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Antifungal activity of selected sea cucumber species from Tukuran, Zamboanga del Sur, Mindanao, Philippines using modified SPOTi assay

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Abstract. *Ereguero MG, Cordero MA, Dalayap R, Tabugo SR. 2022. Antifungal activity of selected sea cucumber species from Tukuran, Zamboanga del Sur, Mindanao, Philippines using modified SPOTi assay. Biodiversitas 23: 6049-6055.* Marine organisms have become essential candidates for discovering novel compounds that can be medically and commercially important. They are believed to yield novel compounds with unique chemical structures and significant biological activities since they managed to survive a milieu of pathogenic microorganisms and essential predators. The quest against fungal infections has been challenging due to acquired resistance to commercially available drugs hence, novel sources to treat infectious diseases caused by fungi have been investigated. Sea cucumbers have been recognized in Asian countries as a rich food source. Apart from that, they have also been studied for their medicinal properties. This study aimed to evaluate the antifungal activity of the selected sea cucumber species found in Brgy. Sugod, Tukuran, Zamboanga del Sur, Mindanao, Philippines using the modified SPOTi assay. Methanol: ethyl acetate crude extracts of *Holothuria scabra*, *Stichopus* sp., and *Holothuria atra* were tested for their antifungal activity against *Aspergillus niger* and *Candida tropicalis*. A 96-well plate assay was used, and the growth of fungi was assessed visually for up to 72hrs. The concentration resulting in 'no fungal growth' was observed and taken as the Minimum Inhibitory Concentration (MIC) value. The average MIC was calculated. Results showed that sea cucumber crude extracts have MIC values of 0.0333-0.1 mg/mL. Tukey's posthoc test confirmed that the antifungal activity of the sea cucumber extracts was comparable to the positive control Fluconazole ($p>0.05$). The present study revealed a potentially effective natural antifungal agent from sea cucumbers.

Keywords: Antifungal activity, crude extracts, fungi, minimum inhibitory concentration, sea cucumbers

INTRODUCTION

The ocean is a reservoir of potent bioactive compounds, some of which are used as food obtained from marine organisms (Kiani et al. 2014). Most recent studies have focused on benthic organisms' ability to produce bioactive metabolites in response to environmental stresses such as competition for space and food, maintenance of unfolded surface, and predator deterrence (Ali and Tamilselvi 2016). The prevalence of invasive fungal infections has increased significantly in the past few years. This increasing number of invasive fungal infections was correlated with the growing number of immunosuppressed patients (Palmieri et al. 2022). The antifungal agents are challenged due to the appearance of resistant fungal strains, and their associated side effects (Ademe 2020). Because of the therapeutic limitations of the current antifungal compounds, there is a necessity to explore possible sources of antifungal agents.

Marine natural products attracted considerable attention since marine organisms produce natural products that are pharmaceutically useful. As a result, research on marine organisms focusing on investigating their antimicrobial properties has increased (Ghadiri et al. 2018). Sea cucumbers are marine benthic organisms with elongated bodies distributed on the sea floor worldwide. They occupy a wide range of marine habitat, from tropical to polar areas and intertidal zone to deep oceans (Yuan et al. 2018); with extreme conditions that require them to adapt to survive. These organisms have remarkable biodiversity in Asia Pacific region, with 1716 species (Bahrami et al. 2014). Their role in marine ecosystems vary among species such as contributing to sediment health, recycling nutrients, influencing seawater chemistry, bolstering high biodiversity through symbiotic relationships, and forming energy transfer pathways in food chains (Purcell et al. 2016). Sea cucumbers are valuable because of their nutritional value, therapeutic and pharmaceutical properties (Komala 2014; Künili and Çolakoğlu 2018). They have been widely used in Asian cuisines and folk medicine (Pangestuti and Arifin 2018). Sea cucumbers have a long history as a food source in Southeast Asia. Sea cucumbers are reported to comprise amino acids such as aspartic leucine, arginine, isoleucine, glutamic, glycine, histidine, lysine, valine, and threonine on a dry weight basis. In addition, sea cucumbers also contain niacin, minerals and trace elements, thiamine, retinol/carotenoid complex, riboflavin, and omega-3 fatty acids. In this framework, sea cucumbers are widely used in Traditional Chinese

Medicine to treat joint and skeletal weakness, kidney system disorders, constipation, circulatory ailments, and poor lipid digestion. They are also used as treatments for wounds, skin diseases, and aches in a cream based on the oil extracts derived from sea cucumbers (Liang et al. 2022).

Due to its high nutrient content of vitamins and minerals, and several distinctive bioactive compounds, sea cucumber has substantial commercial value (Oh et al. 2017). Sea cucumbers are a source of high-value-added compounds with health benefits and effects. Bioactive peptides, vitamins, minerals, fatty acids, saponins, carotenoids, collagens, gelatins, chondroitin sulfates, amino acids, fatty acids and other bioactive compounds are examples of derived functional ingredients of sea cucumbers (Pangestuti and Arifin 2018). Thus, the antimicrobial properties of sea cucumbers can be linked to the presence of a wide array of bioactive substances (Bordbar et al. 2011).

In the Philippines, sea cucumber collection has become a livelihood for small-scale fishermen in coastal communities. The collection of sea cucumber species was usually done in shallow waters. However, the skyrocketing demand for these species causes the collection of the species to be conducted in deeper waters. As a result, the Philippines ranked second in production and export in the world, next to Indonesia, with 20,000 tons of catch per year (Cañada et al. 2020). There are more than 1500 different species of sea cucumber, and about 100 species are recognized to be safe for human consumption. The most notable commercial species are *Acaudina molpadioides, Actinopyga mauritiana, Apostichopus japonicus, Cucumaria frondosa, Cucumaria japonica, Holothuria forskali, Holothuria polii, Holothuria nobilis, Holothuria tubulosa, Isostichopus badionotus*, and *Pearsonothuria graeffei* (Hossain et al. 2020).

Studies reported that some sea cucumber species have important antimicrobial characteristics in their soluble extracts (Nobsathian et al. 2017; Mashjoor et al. 2018). Strong antifungal activity was reported for organic and aqueous extracts of various sea cucumber species due to triterpene glycosides, also commonly known as sea cucumber saponins or holothurians (Husni et al. 2014). Antimicrobial resistance has been a major global threat to the population's health which endangers the prevention and treatment of a wide range of infectious diseases (Iwu-Jaja et al. 2021; Bharadwaj et al. 2022). Therefore, searching for alternative antimicrobial agents from alternative sources became an essential demand (Ibrahim et al. 2020).

Recently, drug susceptibility screening methods have been tested for bacteria and fungi. Increased research activity related to antifungal susceptibility sparks the need for an adequate and undisputable quantitative assay. This led to the development of various methods such as micro- and macro-dilution methods, agar-based using disc diffusion, E-test, colorimetric modifications and bioluminescence assays to enhance Minimum Inhibitory Concentration (MIC) however, a limitation to these assays is the long incubation times (Karaca and Koc 2004; Rizi et al. 2015). Successful development, application and adaptation of an objective assay for the screening of antifungal agents called the SPOTi assay somehow circumvent this problem and paved the way for a faster antifungal screening process. The quantitative results of the SPOTi assay have been validated and coincide with the 'qualitative' ones (disc diffusion tests) as they were performed alongside (Rizi et al. 2015). Thus, the method was adapted and modified to tailor-fit the test organism.

This study explores the potential antifungal activity of selected sea cucumber species found in Brgy. Sugod, Tukuran, Zamboanga del Sur, Mindanao, Philippines by determining the MIC of the crude extracts through modified SPOTi assay. This method for testing microbial growth and viability inhibitors entails a high-throughput solid agar-based assay in a muti-well plate (96 well) (Guzman et al. 2013). It is a fast, reliable, feasible, and reproducible method that can be implemented in academic and industrial settings. The results will serve as baseline information for further studies.

MATERIALS AND METHODS

Specimen collection and identification

Prior to the conduct of the study, the research was approved by the institutional review board (IRB) and biosafety clearance was obtained. Informed consent and permits from the Mayor and the Department of Agriculture, Bureau of Fisheries and Aquatic Resources (DA-BFAR) were also acquired. A total of three different sea cucumber species were collected from Brgy. Sugod, Tukuran, Zamboanga del Sur, Mindanao, Philippines. The collected samples were transferred into a clean container and placed in an ice chest for transport. Finally, the samples were brought to the Molecular Systematics and Oceanography Laboratory in the Premier Research Institute of Science and Mathematics (PRISM), MSU-IIT for experimentation. The samples were morphologically identified using the identification guide of Purcell et al. (2012).

Preparation of sea cucumbers and crude extracts

The collected sea cucumber samples were washed and cleaned with distilled water to remove the unwanted material on their epidermal surface. Twelve (12) pieces of sea cucumbers were grouped morphologically. Prior to the extraction procedure, the samples were cut into small pieces and soaked separately in methanol: ethyl acetate (50:50) for one week. A total of four (4) extracts were obtained since extracts from *Holothuria atra* have two separate layers, upper (1) and lower (2) layers, *Holothuria scabra*, and *Stichopus* sp. Crude extracts were filtered and concentrated *in vacuo* using a rotary evaporator and then exposed to pressurized nitrogen to remove the residues.

Test organisms

Candida tropicalis BIOTECH 2085, and *Aspergillus niger* BIOTECH 3080 were obtained from the University of the Philippines - Los Baños. Test organisms were subcultured on appropriate media as prescribed and incubated at room temperature.

Determination of antifungal activity

Sabouraud's dextrose agar (SDA) and Sabouraud's dextrose broth (SDB) were used as the media in the assay. Aseptic procedures were performed in preparing the cultures. Stock inoculum suspension of the *Aspergillus niger* and *Candida tropicalis* were prepared from 14-day old cultures grown on SDA slants at 35℃. The fungal colonies were covered with 4 mL SDB, and suspensions were obtained by gently probing the agar's surface with the tip of a sterile inoculating loop, generating a mixture of conidial and hyphal fragments. The obtained suspensions were then filtered through four layers of sterile gauze, which retains the hyphal fragments but permits the passage of microconidia. A filtration process was used as this has been shown to provide greater reproducibility and reliability of susceptibility testing. The filtrate was centrifuged. The Optical Density of the test organisms at 0.5-1 absorbance reading at 600 nm was measured using a handheld spectrophotometer (Photopette).

The desired extract of 100 ppm was made by dissolving 1mg of the crude extract into 10 mL sterile distilled water to obtain 0.1 mg/mL extract.

The Fluconazole solution (positive control) was prepared by dissolving 5 mg tablet in 50 mL of sterile distilled water to obtain a 100ppm solution. A vortex mixer was used to ensure a well-dissolved and homogenous solution.

The published method of Rizi et al. (2015) was employed with slight modifications. Each sterile 96-well plate was appropriately labeled under aseptic conditions. A volume of 100µL of the test material was prepared at 100ppm or 0.1 mg/mL of crude extracts (test materials) and pipetted in the plate's first column (row C to row F). Next, a volume of 100 µL Fluconazole was pipetted into the desired column (C1 at row G) and serves as the positive control. Also, a volume of 100 µL sterilized distilled water was pipetted into the desired column (C1 at row A) and serves as the negative control. Then, 100 µL of sterile saline was added to all wells except for column 1. Except for row B, serial dilution was performed using a multichannel pipette such that each well had 50 µL of the test material in descending concentrations, discarding the last 50 µL from the last column. After that, 150 µL of SDA was added to all wells to ensure that the final volume has a single strength of the SD agar. Then, 5 µL of the respective fungal suspension (5×10^6 CFU/mL) was added to each well. The plates were then incubated, and growth was assessed visually for up to 72 hours. Plates were prepared in triplicates. The concentration resulting in 'no fungal growth' was observed and taken as the Minimum Inhibitory Concentration (MIC) value. The average of the three values was calculated.

Data analysis

Paleontological Statistics (PAST) v.2.17 software was used to analyze the data. Statistical differences between the concentrations were analyzed using a One-way Analysis of Variance (ANOVA). Tukey's pair-wise test was also used to check the significant difference between the groups examined and with respect to the positive control.

RESULTS AND DISCUSSION

Due to the rich oceanic biodiversity, marine organisms are valuable sources of nutritious foods and medicine. They represent novel reservoirs of biologically active components, particularly bioactive peptides and antimicrobial, anti-inflammatory, and anticancer agents (Venugopal 2008). Secondary metabolites from marine invertebrates display a wide range of bioactivities, such as antibacterial, antifungal, and cytotoxic effects (Darya et al. 2020). The present study focused on the antifungal activity of sea cucumber crude extracts (*Holothuria scabra*, *Stichopus* sp., and *Holothuria atra*) (Figure 1) against *Aspergillus niger* and *Candida tropicalis*.

In vitro antifungal activity was screened, and after 72h of incubation, the growth of the test organisms was assessed visually. The concentration with 'no fungal growth' was taken as the Minimum Inhibitory Concentration (MIC) value. Fluconazole was used as the positive control. Fluconazole interacts with 14-demethylase, a cytochrome P-450 enzyme responsible for converting lanosterol to ergosterol (Spampinato and Leonardi 2013). Ergosterol forms an essential part of the fungal cell membrane and fluconazole inhibits the synthesis of ergosterol thereby increasing cellular permeability which permits ions to leak from the cell and cause cell death. Four sea cucumber extracts were investigated to evaluate their antifungal activity against two strains of fungi, *Aspergillus niger* and *Candida tropicalis*, using the modified SPOTi assay. Results show a promising potential for the crude extracts examined. Figures 2 and 3 are representative 96-well plate showing the antifungal activity of sea cucumber extracts and Fluconazole against *C. tropicalis* and *A. niger*.

A **B** **C**

Figure 1. Sea cucumber species found in Brgy. Sugod, Tukuran, Zamboanga del Sur, Philippines. A. *Holothuria scabra*; B. *Stichopus* sp.; and C. *Holothuria atra*

The antifungal activity of the sea cucumber extracts was measured and compared to the positive control, Fluconazole. Table 1 shows the MIC value of crude extracts against the test organisms. Sea cucumber crude extracts were observed to inhibit the growth of *A. niger* and *C. tropicalis* with MIC values of 0.0333-0.1 mg/mL with respect to the positive control. It was found that *H. scabra*, *Stichopus* sp., and *H. atra* (1 and 2) crude extracts were able to inhibit the growth of *A. niger* with MIC values of 0.1, 0.0333, 0.0667, and 0.0667 mg/mL, respectively. Moreover, *H. scabra*, *Stichopus* sp., and *H. atra* (1 and 2) crude extracts inhibit the growth of *C. tropicalis* with MIC values of 0.05, 0.0667, 0.0833, and 0.0417 mg/mL, respectively.

One-way Analysis of Variance (ANOVA) was used to compare the antifungal activity of the sea cucumber crude extracts and positive control as shown in Table 2. The one-way ANOVA showed no significant difference between groups since p-value > 0.05. Thus, the antifungal activity exhibited is comparable between groups and to the positive control.

Tukey's pairwise results confirmed that the antifungal activity of sea cucumber crude extracts was comparable to the positive control, as shown in Table 3. Furthermore, *H. scabra* and *H. atra* (1) are comparable with each other with p-value of 1. This implies that they are highly active against test organisms, the only difference is that *H. scabra* is more potent against *C. tropicalis* and *H. atra* (1) is more active against *A. niger*.

The results show that the modified SPOTi assay was helpful in assessing the antifungal activity of the crude extracts against the test organisms since the MICs of the test samples could be reliably determined on the third day (Rizi et al. 2015). Compared to the broth micro-dilution and disc-diffusion method, which requires a seven-day incubation period (Norris et al. 1999), the modified SPOTi assay offers ease of performance with quantitative measures of MICs (Rizi et al. 2015). In this case, a more objective determination of the MICs was made possible.

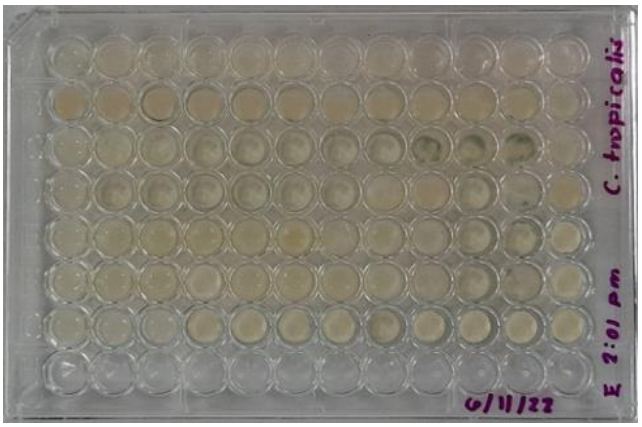

Figure 2. Susceptibility of *C. tropicalis* to sea cucumber crude extracts (rows C, D, E, and F; media+extracts+fungi) and Row G (positive control) (media+Fluconazole+fungi); Row A served as negative control (media+distilled water); Row B (media+fungi;no extract); using the 96-well plate modified SPOTi assay method. Plates were incubated for 72h

Figure 3. Susceptibility of *A. niger* to sea cucumber crude extracts (rows C, D, E, and F; media+extracts+fungi) and Row G (positive control) (media+Fluconazole+fungi); Row A served as negative control (media+distilled water); Row B (media+fungi; no extract); using the 96-well plate modified SPOTi assay method. Plates were incubated for 72h

Table 1. Minimum Inhibitory Concentration (MIC) of sea cucumber crude extracts and positive control against test organisms after 72h

	Minimum Inhibitory Concentration (mg/mL)				
	Extracts/test materials				**Positive control**
Test organisms	*H. scabra*	*Stichopus* sp.	*H. atra* (1)	*H. atra* (2)	Fluconazole
A. niger	0.1	0.0333	0.0667	0.0667	0.025
C. tropicalis	0.05	0.0667	0.0833	0.0417	0.0167

Table 2. One-way Analysis of Variance (ANOVA) of sea cucumber extracts and positive control (Fluconazole) against test organisms

	Sum of sqrs	**df**	**Mean square**	**F**	**p-value**
Between groups:	0.00398611	4	0.000996528	2.174	0.2083
Within groups:	0.00229167	5	0.000458333		
Total	0.00627778	9			

Table 3. Tukey's pairwise comparison of four (4) sea cucumber extracts and positive control (Fluconazole)

	H. scabra	***Stichopus* sp.**	***H.atra* (1)**	***H.atra* (2)**	**Fluconazole**
H. scabra	-				
Stichopus sp.	0.7691	-			
H. atra (1)	1	0.7691	-		
H. atra (2)	0.857	0.9996	0.857	-	
Fluconazole	0.2207	0.672	0.2207	0.5744	-

Noteworthy, Aspergillus and Candida species were used as test organisms because the resistance of Aspergillus and Candida species to commercially available drugs have been increasingly reported due to susceptibility test standards and with increasing use of antifungal agents (Arendrup 2014). *Candida tropicalis* is predominant and causes infections in animals and humans (Lima et al. 2022). Candidiasis is a broad term that refers to mucosal, cutaneous, and deep-rooted organ infections caused by fungi of the Candida genus. At least 15 distinct *Candida* spp. can cause human diseases, which include *C. tropicalis* (Pappas et al. 2018). In Asia and some parts of Latin America, *C. tropicalis* is among the most common species related to candidemia, and mortality rates are usually 40%. A recent study suggests an increasing ubiquity of fluconazole and multidrug resistance of *C. tropicalis* retrieved from humans (Lima et al. 2022). On the other hand, aspergillosis is a severe clinical problem that is caused by Aspergillus species, especially in immunocompromised patients, thus Aspergilli has become an opportunistic fungal pathogen (Shishodia et al. 2019). The Aspergillus genus has the ability to cause a wide range of clinical problems, from simple allergic forms to invasive aspergillosis, with invasive forms having a mortality rate of more than 80% if not treated promptly (Romero et al. 2019). A multitude number of patients have been detected with chronic pulmonary aspergillosis worldwide. Moreover, Aspergillus causes invasive aspergillosis (Shishodia et al. 2019). *A. niger* is distributed everywhere and it mainly causes otomycoses. Furthermore, they are also regarded as the third most common cause of invasive pulmonary aspergillosis (Van Der Linden et al. 2011). *In vitro*, *A. niger* was reported to have a 2.4% rate of invasive diseases among all *Aspergillus* spp. Clinical studies, however, have found that *A. niger* is responsible for 4-8.7% of invasive aspergillosis cases (Ergene et al. 2013). Invasive aspergillosis remains a complex infectious disease to manage. Until recently, species identification was enough to guide antifungal therapy, but the emergence of acquired resistance limits the use of species identification for predicting the activity of antifungal agents (Van Der Linden et al. 2011). Heightened drug-resistance cases of Aspergillus species pose an additional threat to human health (Shishodia et al. 2019).

Results obtained coincide with several studies on the antifungal activity of sea cucumber species. In the study of Mohammadizadeh et al. (2013), *H. scabra* methanol showed a positive result against *A. niger* with MIC values ranging from 3 to 9 µg/mL. At 100 µL concentration of *H. atra* extract recorded maximum antifungal activity towards *T. viride*, *A. niger*, *A. falvis*, *C. albicans*, and *P. chrysogenum* with a diameter of 16 14, 12, 18, and 14 mm, respectively (Dhinakaran and Lipton 2014). The antifungal activity of *Holothuria polii* was investigated and results showed that the ethanol extract of the body wall of the mentioned sea cucumber species has a potent antifungal activity on *A. flavus*, *A. niger*, and *C. albicans* in a concentration of 2.5mg/mL (Omran and Allam 2013). On the other hand, the methanol and aqueous methanol extract of *S. variegatus* has an inhibitory effect against *A. niger* in the concentration of 1-8mg/mL (Shakouri et al. 2017). Adibpour et al. (2014) examined the antifungal activity of *Holothuria leucospilota*. The results presented that the body wall and coelomic fluid extract of the species inhibited the growth of all fungi at 1000 µg/mL and 2000 µg/mL. However, the body wall extract of the species did not inhibit the growth of *A. niger* at 2000 µg/mL. Another study of *H. leucospilota* extracts was examined against *A. niger and C. albicans*. The body wall, gonad and intestine methanol and chloroform extracts of the species have antifungal activity against *A. niger*, but intestine chloroform and gonad methanol extracts had no antifungal activity on *C. albicans* (Farjami et al. 2014). The organic extracts (acetonitrile and ethanol) of the different tissues of *H. leucospilota* had more antifungal activity than the phosphate-buffered saline extracts. The extracts of the respiratory tree had more antifungal activity against Candida strains followed by the body wall and gastrointestinal tract extracts (Ghadiri et al. 2018). In a study by Sarhadizadeh et al. (2014), they reported that *Stichopus herrmanni* methanol and methanol-water extracts have indicated antifungal activity against *A. niger*. The extracts showed antifungal activity with MICs ranging from 3 to 15 µg/mL. The highest antifungal activity was found in the body wall methanol-water extract with a 38 mm inhibition zone at 18 µg/mL extract concentration. In another study by Sahlan et al. (2020), the jelly derived from sea cucumber *H. scabra* have antifungal effect against *C. albicans* with 11 mm zone of inhibition.

Sea cucumbers are a source of high-value-added compounds with health benefits effects. Bioactive peptides, vitamins, minerals, fatty acids, saponins, carotenoids, collagens, gelatins, chondroitin sulfates, amino acids, fatty acids and other bioactive compounds are examples of derived functional ingredients of sea cucumbers (Pangestuti and Arifin 2018). Thus, the antimicrobial properties of sea cucumbers can be linked to the presence of a wide array of bioactive substances (Bordbar et al. 2011).

Numerous antimicrobial components have been isolated from sea cucumbers, such as antimicrobial peptides, complement-like substances, lysozymes, naphthoquinone pigments, polyhydroxylated sterols, and steroidal glycosides. Several holostane-type tripterpene glycosides from *Holothuria fuscocinerea* and three cytotoxic glycosides from *Mensamaria intercedens* Lampert have been isolated and display antibacterial, antifungal, and cytotoxic activity (Adibpour et al. 2014). The triterpene glycosides isolated from *H. scabra, A. lecanora, B. marmorata, and H. axiloga* exhibited notable antifungal activity. The triterpene glycosides isolated from the ethanolic extract of *Psolus patagonicus* indicated an antifungal activity against *Cladosporium cladosporioides*, a phytopathogenic fungus, in a dose-dependent manner. In addition, the jelly contained the hydrolysate of *S. japonicus,* which also has triterpene glycosides, also known as holoxins, exhibited an inhibitory activity against oral Candida (Shi et al. 2015). According to Oh et al. (2017), the antifungal activity of sea cucumbers is due to their adaptability since their bodies are accustomed to fungi since fungi are commonly found in their environment.

Benthic marine environments are popularly described as a soup of microorganisms. Thus, deposit-feeding and sedentary bottom-dwelling organisms such as sea cucumbers are incessantly exposed to harmful pathogens. For this reason, they possess an efficient chemical defense mechanism, which involves the production of secondary metabolites with antimicrobial activities to survive and protect themselves against microbial infections. In this sense, the antimicrobial properties of sea cucumber extracts have been attributed to the presence of saponin. These secondary metabolites have been determined to possess various pharmacological benefits such as antifungal, antibacterial, and antiviral activities (Telahigue et al. 2020).

Sterol plays an important role in maintaining membrane organization and regulating signal transduction of the eukaryotic membranes (Zheng Koh and Saheki 2021). Ergosterol is a significant fungal membrane sterol that controls membrane fluidity, plasma membrane biogenesis, and function. Thus, ergosterol homeostasis is critical for fungal cells. Azole drugs such as fluconazole, are widely utilized in treating fungal infections by inhibiting ergosterol biosynthesis (Yang et al. 2015). Saponins have cytotoxic and other biological activities, such as antimicrobial activity. The antifungal activity of saponin is due to its ability to complex sterols in the fungal membranes and induce loss of membrane integrity through the formation of transmembrane pores (Kim et al. 2018). The antifungal activity of sea cucumbers could be due to bioactive compounds such as saponins.

The search for antimicrobial agents from various sources, including the marine environment, has been employed. In this study, the modified SPOTi Assay proved to be an effective means to test the antifungal activity of selected sea cucumber species. The assay provided a convenient method to determine tested extracts' minimum inhibitory concentration (MIC) values. Results of the study showed that the crude extracts of selected sea cucumber species possess antifungal activity and can be a potential source for novel antifungal agents. However, further studies are needed to identify and verify the components responsible for the antifungal activity of these species.

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