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# Sesquiterpenes from the essential oil of *Laurencia dendroidea* (Ceramiales, Rhodophyta): isolation, biological activities and distribution among seaweeds

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**Abstract:** Two known sesquiterpenes (1*R**,2*S**,3*R**,5*S**,8*S**,9*R**)-2,3,5,9-tetramethyltricyclo[6.3.0.0$^{1,5}$]undecan-2-ol and (1*S**,2*S**,3*S**,5*S**,8*S**,9*S**)-2,3,5,9-tetramethyltricyclo-[6.3.0.0$^{1,5}$]undecan-2-ol were isolated for the first time from the essential oil of the red seaweed *Laurencia dendroidea* collected in the Brazilian coast. These compounds were not active against eight bacteria strains and the yeast *Candida albicans*, but showed some antioxidant activity. Both compounds were also found in other seaweed species showing that they are not exclusive taxonomic markers to the genus *Laurencia*.

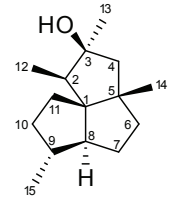
## Introduction

Species of algae from the genus *Laurencia* J.V. Lamour. (Ceramiales, Rhodomelaceae) have been a subject of intensive research since an earlier study of marine natural products (Faulkner, 2000). The genus *Laurencia* is a rich source of secondary metabolites of diverse structures with biological activity (Rashid et al., 1995), which can vary significantly with species and location.

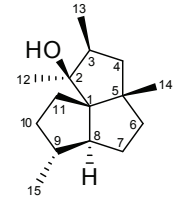
The volatile organic compounds which comprise the essential oil are molecules with low molecular weight, low to moderate hydrophilicity and high vapor pressure. Since these compounds can cross the membrane and be released in the atmosphere, they play an important role as chemical communications for algae in marine ecosystems (Fink, 2007; Gressler et al., 2009; Gressler et al., 2011).

A wide range of volatile secondary metabolites such as halogenated and sulfur compounds (Kladi et al., 2004; Karabay-Yavasoglu et al., 2007), hydrocarbons, alcohols, phenols, aldehydes, ketones, acids, esters, terpenes and other compounds (Gressler et al., 2009), are distributed among the seaweeds. However, few works reported the essential oil composition of seaweeds.

This study describe, for the first time, the isolation of two triquinane alcohols (1*R**,2*S**,3*R**,5*S**,8*S**,9*R**)-2,3,5,9-tetramethyltricyclo[6.3.0.0$^{1,5}$]undecan-2-ol (SPF-1), also known as 7-*epi*-silphiperfolan-6β-ol (**1**), and (1*S**,2*S**,3*S**,5*S**,8*S**,9*S**)-2,3,5,9-tetramethyltricyclo[6.3.0.0$^{1,5}$]undecan-2-ol (SPF-2), also known as silphiperfolan-7β-ol (**2**), from the essential oil of *Laurencia dendroidea.*




**1**




**2**

In addition, this paper describes a screening of these two compounds in a range of 21 species of seaweeds collected in different places and dates, and their antimicrobial and antioxidant properties.

## Materials and Methods

### *Seaweed samples*

The red macroalga *Laurencia dendroidea* was collected at Parati beach in Anchieta, Espírito Santo State, Brazil, in October, 2006 and identified by Dr.

Mutue Toyota Fujii. An exsiccate (SP 399.947) is deposited on Herbário do Estado (SP) "Maria Eneyda P. K. Fidalgo" at Instituto de Botânica de São Paulo. Other 21 algae species distributed in 41 samples were used for analyses. Their description is shown in Table 1.

**Table 1.** Description of algae species used in the distribution analysis of (-)-7-*epi*-silphiperfolan-6β-ol (**1**) and (-)-silphiperfolan-7β-ol (**2**).

| Alga specie | Abbreviation | Place of collection | Date of collection | Herbarium number |
|---|---|---|---|---|
| *Laurencia aldingensis* Saito & Womersley | LA1 | ES, Anchieta, Castelhanos | 11/10/2007 | SP 400.907 |
| *Laurencia aldingensis* Saito & Womersley | LA2 | ES, Anchieta, Ubú | 04/06/2008 | SP 400.211 |
| *Laurencia aldingensis* Saito & Womersley | LA3 | ES, Anchieta, Ubú | 08/04/2009 | SP 400.210 |
| *Laurencia dendroidea* J. Agardh | LD1 | ES, Anchieta, Parati | 03/10/2006 | SP 399.947 |
| *Laurencia dendroidea* J. Agardh | LD2 | ES, Anchieta, Castelhanos | 01/07/2007 | SP 399.945 |
| *Laurencia dendroidea* J. Agardh | LD3 | SP, Ubatuba, Praia Brava | 25/10/2007 | SP 401.375 |
| *Laurencia dendroidea* J. Agardh | LD4 | ES, Anchieta, Castelhanos | 03/06/2008 | SP 399.806 |
| *Laurencia dendroidea* J. Agardh | LD5 | ES, Anchieta, Castelhanos | 08/04/2009 | SP 400.905 |
| *Laurencia dendroidea* J. Agardh | LD6 | ES, Anchieta, Castelhanos | 11/10/2007 | SP 400.906 |
| *Laurencia dendroidea* J. Agardh | LD7 | RN, Natal, Praia Rio do Fogo | 03/12/2009 | n.i. |
| *Laurencia* sp.1 | Lsp1A | ES, Anchieta, Ubú | 30/06/2007 | SP 400.206 |
| *Laurencia* sp.1 | Lsp1B | ES, Guarapari, Meaípe | 04/06/2008 | SP 400.793 |
| *Laurencia* sp.2 | Lsp2 | ES, Anchieta, Ubú | 04/06/2008 | SP 399.936 |
| *Laurencia translucida* Fujii & Cordeiro-Marino | LT1 | ES, Anchieta, Parati | 30/06/2007 | SP 400.213 |
| *Laurencia translucida* Fujii & Cordeiro-Marino | LT2 | ES, Anchieta, Castelhanos | 09/04/2009 | SP 400.830 |
| *Palisada flagellifera* (J. Agardh) K.W. Nam | PF1 | ES, Anchieta, Castelhanos | 03/06/2008 | SP 400.203 |
| *Palisada flagellifera* (J. Agardh) K.W. Nam | PF2 | ES, Anchieta, Castelhanos | 08/04/2009 | SP 401.376 |
| *Palisada perforata* (C. Agardh) K.W. Nam | PP1 | ES, Anchieta, Castelhanos | 11/10/2007 | SP 400.908 |
| *Palisada perforata* (C. Agardh) K.W. Nam | PP2 | ES, Anchieta, Castelhanos | 03/06/2008 | SP 400.204 |
| *Palisada perforata* (C. Agardh) K.W. Nam | PP3 | ES, Anchieta, Castelhanos | 08/04/2009 | SP 401.377 |
| *Ceramium* sp. | Csp | n.i. | n.i. | n.i. |
| *Chondria littoralis* Harvey | CL1 | ES, Anchieta, Parati | 04/06/2008 | SP 401.378 |
| *Chondria littoralis* Harvey | CL2 | ES, Anchieta, Castelhanos | 09/04/2009 | SP 401.379 |
| *Gracilaria birdiae* Palastino & Oliveira | GB | ES, Anchieta, Parati | 03/10/2006 | SP 400.901 |
| *Gracilaria domingensis* (Kützing) Sonder ex Dickie | GD1 | ES, Anchieta, Parati | 03/10/2006 | n.i. |
| *Gracilaria domingensis* (Kützing) Sonder ex Dickie | GD2 | RN, Natal | n.i | n.i. |
| *Plocamium brasiliense* (Greville) M.A. Howe & W.R. Taylor | PB | ES, Anchieta, Ubú | 03/10/2006 | SP 399.944 |
| *Pterosiphonia pennata* (C. Agardh) Sauvageau | PT | SP, Ubatuba, Praia Brava | 27/10/2007 | SP 400.898 |
| *Spyridia aculeata* (C. Agardh ex Decaisne) Kützing | SA1 | ES, Anchieta, Parati | 05/10/2006 | SP 400.903 |
| *Spyridia aculeata* (C. Agardh ex Decaisne) Kützing | SA2 | ES, Anchieta, Parati | 04/06/2008 | SP 400.899 |
| *Wrangelia penicillata* (C. Agardh) C. Agardh | WP | ES, Anchieta, Parati | 03/10/2006 | n.i. |
| *Hypnea musciformis* (Wulfen) J.V.Lamouroux | HM1 | SP, Ubatuba | 26/10/2006 | n.i. |
| *Hypnea musciformis* (Wulfen) J.V.Lamouroux | HM2 | SP, Ubatuba | 26/10/2006 | n.i. |
| *Hypnea nigrescens* Greville ex J. Agardh | HN | SP, Ubatuba, Praia Dura | 13/09/2008 | SP 400.895 |
| *Octhodes secundiramea* (Mont.) M. Howe | OS1 | ES, Serra, Manguinhos | 06/10/2006 | SP 400.748 |
| *Octhodes secundiramea* (Mont.) M. Howe | OS2 | ES, Anchieta, Castelhanos | 03/06/2008 | SP 400.201 |
| *Octhodes secundiramea* (Mont.) M. Howe | OS3 | ES, Anchieta, Castelhanos | 08/04/2009 | SP 400.894 |
| *Solieria filiformis* (Kützing) P.W. Gabrielson | SF | ES, Serra, Manguinhos | 04/10/2006 | SP 400.900 |
| *Pterocladiella capillacea* (S.G.Gmelin) Santelices & Hommersand | PC | SP, Ubatuba, Praia Dura | 10/12/2007 | SP 400.902 |
| *Sargassum* sp C. Agardh | Ssp | SP, Ubatuba, Ilha do Mar Virado | 30/06/2007 | SP 400.896 |
| *Ulva* sp. | Usp | SP, Ubatuba | 10/12/2007 | n.i. |

ES – Espírito Santo State; SP – São Paulo State; RN – Rio Grande do Norte State; n.i.- not informed.

*Distillation of the volatile components*

Four hours of steam distillation of 200 g of fresh material was done in a Clevenger-type apparatus. The obtained distillate was removed from the apparatus with ethyl ether and the water residue was removed with sodium sulfate. The samples were concentrated by rota-evaporation and then diluted 100 times with ethyl acetate prior to analysis by GC/MS.

*Isolation and identification of the compounds*

The two major compounds of the essential oil were separated by preparative TLC (Silica Gel 60 $F_{254}$ 0.5 mm Merck) eluted twice with hexane:ethyl acetate (95:5). The compounds SPF-1 and SPF-2 were analyzed by GC/MS and uni- and bidimensional NMR.

*Gas chromatography/mass spectrometry (GC/MS) analysis*

The essential oil and the isolated compounds were analyzed in a gas chromatograph coupled to a mass spectrometer (GCMS-QP2010 Plus, Shimadzu, Japan). The chromatographic separation was achieved with a Rtx-5MS capillary column (30 m x 0.25 mm x 0.1 µm) and helium was used as carrier gas (with a flow rate of 1 $mL.min^{-1}$). Oven temperature was increased at a rate of 3 °C.$min^{-1}$ from 60 to 260 °C, and hold for 40 min. Injection and transfer line temperatures were 220 and 240 °C. The detection was performed in the full scan mode, with a mass range of 50-650 *m/z*. Electron impact ionization was employed with collision energy of 70 eV, and the mass spectrometer ion source was maintained at 240 °C. The target compounds were identified by GC retention times and from their mass spectra. The relative concentration (%) was determined by the integration of all peaks obtained in the total ion chromatogram (TIC) mode. The proportion between the compounds SPF-1 and SPF-2 in different marine seaweed species was obtained using the extraction of specific fragments, characteristic of each compound (*m/z* 98 for SPF-1 and *m/z* 86 for SPF-2).

*NMR spectra*

$^{1}H$ and $^{13}C$ uni- and bi-dimensional NMR spectra were recorded using a DPX 500 (Bruker, Germany) spectrometer with $CDCl_3$ as solvent and TMS as internal standard.

*HS-SPME extraction and analysis*

The lyophilized algae material (50 mg) were kept in a 10 mL vial. After the equilibration time (15 min at 60 °C) the SPME fiber (PDMS, 100 µm) was exposed during 15 min. The volatile organic compounds composition of the species were analyzed by GC/MS at the same conditions described for the essential oil and isolated compounds.

*Test microorganisms*

*In vitro* antimicrobial studies were carried out against eight bacterial strains (*Staphylococcus aureus* ATCC 29213, *Bacillus subtilis* ATCC 6633, *Enterococcus faecalis* ATCC 29212, *Streptococcus pneumoniae* ATCC 49619, *Klebsiella pneumoniae* ATCC 13883, *Salmonella typhi* ATCC 19430, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853), and one yeast strain (*Candida albicans* ATCC 10231).

*Antimicrobial testing*

The Minimal Inhibitory Concentration (MIC) was determined in 96-well culture plates by a micro-dilution method using a microorganism suspension with a density of $10^5$ $CFU.mL^{-1}$ in Mueller Hinton Broth (MHB) for bacteria, and Sabouraud Broth (SB) for yeast, as recommended by NCCLS (NCCLS M100-S15 and M38-A). The bacterial suspension was incubated for 24 h at 35±1 °C and the yeast suspension for 72 h at 35±1 °C. Proper blanks were assayed simultaneously with samples tested in triplicate. The concentrations tested were 500, 250, 125, 62.5, 31.25 and 16.125 $\mu g.mL^{-1}$.

*Antioxidant testing*

DPPH radical scavenging assay

Radical scavenging activity of *L. dendoidea* essential oil and isolated compounds against stable DPPH (2,2-diphenyl-2-picrylhydrazyl, Sigma-Aldrich Chemie, Steinheim, Germany) was determined spectrophotometrically according to Yen & Chan (1995) with some modifications. The absorbance decrease was measured at 517 nm on a UV-visible light spectrophotometer (Spectrum Power $Wave_x$ 340, Bio-Tec Instruments, INC). Sample solutions were prepared by dissolving the essential oil or the isolated compounds in methanol. The solution of DPPH in methanol (1 mM) was prepared daily, before UV measurements. Ten µL of this solution were mixed with 100 µL sample solution of final concentrations of 500, 250, 100, 50 and 5 $\mu g.mL^{-1}$ in a 96-well plate. The samples were kept in the dark for 20 min at room temperature and then the decrease in absorption was measured. Absorption of blank sample containing the same amount of methanol and DPPH solution was prepared and measured daily. Vitamin E was used as a positive control. The experiment was carried out

in triplicate. Radical scavenging activity was calculated by the following formula:

$$\%Inhibition = \frac{(Abs_{DPPH} - Abs_{sample})x100}{Abs_{DPPH}}$$

where $Abs_{DPPH}$ is the absorption of blank sample (MeOH+DPPH) and $Abs_{sample}$ is the absorption of tested solution+DPPH.

Chemiluminescence determination in the presence of HRP

This method is based on light emission and, for antioxidant determination, solutions of 1 mg.mL$^{-1}$ of the test sample in phosphate buffer 5% DMSO were mixed with $H_2O_2$, yielding a final concentration of 5 x 10$^{-5}$ mol.L$^{-1}$. Then luminol (5-amino-2,3-dihydrophthalazine-1,4-dione, in DMSO) was added to a final concentration of 1.13 x 10$^{-4}$ mol.L$^{-1}$. The 96-well plate was incubated for 3 min at 30 °C and then HRP at a final concentration of 0.2 IU.mL$^{-1}$ was added to initiate the chemiluminescence reaction (Krol et al., 1994). Phosphate buffer/5% DMSO was used as a blank for the maximum luminescence and *N*-acetyl-L-cysteine was used as the positive control. Chemiluminescence was measured for 15 min at 25 °C with a microplate reader (Tecan Infinite®M200). Final concentrations of 500, 250, 100, 50 and 5 mg.mL$^{-1}$ of the solutions were analyzed and the experiment was carried out in triplicate. The antioxidant activity was calculated by the following formula:

$$\%Inhibition = \frac{(A_{control} - A_{sample})x100}{A_{control}}$$

where $A_{control}$ is the area under the curve of the blank (phosphate buffer/5% DMSO+$H_2O_2$+luminol+HRP) during 15 min and $A_{sample}$ is the area under the curve of the sample (sample+$H_2O_2$+luminol+HRP) during 15 min.

## Results and Discussion

The extraction of the essential oil of *L. dendroidea* yielded 0.066% of fresh weight. The chromatogram of the essential oil obtained showed one major peak at 29.5 min (61.67%). Since this peak was not identified by the available libraries (NIST 08 and NIST 08S), the essential oil was submitted to TLC analysis and it was possible to see two strong spots, which were further isolated by preparative TLC. The two isolated compounds were analyzed by GC/MS and NMR techniques and both showed the same retention time (29.5 min) and a 1529 Kovats index.

The $^1$H-NMR spectrum of the compound SPF-1 showed that all proton resonances are between δ 0.87 and 1.86 ppm. Only four signals were useful to structure determination (doublets at δ 0.87 and 0.97, singlets at δ 1.07 and 1.10) with integration equivalent for three hydrogen atoms. Similarly as SPF-1, in the $^1$H-NMR spectrum of the compound SPF-2 all proton resonances were between δ 0.89 and 1.69 ppm. Four methyl resonances at δ 0.89 (d), 0.92 (d), 1.11 (s) and 1.17 (s) with integration equivalent for three hydrogen atoms were also seen.

Table 2 shows the $^1$H and the $^{13}$C NMR data of both non-halogenated sesquiterpenes. A comparison between the values obtained in this study and literature data showed that the compounds are SPF-1 and SPF-2, previously isolated from *Laurencia majuscula* Saito & Womersley and *L. dendroidea* J. agardh (as *L. scoparia*) (Coll & Wright, 1989; Wright et al., 1990; Davyt et al., 2006). However, the $^{13}$C-NMR data published by Wright et al. (1990) have one incorrect signal-carbon number attribution. The authors indicated that the δ 27.8 $^{13}$C-NMR signal is the ethylene group bonded to C3 (δ 81.1 ppm) to SPF-1, but our HMBC-NMR correlation indicates that δ 27.8 ppm signal is bounded to C5. The $^1$H-$^{13}$C long range correlations are also given in Table 2.

Both triquinane alcohols smell camphoraceous and woody and have oil aspect.

To verify if the isolated compounds are molecules that could represent taxonomic markers, the presence of the mixture of these compounds was verified in 21 seaweed species which grow in Brazilian coast by HS-SPME-GC/MS analysis.

As previously informed, both sesquiterpenes have the same retention time (probably because of the similarity in their structure), but different mass spectra (Figure 1). To analyze the proportion of each compound in the peak of a crude sample, *m/z* 98 and *m/z* 86 were chosen as characteristic fragments to identify SPF-1 and SPF-2, respectively. The Table 3 shows the species tested and the relative amount of the mixture.

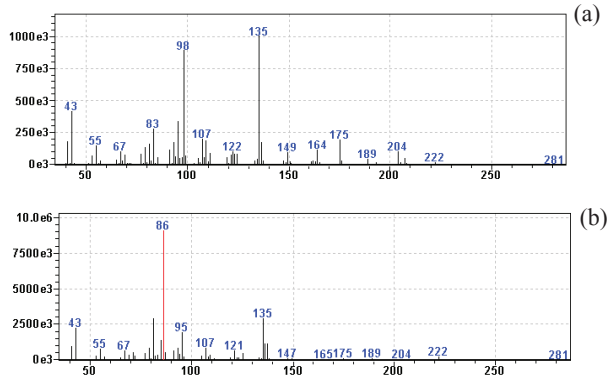



**Figure 1.** Mass spectra of (a) (-)-7-*epi*-silphiperfolan-6β-ol (**1**) and (b) (-)-silphiperfolan-7β-ol (**2**).

**Table 2.** Chemical shifts obtained by NMR analysis of SPF-1 and SPF-2 in $CDCl_3$ at 500 MHz.

| nº C | (-)-7-*epi*-silphiperfolan-6β-ol (SPF-1) | | (-)-silphiperfolan-7β-ol (SPF-2) | |
|---|---|---|---|---|
| | δ $^1$H ppm | δ $^{13}$C ppm | δ $^1$H ppm | δ $^{13}$C ppm |
| 1 | - | 65.9 | - | 69.9 |
| 2 | 1.53 (q, *J* = 7.5 Hz) | 53.9 | - | 85.3 |
| 3 | - | 81.2 | 1.63 (m) | 41.0 |
| 4 | 1.55 (d, *J* = 7.5 Hz), 1.62 (d, *J* = 7.5 Hz) | 55.7 | 1.34 (m), 1.61 (m) | 47.3 |
| 5 | - | 50.2 | - | 49.7 |
| 6 | 1.48 (m), 1.59 (m) | 42.7 | 1.47 (m) | 41.8 |
| 7 | 1.46 (m) and/or 1.55 (m) and/or 1.69 (m) | 26.9 | 1.29 (m), 1.51 (m) | 29.0 |
| 8 | 1.43 (m) | 63.1 | 1.51 (m) | 57.7 |
| 9 | 1.39 (m) | 38.4 | 1.52 (m) | 42.5 |
| 10 | 1.11 (m),1.76 (m) | 37.1 | 1.70 (t,d *J* = 5.5 Hz, *J* = 2 Hz) | 36.4 |
| 11 | 1.46 (m) and/or 1.55 (m) and/or 1.69 (m) | 26.9 | 1.86 (q,d, *J* = 7 Hz, *J* = 2.5 Hz) | 27.9 |
| 12 | 0.89 (d, 3H, *J* = 7.0 Hz) | 8.3 | 1.10 (s, 3H) | 22.3 |
| 13 | 1.17 (s, 3H) | 26.3 | 0.87 (d, 3H, *J* = 6.0 Hz) | 12.5 |
| 14 | 1.11 (s, 3H) | 27.8 | 1.07 (s, 3H) | 26.8 |
| 15 | 0.92 (d, 3H, *J* = 6.5 Hz) | 19.7 | 0.97 (d, 3H, *J* = 6.5 Hz) | 19.6 |
| nº C | $^1$H-$^{13}$C NMR (HMBC) | | | |
| 12 | 1,2,3 | | 1,2,3 | |
| 13 | 2,3,4 | | 2,3,4 | |
| 14 | 1,4,5,6 | | 1,4,5,6 | |
| 15 | 8,9,10 | | 8,9,10 | |

As can be seen in Table 3, both compounds SPF-1 and SPF-2 were observed in different seaweed species, in amounts ranging from 1.12±0.37 to 53.25± 0.21%.

Regarding the proportion of the fragments analyzed, most of the species that have the sesquiterpenes SPF-1 and SPF-2 in his composition showed higher percentage of the *m/z* 86 (in a proportion of 2:1), indicating that the compound SPF-2 is twofold higher. On the other hand, the proportion found between *m/z* 86 and *m/z* 98 was 1.5:1 for *Palisada perforata* (Bory) K.W. Nam (PP3).

Even though both fragments were seen in the *Pterosiphonia pennata* (C. Agardh) Sauvageau (PT) samples, it was not possible to obtain the total amount of SPF-1 and SPF-2 because there was another compound (with a different mass spectra) in the same retention time.

The sesquiterpenes evaluation also showed large occurrence in the Ceramiales group, which is known to have high potential of secondary metabolites production (Fenical & Norris 1975). In the *Laurencia dendroidea*, samples collected in different places at different dates showed that both compounds represented around 50% of composition (53.25±3.21 and 48.67±0.32% for LD3 and LD4, respectively). Among the Gigartinales, only *O. secundiramea*, a known producer of secondary metabolites with various activities (Paul et al., 1988), is capable to produce the referred compound. Sesquiterpenes were not found in *Sargassum* sp., Ulva sp. species (Heteroconthophyta and Chlorophyta group representatives, respectively). Green seaweeds produce mainly acyclic sesqui- and diterpenes while brown seaweeds are known by polyphenols, terpenoids, volatile hydrocarbons and sulfur compounds production (Pereira, 2002). The non occurrence of SPF-1 and SPF-2 might be an indication that these compounds are only observed in red algae.

For some species, like *Gracilaria domingensis* (Kützing) Sonder ex Dickie and *Gracilaria birdiae* Plastino & Oliveira, the presence of the sesquiterpenes was not expected, since no previous evidence of this metabolite class was found in the literature. The very small amount found in GB and GD2 might be explained by the sampling method used. HS-SPME integrates extraction and concentration of volatile compounds into a single step, leading to a high sensitivity (Gressler et al., 2009), not achieved with other extraction methods (like hydrodistillation and solvent extract). In *Palisada perforata* (Bory) K.W. Nam, previous called as *Laurencia papillosa*, the SPF-1 and SPF-2 production was seen in our study, which is consistent with other terpenes already indentified by Kamenarska et al. (2006).

**Table 3.** Relative proportion of (-)-7-*epi*-silphiperfolan-6β-ol (**1**) (*m/z* 86) and (-)-silphiperfolan-7β-ol (**2**) (*m/z* 98) in different marine macroalgae species samples obtained by HS-SPME-GC/MS analysis.

| Samples | Amount (%±SD) | Fragment (%) | | |
|---|---|---|---|---|
| | | *m/z* 98 | *m/z* 86 | SD |
| LA1 | 1.12±0.37 | 21.57 | 78.43 | 3.68 |
| LA2 | - | n.d. | n.d. | n.d. |
| LA3 | - | n.d. | n.d. | n.d. |
| LD1 | 4.43±1.95 | 30.77 | 69.23 | 1.87 |
| LD2 | 1.93±0.32 | 37.86 | 62.14 | 1.64 |
| LD3 | 53.25±3.21 | 30.68 | 69.32 | 0.41 |
| LD4 | 48.67±1.97 | 32.71 | 67.29 | 0.81 |
| LD5 | 1.88±1.30 | 30.49 | 69.51 | 1.65 |
| LD6 | 3.74±1.17 | 30.26 | 69.74 | 8.39 |
| LD7 | 5.60±0.60 | 27.65 | 72.35 | 0.58 |
| Lsp1A | 6.38±0.32 | 35.96 | 64.04 | 2.06 |
| Lsp1B | - | n.d. | n.d. | n.d. |
| Lsp2 | 13.29±8.44 | 26.68 | 73.32 | 0.71 |
| LT1 | - | n.d. | n.d. | n.d. |
| LT2 | - | n.d. | n.d. | n.d. |
| PF1 | - | n.d. | n.d. | n.d. |
| PF2 | - | n.d. | n.d. | n.d. |
| PP1 | 22.18±8.48 | 32.17 | 67.83 | 2.19 |
| PP2 | 11.38±1.26 | 31.03 | 68.97 | 2.04 |
| PP3 | - | n.d. | n.d. | n.d. |
| Csp | 5.18±1.61 | 28.08 | 71.92 | 1.05 |
| CL1 | - | n.d. | n.d. | n.d. |
| CL2 | - | n.d. | n.d. | n.d. |
| GB | 3.80±1.14 | 30.27 | 69.73 | 0.27 |
| GD1 | - | n.d. | n.d. | n.d. |
| GD2 | 1.88±0.35 | 30.43 | 69.57 | 0.60 |
| PB | - | n.d. | n.d. | n.d. |
| PT | n.q. | 34.24 | 65.76 | 1.88 |
| SA1 | 5.42±0.75 | 28.60 | 71.40 | 1.25 |
| SA2 | 12.95±1.03 | 34.97 | 65.03 | 3.77 |
| WP | 2.26±0.27 | 35.71 | 64.29 | 0.47 |
| HM1 | - | n.d. | n.d. | n.d. |
| HN2 | - | n.d. | n.d. | n.d. |
| HN | - | n.d. | n.d. | n.d. |
| OS1 | 1.21±0.47 | 31.57 | 68.43 | 1.25 |
| OS2 | 1.27±0.04 | 28.58 | 71.42 | 0.52 |
| OS3 | - | n.d. | n.d. | n.d. |
| PC | 3.89±0.32 | 30.69 | 69.31 | 0.21 |
| SF | - | n.d. | n.d. | n.d. |
| Ssp | - | n.d. | n.d. | n.d. |
| Usp | - | n.d. | n.d. | n.d. |

SD: standard deviation; n.d.: not detected; n.q.: not quantified.

The essential oil and the two isolated compounds (SPF-1 and SPF-2) were tested to verify their antimicrobial and antioxidant activities. No significant antimicrobial inhibition was observed to the microorganism in the concentration tested. The essential oil of *L. dendoidea* J. Agardh did not show good antioxidant activity (8.10% at 500 $\mu g.mL^{-1}$), however, the isolated compounds SPF-1 and SPF-2 showed higher potencies 27.5 and 30.3% at 500 $\mu g.mL^{-1}$, respectively when analyzed by the DPPH method, Figure 3(a). The antioxidant activity observed by the chemiluminescence method was 14.9, 34.6 and 20.8% at 500 $\mu g.mL^{-1}$ for the essential oil, SPF-1 and SPF-2, respectively, Figure 3(b).

Overall, DPPH assay was fairly consistent with the chemiluminescence assay. The small difference between the results could be consequence of the differential sample solubility in the reactions media (organic medium for DPPH assay and aqueous medium for chemiluminescence assay), difference in sensitivity among both methods (according to Hirayama et al., 1997, chemiluminescence method is more sensitive because of the high light emission produced by the $H_2O_2$/luminol/HRP system) and/or different mechanisms of radical scavenging.

## Conclusions

Both tricyclic sesquiterpenes (SPF-1 and SPF-2) have never been reported in Brazilian seaweed species. Although they were observed in variable quantities in different seaweed samples, they showed to be very common between distinct species. However, more systematic studies (considering environmental physicochemical parameters) should be done to allow a better comprehension of SPF-1 and SPF-2 algae synthesis and seasonal distribution.

Based on the results obtained, it can be inferred that these compounds do not act as taxonomic markers to the genus *Laurencia.*

It was also seen that the isolated compounds were not active against the bacteria and yeast strains tested up to 500 $\mu g.mL^{-1}$. On the other hand, these compounds showed some antioxidant properties.

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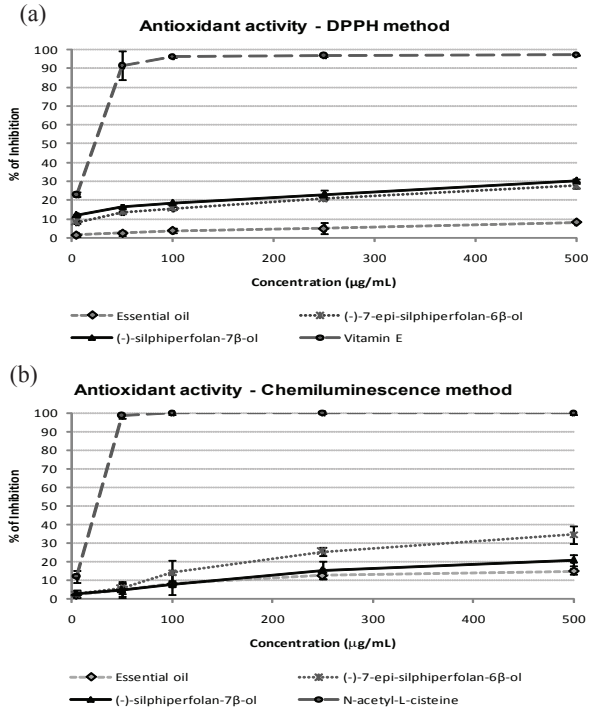


**Figure 3.** Comparative graphs of the antioxidant activity.

## References

Coll JC, Wright AD 1989. Tropical marine algae. III New sesquiterpenes from *Laurencia majuscula* (Rhodophyta, Rhodophyceae, Ceramiales, Rhodomelaceae). *Aust J Chem 42*: 1591-1603.

Davyt D, Fernandez R, Suescun L, Mombrú AW, Saldaña J, Domínguez LD, Fujii MT, Manta E 2006. Bisabolanes from the red alga *Laurencia scoparia. J Nat Prod 69*: 1113-1116.

Faulkner DJ 2000. Marine natural products. *Nat Prod Rep 17*: 7-55.

Fenical W, Norris JN 1975. Chemotaxonomy in marine algae: chemical separation of some *Laurencia* species (Rhodophyta) from the Gulf of California. *J Phycol 11*: 104-108.

Fink P 2007. Ecological functions of volatile organic compounds in aquatic systems. *Mar Freshw Behav Phy 40*: 155-168.

Gressler V, Colepicolo P, Pinto P 2009. Useful strategies for algal volatile analysis. *Curr Anal Chem* 5: 271-292.

Gressler V, Fujii MT, Martins AP, Colepicolo P, Pinto P 2011. Biochemical composition of two seaweed species grown on the Brazilian coast. *J Sci Food Agric,* http://dx.doi.org/10.1002/jsfa.4370.

Hirayama O, Takagi M, Hukumoto K, Katoh S 1997. Evaluation of antioxidant activity by chemiluminescence. *Anal Biochem 247*: 237-241.

Kamenarska Z, Ivanova A, Stancheva R, Stoyneva M, Stefanov K, Dimitrova-Konaklieva S, Popov, S 2006. Volatile compounds from some Black Sea red algae and their chemotaxonomic application. *Bot Mar 49*: 47-56.

Karabay-Yavasoglu NU, Sukatar A, Ozdemir G, Horzum Z 2007. Antimicrobial activity of volatile components and various extracts of the red alga *Jania rubens. Phytother Res 21*: 153-156.

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

Krol W, Czubaa Z, Schellara S, Paradowski Z, Shanitb J 1994. Structure-activity relationship in the ability of flavonols to inhibit chemiluminescence. *J Ethnopharmacol 4*1: 121-126.

National Committee for Clinical Laboratory Standards 1998. Reference method for broth dilution antifungal susceptibility testing of conidium-forming filamentous fungi. Proposed standard M38-P. National Committee for Clinical Laboratory Standards, Wayne, PA, USA.

National Committee for Clinical Laboratory Standards 2005. Antimicrobial Susceptibility Testing. Proposed standard M100-S15. National Committee for Clinical Laboratory Standards, Wayne, PA, USA.

Paul VJ, Hay ME, Duffy JE, Fenical W, Gustafson K 1988. Chemical defense in the seaweed *Ochtodes secundiramea* (Montagne) Howe (Rhodophyta): effects of its monoterpenoid components upon diverse coral-reef herbivores. *J Exp Mar Biol Ecol 114*: 249-260.

Pereira RC 2002. Ecologia Química Marinha. In: Pereira RC & Soares-Gomes A (org.) Biologia Marinha 1. ed. Engenho Novo: Interciência, p. 281-310.

Rashid MA, Gustafson KR, Cardellina JH, Boyd MR 1995. Brominated chamigrane sesquiterpenes produce a novel profile of differential cytotoxicity in the NCI *in vitro* screen. *Nat Prod Lett 6*: 255-259.

Wright AD, Coll JC, Price IR 1990. Tropical marine algae, VII The chemical composition of marine algae from north queensland waters. *J Nat Prod 33*: 845-861.

Yen G-C, Chen H-Y 1995. Antioxidant activity of various tea extracts in relation to their antimutagenicity. *J Agr Food Chem 43*: 27-32.

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