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Isolation and Antimicrobial Assessment of Crude Extract from Aspergillus sp. SO12 Isolated from a Marine Source

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Abstract: The marine environment represents a rich source of microorganisms that have been increasingly explored for their bioactive potential. In this study, we report the isolation of Aspergillus sp. SO12 from a marine source and the subsequent extraction of its crude extract. The bioactive potential of this extract was evaluated through antibacterial assays against various bacterial strains and antifungal assessments against Candida albicans and Aspergillus niger. The isolated strain, Aspergillus sp. SO12, was obtained from a marine sediment sample. Molecular characterization confirmed its identity as a member of the Aspergillus genus, and subsequent cultivation allowed for the production of a crude extract. The extract was obtained using organic solvent. Antibacterial activity of the crude extract was assessed against a panel of clinically relevant bacterial strains, including Gram-positive (Staphylococcus aureus and Bacillus subtilis) and Gram-negative (Escherichia coli and Pseudomonas aeruginosa) bacteria. The extract demonstrated significant antibacterial activity, with notable inhibition zones and minimum inhibitory concentrations (MICs), suggesting the

presence of bioactive compounds with potential therapeutic value against these pathogens. Furthermore, the antifungal potential of the crude extract was evaluated against Candida albicans, a common pathogenic yeast, and Aspergillus niger, a close relative to the producing fungus. The extract displayed potent antifungal activity against both Candida albicans and Aspergillus niger, indicating its capacity to combat fungal infections. In summary, Aspergillus sp. SO12, isolated from a marine source, produces a crude extract with remarkable antibacterial and antifungal properties. The study underscores the potential of marine-derived microorganisms in yielding bioactive compounds with applications in antimicrobial drug development and biotechnology. This research contributes to our understanding of the bioactive potential of marine fungi and suggests promising avenues for future investigations into novel antimicrobial agents.

Keywords: Antimicrobial, G –ve bacteria, G +ve bacteria, Aspergillus, Marine water

1. Introduction

Marine fungi, often residing in the world's oceans and seas, have gained increasing recognition for their diverse ecological roles and their potential as a source of bioactive compounds, including antimicrobial agents. These fungi exhibit remarkable diversity and can be found in various marine niches, such as decaying wood, algae, and sediments [1]. Their unique adaptation strategies enable them to thrive in extreme marine environments characterized by high salinity, high-pressure conditions, and temperature variations, with adaptations including the production of osmolytes, pigments, and enzymes [2]. This adaptation to challenging conditions has led to a growing interest in marine fungi's potential as a source of novel bioactive compounds. They have demonstrated the capacity to produce bioactive molecules with antimicrobial properties, inhibiting the growth of bacteria, fungi, and other microorganisms. These bioactive compounds offer promise in addressing global challenges posed by antibiotic resistance and persistent threats of bacterial and fungal infections [3]. The search for new sources of antimicrobial agents is particularly crucial in the context of increasing antibiotic resistance and the limited availability of effective treatment. In this dynamic field, the exploration of marine fungi's bioactive potential provides an avenue for developing innovative antimicrobial

therapies. As we delve deeper into understanding the diverse ecological roles and bioactive potential of marine fungi, they emerge as a valuable resource in the quest for novel antimicrobial solutions and the development of alternative antibiotics and antifungal agents [4]. The unique adaptations of these organisms and their ability to produce bioactive compounds make them a promising area of study in the ongoing battle against microbial infections and the emergence of multidrug-resistant pathogens.

2. Material and methods

2.1. Sample Collection of Marine Sediment from Hurghada

Marine sediment samples were collected from the coastal region of Hurghada, Egypt, a known hotspot for diverse marine microorganisms. Sampling was conducted in triplicate at a depth of approximately 1-3 meters using a Van Veen grab sampler. Substrate-rich sediment samples were collected from multiple points to ensure representativeness. The samples were immediately transferred into sterile containers to preserve their natural microbial composition and transported to the laboratory under cool, dark conditions.

2.2. Isolation of Fungi Using Serial Dilution Method

Upon arrival at the laboratory, the collected marine sediment samples were processed for the isolation of fungi. Aseptic techniques were employed throughout the isolation process. Each sediment sample was weighed, and aseptically transferred into separate sterile containers. A series of serial dilutions were prepared by suspending a known weight of sediment in sterile saline solution (0.85% NaCl). Dilutions were made up to 10^(-6) to ensure a wide range of fungal concentrations. Next, 100 µL aliquots from appropriate dilutions were spread-plated onto selective agar media suitable for fungal isolation. Sabouraud Dextrose Agar (SDA) supplemented with chloramphenicol (50 µg/mL) was used to inhibit bacterial growth. The plates were incubated at 25°C for a period of 7-10 days, with regular observation for fungal colony development. Distinct fungal colonies were subcultured onto fresh SDA plates to obtain pure cultures, which were then stored at 4°C for further analysis.

2.3. Fermentation on Potato Dextrose Broth Media

Pure fungal cultures obtained from the marine sediment samples were further evaluated for their ability to produce secondary metabolites

and bioactive compounds. For this purpose, each fungal isolate was inoculated onto Potato Dextrose Broth. The PDA plates were incubated at 25°C for a period of 14-21 days to allow the fungi to grow and potentially produce bioactive compounds. Fungal cultures were monitored for growth, sporulation, and the development of any distinct pigments or metabolites.

2.4. Extraction of bioactive metabolites

Following incubation, the fungal mycelia and broth were separated by vacuum filtration through sterile filter paper, yielding a filtrate rich in fungal metabolites in the PDB. Ethyl acetate was selected as the organic solvent for extraction due to its ability to efficiently partition a wide range of fungal metabolites. The filtrate was transferred to a separatory funnel, and an equal volume of ethyl acetate was added. The mixture was thoroughly shaken and allowed to separate into two distinct layers. The ethyl acetate layer, containing the extracted crude fungal metabolites, was carefully separated from the aqueous layer and transferred to a clean flask. To remove any remaining traces of water, the ethyl acetate extract was dried using anhydrous sodium sulfate and subsequently filtered. The solvent was removed from the ethyl acetate extract under reduced pressure using a rotary evaporator, leaving behind a concentrated crude extract of the fungal metabolites. To ensure complete removal of the solvent, the extract was further dried in a desiccator for approximately 24 hours. The obtained crude extract was then stored at -20°C in airtight containers until further analysis. This extraction process allowed for the concentration of bioactive compounds produced by the fungi in liquid culture, providing a valuable resource for subsequent chemical characterization and bioactivity screening.

2.5. Antimicrobial results

To measure the antibacterial activity of the crude and pure compounds. Gram-negative bacteria and, Gram-positive bacteria were used as test organisms. The test was performed in 96-well flat polystyrene plates. 10µl of silver nanoparticles (final concentration of 500 µg/ml) were added to 80 µl of lysogeny broth (LB broth) followed by addition of 10 µl of bacterial culture suspension (log phase), then the plates were incubated overnight at 37°C. After incubation, the positive antibacterial effect of the tested compound observed as clearance in the wells, while compounds that didn't have an effect on the bacteria, the growth media appeared opaque in wells, The control is the pathogen without any treatment.

The absorbance was measured after about 20 h at OD600 in a Spectrostar Nano Microplate Reader (BMG LABTECH GmbH, Allmendgrun, Germany).

2.6. Biofilm inhibitory activity

The biofilm inhibitory activity of the obtained silver nanoparticles was measured using microtiter plate assay (MTP) in 96 well-flat bottom polystyrene titre plates against four clinical microbes (*P. aeruginosa*, *S. aureus*, *E. coli* and *B. subtilis*) according to (Hamed et al., 2020) [5]. Briefly, each well was filled with 180 µL LB broth (tryptone 10g, yeast extract 5 g. NaCl 10 g/L) then inoculation with 10 µL of overnight pathogenic bacterial culture followed by addition of 10 µL of the desired samples *versus* a blank control and incubated at 37 °C for 24 h. After incubation, content in the wells were removed, washed with 200 µL of phosphate buffer saline (PBS) pH 7.2 to remove free floating bacteria. The adherence of sessile bacteria was fixed with 2% sodium acetate and stained with 0.1% crystal violet. Excessive stain was removed by deionized water wash and kept for drying. Further, dried plates were washed with 95% ethanol and optical density (OD) was determined at 595 nm using a microtitre plate reader (BMG LABTECH GmbH, Allmendgrün, Germany).

3. Results and Discussion:

3.1. Isolation of Aspergillus sp. SO12 from Marine Sediment in Hurghada:

In this study, Aspergillus sp. SO12 was successfully isolated from marine sediment collected in the coastal region of Hurghada, Egypt. Marine environments, with their unique ecological niches, provide an ideal habitat for a diverse range of microorganisms, including fungi. The isolation of Aspergillus sp. SO12 from this specific marine source underlines the remarkable biodiversity of marine fungi, which have the potential to produce valuable bioactive compounds.

3.2. Extraction of Crude Extract from Aspergillus sp. SO12 Cultivated in Potato Dextrose Broth:

To assess the bioactive potential of Aspergillus sp. SO12, the fungus was cultivated in Potato Dextrose Broth (PDB), a rich nutrient medium suitable for fungal growth and secondary metabolite production. The crude extract obtained from this culture served as a valuable source of fungal metabolites for further analysis. The choice of ethyl acetate as the extraction solvent was made to efficiently partition a wide range of fungal metabolites.

3.3. Antibacterial Activity Assessment:

The crude extract from Aspergillus sp. SO12 was subjected to antibacterial assays against a panel of clinically relevant bacterial strains, including Pseudomonas aeruginosa, Escherichia coli, Bacillus subtilis, and Staphylococcus aureus. The inhibition ratios against these bacteria were measured and recorded.

The results demonstrated that the crude extract exhibited notable antibacterial activity, with distinct inhibition ratios observed for each bacterial strain. Specifically, the extract displayed significant inhibition against Staphylococcus aureus (59.31%) and Bacillus subtilis (58.25%), highlighting its potential as a source of antibacterial compounds. The antibacterial activity against Pseudomonas aeruginosa (36.02%) and Escherichia coli (18.29%) was also observed, although to a lesser extent **(Table 1, Figure 1)**

3.4. Antifungal Activity Assessment

In addition to antibacterial activity, the crude extract from Aspergillus sp. SO12 was evaluated for its antifungal potential. The antifungal activity was assessed against two important fungal pathogens, Aspergillus niger and Candida albicans. The inhibition ratios against these fungi were recorded **(Table 1, Figure 2)**

The results indicated that the crude extract displayed considerable antifungal activity against both Aspergillus niger (50.65%) and Candida albicans (46.32%). These findings suggest that the fungal metabolites produced by Aspergillus sp. SO12 possess the potential to combat fungal infections caused by these pathogens.

The observed antibacterial activity of the crude extract, particularly against Staphylococcus aureus and Bacillus subtilis, suggests the presence of bioactive compounds with potential as novel antibacterial agents. Additionally, the antifungal activity against Aspergillus niger and Candida albicans underscores the versatility of the fungal metabolites in combatting fungal infections.

These findings open doors for further exploration and characterization of the bioactive compounds present in Aspergillus sp. SO12. Future studies should focus on isolating and identifying specific compounds responsible for the observed antimicrobial activities, as well as their potential applications in drug development and biotechnology. The results of this study contribute to the growing body of knowledge regarding the bioactive potential of marine-

derived fungi and their possible role in addressing microbial infections.

Table 1. antimicrobial activity against test microbes

	Inhibition ratio (%)					
Test microbes	P. aeruginosa	E. coli	B. subtilis	S. aureus	A. niger	C. albicans
Crude	36.02	18.29	58.2580	59.3180	50.65	46.32

100:70: potent 70:50: active 50:30: moderately active 30: 10 : weak

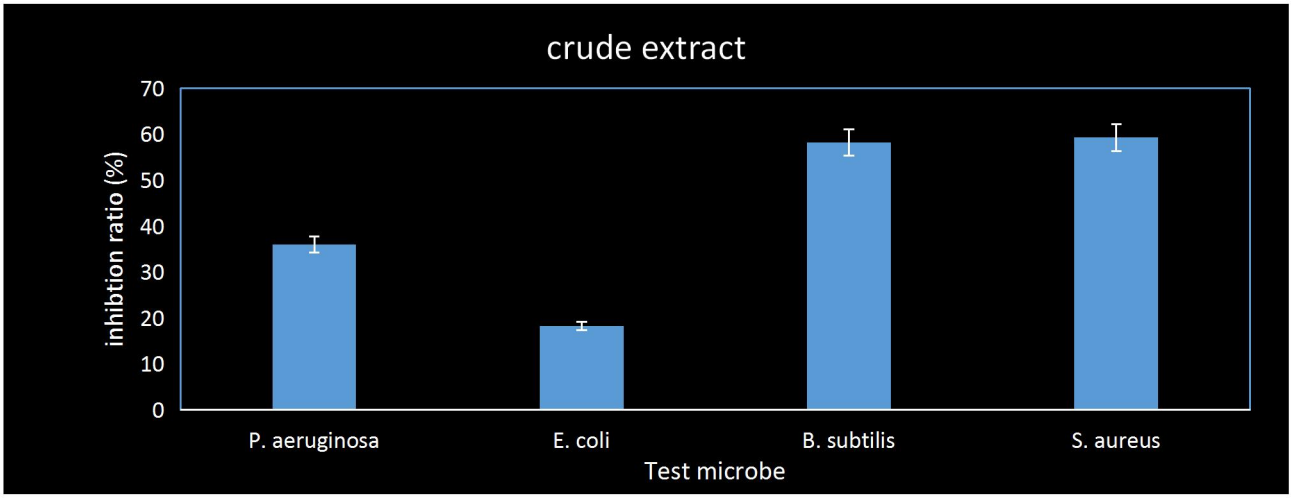

Fig.1. antibacterial activity of tested crude

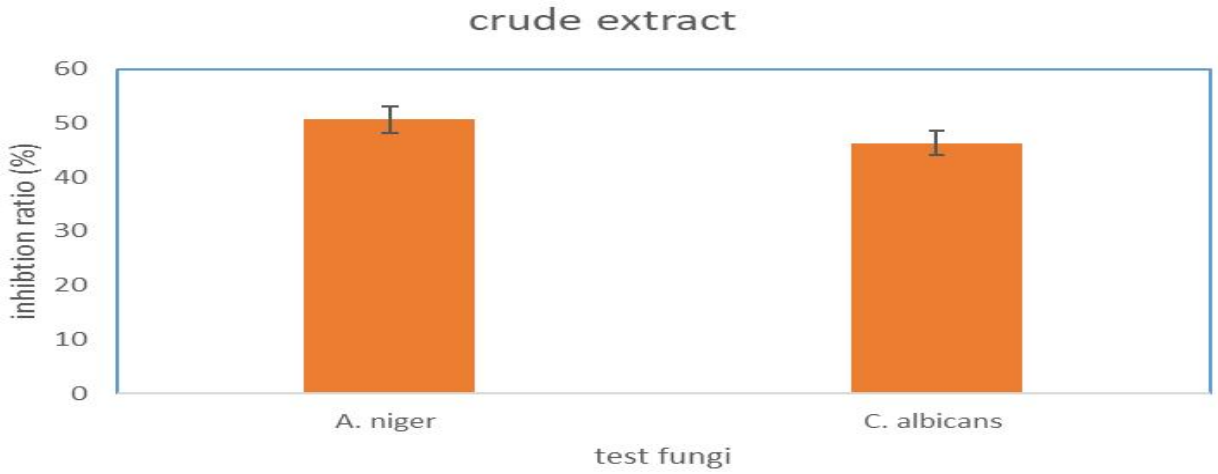

Fig. 2. Antifungal activity of tested crude

The biofilm inhibitory activity of the crude extract obtained from Aspergillus sp. SO12

was assessed against a panel of clinically relevant bacterial strains. The results revealed a significant biofilm inhibitory potential, with varying percentages observed for different bacterial species. Notably, the crude extract exhibited remarkable biofilm inhibitory activity against Staphylococcus aureus, with an inhibition rate of 70.23%. This finding is particularly promising as Staphylococcus aureus is known for its biofilm-forming capabilities, making it a common culprit in chronic infections and antibiotic resistance. Furthermore, the extract displayed substantial biofilm inhibitory activity against Escherichia coli (45.25%), Bacillus subtilis (35.23%), and Pseudomonas aeruginosa (20.02%) **(Table 2, Figure 3).**

The observed biofilm inhibitory activity of the crude extract highlights its potential in combating biofilm-related infections caused by these bacterial species. Biofilm formation is a crucial defense mechanism employed by bacteria, rendering them resistant to conventional antibiotics and host immune responses. The ability of the extract to disrupt biofilm formation in these pathogens underscores its significance in the development of alternative therapies for biofilm-associated infections. The findings open avenues for further investigation into the specific bioactive compounds responsible for this inhibitory effect, potentially paving the way for the development of novel anti-biofilm agents with clinical applications.

Table.2. Biofilm inhibitory ratio

	Biofilm inhibitory ratio (%)			
Compounds	**S. auerus**	**E. coli**	**P. aeruginosa**	**B. subtilis**
Crude	70.23	45.25	20.02	35.23

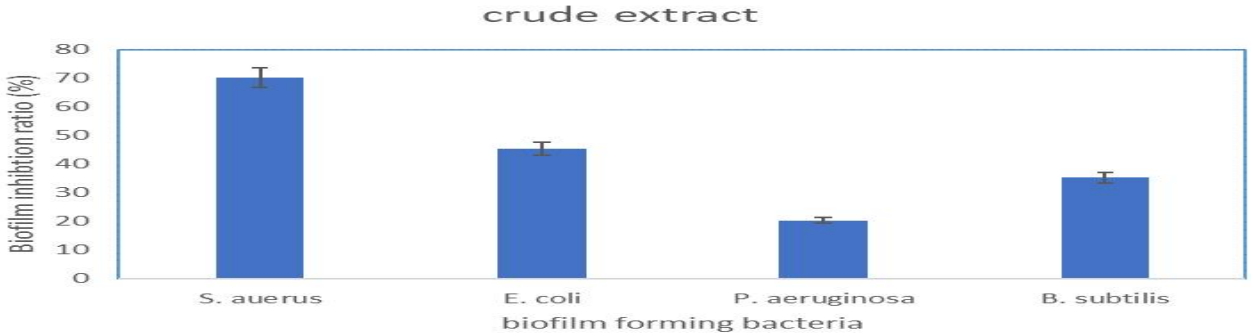

Fig. 3. Biofilm inhibitory ratio

4. References:

1. Jones, E. B. G., Suetrong, S., Sakayaroj, J., Bahkali, A. H., Abdel-Wahab, M. A., & Boekhout, T. (2015). Classification of marine Ascomycota, Basidiomycota, Blastocladiomycota and Chytridiomycota. Fungal Diversity, 73(1), 1-72.
2. Raghukumar, S. (2008). Marine fungal biotechnology: An ecological perspective. Fungal Diversity, 31(1), 19-35.
3. Rateb, M. E., Ebel, R., Jaspars, M., & He, W. (2010). Natural product diversity of actinomycetes and its applications. Natural Product Reports, 27(7), 1126-1164.
4. Desriac, F., Jégou, C., Balnois, E., Brillet, B., Le Chevalier, P., & Fleury, Y. (2010). Antimicrobial peptides from marine proteobacteria. Marine Drugs, 8(3), 823-842.
5. Hamed, A.A.; Kabary, H.; Khedr, M.; Emam, A.N. Antibiofilm, antimicrobial and cytotoxic activity of extracellular green-synthesized silver nanoparticles by two marine-derived actinomycete. RSC Adv. 2020, 10, 10361–10367