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CYTOTOXICITY ASSESSMENT AND ANTIMICROBIAL ACTION OF *Caulerpa taxifolia* (M. Vahl) C.

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ABSTRACT

This study aims to determine the LC50 for Artemia salina Leach and perform a microbial screening two C. taxifolia extracts. In these biological assays have been used larvae of A. salina Leach ob-tained from the incubation of about 20 mg of A. salina cysts, under artificial light for 48 hours. Where groups of 10 met-anauplius were exposed to different concentrations (50 mg / mL to 1000 ug / ml) of methanol extract and hydroalcoholic C. taxifolia . The antimicrobial activity of extracts of C. taxifolia was evaluated by diffusion method in paper disc. The percentage mortality deter-mined after 24 hours of contact. Through the data was calculated LC50. Behavioral analysis of Artemia salina Leach showed a decrease in handling compared to those observed in the control groups. The microbial activity of the extracts were evaluated by measuring the halo of inhibition for two species of gram-positive and two gram-negative bacteria. The extracts showed signifi-cant results for S. aureus and S. spp. This study demonstrates that C. taxifolia has a high cyto-toxicity suggested that its use in cell culture as a molluscicide and can be used in the production of production of antimicrobial drugs.

Keywords: Artemia salina, microbiology, Staphylococcus aureus, Streptococcus spp.

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INTRODUCTION

Many Natural Products laboratories have inserted into their routines simple biological assays in order to select and monitor the plant extracts research in the search for bioactive substances (Nascimento et al., 2008). Among these bioassays, is the toxicity on *Artemia salina* Leach, which is a saltwater microcrustacean commonly used as food for fish. The simplicity with which it can be handled, the speed of the tests and the low cost favors its routine use in several studies, in addition to such lethality tests are widely used in preliminary analyzes of general toxicity (LUNA et al., 2005).

Bacterial resistance emerges as a problem to be solved in the coming decades. Antimicrobial therapy not only markedly reduces the rate of morbidity and mortality caused human infections, and prevents the occurrence of various diseases (Cohen, 1992; Kuhner, Marques. 2003; Silva et al, 2010)

The *Caulerpa* genus consists of green seaweed benthic and their representatives provided with a creeping stem formed by a rhizomatous portion that extends along the substrate, settling through rhizoids structures called and many species are found along the Brazilian coast (SZE, 1998; REVIERS, 2006; Raven et al, 2007;. JOLY, 2011). Some studies have reported biological properties of several species of *Caulerpa* present a broad spectrum of activities, including antibacterial activity (Albuquerque et al, 1983; SRIDHAR et al., 1984), antifungal (Albuquerque et al., 1983), antitumor (NAKAMURA et al, 1997), repellant (Thangam et al., 1993), anticrustáceo (ARA et al., 1999), antiviral, an anticoagulant (PREMANTHAN et al., 1994;... PREMANTHAN et al 1995GHOSH et al., 2004;RODRIGUES; FARIAS, 2005) among others.

C. taxifolia frond features similar to a composite sheet of superior vegetables, with the split blade reminiscent of a fern leaf, a stem with appearance of a stem type stolons and rhizoids structures that are used for fixing the substrate (REVIERS, 2006; Raven et al, 2007).. This study aimed to determine the LC50 of C. taxifolia to Artemia salina Leach, so check with your microbial action.

MATERIALS AND METHODS

Cytotoxicity

It was used to test the methodology of Meyer et al. (1982). In this bioassay was used larvae of Artemia salina Leach obtained from the incubation of 20 mg of Artemia salina cysts in seawater, under artificial light for 48 hours. 10 metanauplius groups were exposed to different concentrations (50, 100, 250, 500, 750 and 1000 ug / ml) of methanol and C. hydroethanolic extract taxifolia and certain percentage mortality after 24 hours of contact. The entire experiment was conducted in triplicate. According to the methodology used, the LC50 is interpolated through the graphical plot of the data. The tests were performed in the Experimental Pharmacology and Oncology Laboratory of the Department of Antibiotics, Federal University of Pernambuco.

Antimicrobian activity

The antimicrobial activity of hydroethanolic and methanol extracts C. taxifolia was evaluated by diffusion method in paper disc (BAUER, 1966) against standard bacteria obtained from the Collection of Microorganisms of the Coleção de Microrganismos do Departamento de Antibióticos da Universidade Federal de Pernambuco Table 1.

The suspensions were seeded into the carpet using "swab" sterile soaked with the same on plates containing Mueller Hinton agar (Staphylococcus aureus, Streptococcus spp., Escherichia coli and Pseudomonas aeruginosa).

On the seeded plates were placed on paper disks of 6 mm in diameter soaked with 10 uL of four concentrations of the extract so that the final concentration obtained was 125; 250, 500 and 1000 ug / disk. The material was incubated at 37 °C. After 24 to 48 hours of incubation, the plates were examined and the diameters of inhibition zones were measured. The entire assay was

performed in triplicate and results were expressed as the arithmetic mean of inhibition halos obtained in the three plates and the standard deviation calculated. As a control, used disks were soaked in Dimethyl Sulfoxide (DMSO) to 100% solvent used to extract and solubilize as standard disks containing the antibiotics gentamicin, chloramphenicol and ketoconazole at concentrations of 10, 50 and 300 ug / disc respectively.

Table 1 - Microorganisms used for the antimicrobial assay.

MICROORGANISMS	CLASSIFICATION
S. aureus	Gram-positive
S. spp	Gram-positive
E. coli	Gram-negative
P. aeruginosa	Gram-negative

Coleção de Microrganismos do Departamento de Antibióticos da UFPE

RESULTS

After 24 hours of exposure to salt A. Leach extracts was observed deaths of all organisms at 1000 mg / mL for hydroethanolic extract and 500 and 1000 mg / mL for the methanol extract.

Behavioral analysis of saline A. Leach in concentrations of 50 mg / mL to 250 mg / mL for the methanol extract and up to 750 mg / mL for hydroethanolic extract showed a decrease in its movement relative to the observed in the control groups, showing that exposure to rates of concentration of C. taxifolia samples influences the movement of the organism studied.

After 48 hours of exposure *A. salina* Leach, it was observed that there was a reduced number of deaths for the lower concentrations of C. taxifolia samples. Thus, from all the observed facts have been proved experimentally that the sample had a LC50 values selected in the range of 122 5µg / mL for the hydroethanolic extract, and 322.5 ug / mL methanolic extract.

Table 2 Antimicrobial activity of hydroethanolic and ethanol extract of *C. taxifolia* against the tested microorganisms.

	hydroethanolic extract				Methanolic extract				DMSO	GEN	CLO	CTZ
Microorganism	125µg	250µg	500 µg	1000 µg	125µg	250µg	500 µg	1000 µg				
S. aureus	6±0,5	8±0,7	11±1,0	16±1,0	4±0,3	7±0,6	14±1,0	26±0,6	-	28±1,0	36±1,0	-
S. spp	4±0,3	5±0,6	7±1,0	9±0,6	5±0,4	6±0,6	13±0,8	14±0,6	-	22±0,6	21±0,6	-
E. coli	0±0,0	0±0,0	0±0,0	0±0,0	0±0,0	0±0,0	0±0,0	0±0,0	-	26±0,6	24±0,6	-
P.aeruginosa	0±0,0	0±0,0	0±0,0	0±0,0	0±0,0	0±0,0	0±0,0	0±0,0	-	25±1,0	32±0,6	-

Average inhibition zones in millimeters. Dashes (-) indicate the absence of inhibition halo. UFPEDA (Microorganisms Collection of the Department of Antibiotics, Federal University of Pernambuco). DMSO (dimethylsulfoxide). GEN (Gentamicin). CLO (Chloramphenicol). CTZ (Ketoconazole).

The results of the antimicrobial activity to determine the Minimum Inhibitory Concentration on solid medium with inhibition halos of Caulerpa taxifolia extracts of *S. aureus*, *S*. spp., *E. faecalis*

and *E. coli* are shown in Table 2. In this assay, it was shown that the samples studied the extracts showed activity against S. aureus and Staphylococcus spp. They were sensitive to methanol extract latter being sensitive also to hydroethanolic extract.

DISCUSSION

According to the World Health Organization (WHO) are considered toxic substances which have LC50 values below 1000 ppm Artemia salina (Meyer et al., 1982). Thus the *C. taxifolia* extracts can be considered toxic since the LC50 determined for the methanol extract was 122.5 mg / mL and the hydroethanolic extract 322.5 mg / mL these values being translated as 122.5 and 322.5 ppm respectively.

Carballo et al. (2002) compared extracts of marine products to the lethality assay for *A. salina* larvae and 2 for cytotoxicity in human cell lines. According to the authors, the results showed a good correlation, as already established for plant extracts (McLaughlin, 1991), suggesting that this bioassay is used to test marine natural products with potential pharmacological activity. To Oliveira-Filho; Paumgartten (2000) and Lima et al (2002) This test can also be used to express the toxicity of an extract with molluscicidal activity against non-target organisms such as small crustaceans and fishes.

Schistosomiasis, caused by trematode Schistosoma mansoni, is an important endemic disease in Brazil and in many other tropical countries. The life cycle of this parasite involves an intermediate host represented in Brazil by the Biomphalaria snails, and the snail B. glabrata the main vector in South and Central America (ALVES et al., 2000). For the control of schistosomiasis, in addition to treating infected patients, it is very important that the populations of snails are controlled as a way of reducing the risk of disease transmission. Aniclosamida is commercially available molluscicide, recommended by the World Health Organization (WHO), and proved to be more effective and less harmful to the environment and human health than other inorganic or synthetic molluscicides (SILVA FILHO et al., 2009). However, the high cost of its application in large areas prevents their use in most developing countries. Thus, the search for natural molluscicides (SILVA et al., 2007a, b) and synthetic (VASCONCELLOS et al., 2005) has gained a new prominence, in order to obtain a cheaper alternative, biodegradable, safe and available for control populations of snails (MEYER et al., 1982). So the *Caulerpa taxifolia* can be an alternative to be used against the populations of snails since obtained a high mortality rate for A. salina and are scientifically proven correlation to the tests (MEYER et al, 1982;. VASCONCELLOS et al., 2005; SILVA et al, 2007a, b).

The front bacterial resistance to antimicrobials is considered as a problem inherent in the antimicrobial therapy; For this reason, it is important to find new therapeutic sources which are more effective for treating infections such as bacterial.

The search for new antimicrobial agents for the control of Staphylococcus aureus is important, since this microorganism was established as an important pathogen because of its virulence, antimicrobial resistance and associated with various diseases, including life threatening systemic disease, skin infections, opportunistic infections and food poisoning (LOWY, 1998; ARDURA, 2009).

Thus, the search for antibacterial properties with more specific plants and substances extracts has been encouraged and intensified (MIGUEL, MICHAEL,1999; RIVERS; RECIO, 2005; BARREIRO; BOLZANI, 2009).

As the agar diffusion technique is a qualitative test based on the diffusion of samples that are performing the action, the lack of antimicrobial activity can be attributed to lack of diffusivity of the extracts.

Studies by Salvaginini et al. (2008) demonstrated the evaluation of the antimicrobial activity through the diffusion well, the ethanol extract obtained from Myrtus communis leaves

L. (Myrtaceae) against strains of *Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Bacillus* subtilis and *Serratia marcescens* having been observed inhibition against S. aureus, *S. epidermidis* and B. subtilis with inhibition zones of around 14 mm diameter. The present study showed significant results for *C. taxifolia* and the inhibition of growth of *S. aureus* and *S*. spp.

According to Alves et al. (2000), whose work presents a classification activity according to the inhibition zone, the extracts to inhibit 13-18 mm were regarded as significant inhibition with and 9-12 mm as partially active. The findings presented in this study of methanolic extracts of C. taxifolia hydroethanolic suggest that both extracts showed significant inhibition of Staphylococcus aureus is an important species being studied since several cases are related to bacterial resistance strains of this species. As the reaction of the front extracts Staphylococcus spp inhibition of methanol extract was considered significant, but the hidroetanoólico extract was considered partially active. The extracts had no activity against gram-negative species studied, which may suggest that the activity displayed across the gram-positive bacteria is related to the cell membrane. Antimicrobial agents have been used since the seventeenth century for the treatment of infectious diseases. An ideal antimicrobial agent exhibits selective toxicity, which means that a substance to be effective against target bacteria, but safe in terms of toxicity to the patient (Davis, 1987; RANG; DALE; RITTER, 2001; BROOKS et al, 2008; SOFIATI, 2009).

CONCLUSION

The *Caulerpa taxifolia* can be an alternative used against the populations of snails since obtained a high mortality rate for *A. salina* (with the extracts studied) and there are scientifically proven correlation for testing.

Through microbial screening, it can be concluded that the methanol extract of *Caulerpa taxifolia* and hydroethanolic has significant activity against *Staphylococcus aureus*, and the methanol extract also considered significant against *Streptococcus* spp. and the partially active hydroethanolic extract showed activity against this species of bacteria. What may suggest further study using other strains of these species and that there was no inhibition compared to the other tested microorganisms.

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