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GREEN SYNTHESIS OF MARINE SPONGE SILVER NANOPARTICLES AND ITS ANTIOXIDANT, WOUND HEALING ACTIVITY

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ABSTRACT

This study explores the collection, extraction, synthesis, characterization, and evaluation of bioactive compounds from marine sponges collected in Rameswaram district, Tamil Nadu, India. The sponges were collected using a grab sample collection method and processed using Soxhlet extraction to obtain ethanolic extracts. These extracts were then utilized in the green synthesis of silver nanoparticles (AgNPs). The synthesis involved combining a 1mM silver nitrate solution with the sponge extract, followed by centrifugation to isolate the nanoparticles. The AgNPs were characterized using High-Resolution Transmission Electron Microscopy (HR-TEM), revealing their size, shape, and crystalline structure. The antioxidant activity of the AgNPs was assessed through a DPPH radical scavenging assay, showing significant free radical neutralization, albeit slightly less effective than ascorbic acid. Additionally, an *in vitro* scratch assay demonstrated the nanoparticles' potential in promoting fibroblast migration, indicating enhanced wound-healing properties. These findings suggest that marine sponge-assisted AgNPs hold promise for applications in antioxidant therapies and regenerative medicine for wound healing.

KEYWORDS

Marine sponge, Extract, $AgNO_3$, Nanoparticles and Wound healing activity.

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INTRODUCTION

The term marine refers to anything related to the sea or ocean. It is a vast and diverse ecosystem that covers more than 70% of the Earth's surface. The marine environment includes a wide range of habitats, from shallow coastal areas to deep ocean trenches. Marine diversity is incredible, both in terms of species and ecosystems[1]. The diversity of marine life is extraordinary[2]. Different species have adapted to thrive in various marine environments. For example, you have fish that can withstand extreme pressures in the deep sea, and others that

are specially adapted to live in coral reefs[3]. The interconnectedness of these species and ecosystems makes the marine environment incredibly dynamic and fragile at the same time. Conservation efforts are crucial to maintaining the health and balance of marine ecosystems. In the vast expanse of our oceans, where mysteries abound and life takes on myriad forms, marine sponges emerge as captivating organisms that have stood the test of time. These humble creatures, often overlooked in the grand tapestry of marine life, possess a remarkable story of growth and survival. This exploration seeks to unravel the secrets of marine sponges, shedding light on their growth patterns, life cycles, and the unique characteristics that define their existence[4].

The vast and varied world beneath the ocean's surface harbors a treasure trove of biodiversity, and among its diverse inhabitants, marine sponges stand out as fascinating organisms with promising pharmaceutical potential. These unassuming creatures, often overlooked, possess a remarkable array of bioactive compounds that exhibit anti-inflammatory properties. The rich diversity of marine sponges, found in different oceanic environments, offers a vast resource for the discovery and production of novel anti-inflammatory agents. Polyketides and Alkaloids: Marine sponges are known to produce a wide range of bioactive compounds, including polyketides and alkaloids. These chemical constituents have demonstrated anti-inflammatory effects, making them attractive candidates for pharmaceutical development. Biodiversity Hotspots: The diverse ecosystems of coral reefs, deep-sea vents, and other marine environments provide unique niches for various sponge species. Each habitat contributes to the synthesis of distinct bioactive compounds, expanding the potential repertoire of anti-inflammatory agents[5].

Enzyme Inhibition: Some compounds from marine sponges have shown the ability to inhibit enzymes involved in the inflammatory process, providing a targeted approach to modulating inflammatory responses. Certain sponge-derived compounds exhibit immunomodulatory effects, influencing the activity of immune cells and cytokines to regulate inflammation. The exploration of marine sponge diversity contributes to drug discovery efforts, with a focus on developing novel anti-inflammatory drugs. These natural products offer potential alternatives to conventional pharmaceuticals. As interest in marine sponge compounds grows, there is a need for sustainable harvesting practices to ensure the conservation of these valuable organisms and their habitats. Advanced bioprospecting technologies, such as genomics and metabolomics, enhance the efficient identification of bioactive compounds from diverse marine sponge species. Researchers from various disciplines, including marine biology, chemistry and pharmacology, collaborate to unlock the full potential of marine sponges. Such interdisciplinary efforts accelerate the discovery and development of anti-inflammatory agents. The application of biotechnological tools, such as bioprospecting and synthetic biology, holds promise for the sustainable production of sponge-derived anti-inflammatory compounds[6]. Compounds derived from marine sponges are progressing through preclinical studies, paving the way for potential clinical trials and the development of new anti-inflammatory medications. the diverse world of marine sponges presents a promising frontier for the production of anti-inflammatory agents. The unique compounds synthesized by these organisms, thriving in varied marine ecosystems, offer a wealth of opportunities for drug discovery and biotechnological applications. As researchers delve deeper into the molecular intricacies of marine sponge diversity, the potential for finding innovative solutions to combat inflammation continues to expand, opening new chapters in pharmaceutical development inspired by the wonders of the ocean[7].

MATERIAL AND METHODS

Marine sponge collection and identification

Marine sponges were collected from the Rameswaram district, Tamil Nadu, India, using a grab sample collection method. The coordinates of

the location are approximately 9°17'18.60″ " N 79°18'45.76"″ E and it is known for its coastal ecosystem. This collection method involves the use of a specialised grab sampler, an instrument designed to collect samples from marine water. The sampler is lowered into the water, and when it reaches the desired depth, it is triggered to close, thus capturing a grab of the sponge.

Extraction by Soxhlet

Using distilled water, the marine sponge was washed, dried after collection, and examined to confirm that it was disease-free. The overlay was cleaned with 0.1% mercury chloride for 20s. The marine sponge was eventually dried in the sun after being cleaned thrice with distilled water. To separate the bioactive compounds, marine sponge was crushed and 500mg of the powder is placed in the "thimble" composed of robust filter paper and put in chamber E of the Soxhlet apparatus. To remove the ethanolic extract from the solution, heat was added to Flask-A and the vapour was condensed in condenser D. The unprocessed substance is sprayed into the needle of the unprepared medicine and withdraws itself when it interacts with the material. When the liquid level in chamber E reached the top of the syphon tube in chamber C, the liquid in chamber E became a soufflé and was placed in flask A. The procedure continued until one drop of the solvent from the syphon tube was completely evaporated. Crude methanolic extract was collected and preserved for phytochemical analysis.

Green synthesis of silver nanoparticles using methanolic extracts

Green synthesis of marine sponge assisted silver nanoparticles

1 mM solution of silver nitrate in double-distilled water provided a controlled source of silver ions for the experiment. The choice of concentration indicated the precision of the approach. A unique aspect of this experiment involved combining the silver nitrate solution with a marine sponge ethanol extract at a specific ratio of 1:5. The marine sponge extract likely contributed distinct properties to the synthesis process. During the experiment, a magnetic mixer set at 500 revolutions per minute was used for mixing. Controlled cooling below the boiling point suggests a deliberate effort to maintain the optimal conditions for the reaction. Recognising the light sensitivity of silver nitrate, the reaction occurs in a dimly lit environment. This precaution was aimed at preventing unwanted reactions and at maintaining the stability of the reaction mixture. Following this, the mixture was centrifuged at 10,000rpm. This step effectively separated the green nanoparticle-containing silver pellet from the rest of the reaction mixture, visually indicating the presence of the nanoparticles. To ensure the purity of the nanoparticle-containing silver pellet, it was washed multiple times with deionised water. This process aims to remove any remaining silver ions and residues from the marine sponge extract, thereby enhancing the quality of the final product. After purification, the nanoparticle-containing silver pellet was moved to a cold, dry, and shadowy location for further analysis. This careful choice of environment suggests a meticulous approach for analysing the synthesised nanoparticles[7].

Characterization of marine sponge assisted silver nanoparticles

High-resolution transmission electron microscopy- marine sponge assisted silver nanoparticles

Exploring the shape and dimensions of the synthesised AgNPs involved high-resolution transmission electron microscopy (HRTEM) with a model JEM 2100F operating at an acceleration voltage of 200kV. For TEM examination, an aqueous AgNP solution was applied to a carbon-coated copper wire mesh, maintaining a temperature similar to that of ambient air. Capturing images of the AgNPs and identifying the presence of Ag metal in the solution were achieved using an Oxford Instruments (UK) EDS system in conjunction with an HRTEM camera.

Antioxidant activity of marine sponge assisted silver nanoparticles

To assess the antioxidant activity of the marine sponge-assisted AgNPs, a modified procedure outlined by Muddukrishnaiah *et al*[16] was employed.

Sample stock solutions were prepared at a concentration of 1.0mg/ml and serially diluted to achieve concentrations of 100, 200, 300, and 400µg/ml. To each concentration, 1 milliliter of 0.3mM DPPH in methanol was added to 2.5 millilitres of the sample solutions, allowing the mixtures to react at room temperature. Following a 30-minute reaction time, the absorbance was measured at 517nm using an ELISA reader. The antioxidant potential percentage (AA) was calculated based on these readings, offering insights into the antioxidant capacity of the extract. Antioxidant activity (I %) = (Abs. blank- Abs. sample)/Abs. blankX100[6].

Marine sponge assisted silver nanoparticles *In vitro* Wound healing activity-scratch assay.

To assess the *in vitro* inflammatory activity of marine sponge-assisted AgNPs using the scratch assay, fibroblast cells were cultured to form a single fusion layer in six-layered plates. A straight line was incised through the single-cell layer using a 200-µL pipette tip. To eliminate impurities, the cells were washed three times with phosphate-buffered saline (PBS). As a control, 0.2% PBS was applied, followed by the addition of 50g of AgNPs to the cells, which were then promptly cultivated. Initial images of the fissures were captured using a phase-contrast microscope. Three culture plates were prepared for each group and the cells were cultured for 48 h at 37°C. Cell growth in the scratch area was evaluated at 0 and 48 h using a phase-contrast microscope[7].

RESULTS AND DISCUSSION

High-Resolution Transmission Electron Microscopy-marine sponge assisted silver nanoparticles

Marine sponge assisted silver nanoparticles characterised by using the HR-TEM analysis and Nanoparticles were formed successfully.

High-Resolution Transmission Electron Microscopy (HR-TEM) is a powerful technique for characterizing the structural details of nanomaterials at the atomic or nanometer scale. When studying marine sponge-assisted silver nanoparticles, HR-TEM plays a crucial role in providing insights into the size, shape and crystalline structure of these nanoparticles. HR-TEM allows for the direct observation of individual silver nanoparticles, providing high-resolution images that reveal their size and morphology. Marine sponge-assisted synthesis methods may result in nanoparticles with unique shapes or sizes and HR-TEM enables the detailed examination of these characteristics. HR-TEM provides information about the crystal structure of silver nanoparticles.

Antioxidant activity of marine sponge assisted silver nanoparticles

The assessment of the antioxidant activity of marine sponge-assisted silver nanoparticles, as indicated by their DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging ability, has yielded intriguing results. The concentrations of marine sponge nanoparticles were compared with the well-established antioxidant, ascorbic acid, at various concentrations. This discussion aims to dissect and interpret the implications of the provided values. At the highest concentration of 125, marine sponge nanoparticles exhibited a DPPH scavenging activity of 68.01%, while ascorbic acid, a known potent antioxidant, displayed a higher activity of 98.01%. This comparison sets the stage for understanding the relative antioxidant potential of marine sponge nanoparticles compared to a standard reference. The observed DPPH scavenging activity suggests a substantial capability of marine sponge nanoparticles to neutralize free radicals, albeit at a lower efficacy than ascorbic acid. As the concentration of marine sponge nanoparticles decreased, a dose-dependent reduction in DPPH scavenging activity was evident. This diminishing trend is expected and aligns with the general principle that antioxidant activity often correlates with the concentration of the antioxidant agent. The concentration of 62.5 displayed a reduction in DPPH scavenging to 58.82%, indicating a decline in antioxidant efficacy compared to the higher concentration. Further reductions in concentration continued to show decreasing DPPH scavenging

activity. At a concentration of 31.25, the marine sponge nanoparticles exhibited a scavenging activity of 52.87%, while ascorbic acid maintained a higher activity at 72.87%. This pattern persisted, with a gradual decrease in DPPH scavenging as the concentration diminished, reflecting the concentration-dependent nature of the antioxidant response. Interestingly, even at lower concentrations, marine sponge nanoparticles displayed notable DPPH scavenging activities. For instance, at a concentration of 1.94625, the scavenging activity was recorded at 26.01%, signifying a residual antioxidant effect even at lower concentrations[9].

The antioxidant activity of marine sponge assisted silver nanoparticles is significant due to its potential applications in various fields, including medicine, biotechnology, and materials science.

The antioxidant activity principle involves the ability of certain compounds to neutralize or counteract the harmful effects of reactive oxygen species (ROS) and free radicals within the body. Antioxidants play a crucial role in maintaining cellular health and preventing oxidative stress, which is associated with various diseases and aging processes. Antioxidants function by scavenging free radicals and ROS. Free radicals are highly reactive molecules with unpaired electrons, which can cause damage to cellular structures by oxidizing lipids, proteins and DNA. Antioxidants neutralize these free radicals by donating electrons, thereby preventing the chain reaction of oxidative damage.

Marine sponge assisted silver nanoparticles *In vitro* inflammatory activity-scratch assay

In this study, the *in vitro* inflammatory activity of Marine sponge-assisted silver nanoparticles was elucidated through a meticulously designed scratch assay. Utilizing skin fibroblast cells as a representative model for cellular response, a confluent monolayer was subjected to controlled scratches, simulating a wound-like environment. The introduction of Marine sponge-assisted silver nanoparticles at a concentration of 50µg revealed a compelling narrative of accelerated cell migration over time. The scratch closure at 12 and 24 hours, as compared to the untreated cells, underscored the nanoparticles' capacity to enhance the migratory capabilities of fibroblasts[10]. Crucially, the inclusion of a positive control validated the experimental setup, affirming its sensitivity to detect enhanced migration. These findings not only suggest a sustained and time-dependent impact of the nanoparticles on cell migration but also hint at their potential anti-inflammatory and wound-healing properties. As the research progresses, delving into the intricate mechanisms underlying this enhanced migration could unveil novel insights into the nanoparticles' mode of action in the context of inflammation. Overall, this study paves the way for considering Marine sponge-assisted silver nanoparticles as a promising candidate for therapeutic interventions in regenerative medicine and dermatology.

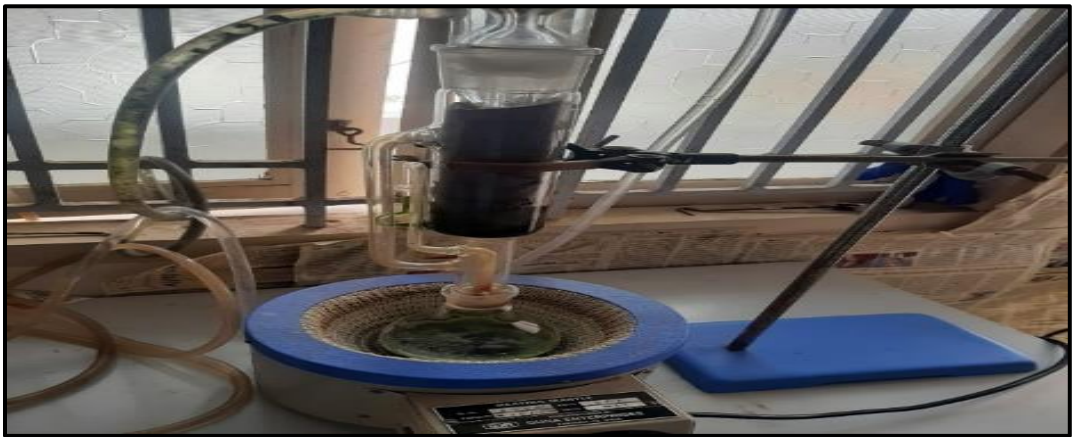

Figure No.1: Ethanolic extract of marine sponge

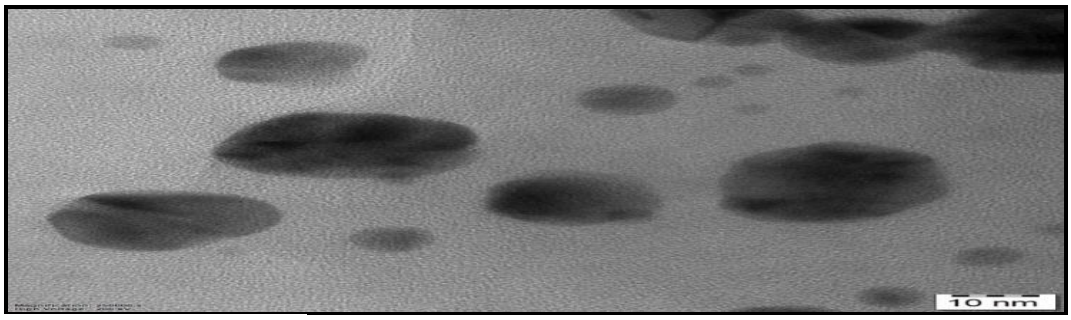

Figure No.2: HR-TEM Spectroscopy marine sponge assisted silver nanoparticles

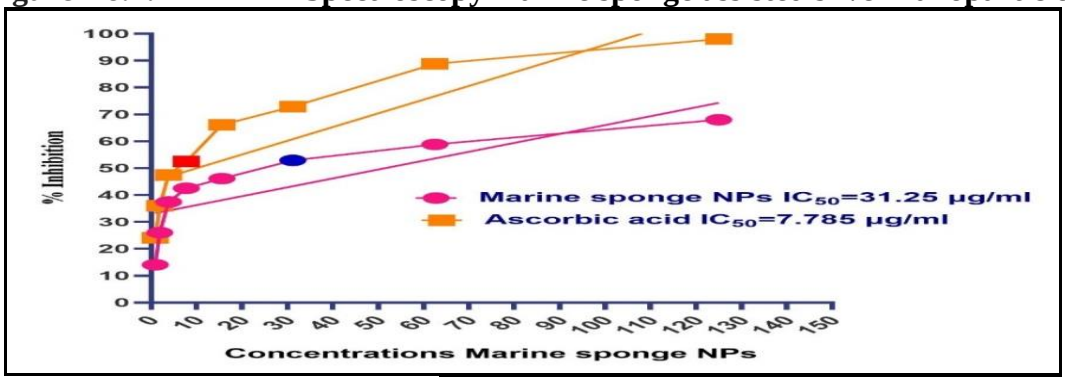

Figure No.3: Antioxidant activity of marine sponge assisted silver nanoparticles-DPPH

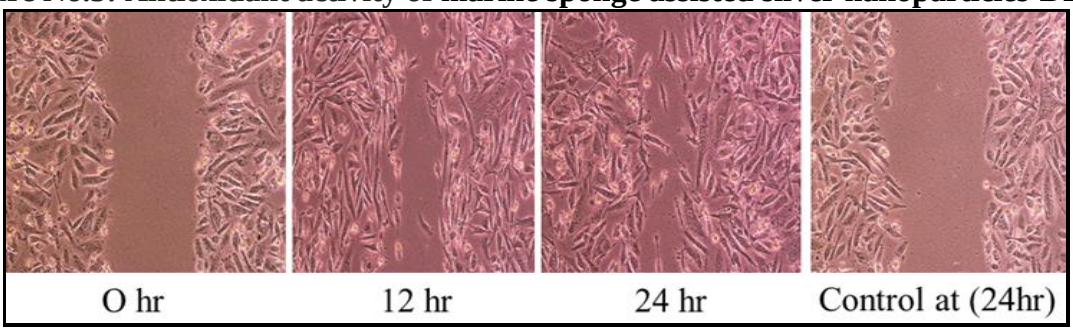

Figure No.4: Marine sponge assisted silver nanoparticles accelerated migration fibroblast in scratch assay. Scratch assay of fibroblast treated with Marine sponge assisted silver nanoparticles for 0, 12 and 24hr

CONCLUSION

The study highlights the successful synthesis and characterization of marine sponge-assisted silver nanoparticles (AgNPs) and their significant antioxidant and wound-healing properties. The antioxidant activity, as demonstrated by the DPPH radical scavenging assay, reveals that the marine sponge nanoparticles exhibit substantial free radical neutralization capabilities, though slightly less potent than the reference antioxidant, ascorbic acid. Furthermore, the in vitro scratch assay underscores the nanoparticles' potential in promoting fibroblast migration, suggesting enhanced wound-healing properties. These findings collectively suggest that marine sponge-assisted AgNPs could be promising candidates for applications in medicine, particularly in antioxidant therapies and regenerative medicine for wound healing.

CONSENT FOR PUBLICATION

Not applicable.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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