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Antimicrobial properties of sea anemone *Anthopleura nigrescens* from Pacific coast of Costa Rica

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ABSTRACT

Objective: To evaluate the antimicrobial and antifungal activities of the aqueous and partitioned extract of sea anemone *Anthopleura nigrescens* (*A. nigrescens*).
Methods: The sea anemone *A. nigrescens* was collected, minced, homogenized, lyophilized and then further partitioned with diethyl ether, acetone, ethanol and water. These fractions were evaluated for antimicrobial activity against bacterial and fungal pathogens.
Results: Acetone extract was found to produce a pronounced inhibition of 7.0 mm against *Proteus vulgaris* and diethyl ether extract inhibited *Pseudomonas aeruginosa* with an inhibition zone of 6.5 mm. In antifungal activity, ethanol extract showed good activity against *Botrytis cinerea*, *Trichoderma harzianum* and *Rhizopus oryzae* compared with other strains. Acetone and ethanol extract of *A. nigrescens* showed activity against all of pathogens tested. Slight activity was observed in the water extract with inhibition zone of 1.5 mm.
Conclusions: The present study revealed that sea anemone *A. nigrescens* may also contain some biologically active agents which have potential activity against pathogenic microorganisms.

1. Introduction

Marine ecosystems have a very large diversity of resources, most of them still partially unknown, and a few others exploited for development of new industrial and toxicological products [1,2]. These natural chemicals have always played an important role in medicine and, in particular, marine metabolites have increasingly become major players in recent drug discovery [3].

Cnidaria is a phylum containing over 10 000 species of animal found exclusively in aquatic (freshwater and marine) environment: they are predominantly marine species, of which 68% belong to the class Anthozoa [4]. Unlike other cnidarians, anthozoans do not have a medusa stage in their development. It means they are sessile marine coelenterates including solitary and colonial polyps, and need to protect themselves against the lethal consequences of environment, including microbial or fungus invasion [5].

Sea anemones, like other coelenterates, produce many biologically active polypeptides and proteins, including pore-forming toxins (cytolysins), phospholipases, proteinase inhibitors and neurotoxins [6–10]. Cytolytic toxins have attracted a great interest of researchers, because they exhibit antitumor, antimicrobial, antiparasitic and other types of biological activities due to the powerful membranolytic action [11,12]. New trends in drug discovery from natural sources have emphasized investigation of the sea anemones ecosystem to explore

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numerous complex and novel chemical entities. These entities are the source of a new lead for treatment of many diseases such as cancer, AIDS, inflammatory condition, arthritis, malaria and a large variety of viral, bacterial and fungal diseases [13,14]. According to Al-Zereini [15], because of increasing demand for the biodiversity in the screening programs seeking therapeutic drugs from natural products, there is now a greater interest in these kinds of marine metabolites.

Acuña *et al.* [16] reported the first record of the sea anemone *Anthopleura nigrescens* (*A. nigrescens*) on the Pacific coast of Central America, extending its distribution to the Pacific coast of Costa Rica. Also, *A. nigrescens* has been reported to produce reducing compounds, saponins, terpenes, steroids, amino acids, and hemolytic sphingomyelin inhibitable cytolytic toxin [17,18]. The present study aimed at determining the antimicrobial activity of tropical sea anemone *A. nigrescens* from Pacific coast of Costa Rica.

2. Materials and methods

2.1. Material collection and identification

Specimens were collected during low tide in a rocky area on the Pacific coast of Costa Rica, specifically in Mata Limón area, province of Puntarenas (latitude 9°55′01.99″ N and longitude 84°42′49.50″ W). Sea anemones were carefully detached from the rock, washed with the sea water, placed in plastic sample containers, and transported at 4 °C to the laboratory. Samples were frozen for transport to prevent decomposition, loss of concentration and their normal activity, as recommended by Haüssermann [19] and Sánchez-Rodríguez *et al.* [20]. A specimen was deposited at the Actiniaria Collection, Department of Marine Science (College of Natural and Exact Sciences, Mar del Plata National University) with reference C.A. 28.

2.2. Preparation and extraction of material

Crude extract was obtained by mincing and homogenizing the whole animal body in distilled water (1:2, w/v). The extract was cold steeped overnight at 4 °C, decanted and then filtered through Whatman No. 1 filter paper. Residues were centrifuged at 13 675 r/min for 20 min at 4 °C in an Eppendorf centrifuge 5418R. The supernatant was collected in separate cleaned glass tubes and stored at −22 °C. The resulting crude extract was lyophilized and partitioned with diethyl ether, acetone, ethanol and water.

2.3. Antimicrobial activity

The antibacterial and antifungal activity was carried out by disc diffusion assay according to the method of Bauer *et al.* [21], and Jorgensen and Ferraro [22]. Antimicrobial activity of sea anemone extract was determined against five bacterial strains *viz.*, *Pseudomonas aeruginosa* (*P. aeruginosa*), *Salmonella enterica* (*S. enterica*), *Klebsiella oxytoca* (*K. oxytoca*), *Escherichia coli* (*E. coli*) and *Proteus vulgaris* (*P. vulgaris*) and five fungal strains *viz.*, *Aspergillus fumigatus* (*A. fumigatus*), *Cladosporium cucumerinum* (*C. cucumerinum*), *Trichoderma harzianum* (*T. harzianum*), *Rhizopus oryzae* (*R. oryzae*) and *Botrytis cinerea* (*B. cinerea*). The bacterial cultures were obtained from Department of Biological Sciences at National University, Costa Rica.

2.3.1. Disc diffusion assay

The strains of microorganisms were inoculated in conical flask containing 100 mL of nutrient broth. These conical flasks were incubated at 37 °C for 24 h and were referred to as seeded broth. Media were carried out using Muller Hinton agar, poured on Petri dishes and inoculated with the test microorganisms from the seeded broth using cotton swabs. Sterile discs of 6 mm width were impregnated with 20 µL of test extract and introduced onto the upper layer of the seeded agar plate. The plates were incubated at 37 °C for 48 h. The susceptibility of the test strain was recorded by measuring the width of inhibition zone around the discs using vernier caliper. Amoxicillin and ketoconazol were the positive standards. Dimethylsulfoxide was used as negative control [23].

3. Results

The results of the antibacterial and antifungal activity of the extracts of sea anemone *A. nigrescens* against the tested pathogens were given in Tables 1 and 2.

3.1. Antibacterial activity

Different degrees of antibacterial inhibition against pathogenic bacteria were obtained with the extracts of *A. nigrescens*. The growth inhibition zone measured ranged from 1.0 to 7.0 mm for all the sensitive bacteria. The acetone extract exhibited the highest antibacterial activity with inhibition zone of 7.0 mm against *P. vulgaris*. Similarly, the diethyl ether extract inhibited the growth of *P. aeruginosa* with an inhibition zone of 6.5 mm.

Table 1
Antibacterial activity of *A. nigrescens* extract against human pathogens.

Pathogens	Zone of inhibition (mm)				
	DE	A	E	W	SD
P. aeruginosa	6.5 ± 0.5	3.0 ± 0.5	4.5 ± 0.5	1.5 ± 0.5	± 2.14
S. enterica	1.5 ± 0.5	3.0 ± 0.5	1.5 ± 0.5	R	± 0.87
K. oxytoca	1.5 ± 0.5	3.5 ± 0.5	3.5 ± 0.5	1.0 ± 0.5	± 1.31
E. coli	3.0 ± 0.5	2.5 ± 0.5	4.5 ± 0.5	1.0 ± 0.5	± 1.44
P. vulgaris	R	7.0 ± 0.5	2.5 ± 0.5	R	± 3.18
SD	± 2.36	± 1.82	± 1.30	± 0.29	

DE: Diethyl ether; A: Acetone; E: Ethanol; W: Water; SD: Standard deviation; R: Resistance; no activity recorded. Vertical SD shows the difference among the pathogens; horizontal SD shows the difference among the solvents.

Table 2
Antifungal activity of *A. nigrescens* extract against fungal pathogens.

Pathogens	Zone of inhibition (mm)				
	DE	A	E	W	SD
A. fumigatus	3.5 ± 0.5	1.5 ± 0.5	2.0 ± 0.5	R	± 1.15
C. cucumerinum	2.5 ± 0.5	3.5 ± 0.5	3.5 ± 0.5	1.0 ± 0.5	± 1.18
T. harzianum	2.5 ± 0.5	2.0 ± 0.5	4.5 ± 0.5	1.0 ± 0.5	± 1.47
R. oryzae	R	2.5 ± 0.5	4.5 ± 0.5	2.0 ± 0.5	± 1.32
B. cinerea	R	1.5 ± 0.5	5.0 ± 0.5	1.5 ± 0.5	± 2.02
SD	± 0.58	± 0.84	± 1.40	± 0.48	

DE: Diethyl ether; A: Acetone; E: Ethanol; W: Water; SD: Standard deviation; R: Resistance; no activity recorded. Vertical SD shows the difference among the pathogens; Horizontal SD shows the difference among the solvents.

As shown in Table 1, the ethanol and acetone extract showed activity against all the pathogens tested. On the other hand, the water extract was practically zero; it showed the minimum activity or slight activity against *K. oxytoca* and *E. coli* (1.0 mm). No activity was recorded in water extract against *S. enterica* and *P. vulgaris*, neither diethyl ether extract against *P. vulgaris*.

3.2. Antifungal activity

The growth inhibition zone measured ranged from 1.0 to 5.0 mm for all the sensitive fungal pathogens. The ethanol extract showed the highest antifungal activity with inhibition zones of 5.0, 4.5 and 4.5 mm against *B. cinerea*, *R. oryzae* and *T. harzianum*, respectively. Water extract showed the minimum activity [(1.0 ± 0.5) mm] against *C. cucumerinum* and *T. harzianum*. As shown in Table 2, the acetone and ethanol extract exhibited activity against all the fungal pathogens tested. No activity was observed in diethyl ether extract against *R. oryzae* and *B. cinerea*, neither water fraction against *A. fumigatus*. Dimethylsulfoxide also had no effect on the growth of any of the 10 microorganisms.

4. Discussion

The antibacterial and antifungal activities were significant in the crude extract of *A. nigrescens*. Pronounced inhibition was conferred by the acetone extract against the bacterial pathogen *P. vulgaris* with zone of 7.0 mm, and the same way, diethyl ether extract of *A. nigrescens* was found to inhibit *P. aeruginosa* with a maximum zone of 6.5 mm. Thangaraj *et al.* [24] reported similar activity in acetone and butanol extract of *Stichodactyla mertensii* against *E. coli* and *Proteus mirabilis* in methanolic extract. Rajak [25] and Balaji [26] found similar properties of sea anemone toxin from *Paracondactylis indicus*, *Paracondactylis sinensis*, *Heteractis magnifica* and *Stichodactyla haddoni* (*S. haddoni*). In the case of antifungal activity, the ethanol extract showed inhibition zones of 5 mm against *B. cinerea*. The antifungal properties of plant essential oils against *B. cinerea* were tested by Wang *et al.* [27] and detailed studies were conducted regarding *in vitro* activity of eugenol on *B. cinerea*. Antifungal activity on *B. cinerea* of flavonoids and diterpenoids isolated from the surface of *Pseudognaphalium* spp. was reported by Cotoras *et al.* [28]. Thangaraj *et al.* [24] reported similar activity of sea anemones *Stichodactyla mertensii* and *Stichodactyla gigantea* against *B. cinerea* in methanolic extract.

John *et al.* [29] reported the antibacterial activity of sea anemone *Anthopleura elegantissima* and *S. haddoni* extracts against human pathogens, which revealed the fact that they have higher potential to produce broad-spectrum antibacterial activity with minimal concentration in non-polar extracts against a wide range of human pathogens. As an earlier report has been made, the crude extract of *S. haddoni* showed good activity against Gram-negative bacteria by Sureshkumar *et al.* [30]. Williams *et al.* [31] reported that the tissue extract of sea anemone showed high inhibition (20 mm) against *Klebsiella pneumonia* and 24 mm with the hexane tissue extract against fish pathogen.

The results of the inhibitory effect were significant with a broad activity in acetone and ethanol extract in antibacterial and antifungal tests. The evidence indicates the metabolites responsible for such effect are soluble in these solvents, which agrees with Saad *et al.* [32], Raja *et al.* [33], John *et al.* [29], Thangaraj *et al.* [24] and Madhumitha and Saral [34], who indicate that the bioassay conducted with different organisms using low polar solvents as extraction solvent showed a better response as antibiotics in general than those performed with ethanol or any other solvent with higher polarity.

Marine organisms collected from Pacific coast of Costa Rica have shown a number of biological activities. This is the first report demonstrating the antibacterial and antifungal activity of *A. nigrescens* with these pathogens. The inhibitory effect of the extracts in *A. nigrescens* against pathogenic bacteria strains can introduce the marine organisms like sea anemones as an alternative in future studies for drugs development for the treatment of ailments caused by these pathogens. Furthermore, the recent research studies demonstrate that the Costa Rican coastline is a potential source to identify new cellular targets for further investigation.

Conflict of interest statement

We declare that we have no conflict of interest.

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