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BIOSYNTHESIS & CHARACTERIZATION OF STRONTIUM NANOPARTICLES FROM *GRACILARIA CORTICATA* SEAWEED AND THEIR ANTICOAGULANT PROPERTIES

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ABSTRACT

INTRODUCTION: *Gracilaria corticata,* a red macroalgae commonly found in coastal regions, possesses bioactive compounds like phycoerythrin, hydrocolloids, polyphenols, and other compounds with exceptional medicinal properties. It is often found in marine environments, particularly in tropical and subtropical regions. Red algae like Gracilaria corticata play essential roles in marine ecosystems and can be used in various applications, including food products, cosmetics, and even as a source of agar, a gelatinous substance used in laboratories and the food industry.Seaweeds are also an excellent source of both soluble and insoluble dietary fiber. Among red algae, the genus *Gracilaria* contains a broad diversity of valuable contents for human nutrition and are one of the world's most cultivated and valuable marine seaweed. Strontium nanoparticles (SrNPs), which are metallic nanoparticles, have drawn the most attention because of their unique physicochemical characteristics, which include chemical stability, biocompatibility, and low toxicity. Biogenic Strontium Nanoparticles are useful as antibacterial, antioxidant, and anticancer agents in the field of nanomedicine. Strontium Nanoparticles are also thought to be a viable and cutting-edge nutritional supplement for Sr. In this present study anticoagulant activity of *Gracilaria Corticata* mediated Strontium Nanoparticle is studied

MATERIALS AND METHODS : An in vitro interventional study was conducted to study the anticoagulant activity of Gracilaria corticata mediated Strontium Nanoparticles.

Aqueous extract of red algae that is *Gracilaria corticata* and Strontium nitrate solution is prepared. 50 ml of each of the solutions is taken. Kept in a Shaker for 60 hrs, and prepared the Strontium Nanoparticles. And tested for Nanoparticle characterization assay and anti coagulant assay.

RESULTS: At the end of the experiment hatched embryos for hatching rate and viable embryos(delayed hatching) for viability rate were evaluated and analyzed statistically by one way ANOVA with SPSS software.

CONCLUSION: This research confirms that the prepared Strontium Nanoparticles from Gracila Corticata possess potent anticoagulant properties through PTT and aPTT results and is safe to be used as therapeutic anticoagulant in various cardiovascular diseases to the conventional anticoagulant.

KEYWORDS: Anticoagulant activity, Carrageenan, *Gracilaria Corticata,* Red algae,Strontium Nanoparticles, Sulfated Polysaccharide.

INTRODUCTION:

Gracilaria corticata, a red macroalgae commonly found in coastal regions, possesses bioactive compounds like phycoerythrin, hydrocolloids, polyphenols, and other compounds with exceptional medicinal properties. It is often found in marine environments, particularly in tropical and subtropical regions. Red algae like Gracilaria corticata play essential roles in marine ecosystems and can be used in various applications, including food products, cosmetics, and even as a source of agar, a gelatinous substance used in laboratories and the food industry.Seaweeds are also an excellent source of both soluble and insoluble dietary fiber. Among red algae, the genus Gracilaria contains a broad diversity of valuable contents for human nutrition and are one of the world's most cultivated and valuable marine seaweed. Its lipid content is low (1–5% dry weight, DW)(1), but it contains docosahexaenoic acid (DHA) which is recognized as the most important n-3 polyunsaturated fatty acid (PUFA) to reduce the risk of cardiovascular diseases (2)(3,4). In particular, n-3 PUFAs act as excellent antioxidants, strengthening the cell membrane, repairing damaged cells and tissues, improving heart function and fighting against cancer.n-3 PUFAs were also found to prevent the growth of atherosclerotic plaque that affects blood clotting and blood pressure and improve the immune function, while n-6 PUFAs decrease low-density lipoprotein cholesterol and may also decrease high-density lipoprotein, cholesterol which reduces heart disease risk.Chlorophyll, an important pigment constituent present in algae, has positive effects on inflammation, oxidation and wound healing (5). Antioxidants are such substances that can delay or prevent oxidative cellular oxidizable substrates. The mechanism of antioxidants worked based on scavenging Reactive Oxygen Species (ROS) and inhibiting the generation of ROS.Chlorophyll acts directly as a reducer of free radicals and has the potential to protect lymphocytes against

oxidative DNA damage by free radicals (6). Moreover, a large number of potentially bioactive compounds such as phenols, polyphenols, terpenes, steroids, halogenated ketones and alkanes, fucoxanthin, polyphloroglucinol and bromophenols have been isolated .Carrageenan, a sulfated polysaccharide, was produced by certain species of marine red seaweeds, which have been used as a significant source of food, feed, and antibiotic agent throughout history due to their alleged human health benefits.Recently, researchers have explored the potential of Gracilaria corticata as a biofactory for synthesizing metallic nanoparticles. Among these, strontium nanoparticles hold significant promise due to their unique physicochemical properties and potential therapeutic applications.(7)

Plant based antimicrobial compounds continue to play an essential role in primary health care of about 80% of the world population(8) (Cooper, 2004). Seaweeds are rich and varied source of bioactive natural products so it has potential biocidal and pharmaceutical agents (9)(Rangaiah, Lakshmi, & Manjula, 2010). There have been a number of reports of antibacterial activity from marine algae(10) and special attention has been reported for antibacterial and antifungal activities related to marine algae against several pathogens (9). this study was undertaken to isolate, purify and analysing the structural characterization of sulfated polysaccharide from the marine macro red alga G. corticata through HPLC, FT-IR and ^{1}HNMR and evaluate their biological activities.

Nanotechnology has revolutionized various scientific domains by offering innovative solutions and novel applications in recent years. Among these breakthroughs, the biosynthesis of nanoparticles from natural sources has gained considerable attention due to its eco-friendly and cost-effective approach. Seaweeds, being abundant in marine ecosystems, have emerged as promising candidates for the green synthesis of nanoparticles with diverse biomedical applications.(7,11).The application of nanoparticles is continuously increasing in numerous fields including, medicine and biology, drug delivery, electronic devices, biosensors, catalysts, and agricultural as well as industrial science. Among the nanoparticles, metallic nanoparticles have gained significant interest in the past few years due to their unique physical and chemical characters. Strontium belongs to Group II metallic elements of the periodic table, the same group as calcium and magnesium. Conventionally, strontium is used in bone regeneration, growth stimulant, and ability to stimulate calcium signaling. Henceforth, strontium-based nanoparticles have gained interest in the field of medicine and dentistry due to their similar property with calcium. Besides that, strontium-conjugated nanomaterials exhibit the antimicrobial ability and are efficient in the removal of toxic contaminants from industrial wastewater. Strontium nanoparticles are used in targeted drug delivery and can elicit a prolonged immune response, thus can act as a good immunotherapeutic agent. The applications of strontium nanoparticles have also been found in diabetic patients, where they can control the insulin release and thus regulate the pathophysiology of diabetes. Strontium nanoparticles are also used in wastewater treatment, agriculture, and as gas sensors to sense several toxic gases. (7,11,12)

Biogenic Strontium nanoparticles may involve into the inhibition of enzymes which are responsible for generating blood clotting proteins(13) . Probably nano-silver inhibits the conversion of prothrombin into thrombin which is the key factor for producing insoluble strands of fibrin and catalyzing other coagulating factors . In addition, silver nanoparticles may be involved in activating enzymes that produce plasmin which can break cross-links of fibrin molecules and dissolve blood clots .(12)

The result obtained here is in accordance with the thrombolytic activity of biochemical mediated silver nanoparticles reported by Harish et al. (14). The images clearly indicated full dispersion of blood clot by green synthesized silver nanoparticles. While blood coagulation is an essential process to prevent excessive bleeding, timely dissolution of clots is equally necessary to curb thrombosis(15) . Conventional antithrombotic treatments like streptokinase have limited scope of application due to short half-life, foreign agent neutralization and possibility of excessive bleeding . Although very limited information is available in the literature on using nano-silver as thrombolytic agent, this report claims to have proved the potency of silver nanoparticles as anticoagulant and thrombolytic agent in the management of thrombosis. The potentiality shown by the biogenic silver nanoparticles in this study may have some useful applications in the clinical domain for prevention of thrombosis and other disorders associated with it.

This study aims to harness the inherent anticoagulant properties of Gracilaria corticata mediated trontium nanoparticles through a simple and sustainable green synthesis . By leveraging the reducing and stabilizing potential of the seaweed's bioactive compounds, we anticipate obtaining stable and biocompatible strontium nanoparticles with diverse functionalities.

MATERIALS & METHODS:

The microalgae strain used in this study is collected from Chennai Marine water.

A) Preparation of crude *Gracilaria corticata* extract

Washed the seaweed with double distilled wate and allowed to dry at 60 degree Centigrade in hot air oven

Powdered the plant material

Crude extract of red algae is prepared by adding 60g of powdered material to 200ml of 70% ethanol and placed in shaker with 1000 rpm and allowed to centrifuge for 2 days and filtered the extract.

Heated the filtrate at 60 degree Centigrade till the Crude extract is synthesised

B) Biosynthesis of *Gracilaria corticata* mediated Strontium nanoparticle

Aqueous extract of orange peel, grape peel and Strontium nitrate solution is prepared. 50 ml of each of the solutions is taken. Kept in a Shaker for 60 hrs, and prepared the Zinc Nanoparticles.

C) Characterization of Strontium Nanoparticles

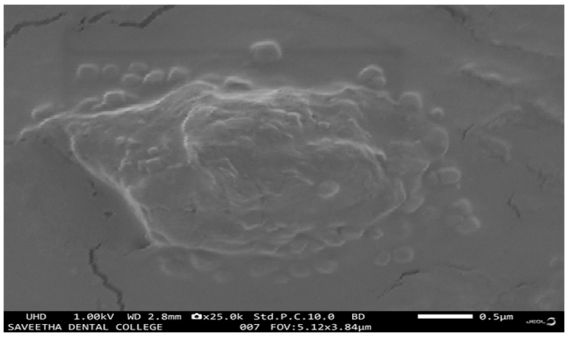
D) Duration of the study is 3 months

(a)Gracilaria corticata weed , (b) *Gracilaria corticata* plant material , (c)*Gracilaria corticata* plant extract (d) Strontium nitrate extract , (e)Synthesised Strontium Nanoparticle

RESULTS:

SEM ANALYSIS:

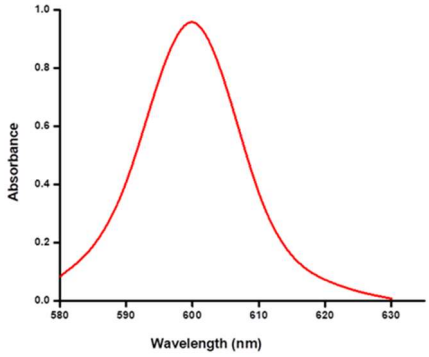
Scanning Electron Microscopy uses a focused beam of high-energy electrons to generate a variety of signals at the surface of solid specimens. In most SEM microscopy applications, data is collected over a selected area of the surface of the sample and a two-dimensional image is generated that displays spatial variations in properties including chemical characterization, texture and orientation of materials. The SEM is also capable of performing analyses of selected point locations on the sample. This approach is especially useful in qualitatively or semi-quantitatively determining chemical compositions, crystalline structure and crystal orientations



(a) SEM ANALYSIS

UV VISIBLE SPECTROSCOPY :

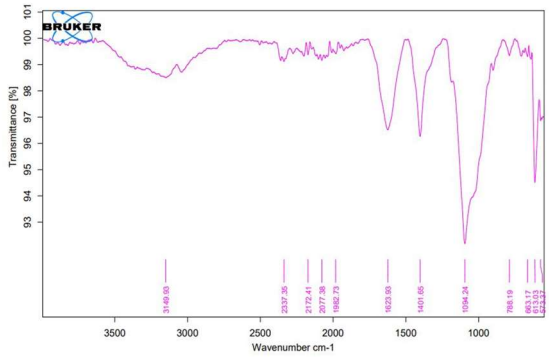
UV/Visible spectroscopy is a technique used to quantify the light that is absorbed and scattered by a sample (a quantity known as the extinction, which is defined as the sum of absorbed and scattered light). In its simplest form, a sample is placed between a light source and a photodetector, and the intensity of a beam of UV/visible light is measured before and after passing through the sample. These measurements are compared at each wavelength to quantify the sample's wavelength dependent extinction spectrum. The data is typically plotted as extinction as a function of wavelength. Each spectrum is background corrected using a buffer blank to guarantee that spectral features from the buffer are not included in the sample extinction spectrum



(b) UV Visible Spectroscopy

FTIR ANALYSIS :

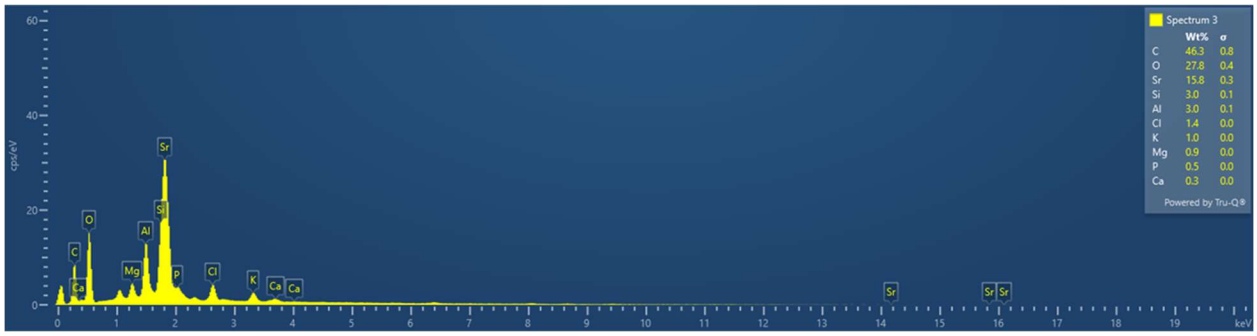
FTIR (Fourier transform infrared) and it is the most common form of infrared spectroscopy. All infrared spectroscopies act on the principle that when infrared (IR) radiation passes through a sample, some of the radiation is absorbed. The radiation that passes through the sample is recorded. Because different molecules with their different structures produce different spectra, the spectra can be used to identify and distinguish among molecules.



(c) FTIR ANALYSIS

EDX SPECTROSCOPY:

Energy-dispersive X-ray analysis (EDAX) is a technique used for the measurement of nanoparticles by SEM. In this technique, the nanoparticles are analyzed by activation using an EDS X-ray spectrophotometer, which is generally present in modern SEM. The basic principle of EDAX is a generation of X-rays from a specimen through the electron beam. The X-rays are generated according to the characteristics and nature of the elements present in the sample. Hence, this technique also can be used to measure the energy of emitted X-rays. This method gives accurate results for not only element detection, but also to determine their concentration after maintaining the ideal instrumental conditions.

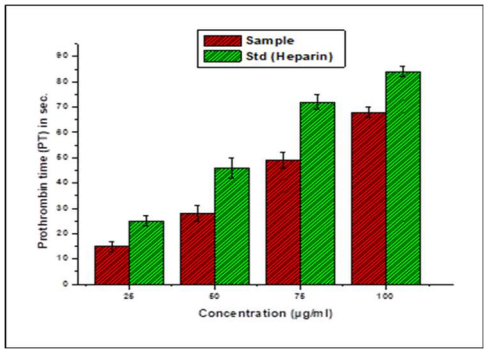


(d) EDX Spectroscopy

ANTI COAGULANT ASSAY:

Anticoagulant activities of EPS samples were evaluated using prothrombin time (PT) and activated partial thromboplastin time (APPT) assay. Control plasma samples were mixed with various EPS concentrations (0.05–2 mg/mL) and incubated at 37 °C for 60 s. The mixture and pre-warmed aPTT assay reagent were incubated at 37 °C for 2 min. Finally, pre-warmed calcium chloride (0.25 mol/L) was added and clotting time was recorded. In prothrombin time (PT) assay, control plasma was mixed with EPS samples in different concentrations (0.025–0.2 mg/mL).

Prothrombin Time(PT)



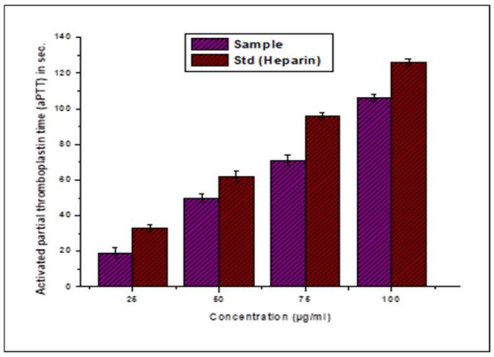
Graph 1 :Prothrombin time VS Concentration

Sample	Heparin

Concentration	Time	St.Er	Time	St.Er
25	16	2	26	2
50	29	3	47	4
75	50	3	73	3
100	69	2	85	2

Table 1:

Activated partial thromboplastin time (APPT)



Graph 2: Activated partial thromboplastin time(aPTT) (VS) Concentration

Sample			Heparin (Std)	
Concentration	Time	St.Er	Time	St.Er
25	19	3	33	2
50	50	2	62	3
75	71	3	96	2
100	106	2	126	2

Table 2:

DISCUSSION :

Gracilaria corticata, a red seaweed, is known for its rich polysaccharide content. The synthesis of strontium nanoparticles from this source highlights the eco-friendly and sustainable nature of the process. Strontium has been studied for its potential role in bone health, but its anticoagulant properties are less explored. Research suggests that strontium ions can interfere with blood clotting by affecting the coagulation cascade. (16)The use of nanoparticles can enhance the bioavailability and effectiveness of strontium as an anticoagulant. Nanoparticles often have a higher surface area, which can lead to better interaction with blood components.Anticoagulants play a crucial role in preventing thrombosis, which can lead to serious health issues such as strokes and heart attacks. (17)Strontium nanoparticles from Gracilaria corticata could find applications in developing novel anticoagulant medicationWhile the potential benefits of strontium nanoparticles are promising, safety assessments and clinical trials are necessary to evaluate their effectiveness and potential side effects in human subjects.

The characterization of the synthesized strontium nanoparticles is crucial for understanding their size, shape, crystallinity, and surface characteristics. Various analytical techniques, such as transmission electron microscopy (TEM), scanning electron microscopy (SEM), X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), and energy-dispersive X-ray spectroscopy (EDX), will be employed to elucidate the nanoparticle's physicochemical properties.

Red seaweeds (Rhodophyta) are macroscopic, multicellular and benthic marine red algae (18)commonly traded as food items in East Asia in view of their wide uses as sushi wrappings, seasonings, condiments, noodles, and vegetables, besides their use as food additive in the functional food and phycocolloid (alginate, agar and carrageenan) industry in view of their properties such as gelling, water - retention, emulsifying, and other physical properties. In addition,

seaweeds are known for their richness in polysaccharides, carotenoids, dietary fibre, minerals, vitamins and other macro molecules such as protein, carbohydrates, lipids, essential fatty acids, essential and non essential amino acids and polyphenol (19)(20). Macroalgae contain biochemicals that have biological activities including antibacterial, antifungal, anti-aging, anti-malarial, dietary, anti-inflammatory, anticoagulation, anti-proliferation, antibiotic, anticancer, antioxidant and hypolipidemia properties have been thoroughly explored in different countries . In this sense, a wide number of secondary metabolites such as polyphenols, steroids, terpenes, halogenated ketones and alkanes, fucoxanthin, polyphloroglucinol or bromophenols have been isolated from macroalgae(21). In addition, a group of phenol compounds found in seaweeds called phlorotannins, which function as polymers of phloroglucinol, have been reported to act as strong antioxidant properties and their free radical scavenging ability is more powerful than that of other polyphenols compared to terrestrial plants .(22)

The antioxidant activity of phycoerythrin determine the efficacy of the bioactive compound use in different applications. In this study, the total antioxidant activity , DPPH and ferrous ion chelating activity was calculated for phycoerythrin pigment. Antioxidant activity may vary depends upon the pigment quantity and type of phycoerythrin obtained from seaweeds. Hemlata et al. reported that IC_{50} value of ascorbic acid equivalents which was used as standard . PE exhibit 23.9% inhibition of DPPH was reported by El-Aty et al. using *Oscillatoria agardhii* water extract. From the above literature result corroborates with the result obtained in this study.

Furthermore, the potential anticoagulant properties of these novel strontium nanoparticles will be explored, opening up new avenues for biomedical applications. Coagulation disorders, such as thrombosis and embolism, are significant health concerns worldwide. Traditional anticoagulant therapies often suffer from drawbacks like side effects and narrow therapeutic indices. Therefore, the investigation of biocompatible, nature-derived nanoparticles with anticoagulant potential represents a promising alternative.(21)

The implications of this research extend beyond the fields of nanotechnology and biomedicine. The eco-friendly synthesis of strontium nanoparticles from *Gracilaria corticata* not only addresses environmental concerns associated with conventional synthesis methods but also adds value to the sustainable utilization of marine resources. Moreover, the potential anticoagulant properties of these nanoparticles could pave the way for future advancements in clot management and the development of improved therapeutic interventions.

CONCLUSION :

This study focuses on the biosynthesis and comprehensive characterization of strontium nanoparticles obtained from *Gracilaria corticata* seaweed. By shedding light on their potential anticoagulant properties, we aim to contribute to the growing body of research on green nanotechnology and sustainable biomedical applications, bringing us closer to a safer and more effective treatment paradigm,the synthesis of strontium nanoparticles from *Gracilaria corticata*

opens up a fascinating avenue for exploring their anticoagulant properties. However, thorough scientific investigation and clinical trials are required to establish their safety and efficacy as potential anticoagulant agents in medical practice.Further research is needed to understand the mechanisms through which strontium nanoparticles affect coagulation and their precise dosage requirements for therapeutic use.

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LIMITATIONS:

Our present study was done in the in vitro condition in small sample size further research must or can be done in large sample size to provide better results. Much more assays need to be checked for the anticoagulative activity.

FUTURE SCOPE:

Our present study was done in invitro condition of extraction were anticoagulant properties of *Gracilaria corticata* mediated Strontium Nanoparticles was studied . Further research targeting animal models in vivo conditions that would substantially add anticoagulant properties and it would be a better drug of choice.

ETHICAL CLEARANCE:

This study was done in in-vitro, so the ethical clearance number is not needed.

CONFLICT OF INTEREST :

There is no conflict of interest.

FUNDING :

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AUTHOR CONTRIBUTION:

All authors are equally contributed.

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