Journal of Pharmaceutical and Biomedical Analysis 52 (2010) 763–769

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Short communication

Trypanocidal, leishmanicidal and antifungal potential from marine red alga *Bostrychia tenella* J. Agardh (Rhodomelaceae, Ceramiales)

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ARTICLE INFO

Article history:
Received 19 August 2009
Received in revised form 8 February 2010
Accepted 16 February 2010
Available online 18 February 2010

Keywords:
Trypanocidal and leishmanicidal activity
Antifungal potential
GC/MS
Marine red algae
Bostrychia tenella

ABSTRACT

Specimens of the red alga *Bostrychia tenella* J. Agardh (Rhodomelaceae, Ceramiales) were collected from the São Paulo coast and submitted to room temperature solvent extraction. The resulting extract was fractionated by partitioning with organic solvent. The n-hexane (BT-H) and dichloromethane (BT-D) fractions showed antiprotozoal potential in biological tests with *Trypanosoma cruzi* and *Leishmania amazonensis* and presented high activity in an antifungal assay with the phytopathogenic fungi *Cladosporium cladosporioides* and *Cladosporium sphaerospermum*. Chromatography methods were used to generate subfractions from BT-H (H01 to H11) and from BT-D (D01 to D19). The subfractions were analyzed by gas chromatography–mass spectrometry (GC/MS), and the substances were identified by retention index (Kovats) and by comparison to databases of commercial mass spectra. The volatile compounds found in marine algae were identified as fatty acids, low molecular mass hydrocarbons, esters and steroids; some of these have been previously described in the literature based on other biological activities. Moreover, uncommon substances, such as neophytadiene were also identified. In a trypanocidal assay, fractions BT-H and BT-D showed IC_{50} values of 16.8 and 19.1 μg/mL, respectively, and were more active than the gentian violet standard (31 μg/mL); subfractions H02, H03, D01 and D02 were active against *L. amazonensis*, exhibiting IC_{50} values of 1.5, 2.7, 4.4, and 4.3 μg/mL, respectively (standard amphotericin B: IC_{50} = 13 μg/mL). All fractions showed antifungal potential. This work reports the biological activity and identification of compounds by GC/MS for the marine red alga *B. tenella* for the first time.

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1. Introduction

Infectious diseases present a serious health problem around the world. Notably, protozoan diseases, particularly malaria, leishmaniasis, Chagas disease and African trypanosomiasis, are major causes of mortality in several tropical and subtropical regions [1,2].

Chagas disease or American trypanosomiasis is endemic to Central and South America. It is estimated that 16–18 million people are currently infected with the protozoan flagellate *Trypanosoma cruzi*, and more than 100 million are exposed to the risk of infection [1,3]. Research for more efficient and less toxic drugs than those currently available for Chagas disease is necessary, since there is a lack of effective medicine against this acute and chronic disease. At present, gentian violet is the only trypanocidal compound used to prevent infection in blood banks, but its use is limited due to toxic effects and the alarming color acquired by the skin and urine of transfusion recipients. Toxic side effects are also evidenced in most of the synthetic compounds used to treat parasitic diseases, as shown by the use of the nitroheterocycles nifurtimox and benznidazole, the unique drugs prescribed in the early stages of trypanosomiasis in America. Benznidazole is currently preferred due to the gastrointestinal and neurological side effects, genotoxicity, and carcinogenic activity in the liver, kidney, urinary bladder and mammary gland of rats caused by nifurtimox use [2,3].

Another very harmful parasitic disease, leishmaniasis, caused by *Leishmania* parasites, is among the six most serious tropical diseases and affects around 12 million people in 88 countries according to the World Health Organization. There are an estimated 1.5 million new cases every year and 350 million people are at risk of infection. In Brazil alone, about 26,000 cases of leishmaniasis are

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doi:10.1016/j.jpba.2010.02.018

registered per year [1,4]. Historically, the chemotherapy of leishmaniasis has been based on the use of pentavalent antimonial drugs. Other medications, such as pentamidine and amphotericin B, have been used as alternative drugs. However, these drugs are not orally active, requiring long-term parenteral administration, and often display serious side effects. There is a significant need for the discovery of new and better therapeutic antileishmanial drugs and macrophage-stimulating compounds, especially those derived from natural sources [4].

There is great interest in screening algae for therapeutic drugs from natural products, as their ability to produce secondary metabolites has been extensively documented [5]. Some research has been carried out concerning marine natural products and their biological effects, such as fungicidal, antimicrobial, antimalarial, antimycobacterial, cytotoxic and antiviral activities. However, only a few studies have investigated the biological potential of Brazilian marine algae (seaweeds) [6]. Among the seaweeds, the genus *Bostrychia* (Rhodomelaceae, Ceramiales) is widespread in tropical and warm temperate environments around the world [7]. A few studies have focused on their chemical composition and detected carbohydrates and polysaccharides. Sorbitol, dulcitol and mannitol, the major carbohydrates in *Bostrychia* species, are biochemical exceptions and are rare in red algae [8]. Notably, the polysaccharides from *Bostrychia montagnei* Harvey showed antiherpetic and anticoagulant activities [9]. In addition, the literature shows the occurrence of ultraviolet sunscreen compounds in some species of genus *Bostrychia* [10].

Recently, our research group reported the isolation of aromatic compounds from *Bostrychia tenella* (J.V. Lamouroux) J. Agardh, including a previously unknown sulfated natural product [11]. Because this marine organism produces pharmaceutically useful compounds, fractions containing volatile metabolites from *B. tenella* were evaluated against the parasites *T. cruzi* and *Leishmania amazonensis* and the phytopathogenic fungi *Cladosporium cladosporioides* and *Cladosporium sphaerospermum*. Herein, we report the biological activity of these fractions and compound identification by GC/MS for the marine red alga *B. tenella*.

2. Experimental

2.1. Algal collection

Samples of *B. tenella* J. Agardh were collected in September 2003 on the coast of Ilha das Couves, Ubatuba, São Paulo, Brazil. The algal material was identified, washed in seawater, selected and maintained at 4 °C. Voucher specimens were deposited at Herbário do Instituto de Botânica-SP, Brazil, with voucher number SP 400217.

2.2. Extraction and chromatographic separation

The fresh algae was dried by freeze-drying method, powdered (178.17 g) and extracted by maceration three times using CH_2Cl_2:MeOH (2:1, v/v) at room temperature. The solvent was evaporated, and the obtained crude extract (8.13 g) was resuspended in MeOH:H_2O (9:1) and partitioned with n-hexane and dichloromethane. The n-hexane (1.10 g) and dichloromethane (0.67 g) fractions were submitted to chromatographic column separation using Silica Gel 60 (70–230 mesh, Merck) with a step-wise gradient (n-hexane, dichloromethane, and methanol), yielding 11 fractions from n-hexane (H-01 to H-11) and 19 fractions from dichloromethane (D-01 to D-19). The n-hexane (BT-H) and dichloromethane (BT-D) fractions as well as some fractions obtained from chromatographic separation (H01, H02, H03, H05, H06, D01 and D02) were evaluated for their trypanocidal, leishmanicidal and fungicidal potential.

2.3. GC/MS analysis

Gas chromatography–mass spectrometry analysis was performed on a Shimadzu GCMS model QP2010 apparatus. The carrier gas (H_2) was adjusted to a constant flow rate (1.62 mL min^{-1}). The DB5-MS column [30 m × 0.25 mm i.d., film thickness 0.25 μm (5% crosslinked phenyl-methylpolysiloxane)] was temperature controlled from 80 (0 min hold) to 200 °C at 15 °C min^{-1}, from 200 °C (5 min hold) to 230 °C at 5 °C min^{-1}, from 230 (10 min hold) to 280 °C at 15 °C min^{-1}, and finally isothermally at 280 °C for 12 min. The injector temperature was set at 250 °C with a split ratio of 1:60. The column outlet was inserted directly into the electron ionization source block operating at 70 eV. The scan range was 40–500 Da. Linear retention indices (RIs) were calculated according to the Kovats method using *n*-alkanes (C_7–C_{28}) as external references. Mass spectral identification was completed by comparison with the commercial mass spectral databases Wiley and NIST.

Additionally, in order to verify the repeatability of volatile compounds, the seven samples (H01, H02, H03, H05, H06, D01, and D02) analyses we carried out in 3-day replicate, each sequence was analyzed twice a day, totalizing six repetitions.

2.4. Trypanocidal assay

The n-hexane and dichloromethane fractions and their subfractions were evaluated in vitro against trypomastigote blood forms of *T. cruzi* (Y strain), which were obtained from parasitic culture in LLCMK2 cells [12]. The cells were cultivated in RPMI 1640 medium, supplemented with 2 nM L-glutamine, 10 nM $NaHCO_3$, 100 U/mL of penicillin, 100 μg/mL of streptomycin and 5% inactivated fetal bovine serum, in culture bottles at 37 °C in an atmosphere with 5% CO_2, and 95% humidity. Infected blood with trypomastigote forms was added to the culture, and the mixture was incubated for 24 h. Following this initial treatment, cells were washed with culture medium, only leaving the parasite-infected cells. After about 5 days, when the process of cellular lysis had started and the trypomastigote forms in the medium were observed, the supernatant containing trypomastigote forms was removed and measured (10^6 parasites/mL) in 96-well microplates. Samples of *B. tenella* in concentrations of 0.5, 2.0, 8.0 and 32.0 μg/mL were added to the mixtures.

After 24 h of incubation, the biological activity was determined using a colorimetric assay with MTT – 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (ACRÓS Organics). First, 10 μL of MTT (5 mg/mL/plate) was added and incubated at 37 °C for 4 h. Subsequently, acid-isopropanol (100 μL of 0.04N HCl in isopropanol) was added to the solutions, and the microplate absorbances were measured in a Sunrise – Tecan apparatus with a 570 nm filter (reference at 630 nm). The data were processed by the program Magelan 3 (TECAN). A DMSO solution was used as the negative control with the same concentrations as the evaluated samples. The activity of the positive control (benznidazole) was verified quantitatively using a previously described methodology [13].

2.5. Antileishmanial activity assay

Promastigote forms of *L. amazonensis* were cultivated at 22 °C in 199 (LGC) medium supplemented with 10% fetal bovine serum, penicillin and streptomycin. These parasite forms (1 × 10^5/mL), obtained by culture in stationary phase, were put in 24-well plates at 22 °C. Different concentrations of algae subfractions (0.5, 2.0, 8.0, 32.0 and 128.0 μg/mL) were added and then evaluated after 24 h. After 24 h, a 100 μL sample of incubated material was withdrawn, and the activity was assayed using MTT (oxidation colorimetric

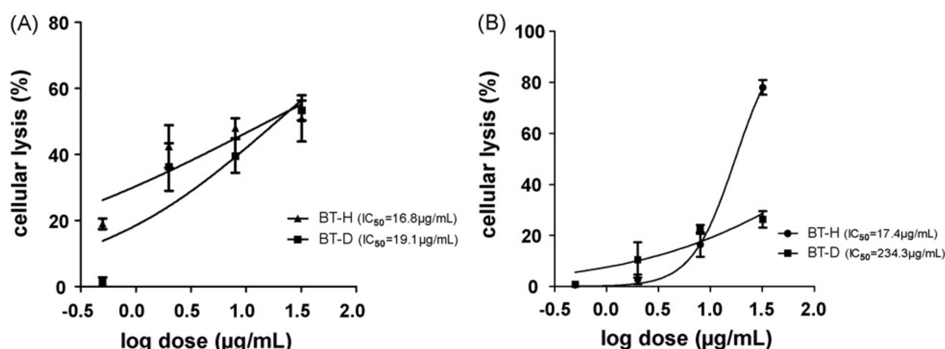

Fig. 1. Dose–response curves of the trypanocidal (A) and leishmanicidal (B) activities of BT-H and BT-D fractions from *B. tenella*. In these assay it was used gentian violet as positive control (IC_{50} = 31 mg/mL) to *Trypanosoma cruzi* an amphotericin B (IC_{50} = 13 μg/mL) to *Leishmania amazonensis*.

assay). A solution of DMSO was used as the negative control in the same concentration applied for the algae subfractions.

2.6. Statistical analysis

The subfractions were prepared in triplicate and measured for their trypanocidal and antileishmanial activity, as expressed by the percentage of activity (%AE) according to the formula:

$$\%AE = \left[\frac{(AE - AEB)}{(AC - ACB)}\right] \times 100,$$

where AE represents the "absorbance of tested plates," AEB is the "absorbance of plates containing medium and sample," AC is the "absorbance of plates containing negative control," and ACB is the "absorbance of plates containing culture medium."

All the IC_{50} values were calculated by nonlinear regression equation, using the computer program GraphPad Prism v.4.02.

2.7. Antifungal activity

The phytopathogenic microorganisms used in the antifungal assays *C. sphaerospermum* (Penzig) SPC 491 and *C. cladosporioides* (Fresen) de Vries SPC 140 were cultured at the Instituto de Botânica, São Paulo, SP, Brazil. The n-hexane and dichloromethane fractions, as well as the subfractions obtained by chromatographic separation, were dissolved in polarity-compatible solvents and applied to pre-coated TLC plates. For the BT-H samples, the TLC plates were developed with n-hexane:ethyl acetate (9:1); the BT-D samples with dichloromethane:methanol (9:1); and the H01, H02, H03, H05, H06, D01 and D02 with n-hexane:ethyl acetate (3:2) as the mobile phase. After eluting, the plates were dried for complete removal of the solvents. The chromatograms were sprayed with a spore suspension of *C. sphaerospermum* or *C. cladosporioides* in a glucose and salt solution and incubated for 72 h in a dark moistened chamber at 25 °C. A clear inhibition zone appeared against a dark background, indicating favorable sample activity. Nystatin was used as the positive control [14].

3. Results and discussion

3.1. Antiprotozoan and antifungal activities of BT-H and BT-D

The BT-H (n-hexane) and BT-D (dichloromethane) fractions contained active substances against the protozoa *T. cruzi* (trypomastigote form), as shown in Fig. 1A. In addition, BT-H was moderately active against the protozoa *L. amazonensis* (promastigote form – Fig. 1B), based on the IC_{50} values of standard drugs used in this experiment (gentian violet IC_{50} = 31 μg/mL and amphotericin B IC_{50} = 13 μg/mL). The BT-D (dichloromethane) fraction was weakly active against *L. amazonensis.* The Rf (retention factor) in thin-layer chromatography, as shown in Table 1, indicated that the BT-H and BT-D fractions consist of more than one active substance. These fractions may contain promising pharmacological properties that should be explored more thoroughly.

With the aim of identifying the substances or groups responsible for the biological activities, the obtained fractions were evaluated after chromatographic separation (see Section 2.2) (Table 1).

After silica-gel chromatography, the fractions displaying high antiprotozoan and antifungal activities were H02, H03, H05, H06, D01 and D02.

In the trypanocidal assay (Fig. 2A), the sample H02 (IC_{50} = 15.8 μg/mL) was stood out, showing higher activity than gentian violet (standard compound), whereas H01 (IC_{50} = 34.5 μg/mL) and D02 (IC_{50} = 36.5 μg/mL) displayed moderate activity. In the leishmanicidal assay (Fig. 2B), samples H02 (IC_{50} = 1.5 μg/mL), H03 (IC_{50} = 2.7 μg/mL), D01 (IC_{50} = 4.4 μg/mL) and D02 (IC_{50} = 4.3 μg/mL) were highly active, presenting IC_{50} values less than amphotericin B (standard compound). Furthermore, two inhibition spots by H02 indicate the presence of at least two substances or groups of substances that might demonstrate antifungal properties.

Fraction BT-D was weakly active against *L. amazonensis* (Fig. 1B), however its D01 and D02 subfractions were active (Fig. 2B). These results indicate that the BT-D fraction contains substances that may be interacting with each other or inhibiting some active compound, and thus appearing inactive. Alternatively, the active substances in the BT-D fraction could be in concentrations insufficient to promote positive activity, but are more concentrated after fractionation and then able to exhibit activity.

Several published reports have demonstrated the antiprotozoan activity of fresh algae and seaweeds. In a study involving the

Table 1
Antifungal inhibition of organic fractions and subfractions from *B. tenella*.

Samples	*C. cladosporioides*		*C. sphaerospermum*	
	Rf	Potential	Rf	Potential
BT-H	0–0.27	3	0–0.16	3
H01	0.31/0.54	1/1	0.27/0.54	1/1
H02	0.29/0.42	1/3	0.27/0.42	1/2
H03	0.3	2	0.25	3
H05	0.25	2	0.24	3
H06	0.21	3	0.15	3
BT-D	0.57/0.64/0.71–1.00	3/2/3	0.81/0.905	3/3
D01	0.32	3	0.29	3
D02	0.24	3	0.25	3

Positive control: Nystatin.
Potential legend: 1 (weak potential); 2 (medium potential); 3 (strong potential).

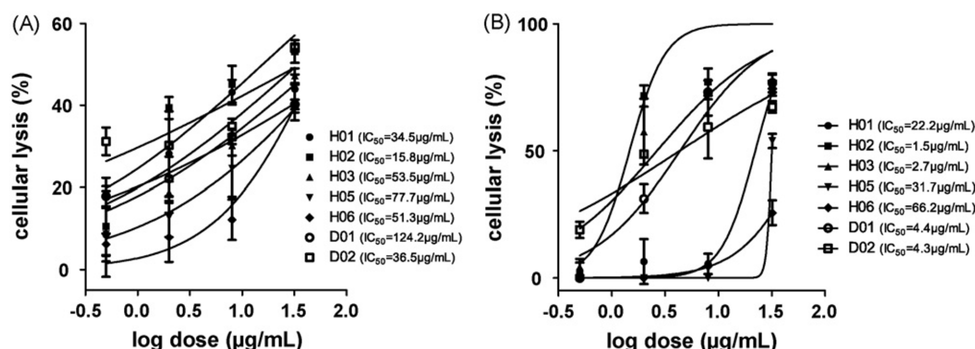

Fig. 2. Dose–response curves of the trypanocidal (A) and leishmanicidal (B) activities of BT-H- and BT-D-derived subfractions from *B. tenella*. In these assay it was used gentian violet as positive control (IC_{50} = 31 mg/mL) to *Trypanosoma cruzi* an amphotericin B (IC_{50} = 13 μg/mL) to *Leishmania amazonensis*.

inhibition of protozoan dihydroorotate dehydrogenase (DHOD), the extracts from two brown algae, *Fucus evanescens* and *Pelvetia babingtonii*, yielded a 59% and 58% decrease in the recombinant DHOD activity, respectively, at a concentration of 50 μg/mL. These results imply that *F. evanescens* and *P. babingtonii* contain inhibitor(s) against the *T. cruzi* DHOD activity and the protozoan infection and proliferation in mammalian cells [15].

The ethanolic extracts of a number of marine macroalgae (*Dictyota dichotoma*, *Halopteris scoparia*, *Posidonia oceanica*, *Scinaia furcellata*, *Sargassum natans* and *Ulva lactuca*) displayed trypanocidal activity against *Trypanosoma brucei rhodesiense*, with *S. natans* being the most active (IC_{50} 7.4 mg/mL). Except for *H. scoparia*, all extracts also demonstrated leishmanicidal potential. The highest antileishmanial activity was exhibited by *U. lactuca* and *P. oceanica* (IC_{50} values of 5.9 and 8.0 mg/mL, respectively) [16]. Organic extracts from *Laurencia microcladia* (Rhodophyta), *D. caribaea*, *Turbinaria turbinata* and *Lobophora variegata* (Phaeophyta) have promising in vitro activity against Leishmania mexicana promastigotes (IC_{50} values ranging from 10.9 to 49.9 μg/mL) [17].

Some samples such as H05 and H06 showed weak activity in the antiprotozoan assay, but contained substances with antifungal potential, suggesting that these samples have selective and active substances for different biological targets. Similar work was performed by Saito et al. [18] using thin-layer chromatographic screening of growth inhibitors for an imperfect fungus in calcareous red algae (Corallinaceae) *Corallina pilulifera*, *Lithothamnium pacificum*, and *Clathromorphum circumscriptum*. In this work, several substances with positive activity against *Cladosporium herbarum* were detected in the alcoholic extracts of the marine plants. The observed differences among the substances can be attributed to the morphological characteristics of seaweeds [18].

3.2. Chemical profile of BT-H and BT-D by GC/MS

Fractions (H01–H06 and D01–D02) with previously observed trypanocidal and antileishmanial activity were submitted to chemical analysis by GC/MS (Table 2) [23–28]. The major compounds in the fractions were identified, and the variation of these compounds in the different fractions is shown in Table 2.

Table 2 shows the diversity of the classes of substances found in the active samples: alkanes, alkenes (including dienes), alcohols (including diol), ketones, aldehydes, aromatics, esters, lactones, long-chain carboxylic acids, amides, imides and cholesterol derivatives. In a study involving some species of red algae from the Black Sea, the volatiles from most of the investigated red algae had moderate activity against *Staphylococcus aureus*. The volatiles from *H. virgatum* were rich in fatty acid methyl esters (76.4% from the total volatiles), whereas the volatiles from *C. elongate* contained phenol, 3,4-dihydroxy benzaldehyde, benzoic acid and the monoterpene eucalyptol. The volatiles from *Polysiphonia denudata* and *P. denudata* f. *fragilis* had similar activities against *S. aureus*, which might be attributed to the presence of aldehydes, free fatty acids, and phenol [19]. The presence of benzeneacetic acid and 4-hydroxy-benzaldehyde has already been established in marine algae [20,21] and may be responsible for the biological potential of fraction D02. Moreover, a series of aromatic aldehydes, including 4-hydroxy-benzaldehyde showed moderate activity against the fungi *Coriolus versicolor* and *Laetiporus sulphureus* [22], which supports our assumption that these compounds, observed in *B. tenella*, can be responsible for the antileishmanicidal activity.

We were also able to detect free fatty acids, which serve as energetic substrates and allelopathic agents [23]. The hexadecanoic acid (palmitic acid) produced by green algae was one of the substances evaluated in the cytotoxic assays involving phytoplankton and cyanobacteria. In a work involving fungal growth in the laboratory, a mixture of palmitic and oleic acids enhanced the growth of continuous-tomato and continuous-cucumber seedlings. Except for oleic acid, the fatty acids inhibited the mycelial growth of one or more tested fungi in a Petri dish assay. The saturated fatty acids showed stronger antifungal activity than the unsaturated fatty acids, suggesting that these fatty acids might be applicable as alternative approaches to the integrated control of phytopathogens [24]. The mode of action of these compounds against molds is not well understood, although it has been suggested that the fatty acids could disrupt the plasma membrane of fungi, thereby inhibiting the formation of apressoria in the infected leafs [25].

Other groups identified in our study include 2,6,10-trimethyl, 14-ethylene-14-pentadecene (neophytadiene), (*E*)-(7R,11R)-3,7,11,15-tetramethyl-2-hexadece-1-ol (phytol), 2,6,10,14-tetramethyl-hexadecadiene (phytadiene), 2,6,10,14-tetramethyl-2-hexadecene (phyt-2-ene) and 6,10,14-trimethyl-2-pentadecanone (hexahydrofarnesyl acetone). Phytol and neophytadiene have already been proposed to have certain antimicrobial activities [26]. The diterpene phytol had clear bactericidal activity and inhibited the growth of *S. aureus*, at a concentration of 0.15 μg/mL, as determined by damage to the cell membranes. The crude methanol extract of the Kenyan shrub *Leucas volkensii* Gürke (Labiatae) displayed antimycobacterial activity against *Mycobacterium tuberculosis* in a radiorespirometric bioassay. The bioassay-guided fractionation of the crude extract led to the identification of (*E*)-phytol as the principal active component with a minimum inhibitory concentration (MIC) of 2 μg/mL, a value also observed for (3*R*,*S*,7/*R*,11*R*)-phytanol, (*Z*)-phytol, and a commercially available 2:1 mixture of (*E*)- and (*Z*)-phytol [27]. Two nitrogenous sterols 7β-aminomethylcholesterol and 7α,β-aminocholesterol were active against *Leishmania donovani* promastigotes. Furthermore, (24R,S)-24-hydroxy-24-methylcholesterol (MEC, 12.5 μM) was the most active compound

Table 2
Compounds detected by GC/MS in active fractions of *B. tenella* (% of the total volatile mixture).

Compound	R Ind[a]	R Ind[b]	Ident	H01	H02	H03	H05	H06	D01	D02
3-Methyl-2-cyclopenten-1-one	–	–	MS[*1]	0.74	3.22	–	0.79	5.13	4.35	3.81
2-Pentanone	–	–	MS[*2]	4.89	13.73	25.00	1.61	29.08	22.38	30.90
3-Hexene-2,5-diol	–	–	MS[*3]	–	–	–	9.98	–	–	–
NI	1091.9	–	–	0.51	–	–	2.80	4.57	4.36	3.00
Nonanal	1101.8	1103[#1]	MS, RI	0.27	–	–	–	–	–	–
NI	1115.8	–	–	–	–	–	–	2.34	2.28	1.61
trans-5-Methyl-2-(1-methylethyl)-cyclohexanone	1147.5	1154[#1]	RI	0.33	–	–	6.83	5.77	5.59	3.16
5-Oxo-hexanoic acid	1159.6	–	MS[*4]	–	–	–	9.78	–	–	10.27
NI	1160.2	–	–	–	–	–	–	–	–	5.67
2-Decanone	1202.9	1192[#2]	RI	–	–	–	0.99	–	–	–
3-Ethyl-4-methyl-1H-pyrrole-2,5-dione	1231.2	1231[#3]	RI	0.53	1.62	–	–	–	–	–
Benzeneacetic acid	1244	1246[#4]	MS, RI	–	–	–	–	–	–	1.20
(*E*)-2-Decenal	1260.4	1260[#5]	MS, RI	–	0.78	–	–	–	–	–
NI	1277.4	–	–	–	–	–	–	1.78	1.68	2.56
4-Hydroxy-benzaldehyde	1363	–	MS[*5]	–	–	–	–	–	–	5.89
3-Hydroxy-2,2,4-trimethylpentyl-isobutanoate	1370	–	RI	0.24	–	–	–	–	–	–
4-Methyl-tetradecane	1452.4	1463[#4]	MS, RI	–	–	–	0.27	–	0.71	–
NI	1481.9	–	–	–	–	–	–	–	1.15	2.54
3-Acetyl-3-methyl-cyclopentene	1483.2	–	MS[*6]	–	2.92	–	–	–	–	–
Pentadecanal	1706.2	1712[#3]	MS, RI	0.20	–	–	–	–	–	–
Tetradecanoic acid	1747.8	1750[#6]	MS, RI	3.18	–	–	1.14	3.13	3.07	–
Hexadecanal	1808	1811[#1]	MS, RI	–	–	–	1.15	–	–	–
Pentadecanoic acid	1810.4	–	MS[*7]	0.24	–	–	–	–	–	–
2,6,10-Trimethyl, 14-ethylene-14-pentadecene	1823.4	1817[#6]	MS, RI	0.85	12.38	14.38	23.29	2.14	1.72	3.09
6,10,14-Trimethyl-2-pentadecanone	1828.5	1824[#6]	MS, RI	0.73	3.73	–	2.50	1.28	1.29	2.19
2,6,10,14-Tetramethyl-2-hexadecene	1846.9	–	MS[*8]	–	2.89	–	6.10	–	–	–
2,6,10,14-Tetramethyl-hexadecadiene	1865.7	1861[#6]	MS, RI	0.34	4.33	6.06	9.28	0.80	0.78	1.34
Methyl-hexadecanoate	1912.2	1910[#6]	MS, RI	–	–	–	–	0.85	0.84	–
Hexadecanoic acid	1950	1960[#7]	MS, RI	61.44	7.08	5.44	4.44	30.14	30.64	2.92
2-Hydroxy-1,3-propanediylhexadecanoate	2000.4	–	MS[*9]	0.17	–	–	–	–	–	–
Heptadecanoic acid	2054.1	2063[#4]	MS, RI	0.18	–	–	–	–	–	–
5-Dodecyldihydro-2(3H)-furanone	2095.1	–	MS[*10]	0.56	–	–	–	–	0.56	–
(*E*)-(7R,11R)-3,7,11,15-Tetramethyl-2-hexadece-1-ol	2100.6	2106[#6]	MS, RI	–	–	1.23	1.92	–	–	–
6-Heptyltetrahydro-2H-pyran-2-one	2126.3	2124[#8]	RI	0.24	–	–	–	–	–	–
Z-9-Octadecenoic acid	2134.5	2139[#7]	MS, RI	2.52	–	–	–	–	–	–
E-9-Octadecenoic acid	2138.8	–	MS[*11]	1.44	–	–	–	–	–	–
Octadecanoic acid	2157.6	2164[#4]	MS, RI	0.80	–	–		–	–	–
Hexadecanamide	2168.4		MS[*12]	–	–	–	0.25	–	–	–
4,8,12,16-Tetramethylheptadecan-4-olide	2333.4	–	MS[*13]	0.42	–	–	–	–	–	–
NI	–	–	–	0.12	–	3.12	–	–	–	–
4,6-Cholestadien-3-β-ol	–	–	MS[*14]	4.37	7.33	10.00	2.99	1.03	0.83	–
NI	–	–	–	0.77	1.47	2.09	0.51	–	–	–
3-β-Cholest-5-en-3-ol	–	–	MS[*15]	5.84	23.58	2.43	0.93	–	–	–
3,5-Cyclocholestan-6-one	–	–	MS[*16]	0.38	–	–	–	–	–	–
Cholesta-3,5-dien-7-one	–	–	MS[*17]	1.01	1.84	7.32	0.82	–	–	–
4,6-Cholestadien-3-one	–	–	MS[*18]	0.51	0.93	–	–	–	–	–
NI	–	–	–	–	5.86	–	–	–	–	–
NI	–	–	–	–	–	5.76	–	–	–	–

#1 to #8: references for RI literature values (Appendix A). *1 to *18: fragmentation profile for substances identified by MS (Appendix B).
[a] Calculated retention index.
[b] Retention index from literature.

against *Trypanosoma brucei brucei* from a series of evaluated oxysterols, including ketosterols and hydroxysterols [28].

This experiment was evaluated based on retention time of majority compounds (more than 5%) in each fraction, expressed as relative standard deviation (RSD). For all substances analyzed, the RSD values were below 1%, indicating the satisfactory repeatability of the proposed method. In addition, the compounds not compared by literature data were submitted to mass spectrometry analysis, confirming the structures by the fragmentation pattern (Table 2).

4. Conclusions

In this work, a general chromatographic procedure to obtain fractions containing volatile compounds from the marine red alga *B. tenella* is presented. The GC/MS method was also applied for the identification of the volatile composition, allowing for the characterization of bioactive extracts that could be potentially used for the pharmaceutical industry. The biological evaluation allowed for a correlation between functional groups and exhibited activity. The possibility of finding functional and interesting compounds from the alga *B. tenella* using this methodology and other assays (phytopathogenic fungi *C. cladosporioides* and *C. sphaerospermum*, and antiprotozoal assays using *T. cruzi* and *L. amazonensis*) looks promising.

Acknowledgements

The authors are grateful to FAPESP for a fellowship (2007/57985-8) and financial support (05/53808-9) and to CNPq for financial support (485421/2006-2 and 420.015/2005-1).

Appendix A.

References for retention index literature values shown in Table 2.

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#7 R.N.S. Torres, J.A.D. Lopes, J.M. Moita Neto, A.M.G.L. Citó, Constituents voláteis de própolis piauense, Quim. Nova 31 (2008) 479–485.
#8 M. Miyazawa, S. Nagai, T. Oshima, Volatile components of the straw of *Oryza sativa* L., J. Oleo Sci. 57 (2008) 139–143.

Appendix B.

The fragmentation profile for the compounds identified by means of mass spectrometry, Table 2:

***1**: 96 (100); 67 (69); 53 (50); 81 (36); 40 (35); 41 (23); 95 (23); 68 (17); 97 (12); 42 (10).

***2**: 43 (100); 58 (17); 86 (16); 41 (9); 71 (7); 42 (6); 55 (6).

***3**: 43 (100); 71 (40); 55 (24); 83 (23); 57 (21); 98 (21); 41 (14); 42 (10); 70 (8); 58 (8).

***4**: 43 (100); 42 (14); 113 (13); 41 (12); 45 (11); 70 (10); 40 (7); 68 (7); 85 (6).

***5**: 121 (100); 122 (90); 65 (41); 93 (41); 63 (10); 66 (7); 123 (6).

***6**: 81 (100); 41 (17); 79 (11); 43 (10); 82 (7); 53 (6); 55 (5); 83 (5).

***7**: 43 (100); 73 (72); 41 (69); 57 (68); 60 (66); 55 (52); 71 (40); 69 (36); 129 (32); 199 (25); 85 (24); 83 (23); 87 (21); 56 (20).

***8**: 82 (100); 81 (98); 68 (88); 43 (86); 95 (85); 57 (84); 41 (69); 69 (59); 123 (54); 55 (54); 67 (51); 71 (44); 83 (43); 79 (36); 96 (36); 96 (36); 97 (31); 124 (31); 124 (31); 109 (30); 56 (22); 110 (19); 70 (19); 111 (18).

***9**: 43 (100); 57 (87); 41 (61); 55 (56); 71 (52); 73 (50); 60 (45); 69 (38); 129 (29); 83 (27); 97 (24); 152 (21); 85 (19); 115 (18); 56 (17); 42 (15); 185 (15).

***10**: 85 (100); 43 (54); 41 (53); 55 (48); 69 (38); 57 (37); 84 (36); 97 (34); 83 (31); 56 (29); 70 (21); 98 (20); 96 (18); 111 (17); 128 (10); 192 (8); 138 (6); 236 (6); 218 (5).

***11**: 55 (100); 41 (80); 69 (74); 43 (62); 83 (46); 97 (45); 57 (37); 56 (30); 70 (29); 84 (29); 67 (27); 98 (27); 98 (27); 73 (24); 96 (23); 81 (21); 82 (20); 60 (20); 111 (20); 71 (20); 68 (19); 125 (10); 264 (9); 137 (7).

***12**: 59 (100); 72 (38); 43 (22); 41 (15); 55 (12); 60 (8).

***13**: 99 (100); 43 (44); 57 (26); 55 (25); 69 (24); 41 (22); 71 (18); 70 (16); 126 (16); 56 (15); 114 (15); 83 (14); 111 (13); 97 (11); 85 (8); 98 (7); 109 (6); 110 (6); 125 (6).

***14**: 135 (100); 366 (89); 143 (70); 43 (52); 81 (48); 119 (43); 149 (41); 95 (41); 247 (37); 55 (36); 41 (36); 57 (35); 129 (34); 105 (33); 91 (32); 145 (31); 158 (31); 144 (30), 69 (30).

***15**: 386 (100); 43 (96); 55 (68); 301 (65); 107 (65); 95 (64); 275 (63); 81 (60); 105 (60); 57 (60); 41 (60); 145 (59); 368 (48); 91 (47); 93 (46); 69 (46); 213 (45); 79 (44); 67 (44); 353 (41); 159 (39); 161 (39); 133 (39); 119 (38); 163 (34); 255 (34); 371 (33); 231 (27); 173 (21); 247 (16).

***16**: 384 (100); 136 (60); 43 (58); 79 (54); 122 (53); 123 (50); 55 (46); 95 (45); 41 (41); 121 (35); 385 (31); 57 (31); 81 (31); 91 (30); 93 (30); 161 (29); 369 (28); 229 (22); 271 (13).

***17**: 174 (100); 382 (47); 161 (27); 159 (25); 187 (23); 43 (21); 175 (16); 91 (16); 41 (15); 81 (15); 269 (13); 367 (7).

***18**: 124 (100); 43 (39); 229 (39); 384 (31); 55 (30); 41 (29); 261 (29); 95 (26); 147 (26); 81 (25); 260 (24); 135 (23); 107 (21); 93 (21); 342 (13); 299 (8); 187 (8).

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