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CRUDE EXTRACT PREPARATION FROM *Halophila Ovalis* SEAGRASS AND ITS ANTICOAGULANT AND ANTI-INFLAMMATORY POTENTIAL

Esakki Amba [1], Monal Yuwanati [2]* and Pitchiah Sivaperumal [3]

[1] Pathology, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Science (SIMATS), Saveetha University, Chennai, Tamil Nadu, India. Email: 152101005.sdc@saveetha.com, ORCID ID: 0009-0002-7381-8957
[2] Professor, Department of Oral Pathology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Science (SIMATS), Saveetha University, Chennai, Tamil Nadu, India. *Corresponding Author Email: monalyuwanati.sdc@saveetha.com
[3] Chief Scientist and Assistant Professor, Marine Biomedical Research Lab & Environmental Toxicology Unit, Cellular and Molecular Research Center, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Science (SIMATS) , Saveetha University, Chennai, Tamil Nadu, India. Email: sivaperumalp.sdc@saveetha.com

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Abstract

In recent years, natural antioxidants have drawn particular interest. Natural antioxidants are hard to get by because manufactured substances are known carcinogens. The marine plants known as seagrasses are currently receiving notice for their secondary metabolites. Seagrasses play an important ecological role in sediment accumulation, stability, and fisheries productivity. It can be used directly to benefit people as food, feed, medicinal, green manure, and vegetation. In the estuaries of south-west Western Australia, *Halophila ovalis* is the predominant seagrass species. Its global range is likewise extensive, encompassing Japan and the tropical Indo-West Pacific regions. The main objectives of this work are to powder the *Halophila ovalis* extract and determine the anti-inflammatory and anticoagulant characteristics of the plant extract. **Materials and Methods:** *Halophila ovalis* crude extract was prepared in order to assess its anticoagulant and anti-inflammatory properties. **Result:** The potent anti-inflammatory properties of the ethanolic extract of *Halophila ovalis* are attributed to its phytochemicals. (66% at a 100 microgram/ml dosage) via preventing protein denaturation. **Conclusion:** Since *halophila ovalis* lengthens the bleeding duration, it can be used as a natural anticoagulant source. It has anti-inflammatory qualities as it has the ability to reduce inflammatory mediators.

Keywords: Anti Inflammatory Activity, Anti Coagulant Activity, Flavonoids, *Halophila Ovalis,* Phenolics, And Sea Grasses.

INTRODUCTION

As a typical defensive reaction to tissue damage, inflammation is caused by a variety of processes, including the release of mediators, cell migration, tissue disintegration, healing, and fluid extravasations.[1] It's a complicated process involving membrane alterations, increased protein denaturation, and vascular permeability that frequently results in discomfort.[2] Hazardous stimuli such as pathogens, irritants, or damaged cells in vascular tissue cause inflammation.[3] Inflammation is an organism's defensive attempt to get rid of harmful stimuli and start the tissue's healing process.[1,4] Conversely, atherosclerosis, rheumatoid arthritis, and vasomotor rhinorrhea can result from untreated inflammation.[5] It is believed that some inflammatory disorders cannot be effectively treated by current treatments, such as opioids and non-steroidal anti-inflammatory medicines (NSAIDS), due to their potency and side effects. [6]Therefore, exploring other options appears wise and crucial. There is an increasing interest in discovering novel anti-inflammatory compounds from natural sources due to the

extensive usage of nonsteroidal anti-inflammatory drugs and commercially accessible analgesics.[7]

Heart disease has been one of the main causes of death in the recent past. Heart attacks and strokes caused by blood clots that have developed in the wrong areas are the most dangerous of these disorders.[8] As a homeostatic mechanism, the body employs blood coagulation to halt bleeding or hemorrhage caused by injury or damage to blood vessels. [9]Conditions such as hemophilia, resulting from a lack of specific coagulation factors, can be exceedingly harmful or even lethal. On the other hand, aberrant blood clots and unnecessary coagulation can impede normal blood flow and prevent oxygen and nutrients from getting to the surrounding tissues.[10,11] In addition to their intended therapeutic effects, most pharmaceutical drugs have side effects that can be experienced. [12]For example, warfarin, a common anticoagulant medication, can cause bleeding gums, red or dark brown urine, red or black bowel movements, nosebleeds, hemoptysis, dyspnea, swelling and discomfort.[13] On the other hand, traditional therapies have a well-established reputation for having fewer adverse effects. Thus, we argue that the use of seagrasses and herbs with few side effects and therapeutic benefits may be the best approach for the prevention and treatment of various ailments, including cardiovascular disorders. [14]

Halophila ovalis is a seagrass belonging to the Hydrocharitaceae family, which is also known by the names spoon grass, paddle weed, and dugong grass.[15]It is a little herbaceous plant that grows well in the Indo-Saltwater Pacific on sea beds and various environments. Little root hairs can be seen all the way along the 800-mm roots. It typically appears on the ocean floor in meadows that cover sandbanks or other areas. [16]The plant's structure supports the ocean floor and provides a haven for a range of species that live both above and below the surface.[17] A multitude of physiologically active metabolites with unique structural traits can be found in marine animals. In addition, a vast array of distinct chemical compounds derived from marine sources and exhibiting a range of biological activities have been discovered.[18] Currently, a lot of these compounds are being studied and developed into novel medications. The primary objectives of this work are to prepare an extract from *Halophila ovalis* and evaluate its inflammatory and anticoagulant qualities.

MATERIALS AND METHODS

Preparation of Ethanolic Crude Extract:

The *Halophila ovalis* seagrass sample was collected in the Tuticorin region of Tamil Nadu. As part of the pre-processing stage, the sample was cleaned and rinsed with distilled water. The preprocessed sample was kept in a hot air oven below 60°C to dry it out. After the sample was dry, a mortar and pestle was used to crush it into a coarse powder. Next, 100g of the sample was added to a conical flask that has been filled with 200 ml of 70% ethanol, and shake for a few days. After that, the extract was filtered using Whatman filter paper. The filtrate samples were swapped out in a water bath that was kept at or below 60 degrees Celsius in order to extract the crude oils.

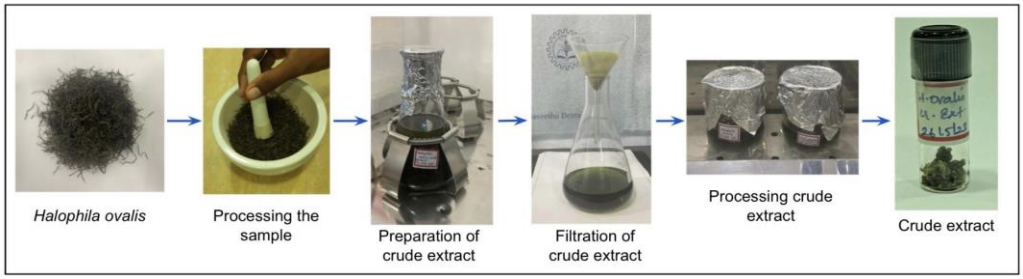

Figure 1: Summary of Preparation of Crude Extract of *H.ovalis*

Anti-coagulant Activity Test:

Prothrombin Time (PT):

According to Osoniyi and Onajobi, The prothrombin time was calculated using Brown's technique. As directed, the calcium thromboplastin reagent was reconstituted with distilled water and pre-warmed in a water bath for about ten minutes at 37 °C. After pipetting 0.1 mL of plasma into clotting tubes, the sample was incubated for two to three minutes at 37°C in a water bath. Next, 25, 50, 75, and 100 µg/mL of plant extract were used for the test, and 0.1 mL of PBS was added as the control. A timer was set, and the calcium thromboplastin reagent (0.1 mL) was quickly added to the mixture. Up until a clot developed, the tubes were progressively slanted at regular intervals (at a 45° angle). The time was recorded at the point the stopwatch was instantly stopped.

Activated Partial Thromboplastin Time (aPTT):

APTT was determined by applying Brown's method, as described by Osoniyi and Onajobi, using a kit of Diagen Kaolin reagents. The partial thromboplastin reagent (phospholipids and kaolin) was reconstituted in compliance with the instructions. The resultant suspension was individually pre-warmed at 37°C in a water bath, as was 0.02mL calcium chloride. After pipetting 0.1 mL of plasma into each clotting tube, the partial thromboplastin reagent (0.1 mL) was quickly added, and the tubes were left in a water bath at 37 °C for two to three minutes. After a further three minutes of incubation, the mixture for the experiment was mixed with 0.1 mL of PBS (as a reference) and an extract solution that included 25, 50, 75, and 100µg/mL of plant fractions. Simultaneously with the addition of 0.1 mL of preheated calcium chloride, a stopwatch was set. The tubes were gradually tilted at regular intervals until a clot formed. The stopwatch was instantly stopped and the time recorded as soon as the clot formed.

Anti-inflammatory Activity Test:

An anti-inflammatory test is the protein denaturation inhibition study. The reaction mixtures were incubated at 37°C ± 2°C for 15 to 20 minutes while submerged in a water bath. The mixture was then heated to 70°C and left for five minutes. The reaction mixture was then allowed to cool at room temperature for fifteen minutes. The absorbance of the reaction mixture was measured before and after denaturation at 680 nm for each concentration (1000µg/mL, 100µg/mL, 10µg/mL, 1µg/mL, 0.1µg/mL, and 0.01µg/mL) using a colorimeter. After every test was run three times, the mean absorbance was determined. The percentage of inhibition was computed on a percentage basis in relation to the control using the following formula.

Percentage of inhibition (%) = Absorbance of control - Absorbance of test

Gas Chromatography-Mass Spectroscopy Analysis:

After separating samples of green and brown algae, the separated fraction was examined using GC-MS. Using a capillary column and a Perkin Elmer Clarus Series Gas Chromatographic System, the phytoconstituents of the seagrass sample were examined. In order to identify the products, retention periods and fragmentation patterns in the library search results kept in the GC-MS program (TurboMass ver 5.4.2) were compared with mass spectra and recognized reference complexes. The following parameters for analysis were applied to the samples: A capillary column containing Elite-5MS (5% phenyl, 95% dimethyl polysiloxane) fused at a thickness of 30 µm and a diameter of 250 µm. The spectroscopic detector for GC-MS was intended to function at 70°C, 6°C to 150°C for two minutes, and 6°C to 290°C for five minutes. This was achieved by using an electron ionization system that ran at 70 eV with helium as the carrier gas flowing at a rate of 1 ml/min. The injectable sample had a split ratio of 10:1 and a volume of one liter. The temperature of the injection and the detector were 250°C. The range of mass spectra that were gathered was between m/z 40 and 500 amu. The GC-MS data were compared with the standard chemical library.

RESULTS

In this present study, prothrombin time, activated partial thromboplastin time, and percentage inhibition of protein denaturation were determined for the crude extract of *Halophila ovalis*.

Prothrombin Time

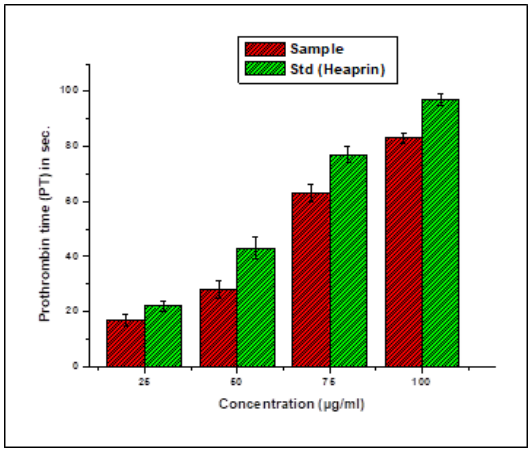

Figure 2: This Graph Shows Prothrombin Time

The anticoagulant activity of seagrass extracts was assessed using the browns technique by analyzing the prothrombin time in seconds. Evaluation of anticoagulant activity at 25 and 50 microgram/mL of standard heparin revealed prothrombin time at 22.5 sec and 42.6 sec, respectively. When the concentration of *Halophila ovalis* extract was increased, anti coagulant capabilities were increased at concentrations of 25 microgram/mL (p 0.05). Between the extract concentrations of 25 microgram/mL (22.5 sec) and 30 microgram/mL (23.7 sec), there was no difference (ns#). The ethanolic extract of *Halophila ovalis* has a maximum anticoagulant activity at a concentration of 100 microgram/mL and that prothrombin time was found to be 80 seconds. Standard heparin at the same concentration (100 micrograms/mL) shows a prothrombin time of 95 seconds.

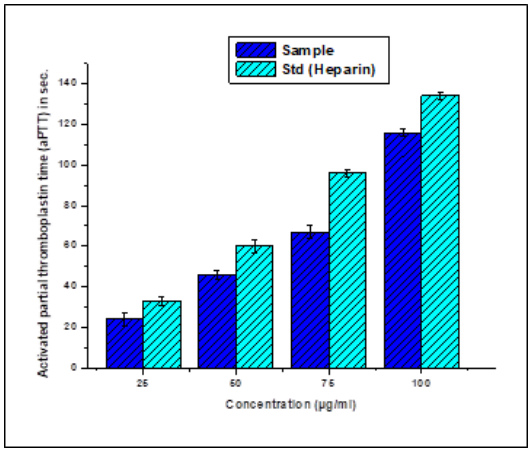

Figure 3: This Graph Shows The Activated Partial Thromboplastin Time

The anticoagulant capacity of seagrass extracts was assessed using the hydrogen peroxide assay by assessing how well they were able to scavenge H2O2 from tissues. Evaluation of anticoagulant activity at 25 and 50 microgram/mL of standard Heparin revealed 28.6 seconds and 60 seconds, respectively. When the concentration of *Halophila ovalis* extract was increased, aPTT increased at concentrations of 25 microgram/mL (p 0.05). Between the extract concentrations of 25 micrograms/mL (21.5%) and 3 micrograms/mL (23%), there was no difference (ns#). The ethanolic extract of *Halophila ovalis* has a maximum aPTT activity of 110 seconds at a concentration of 10 micrograms /mL. Standard heparin at the same concentration (100 microgram /mL) shows 130 seconds of aPTT. The activated partial thromboplastin time (APTT) test is the most commonly used since it provides exact information on the anticoagulant potency of the substance.

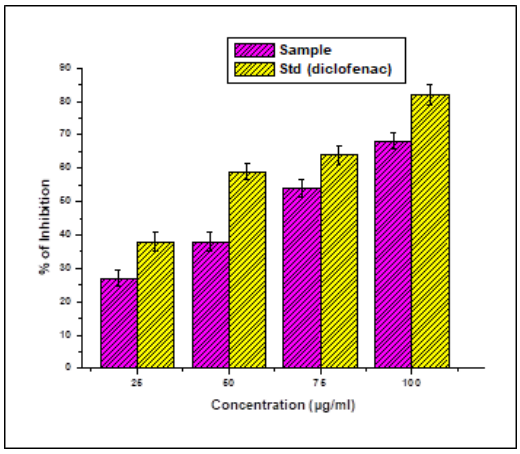

Figure 4: This Graph Displays the Total Anti-Inflammatory Activity, Which was Determined by Calculating the Percentage Inhibition Based on Concentrations

The protein denaturation assay was used to assess the anti-inflammatory efficacy in vitro. For *Halophila ovalis*, there was a concentration-dependent suppression of the protein denaturation activity. Diclofenac sodium was employed as a positive control in the protein-denaturation assay, with dosages ranging from 25 to 100 micrograms/mL. This allowed the percentage inhibition of protein denaturation activity to be shown, which varied from 35% to 80%, respectively. The results presented in figure indicate that the *Halophila ovalis* extract concentrations exhibited percent inhibitions that were

comparable to those of the standard medicine, diclofenac. The extract concentration at 100 micrograms/mL, produced the highest percent inhibition of 66%.

Inhibition of Protein Denaturation

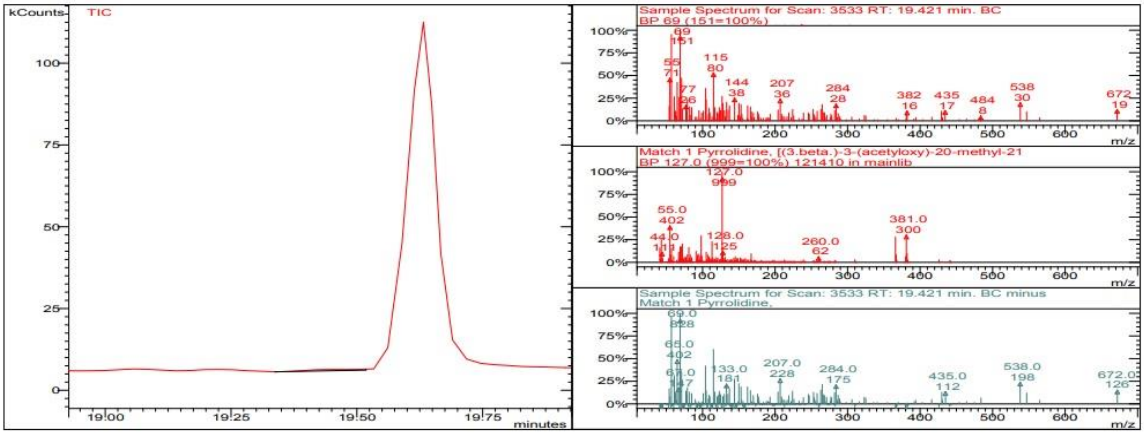

Figure 5: GC-MS Analysis Gas Chromatography Mass Spectrometry - ROS

Secondary metabolites from plants called phytochemicals are vital to the defense mechanism of the plant against grazing animals and other predators. Secondary metabolites have a crucial role in providing protection and resistance to the plant, even if they are not directly engaged in its growth. Following GC-MS analysis, the methanol extract of *Halophila ovalis* revealed a number of bioactive substances, including fatty acids. The GCMS analysis shows a major peak, and that compound is said to be pyrrolidine. Four carbon atoms and one nitrogen atom make up the five-membered ring of pyrrolidine, the parent molecule of the pyrrolidine family.

DISCUSSION

These days, marine natural goods are more advanced since they contain a variety of biologically active components with unique structures and intriguing functional qualities.[19] Sulfated polysaccharides derived from seagrasses have demonstrated various health benefits in the past ten years and are utilized in food, pharmaceuticals, and other consumable products. Sulfated polysaccharides from many seagrasses have been shown to be non-cytotoxic.[20]

Prothrombin time (PT) and activated partial thromboplastin time (APTT) were examined using the instrumental stag diagnostic.[8] PT measures the inhibition of other route factors (Factors X, V, II, and fibrinogen) as well as extrinsic factors (Factor VII). APTT is used to quantify the inhibition of intrinsic factors (Factors XI, XII, IX, and VIII) and other route factors (Factors X, V, II, fibrinogen). [21]The two primary methods used to assess a patient's risk of bleeding are anticoagulant time (aPTT) and prothrombin time (PT). The coagulation profile will be impacted by heparin, an anticoagulant that is frequently administered for a variety of clinical disorders.[22] Calcium chloride is absent from APTT reagents, but activators (silica, ellagic acid, or kaolin) and phospholipids are present. [23]The results of this study demonstrate that an extract of *Halophila ovalis* at a concentration of 100 micrograms/mL was particularly effective in increasing bleeding and delaying blood clotting time when compared to its lower concentrations. [24]For this reason, research on the plant's effects on PT and APTT was continued. Proteoglycan that contains protein and carbohydrates, are most likely the active substance or compounds found in the algal species.[25]

The inhibitory experiments on protein desaturation were employed to examine the anti-inflammatory efficacy of the *Halophila ovalis* ethanol extract in addition to anticoagulant assays.When evaluating the anti-inflammatory properties of lipids derived from seagrass, COX-2 activity and NO levels were the most frequently examined variables. [26]When external and intracellular stimuli are present, COX-2, one of the primary mediators of inflammatory reactions, converts arachidonic acid into prostaglandins.[27] Prostaglandins produced by inflammation have been proven to have immunosuppressive effects, and they are linked to both activation and overexpression of COX-2. Lopes et al. have shown that seagrasses can inhibit COX-2 and reduce prostaglandin synthesis in the same way. [28]Phyla Ochrophyta, Chlorophyta, and Rhodophyta were shown by Da Costa et al. to exhibit COX-2 inhibition by crude extracts of numerous seagrass species, indicating their potential as anti-inflammatory agents[29]. At a dose of 100 micrograms/mL, the highest percentage inhibition of protein denaturation (65%), with a standard error of 2.4 (58 ± 2.4%), was observed in our investigation. When Rhizophora mucronata leaves were assessed for anti-inflammatory activity, they were found to reduce protein denaturation by 33% at a concentration of 100 mg/mL extract. In contrast, at a dosage of 100 micrograms/mL, our extract of *Halophila ovalis* prevented protein denaturation by 65% using the same conventional medication, i.e., ensuring that *Halophila ovalis* has better anti-inflammatory properties.

CONCLUSION

The ethanolic crude extract of *Halophila ovalis* seagrass is a plentiful, sustainable, and unappreciated source of bioactive lipids with strong anti-inflammatory and anticoagulant properties. Because the crude extracts of *Halophila ovalis* exhibit anticoagulant activity, they can be used as a natural source of anticoagulant. The research suggests that focusing on inflammatory pathways could be a novel approach to the management and/or avoidance of a number of chronic metabolic diseases. In addition to being nutrient-rich, seagrasses contain a variety of bioactive substances. These drugs have the potential to control inflammation and lower the risk of a number of NCDs. Consequently, using seagrass from *Halophila ovalis* on a regular basis can offer protection against a range of chronic inflammatory conditions.

Conflict of Interest:

The authors hereby declare that there is no conflict of interest in this study.

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Author Contribution:

A) Amba Esakki - contributed in designing the study, execution of the project, statistical analysis, manuscript drafting.

B) Dr. Monal Yuwanati - contributed in designing the study, execution of the project, statistical analysis, manuscript drafting.

C) Dr. Sivaperumal - contributed in study design, guiding the research work, manuscript correction.

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