Isostichotoxin isolated from *Isostichopus badionotus* (Selenka, 1867) sea cucumber processing's byproducts

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Introduction

Sea cucumbers — mainly from the families Holothuriidae and Stichopodidae — are used both for traditional tonic food and biomedical research (Conand 2006). They constitute an important part of a multi-species invertebrate fishery that has been operating in the Indo-Pacific for traditional and subsistence uses. In the mid- to late-1990s, however, additional markets for sea cucumbers emerged for biomedical research and use for "at home" aquaria (see CITES Secretariat website at: http://www.cites.org 2002). Bioprospectors have become interested in sea cucumbers for natural products research and development. Several commercial products originating from sea cucumber extracts have been marketed in recent years, including ArthiSea and SeaCuMax (arthritis medicines), nutritional supplements, and Sea Jerky (Morgan 2000).

Holothurians contain chondroiton and glucosamine — important cartilage building blocks — and other bioactive substances with anti-inflammatory and anti-tumor activity properties (Mindell 1998; Herecia and Ubeda 1998), as well as fungicidal activity (Darah et al. 1995). Some compounds extracted from holothurians have applications in the prevention and cure of some cancers, and bacterial, fungal or viral infections (Hamel 1997). In Malaysia some medicines and pharmaceutical researches for new products are available from sea cucumbers, such as "gamat oil" "gamat water" "awal gamat" (Baine and Choo 1999; Choo et al. 2004; Zaidnuddin and Kamarruddin 2006).

The bio-actives obtained from *Isostichopus badionotus* processing, began in Cuba in 2003 (Alfonso et al. 2004). These metabolites resulted from processing large amounts of sea cucumbers in boiled sea water as byproduct. This water is usually discarded at sea with no treatment, once cooled. Sea cucumbers are known to produce toxic characteristics and antifungal triterpenoid glycosides of the holostane-type[1-5] with recognized biological actions. Taking into account these results, the current investigation recommends utilizing sea cucumber boiled waste water, and offers a methodology to isolate this metabolite. Other isolated compounds are being currently researched from *I. badionotus* fishery's byproducts.

Boiled sea water is conveniently treated under acidic conditions and the drying process is further submitted to other treatments with solvents (e.g. ethanol and mean polarity eluents), reverse phase conditions with Polycrom–XAD-2, Amberlite and SiO_2 chromatography, thus obtaining the isostichotoxin.

Isolating the toxin

Fifty specimens (15 kg each) of *Isostichopus badionotus* (Fig. 1) were collected in June 2005 by diving at Horiguelas Keyes, located at 20°43.03´N and 78°16.04´W, at depths ranging from 5–12 m and authenticated by Alfonso (*voucher specimen: ISO*-IJ-04), in sampling works. Boiling water (10 L), was filtered, and the aqueous extract was treated, under stirring with 100 mL 10% sulphuric acid (H_2SO_4). The pH was adjusted to 2.8, and the solution was left to stand overnight. The aqueous phase was decanted and ethanol (C_2H_5OH) was added.

The mixture was stirred for 1 h at 25°C, then filtered and the upper layer collected. The pH was then adjusted to 6 and the aqueous phase was concentrated under vacuum and extracted with n-butanol (C_4H_9OH). The butanolic extract was evaporated and separated using column chromatography (XAD-2, Polychrom, 0.5 kg, eluent 0.5–0.7 L 50% ethanol), this eluent was concentrated. The final product (1.0 g) was dissolved in a mixture of ethanol-chloroform-water (100:100:7 v v^{-1} and purified by column chromatography (Silica gel Mesh 60–230, Merck).

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Figure 1. ***Isostichopus badionotus*** **sea cucumber used for processing in Cuba.**

Isostichotoxin,[1] a mixture of natural triterpene glycosides (mp: more than 247°C with decomposition), gives a positive Liebermann-Burchard reaction (steroids and triterpenes), yielding an acid hydrolysis (7% sulphuric acid at 100°C) as a sugar mixture (colored spots with aniline-phosphoric acid reagent). Isostichotoxin has a distinctive maximum in Infrared spectroscopy (FT-IR) at 3300–3500 cm^{-1} and 1745 cm^{-1}. Crude isostichotoxin was purified by recrystallization from methanol (or ethanol) and *in vitro* antifungal activity was tested.

Results were as follows: samples of crystalline isostichotoxin were dissolved as a dimethylformamide aqueous solution of 17.5% to make a 2 mg mL^{-1} solution. The resulting solution was serially diluted with sterile water and added to a series of agar plates, each of which were inoculated with a different organism test. Results are shown in Table 1.

An aqueous alcoholic solution of isostichotoxin at a concentration of 0.01 % was applied to the affected skin of patients, once or several times daily for a period from three days to four weeks. These results are shown in Table 2.

As a preliminary conclusion, isostichotoxin could be used as a potential fungicide mixture for therapeutic treatment of some fungal infections in humans.

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Table 1. **Antifungal activity of *I. badionotus* collected in Cuba's southeastern waters.**

Microorganisms	Minimum inhibitor concentration (mμ mL^{-1})
Trichophyton interdigitale	5.8–6.2
Bacillus subtilis	88
Candida albicans	13.6–16.8
Pseudomonas aeruginosa	79.9–82.3
Mycobacterium tuberculosis	> 100
Microsporus canis	56.3
Escherichia coli	> 100
Torula utilis	2.9
Saccharomyces cerevisiae	2.8

Table 2. **Fungicidal actions on affected skin in patients.**

Effectiveness	Fungus species				
	Pompholyx trichophytia	*Tricophytiaat* (part of lanugo hairs)	*Tinea versicolor*	*Candida erosio interdigitalis*	Number of patients
Very effective	10	1	1	0	12
Effective	7	2	0	1	10
Ineffective	3	1	0	0	4
Total patients	20	4	1	1	26
(%)	77 No side effects were detected during treatment				

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