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## PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITY OF VARIOUS EXTRACTS OF *SARGASSUM MUTICUM*

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### ABSTRACT

Seaweeds or marine macro-algae are potential renewable resource in the marine environment. It has been used as antioxidant, anti-mutagen, anticoagulant and antitumor agent. The medicinal uses of seaweed are vast and range from topical burn therapy to softening of tumors. The brown macro algae, *Sargassum muticum* is a monoecious species generate a large number of propagules and brown algae are distinguished by their color which varies from olive green through light golden to a rather deep shade of brown. Considering the various medicinal potential of seaweeds, the present study was carried out on the preliminary phytochemical screening, free radical scavenging activity and total antioxidant activity of various extracts of *Sargassum muticum*. Phytochemicals were extracted from *Sargassum muticum* using various solvents such as benzene, chloroform, ethanol, ethyl acetate, methanol and petroleum ether. Screening of phytochemical constituents showed positive results for the presence of alkaloids, anthraquinones, carbohydrates, flavonoids, glycosides, saponins, steroids, phenols, terpenoids and tannins. Phytochemicals were extracted best in methanol. Free radical scavenging activity and total antioxidant activity of the various extracts were determined. Among the various extracts methanolic extract was found to have highest DPPH scavenging activity and total antioxidant activity.

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activity

## INTRODUCTION

Seaweeds have been used by humans as medicine and food for at least 13,000 years. Over the past several decades, seaweeds and their extracts have generated an enormous amount of interest in the pharmaceutical industry as a fresh source of bioactive compounds with immense medicinal potential. Seaweeds are rich in antioxidants such as carotenoids, pigments, polyphenols, enzymes and diverse functional polysaccharides<sup>1</sup>. Seaweeds are a part of stable diet in the orient as they are nutritionally rich materials due to a much lesser extent in the rest of the world<sup>2</sup>. The mineral nutrients present in seaweeds are diverse and the main elements being iodine and calcium. The chemical composition of seaweeds varies with species, habitat, maturity and environmental conditions<sup>3</sup>. Seaweeds are excellent source of Vitamin A, B<sub>1</sub>, B<sub>12</sub>, C, D and E. Quality of protein and lipid in seaweeds are most acceptable for consumption compared to other vegetables mainly due to their high content in essential amino acids and relatively high level of unsaturated fatty acids<sup>4</sup>. In contrast to terrestrial plant materials, less research has been conducted on the antioxidant potential of marine seaweeds. Reports on the antioxidant properties of seaweed extracts from India are limited<sup>5</sup>. Seaweed produces various types of antioxidant to counteract environmental stresses. Therefore, seaweed is a potential source of novel antioxidants<sup>6</sup>.

The brown macro algae *Sargassum muticum* is native to Japan and one of the most successful introduced seaweed species<sup>7</sup>. The brown algae are distinguished by their color which varies from olive green through light golden to a rather deep shade of brown. This is because of the presence of a golden brown xanthophylls pigment fucoxanthin (C<sub>4</sub>H<sub>5</sub>O<sub>6</sub>) in their chromatophores<sup>8</sup>.

Considering the immense medicinal properties of seaweeds, the present study was carried to analyze the phytochemical constituents of various extracts of *Sargassum muticum* and to compare the free radical scavenging activity and total antioxidant activity among the various extracts *Sargassum muticum*.

## MATERIALS AND METHODS

### Collection of seaweed:

The seaweed *Sargassum muticum* was collected from Kovalam beach, Kanyakumari district. The plant sample was authenticated by the Department of Botany, Avinashilingam University.

### Preparation of the sample:

The whole algae were washed in sea water and fresh water thoroughly to remove the epiphytes and other contamination. Then the sample was immediately transferred into a polyethylene bag with a small hole to leak out seawater drops and shade dried.

### Qualitative analysis of phytochemicals:

#### Screening test for the presence of phytochemicals:

For the screening of phytochemicals fresh sample was used. Five grams of the fresh sample was weighed and homogenized with 50ml of water, ethanol and hydrochloric acid (1%) separately. The extracts were boiled for 1 hour, cooled and filtered. The filtrate was used to screen for the presence of phytochemicals using standard procedure<sup>9</sup>.

### Preparation of the organic extract of the sample and phytochemical analysis:

The shade dried sample of *Sargassum muticum* was ground to coarse powder. Twenty gram of powder was weighed and wrapped with Whatmann No.1 filter paper and was successively extracted with 200ml of different solvents such as petroleum ether, benzene, chloroform, ethyl acetate, methanol and ethanol with their increasing order of polarity by soxhlation for 12-24 hours. The extracts were analyzed for the presence of phytochemical constituents using standard procedure

### Assay of free radical scavenging activity (DPPH activity):

The stable 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) was used for the determination of free radical scavenging activity of the extracts. Different concentrations (10-100 µg) of each of the extract of *Sargassum muticum* were added with an equal volume of DPPH (0.5 mM) methanolic solution. DPPH solution with methanol was used as positive control and methanol acted as negative control. When DPPH reacts with antioxidant, DPPH was reduced and the colour changed from deep violet to light yellow. After 30

minutes of incubation in dark at room temperature, the absorbance was measured at 517 nm<sup>10</sup>.

The percentage antioxidant activity was calculated by the following formula

$$\% \text{ scavenging activity} = \frac{\text{Absorbance of the sample}}{\text{Absorbance of the control}} \times 100$$

#### Evaluation of total antioxidant capacity by phosphomolybdenum method:

The phosphomolybdenum method was used to evaluate the total antioxidant activity of *Sargassum muticum*. Antioxidants can reduce Mo (IV) to Mo (V) and the green phosphate / Mo (V) compounds, which have an absorption peak at 695 nm, were generated subsequently.

0.3 ml of the sample was mixed with 3.0 ml of the reagent solution (0.6 M Sulphuric acid + 28 mM sodium phosphate + 4 mM ammonium molybdate). Reaction mixture was incubated at 95°C for 90 min in boiling water bath and cooled to room temperature. Absorbance of all the mixtures was measured at 695 nm against blank in UV spectrophotometer before and after the incubation. The blank solution contained 3.0 ml of reagent solution and the appropriate volume of the same solvent used for the sample and it was incubated under same conditions as rest of the sample. Total antioxidant

activity is expressed as the number of equivalents of ascorbic acid in milligrams per gram of extract.

$$\text{Total antioxidant activity} = 100 [ 1 - (A_o - A_t) / (A_o^o - A_t^o) ]$$

Where  $A_o$  is the OD of the sample at time  $t_o$  minutes and  $A_t$  is the time of the sample at time  $t = 90$  minutes.  $A_o^o$  and  $A_t^o$  represent the OD of the control at time  $t = 0$  minutes and  $t = 90$  minutes respectively<sup>11</sup>.

#### RESULT AND DISCUSSION

Nature has been a source of medicinal agent for thousands of years and an impressive number of modern drugs have been isolated from natural sources<sup>12</sup>.

#### Qualitative analysis for the presence of phytochemical:

The preliminary phytochemical investigation showed the presence of phytoconstituents such as carbohydrates, amino acids, phenols, sterols and steroids, saponins, alkaloids, flavonoids, tannin and terpenoids.

The whole part of *Sargassum muticum* was subjected to qualitative analytical tests for the various plant constituents such as amino acids, anthraquinones, alkaloids, carbohydrates, flavonoids, glycosides, saponins, steroids, tannins, terpenoids, phenols, fixed oils and fat. Table 1 shows the phytochemical constituents of various extracts of plant sample.

**Table 1.** Phytochemical constituents of various extracts of *Sargassum muticum*

Phytochemicals	Inference					
	B	C	E	EA	M	PE
Aminoacids	+	+	+	+	+	+
Anthraquinones	+	+	+	+	+	+
Alkaloids	+	+	+	-	+	+
Carbohydrates	+	+	-	+	+	+
Flavonoids	+	+	+	+	+	+
Glycosides	-	+	+	+	+	-
Saponins	+	+	-	+	-	+
Steroids	+	+	+	+	+	+
Tannins	+	+	+	+	+	+
Terpenoids	-	-	-	-	+	-
Phenols	+	+	+	+	+	+
Fixed oils and fats	-	-	-	-	+	-

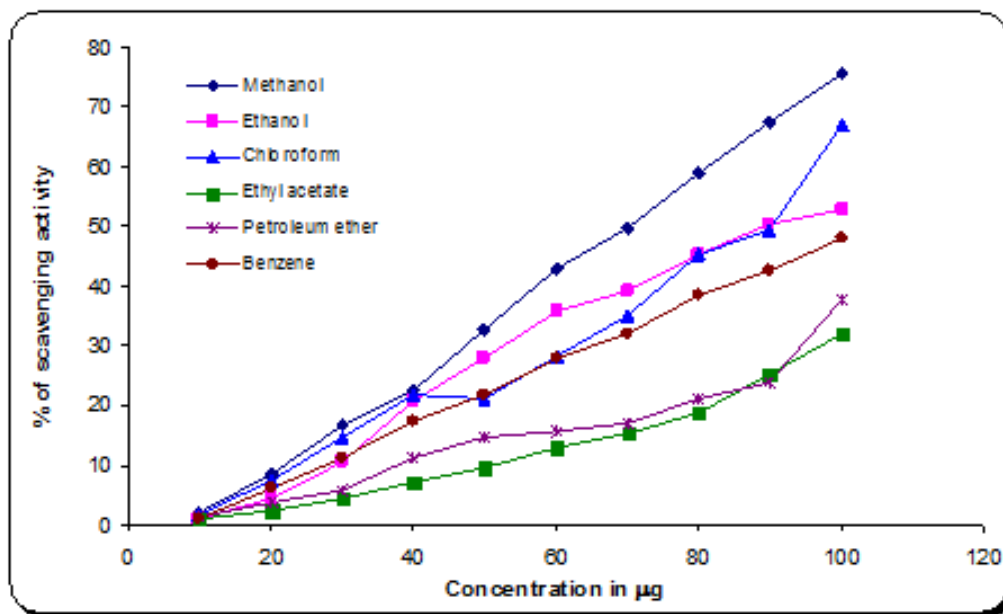
+ Presence; - Absence B = Benzene; C = Chloroform; E = Ethanol; EA = Ethyl acetate; M = Methanol; PE = Petroleum ether

Highest amount of the phytochemical constituents were found in methanolic extract followed by chloroform extract. Flavonoids, steroids and phenols were found to be present in all the extracts.

As per the earlier studies reports, seaweeds contain large amount of polysaccharides but less amount of protein and amino acids<sup>13</sup>.

The total phenol content of edible Irish brown seaweed, *Himanthalia elongata* was found to be at higher level<sup>14</sup>. Studies on brown seaweed, *Sargassum wightii* showed the presence of steroid and flavonoid

**Figure 1.** DPPH radical scavenging activity of various extracts of *Sargassum muticum*



The result showed that the methanolic extract of *Sargassum muticum* gives higher free radical scavenging activity followed by chloroform extract. Very less scavenging activity was seen in ethyl acetate followed by petroleum ether. The free radical scavenging activity increased as the concentration increased in all the extracts.

The efficacies of antioxidants are often associated with their ability to scavenge stable free radicals. Thus, the DPPH radical scavenging activity of methanolic extract demonstrated its oxygen radical absorbance capacity and indicated its potent antioxidant nature.

Studies on seaweeds such as *Fucus vesiculosus* and *Ascophyllum nodosum* found that methanolic extract of

<sup>15</sup>. Seaweeds are low in fats but contain vitamins and bioactive compounds such as terpenoids and sulphated polysaccharide a potential natural antioxidant which are not found in land plants property<sup>16</sup>.

#### Free radical scavenging activity of *Sargassum muticum*

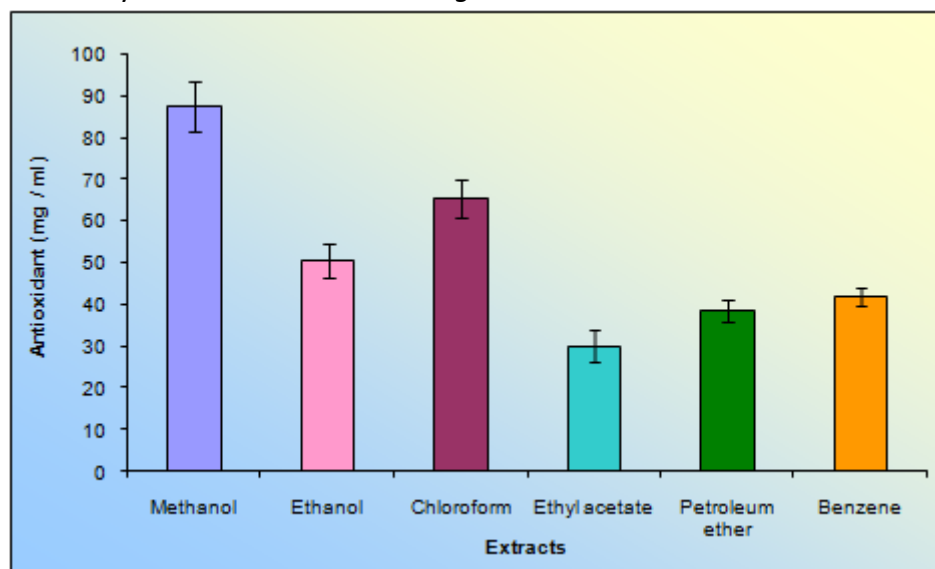
DPPH has been used extensively as a stable free radical to evaluate reducing substance and is a useful reagent for investigating free radical scavenging activity of the component<sup>17</sup>. Figure 1 indicates the DPPH radical scavenging activity of *Sargassum muticum* in different solvent extract with various concentrations.

*Fucus vesiculosus* and *Ascophyllum nodosum* scavenged DPPH radicals by 31.2% and 25.6% respectively. *Sargassum* species are found to have the highest free radical scavenging property<sup>16</sup>.

Investigation on methanolic extract of *Turbinaria conoides* showed significantly higher free radical scavenging activity than ethyl acetate and petroleum ether extracts<sup>11</sup>.

#### Total antioxidant activity of *Sargassum muticum*

Petroleum ether, benzene, chloroform, ethanol, ethyl acetate and methanolic extracts were evaluated for total antioxidant activity. Figure 2 indicates the total antioxidant activity of various extracts of *Sargassum muticum*.

**Figure 2** Total Antioxidant Activity of various extracts of *Sargassum muticum*

Methanolic extract of *Sargassum muticum* exhibited the strongest activity 85.32 mg/ml while ethyl acetate yielded the lowest value  $30.10 \pm 3.62$  mg/ml.

Estimation of the total antioxidant activity of three selected brown seaweeds- viz., *Sargassum marginatum*, *Padina tetrastomatics* and *Turbinaria conoides* indicated that different solvent fractions obtained from total methanolic extract exhibited higher antioxidant activity<sup>18</sup>.

Earlier studies on *Polysiphonia urceolata*<sup>19</sup> showed that the total antioxidant activity was maximum in the methanolic extract of the alga than any other extracts. In recent years a number of studies reported that seaweed extracts demonstrate strong antioxidant properties<sup>20</sup>.

From the study it may be concluded that *Sargassum muticum* is a good source of phytochemicals. Methanolic extract showed the highest DPPH activity and total antioxidant activity among the various organic extracts of *Sargassum muticum*.

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