Inhibitory effect of the red seaweed Plocamium brasiliense against the toxic effects of Lachesis muta snake venom

ARTICLE *in* JOURNAL OF APPLIED PHYCOLOGY · OCTOBER 2014

Impact Factor: 2.56 · DOI: 10.1007/s10811-014-0266-x

8 AUTHORS, INCLUDING:

Rainiomar Raimundo Fonseca
University of the State of Amazonas - Brazil
4 PUBLICATIONS **7** CITATIONS
SEE PROFILE

Eladio Sanchez
Fundação Ezequiel Dias
17 PUBLICATIONS **223** CITATIONS
SEE PROFILE

Valeria Teixeira
Universidade Federal Fluminense
115 PUBLICATIONS **1,488** CITATIONS
SEE PROFILE

Andre Fuly
Universidade Federal Fluminense
56 PUBLICATIONS **973** CITATIONS
SEE PROFILE

J Appl Phycol (2014) 26:2047–2054
DOI 10.1007/s10811-014-0266-x

Inhibitory effect of the red seaweed *Plocamium brasiliense* against the toxic effects of *Lachesis muta* snake venom

Mariana Moreira Claudino · Thaisa Francielle Souza Domingos · Geisiane Alves da Silva · Rainiomar Raimundo Fonseca · Diana Negrão Cavalcanti · Eladio Flores Sanchez · Valéria Laneuville Teixeira · André Lopes Fuly

Received: 3 January 2014 / Revised and accepted: 2 February 2014 / Published online: 18 February 2014

Abstract The ability of extracts and fractions of the red seaweed *Plocamium brasiliense* to inhibit hemorrhagic, edematogenic, hemolytic, clotting and proteolytic activities of *Lachesis muta* snake venom was analyzed. In Brazil, snakebites by *L. muta* are low (2 %) when compared to *Bothrops* genus (90 %); however, their lethality indexes are three times higher than *Bothrops*. Envenomation by *L. muta* venom results in hemorrhage, pain, necrosis, hemolysis, myotoxicity, and death. Since antivenom does not efficiently neutralize local effects, a large number of researchers have attempted to identify molecule(s) from natural sources to inhibit such activities to use them as an alternative treatment for snakebite. We tested four extracts of seaweed *P. brasiliense* obtained with solvents of increasing polarities: *n*-hexane (HEX), dichloromethane (DCM), ethyl acetate (ACE), and hydroalcohol (HYD). Extracts of alga or fractions were incubated with *L. muta* venom, and then, biological assays were performed. The extracts, except the HYD, inhibited all the assays but with different potencies. The DCM extract fully inhibited all activities. Moreover, DCM and HEX extracts inhibited hemolysis induced by a phospholipase A_2 isolated from *L. muta* venom (LM-PLA_2-I). A fraction from HEX enriched in cholesterol isolated from HEX extract inhibited proteolysis by *L. muta* venom and hemolysis by LM-PLA_2-I; in contrast, monoterpenes isolated from DCM extract did not inhibit both activities. Seaweeds may be a promising source of natural inhibitors of the toxic effects caused by snakebite by *L. muta* venom, and they may be used to develop new strategies for antivenom treatment.

Keywords Antivenom · Bioprospecting · *Lachesis muta* · *Plocamium brasiliense* · Seaweed · Snake venom

M. M. Claudino · T. F. S. Domingos · G. A. da Silva · A. L. Fuly (✉)
Department of Cellular and Molecular Biology, Institute of Biology, Federal University Fluminense, Outeiro de São João Batista, s/n, 3º andar, sala 310, Niterói, RJ 24220-008, Brazil
e-mail: andfuly@vm.uff.br

R. R. Fonseca · D. N. Cavalcanti · V. L. Teixeira
Department of Marine Biology, Institute of Biology, Federal University Fluminense, Niterói, RJ 24220-008, Brazil

R. R. Fonseca
Department of Organic Chemistry, Institute of Chemistry, Federal University Fluminense, Niterói, RJ 24220-008, Brazil

E. F. Sanchez
Laboratory of Biochemistry of Proteins from Animal Venoms, Research and Development Center, Ezequiel Dias Foundation, Belo Horizonte, MG 30510-010, Brazil

Introduction

A great number of molecules have been isolated from marine organisms, and their structures and biological functions were deeply investigated. Among such organisms, marine algae deserve attention because they produce many biologically active molecules with antiviral, anticlotting, anti-inflammatory, and other effects. However, the antivenom ability of marine algae has not been studied. Snakebite is an important health problem in the world, mainly in the tropical and subtropical regions as well as in poor countries (Swaroop and Grab 1954; Warrell 1992; Chippaux 1988), and according to the World Health Organization, snakebites are considered a neglected disease.

Snake venom is composed of a complex mixture of proteins that is responsible for a spectrum of symptoms in victims that follow snakebites (Theakston and Reid 1983; MS/Funasa 2001). These effects may be divided into local (necrosis, edema, hemorrhage, and pain) and systemic (hemorrhage,

clotting or anticlotting, and renal or heart failure) (Jorge et al. 1997). In many countries, the regular treatment is the parenteral administration of serum, also called antivenom which is obtained from hyperimmunization of equines (MS/Funasa 2001). Antivenom efficiently neutralizes systemic effects induced by venoms, thus preventing victim death. However, antivenom has some disadvantages: it may induce adverse reactions (from mild fever to anaphylaxis reactions), and it does not neutralize local effects (Cardoso et al. 2003). This fact may lead limb amputations or deformity (Gutiérrez et al. 2009). Therefore, the search for new molecules as an alternative method to the regular antivenom therapy for neutralizing the main biological activities of venoms is important, and several groups are looking for alternative strategies to treat snakebite poisoning. It is estimated that about 80 % of human beings use plants or other natural products to treat several pathologies (Martz 1992; Mors et al. 2000; Soares et al. 2004, 2005), and some of their active principles capable of inhibiting biological activities induced by snake venoms have been isolated (Soares et al. 2005). However, their mechanism of action is still under investigation (Coe and Anderson 2005; Veronese et al. 2005). Besides plants, marine algae produce biologically active molecules with anticlotting (de Andrade Moura et al. 2011a, b; Rocha et al. 2005), antiviral (Cirne-Santos et al. 2008), anticancer (Rocha et al. 2007), and antimicrobial (González del Val et al. 2011) activities. Because of these pharmacological effects, some of them have been used to develop drugs. The genus *Plocamium* Lamouroux (Rhodophiceae) contains more than 40 species that are widely distributed (Saunders and Lehmkuhl 2005). The red seaweed *Plocamium brasiliense* (Greville) Howe and Taylor is abundant in the east–southeast coast of Brazil (Teixeira 2009). These marine algae typically produce acyclic and cyclic halogenated monoterpenes of which more than 100 molecules have been isolated (Kladi et al. 2004, Vasconcelos et al. 2010) and several biological activities have been found, such as antimicrobial, antifungal, ichthyotoxic, cytotoxic, and insecticidal activities (Vasconcelos et al. 2010). It has been suggested that the algal halogenated monoterpenes might be of chemotaxonomic interest and have displayed antiviral activity against herpes virus as well (Ferreira et al. 2010). Moreover, *P. brasiliense* can be considered a potential source of herbicides (Fonseca et al. 2012) or food (Gressler et al. 2011).

However, no antivenom property has been studied. So, the objective of this work was to evaluate the ability of crude extract of the alga *P. brasiliense* to neutralize in vitro (coagulant, proteolytic, and hemolytic) and in vivo (hemorrhagic and edematogenic) activities induced by *Lachesis muta* venom, since *L. muta* has the highest incidence of lethality in Brazil. Furthermore, the effect of three isolated products (two monoterpenes and cholesterol) from alga on proteolysis and hemolysis was also investigated.

Material and methods

Specimens of *Plocamium brasiliense* were collected by snorkeling during October 2010, at Enseada do Forno, in the city of Armação de Búzios, located to the north of Rio de Janeiro State, Brazil (22° 45′ S, 41° 52′ W). They were washed with local seawater and separated from sediments, epiphytes, and other associated organisms. The algae were dried at room temperature (average of 28 °C) for 20 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). The Brazilian algal collection license has the number 10594 (IBAMA/SISBIO).

Preparation of crude extracts and fractions

The crude extract of *P. brasiliense* was triturated in an industrial blender and placed in a plastic tray, yielding 5.16 g of powdered whole algae. The extracts were obtained according to Fonseca et al. (2012), which yielded the following masses: *n*-hexane, 500 mg; dichloromethane, 196 mg; ethyl acetate, 42 mg; and ethanol/water, 250 mg. The extract prepared in *n*-hexane (500 mg) was subjected to silica gel 70–230-mesh column chromatography (4×70 cm) eluted with *n*-hexane, CH_2Cl_2, EtOAc, and MeOH in sequence to give 97 fractions of 10 mL each (F1–F97) and 55 fractions of 20 mL each (F98–F153). Fraction F17 (18 mg) eluted with *n*-hexane/CH_2Cl_2 (9.7:0.3) and fraction F76 (16 mg) eluted with *n*-hexane/CH_2Cl_2 (5:5) afforded the pure halogenated monoterpenes 8-bromo-3,4,7-trichloro-3,7-dimethyl-1*E*,5*E*-octadiene **(1)** and 1,8-dibromo-3,4,7-trichloro-3,7-dimethyl-1*E*,5*E*-octadiene **(2)**, respectively. Fractions F112–F118 eluted with *n*-hexane/CH_2Cl_2 (3.5:6.5) and F119–F125 eluted with *n*-hexane/CH_2Cl_2 (3:7) afforded pure cholesterol (42 mg) **(3)**. The fractions were analyzed according to Fonseca et al. (2010) by HRGC/MS on a HP 5890 series GC system, coupled to a HP 5973 mass selective detector in the EI mode (70 eV) equipped with a HP-1 MS capillary column (30 m×0.25 mm; film thickness 0.25 μm).

Venom and animals

Lachesis muta venom was kindly supplied from the Fundação Ezequiel Dias (FUNED), Belo Horizonte, Minas Gerais state, Brazil, vacuum-dried, and stored at −20 °C until used in the experiments. Male Balb/c mice (*Mus musculus* species), weighing 18–20 g and approximately 4–5 weeks old, were obtained from the Center of Laboratory Animals (NAL) of the Federal Fluminense University (UFF). They were housed under controlled temperature (24±1 °C) and light conditions, and all experiments performed were approved by the UFF Institutional Committee for Ethics in Animal Experimentation (CEUA: 200/10) and were in accordance with the guidelines of the Brazilian Committee for Animal Experimentation (COBEA).

Antihemolytic activity

The degree of hemolysis of *L. muta* venom or the purified PLA_2 (LM-PLA_2-I) was determined by the indirect hemolytic test using human erythrocytes and hen's egg yolk emulsion as substrate (Fuly et al. 2002). The lowest amount of *L. muta* venom or LM-PLA_2-I (μg mL^{-1}) that produced 100 % hemolysis was denoted as minimum indirect hemolytic dose (MIHD). Inhibitory experiments were performed by incubating algal extracts or products with one MIHD for 30 min at room temperature, and then, hemolytic activity was evaluated. Control experiments were performed by incubating venom with DMSO (5 %*v*/*v*) or saline, instead of samples.

Anticlotting activity

The clotting activity of *L. muta* venom was monitored using a digital Amelung coagulometer, model KC4A (Labcon, Germany). Different concentrations of *L. muta* venom were mixed with diluted citrated plasma (1:1 in saline) donated from healthy volunteers collected at a local public blood bank (University Hospital Antônio Pedro of the Federal Fluminense University), and the amount of venom (μg mL^{-1}) that clotted plasma around 60 s was denoted as minimum coagulant dose (MCD). To evaluate the inhibitory effect, algal extracts were incubated for 30 min at room temperature with one MCD, and then, the mixture was added to plasma and clotting time recorded. Control experiments were performed in parallel by incubating venom with DMSO (1 %*v*/*v*) or saline.

Antihemorrhagic activity

Hemorrhagic lesions produced by *L. muta* venom were quantified using a procedure described by Kondo et al. (1960), with modifications. Briefly, samples were injected intradermally (i.d.) into the abdominal skin of mice. Two hours later, the animals were euthanized, and the abdominal skin was removed, stretched, and inspected for visual changes in the inner surface of subcutaneous layers to localize hemorrhagic spots. Hemorrhage was quantified as the minimum hemorrhagic dose (MHD), defined as the amount of venom (μg g^{-1}) able to produce a hemorrhagic halo of 10 mm (Nikai et al. 1984). The inhibitory effect of algal extracts was investigated by incubating them with two MHD of *L. muta* venom for 30 min at room temperature, and then, the mixture was injected i.d. into the mice and hemorrhage was measured. In another set of experiment, *L. muta* venom was firstly injected i.d., and 15 min later, the algal extract was injected i.d. at the same site where venom was injected. Hemorrhagic activity was expressed as the mean diameter (in mm) of the hemorrhagic halo induced by *L. muta* venom in the absence and presence of the alga. Negative control experiments were performed by injecting DMSO (5 %*v*/*v*), saline, or samples alone.

Antiproteolytic activity

Proteolytic activity of *L. muta* venom was determined using azocasein as substrate (0.2 %*w*/*v*, in 20 mM Tris–HCl, 8 mM $CaCl_2$, pH 8.8), with minor modification (Garcia et al. 1978). An effective concentration (EC) was defined as the amount of venom (μg mL^{-1}) able to produce a variation of about 0.2 OD units at 420 nm (spectrophotometer Hitachi U-5100). The inhibitory effect of algal extracts or products was performed by incubating them with two EC of *L. muta* venom for 30 min at room temperature, and then, proteolysis was measured. Similarly, control experiments were conducted by mixing venom with DMSO (5 %*v*/*v*) or saline, and further proteolysis was determined.

Antiedematogenic activity

Edema-inducing activity of *L. muta* venom was determined according to Yamakawa et al. (1976). Groups of five mice received subcutaneously (s.c.) 50 μL of *L. muta* venom in the right paw, while the left paw received 50 μL of saline or DMSO. One hour after injection, edema was evaluated as the percentage increase in weight of the right paw compared to the left one. Antiedematogenic activity was performed by incubating algal extracts with *L. muta* venom for 30 min at room temperature, and then, the mixture was injected s.c. into the mice. Control experiments were performed by mixing *L. muta* venom with DMSO (5 %*v*/*v*) or saline.

Statistical analysis

Results are presented as means ± SE. The statistical significance of differences between tests was evaluated using Student's unpaired *t* test. *p* values of <0.05 were considered statistically significant.

Results

Three products were previously isolated from the alga *P. brasiliense*, and their chemical structure is shown in Fig. 1. These products are as follows: monoterpenes **(1, 2)** and cholesterol **(3)**. All of them were tested against harmful in vitro and in vitro activities induced by *L. muta* venom.

Effect of *P. brasiliense* on *L. muta* venom-induced blood clotting

L. muta venom clotted plasma in a concentration-dependent manner, and the minimum dose coagulant (MDC) was 12 μg mL^{-1}. This venom concentration was incubated for 30 min at room temperature with algal extracts of *P. brasiliense* as well as with NaCl or DMSO, and then, the

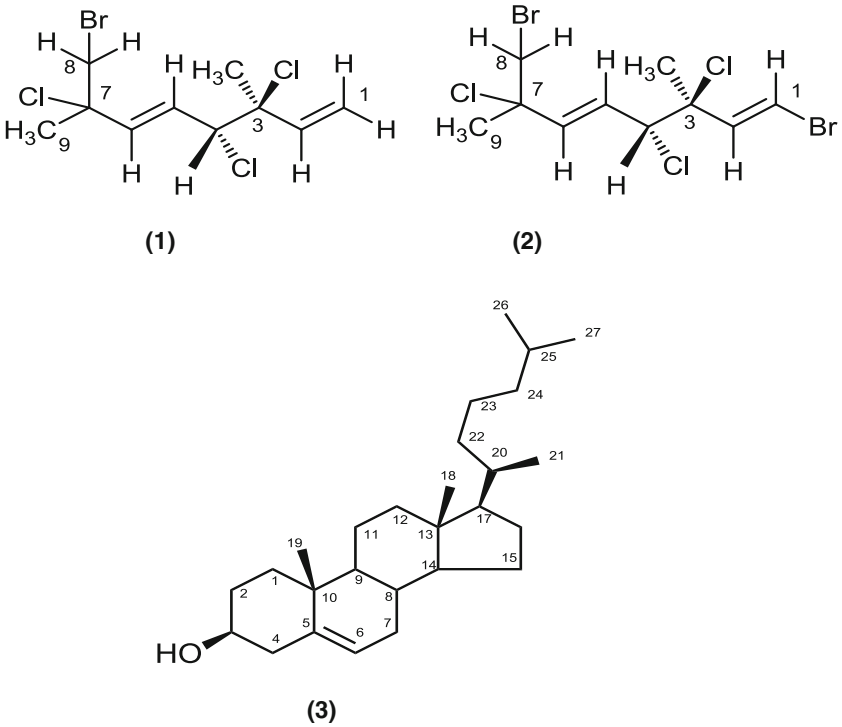

Fig. 1 Chemical structure of monoterpenes 8-bromo-3,4,7-trichloro-3,7-dimethyl-1*E*,5*E*-octadiene **(1)** and 1,8-dibromo-3,4,7-trichloro-3,7-dimethyl-1*E*,5*E*-octadiene **(2)** and cholesterol **(3)**

mixture was added to plasma and clotting monitored. As seen in Fig. 2, the extracts prepared in *n*-hexane (HEX) and dichloromethane (DCM) inhibited clotting induced by *L. muta* venom, whereas the extracts prepared in ethyl acetate (ACE) and hydroalcohol (HYD) did not inhibit clotting (Fig. 2).

Effect of *P. brasiliense* on *L. muta* venom-induced proteolysis

L. muta venom hydrolyzed azocasein with a CE of 5 μg mL^{-1}, and this venom concentration was incubated with either different concentrations of algal crude extracts (50, 125, and 250 μg mL^{-1}) to give a 1:10, 1:25, and 1:50 venom/alga extract ratio (*p*/*p*) or with fractions to give a 1:25 ratio. Then, proteolytic activity was determined. The extract prepared in HEX inhibited proteolysis in a concentration-dependent manner, while the extracts prepared in DCM or ACE did not (Fig. 3a). The DCM extract inhibited 100 % proteolysis at any concentration tested, and the ACE extract inhibited near 100 %. In contrast, the HYD extract inhibited around 15 % proteolysis at any venom/alga ratio (Fig. 3a). Cholesterol **(3)** inhibited 95 % proteolysis, and both monoterpenes **(1)** and **(2)** did not inhibit proteolysis (Fig. 3b).

Effect of *P. brasiliense* on *L. muta* venom-induced hemolysis

L. muta venom lysed erythrocytes in a concentration-dependent manner with a minimum indirect hemolysis concentration (MIHC) of 4 μg mL^{-1} (data not shown). The extracts of *P. brasiliense* (38, 95, and 190 μg mL^{-1}) were incubated with *L. muta* venom (4 μg mL^{-1}), and hemolytic

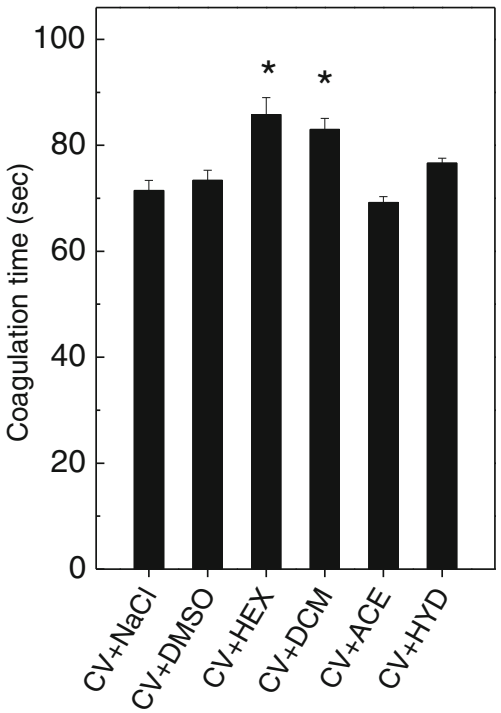

Fig. 2 Effect of *P. brasiliense* on clotting induced by *L. muta* venom. Crude venom (*CV*) of *L. muta* (12 μg mL^{-1}) was incubated with algae extracts (300 μg mL^{-1}) prepared in *n*-hexane (*HEX*), dichloromethane (*DCM*), ethyl acetate (*ACE*), and hydroalcohol (*HYD*), and then, mixtures were added to plasma and clotting monitored. Control groups are as follows: CV incubated with NaCl (*CV+NaCl*) or with DMSO (*CV + DMSO*). Results are expressed as mean ± SE of two individual experiments (n=3). *p<0.05 when compared to CV + NaCl or CV + DMSO groups

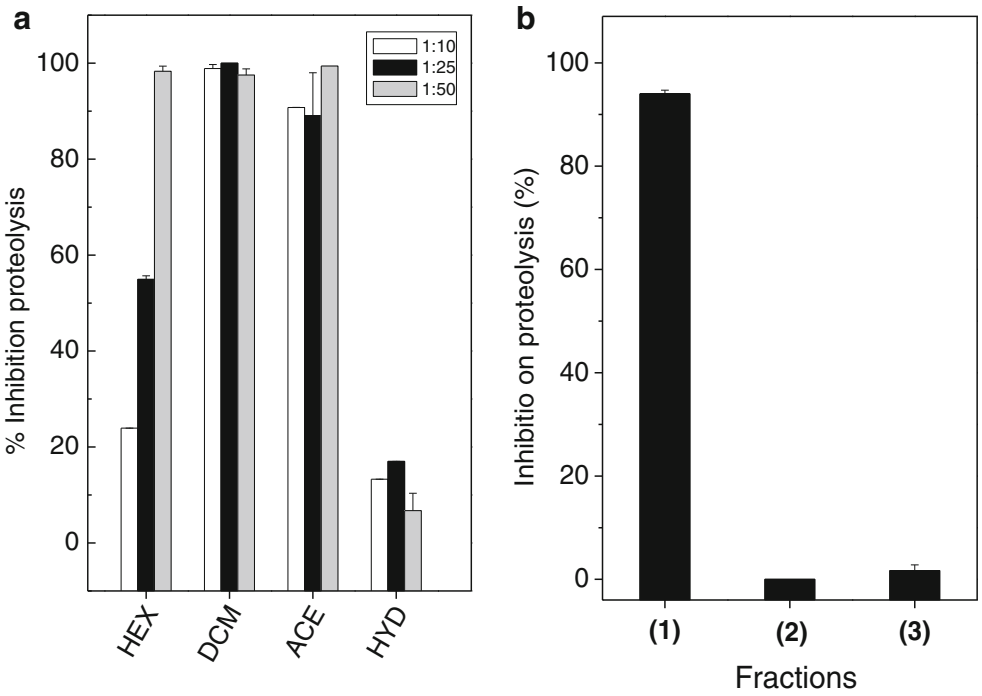

Fig. 3 Effect of *P. brasiliense* on proteolysis induced by *L. muta* venom. **a** Crude extracts of *P. brasiliense* algae (50 μg mL^{-1}, *white columns*; 125 μg mL^{-1}, *black columns*; and 250 μg mL^{-1}, *gray columns*) prepared in *n*-hexane (*HEX*), dichloromethane (*DCM*), ethyl acetate (*ACE*), or hydroalcohol (*HYD*) were incubated with *L. muta* venom (5 μg mL^{-1}). **b** 75 μg mL^{-1} of fractions **(1)**, **(2)**, or **(3)** were incubated with *L. muta* venom. Results are expressed as mean ± SE of two individual experiments (n=3)

assay was further evaluated. As seen in Fig. 4, at any of the tested concentration of the extract prepared in DCM, a 100 % inhibition was achieved, while the HEX extract gave 20, 24, and 65 % inhibition. At the highest algae concentration (190 μg mL^{-1}), the ACE and HYD extracts inhibited by 24 and 7 %, respectively.

All the extracts were tested on the hemolysis caused by a phospholipase A_2 isolated from *L. muta* venom (called LM-PLA_2-I) as well (Table 1). HEX and DCM (190 μg mL^{-1}) showed 100 % inhibition of the hemolysis induced by 12 μg mL^{-1} LM-PLA_2-I. The other extracts (ACE or HYD) and monoterpenes **(1)** and **(2)** did not inhibit LM-PLA_2-I-induced hemolysis. However, cholesterol **(3)** inhibited hemolysis by 80 % (Table 1). DMSO or saline did not interfere in hemolysis induced by LM-PLA_2-I.

Effect of *P. brasiliense* on *L. muta* venom-induced hemorrhage and edema

L. muta venom (2 μg g^{-1}) caused a hemorrhage halo in mice around 20 mm, which corresponds to two MHD (data not shown). The extracts HEX, DCM, and ACE (152 μg g^{-1}) fully protected mice from hemorrhage induced by *L. muta* venom (22 μg g^{-1}); however, the extract HYD (152 μg g^{-1}) failed to protect them (Fig. 5a). Moreover, the effect of extracts of *P. brasiliense* on *L. muta* venom-induced edema was also investigated (Fig. 5b). Extracts (92 μg g^{-1}) were incubated with venom (32 μg g^{-1}), and then, the mixtures were injected into the mice. The extracts HEX, DCM, and ACE inhibited edema around 15 %, and HYD had no inhibitory effect (Fig. 5b).

Discussion

Snakebites represent a serious public health problem in tropical countries because of the frequency, morbidity, and

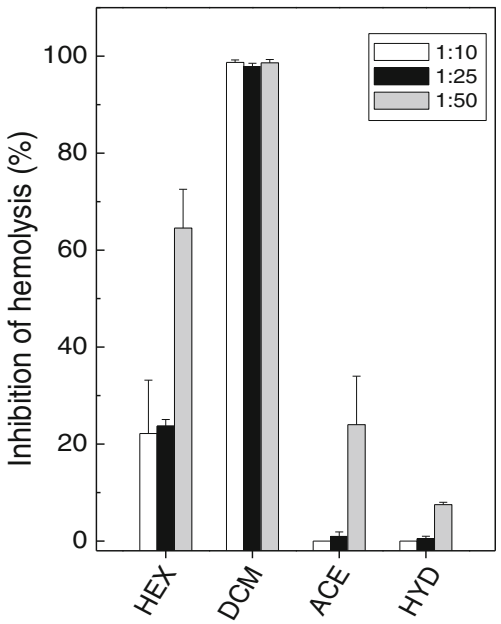

Fig. 4 Effect of *P. brasiliense* on hemolysis induced by *L. muta* venom. Different concentrations of *P. brasiliense* algae extracts (38 μg mL^{-1}, *white columns*; 95 μg mL^{-1}, *black columns*; and 190 μg mL^{-1}, *gray columns*) prepared in *n*-hexane (*HEX*), dichloromethane (*DCM*), ethyl acetate (*ACE*), or hydroalcohol (*HYD*) were incubated with *L. muta* venom (4 μg mL^{-1}). Results are expressed as mean ± SE of two individual experiments (n=3)

Table 1 Inhibitory effect of *P. brasiliense* on hemolysis caused by LM-PLA_2-I

Venom source	Controls/alga source	Inhibition (%)
LM-PLA_2-I	DMSO	0
	Saline	0
	HEX	100
	DCM	100
	ACE	0
	HYD	0
	Monoterpene **(1)**	0
	Monoterpene **(2)**	0
	Cholesterol **(3)**	80

The extracts of *P. brasiliense* (190 μg mL^{-1}) prepared in *n*-hexane (HEX), dichloromethane (DCM), ethyl acetate (ACE), and hydroalcohol (HYD) or 90 μg mL^{-1} of monoterpenes **(1)** or **(2)** and cholesterol **(3)** were incubated with LM-PLA_2-I (12 μg mL^{-1}), and the hemolytic activity was performed as described in the "Material and methods". Results are expressed as means ± SE (n=4). The products, monoterpenes **(1)** or **(2)** and cholesterol **(3)** were also tested, but only cholesterol inhibited hemolysis induced by LM-PLA_2-I

mortality associated with them (Chippaux 1988). Snake venom of the genus *Lachesis* contains a complex mixture of enzymes, such as metalloprotease (Rucavado et al. 1999), phospholipases A_2 (Ferreira et al. 2009), and serine proteases (Magalhães et al. 1997) that are responsible for high levels of hemorrhagic, procoagulant, proteolytic, and phospholipase A_2 activities when compared with several *Bothrops* snake venoms (Fuly et al. 1993). The search for molecules either from natural or synthetic sources able to inhibit such harmful activities of venoms is therefore quite relevant. Previous studies have shown the ability of extracts and diterpenes isolated from the brown marine alga *Canistrocarpus cervicornis* to inhibit some of these activities induced by *L. muta* snake venom (de Andrade Moura et al. 2011a, b; Domingos et al. 2011, 2012). However, the antivenom property has not been studied in the red alga *P. brasiliense*. Several chemical components have been isolated from the Brazilian alga *P. brasiliense* (Ferreira et al. 2010; Vasconcelos et al. 2010; Fonseca et al. 2012) including halogenated monoterpenes (Vasconcelos et al. 2010). The crude extract from *P. brasiliense* and a fraction enriched in halogenated monoterpene had activity against HSV-1 virus (Ferreira et al. 2010). Besides pharmacological activities, such metabolites also have shown other biological activities such as defense against marine herbivores, fouling organisms, and pathogens, as well as protection against radiation and allopathic agents (Pereira et al. 2010; Sakata et al. 1991). The potential allelopathic activity of four extracts (*n*-hexane, dichloromethane, ethyl acetate, and aqueous ethanol) from *P. brasiliense* also has been assessed (Fonseca et al. 2012). In the present study, we showed the ability of extracts prepared in solvents of increasing polarities (*n*-hexane, dichloromethane, ethyl acetate, and hydroalcohol) to inhibit in vivo and in vitro activities of *L. muta* venom. In parallel, the neutralizing property of products isolated from HEX or DCM extracts was tested as well. *P. brasiliense* extracts inhibited clotting that is an effect mediated by serine or metaloproteases in venom. They may affect clotting cascade by cleaving plasma proteins that in turn may lead to hemorrhage (Markland 1998). Similar results were found for the brown marine alga *Spatoglossum schröederi*

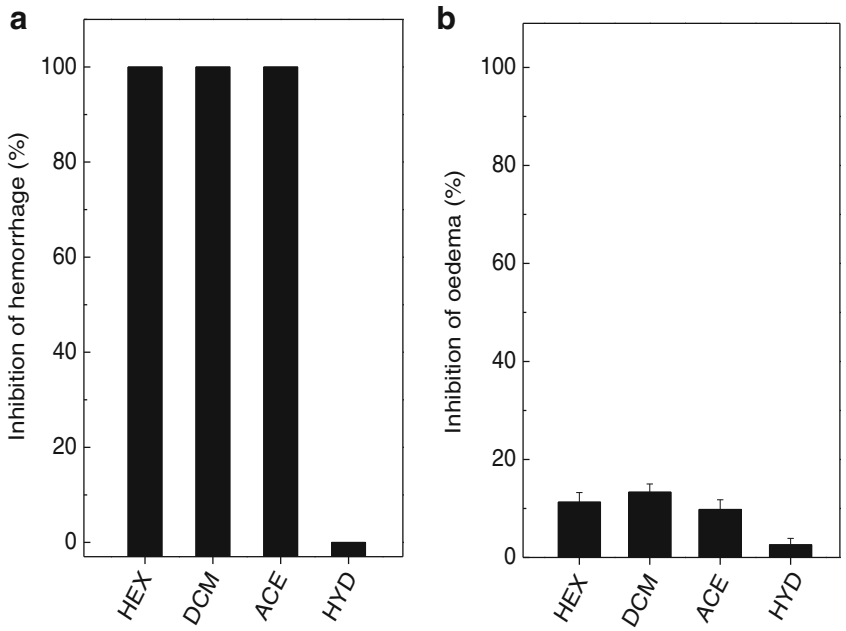

Fig. 5 Effect of *P. brasiliense* on hemorrhage and edema induced by *L. muta* venom. The extracts of *P. brasiliense* prepared in *n*-hexane (*HEX*), dichloromethane (*DCM*), ethyl acetate (*ACE*), or hydroalcohol (*HYD*) were incubated with *L. muta* venom. Then, mixtures were injected into the mice, and hemorrhagic (**a**) or edematogenic (**b**) activity was analyzed. Results are expressed as mean ± SE of two individual experiments (n=5)

(Phaeophyceae, Dictyotales) (Domingos et al. 2012). Besides inhibiting clotting induced by *L. muta* venom, plasma clotting induced by thrombin was also impaired by diterpenes or crude extracts of algae (de Andrade Moura et al. 2011a, b). In fact, algae contain products that may interact with thrombin enzymes, inhibiting their enzymatic activity and preventing plasma clotting. Several symptoms of envenomation by snakes, such as clotting and hemorrhage, are related to the presence of proteases in venoms. As seen, crude extract of *P. brasiliense* prepared in HEX and in DCM as well as some isolated products inhibited proteolysis and hemorrhage as well. Two fractions (F17 and F115) from HEX extract inhibited proteolysis by 100 %, but the other product (F76) from DCM extract did not inhibit such activity. On the other hand, DCM extract fully inhibited proteolysis. So, fractions from DCM should be tested. Some authors suggest that inhibitory mechanism of natural molecules on hemorrhagic activity induced by snake venoms may occur by chelating essential metals ions (Mors et al. 2000; Owuor and Kisangau 2006) or by interacting with their catalytic site (Havsteen 1983; Selvanayagam et al. 1996). HEX, DCM, and ACE extracts did not efficiently inhibit edema. Edema is one of the deleterious and local effect induced by venoms that may be caused by hemorrhagins and phospholipase A_2 (PLA_2) (Kini 2006; de Paula et al. 2009), and in general, it is poorly inhibited by natural molecules as well as by commercial antivenoms (Picolo et al. 2002; da Silva et al. 2007). Besides edema, PLA_2s induce hemolysis, and such extracts inhibited hemolysis induced by *L. muta* venom and by a phospholipase A_2 previously purified from *L. muta* (LM-PLA_2-I) venom (Fuly et al. 1997). Besides their participation in prey digestion, PLA_2s may display pharmacological and toxic effects in victims (Gutiérrez and Ownby 2003; Kini 2003) and therefore may affect a wide variety of systems through different biological effects. These enzymes are one of the most active pharmacological components in venoms, and therefore, several studies have been conducted to search for molecules able to inhibit the PLA_2 enzyme, be from natural or synthetic source (Murakami et al. 2005; Arruda et al. 2002; Melo et al. 1994; Ticli et al. 2005).

In conclusion, red seaweed may be a promising source of natural inhibitors of enzymes involved in biological activities of *L. muta* venom, making them potential organisms to be used in the complementary treatment of envenomation by this snake and/or for guiding the development of new drugs for the treatment of several diseases. The polarity of the solvents used to prepare the algal extracts influenced the inhibition potency of the biological activities tested in this work and should be taken into consideration.

Acknowledgement This work was supported by the International Foundation for Science (IFS-Grant F/4571-1), FAPERJ, CNPq, CAPES, and UFF/PROPPi.

References

Arruda EZ, Silva NM, Moraes RA, Melo PA (2002) Effect of suramin on myotoxicity of some crotalid snake venoms. Braz J Med Biol Res 35:723–736

Cardoso JLC, França FOS, Wen FH, Málaque SA, Haddad VJ (2003) Animais peçonhentos no Brasil; Biologia, Clínica e Terapêutica dos acidentes ofídicos. Editora Sarvier, São Paulo

Chippaux JP (1988) Snake-bites: appraisal of the global situation. Bull World Health Organ 76:515–524

Cirne-Santos CC, Souza TM, Teixeira VL, Fontes CF, Rebello MA, Castello-Branco LR, Abreu CM, Tanuri A, Frugulhetti IC, Bou-Habib DC (2008) The dolabellane diterpene dolabelladienetriol is a typical noncompetitive inhibitor of HIV-1 reverse transcriptase enzyme. Antivir Res 77:64–71

Coe FG, Anderson GJ (2005) Snakebite ethnopharmacopoeia of eastern Nicaragua. J Ethnopharmacol 96:303–323

da Silva NM, Arruda EZ, Murakami YL, Moraes RA, El-Kik CZ, Tomaz MA, Fernandes FF, Oliveira CZ, Soares AM, Giglio JR, Melo PA (2007) Evaluation of three Brazilian antivenom ability to antagonize myonecrosis and hemorrhage induced by *Bothrops* snake venoms in a mouse model. Toxicon 50:196–205

de Andrade Moura L, Bianco EM, Pereira RC, Teixeira VL, Fuly AL (2011a) Anticoagulation and antiplatelet effects of a dolastane diterpene isolated from the marine brown alga *Canistrocarpus cervicornis*. J Thromb Thrombolysis 31:235–240

de Andrade Moura L, Ortiz-Ramirez FA, Cavalcanti DN, Ribeiro SM, Muricy G, Teixeira VL, Fuly AL (2011b) Evaluation of marine brown algae and sponges from Brazil as anticoagulant and antiplatelet products. Mar Drugs 9:1346–1358

de Paula RC, Castro HC, Rodrigues CR, Melo PA, Fuly AL (2009) Structural and pharmacological features of phospholipases A_2 from snake venoms. Protein Pept Lett 16:899–907

Domingos TFS, Vallim MA, Carvalho C, Sanchez EF, Teixeira VL, Fuly AL (2011) Anti-snake venom effect of secodolastane diterpenes isolated from Brazilian marine brown alga *Canistrocarpus cervicornis* against *Lachesis muta* venom. Braz J Pharm 2:234–238

Domingos TFS, Ortiz-Ramirez FA, Villaça RC, Cavalcanti DN, Sanchez EF, Teixeira VL, Fuly AL (2012) Inhibitory effect of a Brazilian marine brown alga *Spatoglossum schröederi* upon biological activities of *Lachesis muta* snake venom. Braz J Pharm 4: 741–747

Ferreira T, Camargo EA, Ribela MT, Damico DC, Marangoni S, Antunes E, De Nucci G, Landucci EC (2009) Inflammatory oedema induced by *L. muta muta* (Surucucu) venom and LmTX-I in the rat paw and dorsal skin. Toxicon 53:69–77

Ferreira WJ, Amaro R, Cavalcanti DN, de Rezende CM, da Silva VA, Barbosa JE, Paixão IC, Teixeira VL (2010) Antiherpetic activities of chemical components from the Brazilian red alga *Plocamium brasiliense*. Nat Prod Commun 5:1167–1170

Fonseca RR, Ortiz-Ramírez FA, Cavalcanti DN, Ramos CJB, Teixeira VL, Souza Filho APS (2012) Allelopathic potential of extracts from marine macroalga *Plocamium brasiliense* and their effects on pasture weed. Braz J Pharm 22:850–853

Fuly AL, Francischetti IM, Zingali RB, Carlini CR (1993) Partial purification and some physicochemical properties of phospholipases A_2 from the venom of the bushmaster snake (*Lachesis muta*). Braz J Med Biol Res 5:459–463

Fuly AL, Machado OL, Alves EW, Carlini CR (1997) Mechanism of inhibitory action on platelet activation of a phospholipase A_2 isolated from *Lachesis muta* (Bushmaster) snake venom. Thromb Haemost 78:1372–1380

Fuly AL, de Miranda AL, Zingali RB, Guimarães JA (2002) Purification and characterization of a phospholipase A_2 isoenzyme isolated from *Lachesis muta* snake venom. Biochem Pharmacol 63:1589–1597

Garcia ES, Guimarães JA, Prado JL (1978) Purification and characterization of a sulfhydryl-dependent protease from *Rhodnius prolixus* midgut. Arch Biochem Biophys 2:315–322

González del Val A, Platas G, Basilio A, Cabello A, Gorrochategui J, Suay I, Vicente F, Portillo E, Jiménez del Río M, Reina GG, Peláez F (2011) Screening of antimicrobial activities in red, green and brown macroalgae from Gran Canaria (Canary Islands, Spain). Int Microbiol 4:35–40

Gressler V, Fujii MT, Martins AP, Colepicolo P, Mancini-Filho J, Pinto E (2011) Biochemical composition of two red seaweed species grown on the Brazilian coast. J Sci Food Agric 91:1687–1692

Gutiérrez JM, Ownby CL (2003) Skeletal muscle degeneration induced by venom phospholipases A_2: insights into the mechanisms of local and systemic myotoxicity. Toxicon 42:915–931

Gutiérrez JM, Lomonte B, León G, Alape-Girón A, Flores-Díaz M, Sanz L, Ângulo Y, Calvete JJ (2009) Snake venomics and antivenomics: proteomic tools in the design and control of antivenoms for the treatment of snakebite envenoming. J Proteome 72:165–182

Havsteen B (1983) Flavonoids, a class of natural products of high pharmacological potency. Biochem Pharmacol 32:1141–1148

Jorge MT, Sano Martins SI, Tomy SC, Castro SC, Ferrari RA, Ribeiro LA, Warrell DA (1997) Snake bite by the bushmaster (*Lachesis muta*) in Brazil: case report and review of the literature. Toxicon 35:545–554

Kini RM (2003) Excitement ahead: structure, function and mechanism of snake venom phospholipase A_2 enzymes. Toxicon 42:827–840

Kini RM (2006) Anticoagulant proteins from snake venoms: structure, function and mechanism. Biochem J 397:377–387

Kladi M, Vagias C, Roussis V (2004) Volatile halogenated metabolites from marine red algae. Phytochem Rev 3:337–366

Kondo H, Kondo S, Ikezawa H, Murata R (1960) Studies on the quantitative methods for determination of hemorrhagic activity of Habu snake venom. Jpn J Med Sci Biol 13:43–52

Magalhães A, Monteiro MR, Magalhães HPB, Mares-Guia M, Rogana E (1997) Thrombin-like enzyme from *Lachesis muta muta* venom: isolation and topographical analysis of its active site structure by means of the binding of amidines and guanidines as competitive inhibitors. Toxicon 35:1549–1559

Markland FS (1998) Snake venoms and the hemostatic system. Toxicon 12:1749–1800

Martz W (1992) Plants with a reputation against snakebite. Toxicon 30: 1131–1142

Melo PA, do Nascimento MC, Mors WB, Suarez-Kurtz G (1994) Inhibition of the myotoxic and hemorrhagic activities of crotalid venoms by *Eclipta prostrata* (Asteraceae) extracts and constituents. Toxicon 32:595–603

Mors WB, Nascimento MC, Pereira BM, Pereira NA (2000) Plant natural products active against snake bite—the molecular approach. Phytochemistry 55:627–642

MS (Ministério da Saúde) / Funasa (Fundação Nacional de Saúde) (2001) Manual de Diagnóstico e Tratamento de Acidentes por Animais Peçonhentos. MS/Funasa, Brasília

Murakami MT, Arruda EZ, Melo PA, Martinez AB, Calil-Eliás S, Tomaz MA, Lomonte B, Gutiérrez JM, Arni RK (2005) Inhibition of myotoxic activity of *Bothrops asper* myotoxin II by the anti-trypanosomal drug suramin. J Mol Biol 350:416–426

Nikai T, Mori N, Kishyda M, Sugihara H, Tu AT (1984) Isolation and biochemical characterization of hemorrhagic toxin f from the venom of *Crotalus atrox* (western diamondback rattlesnake). Arch Biochem Biophys 2:309–319

Owuor BO, Kisangau DP (2006) Kenyan medicinal plants used as antivenin: a comparison of plant usage. J Ethnobiol Ethnomed 2:1–8

Pereira RC, Pinheiro MD, Teixeira VL, da Gama BA (2010) Feeding preferences of the endemic gastropod *Astraea latispina* in relation to chemical defenses of Brazilian tropical seaweeds. Braz J Biol 62:33–40

Picolo G, Chacur M, Gutiérrez JM, Teixeira CFP, Cury Y (2002) Evaluation of antivenoms in the neutralization of hyperalgesia and edema induced by *Bothrops jararaca* and *Bothrops asper* snake venoms. Braz J Med Biol Res 35:1221–1228

Rocha HA, Moraes FA, Trindade ES, Franco CR, Torquato RJ, Veiga SS, Valente AP, Mourão PA, Leite EL, Nader HB, Dietrich CP (2005) Structural and hemostatic activities of a sulfated galactofucan from the brown alga *Spatoglossum schroederi*. An ideal antithrombotic agent? J Biol Chem 280:41278–41288

Rocha FD, Soares AR, Houghton PJ, Pereira RC, Kaplan MA, Teixeira VL (2007) Potential cytotoxic activity of some Brazilian seaweeds on human melanoma cells. Phytother Res 21:170–175

Rucavado A, Sanchez EF, Franceschi A, Magalhães A, Gutiérrez JM (1999) Characterization of the local tissue damage induced by LHF-II, a metalloproteinase with weak hemorrhagic activity isolated from *Lachesis muta muta* snake venom. Toxicon 37:1297–1312

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 feeding inhibitors for marine herbivores. Nippon Suisan Gakkaishi 57:743–746

Saunders GW, Lehmkuhl KV (2005) Molecular divergence and morphological diversity among four cryptic species of *Plocamium* (Plocamiales, Florideophyceae) in northern Europe. Eur J Phycol 40:293–312

Selvanayagam ZE, Gnanavendhan SG, Balakrishna K, Rao RB, Sivaraman J, Subramanian K, Puri R, Puri RK (1996) Ehretian one, a novel quinonoid xanthene from *Ehretia buxifolia* with antisnake venom activity. J Nat Prod 59:664–667

Soares AM, Januario AH, Lourenco MV, Pereira AM, Pereira OS (2004) Neutralizing effects of Brazilian plants against snake venoms. Drugs Future 29:1105–1117

Soares AM, Ticli FK, Marcussi S, Lourenço MV, Januário AH, Sampaio SV, Giglio JR, Lomonte B, Pereira PS (2005) Medicinal plants with inhibitory properties against snake venoms. Curr Med Chem 12: 2625–2641

Swaroop S, Grab B (1954) Snakebite mortality in the world. Bull World Health Organ 10:35–76

Teixeira VL (2009) Produtos Naturais Marinhos. In: Pereira RC, Soares-Gomes A (eds) Biologia Marinha. Interciência, Rio de Janeiro, pp 443–471

Theakston RDG, Reid HA (1983) Development of simple standard assay procedures for the characterization of snake venom. Bull World Health Organ 61:949–956

Ticli FK, Hage LI, Cambraia RS, Pereira PS, Magro AJ, Fontes MR, Stábeli RG, Giglio JR, França SC, Soares AM, Sampaio SV (2005) Rosmarinic acid, a new snake venom phospholipase A_2 inhibitor from *Cordia verbenacea* (Boraginaceae): antiserum action potentiation and molecular interaction. Toxicon 46:318–327

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

Veronese EL, Esmeraldino LE, Trombone AP, Santana AE, Bechara GH, Kettelhut I, Cintra AC, Giglio JR, Sampaio SV (2005) Inhibition of the myotoxic activity of *Bothrops jararacussu* venom and its two major myotoxins, BthTX-I and BthTX-II, by the aqueous extract of *Tabernaemontana catharinensis* A. DC. (Apocynaceae). Phytomedicine 12:123–130

Warrell DA (1992) The global problem of snakebite: its prevention and treatment. In: Gopalakrishnakone P, Tu CK (eds) Recent advances in toxinology research. National University of Singapore, Singapore, pp 1–13

Yamakawa M, Nozaki M, Hokama Z (1976) Fractionation of *Sakishima habu* (*Trimeresurus elegans*) venom and lethal hemorrhagic and edema forming activities of the fractions. In: Ohsaka A, Hayashi K, Sawai Y (eds) Animal, plant and microbial toxins. Biochemistry, New York, pp 97–109