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Exploring the Biotechnological Potential of Marine Sponges: Insights into Endophytic Microbial Diversity, Fungal Biosynthesis of Copper Nanoparticles, and Antimicrobial Properties

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Abstract:

This article presents findings from a study on marine sponges collected from two sites in Hurghada, Red Sea, Egypt, aiming to understand the ecological dynamics and distribution of sponges in the region. The samples, including SA1, SA2, and SA3, were collected at varying depths from different sponge species at distinct sites. This information contributes to our understanding of marine ecosystems in the Red Sea. The study isolated endophytes from these sponges, revealing a diverse array of microbial communities. Particularly, the endophytes from SM1, SM2, SM3, SM4, SM5, SS2, SS3, SN11, SN12, and M56 exhibit potential for biotechnological applications. Sponges host unique microbial communities, potentially biotechnologically relevant. Fungal filtrates, like SM4, successfully synthesize Copper Nanoparticles, enhancing understanding of fungal strains proficient in nanoparticle biosynthesis. Morphological and microscopic examinations of *Aspergillus* sp. help identify and characterize the fungal genus. Different species are distinguished by colony and structure features. Copper nanoparticles show moderate antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*, with potential for antifungal agents in medical and healthcare settings.

Keywords: Marine Sponges**,** Endophytic Fungus, Microbial Diversity, Copper Nanoparticls, Antibacterial, antifungal, Nanobiotechnology, Metallic nanoparticles.

1. Introduction

Nanobiotechnology, positioned at the crossroads of nanotechnology and biology, stands as a transformative field with far-reaching implications for molecular biology and healthcare [1]. Its profound significance lies in its multifaceted applications, spanning drug and gene delivery, biomarker analysis, and targeted drug therapy, all contributing to the advancement of medical science and disease management [2,3]. One intriguing avenue within this dynamic landscape is the exploration of copper (Cu) particles, which emerge as vital structural components in various biological tissues, including elastic tissue, skin, bone, and other organs. These structural roles, however, merely scratch the surface of copper's contributions to biological processes [4].

Copper, as an essential trace element, is intricately involved in a plethora of biological functions at both molecular and cellular levels. Notably, it assumes a pivotal role in the photosynthetic electron transport chain, hormone signaling, cell wall metabolism, protein regulation, mitochondrial respiration, and the oxidative stress response. This diversity of roles highlights copper's versatility as a key player in maintaining the integrity and functionality of biological systems. At the cellular level, copper is indispensable in metabolic pathways, influencing oxidative phosphorylation, signal trafficking, and the mobilization of ions, such as iron, particularly during inflammatory responses [5].

The amalgamation of nanobiotechnology with the intricate and diverse functions of copper opens promising avenues for innovative research and applications. Exploring the interplay between nanotechnology and copper within biological systems not only enriches our understanding of fundamental processes but also holds great potential for the development of novel therapeutic interventions and targeted treatments. As we delve deeper into the synergies between nanobiotechnology and copper, the prospects for advancements in disease diagnosis, treatment, and personalized medicine appear increasingly promising [6].

The biosynthesis of copper oxide using associated fungi represents an intriguing avenue in nanotechnology research. Fungi, owing to their unique metabolic capabilities and interactions with metal ions, have emerged as promising agents for the eco-friendly synthesis of nanoparticles. In this process, fungi play a dual role as both reducing and stabilizing agents, facilitating the transformation of copper ions into copper oxide nanoparticles. The associated fungi contribute to the biotransformation through the secretion of enzymes and biomolecules that participate in the reduction of copper ions and the subsequent stabilization of the formed nanoparticles. This green synthesis approach offers several advantages, including the use of non-toxic and biodegradable agents, mild reaction conditions, and the potential for large-scale production. Understanding the biosynthetic mechanisms involved in copper oxide synthesis using associated fungi not only contributes to the advancement of nanotechnology but also aligns with sustainable practices, minimizing the environmental impact associated with traditional chemical synthesis methods.

2. Material and methods

2.1. Marine Sample collection

The marine sponge samples were collected using SCUBA equipment at 5 and 10 m depths in Hurghada region, Red Sea, Egypt. The sponges were kept at the fridge until used for investigation and analysis.

2.2. Isolation of associated fungi

The marine sample parts were rinsed with sterile sea water and then surface sterilized under sterile circumstances (SSW) for marine samples while normal samples were rinsed with fresh water submerged for one minute in 70% ethanol. The parts were sterilized once more by rinsing with SSW and fresh water, then 1 minute of submersion in 2% sodium hypochlorite, followed by another rinse with SSW and fresh water 3 times. Following that, 4 to 5 drops of the water from the final rinse were inoculated on potato dextrose agar medium to evaluate the effectiveness of the process of surface sterilization. The treated samples pieces were then placed on potato dextrose agar plates, approximately three weeks of incubation at 28 °C were followed by daily checks to look for the emergence of new colonies of fungi.

2.3. Morphological identification of isolated fungi

The study of colony morphology on growth media, including the texture, color, and pattern of fungal colonies, is another essential component of morphological identification. Additionally, the examination of reproductive structures, such as spore-bearing structures, is crucial for accurate classification. Techniques like staining, slide culture preparations, and mounting slides for microscopic examination are commonly employed to enhance visibility and aid in the identification process [7].

2.4. Preparation of fungal culture filtrate

Small-scale fermentation was performed in 250 mL flasks using a potato dextrose broth medium. For the liquid medium, the fungal culture was inoculated into 50 mL of 250 Erlenmeyer flask for 7 days at 28oC at 120 rpm. After incubation, the cells were separated from the culture supernatants by 6,000 rpm, 10-minute centrifugation at 6 °C.

2.5. Screening fungal ability to Biosynthesis of Cu

The ability of the obtained fungus to biosynthesize CuNPs. Concentrations (1mM) of copper sulphate (CuSO4) were prepared and added into fungal filtrate with an equal ratio of 1:1. 25 ml of copper sulfate was mixed with 25 ml of fungal extract and incubated in an arbitrary shaker for 24 h at 30 ° C. Filtrate without CuSO4 was used as a control. The final yield of CuNPs synthesized from the fungal extract was 0.015–0.201 µg/ml quantified through a spectrophotometer.

3. Results

3.1. Marine sample collection

The marine sponges have been collected from two sites at Hurghada, red se, Egypt. SA1, sourced from Site 1, originated from Sponge 1 at a depth of 5 meters. SA2, also from Site 1, was collected from Sponge 2 at a slightly deeper depth of 7 meters. SA3, obtained from Site 2, featured Sponge 3 at the same depth of 7 meters. These findings provide a concise overview of the sampling locations, associated sponge species, and depths, contributing essential information to the understanding of the ecological dynamics and distribution of sponges in the Red Sea **(Table1).**

Table (1) Marine samples data.

Sample code	Site No.	species nature	Depth M
SA1	Site 1	Sponge 1	5
SA2	Site 1	Sponge 2	7
SA3	Site 2	Sponge 3	7

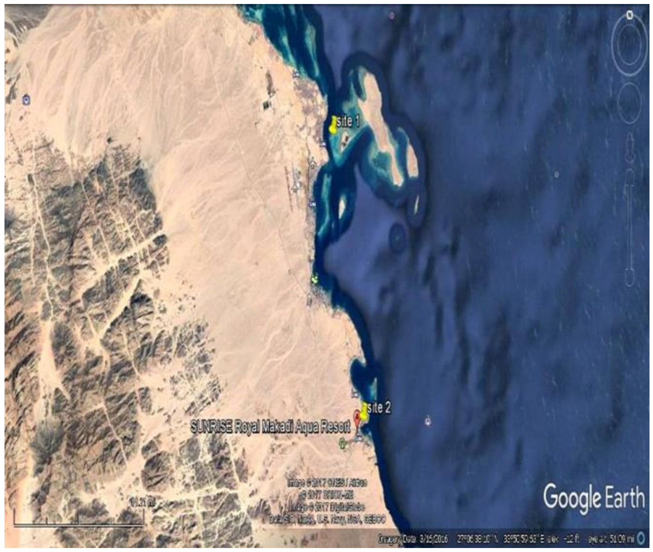

Fig. (1) Site for collection of marine samples.

3.2. Isolation of associated fungi

Table 2 presents the isolated endophytes from various marine samples, including Sponge 1 (SM1, SM2, SM3, SM4, SM5), Sponge 2 (SS2, SS3, SS3), and Sponge 3 (SN11). The location of Sponge 1 is Hurghada (Site 1), Sponge 2 is from Hurghada (Site 2), and Sponge 3 is also from Hurghada (Site 2). The examination of sponges from the Red Sea represents a significant aspect of marine research, given the region's unique ecological characteristics. Sponges, as filter-feeding organisms, play a crucial role in maintaining the balance of marine ecosystems. The diversity of sponge species in the Red Sea contributes to its overall biodiversity, making it a valuable area for ecological studies. Additionally, the isolated endophytes from these sponges, as indicated in Table 2, hold promise for biotechnological applications.

3.3. Screening the isolated fungal filtrate ability to the biosynthesis of CuNPs

The study involved evaluating the potential of the isolated fungal filtrate in biosynthesizing Copper Nanoparticles (CuNPs). The procedure commenced with the preparation of fungal extract using Potato Dextrose Broth. Subsequently, the filtrate derived from the extract was screened for its proficiency in CuNP biosynthesis. Notably, only the fungus labeled as SM4 exhibited the capability for CuNP biosynthesis. Detailed results are presented in Table 3, providing insights into both positive and negative CuNP formation. The assessment criteria included the observation of a blue color indicative of CuNP formation. This thorough analysis not only underscores the success of CuNP biosynthesis by the SM4 fungus but also furnishes valuable information regarding the extent of nanoparticle formation based on precipitate characteristics. These findings enhance our comprehension of specific fungal strains adept at mediating CuNP biosynthesis, opening avenues for potential applications across diverse fields.

Table (2) Isolated endophytes from different marine samples.

Sample code	Endophyte isolate	location of Sponge
Sponge 1	SM1 SM2 SM3 SM4 SM5	Hurghada (Site 1)
Sponge 2	SS2 SS3 SS3	Hurghada (Site 2)
Sponge 3	SN11 SN12 M56	Hurghada (Site 2)

Table (3) Screening of isolated fungi ability to biosynthesis CuNPs

Endophyte isolate	Formation of blue color
SM1	+
SM2	+
SM3	-ve
SM4	+++
SM5	-ve
SS2	-ve
SS3	+
SS3	+
SN11	-ve
SN12	++
M56	++

3.4. Identification of isolated fungus

Morphological and microscopic examinations of *Aspergillus* sp. are essential steps in the identification and characterization of this fungal genus. Morphologically, *Aspergillus* species typically exhibit a distinctive appearance on solid growth media. Colonies often display a filamentous and fluffy structure, with colors ranging from white to various shades of green or yellow. The reverse side of the colony may have a different coloration.

Microscopic examination involves studying the reproductive structures and cellular morphology under a microscope. *Aspergillus* species produce characteristic conidiophores, which are specialized structures that bear conidia. The conidia are often produced in chains, forming structures resembling a brush or broom. The conidia are usually spherical or elliptical, with a smooth or ornamented surface. Additionally, the conidiophores often have a characteristic flask-shaped structure.

Staining techniques, such as lactophenol cotton blue, can enhance the visibility of various structures during microscopic examination. Furthermore, observing the conidial heads, their arrangement, and the size and shape of the conidia aid in distinguishing different Aspergillus species.

3.5. Antimicrobial activity

The results of the antibacterial activity assessments against *Staphylococcus aureus (S. aureus)* and *Escherichia coli (E. coli)* for the different agents, including crude extract, copper nanoparticles (CuNPs), and ciprofloxacin (Cipro, 10 µg/ml), reveal distinct patterns of effectiveness **Table 4 Figure 2.**

The crude extract demonstrated moderate antibacterial activity, with inhibition percentages of 30.3% against *S. aureus* and 50.25% against *E. coli*. This suggests that the crude extract possesses some inherent antibacterial properties, albeit with a more significant effect against *E. coli*.

In contrast, the application of copper nanoparticles (CuNPs) substantially enhanced the antibacterial activity. The CuNPs exhibited a notable increase in inhibition percentages, reaching 78.25% against *S. aureus* and 70.25% against *E. coli*. This enhancement highlights the potential of CuNPs as effective antibacterial agents, particularly against *S. aureus*.

Comparatively, the positive control, ciprofloxacin (Cipro, 10 µg/ml), displayed the highest antibacterial activity among the tested agents. The inhibition percentages were 99.23% against *S. aureus* and 98.58% against *E. coli*, indicating the strong efficacy of ciprofloxacin in inhibiting the growth of both bacterial strains.

The results underscore the promising antibacterial potential of copper nanoparticles, showcasing their ability to significantly enhance the inhibitory effects against both *S. aureus* and *E. coli* compared to the crude extract. While ciprofloxacin remains the most potent antibacterial agent in this study, the findings suggest that copper nanoparticles could serve as valuable alternatives or supplements in the development of antibacterial agents with potential applications in various fields, including medicine and healthcare. Further studies on the mechanisms of action and safety profiles of copper nanoparticles are warranted for a comprehensive assessment of their potential as antibacterial agents.

Table (4) Antibacterial activity of CuNPs

	Antibacterial activity (%)	
	E. coli	***S. aureus***
Crude	50.25	30.3
CuNPs	70.25	78.25
Cipro(10ug/ml)	98.58	99.23

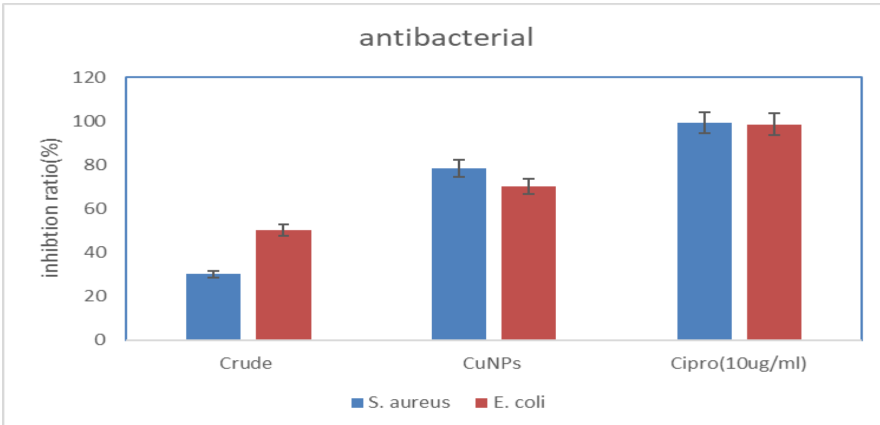

Fig. (2) Antibacterial activity.

3.6. Antifungal activity

The antifungal results against *Candida albicans* (*C. albicans*) demonstrate varying degrees of effectiveness for the tested agents, including crude extract, copper nanoparticles (CuNPs), and nystatin (Nyst, 20 µg/ml) **Table (5) Fig. (3)**

The crude extract exhibited a moderate antifungal activity with an inhibition percentage of 25.68% against *C. albicans*. This suggests that the crude extract possesses some inherent antifungal properties, although the efficacy appears to be relatively limited.

In contrast, the application of copper nanoparticles (CuNPs) significantly enhanced the antifungal activity. The CuNPs demonstrated a notable increase in inhibition percentage, reaching 45.36% against *C. albicans*. This enhancement suggests the potential of CuNPs as effective antifungal agents against *C. albicans*, showcasing their ability to inhibit the growth of the fungus.

Comparatively, the positive control, nystatin (Nyst, 20 µg/ml), displayed the highest antifungal activity among the tested agents. The inhibition percentage was remarkably high at 97.98% against *C. albicans*, indicating the strong efficacy of nystatin in inhibiting the growth of this fungal strain.

The results highlight the promising antifungal potential of copper nanoparticles, suggesting their ability to enhance the inhibitory effects against *C. albicans* compared to the crude extract.

While nystatin remains the most potent antifungal agent in this study, the findings indicate that copper nanoparticles could serve as valuable alternatives or supplements in the development of antifungal agents, with potential applications in medical and healthcare settings. Further investigations into the mechanisms of action and safety profiles of copper nanoparticles are essential for a comprehensive evaluation of their potential as antifungal agents.

Table (5) antifungal activity of biosynthesized CuNPs

	Antifungal activity (%)
Samples	***C. albicans***
Crude	25.68
CuNPs	45.36
Nyst (20 ug/ml)	97.98

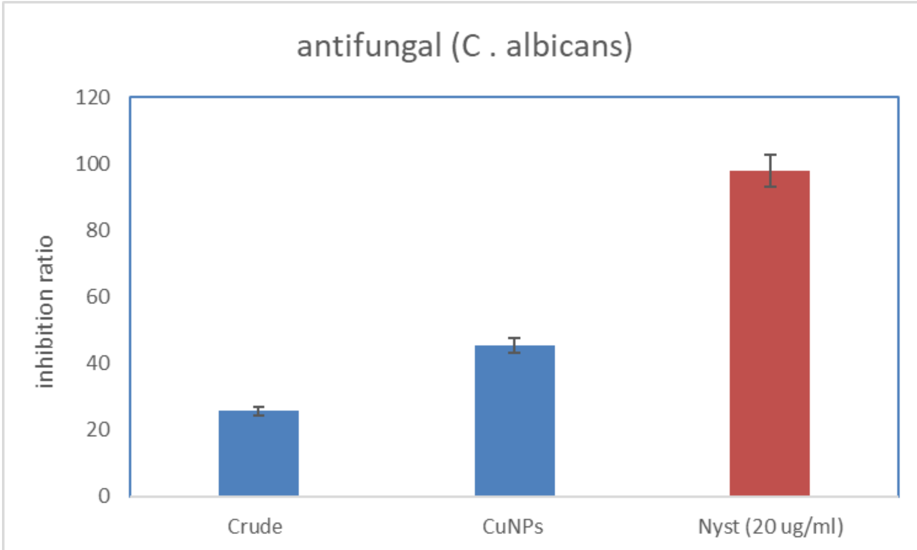

Fig. (3) antifungal activity of biosynthesized CuNPs

4. Discussion

Currently, marine microorganisms are considered an undervalued source of undiscovered active chemicals. Some microbes are planktonic, whereas others are symbiotic. For instance, sponges harbor microbes. Isolating marine fungus from macroorganisms' tissues can be a simple method for obtaining marine fungi [8]. The fungus *Aspergillus flocculosus* 168ST-16.1 was obtained from the algae *Padina sp.* found at a depth of 10 meters in Da Nang, Vietnam [9]. Choi *et al.* identified 25 endophytic fungus from the marine sponge *H. fascigera* in their investigation.

The bioactivity of endophytic fungus was tested against microbial pathogens including *S. aureus*, *E. coli*, and *C. albicans* using the agar diffusion method. Eight isolates demonstrated antimicrobial activity by inhibiting the growth of *S. aureus* with halos measuring between 11 and 16.5 mm based on the test findings. None of the extracts showed activity against the Gram-negative bacteria. We identified 11 endophytic fungi from 3 distinct marine sponge sources in Hurghada, Egypt for our investigation, and the *Aspergillus sp.* was identified based on its morphological characteristics. Table 2 displays the isolated endophytes from several marine samples, such as Sponge 1 (SM1, SM2, SM3, SM4, SM5), Sponge 2 (SS2, SS3, SS3), and Sponge 3 (SN11). This study aimed to produce nanoparticles by a microbial-based method and assess their antibacterial properties. Fungi are excellent candidates for the biogenic production of copper nanoparticles due to their strong metal resistance and ease of handling. *Penicillium citrinum*, *Penicillium aurantiogriseum*, and *Penicillium waksmanii* have been shown to produce spherical Cu-nanoparticles [10].

The work offers a detailed understanding of the environmentally benign production of copper nanoparticles by marine fungi and the assessment of their antimicrobial properties. The fungus identified as SM4 was the only one capable of biosynthesizing CuNPs. Table 4 presents comprehensive results on the creation of both positive and negative CuNP, offering valuable insights. The assessment criteria involved observing a blue tint that indicates the production of CuNP. Antibiotic resistance is a significant health concern that greatly affects human well-being [11]. The study demonstrated diverse efficiency patterns of different treatments, such as crude extract, copper nanoparticles (CuNPs), and ciprofloxacin, against *Staphylococcus aureus* and *Escherichia coli*. The crude extract exhibited moderate antibacterial activity, inhibiting 30.3% of *S. aureus* and 50.25% of *E. coli*. Copper nanoparticles greatly enhanced efficiency, obtaining inhibitory percentages of 78.25% against *S. aureus* and 70.25% against *E. coli*. Ciprofloxacin, the positive control, exhibited the highest antibacterial activity, blocking 99.23% and 98.58% of both bacterial species. The work emphasizes the robust antibacterial characteristics of copper nanoparticles, indicating their potential as alternative or additional antibacterial agents for possible application in medicine and healthcare.

Naqvi *et al.* found comparable outcomes, achieving a 21 mm zone of inhibition (ZOI) against *S. aureus* [12]. A study by Saad *et al.* found that CuNPs exhibited a 16.3 mm Zone of Inhibition (ZOI) against *S. aureus* and a 26 mm inhibitory zone against *E. coli*. Research conducted by Caroling and colleagues found that CuNPs produced inhibitory zones of 10 mm against *E. coli* and 12 mm against *S. aureus* [13-14]. The research confirmed that fungus-synthesized CuNPs are effective antibacterial agents. The level of inhibition depends on the concentration of nanoparticles and the initial concentration of the bacteria. Nanoparticles of small size can strongly adsorb to bacterial cell surfaces, disrupting the membrane and causing intracellular substances to flow, which damages the bacterial cells [15]. The antifungal properties of nanoparticles were tested by comparing the growth rates of *A. niger* exposed to varying concentrations (0.5%, 1%, and 1.5%) of Cu nanoparticles with a control group. The growth rate of *A. niger* at a 0.5% concentration of Cu nanoparticles was detected. This study examined the antifungal effectiveness of crude extract, copper nanoparticles (CuNPs), and nystatin against *Candida albicans*. The crude extract exhibited modest effectiveness by suppressing 25.68% of growth. CuNPs shown a notable increase in efficacy, reaching 45.36%, indicating their potential as potent antifungal agents. Nystatin, the positive control, exhibited the highest antifungal activity by blocking 97.98% of the fungus strain, show causing its powerful ability to suppress the fungus.

Nanobiotechnology plays a crucial role in molecular biology by addressing many diseases through medication and gene delivery, biomarker analysis, and targeted drug therapy [16]. Copper (Cu) particles are a vital component of elastic tissue, skin, bone, and several organs. It plays a role in the photosynthetic electron transport chain, hormone signaling, cell wall metabolism, protein regulation, mitochondrial respiration, and oxidative stress response. At the cellular level, it is crucial for metabolic processes such oxidative phosphorylation, signal trafficking, and ion mobilization, including iron inflammation [17].

5. Conclusion

In conclusion, this comprehensive study sheds light on various aspects of marine samples collected from the Red Sea, offering valuable insights into the ecological dynamics and potential applications of associated microorganisms. The isolation of endophytes from diverse sponge species underscores the microbial richness of these marine ecosystems, providing a promising foundation for future biotechnological endeavors. The successful biosynthesis of Copper Nanoparticles (CuNPs) by the fungus labeled as SM4 presents a noteworthy achievement, indicating the potential of this fungal strain for nanoparticle production. This finding opens avenues for applications in nanotechnology and materials science, emphasizing the ecological and biotechnological relevance of marine-derived fungi.

Morphological and microscopic examinations of *Aspergillus sp.* contribute to the understanding of fungal biodiversity, providing essential information for fungal identification and characterization. This knowledge enhances our ability to explore the roles of specific fungi in various ecological contexts. Antimicrobial assessments reveal the moderate antibacterial activity of crude extracts, while copper nanoparticles (CuNPs) demonstrate significant enhancement, showcasing their potential as effective antibacterial agents. The study suggests that CuNPs could serve as alternatives or supplements to conventional antibacterial agents, particularly against *Staphylococcus aureus* and *Escherichia coli*.Similarly, in antifungal assessments against *Candida albicans*, CuNPs exhibit enhanced antifungal activity compared to crude extracts. Although nystatin remains the most potent antifungal agent, the study suggests that CuNPs hold promise as potential candidates for antifungal applications in medical and healthcare settings.

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