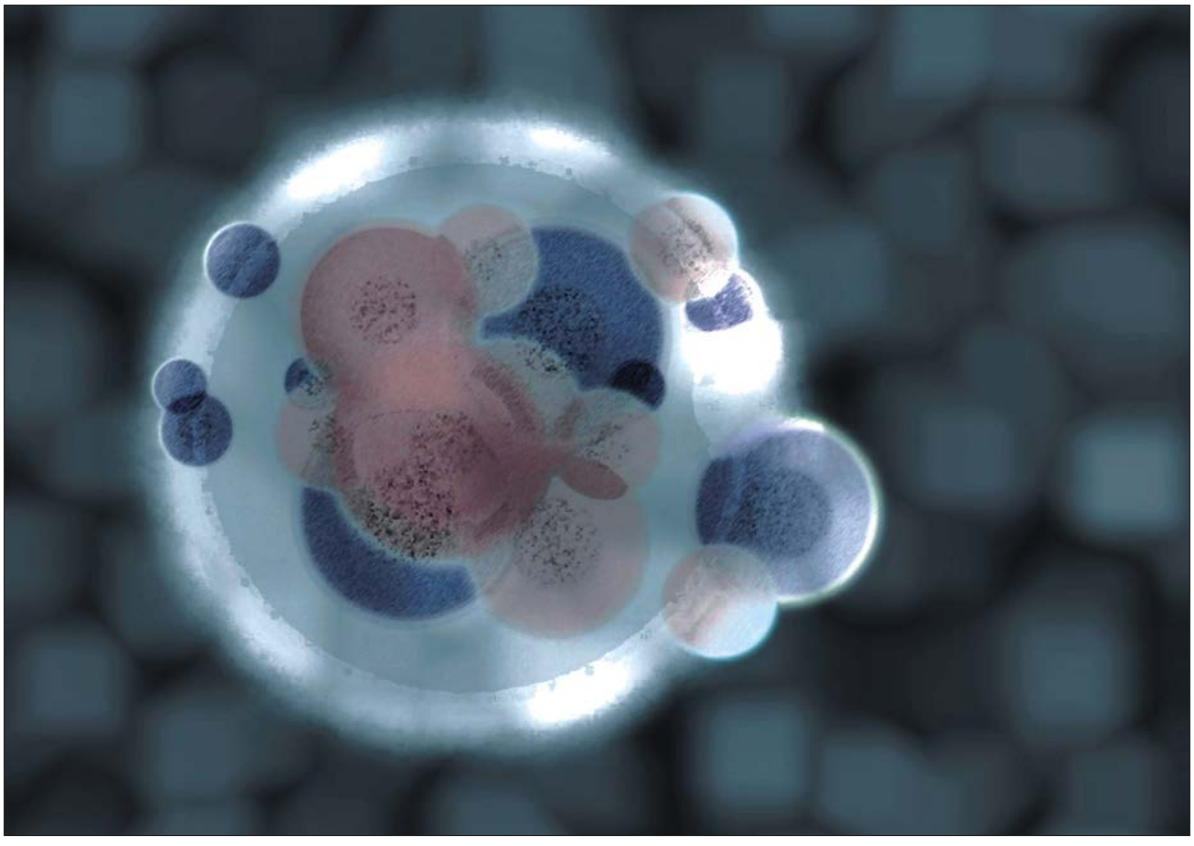

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Short Communication
Biological Activities from the Marine Sponge *Suberites aurantiacus*

[1]Sara García-Davis, [2]Mauricio Muñoz-Ochoa, [1]Catalina Rivas-Morales and [1]Ezequiel Viveros-Valdez

[1]Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León, San Nicolás de los Garza, N.L, México
[2]Instituto Politécnico Nacional, Centro Interdisciplinario de Ciencias Marinas, La Paz, B.C.S, México

Abstract

Background and Objective: Invertebrates comprises more than fifty percent of marine animals. Among them, sponges are the most prolific source of bioactive compounds. *Suberites aurantiacus* (*S. aurantiacus*) is an abundant sponge in Mexican Pacific, which has been scarcely studied, for this reason authors screened it for its bioactivities. The current study proposed to determine the enzymatic inhibition and scavenger, toxic and antibacterial activities from *Suberites aurantiacus*. **Materials and Methods:** *S. aurantiacus* samples were collected in Magdalena Bay, Mexico. The ethanolic extract and its fractions were assayed for their antioxidant effect using DPPH, ABTS and NO assays, evaluated their toxicity against *Artemia salina* (*A. salina*) and their antibacterial activity against *Staphylococcus aureus* (*S. aureus*), *Bacillus subtilis* (*B. subtilis*), *Enterococcus faecalis* (*E. faecalis*), *Enterobacter aerogenes* (*E. aerogenes*), *Escherichia coli* (*E. coli*) and *Klebsiella pneumoniae* (*K. pneumoniae*) as well as their inhibitory effect on α-glucosidase and α-amylase. **Results:** The results showed median lethal doses (LD_{50}) against *A. salina* < 1 mg mL^{-1} for two fractions and a moderate antibacterial activity against Gram positive bacteria. **Conclusion:** In view of these results, *S. aurantiacus* could be considered a potential source of antibacterial compounds.

Key words: Sponge, bactericide, toxicity, antioxidant, enzyme inhibition

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Corresponding Author: Ezequiel Viveros-Valdez, Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León, San Nicolás de los Garza, Nuevo León, CP 66450, México Tel: 52+(81)- 8329 4110

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Natural products have been explored over the years for their potential applications as food, fragrances, pigments, insecticides and drugs[1]. Most of the efforts to discover new bioactive metabolites have been focused in plants due to their relative ease of access. However, since the development of appropriate scuba techniques, about 50 years ago, the marine environment has been intensely explored as a source of new bioactive agents[2].

This environment covers a wide range of temperatures, pressure and nutrients, housing extensive photic and aphotic zones[3], constituting a source of countless products whose properties include antiviral, antibacterial, antiprotozoal, antifungal, cytotoxic and antitumor activities[4].

Marine invertebrates comprise approximately 60% of all marine animals, one of the groups with the highest number of bioactive compounds reported, most of them produced as defense mechanism. Due to their lack of swimming speed and physical defense, synthesizing compounds that can be used to discourage predators confers them an adaptive advantage in marine environment[5].

Sponges (phylum Porifera) are the oldest multicellular animals and they are present in abundance in tropical reefs, polar latitudes, freshwater lakes and rivers[6]. More than 5,300 metabolites has been isolated from sponges[7], representing 48.8% of all the new marine invertebrates natural products discovered between 1990 and 2011[8].

Suberites aurantiacus sponge belongs to the Demospongiae class, the Hadromerida order and the Suberitidae family. It inhabits estuarine environments and semi-enclosed bays and is one of the most abundant species in Mexican Pacific estuaries[9]. In addition, it is known that *Suberites* sponge produce principally sesterpenes[10-14], nevertheless, there is a lack of biological activity studies concerning sponges from the *Suberites* genus in Mexico. For these reasons, current study proposed to determine the enzymatic inhibition and scavenger, toxic and antibacterial activities from *Suberites aurantiacus.*

MATERIALS AND METHODS

Preparation of *Suberites aurantiacus* extract and fractionation: Sponges were collected in June, 2009 in Magdalena Bay, Baja California Sur at 1.5 m of depth. Dr. Enrique Ávila Torres collected and identified the specimens, 675 g of animal material were used to obtain an ethanolic extract by maceration. The ethanolic extract was partitioned with water:butanol (1:1), obtaining an aqueous partition (22.79 g) and butanol (4.72 g). The organic phase was mixed and evaporated with silica gel, then fractionated using a stepwise gradient of CH_2Cl_2:EtOH (100:0-0:100). Posteriorly, a Thin Layer Chromatography silica gel (TLC plates) was performed and all the fractions were compared and grouped it into four fractions: E1Bt1 (1.5 g), E1Bt2 (1.2 g), E1Bt3 (1.4 g) and E1Bt4 (0.66 g).

Antibacterial activity: The antibacterial activity was determined by the disk diffusion method using Mueller Hinton agar. Gram-positive (*S. aureus*, *B. subtilis*, *E. faecalis*) and Gram-negative bacteria (*E. aerogenes*, *E. coli*, *K. pneumoniea*) standardized suspension (1.5×10^8 CFU mL^{-1}) was applied on the solidified culture medium and sterile paper disks (6 mm) were impregnated with 1.5 mg/disk of the samples. The disk were transferred onto the inoculated agar plates and incubated at 37°C/24 h, then the resulting inhibition zone (clear area without bacterial growth including the disk)[15]. Positive and negative controls were gentamicin (15 µg/disk) and dimethyl sulfoxide (DMSO), respectively.

Antioxidant activity: DPPH (2,2-Dipheyl-1-picrylhydrazyl) radical scavenging activity was determined using 100 µL of samples (from 2-0.12 mg mL^{-1}) and 100 µL DPPH (0.12 mg mL^{-1}) in microplate. The reaction was completed after 30 min in the dark at room temperature and the absorbance was read at 517 nm, noting a color to turn from purple to yellow[16]. Positive and negative controls were trolox and methanol, respectively.

To determine ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) radical scavenging activity, the cation was prepared by mixing 10 mM ABTS stock solution with 2.45 mM potassium persulfate and leaving the mixture for 16 h. The ABTS solution was diluted with ethanol to get an absorbance of 0.7 ± 0.05 at 734 nm. The assay was conducted adding 200 µL of ABTS solution and 20 µL of tested samples (0.25-2 mg mL^{-1}), measurements were taken at 734 nm after 6 min in dark[16]. Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was the control.

Nitric oxide (NO) scavenging activity was analyzed using the Griess reaction in microplate. Fifty microliter of sodium nitroprusside (5 mM) was mixed with 50 µL of the samples (2-0.12 mg mL^{-1}) and it was incubated at room

temperature for 2 h. After that, 100 µL of Griess reagent was added and the absorbance was read at 550 nm. Two controls were added: NO production (PBS) and NO inhibition (trolox).

Toxic activity: Toxic activity was determined by *Artemia salina* bioassay. Brine shrimp eggs were hatched in artificial sea water and after 48 h incubation in a warm room (27°C), nauplii were exposed to three different samples concentration (1000, 100 and 10 µg mL^{-1}) containing 10 organisms/5 mL and incubated at 27°C for 24 h. Vials were then examined and the number of dead nauplii in each vial was counted. Controls were artificial sea water, DMSO (1%) and potassium dichromate[15].

Enzyme inhibition assays: The α-glucosidase inhibitory activity was evaluated according to the chromogenic method described by Kaskoos[17] with some modifications. After a pre-incubation at 37°C for 15 min, 50 µL of sample and 50 µL of α-glucosidase (0.8 U mL^{-1}) were incubated in 96 well plates at 37°C for 15 min. After that, 50 µL of 625 mM p-nitrophenyl-α-D-glucopyranoside (PNPG) solution was added to each well and incubated for another 15 min. Then, the reaction was stopped by adding 100 µL of 0.2 M $NaCO_3$ into each well and absorbance reading was recorded at 405 nm.

The α-amylase inhibitory activity was evaluated according to Sudha *et al.*[18] with some modifications. After a pre-incubation at 37°C for 30 min, 50 µL of sample and 50 µL of α-amylase (1 U mL^{-1}) was incubated in 96 well plates at 37°C for 15 min. After that, 50 µL of 0.5% starch solution in phosphate buffer was added to each well and the reaction was incubated at 37°C for 20 min. The reaction was stopped with 20 µL of 1 M HCl, followed by the addition of 50 µL of iodine reagent and absorbance was read at 750 nm.

Statistical analysis: All the experiments were performed in triplicate and the data were expressed as mean±standard deviation (SD). The median lethal dose (LD_{50}), half maximum effective concentration (EC_{50}) and half maximum inhibitory concentration (IC_{50}) values were determined using a probit regression with the SPSS version 17.0 software.

RESULTS

The present results demonstrated the scavenging effect, the carbohydrate hydrolyzing enzymes inhibition and the toxic and bactericide effect of the ethanolic extract and fractions from *S. aurantiacus*. The fraction obtained with CH_2Cl_2:EtOH (2:8) from butanolic phase showed bactericide effect only against Gram-positive bacteria, the best activity was against *S. aureus* and *B. subtilis* with 9 mm of inhibition zone. (Table 1), this sample also showed toxicity in the *Artemia salina* assay with median lethal dose of 637.9±120 µg mL^{-1} (Table 2). Nevertheless, all the samples obtained from *S. aurantiacus* possess a poorly effectiveness as DPPH, ABTS and NO radical scavengers, since the results show EC_{50} higher to 2 mg mL^{-1}. The inhibition of α-amylase and α-glucosidase show IC_{50} higher to 2 mg mL^{-1}, while acarbose IC_{50} were 0.12±0.02 and 0.97±0.08 mg mL^{-1} for α-glucosidase and α-amylase, respectively. The observed biological effect for the fraction E1Bt3 is acceptable, considering that the active compound could be diluted. It is possible that isolating the active compound or compounds will provide better effect.

DISCUSSION

Antibiotic use has contributed significantly to the increased resistance of many bacterial strains and as a consequence of an increasing demand in screening for new therapeutic drugs from natural products. There is a greater interest towards marine organisms[19], only from marine sponges more than 800 antibiotic compounds have been isolated[20]. For these reasons, authors decided to studied the

Table 1: Bactericidal activities from *Suberites aurantiacus*

Fractions	*Staphylococcus aureus*	*Bacillus subtilis*	*Enterococcus faecalis*	*Klebsiella pneumoniae*	*Enterobacter aerogenes*	*Escherichia coli*
Extract	-	-	-	-	-	-
E1Bt1	-	-	-	-	-	-
E1Bt2	7±0	8±1	7±0	-	-	-
E1Bt3	10±2	9±0	9±1	-	-	-
E1Bt4	-	-	-	-	-	-
Gentamicin	19±1	20±2	10±0	15±1	25±3	16±0

Values are in inhibition zone (mm), n = 3±SD, Fractionation obtained with gradients CH_2Cl_2:EtOH: E1Bt1(100:0), E1Bt2 (1:1), E1Bt3 (2:8) and E1Bt4 (0:100)

Table 2: Median lethal doses (LD_{50}) from *S. aurantiacus* against *A. salina*

Fractions	LD_{50} (μg mL^{-1})
Extract	>1000
E1Bt1	235.6±30
E1Bt2	>1000
E1Bt3	637.9±120
E1Bt4	>1000
Potassium dichromate	18.0±4

Medial lethal dose (LD_{50}), n=3±SD. Fractionation obtained with gradients CH_2Cl_2:EtOH: E1Bt1(100:0), E1Bt2 (1:1), E1Bt3 (2:8) and E1Bt4 (0:100)

antibacterial activity of *Suberites aurantiacus* extract and its fractions using the disk diffusion method, which showed a moderate bactericide effect against Gram positive bacteria for fractions E1Bt2 and E1Bt3, this could be due to a minority compound, the sesterpenes have been the more common isolated natural compounds from sponges with bactericidal activity[10-14]. Antibacterial studies from sponges has been reported since marine natural products started, Burkholder and Rutzler[21] assayed antibacterial activity of 31 sponges and found a major inhibition of Gram-positive bacteria, due to the permeability provided by the cell wall or to the membrane accumulation tactics of Gram negative bacteria[22], this was according to current results and may be due to the many sterols present in the *S. aurantiacus* extract[23].

Turk *et al.*[24] reported the antibacterial activity of ethanolic extracts from Antarctic marine sponges, where only 8 out from the 33 sponges were active with Minimum Inhibitory Concentration (MIC) values between 4 and 400 μg mL^{-1}, in contrast to their present study where they studied the aqueous and organic extracts from tropical marine sponges against *B. subtilis* and *E. coli* and most of the organic extracts showed MIC values between 0.1 and 36 μg mL^{-1} for *B. subtilis*[25].

According to literature, sponges produce high levels of cytotoxic chemicals by the emission of mucus containing toxins as defense of predation and against fouling organisms[26], for this reason the toxic effect of *S. aurantiacus* using the brine shrimp lethality assay was determined, which is one of the most useful tools for the preliminary assessment of toxicity/cytotoxic of organic extracts, considered good candidates for the search of antineoplastics when it shows a LD < 1 mg mL^{-1} [15,27]. Current results showed a toxic effect for fractions E1Bt1 and E1Bt3, this could suggest also two different bioactive compounds and being that fraction E1Bt2 were not active. In general, sponge extracts have shown a wide variation of lethal doses, for example, Sonia *et al.*[28], demonstrated the toxic effect from many sponges against *A. salina* nauplii and lethal doses (LD_{50}) fluctuating between 0.5 and 9 mg mL^{-1} was observed.

Natural products have been reported as carbohydrate-hydrolyzing enzymes, associating the observed effects with the phenolic content and antioxidant activity of the extracts[29]. This could explain current results, which did not show carbohydrate-hydrolyzing enzymes inhibition neither antioxidant activity. Orhan *et al.*[30] evaluated biological activity from seven marine sponges from Mediterranean sea and showed a low radical scavenging activity (>2 mg mL^{-1}). Shaaban *et al.*[31] evaluated the antioxidant activity by DPPH and NO methods and the α-glucosidase and α-amylase inhibitory effect of different extracts from four marine sponges and only one extract showed EC_{50} and IC_{50} values <1 mg mL^{-1}. Berne *et al.*[32] assayed 24 Antarctic marine sponges for a range of bioactivities, where four showed inhibitory effect on α-amylase, additionally, the extracts were tested on a variety of pathogenic and multiresistant Gram-negative and Gram-positive bacterial strains whereby all the tested sponge extracts showed weak to moderate inhibitory activities against at least one strain of the bacteria tested, nevertheless, the assay was carry out with 20 mg/disk, obtaining inhibitory zone diameter of 7-13 mm.

Although, sponges have been an interesting source of bioactive compounds, present study results and studies cited formerly, showed moderate Bioactivities from different genus of sponges. Mehbub *et al.*[33] reviewed the marine sponge derived natural products between 2001 and 2010 and reported a total of 1,615 new compounds from different orders of marine sponges with different Bioactivities, which showed some orders with over 200 bioactive compounds, meanwhile there are orders with less than 10 bioactive molecules. From Hadromerida order, the review showed 74 bioactive molecules, 2 and 25 out of them with antibacterial and cytotoxic activities, respectively.

These studies were according with current study, in which ethanolic extract and fractions of *S. aurantiacus* is not as promising as antiradical and α-glucosidase and α-amylase inhibitor. Nevertheless, it is moderately active against Gram positive bacteria and *A. salina* nauplii.

CONCLUSION

Marine invertebrates have been an important source of bioactive metabolites with a wide spectrum of interesting activities, in relation to the above, the marine sponge *S. aurantiacus* possesses compounds with bactericidal effect against Gram negative bacteria and toxic/cytotoxic activity. The best effect was showed by the fraction E1Bt3 proposing the isolation of bactericidal and/or cytotoxic compounds from these fraction for futures studies.

SIGNIFICANCE STATEMENT

The present study reports the bactericidal and toxic activities of the fractions from *Suberites aurantiacus* extract, which although it is an abundant specie in pacific coast of Mexico, it had not been study before for these bioactivities. It aims to expand upon the knowledge concerning the bioactive profile of sponges from Baja California Sur, what opens the possibility for the technological use of regional biodiversity.

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