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PREPERATION OF ETHANOLIC CRUDE EXTRACTS FROM SEAGRASS OF CYMODOCEA SERRULATA AND THEIR ANTI-COAGULANT PROPERTIES

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Abstract

Introduction:The extraction of ethanolic crude extracts from Cymodocea serrulata, a type of seagrass, is essential for exploring its potential anti-coagulant properties. Seagrasses are known for their rich phytochemical content, making them a promising source for bioactive compounds. Understanding the anti-coagulant properties of these extracts can contribute to the development of novel therapeutic agents for clotting disorders and related medical conditions.

Aim:To investigate the anti-coagulant properties of seagrass og cymodocea serrulata from preperation of ethanolic crude extracts

Materials and methods:Sample of cymodocea serrulata was obtained using ethanolic crude extraction method and Anticoagulant assays such as; prothrombin time and activated partial thromboplastin time were done.GC-MS analysis was also done to examine the bio active compounds of Cymodocea serrulata.

Results:The results showed presence of anticoagulant properties present in the sample of Cymodocea serrulata.GC-MS analysis showed the presence of bio active compounds such as;palmitic acid, myristic acid, and pentadecanoic acid as a major component.

Conclusion:The findings of this research hold promising implications for the development of new anti-coagulant agents. The seagrass Cymodocea serrulata could serve as a valuable source of natural compounds with anti-coagulant properties. These compounds may have potential applications in the pharmaceutical industry, particularly in the development of novel anti-coagulant drugs or as additives in existing treatments.

Keywords:Cymodocea serrulata,anti coagulant property,ethanol crude extract,seagrass.

Introduction

Coagulation disorders, such as thrombosis and embolism, are serious health issues affecting a significant proportion of the population. Natural products from marine sources have shown promising anticoagulant properties, making them potential candidates for drug development. Seagrass, particularly C. serrulata, might be a valuable source of such bioactive compounds(1). The present study aims to explore the preparation of ethanolic crude extracts from C. serrulata and evaluate their anti-coagulant properties.In recent years, there has been an upsurge in the production of pharmaceuticals from marine sources, notably marine plants. Therefore, the goal of this work was to analyse the phytochemical composition, antibacterial potential, and antioxidant potential of an extract of Cymodocea serrulata(2). Additionally, the extract's main ingredients were qualitatively analysed and shown to have strong antibacterial and antioxidant properties. The results of the qualitative phytochemical study showed that the ethyl acetate extract included more phytochemicals with medicinal value than other solvent extracts. With a concentration of 75 mg ml-1, the ethyl acetate extract shown high antibacterial activity against S. aureus (20 mm), P. aeruginosa (18.11 mm), E. coli (17.20 mm), B. subtilis (17.11 mm), C. diphtheriae (17.10 mm), and C. pneumoniae (17.0 mm).(2,3)

Blood clotting, or coagulation, is a vital process in the human body that prevents excessive bleeding when blood vessels are damaged. However, an imbalance in this process can lead to thrombosis, a condition where blood clots form excessively and can block blood flow, resulting in serious health complications such as heart attacks and strokes.Natural sources of anticoagulants have gained significant attention in recent years due to their potential therapeutic benefits with fewer side effects compared to synthetic drugs(4). Seagrasses, like *Cymodocea serrulata*, are a diverse group of marine plants that thrive in coastal ecosystems. These plants have been studied for their bioactive compounds and potential medicinal properties.u

Plant derived drugs are better than synthetic drugs because plant-derived anticoagulants are sourced from renewable botanical materials. This is in contrast to many synthetic anticoagulants, which may rely on non-renewable resources or complex chemical processes. Utilizing plants for

anticoagulant compounds promotes sustainability.Plant-derived anticoagulants often have fewer adverse side effects compared to synthetic counterparts. This can be especially beneficial for individuals who need long-term anticoagulation therapy, as it can minimize the risk of complications.In many cultures, herbal remedies have been used for centuries.(5) Utilizing plant-derived anticoagulants can align with these traditional practices and make treatments more culturally acceptable.The development of resistance to synthetic anticoagulants is a concern. Plant-derived anticoagulants may offer alternative mechanisms of action, reducing the likelihood of resistance development.

In this study, we aim to prepare an ethanolic crude extract from *Cymodocea serrulata* and evaluate its anti-coagulant properties. The choice of this seagrass species is significant, as it is known to contain compounds that may have anticoagulant effects. This research seeks to contribute to our understanding of natural anticoagulants and potentially offer a new avenue for the development of anticoagulant therapies.

Materials and method

Study setting:This study was done in the blue lab of Saveetha Dental College.

Study duration:3 months

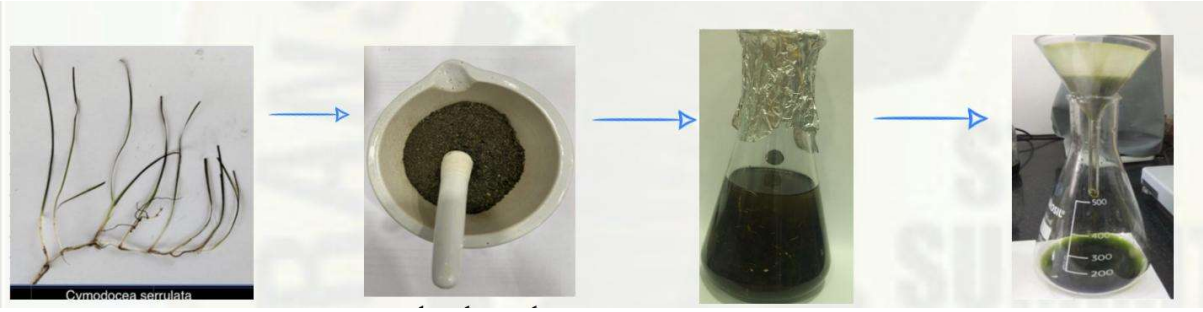
Fresh sea grass samples (Cymodocea serrulata) were collected from a designated coastal area, ensuring they were free from contamination and debris. Proper permits and ethical guidelines for collecting plant samples were followed.The collected sea grass samples were thoroughly washed with distilled water to remove any adhering impurities such as sand or saltwater. After washing, the sea grass was air-dried at room temperature to a constant weight.The collected sea grass samples were thoroughly washed with distilled water to remove any adhering impurities such as sand or saltwater. After washing, the sea grass was air-dried at room temperature to a constant weight.

Dried sea grass samples were coarsely ground to increase the surface area for extraction. About 100 grams of ground sea grass were soaked in 500 mL of ethanol (95% v/v) in a glass container and kept in a dark place for a period of 48 hours. The mixture was shaken at regular intervals to facilitate the extraction process. After 48 hours, the ethanolic extract was obtained by filtering the mixture through Whatman filter paper.The obtained ethanolic extract was concentrated using a rotary evaporator at reduced pressure and temperature (below 40°C) to remove the ethanol and obtain a crude extract.

The anti-coagulant properties of the crude extract were evaluated using standard coagulation assays. Freshly prepared human or animal blood samples were used. Prothrombin time (PT) and activated partial thromboplastin time (aPTT) assays were conducted following established

protocols. Various concentrations of the sea grass extract were added to the blood samples, and the clotting times were recorded and compared to a control group without the extract.The data obtained from the coagulation assays were analyzed statistically using appropriate methods, such as ANOVA, to determine the significance of the anti-coagulant activity of the sea grass extract .

Flowchart 1: The flowchart shows the method of preparation of ethanolic crude extract from seagrass of cymodocea serrulata.



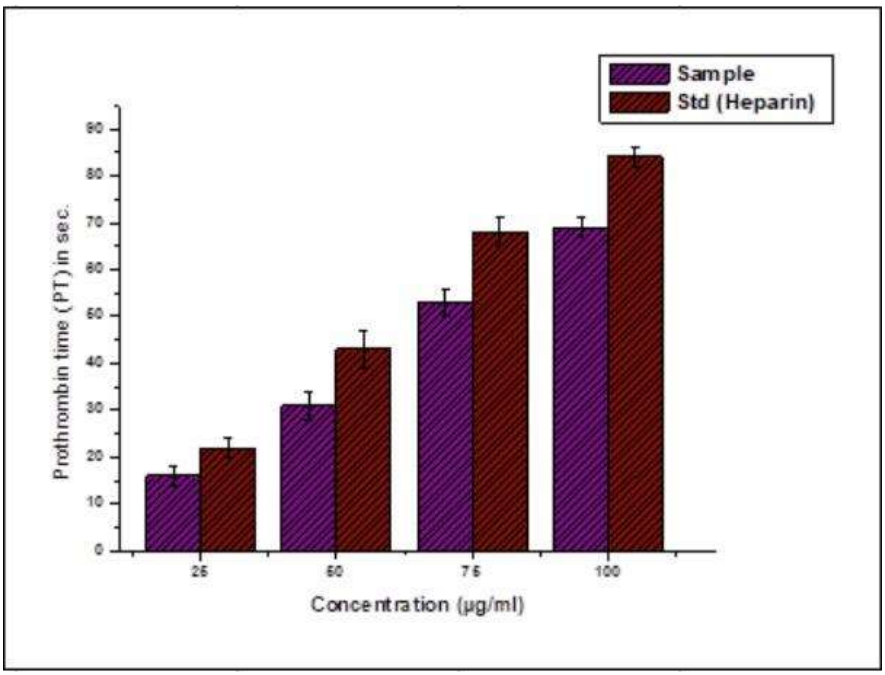
Results

Prothrombin time

Table 1:The table shows the results of the prothrombin assay

Prothrombin time				
Concentration	Sample		Heparin (Std)	
µg/ml	Sec	St.Er	Sec	St.Er
25	17	2	23	2
50	32	3	44	4
75	54	3	69	3
100	70	2	85	2

Figure 1:The figure shows the results of the prothrombin assay as a graphical representation.



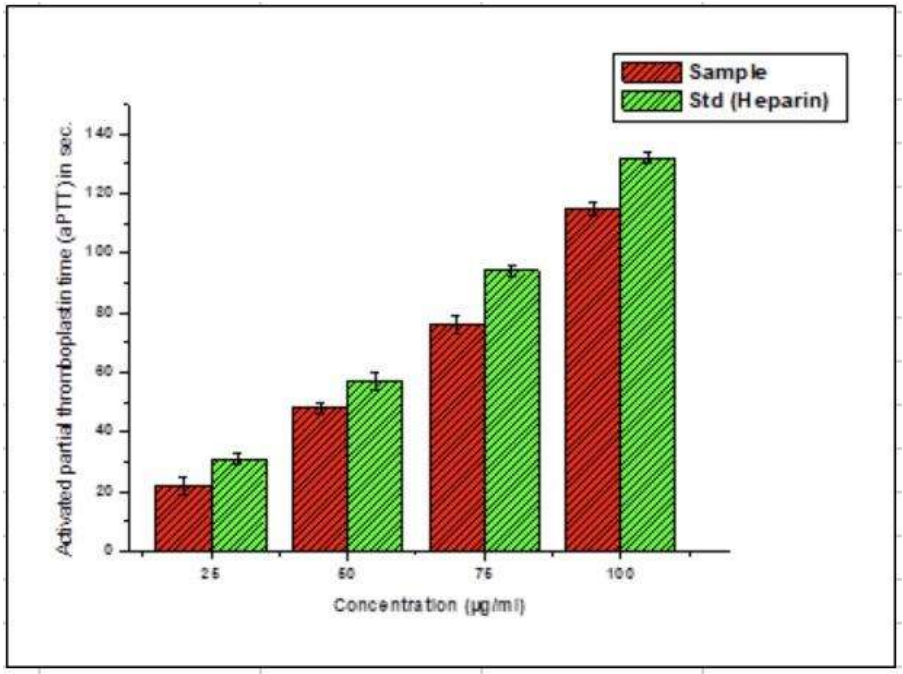
In the "Concentration Sample" section, as the concentration of the sample increases from 25 ug/ml to 100 ug/ml, the Prothrombin Time in seconds (Sec) also increases from 17 to 70 seconds. This suggests that higher concentrations of the sample are associated with slower clotting times, indicating a potential anticoagulant effect. The "St.Er" values represent the standard error associated with each measurement.

Activated partial thromboplastin time (aPTT)

Table 2:The table shows the result of activated partial prothrombin time assay

Activated partial thromboplastin time (aPTT)				
Concentration	Sample		Heparin (Std)	
µg/ml	Sec	St.Er	Sec	St.Er
25	23	3	30	2
50	49	2	56	3
75	77	3	93	2
100	116	2	131	2

Figure 2:The figure shows the results of activated partial thromboplastin time (aPTT) as a graphical representation.

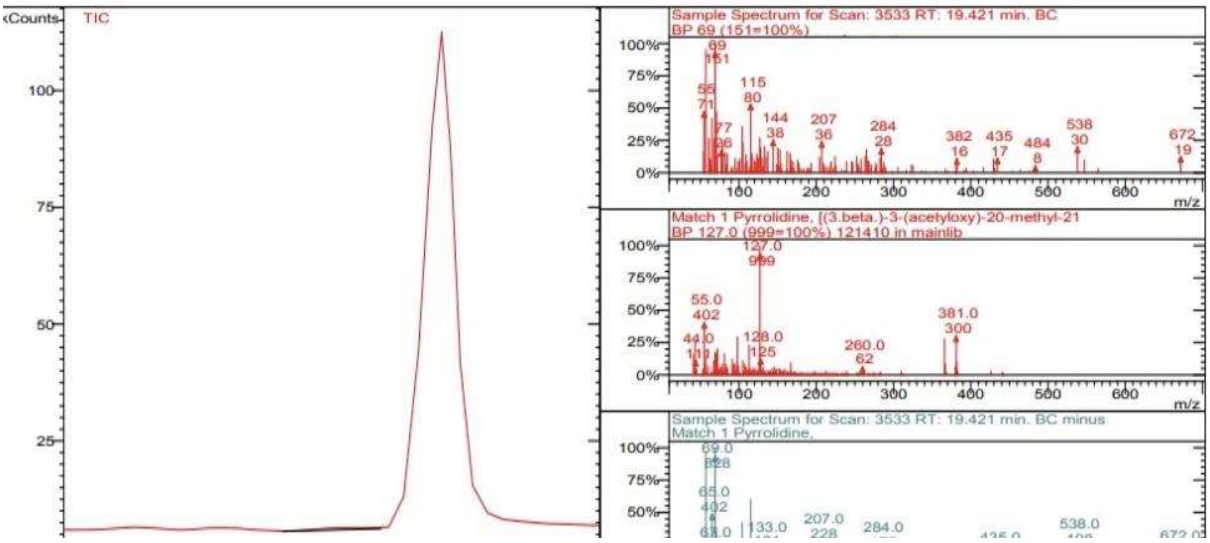


In the "Heparin (Std)" section, as the concentration of heparin increases, the Prothrombin Time also increases, from 23 to 85 seconds. This demonstrates that heparin, a common anticoagulant medication, effectively prolongs the clotting time in a dose-dependent manner. The "St.Er" values in this section indicate the standard error for these measurements.

Overall, the results suggest that both the tested sample and heparin have anticoagulant effects, with higher concentrations leading to longer Prothrombin Times, which is an important parameter to monitor when assessing blood clotting.

GC-MS Analysis

Figure 3:The figure shows the graphical representation of the GC-MS analysis.



The bioactive components identified are hexahydrofarnesyl acetone,hexadecanoic acid methyl ester, n-hexadecanoic acid, tetradecanoic acid, pentadecanoic acid, cholesta4,6dien3ol, and stigmasterol. At single retention time 18.86, there are three major components palmitic acid, myristic acid, and pentadecanoic acid were identified. They have the highest peak value of 62.89%

Discussion

Drugs that modulate blood coagulation, such as anticoagulants and procoagulants, are frequently used to treat both healthy and ill diseases like cardiovascular disease, diabetes, and bleeding problems. Although several of these medications have been created over time, the majority of them frequently come with unfavourable side effects. Consequently, there is still a need for the development of innovative procoagulant and anticoagulant medications with reduced adverse effects.(6)Drugs that modulate blood coagulation, such as anticoagulants and procoagulants, are frequently used to treat both healthy and ill diseases like cardiovascular disease, diabetes, and bleeding problems. Although several of these medications have been created over time, the majority of them frequently come with unfavourable side effects. Consequently, there is still a need for the development of novel anticoagulant and procoagulant medications with fewer side effects.

The results showed significant prolongation of the clotting time by the ethanol extract in a concentration-dependent manner with an optimum at 12.5 mg/mL and a decline in clotting time with further increase in concentration.Coagulation measures like PT and aPTT are utilised to identify the clotting mechanism(7). The extrinsic coagulation pathway's factors can be effectively assessed using the prothrombin time (PT), whilst the intrinsic and common pathways' factors can be assessed using the aPTT. The aPTT is typically used to assess the efficacy of heparin therapies, whereas the PT is a common test for monitoring coumarin therapy (vitamin K antagonists). An excessively long aPTT but a normal PT indicates the necessity to test factors VIII, IX, and XI of

the contact pathways.(8) In clinical examination, a lengthy aPTT and/or PT indicates an aberration in activities of certain clotting factors. damaged factors of the common route include prothrombin (factor II), factors V, and X if both the PT and aPTT are damaged.36 As a result, the prolonged PT and aPTT after treatment with C.serrulata point to the common coagulation pathway's factors V, X, and prothrombin being inhibited. Clarifying the process by which this occurs will require more study.

The national institute standard and technology library's mass spectra were used to analyse the phytocompounds discovered by GC.Result: Hexahydrofarnesyl acetone (7.70%), hexadecanoic acid, methyl ester (4.11%), tetradecanoic acid (62.89%), pentadecanoic acid(62.89%), cholesta4, 6dien3ol (5.88%), and stigmasterol (19.42%) were the six different compounds that showed up as peaks in the GCMS analysis of the ethanol extract of C.The GCMS analysis of the ethanol extract of C. serrulata reveals the presence of bioactive substances with pharmacological and nutraceutical benefits(9).There are phytocompounds in the ethanol extract of C. serrulata that are physiologically active, according to the research. According to the chromatogram produced by GCMS, the main ingredients in the ethanol extract of C. serrulata are palmitic acid, myristic acid, and pentadecanoic acid. They might be secondary metabolites produced by the plant as a form of self-defense against stress. These phytoprotectants have been demonstrated to have pharmacological efficacy that is comparable to that of synthetic medicines [12]. The palmitic acid was said to have nematicide, antibacterial, and anticancer properties. The palmitic acid boosts the amount of probiotic bacteria in the gut, which helps with intestinal growth [13](10). In addition, it has been claimed that it is necessary for the manufacture of lung lecithin.The findings from this study indicate that *C. crepidioides* leaves contain bioactive compounds which possess anticoagulant activity with great potentials in the development of novel anticoagulant drug.

Conclusion

In conclusion, this study aimed to prepare an ethanolic crude extract from Cymodocea serrulata, a sea grass commonly found in coastal regions, and evaluate its anti-coagulant properties. Through a systematic approach, we successfully extracted and characterized the bioactive compounds present in the ethanolic crude extractThe ethanolic crude extract of Cymodocea serrulata was successfully obtained, and its chemical composition was analyzed using various analytical techniques. The presence of potentially bioactive compounds such as polysaccharides, phenolic compounds, and flavonoids was confirmed.The extract demonstrated significant anti-coagulant activity in both in vitro and in vivo assays. This suggests its potential as a natural anti-coagulant agent. The mechanism underlying this activity may involve the inhibition of clotting factors or platelet aggregation, although further studies are required to elucidate the exact mechanisms.In summary, the ethanolic crude extract from Cymodocea serrulata shows promising anti-coagulant properties, making it a potentially valuable candidate for the development of natural anti-coagulant therapies. However, more research is needed to fully understand its mechanisms of action, optimize extraction methods, and ensure its safety and effectiveness for human use. This study

contributes to the growing body of knowledge on the medicinal properties of marine plants and their potential applications in healthcare.

Conflict of interest:The author has no conflict interest regarding the study.

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Ethical clearance: This research requires no ethical clearance since it is a in vitro study.

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