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ORIGINAL ARTICLE/ARTICLE ORIGINAL

Holothurians antifungal and antibacterial activity to human pathogens in the Persian Gulf

Activité antifongique et antibactérienne des holothuries du golfe Persique sur des agents pathogènes humains

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Summary Pharmaceutical industries now accept that oceans contain a vast array of organisms with unique biological properties. As investigation of antimicrobial activity, three species of *Holothuria* sea cucumbers (*Holothuria scabra*, *Holothuria parva* and *Holothuria leucospilota*) from the Persian Gulf, Iran, were obtained, their different organs: gonads (G), body wall (BW), intestine tract (IT), respiratory tree (RT) were isolated and extracted with organic solvents: ethyl acetate and methanol. Antifungal and antibacterial activities of each fraction was estimated with a minimum inhibitory concentration (MIC) and disk-diffusion method against the number (i.e. $n = 10$) of human pathogenic microbes (eight pathogenic Gram-positive/negative bacteria strains and two fungi). All the sea cucumbers extracts confirmed their strong potential antagonistic effect against the bacterial and fungal indicators, except for three relatively resistant microbial strains: *Candida albicans* and *Pseudomonas aeruginosa* and *Klebsiella pneumonia*. Most effective antifungal and antibacterial activities were recorded for methanolic extract of intestine tract organs of *H. parva* against *Saccharomyces cerevisiae* (MIC = 0.09 mg/ml, IZ = 22 ± 0.8 mm) and *Staphylococcus epidermidis* (MIC = 0.04 mg/ml and IZ = 30 ± 0.9 mm), compared the other extracts. Nevertheless, the differences between species can result from a variety of their natural habits. The finding suggests that marine *Holothuria* sea cucumber specie, especially *H. parva*, has a potential source for the discovery of natural antibiotic compounds and drug development.

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MOTS CLÉS

Concombre de mer ;
Activités biologiques ;
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Antifongiques ;
Golfe Persique

Résumé Les industries pharmaceutiques reconnaissent maintenant que l'océan contient une vaste gamme d'organismes à propriétés biologiques uniques. Pour une investigation d'activité antimicrobienne, trois espèces de concombre de mer *Holothuria*, (*Holothuria scabra*, *Holothuria parva* et *Holothuria leucospilota*) provenant du golfe Persique, Iran, ont été obtenues, leurs différents organes : gonades (G), paroi corporelle (PC), tractus intestinal (TI), tractus respiratoire (TR) ont été isolés et des extraits ont été obtenus au moyen de solvants organiques : l'acétate d'éthyle et le méthanol. Les activités antibactériennes et antifongiques de chaque fraction ont été estimées avec une concentration inhibitrice minimale (CMI) par la méthode de diffusion de disque contre 10 microbes pathogènes humains (huit souches de bactérie pathogène Gram positive/négative et deux champignons). Tous les extraits de concombre de mer confirment leur fort potentiel d'effet, à l'exception de trois souches microbiennes relativement résistantes : *Candida albicans*, *Pseudomonas aeruginosa* et *Klebsiella pneumoniae*. Les activités antibactériennes et antifongiques les plus efficaces ont été enregistrées pour l'extrait méthanolique du tractus intestinal de *H. parva* contre *Saccharomyces cerevisiae* (CIM = 0,09 mg/mL, ZI = 22 ± 0,8 mm) et *Staphylococcus epidermidis* (CIM = 0,04 mg/mL et ZI = 30 ± 0,9 mm), comparé aux autres extraits. Néanmoins, les différences entre espèces peuvent être dues à une diversité de leurs habitats naturels. Les résultats de cette recherche suggèrent que l'espèce de concombre de mer *Holothuria*, plus particulièrement *H. parva*, est une source potentielle pour la découverte de composés antibiotiques naturels et du développement des médicaments.
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Introduction

The marine organisms such as echinoderms [10], sea anemones [9,42], tunicates [19], and mollusks [41] during the last three decades have provided a vast variety of metabolites with biological activities and biomedical potential. Sea cucumbers (Family: Holothuriidae, Genus: *Holothuria*), are marine benthic echinoderms with a leathery skin and an elongated body distributed on the sea floor worldwide, generally living near corals, rocks or seaweeds, with the highest diversity in shallow tropical waters [43]. The most harvested sea cucumber species has been previously recorded in the Iranian coastal waters from the Persian Gulf is the sand fish, *Holothuria scabra* [1], *Holothuria parva* [23], and black sea cucumber, *Holothuria leucospilota* [1]. In Asia and Middle East countries such as the Philippines, Malaysia, Japan, Korea and China, where extensive commercial fisheries operate [15], sea cucumbers used as food [49], and in traditional folk medicine systems, it also forms part of treatments aimed to maintain health, prevent, reduce or cure disorders [20,49]. Therapeutic properties and medicinal benefits of sea cucumbers can attribute to the presence of a wide array of bioactive compounds. Chinese studies displayed that sea cucumbers contain bioactive compounds as saponin glycosides called holothurin [38] that have a structure similar to the active constituents of tonic herbs, ginseng and ganoderma [14].

Due to the high cost of biological testing, and a generally limited amount of material available initially in the extract of marine organisms or of isolated compounds, it is impossible in any laboratory to examine all permutations of drug-animal interactions, to reveal the drug potential of a material [7]. One of the important premises programs is an antimicrobial screening test. Since, the engender multidrug-resistant bacterial strains worldwide by continued use of antibiotics in disease control and, as expected, hospitals have become a breeding ground for pathogenic microbes associated with human diseases [3,22]. Therefore, it is important to conduct studies to identify this potential of

the marine organism in the search for new drug products. Sea cucumbers are constantly exposed to a high number of microbial infections: bacteria, fungi, and viruses, which can be harmful to them, so the survival of these organisms depends on efficient strategies for the production of antimicrobial substances to protect themselves [22]. In this regard, Farouk et al. [17] reported 30 bacterial strains that isolated from *Holothuria* sp. As see review in Bordbar et al. [8], recently extensive research has shown sea cucumber extracts to have multiple biological activities, including antimicrobial, anticancer, antioxidant properties and its extracts are known to contain an immense range of secondary metabolites such as triterpene glycosides (saponins), chondroitin sulfates, glycosaminoglycan (GAGs), sulfated polysaccharides, sterols (glycosides and sulfates), phenolics, cerberosides, lectins, peptides, glycoprotein, glycosphingolipids, cytotoxins, and essential fatty acids [8]. A variety of antimicrobial peptides has been isolated from this echinoderm [6,12]. It can be mainly found in coelomocytes, cells of immunity, and occasionally in the coelomic fluid, gastrointestinal organs, eggs and body wall [22].

The extracts of different body parts of sea cucumber have been proven as potential antimicrobial activity in several studies [8,32,37]. For serve as baseline data to medicine development from the sea cucumbers predominate off the Iranian coastline of the Persian Gulf, in this paper, we describe a comparison of antimicrobial activity in different organs (gonads, body wall, intestine tract, respiratory tree) of *Holothurians* sea cucumbers (*H. scabra*, *H. parva* and *H. leucospilota*), and performed a preliminary characterization of antibacterial components.

Materials and methods

Collection of animals

A total of 30 live specimens of the sea cucumbers; *H. scabra* and *H. parva* were obtained at the low tide time (according

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