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ORIGINAL ARTICLE/ARTICLE ORIGINAL

Antifungal activity of aqueous and methanolic extracts from the Mediterranean sea cucumber, *Holothuria polii*

Activité antifongique des extraits aqueux et méthanoliques du concombre de mer, *Holothuria polii*

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Abstract Crude extracts and semi-purified fractions (F5, F6) from the Mediterranean sea cucumber, *Holothuria polii*, collected from the bay of Tabarka (Tunisia coast) were evaluated for their antifungal activity against filamentous fungi and yeast. The activity was determined in vitro, using the well diffusion test in the casitone agar medium. Both the aqueous and the methanolic extracts were found to produce, in a concentration-related manner (600—1500 μg/well) a significant antifungal activity. The semi-purified fractions (F5, F6) of both extracts exhibited also a significant antifungal activity in a concentration-related manner (150—300 μg/well). The strains of *Aspergillus fumigatus* were more susceptible to these extracts and derived fractions, while those of *Trichophyton rubrum* were found to be less susceptible. No activity was observed against strains of *Candida albicans*. These findings suggest that the polar active fractions (F5, F6) obtained from aqueous and methanolic extracts could contain a new antifungal compound(s). The purification and the determination of chemical structure of compound(s) of these active fractions are under investigation.

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MOTS CLÉS
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Activité antifongique ;
Extrait aqueux ;
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Purification ;
Fractions semi-purifiées

Résumé Les extraits bruts et les fractions semi-purifiées (F5, F6) du concombre de mer, *Holothuria polii*, récolté dans la baie de Tabarka (Tunisie), ont été évalués in vitro pour leur activité antifongique, sur des souches de champignon filamenteux et de levures, en utilisant la méthode des puits. Les deux extraits, aqueux et méthanolique, ont montré une activité antifongique significative, concentration dépendante (600—1500 μg/puit). Les fractions semi-purifiées (F5, F6) des deux extraits ont montré aussi une activité antifongique significative, concentration dépendante (150—300 μg/puit). Les souches d'*Aspergillus fumigatus* se sont révélées plus sensibles aux deux extraits et leurs fractions, alors que *Trichophyton rubrum*, était moins sensible. Aucune activité n'a été observée contre les souches de *Candida albicans*. Ce résultat suggère que les fractions polaires (F5, F6), issues aussi bien de l'extrait aqueux que méthanolique, pourraient contenir une nouvelle substance antifongique. La purification et la détermination de la structure chimique de(s) substance(s) de ces deux fractions actives sont en cours d'investigation.

Introduction

The increase of the incidence of fungal infections is due to the emergence of resistant pathogens and their nosocomial dissemination, especially among immunocompromised or neutropenic patients. Scientific efforts to discover new potential antifungal drugs are principally leaned towards synthetic and natural products of plant origin. Since the few last decades, marine environment have been recognized to be a rich sources of bioactive metabolites with varied biological and pharmacological activities. Some compounds possessing antifungal properties have been isolated from marine organisms [4,5].

For a long time, sea cucumbers have been limited to their physiological and ecological aspects [6]. However, since the potential of the echinoderm sea cucumbers as a source of food [18] have been recognized by native Asian countries, scientific researchers started to investigated their medicinal properties [8,9,11,13,15] and they have demonstrated the high pharmacological potential of triterpene glycosides, isolated from different species of the sea cucumbers: *Cucumaria japonica* [7], *Hemoiedema spectabilis* [10], *Holothuria pervicax* [19]. These glycoside compounds possess antifungal [3,7,10,14,16], anti-inflammatory [2,12] and cytotoxic [10,20] activities.

In this study, we report the antifungal activities of crude extracts of body fluid and body wall from the Mediterranean specie of sea cucumber, *Holothuria polii*. The semi-purified fractions by chromatography of these extracts are also tested.

Materials and methods

Biological materials

Sample collection

The sea cucumbers, *H. polii*, were collected from the Mediterranean sea, around the rocky shore of the bay of Tabarka (Tunisia), in June 2003. The collected samples were cleaned by rinsing with seawater and distilled water and transported in cool box to the laboratory where the internal organs were removed; then the body fluid and body wall were recuperated in separated labeled plastic bags, and kept frozen at −20 °C until extraction. Identification of specimens was carried out in the National Institute of Marine Sciences and Technologies, Salamboo, Tunisia.

Preparation of the extracts

The samples of body fluid and body wall were defrosted before use. The body fluid recuperated was homogenized with stirring using the magnetic stirrer for 15 min, and filtered using some cotton wool followed by passage through a Whatman filter paper#1. After centrifugation (15 min, 30,000 × *g*, 4 °C), the supernatant was lyophilized to give the aqueous extract.

The body wall recuperated was cut into small pieces (about 2 cm), homogenized using a blender and suspended with stirring in 80% methanol during one night at 4 °C. After filtration using some cotton wool followed by passage through a Whatman filter paper#1, and centrifugation (15 min, 30,000 × *g*, 4 °C), the methanolic extract was evaporated under vacuum at 45 °C, and distilled water was then added to the residue. The aqueous phase then was collected and lyophilized to give the methanolic extract. The powdered extracts of each sample was stored at −20 °C until use.

Purification of the semi-purified fractions

Often, bioactive compounds constitute a very minor part of the crude extract. In order to localize the active fraction, aqueous and methanolic extracts of *H. polii* were purified, using C18 cartridges (Sep-pack, Supelco), by gradient elution with methanol—water mixture (0%, 20%, 40%, 60%, 80%, and 100% methanol) to give six fractions (F1—F6). Methanol solvents were removed from fractions recuperated using rotating evaporator at 45 °C and distilled water was then added to the residues and the aqueous phases were lyophilized. The powdered fractions were stored at −20 °C until use.

Screening of antifungal activities

Five fungal species, obtained from the collection of fungi of Pasteur Institute, were used as test strains: three filamentous fungi (*Aspergillus fumigatus* IP 1082.72, *A. fumigatus* IP 2279.94, and *Trichophyton rubrum* IP 2043.92) and two yeast species (*Candida albicans* IP 48.72, and *Candida albicans* IP 884.65). All strains were maintained on Sabouraud's Agar or on Czapek Agar (for *A. fumigatus*) at 28 °C.