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POTENTIAL ANTIOXIDANT ACTIVITY FROM METHANOLIC EXTRACT OF *HALOPHILA OVALIS*

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ABSTRACT:

Seagrasses play an important ecological role in fisheries production, sediment buildup and stabilization. It has direct value to people as food, feed, green, manure, and medicine. Seagrass species have been found to be potential sources of antibacterial, antifungal and anti-inflammatory agents as well as anti-cancer substances by phytochemical investigations. The main aim of this study is to prepare *Halophila ovalis* extract powder and to determine the antioxidant activities of methanolic plant extract.

Materials and Methods:

The antioxidant activity of methanolic extract of *Halophila ovalis* was investigated by TAA, DPPH and H2O2 assay.

Result:
The study revealed that methanol extract of *Halophila ovalis* has greater antioxidant activity. Flavonoid content was found highest in methanol extract of *Halophila ovalis.* It showed high radical scavenging activity.
Conclusion:
The study revealed the potential of the *Halophila ovalis* as natural sources of antioxidants having considerable commercial importance.

KEYWORDS: Antioxidant activity, flavonoids, *Halophila ovalis,* phenolics and sea grasses.

INTRODUCTION:

Seagrasses were found predominantly within the areas of intertidal region, with an approximate land surface area of vertical distribution ranging between the level of 0.2 m below lowest astronomical tide (LAT) and 1.4 m above LAT in a region that makes us experience a tidal range of 3 m in common (1). The dominant seagrass or a major contributor in the area was *H.ovalis*, a small or tiny in height broad-leafed seagrass that occurred or found as an understorey species with a low degree of biomass and a patchy distribution. Environmental changes are extremely pronounced for intertidal seaweeds. Production or release of ROS like the superoxide radical is a significant reaction to stress by aerobic cells that use oxygen-oriented cells. (2)

Halophila ovalis, often called paddle weed, spoon grass, or dugong grass, is a member of the Hydrocharitaceae family of seagrasses. It is a tiny herbaceous plant that grows in the Indo-saltwater Pacific's habitats, including sea beds. The plant grows on soft sand or mud substrates near reefs, estuaries, islands, and intertidal zones. The stems that protrude from the rhizome beneath the sand bear ovate-shaped leaves. The roots grow up to 800 mm long and have tiny root hairs all over them. It frequently grows in meadows that cover a sand bank or other area of the ocean floor. The layout of the plant, both above and below the surface, stabilises the ocean floor and offers habitat for other species. It is consumed by dugong.

All aerobic organisms produce or secrete ROS, which have the potential to easily react with the majority of biological molecules, particularly proteins, lipoproteins, and DNA. This can result in a wide range of patho-physiological disorders or disabilities, including diabetes, inflammation, cancer, and genotoxicity. Reactive oxygen species (ROS) that are physiologically harmful can be scavenged or removed by antioxidants.(3)

The most common ROS is hydroxide ion and have the shortest period of half-lives. Moreover, H2O2 peroxide ion converts into 1O2 and HO- radicals, which then later on become very powerful oxidizing agents among other agents . Not only 1O2 and HO-, but also H2O2 can cross membranes and may oxidize a number of compounds (4).

Biologically active metabolites with new structural properties can be found in abundance in marine species. Numerous marine-derived, chemically distinct compounds with diverse biological activities have been discovered to date. A number of these compounds are currently the subject of research and are being developed into novel medications (5).

Angiosperms that live in brackish or marine environments include seagrass. It is a rare plant that can withstand extreme salinity, immersion, sporadic dedication, anchoring to the seafloor, and hydrophilic pollination (6). It is well recognised that seagrass habitats are extremely productive and have a significant ecological role as spawning grounds for fish and crustaceans like shrimp, as a source of food and a place to hide for a variety of creatures, and in the recycling of nutrients. Additionally, it has been demonstrated that phytochemicals have antibacterial properties and are utilized to treat a variety of bacterial and fungal illnesses. Finding novel antimicrobial compounds with an effective chemical structure as a treatment for microbial infections is urgently needed today. (7)

In biological systems, antioxidants serve a variety of purposes, including preventing oxidative damage and assisting the main cell signalling pathways. Antioxidants' main function in cells is to stop the harm that reactive oxygen species can do to cells (ROS) During excessive metabolism, living things produce reactive oxygen species (ROS), comprising peroxide radicals, hydroxyl radicals, superoxide radicals, and nitric acid radicals. Oxidative stress, which is brought on by high ROS levels, can lead to a number of pathological disorders, including cancer, aging, and cardiovascular disease. (8)

As a result, much recent research has concentrated on antioxidant phytochemicals like flavonoids. Polyphenols are particularly significant antioxidants because of their high redox potentials, which enable them to function as reducing agents, hydrogen donors, and singlet oxygen molecules.(9)

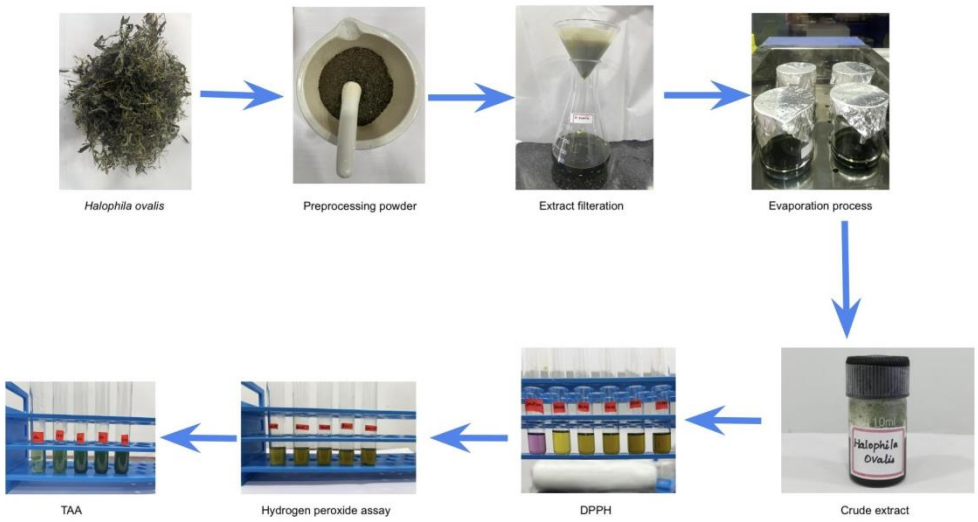
Seagrasses are a rich source of antioxidant activity, according to data from more recent times. A variety of screening assays, including but not limited to high overall relative DPPH radical scavenging capacity assay and HO radical scavenging capacity assay, were created and utilised to search for prospective antioxidants. (10). Our team has extensive knowledge and research experience that has translate into high quality of publications (11–24)

The main aim of this study is to prepare *Halophila ovalis* extract powder and to determine the antioxidant activities of methanolic extract.

MATERIALS AND METHODS:

Preparation of Methanolic crude extract:

The seagrass sample of *Halophila ovalis* was collected at Tuticorin district, Tamilnadu. The pre-processing of the sample includes cleaning and washing using distilled water. The pre-processed sample was kept for drying in a hot air oven at below 60C. After drying, the sample was crushed into a coarse powder, using mortar and pestle. Then add 100g of sample in 200ml of 70% methanol in a conical flask and then place in a shaker for a couple of days. Then Whatman filter paper was used to filter the extract through.The filtrate samples were replaced in water bath below 60C to concentrate and get the crude extracts.



Summary of preparation of crude extract of *H.ovalis*

RESULTS:

In this present study, Total Antioxidant activity, DPPH radical scavenging activity, Hydrogen peroxide radical scavenging activity were determined for methanolic extract of *Halophila ovalis*.

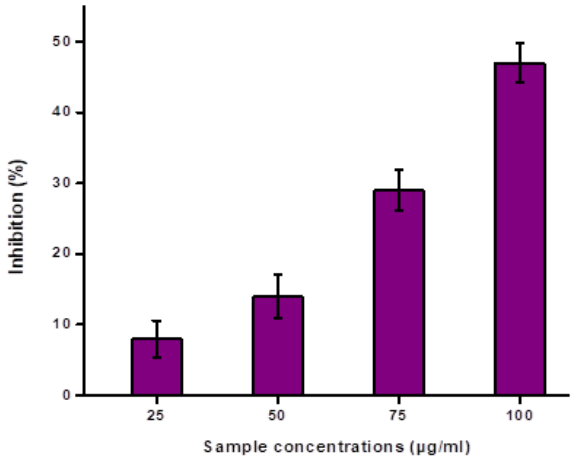


Figure 1: This graph shows total anti oxidation activity which is calculated with the help of percentage of inhibition in accordance with concentrations. If the concentration increases, the percentage of inhibition increases .

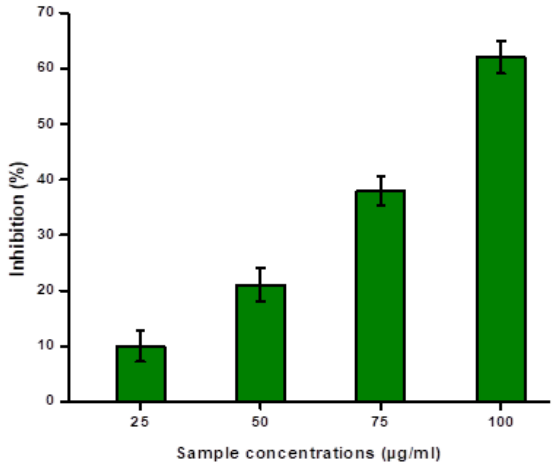


Figure 2: DPPH radical scavenging assay , it is an accepted method to screen the antioxidant activity of plant extracts . Violet Color DPPH is reduced to yellow in a concentration dependent manner.

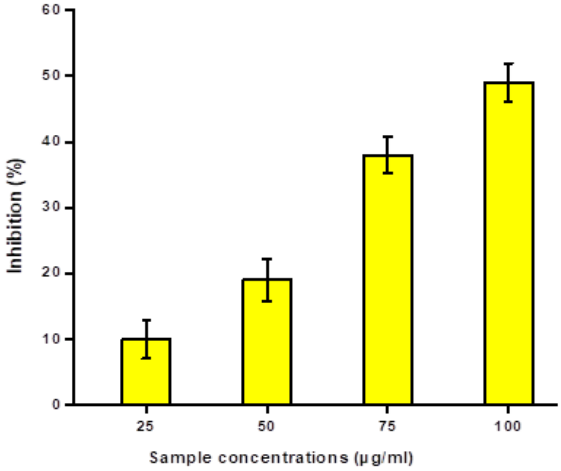


Figure 3: The classical method to determine the hydrogen peroxide scavenging activity of plant extracts is evaluated by measuring the disappearance of hydrogen peroxide at a wavelength of 230 nm.

µg/ml	**TAA**	**S.Er**	**DPPH**	**S.Er**	**H_2O_2**	**S.Er**
25	10	2.8	8	2.6	10	2.9
50	21	3	14	3.1	19	3.2
75	38	2.6	29	2.9	38	2.8
100	62	2.9	47	2.8	49	2.9

Table 1: The concentration and the percentage of inhibition of plant extracts in every assays in a concentration dependent manner

DISCUSSION:

Through the TAA, DPPH, and H_2O_2 assays, the antioxidant activity of *Halopila ovalis* plant extract was investigated. *Halophila ovalis* methanolic extracts were tested for their antioxidant capacity. At a concentration of 100 g/ml, the extracts demonstrated significant radical blocking properties. Calculations were made to compare *Halophila ovalis* radical-scavenging capacity to that of regular ascorbic acid. The outcome demonstrated that as sample concentration increased, scavenging activity also did so. (25)

The extract's ability to lower stable free radicals can be linked to its increased phenolic and flavonoid content, which are known to have antioxidant activity. It is widely known that the ability of plant extracts containing polyphenol components to act as electron or hydrogen donors and to snare free radicals accounts for the antioxidant action of these extracts. Therefore, it may be inferred that the increased phenolic and flavonoid content of the methanolic extract of *Halophila ovalis* may be the cause of its enhanced antioxidant activity. (26)

Several metabolites with a variety of biological functions have been obtained from seagrasses. Phytoconstituents like vitamins especially C and phenolics like tannin, have been linked to seagrasses, terrestrial plants, and algae as antioxidants that scavenge radicals like H2O2. (27)

In addition to their primary defense function, phenolic compounds, which are frequently found in plants, have been reported to have a number of biological actions, including potential as antioxidants and free radical scavengers. The primary mechanism of action of polyphenols is thought to be their redox potentials, which are critical in absorbing and neutralizing free radicals, quenching singlet oxygen, or breaking down peroxides. According to earlier studies, seagrasses, particularly their polyphenols, show antioxidant action. (28)

The acronym DPPH is frequently used to refer to the organic molecule 2,2-diphenyl-1-picrylhydrazyl. It is a crystalline powder with a dark tint that is made up of stable free radical molecules. DPPH has two main uses, both in laboratory research: one is a standard for the location and strength of electron paramagnetic resonance signals, and the other is a monitor of chemical reactions involving radicals, most notably it is a frequent antioxidant assay. In plant biochemistry, the 2,2-diphenylpicrylhydrazyl (DPPH) assay is frequently used to assess a plant's constituent's capacity to scavenge free radicals. The technique is based on spectrophotometric analysis of the change in DPPH concentration brought on by the interaction with an antioxidant. For this assay, several methods have been employed under various conditions, including various reaction periods, solvents, pH levels, and various chemicals used as antioxidant standards.

Free radical donor DPPH has been extensively utilized to assess the capacity of natural antioxidants to scavenge free radicals. Positive correlations between total phenolic compound and radical scavenging activity have been documented by numerous researchers. It has been

found that as the quantity of phenolic compounds grew, so did the activity of radical scavenging. Ascorbic acid, the positive control, demonstrated much stronger antioxidant activity.
Through a SET mechanism, the Total Antioxidant Capacity (TAC) Assay calculates the total antioxidant capacity of biomolecules from various samples. Antioxidants cause copper(II) to be converted to copper (I). The chromogen and copper (I) ions then interact to create a hue with a peak absorbance at 490 nm.

The simplest peroxide and a reactive oxygen species with an oxygen-oxygen single bond is hydrogen peroxide. When exposed to light, it breaks down gradually, but when organic or reactive substances are present, it breaks down quickly. It is normally kept in a dark bottle with a stabilizer in a weakly acidic solution. In biological systems, including the human body, hydrogen peroxide is present. Peroxidases are a subclass of enzymes that utilise or break down hydrogen peroxide.
The H2O2 approach outperformed the NO method in scavenging most marine species' methanol and water extracts.(29)
This discovery has ramifications for both the environment and medicine. According to reports, several of these antioxidants function as UV biofilters and lessen the negative effects of UV light.. (30)

CONCLUSION:

The results of this study showed that the *Halophila ovalis* methanol extract has high antioxidant activity. The methanol extract of *Halophila ovalis* was high in phenol and flavonoids, which may be due to its strong antioxidant activity. Our study has shown that seaweed is a new source of antioxidants, consistent with previous studies, and is endowed with the properties of dietary supplements. These noble antioxidant vitamins and especially phenolic compounds in seaweed, can help reduce the ill effects of pollution and help manage coastal areas.

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Author Contributions:

Amba Esakki: Literature search, Data collection analysis, Manuscript drafting
Dr.R. Gayatri Devi : Data Verification, Manuscript draft
Dr.J.Selvaraj: Data collection analysis, Data Verification, Manuscript draft

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