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Full Paper

Radical-scavenging Potential of Phenolic Compounds from Brazilian Lichens

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Abstract: Lichens produce a wide range of phenolic substances, mostly depsides and depsidones. As part of our ongoing study of lichens from the Cerrado biome in Mato Grosso do Sul state, the present article reports novel findings on the radical-scavenging activity of two depsides, five depsidones, usnic acid, and lichexanthone that were evaluated against 0.1 and 0.3 mM 2,2'-diphenyl-1-picrylhydrazyl radical concentrations. These substances were isolated from the lichens *Parmotrema tinctorum* (Nyl.) Hale, *Parmotrema dilatatum* (Vain.) Hale, *Pseudoparmelia sphaerospora* (Nyl.) Hale, *Parmotrema lichexanthonicum* Eliasaro & Adler, *Ramalina anceps* Nyl. *Usnea subcomosa* Vain. and *Usnea jamaicensis* Ach. Usnic acid (EC_{50} = 3.34 ± 1.44 and 5.97 ± 1.91 mM, respectively) and atranorin (2.48 ± 1.18 and 10.10 ± 1.18 mM, respectively) proved the most active unmodified compounds. Lecanoric and protocetraric acids exhibited significant EC_{50} differences between DPPH concentrations. Besides these, nine 9'-*O*-alkyl protocetraric acid derivatives were also evaluated. 9'-*O*-methyl protocetraric and 9'-*O*-iso-propyl protocetraric acids (with respective EC_{50} values of 1.74 ± 0.83 and 1.03 ± 1.0 mM, both against 0.1 mM DPPH) were the most active compounds evaluated. Except for 9'-*O*-methyl protocetraric acid, chain elongation correlated with increased scavenging activity in the linear series from 9'-*O*-ethyl to 9'-*O*-*n*-hexyl protocetraric acid.

Keywords: lichens; scavenging activity; phenolic compounds; DPPH concentration

1. INTRODUCTION

Phenolic compounds are largely distributed in nature, being overwhelmingly synthesized by algae, fungi, lichens, mosses, higher plants, and other organisms. Their large structural diversity provides them with a wide range of biological and/or pharmacological properties. In the lichens, usnic acid, atranorin and some pigments occur in the cortex while most of the phenolic compounds occur in the medullary layer. Some of these compounds play important roles, such as, regulatory effect of the biosynthetic pathways as responses of the lichens to their variable environments; others are photo-protective against UV radiation. Besides many compounds act in the defense against microorganisms, insects and molluscs, among others [1, 2].

Increasing evidence has been gathered of the involvement of oxidative stress induced by active oxygen and nitrogen species in several chronic human disorders, such as cancer and heart disease [3]. Numerous studies about the biological activities of phenols have revealed noteworthy antioxidative and free-radical-scavenging properties of these compounds and their strong inhibition of oxidative damage [4, 5].

Although many phenolic compounds from natural sources are potent antioxidants, a number of others exhibit lower activity. Structural modification and/or functionalization, however, allow the activity of several compounds to be improved. The activity of modified substances has been investigated to establish

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the relationships between structure and antioxidant activity [6–9].

Antioxidants can be natural or synthetic, and restrictions apply to the use of the latter because of their carcinogenicity [10, 11], a detrimental property that has stimulated the search for natural counterparts.

Lichens, symbiotic associations of an alga (the phycobiont) and a fungus (the mycobiont), can generate a wide range of phenolic substances, mostly depsides and depsidones. Investigations of the antioxidant activity of lichen compounds has typically been conducted with extracts [12–16], but pure substances isolated from lichens and their derivatives have also been employed [9, 12, 17–22]. As part of our study of lichens from the Cerrado biome in Mato Grosso do Sul state, Midwest Brazil, the present article reports novel findings on the radical-scavenging activity of two depsides (atranorin and lecanoric acid), five depsidones (protocetraric, hypostictic, norstictic, salazinic, and psoromic acids), usnic acid, lichexanthone, and derivatives of 9'-*O*-alkyl protocetraric acid.

2. MATERIAL AND METHODS

General procedures

NMR spectra were recorded on a Bruker PX 300 spectrometer and the substances were solubilized in $CDCl_3$ or DMSO-d_6. Solvent resonances were used as internal references. Mass spectra (EI, 70 eV) were obtained using a Shimadzu CGMS QP 2010 Plus gas chromatograph–mass spectrometer in direct injection mode, and melting points were recorded on a Uniscience do Brasil model 498 apparatus. Si-gel (Carlo Erba, 70-270 mesh, and Merck, 230-400 mesh) was employed for column chromatography.

Plant material

Parmotrema dilatatum (Vain.) Hale (CGMS 49840), *Parmotrema tinctorum* (Nyl.) Hale (CGMS 49842), *Pseudoparmelia sphaerospora* (Nyl.) Hale (CGMS 49837), and *Usnea subcomosa* Vain. (CGMS 49841) were collected near Piraputanga village in Aquidauana county, Mato Grosso do Sul state, Brazil (20°27′21.2″S, 55°29′00.9″W; alt. approx. 200 m; *Ramalina anceps* Nyl. (CGMS 49839), *Parmotrema lichexathonicum* Eliasaro & Adler (IbtSP 381512) and *Usnea jamaicensis* Ach. (CGMS 49838) were obtained from home decor stores. *Parmotrema dilatatum* and *P. tinctorum* were identified by Mariana Fleig, of the Universidade Federal do Rio Grande do Sul; *Usnea jamaicensis*, by Phillipe Clerc, of the Herbarium of Geneva, Switzerland. Identification of the remaining species was performed by M.P.M. and A.A.S., of the present study.

Extraction and isolation of compounds

Extractions of atranorin (**1**), protocetraric acid (**2**) (from *P. dilatatum*), lecanoric acid (**3**) (*P. tinctorum*), usnic acid (**4**) (from *U. subcomosa*), lichexanthone (**5**) salazinic acid (**6**) (both from *P. lichexanthonicum*), hypostictic acid (**7**) (from *P. sphaerospora*), norstictic acid (**8**) (from *R. anceps*), and psoromic acid (9) (from *U. jamaicensis*) were carried out according to Honda et al. [23] and Micheletti et al. [24]. Their structures were confirmed by ^{1}H, ^{13}C, DEPT 135°, gHMQC, and gHMBC spectra. Spectroscopic data of compounds **1–9** (Figures 1S–9S) were in accordance with the literature [24–29].

Derivatives

Protocetraric acid derivatives **10–18**: A mixture of protocetraric acid (200 mg, 0.53 mmol) and 50 mL of an alcohol (methanol, ethanol, *n*-propanol, *n*-butanol, *n*-hexanol, *iso*-propanol, *sec*-butanol, or *tert*-butanol) was heated in a reaction flask at 50-60 °C. The reaction mixtures were monitored by TLC. After solvent evaporation, the products of methanol (**10**), ethanol (**11**), *n*-propanol (**12**), and *iso*-propanol (**16**) were treated with a small volume of acetone in an ice bath and centrifuged for 10 min at 3000 rpm, while the reaction products of the *n*-butanol (**13**), *n*-pentanol (**14**), *n*-hexanol (**15**), *sec*-butanol (**17**), *tert*-butanol (**18**) were treated with hexane, and their respective mixtures were centrifuged for 10 min at 3000 rpm. The procedure was sustained until a pure product was obtained. The chemical shift attributions for ^{1}H and ^{13}C present in the chains inserted into the protocetraric acid molecule were compared with those described by Lopes et al. [9] and Micheletti et al. [24] (Figures 11S–18S). All compounds were 95-98% pure, as determined by TLC and NMR.

Evaluation of antioxidant activity by DPPH scavenging

The antioxidant activity of the phenolic compounds, measured as their ability to scavenge DPPH, was determined as per Taskova et al. [30], with modifications. Solutions of compounds (1) – (18) (1.0 mL) in DMSO at different concentrations were added to 2.0 mL of a DPPH solution in DMSO prepared daily and protected from light. Each mixture was incubated at room temperature in the dark for 30 min, followed by absorbance readings at 517 nm using a spectrophotometer. DPPH concentrations of 0.1 mM (4 mg%) and 0.3 mM (12 mg %) (67 and 200 μM in reaction medium, respectively) were employed. The DPPH scavenging effect (%) was calculated as follows:

$$\text{DPPH scavenging effect (\%)} = (A_{blank} - A_{sample})/A_{blank} \times 100$$

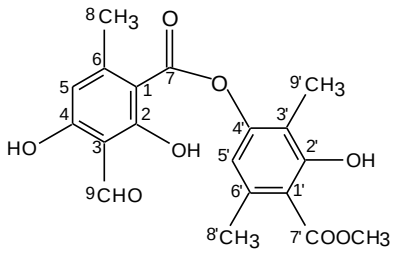
where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance of the test compound.

Compound concentrations capable of inhibiting DPPH activity by 50% (EC_{50}) were calculated from a graph plotting inhibition percentages against solution concentrations [31]. All assays were carried out in triplicate. Each phenolic compound was tested at six concentrations to investigate response linearity and establish EC_{50} values within a suitable linear range.

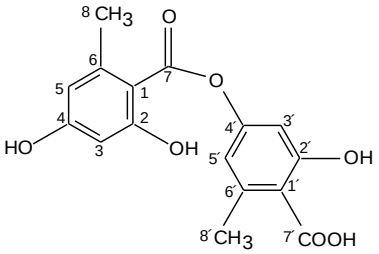
Testing DMSO against DPPH yielded no absorbance changes at 517 nm. Gallic acid was used as a positive control.

3. RESULTS AND DISCUSSION

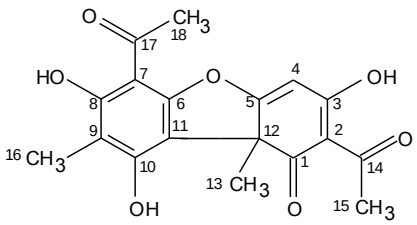
The depsides atranorin (**1**) and lecanoric acid (**3**), the depsidones protocetraric (**2**), salazinic (**6**), hypostictic (**7**) norstictic **(8)**, and psoromic (**9**) acids, the xanthone lichexanthone (**5**), and usnic acid (**4**) isolated from lichen species were evaluated for their free-radical-scavenging activity towards 2,2'-diphenyl-1-picrylhydrazyl (DPPH, a stable free radical) at 0.1 and 0.3 mM concentrations. Nine derivatives (10–18) of 9'-*O*-alkyl protocetraric acid were also evaluated (Figure 1).



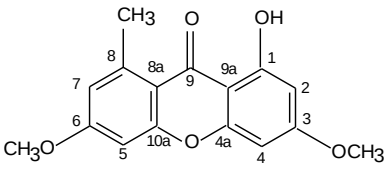
Atranorin (**1**)



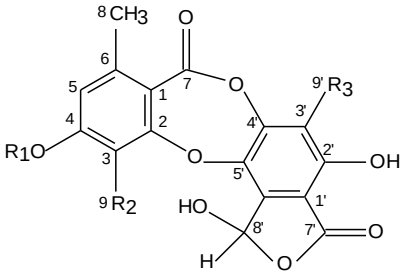
Lecanoric acid (**3**)



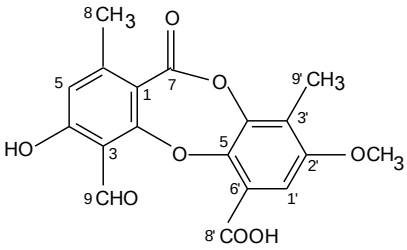
Usnic acid (**4**)



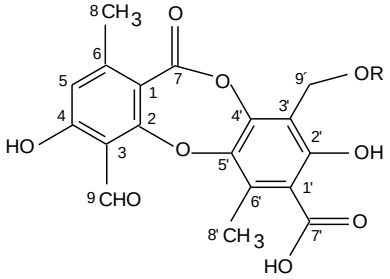
Lichexanthone (**5**)



$R_1 = H$; $R_2 = CHO$; $R_3 = CH_2OH$, salazinic acid (**6**)
$R_1 = CH_3$; $R_2 = CH_3$; $R_3 = CH_3$, hypostictic acid (**7**)
$R_1 = H$; $R_2 = CHO$; $R_3 = CH_3$, norstictic acid (**8**)



Psoromic acid (**9**)



R = H = Protocetraric acid (**2**); = CH_3 (**10**);
= CH_2CH_3 (**11**); = $CH_2CH_2CH_3$ (**12**);
= $CH_2(CH_2)_2CH_3$ (**13**); = $CH_2(CH_2)_3CH_3$ (**14**);
= $CH_2(CH_2)_4CH_3$ (**15**); = $CH(CH_3)_2$ (**16**);
= $CH(CH_3)CH_2CH_3$ (**17**); = $C(CH_3)_3$ (**18**)

Figure 1. Structures of the compounds isolated from lichens (**1**–**9**) and derivatives of protocetraric acid (**10**–**18**) evaluated against DPPH.

The compounds isolated are derived from orsellinic or β-methyl orsellinic acids. In addition to a phenolic OH group, the structure of those compounds may contain other substituents, such as -COOH, -CHO, $-CH_2OH$, $-CH_3$, and other alkyl groups. The phenolic OH group may be methylated. The hydrogen atom of this group can be donated to free radicals, and the antioxidant capacity of polyphenols is related to the effect of other substituents and their positions in the molecule [8, 32]. In compounds **1**–**9**, hydrogen bonds can be formed between the oxygen of a carbonyl and the hydrogen of a hydroxyl group in an *ortho* position. ^{1}H-NMR spectra of these compounds showed signals for an aromatic hydroxyl in the δ range of 10.0 to 13.4 ppm, while an OH group from usnic acid appears at δ 18.82 ppm (Table 1). These signals shifted further downfield, compared with that of OH in orcinol (near 8.0 ppm), indicating an intermolecular hydrogen bond [9, 33].

Table 1. Chemical shifts (δ) for OH groups in orcinol and in compounds **1**–**9**.

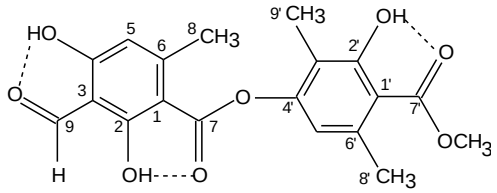
Compound	δ (ppm)
Orcinol	8.08
Atranorin (**1**)[a]	12.55 (C-2), 12.50 (C-4), 11.95 (C-2')
Protocetraric acid (**2**)[b]	11.94 (C-4)
Lecanoric acid (**3**)[b]	10.02 (C-4, C-4'), 10.48 (C-2, C-2').
Usnic acid (**4**)[a]	11.00 (C-10), 13.29 (C-8), 18.82 (C-3)
Lichexanthone (**5**)[a]	13.36 (C-1)
Salazinic acid (**6**)[b]	12.04 (C-4)
Hypostictic acid (**7**)[b]	10.0 (C-2')
Norstictic acid (**8**)[b]	10.23 (C-2'), 12.04 (C-4)
Psoromic acid (**9**)[b]	12.15 (C-4)

[a]$CDCl_3$, [b]DMSO-d_6.

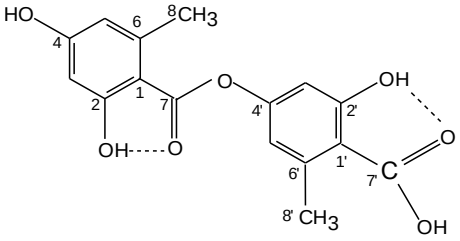
Of the compounds tested, atranorin (**1**), usnic acid (**4**), and lecanoric acid (**3**) contain three phenolic OH groups, while protocetraric (**2**), salazinic (**6**), and norstictic (**9**) acids contain two, and hypostictic acid (**7**) and lichexanthone (**5**) have one single OH phenolic group. Although lecanoric acid (**3**) has the same number of phenolic OH groups as atranorin (**1**) and usnic acid (**4**), it proved the least active (against 0.3 mM DPPH) of the compounds tested. Compounds **1**, **3**, and **4** have the same numbers of hydroxyl groups as gallic acid, but exhibited much lower antioxidant activity against DPPH—a feature possibly related to differences in the positions of OH groups and formation of hydrogen bonds between phenolic OH and carbonyl groups the aldehyde, carboxyl, or ketone groups present in *ortho* position in the structures of compounds **1**, **3**, and **4**. Hydrogen bonds occur in all substances tested (see Figure 2 for compounds **1**–**9**).

-COOH groups have electron-withdrawing properties detrimental to the H-donating ability of hydroxyl acids. In contrast, carboxylate groups ($-COO^-$), which are electron-donating, favor H-atom transfer and electron donation based on radical scavenging [34–36]. Depsides **1** and **3** have phenolic groups at C-2, C-4, and C-2'. C-2 is in *ortho* position to an ester group (C-7) in ring A and to an aldehyde at C-3 in the atranorin structure. Lecanoric acid (**3**) has one phenolic group each at C-2 and C-4. Depsidones **2**, **6**, **8**, and **9** have a phenolic group at C-4 *ortho* to an aldehyde (C-3). Except in psoromic acid, the phenolic group present at C-2' in all compounds evaluated is also *ortho* to a carboxyl, ester, or carbonyl of the lactol groups at C-1' and *ortho* to $-CH_3$ or $-CH_2OH$ at C-3'. Usnic acid (4) contains a $-CH_3$ group at C-9 in *ortho* position to OH at C-8 and C-10, while atranorin (**1**) has a $-CH_3$ group at C-3'. The same applies to hypostictic (**7**) and norstictic (**8**) acids. Salazinic (**6**) and protocetraric (**2**) acids have a $-CH_2OH$ group at

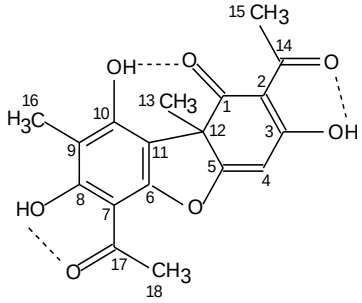
C-3'. For these compounds, interpreting the effect of substituent groups on scavenging ability is a complex task, given the number of substituents that simultaneously exert electron-withdrawing and electron-donating effects.



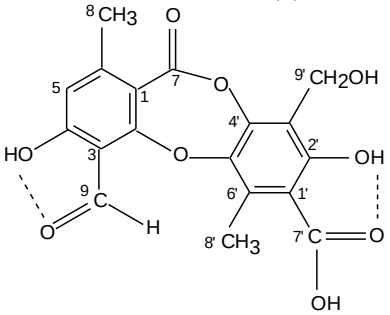
Atranorin (**1**)



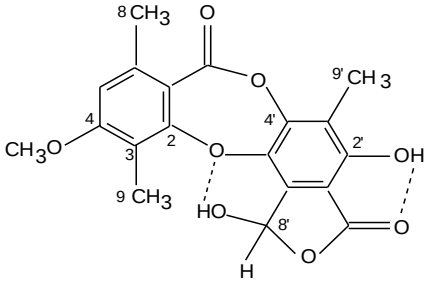
Lecanoric acid (**3**)



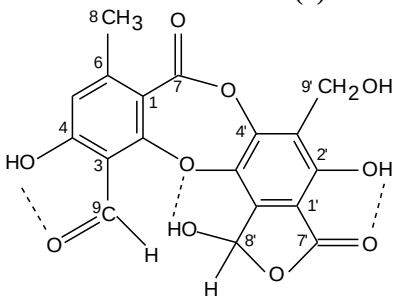
Usnic acid (**4**)



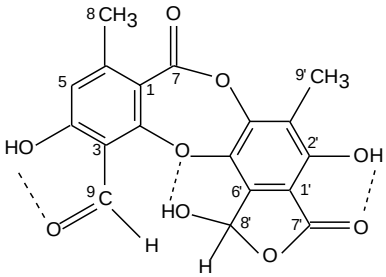
Protocetraric acid (**2**)



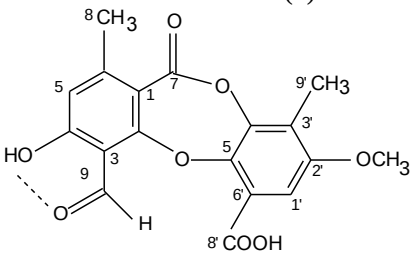
Hypostictic acid (**7**)



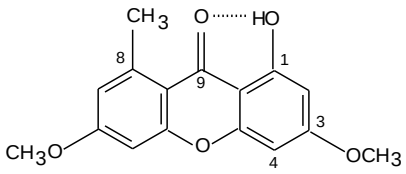
Salazinic acid (**6**)



Norstictic acid (**8**)



Psoromic acid (**9**)



Lichexanthone (5)

Figure 2. Structures of compounds **1-9**, depicting intramolecular hydrogen bridges.

Of natural substances **1–9**, usnic acid (**4**) and the depside atranorin (**1**) proved the most active, followed by the depsidones protocetraric (**2**), salazinic (**6**), hypostictic (**7**), and norstictic (**8**) acids, three of which **6**, **7** and **8** have a lactol ring. Protocetraric acid (**2**) exhibited higher activity than other depsidones tested against 0.1 mM DPPH, while the depside lecanoric acid (**3**) was the least active compound (against 0.3 mM DPPH) (Table 2).

Table 2. Antioxidant activity of compounds (1) – (9) against DPPH.

Compounds	*log *P*	EC_{50} (mM) against 0.1 mM DPPH (4 mg %)	EC_{50} (mM) against 0.3 mM DPPH (12 mg %)
Atranorin (**1**)	6.14 ± 0.49	2.48 ± 1.18	10.10 ± 1.18
Protocetraric acid (**2**)	2.83 ± 1.38	5.08 ± 2.25	38.06 ± 0.83
Lecanoric acid (**3**)	4.39 ± 0.42	7.48 ± 5.10	68.91 ± 0.98
Usnic acid (**4**)	1.27 ± 0.75	3.34 ± 1.44	5.97 ±1.91
Lichexanthone (**5**)	3.06 ± 0.85	13.85 ± 1.79	28.93 ± 0.79
Salazinic acid (**6**)	0.69 ± 1.48	10.66 ± 1.33	28.92 ± 0.59
Hypostictic acid (**7**)	2.63 ± 1.43	13.50 ± 1.4	19.10 ± 2.09
Norstictic acid (**8**)	2.33 ± 1.48	28.3 ± 0.99	21.68 ± 0.38
Psoromic acid (**9**)	2.98 ± 1.36	17.36 ± 2.97	n.d.
Gallic acid	0.91 ± 0.33	$(23.9 \pm 0.99) \times 10^{-3}$	0.030 ± 0.0014

n.d. = not determined.
EC_{50} is the concentration of inhibitor that reduces by 50% the concentration of DPPH in the reaction medium.
*log *P* values were calculated using ACDLABS 12.0 software.

Tested against both DPPH concentrations, compounds **1–9** yielded different EC_{50} values. Except for norstictic acid (**8**), all activity levels were higher against 0.1 mM DPPH (66.7 μM in reactional medium) than against 0.3 mM DPPH (200 μM in reactional medium).

In addition to structural features, the effect of solvent and DPPH concentration should be taken into account. Hydrogen transfer depends on the medium employed, and its rate is regulated by two factors: strength of the phenolic O–H bond and magnitude of the kinetic solvent effect, which depends on the intensity of interactions taking place between solvent and the hydrogen atom being donated to the radical. The stronger these interactions and their number, the greater the number of solvent molecules to be displaced before hydrogen can be abstracted by the free radical. Solvent interaction with hydroxyls reduces the reaction rate, a decrease reflected in lower antioxidant activity. In the present investigation, compounds **1–18** exhibited higher solubility in DMSO than in other solvents previously tested. This solvent was therefore selected for the study of scavenging activity using two DPPH concentrations [37–39].

The amount of unreacted DPPH had a marked influence on EC_{50} values. Figure 3 depicts the correlations between EC_{50} values of compounds **1–9** and log *P* values for both DPPH concentrations. The most significant differences in EC_{50} as a function of DPPH concentration were found for lecanoric (**3**), protocetraric (**2**), and salazinic (**6**) acids and lichexanthone (**5**).

Of the 9'-*O*-alkyl protocetraric acid derivatives (**10–18**), only 9'-*O*-methyl protocetraric (**10**) and 9'-*O*-*iso*-propyl protocetraric (**16**) acids were more active than any other compound tested. The 9'-*O*-ethyl (**11**), *n*-propyl (**12**), *n*-butyl (**13**), *n*-pentyl (**14**), *sec*-butyl (**17**), and *tert*-butyl (**18**) derivatives of protocetraric acid proved less active that protocetraric acid (**2**). However, the linear series from 9'-*O*-ethyl protocetraric acid (**11**) to 9'-*O*-*n*-hexyl protocetraric acid (**15**) exhibited growing activity with chain elongation (Table 3). In terms of EC_{50} and log *P* values, this linear correlation can be observed in Figure 4.

Figure 4 also depicts linear correlation between EC_{50} and log *P* values for compounds **11–15**. 9'-*O*-methyl-protocetraric (**10**), 9'-*O*-*iso*-propyl-protocetraric (**16**), 9'-*O*-*sec*-butyl-protocetraric (**17**), and 9'-*O*-*tert*-butyl-protocetraric (**18**) acids behave as outliers relative to compounds **11–15**, as regards linear carbon chain elongation.

Studies based on DPPH scavenging have long been conducted, yet the approach continues to yield new findings, as the remarkable stability of this radical makes it suitable for the investigation of extracts or pure substances with potential antioxidant activity. The method has been employed with numerous variations, including changes in reaction conditions, as recently described by Fadda et al. [40] who evaluated the reaction kinetics of DPPH using lemon juice, pomegranate juice, green tea infusion, and rosemary essential oil.

Among other investigators of the antioxidant properties of lichen-derived compounds, Jayaprakasha et al. [12] employed a β-carotene linoleate model system to demonstrate the moderate antioxidant activity of methyl orsellinate, orsellinic acid,

atranorin, and lecanoric acid isolated from *Parmotrema stuppeum* (Nyl.) Hale.

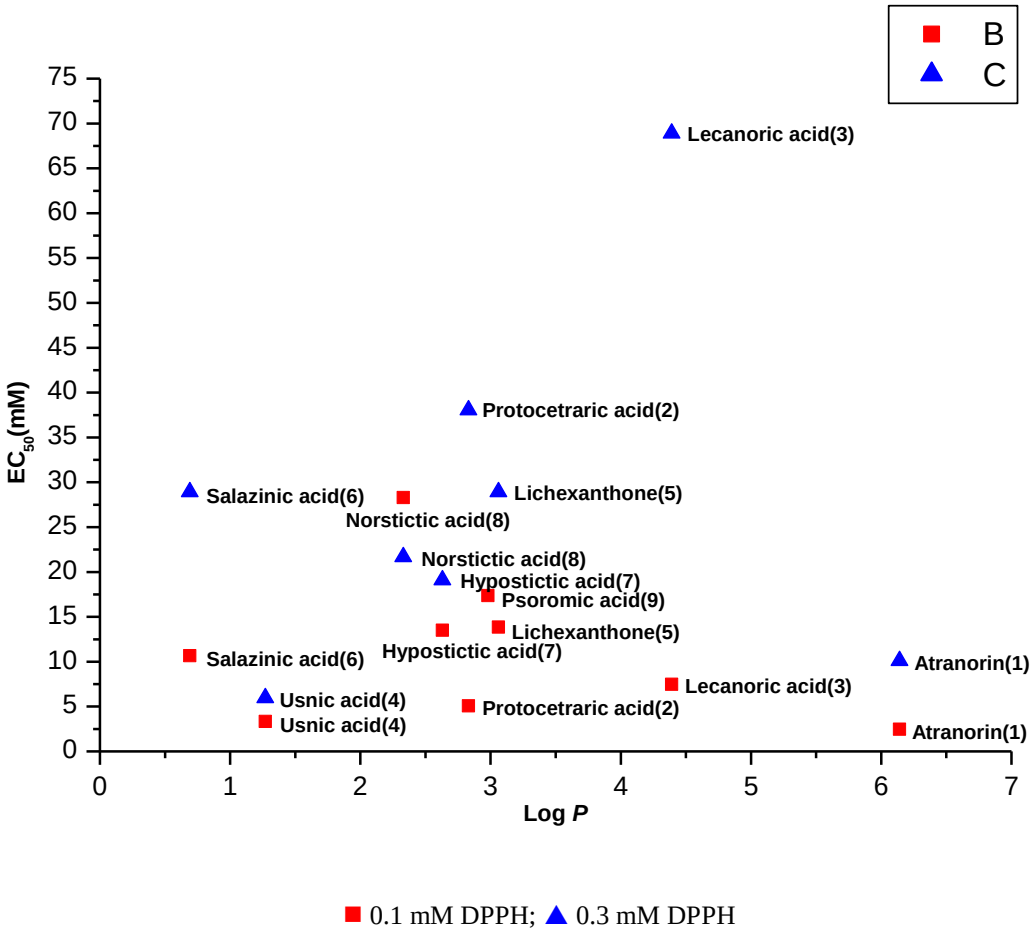


Figure 3. EC_{50} values for compounds **1–9** tested against DPPH in a DMSO solution. Values of log *P* were calculated using ACD-Log*P* software (95% confidence interval).

Table 3. EC_{50} and log *P* values of compounds **10–18** tested against 0.1 mM DPPH (4 mg %).

Compounds	***log *P***	**EC_{50} (mM)**
9'-*O*-methyl protocetraric acid (**10**)	3.75 ± 1.39	1.74 ± 0.83
9'-*O*-ethyl protocetraric acid (**11**)	4.28 ± 1.39	17.74 ± 1.64
9'-*O*-*n*-propyl protocetraric acid (**12**)	4.81 ± 1.39	12.47 ± 0.69
9'-*O*-*n*-butyl protocetraric acid (**13**)	5.34 ± 1.39	10.43 ± 2.12
9'-*O*-*n*-pentyl protocetraric acid (**14**)	5.88 ± 1.39	7.89 ± 0.93
9'-*O*-*n*-hexyl protocetraric acid (**15**)	6.41 ± 1.39	4.14 ± 2.26
9'-*O*-*iso*-propyl protocetraric acid (**16**)	4.63 ± 1.39	1.03 ± 1.0
9'-*O*-*sec*-butyl protocetraric acid (**17**)	5.16 ± 1.39	18.34 ± 1.93
9'-*O*-*tert*-butyl protocetraric acid (**18**)	4.98 ± 1.39	22.16 ± 0.99
Gallic acid	0.91 ± 0.33	(23.9 ± 0.99) × 10^{-3}

*Values of log *P* were calculated using ACDLABS 12.0 software.

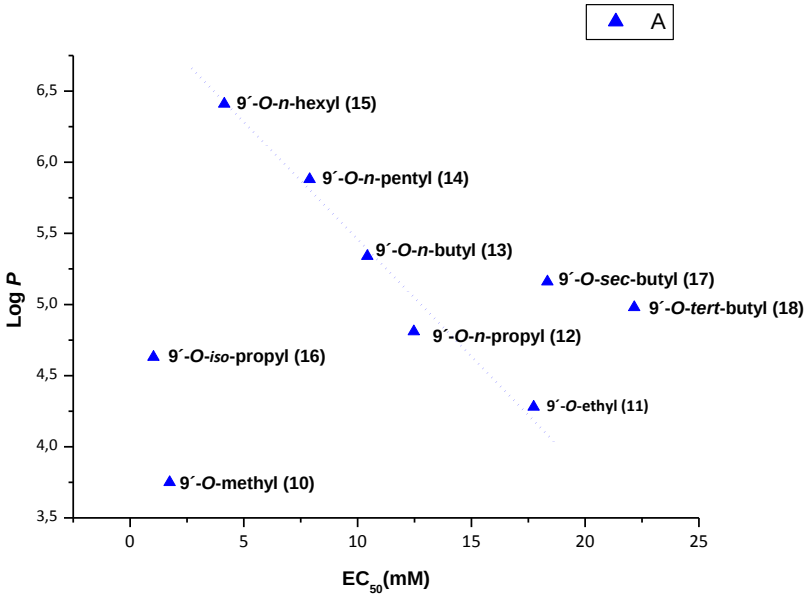


Figure 4. Correlation between EC_{50} and log *P* for protocetraric acid derivatives **10**–**18**.

Usnic acid, methyl β-orcinol carboxylate, ethyl hematommate, atranorin, diffractaic acid, gyrophoric acid, and (+) protolichesterinic acid isolated from *Parmelia nepalensis* Tayl. were evaluated for their ability to inhibit non-enzymatic lipid peroxidation in model membranes and to react with DPPH, but none of the compounds proved active in either test [17]. Papadapoulon et al. [21] evaluated the radical-scavenging activity of compounds isolated from *Hypotrachyna revoluta* (Flörke) Hale using luminol chemiluminescence, with superior results for 8'-methyl menegazziaic acid, atranorin, and deoxystictic acid. From *Usnea articulata* (L.) Hoffm., depsidones pertaining to the stictic acid chemosyndrome were isolated (including stictic, norstictic, peristictic, cryptostictic, menegazziaic, constictic, and 3-*O*-methyl-consalazinic acids), in addition to two new depsidones (one being the open-ring form of stictic acid and the other structurally very similar to this acid, but exhibiting a CH_2OH group at C-3 and lacking the OH group at C-8'). In addition to these compounds, fumarprotocetraric, barbatic, and usnic acids and atranorin, methyl β-orcinol carboxylate, and ergosterol peroxide were isolated. Dévéhat et al. [20] found two new depsidones and fumarprotocetraric acid to inhibit 3000 μM DPPH by 30%, 18%, and 20%, respectively. Luo et al. [41] reported on the DPPH scavenging activity of sekikaic and homosekikaic acids (IC_{50} of 0.082 and 0.276 mg/mL, respectively).

Although the antioxidant properties of atranorin (1), lecanoric (3), and usnic (4) acids have been well established differences in methods and conditions may preclude direct comparisons [12, 17, 19–21]. With the present investigation, the antioxidant activity of protocetraric acid derivatives (10) – (18) against DPPH is being reported for first time.

4. CONCLUSION

In conclusion, in this work we have studied the radical-scavenging activity of phenolic compounds from lichens with the aim of comparing their activities on two different DPPH concentrations. Atranorin and usnic acid were the compounds most active in both DPPH concentrations. Except norstictic acid, the activities of the compounds **1**–**7** were higher against 0.1 mM DPPH. The most significant differences in EC_{50} as a function of DPPH concentration were found for lecanoric, protocetraric, salazinic acids and lichexanthone. The 9'-*O*-methyl protocetraric and 9'-*O*-*iso*-propyl protocetraric acids were more active than any other compound here tested. The linear series from 9'-*O*-ethyl protocetraric acid to 9'-*O*-*n*-hexyl protocetraric acid exhibited growing activity with chain elongation.

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Supplementary data

Supplementary data associated with this article can be found at http://www.orbital.ufms.br/index.php/Chemistry/rt/suppFiles/721/0