

CHEMICAL CONSTITUENTS AND ANTIOXIDANT PERSPECTIVES OF EXTRACTS OF *CHARA GLOBULARIS* AND *CLADOPHORA* SPECIES (STRING ALGAE) SPECIESKARPAKAVALLI M^{1*}, SANGILIMUTHU AY², MOHAN S³

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ABSTRACT

Objectives: Marine organisms are capable of producing unusual bioactive compounds that are not observed in terrestrial sources. Algae are now drawing a greater interest following the increase in demand for biodiversity in the screening programs seeking therapeutic drugs from natural products.

Methods: Hot acid extraction and cold alkali extraction of *Chara globularis* and *Cladophora* species (string algae), respectively, were carried out successfully. The extracted materials were tested for the qualitative reactions and subjected for thin-layer chromatography, ultraviolet, and infrared spectral studies so as to characterize the extracted materials of two algae. They were estimated for their antioxidant perspectives by hydrogen peroxide radical scavenging and reducing power assay methods.

Results: Percentage yield of the extracts was found to be 80% and 40%, respectively, for the two algal species. The IC₅₀ value of hydrogen peroxide scavenging activity of *C. globularis* and *Cladophora* species was found to be 30 µg/ml and 20 µg/ml, respectively, and the same for the standard ascorbic acid was 20 µg/ml. The results were found to be dose dependent, i.e., higher the concentration, and more was the scavenging activity.

Conclusion: Despite the widespread uses and claimed advantages of the algae, only a few investigations on the chemical composition have been reported. A good correlation between the structure and activities of these most popular categories can be brought about.

Keywords: *Chara Globularis*, *Cladophora* species (string algae), Extraction, Phytochemical analysis, Antioxidant activity.

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INTRODUCTION

Algae are photosynthetic organisms and are commercially cultivated for pharmaceuticals, nutraceuticals, cosmetics, and aquaculture purpose [1]. *Chondrus crispus* is used as "carrageen," an excellent stabilizer in milk products. Alginates in creams and lotions are absorbable through the skin [2]. One more exciting application in which algae is used extensively today is in the manufacture of cosmetics in the forms such as algal flakes, algal oil, algal powder and algal flour, algal soaps, algal clay mask, algae beauty serum, algal beauty oil, algal oil/salt scrub, algal whole cell shampoo and conditioner, and algae anti-aging creams [3-6].

Sea nutrients such as algae and sea kelp are loaded with essential minerals and vitamins. More over offer rejuvenating properties to prevent the aging process and help in activating the cell renewal. Dichloromethane, ethanol, and boiling water extracts of the brown seaweeds *Sargassum fulvellum* and *Sargassum thunbergii* were examined for antipyretic, analgesic, and anti-inflammatory activities in mice by Kang *et al.* Other activities of algae reported include wound healing, anti-inflammatory, antidiabetic [9], antioxidative, anticancer [10], anticoagulants, antibiotics, antihypertensives [11], dilatory agents, blood cholesterol reducers [12], insecticides, antitumorogenic agents [13], nutritional supplements, and pharmaceutical applications [14].

Chara globularis though appears to be a plant but is actually a multicellular macroalga. Chara grows attached to the bottoms of ponds, lakes, rivers, and ditches and can form submersed beds of vegetation. Individual plants can range in size, from a few inches in length to several feet in length [21]. Chara has whorls of 6-8 branchlets that arise from nodes along the stem. Monoecious and dioecious species exist, but vegetative plants persist year-round. During times of reproduction,

dark, ball-like sporangia appear seed-like along the branchlets. Chara is also known to have a strong garlic odor. This macroalga has no true "leaves," only branches and branchlets [22] (Table 1).

The thallus of Chara is branched, multicellular, and macroscopic, which is mainly differentiated into rhizoids and main axis [23]. A string alga (*Cladophora*) is caused by a filamentous species of algae, which grows in long strands. These algae eventually tangle together and forming thick mats that can double their weight within 24 h. Blanket weed or string algae tend to adhere to rocks and waterfalls, which can be unsightly [24].

General procedures for extracting the algae usually involve conventional/classical extraction, gentle cold extraction, extractions involving vacuum drying or spray drying, and extraction followed by sorting steps or even extraction followed with multisteps filtration as main filtration, safety filtration, and polishing filtration to assure for better and purer isolates. Apart from these, other conventional and easier techniques of extractions such as acid or alkali or aqueous methods can be entertained for extraction of algae.

The antioxidant activity of putative antioxidant has been attributed to various mechanisms, among which are reduction of free radicals, prevention of chain initiation, binding of transition metal ion catalysts, chelating, decomposition of peroxides, prevention of continued hydrogen abstraction, and as oxygen scavengers [25] and thus can be utilized to scavenge the excessive free radicals generated from human body [26]. There are several methods available to assess antioxidant activity of compounds. The easy, rapid, and sensitive methods for screening-free radical scavenging activity are hydrogen peroxide, nitric oxide, alkaline dimethyl sulfoxide, and reducing power assay methods [27].

METHODS

Chemicals and instruments

All chemicals and solvents used were of analytical grade and obtained from S D fine chemicals, Chennai, India. The purity of the compounds was checked by thin-layer chromatography (TLC) using Silica gel-G (E-Merck). Ultraviolet (UV) spectral studies were done on Shimadzu UV spectrophotometer (Model No. UV- 2400 PC). I.R. spectra were recorded in KBr on Shimadzu spectrophotometer.

Extraction methods

Acid extraction

The algae species of *C. globularis* and *Cladophora* species were collected from in and around the places of our college area, i.e., Othakkalmandapam, Coimbatore district, Tamil Nadu, and were dried properly under sunshades. Well-dried algae species were then triturated into fine powders using powder mill and weighed to a specific quantity which in turn subjected for acid extraction, i.e., with hydrochloric acid (0.1 N). Reaction conditions followed were 1-h and 3-h time intervals and at temperature of 70°C. Different extracts of this acid extraction were then filtered and evaporated. The yield of the obtained crude extract was then calculated (Table 4).

Alkali extraction

The algae species of *C. globularis* and *Cladophora* species were collected and were dried properly under sunshades. Well-dried harvested seaweeds were then cut and triturated into fine powders using powder mill. They were then dried above 120°C and weighed to a specific quantity for subjecting them to alkali extraction, i.e. with sodium hydroxide solution (0.1 N). Alkali extract

was then filtered and evaporated to dryness. The yield of the alkali extract was calculated (Table 4). Solubility parameters of both the extracts were checked with different organic solvents and were tabulated (Table 2).

Preliminary physical data and spectral characterization

- Dissolved the sample solution (0.5 ml) in concentrated acid (2.0 ml) and distilled water (3 ml) and cooled the solution to 0–5°C in an ice bath for 5 min. A cold solution (ice bath) of sodium nitrite (0.5 g) was added in distilled water (2.0 ml) from a dropper, with swirling of the test tube and keeping in the ice bath.
- Placed the compound (0.5 g), sodium hydroxide (5%, 10 ml) and benzenesulfonyl chloride (1 ml) in a test tube and stoppered the tube and shaken until the odor of the sulfonyl chloride had disappeared. The solution must be kept alkaline. No reaction found to be occurring in the precipitate formation, and thus, the substance is a tertiary amine (Table 3).

A UV spectrum of compound (20 µg/ml) was recorded using spectral grade distilled water. UV spectral data of the compound is given in Table 4. The infrared (IR) spectral study was done on Shimadzu IR spectrophotometer by KBr disc method, and the relevant data of the compound is given in Table 4.

Antioxidant activity

Hydrogen peroxide radical scavenging assay

Glucose oxidase in the presence of glucose produces hydrogen peroxide. Gulcin *et al.* 2004 method [28] were followed to

Table 1: Antioxidant compounds from algae and their health benefits

S. No.	Antioxidant compounds	Perceived health benefits
1.	β-carotene, lutein	Protective against breast cancer
2.	Bromophenol, carrageenan, oligosaccharides Fucoidan	α-glycosidase inhibition[15] Antitumor[16] Anti-HIV Ameliorates hyperoxaluria, anticancer protection
3.	Fucophlorethols	Chemopreventive [17]
4.	Fucoanthin	Antiangiogenic, to treat retinol deficiency
5.	Galactan sulfate	Antiviral
6.	Phenolic compounds	Antiproliferative [18]
7.	Phlorotannins	Anti-inflammatory, bactericide, hypertension, photochemopreventive effect [19]
8.	Phycocerythrin	Amelioration of diabetics
9.	Polyphenols	Vascular chemoprotection [20], antimicrobial, α-glycosidase inhibition
10.	Porphyran, shinorine	Antiaging

Table 2: Solubility parameters of *Chara globularis* and *Cladophora* species

S. No.	Solvents	<i>Chara globularis</i>	<i>Cladophora</i> species
1	Distilled water	Soluble	Soluble
2	Dilute Hydrochloric acid	Soluble	Soluble
3	Ethanol	Soluble	Soluble
4	Pyridine	Insoluble	Soluble
5	Benzene	Insoluble	Insoluble
6	Chloroform	Insoluble	Insoluble

Table 3: Phytochemical analysis of *Chara globularis* and *Cladophora* species

S. No.	Phytoconstituents	<i>Chara globularis</i>		<i>Cladophora</i> species	
		Acid extraction	Alkali extraction	Acid extraction	Alkali extraction
1	Carbohydrates	+	+	+	+
2	Glycosides	-	-	-	-
3	Proteins	+	+	-	-
4	Fixed oil and fats	-	-	-	-
5	Tannins and phenolic compounds	+	+	+	+

determine the hydrogen peroxide scavenging activity of the crude extracts of *C. globularis* and *Cladophora* species. A solution of hydrogen peroxide (20 mM) was prepared in phosphate buffer saline (PBS), pH 7.4. The various concentrations (30–150 µg/ml) of the test samples and standard ascorbic acid in methanol (each 1 ml) were added to 2 ml of hydrogen peroxide solutions in PBS. The absorbance was measured at 230 nm in the UV spectrophotometer (Shimadzu, UV-2450) after 10 min of incubation at 37°C against a blank solution that contained samples in PBS without hydrogen peroxide. The percentage of scavenging of hydrogen peroxide was calculated using the formula:

$$\% \text{ Scavenged } [H_2O_2] = [(A_0 - A_1)/A_0] \times 100,$$

Where A_0 is the absorbance of the control, and A_1 is the absorbance of the sample/standard (Table 5).

Reducing power assay

Any substances which are having reduction potential react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which again react with ferric chloride to form ferric ferrous complex. They have an absorption maximum at 700 nm. Different concentrations of crude extracts were taken separately and mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml potassium ferricyanide (1%). The mixture was incubated at 50°C for 20 min and added 2.5 ml of trichloroacetic acid (10%). The given mixture was centrifuged at 3000 rpm for 10 min. Finally, 2.5 ml of the supernatant solution was mixed with 2.5 ml of distilled water and 0.5 ml $FeCl_3$ (0.1%). The absorbance was measured at 700 nm in UV-visible spectrophotometer. Here, ascorbic acid was used as standard and phosphate buffer as blank solution (Table 5). The reducing power was calculated using the given formula:

$$\% \text{ increase in reducing power} = \frac{A_{\text{test}}}{A_{\text{Blank}}} - 1 \times 100$$

Statistical analysis

Statistical analysis was carried out employing analysis of variance and Dunnett's multiple comparison tests.

RESULTS

A great deal of interest has been developed in the nutraceutical and pharmaceutical industries to isolate natural medicinal compounds from marine resources. Among marine resources, marine algae are valuable sources of novel bioactive compounds with pharmacological effect [29]. The nutrient density (amino acids, mineral salts, trace elements, and vitamins) of the all-round talent is impressive. Minerals and trace elements of algae actually have a similar distribution of cells as those in the human body. The amino acid content of the green algae is very similar to the human collagen.

Algae are one of the richest sources for many known and novel bioactive compounds with a lot of varied pharmacological activities, namely, anticoagulant, antithrombotic [4], antiviral, antitumor [5], antioxidant, anti-inflammatory, antimicrobial, reducing blood lipids, therapeutic potential in surgery, immunomodulatory activity [30-32] and against hepatopathy, and uropathy [33]. Hot acid extraction and cold alkali extraction of *C. globularis* and *Cladophora* species (string algae), respectively, were carried out successfully and were found to be yielding 80% and 40% extracts, respectively. The lower percentage yield of the latter was found to be due to the dampened nature of *Cladophora* species. The percentage yield of said extracts could be improved by advanced extraction techniques such as extraction with an aid [34] of ultrasound, pressurized solvent, supercritical fluid chromatography, or countercurrent chromatography.

The crude extracts of both algae were tested for their solubility in various solvents and were found to be soluble in distilled water, dilute hydrochloric acid, and alcohol and were found to be insoluble in pyridine, benzene, and chloroform. The extracted materials of both algae species were tested for the qualitative reactions and were found

Table 4: Physical and spectral data of *Chara globularis* and *Cladophora* species

S. No.	Name of the algae species	Weight of herbal materials taken in g	Weight of crude extract	% of crude extract obtained	Rf value	λ_{max}	IR spectra
1	<i>Chara globularis</i>	500	4	80	0.56	291.5	3500–2500/cm OH stretching; C-C stretching; 1420/cm; CH ₂ -C=O acyclic; 1190/cm C-O stretching
2	<i>Cladophora</i> species	500	2	40	0.34	290.5	3500–2500/cm OH stretching; 1581/cm; C-C stretching; 1420/cm; CH ₂ -C=O acyclic

Table 5: The free radical scavenging property of *Chara globularis* and *Cladophora* species by hydrogen peroxide assay and reducing power assay method

Sl. No.	Name of the method	Concentration in µg/ml	Absorbance of ascorbic acid	Absorbance of <i>Chara globularis</i>	% inhibition of free radicals	Absorbance of <i>Cladophora</i> species	% inhibition of free radicals
1	Hydrogen peroxide assay	10	3.5	2.474	70.68	3.123	89.22
		20	3.2	2.445	78.87	2.614	96.81
		30	3.0	2.428	80.93	3.012	97.16
		40	2.8	2.418	86.35	2.992	99.73
		50	2.7	2.404	89.03	2.661	97.10
2	Reducing power assay	10	0.9	0.774	60.06	0.817	70.75
		20	1.2	0.810	63.38	0.849	73.00
		30	1.3	0.824	65.37	1.038	75.18
		40	1.5	0.901	67.50	1.095	79.84
		50	1.62	1.059	79.33	1.218	90.77

Values are expressed as mean+ SD; Values are from triplicate readings; and are statistically significant at $p < 0.05^*$, $p < 0.01^{**}$, $p < 0.001^{***}$, when compared to the standard

to be containing the phytochemicals of the classes of carbohydrates, proteins, tannins, and phenolic compounds and were subjected for TLC, UV, and IR spectral studies so as to characterize the extracted materials of two algae (Table 4). TLC techniques were performed for the extracted materials of *C. globularis* and *Cladophora* species. Detection is done using iodine vapor and UV light. Both types' algae extracts showed a single spot whose Rf values are shown in Table 4 and Fig. 1. The absorption maxima, λ_{max} (Table 3) of the individual extracts, were recorded using distilled water as solvent. IR spectra were taken for the two different extracts of two different algae, and the characteristic absorption peaks were observed for all relevant groups (Table 4).

An imbalance between the amount of reactive oxygen species and antioxidant enzymes is a problem for our health. Hence, the daily intake of foods with antioxidant is necessary (Halliwell). Superoxide radical O_2^- a highly reactive and toxic species is generated by numerous biological and pathological reactions by reacting with many substrates, produced in various metabolic processes. Both the aerobic and anaerobic organisms possess superoxide dismutase which catalyzes the breakdown of superoxide radical [35,36]. It is well known that superoxide anions damage biomacromolecules directly or indirectly by forming H_2O_2 , OH^- , peroxy nitrite, or singlet oxygen during pathophysiological events. It can cause oxidation or reduction of solutes depending on their reduction potential [37].

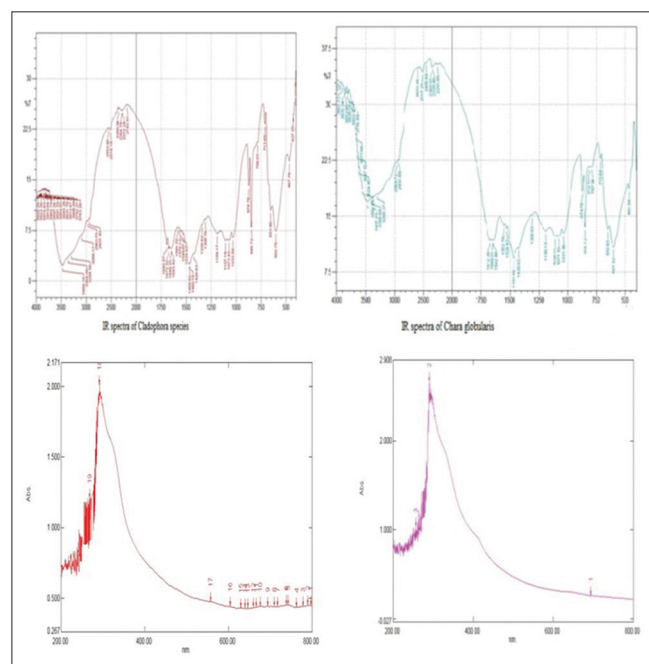


Fig. 1: Infrared and ultraviolet spectral data of respective *Chara globularis* and *Cladophora* species

The antioxidant activity of the acid- and alkali-extracted *C. globularis* and *Cladophora* species was assessed using various antioxidant systems such as hydrogen peroxide scavenging activity and reducing power assay. Results revealed that *C. globularis* showed better and equipotent activity to that of the standard ascorbic acid when compared to *Cladophora* species. The IC50 value of hydrogen peroxide scavenging activity of *Chara globularis* and *Cladophora* species was found to be 30 $\mu\text{g}/\text{ml}$ and 20 $\mu\text{g}/\text{ml}$, respectively, and the same for the standard ascorbic acid was 20 $\mu\text{g}/\text{ml}$ (Table 5). The results were found to be dose dependent, i.e., higher the concentration, and more was the scavenging activity.

DISCUSSION

Algae are the undisputed treasures of sea. Algae have been used as a food source for many centuries by traditional culture. They

are used in the manufacture of local food products such as crisps, baked goods, pasta, and noodles. They are also believed to have many important health benefits such as rejuvenation, induction of appetite, to soothe stomach ulcers, and to get recovery from common maladies. Marine algae-derived phlorotannins and SPs have great potential for developing as anticoagulant drugs in nutraceutical and pharmaceutical areas. Although most of the countries in the world are recognizing the significance of seaweeds various fields such as textile and food industry, pharmaceuticals, and cosmetics, we lack awareness regarding the nutritional, commercial, and pharmaceutical importance of seaweeds.

CONCLUSION

To overcome this, our research focus might lead to an ensured pavement for the same. Standardization process of these medicinally important isolates becomes possible. More reliability on the quality of compounds was obtained from these species and thereby for use as a category in life-saving pharmaceutical fields.

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CONFLICTS OF INTEREST

There are no conflicts of interest.

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