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Antioxidant activity and total phenolic contents of selected seaweeds from Okha coast of Gujarat

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Abstract

India has 7,500 km of coastline bounded to the south by the Indian Ocean to the west by the Arabian Sea to the east by the Bay of Bengal. Gujarat covers nearly 1600 km of coastal area. The marine macroalgae are a diverse group of organisms. In the present study, a total of 69 species of green, red, and brown seaweed were collected at the Okha coast (22° 28' 1 N, 69° 04' 44 E). The methanolic extracts were prepared from the collected seaweeds. Further, methanolic extracts of the seaweeds were assessed for their antioxidant activity using the DPPH radical scavenging assay using the microplate reader. Among all the selected seaweed *Hypnea musciformis* has the highest antioxidant activity compared to others. Folin–Ciocalteu method was used to determine the total phenolic content. *Gelidium coulteri* had content higher amount of phenolic content compared to other seaweeds. The results suggest that selected seaweeds possess antioxidant potential and phenolic content which could be considered for future applications in pharmaceutical, cosmetics and food industries.

Keywords: Seaweeds, Okha coast, antioxidant, phenolic content

Introduction

India has 7,500 km of coastline bounded to the south by the Indian Ocean to the west by the Arabian Sea to the east by the Bay of Bengal. The marine system with diverse habitats and rich biota is being studied by the number of workers for their tremendous economic uses (Rotter *et al.*, 2021) [1]. According to the report of the Food and Agriculture Organization of United Nations (2022), approximately 30-35 million metric tons of seaweed are cultivated and harvested annually worldwide. Seaweeds flourish abundantly along the Indian coastline, particularly in rocky shore regions. They create lush beds spanning from Visakhapatnam on the eastern coast to Mahabalipuram, the Gulf of Mannar, Tiruchendur, Tuticorin, and Kerala on the southern coast. Additionally, they thrive in Veraval and the Gulf of Kutch on the western coast, as well as in the Andaman and Nicobar Islands and Lakshadweep (Vijay *et al.*, 2017) [2]. Earlier Oza and Zaidi (2011) [3] reported a total of 841 species of seaweeds along the Indian coast of which 216 species of Chlorophyta, 191 species of Phaeophyta, and 434 species of Rhodophyta. Among the Indian maritime states, Gujarat state has the longest coastline extending up to 1600 km. Jha *et al.*, (2009) [4] reported 198 species of seaweed along the Gujarat coast of which 109 species of Rhodophyta, 54 species from the Chlorophyta, and 35 species belonging to Phaeophyta.

Seaweeds are rich sources of nutrients and natural bioactive compounds, such as carotenoids, dietary fibers, proteins, essential fatty acids, vitamins, and minerals (Xu *et al.*, 2023) [5]. These compounds are interesting for the pharmaceutical, medical, cosmetic, nutraceutical, food, and agricultural industries (Hughes *et al.* 2018; Lozano Muñoz and Díaz. 2022) [6, 7]. Bioactive compounds from seaweeds possess a wide assortment of pharmacological properties, like anticancer, antibacterial, antifungal, antiviral, anti-inflammatory, anticoagulant, antioxidant, bone regenerating, hepatoprotective and neuroprotective properties (Lomartire and Gonçalves, 2022) [8]. The antioxidant property of seaweeds is due to the phenolic compounds. Phenolic compounds or polyphenols exhibit various physiological properties, including antioxidant, antimutagenic, antitumor, and anticancer activities (Kumar *et al.*, 2014) [9]. Many studies have shown that a high dietary intake of natural phenolics is strongly associated with longer life expectancy, reduced risk of

developing some chronic diseases, various types of cancer, diabetes, obesity, improved endothelial function, and reduced blood pressure (Rahman *et al.*, 2022) ^[10]. Seaweeds play the role of free radical scavengers; they prevent or repair damages caused by oxidative stress and have a high potential for the treatment of various diseases (Liu and Sun, 2020) ^[11]. The antioxidant characteristics of these seaweeds have been associated with their phenolic composition. Considering this, in the present study 69 seaweed species belonging to Chlorophyta, Phaeophyta, and Rhodophyta were collected from Okha Coast to assess their antioxidant activity and estimate phenolic content. The antioxidant potential and phenolic content of extracts from these seaweeds hold significant potential for applications in the food and pharmaceutical industries.

Methods and Materials

Collection of Seaweed

Seaweeds were collected from Okha Coast (22° 28' 45"N, 69° 04' 42"E) Saurashtra from November to March (2023-2024). A total of 69 algae samples were collected from Chlorophyta, Phaeophyta, and Rhodophyta (Plate 1-4). The algae were cleaned, washed in distilled water, air dried at room temperature, powdered, and stored.

Extraction of phenolic compound from the seaweeds

The 500 mg of dry seaweed powder was taken and crushed with 10 ml of 80% Methanol. The mixer was kept under dark for 48 h on shaking condition. The next day the extracts were centrifuged at 5000 g for 15 min. The supernatant was collected in a Petri plate and allowed for evaporation. The remaining dry residue was dissolved in sterile distilled water (5 ml) and used as a source of phenols. The methanol extract was used as an antioxidant measurement.

Total Phenolic content

The concentration of total phenols was determined according to Swain and Hills (1959) ^[12]. The reaction mixture contained 100 µl of extract, 10 µl of 1N Folin-Ciocalteu's reagent and 200 µl of saturated sodium carbonate. The reaction mixture was incubated at room temperature for 5 minutes. The absorbance was measured at 660 nm using Biotek microplate reader µQuant. Measurements were carried out in triplicate and phenol content was calculated using a calibration curve of chlorogenic acid (100-1000 µg/ml).

DPPH free radical scavenging assay

Radical scavenging assay was determined according to Madhar *et al.*, (2023) ^[13]. DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging assay is a stable free radical and is widely used to assess the radical scavenging activity of antioxidant compounds. This method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen-donating antioxidant due to the formation of the nonradical form DPPH-H by the reaction. This transformation results in a color change from purple to yellow, which is measured on a microplate reader (µ Quant, Bio Teak, USA). The disappearance of the purple color is monitored at 517 nm. The reaction mixture (200 µl) consists of 100 µl of DPPH (1 mM) and 100 µl methanol extract. It is incubated for 20 minutes at room temperature in the dark and then the absorbance is measured at 517 nm. Activity

was expressed as mg equivalent to ascorbic acid/g dry powder, the experiment was performed in triplicate.

Results and Discussion

Free radicals play an important role in affecting human health by causing many diseases. Antioxidants scavenge free radicals from the cells and prevent or reduce the damage caused by oxidation. Seaweeds can serve as a good source of antioxidants. The protective effect of antioxidants continues to be studied around the world (Chakraborty *et al.*, 2013; Liu and Sun, 2020) ^[11, 14].

In the present work, radical scavenging activity was measured in mg/g dry powder and measured by DPPH free radical scavenging method. The stable free radical DPPH was reduced and decolorized. The antioxidant activity of 45 species of Rhodophyta seaweed is presented in Figure-1. The antioxidant activity was expressed in mg/gram dry powder. The radical scavenging activity of Rhodophyta species was observed in the range of 22 - 52.9 mg/g dry powder.

The antioxidant activity of the *Halymenia* genus group *H. esperi* (44 mg) was given the highest activity compared to *H. macrocarpa* (40.33 mg), *H. floresii* (37.69 mg), *H. porphyraeformis* (32.49 mg), *H. durvillei* (25 mg) and *H. maculata* (24 mg). The *Hypnea musciformis* (52.92 mg) was given the maximum amount of antioxidant activity followed by *Hypnea valentiae* (38 mg), *H. spinella* (35.69 mg), *H. esperi* (31.26 mg) and *H. cervicornis* (30.14 mg). From the group of Laurencia genera, a moderate amount of activity was observed in *L. obtuse* (42.53 mg), *L. platyclade* (38.7 mg), *L. perforata* (33.86 mg), *L. glandulifera* (32.09 mg), *L. papillosa* (30.59 mg) and *L. cruciata* (30 mg). The *Gracillaria* genera were given antioxidant activity in the range (30.55- 36 mg). The *G. foliferi* (36 mg) was given higher activity compared to other species of the genus. The *Scinaia fascicularis* (51 mg) was given the maximum amount of antioxidant activity compared to others *S. confusa* (41.77 mg) and *S. complanate* (22 mg).

The higher to moderate amount of free radical scavenging was observed in all seaweeds such as *Acanthophora spicifera* (35.6 mg), *Amphiroa anceps* (28.5 mg), *Amphiroa fragilissima* (31.2 mg), *Botryocladia leptopoda* (36.6 mg), *Centroceras clavulatum* (30.0 mg), *Champia indica* (44.0 mg), *Coelarthrum opuntia* (31.3 mg), *Coralina elongate* (39.0 mg), *Dermonema virens* (32.0 mg), *Gelidiella acerosa* (38.1 mg), *Gelidiopsis variabilis* (27.0 mg), *Gelidium coulteri* (40.0 mg), *Gelidium micropterum* (47.0 mg), *Halopegma duperreyi* (30.71 mg), *Liagora ceranoides* (31.90 mg), *Rhodymenia dichotoma* (23 mg) and *Sarconema filiforme* (42.53 mg).

These red algae have high amounts of pigments such as chlorophyll (*a* and *d*), carotenoids (β-carotene, fucoxanthin, astaxanthin, xanthophyll), and other pigments such as phycoerythrin, phycocyanin, and allophycocyanin (Cian *et al.*, 2015) ^[15]. Rhodophyta usually presents a higher content of proteins, carbohydrates, and minerals than green and brown algae (Belghit *et al.*, 2017; Torres, 2019; Øverland *et al.*, 2019) ^[16, 17, 18]. Numerous studies have confirmed that Rhodophyta has high contents of bioactive compounds (~1600 described) (Leal *et al.*, 2013) ^[19]. Regarding their properties, antioxidant, anti-inflammatory, anti-tumor, antiviral, anti-hyperlipidemic, anticancer, and immunomodulatory activities have been described both on *in vitro* and *in vivo* experiments (Silva *et al.*, 2012;

Rudtanatip *et al.*, 2018) ^[20, 21]. The antioxidant compounds in seaweeds, like carotenoids, polysaccharides, and polyphenols, can neutralize ROS through their oxidation process since their affinity to those oxidative compounds is very high (Moubayed *et al.*, 2017) ^[22].

The antioxidant activity of 14 species of Chlorophyta seaweed is presented in Figure 2. The green algae *Chaetomorpha antennina* was given the higher antioxidant activity (54 mg) compared to *Acrosiphonia orientalis*, *Bryopsis plumosa*, *Caulerpa racemosa*, *Caulerpa verticillate*, *Chaetomorpha aerea*, *Chaetomorpha crassa*, *Cladophoropsis javanica*, *Enteromorpha flexuosa*, *Enteromorpha linza*, *Ulva lactuca*, *Valonia aegagropila*, *Halimeda macroloba* and *Acrosiphonia arcta*.

The antioxidant activity of 10 species of Phaeophyta seaweeds is presented in Figure -3. The brown algae *Padina boryena* (47 mg) produced the maximum amount of antioxidant activity compared to *Dictyopteris delicatula* (38 mg), *Dictyota dichotoma* (35 mg), *Padina tetrastomatica* (33 mg), *Spatoglossum asperum* (42 mg) and *Cystoseira indica* (30 mg). The *Sargassum* sp. was given antioxidant activity in range (31-41 mg/ g dry powder).

Algae exhibit higher antioxidant activity due to a higher content of nonenzymatic antioxidant components, such as ascorbic acid, reduced glutathione, phenols, and flavonoids (Wu *et al.*, 2010) ^[23]. Previous research has documented the antioxidant potential of numerous seaweeds. The study of antioxidant metabolites in algal physiology suggested the involvement of a large number of secondary metabolites (Souza *et al.*, 2007) ^[24]. In recent studies, laminarin extracted from brown seaweed has demonstrated significant antioxidant properties (Rajauria *et al.*, 2021) ^[25]. The genus *Halimeda* contains phytopharmaceuticals and possess pharmacological properties, such as antimicrobial activity, stimulation of apoptosis, and anti-trichomonal and anti-inflammatory activity (Silva *et al.*, 2017) ^[26]. Dietary supplementation of seaweeds (*Sargassum* spp.) and their derivatives enhanced the immune responses and antioxidant capabilities (Telles *et al.*, 2018) ^[27]. As a result, many marine bio-sources in the last decades have attracted attention for their potential to discover natural bioactive compounds for the development of novel drugs and nutritious foods.

Phenolic compounds represent a broad and varied category of molecules encompassing various families of aromatic secondary metabolites found in plants (Waterman and Mole, 2005) ^[28]. These compounds have been recognized for their diverse biological activities, including antioxidative properties. Previous studies have indicated that extracts from marine seaweeds, particularly their polyphenols, exhibit antioxidant activity (Yan *et al.*, 1999; Kuda *et al.*, 2005) ^[29, 30]. Phlorotannins and fucoxanthin are reported as the primary active compounds in different seaweed extracts (Yan *et al.*, 1999) ^[31].

In the present work, phenolic content was measured in mg/g dry powder. The phenolic content of 45 species of Rhodophyta seaweed is presented in Figure -4. The nine red algae were produced the higher amount of phenolic content such as *Gelidium coulteri* (75.67 mg), *Grateloupia turuturu* (58.55 mg), *Grateloupia elliptica* (47.38 mg), *Acanthophora spicifera* (40.88 mg), *Laurencia perforate* (40.17 mg), *Laurencia platyclade* (38.90 mg), *Hypnea musciformis* (35.60 mg) and *Gracilaria corticate* (33.08 mg).

The thirty species of red algae were produced the moderate amount of phenolic content i.e. *Rhodymenia dichotoma* (31.45 mg), *Grateloupia filicina* (30.67 mg), *Halymenia floresii* (29.73 mg), *Scinaia confuse* (29.67 mg), *Halymenia esperi* (29.47 mg), *Laurencia papillosa* (28.61 mg), *Botryocladia leptopoda* (27.81 mg), *Dermonema virens* (27.67 mg), *Hypnea spinella* (27.64 mg), *Gracilaria folifera* (27.47 mg), *Hypnia cervicornis* (27.39 mg), *Halymenia durvillei* (26.37 mg), *Gracilaria multipertita* (25.90 mg), *Laurencia obtuse* (25.65 mg), *Laurencia cruciate* (25.47 mg), *Gelidium micropterum* (24.97 mg), *Laurencia glandulifera* (24.87 mg), *Gelidiella acerosa* (24.20 mg), *Scinaia fascicularis* (21.83 mg), *Gracilaria foliferi* (21.65 mg), *Liagora ceranoides* (21.28 mg), *Amphiroa fragilissima* (20.22 mg), *Hypnea esperi* (19.99 mg/ g dry powder), *Hypnea valentiae* (19.83 mg), *Champia indica* (19.3 mg), *Coelarthrum opuntia* (18.20 mg), *Gracilaria fergusonii* (17.22 mg), *Halymenia macrocarpa* (16.69 mg), *Sarconema filiforme* (15.77 mg), *Scinaia complanate* (15.04 mg).

The eight species of Rhodophyta showed low level of phenolic contents such as *Haloplegma duperreyi* (13.26 mg), *Gelidiopsis variabilis* (12.61 mg), *Halymenia porphyraeformis* (11.89 mg), *Halymenia maculate* (11.092 mg/ g dry powder), *Amphiroa anceps* (10.34 mg), *Hypnea cervicornis* (9.50 mg/ g dry powder), *Coralina elongate* (8.6 mg) and *Centroceras clavulatum* (7.49 mg).

The phenolic contents of Chlorophyta seaweed are presented in Figure -5. The *Acrosiphonia orientalis* produced the highest phenolic content (67.6 mg) compared to *Bryopsis plumosa* (24.7 mg), *Caulerpa racemosa* (40.5 mg), *Caulerpa verticillate* (27.0 mg), *Chaetomorpha aerea* (18.3 mg), *Chaetomorpha antennina* (17.3 mg), *Chaetomorpha crassa* (30.3 mg), *Cladophoropsis javanica* (32.1 mg), *Enteromorpha flexuosa* (14.8 mg), *Enteromorpha linza* (12.3 mg), *Ulva lactuca* (21.8 mg), *Valonia aegagropila* (19.8 mg), *Acrosiphonia arcta* (20 mg) and *Halimeda macroloba* (13.71 mg).

The phenolic content of Phaeophyta seaweed is presented in Figure -6. The phenolic content was found in range (67.31 - 13.48 mg). The maximum amount of phenolic content was showed the *Sargassum* sp. (67.31 mg) compared to *Cystoseira indica* (13.48 mg), *Dictyopteris delicatula* (35.97 mg), *Dictyota dichotoma* (22.62 mg), *Padina boryena* (27.64 mg), *Padina tetrastomatica* (27.58 mg), *Sargassum cinereum* (18.73 mg), *Sargassum* sp. (24.20 mg), *Sargassum* sp. (38.09 mg) and *Spatoglossum asperum* (47.32 mg).

Phenolic substances are generally obtained from all red, green, and brown seaweeds. Phenolic compounds found in seaweeds include simple phenols such as phenolic acids (Rodríguez-Bernaldo *et al.*, 2010) ^[32]. Additionally, seaweeds contain distinct polyphenols such as phenolic terpenes like rosmanol, carnosol, and carnosic acid (Zhong *et al.*, 2020) ^[33], as well as terpenoids like chromene, chromanol, and plastoquinone (Cotas *et al.*, 2020) ^[34]. Bromophenols such as 2,4-bromophenol, 2,6-bromophenol, and 2,4,6-tribromophenol are also present in seaweeds (Rodríguez-Bernaldo *et al.*, 2010; Cotas *et al.*, 2020) ^[32, 34]. Seaweeds are rich in hydrophilic polyphenolic compounds including epigallocatechin gallate, epicatechin, and phlorotannins (Boonchum *et al.*, 2011) ^[35]. Several studies suggest that polyphenols play a role in preventing cardiovascular diseases, cancers, inflammation, and osteoporosis (Das., 2012) ^[36]. Furthermore, polyphenols appear to be involved in hindering neurodegenerative

diseases and diabetes mellitus (Scalbert *et al.*, 2005) [37]. Phenolic compounds produced from marine algae have been investigated for their anticancer, antibacterial, anti-

inflammatory, and antioxidant properties (Wang *et al.*, 2017) [38].

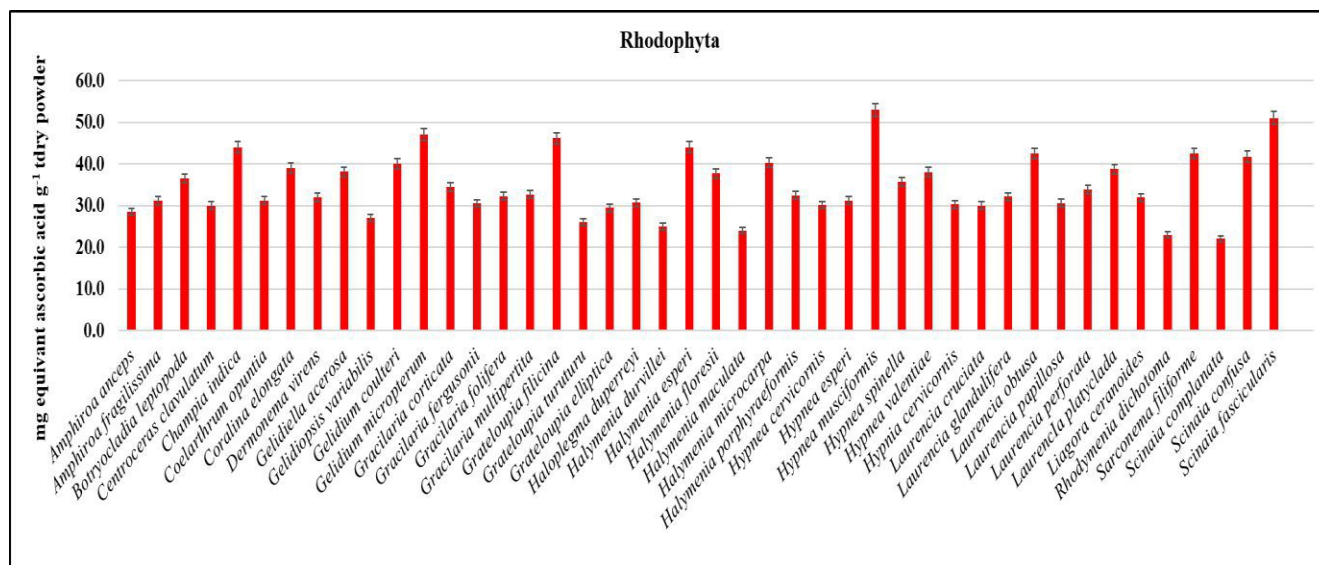


Fig 1: Antioxidant activity of selected Seaweeds from Rhodophyta class

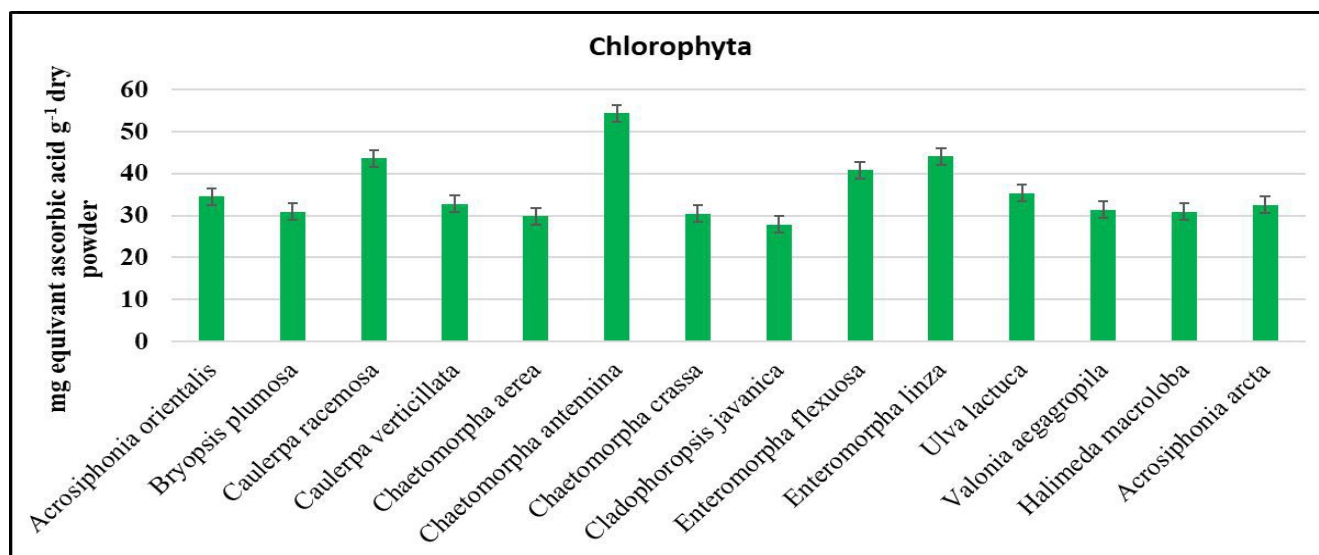


Fig 2: Antioxidant activity of selected seaweeds from Chlorophyta class

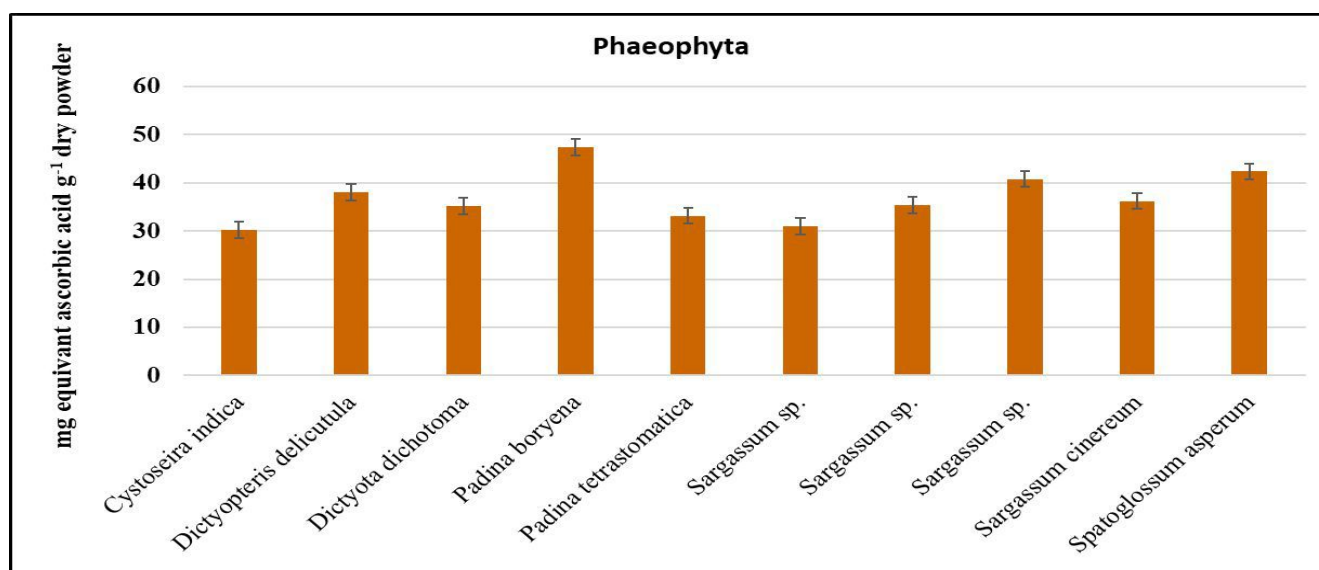


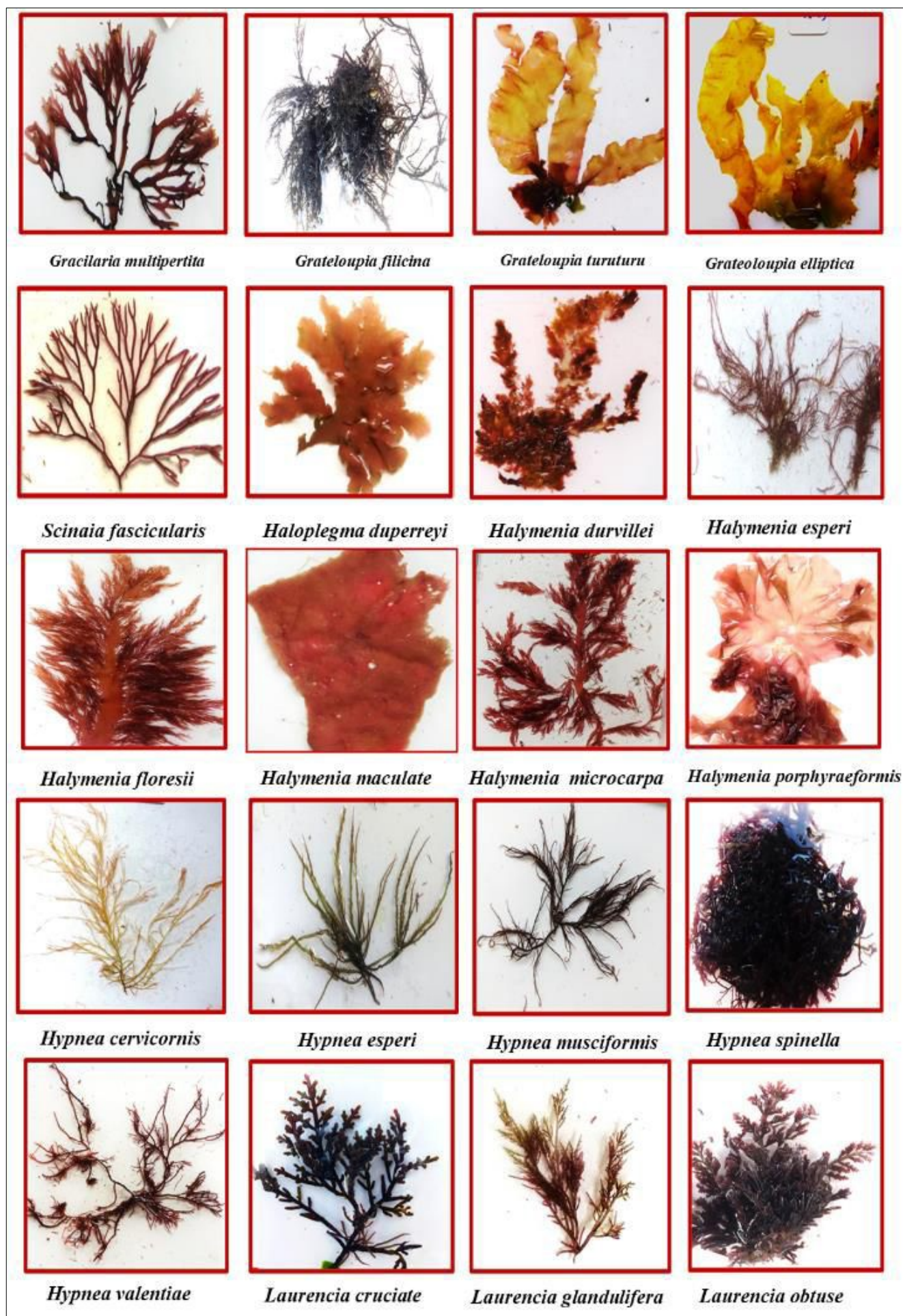
Fig 3: Antioxidant activity of selected seaweeds from Phaeophyta class



Plate 1: Seaweeds Collected from Okha Coast



Plate 2: Seaweeds Collected from Okha Coast

**Plate 3:** Seaweeds Collected from Okha Coast

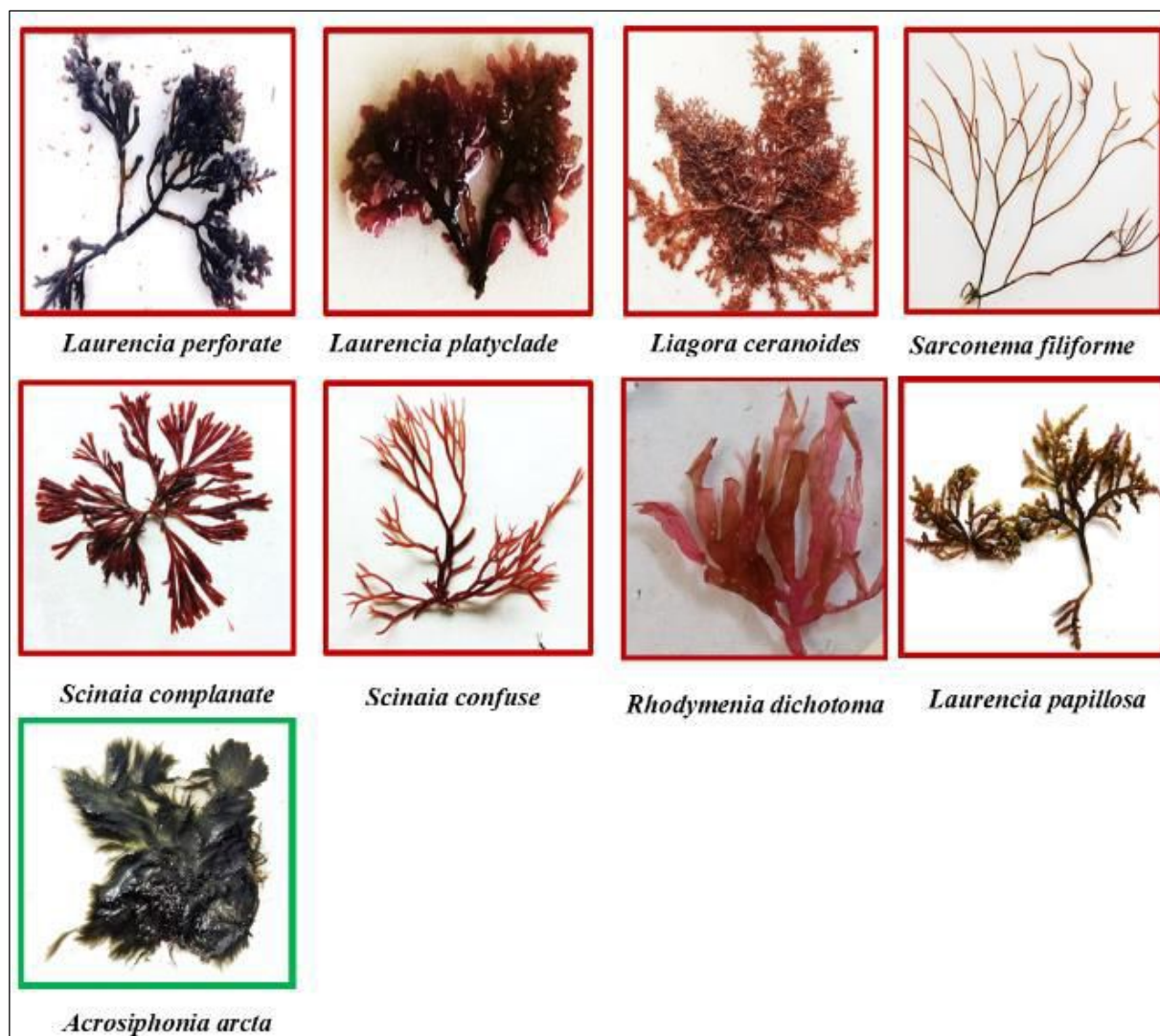


Plate 4: Seaweeds Collected from Okha Coast

Conclusion

The results indicated that all the tested seaweeds in this investigation possess antioxidant activity and total phenolic content. The *Hypnea musciformis* (Rhodophyta), *Chaetomorpha antennina* (Chlorophyta), and *Padina boryana* (Phaeophyta) possess higher antioxidant activity. The *Gelidium coulteri* (Rhodophyta), *Acrosiphonia orientalis* (Chlorophyta), and *Sargassum sp.* (Phaeophyta) possess a higher amount of phenolic content compared to other seaweeds. Most seaweeds are given the maximum amount of antioxidant and phenolic contents. These seaweeds can be used as a good source of natural antioxidants. Further study is required to find their biologically active compounds.

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