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Antiviral activity of extracts from Brazilian seaweeds against herpes simplex virus

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Abstract: Organic extracts of 36 species of marine algae (sixteen species of Rhodophyta, eight species of Ochrophyta and twelve species of Chlorophyta) from seven locations on the Brazilian coast were evaluated for their anti-HSV-1 and anti-HSV-2 activity resistant to Acyclovir (ACV). Activity tests in crude extracts, followed by the identification of the major compounds present, were performed for all species. The chemical profiles of all crude extracts were obtained by ^{1}H-NMR and ^{13}C-NMR spectroscopy. The percentage of extracts with antiviral activity was higher for HSV-1 (86.1%) than for HSV-2 (55.5%). The green algae *Ulva fasciata* and *Codium decorticatum* both showed the highest activity (99.9%) against HSV-1, with triacylglycerols and fatty acids as the major components. The red alga *Laurencia dendroidea* showed good activity against HSV-1 (97.5%) and the halogenated sesquiterpenes obtusol and (-)-elatol were identified as the major components in the extract. Against HSV-2, the green alga *Penicillus capitatus* (Chlorophyta) and *Stypopodium zonale* (Ochrophyta) were the most active (96.0 and 95.8%). Atomaric acid, a meroditerpene, was identified as the major secondary metabolite in the *S. zonale* extract. These results reinforce the role of seaweeds as important sources of compounds with the potential to enter into the pipeline for development of new drugs against herpes simplex.

Introduction

Seaweeds provide a rich source of structurally diverse secondary metabolites. These are mainly terpenes, acetogenins and polyphenols, including many halogenated compounds (Maschek & Baker, 2008). These secondary metabolites provide defense against herbivores (Pereira et al., 2004b; Lima et al., 2008), fouling organisms (Da Gama et al., 2008) and pathogens (Paul & Ritson-Williams, 2008); they also play a role in reproduction (Amsler & Fairhead, 2005), protection from UV radiation (Gomez et al., 1998) and as allelopathic agents (Beach et al., 2003). These compounds have shown some interesting pharmacological activities such as: antitumoral (Barbier et al., 2001), antiparasitic (Davyt et al., 2001), antibacterial (Vairappan, 2003), antiviral (Santos et al., 1999; Pereira et al., 2004a; Soares et al., 2007), antioxidant (Nahas et al., 2007), and antifungal activity (de Oliveira et al., 2008). In particular, antiviral effects of sulfated polysaccharides and terpenes against a variety of enveloped viruses, such as Herpes Simplex Virus type 1 (HSV-1) and 2 (HSV-2), Human Immunodeficiency Virus (HIV), human cytomegalovirus, dengue viruses, respiratory syncytial and influenza viruses have been reported (Laillea et al., 1998; Ghosh et al., 2004; Cirne-Santos et al., 2008; Hidari et al., 2008).

At present, the availability of safe and potent antiviral agents against herpes viruses is far from ideal. Acyclovir (ACV) is the compound chosen for clinical use against HSV-1 and HSV-2 in systemic or topical therapy (Brown et al., 2002). Other ACV-related nucleoside analogs, all targeted against viral DNA synthesis, have recently been approved for human use (De Clercq, 2005). Although these compounds are potent and contribute to

the overall reduction of morbidity associated with viral infection, the emergence of viral resistant variants after prolonged treatment in immunocompromised patients still occurs, which justifies the continuous search for novel antiherpetic agents (Jerome, 2005).

In this context, metabolites from algae represent interesting types of compounds to assay as promising antiviral agents. This study presents the in vitro antiherpetic properties and the chemical profiles of most active crude extracts of seaweeds from the Brazilian coast.

Materials and Methods

Plant material

Thirty-six species of macroalgae, belonging to three algal divisions (Rhodophyta, Ochrophyta and Chlorophyta), were collected from six sites in Rio de Janeiro state, on the southeastern Brazilian coast: Forno beach (22°44'31.70"S, 41°52'35.97"O), Rasa beach (22°44'3.15"S, 41°57'30.15"O), Francês Island (22°24'6.46"S, 41°41'37.16"O), Tatagiba beach (21°23'31.11"S, 40°59'9.64"O), Cavaleiros beach (22°24'18.50"S, 41°47'42.48"O) and Cabo Frio Island (23°0'10.02"S, 42 0'24.43"O), between February, 2006, and March, 2007. Five species of Chlorophyta were collected in Bahia state, on the northeast coast (17°6'38.86"S, 39°10'54.95" W), in March, 2009 (Table 1). All algae were collected by A. R. Soares and identified by L. M. S. Gestinari and Y. Yoneshigue-Valentin. The algae were washed in seawater to eliminate associated organisms and air-dried. Voucher specimens were deposited at RFA (Thiers, 2008).

Chemical analysis

The air-dried algal material was powdered and extracted three times with dichloromethane:methanol (1:1) at room temperature, except the material collected in Bahia state, which was extracted three times with dichloromethane. After the evaporation of the solvent, all the crude extracts were analyzed by ^{1}H-NMR (Nuclear Magnetic Resonance) (300 MHz, $CDCl_3$) and ^{13}C-NMR (75 MHz, $CDCl_3$) spectroscopy (Bruker Avance spectrometer using tetramethylsilane (TMS) as internal standard) and thin layer chromatography (Silica gel GF_{254} TLC plates, Merck) with 2% $Ce(SO_4)_2$ in sulphuric acid as the spray detection reagent and heating the TLC plates at 100°C. The crude extracts were used to perform antiherpetic activity evaluation.

Table 1. The checklist of the benthic marine macroalgae collected in this study.

Phyllum	Sampling Site Habitat	Species	Order	Family
RHODOPHYTA	Cabo Frio Island (23° 0'10.02"S, 42 0'24.43"O) Intertidal	*Laurencia dendroidea* J. Agardh	Ceramiales	Rhodomelaceae
	Cavaleiros Beach (22°24'18.50"S, 41°47'42.48"O) Intertidal	*Corallina panizzoii* Schnetter & U. Richt.	Corallinales	Corallinaceae
		Jania crassa J.V. Lamour.	Corallinales	Corallinaceae
		Centroceras clavulatum (C. Agardh in Kunth) Mont. in Durieu de Maisonneuve	Ceramiales	Ceramiaceae
	Forno Beach (22°44'31.70"S, 41°52'35.97"O) Intertidal	*Pterocladiella capillacea* (S.G. Gmel.) Santel. & Hommers.	Gelidiales	Gelidiaceae
		Hypnea musciformis	Gigartinales	
		Laurencia dendroidea J. Agardh	Ceramiales	
	Francês Island (22°24'6.46"S, 41°41'37.16"O) Intertidal	*Jania adhaerens* J.V. Lamour.	Corallinales	Corallinaceae
		Hypnea spinella (C. Agardh) Kütz	Gigartinales	Cystocloniacae
		Spyrdia clavata Kütz	Ceramiales	Ceramiaceae
	Rasa Beach (22°44'3.15"S, 41°57'30.15"O) Intertidal	*Acantophora spicifera*		
		Bostrychia radicans (Mont.) Mont. in Orbigny	Ceramiales	Rhodomelaceae
		Cryptonemia seminervis (C. Agardh) J. Agardh	Halymeniales	Halymeniaceae
		Gracilaria domingensis (Kütz.) Sond. Ex Dickie	Gracilariales	Gracilariaceae

		Hypnea musciformis		
		Plocamium brasiliense (Grev. in J.St.-Hil.) M. Howe & W.R.Taylor	Plocamiales	Plocamiaceae
		Osmundaria obtusiloba (C. Agardh) R. E. Norris	Ceramiales	Rhodomelaceae
		Tricleocarpa cylindrica (J. Ellis & Sol.) Huisman & Borow.	Nemaliales	Galaxauraceae
	Tatagiba Beach (21°23'31.11"S, 40°59'9.64"O) Intertidal	*Hypnea musciformis* (Wulfen in Jacquin) J.V. Lamour.	Gigartinales	Cystocloniacae
		Corallina oficinallis		
		Gracilaria cearensis	Gracilariales	Gracilariaceae
		Chondracanthus acicularis (Roth) Fredericq	Gigartinales	Gigartinaceae
OCHROPHYTA	Cabo Frio Island (23° 0'10.02"S, 42° 0'24.43"O) Infralittoral	*Sargassum polyceratium* Mont.	Fucales	Sargassaceae
	Forno Beach (22°44'31.70"S, 41°52'35.97"O) Infralittoral fringe	*Lobophora variegata* (J.V. Lamour.) Womersley ex E.C. Oliveira	Dictyotales	Dictyotaceae
	Forno Beach (22°44'31.70"S, 41°52'35.97"O) Infralittoral	*Stypopodium zonale* (J.V. Lamour.) Papenf.	Dictyotales	Dictyotaceae
	Rasa Beach (22°44'3.15"S, 41°57'30.15"O) Intertidal	*Padina gymnospora* (Kütz.) Sond.	Dictyotales	Dictyotaceae
	Rasa Beach (22°44'3.15"S, 41°57'30.15"O) Infralittoral fringe	*Dictyopteris delicatula* J.V. Lamour.	Dictyotales	Dictyotaceae
		Dictyota menstrualis (Hoyt) Schnetter, Hörning & Weber-Peukert	Dictyotales	Dictyotaceae
		Sargassum cymosum C. Agardh	Fucales	Sargassaceae
		Sargassum vulgare C. Agardh	Fucales	Sargassaceae
CHLOROPHYTA	Cavaleiros Beach (22°24'18.50"S, 41°47'42.48"O) Intertidal	*Cladophora prolifera* (Roth) Kütz.	Cladophorales	Cladophoraceae
	Forno Beach (22°44'31.70"S, 41°52'35.97"O) Infralittoral fringe	*Caulerpa racemosa* (Forsskål) J. Agardh	Bryopsidales	Caulerpaceae
	Forno Beach (22°44'31.70"S, 41°52'35.97"O) Intertidal	*Codium decorticatum* (Woodw.) M. Howe	Bryopsidales	Codiaceae
	Francês Island (22°24'6.46"S, 41°41'37.16"O) Intertidal	*Chaetomorpha antennina* (Bory) Kütz.	Cladophorales	Cladophoraceae
	Francês Island (22°24'6.46"S, 41°41'37.16"O) Infralittoral	*Codium spongiosum* Harv.	Bryopsidales	Codiaceae
	Rasa Beach (22°44'3.15"S, 41°57'30.15"O) Intertidal	*Bryopsis* sp.	Bryopsidales	Bryopsidaceae
	Rasa Beach (22°44'3.15"S, 41°57'30.15"O) Intertidal	*Ulva fasciata* Delile	Ulvales	Ulvaceae
	Centro Beach (17°6 38.86" S3910' 54.95"W) Intertidal	*Avrainvillea elliotti* A. Gepp & E.S. Gepp	Bryopsidales	Udoteaceae
		Udotea flabellum (J. Ellis & Solander) M. A. Howe	Bryopsidales	Udoteaceae
		Halimeda opuntia J.V. Lamouroux	Bryopsidales	Halimedaceae
		Halimeda tuna tuna (J. Ellis & Solander) J.V. Lamouroux	Bryopsidales	Halimedaceae
		Penicillus capitatus Lamarck	Bryopsidales	Udoteaceae

Cells and viruses

Vero cells were grown in Eagle's minimum essential medium (MEM) supplemented with 2 mM L-glutamine, 50 µg/mL gentamicin, 2.5 µg/mL fungizon, plus 10% of heat-inactivated fetal bovine serum (FBS) (Schmidt, 1979) and kept at 37 °C in an atmosphere of 5% CO_2. Acyclovir-resistant HSV-1 and HSV-2 strains isolated from typical oral and genital lesions, respectively, were used. The isolates were typed by the polymerase chain reaction (PCR) using specific primers to identify HSV-1 and HSV-2 (Markoulatos et al., 2001) and evaluated with regard to sensibility to Acyclovir (De La Iglesia et al., 1998).

Cytotoxicity

The algal extracts were solubilized in dimethylsulfoxide (final concentration 1%) and diluted in water to a concentration of 400 µg/mL, sterilized by filtration through a Millipore membrane filter (0.22 µm) and frozen at -20 °C until use. The cytotoxicity assay was performed by incubating, in triplicate, Vero cell monolayers cultivated in 96-well microplates with two-fold serial dilutions of the extracts for 48 h at 37 °C. Morphological alterations of the treated cells were observed in an inverted optical microscope (Leitz-Germany 633456), and the maximum non-toxic concentrations (MNTC) were determined (Walker et al., 1972). Cellular viability was further evaluated by the neutral red dye-uptake method (Neyndorff et al., 1990). The 50% cytotoxic concentration (CC50) was defined as the dilution that caused a reduction of 50% in the number of viable cells.

Antiviral assays

Anti-HSV activity was evaluated by reduction of the virus titers using TCID50 (50% tissue culture infective dose) determinations. Vero cell monolayers cultivated in 96-well microplates were treated with the algal extracts at the MNTC. Immediately after, logarithmical dilutions of HSV-1 and HSV-2 suspensions were added to treated and untreated cell cultures and incubated in a 5% CO_2 atmosphere for 48 h at 37 °C. Following incubation, the virus titers were calculated using the Reed & Muench (1938) statistical method and expressed as TCID50 values. Results of the antiviral activity were expressed as Percentage of Inhibition (PI) (Nishimura et al., 1977) using antilogarithmic values of the TCID50 values as follows: PI=[1-(antilogarithm of the test value/antilogarithm of the control value)]x100.

Results

A total of 36 macroalgae species from seven sites along the Brazilian coast were tested against acyclovir-resistant HSV-1 and HSV-2. Of these, sixteen species were Rhodophyta (44.4%), eight species were Ochrophyta (22.2%) and twelve species were Chlorophyta (33.3%, Table 1). The results of the antiviral activity were expressed as PI. All results are reported in Table 2.

Out of all the crude extracts, 31 (86.1%) showed some activity against HSV-1 (PI values ranging from 20.6 to 99.9%) and 20 (55.5%) some activity against HSV-2 (PI values ranging from 20.6 to 96.0%). Among the three Phyla, the Ochrophyta showed the highest percentages of active extracts against HSV-1, with 100% of the extracts exhibiting activity. The Chlorophyta and Rhodophyta represented 91.6 and 75.0%, respectively of the active extracts. However, against HSV-2, Chlorophyta exhibited the highest percentage of active extracts (66.7%), followed by Ochrophyta (62.5%) and Rhodophyta (43.7%). A strong anti-herpetic activity was considered for extracts with PI>90%. Eleven species (30.5%), listed in Table 2, showed anti-HSV-1 activity with a PI superior to 90%. Among these, *L. dendroidea, S. zonale, S. cymosum, U. fasciata* and *C. decorticatu* showed very high activities (97.5, 96.8, 98.2, 99.9 and 99.9% respectively). On the other hand, strong anti-HSV-2 activity (PI>90%) was observed in only four species (11.1%), *i.e., S. zonale, S. cymosum, C. acicularis* and *P. capitatus*, with *P. capitatus* being the most active (96.0%). All algal extracts with strong anti-HSV activity (PI>90%) presented no toxicity to Vero cells (CC50>200 µg/mL), except the extract from *Laurencia dendroidea* (CC50 48.2 µg/mL).

All the crude extracts were analyzed by ^{1}H-NMR and ^{13}C-NMR spectroscopy. The major constituents of the most active extracts (*L. dendroidea, S. zonale, S. cymosum, U. fasciata, C. decorticatum* and *P. capitatus*) were identified. Characteristic signals for terpenoids were observed in the crude extracts from the algae *L. dendroidea* and *S. zonale*. Comparison of the spectroscopic data with previously reported data allowed the identification of the halogenated sesquiterpenes obtusol (**1**) and (-)-elatol (**2**) from *L. dendroidea* and the meroditerpenoid atomaric acid (**3**) from *S. zonale* (González et al., 1979; Wessels et al., 1999; Soares et al., 2003; Machado et al., 2011). The phenolic composition of the *S. cymosum* extract was suggested by the signals in the ^{1}H NMR spectrum at δ 6.54 (bs), 6.51 (d, *J*=3.0 Hz), 6.48 (d, *J*=3.0 Hz) and 6.45 (bs), characteristic to two coupled aromatic protons meta to each other, and a group of the signals at 160.0-120.0 ppm in the ^{13}C NMR spectra, characteristic of a phenolic moiety. The presence of triacylglycerols and fatty acids as the major components from the algae *U. fasciata, C. decorticatum* and *P. capitatus* is indicated by the strong ^{1}H NMR signals at δ 4.29 ppm

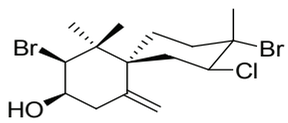
Table 2. Benthic marine macroalgae extract activities against acyclovir resistant *Herpes simplex* viruses (HSV-1-ACVr; HSV-2-ACVr). CC50 or 50% cytotoxic concentration is the concentration required to reduce the number of viable Vero cells by 50% after 48 h of incubation with the extracts. MNTC or maximum non-toxic concentration is the maximum concentration that did not cause morphologic alterations of the treated Vero cells. PI: percentage of inhibition.

Species	CC50 (µg/mL)	CMNT (µg/mL)	PI (%) (HSV-1-ACVr)	PI (%) (HSV-2-ACVr)
Rodophyta				
Corallina panizzoii	175.8	100	68.4	Zero
Jania adhaerens	>200	50	Zero	Zero
Jania crassa	173.5	100	43.8	43.8
Tricleocarpa cylindrica	>200	200	83.4	Zero
Bostrychia radicans	>200	200	86.5	Zero
Centroceras clavulatum	>200	200	zero	Zero
Laurencia dendroidea	48.2	3.1	97.5	43.8
Osmundaria obtusiloba	>200	100	90	Zero
Spyrdia clavata	>200	200	85.9	20.6
Pterocladiella capillacea	>200	50	68.4	Zero
Hypnea musciformis	>200	100	57.3	74.9
Hypnea spinella	>200	200	92	Zero
Chondracanthus acicularis	>200	100	68.4	92.4
Gracilaria domingensis	>200	100	Zero	43.8
Cryptonemia seminervis	>200	100	Zero	Zero
Plocamium brasiliense	>200	200	43.8	77.6
Ochrophyta				
Dictyopteris delicatula	>200	100	82.2	77.6
Dictyota menstrualis	94.5	12.5	20.6	Zero
Lobophora variegata	33.8	6.2	92	Zero
Padina gymnospora	>200	100	85.9	43.8
Stypopodium zonale	>200	50	96.8	95.8
Sargassum cymosum	124.4	50	98.2	90
Sargassum polyceratium	194.5	100	86.8	Zero
Sargassum vulgare	128.8	50	76	39.7
Chlorophyta				
Ulva fasciata	>200	200	99.9	Zero
Chaetomorpha antennina	85.8	100	55.3	85.9
Cladophora prolifera	>200	50	90	Zero
Bryopsis sp.	>200	200	82.2	87.4
Codium decorticatum	>200	200	99.9	Zero
Codium spongiosum	>200	50	55.3	55.3
Caulerpa racemosa	>200	50	57.3	Zero
Avrainvillea elliottii	>200	125	Zero	60.2
Udotea flabellum	>200	125	90	75
Halimeda opuntia	>200	62.5	73.1	68.4
Halimeda tuna	>200	250	84.1	82.2
Penicillus capitatus	>200	250	93.0	96

(dd, *J*=7.4; 14.6 Hz), 4.16 ppm (dd, *J*=6.0; 12.4 Hz) and 5.36 ppm (m), characteristic of triacylglycerols. The strong signal from the terminal methyl groups of the fatty acid esters were clearly observed at δ 0.88 ppm (t, *J*=7.2 Hz). The signals at δ 2.31ppm (t, *J*=7.4 Hz) and 1.60 ppm (m) correspond to the methylene protons α- and β- to the carbonyl groups, respectively. Peaks at δ 2.80 ppm and 2.02 ppm are attributed to methylene protons adjacent to double bonds. A strong peak for the internal methylene groups of the long chain of the fatty acid esters was observed at δ 1.26 ppm (bs). The ^{13}C NMR spectra of the extracts showed resonances of fatty acid ester carboxyl groups and the signals at 60-70 ppm indicated the presence of the glycerol moiety. All of the NMR data are shown in Table 3.

Discussion

Several molecules extracted from marine algae possess a broad spectrum of antiviral activity. Chemical classes for these compounds include sterols, terpenes, acetogenins, polyssacharides, fatty acids and polyphenols (Pereira et al., 2004a; Maschek & Baker, 2008; Hidari et al., 2008). In this study, we investigated the anti-herpetic activity against acyclovir-resistant HSV-1 and HSV-2 of lipophilic extracts from 36 Brazilian seaweeds. Of all the crude extracts, 31 (86.1%) showed some activity against HSV-1 and 20 (55.5%) some activity against HSV-2. The most active anti-HSV extracts were obtained from the species *L. dendroidea, U. fasciata, C. decorticatum, S. zonale, S. cymosum, C. acicularis* and *P. capitatus.*



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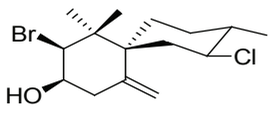

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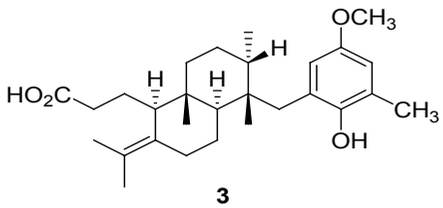

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Table 3. ^{1}H- ($CDCl_3$, 300 MHz) and ^{13}C-NMR ($CDCl_3$, 75.5 MHz) data for the major components of the crude extracts from *Laurencia dendroidea, Stypopodium zonale, Ulva fasciata, Codium decorticatum, Penicillus capitatus* and *Sargassum cymosum.*

Compound	Assignment	^{1}H (ppm)	Multiplicity: *J* (Hz)	^{13}C (ppm)
		L. dendroidea		
obtusol (**1**)	CH-1	1.74	*m*	25.5
	CH-2	2.30	*dm* (12.6)	40.5
	CH-3	-	-	67.6
	CH-4	4.70	*dd* (10.8 and 2.9)	68.0
	CH-5			37.1
	CH-6	-		50.0
	CH-7	-		141.8
	CH-8	2.62	*d* (14.0)	38.5
		2.50	*d* (14.2)	
	CH-9	4.10	*bs*	72.0
	CH-10	4.48	*d* (3.0)	70.1
	CH-11	-		44.0
	CH-12	1.08	*s*	24.0
	CH-13	1.08	*s*	20.8
	CH-14	5.39	*bs*	117.8
		5.05	*bs*	
	CH-15	1.83	*s*	23.6
(-)-elatol (**2**)	CH-1	2.58	*dl* (17.5)	25.5
		2.36	*dl* (17.5)	
	CH-2	-	-	128.0
	CH-3	-	-	124.1
	CH-4	1.98	*m*	29.5
	CH-5	1.63	*m*	25.6
	CH-6	-	-	49.4

	CH-7	-		140.8
	CH-8	2.62	*dd* (14.4 and 2.71)	33.5
		2.49	*dm* (14.5)	
	CH-9	4.14	*d* (2.9)	72.2
	CH-10	4.60	*d* (2.9)	70.4
	CH-11	-	-	43.2
	CH-12	1.05	*bs*	20.7
	CH-13	1.06	*bs*	24.2
	CH-14	5.12	*bs*	115.2
		4.80	*s*	
	CH-15	1.70	*s*	19.4
		S. zonale		
atomaric acid (**3**)	CH-1	2.84	*d* (13.8)	34.7
		2.25	*d* (14.3)	
	CH-2	-		40.0
	CH-3	1.73	*m*	35.1
	CH-4	1.26	*d* (14.4)	24.9
	CH-5	1.49	*m*	36.1
	CH-6	-		38.5
	CH-7	1.38	*dd* (12.0 and 6.0)	41.8
	CH-8	1.74	*m*	22.2
	CH-9	2.39	*m*	23.3
	CH-10	-	*m*	122.3
	CH-12	1.81	*m*	
	CH-12	1.81	*m*	
	CH-13	2.26	*m*	32.7
	CH-14	-		174.9
	CH-15	-		133.3
	CH-16	1.68	*s*	20.4
	CH-17	1.66	*s*	20.6
	CH-18	1.02	*s*	17.8
	CH-19	0.93	*s*	20.4
	CH-20	1.15	*d* (6.9)	15.5
	CH-1’	-		147.5
	CH-2’	-		128.3
	CH-3’	6.69	*d* (3.0)	113.6
	CH-4’	-		151.7
	CH-5’	6.54	*d* (2.7)	112.9
	CH-6’	-		125.5
	CH-7’	2.22	*s*	17.5
	CH-8’	3.73	*s*	55.0
		U. fasciata, C. decorticatum and *P. capitatus*		
triacylglycerols	CH *sn*-2	5.26	m	69.0
	CH_2 *sn*-1,3	4.29	*dd*, (7.4 and 14.6)	62.0
		4.16	*dd* (6.0 and 12.4)	
fatty acids	COO	-	-	172.8
	ω-CH_3	0.88	*t* (7.2)	14.2
	α-CH_2	2.31	*t* (7.4)	
	β-CH_2	1.60	*m*	

	CH_2-C=	2.80	*m*
		2.02	*m*
	$(CH_2)_n$-CH_3	1.26	*bs*
		S. cymosum	
phenolic compounds	6.54	*bs*	
	6.51	*d* (3.0)	
	6.48	*d* (3.0)	
	6.45	*bs*	

Red algae of the genus *Laurencia* are found in tropical and subtropical regions throughout the world and are an extremely rich source of secondary metabolites with diverse structural features, mainly halogenated terpenes and C15-acetogenins, with a broad spectrum of biological activity (Machado et al., 2010). The halogenated sesquiterpenes obtusol (**1**) and (-)-elatol (**2**) were identified as the major compounds of *L. dendroidea.*

The green algae *U. fasciata, C. decorticatum* and *P. capitatus* showed high activity against HSV-1. These genera had high concentrations of polysaccharides and fatty acids (Pope et al., 1996). These compounds may be responsible for the observed activity. Fatty acid-treated cells are resistant to infection by a variety of lipid-enveloped viruses, including herpes viruses (Pope et al., 1998). The chemical profiles of the crude extracts, obtained by the use of ^{1}H-NMR and ^{13}C-NMR spectroscopy, showed the presence of triacylglycerols and a mixture of fatty acids as the major components in these extracts.

The brown algae of the genera *Stypopodium* (Dictyotales) and *Sargassum* (Fucales) are abundantly found along the Brazilian coast. Both genera are known to produce meroditerpenes (mixed biogenesis diterpenes). Other metabolites of structural classes such as glycerides (Tang et al., 2002a), steroids (Tang et al., 2002b), dipeptides (Liu et al., 2009) and flavonoids (Liu et al., 2009) were isolated from the genus *Sargassum.* The species *S. cymosum* together with *S. zonale* were the only species that were highly active against both the viruses HSV-1 and HSV-2. The meroditerpenoid atomaric acid (**3**) was identified as the major secondary metabolite in the S. zonale extract. Meroditerpenes from *S. zonale* are known to have diverse biological activities (Wessels et al., 1999; Sabry et al., 2005), including anti-HSV-1 activity (Soares et al., 2007). The ^{1}H-NMR spectroscopic data of the crude extract of *S. cymosum* showed signals characteristic of phenolic compounds as the major constituents. Phenolic compounds have received considerable attention because of their therapeutic effects and their favorable antiviral activity (Quideau et al., 2004; Likhitwitayawuid, 2005; Tareq et al., 2007).

Although it is not possible to determine whether only one or a combination of several molecules are responsible for the observed anti-HSV-1 and anti-HSV-2 activity of the extracts, the presence of terpenes, fatty acids and phenolic compounds is consistent with the observed anti-herpetic activity since these types of metabolites, isolated from marine and terrestrial sources, have already been shown to have anti-herpetic activity (Khan et al., 2005; Hayashi et al., 2008).

The results of the present study indicate that different crude extracts from marine algae exhibit high anti-herpetic activity. The present findings provide a basis for further experiments on the identification and characterization of specific compounds with high anti-herpetic activities.

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