ANTIOXIDANT ACTIVITY FROM *CYMODOCEA SERRULATA* SEAGRASS CRUDE EXTRACT

ABSTRACT:

INTRODUCTION: *Cymodocea* can be found in clear water and in the high intertidal areas.The antioxidant study of the extract of *C. serrulata* shows the highest free radical scavenging property on ethanol extract. This may be due to the presence of high phenolic compounds. The study brings out the medicinal value of *C. serrulata* which can be used as a nutraceutical compound in various food and pharmaceutical industries.Antioxidants are defending your body cell from damage caused by free radicals when they accumulated may cause oxidative stress. The synthetic antioxidants are producing more side effects, while natural antioxidants play a major role in scavenging free radicals without side-effects.

AIM: To analyse the antioxidant activity from *cymodocea serrulata* sea grass from crude extract.

MATERIALS AND METHODOLOGY : The fresh leaves of *Cymodocea serrulata* were collected from parangipettai coastal area, Tamil Nadu. The seagrass are washed thoroughly with tap water then shade dried on table tissue paper for 2-3 weeks and turn into a fine power. And 10g of dried powdered seagrass samples were mixed with 100ml of methanol/Ethanol.

Using DPPH assay, to extract 0.1ml add equal volume of DPPH 0.1 ml . After 20 min , absorb at 517 nm. Ascorbic acid is used as a standard concentration and DPPH was calculated.

RESULT: The result concludes that *Cymodocea serrulata* showed good antioxidant activity. when concentration increases the percentage of the zone of inhibition is also increased, which shows that it shows a good antioxidant activity.

CONCLUSION: In this study antioxidant activity was checked by using *Cymodocea serrulata*. Using DPPH assay , it has concluded that it has strong antioxidant activity from crude extract. In the future it can be used for medication and further studies can be done by using individual components for various particles.

KEYWORD: antioxidant activity, *cymodocea serrulata*, sea grass, DPPH assay, marine.

INTRODUCTION:

Sea grass is an endophytic fungus which grows in the southern region . It produces anti metabolite which grows from the marine plant *cymodocea serrulata(1)*. In marine ecosystems, fish depend on the marine plant and vegetation. The *cymodocea* is a ribbon grassy leaf which is a terrestrial and flowering plant which is submerged in the water and it can see more in the subtropical coastal area and it will grow well in muddy and Sandy regions. And it has antibacterial activity. Plant related products are useful in medicinal purposes such as anti inflammatory, anti cancer, antibiotic (2). Ethanolic leaf shows antioxidant and antibacterial activity. The bioactivity of sea grass is assessed on lung fibroblast cell lines. In this it contains a high level of carotenoids, mainly xanthophylls with antioxidant roles (3). Endophytic fungi are mostly monocotyledon and dicotyledon and seagrass are monocotyledon and flowering plants (4). with diphenyl 1- picryl hydrazyl radical it has reduced power on marine extract. Marine algae act as food material and pharmaceutical for treating oxidative disease (5).

Seagrass of the mandapam coast region has high levels of phenol and high reducing power. Antioxidant activity has a high percentage of DPPH radical scavenging activity (6). And this is used for various oxidative stress related diseases . DPPH activity is better than vitamin -c ofthe sea grass . and it produces the free radical which has an antioxidant activity (7). High phenolic and flavonoid content was found in the plant species . and it is found in many herbaceous plants (8). The antibacterial property of *cymodocea serrulata* was tested against the human pathogen in that ethyl acetate shows maximum activity against the pathogen (9). It is a potential bio reductant. It grows rapidly and is eco-friendly towards cancer therapy (9,10). It is more tolerant to burial and light attenuation; it can form a canopy in a higher position above the bottom due to the presence of vertical rhizomes (11). Chloroform and methanol extract showed effective

inhibition against alpha amylase. It shows glucosidase inhibition which is mainly responsible for antidiabetic action (12) .

Antioxidant activity of water extract plants was evaluated in that it shows strong antioxidant activity. The plant extracts the highest superoxide radical scavenging activity and acts as a natural antioxidant (12,13). *Cymodocea spp* used as tranquilizer babies during pregnancy and were used for cough and malaria and leprosy (14). It shows the reduced MIC and shows no inhibition. In methanol and ethyl acetate it shows maximum pathogen. Natural products produce important sources for antibacterial agents. Marine species comprise total global biodiversity and antimicrobial seagrass encourage travelling organisms. Isolation of sea grass produces biological molecules which prove to produce new drug discovery (15).

There was no study conducted about the antioxidant activity *cymodocea serrulata* in the previous studies. Our team has extensive knowledge and research experience that has translated into high quality publications (16–20),(21),(22),(16),(23),(24),(25),(26)(18,27,28),(29–33). In future , further studies can be done with other activities of *cymodocea serrulata* from different regions and with different activities. And the aim of this study is to determine the antioxidant activity of *cymodocea serrulata* sea grass from crude extract.

MATERIALS AND METHODOLOGY:

Collection of plant material and preparation

The fresh leaves of *Cymodocea serrulata* were collected from Parangipettai coastal area, Tamil Nadu. The seagrass is washed thoroughly with tap water then shade dried on table tissue paper for 2-3 weeks and turned into a fine powder.

Preparation of extraction: 10g of dried powdered seagrass samples were mixed with 100ml of methanol/Ethanol (V/V) and allowed to place for 24 hours at ambient temperature. Then the mixture was passing through whatman filter paper (No.4) then the filtrate was centrifuged at 3000 rpm for 10min and further filtered by 0.45µm syringe micro filter. At last, the solvents are

evaporated via vacuum rotary evaporator until samples are obtained in powder form. Then the sample was stored in a shadowy aluminum container at 4ºC for further analysis.

Total antioxidant activity: Total antioxidant activity of the crude seagrass extract was determined by following method: 0.3 ml of sample was prepared in different concentrations (0.5– 3 mg/ml) with 3 ml of reagent solution (0.6 M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). Reaction mixture was incubated at 95ºc for 90 minutes in a water bath. Absorbance of all sample mixtures was measured at 695 nm. Total antioxidant activity has been expressed as the number of equivalents of ascorbic acid.

DPPH Assay : The antioxidant potential of seagrass crude extract was determined on the basis of their scavenging activity of the stable 1,1- diphenyl-2-picryl hydrazyl (DPPH) free radical. Different concentrations (0.5-3mg/ml) of samples were mixed with 2.9ml diphenylpicrylhydrazyl (DPPH) solution (120µM) in methanol and incubated in darkness at 37°c for 30 minutes. The absorbance was recorded at 517 nm. Inhibition of free radical by DPPH in percentage (I %) was calculated with the following equation:

$$\text{Percentage of Inhibition (I \%)} = (A_{blank} - A_{sample}) / A_{blank} \times 100$$

Where, A_{blank} is the absorbance of the control reaction and A_{sample} is the absorbance of the test compound. The values of inhibition were calculated for the various concentrations of the sample.Ascorbic acid was used as positive control (Kamala et al., 2015) and all the tests were carried out in triplicate.

Total reducing power

Reducing capacity of crude extract obtained from the seagrass extract were determined by following method: Briefly, 1 ml of Benzene: chloroform (2:1) containing different concentrations of extract (0.5-3mg/ml) were mixed with 2.5 ml of benzene: chloroform and 2.5 ml potassium ferricyanide (1%) reaction mixture was incubated at 50ºC for 20 min. After incubation, 2.5 ml 10% trichloroacetic acid was added and centrifuged at 10,000 rpm for 10 min

2.5ml. The upper layer was mixed with 2.5ml distilled water and 0.5ml $FeCl_3$ (0.1%) and the absorbance was measured at 700 nm.

RESULTS:

Figure:1

The fresh leaves of *Cymodocea serrulata* which are collected from parangipettai coastal area, Tamilnadu.

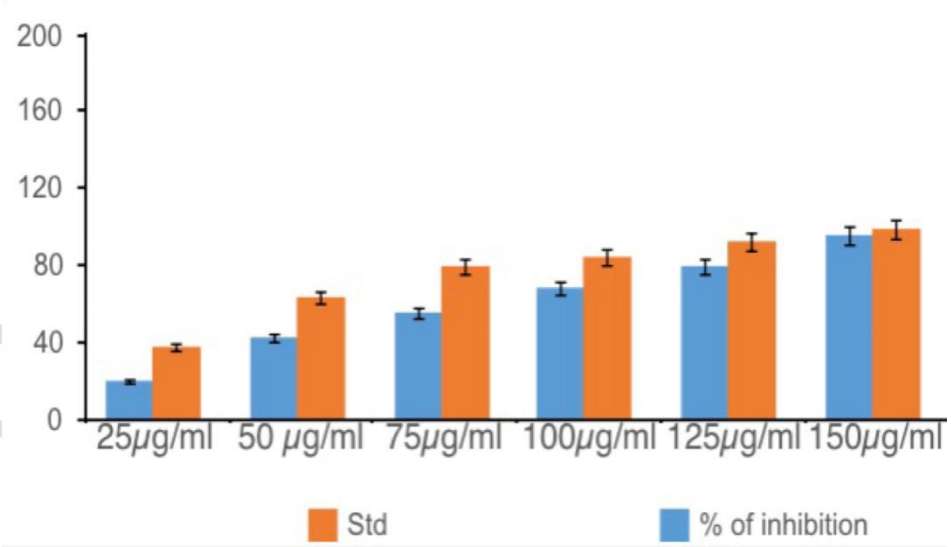

Figure 2: Graph representing the antioxidant activity of *cymodocea serrulata* sea grass. In this x-axis represents the concentration level and y- axis represents the % DPPH inhibition, data implies as mean±SEM .

In this orange denotes standard solution and blue denotes the percentage of inhibition. When the concentration increases the percentage of inhibition also increases which shows a good antioxidant activity.

TAA	AAE
25µg/ml	36.85±1.22
50 µg/ml	54.69±1.3
75µg/ml	72.84±0.9
100µg/ml	98.61±1.22
125µg/ml	114.75±1.31
150µg/ml	137.65±1.3

Table :1 Total antioxidant activity of *cymodocea serrulata* (sea grass).

TRP	AAE
25µg/ml	12.62±1.21
50 µg/ml	31.49±1.3
75µg/ml	42.68±0.9
100µg/ml	57.29±1.23
125µg/ml	68.38±1.31
150µg/ml	81.57±1.3

Table:2 Total reducing property of *cymodocea serrulata* sea grass.

DPPH	% of inhib	Std
25µg/ml	19.27	37.3
50 µg/ml	41.52	62.7
75µg/ml	54.38	78.52
100µg/ml	67.24	83.59
125µg/ml	78.65	92.4
150µg/ml	94.38	98.6

Table 3: shows the antioxidant activity of *cymodocea serrulata* by DPPH assay.

DISCUSSION:

In the current study the result shows that *cymodocea serrulata* seagrass show good antioxidant activity from the crude extract. In fig -2 the graph represents the antioxidant activity of *cymodocea serrulata* sea grass from the crude. In this x-axis represents the concentration level and y- axis represents the % DPPH inhibition. In this orange denotes standard solution and blue denotes the % of inhibition. When the concentration increases the percentage of inhibition also increases which shows a good antioxidant activity. The antioxidant activity of the plant shows a percentage of 94% which is not as good as the standard but can be used as a potent antioxidant. Similar studies on the same species show a similar range of antioxidant activity.Previous studies on nutmeg shows that it was an antioxidant of 89.37 percent which is in comparison with the standard of 98.61% doing that these steps can be used as a potent antioxidant (34). The active extract of *C.serrulata* showed maximum inhibition against *E.coli* and the compound phenyl thioketone was analysed for its antibacterial activity and was proved good and can be used for therapeutic purposes (35,36).

The bioactive compounds from endophytic bacteria showed maximum sensitivity with minimum concentration than the bioactive compounds from epiphytic bacteria and other biological origin. Hence, steps have been undertaken to find out the reason for the maximum activity of endophytic bacteria from seagrasses (37). In previous studies it is reported that the application of the extract of seagrass proves toxic less and does not produce any harmful effect and it has better free radical scavenging activity which justifies the results of our present study (38) & (39). In previous studies it is also reported that the extract has significant Minimum inhibitory concentration and Minimum bacterial concentration against all the bacteria; pathogens and it is more sensitive against *Pseudomonas aeruginosa* (40). Our team has extensive knowledge and

research experience that has translated into high quality publications (41) (42) (43) (44) (45) (46) (47) (48) (49) (50) (51) (52) (53) (54).

There are some potential limitations, that is the study is taken into consideration with only one marine plant and it should be considered to be done on a large scale. And there is a high possibility of occurrence of error. The study was carried out in-vitro and it cannot be assumed that the result of antioxidant activity could be translated into clinical effectiveness which proves to be a limitation. In future clinical trials and animal experiments can be done to check the toxicity of the extract. In the future it can be formulated as an alternative drug and commercial products can be prepared which will possess a great and potential value in the herbal markets.

CONCLUSION:

Using DPPH assay , *cymodocea serrulata* sea grass has strong antioxidant activity from crude extract. In the future it can be formulated for medication. It consists of methanolic compounds, phenolic compounds, flavonoids and isolated components(55-64). Each of these properties plays a key role in the advancement of human health. From the present study it is evident that Sea grass possesses a good antioxidant activity. The significant health benefits of sea grass have been explored. Further investigations are necessary to provide additional clinical evidence against the free radical scavenging activity.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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