



# *Astichopus multifidus* and *Holothuria floridana* from Yucatecan Coast as potential source of antifungal agents.

*Astichopus multifidus* y *Holothuria floridana* de la costa yucateca como fuente potencial de agentes antifúngicos

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## Highlights

- First report on antifungal properties of *Astichopus multifidus* and *Holothuria floridana*.
- Butanolic body extracts show low MICs against *Cryptococcus neoformans*, *Trichophyton rubrum*, and *Aspergillus niger*.
- Nearly 50% of sea cucumber extracts exhibited antifungal activity.

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## ORIGINAL RESEARCH

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## ABSTRACT

**Introduction.** Infections caused by pathogenic fungi present a persistent public health challenge. Marine invertebrates, such as sea cucumbers (holothurians), are recognized for their high protein content and various medicinal properties. They also serve as reservoirs of bioactive compounds. In the territorial waters of México, six sea cucumber species, including *Astichopus multifidus*, *Isostichopus badionotus*, and *Holothuria floridana*, are found along the Yucatán Peninsula. However, overexploitation threatens these species, potentially diminishing valuable sources of antimicrobial agents. **Objectives.** This study aims to evaluate the antifungal activity of organic extracts from *A. multifidus* and *H. floridana* against various fungal strains, using minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) assays. **Materials and Methods.** Organic extracts from *A. multifidus* and *H. floridana* were obtained and tested for antifungal activity using a microdilution method. The MIC and MFC values were determined against a panel of fungal strains, including *Trichophyton rubrum*, *Aspergillus niger*, and *Cryptococcus neoformans*. Extracts from *Isostichopus badionotus* were also evaluated. **Results and Discussion.** Approximately 50% of the extracts exhibited antifungal activity. Butanolic body extracts from *A. multifidus* and *H. floridana* displayed the lowest MIC values (15.6 µg/mL) against *T. rubrum* and *A. niger*, respectively. Additionally, these extracts demonstrated antifungal activity (31.2 µg/mL) against *C. neoformans*. In contrast, extracts from *I. badionotus* showed no activity. These results suggest that *A. multifidus* and *H. floridana* contain bioactive compounds with antifungal potential. **Conclusions.** The antifungal properties of *A. multifidus* and *H. floridana* highlight their potential as sources of novel antifungal agents. Their sustainable use could contribute to the development of new antifungals.

## RESUMEN

**Introducción.** Las infecciones causadas por hongos patógenos representan un desafío persistente para la salud pública. Los invertebrados marinos, como los pepinos de mar (holoturias), son reconocidos por su alto contenido proteico y diversas propiedades medicinales. Además, actúan como reservorios de compuestos bioactivos. En las aguas territoriales de México, se encuentran seis especies de pepinos de mar, entre ellas *Astichopus multifidus*, *Isostichopus badionotus* y *Holothuria floridana*, distribuidas a lo largo de la península de Yucatán. Sin embargo, la sobreexplotación amenaza a estas especies, lo que podría reducir fuentes valiosas de agentes antimicrobianos. **Objetivos.** Este estudio tiene como objetivo evaluar la actividad antifúngica de extractos orgánicos de *A. multifidus* y *H. floridana* frente a diversas cepas fúngicas, mediante ensayos de concentración mínima inhibitoria (CMI) y concentración mínima fungicida (CMF). **Materiales y métodos.** Se obtuvieron extractos orgánicos de *A. multifidus* y *H. floridana*, los cuales fueron evaluados mediante el método de microdilución para determinar su actividad antifúngica. Se establecieron los valores de CMI y CMF frente a un panel de cepas fúngicas, incluyendo *Trichophyton rubrum*, *Aspergillus niger* y *Cryptococcus neoformans*. También se evaluaron extractos de *Isostichopus badionotus*. **Resultados y discusión.** Aproximadamente el 50 % de los extractos mostró actividad antifúngica. Los extractos butanólicos del cuerpo de *A. multifidus* y *H. floridana* presentaron los valores más bajos de CMI (15,6 µg/mL) frente a *T. rubrum* y *A. niger*, respectivamente. Además, estos extractos mostraron actividad antifúngica (31,2 µg/mL) contra *C. neoformans*. En contraste, los extractos de *I. badionotus* no presentaron actividad. Estos resultados sugieren que *A. multifidus* y *H. floridana* contienen compuestos bioactivos con potencial antifúngico. **Conclusiones.** Las propiedades antifúngicas de *A. multifidus* y *H. floridana* destacan su potencial como fuentes de nuevos agentes antifúngicos. Su uso sostenible podría contribuir al desarrollo de nuevos tratamientos antimicrobianos.

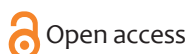


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## INTRODUCTION

Infections caused by pathogenic fungi have remained for many years as an important public health problem <sup>(1)</sup>. In recent years, there has been a drastic rise in the incidence of fungal infections, which is attributed to the susceptibility of the population to opportunistic infections mainly due to immunosuppression closely linked to hematologic diseases, inflammatory auto-immune conditions, human immunodeficiency virus (HIV) infection, malnutrition or aggressive medical practices, like anticancer chemotherapy or procedures for organ transplants <sup>(2)</sup>.

Nowadays, candidiasis, aspergillosis and cryptococcosis are the most frequent invasive fungal infections <sup>(3)</sup>. Cryptococcosis, caused by encapsulated yeasts of the *Cryptococcus* genus, affects the central nervous system producing meningitis and meningoencephalitis with a mortality rate of 80 % <sup>(4)</sup>. Annually, more than one million people are infected by *Cryptococcus neoformans* (patients with acquired immunodeficiency syndrome (AIDS), cancer, and organ transplants) or *Cryptococcus gatti* (immunocompetent hosts) <sup>(5)</sup>. Cryptococcal meningitis is the leading cause of meningitis among adults infected with HIV and is increasingly being diagnosed in individuals who are HIV-seronegative <sup>(6)</sup>.

Invasive candidiasis is an emerging infection closely linked to advancements in medical technology and is widely recognized as a significant contributor to morbidity and mortality in healthcare settings <sup>(7)</sup>. *Candida albicans* is the most prevalent species involved in invasive candidiasis, however over past years there has been reports on the implication of other species like *C. glabrata*, *C. krusei*, *C. lusitaniae*, *C. parapsilosis*, and *C. tropicalis* <sup>(8)</sup>.

Species belonging to *Aspergillus* genus are the causal agents of aspergillosis. This opportunistic infection, acquired through the respiratory tract, leads to invasive pulmonary aspergillosis, which is associated with high morbidity and mortality rates <sup>(9-10)</sup>. *Aspergillus fumigatus* has been reported to be the species that most commonly causes invasive pulmonary aspergillosis, followed by *A. flavus* and *A. niger* <sup>(11-12)</sup>.

Cutaneous fungal infections, induced by both dermatophyte and non-dermatophyte fungi, present considerable obstacles to worldwide public health. Dermatophyte infections predominantly impact the skin, hair, and nails. Pathogens like *Trichophyton rubrum*, are the most common classes of fungal agents responsible for these infections <sup>(13)</sup>.

Three classes of antifungal drugs are currently available for the prevention and treatment of invasive fungal diseases triazoles, polyenes, and echinocandins. However, the use of these agents is often limited by drug toxicity, drug-drug interactions, and the lack of oral formulations.<sup>(14)</sup> These observations highlight the ongoing need to discover novel, effective, and safe antifungal agents with minimal or acceptable adverse effects. Natural products are promising sources of such bioactive compounds, and oceanic environments, with their extensive biodiversity and numerous ecological interactions among macro and microorganisms, serve as a significant reservoir of these compounds <sup>(15-16)</sup>.

Sea cucumbers or holothurians are spiny-skinned marine invertebrates commercially exploited fresh or in dehydrated form (*bêche de mer*, trepang, gamat) in Asian markets, mainly in China, Korea, Indonesia, and Japan as functional foods, because of their high-protein content and their putative aphrodisiac effect, tonic and medicinal properties<sup>(17)</sup>. Asian folk medicine describes the use of alcoholic extracts from body of certain holothurians species to treat tumors, fungal infections, control high blood pressure, arthritis and muscular disorders as well as the application of cataplasms prepared with the sea cucumber body for treating minor wounds<sup>(18-21)</sup>.

These animals are a significant source of biologically active constituents, including cerberosides, glycosaminoglycans, lectins, fucosylated chondroitin sulfate, polysaccharides, phospholipids, and particularly, triterpene glycosides. These triterpene glycosides exhibit inherent antifungal and antioxidant properties<sup>(22-23)</sup>.

Extracts and compounds from several sea cucumber species have been studied to determine their potential as sources of antifungal drugs. Among them, those obtained from *Actinopyga lecanora*<sup>(24-25)</sup>, *Apostichopus japonicus*<sup>(26)</sup>, *Holothuria leucospilota*<sup>(27)</sup>, *H. polii*<sup>(28)</sup>, *H. scabra*<sup>(29)</sup>, and *Psolus patagonicus*<sup>(30)</sup> have exhibited significant activity against both yeasts and molds.

In these contexts, six species of sea cucumbers in Mexican territorial waters are subject to intense fishing. Two of these species are found in the Gulf of California within the Pacific Ocean, while the remaining species inhabit the shallow waters (0-80 m depth) of the Yucatán Peninsula. Recently, *Astichopus multifidus* (Sluiter, 1910), *Isostichopus badiionotus* (Selenka, 1867), and *Holothuria* (Halodeima) *floridana* (Pourtalés, 1851), three species from the littoral zone of the Yucatán Peninsula, have been extensively harvested to meet international trade demands. This overharvesting threatens holothurian populations, leading to a dangerous decline and the potential loss of a source of new antifungal drugs<sup>(31)</sup>.

Therefore, with the aim of evaluate the potential of sea cucumber species from the Yucatán Peninsula coasts, as a source of antifungal compounds, and to contribute to their conservation; eighteen organic extracts obtained from the body and visceral organs from *A. multifidus*, *I. badiionotus*, and *H. floridana* were investigated against eight reference fungal strains, five yeast and three molds.

## MATERIALS AND METHODS

### Animal materials

Specimens of *Astichopus multifidus*, *Holothuria floridana* and *Isostichopus badiionotus* were collected at coasts of Progreso (N 21° 27.3'–W 89° 42.7') in the state of Yucatán (México), by scuba at depths ranging from 0.6 to 16 m. All animals were promptly placed in plastic bags containing ice water and transported to the laboratory, where they were washed, manually cleaned, and eviscerated; body wall and viscera were stored separately at –17 °C until use. M. Sc. Carlos Zetina Moguel authenticated the specimens according to the identification procedure established from Hendler<sup>(32)</sup>. The voucher specimens (*A. multifidus* YUC-CC-250-11/CAR/19445,

*H. floridana* YUC-CC-250-11/CAR/19444, *I. badiionotus* YUC-CC-250-11/CAR/19452), were deposited in the zoological collection of the Campus de Ciencias Biológicas y Agropecuarias, Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Yucatán (UADY), Mérida, Yucatán, México. The collected organisms were cleaned of any foreign material, and the body wall was separated from the viscera, as well as the coelomic fluid. The evisceration of each organism was carried out by making a longitudinal cut in the ventral region of the organism from the anus to the mouth.

### Fungal Strains

The fungi used in this study were *Candida albicans* (ATCC 10231), *Candida krusei* (ATCC 6258), *Candida parapsilosis* (ATCC 10232), *Candida tropicalis* (ATCC 20005), *Cryptococcus neoformans* (ATCC 11240), *Aspergillus niger* (ATCC 16888), *Aspergillus fumigatus* (ATCC 1022), and *Trichophyllum rubrum* (ATCC 10218). All of them were acquired from the American Type Culture Collection. Before testing, the strains were cultured on Sabouraud dextrose agar; the filamentous fungi were maintained at room temperature for 5 days, while the yeasts were maintained for 24 h at 37°C.

### Extraction procedure

The viscera and coelomic fluid were collected and weighed as well as the body wall (400 grams of each species in both cases); later, they were placed in labeled plastic bags indicating collection data and stored at -10°C until their use. The body wall was cut into small pieces, ground, and macerated in distilled methanol. The ratio between the sample and the solvent methanol (MeOH) was 1:2 (weight:volume). The maceration time was seven days, after which it was filtered through a glass funnel with Whatman filter paper no.1. The obtained methanolic extract was concentrated under reduced pressure until complete removal of the solvent.

The residue of the MeOH extract was suspended in 250 mL of water and subjected to liquid-liquid extraction using 250 mL of hexane (Hex), repeated four times (4×). The remaining aqueous phase was subsequently extracted with ethyl acetate (AcOEt) and then with butanol (BuOH), following the procedure described above. Each organic extract (hexane, ethyl acetate, and butanol) was concentrated under reduced pressure until complete removal of the solvent. The final aqueous phase, which remained after all extractions, was retained and labeled as the aqueous extract (H<sub>2</sub>O), this residue was suspended in a methanol-acetone solution (1:1, v/v), producing a precipitate that was collected by filtration through a glass funnel with Whatman No. 1 filter paper. The precipitate was washed three times with the same solvent mixture, and the combined washings and filtrate were concentrated under vacuum.

## Antimicrobial Susceptibility Testing

The susceptibility tests were carried out following the Clinical and Laboratory Standards Institute (CLSI) microdilution techniques, M27-A3 and M38-A, for yeasts and filamentous fungi, respectively (33, 34). The inoculum concentration was  $1 \times 10^3$  UFC/mL for yeasts or  $1 \times 10^6$  UFC/mL for molds. The minimum inhibitory concentration (MIC) was determined for each extract using the microplate method. Samples of 100  $\mu$ L of the dimethyl sulfoxide (DMSO) test solution of each extract were 2-fold serially diluted with Sabouraud dextrose broth and 100  $\mu$ L of a culture of microorganisms were added to a 96-well microplate. Final extracts concentrations ranged from 500 to 0.975  $\mu$ g/mL. The microplates were incubated 24 h at 36°C for yeasts and 5 days at room temperature for molds. The concentration of DMSO in test solutions of each extract never exceeded 1% (v/v). Itraconazole and fluconazole (ranging from 8 to 0.015  $\mu$ g/mL) were used as positive control while DMSO was used as negative controls. To indicate microbial growth, after incubation period 40  $\mu$ L of a 0.02 % aqueous solution of *p*-iodonitrotetrazolium violet were added to the microplates and incubated at 37 °C for 30 min.

The MICs values were established as the lowest concentration of the extract capable of inhibiting visible microorganism growth. The minimum fungicidal concentrations (MFC) were determined by subculturing 100  $\mu$ L from each well without visible growth onto Sabouraud dextrose agar plates. MFC was established as the lowest extract concentration which showed no fungal growth after 48 h of incubation at 35 °C.

## RESULTS

The obtained yields resulting from the liquid-liquid extraction process (Hex, AcOEt, BuOH, and H<sub>2</sub>O) were assessed for each individual extract. It was noted that in the case of *Astichopus multifidus*, the yields of the obtained extracts (AmBHex, AmBAcOEt, AmBBuOH, AmBH<sub>2</sub>O, AmVHex, AmVAcOEt, AmVBuOH, AmH<sub>2</sub>O) exhibited a variation ranging from 1.43% to 0.01% relative to the overall weight. For *Isostichopus badionotus* extracts (IbBHex, IbBAcOEt, IbBBuOH, IbBH<sub>2</sub>O, IbVHex, IbVAcOEt, IbVBuOH, IbH<sub>2</sub>O), the yields ranged from 0.01% to 0.02%. In the instance of *Holothuria floridana*, the yields spanned from 0.75% to 9.3%. (Table 1).

**Table 1. Extract yield obtained from the sea cucumbers**

Species	Animal part	Extract yield			
		Hex (%)	AcOEt (%)	BuOH (%)	H <sub>2</sub> O (%)
<i>Am</i>	B	0.001	0.06	0.20	1.43
	V	0.003	0.06	0.13	0.54
<i>Ib</i>	B	0.010	0.01	0.01	0.02
	V	0.010	0.01	0.01	0.02
<i>Hf</i>	B	2.700	0.99	7.50	9.30
	V	2.150	0.75	0.78	3.39

Am, *Astichopus multifidus*; Ib, *Isostichopus badionotus*; Hf, *Holothuria floridana*; Hx, hexane; EtOAc, ethyl acetate; BuOH, butanol; H<sub>2</sub>O, water; B, body; V, viscera.

The significant antifungal activity results of the organic extracts from *A. multifidus* and *H. floridana* are presented in (Tables 2 and 3). In contrast, the activity observed in extracts obtained from *I. badiionotus* was not considered relevant and is omitted.

**Table 2. Antifungal activity of the extracts of holothurians on yeasts**

Species	Animal Part	Extract	Yeast MIC (MFC) µg/mL			
			<i>Ca</i>	<i>Ck</i>	<i>Cp</i>	<i>Cn</i>
<i>Am</i>	B	Hx	-	125(500)	-	-
		EtOAc	-	-	125 (250)	62.5 (62.5)
		BuOH	125 (250)	125 (250)	62.5 (125)	31.2 (62.5)
<i>Hf</i>	V	BuOH	-	-	-	125 (-)
		B	Hx	-	-	-
	BuOH	-	-	62.5 (250)	31.2 (31.2)	
	V	Hx	-	-	-	-
		BuOH	-	-	-	-
	Fluconazole	0.5	32	2	2	

*Am*, *Astichopus multifidus*; *Hf*, *Holothuria floridana*; *Ca*, *Candida albicans*; *Ck*, *Candida kerusei*; *Cp*, *Candida parapsilopsis*; *Cn*, *Cryptococcus neoformans*; MIC, Minimum Inhibitory Concentration; MFC, Minimum Fungicidal Concentration; Hx, hexane; EtOAc, ethyl acetate; BuOH, butanol; b Body; V viscera; -, >500 µg/mL.

**Table 3. Antifungal activity of the extracts of holothurians on molds.**

Species	Animal Part	Extract	Molds MIC (MFC) µg/mL		
			<i>Af</i>	<i>An</i>	<i>Tr</i>
<i>Am</i>	B	Hx	-	-	-
		EtOAc	31.2 (62.5)	125 (250)	31.2 (62.5)
		BuOH	31.2 (125)	62.5 (250)	15.6 (31.2)
<i>Hf</i>	V	BuOH	62.5 (125)	-	-
		B	Hx	62.5 (125)	125 (250)
	BuOH	31.2 (125)	15.6 (500)	62.5 (62.5)	
	V	Hx	125 (500)	125(500)	-
		BuOH	125 (500)	-	-
	Itraconazole	0.5	0.25	0.06	

*Am*, *Astichopus multifidus*; *Hf*, *Holothuria floridana*; *Af*, *Aspergillus fumigatus*; *An*, *Aspergillus niger*; *Tr*, *Trichophyton rubrum*; MIC, Minimum Inhibitory Concentration; MFC, Minimum Fungicidal Concentration; hx, hexane; EtOAc, ethyl acetate; BuOH, butanol; B body; V viscera; -, >500 µg/mL.

As published (35), a crude extract can be considered a candidate for further bio-guided studies if it shows activity at  $\leq 100$  µg/mL, the present study considers extracts with MIC values  $\leq 125$  µg/mL as active. Being that forty-four percent of the investigated extracts showed some degree of antifungal activity. The butanolic extract of *A. multifidus* body walls was the only extract active against yeasts and molds, while the same type of extract obtained from *H. floridana*, was active mainly against mold strains. It is important remark that both extracts exhibited the most significant antifungal activity, MICs of 15.6 µg/mL against *T. rubrum* and *A. niger*.

The ethyl acetate and butanolic extracts from body walls of *H. floridana* and *A. multifidus*, respectively, showed the same pattern of antifungal activity. These extracts were active against *Candida parapsilosis*, *Cryptococcus neoformans*, *Aspergillus fumigatus*, *A. niger*, and *Trichophyton rubrum*. Besides, the values of the obtained MICs were similar. The extract obtained with butanol from the viscera of *A. multifidus*, together with the lipophilic extract of viscera and body walls of *H. floridana* showed activity mainly against molds *A. fumigatus* and *A. niger*, their MICs ranging from 62.5 to 125 µg/mL.

The non-polar extract of *A. multifidus* body walls as well as the butanolic extract of viscera of *H. floridana* exhibited the narrowest spectra of antifungal activity and the highest values of MIC (125 µg/mL each one). The first-mentioned extract was active against *C. krusei*, while the second one inhibited the growth of *A. fumigatus*.

*A. fumigatus* was the most susceptible mold strain to the sea cucumber extracts, showing highest susceptibility to the extracts of *A. multifidus*. Regarding yeasts, *C. neoformans* was susceptible to 62% of the investigated holothurian extracts.

## DISCUSSION

The yield of extraction is influenced by factors such as the solvents used, the duration of extraction, temperature, and the chemical composition of the sample<sup>(36)</sup>. In this study, polar and non-polar solvents, as well as water, were used to obtain extracts for the determination of biological activity. The highest yields were found in the aqueous extracts and those obtained from the body, which is characteristic of the holothuroid species<sup>(37-39)</sup> furthermore, *Holothuria floridana* exhibited the most pronounced yields ( $\geq 0.75\%$ ) relative to the other holothuroid species.

Regarding antifungal activity, and based on the cutoff value established in this study, extracts from *Isostichopus badiionotus* did not exhibit significant activity against either the yeast or mold strains tested. This finding is consistent with previous studies on this sea cucumber<sup>(40)</sup>.

Although this study is the first to assess the antifungal activity of extracts from *Astichopus multifidus* and *H. floridana*, our results can be compared to those reported for other members of the family Holothuriidae by other researchers.

The present study evaluated the antifungal activity of sea cucumber extracts using a microdilution method. Comparable results from prior research were observed with extracts from *Actinopyga lecanora*, *Holothuria scabra* and *H. atra*. In these studies, whole-body extracts prepared with ethyl acetate, butanol, and methanol exhibited similar antifungal activity against *Candida albicans*, *C. tropicalis*, *Cryptococcus neoformans*, *Trichophyton mentagrophytes*, and *Aspergillus niger*, with minimum inhibitory concentrations (MICs) ranging from 33.5 to 500 µg/mL<sup>(41-42)</sup>. Likewise, similar antifungal activities were reported for a crude mixture of components obtained from *Neothyone gibbosa*, *Stichopus japonicus*, using ethanol, butanol and water, where MICs from 8 to 125 µg/mL were established against *C. albicans*, *C. tropicalis*, *C. neoformans*, *A. niger*, *A. fumigatus*, *T. mentagrophytes*, and *T. rubrum*<sup>(43-44)</sup>.

Extracts obtained from *H. atra*, *H. polii*, and *H. leucospilota* were also tested for their antifungal activity using the disk diffusion method. The MICs ranged from as low as 3 µg/mL to as high as 5000 µg/mL against *C. albicans*, *C. tropicalis*, *C. neoformans*, *A. niger*, *A. fumigatus*, *T. mentagrophytes*, and *T. rubrum*. This variability can be attributed to differences in the extraction methods used, which included both hot and cold conditions, as well as variations in the parts of the sea cucumber used for extract preparation<sup>(45-49)</sup>. Similarly, using the same method, extracts from other holothurians, such as *Stichopus hermanni*, *S. chloronotus*, and *S. (variegatus) horrens*, displayed a similar pattern of activity, with MICs ranging from 3 to 1000 µg/mL against *C. albicans*, *A. niger* and *A. fumigatus*<sup>(50-52)</sup>. Other methods for determination of activity have established the antifungal behavior, from *H. polii*, where a macrodilution method showed the effect of metanolic extract at 2500 µg/mL against *A. niger*<sup>(53)</sup>.

As established, although this study is the first to report on the antifungal activity of extracts obtained from *A. multifidus* and *H. floridana*, the observed results can be plausibly explained by the presence of metabolites isolated from these species and related members of their family. Notably, the triterpenoid glycosides astichoposide C and stichloroside B2 have been reported as being isolated from the body walls of these species<sup>(54-55)</sup>. Among these, only astichoposide C has been tested against fungal strains, showing MICs of 25, 12.5, and 6.25 µg/mL against *C. albicans*, *A. niger*, and *T. rubrum*, respectively<sup>(56)</sup>. These values are closely similar to those obtained in the present study, where the butanolic extract derived from the body walls of *A. multifidus* inhibited the growth of the same three fungal species at concentrations of 125, 62.5, and 15.6 µg/mL, respectively.

The activity of these same extracts against *C. neoformans* and *A. fumigatus* (MICs of 31.2 µg/mL) can also be attributed to the behavior of triterpene glycosides isolated from related species. For instance, stichloroside C, which exhibits strong antifungal activity, has been reported with MIC<sub>80</sub>s (Minimum Inhibitory Concentrations required to inhibit the growth of 80% of tested organisms) ranging from 1.4 to 22.3 µM against *C. albicans*, *C. neoformans*, *C. tropicalis*, *T. rubrum*, and *A. fumigatus*<sup>(26)</sup>.

Other compounds that may explain the results obtained from butanolic extract of the body walls of *A. multifidus*, which showed antifungal activity against two yeast strains and three mold species at concentrations ranging from 31.2 to 125 µg/mL, include variegatusides, a family of triterpene glycosides isolated from what was formerly known as *Variegates semper* and is now classified as *Stichopus horrens*. Additionally, marmoratoside A, another triterpene glycosides, has demonstrated antifungal activity with MIC<sub>80</sub>s ranging from 0.7 to 3.4 µg/mL against *C. albicans*, *Candida pseudotropicalis*, *Candida parapsilosis*, *T. rubrum*, and *A. fumigatus*<sup>(57-58)</sup>.

In contrast, it is important to note that both *C. albicans* and *Candida krusei* showed no susceptibility to the ethyl acetate extract obtained from the body walls of *A. multifidus*. This lack of activity may be attributed to either the absence of biologically active compounds or their presence in concentrations too low to exhibit detectable activity. Given the well-documented isolation of triterpene glycosides from sea cucumbers through butanol-based tissue extracts, it is clear that the antifungal efficacy observed in the ethyl acetate extract cannot be attributed to compounds within this chemical category.

The extracts derived from the viscera and body walls of *H. floridana* (Holothuriidae), a sea cucumber species distributed from the Gulf of México to the Western Caribbean<sup>(59)</sup>, exhibited reduced potency against a smaller range of fungal strains compared to the extracts from *A. multifidus*. Additionally, the antifungal spectrum of the butanolic extract from the body walls of *H. floridana* was notably narrower than that observed in *A. multifidus*. However, this activity can also be explained by previously reported metabolites isolated from this sea cucumber.

The triterpenoid glycosides holothurins A1, A2 (also known as echinoside A), B1 (referred to as echinoside B), and the holotoxins A, B, and C, isolated from the muscular tissues of *H. floridana*, have been reported to exhibit antifungal activity against *C. albicans*, *A. niger*, and *T. rubrum* with MICs ranging from 0.78 to 6.25 µg/mL<sup>(60-62)</sup>. However, other studies have reported a MIC value of 1.5 mg/mL for holothurin A1 against *C. albicans*<sup>(63)</sup>. Additionally, polysaccharides such as fucan sulfate and fucoidan, which have demonstrated biological activity, have also been isolated from *H. floridana*. Although these compounds have not been extensively tested against fungal strains, they might contribute to the antifungal activity observed in this study<sup>(64)</sup>.

The coastal waters of the Yucatán Peninsula are home to various species of sea cucumbers that represent a promising source of antifungal compounds. Among the six species identified in the region, *A. multifidus* and *H. floridana* stand out for their potential medicinal value. These marine invertebrates, beyond their commercial appeal, harbor bioactive compounds that may offer novel antifungal properties. With increasing threats of overexploitation, it is critical to explore the biochemical diversity of sea cucumbers in Yucatán's waters, which could provide the basis for developing new antifungal medications

## CONCLUSIONS

The antifungal activity observed in the butanolic extracts, particularly those obtained from the body walls of *Astichopus multifidus* and *Holothuria floridana*, demonstrated minimum inhibitory concentrations (MICs)  $\leq 125$  µg/mL, which qualifies them as active extracts under the criteria established in this study. These results indicate the presence of bioactive compounds with potential antifungal properties. This is the first report identifying such activity in these two holothurian species from the Yucatecan coast. Future studies should focus on isolating and characterizing the specific compounds responsible for this activity, with special emphasis on triterpene glycosides, in order to explore their efficacy against a broader panel of fungal pathogens, including resistant strains.

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## ETHICAL CONSIDERATIONS

The study was conducted in accordance with national and international ethical standards for the use of marine resources. The collection of holothurian species was authorized by the Dirección General de Ordenamiento Pesquero y Acuícola of SAGARPA under permit number 01038.190210.0574.

## DECLARATION OF COMPETING INTEREST

The authors have declared no conflict of interest.

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