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Allelopathic potential of extracts the from marine macroalga *Plocamium brasiliense* and their effects on pasture weed

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Abstract: Four extracts from the marine red alga *Plocamium brasiliense* (Greville) M.A.Howe & W.R.Taylor were prepared to identify and characterize their potential allelopathic effects on seed germination, radicle elongation and hypocotyl development of the weeds *Mimosa pudica* L. and *Senna obtusifolia* (L.) Irwin & Barneby. The four extracts were prepared in a sequence of solvents of increasing polarity: *n*-hexane, dichloromethane, ethyl acetate and ethanol/water (7:3). The germination bioassay was carried out at 25 °C with a 12 h photoperiod and the radicle elongation and hypocotyl development at 25 °C with a 24 h photoperiod. The dichloromethane extract showed inhibitory effects on seed germination of both plants (35 and 14%, respectively, in *M. pudica* and *S. obtusifolia*), radical germination (52 and 41.7%, respectively) and hypocotyl development (17.1 and 25.5%, respectively). Given the high sensitivity of this parameter to the potential allelopathic effects and the insufficient number of references found in the literature, these results are expected to stimulate new tests with other species of marine algae. Given the high sensitivity of the method for the detection of allelopathic potential, the species *P. brasiliense* emerges as a possible source of allelopathic substances against weed species. The results are attributed to the chemical composition, especially in relation to the presence of halogenated monoterpenes.

Introduction

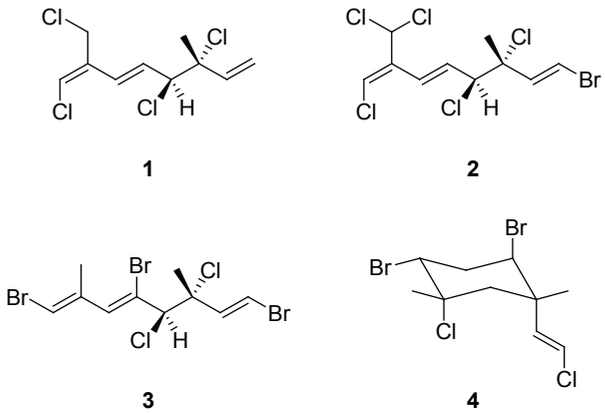
The inadequate use of fire and mowing, along with the application of synthetic herbicides, has become a very common practice for controlling weeds in Brazilian pastures. Nonetheless, these procedures have been demonstrated to be inefficient (Souza Filho et al., 2006). The synthetic herbicides currently utilized in agriculture have organochlorine and organophosphate chemicals in their composition. Thus, despite the importance of such herbicides for the control of invasive plants, there has been ample discussion concerning the damage they cause to both man and the environment.

Therefore, the investigation of the allelopathic potential of various living organisms might be a more viable alternative when it comes to controlling invasive plants. In fact, the liberation of chemical substances that have phytotoxic activities can have a significant influence on the population dynamics of plants in the countryside, since these substances may alter the pattern and the density of the vegetation (Souza Filho & Alves, 2000, Souza Filho, 2002, Souza Filho et al., 2006; Souza et al., 2006). Taking this into consideration, our study aims to enable quantitative and qualitative improvements in the production of food on both small and large agricultural properties in Brazil.

In this context, Brazil has a large biological diversity, especially in marine environments, where macroalgae are especially relevant since they represent a natural strategic resource for the development of biotechnology. The genus *Plocamium* Lamouroux has approximately 42 species and more than 100 monoterpenes have been isolated from twelve species (Teixeira, 2009). *P. brasiliense*, the only species on the Brazilian coast, is distributed in North America, the Caribbean Islands

(Taylor, 1960; Schneider & Searles, 1991), Trinidad & Tobago (Duncan & Lee Lum, 2006) and South America (Taylor, 1930, 1960; Joly, 1957, 1965; Ganesan, 1990; Schneider & Searles, 1991; Díaz-Pulido & Díaz-Ruíz, 2003).

Previous studies of the chemical components isolated from Brazilian *P. brasiliense* have been performed by our group (Ferreira et al., 2010; Vasconcelos et al., 2010). The halogenated monoterpenes **1**, **2** and **3** were isolated from *P. brasiliense* in a chemotaxonomic analysis (Vasconcelos et al., 2010). In another study, the crude CH_2Cl_2 extract (containing monoterpenes **1**, **2** and **4**) and a fraction enriched with the halogenated monoterpene **2** from *P. brasiliense* were evaluated for cytotoxicity and against the virus HSV-1 (Ferreira et al., 2010).



Studies showed that these metabolites represent a means of defense against marine herbivores, fouling organisms and pathogens, as well as protection against radiation and allopathic agents (*e.g.*, Sakata et al., 1991). Based on these conclusions, the present study assesses the potential for allelopathic activity of four extracts (n-hexane, dichloromethane, ethyl acetate and aqueous ethanol) obtained from the marine alga *Plocamium brasiliense* (Rhodophyta).

Materials and Methods

Plant material

Specimens of *Plocamium brasiliense* (Greville) M.A.Howe & W.R.Taylor were collected in October, 2010, in the sublittoral of Enseada do Forno, Armação de Búzios (RJ) (22°45'S e 41°52'W). The seaweeds were washed with local seawater and separated from sediments, epiphytes and other associated organisms. The algae were dried for twenty days and extracted with the appropriate solvent. Voucher specimens were deposited in the Herbarium of the Universidade do Estado do Rio de Janeiro (HRJ 10331-32).

Receptor species

The receptor plants used in the bioassays were two of the main weeds that occur in areas of cultivated pastures in the Amazon region, which are known as malícia (*Mimosa pudica*) and mata-pasto (*Senna obtusiloba*). The seeds of these two plants were collected in the municipality of Castanhal-PA. The seeds were cleaned and purged and placed in seed conservation chambers. The seed dormancy was overcome as established by Souza Filho (2002) and Souza Filho et al. (2006).

Preparation of crude extracts

The algae *P. brasiliense*, collected and dried at room temperature (average of 28 ºC), were triturated in an industrial blender and placed in a plastic tray, yielding 5.16 g of powdered whole algae. The extracts were obtained by an exhaustive extraction using a decanting funnel (1 L). The procedures involved the sequential utilization of solvents of increasing polarity. Therefore, the order in which the solvents were used was: *n*-hexane, dichloromethane, ethyl acetate and ethanol/water (7:3). In the first step, 5.16 g of dry algae were placed in the decantation funnel with 400 mL of *n*-hexane for eight days. This process was repeated twice. The extracts were concentrated on a rotary evaporator, obtaining 122 mg of n-hexane extractable material. The sequence of extraction was repeated for the other solvents, which generated the following masses: dichloromethane, 196 mg; ethyl acetate, 42 mg; and ethanol/water, 250 mg.

Assays

The seed germination bioassays were developed under controlled conditions of constant temperature (25 °C) and a photoperiod of 12 h. Germination was monitored for fifteen days with daily counts and elimination of the germinated seeds. Germinated seeds were considered to be those that had a root length equal to or greater than 2.00 mm (Souza Filho, 2002, Souza Filho et al., 2006). Each transparent Petri dish, 9.0 cm in diameter, received twenty seeds. The radicle elongation biossays were performed under controlled conditions of constant temperature (25 °C) and a photoperiod of 24 h in a germination chamber with cool white fluorescent lamps and and a luminous flux of approximately 10 $\mu mol.m^{-2}.s^{-1}$. We used six seeds pre-germinated for approximately three days in a transparent Petri dish 9.0 cm in diameter. At the end of a period of ten days of growth, we measured the length of the radicles. Each Petri dish was covered with quantitative filter paper (Souza Filho, 2002).

A concentration of 1% m/v of each crude extract was added at the beginning of each bioassay. Since then, only distilled water was added to the system when

necessary. The test solutions were added only once, at the beginning of the bioassays, and thereafter distilled water added only when necessary.

Statistical analysis

The statistical test for all experiments was completely randomized with three repetitions. Data were submitted to the F test for analyses of variance, while the means were compared by the Tukey test (p<5%). All analysis was performed with the program SAS for Windows (Sas, 1989).

Results and Discussion

The results for the effect of the crude extracts on the germination of *M. pudica* and *S. obtusifolia* are summarized in Table 1. No seaweed extract promoted a significant allelopathic effect on the seeds of *S. obtusifolia*, the best result (14% of inhibition) being that of the dichloromethane extract. By comparison, the allelopathic effects of the extracts on *M. pudica* were more significant than those obtained with S. obtusifolia. The dichloromethane extract was the most active, with the greatest inhibition of the germination of the seeds (25%).

Table 1. Inhibitory effects of the different crude extracts of the algae *Plocamium brasiliense* on the germination of two species of weeds. Data expressed as percentage of inhibition compared to the control.

Receptor plants	Extracts			
	n-hexane	dichloromethane	ethyl acetate	ethanol/ water
Mimosa pudica	10.0±3.4	25.0±5.4	13.0±4.1	14.0±2.7
Senna obtusiloba	4.0±1.3	14.0±3.9	6.0±2.6	7.0±2.8

The analysis of the effect of each extract on radicle elongation is presented in Table 2. The results show that the dichloromethane extract of *P. brasiliense* contains active components that inhibited 52.0 and 41.7%, respectively, of the radicle elongation of *M. pudica* and *S. obtusiloba*. However, in the radicle elongation experiments, the ethyl acetate extract also showed 32.0% inhibition of *M. pudica* and 21.1% for *S. obtusiloba*. These expressive values in the radicle elongation bioassay demonstrate that the sensitivity to potential allelopathic effects is much greater in the radicle elongation phase than in seed germination.

In the analysis of the effect of each extract on hypocotyl development of the weeds, the ethanol/ water and dichloromethane extracts were more effective with *S. obtusifolia* than with *M. pudica* (Table 3). The dichloromethane extract was again the greatest inhibitor (17.1% for *M. pudica* and 25;5% for *S. obtusifolia*), followed by the ethanol/water extract (13.4% for *M. pudica* and 14.8% for *S. obtusifolia*). The crude dichloromethane extract was the most efficient and, therefore, the extract that represents the main source of secondary metabolites with allelopathic activity present in *P. brasiliense.* In addition, the *P. brasiliense* extracts tested were more efficient for *M. pudica* in the germination and radicle development bioassays, whereas *S. obtusifolia* showed better results in the hypocotyl experiment since it was more sensitive to the allelochemicals present in the dichloromethane and ethyl acetate extracts.

These results also indicate that the dichloromethane extract is more likely to inhibit the development of invasive plants than their germination, because the bioassay for radicle development presented results of greater magnitude.

Table 2. Inhibitory effects of the different crude extracts of the algae *Plocamium brasiliense* on the development of radicles. Data expressed as percentage of inhibition compared to the control treatment.

Receptor plants	Extracts			
	n-hexane	dichloromethane	ethyl acetate	ethanol/ water
Mimosa pudica	11.6±5.4	52.0±5.2	32.0±4.4	29.3±5.2
Senna obtusiloba	8.4±2.8	41.7±4.8	21.1±3.9	18.6±3.7

Table 3. Inhibitory effects of different crude extracts of the algae *Plocamium brasiliense* on the development of the hypocotyl. Data expressed as percentage of inhibition compared to the control treatment.

Receptor plants	Extracts			
	n-hexane	dichloromethane	ethyl acetate	ethanol/ water
Mimosa pudica	8.7± 3.1	17.1±3.2	10.4±2.8	13.4±3.0
Senna obtusiloba	6.4±2.7	25.5±3.8	9.8±3.0	14.8±3.8

The results obtained with the extracts point to the importance of a search for the chemical components responsible for these phytotoxic activities. In any event, the red marine alga *P. brasiliense* can now be considered to be a potential source of herbicides.

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References

Díaz-Pulido G, Díaz-Ruíz M 2003. Diversity of benthic marine algae of the Colombian Atlantic. *Biota Colombiana 4*: 203-246.

Duncan EJ, Lee Lum LM 2006. A checklist of the marine macroalgae of the Republic of Trinadad and Tobago. *Caribbean Mar Studies 7*: 1-96.

Ferreira WJ, Amaro R, Cavalcanti DN, Rezende CM, Giongo VA, Barbosa JE, Paixão ICNP, Teixeira,VL 2010. Anti-herpetic activities of chemical components from the Brazilian red alga *Plocamium brasiliense. Nat Prod Commun 5*: 1167-1170.

Ganesan EK 1990. *A catalog of benthic marine algae and seagrasses of Venezuela*. Caracas: Fondo Editorial Conicit, p 1-237.

Joly AB 1957. Contribuição ao conhecimento da flora ficológica marinha da Baía de Santos e Arredores. *Bol Fac Fil Ci Letras Univ S Paulo, Bot 14*: 3-199.

Joly AB 1965. Flora marinha do litoral norte do estado de São Paulo e regiões circunvizinhas. *Bol Fac FilCi Letras Univ S Paulo, Bot 21*: 5-393.

Sakata K, Iwase Y, Ina K, Fujita D 1991. Chemical studies on feeding inhibitors for marine herbivores. 2. Halogenated terpenes isolated from the red alga *Plocamium leptophyllum* as fedding inhibitors for marine herbivores. *Nippon Suisan Gakkaishi 57*: 743-746.

SAS 1989. User's Guide. Version 6.12, SAS Inst. Inc., Cary, NC.

Schneider CW, Searles RB 1991. *Seaweeds of the southeastern United States - Cape Hatteras to Cape Canaveral.* Durrham & London: Duke University Press, Durham, p 1-553.

Souza Filho APS, Alves SM 2000. Potencial alelopatico de plantas de acapu (*Vouacapoua americana*): efeitos sobre plantas daninhas de pastagens. *Planta Daninha 18*: 435-441.

Souza Filho APS, Santos RA, Santos LS, Guilhon GMP, Santos AS, Arruda MSP, Muller AH, Arruda AC 2006. Potencial alelopático de *Myrcia guianensis*. *Planta Daninha 24*: 649-656.

Souza Filho APS 2002. Atividade potencialmente alelopática de extratos brutos e hidroalcoólicos de feijão-de-porco (*Canavalia ensiformis*). *Planta Daninha 20*: 357-364.

Souza LS, Velini ED, Martins D, Rosolem CA 2006. Efeito alelopático de capim-braquiária (*Brachiaria decumbens*) sobre o crescimento inicial de sete espécies de plantas cultivadas. *Planta Daninha 24*: 657-668.

Taylor WR 1930. A synopsis of the marine algae of Brazil. *Revue Algologique 5*: 1-35.

Taylor WR 1960. *Marine algae of the eastern tropical and subtropical coasts of the Americas*. Ann Arbor, The University of Michigan Press, p 1-870.

Teixeira VL 2009. Produtos Naturais Marinhos. In *Biologia Marinha.* Pereira RC, Soares-Gomes A (Eds), Rio de Janeiro, Interciência, p 443-471.

Vasconcelos MA, Ferreira WJ, Pereira RC, Cavalcanti DN, Teixeira VL 2010. Chemical constituents from the red alga *Plocamium brasiliense* (Greville) M. Howe and W.R. Taylor. *Biochem Syst Ecol 38*: 119-121.

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