کارگاه‌های آموزشی مرکز اطلاعات علمی

مقاله نویسی علوم انسانی

اصول تنظیم قراردادها

آموزش مهارت های کاربردی در تدوین و چاپ مقاله
Antibacterial and Antifungal Activity of *Holothuria leucospilota* Isolated From Persian Gulf and Oman Sea

Neda Adibpour 1,2; Farhad Nasr 1; Fatemeh Nematpour 3; Arash Shakouri 3; Abdolghani Ameri 1,4,*

1Marine Pharmaceutical Sciences Research Center, School of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, IR Iran
2Department of Medicinal Chemistry, School of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, IR Iran
3School of Marine Sciences, Chabahar Maritime University, Chabahar, IR Iran
4Department of Food and Drug Control, School of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, IR Iran

*Corresponding author: Abdolghani Ameri, Marine Pharmaceutical Sciences Research Center, School of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, IR Iran.
Tel: +98-6113738378, Fax: +98-6113738381, E-mail: kazem9020@hotmail.com

Received: October 22, 2012; Revised: January 23, 2013; Accepted: February 23, 2013

Background: Emergence of antimicrobial resistance toward a number of conventional antibiotics has triggered the search for antimicrobial agents from a variety of sources including the marine environment.

Objectives: The aim of this study was to evaluate the antimicrobial potential of *Holothuria leucospilota* from Qeshm and Kharg Islands against some selected bacteria and fungi.

Materials and Methods: In this investigation, sea cucumbers from two coastal cities of Persian Gulf were collected in March and May 2011 and identified by the scale method according to the food and agriculture organization of the United Nations. Antimicrobial activity of hydroalcoholic extracts of the body wall, cuvierian organs and coelomic fluid against *Staphylococcus aureus* and *Escherichia coli* was evaluated by the spot test. In addition, their antifungal activity was assessed by the broth dilution method.

Results: The displayed effect was microbiostatic at concentrations of 1000 and 2000 μg/mL rather than microbicidal. The highest activity of hydroalcoholic extracts was exhibited by body wall, cuvierian organs and coelomic fluid against *Staphylococcus aureus* and *Salmonella typhi*, *Streptococcus pyogenes* and *Pseudomonas aeruginosa*. *Aspergillus niger*, *A. flavus* and *A. brasiliensis*. However, none of the methanol, chloroform and n-hexane extracts showed appreciable effects against *Shigella dysenteriae*, *Proteus vulgaris*, *Bacillus cereus*, *S. epidermidis* and *Candida albicans*. Moreover, cuvierian organs did not possess any antifungal potential.

Conclusions: Our data indicated that water-methanol extracts from the body wall of *H. leucospilota* possess antibacterial and antifungal activity. However, additional and in-depth studies are required to isolate and identify the active component(s).

Keywords: *Holothuria leucospilota*; Anti-Infective Agents; Persian Gulf

1. Background

Emergence of antimicrobial resistance toward a number of conventional antibiotics has stimulated the search for antimicrobial agents from a variety of sources including the marine environment. Sea cucumbers are echinoderms from the class *Holothuroidea*. In Vietnamese traditional medicine, sea cucumbers had been used as tonics and delicacies (1). In Malaysia, different species of sea cucumbers are used to relieve pain and skin irritations and treat eczema and arthritis (2). Antimicrobial activities of several species of echinoderms from the Gulfs of California, Mexico and Caribbean Islands and the Coast of Norway have been reported (3-5).

Various antimicrobial components including steroidal glycosides (6), polyhydroxylated sterols (7), naphthoquinone pigments (8), lysozymes (9, 10), complement-like substances (11) and antimicrobial peptides (12) have been isolated from the sea cucumbers. Additionally, several holostane-type triterpene glycosides (from *Holothuria fusco-cinerea*) (13) and three new cytotoxic triterpene glycosides (from *Mensamaria intercedens* Lampert) displaying broad range of antibacterial, antifungal and cytotoxic activity (14), have been isolated.

Although the focus of study on marine organisms such as echinoderms and holothuroids is increasing, information regarding exploitation and fishing techniques in Iran is scanty and recent. However, due to the expansive coastal area of Iran, most coastal cities have some species of Holothurians (15). Since sea cucumbers are not popular in Iran, out of 1400 globally-recorded (16) Holothurian species, so far only 20 have been recorded in Iran (17). The most harvested sea cucumber in Iran is the sandfish,
H. scabra or Khiar Daryael (as it is called in the local language), harvest of which began in 2004 at Qeshm Island (18).

2. Objectives

Since the published data about the antimicrobial activity of sea cucumbers in Persian Gulf and Oman Sea is very scarce, the aim of this study was to evaluate the antimicrobial potential of the sea cucumber, *H. leucospilota*, collected from Qeshm and Kharg islands in Persian Gulf and Oman Sea.

3. Materials and Methods

3.1. Collection of Samples

Sea cucumbers were harvested freshly from fixed sites of two coastal cities of Kharg and Qeshm in the Persian Gulf and also from the rocky beach of Tis village in Oman Sea during March and May of 2011. Samples were rinsed with distilled water to remove debris and foreign particles. All samples were identified according to the food and agriculture organization of the United Nations (19). Following identification, all samples were maintained at -20°C until the usage time.

3.2. Processing of Samples

Initially, body wall, cuvierian organs, and coelomic fluid were separated. Then, each part was cut into several pieces and dried at room temperature in the dark, then milled to a fine powder.

3.3. Extraction of Samples

Crude methanol extracts of *H. leucospilota* were prepared by maceration of different body parts in appropriate amounts of methanol-water (50:50), mixing, and maintenance for 16 hours. Then, the mixture was filtered and the process was repeated for the second time. Finally, the two portions were pooled together. The filtrate was concentrated to dryness by rotary evaporation. The obtained powder was subjected to extraction with n-hexane, chloroform and ethyl acetate and then all the obtained powder was subjected to extraction with n-hexane. The filtrates were dried by flash evaporation.

3.4. Test Microorganisms

Test bacteria used in the study included standard strains of *Shigella dysenteriae* (ATCC 13313), *Proteus vulgaris* (ATCC 29905), *Escherichia coli* (ATCC 8739), *Salmonella typhimurium* (ATCC 19430), *Pseudomonas aeruginosa* (ATCC 9027), *Staphylococcus aureus* (ATCC 29737), *S. epidermidis* (ATCC 12229), and *Bacillus cereus* (ATCC 11778). Fungi included clinical isolates of *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *A. nidulans*, *A. restatus* and standard strains of *A. brasilienisis* (ATCC 9029) and *Candida albicans* (ATCC 10231). All standard ATCC microorganisms were procured from the institute for collection and maintenance of pathogenic and industrial bacteria and fungi of Iranian research organization for science and technology (IROST), Tehran, Iran.

3.5. Antibacterial Assay

Various extracts of different body parts were assayed against the test bacteria using the spot test (20). Initially, various concentrations (2000 - 40000 µg/mL) of different dried extracts were prepared in sterile distilled water, then 1 mL of each extract was transferred to a sterile petri plate, 19 mL of molten Mueller-Hinton agar medium was added, the contents of plates were gently rotated and allowed to solidify to give the concentration range of 100 - 2000 µg/mL. Microbial suspensions for each test microorganism were prepared and adjusted according to 0.5 McFarland standard. Ten microliters of each tested bacteria was deposited on the surface of each plate. Bacteria were incubated at 37°C for 24 hours. At the end of the incubation period, plates were examined for growth and minimal inhibitory concentration of each extract was determined. All assays were repeated two times.

3.6. Antifungal Assay

Before starting the antifungal assay, each test strain was cultured on Sabouraud’s dextrose agar and incubated for 1 - 2 days (*C. albicans*) or 2 - 3 days (*Aspergillus spp.*) at 37°C. For *Aspergillus* spp, suspensions of each strain in 3 mL of sterile distilled water containing 500 ppm Tween 80 (Merck, Germany) were prepared by vortex mixing. After filtration through a sterile celfte to remove the hyphal fragments and residual agar, the starting inocula were adjusted to 10⁴ CFU/mL by sterile distilled water using spectrophotometry at 530 nm. Preparation of *C. albicans* was done in the same manner without using Tween 80 (21, 22).

Stock solutions of the extracts were prepared in sterile water. The resulting solutions were progressively double diluted with the test medium (RPMI 1640 (Sigma, USA) supplemented with L-glutamin without sodium bicarbonate and buffered to pH 7 with 0.165 M (35.54 g/L) morpholinesulfonic acid (MOPS)) to give the final concentration range of 100 - 2000 µg/mL. Blanks were prepared in the test medium using the same quantities of water, but without the test extracts. One milliliter of each strain was mixed with 1 mL of media-containing test extracts or in 5 mL culture tubes and tubes were incubated at 37°C for 48 hours. The MIC values were determined as the lowest concentration of the test extract with no visible growth.

4. Results

Hydroalcoholic extract (50%) of *H. leucospilota* exhibited antibacterial and antifungal activity against some of the test microorganisms (Table 1). Results with antibacterial
effects showed microbistatic effects rather than micro-
cidal. The highest activity was exhibited by the body wall, 
Cuvierian organs, and coelomic fluid against E. coli, S. 
typhi, S. aureus and P. aeruginosa. Coelomic fluid also in-
hibited the growth of these bacteria, but at a slower rate. 
Hydroalcoholic extract of the three examined body tissues 
did not inhibit the growth of S. dysenteriae, P. vulgaris, B. 
ceerus and S. epidermidis.

Table 2 depicts the results of all extracts examined for 
antifungal activity. As presented in this Table, cuvierian 
extracts did not inhibit the fungal growth even at a higher 
concentration (2000 µg/mL). However, the body wall 
and coelomic fluid inhibited the growth of all fungi at both 
concentrations; nonetheless, body wall extract at 1000 µg/mL did not inhibit the growth of A. niger.

No significant difference of antibacterial and antifun-
gal activity was observed between the H. leucospilota 
collected from Persian Gulf and Oman Sea.

Table 1. Antimicrobial Activity of Various Extracts of H. leucospilota Against Different Species of Bacteria

| Test Organism | Body Wall | Cuvierian Organs | Coelomic Fluid |
|---------------|-----------|------------------|----------------|
| B. cereus     | 2000      | 1000             | 2000           |
| S. epidermidis| 2000      | -                | -              |
| S. aureus     | 2000      | -                | -              |
| S. dysenteriae| 2000      | -                | -              |
| S. typhi      | 2000      | -                | -              |
| P. proteus    | 2000      | -                | -              |
| E. coli       | 2000      | -                | -              |
| P. aeruginosa | 2000      | -                | -              |

a: no activity. 
b: antimicrobial activity.

5. Discussion

Hydroalcoholic (50%), n-hexane, chloroform, and meth-
anol extracts from different tissues and organs of the sea 
cucumber, H. leucospilota, were screened for antimicro-
bial activities against an array of Gram positive and Gram 
negative bacteria as well as fungi and molds. From all 
extracts prepared with different solvents, only the hydroal-
coholic extracts of the body wall, Cuvierian organs, and 
coelomic fluid exhibited antibacterial and antifungal ac-
tivities in vitro. Previous studies reported antimicrobial 
activities from various species of echinoderms (2, 4, 6). 
In most of the studied species, the whole bodies or body 
walls were tested. Other studies reported antimicrobial 
activities of egg extracts of Paracentrotus lividus (23) and 
Marthasterias glacialis (10). In the latter study, the active 
compound was reported to be a lysozyme. 

Our study indicates that the component(s) responsible 
for antimicrobial activity appear(s) to be concentrated 
mainly in the body wall and coelomic fluid; but little or 
no activity was observed in the cuvierian organs since 
there was moderate activity toward some species of bac-
teria and none against any of the tested fungi and molds. 
The methanol-water extracts of tissues/organs exhibited 
antimicrobial activities against some selected species of 
Gram positive and Gram negative bacteria as well as fun-
gi, suggesting that multiple factors are responsible for 
these activities. Thus, additional in-depth chemical analy-
ses are required for isolation and purification of the ac-
tive compound(s) as well as identification of their chemi-

cal nature and evaluation of their potential strength for 
novel drugs.

In conclusion, the body wall and coelomic fluid of Hol-
thuria leucospilota collected from Persian Gulf and Oman 
Sea showed weak antibacterial and antifungal effects 
against few species of human pathogenic bacteria and 
fungi undertaken in this study.

Acknowledgements

The contents of this paper have been extracted from the 
Pharm D. thesis No. 798.

Authors’ Contribution

Neda Adibpour and Abdolghani Ameri developed the 
original idea and protocol and contributed to the prepa-
ration of the manuscript; Frahad Nasr and Fatemeh 
Nematpour conducted the antimicrobial tests and prepa-
ration of the samples; collection and identification of Ho-
lothuria leucospilota was done by Arash Shakouri.

Financial Disclosure

There is no financial disclosure.

Funding/Support

This study was supported by grant No. U-90280 from 
Vice Chancellor of Research of Ahvaz Jundishapur Uni-
versity of Medical Sciences.
References

1. Bich DH, Chung DQ, Chuong BX, Dong NT, Dam DT, Hien PV, et al. The medicinal plants and animals in Vietnam. Hanoi Sci Technol Pub House. 2004;4:76.

2. Ridzwan BH, Kaswandi MA, Azman Y, Fuad M. Screening for antibacterial agents in three species of sea cucumbers from coastal areas of Sabah. Gen Pharmacol. 1995;26(7):353-43.

3. Reinhart KL, Shaw PD, Shield LS, Glover JB, Harbour GC, Koker MES, et al. Marine natural products as sources of antiviral, antimicrobial, and antineoplastic agents. Pure appl chem. 1981;53(4):795-817.

4. Bryan PJ, Mc Clintock JB, Watts SA, Marion KR, Hopkins TS. Antimicrobial activity of ethanolic extracts of echinoderms from the northern Gulf of Mexico. In: David B, Guille A, Feral JP, Roux M editors. Echinoderms. Rotterdam: Through Time Balkema; 1994. p. 17-23.

5. Haug T, Kjul AK, Styvold OB, Sandsdalen E, Olsen OM, Stensvag K. Antibacterial activity in Strongylocentrotus droebachiensis (Echinoidea), Cucumaria frondosa (Holothuroidea), and Aste rias rubens (Asteroidea). J Invertebr Pathol. 2002;81(2):94-102.

6. Andersons L, Bohlin L, Iorizzi M, Riccio R, Minale L, Moreno Lopez W. Biological activity of saponins and saponin-like compounds from starfish and brittle-stars. Toxicon. 1989;27(2):179-88.

7. Iorizzi M, Bryan P, Mc Clintock J, Minale L, Palagiano E, Maurelli S, et al. Chemical and biological investigation of the polar constituents of the starfish Luidia clathrata, collected in the Gulf of Mexico. J Nat Prod. 1995;58(5):653-7.

8. Service M, Wardlaw AC. Echinochrome-A as a bactericidal substance in the coelomic fluid of Echinus esculentus (L.). Comp Biochem Physiol B Biochem Mol Biol. 1984;79(2):366-165.

9. Canicatti C, Roch P. Studies on Holothuria polii (Echinodermata) antibacterial proteins. I. Evidence for and activity of a coelomocyte lysozyme. Experientia. 1989;45(8):756-759.

10. Leonard IA, Strandberg JD, Winkelstein JA. Complement-like activity in the sea star, Asterias forbesi. Dev Comp Immunol. 1990;14(1):39-30.

11. Services M, Wardlaw AC. Echinochrome-A as a bactericidal substance in the coelomic fluid of Echinus esculentus (L.). Comp Biochem Physiol B Biochem Mol Biol. 1984;79(2):366-165.

12. Beauregard KA, Tuong NT, Zhang H, Lin W, Beck G. The detection and isolation of a novel antimicrobial peptide from the echinoderm, Cucumaria frondosa. Adv Exp Med Biol. 2000;484:55-62.

13. Zhang N, Yi YH, Tang HF. Bioactive triterpene glycosides from the sea cucumber Holothuria fuscocinerea. J Nat Prod. 2006;69(10):1492-5.

14. Kou ZR, Yi YH, Wu HM, Wu JH, Liaw CC, Lee KH. Interceden sides A-C, three new cytotoxic triterpene glycosides from the sea cucumber Mensamaria intercedens Lampert. J Nat Prod. 2003;66(8):1055-60.

15. Shakouri A, Aminrad T, Nabavi MB, Kochanian P, Savari A, Safaihey A. New Observation of Three Species of Sea Cucumbers from Chaba har Bay (Southeast Coasts of Iran). J Biol Sci. 2009;9(2):184-187.

16. Pawson DL. Phylum Echinodermata Zootaxa, 2007; 1668.

17. Dabbagh AR, Sedaghat MR, Rameshi H, Kamrani E. Breeding and larval rearing of the sea cucumber Holothuria leucospi lata (Brandt) (Holothuria veugabunda Senlena) from the northern Persian Gulf, Iran. SPC Eche de mer Inf Bull. 2011;31:35-38.

18. Alkhami M, Elsayenough M, Khazaali A, Kamrani M, Mokhlesi A, Bastami KD. Sea cucumber fisheries of Qeshm Island, Persian Gulf. SPC Eche de mer Inf Bull. 2012;32:60-61.

19. Kelman D, Kushman Y, Rosenberg E, Kushmoro A, Loya Y. Antimicrobial activity of Red Sea corals. Marine Biol. 2006;149(2):357-361.

20. Torian V. Antibiotics in Laboratory Medicine. Baltimore: Williams and Wilkins; 2005.

21. Bolens EG, Richardson MD. Medical mycology a practical approach. New York: Oxford; 1989.

22. National Committee for Clinical Laboratory Standards Reference. Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts: Approved Standard. Villanova, PA; 1997.

23. Stabili I, Lasaguas M, Pastore M. Preliminary Study on the Antibacterial Capabilities of Eggs of Paracentro tus lividus(Echinodermata: Echinoidea). J Inver Pathol. 1996;67(2):180-182.
کارگاه‌های آموزشی مرکز اطلاعات علمی

مقاله نویسی علوم انسانی

اصول تنظیم قراردادها

آموزش مهارت های کاربردی در تدوین و چاپ مقاله