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Biological activity and chemical characterization of *Gracilaria caudata* J. Agardh (Gracilariaceae) extracts

Atividade biológica e caracterização química dos extratos da *Gracilaria caudata* J. Agardh (Gracilariaceae)

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The growing incidence of antimicrobial resistance poses a significant threat to global public health, a factor that has intensified the search for new natural sources with therapeutic potential. In this scenario, seaweeds stand out for their rich composition of secondary metabolites with diverse pharmacological properties. In this study, the biological activity and chemical composition of extracts from *Gracilaria caudata* J. Agardh were evaluated. Microbiological tests demonstrated a broad spectrum of antimicrobial action, with emphasis on the hexane extract, which proved effective against *Candida albicans, Candida glabrata, Escherichia coli, Salmonella choleraesuis, Staphylococcus aureus, Bacillus subtilis,* and *Bacillus cereus*, presenting inhibition halos ranging from 7.2 mm to 18.8 mm. In addition, the extracts did not show cytotoxicity in sheep erythrocytes in the preliminary evaluation, reinforcing their safety for future applications. ^{1}H NMR spectroscopic analysis revealed the predominance of nonpolar metabolites, such as fatty acids. These results highlight the biotechnological potential of *Gracilaria caudata* as a promising source of marine-derived antimicrobial agents.
Keywords: antimicrobials, bioprospecting, natural products.

A crescente incidência da resistência antimicrobiana representa uma ameaça significativa à saúde pública global, fator que tem intensificado a busca por novas fontes naturais com potencial terapêutico. Nesse cenário, as macroalgas marinhas destacam-se por sua rica composição de metabólitos secundários com propriedades farmacológicas diversas. Neste estudo, avaliou-se a atividade biológica e a composição química dos extratos de *Gracilaria caudata* J. Agardh. Os ensaios microbiológicos demonstraram um amplo espectro de ação antimicrobiana, com ênfase no extrato hexânico, que se mostrou eficaz contra *Candida albicans*, *Candida glabrata*, *Escherichia coli*, *Salmonella choleraesuis*, *Staphylococcus aureus*, *Bacillus subtilis* e *Bacillus cereus*, apresentando halos de inibição variando de 7,2 mm a 18,8 mm. Além disso, os extratos não demonstraram citotoxicidade em eritrócitos de carneiro na avaliação preliminar, reforçando sua segurança para aplicações futuras. A análise espectroscópica de RMN ^{1}H revelou a predominância de metabólitos apolares, como ácidos graxos. Esses resultados evidenciam o potencial biotecnológico de *Gracilaria caudata* como uma fonte promissora de agentes antimicrobianos de origem marinha.
Palavras-chave: antimicrobianos, bioprospecção, produtos naturais.

1. INTRODUCTION

Gracilaria caudata is a species of seaweed belonging to the phylum Rhodophyta (red algae), class Florideophyceae, order Gracilariales, and family Gracilariaceae [1]. Seaweed is an important source of bioactive metabolites for the pharmaceutical industry, many of which can be used to treat diseases such as cancer, inflammation, oxidative stress, allergies, thrombosis, hypertension, pain, arthritis, as well as viral, bacterial and fungal infections [2, 3].

In recent years, the growing incidence of antimicrobial resistance has become a significant threat to global public health, compromising the effectiveness of essential medical treatments and promoting the spread of infectious diseases [4]. Despite the wide variety of antimicrobials used to combat pathogenic microorganisms, the emergence of resistant strains has driven the search for new therapeutic agents that have a broad spectrum of action, low cost, and less potential for inducing resistance [5, 6].

In this scenario, bioprospecting of natural compounds emerges as a promising strategy. Among marine organisms with bioactive potential, seaweed of the genus *Gracilaria* stand out for their diversity of secondary metabolites, which may represent effective sources for the development of new antimicrobial agents [7, 8].

Thus, the present study aimed to perform phytochemical screening, evaluate cytotoxicity, and investigate the antimicrobial activity of extracts from *Gracilaria caudata*.

2. MATERIAL AND METHODS

2.1 Material collection

The collections were authorized by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio), through the Sistema de Autorização e Informação em Biodiversidade (SISBIO), under number 25423-15. An exsiccate of the species collected was prepared and is deposited in the Herbarium of the State University of Bahia (HUNEB), under registration number 34014. The collections were made during low tides, following conventional techniques for studying seaweeds [9].

2.2 Preparation of crude extracts

The botanical material was separated manually and washed with distilled water to remove impurities and excess salt. Seaweeds were then dried in an oven at 40°C and ground into a fine powder. To prepare the extracts, 251.91 g of dry biomass of *Gracilaria caudata* was used. The extracts were obtained by maceration using solvents in ascending order of polarity: hexane and a binary solution of ethyl acetate and ethanol in a ratio of 9:1 (v/v). The process consisted of sequential extraction, in which the same biomass was successively subjected to each solvent (500 mL). For each extracting solution, three consecutive macerations were performed at 72-hour intervals, followed by filtration. After evaporation of the solvent at room temperature, the crude extracts were stored at 4 °C until biological analysis was performed. The entire preparation methodology was adapted from the protocol described by Rocha et al. (2021) [10].

2.3 Phytochemical screening

The phytochemical screening of the extracts was carried out using hydrogen proton nuclear magnetic resonance spectroscopy (^{1}H NMR). The analyses were carried out on an INOVA spectrometer operating at 500 MHz, using tetramethylsilane (TMS) as an internal standard for chemical calibration [10].

2.4 Evaluation of hemolytic activity

The hemolytic activity of the extracts was evaluated using the disk diffusion method with blood agar, as described by Rocha et al. (2025) [11] with adaptations. Four concentrations (10.000, 1.000, 100, and 10 µg mL^{-1}) were prepared by resuspending specific masses in different volumes of dimethyl sulfoxide (DMSO, 2%) to achieve the desired concentrations for each treatment. Aliquots of 10 µL of each concentration were applied to sterile filter paper discs (5 mm in diameter) in triplicate. The impregnated discs were distributed on Petri dishes containing blood agar (Laborclin®). DMSO (2%) was used as a negative control, while the positive control consisted of Tween 80 (100%). The plates were incubated at 37°C for 24 hours. After the incubation period, the presence or absence of hemolysis halos around the discs was evaluated, which were measured with a millimeter ruler and expressed in millimeters (mm) ± standard deviation.

2.5 Microorganisms

To evaluate the antimicrobial activity of the extracts, standard strains from the American Type Culture Collection (ATCC) and the Tropical Culture Collection (CCT) were used: *Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis* (ATCC 6633), *Bacillus cereus* (CCT 0096), *Micrococcus luteus* (ATCC 10240), *Escherichia coli* (ATCC 94863), *Salmonella choleraesuis* (ATCC 14028), as well as fungal strains of *Aspergillus niger* (ATCC 16404), *Candida glabrata* (CCT 0728), and *Candida albicans* (ATCC 18804). The selected microorganisms are widely used in antimicrobial susceptibility testing because they are associated with human infections and show significant resistance to several commercially available antimicrobials.

2.6 Evaluation of antimicrobial activity

Antimicrobial activity was verified by the disk diffusion method according to standard M07-A10 [12]. Bacteria were cultured on Mueller-Hinton agar at 37°C for 24 hours, while fungi were cultured on Sabouraud Dextrose agar at the same temperature for 48 hours. Colonies of freshly cultured microorganisms were transferred directly from the plates to tubes containing sterile saline solution (0.9%). The suspension was adjusted according to the MacFarland-0.5 standard, corresponding to a concentration of 1.5×10^8 CFU/mL.

The suspended inoculum was transferred to Petri dishes using a swab previously soaked in the microbial suspension. To prepare the test solutions, the extracts were initially resuspended in DMSO (5%), used as a diluent, until reaching a concentration of 100 mg mL^{-1}. Filter paper discs (5 mm in diameter) containing 10 µL of each extract were then applied to the plates. DMSO (5%) was used as a negative control and tetracycline (30 µg/disc) as a positive control for bacteria. For fungal strains, the positive control used was Ciclopirox Olamine (0.1%). The plates were incubated at 37°C (24 hours for bacteria and 48 hours for fungi). The assays were conducted in triplicate, and the results were expressed in millimeters (mm) ± standard deviation corresponding to the diameters of the inhibition halos.

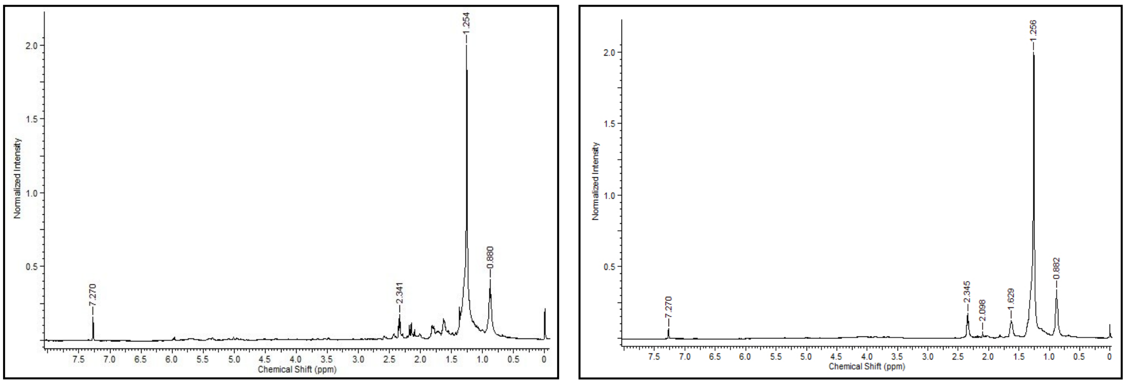
2.7 Statistical analyses

Statistical analyses were performed using GraphPad Prism 8 software. Data normality was verified using the Shapiro-Wilk test. Normally distributed data were analyzed using one-way ANOVA, followed by Tukey's multiple comparison test. All tests were conducted with a significance level of 5% ($p \leq 0.05$).

3. RESULTS

3.1 Phytochemical screening

The ^1H NMR spectra of hexane and ethyl acetate/ethanol (9:1, v/v) extracts of *Gracilaria caudata* revealed a set of signals indicative of the presence of apolar components (Figures 1A-B).



A B

Figure 1: (A) [1]H NMR spectra (500 MHz) of the hexane extract of Gracilaria caudata; (B) [1]H NMR spectra (500 MHz) of the ethyl acetate/ethanol extract (9:1, v/v) of Gracilaria caudata.

Based on the chemical shifts observed, the hexane extract (A) is predominantly composed of lipid substances. The high signal intensity at δ 1.254 ppm, together with the signals at δ 2.341 ppm and δ 0.880 ppm, suggests the abundant presence of fatty acids (free or esterified), triglycerides, and other lipids with long saturated aliphatic chains. A high-intensity signal at δ 1.25 ppm was observed in both extracts, suggesting the presence of a long CH_2 chain, while the signal at δ 0.880 ppm indicated the presence of terminal methyl groups (CH_3) of fatty acids.

The acetate and ethanol extract (B) is also predominantly composed of lipid substances, with a strong presence of fatty acids and their derivatives, evidenced by the high intensity signals at δ 1.256 ppm and δ 0.882 ppm. The additional signals at δ 2.088 ppm and δ 1.629 ppm, compared to Extract A, may suggest a greater diversity of lipids, possibly including fatty acids with a higher degree of unsaturation or the presence of other functional groups that influence the chemical environment of the methylene protons.

3.1 Evaluation of hemolytic activity

No hemolytic effect was observed for any of the *Gracilaria caudata* extracts at the concentrations tested (10 – 10.000 µg mL^{-1}), indicating the absence of hemolysis under the conditions evaluated. In contrast, the positive control (Tween 80, 100%) produced halos of 18.0 ± 0 mm (mean ± standard deviation), confirming the sensitivity of the assay. The negative control (DMSO, 2%) showed no hemolytic activity. Taken together, these results demonstrate that the extracts are not hemolytic in the concentration range evaluated.

3.2 Evaluation of antimicrobial activity

The evaluation of *Gracilaria caudata* extracts by disk diffusion showed activity against gram-positive bacteria (*Bacillus subtilis, Micrococcus luteus, Staphylococcus aureus, Bacillus cereus*), gram-negative bacteria (*Escherichia coli, Salmonella choleraesuis*), and fungal strains (*Candida albicans, Candida glabrata, Aspergillus niger*) (Table 1).

Table 1: Antimicrobial activity of extracts at a concentration of 100 mg mL-1 of the seaweed Gracilaria caudata.

	Inhibition zone (mm)			
Bacterial strains	**HEX**	**ACET/ETOH**	**Controls**	
			Tetracycline (30µg)	**DMSO (5%)**
B. subtilis	11 ± 0,63[a]	8,5 ± 0,55[a]	26,7 ± 0,58[b]	0,0 ± 0,0
M. luteus	7,2 ± 1,17[a]	7,7 ± 1,21[a]	34,0 ± 1,0[b]	0,0 ± 0,0
S. aureus	10,3 ± 1,63[a]	8,2 ± 0,75[a]	25,7 ± 0,58[b]	0,0 ± 0,0
B. cereus	10,8 ± 0,41[a]	8,3 ± 1,21[a]	25,7 ± 0,58[b]	0,0 ± 0,0
E. coli	11,8 ± 0,75[a]	10,2 ± 1,47[a]	22,7 ± 0,58[b]	0,0 ± 0,0
S. choleraesuis	11,3 ± 1,37[a]	7,7 ± 0,82[a]	19,3 ± 0,58[b]	0,0 ± 0,0
Fungal strains			**Ciclopirox Olamine (0.1%)**	
C. albicans	18,8 ± 1,79[a,b]	14,5 ± 3,94[b]	15,0 ± 0[b]	0,0 ± 0,0
C. glabrata	13,5 ± 2,43[a]	NT	15,0 ± 0[a]	0,0 ± 0,0
A. niger	10,4 ± 0,55[a]	6,3 ± 0,52[a]	15,7 ± 0[b]	0,0 ± 0,0

Hex - Extract in hexane; Acet/EtOH - Extract in ethyl acetate/ethanol (9:1, v/v); DMSO - Dimethylsulfoxide; NT - Not tested. Results are expressed as mean ± standard deviation (n=3). Means followed by the same letters in the lines do not differ statistically from each other by Tukey's comparison test (p >0.05).

A concentration of 100 mg mL^{-1} was used as the initial screening condition for the preliminary evaluation of the antimicrobial activity of the extracts, following an approach similar to that adopted by Bomfim et al. (2020) [13] and Rocha et al. (2025) [11]. The highest antifungal activity was observed for the hexane extract against *Candida albicans*, with an inhibition halo of 18.8 mm. Regarding bacteria, the best results were also obtained with the hexane extract, highlighting the inhibition of *Escherichia coli* and *Salmonella choleraesuis*, with halos of 11.8 mm and 11.3 mm, respectively.

The ethyl acetate/ethanol (ACET/ETOH) extract was not evaluated against the *Candida glabrata* strain due to the limited amount of extract obtained after the extraction process, which resulted in a reduced yield.

4. DISCUSSION

Similar to other species of the genus *Gracilaria*, extracts of *Gracilaria caudata* showed remarkable biotechnological potential, suggesting the presence of biologically active compounds. Consistently, the extracts evaluated demonstrated a broad spectrum of antimicrobial activity against the strains tested, reinforcing the chemical and biological relevance of the species. The hexane and ethyl acetate/ethanol extracts exhibited antimicrobial activity, but with significantly lower effects than those observed for the antibiotic used as a positive control, as indicated by statistical analysis ($p<0.05$). This result is consistent with what is expected when comparing crude extracts – complex mixtures containing dozens or hundreds of metabolites in low concentrations, subject to synergistic, additive, or antagonistic interactions – with purified and formulated drugs, which contain isolated compounds of high purity and high molecular affinity for their biological targets [14, 15].

The microorganisms tested comprise a clinically relevant group due to their high resistance to conventional antimicrobials and their potential to cause opportunistic and difficult-to-treat infections [16, 17]. These findings are consistent with previous studies involving species of the same genus [18-20].

As discussed by Capillo et al. (2018) [18] and Torres et al. (2019) [8], antimicrobial activity in species of the genus *Gracilaria* is widely attributed to the presence of structurally diverse secondary metabolites. In the study by Capillo et al. (2018) [18], for example, extracts of *G. gracilis* exhibited limited activity, with inhibition restricted to *Bacillus subtilis*, indicating a relatively narrow spectrum of action.

In contrast, the *Gracilaria caudata* extracts analyzed in the present study demonstrated a considerably broader spectrum, with activity against Gram-positive and Gram-negative bacteria, as well as yeasts and filamentous fungi, evidencing a more robust bioactive profile. Among the compounds plausibly involved in this activity are fatty acids, polyphenols, bromophenols, lipids, and sterols, classes recognized for interfering with essential cellular processes of pathogenic microorganisms, conferring inhibitory potential against clinically relevant strains [21, 22].

The observation of the antifungal effect of *Gracilaria caudata* extracts was also significant. The absence of statistical difference between the extract and the antifungal agent ($p > 0.05$) in the *Candida* species evaluated indicates that, at the concentration tested, the *Gracilaria caudata* extract showed efficacy comparable to the reference drug – a finding that reinforces its high biotechnological potential. This performance suggests the presence of metabolites with recognized antifungal activity, including long-chain fatty acids, steroids, terpenoids, and phenolic compounds, widely associated with the inhibition of *Candida* species [23, 24].

For *Aspergillus niger*, the extracts tested exhibited significantly smaller inhibition halos than those produced by the reference antifungal agent ($p<0.05$), indicating lower potency at the screening concentration used (100 mg mL^{-1}). This difference is consistent with what is reported in the literature, since filamentous fungi tend to have greater intrinsic resistance due to the complex composition of their cell wall and the action of efficient detoxification mechanisms. These factors may require higher concentrations or more purified fractions of the extracts to produce effects comparable to those observed with purified drugs [25, 26].

The literature corroborates this potential within the *Gracilaria* genus. Shojaee et al. (2023) [27] demonstrated strong antifungal activity of *G. corticata* against dermatophytes (*T. mentagrophytes, M. canis,* and *M. gypseum*), attributing part of this effect to the action of florotanins capable of compromising the cell wall, membrane, and fungal mitochondrial processes [28]. Complementarily, Sampaio et al. (2022) [29] reported fungistatic activity of *Gracilaria cervicornis* against *C. albicans*, *C. tropicalis*, and *C. krusei*, in addition to the extract's ability to potentiate fluconazole, indicating its viability for use in combination therapies.

The discovery of antifungal activity is extremely relevant in the clinical field, given the pathogenic implications related to the *Candida* genus [30]. Species of this genus are classified as commensal microorganisms that become pathogenic in situations of reduced host immunity and are therefore classified as opportunistic pathogens [31, 32]. In view of this, the effective antifungal inhibition of the extracts indicates the feasibility of creating new therapies or improving existing treatments to combat infections caused by these microorganisms.

In the cytotoxicity assay, the absence of hemolysis halos indicates that extracts from the seaweed *Gracilaria caudata* do not contain hemolytic components, or contain them only in very low levels. The literature describes that hemolysis induced by plant extracts is generally associated with the interaction of certain metabolites, especially saponins, with the lipids present in the red blood cell membrane [33]. As discussed by Flek et al. (2019) [34], saponins have a high affinity for cholesterol in erythrocyte membranes, leading to disruption of the lipid bilayer, increased permeability, and, consequently, cell rupture.

However, although compounds with detergent properties – such as saponins themselves – are classic inducers of hemolysis through complexation with cholesterol [35, 36], the phytochemical analysis performed in this study did not detect the presence of saponins in the extracts evaluated. In contrast, a predominance of fatty acids and other lipids was found in the extracts. This phytochemical composition provides a coherent explanation for the observed dissociation between antifungal activity and the absence of hemolysis.

Fatty acids are widely recognized for their ability to destabilize fungal membranes [37-40], whose predominant sterol is ergosterol. Although ergosterol is functionally similar to cholesterol, structural differences confer selectivity to interactions with these compounds [41]. Thus, the extracts exhibit antifungal activity by preferentially interacting with ergosterol-rich membranes, without exhibiting the same lysis potential on erythrocytes, whose membrane is rich in cholesterol.

In addition to evidence of antimicrobial activity and the absence of cytotoxicity, the chemical composition of *Gracilaria caudata* further reinforces its bioactive potential, especially due to the presence of several secondary metabolites detected in the extracts. Among these, lipid compounds – in particular fatty acids – stand out as the majority in ^{1}H NMR analysis, strongly suggesting their involvement in the biological activities observed.

The formation of fatty acids occurs when acetyl-CoA, instead of proceeding to the citric acid cycle, is diverted to the malonic acid pathway, where it is converted to malonyl-CoA and used by the fatty acid synthase complex. This process derives directly from primary metabolism and generates lipids that can act structurally or as bioactive metabolites [42]. In addition to this route, other pathways also contribute to the production of fatty compounds, such as the mevalonic acid (MVA) and MEP pathways, which are responsible for the synthesis of terpenoids and sterols, expanding the diversity of lipids produced by organisms [43] in response to environmental variations.

The fatty acid composition in species of the genus *Gracilaria* is strongly influenced by environmental factors, especially light intensity and temperature, which modulate the synthesis and proportion of these lipids [44]. Jayasankar and Kulandaivelu (1999) [44] demonstrated wide qualitative and quantitative variation in the main fatty acids – such as lauric, myristic, myristoleic, palmitic, palmitoleic, stearic, oleic, and linoleic – among seven specimens analyzed, belonging to three distinct species of *Gracilaria* (*G. edulis*, *G. corticata*, and *G. crassa*). Even individuals collected in the same area showed significant differences, mainly attributed to light availability, highlighting the metabolic plasticity of the genus as an adaptive strategy, allowing the maintenance of membrane fluidity and functionality under variable environmental conditions [45].

Although the mechanism of action of fatty acids is not yet fully understood, evidence suggests that their main target is the membranes of microorganisms [46]. According to Ruffel et al. (2016) [47] and Debois and Smith (2010) [48], the structure of fatty acids allows them to act as detergents (lipophilicity), promoting the formation of pores in the bacterial membrane, which compromises its integrity and interferes with essential processes, such as the electron transport chain and oxidative phosphorylation, affecting energy production. In fungi, fatty acids cross the cell wall and interact with the ergosterol-rich plasma membrane, increasing its fluidity and promoting changes in membrane proteins, which leads to cytoplasmic disintegration and loss of vital cellular functions [49].

5. CONCLUSION

The extracts of *Gracilaria caudata* showed promising bioactive potential, with emphasis on the hexane extract, which demonstrated greater efficacy in the tests performed. The results indicate a broad spectrum of antimicrobial action and no toxicity in biological models, suggesting that lipophilic compounds present in seaweed can contribute significantly to the development of new antimicrobial agents. Considering the complexity of the chemical matrix involved, future studies should prioritize the isolation of active substances, with the aim of increasing selectivity and elucidating the mechanisms of action involved.

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