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SYNTHESIS, CHARACTERISATION AND ANTI INFLAMMATORY POTENTIAL OF SILVER NANOPARTICLES FROM HALOPHILA DECIPIENS SEAGRASS

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ABSTRACT

AIM: To synthesise silver nanoparticles using *Halophila decipiens* seagrass and to evaluate the anti-inflammatory potential of the obtained formulation.

INTRODUCTION:

Silver nanoparticles have unique properties and are being tested for biosafety and bio distribution. They have good antimicrobial potential and anti viral potential.*Halophila decipiens* is a pan tropical species of Caribbean seagrass. It is a potential antioxidant and is a promising source of therapeutic products.

MATERIALS AND METHODS:

Dried and powdered sample of *Halophila decipiens* seagrass was taken and an aqueous extract was prepared.A crude extract of *Halophila decipiens seagrass* was prepared by heating in a water bath at 60 degrees celsius.The nanoparticles were biosynthesised using the crude extract.

The nanoparticle formulation was subjected to protein denaturation assay to check its anti inflammatory activity

RESULTS:

The effect of crude extract of *Halophila decipiens* was evaluated using protein denaturation assay.The denaturation was seen as a marked attenuation in a concentration dependent manner.

DISCUSSION:

The results imply that the crude extract, the total flavonoid contents and the aqueous fraction of *Halophila decipiens* exhibited protein denaturation. The isolation of secondary metabolites will be beneficial in understanding the chemical process involved in presenting anti-inflammatory activity.

CONCLUSION:

The findings suggest that *Halophila decipiens* seagrass extracts have anti-inflammatory potential. It can also be implied that the phytochemical contents of the seagrass extracts were key in prevention of protein denaturation.

Keywords: Silver nanoparticles, biosynthesis, seagrass,anti inflammatory effect,halophila decipiens

INTRODUCTION:

The field of nanotechnology has witnessed tremendous growth in recent years, with an increasing focus on the synthesis and characterization of nanoparticles for various applications in medicine and industry. Among the various types of nanoparticles, silver nanoparticles (AgNPs) have garnered significant attention due to their unique properties and potential applications in biomedicine. AgNPs have demonstrated remarkable antimicrobial, anti-inflammatory, and wound-healing properties, making them promising candidates for therapeutic interventions.(1)

Nanoparticles (NPs) are con- sidered as particles with a size of up to 100 nm, which exhibit complete- ly new or improved properties as compared to the larger particles of the bulk material with specific characteristics, such as size, distribution and morphology. (2)Nanoparticles from the noble metals such as gold, silver and platinum are being combined with products that were in direct contact with the human body e.g., shampoos, detergents, soaps, shoes, cosmetic products and toothpaste, besides medical and pharmaceutical applications.(3)(4)

Marine organisms, including seagrasses, have emerged as an intriguing source for the synthesis of nanoparticles due to their rich bioactive compounds and unique environmental adaptations. *Halophila decipiens,* a species of seagrass found in coastal areas, has drawn attention for its medicinal properties and potential as a source of nanoparticles.(5)

This research aims to synthesise and characterise silver nanoparticles using *Halophila decipiens* seagrass extract. The process involves reducing silver ions present in a silver precursor to produce AgNPs, utilising the reducing potential of the seagrass bioactive compounds. The resulting nanoparticles will be characterised using various analytical techniques to understand their size, shape, and stability.

Furthermore, the study will explore the anti-inflammatory potential of the synthesised silver nanoparticles. Inflammation is a complex biological response that occurs in response to harmful stimuli such as pathogens or tissue damage. While acute inflammation is a vital defence mechanism, chronic inflammation can lead to various diseases, including rheumatoid arthritis, atherosclerosis, and cancer. Therefore, there is a growing need to develop effective anti-inflammatory agents with minimal side effects.

The anti-inflammatory potential of silver nanoparticles has been observed in previous studies, where AgNPs have shown the ability to inhibit proinflammatory cytokines, enzymes, and mediators.(4) However, the anti-inflammatory effects of AgNPs derived from *Halophila decipiens* seagrass have not been extensively investigated. This research will fill this knowledge gap and provide valuable insights into the potential therapeutic applications of these nanoparticles.

The study will involve in vitro experiments to evaluate the effect of AgNPs on inflammatory cells and their ability to modulate the production of inflammatory markers. The results obtained will be analysed and discussed in the context of developing novel anti-inflammatory agents.

In summary, this research will contribute to the growing body of knowledge on the synthesis and characterization of silver nanoparticles from *Halophila decipiens* seagrass. Additionally, it will shed light on the anti-inflammatory potential of these nanoparticles, potentially paving the way for the development of new and effective therapeutic agents for inflammatory-related diseases.

MATERIALS AND METHODS:

The study was conducted in the Blue lab of Saveetha Dental College,SIMATS,Chennai for a duration of 3 months. The crude extract was prepared and followed by synthesis of nanoparticles. The obtained formulation was subjected to protein denaturation assay to assess its anti inflammatory property.

Preparation of crude extract

100g of dried and powdered sample of *Halophila decipiens* seagrass was taken as the sample.The samples were collected from a marine source.

Crude extract was prepared from *Halophila decipiens* seagrass. 100g of the sample was kept in 70% ethanol.The sample was left in the orbital shaker for 2 days.The seagrass sample was filtered through Whatmann filter paper No.1.After filtration, the sample was transferred to a 100mL

beaker.The beaker was placed in a water bath at 60 degrees celsius until a crude extract was obtained.

Synthesis of silver nanoparticles

Silver nanoparticles were synthesised in the extract by adding 1mMol of silver nitrate solution.A dark brown colouration indicated the successful synthesis of the particles.

Albumin denaturation assay was performed to determine the anti-inflammatory activity of *Halophila decipiens* seagrass.

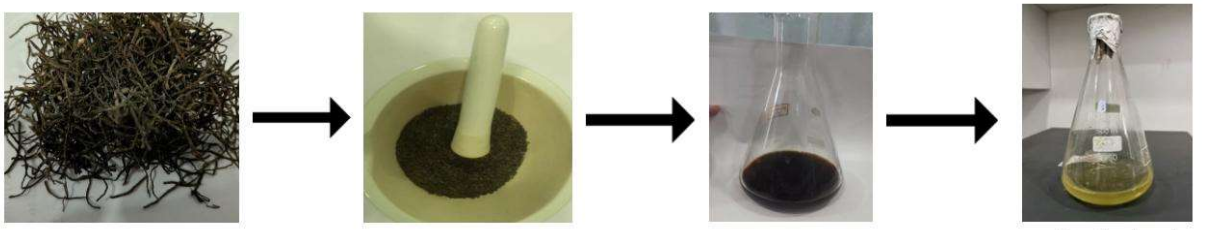
Assessment of anti inflammatory potential using protein denaturation assay

The reaction mixture consisted of 0.2 ml of egg albumin, 2.8 ml of phosphate buffered saline (pH 6.4) and 2 ml of varying concentrations of the test extract, by which the concentrations (µg/ml).Similar volume of double-distilled water served as control. Then the mixtures were incubated at 37°C ± 2°C in a biological oxygen demand incubator for 15 min and then heated at 70°C for 5 min. After cooling, their absorbance was measured at 660 nm (Systronix Spectrophotometer 150) by using the vehicle as blank. Diclofenac sodium at the final concentration (µg/ml) was used as reference.

Anti inflammatory activity assay was performed at 5 different concentrations of 10 µl,20 µl,30 µl,40 µl and 50 µl. Bovine serum was added to all test tubes.

The percentage of inhibition of protein was determined on a percentage basis with respect to control.

Formula: Percentage inhibition(%)= Absorbance of control-absorbance of test *1000



Halophila decipiens seagrass | Powdered sample | Crude extract | Visualisation of nanoparticle synthesis

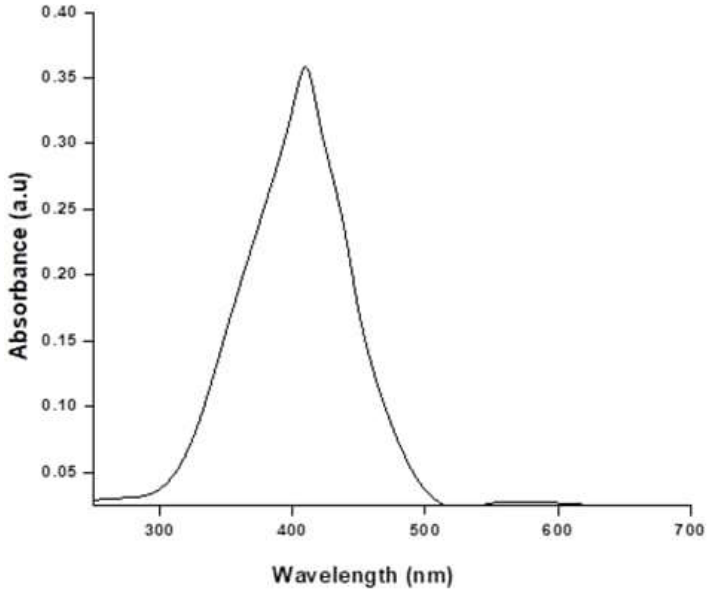
RESULTS:

The present study was undertaken to synthesise silver nanoparticles using *Halophila decipiens* seagrass extracts. Further, the anti-inflammatory potential of the synthesised nanoparticles was assessed to evaluate the properties of the obtained formulation.

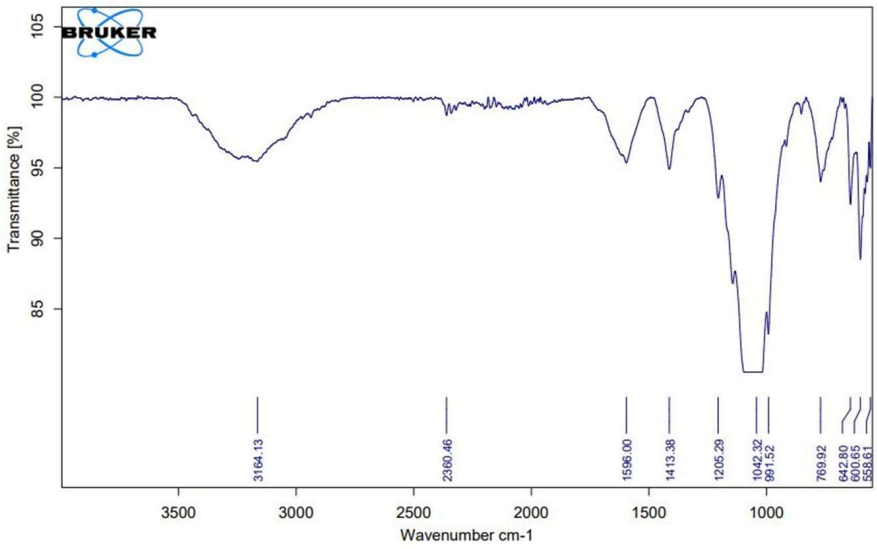
The synthesised nanoparticles were subjected to various tests to assess its size,shape,morphology and to confirm its formation.

Table 1:Protein denaturation assay to test anti inflammatory effects:

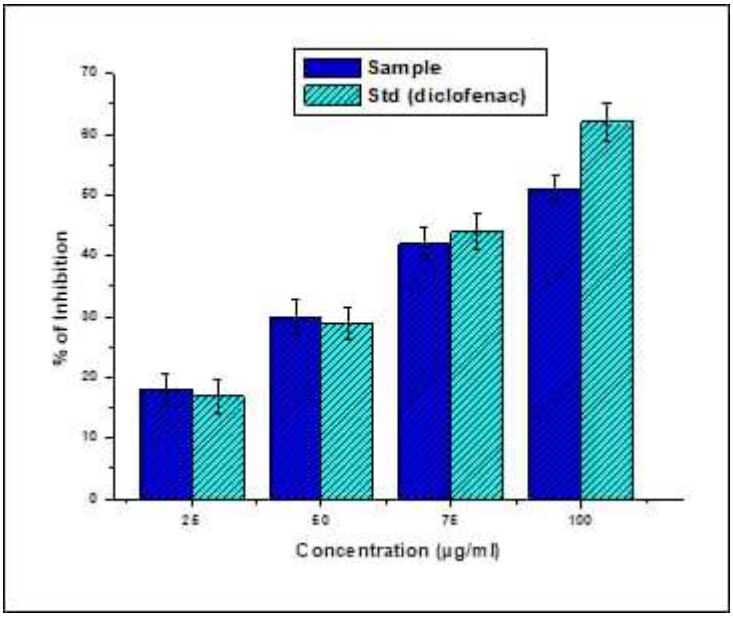
µg/ml	Prt. Den.inbt	Diclofenac
25	18(+/-2.5)	17(+/-2.8)
50	30(+/-2.9)	29(+/-2.6)
75	42(+/-2.7)	44(+/-2.9)
100	51(+/-2.4)	62(+/-3.2)



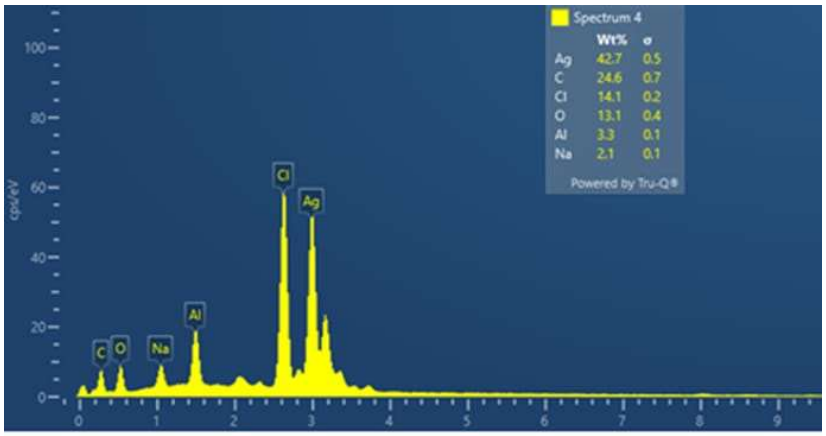
Graph 1: UV Visible nanoparticles



Graph 2: FTIR analysis



Graph 3: Protein denaturation assay



Graph 4: SEM image

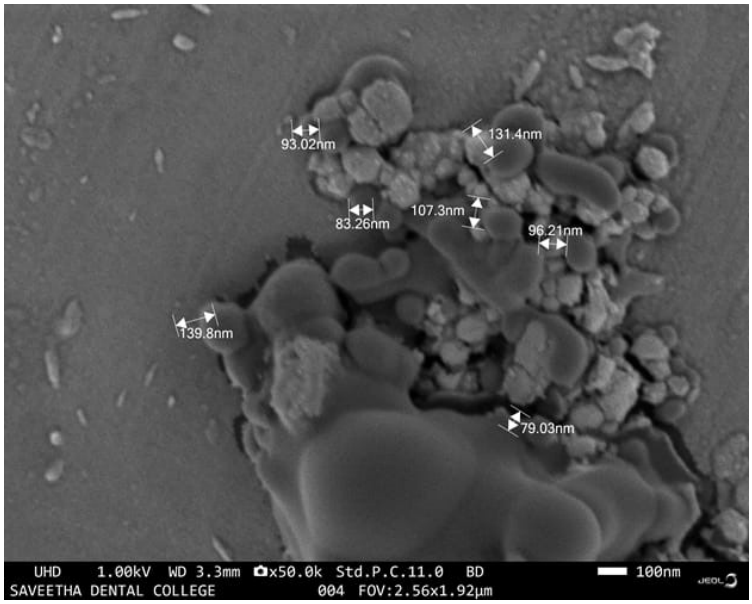


Figure 1: Visualisation of nanoparticle

DISCUSSION:

The silver nanoparticles were synthesised using *Halophila decipiens* seagrass.The nanoparticles so formed were subjected to various assays to check its various properties.

The UV spectroscopy assay confirms the silver nano-particle formation and plasmonic resonance can be found by analysing the absorbance data. The FTIR assay is used to identify and confirm the formation of products through discrimination of similar materials. The SEM images are used to identify the size,shape and morphology of the synthesised nanoparticles. Further, the protein denaturation assay is done to test the anti-inflammatory activity of the silver nanoparticles synthesised using *Halophila decipiens* seagrass.

The protein denaturation assay was performed to check the anti-inflammatory activity of the nanoparticles.(5,6) The reaction mixture consisted of 0.2 ml of egg albumin, 2.8 ml of phosphate buffered saline (pH 6.4) and 2 ml of varying concentrations of the test extract, by which the concentrations (µg/ml).Similar volume of double-distilled water served as control. Then the mixtures were incubated at 37°C ± 2°C in a biological oxygen demand incubator for 15 min and then heated at 70°C for 5 min. After cooling, their absorbance was measured at 660 nm (Systronix Spectrophotometer 150) by using the vehicle as blank. Diclofenac sodium at the final concentration (µg/ml) was used as reference.

The anti-inflammatory potential of silver nanoparticles has been observed in previous studies, where AgNPs have shown the ability to inhibit proinflammatory cytokines, enzymes, and mediators.(7) The denaturation was seen as a marked attenuation in a concentration dependent manner.The results imply that the crude extract, the total flavonoid contents and the aqueous fraction of *Halophila decipiens* exhibited protein denaturation. The isolation of secondary metabolites will be beneficial in understanding the chemical process involved in presenting anti-inflammatory activity.

Biosynthesis of nanoparticles have shown to have less toxic effects. It is also an eco-friendly production method and reduces wastage by usage of the parts commonly discarded by people,i.e, fruit peels. (8)Biosynthesised nanoparticles also have higher antibacterial activity as they have stronger surface interactions with bacteria.The anti-inflammatory potential of silver nanoparticles has been observed in previous studies, where AgNPs have shown the ability to inhibit proinflammatory cytokines, enzymes, and mediators. However, the anti-inflammatory effects of AgNPs derived from *Halophila decipiens* seagrass have not been extensively investigated

Studies done by Asharani et al (9,10)suggest that the toxicity of silver nanoparticles depends on the concentration of treatment particles. They concluded that the deposition of nanoparticles inside the nucleus of the cells led to the observed toxicity through various mechanisms. Therefore, further studies focused on the adverse effects of the Ag NPs are required and the products should be promoted only after detailed studies are done on all aspects of the nanoparticles.

Investigation of the potential toxicity of the seagrass extract should be done in order to ensure their bio safety.Conducting clinical trials to evaluate the anti inflammatory effects of the seagrass extracts in human subjects is of prime importance, thereby emphasising the need for conduction in vivo and toxicology studies to check its efficacy before use on humans.

CONCLUSION

The effect of crude extract of *Halophila decipiens* was evaluated using protein denaturation assay.The denaturation was seen as a marked attenuation in a concentration dependent manner.The results imply that the crude extract, the total flavonoid contents and the aqueous fraction of *Halophila decipiens* exhibited protein denaturation. The isolation of secondary metabolites will be

beneficial in understanding the chemical process involved in presenting anti-inflammatory activity.

Therefore, it can be concluded that *Halophila decipiens* seagrass extracts have anti inflammatory potential. It can also be implied that the phytochemical contents of the seagrass extracts were key in prevention of protein denaturation.

Investigation of the potential toxicity of the seagrass extract should be done in order to ensure their bio safety.Conducting clinical trials to evaluate the anti inflammatory effects of the seagrass extracts in human subjects is of prime importance, thereby emphasising the need for conduction in vivo and toxicology studies to check its efficacy before use on humans.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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- Saveetha university
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ETHICAL CLEARANCE :

Since it is an in vitro study, ethical clearance is not required

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