

ANTIBACTERIAL ACTIVITY OF EXTRACTS OF SIX MACROALGAE FROM THE NORTHEASTERN BRAZILIAN COAST

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SHORT COMMUNICATION

ABSTRACT

Hexane, chloroform and ethanol extracts of six marine macroalgae (Rhodophyta and Chlorophyta) from North Ceará coast (Northeast Brazil) were evaluated for antibacterial activity by the single disk method. Best results were shown by the hexane extracts of *Amansia multifida* against enteric Gram-negative strains such as *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *S. cholerae-suis*, *Serratia marcescens*, *Vibrio cholerae* and the Gram-positive bacteria *Bacillus subtilis* and *Staphylococcus aureus*.

Key words: antibacterial activity, algae, lipid-soluble extracts

More than 150 000 macroalgae or seaweed species are found in the oceans of the globe but only a few of them were identified (9). Secondary or primary metabolites from these organisms may be potential bioactive compounds of interest for the pharmacological industry (1). Special attention has been reported for antiviral, antibacterial and/or antifungal activities related to marine algae against several pathogens (3,5,6,7,8,13,14,17,20). As an efficient strategy of investigation, organic solvents have been used to extract the possible lipid-soluble active principles from macroalgae (11). Since Brazil has an extensive coast where algae from virtually all groups are present, the goal of the present work was to test six macroalgae from Northeastern Brazil for antibacterial activity.

Samples of algae were collected during low tide at Pacheco Beach, Ceará State, from May to June 1995. Ecological damage during harvesting was prevented by not removing the algae stems. All samples were brought to laboratory in plastic bags containing sea water to prevent evaporation and then washed with distilled water to separate potential contaminants. Algae were identified following Harvey (9), and belonged to two

families: Chlorophyceae (*Ulva fasciata* DELILE, *Caulerpa cupressoides* WEST IN VAHL, *Caulerpa prolifera* FORSSKAL) and Rhodophyceae (*Gracilaria domingensis* SONDER, *Gracilaria* sp., *Amansia multifida* LAMOUROUX).

Samples were dried in oven at 37°C and ground in an electric coffee mill. Resulting powder was submitted to lipid-soluble extraction with hexane, chloroform and ethanol 1:15 (p:v) using a Soxhlet extractor at 55-60°C. All samples were refluxed until saturation (24 h) and the respective extracts were dried in an oven at 50°C (chloroform and ethanol extracts) or rotavapor (hexane extracts). Subsequently, the residual extracts were suspended in the respective solvents to final concentration of 1mg/20 µl.

Antibacterial activity was tested against the pathogenic Gram-negative strains of *Citrobacter freundii*, *Escherichia coli* (ATCC – 13863), *Enterobacter aerogenes*, *Klebsiella pneumoniae* (ATCC – 10031), *Morganella morganii*, *Pseudomonas aeruginosa* (ATCC – 25619), *Salmonella typhi*, *S. enterica* subsp. Typhimurium, *S. enterica* subsp. Enteritidis, *S. cholerae-suis*, *Serratia marcescens* and *Vibrio cholerae* or

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Gram-positive strains of *Bacillus subtilis* (ATCC – 6633), *Staphylococcus epidermidis* and *S. aureus* (ATCC – 6538). Single disk method as described by Bauer *et al* was used (4). The bacterial strains used in this work (others than ATCC strains) were isolated from human beings and belong to the laboratory collection of the Department of Biology from Federal University of Ceará. All bacteria were grown (10^8 cells/ml) in nutrient broth incubated at 37°C for 24 h and plated, using a sterile swab, onto Petri dishes containing Müeller-Hinton agar. At the same time, sterile discs of 5 mm diameter were embedded with 20 µl of the algal lipid-soluble extracts – experimental group – or with the solvent – control group – and added to the cultured dishes. Toxicity of the macroalgae extracts against microorganisms was determined after 24 h at 37°C by measuring the diameter of the halo around the discs, using a pachymeter. All experiments were performed at least in duplicate. Representative halos were those measuring a diameter superior to 10 mm.

Results are described in Table 1. The largest halos were achieved by the hexane extract of the Rhodophyceae *Amansia multifida* against *E. aerogenes*, *K. pneumoniae*, *P. aeruginosa*, *S. typhi*, *S. cholerae-suis*, *Ser. marcescens*, *Vibrio cholerae*, *B. subtilis* and *Staphylococcus aureus*. In addition, *Gracilaria* sp. produced a discrete inhibition halo against *B. subtilis*. Presence of representative halos in Chlorophyceae hexane extracts was shown only by *C. cupressoides* against *M. morgani*, *B. subtilis* and *S. epidermidis*. Other algae hexane, chloroform and ethanol extracts achieved halos not superior to

10 mm or were not identified (data not shown). Likewise, solvents used alone (controls) produced no halo (data not shown).

Lipid-soluble extracts from marine macroalgae have been investigated as a source of substances with pharmacological properties. Moreover, several different organic solvents have been used to screening algae for antibacterial activity. Early, Olessen *et al.* (12) related antibacterial activity in the chloroform and acetone extracts of *Falkenbergia billebrandii* against *S. aureus*. Kamat *et al.* (10) reported antiviral activities in ethanol extracts from 17 macroalga species of Indian Coast from 31 species tested. Moreover, antiherpes and antiinfluenza activities have been recently reported (18,19). Sastry *et al.* (16) showed antibacterial activity against Gram-positive and Gram-negative pathogenic strains after successive extraction with benzene, chloroform and methanol. Likewise, Mahasneh *et al.* (11) have shown antibiotic activity in organic extracts of six species of marine algae against multi-antibiotic resistant bacteria.

Antimicrobial activity depends on both algal species and efficiency on extraction of their active(s) principle(s). For example, water extracts from *U. fasciata*, *C. prolifera* and *G. domingensis* were all effective against *Proteus vulgaris* but the same did not happen with *A. multifida* (21). Likewise, lipid-soluble extracts of *A. multifida* (chloroform-methanol 2:1, 30 min reflux) tested against *P. aeruginosa*, *E. coli*, *B. subtilis* and *S. aureus* have shown no activity (2). On the other hand, from the 6 algae used in the present study, only the hexane extract of the Rhodophyceae *A. multifida* was effective against all bacteria tested (Table 1).

Table 1. Inhibition halo diameter (mm) of hexane extracts of marine macroalgae from Northeastern Brazilian coast

Microorganisms	Algae						
	Chlorophyceae			Rhodophyceae			
	<i>Ulva Fasciata</i>	<i>Caulerpa cupressoides</i>	<i>Caulerpa prolifera</i>	<i>Gracilaria domingensis</i>	<i>Gracilaria</i> sp.	<i>Amansia multifida</i>	
Gram positive	<i>Bacillus subtilis</i>	-	13.0 ± 0.1	-	-	1.0 ± 0.1	16.5 ± 0.7
	<i>Staphylococcus epidermidis</i>	-	17.0 ± 0.1	-	-	-	NT
	<i>Staphylococcus aureus</i>	-	-	-	-	-	18.5 ± 0.7
Gram negative	<i>Citrobacter freundii</i>	-	-	-	-	-	NT
	<i>Escherichia coli</i>	-	-	-	-	-	-
	<i>Enterobacter aerogenes</i>	-	-	-	-	-	14.5 ± 0.7
	<i>Klebsiela pneumoniae</i>	-	-	-	-	-	-
	<i>Morganella morgani</i>	-	11.0 ± 0.2	-	-	-	NT
	<i>Pseudomonas aeruginosa</i>	-	-	-	-	-	-
	<i>Salmonella typhi</i>	NT*	NT	NT	NT	NT	17.5 ± 0.7
	<i>Salmonella typhimurium</i>	-	-	-	-	-	NT
	<i>Salmonella enteritidis</i>	-	-	-	-	-	NT
	<i>Salmonella cholerae-suis</i>	NT	NT	NT	NT	NT	18.0 ± 0.7
	<i>Serratia marcescens</i>	NT	NT	NT	NT	NT	17.0 ± 1.4
<i>Vibrio cholerae</i>	NT	NT	NT	NT	NT	18.5 ± 0.6	

* NT: Not tested.

The chemical nature of active principles in lipid-soluble extracts of marine algae is not so far totally identified. Rossel *et al.* (15) associated antibiotic activity from 10 *Xantophyta* to the presence of unsaturated fatty acids, organic acids and phenol compounds. Our preliminary results suggest that antibacterial activity observed in *A. multifida* hexane extract against Gram-positive as well Gram-negative bacteria could be due to more than one active principle. This hypothesis will be further investigated.

Finally we conclude that macroalgae from the Brazilian coast are potential sources of bioactive compounds and should be investigated for natural antibiotics.

RESUMO

Avaliação de atividade antibacteriana de extratos de seis macroalgas do nordeste brasileiro

Extratos lipídicos de seis macroalgas (Rhodophyta e Chlorophyta) coletadas na costa do Estado do Ceará foram avaliadas para atividade antibacteriana pelo método de difusão em disco. Os extratos de *Amansia multifida* (obtidos a partir dos solventes orgânicos hexana, clorofórmio e etanol) demonstraram os melhores resultados contra as espécies entéricas Gram negativas de *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *S. cholerae-suis*, *Serratia marcescens*, *Vibrio cholerae* e bactérias Gram positivas *Bacillus subtilis* e *Staphylococcus aureus*.

Palavras-chave: atividade antibacteriana, algas, extratos lipídicos.

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