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Research Article

Antioxidant capacity of fungi associated with corals and sponges of the reef system of Veracruz, Mexico

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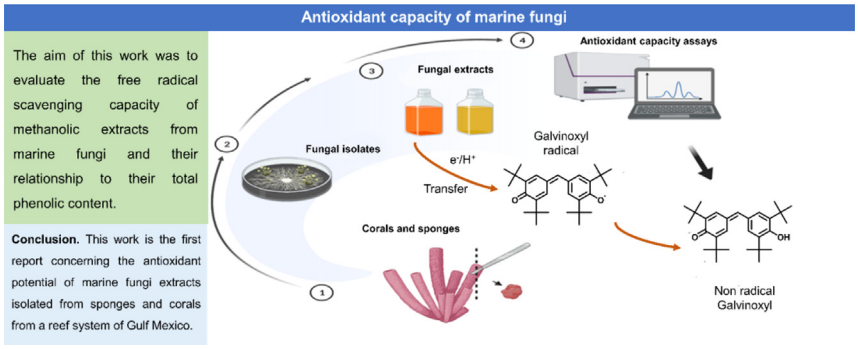
GRAPHICAL ABSTRACT



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ABSTRACT

Background: Marine fungi are considered as a promising source of pharmacologically important extracts and compounds owing to the new chemical structures that they can synthesize due to the environmental conditions of their habitat. The aim of this work is to evaluate the free radical scavenging capacity of methanolic extracts from marine fungi and their relationship to their total phenolic content. For this, the radical tests ABTS, DPPH, and Galvinoxyl were used, comparing these results with the antioxidant Trolox as reference. The total phenol content was quantified using the Folin-Ciocalteu method. All data were analyzed by ANOVA followed by Tukey's post hoc tests ($p < 0.001$).

Results: The results indicate that *Fusarium oxysporum* broth extract (Apl) showed a greater capacity for free radical scavenging when compared with the Trolox standard (128% ABTS) as well as the biomass extract of *Cladosporium cladosporioides* (A.c) with values of (107 % ABTS and 102% Galvinoxyl). In addition, the variation found in the total phenolic content for each bioactive extract suggests that their antioxidant activity is not exclusively related to phenolic compounds and hence might be attributed to other types of metabolites.

Conclusions: This work is the first to report the antioxidant capacity of marine fungi isolated from sponges and corals in Mexico. These results, we consider, support the selection, conservation, and use of marine fungi as an alternative source of phenolic and non-phenolic compounds that could be used in pathologies such as oxidative stress and cancer, among others.

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1. Introduction

Oxidative stress is defined as an increase in the concentration of oxidative species in biological systems due to uncontrolled production or decrease in consumption of these species, which is directly or indirectly related to damage to biologically important molecules such as proteins, nucleic acids, and lipids [1,2,3,4,5]. The term "antioxidant activity" originally comes from the field of chemistry and later was adapted to biology, medicine, epidemiology, and nutrition fields. The concept is employed to describe the capacity of reducing the concentration of oxidant species by certain molecules in food and biological systems [6,7,8]. This molecular property can be explained by two mechanisms: primarily, where the concentration of the oxidant is reduced through a direct reaction (e.g., polyphenols or some enzymes such as superoxide dismutase), and on the secondary level, when an antioxidant molecule reduces the concentration of oxidants by inhibiting the production routes of such molecules (e.g., metal chelators) [9,10,11,12].

Among natural antioxidants, we can find phenolic compounds. These compounds have a wide array of useful properties such as anti-cancer, anti-inflammatory, anti-microbial, anti-viral, and anti-osteoporotic activity; it has also been reported that antioxidants offer cardiovascular protection and they could reduce the risk and complications of diabetes [9,10,11,12,13,14,15]. Because of this, the interest and the use of natural resources to carry out bioprospecting studies of medicinal, marine, and edible fungi that show important antioxidant properties have increased [16,17,18,19,20]; as an example, the aqueous and methanolic extracts from *Lentinus edodes* and *Volvariella volvacea* show antioxidant activity which is positively related to the phenolic compound content [21]. There have also been reports that the free radical scavenging capacity of the methanolic or aqueous extracts of species such as *Flammulina velutipes*, *Pleurotus djamor*, *Cantharellus cibarius,* and *Agaricus lanipes*, relating to their ability to inhibit lipidic peroxidation, was explained by their flavonoid and polyphenol content [22,23]. With regard to fungi associated with marine organisms, biologically active compounds that include antioxidant properties have also been reported. Since 2017, fungi associated with cnidarians (like corals) and sponges have been responsible for 35% of new marine natural products reported [24,25,26,27]. An example of this is the antioxidant capacity in vitro observed by non-phenolic antioxidant metabolites derived from lactones, xanthones, quinones, and hydroantraquinones isolated from marine fungi [28]. In this regard, it is known that some natural antioxidants are produced by *Penicillium roquefortii*, *Aspergillus candidus*, *Mortierella* sp., *Emericella falconensis,* and *Acremonium* species [29].

Based on the above, in this study, the free radical scavenging capacity of extracts obtained from marine fungi isolated from sponges and corals of the National Park Sistema ArrecifalVeracruzano (NPSAV) in the Gulf of Mexico was assessed, and the relationship between their antioxidant capability and the phenolic compound content of the extracts was discussed.

2. Materials and methods

2.1. Biological material and culture

The free radical scavenging potential of eight marine fungi from the culture collection of the "Centro de Investigación en Micología Aplicada" was evaluated. The identification and bioactivity from these strains on the inhibition of bacterial quorum sensing and antiproliferative activity against solid human tumor cell lines were previously reported [30,31]. The strains were as follows: *Fusarium oxysporum* isolated from *Aplysina* sp.; *F. oxysporum* isolated from *Diploria strigosa*; *Fusarium* sp., *Cladosporium cladosporioides* isolated from *Amphimedon compressa; C. cladosporioides* isolated from *Plexaura flexuosa*; *Curvularia trifolii* from *Amphimedon compressa*; *Sarocladium strictum* from *Agelas* sp.; and *Nigrospora sphaerica* from *Plexaura flexuosa*. Fungal strains were cultured and maintained in a culture media composed of (g/L): yeast extract (4), soluble stark (10), polypeptone (2), bacteriological agar (15), and marine water (75%).

2.2. Liquid fermentation and extraction

A liquid fermentation of 500 mL in Erlenmeyer flasks of 500 mL (5 × 100 mL of culture medium) was used for each strain. The flasks were inoculated with small pieces of agar around 0.5 cm in diameter with mycelium-spores of the fungus and were then incubated in an orbital shaker for 14 d at 25 ± 2 °C. After the incubation period, the biomass produced and the culture broth were separated by vacuum filtration, yielding two methanol:chloroform (1:1) extracts for each strain, one for each of the previously separated fractions [31]. The extracts were dried in a rotatory evaporator at a reduced pressure, then resuspended in methanol and employed in the free radical scavenging capability and phenol content assays.

2.3. Trolox equivalents antioxidant capacity (TEAC)

2.3.1. ABTS [azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)]

The TEAC employing *azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)* (ABTS) was carried out according to the method described by Thaipong et al. [32], with some modifications. A working solution made of equal amounts of ABTS 7.4 mM and potassium persulfate ($K_2S_2O_8$) 2.6 mM was used. Once the two solutions were mixed, the mixture was allowed to react for 12 to 16 h to form the $ABTS^+$ radical. The working solution was diluted 1:60 in methanol to be used in the assay. For each sample, 2.85 mL of the working solution was mixed with 0.15 mL of fungi extract or Trolox standard and left to react for 5 min. After that time, the decrease in absorbance was measured at 734 nm. For the calibration curve, a standard Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used for the following concentrations: 6.25, 12.5, 25, 50, and 100 μM. The extracts were tested at a concentration of 1 mg/mL. The results were obtained from the linear equation and were expressed as μM Trolox/mg extract.

2.3.2. DPPH (1,1-diphenyl-2-picrylhydrazyl)

With minor modifications, *1,1-diphenyl-2-picrylhydrazyl* (DPPH) measurement was performed in accordance with the report of Floegel et al.[8]. A DPPH 0.1 mM solution in methanol/water (80% v/v) was used, and it should be stirred for 40 min at room temperature and protected from light. After that, at room temperature and in the dark for 30 min, a reaction between 2.85 mL of DPPH solution and 0.15 mL of fungi extract or Trolox standard took place. The decrease in absorbance was measured at 517 nm. A curve of Trolox was built by employing concentrations of 25, 50, 75, 100, 150, and 200 μM. The results are expressed as μM Trolox/mg extract and were obtained from the Trolox standard curve. The samples were tested at a concentration of 1 mg/mL.

2.3.3. Galvinoxyl (2,6-Di-tert-butyl-α-(3,5-di-tert-butyl-4-oxo-2,5-cyclohexadiene-1-ylidene)-p-tolyloxy)

This assay is recommended for electron and hydrogen donor compounds and is compared with the DPPH assay. The galvinoxyl radical was found to be more reactive against phenolic compounds [33]. This technique was carried out as described by Palanisamy et al. [34] with some modifications where 2.7 mL of galvinoxyl 10 μM solution (methanolic) was mixed with 0.27 mL of fungi extract or Trolox standard and allowed to react for 20 min in the

dark at room temperature. After that, the decrease in absorbance was measured at 432 nm. The standard curve was made with Trolox concentrations of 5, 10, 15, 20, 25, and 30 μM. The samples were tested at 1 mg/mL, and the results were expressed as μM Trolox/mg extract.

2.4. Total phenolic content (TPC)

The determination of the phenolic content of the tested extracts was made using the Folin-Ciocalteu method. According to the technique, 0.2 mL of the sample or gallic acid standard was mixed with 2.6 mL of deionized water and 0.2 mL of Folin-Ciocalteu reagent, and after 6 min, 2 mL of sodium carbonate 10.75% (w/v) was added to the mix. The mixture reacts for 30 min, and the decrease in absorbance was measured at 760 nm. The blank for the assay was methanol 50% (v/v). A standard curve of gallic acid was made employing concentrations of 10, 20, 50, 70, 100, and 200 (mg/L) diluted in methanol 50%, and the samples were tested at a concentration of 1 mg/mL and diluted in MeOH. The results were expressed as Gallic Acid Equivalents per mg of extract (GAE/mg extract).

2.5. TEAC ratio

The TEAC ratio was calculated in an attempt to standardize the results and thus allow a better comparison between the three radical scavenging assays, similar to that published by Foti et al. [35], for which the following equation was employed:

$$(\%)TEAC = \frac{TEAC_{extract}}{IC_{50(assay)}} * 100$$

where: $TEAC_{extract}$ are the values in Table 1 and $IC_{50(assay)}$ (mean inhibitory concentration) are the values calculated previously for each antioxidant assay. The IC_{50} values were calculated from the regression equation of a Trolox standard.

2.6. Statistical analysis

Each individual assay was run in triplicate. Shapiro-Wilk tests were used to assess the normality assumption ($p < 0.05$). The differences between treatments were analyzed by using ANOVA, followed by Tukey post hoc tests ($p < 0.001$). All statistical analyses were performed with R software v3.2.1 (R Core Team, 2015).

3. Results and discussion

The free radical scavenging capacity was measured as Trolox micromolar solution equivalents per mg of extract (TEAC/mg extract) in three different assays (ABTS, DPPH, and galvinoxyl free radicals). In addition, the total phenolic content was measured as gallic acid equivalents per mg of extract (GAE/mg) of 16 fungal extracts corresponding to the culture broth and biomass produced by each of the 8 fungal strains derived from the NPSAV in the Gulf of Mexico. These were tested, and the results are shown in Table 1. The linear interval of each TEAC calculation performed was optimized (R^2_{DPPH} = 0.9998, R^2_{ABTS} = 0.9996, $R^2_{Galvinoxyl}$ = 0.9931) as was the TPC assay (R^2_{TPC} = 0.9973) employing Trolox and gallic acid as standards, respectively. The IC_{50} values employed in the TEAC calculation for the ABTS, DPPH, and galvinoxyl radicals were 83.5 ± 3.1; 90.6 ± 5.1 y, and 17.5 ± 2.0 μM of Trolox, respectively.

The TEAC results for the $ABTS^+$ radical showed that the biomass extracts are more active in *C. cladosporioides* (A.c) ($p < 0.001$), *F. oxysporum* (D.s) ($p < 0.001$), *Fusarium* sp. ($p < 0.001$), and *Sarocladium strictum*($p < 0.01$), with values of 89.0, 63.9, 56.6, and 54.2 μM of Trolox equivalents per mg of extract, respectively. Similarly, the broth extracts of *F. oxysporum* (Apl) ($p < 0.001$), *S. strictum* ($p < 0.01$), and *C. cladosporioides* (P.f) ($p < 0.001$) had the highest TEAC/mg of extract against the $ABTS^{+}$ radical, with values of 107.0, 74.1, and 72.8 μM, respectively. Thus, the extract with the highest TEAC against $ABTS^{+}$ was that obtained from the culture broth of *F. oxysporum* (Apl) ($p < 0.001$). Furthermore, the TEAC study with DPPH radicals shows that the most effective extracts were from the *C. cladosporioides* (P.f) biomass extract (43.4 μM) and *Fusarium oxysporum* (D.s) both broth and biomass extracts ($p < 0.001$), showing values of 44.9 and 41.6 μM of Trolox equivalents per mg of extract, respectively. Meanwhile, the TEAC results of the galvinoxyl radical assay showed that *Cladosporium cladosporioides* (A.c) biomass and *Fusarium* sp. broth extracts presented the highest radical scavenging capacity ($p < 0.001$), with 17.8 and 17.4 μM of Trolox equivalents per mg of extract, respectively. The values of total phenolic compounds of the biomass and broth for all fungal isolates were measured, but their results were not statistically significant ($p > 0.1$), see Table 1.

Thus, the statistical analysis showed significant differences for the ABTS and galvinoxyl assays, both for the fungal strain and for the type of extract, while the DPPH assay results showed an effect only among the fungal strains, with no statistical differences

Table 1
Trolox equivalent antioxidant capacity (TEAC) employing ABTS, DPPH and galvinoxyl radical methods, also total phenolic content (TPC) expressed as gallic acid equivalents (GAE) of the 8 marine fungi studied, the average values were obtained from the regression equation with $N = 3$. A.c. = *Amphimedon compressa*, Age = *Agelas* sp. Apl = *Aplysina*sp. D. s. = *Diploria strigosa*, P.f. = *Plexaura flexosa*. ABTS (a is the lowest value and j the highest); DPPH (a is the lowest value and c the highest); galvinoxyl (a is the lowest value and c the highest). Superscripts after values indicated with the same letters in each column are not different from each other at the 0.001 probability level.

Marine fungi	Extract type	TEAC(μM Trolox/mg extract)			TPC GAE/mg extract
		ABTS	DPPH	Galvinoxyl	
Cladosporium cladosporioides (A.c)	Biomass	89.0 ± 0.9^{i}	34.2 ± 0.6^{bc}	17.8 ± 1.4^{c}	78.8 ± 16.2
	Broth	59.2 ± 6.9^{fh}	29.5 ± 6.5^{ac}	16.0 ± 0.5^{bc}	72.2 ± 3.5
Curvularia trifolii (A.c)	Biomass	33.1 ± 2.5^{bc}	25.1 ± 7.0^{ab}	3.1 ± 1.4^{a}	34.9 ± 9.0
	Broth	42.6 ± 2.4^{cde}	16.3 ± 2.7^{a}	13.5 ± 2.8^{bc}	50.4 ± 5.4
Sarocladium strictum (Age)	Biomass	54.2 ± 6.0^{dfg}	33.5 ± 1.3^{bc}	5.2 ± 0.2^{a}	123.2 ± 7.3
	Broth	74.1 ± 2.8^{hi}	34.4 ± 9.8^{bc}	15.5 ± 2.2^{bc}	76.7 ± 15.2
Fusarium oxysporum (Apl)	Biomass	27.3 ± 0.3^{ab}	35.9 ± 0.9^{bc}	15.9 ± 1.3^{bc}	42.0 ± 5.1
	Broth	107.0 ± 11.1^{j}	35.7 ± 1.9^{bc}	4.1 ± 0.7^{a}	139.7 ± 9.1
Fusarium sp. (D.s)	Biomass	56.6 ± 1.7^{efg}	23.5 ± 1.2^{ab}	13.5 ± 0.4^{bc}	50.8 ± 1.6
	Broth	16.0 ± 3.7^{a}	34.3 ± 0.2^{bc}	17.4 ± 2.8^{c}	68.0 ± 6.8
Fusarium oxysporum (D.s)	Biomass	63.9 ± 2.0^{gh}	41.6 ± 6.6^{c}	15.9 ± 2.0^{bc}	39.8 ± 3.2
	Broth	40.2 ± 2.8^{bd}	44.9 ± 5.2^{c}	13.0 ± 0.7^{bc}	84.7 ± 1.5
Nigrospora sphaerica (P.f)	Biomass	44.5 ± 10.3^{cdf}	29.4 ± 2.2^{ac}	2.0 ± 0.2^{a}	27.4 ± 2.4
	Broth	25.2 ± 2.8^{ab}	29.0 ± 4.3^{ac}	14.6 ± 2.7^{bc}	65.1 ± 12.1
Cladosporium cladosporioides (P.f)	Biomass	25.4 ± 5.2^{ab}	43.4 ± 6.7^{c}	14.0 ± 2.4^{bc}	22.5 ± 1.8
	Broth	72.8 ± 0.6^{h}	36.2 ± 11.0^{bc}	11.4 ± 2.7^{b}	67.6 ± 10.0

between the biomass and broth extracts ($p < 0.001$). The TPC test results, on the other hand, were not statistically significant, suggesting a variation in the polyphenol contents of each extract. That is also supported by the Pearson correlation analysis, where TPC and ABTS had a R value of 0.59, while our values indicated that TPC and galvinoxyl had a R value of −0.18 and TPC and DPPH had a R value of 0.14.

In this sense, to represent the antioxidant capacity of the extracts vs Trolox, where those extracts with % TEAC ≥ 80 are considered efficient, we transform the data to percentage values according to the equation formulated in 2.5 TEAC ratio. The results of these analyses are shown in Table 2, the biomass extract of *C. cladosporioides* (A.c) exhibited a greater antioxidant capacity than Trolox against ABTS radicals, whereas the broth extracts from *F. oxysporum* (Apl), *S. strictum,* and *C. cladosporioides* (P.f) exhibited a %TEAC higher than 80% also against ABTS. With the exception of the broth extract from *C. trifolii*, all fungal extracts showed greater activity as DPPH scavengers than Trolox. Also, the biomass extract from *C. cladosporioides* (A.c.) presents greater radical scavenging activity than Trolox against the galvinoxyl radical, while the biomass extracts from *F. oxysporum* (Apl), *F. oxysporum* (D.s), *C. cladosporioides* (P.f), and broth extracts from *C. cladosporioides* (Apl), *S. strictum* and *Fusarium* sp. showed a %TEAC higher than 80% against the galvinoxyl radical (Fig. 1). Therefore, extracts with this value are considered efficient free radical scavengers, for example, the biomass extract of *C. cladosporioides* (A.c) has a %TEAC higher than 100%, suggesting that this extract is more active than Trolox (Table 2). In addition, the broth extract from *S. strictum* shows a %TEAC higher than 80% against the three assayed radicals. Among the three antioxidant assays performed, the galvinoxyl radical has major biological relevance because, in this case, the radical is in an oxygen atom, as well as in the reactive oxygen species, which are of biological relevance [36]. Therefore, the TEAC against that radical is considered as a criterion for indicating good antioxidant activity, thus the broth extracts from *C. cladosporioides* (A.c), *Fusarium* sp. and *N. sphaerica,* and the biomass extracts from *F. oxysporum* (Apl) and *C. cladosporioides* (P.f) fulfill this criterion.

Despite the fact that the TEAC was evaluated in three different radicals as ABTS, DPPH, and galvinoxyl, in the present study, no selectivity against the DPPH radical could be observed in comparison to the galvinoxyl and ABTS radicals. Nevertheless, it is considered that all three radicals react via hydrogen transfer and by electron transfer reactions, with the latter possibly participating via sequential proton loss electron transfer (SPLET) and sequential electron proton transfer (SEPT), both of which are in competence and the prevalence of which depends on several factors (Fig. 2). The structure of the antioxidant molecule is also a determinant factor in the preference of one of the mechanisms. The solvent in which the reaction is carried out is also a determinant factor, because it is well known that the reactions of hydrogen atom transfer are more relevant in non-polar media, while the reactions of electron transfer are more significant in an aqueous medium [37]. The assays carried out in this study consider the differences in the solvent, using absolute methanol in the case of ABTS and galvinoxyl radicals, while in the solvent in the DPPH assay was a mixture of methanol and water in an 8:2 ratio. This difference could affect the main mechanism, favoring the transfer of a hydrogen atom in ABTS and galvinoxyl radicals, while favoring electron transfer in DPPH radicals.

The pharmacological relevance of marine natural products is based on the potent biological activity derived from adaptation to their ecological environment. Therefore, they serve as a reservoir of ecological resources in which these microorganisms are can produce secondary metabolites with unreported bioactivities or chemical compounds of novel structures. Endophytic fungi metabolites *in vitro* antioxidant capacity may be related to the fungi complex ecological interaction with their marine host [28]. It has been common to report a positive ratio between total phenolic content and the antioxidant capacity of a large number of plant species [38]. This behavior has also been observed in fungi in which a relationship between the total phenolic content and the antioxidant capacity against DPPH radical of *Flammulina velupies* extract was observed [22]. It was also found that the total phenolic content of the methanolic extract of *Cantharellus cibarus* has a high antioxidant capacity for lipid peroxidation and chelant capacity [23]. Furthermore, there are reports of the production of anti-inflammatory and antioxidant compounds from extracts of the genus *Gymnascella*, *Engyodontium*, *Chaetomium,* and *Nigrospora* isolated from the marine sponge *Hippospongia communis* [39].

In our results, it can be observed that the biomass extract of *C. cladosporioides* (Apl) shows the best TEAC values for all radicals assayed, also it has a medium value of TPC and this extract also presents the highest TEAC in the galvinoxyl radical assay. *Fusarium* sp. culture broth was the second-best extract in terms of TEAC against galvinoxyl radical. The TEAC in DPPH and ABTS assays of broth extracts from *C. cladosporioides* (P.f) and *S. strictum* were related to a medium value of TPC. In the DPPH radical assay, the highest TEAC among all the fungal strain tested was that of

Table 2
TEAC Percentage of marine fungi extracts using three different assays of antioxidant activity. 100% is equal to the IC_{50} value for Trolox in each of the assays (ABTS 83.5 ± 3.1; DPPH 90.6 ± 5.1; and Galvinoxyl 17.5 ± 2.0 μM).

Marine fungi	Extract type	(%) TEAC		
		ABTS	DPPH*	Galvinoxyl
Cladosporium cladosporioides (A.c)	Biomass	107	196	102
	Broth	71	169	91
Curvularia trifolii (A.c)	Biomass	40	143	18
	Broth	51	93	77
Sarocladium strictum (Age)	Biomass	65	192	30
	Broth	89	197	89
Fusarium oxysporum (Apl)	Biomass	33	205	91
	Broth	128	204	23
Fusarium sp. (D.s)	Biomass	68	134	77
	Broth	19	196	99
Fusarium oxysporum (D.s)	Biomass	77	238	91
	Broth	48	257	74
Nigrospora sphaerica (P.f)	Biomass	53	168	11
	Broth	30	166	83
Cladosporium cladosporioides (P.f)	Biomass	30	248	80
	Broth	87	207	65

*The values shown were calculated with an extra factor of 5.2 of Trolox due to a concentration adjustment between DPPH and the other two radicals.

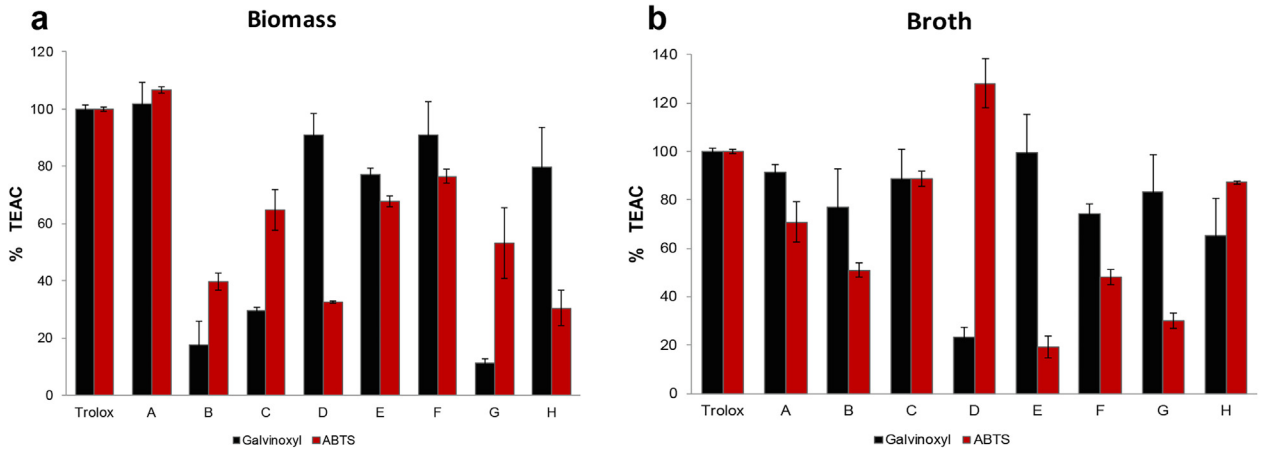

Fig. 1. TEAC Percentage of ABTS and galvinoxyl radical assays from biomass (a) and broth (b) of marine microscopic fungi extracts. Each value is the mean ± standard deviation (N = 3). Trolox value of 100% is equal to the IC_{50} (ABTS 83.5 ± 3.1 and Galvinoxyl 17.5 ± 2.0 Trolox μM), A. *Cladosporium cladosporioides*, B. *Curvularia trifolii* (both from *Amphimedon compressa*), C. *Sarocladium strictum* (from *Agelas* sp.), D. *Fusarium oxysporum* (from *Aplisina* sp.), E. *Fusarium* sp. F. *Fusarium oxysporum* (both from *Diploria strigosa*), G. *Nigrospora sphaerica*, H. *Cladosporium cladosporioides* (both from *Plexaura flexuosa*).

F. oxysporum (D.s), also the biomass extract of *C. cladosporioides* (P.f) showed high activity in these tests in both cases, with a medium value of TPC. Furthermore, *F. oxysporum* (Apl) showed a good TEAC against DPPH and ABTS radicals, particularly the broth extract, which presented the best TEAC in ABTS assay and is directly related to the highest TPC among all the assayed extracts.

Because there is a significant difference in the TEAC values between the biomass and broth extracts for the ABTS and galvinoxyl assays, it is reasonable to assume that such a difference is due to the chemical nature of each extract type. Meanwhile, the lack of difference between broth and biomass for the DPPH assay might be explained by the peculiarities of its antioxidant mechanism compared with the other assays. The variation found in the total phenolic content for each treatment suggests that their antioxidant activity is not related to polyphenols and hence might be attributed to other types of metabolites. This is also supported by the results of the correlation analysis, which show that there is no high Pearson R value.

It has been reported that antioxidant capacity depends not only on the concentration of phenolic compounds, but also on the interaction involved with other components and the applied methodology employed [40]. Hydroquinones such as acremonin A and acremonin A glucoside, as well as 2-(1-hydroxy-1-methyl)-2,3-dihydrobenzofuran-5-ol and 2,2-dimethylcromone-3,6-diol, all these isolated from the marine fungus *Acremonium* sp. are reported to have shown antioxidant activity [29]. In addition, compounds with antioxidant activity such as 4-(3,4-dihydroxybenzamide) methyl butanoate, 5-O-methylsulochine, and 4-(3,4-dihydroxybenzamide) butanoic acid were isolated from the ethyl acetate extract of the marine fungi *Aspergillus wentii* EN-48 [41]. Similarly, there are reports regarding the antioxidant activity of hydro-antraquinones isolated from the marine

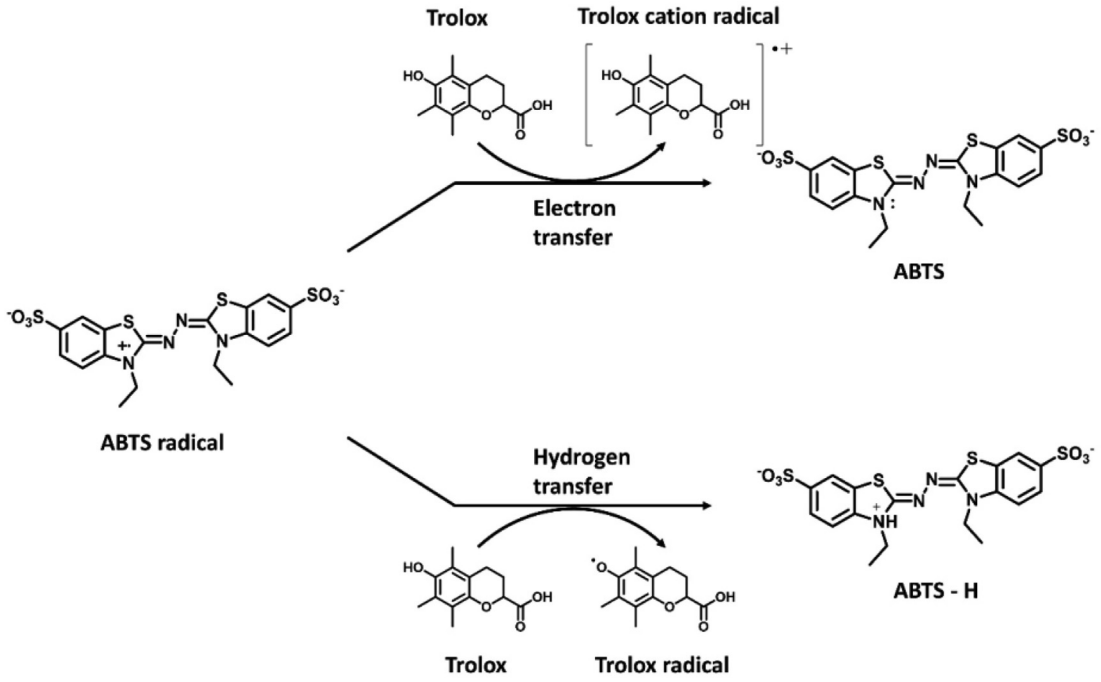

Fig. 2. Reactions of the radical ABTS with Trolox via electron transfer and hydrogen transfer.

endophytic fungi *Talaromyces islandicus* EN-501 [13]. So, after comparing the TEAC and TPC obtained, the results suggest that, in addition to phenolic compounds, the extracts may contain non-phenolic antioxidants such as lactone derivates, xantones, quinones, and hidroantraquinones, coinciding with what was reported by König et al. [28], who mentioned this group of compounds as new antioxidants derived from marine microorganisms.

4. Conclusions

Marine fungi present pharmacological relevance because their biological activity is related mainly to their anti-proliferative and antibacterial properties [24]. This is the first report on the antioxidant potential of marine fungi extracts isolated from sponges and corals in the Gulf of Mexico's NPSAV. The results indicated that the biomass extract of *C. cladosporioides* (Ac) has a slightly higher free radical scavenging capacity than Trolox (7% for the ABTS assay and 2% for the galvinoxyl assay), whereas the broth extract of *S. strictum* showed more than 80% of this capacity, both extracts against the free radicals ABTS and galvinoxyl. These results support the selection and usage of marine fungi in the purification and elucidation of antioxidant (phenolic and non-phenolic) compounds that may be useful in the treatment of pathologies caused by an increase in the free radical concentration or related to oxidative stress, as well as the importance of coral reef conservation as an important source of microorganisms with biological potential.

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Conflict of interest

All authors declare that they have no conflict of interest.

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