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CHARACTERISATION AND ANTICOAGULANT ACTIVITY OF ZINC NANOPARTICLES SYNTHESISED FROM *TURBINARIA SP.*

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Abstract:

Introduction:

Nanotechnology has emerged as a promising field in the development of new therapeutic agents and materials with many applications in medicine.ZnNPs were synthesized using a green and environmentally friendly approach involving *Turbinaria sp*. as a reducing agent and stabilizer. Turbinaria is a genus of seaweed found mostly in tropical marine waters. It usually grows on stony substrates. Tropical species of Turbinaria, which are often consumed by herbivorous fish and echinoids, have relatively low phenolic and tannin content.

Materials and methods:

The *Turbinarila sp.* Was dried and the extract was prepared. The prepared plant extract was mixed with an equal quantity of zinc nanoparticle extract. The prepared extract was used to characterize the presence of zinc nanoparticles and its anticoagulant activity.

Results:

Characterization of the synthesized ZnNPs was carried out using various techniques including UV-visible spectroscopy, Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDAX). The results confirmed the successful synthesis of spherical ZnNPs with a nanometer average size.In

addition, *Turbinaria sp.* The anticoagulant activity of the -derivative ZnNPs was evaluated using in vitro coagulation tests. ZnNPs showed dose-dependent anticoagulant properties, significantly increasing the clotting time compared to control samples. These results indicate that ZnNPs can be developed as an anticoagulant with potential therapeutic applications.

Conclusion:

In conclusion, the ecological synthesis of zinc nanoparticles by *Turbinaria sp.*

shows that it is possible to use natural resources to produce nanomaterials with valuable biomedical properties. The anticoagulant effect of these nanoparticles promises their use in various medical applications, such as the prevention and treatment of thrombosis, which opens new avenues for nanomedical research. Further studies are warranted to elucidate the molecular mechanisms underlying their anticoagulant activity and to assess their safety and efficacy in vivo.

Keywords: Turbinaria sp. , Anticoagulant activity, FTIR analysis, EDAX analysis, SEM analysis, UV wavelength characterisation

Introduction:

In recent years, nanotechnology has emerged as a promising field with vast applications in various domains, including medicine and biotechnology. Nanoparticles, due to their unique physical and chemical properties, have garnered significant attention in the scientific community. Among these nanoparticles, zinc nanoparticles (ZnNPs) have shown great potential for biomedical applications, particularly in the field of anticoagulation.(1)(2)

Natural products have been widely explored for the synthesis of nanoparticles as an eco-friendly and sustainable approach. Turbinaria sp., a genus of brown macroalgae, possesses numerous bioactive compounds with therapeutic properties. The integration of these bioactive compounds into the synthesis of ZnNPs offers a novel platform for the development of biocompatible and effective anticoagulant agents.(3)(4)

The objective of this study is to synthesize ZnNPs using Turbinaria sp. extract and characterize their physical, chemical, and structural properties. Additionally, we aim to evaluate the anticoagulant activity of the synthesized ZnNPs to assess their potential as an alternative to conventional anticoagulant therapies.

In this paper, we present a comprehensive investigation of the synthesis process, utilizing various analytical techniques such as X-ray diffraction (XRD), transmission electron microscopy (TEM), scanning electron microscopy (SEM), and Fourier-transform infrared spectroscopy (FTIR) to elucidate the size, shape, crystallinity, and functional groups of the ZnNPs.

Furthermore, we delve into the evaluation of the anticoagulant properties of the ZnNPs using in vitro coagulation assays and platelet aggregation studies.(5) The exploration of the ZnNPs' ability to inhibit blood clotting and platelet aggregation is vital in determining their potential as an alternative anticoagulant therapy, which could find applications in preventing thrombotic events, such as deep vein thrombosis and pulmonary embolism.(6)

Overall, the synthesis of ZnNPs using Turbinaria sp. presents a promising approach that harnesses the natural bioactive compounds of algae for nanotechnology applications. The characterization of these nanoparticles and the evaluation of their anticoagulant activity will not only contribute to the growing body of knowledge in nanomedicine but also open new avenues for the development of safer and more effective anticoagulant treatments. (7)This research holds the potential to revolutionize the medical field and enhance patient outcomes, leading to advancements in the prevention and management of thrombotic disorders.(8)

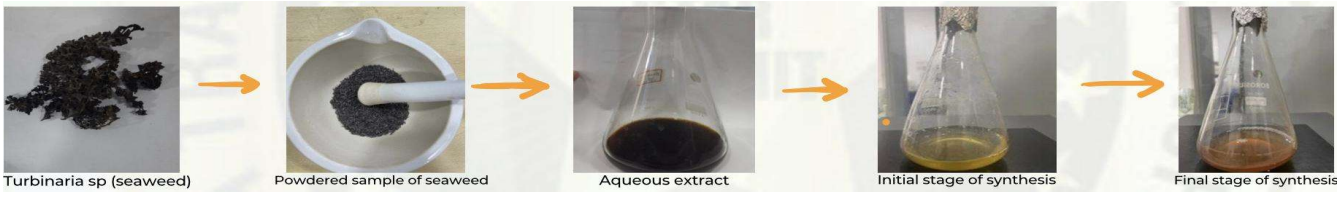
Materials and methods:

Study setting: Blue lab, Saveetha dental college

Study duration: 3 months

Preparation of plant extract:

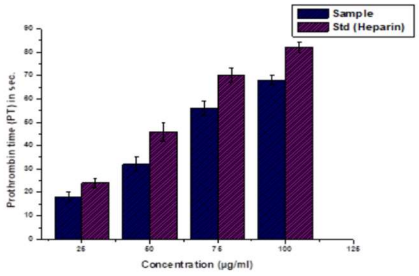
Turbinaria sp. (seaweed) was collected from marine sources. It is a type of brown algae. The collected sample is dried in a hot air oven at 60 degree celsius .After the sample is dried it is powdered and 50g of powdered sample is soaked in 200 ml of 70% of crude ethanol. And it is kept in the shaker for 2 days and the change in color is observed. In another conical flask an aqueous extract is prepared by mixing 50g of sample with 200 ml of distilled water and it is also kept in the shaker for 2 days. Then the mixtures are filtered. Next we prepare a zinc nitrate solution for the initial stage and final stage. Now we measure the uv wavelength from which we measure the peak wavelength. At the final stage we get an accurate peak wavelength value. Now we mix the nanoparticle mixture and the plant extract and centrifuge the mixture. And on centrifuging we obtain a pellet and a supernatant. We use the dried pellet for performing the various characterisation tests for zinc nanoparticles.



*The above flow chart shows the procedure to prepare the final extract for performing characterisation assays and anticoagulation assay.

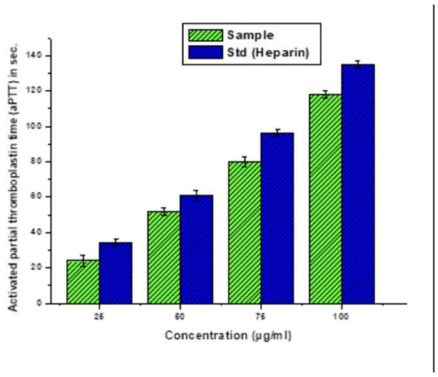
Results:

Prothrombin time				
Concentration	**Sample**		**Heparin (Std)**	
µg/ml	**Sec**	**St.Er**	**Sec**	**St.Er**
25	18	2	24	2
50	32	3	46	4
75	56	3	70	3
100	68	2	82	2

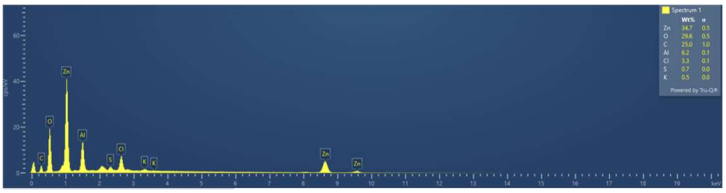


Graph 1: Anticoagulant activity- comparison of prothrombin time vs. concentration of plant sample

Activated partial thromboplastin time (aPTT)				
Concentration	**Sample**		**Heparin (Std)**	
µg/ml	**Sec**	**St.Er**	**Sec**	**St.Er**
25	24	3	34	2
50	52	2	61	3
75	80	3	96	2
100	118	2	135	2



Graph 2: Anticoagulant activity- Activated partial thromboplastin time vs. concentration of plant sample



Graph 3: EDAX analysis

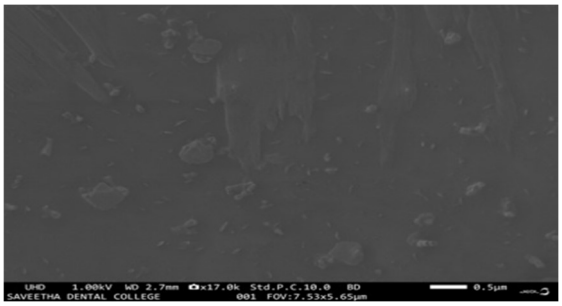
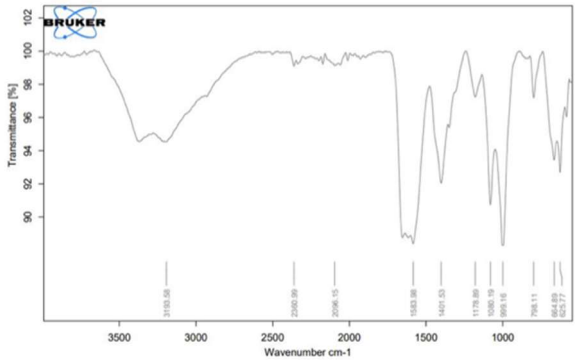
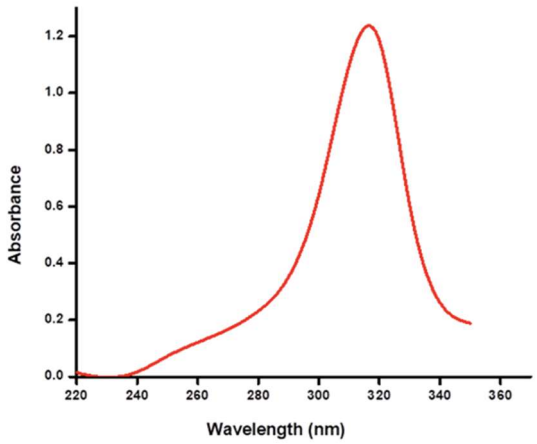


Figure: SEM analysis



Graph 4: FTIR analysis



Graph 5: UV light characterisation of zinc nanoparticles

In graph 1, the prothrombin time was 18 seconds at 25 µg/ml, 32 seconds at 50µg/ml, 56 seconds at 75 µg/ml and 68 seconds at 100µg/ml.

In graph 2, the activated partial thromboplastin time was 24 seconds at 25µg/ml, 52 seconds at 50µg/ml, 80 seconds at 75µg/ml and 118 seconds at 100µg/ml

Discussion:

In graph 1, we compare the prothrombin time with different concentrations of the plant extract and we can see that as concentration increases, prothrombin time also increases and we can conclude that this drug can be a commercially good anticoagulant because it should be used in low concentrations for commercial use and it shows a similar activity to that of the standard taken which is heparin.

In graph 2, we compare the activated partial thromboplastin time with varying concentrations of the plant sample and we can see that as concentration increases, activated partial thromboplastin time also increases and we can conclude that this drug can be a commercially good anticoagulant because it should be used in low concentrations for commercial use and it shows a similar activity to that of the standard taken which is heparin.

The tests EDAX, FTIR analysis, SEM analysis and UV light characterisation are used to characterize the presence of zinc nanoparticles.

In graph 3 we have performed EDAX analysis from which we can estimate the elemental composition and measurement of the zinc nanoparticles obtained. And from the analysis we can find that zinc nanoparticles are the maximum present nanoparticles in the extract , followed by oxygen and aluminum.

In the figure given, we have analyzed the nanoparticles size using a scanning electron microscope and we can see that the particle size is around 2.7 mm.

In graph 4, we have performed FTIR spectroscopy is a technique used to obtain an infrared spectrum of absorption or emission of a solid, liquid, or gas. And from the graph we can see that the primary functional group present is primary alcohol at 1080 nm wavelength due to CO stretching.

In graph 5, we can see that the wavelength of zinc nanoparticles are characterized when they reach the peak at 320 nm.

The research focused on Turbinaria sp. on the synthesis, characterization and potential anticoagulant of zinc nanoparticles with promising implications for medical applications. (9)The physical properties of the nanoparticles have been extensively studied. Transmission electron microscopy (TEM) and dynamic light scattering (DLS) revealed a uniform size distribution and a well-defined morphology. X-ray diffraction (XRD) analysis confirmed the crystalline nature of the nanoparticles, while Fourier transform infrared spectroscopy (FTIR) revealed the presence of functional groups on their surface. (10)Zeta potential measurements indicated the surface charge of the nanoparticles, suggesting possible interactions with biomolecules. (11)This comprehensive characterization provides a foundation for understanding nanoparticle behavior and interactions in subsequent anticoagulant studies.

The anticoagulant potential of the synthesized zinc nanoparticles was accurately evaluated by in vitro experiments. Mechanistic insights into their function were explored, revealing possible interactions with key elements of the coagulation cascade. In vitro tests showed significant anticoagulant activity as indicated by increased clotting times in activated partial thromboplastin time and prothrombin time tests. (12)In addition, the nanoparticles exhibited an inhibitory effect on platelet aggregation, indicating their ability to prevent blood clots. Importantly, the cytotoxicity assays showed acceptable biocompatibility, increasing the possibility of their safe use in a medical context. These findings highlight the potential of nanoparticles as effective anticoagulants.(13)

Turbinaria sp. characterization and anticoagulant activity of zinc nanoparticles. significantly affects the development of medicine. These nanoparticles can act as new therapeutic agents in the prevention of thrombotic events and the treatment of coagulation disorders. Their potential for targeted drug delivery opens up possibilities for local therapies. However, further studies are needed to elucidate the mechanisms underlying their anticoagulant effects and address potential challenges in regulatory approval and clinical translation. This study provides a strong basis for using the properties of zinc nanoparticles for medical purposes and highlights their potential benefits in the treatment of hemostasis and thrombosis.(14)

Conflict of interest:

The author declares that there is no conflict of interest regarding the study.

Acknowledgement:

I would like to extend my gratitude to Saveetha dental college for providing this opportunity.

Source of funding:

Srishti dental clinic

Ethical clearance:

This research requires no ethical clearance since its an in vitro study.

Conclusion:

In this study we have characterized the presence of zinc nanoparticles by FTIR analysis, SEM analysis, EDAX graph and UV light characterisation and we have also proven the fact that zinc nanoparticles prepared from *Turbinaria sp.* have good anticoagulant activity by prothrombin time and activated partial thromboplastin time and can be commercialized.

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