

ANTIOXIDANT ACTIVITY OF PORIFERANS FROM THE COLOMBIAN CARIBBEAN ROCKY COAST BY DPPH AND ABTS METHOD

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Abstract

Marine invertebrates, especially the species belonging to the Phylum Porifera represents a rich source of new biological and chemical compounds of great interest. Many of these bioactive molecules have been considered to have great potential, such as antibacterial, anticancer, anti-inflammatory and antioxidant, among other potential biological activities. The main goal of this study was to evaluate the antioxidant activity of aqueous extracts from the Colombian Caribbean Poriferans: *Suberites aurantiacus*, *Desmapsamma anchorata* and *Ircinia campana*, using the in vitro models DPPH and ABTS; and the results were compared against the results obtained with the antioxidant commercial, hydroquinone. In the ABTS assay, the aqueous extracts and fractions containing precipitated proteins exhibited high inhibitory activity of radical cation showing more than 77% of inhibition in comparison with the hydroquinone, while the free radicals captation by DPPH in the *I. campana* (aqueous extract) was 146,73% when compared to the positive control (91,01%). The results suggest that the marine sponges of the rocky coast of the Colombian Caribbean are promising sources of antioxidants and others molecules with useful potential to be used in biotechnological processes.

Keywords: Bioprospecting; Antioxidant Activity; *Suberites aurantiacus*; *Desmapsamma anchorata*; *Ircinia campana*.

Introduction

Porifera, a phylum within the kingdom Animalia, constitutes a group of sessile organisms, whose main function is to filter the water [1], looking for food to survive, a process through which these organisms are exposed to themselves to a large variety of microorganisms, including bacteria, fungi and viruses, that are deleted during their passage through the sponge channels [2]. It is presumed that this interaction with other organisms facilitates the production of secondary metabolites by the sponge [3], allowing them to achieve a natural coexistence with potentially pathogenic micro-organism due to the presence of mechanisms of defenses as the innate immunity that these organisms have. There is no doubt that marine invertebrates represent produce an arsenal of new and innovative substances of great diversity [4]. This variety of bioactive compounds have been subject of studies either from a chemical point of view (alkaloids, lectin, saponin, macrolides, terpenoids, nucleoside derivatives, peptides, etc.), or in relation with their biological properties, in which there are a wide range of effects, such as: antimicrobial, antiprotozoal, antiparasitic, anticarcinogenic, antioxidant, antitumor, among others [5-12]. The Poriferans are considered a promising source of new therapeutic products [13], as natural dietary supplements, or for the cosmetology industry among others potential uses [14,15]. Besides, these study of these compounds represent a significant contribution to knowledge of biological and chemical compounds from marine animals sources to be used in medicine. The last years, the selection of antioxidant molecules that can contribute to delay the natural process of aging and help in the understanding of this biological event in the human body has increased considerably, because it is known these compounds can prevent or reduce the occurrence of cardiovascular, brain, and immune system-related diseases, among others; contributing to a better quality of life [16]. Therefore, we selected the sponges *Suberites aurantiacus*, *Desmapsamma anchorata* and *Ircinia campana* from Colombian Caribbean rocky coast, in order to evaluate antioxidant activity of their aqueous extracts.

Material and Methods

Biological Material

The sponges were collected through scuba diving in rocky shores of the Colombian Caribbean located in the town Taganga (Santa Marta – Magdalena, 11°16' 08. 89" N, 74° 11' 50. 21" O) and the

neighborhood El Laguito (Cartagena – Bolivar, 10°23'45.89", 75°33'40.33"). All samples were transported at 4°C to the laboratory; cut into small pieces, washed thoroughly with distilled water and frozen at -20°C for further analyses. The sponge identification was carried out by the Sven Zea Ph.D., Professor from Universidad Nacional de Colombia (Santa Marta/Magdalena), based on skeletal characteristics (size and shape of the spikes), features consistency and pigmentation of the specimen. Sponge species investigated in this study were: *Suberites aurantiacus 1*, *Suberites aurantiacus 3*, *Desmapsamma anchorata* and *Ircinia campana* (Figure 1). Specimens were deposited in the Porifera collection of the Marine Natural History Museum of Colombia (MHNMC, INVEMAR, Santa Marta, Colombia).

Extract preparation

Twenty grams of animal tissue were weighed (wet weight) and macerated with a mortar using 0.5 M Tris-HCl buffer solution (pH 7.5). The resulting soluble extract was centrifuged at 2400 g for 15 minutes and the supernatant recovered labeled as crude extract (EC). *S. aurantiacus* crude extract was submitted to fractionation with ammonium sulfate following a saturation gradient: 30%, 50%, 70%, 90%, centrifuged at 9600 g for 30 min at 4°C, at the final fraction dialyzed against distilled water using 3500 MWCO membrane for 24 hours [17]. Protein concentration was determined by Bradford method [18]. This assay was executed in triplicate, by adding 20 µL of EC plus 180 µL of Bradford reagent to each sample followed by 5 min of incubation at room temperature. Finally, sample absorbances were read at 595 nm using a spectrophotometer T80 + UV/VIS and compared with a BSA curve standard.

Free radical scavenging activity: DPPH test

The supernatant from Poriferans samples as well as all fractions that were described above, were diluted in proportion 1:20 (v/v) in distilled water, and centrifuged at 2400 g for 5 minutes. Due to the fact 1,1-diphenyl-2-picril-hidrazilo (DPPH) is rapidly degraded by light [19], it was prepared in methanol analytical grade minutes before the free radical DPPH procedure was started. Test was set up using 25 µL of each sample diluted (1:20) in water and 100 µL of DPPH dissolved in methanol. After 30 minutes of incubation under dark at room temperature conditions, the capacity of the samples to trap the radical DPPH at 517 nm was measured, using a spectrophotometer Multiskan Go (Skandit Software Version 3.2.1.4 RE 10040) [14,20-22], As blank was

prepared the crude extract with ultra-pure water and DPPH solution in order to eliminate the absorbance of the extracts at this wavelength. Discoloration was compared with a solution of hydroquinone in methanol 1000 mg/L (positive control) [23]. The assay was performed in triplicate and the negative control used in this case was DPPH and water.

Results were expressed in the percentage of discoloration of DPPH by using the following expression:

$$\%A.A = \frac{\text{Abs. Control (-)} - \text{Abs. Extract}}{\text{Abs. Control (-)}} \times 100$$

Inhibitory activity of the radical cation (ABTS)

The inhibitory activity of the radical cation ABTS by aqueous extracts and fractions from Poriferans samples were conducted following the method described by Re and collaborators (1999) [24] with some modifications. Initially, ABTS reagent was dissolved in water at a concentration 3.5 mM and mixed with 2.45 mM potassium persulfate. This solution was made 12 hours prior to testing, kept at room temperature and covered with aluminum to protect the solution from light. Absorbance of ABTS solution was adjusted to 0.7 ± 0.02 at 732 nm using ethanol. Each test was assayed using 6 μ L of sample and 194 μ L of ABTS solution. The solvent was used as negative control, and a solution of Hydroquinone (1000 mg/L) in ethanol was used as positive control. Absorbance was read after 30 minutes at 732 nm using a spectrophotometer Multiskan Go. Antioxidant capacity was calculated with the equation described above.

Statistical

For statistical analysis, descriptive analysis, normal distribution, homogeneity of variance and difference between treatments unadjusted p-value were performed using R (R Core Team 2015). Plots were developed using the package ggplot2 to R statistical program.

Results and Discussion

Natural extracts have been considered an important source of compounds with potential to be used for manufacturers of food, cosmetics and pharmaceuticals. For this reason, it is important to evaluate aqueous extracts of Poriferans for the presence of these kind of metabolites in order to provide alternatives and solutions to these needs. Antioxidants have multiple mechanisms of action,

depending on the reaction system or the radical source [14,25]. The aim of this study is to evaluate the antioxidant properties (ABTS and DPPH) of sponges from Colombian Caribbean Rocky Coast.

Protein concentrations of the aqueous extracts showed that *D. anchorata* had 1.4 mg/mL, *I. campana* 3.1 mg/mL, *S. aurantiacus 1* 1.9 mg/mL, *S. aurantiacus 3* 4.6 mg/mL and *S. aurantiacus* fractions 30, 50, 70 y 100, were of 0.179 mg/mL, 0.282 mg/mL, 0.400 mg/mL and 0.077 mg/mL, respectively. All aqueous extracts and fractions were initially assessed by their antioxidant capacity using the radical cation ABTS. The spectrophotometric-based method is the most applied to determine total antioxidant activity of pure substances, aqueous mixtures and beverages [24]. Likewise, this is one of the most effective techniques when working with aqueous extracts and samples precipitated from Porifera. During the reaction the blue radical ABTS became colorless (neutral) to the aqueous extracts from *S. aurantiacus 1*, *S. aurantiacus 3*, *D. anchorata* and *S. aurantiacus* fractions 30%, 50%, 70% and 100%. In this case, all samples showed antioxidant activity greater than Hydroquinone (99,84%) used as positive control. However, *I. campana* showed 77,26% (Figure 2). Extracts and fractions used in the ABTS test in vitro showed free radical scavenger activity, a result that is similar to ABTS radical antioxidant activity of 80% with 670 μ g/mL reported by Núñez *et al.* (2006) [26], for hydroalcoholic extract obtained from sponge *Ircinia* sp. an specie from the Caribbean Sea considered of biomedical interest. In addition, Montañó and Santafé (2011) [7] mentioned that marine organisms from Colombian Caribbean and especially sponges of the genus *Ircinia* sp. are promising source of compounds with potent antioxidant activity as evidenced in its preliminary test, where the percent inhibition of radical ABTS showed by methanol extracts of the sponges *Ircinia felix*, *Amorphinopsis atlantica* and *Mycale microsigmatosa* were 60.8%, 53.1% and 66.9%, respectively.

The antioxidant capacity was also assessed by the DPPH method. Hydroquinone, used as positive control in our assays showed 91.01% of antiradical efficiency, while the aqueous extract *I. campana* showed superior activity than the positive control with 146.73% and the fractions 30% and 50% exhibited free radical scavenging activity higher than 50% (Figure 3). In contrast, *S. aurantiacus 1*, *D. anchorata* and both 70% and 100% fractions showed low antiradical efficiency ranged between 21.27% and 48.47%. In synthesis, *S. aurantiacus 3*, *I. campana* and fractions at 30% and 50% of saturation

showed antioxidant activity higher than 50%, corresponding to half of the activity presented in the positive control (Hydroquinone) (Figure 3). Similarly, when extracts from *Ircinia sp.* were tested by DPPH assay the activity results showed 83.0 mg/mL and reducing the ferric ion with 52.0 ± 0.8 mg AA/g [27]. Another research conducted using chloroform and an aqueous extract of poriferan *Callyspongia sp.* from Mandapam coast (Tamil - Nadu) also demonstrated to be able to reduce the stable radical DPPH to yellow, indicating the presence of antioxidant molecules [28]. Moreover, in the organic extract (ethyl acetate) of the Hyrtios sponge three alkaloids called Hyrtioerectines were isolated. Compounds D and F from this red sea specimen showed free radical scavenging of 45% and 42%, respectively by using the DPPH method. Compound E only showed 31% of antioxidant capacity which is considered moderately active [29]. During search of antioxidants from ethanolic extract of *Iotrochota sp.* it was possible to observe purple points on a thin layer chromatography which reduced the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). ($IC_{50} = 183.97 \mu\text{g/mL}$), allowing the isolation of a purpurone-like compound which has potent antioxidant activity $IC_{50} = 19 \mu\text{m}$ [30].

In general, antioxidant activity treatments did not come from a normal distribution; therefore, Kruskal-Wallis (non-parametric test) was used to determine the differences. The results show that there are significant statistical differences between treatments in each method for ABTS and DPPH with p-values of 0.005 and 0.001, respectively. In addition, no significant statistical differences between treatments to DPPH ($p < 0.100$) and ABTS methods ($p < 0.100$) were observed when Wilcoxon test for multiple comparisons was performed.

It was found that aqueous extracts and fractions evaluated resulted highly promising according to the techniques that were evaluated. Although, each method has different reaction conditions and solubility [14]; for these reasons, some results showed free radical scavenging (DPPH) below 50% for the samples *S. aurantiacus 1*, *D. anchorata* and *S. aurantiacus* fractions 70% and 100%, while all treatments except *I. campana* showed activity values higher than 75% by ABTS assay. It could be deduced that extracts from these poriferans contains phenolics compounds due to the fact that ABTS strongly reacts with a hydrogen donor atom, such as phenols which transforms the substance, a chemical reaction that is evident from the change from blue-green to a colorless substance. It is important to mention that the ABTS is fairly stable

in the absence of phenolic compounds, although probably DPPH is more selective than the ABTS in the reaction with donors of hydrogen and it has been seen that in the case of the polyphenols, the antioxidant capacity significantly changes between a method and another [31]. Extracts and fractions evaluated in this study showed antioxidant activity by using two different methods (DPPH and ABTS). The results obtained in this study show that aqueous extracts of *S. aurantiacus*, *D. anchorata* and *I. campana* promise to be a natural and valuable source of antioxidants as evaluated in both methods (DPPH and ABTS). It is important to point out that these extracts are complex mixtures of secondary metabolites that could become the target of future research approaches focused on the isolation and characterization of compounds with significant biological activity against the radical ABTS and DPPH. No researches related with the antioxidant capacity of aqueous extracts from the genera *Suberites*, *Desmapsamma* and *Ircinia* have been reported so far. Although, numerous reports with organic solutions were found, showing evident activity at various concentrations and significant differences between them. These results could be the consequence of different factors such as: geographical areas, depth, salinity and temperature of those areas where samples were collected.

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Figure 1. Poriferans samples collected from Rocky Shores of the Colombian Caribbean. **A.** *Suberites aurantiacus1*, **B.** *Suberites aurantiacus3*, **C.** *Desmapsamma anchorata*, **D.** *Ircinia campana*

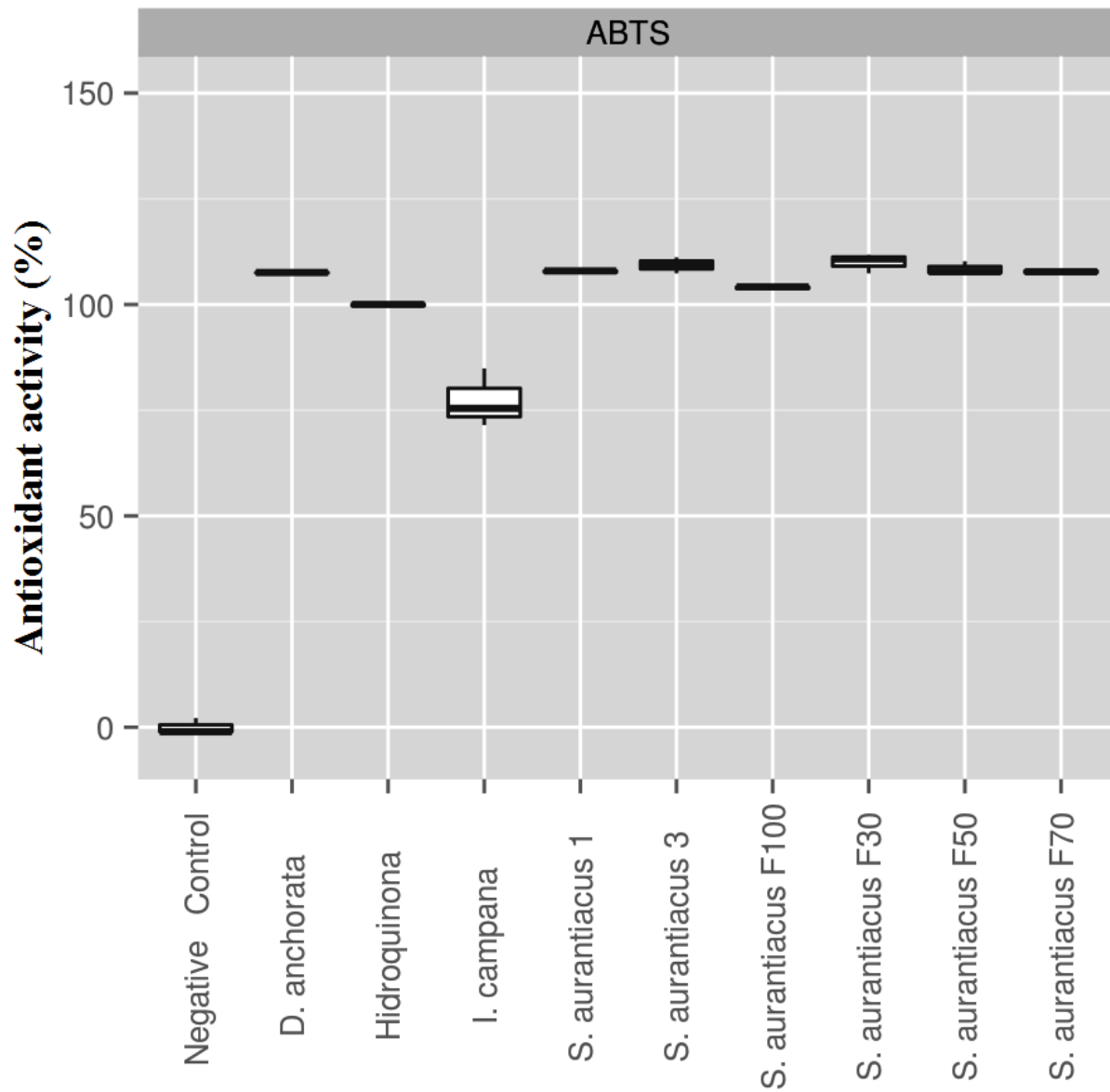


Figure 2. ABTS radical cation scavenging of the aqueous extracts and fractions from Poriferans selected. Hydroquinone was used as positive control.

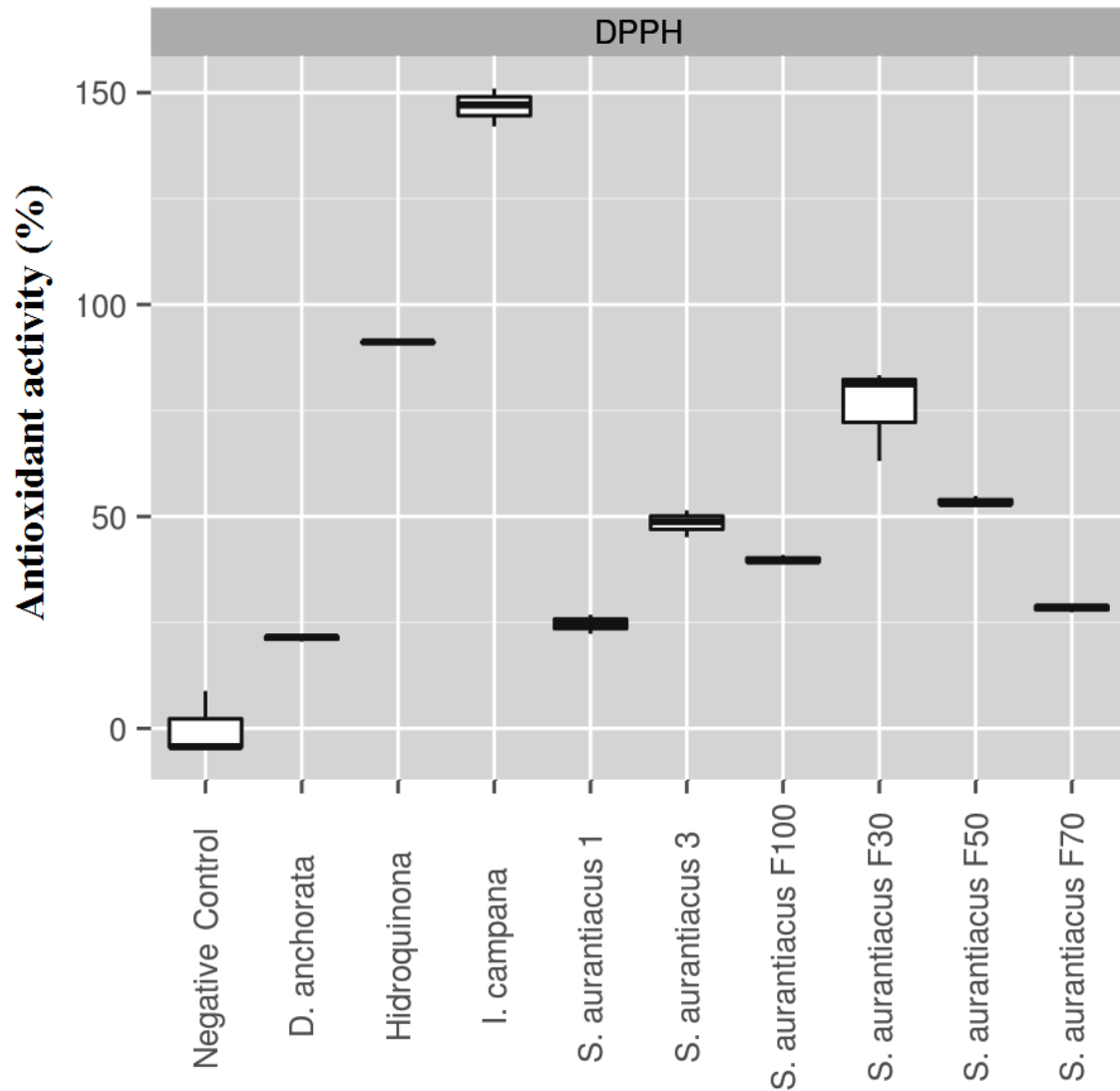


Figure 3. Free radical scavenging activity on DPPH test of the aqueous extracts and fractions from Poriferans selected. Hydroquinone was used as positive control.