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ORIGINAL ARTICLE

Antioxidant Potentials & Total phenolic content of brown and red seaweeds harvested from the Sindhudurga Coastal area, Maharashtra India

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ABSTRACT
Evaluation of natural compound obtained from phytochemical screening provides keyidea for investigating new drug. Corallina officinalis and Sargassum ilicifolium red &brown seaweedshave reported to exhibit different biological activities such as anticancer, antimicrobial, antidiabetic, anti-inflammatory, and antioxidant etc. This study intent to explore the antioxidant activity of crude extracts of the seaweed. Ethenolic and Acetone extracts of two seaweeds, collected from Sindhudurga coastal area, Maharashtra, India were evaluated using various in vitro methods. DPPH scavenging method & Hydrogen peroxide scavenging assay showed significant antioxidant activity. Folin–Ciocalteu method was used to determine the total phenolic content of the extracts/fractions, and the results were expressed as mg of gallic acid equivalent (GAE)/g of the seaweed extracts. Since these alga being used as food during ancient time we may conclude that these macroalgae could be further developed as food additives so as to reduce illness naturally and the extracts can be used to hostities various disorders.
Keywords: *Antioxidant activity, Red seaweeds, Corallina,Sargassum, DPPH Assay*

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INTRODUCTION

Since macroalgae have attracted attention being natural reservoir of pharmaceutically active molecule with numerous applications in an industrial field such as textile, biomedical, cosmetic, food and Pharmaceutical. Algae have been vital part of human civilization since ancient time. Traditionally algae have been part of the cuisine in East Asia, especially Japan, Korea, the Philippines, Vietnam, Taiwan & China[2].Marine floras have been used for medicinal purpose in India, China, and Near East & Europe since ancient times. Having rich in antioxidants alga has gained so much attention and intended towards the development of ethno medicines due to having constituents such as phenols, flavonoids, alkaloids, tannins, vitamins, terpenoids and many more phytochemicals responsible for different pharmacological activities[3].

Occupying almost 71% of globe, the ocean is rich in biodiversity and the microflora and microalgae alone constitute more than 90% of oceanic biomass. [4].This vast marine floral resource will offer a great scope for discovery of new drugs. It is increasingly recognized that ocean contains a huge number of natural products and novel chemical entities with unique biological activities that may be useful in finding the potential drugs with greater efficacy and specificity for the treatment of human diseases.[14, 15].This vast marine floral resource will offer a great scope for discovery of new drugs. Marine floras have been used for medicinal purposes in India, China, the Near East and Europe, since ancient times. The people of China and Japan have been using seaweeds for consumption. The seaweeds especially brown seaweeds are rich in iodine and hence there is a least incidence of goiter and glandular diseases. [6]. History reveals that

many countries have been using seaweeds as vermifuge, anesthetics and ointment as well as for the treatment of cough, wounds, gout, goiter, venereal disease, and so forth. Seaweed dietary fibers perform varied range of functions such as antioxidant, ant mutagenic, anticoagulant, and antitumor. [7].

The roles of free radicals in the biology become an area of interest. It is generally accepted that radicals play an important role in the development of tissue damage and pathological events in the living organism. Free radicals are defined as an atoms or molecules that Contain one or more unpaired electrons. making them unstable and highly reactive most important ROS are the superoxide anion radical O',Hydrogen peroxide(alkoxyl (RO), peroxyl (ROO) [8]. hydroxyl radical (OH) and hypochlorus acid (HOCI), Other nonoxygen reactive species exist such as reactive nitrogen species (RNS), such as nitric oxide (NO) [9]. A number of major cellular antioxidant defense mechanism exist to neutralize the damaging effects of free radicals, Enzymatic antioxidant system Super-oxide dismutase (SOD). catalase, glutathione (GSH). peroxidase (GPX) and GSH reductase function by direct or sequential removal of ROS, thereby terminating their activities [11].

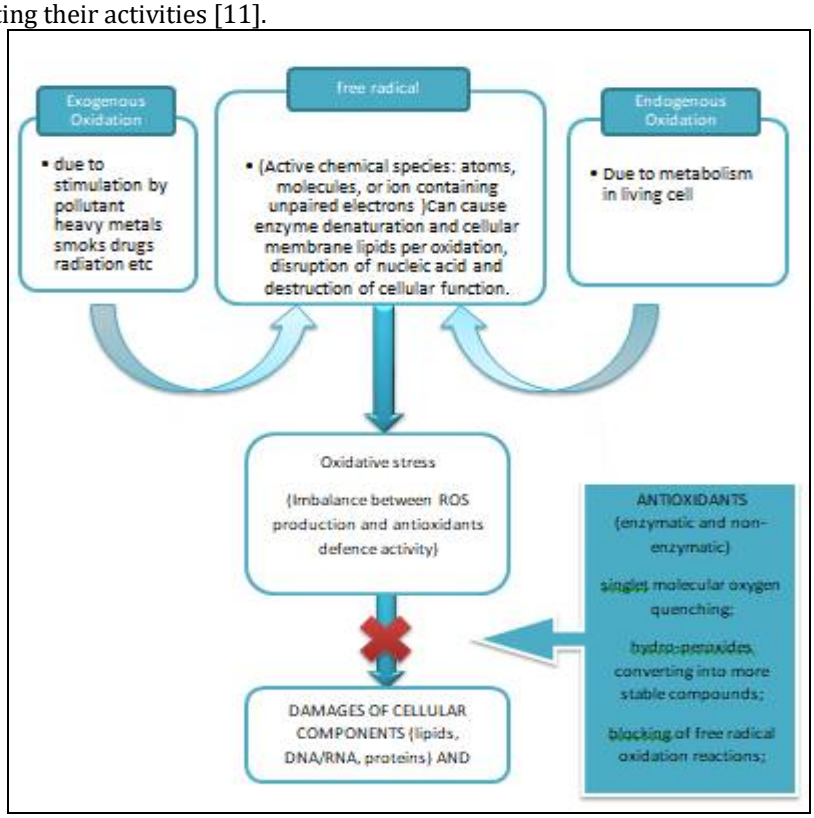


Fig.1. Oxidation process & effect of oxidation

MATERIAL AND METHODS

Seaweed material

Red and Brown seaweeds used in this study were *Corallina officinalis. (*Linnaeus) (Family: Corallinaceae, Order: Corallinales), Sargassum *ilicifolium. (*Turner) (Family: Sargassaceae , Order: Fucales respectively [1].Algae were freshly collected from the Sindhudurga coastal area, Maharashtra India (Fig. 2). Samples collected were washed in running water for 10 min, transported to the laboratory and shade dried (35 ± 3 °C) for 36 h. The shade dried seaweeds were powdered and used for further experiments.

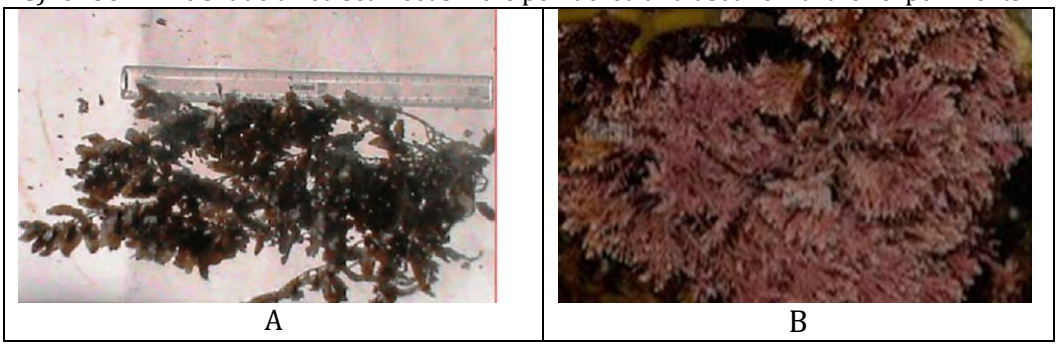


Fig. 2. *A) Sargassum sp.B) Corallina sp.*

Preparation of seaweed extracts

The powdered seaweed samples (100 g) were extracted with ethanol as well acetone using soxhlet extraction.The extract was concentrated using Rotary evaporator ABC/INC Biomedica BMI776,Indiaand then it was subjected for total phenolic content determination by the yellow Folin-Ciocalteu reagent and Antioxidant activity[31].

Chemicals and reagents

All solvents used were of analytical grade. 1,1-dipheny1–2-picrylhydrazyl (DPPH·), 2-thiobarbituric acid (TBA), trichloroacetic acid (TCA), 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-4′,4′-disulfonic acid sodium salt (ferrozine), Folin-Ciocalteu reagent, ABTS (2,2′-azino-bis-(3-ethylbenzothiozoline-6-sulfonic acid diammonium salt), gallic acid, acetyl acetone, ammonium acetate, ascorbic acid, ethylene diaminetetra acetic acid (EDTA), ferrous ammonium sulfate, $FeCl_3$, potassium ferricyanide, and $FeCl_2$ were purchased from New Neeta Chemicals,Pune.

Estimation of phenolic content

Folin– Ciocalteu reagent according to (Singleton & Rossi, 1965) method was used to evaluate the amount of total phenols in seaweeds extracts. The developed color was read at 760 nm with Gallic acid stock solution (10 mg/10 ml) as standard. The results were expressed as milligram Gallic acid equivalent (mg GA/g dry weight of seaweed). [12]. Brieflyvarious concentrations 2-10ug/ml of gallic acid were prepared in triplicate from stock solution and 1 ml was transferred in each test tube.The volume was made 3 ml with distilled water 0.5 ml of Folin– Ciocalteu reagent was added to test tubes containing standard solution and test solutions of acetone and Ethenolic extract respectively. The solutions were mixed thoroughly and absorbance was measured at 760 nm with reagent blank after an hour. The concentrations of total phenolic compounds in each extract were determined as milligram of gallic acid equivalent (mg GAE)/g of the extracts by using the regression equation from the calibration curve of the gallic acid standard. All determinations were performed in triplicate[13].

Table 1.Total phenolic contents of *Sargassum*&*Corallina* extracted in different solvent at different concentrations.

Concentration (ug/ml)	Phenolic content mg/GAE/ml of Extract *Sargassum*		Phenolic content mg/GAE/ml of Extract *Corallina*	
	Acetone	Ethanol	Acetone	Ethanol
2	0.149 ±0.001	0.195 ±0.001	0.123 ±0.001	0.157 ±0.001
4	0.224 ±0.002	0.258 ± 0.002	0.201 ±0.002	0.265 ±0.002
6	0.307 ±0.002	0.324±0.002	0.300 ±0.002	0.384±0.002
8	0.372 ±0.003	0.410±0.003	0.364 ±0.003	0.435±0.003
10	0.454 ± 0.002	0.518±0.001	0.432 ±0.002	0.542±0.001

Expressed Data has mean ± SD, n=3. Means on the same column with different superscripts are significantly different at ($p < 0.05$)

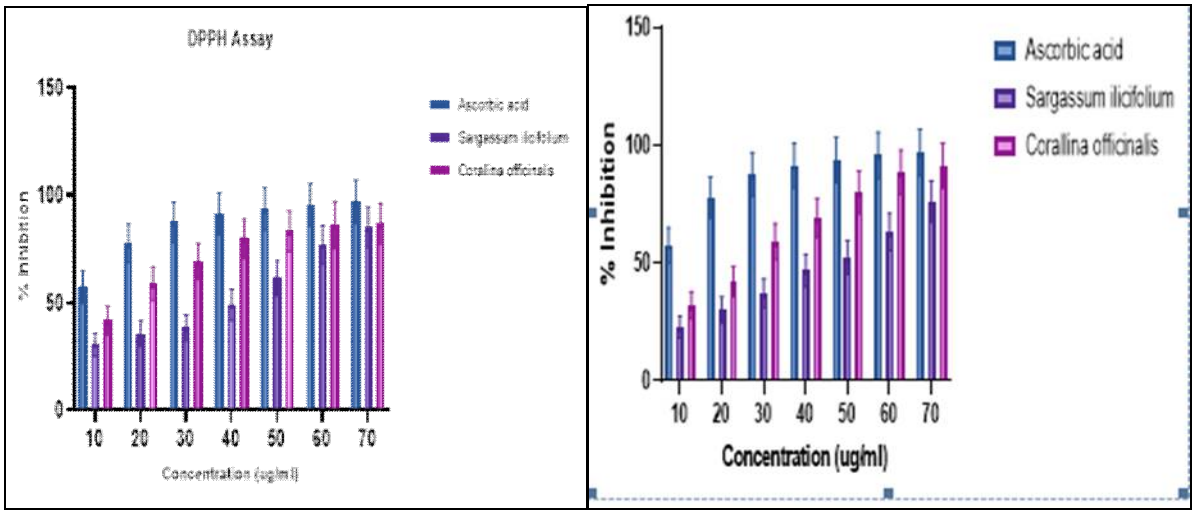
Antioxidant activity

DPPH Assay (1, 1-Diphenyl-2-picryl- Hydrazil radical scavenging activity)

Stable radical, DPPH·, as a standard reagent is being used to determine the antioxidant activity of extracts using the method described earlier by (Lim etal. 2007) with suitable modifications. Briefly, stock solutions of the extracts were prepared in EtOH and Acetone. Dilutions were made to obtain concentration ranging from 0.1 to 1.0 mg/ml. Diluted solutions (2.0 mL) were mixed with 2.0 mL of 0.16 mM DPPH in EtOH. The mixtures were shaken vigorously and maintained for 30 min at circling temperature (30 °C) in the dark. The absorbance of mixtures was measured at 517 nm against a reagent blank by using a UV–Vis spectrophotometer.[16,17] The absorbance of the control (2 mL DPPH solution + 2 ML EtOH) and (2 ml DPPH solution + 2 mlAcetone) the samples were measured spectrophotometrically. The experiment was performed in triplicate. The scavenging activity can be calculated by using the following equation [19].

DPPH · scavenging activity (%) = (A517 of control − A517 of sample) × 100/A517 of control.

The plot of scavenging activity on DPPH· was recorded (concentration of the sample to scavenge 50 % of the DPPH radicals; ug/ ml) was then calculated.

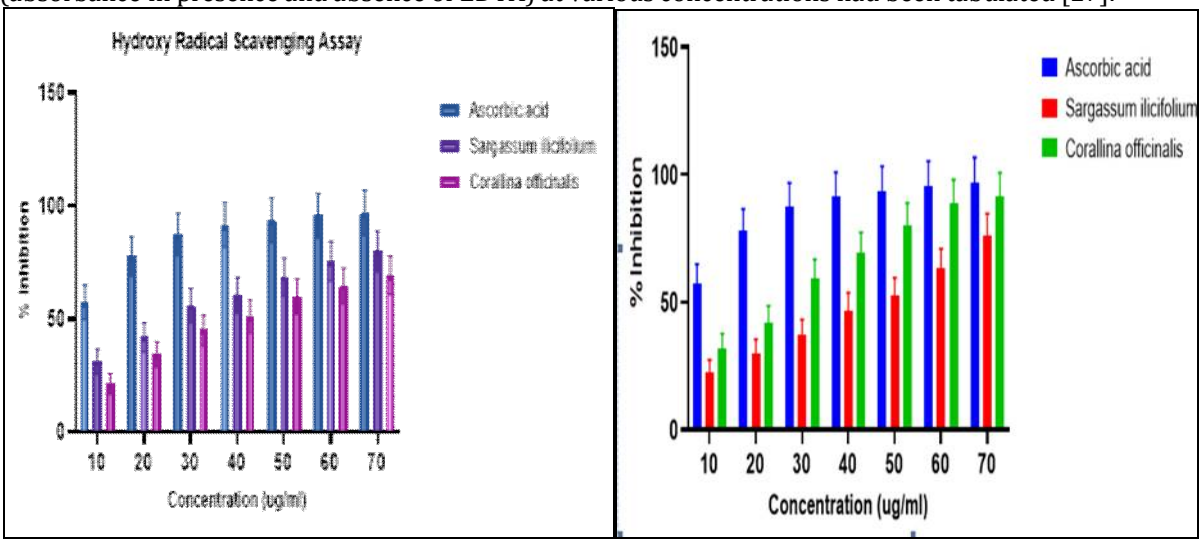


Acetone extract **Ethanol extract**

Fig 1: DPPH Assay

Hydroxyl Radical Scavenging Activity

The hydrogen peroxide radical scavenging activity was determined using the method described by Aruoma and Halliwell [26]. The deoxyribose method was used to determine the hydroxyl radicals trapping capacity of algal extract, as per standard method. In this procedure FeCl3-EDTA-ascorbic acid was used to evolve OH radicals, as detailed below. It was performed in 2 conditions, in presence of EDTA, (non-site-specific) to determine its OH trapping capacity and in absence of EDTA (site specific) to assess its metal chelation property. This protocol was performed to check the effect of algal extract on hydroxyl (OH.) radical's trapping potential. Different concentrations (10–70 μg/mL) of extract were added to the reaction mixture in a final volume of 1 mL in potassium phosphate buffer (10 mM, pH 7.4). It was incubated at 37◦ C for 1 h and then mixed with 1 mL of 2.8% TCA (w/v in water) and 1 mL of 1% thiobarbituric acid (TBA) (w/v). It was then heated in a boiling water bath for 15 min and cooled and absorbance was taken at 532 nm. In an experiment, thiourea was taken as positive control. Thiourea was used as standard as hydroxyl radical scavenger [21].To assess the metal chelation property of plant extract the above experiment was repeated in absence of EDTA. The difference between 2 readings (absorbance in presence and absence of EDTA) at various concentrations had been tabulated [27].



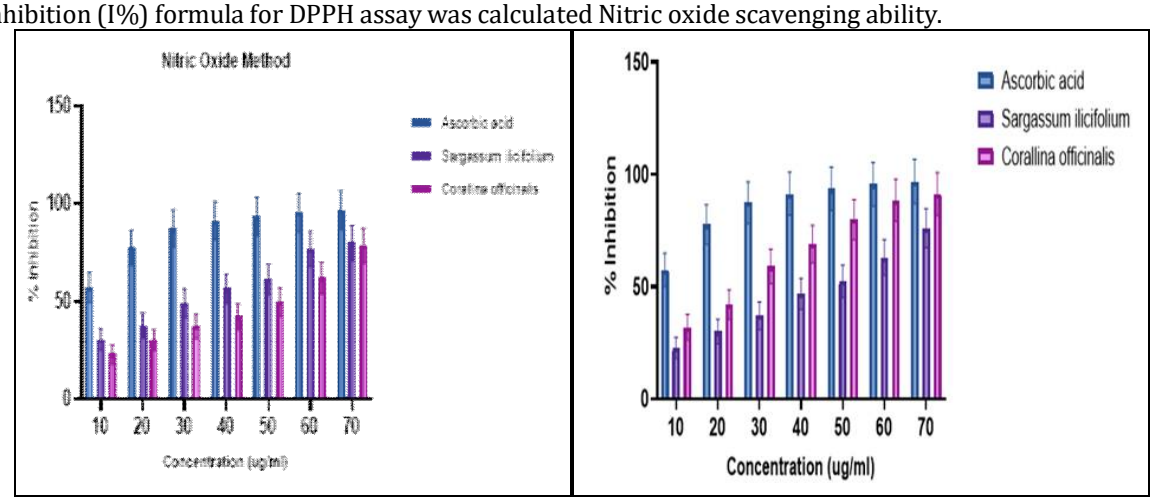
Acetone extract **Ethanol extract**

Fig 2: Hydroxyl Radical Scavenging Activity

Nitric Oxide Scavenging Ability

The (SNP) aqueous sodium nitroprusside solution produced from Nitric oxide interacts with oxygen to produce nitrite ions, which may be determined according to Griess Illosvoy reaction [30]. It consistsof 10 mM SNP in 0.5 M phosphate buffer (pH 7.4) and various concentrations (100–1000 μg/mL) of the algal extract in a final volume of 3 mL. upon incubation for 60 min at 37◦ C, 0.1% α-napthyl-ethylenediamine in

water and 1% sulphanilic acid in 5% H3PO4was added (Griess reagent.) The chromophore (pink) generated during nitrite ions diazotization with sulfanilamide and subsequent coupling with *α*-napthylethylene diamine were measured at 540 nm. positive control was used as Ascorbic acid. percent inhibition (I%) formula for DPPH assay was calculated Nitric oxide scavenging ability.

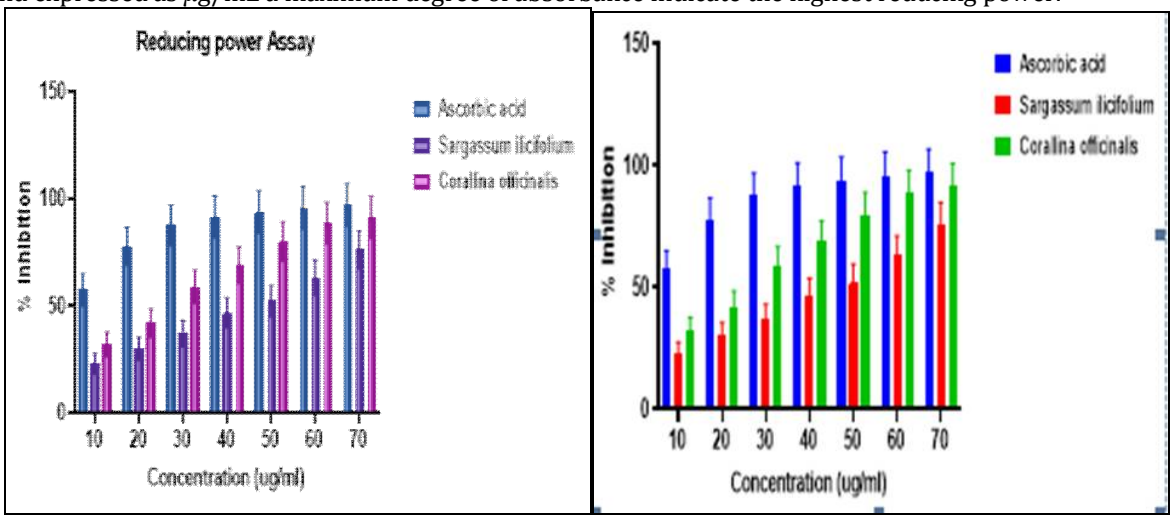


Acetone extract **Ethanol extract**

Fig 3: Nitric Oxide Scavenging Ability

Reducing Power Assay

The Reducing Power Assay determined the reducing capacity by transformation of Fe^{3+}- Fe^{2+}in presence of algal extractas per the developed method [18]. In a test protocol 2.5 mL solution of extract (10–70ug/mL) was mixed with equal volume of phosphate buffer (0.2 M, pH 6.6) and 1% potassium ferricyanide and kept in water bath at 50◦ C for 20 min. Thereafter it was cooled rapidly and 2.5 mL of 10% trichloroacetic acid was mixed and vortexed. It was centrifuged at 3,000 rpm for 10 min and its 5 mL supernatant was mixed with equal volume of distilled water and 1 mL of 0.1% ferric chloride. Andincubated at room temperature for 10 min absorbance was taken at 7oo nm. The reducing property of algal extract was standardized against ascorbic acid and expressed as difference in optical density (OD) from control as well as test as 0.1 and expressed as *μ*g/mL a maximum degree of absorbance indicate the highest reducing power.



Acetone extract **Ethanol extract**

Fig4: Reducing Power Assay

RESULTS

The antioxidant activity of Ethanolic extract was found to be more potent compared with the Acetone extract of both the seaweeds. The yields of the Ethanolic extracts, of both the seaweed exhibited higher (9 g/100 g,8.5 g/100 g dry sample) followed by Acetone extract (4.8 g/100 g&; 5.2 g/100 g dry sample, respectively). Antioxidant activity using Nitric oxide has higher result for Sargassum compared with standard ascorbic acid. It's mainly attributed toward the fact that sulfated polysaccharides are abundant in brown algae.Presentlyin this study, the total phenolic content of Sargassum & Corallina extracted in different solvents are presented in Table-1. In Acetone extract, TPC varied from 0.123 to 0.454 mg GAE/ml

of *Sargassum*and *Corallina* respectively while Ethenolic extract have showed increased TPCThis study reveals fact that there is significantly increase in the yields of phenolic content with increase in the polarity of solvents.

DISCUSSION

Seaweeds are traditionally being used by tribal communities as food and natural medicine. [7]. Now upon understanding algal constituents are considered as first line of defense against ROS and SOD, antioxidantsare under major consideration. Their daily intake protects the immune system and slows down the age retardation. Peptides, amino acids, polyphenols, terpenoids, etc., aresome antioxidants already in use in the cosmetics, pharmacy, and food industries. The work is being extensively carried on investigation of antioxidant substances derived from marine-seaweed *Sargassum* & *Corallina*[20,32].Current work enhances knowledge of the potential uses of seaweeds from Sindhudurga west coast, because such information is lacking for this geographical region. Thailand, and the Philippines, are the leading producers and consumers of edible seaweeds that contain these antioxidants in high amounts [8]. Our results shows that these seaweeds could bee xplore as good natural antioxidant for enhancing the nutraceutical products as well as promising alternate in pharmaceuticals .[23]

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CONFLICT OF INTEREST DECLARATION: None

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