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# Exploration of beneficial activities of marine algae for cosmeceuticals

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**ABSTRACT**

Algae play an important role in many ecosystems which provide the foundation for the aquatic chains livelihood of all fisheries in the ocean and inland and moreover produce about 70 percent of all the air we breathe. Algae contain bioactive compounds which play an important role against various diseases and ageing process. Algae are chief source of antioxidants used in the pharmaceutical, medical, nutraceutical, agricultural and food industries. Some species of algae were collected from Pulicat Lake, Kovalam and Mamallapuram in the east coast of India. The antioxidant potency and anti-inflammatory activity were determined for the following five algal species: *Chaetomorpha brachygona, Enteromorpha compressa, Gracilaria verrucosa, Gratiloupia lithophila* and *Ulva lactuca*. Among them *Gracilaria verrucosa*, which is found to have the highest biding properties, can be used for anti-aging, anti-tanning, pigmentation, pimple reduction and skin whitening. A dermal cream was prepared using *Gracilaria verrucosa* extract and the cell viability test was done. As the cream has not affected cell viability, it has become clear that *Gracilaria verrucosa* can be a substitute for other nutritious fruits and vegetables of various applications in cosmetic industry.

## Introduction

Algae are very peculiar and found relatively everywhere on the planet. They play an important role in many ecosystems which provide the foundation for the aquatic chains livelihood of all fisheries in the ocean and inland. Further they produce about 70 percent of the air we breathe. Algae are a chief source of antioxidants and they are taken advantage of mostly in the fields of pharmaceutical, medical, nutraceutical, agricultural and food industries. In algae, natural antioxidants contain bioactive compounds which play an important role against different diseases and ageing processes. In shallow water habitats, marine algae can be manifested to a composite of ultraviolet light and air which leads to the formation of free radicals and other reactive oxygen species (ROS). Inspite of their exposure to harmful ROS, algae have absence of oxidative stress in their structural components and abstain from oxidation during storage which indicates the presence of antioxidant protection system in their cells. Antioxidants neutralize free radicals to donate an electron which oxidize biomolecules that lead to cell death and tissue damage. This paves the way for utilization of algae in cosmetics. Again in nutraceuticals, they can be used to enrich the nutritional value of food and animal feed because of their well-balanced composition. Antioxidants play a vital role in food industry due to its act against lipid peroxidation. In animals, algal biomass can enhance the immune response which results in growth promotion, disease resistance, enhance gut function, antiviral and antibacterial action, reproductive performance and weight control(Neelma Munir *et al.*)

Some species of algae such as *Chaetomorpha brachygona*, *Enteromorpha compressa*, *Gracilaria verrucosa*, *Gratiloupia lithophila* and *Ulva lactuca* were collected from Pulicat Lake, Kovalam and Mamallapuram in the east coast of India. Methanolic extracts of the species were prepared by cold percolation method. The free radical scavenging activity using DPPH assay, Ferric Thiocyanate Assay, Thiobarbituric acid assay, Ferric Reducing Antioxidant assay, ABTS radical scavenging assay, anti-inflammatory activity and cytotoxicity of the collected algae from different localities were evaluated and compared. Later the properties were applied to form a commercial toxicity free cosmetic product.

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## Materials and Methods

Collection and identification

Five species of algae were collected from Pulicat Lake, Kovalam and Mamallapuram in the east coast of India during the early rainy season in the month of June (Plate-1). They were identified as *Chaetomorpha brachygona*, *Enteromorpha compressa*, *Gracilaria verrucosa*, *Gratiloupia lithophila* and *Ulva lactuca* species based on their habitat conditions, morphology and their prevalence in the area where the samples were collected.

Preparation of crude algal extract

The crude from the algae were extracted through the cold percolation method. Aqueous methanol mixture of 1:10 ratio was used as the solvent. 20g of each algal sample in 200ml of methanol was taken and was placed in a controlled shaker for 48minutes at a temperature of 30±2°C. The extract was filtered and then concentrated for determining the antioxidant, anti-inflammatory and cytotoxicity activity (Udaya Prakash *et al.*)

Antioxidant assays

Aqueous methanol extracts obtained from each algal species were used to study the anti oxidat roperty by various assays.

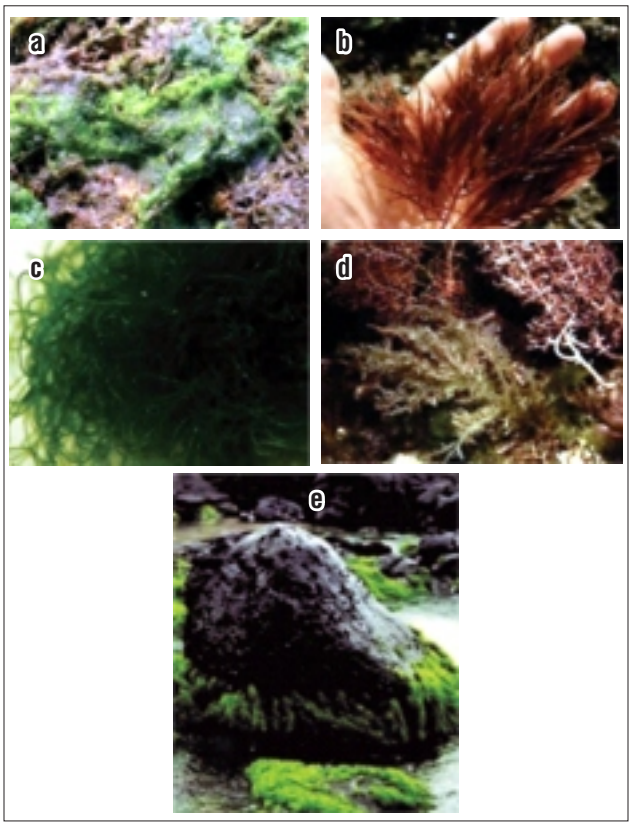


Plate-1. *Algal species collected - a.* Cheetomorpha brachygona, *b.* Gracilaria verrucosa, *c.* Enteromorpha compressa, *d.* Gratiloupia lithophila *and e.* Ulva lactuca

DPPH free radical scavenging assay

Different concentration of extracts in the range of 10, 20, 30, 40 and 50 mg/mL were taken in test tubes. The extracts were made upto 1 mL with methanol and 1mL of 0.01mM DPPH was added in each test tube. Identical solution of DPPH was added to the Butylated Hydroxyanisole (BHA) and it was used as the reference. Pure methanol was used as the blank. The samples were 'incubated in dark at room temperature for 30 minutes. Then the absorbance at 517nm was read. The percent inhibition was calculated by using the following formula:

Effective concentration% = {(control absorbance – test absorbance) / control absorbance} × 100

Ferric thiocyanate assay

The inhibitory effect of algae against oxidation by peroxide was determined by Ferric thiocyanate assay. 120 µL of 98% ethanol, 100µL of 2.51% of linoleic acid in ethanol and 9mL of 40mMphosphate buffer at PH7 were added to 100µL of the algal extract. The solution was kept in dark condition at 40°C for a few minutes. 100µL of the above mixture was added to 9.7mLof 75% ethanol, 100µL of 30% ammonium thiocyanate and 100µL of 20Mm $FeCl_3$ in 3.5% HCl solution and incubated for 3 minutes. The absorbance at 500nm was observed. (UdayaPrakash *et al.*)

The percentage inhibition was calculated with tannic acid as reference:

Effective concentration% = {(control absorbance – test absorbance)/ control absorbance} × 100

Thiobarbituric acid assay

2mL of each 20% trichloroacetic acid, 0.67% thiobarbituric acid, 1mL of 2.51% linoleic acid and 1mL of algal extract were taken and mixed together. The solution was kept in boiling water bath for 10 minutes and then cooled. After cooling, the solution was centrifuged at 3000rpm for 5minutes and the absorbance of the supernatant at 532nm was measured. (UdayaPrakash *et al.*). The percentage inhibition was calculated with the standard solution of Butylated hydroxyl toluene (BHT) as reference.

Effective concentration% = {(control absorbance – test absorbance)/ control absorbance} × 100

Ferric Reducing Antioxidant Power Assay

1mL of algal extract was added to 2.5mL of 0.2M phosphate buffer at pH 7. 2.5mL of 1% potassium ferricyanide added to it and the mixture was incubated at 50°C for 30minutes. 2.5mLof 10% trichloroacetic acid was added to it and the total mixture was centrifuged for 10minutes at 6500 rpm. 2.5mL of the supernatant, 2.5mLof distilled water and 0.5mL of 0.1% $FeCl_3$ were mixed and the absorbance at 700nm was observed. (UdayaPrakash *et al.*). The percentage inhibition was calculated with the standard solution $FeSO_4$ as reference.

Effective concentration% ={(control absorbance – test absorbance)/ control absorbance} × 100

ABTS assay

7mM of ABTS solution [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)] was added to 2.45mM potassium persulphate and incubated in dark for 12-16 hours. The solution was diluted with ethanol and then the absorbance reached 0.7±0.2 at 734nm. Then 1mL of the above diluted solution was added with 100µL of algal extract and incubated for 6minutes. The absorbance at 734nm was then observed. (UdayaPrakash *et al.*) The percentage inhibition was calculated with the standard solution tannic acid as reference.

Effective concentration% ={(control absorbance – test absorbance)/ control absorbance} × 100

Anti –inflammatory assay

The algal extract 100µL was added to 500µL of 1% bovine serum albumin. This mixture was kept at room temperature for 10 minutes and then heated at 51$^0$C for 20 minutes. Then it is allowed to cool to room temperature for a few minutes. Finally the absorbance was measured at 660nm. (Reshma *et al.*). The percentage of denaturation inhibition was calculated with the standard solution acetyl salicylic acid as reference.

Effective concentration% ={(control absorbance – test absorbance)/ control absorbance} × 100

Invitro assay for cytotoxicity (MTT assay)

Cells (1×10$^5$well) were plated in 24 well plates and then incubated at 37$^o$C with 5% $CO_2$ condition. After the cell reached the confluence, various concentrations of the samples were added and incubated for 24 hours. After incubation, the sample was removed from the well and washed with phosphate buffer saline at pH7.4 or MEM without serum. 100µL/well (5mg/mL) of 0.5% 3-(4,5dimethyl-2-thioazyl)-2,5 diphenyl—tetrazolium bromide (MTT) was added and incubated for 4 hours. After incubation, 1ml of DMSO was added in all the wells. The absorbance at 570nm with UV-spectrophotometer by using DMSO as the blank was measured. After the absorbance was measured, concentration required for 50% inhibition (IC50) was found graphically. The percentage of cell viability was calculated by using the following formula:

%cell viability = A570 of treated cells/A570 of control cells ×100

Graphs are plotted by using the %cell viability at Y-axis and concentration of sample in X-axis.

Cream Formulation

Paraffin wax -10 g, Microcrystalline wax -4g

Liquid paraffin -5ml Olive oil - 4ml

The above ingredients are mixed well in a beaker and kept in a water bath for melting of the substances. 20ml distilled water was boiled in a water bath at 80$^0$C for 5 minutes and added into the beaker slowly. Then 4g of borax in 10ml of distilled water was added into the above mixture and mixed vigorously. The mixture was stored in the refrigerator for 30 minutes. The algal extract was added into the mixture and mixed vigorously by using a vortex mixer. Aloe vera can be added for combined and enhanced medical properties.Finally, the colorant and fragrance oil were added to form the final cream product (Plate-2).

## Results and Discussion

DPPH free radical scavenging assay

The DPPH assay was used to determine the free radical scavenging activity. 1,1 Diphenyl 2- PicrylHydrazyl is stable free radical which is red in color and it turns yellow in the presence of anti-oxidant sample where scavenging by antioxidant compounds represents the degree of discolouration. Among the five species of algae, *Gracilaria verrucosa* showed the highest free radical scavenging activity of 59.24% inhibition at 50mg/ml concentration of algal sample (Table-1).


Plate-2. *Final cream product prepared from* Gracilara verrucosa

Table-1. *Inhibition results of DPPH assay(in percentage)*

| Species / Concentraion | 10 mg/mL | 20 mg/mL | 30 mg/mL | 40 mg/mL | 50 mg/ml |
|---|---|---|---|---|---|
| *Chaetomorpha brachygona* | 27.67 | 34.33 | 42.11 | 49.69 | 58.72 |
| *Enteromorpha compressa* | 11.25 | 16.54 | 22.71 | 26.89 | 33.33 |
| *Gracilaria verrucosa* | 36.67 | 42.42 | 49.16 | 54.78 | 59.24 |
| *Gratiloupia lithophila* | 7.34 | 11.11 | 14.72 | 16.52 | 21.78 |
| *Ulva lactuca* | 16.64 | 22.78 | 29.68 | 36.67 | 44.54 |

Ferric thiocyanate assay

Iron (III) ions react with thiocynate ions to form an intense red colored complex ion. But due to the anti – oxidant activity of Iron (III) ions are converted into Iron(II) and the red colored compound is not formed. Thus on measuring colorimetric values, the anti-oxidant activity of the algal samples are found. Among them, *Gracilaria verrucosa* was found with average higher percent inhibition of 69.54 at 50mg/ml concentration (Table-2).

Thiobarbituric acid assay

Thiobarbituric acid assay is based on the principle of lipid peroxidation. Thiobarbituric acid reacts with MDA to produce pink colored compound. Among the five species of algae, *Gracilaria verrucosa* showed the higher antioxidant activity at varying concentrations with maximum inhibition of 51.78 % at 50mg/ml concentration (Table-3).

Ferric reducing antioxidant power assay

Ferric reducing antioxidant power assay is used to determine reducing power of electron donating antioxidants. Here the ferric tripyridyl triazine complex [Fe(III)] is reduced to ferrous [2,4,6-tripyridyl-s-triazine] Fe(II) by the sample. Among the five algal species, *Gracilaria verrucosa* has shown the maximum activity of reducing power with 70.12 % inhibition at 50mg/ml concentration (Table-4).

ABTS radical scavenging assay

The ABTS assay is used to determine the free radical scavenging activity. The ABTS radical cation is reactive towards the most antioxidants which includes the phenolics, thiols and vitamin C. The ferryl myoglobin radical was formed from met-myoglobin and hydrogen peroxide and the blue color of ABTS changes to a colourless form. *Gracilaria verrucosa showed* the highest free radical scavenging activity with inhibition of 96.67% at 50mg/ml concentration among all the algal species (Table-5).

Anti-inflammatory assay

The anti-oxidant potency of the collected algal species were determined at invitro-conditions based on the prevention of bovine serum albumin protein degradation and the *Gracilaria verrucosa* has shown comparatively higher of inhibition of 43.82% at 50mg/ml concentration (Table-6).

Cytotoxicity assay

The viability of the cells are checked to determine the cyto-toxicity of the cream product and it is found to have cell viability of 61.96% at 1000 µg/mL concentration (Table-7)

## Discussion

Comparing the values of the five collected species, *Gracilaria verrucosa* has as shown the highest anti-oxidant value followed by *Chaetomorpha brachygona*, *Enteromorpha compressa*, *Ulva lactuca* and *Gratiloupia lithophila*. The $EC_{50}$ value (mg/ml) for the *Gracilaria verrucosa* was calculated as 33.68 by DPPH assay. This is higher than the value of ripened *Carica papaya* fruit and its leaves whose $EC_{50}$ values (mg/ml) are 6.5 and 7.8 respectively (Maisarah *et al.*). However it shows

Table-2. *Inhibition results of Ferric Thiocyanate assay(in percentage)*

| Species / Concentraion | 10 mg/mL | 20 mg/mL | 30 mg/mL | 40 mg/mL | 50 mg/ml |
|---|---|---|---|---|---|
| *Chaetomorpha brachygona* | 33.78 | 44.74 | 56.24 | 65.79 | 76.67 |
| *Enteromorpha compressa* | 19.67 | 27.78 | 35.24 | 44.54 | 51.33 |
| *Gracilaria verrucosa* | 41.35 | 48.78 | 56.86 | 62.67 | 69.54 |
| *Gratiloupia lithophila* | 13.78 | 19.45 | 26.44 | 31.27 | 39.42 |
| *Ulva lactuca* | 24.72 | 31.67 | 39.78 | 45.45 | 53.51 |

Table-3. *Inhibition results of thiobarbituric acid assay(in percentage)*

| Species / Concentraion | 10 mg/mL | 20 mg/mL | 30 mg/mL | 40 mg/mL | 50 mg/ml |
|---|---|---|---|---|---|
| *Chaetomorpha brachygona* | 21.64 | 26.67 | 30.14 | 37.67 | 42.42 |
| *Enteromorpha compressa* | 10.17 | 12.45 | 15.67 | 19.78 | 24.27 |
| *Gracilaria verrucosa* | 29.68 | 33.56 | 38.67 | 44.27 | 51.78 |
| *Gratiloupia lithophila* | 5.64 | 11.67 | 20.73 | 31.45 | 43.45 |
| *Ulva lactuca* | 13.67 | 19.45 | 27.27 | 33.89 | 42.54 |

Table-4. *Inhibition results of Ferric reducing anti-oxidant power assay(in percentage)*

| Species / Concentraion | 10 mg/mL | 20 mg/mL | 30 mg/mL | 40 mg/mL | 50 mg/ml |
|---|---|---|---|---|---|
| *Chaetomorpha brachygona* | 38.16 | 41.45 | 49.78 | 57.67 | 68.89 |
| *Enteromorpha compressa* | 20.14 | 29.68 | 41.44 | 53.67 | 62.67 |
| *Gracilaria verrucosa* | 42.24 | 49.56 | 55.78 | 62.67 | 70.12 |
| *Gratiloupia lithophila* | 21.17 | 28.78 | 36.67 | 44.44 | 52.89 |
| *Ulva lactuca* | 19.17 | 25.63 | 32.78 | 40.67 | 49.89 |

Table-5. Inhibition results of ABTS radical scavenging assay (in percentage)

| Species / Concentraion | 10 mg/mL | 20 mg/mL | 30 mg/mL | 40 mg/mL | 50 mg/ml |
|---|---|---|---|---|---|
| *Chaetomorphabrachygona* | 81.56 | 84.78 | 87.67 | 89.45 | 92.11 |
| *Enteromorphacompressa* | 77.67 | 80.12 | 82.89 | 86.45 | 90.27 |
| *Gracilaria verrucosa* | 89.89 | 90.54 | 91.89 | 93.56 | 96.67 |
| *Gratiloupia lithophila* | 56.67 | 61.45 | 65.67 | 68.78 | 73.27 |
| *Ulva lactuca* | 78.16 | 81.45 | 84.78 | 88.67 | 92.57 |

Table-6. *Inhibition results of anti-inflammatory assay(in percentage)*

| Species / Concentraion | 10 mg/mL | 20 mg/mL | 30 mg/mL | 40 mg/mL | 50 mg/ml |
|---|---|---|---|---|---|
| *Chaetomorphabrachygona* | 31.45 | 32.55 | 34.00 | 39.45 | 43.45 |
| *Enteromorpha compressa* | 26.27 | 27.55 | 34.09 | 37.73 | 38.09 |
| *Gracilaria verrucosa* | 34.73 | 38.91 | 41.27 | 43.27 | 43.82 |
| *Gratiloupia lithophila* | 15.27 | 16.55 | 23.09 | 26.73 | 27.09 |
| *Ulva lactuca* | 27.27 | 28.18 | 32.36 | 35.27 | 42.91 |

Table-7. *Cell viability in cyto-toxicity(in percentage)*

| Concentration (µg/mL) | Dilutions | Absorbance | Cell viability (%) |
|---|---|---|---|
| 1000 | Neat | 0.318 | 61.96 |
| 500 | 1:1 | 0.347 | 67.64 |
| 250 | 1:2 | 0.381 | 74.26 |
| 125 | 1:4 | 0.409 | 79.72 |
| 62.5 | 1:8 | 0.434 | 84.60 |
| 31.2 | 1:16 | 0.466 | 90.83 |
| 18.6 | 1:32 | 0.491 | 95.71 |
| 7.8 | 1:64 | 0.501 | 97.66 |
| Control | - | 0.513 | 100 |

comparatively lower value than other widely used plant species like *Aloe vera* ($EC_{50}$ value =58.36 µg/mL) and tea ($EC_{50}$ value = 13.36 µg/mL)(Tin A. Khaing). Cosmetics have found a great market in the present economy. Dermal care products contribute above 3/4 $^{th}$ of the total cosmetic products. Highly nutritious fruits and vegetables are exploited for their anti-oxidant and anti-inflammatory properties in the production of body lotions, creams and soaps. But from the above assay results, it becomes clear that *Gracilaria verrucosa* with premier anti-oxidant and anti-inflammatory properties can be used as an alternative source for other fruits and vegetables. The cream product obtained was also identified to be cyto-toxicity free. It is evident from the different assays done, that the marine alga *Gracilaria verrucosa* has immense anti-oxidant and anti-inflammatory properties and this species is found to be abundant along the eastcoast of India. The cosmetic cream produced was also found to be cyto-toxicity free. So, this species is recommended for usage in cosmetic industry as a replacement for many nutritious fruits and vegetables.

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