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Phytochemical analysis and Antioxidant activity of *Hypnea cervicornis* J. Agardh

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Abstract

Marine algae inhabit diverse marine environments, from shallow coastal waters to deep-sea habitats. Their adaptation to these varied and often challenging ecosystems has resulted in a unique array of phytochemicals, many of which exhibit potent antioxidant activity. The present study explores the rich tapestry of phytochemicals in *Hypnea cervicornis* J. Agardh and its contribution to antioxidant activity. Antioxidants neutralize free radicals, reducing oxidative stress, prevents cell ageing and lowers the risk of chronic diseases. The ethanolic extract showed the presence of terpenoids, carbohydrates, quinones and steroids in the phytochemical analysis. The antioxidant activity was analysed by doing DPPH (1- Diphenyl 2-picrylhydrazyl) radical scavenging activity, ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) Radical Cation Scavenging Assay and Ferric Reducing Antioxidant Power assay (FRAP) which showed that the ethanolic algal extract has high potent antioxidant activity.

Key words : Phytochemical analysis, antioxidant activity, ethanolic algal extract, *Hypnea cervicornis.* DPPH, ABTS, FRAP.

Marine algae have gained global scientific attention due to their ecological significance and the vast array of bioactive compounds they produce. Among these, red algae (phylum *Rhodophyta*) represent one of the most diverse and biochemically rich groups. A notable member of this group is *Hypnea cervicornis*, a red macroalga belonging to the class *Florideophyceae*, order *Gigartinales*, and family *Cystocloniaceae*[7]. This species is easily recognized by its characteristic antler-like branching and firm, cartilaginous texture.

Classification

Kingdom : Plantae

Phylum : Rhodophyta

Class : Florideophyceae

Order : Gigartinales

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Family : Cystocloniaceae
Genus : *Hypnea*
Species : *Hypnea cervicornis* (*J. Agardh*)

Geographical Distribution :

Hypnea cervicornis is widely distributed across tropical and subtropical marine environments, particularly in shallow intertidal and subtidal zones. It thrives in rocky coastal habitats, often forming dense mats or growing epiphytically on other seaweeds. The species has been reported in the Caribbean Sea, Atlantic coast of South America, Southeast Asia, and parts of the Indian Ocean, reflecting its ecological adaptability and wide biogeographic range[5,18].

One of the most studied aspects of *Hypnea cervicornis* is its rich phytochemical profile and antioxidant potential. Red algae, in general, are known for their abundant secondary metabolites, including phenolics, flavonoids, sulfated polysaccharides, and pigments such as phycoerythrins and carotenoids, which are potent antioxidants[19]. In the case of *H. cervicornis*, several studies have demonstrated its free radical scavenging activity, indicating a strong potential to combat oxidative stress, which is implicated in the pathogenesis of numerous chronic diseases including cancer, neurodegenerative disorders, and cardiovascular diseases[1,15]. The antioxidant activity has been primarily attributed to the presence of polyphenols and carrageenans sulfated galactans unique to red algae known for their role in modulating immune responses and protecting against cellular damage[4].

Apart from its antioxidant activity, *Hypnea cervicornis* exhibits a range of other biological activities. Extracts of the alga have shown antimicrobial, antiviral, anti-inflammatory, and anticancer properties in vitro, which highlight its promise as a natural source of therapeutic agents[11,16]. Moreover, its antiviral potential, particularly against Herpes simplex virus and Human papillomavirus, has opened avenues for research into algal-based antiviral formulations[9].

From an industrial perspective, *H. cervicornis* is an important source of carrageenan, a water-soluble sulfated polysaccharide extensively used in the food, cosmetic, and pharmaceutical industries. Carrageenan derived from *Hypnea* species serve as gelling, thickening, and stabilizing agents in products such as dairy desserts, toothpaste, and drug delivery systems[14]. Its biodegradable and non-toxic nature also makes it a preferred additive in green packaging and biomedical hydrogels. Furthermore, the increasing interest in functional foods and nutraceuticals has placed *H. cervicornis* under the spotlight as a potential ingredient for antioxidant-rich dietary supplements and health-promoting products.

Phytochemical analysis plays a crucial role in identifying and quantifying the bioactive compounds present in algal extracts, providing valuable insights into their potential health benefits. In the present study, the phytochemical screening of *Hypnea cervicornis* was performed to identify the phytochemical constituents and to investigate the antioxidant potentials of *Hypnea cervicornis*.

Collection of sample :

The fresh sample was collected from

the Mandapam sea shore Latitude: 9°19.010' N Longitude: 79°19.948' E, Ramanathapuram district, Tamil Nadu (Map:1). The fresh specimen was sent to Botanical Survey of India (BSI), Southern Regional Center, Coimbatore for identification. It was identified as *Hypnea cervicornis* (J. Agardh). Sample materials (Fig. 1) were washed under running tap water, to remove dirt and debris and shade dried. It is then ground to coarse powder and stored in airtight bottles which are kept in the dark.

Preparation of extract :

Maceration:10 g of dried algal powder is kept in 100ml ethanol for 72 hours. The extract is filtered and kept in refrigerator for a week.

Phytochemical analysis :

The different phytochemicals test were performed with the extract to understand its chemical composition. The following tests were carried out individually for the crude extract to detect various phyto constituents present in the extract.[17]

Detection of alkaloids :

Dragendorff's test :

To the 500 µL of extract, few drops of con. HCl was added and shaken well. Then few drops of Dragendorff's reagent were added. A prominent orange to red precipitate indicates the test is positive. [17]

Detection of Terpenoids :
Salkowski test :

To the 500 µL of extract, 1 mL of chloroform was added and mixed well. Then few drops of conc. H_2SO_4 were added carefully along the sides of the test tube to form a reddish brown layer, which indicates the test is positive.[17]

Detection of phenolic compounds :

Ferric chloride test :

The 500 µL of extract was dissolved in 1 mL of distilled water. To this, few drops of neutral 5% ferric chloride solution were added. A dark green colour or violet colour indicates the presence of phenolic compounds.[17]

Detection of Flavonoids :

To the 500 µL of extract, few drops of NaOH solution were added and shaken well. Bright yellow color appears and addition of excess amount of con. H_2SO_4, the yellow color disappears indicates the presence of flavonoids.[17]

Detection of Tannins :

Lead acetate test :

The 500 µL of extract was dissolved in distilled water and to this; 1 mL of 10% lead acetate solution was added. A bulky white precipitate indicated the presence of tannins.[17]

Detection of carbohydrates :

Molisch *test :*

To the 500 µL of extract, two drops of alcoholic α-naphthol solution was added and shaken well. To this, few drops of Conc.H_2SO_4

was added along the sides of the test tube. Formation of violet ring indicated the presence of carbohydrates[17].

Detection of saponins :

Foam test :

The 500 µL of extract was diluted with distilled water and made up to 5 mL. The suspension was shaken well vigorously. A persistent foam indicated the presence of saponins.[10]

Detection of glycosides :

Legal's test :

The 500 µl of extract was dissolved in 500 µl of pyridine. Then, 500 µl sodium nitroprusside solution was added followed by few drops of NaOH solution to make it alkaline. Formation of blood red colour indicated the presence of glycosides[17].

Detection of Quinone :

Sulphuric acid test :

The 500 µl of extract, 1 ml of methanol was added and mixed well. Then few drops of conc.H_2SO_4 were added carefully along the sides of the test tube to form a red ring, which indicates the test is positive[8].

Detection of Steroids :

Sulphuric acid test :

The 500 µl of extract, 1 ml of acetic anhydride was added and mixed well. Then few drops of conc.H_2SO_4 were added carefully along the sides of the test tube to form a red ring, which indicates the test is positive[17].

Antioxidant activity :

DPPH· Radical Scavenging Activity :

The radical scavenging capacity of the extract was measured based on DPPH(1,1-diphenyl 2-picrylhydrazyl) radical scavenging activity. One mL of 0.1 mM DPPH solution in methanol was mixed with 1 ml of various concentrations (50300 µg/ml) of samples. The mixture was then allowed to stand for 30 min incubation in dark. One ml methanol mixed with 1 ml DPPH solution was used as the control[3]. The decrease in absorbance was measured at 517 nm. The percentage of inhibition was calculated as

$$\%\ \text{of DPPH}^{\cdot}\ \text{radical inhibition} = \left(\frac{\text{Control}-\text{Sample}}{\text{Control}}\right)\times 100$$

ABTS Radical Cation Scavenging Assay :

The antioxidant capacity was estimated in terms of the ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation scavenging activity following the procedure described by Delgado-Andrade *et al.*,[6]. ABTS was obtained by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulfate and the mixture was left to stand in the dark at room temperature for 12-16 h before use. The ABTS solution (stable for 2 days) was diluted with 5mM phosphate-buffered saline (pH 7.4) to an absorbance of 0.70±0.02 at 730 nm. After the addition of methanol extract of varying concentrations (10-60 µg/mL) to 1 mL of diluted ABTS solution, the absorbance was measured after

Fig. 1. *Hypnea cervicornis* J. Agardh

Map1: Location site - Mandapam

(Pic courtesy: Mandapam Tide Station Location Guide)

Fig. 2.1. Phytochemical Analysis

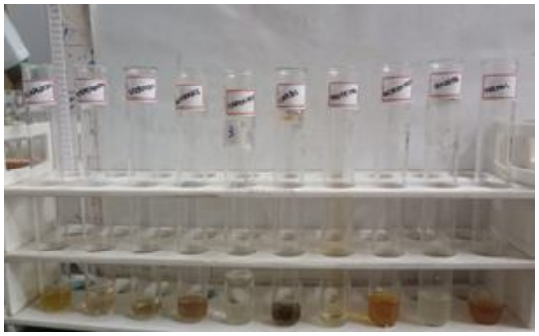


Table-2.2. Phytochemical analysis

S.no	Test	Result
1.	Alkaloids	-
2.	Terpenoids	+
3.	Phenolic Compounds	-
4.	Flavonoids	-
5.	Tannins	-
6.	Carbohydrates	+
7.	Saponins	-
8.	Glycosides	-
9.	Quinone	+
10.	Proteins	-
11.	Steroids	+

Fig. 3.1. DPPH˙ Radical Scavenging Activity

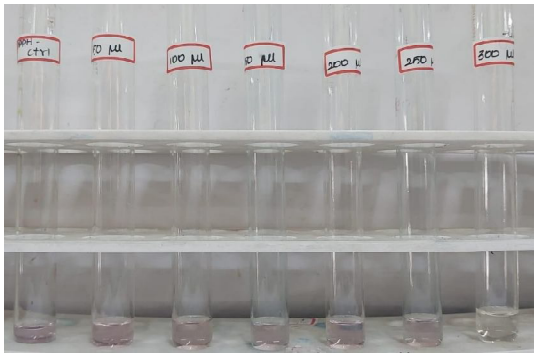
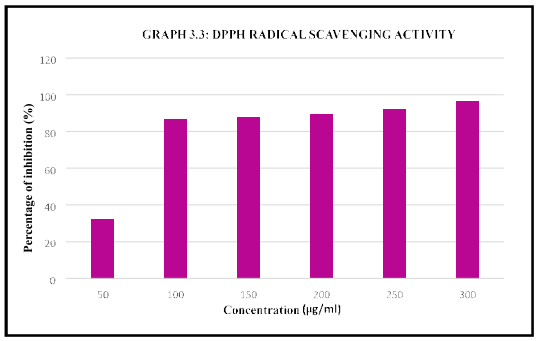


Table-3.2. DPPH˙ Radical Scavenging Activity

S.no.	Concen-tration (µg/ml)	Absor-bance @ 517nm	Percentage of Inhi-bition (%)
1.	Control	0.324	-
2.	50	0.219	32.40
3.	100	0.043	86.72
4.	150	0.039	87.96
5.	200	0.035	89.19
6.	250	0.026	91.97
7.	300	0.011	96.60

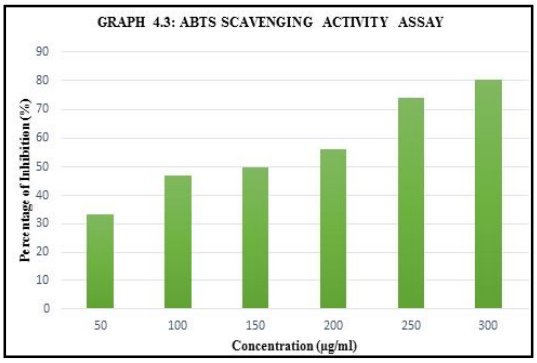


IC_{50} value = 77.16 µg/ml

Fig. 4.1. ABTS Radical Cation Scavenging Assay.

Table-4.2. ABTS Radical Cation Scavenging Assay

S. no.	Concen-tration (µg/ml)	Absor-bance @730nm	% of Inhibi-tion
1.	Control	0.130	-
2.	50	0.087	33.07
3.	100	0.069	46.92
4.	150	0.065	50.00
5.	200	0.057	56.15
6.	250	0.034	73.84
7.	300	0.026	80.00



IC_{50} value = 150 µg/ml

Fig. 5.1. Ferric Reducing Power Assay.

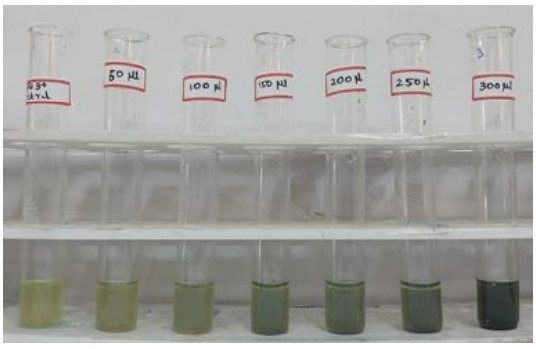
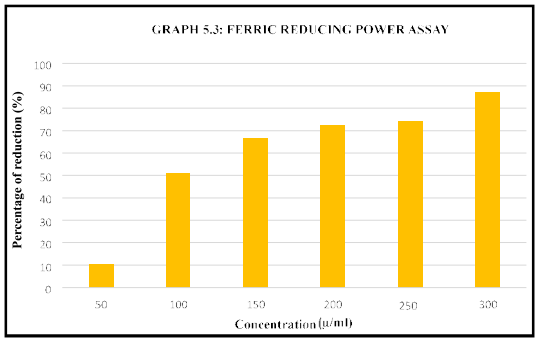


Table-5.2. Ferric Reducing Power Assay.

S. no.	Conc. (µg/ml)	Absor-bance @700nm	% of Reduc-tion
1.	Control	0.155	-
2.	50	0.173	10.40
3.	100	0.317	51.10
4.	150	0.464	66.59
5.	200	0.565	72.56
6.	250	0.607	74.46
7.	300	1.207	87.15



IC_{50} value = 97.84 µg/ml

10 min. The ABTS radical-scavenging activity of the samples was expressed as

$$\%\ \text{of ABTS}^{\cdot}\ \text{inhibition} = \left(\frac{\text{Control} - \text{Sample}}{\text{Control}}\right) \times 100$$

Ferric Reducing Power Assay :

Ferric Reducing Antioxidant Power (FRAP) assay method that uses antioxidants as reductants in a redox-linked colorimetric reaction. Different concentrations of the extracts (50-300µg/mL) were mixed with 1

mL of phosphate buffer (0.2 M, pH 6.6) and 1mL of 1% potassium ferric cyanide. The mixture was incubated at 50°C for 20 minutes. 1 mL of 10% trichloroacetic acid was added to the mixture. Then 1 mL of 0.1% of freshly prepared ferric chloride was added and the absorbance of the resultant solution was measured at 700 nm (Ferriera *et.al*., 2007).

$$\% \text{ of Reduction} = \left(\frac{\text{Control} - \text{Sample}}{\text{Control}}\right) \times 100$$

Morphology : The algae is highly branched filamentous structure. The colour ranges from deep reddish brown to light yellowish green in colour. It looks cylindrical, stiff, slightly slimy and smooth branch and branchlets. It has earthy fishy odour because of the marine environment.

Phytochemical Analysis :

The phytochemical screening was done using the ethanolic extract of the algae which showed the presence of Terpenoids, Quinones, Carbohydrates and Steroids (Table 2.2). Qualitative analysis revealed the presence of various bioactive compounds, including phenolics, flavonoids, alkaloids, terpenoids, carbohydrates, proteins, and lipids, while saponins were absent. Among these, phenolic and flavonoid compounds were observed in higher concentrations, aligning with previous studies on red algae that have reported phenolic-rich profiles in Hypnea species[12,15].

Antioxidant activity :

DPPH˙ Radical Scavenging Activity :

The DPPH radical activity was done using the ethanolic algal extract (Fig. 3.1). The maximum inhibition percentage 96.60 % was observed in the highest concentration 300µg and the minimum inhibition percentage 32.40% was observed in lowest concentration 50µg of the sample**.** IC_{50} value is 77.16 µg/ml**.** (Table 4.2 and Graph 4.3).

ABTS Radical Cation Scavenging Assay :

ABTS radical scavenging assay was done using the ethanolic algal extract (Fig. 4.1) The maximum inhibition percentage 80% was found in the highest concentration of the algal sample (300µg) and minimum inhibition percentage 33.07% was found in the lowest concentration of the sample (50µg). IC_{50} value = 150 µg/ml (Table 4.2 and Graph 4.3).

Ferric Reducing Power Assay :

Ferric Reducing Antioxidant Power assay method was done using the ethanolic algal extract (Fig. 5.1). This showed that the maximum percentage 87.15% of reduction was observed in the concentration 300µg and minimum percentage 10.4% found in 50µg. IC_{50} value = 97.84 µg/ml (Table 5.2 and Graph 5.3).

The presence of carrageenan, a sulfated polysaccharide characteristic of *Hypnea*, may also contribute to the antioxidant effect, either directly through radical scavenging or indirectly through modulation of cellular antioxidant pathways[4]. In contrast, the relatively low levels of tannins and absence of saponins suggest that while *H. cervicornis* is not a broad-spectrum phytochemical source, its dominant constituents are especially potent in antioxidant-related activity.

When compared to other red algal species, such as *Gracilaria edulis* or *Gelidium amansii, H. cervicornis* demonstrated comparable or superior antioxidant activity, especially in terms of DPPH inhibition. This may be attributed to differences in geographic origin, environmental factors, and seasonal variations, all of which are known to affect phytochemical expression in macroalgae[2,19]. Additionally, the method of extraction (*e.g.*, methanol vs. aqueous) can significantly influence the yield and efficacy of bioactive compounds, as polar solvents tend to extract more phenolics and flavonoids.

The demonstrated antioxidant potential, combined with the presence of carrageenan and other valuable compounds, highlights *H. cervicornis* as a multifunctional bioresource. Its extracts could be developed into natural antioxidant supplements, functional food additives, or cosmeceutical ingredients aimed at preventing oxidative damage. Moreover, its established use as a carrageenan source for industrial gelling and thickening suggests that pharmaceutical or food applications could be dual-purpose—offering both structural and health-promoting benefits.

The present study investigated the antioxidant activity and phytochemical profile of *Hypnea cervicornis*, a red macroalga known for its rich biochemical composition and pharmacological potential. The findings indicate a significant correlation between the phytochemical constituents and the antioxidant activity of the algal extracts, supporting the hypothesis that *Hypnea cervicornis* serves as a promising source of natural antioxidants.

In light of its broad distribution, ecological resilience, and rich biochemical composition, *Hypnea cervicornis* stands out as a valuable marine resource. The demonstrated antioxidant activity and therapeutic potential, combined with its industrial relevance, make it a subject of increasing interest in pharmaceutical, nutraceutical, and biotechnological research. Ongoing investigations into its phytochemical constituents and mechanisms of bioactivity are likely to yield novel applications and enhance our understanding of marine natural products.

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