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Exploring Marine Fungal Crude Extracts for Antidiabetic Potential Samah M. El-fiqy¹, Dina M. Barka¹, Ham M. El-shora², Mohamed E. El awady ³, Ahmed A. Hamed⁴

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Abstract

This study focused on the exploration of antidiabetic potential within marine fungal communities collected from diverse locations in Hurghada. Various fungi were isolated from Sponge 1, 2, and Sea Grass, exhibiting distinct morphologies. Notable isolates included s289 (white-gray) from Sponge 2, s313 (black) from Sponge 1, and s213 (black) from Sponge 2. Small-scale fermentation and extraction processes were employed to obtain crude extracts, concentrating bioactive secondary metabolites. Evaluation of antidiabetic activity through α -glucosidase inhibition revealed distinct inhibitory potency levels among the extracts. Notably, s313 displayed potent activity (IC50: 0.505 mg/ml), suggesting promising antidiabetic potential. Moderately inhibitory activities were observed for s289 (IC50: 0.972 mg/ml) and s213 (IC50: 0.617 mg/ml). The comparison with the reference drug Acarbose (IC50: 0.375 mg/ml) highlighted the competitive nature of the fungal extracts. This study underscores the diverse bioactivity of marine fungal crude extracts and emphasizes their potential as alternative or complementary antidiabetic agents, encouraging further exploration of natural sources for effective treatments.

Key words: Relevant Terms: antidiabetic agent, marine fungi, secondary metabolites.

Introduction

The escalating prevalence of diabetes mellitus worldwide underscores the critical need for innovative therapeutic strategies and novel pharmacological agents. Natural products, particularly those derived from fungi, have emerged as promising sources of bioactive compounds with diverse medicinal properties [1]. Fungi inhabit a myriad of ecological niches, displaying an exceptional ability to synthesize secondary metabolites as part of their adaptive mechanisms [2]. These bioactive secondary metabolites have garnered considerable attention for their potential antidiabetic properties, making fungi an intriguing reservoir for drug discovery in the realm of diabetes management [3].

The unique chemical diversity inherent in fungal secondary metabolites offers a vast repertoire of compounds with varied pharmacological activities. As part of their ecological roles, fungi produce an array of bioactive molecules to navigate their complex interactions within diverse ecosystems [4]. Harnessing the therapeutic potential of these compounds could pave the way for the development of novel antidiabetic agents. In this context, our study focuses on the comprehensive evaluation of bioactive secondary metabolites from fungi, specifically exploring their potential as antidiabetic agents through the inhibition of α -glucosidase, a key enzyme implicated in glucose metabolism [5].

The rich biodiversity of marine ecosystems, including sponges and sea grass, serves as a unique reservoir for fungal diversity. These ecosystems harbor

fungi with distinct adaptations, potentially leading to the synthesis of unique bioactive compounds [6]. By delving into the depths of marine fungal diversity, we aim to uncover novel secondary metabolites with potent antidiabetic properties. The integration of traditional knowledge with modern techniques in fungal isolation, fermentation, and extraction positions our study at the forefront of antidiabetic drug discovery, offering new avenues for the development of therapeutics targeting diabetes [7].

Understanding the intricacies of fungal secondary metabolites and their potential antidiabetic effects requires a multidisciplinary approach. By combining molecular biology, microbiology, and pharmacology, we endeavor to elucidate the mechanisms of action of these bioactive compounds [8]. This research not only contributes to expanding the scientific understanding of fungal secondary metabolites but also holds the promise of providing valuable insights into the development of antidiabetic drugs with enhanced efficacy and reduced side effects[9].

In summary, this study embarks on a journey into the realm of fungal secondary metabolites, unveiling their potential as antidiabetic agents. By exploring the unique fungal diversity within marine ecosystems and employing advanced methodologies, we aim to contribute to the growing body of knowledge in diabetes research and foster the development of innovative therapeutic solutions.

Materials and Methods Collection of Samples from Plants:

Marine fungi were meticulously sampled from diverse ecological niches, including sponges and sea grass, situated in different locations within the vibrant marine ecosystem of Hurghada. The samples were systematically identified and labeled as follows: Sponge 1, Sponge 2, and Sea Grass, obtained from Locations 1, 2, and 3, respectively.

Isolation of Fungi from Collected Samples:

The collected samples underwent a rigorous process of fungal isolation to ensure the acquisition of distinct fungi isolates. The isolated fungi were meticulously labeled as s289, s313, s259, s35, s213, and s289, each corresponding to the samples derived from Sponge 2, Sponge 1, Sponge 2, Sea Grass, Sponge 2, and Sponge 2, respectively. Detailed morphological characteristics, encompassing color variations and surface textures, were meticulously observed to ascertain the diversity of the fungal isolates.

Small-Scale Fermentation and Extraction of Crude Extracts:

The isolated fungi were subjected to a controlled small-scale fermentation process, allowing for the cultivation of these microorganisms under specific conditions conducive to secondary metabolite production. Subsequently, crude extracts were meticulously obtained through extraction processes, employing appropriate solvents to selectively capture the diverse bioactive secondary metabolites synthesized during fermentation. The extraction procedures were optimized to ensure the concentration and preservation of the compounds of interest for subsequent analytical endeavors.

Screening of α-Glucosidase Assay for Isolated Fungi Crude Extracts:

The antidiabetic potential of the isolated fungi crude extracts was systematically assessed through the screening of α -glucosidase inhibitory activity. The assay encompassed several intricately designed steps, The preparation of highly concentrated crude extracts, ensuring the inclusion of a broad spectrum of bioactive compounds. A comprehensive screening for α glucosidase inhibitory activity, meticulously evaluating the potential of each crude extract. The precise determination of IC50 values for each isolated fungi crude extract, provides quantitative insights into their inhibitory potency. A robust statistical analysis of the obtained data, including the calculation of standard deviations (SD), to discern variations and trends.A comparative evaluation with the positive control (Acarbose), a well-established antidiabetic agent.

Results

Collection of Marine Samples:

Marine samples were systematically collected from diverse locations in Hurghada, including Sponge 1 (Location 1), Sponge 2 (Location 2), and Sea Grass (Location 3). These samples were chosen to encompass varied marine environments and their associated microbial communities (Table 1)

Isolation of marine associated Fungi

The table presents the morphological characteristics of fungal isolates obtained from marine samples, specifically sponges 1 and 2, as well as seagrass. Each fungal isolate is assigned a unique code, and its corresponding morphology is described.For sponges 1, three distinct fungal isolates were identified: s287, s288, and s289. Fungal isolate s287 exhibited a whitegray coloration, while s288 appeared green, and s289 was black. These variations in coloration suggest the presence of different fungal species or strains within the sponge 1 sample, each potentially adapted to its specific ecological niche within the sponge matrix.

In sponge 2, a greater diversity of fungal isolates was observed, with five distinct morphologies represented by isolates s313, s314, s315, and s316. Fungal isolate s313 appeared black, while s314 was described as gray, and s315 exhibited a white coloration. Interestingly, isolate s316 was identified as black, indicating the presence of a second black-colored fungal species or strain within sponge 2.

In contrast, the fungal isolates obtained from seagrass consisted of two distinct morphologies represented by isolates s213 and s214. Isolate s213 appeared black, while s214 exhibited a green coloration.

The diversity in fungal morphology observed across the marine samples suggests a complex microbial community present within these environments. Factors such as substrate composition, nutrient availability, and environmental conditions likely influence the diversity and distribution of fungal species within marine habitats. Further characterization of these fungal isolates, including molecular analysis and functional studies, could provide valuable insights into their ecological roles and potential biotechnological applications in marine ecosystems.

 Table (1) Marine sample collection.

Marine samples	Location
Sponge 1	Location 1 (Hurghada)
Sponge 2	Location 2 (Hurghada)
Sea grass	Location 3 (Hurghada)

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	Fungal code isolate	Morphology of fungus
Sponge 1	s287	White gray
	s288	Green
	s289	Black
Sponge 2	s313	Black
	s314	Grey
	s315	White
	s316	black
Seagrass	s213	Black
	s214	Green

Table (2) Isolated fungi from marine samples.

Small-Scale Fermentation and Extraction

The utilization of small-scale fermentation procedures served as a crucial step in the isolation and concentration of bioactive secondary metabolites produced by the fungal isolates. This process aimed to extract and concentrate the metabolites present in the fungal cultures, facilitating further analysis and characterization of their bioactivity.

Table 3 provides insight into the yields of crude extracts obtained from each fungal isolate following the fermentation process. The weights of the crude extracts varied across the different fungal isolates, ranging from 1.02 to 1.52 units.

Among the fungal isolates from sponge samples (s287, s288, s289, s313, s314, s315, s316), isolate s289 exhibited the highest yield of crude extract at 1.52 units, followed closely by isolate s316 with a yield of 1.41 units. These results suggest that these particular fungal isolates may be prolific producers of bioactive

secondary metabolites, potentially possessing significant pharmaceutical or biotechnological relevance.

Similarly, fungal isolates obtained from the seagrass sample (s213, s214) also yielded notable amounts of crude extract, with isolate s213 yielding 1.24 units and isolate s214 yielding 1.12 units. These findings indicate the potential richness of bioactive metabolites present within fungal communities associated with marine environments.

The variations in crude extract yields among the fungal isolates may be attributed to several factors, including differences in fungal species, metabolic activity, and growth conditions during fermentation. Optimization of fermentation parameters such as culture media composition, pH, temperature, and incubation time could potentially enhance the yield of bioactive metabolites from these fungal isolates.

Table (3) Small-scale fermentation and obtaining fungal crude extract.

Fungal code isolate	Crude extract weight
s287	1.02
s288	1.13
s289	1.52
s313	1.32
s314	1.12
s315	1.11
s316	1.41
s213	1.24
s214	1.12



Fig. (1) α -glucosidase inhibition screening of fungal crude extracts.

Screening of α-Glucosidase Assay

The evaluation of the antidiabetic activity of fungal crude extracts (s287, s288, s289, s313, s314, s315, s316,s213, s214) and Acarbose, as determined by their α -glucosidase inhibition, presents intriguing insights into potential therapeutic avenues for diabetes management. The observed IC50 values indicate distinct levels of inhibitory potency among the tested extracts. Notably, s313 emerges as a particularly promising candidate, displaying a comparatively lower IC50 value of 0.505 mg/ml. signifying potent α -glucosidase inhibition. This suggests the presence of bioactive compounds within s313 that may merit further exploration for their antidiabetic potential. While s289 and s213 exhibit moderate inhibitory activities with IC50 values of 0.972 mg/ml and 0.617 mg/ml, respectively, their intermediary positions suggest the need for a more in-depth analysis to uncover the specific compounds responsible for the observed effects. The inclusion of Acarbose as a reference, with a robust IC50 value of 0.375 mg/ml, underscores the competitive nature of the fungal extracts and hints at their potential as alternative or complementary antidiabetic agents. Overall, these findings not only highlight the diverse bioactivity of fungal crude extracts but also emphasize the significance of exploring natural sources in the search for effective antidiabetic treatments.

Discussion

Marine natural products are key sources of biologically active molecules that have been shown to modulate a variety of biological functions, including antioxidant, antimicrobial, and anticancer properties [10]

The comprehensive investigation into marine samples collected from diverse locations in Hurghada, including Sponge 1, Sponge 2, and Sea Grass, followed by the isolation of fungi, has yielded valuable insights into the potential antidiabetic properties of fungal crude extracts. The chosen locations were intended to capture a range of marine environments and their associated microbial communities, contributing to the diversity of the collected samples [11].

Upon isolation, several fungi were identified, each exhibiting distinct morphologies. Notable among these fungal isolates were s289 from Sponge 2, displaying a white-gray morphology, s313 from Sponge 1 with a black morphology, and s213 from Sponge 2, also exhibiting a black morphology. The diversity in fungal morphologies reflects the richness of the microbial communities in these marine environments [12].

Subsequent small-scale fermentation procedures were employed to extract crude extracts from the isolated fungi, aiming to concentrate the bioactive secondary metabolites produced by these fungi. The focus on small-scale fermentation allows for an initial exploration of the potential bioactivity of these fungi in a cost-effective manner [13].

The evaluation of the antidiabetic activity of the fungal crude extracts (s289, s313, s213) and the reference drug Acarbose through α -glucosidase inhibition has revealed intriguing results. Particularly noteworthy is the promising antidiabetic candidate s313, which exhibited a significantly lower IC50 value of 0.505 mg/ml, indicating potent α -glucosidase inhibition [14]. This suggests the presence of bioactive compounds within s313 that may warrant further investigation for their antidiabetic potential.

In contrast, s289 and s213 exhibited moderate inhibitory activities with IC50 values of 0.972 mg/ml and 0.617 mg/ml, respectively. The intermediary positions of these extracts in terms of inhibitory potency suggest the need for a more in-depth analysis to identify and characterize the specific compounds responsible for their observed effects on α -glucosidase activity [15].

The inclusion of Acarbose as a reference with a robust IC50 value of 0.375 mg/ml serves as a benchmark, highlighting the competitive nature of

the fungal extracts. This comparison suggests that the fungal extracts may possess potential as alternative or complementary antidiabetic agents, showcasing the significance of exploring natural sources for effective treatments.

In conclusion, the investigation into the antidiabetic activity of fungal crude extracts derived from marine samples emphasizes the diverse bioactivity present in these natural sources [16]. The findings underscore the potential of marine fungi as a valuable reservoir for antidiabetic compounds and emphasize the importance of continued exploration in the search for effective and sustainable antidiabetic treatments.

Conclusion

In summary, our study aimed to explore microbial diversity within marine environments by systematically collecting samples from various locations in Hurghada, including Sponge 1, Sponge 2, and Sea Grass. Analysis of marine-associated fungi revealed diverse morphological characteristics, reflecting the complex microbial communities inhabiting these habitats. The observed diversity highlights the impact of substrate composition, nutrient availability, and environmental conditions on fungal distribution and adaptation in marine ecosystems.

Further characterization of fungal isolates through molecular analysis and functional studies offers insights into their ecological roles and potential applications in biotechnology. Utilizing small-scale fermentation and extraction techniques, we isolated and concentrated bioactive secondary metabolites from fungal cultures. Varied yields of crude extracts among isolates indicate the potential of certain fungi as prolific producers of bioactive compounds with pharmaceutical or biotechnological relevance. Optimizing fermentation parameters holds promise for enhancing the yield of bioactive metabolites, opening avenues for future research to harness the untapped potential of marine-associated fungi across diverse applications.

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