

Evaluation of antibacterial, antifungal and modulatory activity of methanol and ethanol extracts of *Padina sanctae-crucis*

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Abstract

Background: Multi-resistant microorganisms such as *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida tropicalis* e *Candida krusei* are the main causes of microbial infections. *Padina sanctae-crucis* is a seaweed often used to check the contamination of ecosystems by materials such as heavy metals, but studies of the antimicrobial activity of the same seaweed were not found.

Methods: The tests for the minimum inhibitory concentration and modulation of microbial resistance, with the use of ethanolic and methanolic extracts of *Padina Sanctae-crucis* combined with drugs of the class of aminoglycosides and antifungal were used to evaluate the activity against the cited microorganisms.

Results: Was observed a modulation of antibiotic activity between the natural products and the *E. coli* and *S. aureus* strains, indicating a synergism and antagonism respectively.

Conclusions: The results showed a moderate modulatory effect against some microorganisms studied.

Keywords: multi-resistant microorganisms, modulation, *Padina Sanctae-crucis*, antimicrobial activity.

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Introduction

The World Health Organization (WHO)¹ defines medicinal plants as any plant which in one or more of its organs contains active ingredients which can be used for therapeutic purposes or contain compounds that can be used for the synthesis of useful drugs¹.

The population of the poorest countries uses medicinal plants by symbolic tradition associated with low cost of this treatment, but in most developed countries there is an increased use of herbal medicines influenced by fad consumption of natural products^{2,3}.

Medicinal plants and their products have been used as self-medication based on traditional knowledge^{4,6}. The ethnopharmacological studies have cataloged lately a large number of plants used by the same people who got their medicinal activities confirmed^{4,6}.

The growing interest in medicinal plant research in recent decades is due to an increasing apparition of adverse effects of conventional drugs and the increase of microbial resistance to antibiotics, the researchers are looking more carefully the natural products that could provide effective treatment for diseases and infections and a low toxicity to the patient^{7,8}.

Two main factors are crucial to microbial resistance: the emerging and re-emerging diseases and the resistance to antibiotics⁹. The resistance to antibiotics is a process of natural selection, selecting the most resistant microorganisms of a group or selecting those that have the ability to adapt, either by mutation or obtaining resistance factors transmitted by another microorganism⁹. Several chemical compounds, synthetic or from natural sources, have direct activity against many species of bacteria, enhancing the activity of a specific antibiotic, reversing the natural resistance of bacteria to specific antibiotics, causing the elimination of plasmids, and inhibiting the active efflux of antibiotics through the plasma membrane^{10,11}. The potentiation of antibiotic activity or the reversal of antibiotic resistance allows the classification of these compounds as modifiers of antibiotic activity^{10,11}.

This search for more effective active ingredients and less dangerous to humans and for a greater effectiveness against some microorganisms

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puts the herbal medicines studies in a significant level in the scientific research, opening up opportunities for ethnopharmacological studies, phytochemical and microbiological among any other areas⁷.

Padina sanctae crucis is part of the *Eukaryotic* group, being found as integrant of kingdom *Chromista*, in which is classified part of the phylum *Ochrophyta* and subphylum *Phaeista*, is also classified as a integrant of class *Phaeophyceae* and is within the order *Dictyotales*, *Dictyotaceae* family belonging to the genus *Padina*¹².

The specific taxonomic study of gender *Padina* is usually very difficult due to the the lack of uniformity that is found in the literature about the characteristics that should be taken into consideration for species differentiation. In Brazil, by the year 2000, there were no taxonomic studies that explain about only this genus¹³.

The research objective was evaluate the action of spectrum and the minimum inhibitory concentration (MIC) of extracts obtained from leaves of *Padina sanctae crucis* against pathogenic strains of fungi and bacteria. Besides that, determine the effectiveness of the extracts in the modulation of fungal and bacterial resistance to aminoglycosides and antifungal.

Methods

Microbial strains

The bacteria used in test of Minimum Inhibitory Concentration (MIC) were the standard strains of *Escherichia coli* ATCC 10536, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 15442. To evaluate the modulatory activity of the natural products were used the follow multiresistants bacterial strains isolated from clinical environments: *Pseudomonas aeruginosa* 03, *Escherichia coli* 27 and *Staphylococcus aureus* 358.

The fungal strains utilized were: *Candida tropicalis* 40042: *Candida krusei* 2538. All the strains were maintained in slants with Heart Infusion Agar (HIA; Difco Ltda) and before the tests the cells were cultured by 24 hours at 37°C in brain heart infusion. (BHI, Difco Laboratories Ltda).

Drugs and Natural Products

The methanol and ethanol extracts were obtained from Dr. Emídio V. L. da Cunha, Laboratory of Pharmaceutical Technology – UFPB, Brazil. The drugs used in the tests were the aminoglycosides: Amikacin, Gentamicin and Neomycin (Sigma Co., St. Louis, USA). All the drugs were diluted using sterile water. The antifungal drugs used in the tests were: Anphotericin B; Nystatin; Mebendazole and Benzoilmetronidazol, the

antifungal drug solutions were prepared according to the manufacturer's instructions.

Phytochemical prospecting

The qualitative phytochemical tests to detect the presence of phytocompounds were performed according to the method described by Matos¹⁴. The tests were based on the visual observation of a change in color or formation of precipitate after the addition of specific reagents.

Minimal Inhibitory Concentration

The method used was microdilution in broth. The methanolic and ethanolic solution of *Padina sanctae-cruces* was solved using DMSO and diluted to 1024 µg/mL using sterile distilled water. The bacterial inoculum was diluted using BHI 10% to a final concentration of 10⁵UFC/mL. 100µL of the inoculums were distributed on each well of a microtiter plate with 96 wells and then submitted to a twofold serial dilution using 100µL of the extract, ranging the concentrations between 512 to 8 µg/mL. The plates were incubated for 24 hours at 35°C¹⁵. The determination of bacterial MIC was made using the indicator resazurin, while the MIC of fungi were determined by the turbidity. The MIC was defined as the lowest concentration where no growth can be observed, according CLSI¹⁶.

Test of Drugs Modulation

To verified if the natural product would alter the action of antimicrobial drugs against the strain tested, was used the method proposed by Coutinho *et al.*¹⁰. The Methanolic and ethanolic extract of *Padina sanctae-cruces* was tested in sub-inhibitory concentration (MIC/8 = 128 µg/mL). Were distributed 100µL of a solution containing BHI, the microbial inoculums and extract in each well. After this, 100µL of the antimicrobial drug was mixed with the first well, following the twofold dilution, ranging the concentrations of aminoglycosides and antifungals between 2500 to 2.44 µg/mL and 512 to 2 µg/mL respectively.

Results and discussion

The phytochemical prospection shows the presence of phytocompounds, such as phenols, tannins, flavonoids and terpenes. The phenols and tannins have a very reported antimicrobial activity associated with the hydrolysis of an ester bond with gallic acid, thereby serving as a mechanism of natural defense against microbial infections¹⁷.

Flavonoids are synthesized by plants in response to microbial infection and are effective against a broad

range of microorganisms¹⁸. The activity is probably due to their capacity to form complexes with extracellular soluble proteins, linked with the bacterial cell wall and with the cell membrane. Terpenes occur in the form of diterpenes, triterpenes, tetraterpenes as well as hemiterpenes and sesquiterpenes, all with several reports about the antibacterial activity¹⁹.

In the last years, various studies have been conducted in different countries, demonstrating the efficacy of biological activities of medicinal plants²⁰. There are few reports about the bioactivities of seaweeds from the species *Padina sanctae crucis*, being this work the first report about the antimicrobial and modulatory-antibiotic activities of *Padina sanctae crucis*²¹.

Were found for MIC tests against bacterial strains of: (*Escherichia coli* ATCC10536; *Staphylococcus aureus* ATCC25923 and *Pseudomonas aeruginosa*

ATCC15442), and against the fungal strains of: (*Candida tropicalis* ATCC40042 and *Candida krusei* ATCC2538), results that were shown at a concentration of ≥ 1024 . Those tests that were seen above or equal to 1024 do not represent a clinically significant result, but this characteristic can be changed in the modulation tests.

Several natural products such as extracts and essential oils from plants had been shown to modify antibiotic activity, using the contact direct method, as *Turnera ulmifolia*, *Momordica charantia*, *Mentha arvensis*, *Cordia verbenaceae*, and others^{10,22}.

The results in Table 1 and Table 2 were found for the tests of modulation of microbial resistance against multiresistant bacterial strains of: *Escherichia coli* 27 (EC27), *Staphylococcus aureus* 358 (SA358), *Pseudomonas aeruginosa* 22 (PA22) and two fungal strains: *Candida tropicalis* ATCC40042 and *C. krusei* ATCC2538.

Table 1. Modulation of antibiotic activity of EEPSC and MEPSC combined with aminoglycosides (g.mL⁻¹).

EC27	EEPSC	MEPSC	Antibiotic alone
Amikacin	78,1	9,8	78,1
Neomycin	9,8	19,5	78,1
Gentamicin	2,4	2,4	2,4
SA358	EEPSC	MEPSC	Antibiotic alone
Amikacin	19,5	19,5	19,5
Neomycin	39,1	39,1	4,8
Gentamicin	2,4	2,4	2,4
PA22	EEPSC	MEPSC	Antibiotic alone
Amikacin	312,5	312,5	312,5
Neomycin	312,5	312,5	312,5
Gentamicin	19,5	19,5	19,5

The results were relevant in modulating the resistance of the strain of EC27 when EEPSC was associated with neomycin and MEPSC when associated with amikacin. In both cases was observed a reduction of 3 points of MIC as can be seen comparing the results with the control and the reference values.

The seaweed can produce several secondary metabolites and consequently express many biological activities. They are capable to synthesize halogens metabolites that can belong practically to all chemical classes from short-chain hydrocarbon, simple ketones, phenols and acetogenins up sophisticated terpenes²³.

The seaweed are similar to land plants in relation to the production of substances generically called secondary metabolites, such as terpenes, compounds aromatics, acetogenins, amino acid derivatives and

especially polyphenols (halides and sulfates). These compounds give the seaweed stronger antioxidant activity. And for that reason may perhaps be associated with a possible antimicrobial activity^{24,25}.

The inhibition mechanisms of the growth of microorganisms are due in part to the hydrophobic nature of some components. As a result, they can interact with the cell membrane, affecting the respiratory chain and the production of energy or even make the cell more permeable to antibiotics²². These mechanisms of action can be obtained by the combination of antibiotic with extract at a sub-inhibitory concentration applied directly to the culture medium^{20,25}.

This strategy is called "potentiation of activity multi-effect targeting" and refers to the utilization of plants and drugs in an approach using combinations,

affecting various targets, where the different therapeutic components collaborate in a potential mechanism of action manner. This approach is not only

for combinations of extracts; combinations between natural products or extracts and synthetic products or antibiotics are also possible²⁵.

Table 2. Modulation of antifungal activity of EEPSC and MEPSC combined with antifungals (g.mL⁻¹)

CT-ATCC40042	EEPSC	MEPSC	Antifungal alone
Mebendazole	≥ 1024	≥ 1024	≥ 1024
Anphotericin B	≥ 1024	≥ 1024	≥ 1024
Nystatin	≥ 1024	≥ 1024	≥ 1024
Benzoilmetronidazol	≥ 1024	≥ 1024	≥ 1024
CK-ATCC2538	EEPSC	MEPSC	Antifungal alone
Mebendazole	≥ 1024	≥ 1024	≥ 1024
Anphotericin B	≥ 1024	≥ 1024	≥ 1024
Nystatin	≥ 1024	≥ 1024	≥ 1024
Benzoilmetronidazol	≥ 1024	≥ 1024	≥ 1024

It was possible observe that the strain of SA358 there was a case of antagonism of the product with neomycin, because there was an increase in bacterial resistance quantified in 3 points of MIC as can be seen comparing the results with the control and with the reference values. In the modulation of the fungi it was observed that the both natural products had no effect even when combined with the antifungal drugs because the results presented all equal to those found in control, thereby making the result irrelevant. In the literature there was not found any research that cite the antifungal activity of the *Padina sanctae crucis*.

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