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Secondary metabolites from marine fungi as anticandidia Samuel N. Wesa¹, Mervat G. Hassan¹, Sabah A. AboElmaaty¹, Mohamed E. Elawady² and Ahmed A Hamed³

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Abstract

Background: Most fungal pathogen-induced infections in humans are caused by species of Candida. Members of these species include Candida albicans, which is the most common cause of opportunistic infections.

Purpose: to determine whether bioactive compounds isolated from thirteen different fungal strains have anticandida properties against a variety of Candida isolates obtained from urine and vaginal samples. The findings demonstrate a marked variation in the inhibitory responses amongst various strains of Candida, indicating a complex interaction between fungal strains and their bioactive compounds. There were clear strain-specific variations, with some fungal strains showing strong efficacy and others showing little to no inhibitory effects. Interestingly, Fungal Strain 8 continuously exhibited strong inhibitory responses, highlighting the significant influence of particular fungal strains. Different Candida isolates had varying levels of sensitivity to bioactive compounds, highlighting the need for specialized antifungal treatments. Clinical relevance can be drawn from the observed diversity in anticandida activity, which points to possible directions for the development of targeted antifungal drugs. Subsequent investigations may delve into fundamental mechanisms, pinpoint pivotal bioactive compounds, and facilitate the advancement of innovative antifungal medication discovery. Conclusion: this research provides significant understanding of the intricate dynamics of anticandida activity, laying the groundwork for future developments in specialized treatments to combat Candida infections.

Key words: Relevant Terms: Candida albicans, marine fungi, secondary metabolites

1. Introduction

Bioactive secondary metabolites are a broad class of organic compounds with unique chemical structures and functions that are produced in large quantities by fungi [1]. These secondary metabolites, also known as natural products [1], are vital to the survival and adaptation of fungi in their ecological interactions[2]. A lot of attention is focused on these bioactive secondary metabolites' possible anticandida properties, or their capacity to fight infections brought on by Candida species[3].

Candida is a commensal fungus that grows on many of the mucosal surfaces of the human body[4-5]. Candida albicans is the most common species[6]. Although Candida usually coexists benignly as a component of the normal flora, in certain situations it can become an opportunistic pathogen that can cause infections ranging from superficial mucocutaneous manifestations to potentially fatal systemic manifestations[7-8]. Bioactive secondary metabolites from fungi are an interesting subject of study because of the need to explore alternative agents due to the increasing antifungal resistance and the limitations of current therapeutic options [9]

Candida infections can occur under certain circumstances, including pregnancy, diabetes, and the use of antibiotics [10-11]. Traditionally, treatment consists of a small class of synthetic medications such as fluconazole [12], nystatin [13], and ketoconazole [12]. But misusing them can result in resistance [14]. Continuous research into natural compounds as alternatives is required to address this. Rich microbial diversity can be found in aquatic

environments[15], especially when these habitats are inhabited by multicellular aquatic organisms. Researchers are drawn to microorganisms because of their distinctive physicochemical properties, particularly those of those that survive in harsh environments [16-19]. The primary producers of antibiotics, actinomycetes are linked to marine life and can be found in marine sediments [20].

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Material and Methods Sample Collection

Three carefully chosen marine samples from various locations were taken in order to investigate the microbial diversity found in a Mediterranean Sea lake. The goal of these samples was to demonstrate the diversity of the microbial community living in the lake's various ecological niches.

Marine Fungi Isolation from Isolated Marine Samples

Potato Dextrose Agar [PDA]: This adaptable medium was used for two purposes: it made it easier to separate the fungi from the lake samples that were collected and to inoculate them. One milliliter of seawater from the lake samples was carefully added to three petri dishes as part of an inoculation procedure. The uniform distribution of these inoculation dishes on PDA's surface produced an environment that was favorable to the development of marine fungi. The obtained fungi were incubated for five days at 28°C before being subcultured for an additional five days on fresh PDA. Subculturing was

done on PDA plates with the goal of guaranteeing the purity of the isolated strains.

Collection of Candida Isolates

Urine samples and a vaginal swab were systematically collected from various locations in order to expand our understanding of the microbiological landscape. With the careful serial dilution method used to plate these biological samples on PDA media, a favorable substrate for the growth of Candida species was created. Candida isolates were able to proliferate and separate during the 24-hour incubation period, which was maintained at 32°C.

Merely Fermenting on a Small Scale and Acquiring Fungal Crude Extract

Small-scale fermentation was used to produce the concentrated reservoir of potentially bioactive compounds that is the isolated fungal crude extract. Here, the growing medium for the fungus was 50 grams of rice medium combined with 50 milliliters of distilled water that had been sterilized. For a period of 15 days at 28°C, the mixture was carefully cultivated to allow the synthesis and accumulation of metabolites within the fungal biomass.

Fungal Bioactive Compound Extraction with Ethyl Acetate

The bioactive compounds of thirteen carefully chosen fungal isolates were extracted after they were cultivated for 15 days at 28°C. The cultures were exposed to ethyl acetate, which is well-known for its effectiveness in extracting a wide variety of

Table (1) Fungal isolation from gathered samples

compounds. To ensure the effective extraction of bioactive compounds, the mixture was thoroughly shaken for 30 minutes. The extracts were then allowed to dry at room temperature, producing concentrated extracts that were carefully selected for additional in-depth examination.

Assessing Candida Sensitivity:

Sterilized disks were carefully prepared for the disk diffusion method in order to assess sensitivity. Every fungal extract was examined closely, enabling a thorough evaluation of its effectiveness against isolates of Candida. This step gave important information about how the isolated fungal compounds might be used to treat infections caused by Candida.

Results RESULTS AND DISCUSSION Collecting Marine Samples

In the Mediterranean Sea Lake, three marine samples (SAM1, SAM2, and SAM3) were successfully gathered from various locations. The deliberate choice of sites for sampling was intended to encompass the wide range of microbial diversity found in the lake.

1. Fungal Isolation from Marine Samples:

From the three marine samples, thirteen fungal strains (designated as SAM11 to SAM313) were extracted. The richness and complexity of the fungal community thriving in the lake environment were highlighted by the wide range of isolated strains Table (1)

Marine sample code	Isolate fungal code
SAM1	SAM11
	SAM12
	SAM13
	SAM14
	SAM15
	SAM16
SAM2	SAM27
	SAM28
	SAM29
	SAM210
SAM3	SAM311
	SAM312
	SAM313

2. Candida Strain Isolation

Three pathogenic Candida strains successfully isolated from one vaginal sample, two urine samples (Urine 1 and Urine 2) and one urine sample. By taking this step, the inclusion of clinically relevant species of Candida in the ensuing analysis was guaranteed.

3. Isolated Fungal Culture on Rice

Rice medium was used to cultivate the isolated fungal strains, giving them an appropriate substrate for growth and metabolite production. Using the potential bioactive compounds that these fungi produce was the goal of this step.

4. Bioactive Compound Isolation:

Bioactive compounds were efficiently extracted from the cultivated fungi using ethyl acetate. Concentrating the potentially medicinal compounds that the isolated fungi produced required this extraction procedure.

5. Measuring Anticandida Activity

The anticandida activity of the bioactive compounds was methodically assessed against three isolates of Candida [coded 13 to 1] obtained from urine and vaginal samples. The clear zones surrounding the fungal colonies, which are a sign of the compounds' ability to inhibit Candida growth, were used to quantify the results.

The results of the anticandida activity assessment are displayed in figure 1, which also includes the measured clear zones surrounding fungal colonies in response to bioactive compounds that were isolated from different strains of fungal. This talk explores the trends and implications that can be seen in the data.

There is a clear variation in how the various Candida strains react to the fungal bioactive substances. The clear zones that are observed, which indicate inhibitory activity, vary in size from 0 to 2 mm. This indicates that the compounds that were tested have varying degrees of efficacy against different strains of Candida.

Responses to the bioactive substances derived from fungal strains vary amongst Candida strains. Notably, Strain 1 exhibits high susceptibility, displaying distinct zones measuring 1.5 mm and 2 mm against isolates from the vagina and urine, respectively. Conversely, the lack of distinct zones indicates that strains 9 and 4 have little to no inhibitory activity.

It becomes clear how fungal strains affect the activity of anticandida agents. In both urine1 and vaginal isolates, fungal strain 8 consistently elicits inhibitory responses against strains of Candida. Conversely, Fungal Strains 9 and 3 seem to be less successful, showing either negligible or no inhibitory

Variations in the sensitivity of Candida isolates to bioactive compounds underscore the necessity of a nuanced approach in the development of antifungal therapies. Particularly, urine 2 isolates exhibit varying degrees of susceptibility, indicating possible strain-specific variations in response.

Clinical significance can be derived from the observed variation in anticandida activity, which highlights the potential of particular fungal strains and their bioactive compounds as sources for the development of targeted antifungal agents. Comprehending the responses specific to a strain helps to customize therapeutic interventions for better treatment results.

These findings open up new avenues for research. Subsequent research endeavors may delve into the fundamental principles underlying strainspecific reactions, pinpoint the principal bioactive constituents accountable for inhibitive impacts, and evaluate the feasibility of transforming these compounds into innovative antifungal medications. The thorough assessment of anticandida activity offers insightful information that will help research into targeted treatments for Candida infections advance.

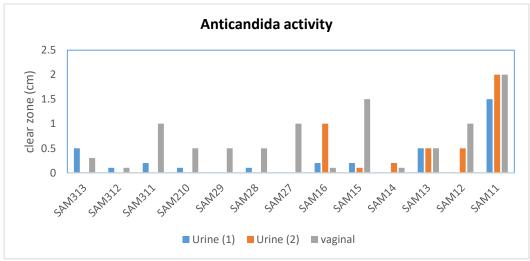


Fig. (1) The isolated fungal crude extract's anticandidal activity

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