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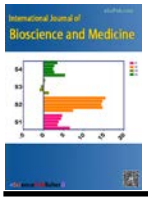

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Evaluation of the antioxidant activity of extracts obtained from the seaweed Caulerpa taxifolia

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ABSTRACT

This study aimed to evaluate the antioxidant activity of the extracts of marine seaweed Caulerpa taxifolia by two methods of study of antioxidant activity. The antioxidant activity of the extracts hidroalcolico, methanol and hidroalcolico was evaluated according to Mitsuda (1967). The ability of the extracts to kidnap hydrogen peroxide was determined by Ruch method (1989). The analysis carried out by reading the samples in octoplicata in spectrophotometer, it was possible to calculate the antioxidant and scavenging activities of the extracts hydrogen peroxide. The antioxidant activity presented by the three extracts from C. taxifolia by the ferric thiocyanate method varied each other, showing the methanol extract greater activity than the alcohol and aqueous extracts hydro, may be due to the presence of secondary metabolites like terpenoids. The kidnapper power of hydrogen peroxide of Caulerpa taxifolia strata known significant values when compared to the reference drug.

Keywords: Green algae, Ferric thiocyanate, Hydrogen peroxide.

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INTRODUCTION

Antioxidants are able to stabilize or deactivate free radicals before they attack biological targets in cells. The radicals formed from antioxidants are not reactive to propagate the chain reaction, being neutralized by reaction with another radical, forming stable products or can be picked up by other antioxidants (Andrade et al, 2007). In the present study, the use of free radicals and other oxidants induces oxidative damage in biomolecules and has been associated with the aging and development of many chronic and degenerative diseases such as cancer, cardiovascular diseases, atherosclerosis, Alzheimer's disease, Parkinson's disease (ROESLER et al., 2007).

Natural products have been an alternative to oxidative damage at the cellular level, and phenolic compounds are known to have antioxidant activity mainly due to their reducing properties and chemical structure (SOUSA et al., 2007; YESILYURT, 2008). These compounds are well distributed by the plant kingdom, being found in terrestrial, aquatic plants and marine algae.

Green algae have several compounds of great interest to the pharmaceutical, food and cosmetic industries. The addition of antioxidants confers protection not only to food but also to animal organisms, since antioxidants are ingested, fight free radicals and their repercussions contributes to reducing the risk of pathologies (VULCAIN et al., 2005).

The genus Caulerpa is composed of benthic green seaweed and its representatives endowed with a creeping stem formed by a rhizomatous portion that expands along the substrate, being fixed through structures called rhizoids. Many species of this genus are found in the Brazilian coast (SZE, 1998; JOLY, 2005). A few studies have reported biological properties, such as antiviral and anticoagulant (MATSUBARA et al., 2001; GHOSH et al., 2004; RODRIGUES, FARIAS, 2005).

The species C. taxifolia is similar to a leaf composed of the upper vegetables, with the leaf-shaped limb resembling a fern, a stem with the appearance of a stolon-like stem, and rhizoids which are structures used to fix it to the substrate (REVIERS, 2006). A series of secondary metabolites in this plant may be identified that may be an alternative for the development of products and medications, which may bring improvements to the treatment of pathologies (MOURA et al., 2012). The present study aimed to evaluate the antioxidant activity of extracts obtained from marine macroalgae C. taxifolia by two methods of antioxidant activity study.

MATERIAL AND METHODS

The respective work is of the quantitative type and was developed in the laboratory of Physiology and Animal Pharmacology of the Federal Rural University of Pernambuco. The marine macroleaf Caulerpa taxifolia, native to the Indian Ocean, was collected for the present study on the "Carneiros" beach, south coast of the State of Pernambuco, with geographic coordinates: -8.703570 and -35.079224, latitude and longitude respectively, is 113 km from the capital Recife. The species collected in order to produce an exsicata where it was deposited in the Herbarium Geraldo Mariz, Federal University of Pernambuco, is registered with the number: 68.863, identified by Curator Marlene Barbosa.

Evaluation of the antioxidant activity by the Ferric Thiocyanate method

The antioxidant activity of hydroalcoholic, methanolic and hydroalcoholic extracts was evaluated according to the methodology described by Mitsuda et al. (1967). Mixture containing 1 mL of the algal extract, 1.1 mL of 2.51% (w / v) linoleic acid in ethanol (99.0% v / v), 2 mL of 0.05 M phosphate buffer (pH 7.0) and 0.9 mL of distilled water was placed in an amber glass vial with screw cap and stowed in a forced air circulation oven at 50.0 ± 0.5 ° C. To 0.1 mL of this solution was added 5 mL of 75% (v / v) ethanol, 0.1 mL of 30% (w / v) ammonium thiocyanate and 0.1 mL of 0.02 M ferrous chloride. After, exactly 3 minutes of reaction at

room temperature (25.0 ± 0.5 ° C), the absorbance at 500 nm was carried out on a Hewlet Pakard model 8452A UV-VISIVEL spectrophotometer, which was repeated every 24 hours until that the purple color of the control reached a maximum value. The concentration of the extracts and the synthetic antioxidant hydroxybutylanisole (BHA) was 0.01% (w / v). The percentage of inhibition in lipid oxidation was calculated according to the algebraic expression:% inhibition = {[abs. mean of control - abs. final sample mean] / abs. final control mean x100.

Evaluation of the hydrogen peroxide hijacking power.

The ability of extracts to sequester hydrogen peroxide was determined according to the method of Ruch et al. (1989). A solution (4 mM) of hydrogen peroxide was prepared in phosphate buffer (pH 7.4). The concentration of hydrogen peroxide was determined spectrophotometrically at the wavelength of 230 nm using molar absorptivity of 81-1 cm -1 (BEERS; SIZER, APUD YEN; CHEN, 1995). To the extract (10400 mL, in 4 mL of distilled water) was added hydrogen peroxide solution (0.6 mL). After 10 minutes of reaction at room temperature (25.0 ± 0.5 ° C), a blank solution containing the extract in buffer without hydrogen peroxide was performed.

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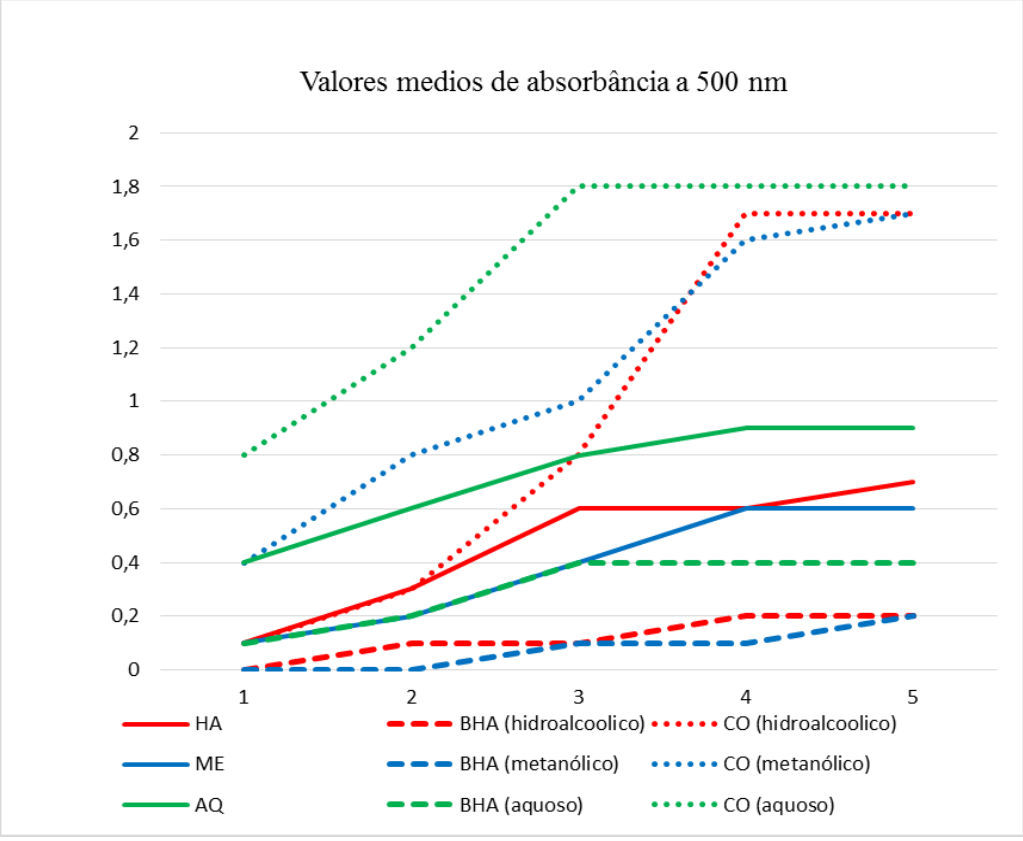
RESULTS

Evaluation of the antioxidant activity by the Ferric Thiocyanate method

The average values of absorbance presented by the hydroalcoholic extract of C. taxifolia (Graph 1) allowed to obtain the percentage of antioxidant activity according to the formula mentioned in materials and methods, where the values expressed in table 1 were obtained.

Mean values of absorbance of samples containing hydroxylic extract of C.taxifolia (0.01%) obtained in the ferric thiocyanate test at 0, 24, 48, 72 and 96 hours



Graph 1 - mean values of absorbance at 500 nm of the hydroalcoholic extract, methanolic extract, and the aqueous extract of the marine macroalga C. taxifolia.

Table 1 - Percentages of antioxidant activity by the Ferric Thiocyanate method expressed by the hydro alcohol extract, methanolic extract, and the aqueous extract of C. taxifolia.

Extract / Time	0 h	24 h	48 h	72 h	96 h
HA	0,00 % ± 0,000**	0,00 % ± 0,000**	25,00 % ± 0,003**	64,7 % 0,006**	58,82 % 0,007**
BHA (hydroalcoholic)	0,00 % ± 0,000**	66,00% ± 0,002**	87,50 % ± 0,001**	88,23 % ± 0,002**	88,23 % ± 0,003**
ME	75,00 % ± 0,001**	75,00 % ± 0,009**	60,00 % ± 0,005**	62,5 % ± 0,004**	64,7 % ± 0,003**
BHA (methanolic)	100,00 % ± 0,003**	100,00 % ± 0,001**	90,00 % ± 0,007**	93,75 ± 0,002**	88,23 ± 0,001**
AQ	50,00 % ± 0,002**	50,00 % ± 0,000**	55,55 % ± 0,001**	50,00 % ± 0,001**	50,00 % ± 0,000**
BHA (aqueous extract)	87,5 % ± 0,002**	83,33 % ± 0,003**	77,77 ± 0,004**	77,77 ± 0,006**	77,77 ± 0,003**

* significant for values of p≥0.05, ** significant for values of p≥0.01 (ANOVA).

Graph 1 shows the mean absorbance values for the methanolic extract of C. taxifolia, which served as the basis for the calculation of the percentage of antioxidant activity presented in table 1 on the methanolic extract. Mean values of absorbance of the samples containing methanolic extract of C. taxifolia (0.01%) obtained in the ferric thiocyanate test at 0, 24, 48, 72 and 96 hours.

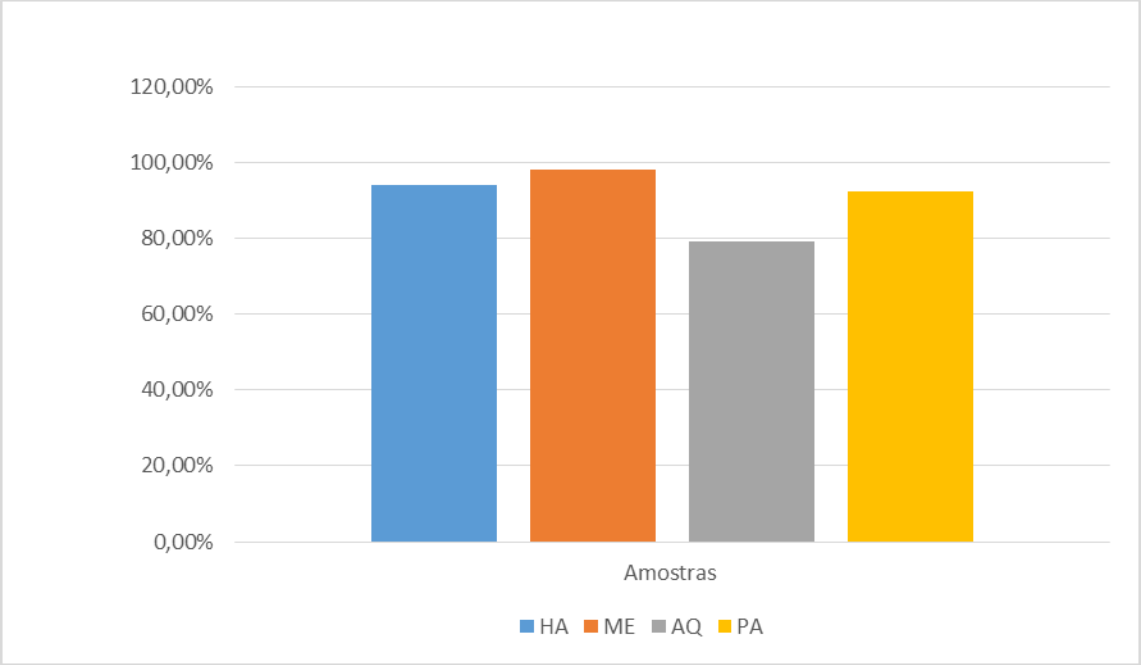
Evaluation of the hydrogen peroxide sequestering power of methanolic extracts

From the analyzes performed by reading the samples in triplicate in a spectrophotometer with wavelength λ = 230 nm, it was possible to calculate the mean values expressed in table 2, and with these data it was possible to apply the formula to check the antioxidant activity of hydroalcoholic extracts , methanolic and aqueous of C. taxifolia whose percentages are expressed in figure 2.

Table 2 - Absorbance values presented by the samples

Samples	**HA**	**Me**	**AQ**	**PA**	**CO**
Absorbance	**0,07**	**0,02**	**0,25**	**0,09**	**1,2**

* significant for values of p≥0.05, ** significant for values of p≥0.01 (ANOVA).



Graph 2 - Antioxidant potential presented by extracts of Caulerpa taxifolia to sequester hydrogen peroxide.

DISCUSSION

Evaluation of the antioxidant activity by the Ferric Thiocyanate method

The antioxidant activity presented by the hydroalcoholic extract was lower than the values presented by the BHA, where the activity of this varied from 23.53 to 66.00% higher than the hydroalcoholic extract, and with the methanolic extract the BHA superiority ranged from 23.53 to 31 , 25%, when compared to the aqueous extract the difference in activity ranged from 22.77 to 37.50%. The antioxidant activity of the extracts of C. taxifolia was higher than the values of the activities of the marine green algae species tested by Raymundo (2004) and the values found for these extracts being much lower than the values found with the reference substance. of superior vegetables tested by Negri (2009).

The antioxidant activity presented by the three extracts obtained from this organism by the ferric thiocyanate method varied among them, showing the methanolic extract an activity greater than the hydroalcoholic and aqueous extracts, perhaps this is due to the composition of secondary metabolites present in the extract which can suggest that the antioxidant activity of C. taxifolia may be due to the presence of phenolic compounds, since these compounds are more easily extracted from vegetal material with methanol extraction. Seaweed research has already resulted in the isolation of various terpenoid compounds, and this tropical algae is one such example. It was known for its damage to the ecosystem, due to the release of a toxic sesquiterpene called caulerpenine (MOZZACHIODI et al., 2001). Caulerpenine (1,4-diacetoxybuta-1,3-diene, according to IUPAC) is an acyclic compound and in its structure we found 3 acetoxy groups (AMICO et al., 2009).

Marine organisms are also a considerable source of terpenes. Structurally this class of organic compounds is formed by units of bound unsaturated isoprenes, and the number of carbon units are classified for these compounds: moterpenes (10 Carbonites), sesquiterpenes (15 carbons), diterpenes (20 carbons) (ABAD and BERMEJO, 2007). Marine macroalgae are always subject to rapid variations in light intensity and concentrations of O2 and CO2 along the water column and thus their survival depends on an efficient response to oxidative stress. For this reason, these organisms may

represent an important source of natural antioxidant substances for both Food and Pharmaceutical Industries (MATSUKAWA et al., 1997).

Evaluation of the hydrogen peroxide hijacking power

When the 200 mg dose was considered, the aqueous extract presented values much lower than that presented by hydrogen peroxide, with the efficiency of the extract being approximately 14% lower than that presented by the reference solution for this activity. The antioxidant effectiveness of natural sources was attributed by several authors to phenolic compounds, which occur naturally in terrestrial and aquatic plants (HAYASE; KATO, 1984). These compounds are mainly responsible for the antioxidant activity of plants, playing a key role in the inhibition of auto oxidation in oils (RAMARATHNAM et al., 1986).

The metabolism of oxygen in living cells, in addition to producing the necessary energy for the activities of the cells, also causes the formation of radicals (ROESLER et al., 2007). Phenolic compounds act as antioxidants, not only for their ability to donate hydrogen or electrons, but also by virtue of their stable intermediate radicals, which prevent the oxidation of various food ingredients, particularly lipids. Carotenoids are constituted of chains of polyenes, in a long system of conjugated double bonds, rich in electrons, responsible for the antioxidant activity of these compounds: both in the absorption of singlet oxygen and free radicals, to interrupt the chain reactions where they are involved.

The natural occurrence of substituted phenols and polyphenols in algae has been scientifically disseminated and expanded over the years (PEDERSEN, 1978). Although this class of compounds includes effective antioxidants (DENISOV, 2009).

The respiratory process and various oxidative reactions of aerobic cells lead to the formation of free radicals, which contribute to the appearance of several diseases. Human cells depend on their antioxidant ability to provide protection against the damaging effects of free radicals and reactive oxygen species, which are inevitable consequences of aerobic life. Several epidemiological studies indicate that high intake of plant products is associated with a reduced risk of a variety of chronic diseases such as atherosclerosis and cancer, effects that have been particularly attributed to compounds that have antioxidant activity in plants: vitamins C and E, phenolic compounds, especially flavonoids, and carotenoids.

CONCLUSION

Marine algae represent a very promising alternative source, since they presented important biological activities, which may contribute to investigations of new drug therapies.

CONGRATULETER

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