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Biotechnological Activities of *Holothuria papillifera* Mortensen, 1938 Inhabiting the Suez Gulf (Northern Red Sea), Egypt

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ABSTRACT

Marine organisms are vast sources of bioactive components, with holothorians, or sea cucumbers being one of these sources. Therefore, sea cucumber specimens were examined as a promising source of natural substances that can be used as antibacterial, antifungal, anti-inflammatory, antioxidant, antiviral, and antitumor agents. *Holothuria papillifera* was collected from Zaafarana, Ras Ghareb, and Altur stations and employed as a biological weapon against some pathogens. *H. papillifera* extracts showed high activity as antibacterial extracts against *Staph. aureus* and *Enterococcus faecalis* (Gram+ve), *Escherichia coli*, *Salmonella typhi*, and *Klebsiella pneumoniae* (Gram-ve). It also exhibited positive antifungal efficacy against *Candida albicans* and revealed moderate activity against liver cancer cells and a weak activity against breast cancer cells. Alternatively, *H. papillifera* crude extract has weak antiviral activity against CoxB4 and very weak antiviral efficacy against HAV (hepatitis A virus). Moreover, our results proved that *H. papillifera* extract had anti-inflammatory effects and exhibited antioxidant power. In conclusion, *H. papillifera* has broad antimicrobial activity against many pathogens, as well as antioxidant, antitumor, anti-inflammatory, among others. It is recommended to explain and characterize the composition of the bioactive compounds of *H. papillifera* to be included in the pharmaceutical industry

INTRODUCTION

Among echinoderms, there are organisms commonly known as sea cucumbers; they are marine animals that belong to the family Holothuroidea and encompass about 1200 extant species. Sea cucumbers (Echinodermata: Holothuroidea) live in a variety of marine environments, including soft, rock bottoms and coral reefs in shallow and deep waters in both temperate and tropical regions **(Elbahnasawy *et al.*, 2023; Hasaballah *et al.*, 2023)**.

From an ecological point of view, holothurians are considered a diverse group and key creatures in marine ecosystems due to their significant ecological importance **(Mona *et al.*, 2019)**. They greatly affect the physical and chemical processes of the soft bottom and coral reef ecosystems through their benthic bioturbation activity and excretion of

inorganic phosphorus and nitrogen, which increases the benthic biota productivity **(Mangion *et al*., 2004; Purcell *et al*., 2016)**.

Sea cucumbers have several benefits in more than one domain. For many centuries, they have been used as a food and have been a delectable cuisine and medicinal remedy for the Asians **(FAO, 2008)**.

As seafood, sea cucumbers and their food products are usually processed into a dried product known as namako in Japanese, trepang in Indonesian, gamat in Malaysian, and balatan in the Philippines **(Omran & Allam, 2012; El-Naggar *et al.*, 2022a)**.

Based on factors such as abundance, species, appearance, thickness of the body wall, color, odor, and primary market demand, sea cucumbers can be classified as having high, medium, or low commercial value economically **(Lo, 2005)**.

Moreover, holothuroids have a significant nutritional value with a high level of protein and low levels of fat. They have similar amino acid contents, while fatty acid profiles differ among species (**Wen *et al.*, 2010)**. Moreover, it is believed to have aphrodisiac properties similar to those of oysters **(Singh, 1980)** as a rich source of bioactive components such as chondroitin sulphate and mucopolysaccharides, and holothurin or saponins (triterpene glycosides) **(Vieira *et al.*, 1991)**. These substances resemble the active ingredients of ganoderma, ginseng, and other well-known tonic plants in terms of structure **(Chen, 2003)**. Functional food that contains bioactive substances is a significant source for the management, prevention, and treatment of diseases and pathogens **(Omran, 2006)**.

As a well-known traditional medicine, the sea cucumbers are able to undergo tissue regeneration after being cut up and returned to the sea. In Malaysia, it is widely used as a traditional remedy for rheumatism, sinus, hypertension, burns, asthma, and cuts. Furthermore, they are utilized for wound healing, especially after clinical surgery and caesarian operations **(Fredalina *et al*., 1999; Qi *et al*., 2007)**. The most important and valuable portion among the profits of sea cucumbers on which we need to focus is their pharmacological usage. Sea cucumber extracts have shown antitumor, antibiotic, antifungal, anti-inflammatory, anticancer, anti-arthritic, and antioxidant activities **(Idid *et al*., 2001; Qi *et al*., 2007)**. Furthermore, it has been explained that sea cucumbers are used in the treatment of high blood pressure, gastric ulcers, and asthma. Moreover, they have an antiparasitic and broad-spectrum antimicrobial effects **(Mona *et al*., 2012; Omran & Allam, 2012; Cui *et al*., 2020)**.

Despite all the above information, especially the last pharmaceutical one, holothurins are an unusual food in Egypt, and people are estranged from their eating. Therefore, the current study aimed to identify the bioactive value of some local Egyptian sea cucumbers inhabiting the Gulf of Suez by making extracts from them and testing those extracts as antimicrobial, anti-inflammatory, antioxidant, and antitumor in order to evaluate their quality and their remedial importance.

MATERIALS AND METHODS

1. Sampling and identification of sea cucumber

During winter 2021, six specimens of sea cucumber were collected along the Red Sea from the Suez Gulf at Zaafarana and Ras Ghareb stations, and they were identified as *Holothuria papillifera* **(Mortensen, 1938)**.

2. Preparation of sea cucumber crude extracts

Sea cucumber specimens were properly cleaned with sea water after collection. One hundred grams of cucumber specimens were macerated in three hundred milliliters of 70% aqueous ethanol in the lab. Whatman 542 filter paper was used for filtering after it had been soaked for ten days while being shaken by the paddle. To get soluble extracts, the solvent was evaporated using a rotary evaporator **(Ballantine *et al*., 1987)**.

3. Antimicrobial assay

3.1. Microbial indicator strains

Eight species of pathogenic microorganisms; two fungi, *Candida albicans* (ATCC 10221), and *Mucor circinelloid* (AUMMC 11656), three Gram-positive bacteria, *Bacillus subtilis* (ATCC 6633), *Enterococcus faecalis* (ATCC 10541), and *Staph. aureus* (ATCC 6538), in addition to the three Gram-negative bacteria, *Escherichia coli* (ATCC 8739), *Salmonella typhimurium* (ATCC 6539), and *Klebsiella pneumoniae* (ATCC 13883), were obtained from the Regional Center of Mycology and Biotechnology. All the bacterial pathogens were grown on nutrient agar and maintained at 4°C.

3.2. Media and bacterial cultures

Atlas (1997) identified the following components of nutritional broth (gl-1): 2 yeast extracts, 1 beef extract, 5 peptone, and 5 sodium chloride. Nutrient agar was made by adding agar (15–20). Nutrient agar slants were used to maintain every harmful bacteria strain. The preparation of the bacterial inoculate involved inoculating 100 milliliters of nutrient broth medium, followed by a 24-hour shaken (250rpm) incubation at 30˚C until the late logarithmic phase of growth (A550 = 1). Nutrient agar plates were used to test the antibacterial properties.

3.3. Antimicrobial activity

The agar-well diffusion method is a popular technique for assessing a plant's or microbial extract's antibacterial activity. The process of inoculating the agar plate surface involves covering the entire surface with a volume of the microbial inoculum, much like in the disk-diffusion approach. The antimicrobial agent or extract solution at the appropriate concentration is then added to the well in a volume of 20– 100mL after an aseptic hole with a diameter of 6– 8mm is punched using a sterile cork borer or a tip. After that, agar plates are incubated in the appropriate environment for the test microorganism. The microbial strain under investigation is prevented from growing by the antimicrobial agent, which diffuses throughout the agar medium **(Magaldia *et al*., 2004)**. A quantitative result (zones of inhibition in millimeters) and a qualitative interpretive categorization (e.g., susceptible or resistant) are obtained from the well diffusion test. A well-developed diffusion method for assessing mold susceptibility has been proposed by the CLSI. The fact that results can be achieved within 16 to 48 hours of

incubation, a shorter incubation period than using the M38, is a key benefit of this disk diffusion technology **(Ingroff *et al.*, 2011)**. Mueller-Hinton agar without supplements, or a regular bacteriology laboratory Mueller-Hinton agar plates (pH, 7.2–7.4 after gelling) are used in the CLSI disk method because they allow mold to develop optimally for either 24 or 48 hours. Any fresh batch of Mueller-Hinton medium should be suitably tested by adhering to CLSI guidelines, as some batches might not be able to support the development of specific organisms sufficiently. According to **Saintigny *et al*. (2011)**, zones found using the well (disk) diffusion test are typically larger than anticipated and may even be above the allowable control limits. The well method uses an inoculum suspension that has been adjusted in accordance with the guidelines for the broth dilution standard method. The agar plates must be infected within 15 minutes of the suspension being adjusted. Three equal streaks were applied to the whole dried agar surface. After letting the agar surface to dry for no more than 15 minutes, a volume (20–100uL) of the antimicrobial agent or extract solution at the necessary concentration was added to the well through an aseptic hole punched with a sterile cork borer or tip. Plates were incubated for 15 minutes after the extract solution was placed and disposed. The inhibition zone diameters (in mm) surrounding the wells after incubation times of 16 to 24 hours (Mucoraceae), 24 hours (*A. fumigatus, A. flavus,* and *A. niger*), or 48 hours (other species) were measured to the nearest full millimeter at the point at which there was a noticeable reduction in growth. The plates should be re-incubated and read later if there is not enough growth at the suggested dates **(Ingroff *et al*., 2011)**.

The antimicrobial activity produced by the ethanolic crude extract of *Holothuria papillifera* was screened against several human and fish pathogens; they are eight species of pathogenic microorganisms: two fungi, *Candida albicans* (ATCC 10221), and *Mucor circinelloid* (AUMMC 11656), three Gram-positive bacteria, *Bacillus subtilis* (ATCC 6633), *Enterococcus faecalis* (ATCC 10541), and *Staph. aureus* (ATCC 6538) in addition to the three Gram-negative bacteria, *Escherichia coli* (ATCC 8739), *Salmonella typhimurium* (ATCC 6539), and *Klebsiella pneumoniae* (ATCC 13883).

4. Antitumor activity

After 72 hours of incubation, the cytotoxicity of extracts at different concentrations (31.25–1000 µg/ml) was evaluated for MCF-7 and HepG2 using the 3-(4, 5-dimethylthiazol2-yl)–2, 5-diphenyl tetrazolium bromide (MTT), albeit with some slight modifications. A spectrophotometer set to 520nm was used to read the assay plates. The extracted concentration needed to kill 50% of the cell population (LC50) was calculated by plotting the produced data on a dose-response curve (**Saintigny *et al*., 2011**).

Cell viability (%) = mean OD/control OD × 100 mean

Abs control, where: Abs absorbance at 490 nm

5. Antiviral activity

Vero cells, which come from the kidney of a typical African green monkey, were acquired from the continuous cell line of the American Type Culture Collection (ATCC), which was founded by **Yamamura and Kawakita (1963)**. The vero cell line was created to isolate and multiply hepatitis A and other enteroviruses. The vero cells used in this

investigation were cultivated and cultured in Dulbecco's modified Eagle's medium (DMEM) with Hanks salt base, 10% fetal calf serum, and 50µg/ ml gentamycin antibiotic solution. Hepatitis A virus H-10 strain, which grows quickly and causes cytopathic effect (CPE) in vero cell cultures in three days, was used in this investigation.

The cytotoxicity of sea cucumber crude extracts was assessed by comparing the morphological changes of vero cells treated with various extracts to those of an untreated control group. The MTT test was used to measure the anti-proliferative activity. The dehydrogenase in a viable cell served as the foundation for the MTT kit, which used a colorimetric technique to lower the coloring reagent to assess cell viability. In conditions supplemented with 10% inactivated fetal bovine serum, vero cells were cultivated as monolayers. Before being treated with the extracts to allow cell attachment to the plate, the monolayers of 10,000 cells were plated (104 cells/well) in 96-well tissue culture plates and incubated for 24 hours at 37°C in a humidified incubator with 5% CO_2. Three wells were left empty as blanks. The cell monolayer was treated with varying quantities of sea cucumber crude extracts (31.25, 62.5, 125, 250, 500, and 1000µg/ l). Every concentration was conducted in triplicate, with the exception of three wells that served as a negative control and contained no extract. The plates were placed in CO_2 incubator with 5% CO_2 at 37°C for 48 hours. Before finishing the experiment, the cells were examined utilizing an inverted microscope for 48 hours to detect how the morphology of the treated and control cells differed at various drug concentrations. After decanting the cell culture medium containing various concentrations of the tested extracts and dead cells, viable adherent cells were left in the tissue culture plate. PBS was used twice to clean the plate containing live cells. The MTT reagent was applied in 50 microliters to each well, except the blank and negative control wells.

Following the addition of the MTT reagent, the plates were left in the dark for four hours to allow the dehydrogenase activity in the mitochondria of living cells to reduce the MTT to create azan, or purple needle color. Each well received 100 microliters of DMSO in order to dissolve the purple formazan crystals. The absorbance was determined using the SynergyTM 2 Multi-Mode Microplate Reader (BioTek Inc., Vermont, and USA) at 620nm (A620) and 570nm (A570). The maximum nontoxic concentration (MNTC) was determined, and the percentage of cell survival was calculated using the following equation:

Cell Survival (%)=(Absorbance of Control Cells/Absorbance of Treated Cells)×100

6. Antioxidant assay

The DPPH radical-scavenging capacity of the extract was assessed using the methodology described by **Yen and Duh (1994)**. The antioxidant capacity of various plant leaf extracts was assessed using 2, 2-diphenyl-1-picrylhydrazyl (DPPH). Briefly, a 0.1mM DPPH solution in ethanol was made. Three milliliters of various extracts in ethanol at varying concentrations (3.9, 7.8, 15.62, 31.25, 62.5, 125, 250, 500, and 1000µg/ ml) were mixed with one milliliter of this solution. Only extracts with the potential to dissolve in ethanol were employed here, and the dilution procedure was used to create the various concentrations of the extracts. After giving the mixture a good shake

and letting it remain at room temperature for thirty minutes, the absorbance at 517nm was measured using a spectrophotometer (UV-VIS, Milton Roy). The experiment was conducted in triplicate, using ascorbic acid as the reference standard component. Using the log dosage inhibition curve, the sample's IC 50 value—the concentration of the sample needed to inhibit 50% of the DPPH free radical—was determined. Increased free radical activity was shown by the reaction mixture's lower absorbance.

The percent DPPH scavenging effect was calculated by using the following equation:

DPPH scavenging effect (%) or percent inhibition = A0 - A 1 / A0 × 100

Where, A0 is the absorbance of the control reaction, and A1 atands for the absorbance in the presence of the test or standard sample.

7. Anti-inflammatory assay

After being drawn from healthy volunteers in 3ml heparinized tubes, the blood was centrifuged for 10 minutes at 3000rpm. To dissolve the red blood pellets, the same volume of normal saline was utilized as that of the supernatant. After measuring the volume of the dissolved red blood pellets, a 40% v/v suspension was made using an isotonic buffer solution (10mM sodium phosphate buffer, pH 7.4). In one liter of distilled water, the buffer solution included 0.2g of NaH_2PO_4, 1.15g of Na_2HPO_4, and 9g of NaCl. As such, the red blood cells previously reconstituted (resuspended supernatant) were utilized.

Samples of the extract used in this experiment were dissolved in a hypotonic solution of distilled water. Duplicate pairs (per dose) of centrifuge tubes were filled with the hypotonic solution (5ml) containing graded doses of the extracts (100, 200, 400, 600, 800, and 1000µg/ ml). Additionally, duplicate pairs (per dose) of the centrifuge tubes were filled with isotonic solution (5ml) containing graded doses of the extracts (100–1000µg/ ml). Five milliliters of each vehicle (distilled water) and indomethacin at 200µg/ ml were found in the control tubes. Each tube was filled with 0.1ml of erythrocyte suspension, which was well mixed. The solutions were centrifuged at 1300g for three minutes after being incubated for one hour at room temperature (37°C). The hemoglobin concentration of the supernatant was measured at 540nm using a spectronic (Milton Roy) spectrophotometer to determine the absorbance (OD). By assuming that 100% of the heamolysis was created in the presence of distilled water, the percentage of heamolysis was computed. The extract's percentage of hemolysis inhibition was computed as follows:

% Inhibition of Hemolysis = 1- (OD2-OD1)/ (OD3-OD1)*100

Where, OD1 = absorbance of test sample in isotonic solution

OD2 = absorbance of the test sample in a hypotonic solution

OD3 = absorbance of the control sample in a hypotonic solution

RESULTS

1. Sea cucumber identification

Holothuria papillifera **Mortensen, 1938**,

- ✓ **Habitat:** *H. papillifera* occupies the soft substrate, sand, and seagrass areas and is never found at the reef slope or reef crest.
- ✓ **Status:** Rare species.
- ✓ **Distribution:** *H. papillifera* is endemic to the Red Sea.
- ✓ **Remarks:** *H. papillifera* has a very limit distribution in the Red Sea and recorded only from the Marine Biology Station, Hurgada, at the northern part of the Red Sea by **Hasan (2001)**, from the Nuwbia area at the Gulf of Aqaba by **Hasan and Hasan (2004)**, and from the El-Khokhah area (the most southern part of the Red Sea) by **Hellal (2010)**. The presence of *H. papillifera* at Zaafarana, and Ras Ghareb stations from the Suez Gulf indicates that there is a northern extension in their distribution. Hence, it is considered to be a new record in such an area for the first time.
- ✓ **Description:** Body club-shaped, narrow anteriorly, much larger posteriorly. The color of living specimen is uniform grayish brown. Color in alcohol: brown dorsally, beige to light brown ventrally. Mouth ventral, anus terminal. Twenty short, yellowish tentacles surrounded by a circle of papillae. Dorsally, there are numerous large conical papillae without alignment. Ventrally, tube feet are densely crowded in the ambulacral as well as in the interambulacral areas.

2. Antimicrobial activity of *Holothuria papillifera* extract

The data in Table (1) reveal that ethanolic crude extract showed a positive activity against all mentioned microorganisms, except for *Mucor circinelloid* and *Bacillus subtilis*, which showed a negative activity.

Table 1. Antimicrobial activity of *Holothuria papillifera* extract

Pathogenic microorganism	Inhibition zone (mm)	
	Control	*Holothuria papillifera*
Bacillus Subtilis (ATCC 6633)	32±0.2	31±0.1
Enterococcus faecalis (ATCC 10541)	25±0.1	28±0.1
Staph.aureus (ATCC 6538)	24±0.1	33±0.2
Escherichia coli (ATCC 8739)	22±0.2	24±0.18
K. pneumoniae (ATCC13883)	19±0.2	25±0.2
Salmonella typhi (ATCC 6539	17±0.15	23±0.1
Candida albicans (ATCC 10221)	27±0.3	31±0.2
Mucor circinelloid (AUMMC 11656)	21±0.1	17±0.1

3. Antitumor activity of *Holothuria papillifera* extract

The data presented in Fig. (1a) demonstrate that *Holothuria papillifera*'s ethanolic extract exhibited an anticancer activity, with an LC50 value of 109.92± 4.28µg/ m, capable of eliminating half of the HepG2 tumor cells. Conversely, the same *H. papillifera extract* exhibited an anticancer activity on the Mcf-7 cell line, with an LC50 value of 173.5 ± 2.9µg/ ml that was able to kill almost half of the tumor cells (Fig. 1b). Two

extracts were shown to have a highly potent anti-breast cancer cell activity in this investigation.

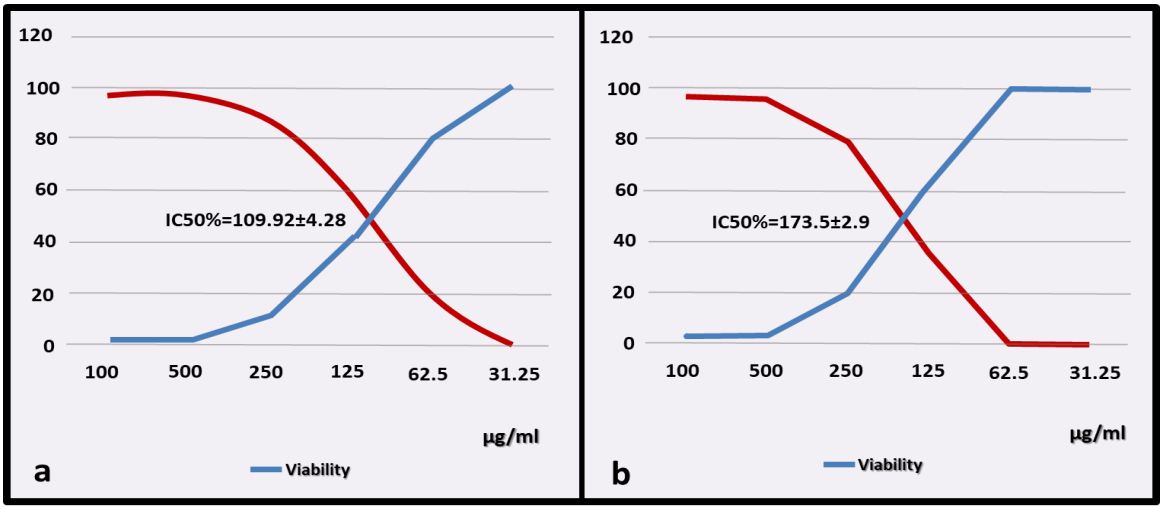

Fig. 1. Effect of *Holothuria papillifera* ethanolic extract on a) HepG2 and b) Mcf-7 cells

4. Antiviral activity of *Holothuria papillifera* extract

With the exception of the final two dilutions, the *Holothuria papillifera* extract was cytotoxic to vero cells; this experiment revealed that its minimum inhibitory concentration (MIC) is 125µg/ ml. The extract's impact on vero cells is amplified during the incubation phase. Finally, using the MTT assay for more research, MNTC was found to be 62.5µg/ mL. The maximum non-toxic concentration of *H. papillifera* extract was 62.5µg/ ml (Table 2 & Fig. 2). The extract's potential against CoxB4 was then assessed, and upon implementing the method used, CoxB4 was injected into vero cells, causing toxicity for 53.56 of them, or 100% of its actual virulent power. The toxicity of the virus to vero cells was reduced to 51.13 by applying *H. papillifera* crude extract, with reflection. Conversely, data in Table (3) assess that the extract of *H. papillifera* had a maximum non-toxic concentration of 62.5µg/ ml. These extracts were then investigated to assess their potential against HAV (hepatitis A virus). Using the applied method, HAV was injected into vero cells, causing toxicity in 48 of them, which reflects 100% of its actual virulent power. The toxicity of the virus to vero cells was reduced to 47.9 by applying *H. papillifera* crude extract, which accounted for 99.79% of its true power.

Table 2. Antiviral effect of the crude extract of *Holothuria papillifera* on CoxB4 during this study

Test	Dilution extract mg/ml	Viability	toxicity	Viral activity %	Anti- viral effect %
Control vero		100	0		
CoxB4		46.44	53.56	100	0
Holothuria papillifera	62.5	48.87	51.13	95.48	4.52

Table 3. Antiviral effect of the crude extract of *Holothuria papillifera* on HAV during this study

Test	Dilution extract mg/ml	viability	toxicity	Viral activity %	Anti- viral effect %
Control vero		100	0		
HAV		51.99	48.01	100	0
Holothuria papillifera	62.5	52.09	47.91	99.79	0.21

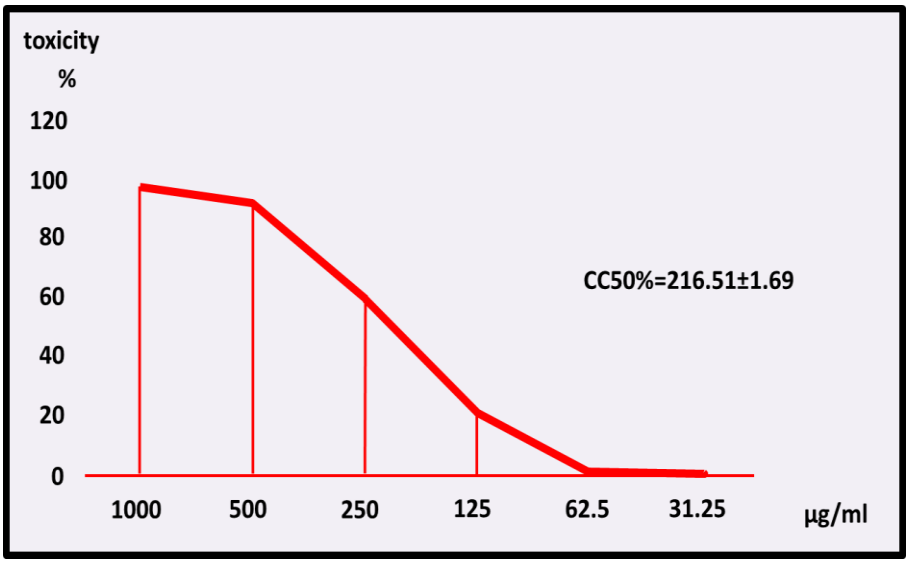

Fig. 2. Effect of *Holothuria papillifera* ethanolic extract on vero cells at different concentrations

5. Antioxidant activity of *Holothuria papillifera* crude extract

As demonstrated by the data in Table (4) and Fig. (3), the antioxidant activity of *Holothuria papillifera* extract was 1.89µg/ ml as the IC50, corresponding to the ascorbic acid standard. In a similar vein, the extracts of *Holothuria papillifera* determined an antioxidant activity with a value of 88.38µg/ ml as IC50, indicating that ascorbic acid's antioxidant power is greater than that of this sample.

Table 4. Antioxidant activity of the crude extract of *Holothuria papillifera*

Extract	Concentration (µg/ml)										
	0	**1.95**	**3.9**	**7.81**	**15.62**	**31.25**	**62.5**	**125**	**250**	**500**	**1000**
Ascorbic acid	0.0	46.6	54.3	62.3	69.5	75.8	82.2	88.5	92.4	94.4	98.2
Holothuria papillifera	0.0	13.9	20.2	25.8	32.5	39.3	46.9	53.3	60.0	66.8	74.1

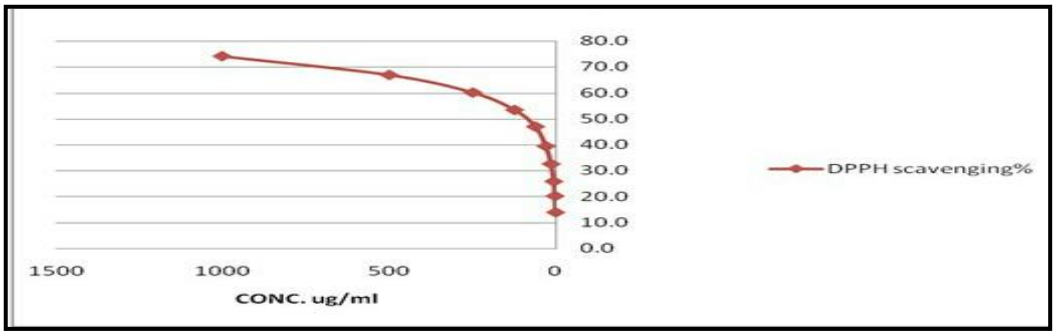

Fig. 3. DPPH scavenging % of *Holothuria papillifera* crude extract

6. Anti-inflammatory activity of *Holothuria papillifera* crude extract

The ethanolic crude extract of the investigated sea cucumber species was examined as an anti-inflammatory agent. The results in Table (5) reveal that the investigated extract had a hemolysis effect (with a value of 73µg/ ml as IC50) almost equal to the hemolysis effect of the standard indomethazone (53.3µg/ ml as IC50).

Table 5. Anti-inflammatory activity of *H. papillifera* crude extract during the study

Extract		Concentration (µg/ml)						
		control	100	200	400	600	800	1000
Hemolysis Inhibition %	Stander (Indo)	0.0	94.4	95.1	95.9	97.7	99.0	99.6
	Holothuria papillifera	0.0	63.8	77.7	82.1	88.9	94.8	97.0

DISCUSSION

Among marine organisms, the sea cucumber is an interesting natural source of novel functional materials with biological activities that could be used in the food and biomedicine industries **(Pangestuti & Arifin, 2018)**.

In Egypt, the small-scale sea cucumber fisheries began operating in the 1990s. The sea cucumbers were harvested and processed by fishermen, and the finished goods were sold to exporters for the Singaporean and Hong Kong SAR (China) markets **(Lawrence *et al.*, 2009)**. Notably, Egypt is now one of the major exporters of the sea cucumbers as a result, particularly in light of the depletion observed in other regions **(Lovatelli & Conand, 2004; Bruckner, 2006)**.

The Red Sea has a diverse sea cucumber population with great potential for commercial, aquaculture activities, fisheries, nutritional, and bioactive value. One of the common sea cucumber species inhabiting the Egyptian Red Sea is *H. papillifera* **(El-Naggar *et al.*, 2022b)**. However, detailed studies showing the bioactivity of sea cucumbers are still lacking, hence this paper aimed to contribute to this area of research.

One of the five genera in the family Holothuriidae that is widely recognized is Actinopyga. Currently, sixteen species in this tiny genus are recognized as legitimate **(Samyn *et al.*, 2006)**.

Holothuria papillifera Mortensen, 1938, is commonly found in the Indo-Pacific region, where it usually resides on coral flats with hard substrates. This species is

virtually exclusively found in high-energy reef crest zones, which sets it apart from most other tropical aspidochirotes **(Conand, 1997)**. These environments are vulnerable to powerful waves and currents and are between one and three meters deep **(Hopper *et al*., 1998)**. These holothurians feed mostly on plant debris-containing epifaunal algal films as well as the brown and blue-green algae that are typical of the hard surfaces they live on **(Ramofafia *et al*., 1997)**. Due to *A. mauritiana*'s shallow, sub-littoral, and intertidal environment as well as its comparatively high commercial value, overexploitation has occurred in several nations. It plays a significant role in the Indo-Pacific region's fisheries **(Jane *et al*., 2004)**.

Wen *et al*. (2010) showed that *A. mauritianars* is one of the sea cucumbers that has more nutrients than other types of the sea cucumber. *A. mauritianars* primarily consists of protein (63.30%± 0.43%), with a negligible amount of lipids (1.40%± 0.02%). *H. papillifera* has a high protein content that can be hydrolyzed to produce bioactive peptides. **Omran *et al*. (2020)** found that the examined sea cucumber, *A. mauritianars*, has a low fat level and high protein content. The sea cucumbers' beneficial substances also explain why they are effective in treating inflammatory illnesses and tissue regeneration. According to a different study, *H. papillifera* contains antioxidant properties and can protect against various forms of cancer **(Krupodorova *et al*., 2012)**.

The results obtained in the present study showed that the ethanolic extract of *H. papillifera* revealed positive antibacterial activity against *Enterococcus faecalis, Staph. aureus, Escherichia coli, K. pneumonia, Salmonella typhi, Candida albicans,* and weak antibacterial activity against *Bacillus subtilis* and *Mucor circinelloid*. These findings are consistent with those of **Shimada (1969)**, who reported that *Holothuria papillifera*'s holothurin extract has antifungal properties against a range of infections, including those affecting vegetables. Furthermore, **Murray *et al*. (2001)** discovered that *Psolus patagonium* extract possesses antifungal properties against *Cladosporium cucumerinum*, a pathogenic fungus. Furthermore, *H. atra*, *Stichopus chloronotus*, and *Stichopus variegatus* methanol extracts have antifungal properties against Trichophyton mentagrophytes and *Candida glabrata*, according to **Kaswandi *et al*. (1999)**. Furthermore, 11 species of the sea cucumbers obtained from the Egyptian Red Sea coast and their methylene chloride/methanol extract were reported to have all-natural antifungal properties against *Candida albicans*, as reported in the study of **Lawrence *et al*. (2009)**. Additionally, **Elbandy *et al*. (2011)** postulated that the majority of the sea cucumbers *Bohadschia cousteau*'s isolated chemicals exhibit a strong antifungal activity against *Candida albicans*. In order to achieve the desired outcome, **Hua *et al*. (2009)** isolated and refined novel active triterpene glycosides from *Holothuria scabra* sea cucumbers, which exhibited antifungal properties against seven strains: *Candida pseudotropicalis, C. albicans, Cryptococcus neoformans, Trichophyton rubrum, Fonsecaea compacta, Aspergillus fumigatus*, and *Microsporum gypseum*. As positive controls, itraconazole, FCZ, and terbinafine were utilized.

Reviewing the findings of the extract's antibacterial activity, it was obvious that *H. papillifera* extract exhibited an antibacterial activity against every pathogenic bacterium

that was studied. While **Ridzwan *et al*. (1995)** discovered that phosphate-buffered saline extracts of both *H. atra* and *H. papillifera* prevented the development of Gram-positive and Gram-negative bacteria, few other investigations support the current findings. Furthermore, the solid-phase extract of the body wall of *Cucumaria frondosa* has been shown to have antibacterial action against Gram-positive bacteria by **Haug *et al*. (2009)**. Furthermore, *Stichopus badionotus* methanolic extract demonstrated an antibacterial activity against *S. aureus* **(Mariana *et al*., 2009)**. **Shimada (1969)**, on the other hand, discovered that the *S. japonicus* extract is inert against both Gram-positive and Gram-negative bacteria. Furthermore, lipid and methanol extracts of *H. atra*, *H. scarba*, and *H. papillifera* were reported to have no antibacterial action, as reported in the work of **Ridzwan *et al*. (1995)**. Similarly, **Omran (2006)** argued that there was no antibacterial action of the tegument ethanol extracts of *Holothuria leucospilota*, *H. polii*, *Bohadschia vitiensis*, and *Holothuria papillifera* against Gram-positive *B. subtilis* and Gram-negative *E. coli*. Furthermore, the methylene chloride/methanol extract of 11 species of the sea cucumbers obtained from the Red Sea coast of Egypt, according to **Lawrence *et al*. (2009)**, shows no antibacterial properties.

These inconsistent results gave the impression that the extraction technique could alter the extract's activity, which could account for the studied extract's higher efficacy. In addition, the results showed that all of the tissue isolates were Gram-negative bacteria, which is consistent with the extract's antibacterial efficacy against two Gram-negative bacterial strains (*A. hydrophila* and *S. choleraesuis* ATCC 14028). It is important to note that bacterial cell secretions, rather than the sea cucumber itself, may be responsible for the antibacterial activity. By filtering the antibacterial fluid of *H. atra* using a 45-µl syringe filter and comparing its efficacy with that of the bacteria extracted from the cucumber-isolated bacteria, **Farouk *et al*. (2007)** demonstrated the validity of this opinion. Interestingly, they discovered that the coelomic fluid exhibited no activity, indicating that the antibacterial activity originates from the secretions of bacterial cells rather than the sea cucumber itself.

The ethanolic extract of *H. papillifera* killed half the number of tumor cells in HepG2. This is considered an evidence that *H. papillifera's* is an antitumor agent with a value of 109.92± 4.28µg/ m as LC50. In this context, the same extract of *H. papillifera* displayed an antitumor effect on the Mcf-7 cell line, with a value of 173.5± 2.9µg/ ml as LC50, which was able to kill about half the number of tumor cells in Mcf-7, indicating that *H. papillifera* extract has a weak activity against breast cancer cells. Additionally, for the impact of *H. papillifera* extract on the liver cancer cells, the experiment showed a moderate activity.

The extracts of *H. polii* and *A. mauritiana* showed a somewhat negative effect on the viability of all investigated cells, as validated by **Kandeil and Atlam (2023)**. The methanolic fractions n-Hexane and ethyl acetate were the primary subjects of earlier research on the cytotoxicity of the Holothurian sea cucumber extracts **(Mashjoor & Yousefzadi, 2019)**. On the other hand, the current findings evidenced the excellent efficacy of ethanolic extracts made from the cucumber's body wall.

HePG2 and MCF-7 cell lines were shown to be significantly inhibited in their ability to proliferate by the Cuvierian tubules of *A. mauritiana* and the body wall extracts of *H. leucospilota* and *B. marmorata* **(Eissa *et al.*, 2021)**. Time-dependent, strong cytotoxic effects on lung cancer cells (the A549 cell line) are exhibited by saponins derived from *H. leucospilota* **(Soltani *et al.*, 2014)**. The cytotoxic effects lead to apoptosis, decrease metabolism, and impede cell development **(McLaughlin, 2008)**. With inhibitory doses ranging from 9.6 to 14.3µg/ ml, the ethanol extract of *H. atra* demonstrated a cytotoxic action against four cell lines (T47D, MCF7, WiDr, and HeLa) **(Nursid *et al.*, 2019)**. They concluded that after being treated with ethanol extract, the T47D cell experienced apoptosis, as seen by the results of the flow cytometry analysis. Significant bioproducts found in the sea cucumbers' bodies, such as phenols and triterpene glycosides, may be responsible for the bioactivity of sea cucumber extracts.

During the current experiment, the crude extract of *H. papillifera* has shown a slight antiviral activity against CoxB4. On the other hand, the extracts' highest non-toxic concentrations of *H. papillifera* was 62.5µg/ ml. They were then tested for their ability to neutralize the hepatitis A virus (HAV). Using the technique used, 48 vero cells were injected with HAV, resulting in toxicity, which is equivalent to 100% of the virus's true pathogenic capacity. The toxicity of the virus to vero cells was reduced to 47.9 by applying *N. magnifica* crude extract, which accounted for 99.79% of its true potency. This indicates that *Holothuria papillifera* crude extract has an imperceptible antiviral efficacy against HAV (hepatitis A virus). The capacity of *H. papillifera* extract to inhibit inflammatory illnesses was remarkable throughout research on anti-inflammatory effects. The antioxidant activity of *H. papillifera* is larger than the ascorbic acid LC50, indicating that the ascorbic acid standard has a higher antioxidant potency than this sample, with an LC50 of 88.38µg/ ml.

Numerous investigations have been conducted to examine the advantages of the sea cucumbers. According to these studies, the sea cucumbers have anti-oxidant properties, which will lessen the harm done to the body's organs. However, the sea cucumber also offers additional health advantages, such as antimicrobial properties, the ability to stop the growth of tumor cells, the ability to repair wounds, and an antithrombotic impact that helps prevent stroke and heart disease **(Herliany *et al.*, 2016)**. It was demonstrated by the surf redfish group's greatest dose of improved collagen and fibroblast density. Numerous phytochemicals, including alkaloids, flavonoids, glycosides, saponins, and steroids, may be the cause of this improvement. These phytochemicals might have anti-diabetic and antioxidant properties. As a result, this surf redfish's antioxidant action creates a favorable milieu for wound healing and lowers blood glucose levels, which speeds up the healing process **(Lesmana *et al.*, 2022)**.

The ethanol extract from the surf redfish contains a variety of phytochemicals, particularly flavonoids and saponins. Antioxidant and anti-diabetic effects are attributed to flavonoids and saponins. By blocking the enzyme that aids in the production of reactive oxygen species (ROS), flavonoids lessen the production of ROS. Meanwhile, by giving free radicals an electron or a hydrogen atom, saponin lessens the effect of free

radicals. Additionally, saponin raises the concentration of the enzymes SOD and CAT, which neutralize free radicals.

To sum up, *H. papillifera* is a good substrate for a variety of bacteria that boost the production of a bioactive chemical with antioxidant, anticancer, anti-inflammatory, and broad antimicrobial activity against various pathogens. Lastly, it is advised to describe and set up the structure of *A. mauritiana's* active ingredient so that it can be used in pharmaceuticals.

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