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AN ASSESSMENT OF ANTI-OXIDANT ACTIVITY OF MARINE ALGAL POLYPHENOLS RETRIEVED FROM *SARGASSUM WIGHTII, TURBINARIA ORNATA, GRACILARIA CORTICATA* AND *GELIDIELLA ACEROSA*

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Keywords:

Seaweeds, DPPH, FRAP, FICA, TAC, Phenolic content, Anti-oxidant activity

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ABSTRACT: Objective: To assess the anti-oxidant activity of different seaweed extract. **Methods:** Different seaweeds as *Sargassum wightii*, *Turbinaria ornata*, *Gracilaria corticata* and *Gelidiella acerosa* were collected from Mandapam, Ramanathapuram District, Tamil Nadu, India and assessed for their suitability as anti-oxidant compounds. Seaweeds were cleaned from epiphytes, washed, dried in shade and powdered. Algae extractions were performed using different solvents such as methanol, ethanol and hexane. Anti-oxidant potential of marine algal polyphenol from methanol, Ethanol and Hexane extract(s) of seaweed were determined using different method as DPPH radical scavenging assay, Ferric reducing anti-oxidant property (FRAP), Ferrous Ion Chelating Activity (FICA), Reducing power, Total Anti-oxidant Capacity (TAC). The total phenolic content from different seaweeds considered for its anti-oxidant activity. **Results:** The DPPH radical scavenging activity in methanol extract of brown seaweed *Turbinaria ornata* (83.8%) is most noteworthy. In *Gracilaria corticata* it was 79.07 % in ethanol extract. In *Sargassum wightii* brown seaweed, high activity in methanol and ethanol extracts (75.51% and 70.16%) respectively were observed. The DPPH activity in ethanol extract of *Sargassum wightii*, *Turbinaria ornata* and *Gelidiella acerosa* was low to moderate. The most elevated reducing power activity was observed in methanol extract of *Sargassum wightii* than in ethanol extract. The total anti-oxidant activity is highest in methanol extract of *Turbinaria ornate* (105.78 mg/g). In present study methanol extraction of seaweed was found to advance the anti-oxidant activity.

INTRODUCTION: Marine biological systems spread over 70% of the globe's surface. These living spaces are involved by an extraordinary assorted variety of marine life forms that produce profoundly structurally diverse metabolites as an own defense system [1].

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The marine biological system gives a huge territory to microalga networks as they involve the rough shores and lowered intertidal zone. The coastline of Tamil Nadu, India has a length of around 1076 km, while Chennai covers 19 km of waterfront zone, Thiruvallur and Ramanathapuram covers 27.9 km and 236.8 km respectively [2].

Seaweeds are marine, benthic, autotrophic, belonging to non-blooming plants and occupy the marine environment. In light of morphology, cell wall and pigment composition, they are ordered into green (Chlorophyta), brown (Phaeophyta), and red (Rhodophyta) algae.

Seaweeds occupy 90% of marine plant species and globally, half of photosynthesis was done by seaweeds [3]. Naturally visible marine algae, prevalently known as seaweeds, establish one of the significant living assets of the sea. They were discovered joined to the bottom, in generally shallow coastal waters zones up to 180 meter profundity, on strong substrate, for example, rocks, dead corals, stones, pebbles, shells and plants.

The marine macro flora of India is profoundly differentiated and involves for the most part of tropical species, on the whole, 271 genera and 1153 types of marine algae which incorporate structures and assortments forms and varieties [4]. Seaweeds contain huge amounts of macronutrients and micronutrients just as other bioactive compounds some of which are pharmacologically dynamic, for example, polyphenols, terpenes and carotenoids, which have anti-oxidant, antimicrobial and anticancer activities [5]. Free radicals have been professed to assume a significant role in influencing human wellbeing by causing numerous diseases (*e.g.*, heart diseases, cancer, hypertension, diabetes, and atherosclerosis). In the previous decade, anti-oxidants have indicated their pertinence in the prevention of different illnesses, where free radicals are ensnared [6].

Free radicals discharged summon inflammatory reactions by harming the significant macro-molecules and membrane system of cells. Anti-oxidants can neutralize these free radicals, in this manner shielding them from such diseases. The commercially available synthetic anti-oxidants are found to exert destructive impacts, and hence, there is a quest for exploring natural anti-oxidants [7]. Seaweeds are known to be a rich source of anti-oxidant compounds.

Anti-oxidant activity of marine algae may emerge from pigments such as chlorophylls and carotenoids, nutrient and vitamin antecedents including α-tocopherol, β-carotene, niacin, thiamine and ascorbic acid, phenolics such as polyphenols and hydroquinone and flavonoids, phospholipids especially phosphatidylcholine, terpenoids, peptides and other anti-oxidative substances, which directly or indirectly contribute to the inhibition or retardation of oxidation processes [8].

Marine algae, like other photosynthesizing plants, are exposed to intense light and high oxygen concentrations that lead to the formation of free radicals and other strong oxidizing agents. The nonappearance of oxidative harm in their basic segments (polyunsaturated unsaturated fats) and the stability during storage proposes that their cells possess defensive anti-oxidative systems [9].

Starting at yet, only a few reports on the anti-oxidant activity of seaweeds have been published since marine algae are opulent in Phenolic compound such as flavonoids, phenolic acids, and tannins are viewed as the significant contributor to the anti-oxidant capacity. In edible seaweeds, anti-oxidant properties have been associated with their phenolic content. In continuation of our studies toward the isolation of bioactive, we initiated an investigation to assess anti-oxidant properties of algal extracts. The study focused on species that are among the most bounteous in the collection site, in general, and that could, in principle, become a natural hotspot for the extraction of metabolites with anti-oxidant properties for use in the pharmaceutical and food industry.

MATERIALS AND METHODS:

Materials: Analytical-grade chemicals were utilized in all the studies. The chemicals and analytical grade reagents were bought from HiMedia and Sisco Research Laboratories, Mumbai and Chennai, India.

Collection and Processing of Seaweed Materials: Seaweed samples of *Sargassum wightii*, *Turbinaria ornata* (brown algae), *Gracilaria corticata*, *Gelidiella acerosa* (red algae)were collected from Mandapam rocky shores (Lat. 9 °C 27' 70 N, Long. 79 °C 12' 52 E) -Rameshwaram, Ramanathapuram district, Tamil Nadu. The collected seaweeds were identified in the Centre for Advanced Studies in Botany, University of Madras, Chennai Tamil Nadu, India.

The algae were washed thoroughly in seawater, followed by tap water until all epiphytes, sand particles, associated fauna\, and other extraneous materials were expelled. Seaweeds were shade dried for 2-3 weeks, and the dry weight of the sample was determined.

The material was hand crushed and ground using an electronic mixer grinder (Philips HL 1643/04 Vertical Mixer Grinder, India).

Preparation of Extracts: The dried powder of macroalgae (seaweeds) 5gm was extracted with 100ml various solvents *viz.* methanol, ethanol, and hexane. The sample was kept in the dark for 7 days with intermittent shaking. After incubation, the solution was decanted and filtered through Whatman No.1 filter paper. The clear extract was subsequently concentrated under reduced pressure using a rotary evaporator (40-450 °C) and kept in the dark bottles at 4 °C until use. Subsequent to obtaining the extracts in the crude form, the percentage yield of the extracts was calculated and check for maximum percent yield.

Anti-oxidant Activity

DPPH Radical Scavenging Activity: The effect of the seaweed extracts on 1, 1-diphenyl-2-picrylhydrazyl (DPPH) was assessed as described by Ak and Turker [5]. Each sample was diluted in methanol before the analysis (1 mg/ml). The DPPH solution was added to the diluted sample, thoroughly mixed, then left for 30 min for the reaction to occur. After that, the absorbance of the sample as measured a 515 nm by using a UV-VIS Spectrophotometer (Shimadzu UV1800). The absorbance of DPPH solution in methanol, without any anti-oxidant (control), was also measured. The percentage of DPPH radical scavenging activity was calculated by using the following equation:

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

Where 'A sample' is the absorbance of the sample after the time necessary to reach the plateau (30 min) and 'A control' is the absorbance of DPPH.

Ferric Reducing Antioxidant Property (FRAP): FRAP assay was performed by the technique given by Jones A *et al.* [10]. Anti-oxidant activity of the standard was estimated by measuring increased absorbance brought about by generated ferrous ions. The working FRAP reagent contained 0.3M acetate buffer (pH 3.6), 10 mM TPTZ (2, 4, 6-tri(2-pyridyl)-S-triazine), 40 mM HCL and 20 mM $Fecl_3$. $6H_2O$ in the ratio of 10:1:1 (freshly prepared and warmed to 37 °C). 2.7 ml of this working solution was mixed with 100 µl of algal extract to initiate the reaction. Absorbance was recorded after 10 min. at 593 nm. Anti-oxidant capacity is expressed as ascorbic acid equivalent.

Ferrous Ion Chelating Activity (FICA): Ferrous ion chelating ability was determined according to the method of Arosio P, Elia L & Poli M [11, 12]. 0.5 ml of individual algal extract was mixed with 0.1 ml of 2 mM $Fecl_2$, 0.2 ml of 5 mM ferrozine solution, and the reaction mixture was incubated for 10 min. at room temperature. Absorbance was measured at 562 nm by using UV-VIS Spectro-photometer (Shimadzu UV1800). The percentage of chelating ability was determined utilizing the following equation.

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

Reducing Power: Reducing power of the extract was evaluated according to the method of K Habeebullah SF *et al.* [13]. This assay gauges the total anti-oxidant capacity of the sample evaluating the redox potentials of the compounds. The extract was mixed with one ml phosphate buffer (0.2M, pH 6.6) and 1 ml 1% potassium ferricyanide and incubated at 50 °C for 20 min. Subsequent to cooling, it was mixed with 1 ml of trichloroacetic acid (10%). 1.5 ml of this mixture was transferred to other test tube to which 1.5 ml distilled water and 0.5 ml $Fecl_3.6H_2O$ (0.1%) were added. The mixture was centrifuged and kept at room temperature for 10 min. Absorbance was perused at 700 nm on a spectrophotometer. The anti-oxidant activity was expressed in terms of ascorbic acid equivalent and expressed as mg/g.

Total Anti-oxidant Capacity: Total anti-oxidant capacity (TAC) was determined according to Kasangana, Haddad and Stevanovic [14]. The extract (100 mg/ml) was mixed with 3 ml reagents containing 0.6 M H_2SO_4, 28 mM sodium phosphate, and 4 mM ammonium molybdate and incubated at 95 °C for 90 min. in a water bath. The absorbance was recorded at 695 nm. Ascorbic acid (0.1 mg/ml in distilled water) was used as a standard, and values were expressed as mg/g.

Determination of Total Phenolic Content: Total polyphenol content (TPC) of algal extracts was determined spectrophotometrically using Folin Ciocalteu reagent according to the strategy

described by Dinesha *et al.* [15]. Extract (0.5 ml) was diluted to 3 ml with distilled water and mixed with 0.5 ml of folin ciocalteu reagent. After three min 2 ml of 20% sodium carbonate were added and the contents were altogether blended. Then it was placed in a boiling water bath for exactly one min., cooled, and afterward, absorbance was recorded at 650 nm against a reagent blank. The total amount of phenols was calculated based on a standard curve of catechol and expressed in mg/100gm of dry weight.

RESULTS: The weights of solvent extracts obtained from seaweeds *Sargassum wightii*, *Turbinaria ornata*, *Gracilaria corticata,* and *Gelidiella acerosa* were tabulated in **Table 1**. Anti-oxidant potential of methanol extract of seaweed were determined using a different method as DPPH radical scavenging assay, Ferric reducing antioxidant property (FRAP), Ferrous Ion Chelating Activity (FICA), Reducing power, Total Anti-oxidant Capacity (TAC) along with total Phenolic content were represented in **Table 2**.

Similarly, anti-oxidant potential of ethanol extract and hexane extracts of seaweed were tabulated in **Tables 3** and **4**, respectively.

TABLE 1: THE WEIGHT (IN GRAM) OF SOLVENT EXTRACTS OBTAINED FROM THE FOUR SEAWEEDS

S. no	Solvents	*Sargassum wightii* (g)	*Turbinaria ornata* (g)	*Gracilaria corticata*(g)	*Gelidiella acerosa* (g)
1.	Methanol	0.85	0.70	0.67	0.65
2.	Ethanol	0.67	0.69	0.82	0.73
3.	Hexane	1.15	0.96	0.80	0.75

TABLE 2: ANTI-OXIDANT PROPERTIES OF SEAWEEDS IN METHANOL EXTRACT

S. 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.	*Sargassum wightii* (g)	75.51± 0.10	0.941± 0.006	1.021 ± 0.002	51.01 ± 0.10	99.13 ± 0.21	3.60 ± 0.005
2.	*Turbinaria ornata* (g)	83.80± 0.10	0.843± 0.004	0.766 ± 0.005	47.30 ± 0.10	105.78 ± 0.032	3.91 ± 0.032
3.	*Gracilaria corticata*	52.41± 0.11	1.046±0.001	0.662 ± 0.001	46.20 ± 0.10	95.44 ± 0.52	2.77 ± 0.005
4.	*Gelidiella acerosa*	54.46± 0.63	0.811± 0.002	0.73 ± 0.002	47.20 ± 0.15	91.81 ± 0.38	3.41 ± 0.02

Values represent mean of three observations with standard deviation (SD) ±

TABLE 3: ANTI-OXIDANT PROPERTIES OF SEAWEEDS IN ETHANOL EXTRACT

S. 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	*Sargassum wightii* (g)	70.16 ± 0.15	0.531± 0.001	0.57 ± 0.00051	62.96 ± 0.30	75.85± 0.27	2.23 ± 0.005
2	*Turbinaria ornata* (g)	71.07 ± 0.10	0.616± 0.001	0.41 ± 0.0001	66.86 ± 0.20	74.18 ± 0.5	2.51 ± 0.001
3	*Gracilaria corticata*	79.07 ± 0.10	0.733± 0.005	0.245 ± 0.001	77.13 ± 0.35	79.23 ± 0.40	1.75 ± 0.001
4	*Gelidiella acerosa*	66.11 ± 0.15	0.785±0.0010	0.149 ± 0.005	76.20 ± 0.55	75.01 ± 0.35	1.89 ± 0.001

Values represent mean of three observations with standard deviation (SD) ±

TABLE 4: ANTI-OXIDANT PROPERTIES OF SEAWEEDS IN HEXANE EXTRACT

S. 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	*Sargassum wightii* (g)	36.66 ± 0.15	0.583± 0.001	0.454± 0.0015	28.70± 0.10	45.10 ± 0.005	2.05 ± 0.001
2	*Turbinaria ornata* (g)	46.17 ± 0.10	0.753 ± 0.005	0.466 ± 0.001	52.53 ± 0.35	39.23 ± 0.001	1.94 ± 0.00
3	*Gracilaria corticata*	37.54 ± 0.15	0.68 ± 0.003	0.108 ± 0.0005	53.10 ± 0.36	52.97 ± 0.28	1.53 ± 0.00
4	*Gelidiella acerosa*	31.39 ± 0.15	0.55 ± 0.0032	0.321 ± 0.0005	46.53 ± 0.58	58.17± 0.86	1.64 ± 0.005

Values represent mean of three observations with standard deviation (SD) ±

DPPH Radical Scavenging Activity: DPPH assay is used to test the ability of the anti-oxidant compounds present in the seaweed extract to function as radical proton scavengers or hydrogen donors. In the current study, the anti-oxidant activities of four seaweed extracts were evaluated. DPPH activity in methanol extract is as *Turbinaria ornate* > *Sargassum wightii* > *Gelidiella acerosa* > *Gracilaria corticata* and in the event of ethanol extract it as *Gracilaria corticata* > *Turbinaria ornate* > *Sargassum wightii* > *Gelidiella acerosa* and slightly lower activity observed in hexane extract. All the four seaweeds in methanol extract showed the highest DPPH activity, more than 50%, and slightly less activity in ethanol extract. In the present study, it was intriguing to take note that ethanol extract of *Gracilaria corticata*, and *Gelidiella acerosa* shows more DPPH activity than methanol extract. The results indicated that all the tested seaweeds in this investigation possessed anti-oxidant activity and were depicted in **Fig. 1**.

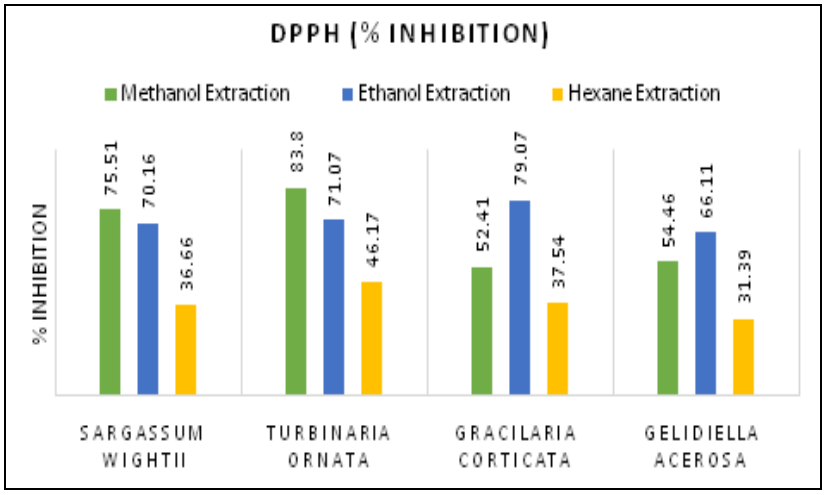

FIG. 1: DPPH RADICAL SCAVENGING ACTIVITY (% INHIBITION)

Ferric Reducing Antioxidant Property (FRAP): The FRAP assay uncovered a maximum anti-oxidant activity in all the four seaweed methanol extract, and it is most noteworthy in red algae *Gracilaria corticata* seaweeds (1.046 mg/g). Ethanol and hexane extract also shows anti-oxidant activity of more than 50%, and ethanol extract of red algae *Gelidiella acerosa* shows a maximum (0.785 mg/g). The outcome of the FRAP assay was delineated in **Fig. 2**.

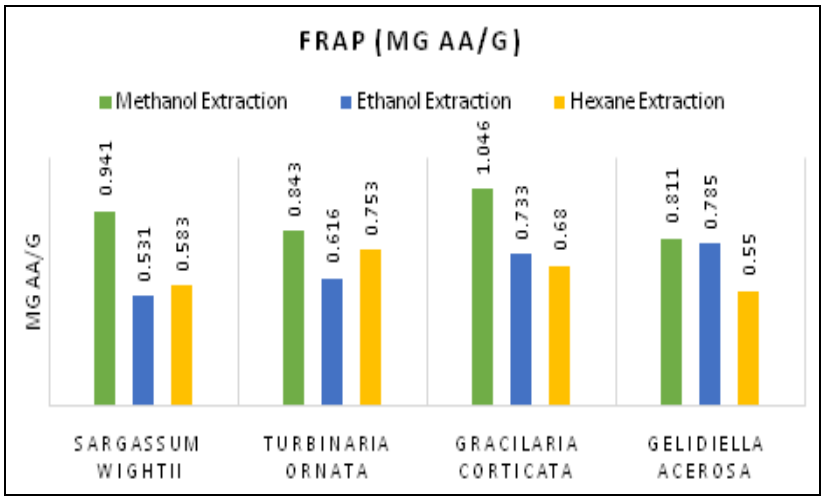

FIG. 2: FERRIC REDUCING ANTIOXIDANT PROPERTY (FRAP)

Reducing Power: Reducing power was high in methanol extract, and it shifts from 0.66 to 1.021 mg/g. Reducing power in different seaweed methanol extract is as *Sargassum wightii*> *Turbinaria ornate* > *Gelidiella acerosa*>*Gracilaria corticata* **Fig. 3**. The ethanol and hexane extract

has less reducing power as contrast with methanol extract. The reducing property demonstrated that the anti-oxidant compounds are electron donors and can reduce the oxidized intermediates of the lipid peroxidation process. So that they can act as primary and secondary anti-oxidants. The reducing power of methanol, ethanol and hexane extract(s) from different seaweeds were depicted in **Fig.3**.

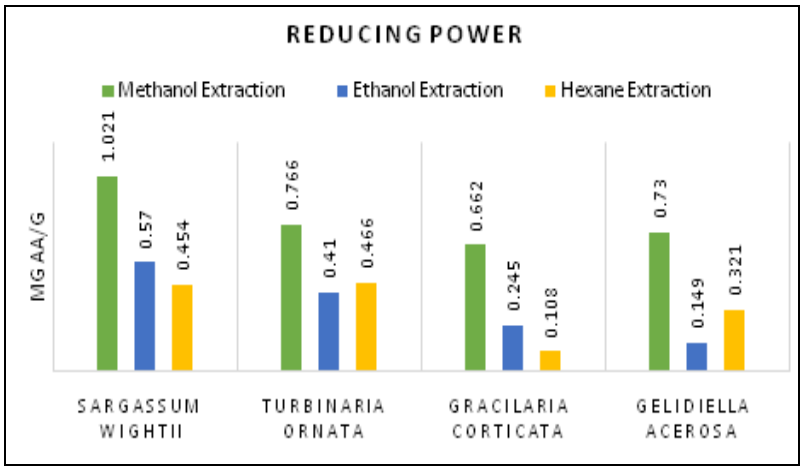

FIG. 3: REDUCING POWER OF DIFFERENT SOLVENT EXTRACTS FROM FOUR SEAWEEDS

Ferrous Ion Chelating Ability (FICA): Metal chelating ability of seaweed extract was tried. In ethanol extract FICA % is (62.96 to 77.13%), in methanol extract it is (46 to 51%) and in hexane extract it is (28.7 to 53%). The FICA was highest in ethanol extract for all seaweeds and was all together as *Gracilaria corticata* > *Gelidiella acerosa* > *Turbinaria ornate* > *Sargassum wightii*. The ethanol extracted seaweed demonstrated a higher ferrous ion chelating ability than the methanol and hexane extract. An extract with higher binding ability would inhibit or repress reaction such as Fenton type reaction, which generates reactive hydroxyl radicals. The outcomes of the ferrous ion chelating ability of different solvent extracts from collected seaweed (s) were delineated in **Fig. 4**.

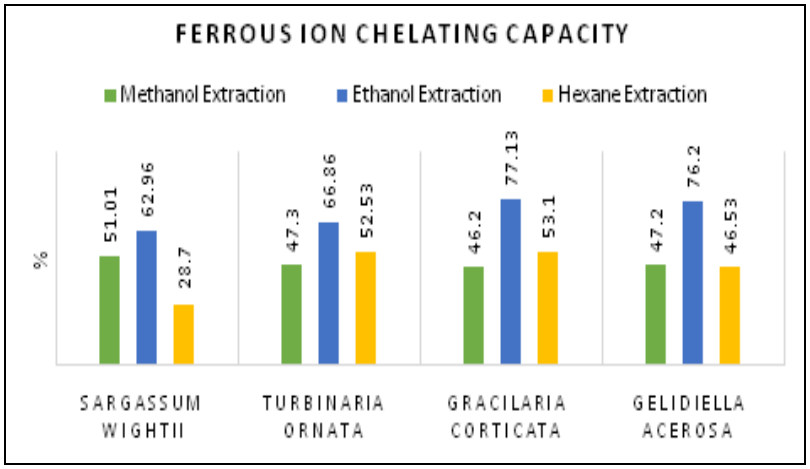

FIG. 4: FERROUS ION CHELATING ABILITY OF DIFFERENT SOLVENT EXTRACTS FROM FOUR SEAWEEDS

Total Anti-oxidant Activity (TAC): The total anti-oxidant capacity was high in methanol extract (91.81 to 105.78 mg/g), ethanol extract has the less anti-oxidant capacity (74.18 to 79.23 mg/g) compared to methanol extract, yet it is more than hexane extract (39.23 to 58.17 mg/g). Hexane extracts show the lowest anti-oxidant capacity in all three extracts. The maximum value was scored in methanol extract of brown algae *Turbinaria ornate* and *Sargassum wightii* (105.78 mg/g and 99.13 mg/g, respectively). The results of total anti-oxidant activity were plot in **Fig. 5**.

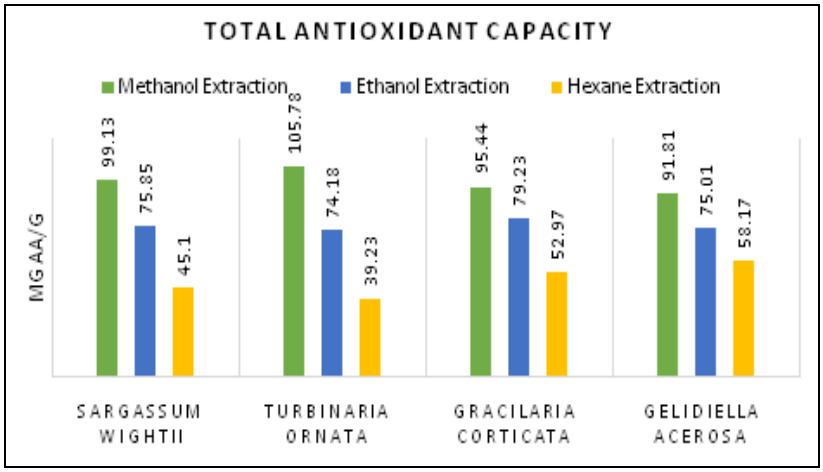

FIG. 5: TOTAL ANTI-OXIDANT CAPACITY OF DIFFERENT SOLVENT EXTRACT(S) OF *SARGASSUM WIGHTII, TURBINARIA ORNATA, GRACILARIA CORTICATA,* AND *GELIDIELLA ACEROSA*

Determination of Total Phenolic Content: In the present study, total phenolic content was discovered higher in methanol extracts than in ethanol extract and was tabulated in **Table 2** and **Table 3**. Maximum phenols were present in the methanol extract of *Turbinaria ornate* (3.91 mg/g) and *Sargassum wightii* (3.6 mg/g). *Turbinaria ornatadis* played higher activity than *Sargassum wightii* and *Gelidiella acerosa*. The total phenolic content of different solvent extract(s) of *Sargassum wightii*, *Turbinaria ornata*, *Gracilaria co*rticata, and *Gelidiella acerosa* was depicted in **Fig. 6**.

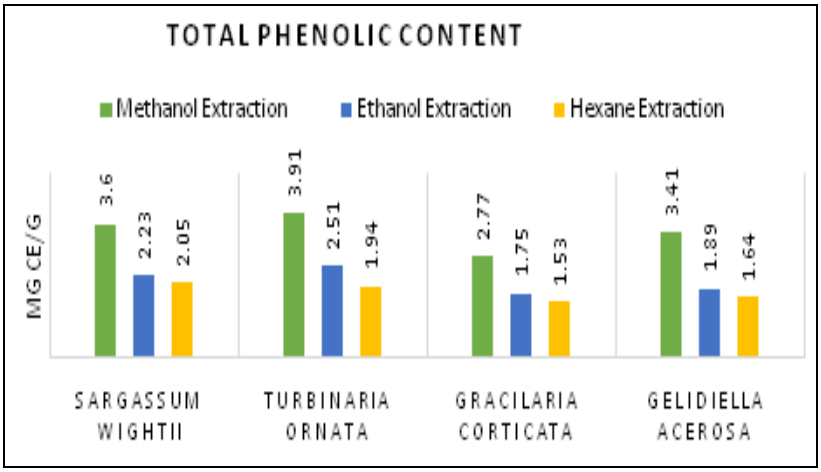

FIG. 6: TOTAL PHENOLIC CONTENT OF DIFFERENT SOLVENT EXTRACT(S) OF *SARGASSUM WIGHTII, TURBINARIA ORNATA, GRACILARIA CORTICATA* AND *GELIDIELLA ACEROSA*

DISCUSSION: The DPPH radical scavenging activity in methanol extract of brown seaweed *Turbinaria ornata* (83.8%) was most noteworthy among all extracts. The DPPH activity in *Gracilaria corticata* was uncovered to 79.07% in ethanol extract. In *Sargassum wightii* brown seaweed, high activity in methanol and ethanol extracts (75.51% and 70.16%) respectively were observed. In ethanol extract of *Sargassum wightii*, *Turbinaria ornata*, and *Gelidiella acerosa*, the DPPH activity was low to moderate. The FRAP assay displayed the greatest anti-oxidant activity in red algae *Gracilaria corticata* seaweeds (1.046 mg/g). The most elevated reducing power activity in methanol extract of *Sargassum wightii* than in ethanol extract. The higher ferrous ion chelating ability was appeared by ethanol extracted seaweed than the methanol and hexane extract. Total anti-oxidant activity is highest in methanol extract of *Turbinaria ornate* (105.78 mg/g). In the present study, methanol extraction of seaweed was found to favor the anti-oxidant activity.

CONCLUSION: DPPH, FICA, and TAC were good in all the seaweed extract. Most of the seaweed extract showed prominent results by

DPPH activity and was maximum in methanol extracted seaweeds as compared to ethanol and hexane extracts. The anti-oxidant activities of the extracts of *Sargassum wightii, Turbinaria ornata, Gracilaria corticata,* and *Gelidiella acerosa*, were evaluated. The outcomes plainly showed that all the tested seaweeds in this investigation possess anti-oxidant activity. The methanol extract of *Turbinaria ornata* demonstrated greater anti-oxidant activity (105.78) and high phenolic content (3.91). Positive and significant correlations between DPPH radical scavenging activity and phenolic content displayed that phenols are the primary contributors to anti-oxidant activity in these seaweed extracts.

The results recommend that seaweeds possess anti-oxidant potential, which could be considered for future applications in the food and pharmaceutical industry. Future researches should be a focus on the effects of anti-oxidant activities of seaweed extracts on anti-oxidant enhancement.

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CONFLICTS OF INTEREST: The author(s) declare that there is no conflict of interest.

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