

# In Vitro Cytotoxic Activity of *Isostichopus badionotus*, a Sea Cucumber from Yucatan Peninsula Coast

Aida R. Pérez-Espadas<sup>1</sup>, María J. Verde-Star<sup>1</sup>, Catalina Rivas-Morales<sup>1</sup>, Azucena Oranday-Cárdenas<sup>1</sup>, María E. Morales-Rubio<sup>1</sup>, Lorena V. León-Deniz<sup>2</sup>, Jaqueline Canul-Canché<sup>3</sup> and Leovigildo Quijano<sup>4,\*</sup>

<sup>1</sup>Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León, Av. Pedro de Alba s/n, Ciudad Universitaria, CP 66450, Nuevo León, México

<sup>2</sup>Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Yucatán, Carr. Xmatkuil, Km. 15.5, Apartado Postal No. 116, CP 97315, Yucatán, México

<sup>3</sup>Facultad de Química, Universidad Autónoma de Yucatán, C. 41 No. 421 Col. Industrial, CP 97150, Mérida, Yucatán, México

<sup>4</sup>Instituto de Química, Universidad Nacional Autónoma de México, Circuito Exterior, Ciudad Universitaria, 04510, México, D. F., México

**Abstract:** The *in vitro* cytotoxic activity of hexane, ethyl acetate and butanol extracts of the sea-cucumber *Isostichopus badionotus* (Holothuroidea) was tested against normal cells (Vero), human cervical carcinoma (HeLa) and breast adenocarcinoma (MCF-7 and MDA-MB-231) ATCC cells by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. Hexane extracts from body walls and viscera showed high cytotoxic activity against HeLa cells ( $IC_{50}$ 's = 48.5 and 42.5  $\mu\text{g mL}^{-1}$ , respectively), while the ethyl acetate extract of body walls was considered low active ( $IC_{50}$  = 98.3  $\mu\text{g mL}^{-1}$ ). In addition, the body walls hexane extract showed a good selectivity index value of 12.0.

**Keywords:** Holothurian, organic extracts, selectivity index, HeLa cells.

## INTRODUCTION

Cancer is a leading cause of death around the world with 12.7 million new cases and 7.6 million deaths worldwide reported in 2008, and it is estimated that the death toll will be 13.1 million deaths in 2030 [1]. Presently, more than 100 types of cancer have been identified, and despite enormous advances in treatment of cancer patients, a growing body of clinical and experimental evidence has revealed a strong impact of drug resistance on clinical outcomes in cancer chemotherapy. Therefore, the need for finding novel agents to improve anticancer drug therapy is imperative [2,3].

Natural products, defined as small-molecule secondary metabolites produced for organisms, have proved to be very useful in development of anticancer drugs over last five decades. Currently, a significant numbers of compounds obtained from marine environment are in clinical trials; hence marine organisms are considered valuable sources of cytotoxic, antiproliferative, antitumor or anticancer compounds [4-7]. Therefore, several sea cucumbers species collected worldwide have been studied in order

to determine its potential as sources of anticancer compounds.

Sea cucumbers species are 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 [8-11] because of their high-protein content and their putative aphrodisiac, tonical and medicinal properties [12,13]. However, some species have been overexploited; which may result in a population collapse and the loss of significant potential source of anticancer drugs for the future.

*Isostichopus badionotus* (Selenka, 1867), is distributed mainly in Gulf of Mexico, Caribbean Sea, and the joining of these two outstanding marine ecosystems, the Yucatan Channel [14, 15]. It is one of four species of sea cucumbers overexploited in Yucatan, Mexico, due to illegal fishing. A literature survey indicated that there is too little information about the metabolites of this species and their biological activity, since only one study about cytotoxicity of a hydrolysate of *I. badionotus* against colorectal HT-29 cancer cells ( $IC_{50}$  = 1450  $\mu\text{g mL}^{-1}$ ) is reported [16].

In consequence, the present study was conducted to test the activity of body walls and visceral organ extracts from *I. badionotus* against Vero, HeLa, and

\*Address correspondence to this author at the Instituto de Química, UNAM, Circuito Exterior, Ciudad Universitaria, 04510, D.F. México; Tel: (+52) 55 56224411; Fax: (+52) 55 56162203; E-mail: quijano@unam.mx

breast adenocarcinoma (MCF-7 and MDA-MB-231) ATCC cells.

## MATERIALS AND METHODS

### Animal Material and Extraction Procedure

Specimens of *Isostichopus badionotus* (27.8 kg wet weight) were collected by scuba diving at depths ranging from 14.5 to 16 m in the coast of Progreso (N 21° 27.3' – W 89° 42.7'), Yucatan (Mexico) in June and November 2010. The specimens were collected and authenticated by M. Sc. Carlos Zetina Moguel and voucher specimen (YUC-CC-250-11/CAR/293) was deposited in the collection of Campus de Ciencias Biológicas y Agropecuarias, Universidad Autónoma de Yucatán, in Mérida, Yucatán, México.

The animals were immediately placed in ice water in sealed plastic bags and transported to the laboratory where they were dissected. Body walls (21.6 kg wet weight) and visceral organ (6.2 kg wet weight, gut content included) were stored separately at  $-17^{\circ}\text{C}$ .

Both body walls and visceral organ samples underwent the same analytical procedure. The frozen tissues were homogenized, chopped and extracted exhaustively with methanol at room temperature. The extracts were subsequently evaporated at reduce pressure for removal of the solvent. The syrup was successively extracted three times each with hexane (Hx), ethyl acetate (EtOAc) and n-butanol (BuOH).

### Cytotoxicity Assay

Breast adenocarcinoma (MCF-7 and MDA-MB-231), human cervical carcinoma (HeLa), and African green monkey kidney (Vero) ATCC cells were used. The cells were cultivated in D-MEM (Invitrogen, México), supplemented by 10% fetal calf serum and antibiotics (100 units  $\text{mL}^{-1}$  penicillin and 100  $\mu\text{g mL}^{-1}$  streptomycin), at  $37^{\circ}\text{C}$  in a humid atmosphere containing 5%  $\text{CO}_2$ .

The cytotoxicity test [17] was conducted by using 96-well microtiter plates containing 2,500 cells per well. The cell suspensions were incubated for 24 h under the conditions mentioned above. After incubation 18.75, 37.5, 75, 150 and 300  $\mu\text{g mL}^{-1}$  of each extract were added per well. The microplates were further incubated for 48 h and then 10  $\mu\text{L}$  of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) (Sigma-Aldrich, USA) solution (5  $\text{mg mL}^{-1}$ ) was added to each well. After 4 h additional incubation, the liquid was

discarded and replaced with 100  $\mu\text{L}$  of dimethyl sulfoxide (DMSO) (Sigma-Aldrich, USA) and the absorbance measured in a microplate reader (Multiskan Asent) at 550 nm. Each assay was carried out in three independent experiments. The cell viability in response to treatment was calculated using Origin 8.6 software. The selectivity index (SI) was calculated with the equation:  $\text{SI} = \text{IC}_{50}\text{normal cells} / \text{IC}_{50}\text{cancer cells}$ .

## RESULTS AND DISCUSSION

The cytotoxic activity of hexane, ethyl acetate and n-butanol extracts from body walls and viscera of *Isostichopus badionotus* collected at the Yucatan Channel were tested *in vitro* against human tumor cell lines of cervix (HeLa) and breast adenocarcinoma (MCF-7 and MDA-MB-231). In order to evaluate the level of harmfulness of the extracts, the selectivity index was determined on African green monkey kidney normal cells (Vero). Results are showed in Table 1.

Based on Manosroi *et al.* [18] screening, in the present study the activity of extract was ranked in two levels: high ( $\text{CI}_{50} \leq 50 \mu\text{g mL}^{-1}$ ), and low ( $\text{CI}_{50} 51\text{-}100 \mu\text{g mL}^{-1}$ ), and the selectivity according to Vonthron-Sénécheau *et al.* [19] who consider that an  $\text{SI} \geq 10$  indicated that biological efficacy of the extract is not due to cytotoxicity.

The HeLa cell line was the most susceptible to non-polar extracts of body walls and viscera of *I. badionotus*, while MCF-7 and MDA-MB231 were not affected by any of the tested extracts with exception of the EtOAc extract, which inhibited the cellular growth at 105.4 and 101.2  $\mu\text{g mL}^{-1}$ , respectively. The hexanic extract of body walls exhibited cytotoxic activity at 48.5  $\mu\text{g mL}^{-1}$  and the one from viscera at 42.5  $\mu\text{g mL}^{-1}$ . The polar extract (BuOH) did not show cytotoxic activity against the three cancer cell lines (HeLa, MCF-7, and MDA-MB231), while the normal cell line (Vero) was not susceptible to any extract. Most of the extracts showed selectivity indexes in the range of 1.0 – 3.8 with exception of hexane extracts, which exhibited selectivity indexes of 12 and 5.2 against HeLa cells, respectively.

According to the ranks described above, the cytotoxic activity of hexane extracts of body walls (48.2  $\mu\text{g mL}^{-1}$ ) and viscera (42.5  $\mu\text{g mL}^{-1}$ ) of *I. badionotus* against HeLa cells were considered high, while the cytotoxic activity of EtOAc extract of body walls was low (98.3  $\mu\text{g mL}^{-1}$ ).

**Table 1: Cytotoxic Activity and Selectivity Index of Extracts from *Isostichopus badionotus***

Animal part	Extract	Cell lines			
		IC <sub>50</sub> <sup>a</sup> µg mL <sup>-1</sup> ± SD <sup>b</sup> (Sf <sup>c</sup> )			
		HeLa	MCF-7	MDA-MB231	Vero
Body walls	Hex	48.5 ± 1.9 (12.0)	197.5 ± 11.5 (3.0)	152.5 ± 4.8 (3.8)	582.8 ± 21.5
	EtOAc	98.3 ± 5.0 (1.6)	105.4 ± 12.0 (1.5)	101.2 ± 12.1 (1.6)	158.2 ± 21.3
	BuOH	> 600	> 600	> 600	> 600
Viscera	Hex	42.5 ± 2.0 (5.2)	167.1 ± 10.0 (1.3)	152.8 ± 11.0 (1.4)	218.9 ± 13.7
	EtOAc	166.7 ± 8.9 (1.3)	204.7 ± 7.9 (1.0)	117.7 ± 7.9 (1.8)	209.3 ± 2.3
	BuOH	> 600	> 600	> 600	> 600
	Paclitaxel	0.08 ± 0.01 (15.5)	0.07 ± 0.02 (17.7)	0.03 ± 0.01 (41.3)	1.24 ± 0.01

<sup>a</sup>Inhibitory Concentration 50.<sup>b</sup>Standard Deviation.<sup>c</sup>Selectivity Index.

A study of the less polar lipid fraction of the chloroform–methanol extract from body walls of *Cucumaria frondosa*, a North Atlantic commercially harvested sea cucumber, resulted in the isolation of cerebroside, which exhibited an antiproliferative effect on Caco-2 colon cancer cells (IC<sub>50</sub> = 4.13 µg mL<sup>-1</sup>) [20]. Probably, the cytotoxic activity of hexane extracts of viscera and body walls of *I. badionotus* could be explained by the presence of cytotoxic cerebroside.

Although, the cytotoxic activity of non-polar extract of *I. badionotus* body walls was classified as high, its SI value was 12.0 unlike the other extracts which showed poor selectivity (1.0–5.2). Besides, it is remarkable that the selectivity showed by the hexane extract of body walls of *I. badionotus* was close to the SI of paclitaxel (15.5), the commercial anticancer drug used as positive control.

On the other hand, the lack of cytotoxic activity of BuOH extracts was unexpected because it is known that triterpene glycosides (saponins), commonly isolated from polar extracts of holothurians, shown cytotoxic activity which is related to membranotropic action due to the presence of an 18(20)-γ-lactone in the aglycon moiety in most cases, as well as a linear tetrasaccharide fragment. Moreover, oligoglycosides containing quinovose instead of glucose or xylose as second monosaccharide unit usually exhibited a high bioactivity [21].

Regarding the presence of saponins in *I. badionotus*, up today, only a mixture of D-xylosides of 3β-hydroxy sterols of the cholestane series has been isolated from the body walls of this species [22]. Therefore, the non-cytotoxic activity of the BuOH extracts of *I. badionotus* might be related with absence

of saponins that fulfill the characteristics above described.

## CONCLUSIONS

Only the non polar extracts from *I. badionotus* were considered significantly active and selective against human tumor cell lines of cervix (HeLa). The good selectivity index exhibited by the hexane extract of the body walls of *I. badionotus* encouraging further research on the isolation of the cytotoxic compounds.

On the other hand, the lack of cytotoxic activity of butanolic extracts might be related with absence of holostan-type triterpene glycosides.

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## REFERENCES

- [1] Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Maxwell D. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer* 2007; 127: 2893-2917. <http://dx.doi.org/10.1002/ijc.25516>
- [2] Nussbaumer S, Bonnabry P, Veuthey JL, Fleury-Souverain S. Analysis of anticancer drugs: A review. *Talanta* 2011; 85: 2265-2289. <http://dx.doi.org/10.1016/j.talanta.2011.08.034>
- [3] Rebutti M, Michiels C. Molecular aspects of cancer cell resistance to chemotherapy. *Biochem Pharmacol* 2013; 85: 1219-1226. <http://dx.doi.org/10.1016/j.bcp.2013.02.017>

- [4] Blunden G. Biologically active compounds from marine organisms. *Phytother Res* 2001; 15: 89-94. <http://dx.doi.org/10.1002/ptr.982>
- [5] Blunt JW, Copp BR, Munro MHG, Northcote PT, Prinsep MR. Marine natural products. *Nat Prod Rep* 2004; 31: 160-258. <http://dx.doi.org/10.1039/c3np70117d>
- [6] Simmons TL, Andrianasolo E, McPhail K, Flatt P., Gerwick WH. Marine natural products as anticancer drugs. *Mol Cancer Ther* 2005; 4: 333-342.
- [7] Conand C., Byrne M. A review of recent developments in the world sea cucumber fisheries. *Marine Fisheries Review* 1993; 55: 1-15.
- [8] Chen J. Overview of sea cucumber farming and sea ranching practices in China. *SPC Beche-de-mer Information Bulletin* 2003; 18: 18-23.
- [9] Chen J. Present status and prospects of sea cucumber industry in China. In: Conand C, Purcell S, Uthicke S, Hamel JF, Mercier A, editors. *Advances in sea cucumber aquaculture and management*. FAO Fisheries Technical Paper No. 463 Rome: FAO 2004; p. 25-38.
- [10] Park SY, Lim HK, Lee S, Hwang HC, Cho SK, Cho M. Pepsin-solubilized collagen (PSC) from red sea cucumber (*Stichopus japonicus*) regulates cell cycle and the fibronectin synthesis in HaCat cell migration. *Food Chem* 2012; 132: 487-492. <http://dx.doi.org/10.1016/j.foodchem.2011.11.032>
- [11] Wen W, Chaoqun H, Zhang L, Luo P, Zhao Z, Fan S, Su T. The application of PCR-RFLP and FINS for species identification used in sea cucumbers (*Aspidochirotida: Stichopodidae*) products from the market. *Food Control* 2010; 21: 403-407. <http://dx.doi.org/10.1016/j.foodcont.2009.06.014>
- [12] Aydin M, Sevgili H, Tufan B, Emre Y, Köse S. Proximate composition and fatty acid profile of three different fresh and dried commercial sea cucumbers from Turkey. *International Journal of Food Science and Technology* 2011; 46: 500-508. <http://dx.doi.org/10.1111/j.1365-2621.2010.02512.x>
- [13] Bordbar S, Anwar F, Saari N. High-Value components and bioactives from sea cucumbers for functional foods- A review. *Mar Drugs* 2011; 9: 1761-1805. <http://dx.doi.org/10.3390/md9101761>
- [14] Guzmán HM, Guevara CA. Population structure, distribution and abundance of three commercial species of sea cucumber (Echinodermata) in Panama. *Caribb. J. Sci.* 2002; 38: 230-238.
- [15] Purcell, SW, Samyn Y, Conand C. *Commercially important sea cucumbers of the world*. FAO Species Catalogue for Fishery Purposes No. 6. Rome: FAO; 2012.
- [16] Pérez-Vega JA, Olivera-Castillo L, Gómez-Ruiz JA, Hernández-Ledesma B. Release of multifunctional peptides by gastrointestinal digestion of sea cucumber (*Isostichopus badionotus*). *J Funct Foods* 2013; 5: 869-877. <http://dx.doi.org/10.1016/j.iff.2013.01.036>
- [17] Schlie-Guzmán M, García-Carrancá A, González-Esquinca A. *In Vitro* and *In Vivo* Antiproliferative Activity of Laherradurin and Cherimolin-2 of *Annona diversifolia* Saff. *Phytother Res* 2009; 23: 1128-1133. <http://dx.doi.org/10.1002/ptr.2760>
- [18] Manosroi J, Dhumtanom P, Manosroi A. Anti-proliferative activity of essential oil extracted from Thai medicinal plants on KB and P388 cell lines. *Cancer Lett* 2006; 235: 114-120. <http://dx.doi.org/10.1016/j.canlet.2005.04.021>
- [19] Vonthron-Sénécheau C, Weniger B, Ouattara M, *et al.* *In vitro* antiplasmodial activity and cytotoxicity of ethnobotanically selected Ivorian plants. *J Ethnopharmacol* 2003, 87: 221-225. [http://dx.doi.org/10.1016/S0378-8741\(03\)00144-2](http://dx.doi.org/10.1016/S0378-8741(03)00144-2)
- [20] Xu J, Guo S, Du L, Wang YM, Sugawara T, Hirata T, Xue CH. Isolation of cytotoxic glucoerebrosides and longchain bases from sea cucumber *Cucumaria frondosa* using high speed counter-current chromatography. *J Oleo Sci* 2013, 62: 133-142. <http://dx.doi.org/10.5650/jos.62.133>
- [21] Kalinin V, Aminin DL, Avilov SA, Silchenko AS, Stonik VA. Triterpene glycosides from sea cucumbers (Holothuroidea, Echinodermata). Biological activities and functions. In: Attaur-Rahman editor. *Studies in Natural Products Chemistry*. Elsevier B.V. 2008; p. 135-196.
- [22] Elyakov GB, Kuznetsova TA, Conde C, Kalinovskaya NI, Kalinovskii AI, Smetanina OF. Glycosides of marine invertebrates VIII. Steroid glycosides of the holothurian *Isostichopus badionotus*. *Chemistry of Natural Compounds* 1979, 6: 799-708.

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