Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 27(1): 217 – 233 (2023)
www.ejabf.journals.ekb.eg

A Comparative Study on Hemolytic and Cytotoxic Perspectives of Saponin from Some Egyptian Sea Cucumber Species

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ARTICLE INFO

Article History:
Received: Jan. 27, 2023
Accepted: Feb. 7, 2023
Online: Feb. 12, 2023

Keywords:
Sea cucumbers,
The brine shrimp,
Hemolytic activity,
Hela,
HEP2,
PC3,
HCT-116

ABSTRACT

There are intense efforts from pharmaceutical manufacturers to discover novel approaches to find new drug candidates for cancer treatment. Sea cucumber is widely consumed in traditional medicine. Its anticancer potential is associated with the content of a bioactive compound known as saponin. The goal of this paper was to compare the hemolytic activity and cytotoxicity of six sea cucumber extracts. The hemolytic assay confirmed the presence of saponins. Two methods examined the cytotoxicity of extracts: (i) lethality assay in brine shrimp (*Artemia salina*) (BSA) and (ii) tetrazolium dye-based colorimetric (MTT) assay in human cancer cell lines, such as colorectal carcinoma (HCT-116), epitheliod carcinoma (Hela), epidermoid carcinoma (HEP2) and human prostate cancer (PC3). Hemolytic activity was observed in *H. atra*, *H. edulis*, *B. marmorata* and *A. mauritiana*, while *H. polii* and *H. leucospilota* showed no hemolytic activity. The data in the BSA bioassay showed high and moderate cytotoxic activity. The more effective extracts were *H. atra* and *B. marmorata*. The average IC_{50} of *H. atra* extract against *Hela*, *HeP2*, HCT-116 and *PC3* cells were 9.14±0.8, 10.39±0.9, 11.43±1, 17.90±1.5µg/ ml, respectively. The data confirmed that the Egyptian sea cucumber species may serve as an exciting source for discovering novel anticancer drugs.

INTRODUCTION

Marine organisms produce secondary metabolites used as lead components in discovering drugs. Sea cucumbers belonging to the class Holothuroidea have a medicinal perspective for discovering drugs due to containing bioactive secondary metabolites **(Bordbar *et al.*, 2011)**. Sea cucumbers are marine invertebrates known as bêche-de-Mer, gamat or trepang used to make delicious soups **(Althunibat *et al.*, 2009)**. They are essential, particularly in several parts of Asia, such as food and traditional drug systems **(Taiyeb-Ali *et al.*, 2003; Bordbar *et al.*, 2011)**. Additionally, they have an exciting outline of distinctive bioactive fractions and a medical potential to display the origin of valuable antioxidant **(Althunibat *et al.*, 2009)**, anticancer and antiproliferative **(Soltani & Baharara, 2014)**, antitumor (**Wang *et al.*, 2014; Assawasuparerk *et al.*, 2016)** and antimicrobial

(Mashjoor & Yousefzadi, 2017; Eissa *et al*., 2021). The body wall and Cuvierian tubules of various sea-cucumber species contain saponin (triterpene glycosides), fatty acids, polysaccharides, glycosaminoglycans, chondroitin sulfates and phenolics **(Bordbar *et al*., 2011; Omran *et al*., 2020)**.

BSA is a minute crustacean used to assess the toxicity of a wide range of products. It is susceptible to toxins and shows the most significant sensitivity to test compounds at the early nauplii stages **(Lewis, 1995)**. A lethality bioassay of BSA was used in natural product discovery, indicating cytotoxicity and a wide range of pharmacological activities **(Meyer *et al*., 1982)**. The previous authors used BSA to determine the toxicity of plant extracts. BSA lethality bioassay was one of the most effective techniques for preliminary toxicity assessment **(Sam, 1983)**. Besides, this assay can be inducted for the toxicity of the cell line and antitumor activity of marine and terrestrial organisms **(Anderson, 1991; Mackeen *et al*., 2000)**.

Cytotoxic agents are the typical therapies inhibiting cancer cells by interfering with DNA replication and preventing cellular division **(Mazzaferro *et al*., 2013)**. These agents have a significant negative aspect of killing both cancer and healthy cells and have disorders associated with their effectiveness **(Bruce Pyenson, 2010)**. Thus, biomedical authors attempt to investigate novel natural products involving safe and effective anticancer agents. Thus, the application of sea cucumbers nowadays has attracted much attention among scientists and consumers because of their prospective health advances.

Our study was performed to explore the cytotoxic activities of the organic crude extraction from some Egyptian sea cucumber species (*A. mauritiana, B. marmorata*, *H. atra*, *H. edulis*, *H. leucospilota* and *H. polii*) using BSA lethality assay and *in vitro* cytotoxic methods against the HeP2 (epidermoid carcinoma), HCT-116 (colorectal carcinoma), Hela (epithelioid carcinoma) PC3 and (human prostate cancer) cell lines.

MATERIALS AND METHODS

Sample processing and extraction

A. mauritiana, *B. marmorata*, *H. atra*, *H. edulis*, *H. leucospilota* and *H. polii* belonging to Order Aspidochirotida and Family Holothuriidae were accumulated from different locations on the Egyptian coasts and identified previously by **Eissa *et al*. (2017)**. Samples were thoroughly rinsed, and their body fluids and interior organs were eliminated through an abdominal incision. Sample body walls were sliced into fine pieces and then extracted at a ratio of 3:1 v/w with 96% ethanol solvent. The mixture was soaked, then the homogenization was extracted twice for one day at room temperature. The extract of sea cucumbers was eliminated after squeezing and filtration through filter paper of 0.45μm. Afterward, the solvent was evaporated at low pressure using a rotary evaporator at 35°C. The obtained extracts were stored in the dark at 4°C until use. The purity of the crude extracts was tested on thin-layer chromatography plates. The spots

were examined under UV light and by adding sulfuric acid (10%) up to the formation of maroon-dark purple spots **(Eissa *et al*., 2021)**, indicating the presence of saponin. Ultra Performance Liquid Chromatography-Mass Spectrometry was previously used in the study of **Omran *et al*. (2020)** to identify the metabolic compounds of each extract.

Hemolytic activity assay

The hemolysis assessment was investigated using blood obtained from a woman with an O^+ blood group. The blood was accumulated in tubes containing an anticoagulant such as ethylenediaminetetraacetic acid (EDTA), then pelleted after centrifugation for 15 min at 800 ×*g* to eliminate the plasma. The pellet, which contains erythrocytes, was cleansed in phosphate-buffered saline (PBS) (pH=7) and suspended in PBS to a final concentration of 3% **(Bondoc *et al*., 2013)**. PBS and standard saponin were used as negative and positive controls, respectively. The hemolytic activity was performed within 96-well plates in triplicate. To begin with, 100µl of PBS was added to each well, followed by a volume of 100µl of each extract. Finally, 100µl of the blood suspension (3%) was added to each well. The absorbance of each sample was measured at 650nm after 4h of incubation at room temperature [19].

Cytotoxicity against BSA

Cytotoxicity of six Holothuroids (*A. mauritiana*, *B. marmorata*, *H. atra*, *H. leucospilota*, *H. edulis*and, *H. polii*) against biological organisms BSA was used to determine the most cytotoxic extracts according to **Mashjoor and Yousefzadi (2019)**. Cysts of BSA were hatched in artificial seawater (pH = 8.8 salinity = 35%) for 48h. The recently hatched nauplii larvae were collected with a pipette, and 20 nauplii were transferred to containers filled with artificial seawater (2 ml). Four dilutions (1000, 500, 250 and 125mg/ ml) of every extract were prepared by dissolving sea cucumber extracts in distilled water integrated into the vials containing artificial seawater and BSA. After 24h of exposure, the number of alive nauplii was calculated. The percentage of mortality at each dosage and control seawater was defined by the lack of regulated forward motion during 30 seconds of examination. The mortality percentage was calculated using the following formulae:

% Mortality = Sum of dead nauplii/Original number of alive nauplii × 100.

LC_{50} for each test dilution was determined after 24h via statistical probit analysis **(Finney, 1971)**.

Cytotoxicity assay against human cell lines

The cytotoxic activity of the tested extracts was examined in the Pharmcogency Laboratory, Faculty of Pharmacy, Mansoura University.

Cell line

Epitheliod carcinoma (Hela), epidermoid carcinoma (HEP2), colorectal carcinoma (HCT-116) and human prostate cancer (PC3) were the tested human cancer cell lines. The cell line was purchased from the American Type Culture Collection through the Holding Company for biological products and vaccines, Cairo, Egypt.

Chemical reagents

Tetrazolium dye (MTT), Roswell Park Memorial Institute medium (RPMI-1640 medium), dimethyl sulfoxide (DMSO) and doxorubicin (Dox) were obtained from Sigma co., St. Louis, USA. Dox was used as a standard anticancer drug for comparison. Fetal bovine serum was purchased from GIBCO, UK.

MTT assay

The cell lines were applied to assess the inhibitory effects of sea cucumbers on cell growth using the MTT assay according to previous studies **(Denizot & Lang, 1986; Eissa *et al*., 2021)**. This colorimetrical test is based on changing tetrazolium bromide color from yellow to a purple formazan derivative in viable cells by mitochondrial succinate dehydrogenase. The cell lines were cultured in RPMI-1640 medium with 10% fetal bovine serum. The cells were cultured in a 96-well plate at 1.0 × 104 cells/well density for 48h at 37°C under 5% Co_2. An addition of one hundred units/ml of antibiotics penicillin as well as 100µg/ ml of streptomycin was performed in a 5% Co_2 incubator at 37°C. Following incubation, the cells were exposed to the serial concentrations of each extract (100, 50, 25, 12.5, 6.25, 3.125 and 1.56µg/ ml) then incubated for 24h. After the treatment, 20µl of MTT (5 mg/ml) solution was added and incubated for 4h. A 100µl of DMSO was added to each well to dissolve the purple formazan. The colorimetric assay was measured at 570nm absorbance using a plate reader (EXL 800, USA). The relative cell viability percentage was determined (A570 of treated samples/A570 of the untreated sample × 100).

Statistical analysis

A statistical analysis system, SPSS Version 17, was used to calculate the mean values ± standard deviation (SD). All data were repeated thrice. One-way analysis of variance (ANOVA) was utilized with the post hoc test to evaluate hemolytic activity between the studied groups and compared groups. Means< 0.001 was considered significant.

RESULTS

1. Hemolytic activity of sea cucumber extracts

The hemolytic action of extracted saponin from *H. polii, H. atra*, *H. edulis*, *H. leucospilota, A. mauritiana, B. marmorata* and standard saponin was assessed using

human red blood cells (Fig. 1). It was only observed in *H. atra*, *H. edulis*, *A. mauritiana*, *B. marmorata* and standard saponin. Conversely, *H. polii* and *H. leucospilota* showed no hemolytic activity.

2. Cytotoxicity against brine shrimp

Brine shrimp mortality test was on a newly hatched *A. salina* incubated with four different doses for 24h, with three replicates for each dose. The mean lethal concentration (LC_{50}) for various test concentrations was calculated after 24h by plotting a log of % mortality vs. log concentration (Figs. 2- 7).

The results in Table (1) demonstrate that *H. atra*, followed by *B. marmorata* and *H. edulis* exhibit the highest toxicity towards BSA larvae with LC_{50}=15.52±2.88, 31.7±5.02, and 43.3±2.83 µg/ml, respectively. Conversely, *H. leucospilota* followed by *H. polii* and *A. Mauritiana* showed low toxicity (LC_{50}=117.31±6.96, 73.9±3.03, 71.6±4.2µg/ ml, respectively). The present data in the BSA bioassay indicate that sea cucumber's body wall ethanolic extract exhibits high and moderate cytotoxic activity in a dosage-dependent way.

3. Inhibitory effect of sea cucumber extracts on the viability of HCT-116, PC3, HeP2 and *Hela* cells

Experiments were performed to evaluate cell survival using an MTT assay. Doxorubicin (DOX) is a standard anticancer medicine utilized for comparison. The viability of cancer cells is reduced rapidly in a dosage-dependent method. The results in Table (2) and Figs. (8- 11) show a significant cytotoxic performance in the culture cancer cell that was noticed in 100µg/ ml (maximum concentrations) for all sea cucumber extracts. *H. atra* and *B. marmorata* extracts exhibited a robust inhibitory effect on the viability of all tested cells (Hela, HeP2, HCT-116 and PC3). The average IC_{50} of *H. atra* extract against these cells was 9.14±0.8, 10.39±0.9, 11.43±1 and 17.90±1.5µg/ ml, respectively. The average IC_{50} of *B. marmorata* extract against the same cells was 21.36±1.8, 13.89±1.1, 7.74±0.6 and 28.83±2.3µg/ ml, respectively. Moreover, *H. leucospilota* extract showed high cytotoxicity against *HeP2* cells, with IC_{50}=18.12±1.4µg/ ml. Moreover, *H. edulis* extract obtained high cytotoxicity against *HeP2* and PC3 cells, with IC_{50}=24.51±1.9 and 27.33±2.2, respectively.

On the other hand, *H. leucospilota* exhibited a moderate inhibitory influence on the viability of three cell lines (HCT-116, *PC3* and *Hela* cells); whereas, *H. edulis* extract obtained a moderate cytotoxicity against two cell lines (HCT-116 and *Hela* cells). In contrast, the body wall extract of *H. polii* and *A. mauritiana* displayed a moderate inhibitory effect on the viability of all tested cells.

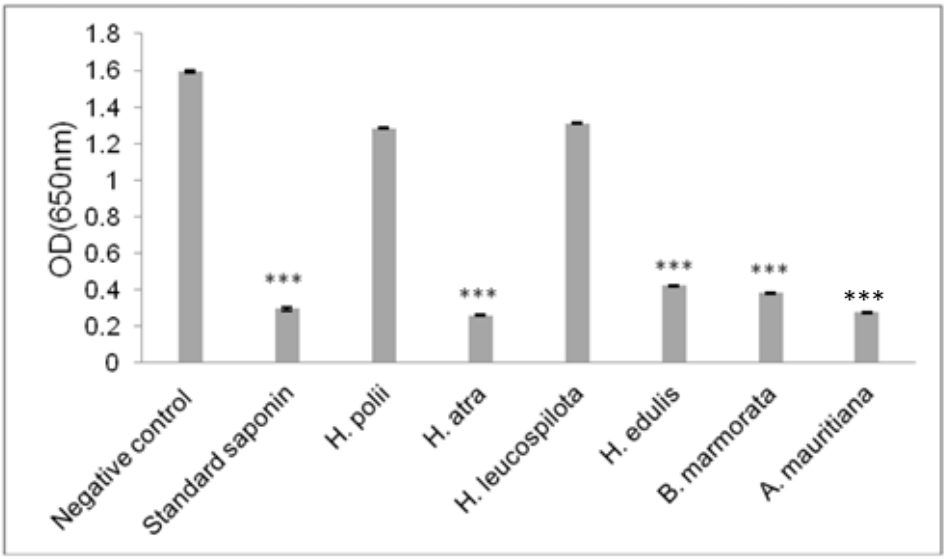

Fig. 1. Hemolytic effect of the sea cucumber species
Values are statistically significant at ****P*< 0.001.

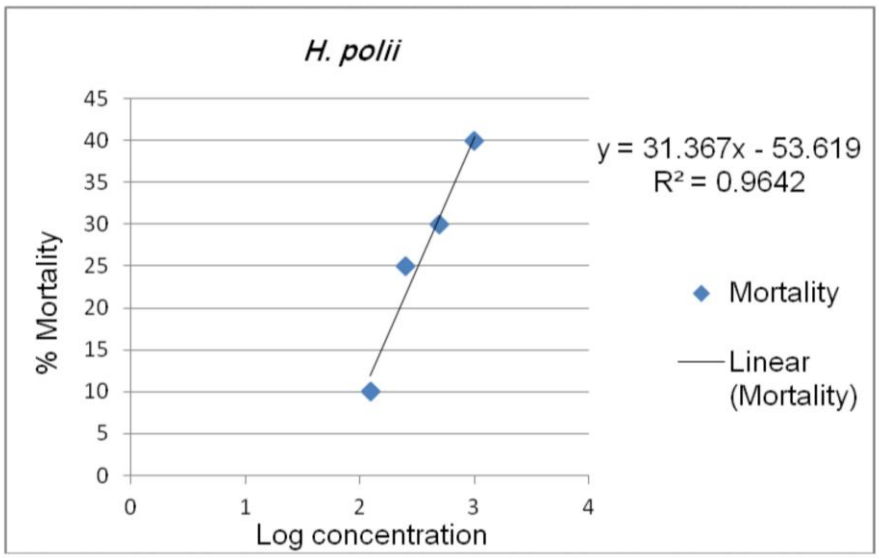

Fig. 2. Linear regression of Probit mortality of brine shrimp against log concentration of *H. polii* extracts after 24h exposure

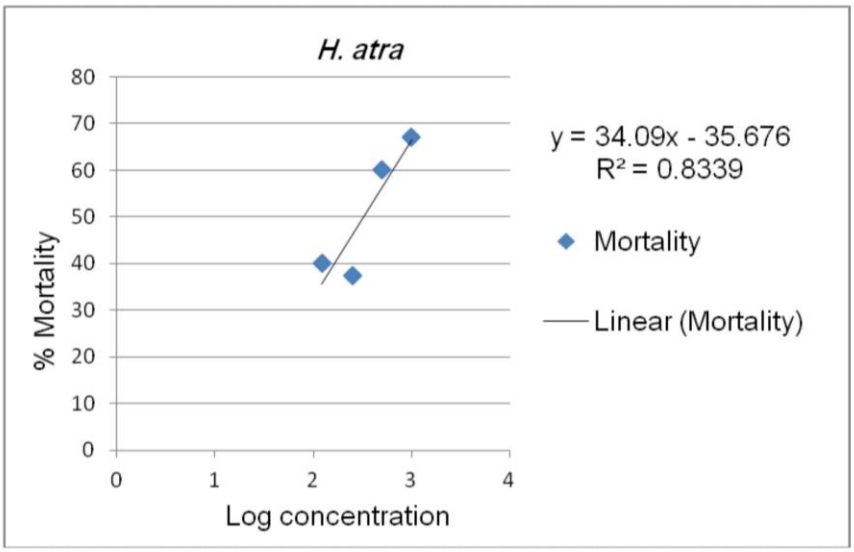

Fig. 3. Linear regression of Probit mortality of brine shrimp against log concentration of *H.atra* extracts after 24h exposure

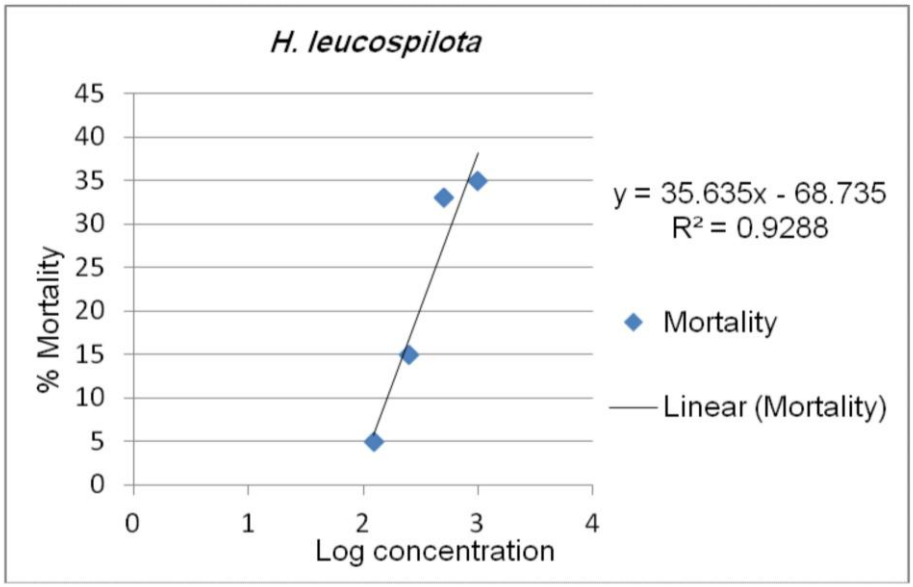

Fig. 4. Linear regression of Probit mortality of brine shrimp against log concentration of *H. leucospilota* extracts after 24h exposure

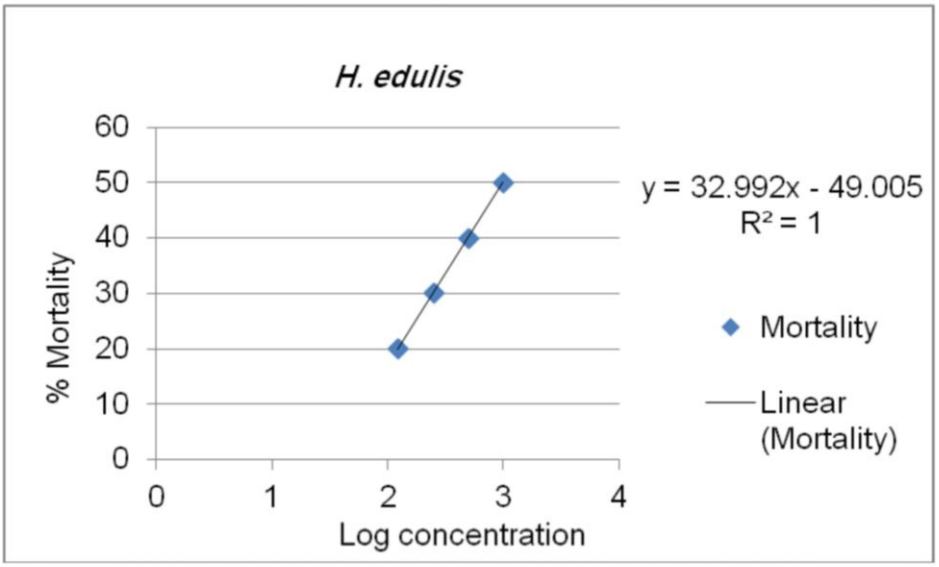

Fig. 5. Linear regression of Probit mortality of brine shrimp against log concentration of *H. edulis* extracts after 24h exposure

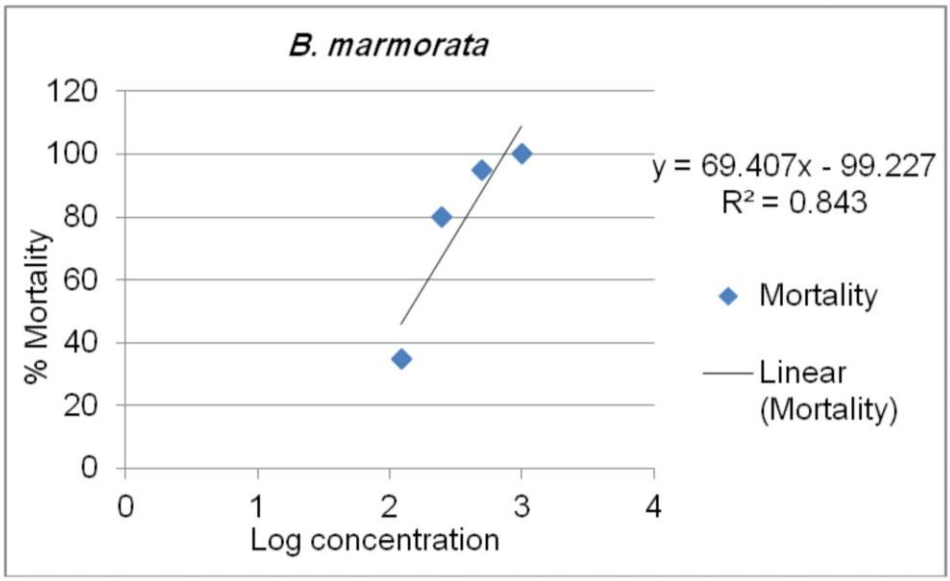

Fig. 6. Linear regression of Probit mortality of brine shrimp against log concentration of *B. marmorata* extracts after 24h exposure

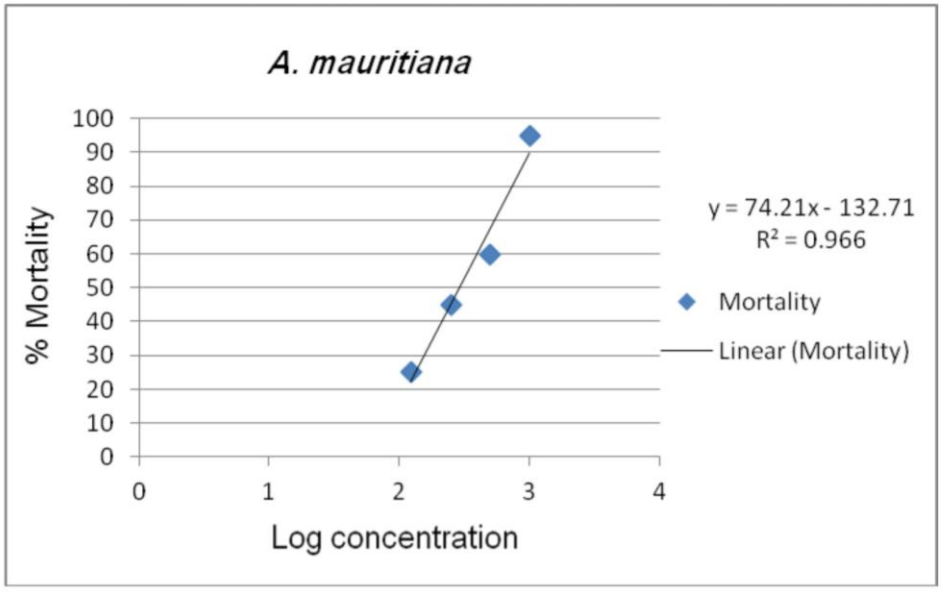

Fig. 7. Linear regression of Probit mortality of brine shrimp against log concentration of *A. mauritiana* extracts after 24h exposure

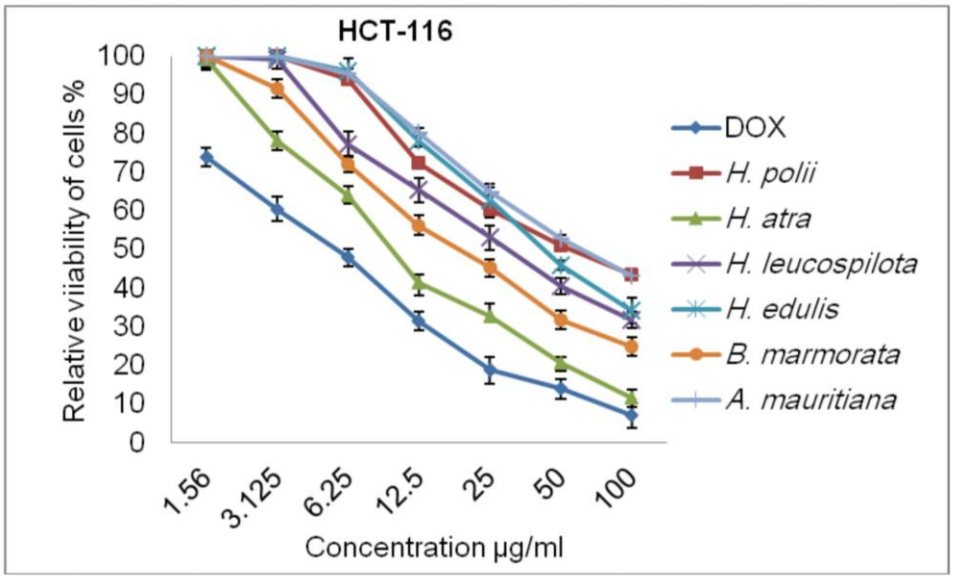

Fig. 8. The Growth-inhibitory effect of the extracts on proliferation of HCT-116 cells
Values are expressed as mean± SD.

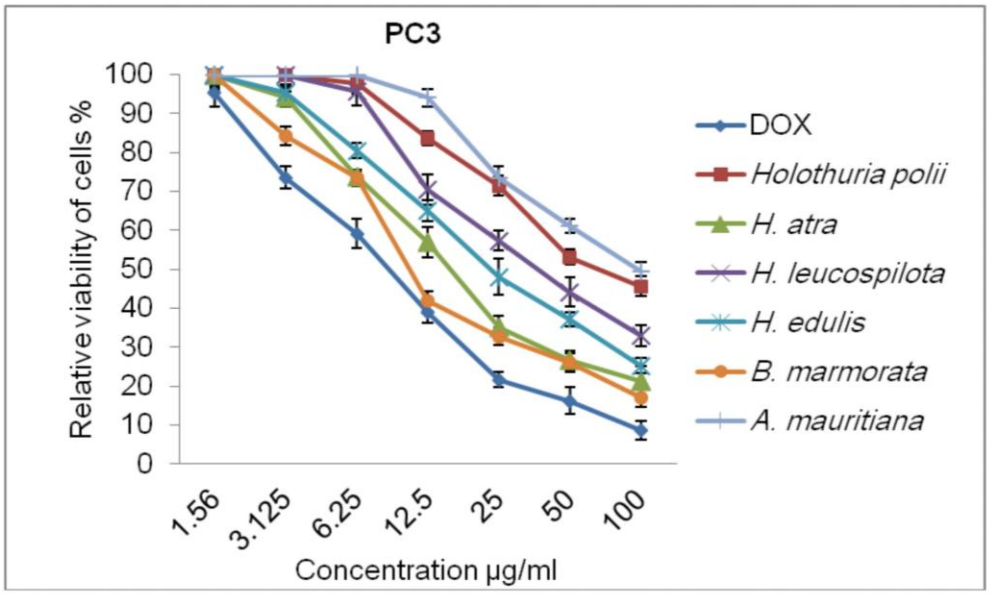

Fig. 9. The Growth-inhibitory effect of extracts on proliferation of PC3 cell line

* Values are expressed as mean±SD.

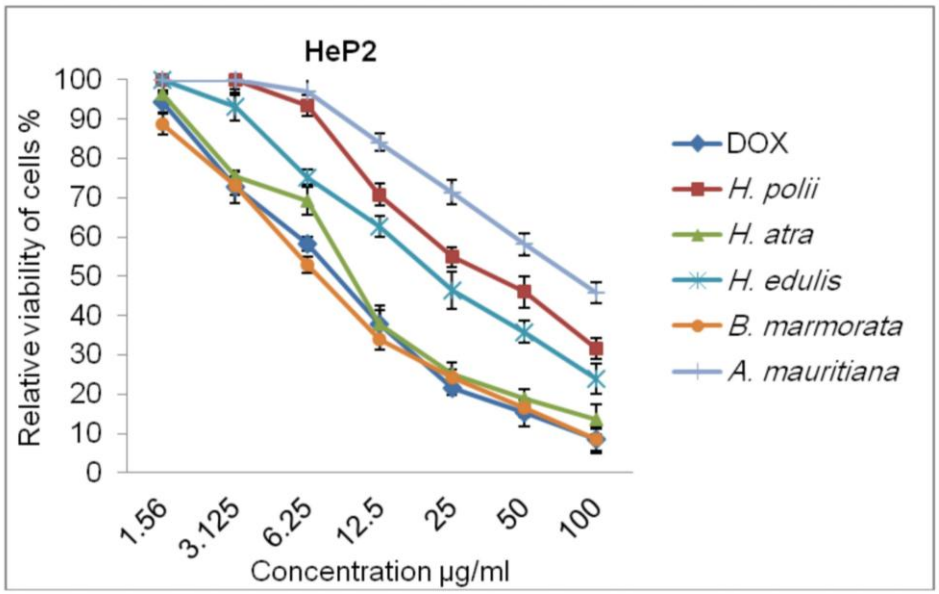

Fig. 10. The Growth-inhibitory effect of the extracts on the proliferation of HeP2 cells
Values are expressed as mean±SD.

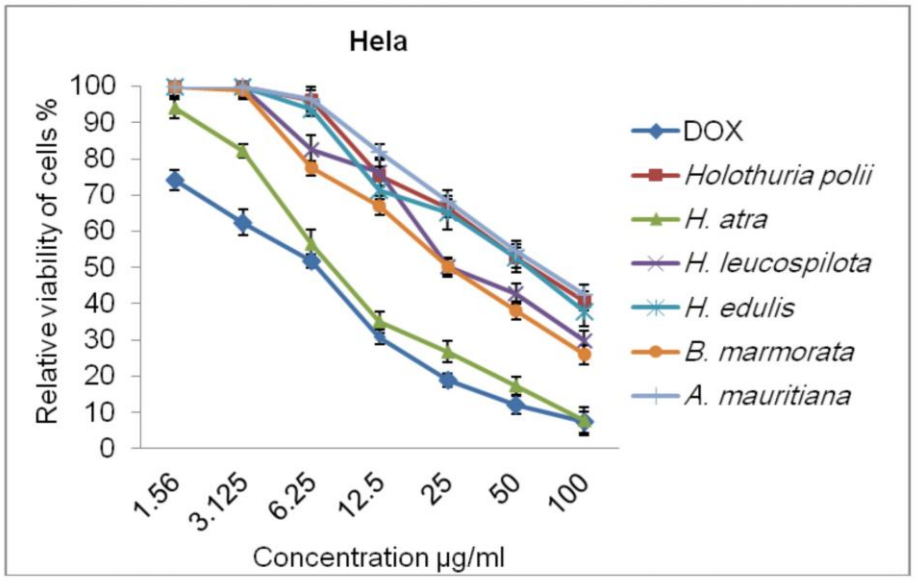

Fig. 11. The Growth-inhibitory effect of the extracts on the proliferation of *Hela* cells
Values are expressed as mean± SD.

Table 1. Lethality assay of sea cucumber extracts against brine shrimp

Group	LC_{50} (µg/ml)
H. polii	73.9±3.03
H. atra	15.52±2.88
H. leucospilota	117.31±6.96
H. edulis	43.3±2.83
B. marmorata	31.7±5.02
A. mauritiana	71.6±4.2

Abbreviations: A: Actinopyga; B: Bohadschia, and H: Holothuria.

Table 2. Cytotoxic activity of the investigated sea cucumber against four human cancer cells

Group	*In vitro* Cytotoxicity IC_{50} (µg/ml)			
	HCT-116	**PC3**	**HeP2**	**Hela**
DOX	5.23±0.3	8.87±0.6	8.54±0.6	5.57±0.4
H. polii	55.73±3.1	68.84±3.8	39.31±2.6	57.90±3.3
H. atra	11.43±1.0	17.90±1.5	10.39±0.9	9.14±0.8
H. leucospilota	32.44±2.4	40.31±2.7	18.12±1.4	34.98±2.5
H. edulis	46.06±2.9	27.33±2.2	24.51±1.9	53.40±3.0
B. marmorata	21.36±1.8	13.89±1.1	7.74±0.6	28.83±2.3
A. mauritiana	61.19±3.6	85.15±4.5	74.67±3.8	64.09±3.7

Abbreviations: DOX: Doxorubicin; Hela: Epitheliod Carcinoma; HEP2: Epidermoid Carcinoma; PC3, Human Prostate Cancer and HCT-116: Colorectal Carcinoma.

DISCUSSION

Bioactive composites in sea cucumbers have pharmaceutical properties such as antiproliferative and anticancer properties, making them an attractive source of bioactive compounds. This role of sea cucumber extracts may be attributed to principal amounts of essential compounds, including fatty acids, chondroitin sulfates, glycosaminoglycans, saponins (triterpene glycosides), phenolics and polysaccharides **(Bordbar *et al.*, 2011; Omran *et al.*, 2020)**. These compounds are essential antioxidants protecting from progressive disorders such as certain cancers and oxidative stress **(Althunibat *et al.*, 2009; Husni *et al.*, 2009)**. The analyzed specimen of the studied species contained several triterpene glycosides, such as holothurin B1, bivittoside C&D and 24-dehydroechinoside A **(Omran *et al.*, 2020)**.

The hemolytic activity of current species against human erythrocytes is widespread in extracts that showed antibacterial activity and is an immediate indication of toxicity against mammalian cells **(Abubakar *et al.*, 2012)**. The hemolysis test indicates the occurrence of saponins in the sea cucumber extract. Ethanol extracts of *A. mauritiana*, *B. marmorata*, *H. atra* and *H. edulis* showed a significant hemolytic effect, compared to the negative control and positively correlated to a standard saponin control group. Conversely, *H. polii* and *H. leucospilota* illustrated no hemolytic activity. Our

result disagrees with the study of **Soltani *et al.* (2014**). They postulated that saponins extracted from the Iranian *H. leucospilota* display moderate hemolytic effects on human blood cells.

The hemolytic action of some sea cucumber extracts has been associated with the existence of saponins in their body wall. Saponins have been confirmed to have a high surface activity, permitting them to disturb cell membranes. The ethanolic and methanolic extracts of the Persian Gulf *H. parva* showed a high-level hemolytic activity **(Shadi & Oujifard, 2018)**. The previous authors attributed the lytic action to producing complexes with cholesterol in the cell membrane.

This study investigated the cytotoxic activity of the ethanolic body wall crude extract of different sea cucumber species (*A. mauritiana*, *B. marmorata*, *H. atra*, *H. leucospilota*, *H. edulis* and *H. polii*). BSA was used as *in vitro* cytotoxicity models and four human cancer cells such as Hela, HEP2, PC3, and HCT-116.

For the primary screening of sea cucumber extracts using BSA lethality assay, we found that *H. atra*, *B. marmorata,* and *H. edulis* with LC_{50} =15.52±2.88, 31.7±5.02, and 43.3±2.83 µg/ml, respectively were more toxic to the larvae than other sea cucumber extracts.

A previous study confirmed that four extracts from *A. miliaris*, *B. argus*, *B marmorata,* and *H. leucospilota* collected from Penjaliran Timur Island are active in BSA **(Albuntana *et al*., 2011)**. They mentioned that *B. argus* is the most highly active species, indicated by the LC_{50} value of 69.254 µg/ml.

The current results towards *in vitro* cytotoxic activity revealed that all the ethanolic extracts from the recent sea cucumbers exhibited cytotoxic performance in the cancer cell cultures. In comparison, *H. atra* and *B. marmorata* extracts showed a significant inhibitory effect on the viability of all tested cells (Hela, HeP2, HCT-116, and PC3). The viability of HCT-116, PC3, HeP2 and Hela cells decreased rapidly with concentrations. *H. leucospilota* extract showed high cytotoxicity against only HeP2 cells, with IC_{50}= 18.12±1.4µg/ ml. However, it exhibited a moderate inhibitory influence on the viability of HCT-116, PC3 and Hela cells. Moreover, *H. edulis* extract obtained high cytotoxicity against HeP2 and PC3 cells, with IC_{50}=24.51±1.9 and 27.33±2.2, respectively, although it showed moderate cytotoxicity against two cells (HCT-116 and Hela cells). However, the extract of *H. polii* and *A. mauritiana* displayed a moderate inhibitory effect on the viability of all tested cells.

Previous studies on the cytotoxicity of *Holothurian* sea cucumber extracts were mainly focused on the methanolic fraction n-Hexane and ethyl acetate **(Mashjoor & Yousefzadi, 2019)**. Conversely, the present results and the study of **Omran *et al.* (2020)** showed the high efficiency of ethanolic extracts from the body wall.

The Cuvierian tubules of *A. mauritiana* and the body wall extracts of *H. leucospilota* and *B. marmorata* showed a strong inhibitory effect on the viability of both

HePG2 and MCF-7 cell lines **(Eissa *et al*., 2021)**. Saponins obtained from *H. leucospilota* have potent cytotoxic effects on lung cancer cells (A549 cell line) and are time dependent **Soltani *et al*. (2014**). The cytotoxic effects reduce metabolism, inhibit cell growth, and cause apoptosis **(McLaughlin, 2008)**. Ethanol extract of *H. atra* showed cytotoxic activity against four cell lines (T47D, MCF7, WiDr, and HeLa) with inhibition concentrations ranging from 9.6 to 14.3 µg/ml **(Nursid *et al*., 2019)**. They mentioned that flow cytometry investigation revealed that the T47D cell underwent apoptosis following treatment with ethanol extract.

The bioactivity of sea cucumber extracts may be ascribed to substantial bioproducts such as triterpene glycosides and phenols in their body.

CONCLUSION

The current sea cucumber species exhibited cytotoxic behavior in the cancer cell cultures. We assumed that *A. mauritiana*, *B. marmorata*, *H. atra*, and *H. edulis* exhibited hemolytic activity, while *H. polii* and *H. leucospilota* showed no hemolytic activity. *H. atra* and *B. marmorata* extracts showed a strong inhibitory effect on the viability of all tested cells (HCT-116, PC3, HeP2, and Hela). The study revealed that Egyptian sea cucumber species may be a source for discovering anticancer novel drugs.

List of abbreviations

A: Actinopyga; B: Bohadschia; BSA: brine shrimp *Artemia salina*; DMSO: Dimethyl sulfoxide; EDTA: Ethylenediaminetetraacetic acid; H: Holothuria; HCT-116: Colorectal carcinoma; Hela: Epitheliod Carcinoma; HEP2: Epidermoid Carcinoma; MTT: tetrazolium dye; PBS; phosphate-buffered saline; RPMI-1640 medium: Roswell Park Memorial Institute medium; PC3: Human prostate cancer.

Acknowledgments

We express our sincere thanks to members of Zoology Laboratory, Faculty of Science, Tanta University.

Author Contribution

Manar kandeil: conceptualization, data curation, methodology, software, validation, formal analysis, investigation, resources, writing-original draft preparation, writing-review, and editing, visualization, supervision. Aalaa Atlam: data curation, methodology, investigation, resources, writing-original draft preparation, writing-review, and editing, visualization, supervision. All authors have read and agreed to the published version of the manuscript.

Ethical Clearance

Tanta University's Faculty of Science and institutional Animal Care authorized the experimental protocols and procedures. Experimental procedures followed the

International Laboratory Animal Care and Use guidelines. The ethical approval number is 1ACUC-SCI-TU-0289.

Declaration of Competing Interests

The authors declare that they have no competing interests.

REFERENCES

Abubakar, L.A.; Mwangi, C.M.; Uku, J.U. and Ndirangu, S.N. (2012). Antimicrobial activity of various extracts of the sea urchin *Tripneustes gratilla* (Echinoidea). Afr. J. Pharmacol. Ther., 1: 19–23.

Albuntana, A.; Yasman, Y. and Wardhana, W. (2011). Toxicity Test of Extracts of the Four Sea Cucumber (Family Holothuriidae) From East Penjaliran Island, Seribu Islands, Jakarta Based on the Brine Shrimp Lethality Test (Bslt). Jurnal Ilmu dan Teknologi Kelautan Tropis., 3: 65-72. https://doi.org/10.29244/jitkt.v3i1.7835

Althunibat, O.Y.; Ridzwan, B.H.; Taher, M.; Daud, J.M.; Ikeda, M.A. and Zali, B.I. (2009). In vitro antioxidant and antiproliferative activities of three Malaysian sea cucumber species. Eur. J. Sci. Res., 37: 376-387.

Anderson, J.E.; Goetz, C.M.; McLaughlin, J.L. and Suffness, M. (1991). A blind comparison of simple bench-top bioassay and human tumour cell cytotoxicities as antitumor prescreens. Phytochem. Analysis., 2: 107-111.

Assawasuparerk, K.; Rawangchue, T. and Phonarknguen, R. (2016). Scabraside D derived from sea cucumber induces apoptosis and inhibits metastasis via iNOS and STAT-3 expression in human cholangiocarcinoma xenografts. Asian Pac. J. Cancer Prev., 17: 2151-2157.

Bondoc, K.G.V.; Lee, H.; Cruz, L.J.; Lebrilla, C.B. and Juinio-Meñez, M.A. (2013). Chemical fingerprinting and phylogenetic mapping of saponin congeners from three tropical holothurian sea cucumbers. Comp. Biochem. Physiol. B., 166: 182-93.

Bordbar, S.; Anwar, F. and Saari, N. (2011). High-value components and bioactives from sea cucumbers for functional foods-A Review. Mar. Drugs., 9: 1761-1805.

Bruce Pyenson, F.S.A (2010). Parity for oral and intravenous injected cancer drugs. Milliman Inc., New York, pp.1-20.

Denizot, F. and Lang, R. J. (1986). Rapid Colorimetric Assay for Cell Growth and Survival. Modifications to the Tetrazolium Dye Procedure Giving Improved Sensitivity and Reliability. Immunol. Methods., 8: 271-277. https://doi.org/10. 1016/ 0022- 1759(86)90368-6

Eissa, S.H.; Omran, N.E.E.; Salem, H.K.; Kabbash, A.K. and Kandeil, M.A. (2017). Surveillance study on the most common sea-cucumbers in some Egyptian coasts. Egypt. J. Exp. Biol. (Zool.)., 13(2): 300-308. https://doi.org/10.5455 /egysebz.20171113105752

Eissa, S.H.; Salem, H.K.; Omran, N.E.; Kabbash, A.M.; Amer, W.H. and Kandeil, M.A. (2021). Comparative study of antimicrobial activity and cytotoxicity of saponin extracted from six Egyptian sea-cucumber species, licorice, and ginseng. The Nat. Prod. J., 11(4): 491-506.

Finney, D. J. (1971). Probit Analysis. 3rd ed. Cambridge University Press, New Delhi, 333 pp.

Husni, A.; Shin, I-S.; You, S. and Chung, D. (2009). Antioxidant properties of water and aqueous ethanol extracts and their crude saponin fractions from a far-eastern sea cucumber, Stichopus japonicus. Food Sci. Biotechno l., 18: 419–424.

Lewis, G.E. (1995). Testing the toxicity of extracts of southern African plants using brine shrimp (Artemia salina). S. Afr. J. Anim. Sci., 91: 382-384.

Mackeen, M.M.; Ali, A.M.; Lajis, N.H.; Kawazu, K.; Hassan, Z.; Amran, M.; Habsah, M.; Mooi, L.Y. and Mohamed, S.M. (2000). Antimicrobial, antioxidant, antitumour-promoting and cytotoxic activities of different plant part extracts of Garcinia atroviridis Griff. ex T. Anders. J. Ethnopharmacol., 72: 395–402.

Mashjoor, S. and Yousefzadi, M. (2017). Holothurians antifungal and antibacterial activity to human pathogens in the Persian Gulf. J. Mycol. Méd., 27: 46-56.

Mashjoor, S.and Yousefzadi, M. (2019). Cytotoxic effects of three Persian Gulf species of Holothurians. IJVR., 20 (1): 19-26.

Mazzaferro, S.; Bouchemal, K. and Ponchel, G. (2013). Oral delivery of anticancer drugs I: general considerations. Drug Discov., Today. 18: 25-34.

McLaughlin, J.L. (2008). Paw paw and cancer: annonaceous acetogenins from discovery to commercial products. J. Nat. Prod., 71: 1311-1321.

Meyer, B.N.; Ferrigny, N.R.; Putnam, J.E.; Jacobsen, L.B.; Nichols, O.E. and McLaughlin, J.L. (1982). Brine shrimp: A convenient general bioassay for active plant constituents. Planta. Medica., 45: 31-34.

Nursid, M.; Marraskuranto, E. and Chasanah, E. (2019). Cytotoxicity and apoptosis induction of sea cucumber Holothuria atra extracts. Phcog Res., 11: 41-6.

Omran, N.E.; Salem, H.K.; Eissa, S.H.; Kabbash, A.M.; Kandeil, M.A. and Salem, M.A. (2020). Chemotaxonomic study of the most abundant Egyptian sea-cucumbers using ultra-performance liquid chromatography (UPLC) coupled to high-resolution mass spectrometry (HRMS). Chemoecol., 30(1): 35–48.

Sam, T.W. (1993). Toxicity testing using the brine shrimp: Artemia salina. In: "Colegate SM, Molyneux Rj, editors. Bioactive natural products: Determination, isolation and structural determination." CRC Press., Boca Ratón, FL, pp. 441–456.

Shadi, A. and Oujifard, A. (2018). Antibacterial, cytotoxic and hemolytic activity of Holothuria parva sea cucumber from north Persian Gulf. Int. J Environ. Sci. Technol., 16(1).

Soltani, M. and Baharara, J. (2014). Antioxidant and antiprolifereative capacity of dichloromethane extract of Holoturia leucospilota sea cucumber. Int. J. Cel. Mol. Bio., 2014: 1-9.

Soltani, M.; Parivar, K.; Baharara, J.; Kerachian; M.A. and Asili, J. (2014). Hemolytic and cytotoxic properties of saponin purifed from Holothuria leucospilota sea cucumber. Rep. Biochem. Mol. Biol., 3(1): 43-50.

Taiyeb-Ali, T.B.; Zainuddin, S.L.A.; Swaminathan, D. and Yaacob, H. (2003). Efficacy of "Gamadent" toothpaste on the healing of gingival tissues: a preliminary report. J. Oral. Sci., 45: 153-159.

Wang, J.; Han, H.; Chen, X.; Yi, Y. and Sun, H. (2014). Cytotoxic and apoptosis-inducing activity of triterpene glycosides from Holothuria scabra and Cucumaria frondosa against HepG2 cells. Mar. Drugs., 12: 4274-4290.