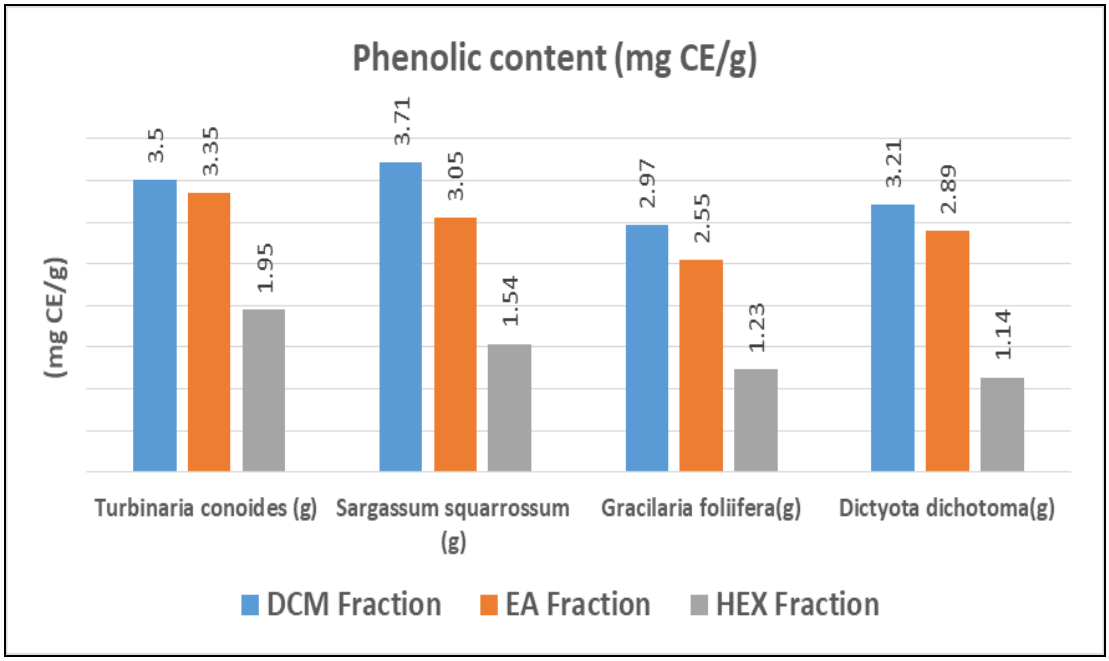

Fig 6: Total phenolic content of different extract fraction

Conclusion

These seaweeds, which were extracted using traditional procedures, showed excellent antioxidant activity and have a wide range of biological, pharmaceutical, and nutraceutical applications. According to the findings, there was a substantial variation in TPC, TFC, and antioxidant activity across four algae species (ABTS, DPPH and FRAP). However, the antioxidant potential of the various algal extracts fraction was discovered to be remarkably variable. All of the seaweed extracts had high levels of DPPH, FICA, and TAC. When comparing the DCM fraction seaweeds to the ethyl acetate and hexane fraction seaweeds, DPPH activity was highest in DCM fraction seaweeds. In comparison to ethyl acetate and hexane fractions, DCM fraction of all seaweeds has strong antioxidant activity and total phenolic content. The results showed that all of the seaweeds studied in this study exhibit anti-oxidant properties. Phenols are the key contributors to anti-oxidant activity in these seaweed extracts, as evidenced by positive and substantial associations between DPPH radical scavenging activity and phenolic concentration.

The findings suggest that seaweeds have anti-oxidant properties, which could be useful in the food and pharmaceutical industries in the future. The impacts of marine extract anti-oxidant activities on anti-oxidant development should be the focus of future research. These natural economic resources must be tapped and developed, which will necessitate several more studies in the future.

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Conflicts of Interest

The author has claimed that he does not have any competing interests (s).

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