© 2014 Journal of Pharmacy & Pharmacognosy Research, 2 (5), 138-147
ISSN 0719-4250
http://jppres.com/jppres

Original Article | Artículo Original

Antioxidant activity, total phenols and flavonoids of lichens from Venezuelan Andes

[*Actividad antioxidante, fenoles y flavonoides totales en líquenes de los Andes venezolanos*]

Claudia M. Plaza[a,b,e *], Lorena E. Díaz de Torres[c,e], Robert K. Lücking[f], Marietta Vizcaya[a,d,e], Gerardo E. Medina[a,b,e]

[a]Research Institute; [b]Biotechnology Section; [c]Department of Pharmacognosy and Organic Medications; [d]Organic Biomolecular Research Group; [e]Facultad de Farmacia y Bioanálisis. Universidad de los Andes (ULA). Edif. Carlos Edmundo, 3er Piso, Instituto de Investigaciones. Sección de Biotecnología. Mérida, Venezuela.
[f]Department of Botany, The Field Museum, 1400 South Lake Shore Drive, Chicago, IL 60605-2496, USA
[*]E-mail: claudiampz@gmail.com

Abstract

Context: The biological potential of lichens has been documented through their use in traditional medicine. Secondary lichen metabolites exert a wide variety of biological actions, including their use as antioxidants.

Aims: To evaluate the antioxidant activity, total phenol content, and flavonoids of four lichen fungal taxa collected in Mérida (Venezuela), and statistically evaluate the correlation between the antioxidant activity and the amount of phenols and flavonoids in the samples.

Methods: Extracts were prepared with water, ethanol and dichloromethane from *Cladonia* aff. *rappii, Cora* aff. *glabrata, Peltigera laciniata* and *Thamnolia vermicularis.* The antioxidant capacity assessment was determined using DPPH• radical method and reducing power, with ascorbic acid as control. Total phenols were determined by means of the Folin-Ciocalteu method with gallic acid. Total flavonoids were estimated according to the modified Dowd method, using quercetin as standard.

Results: The ethanolic extracts of the tested lichens showed the highest scavenging activity and reducing power compared to water and dichloromethane extracts at 4 mg/mL. The highest antiradical power value was found in ethanolic extract of *Peltigera laciniata* (2.28 mL/mg) and the lowest in dichloromethane extract of *Cora* aff. *glabrata* (0.30 mL/mg). The correlation between antioxidant activity and total phenolic content was moderate. The flavonoids content of ethanolic extracts was highly significant but negative ($p < 0.05$). There was good correlation in dichloromethane extracts. The ethanolic extract of *P. laciniata* exhibited the highest antiradical activity despite showing the lowest flavonoid content.

Conclusions: The ethanolic extracts of lichens tested showed to have the higher antioxidant activity and may be used as natural sources of new antioxidants.

Keywords: Antioxidan; *Cladonia*; *Cora*; *Peltigera*; *Thamnolia*; *Venezuelan lichens*.

Resumen

Contexto: El potencial biológico de los líquenes se ha demostrado a través de su uso en la medicina tradicional. Los metabolitos secundarios liquénicos ejercen una amplia variedad de acciones biológicas incluyendo su uso como antioxidantes.

Objetivos: Evaluar la actividad antioxidante, el contenido de fenoles y flavonoides totales de cuatro líquenes colectados en Mérida (Venezuela) y evaluar estadísticamente la correlación entre la actividad antioxidante y la cantidad de fenoles y flavonoides en las muestras.

Métodos: Se prepararon extractos con agua, etanol y diclorometano de *Cladonia* aff. *rappii*, *Cora* aff. *glabrata*, *Peltigera laciniata* y *Thamnolia vermicularis*. La capacidad antioxidante se determinó mediante los métodos del radical DPPH• y poder reductor con acido ascórbico como control. Los fenoles se determinaron por el método de Folin-Ciocalteu con ácido gálico. Los flavonoides se estimaron por el método modificado de Dowd, usando quercetina como estándar.

Resultados: Los extractos etanólicos de los líquenes, mostraron la mayor actividad comparada con los extractos acuosos y diclorometánicos a 4 mg/mL. El valor más alto del poder antiradical fue para el extracto etanólico de *Peltigera laciniata* (2.28 mL/mg) y el más bajo para el extracto diclorometánico de *Cora* aff. *glabrata* (0.30 mL/mg). La correlación entre la actividad antioxidante y el contenido fenólico fue moderada. El contenido de flavonoides de los extractos etanólicos fue altamente significativo pero negativo ($p < 0,05$). Hubo buena correlación en los extractos diclorometánicos. El extracto etanólico de *Peltigera laciniata* exhibió la mayor actividad anti-radical a pesar de mostrar el contenido de flavonoides más bajo.

Conclusiones: Los extractos etanólicos de los líquenes ensayados mostraron tener la mayor actividad antioxidante y pudieran ser utilizados como fuentes naturales de nuevos antioxidantes.

Palabras Clave: Antioxidante; *Cora*; *Cladonia*; líquenes venezolanos; *Peltigera*; *Thamnolia*.

ARTICLE INFO

Received | Recibido: August 12, 2014.
Received in revised form | Recibido en forma corregida: September 29, 2014.
Accepted | Aceptado: October 09, 2014.
Available Online | Publicado en Línea: October 13, 2014.
Declaration of interests | Declaración de Intereses: The authors declare no conflict of interest.
Funding | Financiación: The authors confirm that the project have not funding or grants.

INTRODUCTION

The use of oxygen as part of the process for generating metabolic energy produces reactive oxygen species (ROS), as a product of normal cell metabolism (Raha and Robinson, 2000). These include hydrogen peroxide (H_2O_2) and free radicals such as superoxide anion (O_2^-) and hydroxyl radical (HO•) (Sharma and Kalikotay, 2012). Though oxygen is essential for aerobic forms of life, oxygen metabolites are highly toxic, when they are present excessively in the human body (Kumar et al., 2010). They can attack biological molecules leading to the development of degenerative diseases such as cardiovascular diseases, atherosclerosis, chronic inflammation, premature aging, and cancer (Gutteridge, 1993; Knight, 1995).

Antioxidants are compounds that can inhibit or delay the oxidation of an oxidizable substrate in a chain reaction. Synthetic antioxidants are widely used, but their use is being restricted nowadays because of their toxic and carcinogenic side effects (Verma et al., 2008). For this reason, currently there is a growing interest toward natural antioxidants from herbal resources, able to protect organisms from damage induced by oxidative stress.

Lichens are a symbiotic association between a fungus (mycobiont) and an algal partner, namely a green alga or a cyanobacterium (Kekuda et al., 2011). They produce unique secondary metabolites and exert a wide variety of biological actions including antibiotic, antimycobacterial, antiviral, antiinflammatory, analgesic, antipyretic, antiproliferative and cytotoxic effects (Verma et al., 2008; Manojlovi et al., 2010; Esteban, 2012).

The biological potential of lichens has been proven through their use in traditional medicine (Esteban, 2012). For example, species of the genus *Cladonia* called "reindeer lichens" have been used in teas to treat colds, arthritis, fevers and other problems (Pérez-Llano, 1944). The genus *Peltigera* was used against rabies and hydrophobia (Esteban, 2012). *Thamnolia vermicularis* was employed as anthelmintic and in medicated teas in traditional Chinese medicine (Upreti et al., 2005), and species of *Dictyonema* by the Waorani Indians in Amazonian Ecuador as hallucinogen (Davis and Yost, 1983). *Dictyonema* and its relatives, including the genus *Cora*, are among the few genera of lichenized basidiomycetes (Lücking, 2008; Dal-Forno et al., 2013; Lücking et al., 2013; 2014), while all other lichens mentioned here are formed by ascomycetes.

Just as there are many properties in lichens, their potential as resources of natural antioxidants has been investigated by some researchers (Odabasoglu et al., 2005; Behera et al., 2005; Gulluce et al., 2006; Verma et al., 2008). All of these studies show that lichens and lichen substances might be novel sources of natural antioxidants.

Venezuela is one of the areas richest and most diverse in lichens in the world (Marcano, 1994; Lücking et al., 2009), with 1320 species reported by Feuerer (2008) and probably close to 4000 expected (Lücking et al., 2009). Although, despite the potential importance of lichens in medical, environmental and chemical applications, the Venezuelan lichen biota is poorly known (Hernández, 2010). The aim of this study was to evaluate the antioxidant activity, the total phenol content, and the flavonoid content of four lichen fungal taxa in the genera *Cladonia*, *Peltigera*, *Thamnolia* and *Cora* collected in Mérida (Venezuela) and statistically evaluates the correlation between total phenolic and flavonoids content and the antioxidant activity of studied lichen extracts.

MATERIALS AND METHODS

Collection and identification of lichens samples

Lichen samples of *Cladonia* aff. *rappii* A. Evans (Cladoniaceae), *Cora* aff. *glabrata* (Spreng.) (Hygrophoraceae), *Peltigera laciniata* G. Merr. Gyeln. (Peltigeraceae) and *Thamnolia vermicularis* Sw. Schaer (Icmadophilaceae) were collected in February 2013 from two different locations between 2 395 and 3 999 m altitude in the Mérida state, Venezuela. Initial determination of the lichen samples was done using several identification keys, e.g., López (1986), Nash et al. (2002; 2004) and Sipman (2005; Sipman et al., 2008). Selected samples were subsequently subjected to molecular phylogenetic analysis, which revealed that

the material commonly identified as *Cladonia rappii* and *Cora glabrata* represents undescribed species (no published data). Voucher samples were deposited in the herbarium MERF of the Facultad de Farmacia y Bioanálisis of the Universidad de los Andes and in the Field Museum (F), with the following numbers: C2, C4, C11 and C17.

Preparation of lichens extracts

The lichen material was air-dried at room temperature for one week, after which it was ground to an uniform powder. The extracts (in water, ethanol and dichloromethane) were prepared by soaking 10 g of material of each species separately with 250 mL of each solvent at room temperature. Aqueous extracts were obtained left overnight, ethanol and dichloromethane extracts for seven days. All extracts were filtered using Whatman No.1 filter paper, and ethanol and dichloromethane extracts were concentrated by evaporating the solvent at room temperature.

Reagents and standards

2,2-Diphenyl-1-picrylhydrazyl (DPPH•), potassium ferrocyanide [$K_3Fe(CN)_6$], trichloroacetic acid, ferric chloride ($FeCl_3$), quercetin, gallic acid and Folin-Ciocalteus's phenol reagent were obtained of Sigma-Aldrich (USA); phosphate buffer (dibasic sodium phosphate anhydrous and monobasic sodium phosphate dihydrate), sodium carbonate, ascorbic acid, and aluminum chloride ($AlCl_3$) were obtained from Merck (Germany). All the chemicals used including the solvents were of analytical grade.

DPPH• radical scavenging assay

The effect of the extracts on DPPH• radical was estimated using the method described by Díaz et al. (2011). A solution of DPPH• (6 x 10^{-2} mM) in methanol was prepared, and 2.8 mL of this solution was mixed with 0.2 mL of each extract in methanol at 4 mg/mL. The reaction mixture left in the dark at room temperature for 30 min. The absorbance of the mixture was measured with a spectrophotometer (Spectronic GENESYS™ 10 Bio) at 517 nm. A solution of 2.8 mL of DPPH and 0.2 mL of methanol was used as control. Ascorbic acid was used as a reference antioxidant for this test at 0.176 mg/mL. The inhibition percentage (% INH) of the DPPH• free radical was calculated by the following equation: % INH = [(Abs DPPH• – Abs sample/ Abs DPPH•) x 100], where Abs DPPH• is the absorbance of DPPH• radical + methanol and Abs sample is the absorbance of DPPH• radical + sample extract /or ascorbic acid.

Determination of inverse of effective concentration (ARP)

Samples that showed a greater %INH than 50% were evaluated at different concentrations (0.1, 0.25, 0.5, 1, 2 and 4 mg/mL); the concentration required to obtain 50% of the maximum capacity to capture free radicals (effective concentration, EC_{50}) was then calculated by linear regression. For reasons of clarity, results are expressed in terms of $1/EC_{50}$ or antiradical power ARP = ($1/EC_{50}$); the larger the ARP, the most efficient is the antioxidant. The antiradical activity was defined as the amount of antioxidant per microlitre of extract necessary to obtain an antioxidant activity of 50% (Goupy et al., 1999).

Reducing power

The reducing power of extracts was determined by the method described by Diaz et al. (2011). An aliquot of 200 µL of extracts (4 mg/mL) in ethanol was mixed with 400 µL of phosphate buffer (0.2 M at 6.6 pH) and 400 µL of potassium ferricyanide [10g/L (1%)]. The mixtures were incubated at 50ºC for 30 min before 400 µL of trichloroacetic acid (10%) was added and the mixture centrifuged at 3000 rpm for 10 minutes. Finally, 400 µL of the supernatant solution was mixed with 400 µL distilled water and 100 µL ferric chloride (0.1%). The absorbance of the solution was measured at 700 nm in a spectrophotometer (Spectronic GENESYS™ 10 Bio). Blank was prepared with all the reaction agents without extract. The increase in absorbance of the mixture indicated an increase in the reducing power. Ascorbic acid was used as a positive control at 0.176 mg/mL.

Determination of total phenolic content

The total phenolic content of each extract was determined by the Folin-Ciocalteu method as described in Lamien-Meda et al. (2008). An aliquot of 50 µL of each extract at 0.5 mg/mL in methanol was mixed with 450 µL of water and 250 µL of Folin Ciocalteu reagent (0.2 N in water). This mixture was allowed to stand at room temperature for 5 min and then sodium carbonate solution (7.5% in water, 1250 µL) was added. After 2 h of incubation, the absorbances were measured with a spectrophotometer (Spectronic GENESYS™ 10 Bio) at 760 nm against a blank (water + methanol). A calibration curve was plotted using gallic acid as a standard phenolic compound (0 - 32 µg/mL) in intervals of 4 µg/mL. The results were expressed as µg of gallic acid equivalents (GAE)/mg dry weight (dw) extract.

Determination of total flavonoid content

Total flavonoid content was estimated according to the modified Dowd method described by Lamien-Meda et al. (2008). A methanolic solution (1 mL) of each extract at 0.5 mg/mL was mixed with a solution (1 mL) of $AlCl_3$ in methanol (2%). The absorbance was read at 415 nm after 10 min against a blank sample consisting of methanol – extract (1:1) without $AlCl_3$. A calibration curve was plotted using quercetin as reference flavonoid (0 - 32 µg/mL) in intervals of 4 µg/mL. The results were expressed as µg of quercetin equivalents (QE)/mg dw extract.

Statistical analysis

Statistical analyses were performed with Excel (Microsoft, Redmond, WA, USA), SPSS (SPSS Inc., Chicago, IL, USA), and Statistica 6.0™ (StatSoft, Tulsa, OK, USA). In order to determine the statistical significance of differences in antioxidant activity, Student's t-test for comparison between two means and a one-way analysis of variance (ANOVA) for comparison of more than two means, as well as main effects ANOVA to analyse the simultaneous effects of two independent variables. Pearson's bivariate correlation test was applied to calculate linear correlation coefficients (r) between the total phenolic and flavonoid content of each extract and antioxidant activity in terms of ARP. All results were expressed as mean and standard deviation (SD) values of three parallel measurements.

RESULTS

DPPH• radical scavenging assay

The DPPH• radical scavenging assay of the studied lichen extracts was expressed as percent of inhibition (%INH); (Fig. 1). Scavenging activity of lichen extracts ranged between 5.11% and 95.65%. Activity was significantly different ($p<0.05$) between extracts or lichen species.

Antiradical power as inverse of effective concentration

The antioxidant activity was expressed as antiradical power (ARP) (Table 1) in samples with a % INH higher at 50% (Fig. 1). The results of ARP obtained from linear regression showed a high correlation ($r^2 \geq 0.98$), where highest values of ARP indicate highest antioxidant power. Measured values of ARP varied from 0.30 to 2.72 mL/mg (Table 1).

Reducing power capacity

The results of the reducing power assay of lichen extracts are shown in Fig. 2. Measured values of absorbance varied from 0.11 to 1.22. The experiment of the reduction power showed that the highest reduction power had been in ethanolic extracts of *Peltigera laciniata* and *Thamnolia vermicularis* and dichloromethane extract of *Thamnolia vermicularis*. The reducing power is increased if absorbance of the reaction mixture increased (Kosanić and Ranković, 2011a).

Correlation between antioxidant activity and their total phenols and flavonoids content

The amount of total phenols content in extracts was determined according to the equation obtained from the standard gallic acid graph ($y = 0.0243 x + 0.0064$, $r^2 = 0.9988$) and varied from 4.85 to 23.97 µg of GAE/mg dw extract (Table 1).

Table 1. Total phenol and flavonoid contents and antiradical power (ARP) of lichen extracts.

Lichens	Extract solvent	ARP† (mL/mg dw extract)	Total phenols (µg GAE/mg dw extract)	Total flavonoids (µg QE/mg dw extract)
Cladonia aff. *rappii*	Water	nt	nt	nt
	Ethanol	0.40 ± 0.00	11.11 ± 0.15*	20.77 ± 0.04
	Dichloromethane	nt	nt	nt
Cora aff. *glabrata*	Water	nt	nt	nt
	Ethanol	0.33 ± 0.00	7.29 ± 0.20	19.42 ± 0.07
	Dichloromethane	0.30 ± 0.00	4.85 ± 0.10	16.04 ± 0.04
Peltigera laciniata	Water	nt	nt	nt
	Ethanol	2.72 ± 0.01	12.86 ± 0.14*	9.73 ± 0.07**
	Dichloromethane	nt	nt	nt
Thamnolia vermicularis	Water	nt	nt	nt
	Ethanol	0.95 ± 0.01	12.48 ± 0.06*	12.37 ± 0.07
	Dichloromethane	1.88 ± 0.01	23.97 ± 0.16	37.07 ± 0.08**
Ascorbic acid		28.32 ± 0.23	nt	nt

GAE: Gallic acid equivalents; QE: Quercetin equivalents; nt: not tested; dw: dry weight. †Antioxidant activity in terms of ARP = $1/EC_{50}$. *p <0.05 represents the statistical difference between total phenols *vs* ARP. **p <0.05 represents the statistical difference between total flavonoids *vs* ARP. Data represented as mean ± SD of three independent readings.

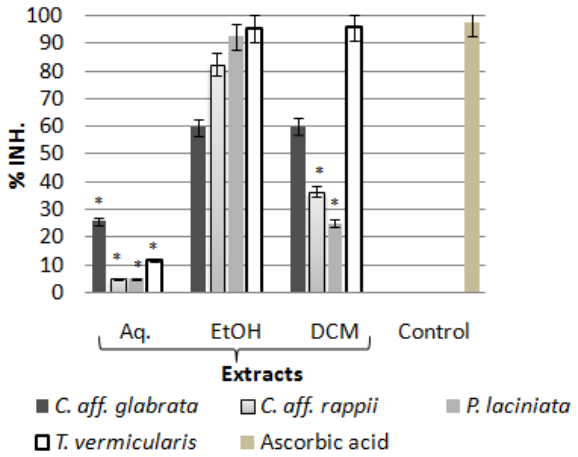

Figure 1. Evaluation of % inhibition of the aqueous, ethanolic and dichloromethane extracts of lichens studied at 4 mg/mL and ascorbic acid as control. Each column represents a lichen: *Cora* aff. *glabrata*; *Cladonia* aff. *rappii*; *Peltigera lacinita* and *Thamnolia vermicularis*, according to the solvent used. Aq. (aqueous extracts): 25.97, 5.22, 5.11, and 11.86%; EtOH (ethanolic extracts): 59.64, 82.31, 92.59, and 95.23%; DCM (dichloromethane extracts): 60.06, 36.60, 25.17, and 95.65%; Control: 97.72%. *p<0.05 represents the statistical difference between Aq. and DCM extracts vs. EtOH and DCM extracts of *T. vermicularis*.

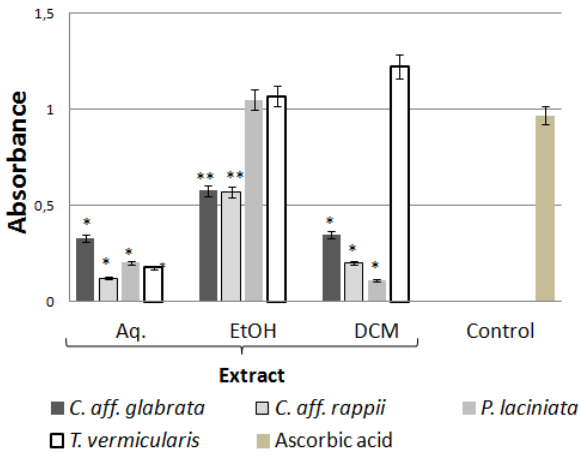

Figure 2. Evaluation of absorbance of aqueous, ethanolic and dichloromethane extracts of lichens at 4 mg/mL and ascorbic acid as control. The value of the absorbance reflects the reducing power of each extract. Each column represents a lichen: *Cora* aff. *glabrata*; *Cladonia* aff. *rappii*; *Peltigera lacinita* and *Thamnolia vermicularis*, according to the solvent used. Aq. (aqueous extracts): 0.12, 0.33, 0.20, and 0.18; EtOH (ethanolic extracts): 0.57, 0.58, 1.05, and 1.07; DCM (dichloromethane extracts): 0.20, 0.35, 0.11, and 1.22; Control: 0.97. *p<0.05 represents the statistical difference between Aq. and DCM extracts vs. EtOH extracts and DCM extract of *T. vermicularis* and **p<0.05 the statistical difference between EtOH extracts whit respect to DCM extracts.

The highest level of phenolic compounds was detected in dichloromethane extracts of *Thamnolia vermicularis* at 23.97 ± 0.16 µg of GAE/mg dw extract, whereas dichloromethane extracts of *Cora* aff. *glabrata* showed the lowest content at 4.85 ± 0.10 µg of GAE/mg dw extract. The total flavonoids content was determined according to the equation obtained from the standard quercetin graph (y = 0.0227 x + 0.0294, r^2 = 0.995). Highest flavonoids content was found in ethanolic extracts of the *T. vermicularis* at 37.07 ± 0.08 µg of QE/mg dw extract, while *Peltigera laciniata* showed the lowest content at 9.73 ± 0.07 µg of QE/mg dw extract (Table 1).

The correlation between the antioxidant activity in terms of ARP of ethanol and dichloromethane extracts and their phenols and flavonoids content were analyzed (Table 1) and is shown in Figs. 3 and 4.

DISCUSSION

Free radical scavenging action is considered to be one of the various mechanisms for antioxidation (Bhoyar et al., 2011). Being used widely as a preliminary test, which provides information on the reactivity of test compounds with a stable free radical. DPPH• assay gives a strong absorption band at 517 nm (purple colour). When it is quenched by the extract there is a decrease in absorbance and discoloration from purple to yellow, due to the formation of the non-radical form DPPH-H by hydrogen donated by the antioxidant (AH), following the reaction DPPH• + AH → DPPH-H + A• (Argolo, 2004; Sheetal et al., 2008).

In this study, a significant decrease in the concentration of the DPPH• radical due to the scavenging ability of extracts from the lichens *Cladonia* aff. *rappi*, *Cora* aff. *glabrata, Peltigera laciniata*, and *Thamnolia vermicularis* was observed. The percentage inhibition oxidative (%INH) of lichen extracts in general decreased in the following order: ethanolic > dichloromethane > aqueous. Aqueous extracts show the weakest antioxidant effect. That is probably because the active components produced by lichens are not very soluble in water (Kinoshita et al., 1994; Huneck and Yoshimura 1996), although they could still partially pass into an aqueous solution (Zagoskina et al., 2013). Nevertheless, across all four lichen species, the results were not uniform.

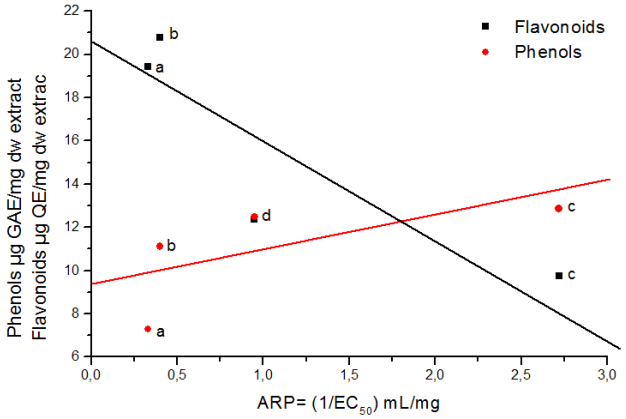

Figure 3. Correlation between the antioxidant activity and total phenol and flavonoid contents in the ethanolic extracts across species. Phenols: p<0.05 represents the statistical difference between ARP and total phenols, with a moderate correlation (r= 0.68). Flavonoids: p<0.05 represents the statistical difference between ARP and flavonoids content, with a negative correlation (r = -0.89). Each vowel means a lichen species: **a:** *Cora* aff. *glabrata*; **b:** *Cladonia* aff. *rappii*; **c:** *Peltigera lacinita* and **d:** *Thamnolia vermicularis*.

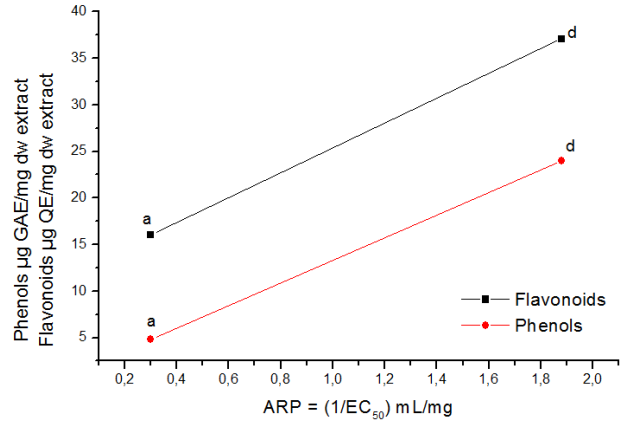

Figure 4. Correlation between the antioxidant activity and total phenol and flavonoid contents in the dichloromethane extracts across species. Phenols: p<0.05 represents the statistical difference between ARP and total phenols, with a high correlation (r= 0.99). Flavonoids: p<0.05 represents the statistical difference between ARP and flavonoids content; also had a high correlation (r = 0.99). Each vowel means a lichen species: **a:** *Cora* aff. *glabrata*, and **d:** *Thamnolia vermicularis*.

Thamnolia showed a consistently high effect with both ethanol and dichloromethane extract, and the same was observed for *Cora*, although at a lower level. Yet, in both *Cladonia* and *Peltigera*, the dichloromethane extract showed substantially lower effects than the ethanol extract.

The ARP of lichen extracts studied showed the proton-donating ability. The ethanolic extract from *Peltigera laciniata* and dichloromethane extract from *Thamnolia vermicularis* showed the largest ARP values. The intensity of the antioxidant activity depended on the tested lichen species, and the solvent used for extraction (Behera et al., 2005).

The reduction capacity of a compound may serve as a significant indicator of its antioxidant activity (Sharma et al., 2012). Antioxidant compounds can donate electrons to reactive radicals, reducing them into more stable and unreactive species (Gulluce et al., 2006). The reduction of ferric ion (Fe^{3+}) to ion ferrous (Fe^{2+}) is measured by the strength of the green-blue color of the solution which absorbs at 700 nm. The method states that the absorbance is directly proportional to the reducing power. In this study, it was interesting confirm the antioxidant effect by two mechanisms, obtained by both methods the highest effect in ethanolic extracts, followed by dichloromethane and finally aqueous. This suggests that the substances responsible for the effect are best extracted with ethanol. A high absorbance of extracts means high reducing power. The reducing properties are generally associated with the presence of reductones. According Gordan (1990), the antioxidant action of reductones is based on the breaking of the free radical chain by donating a hydrogen atom. The results indicate that the reducing power activity of extracts seem to be due to presence of polyphenols which may act in a similar way as reductones by donating the electrons and reacting with free radicals to turns them into more stable products and abort free radical chain reactions (Sasikumar et al., 2010).

Several researchers have found high correlations between the antioxidant activity and phenolic content (Behera et al., 2005; Gulluce et al., 2006; Ranković et al., 2010). However, our study showed only a moderate correlation between the ARP and total phenolic content of studied extracts (r = 0.68), i.e close values in the phenolic content from ethanolic extracts of *Cladonia* aff. *rappii*, *Thamnolia vermicularis* and *Peltigera laciniata* corresponded with different ARP. In addition, the dichloromethane extracts of *T. vermicularis* and *Cora glabrata* showed a better correlation in the antioxidant activity for both as phenols as flavonoids.

Previous research on antioxidant activity of the genus *Cladonia* (Ranković et al., 2010; Kosanić and Ranković, 2011b; Mitrović et al., 2011) showed slight to moderate effect with respect to other lichens species tested. In contrast, strong antioxidant activity in *Thamnolia vermicularis* has been reported previously (Luo et al., 2006). The thamnolic acid has been known to be the main secondary metabolite of this lichen; this compound may be responsible for the high antioxidant activity of the extract of *T. vermicularis* (Luo et al., 2006).

On the other hand, our results show correlation highly significant but negative between antioxidant activities and flavonoids content for ethanolic extracts (r = –0.89). For example, the extract of *Peltigera laciniata*, which had the lowest flavonoid content, showed the highest ARP. In accordance with previous results with *P. rufescens* finding from Odabasoglu et al. (2005) suggest that the antioxidant activity of some tested extracts from this lichen might be attributed by the participation of non-phenolic compounds. It is interesting that peltigeralean lichens, in contrast to lecanorolean taxa, contain few specific lichens substances but have high laccase and tyrosinase activities (Zavarzina and Zavarzin, 2006; Laufer et al., 2009), also lectins are frequently found in extracts of thalli of cyanolichens (Feoktistov et al., 2009).

The opposite occurred with *Cora glabrata*, which showed high flavonoid content but low ARP. Elifio et al. (2000) also isolated lectins with hemagglutinating activity from *Cora* cf. *glabrata* (as *Dictyonema glabratum*) and relatives, and Peña et al. (2012) determined ferric reducing power and the power inhibitor of lipid peroxidation in extracts from *Cora* cf. *glabrata* (as *D. glabratum*) shown be active. The correlation of dichloromethane extracts between ARP and total

phenols content and flavonoids were for both cases, highly significant and positive (r = 0.99).

The results of our study suggest that the distinct behavior of the species and extracts depend precisely of the different levels of solubility in each extract to capture the active substances in each species. The antioxidant effects of extracts of *Peltigera laciniata* and *Cora* aff. *glabrata* are probably due to some other unknown components that may also interact complementarily producing synergism between phenols and other compounds like carbohydrates, proteins, enzymes, etc. Some of these substances are apparently soluble in water. Subsequent studies should focus on the isolation and identification of novel antioxidative components from lichen.

According to the literature, no reports of antioxidant activity of the lichen fungi *Cladonia* aff. *rappii*, *Peltigera laciniata* and *Cora* aff. *glabrata* have been reported, so this study can be considered pioneering for these organisms.

CONCLUSIONS

The ethanolic extracts of the Venezuelan lichens tested were shown to have high antioxidant activity. Based on these results, lichens appear to be good natural sources of novel antioxidants and could be of significance in human therapy.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENT

We are grateful to the staff of Research Institute at Facultad de Farmacia y Bioanálisis of the Universidad de los Andes (ULA) to allow the development of this research. The author thanks to colleagues from Department of Chemistry of Natural Products at Facultad de Farmacia y Bioanálisis-ULA, especially Gabriela Rodriguez-Castillo and Billmary-Z Contreras-Moreno, for their support and cooperation on this work and in others previous scientific projects.

REFERENCES

Argolo ACC, Sant'Ana AEG, Pletsch M, Coelho LCBB (2004) Antioxidant activity of leaf extracts from *Bauhinia monandra*. Bioresour Technol 95: 229-233.

Behera BC, Verma N, Sonone A, Makhija U (2005) Antioxidant and antibacterial activities of lichen *Usnea ghattensis* in vitro. Biotechnol Lett 27(14): 991-995.

Bhoyar M, Mishra G, Naik P, Srivastava R (2011) Estimation of antioxidant activity and total phenolics among natural populations of Caper (*Capparis spinosa)* leaves collected from cold arid desert of trans-Himalayas. Aust J Crop Sci 5(7): 912-919.

Dal-Forno M, Lawrey JD, Sikaroodi M, Bhattarai S, Gillevet PM, Sulzbacher M, Lücking R (2013) Starting from scratch: evolution of the lichen thallus in the basidiolichen *Dictyonema* (Agaricales: Hygrophoraceae). Fungal Biol 117: 584–598.

Davis E, Yost J (1983) Novel hallucinogens from eastern Ecuador. Bot Mus Leaf, Harv Univ 29(3): 291-295.

Díaz L, De-Montijo **S**, Medina A, Meléndez P, Laurence V and Marti-Mestres G (2011) Activity of ethanolic extracts leaves of *Machaerium floribundum* against acne-inducing bacteria, and their cytoprotective and antioxidant effects on fibroblast. Rev Peru Biol 18(2): 153–158.

Elifio S, Da-Silva M, Iacomini M, Gorin P (2000) A lectin from the lichenized Basidiomycete *Dictyonema glabratum.* New Phytol 148: 327-334.

Esteban I (2012) Líquenes usados en medicina tradicional. Bol Soc Micol Madrid 36: 163-174.

Feoktistov A, Kitashov A, Lobakova E (2009) The Characterization of lectins from the thripartite lichen *Peltigera aphthosa* (L.) Willd. Vestn Mosk Univ (Biol.) 64(1): 26–30.

Feuerer T (2008) Checklist of lichens and lichenicolous fungi of Venezuela. Version 1 May 2008. http://www.checklists.de [Consulted: 08/2008].

Gordan MH (1990) Food antioxidants. London/ New York: Elsevier pp 1-18.

Goupy P, Hugues M, Boivin P, Amiot M (1999) Antioxidant composition and activity of barley (*Hordeum vulgare*) and malt extracts and of isolated phenolic compounds. J Sci Food Agric (79): 1625-1634.

Gulluce M, Aslan A, Sokmen M, Sahin F, Adiguzel A, Agara G, Sokmen A (2006) Screening the antioxidant and antimicrobial properties of the lichens *Parmelia saxatilis*, *Platismatia glauca*, *Ramalina pollinaria*, *Ramalina polymorpha* and *Umbilicaria nylanderiana.* Phytomedicine 13: 515–521.

Gutteridge JM (1993) Free radicals in disease processes: a compilation of cause and consequence. Free Radic Res 19: 141-158.

Hernández J (2010) Líquenes del Herbario Nacional de Venezuela (Ven) y sus muestras tipo. Acta Bot Venez 33(2): 363-376.

Huneck S, Yoshimura I (1996) Identification of lichen substances. Berlin: Springer pp. 11-123.

Kekuda P, Yaka V, Swathi D, Suchitha Y, Venugopal T, Mallikarjun N (2011) Mineral composition, total phenol content and antioxidant activity of a macrolichen *Everniastrum cirrhatum* (Fr.) Hale (Parmeliaceae). E-J Chem 8(4): 1886-1894.

Kinoshita K, Matsubara H, Koyama K, Takahashi K, Yoshimura I, Yamamuto Y, Miura Y, Kinoshita Y, Kawai KI (1994) Topics in the chemistry of lichen compounds. J Hattori Bot Lab 76(2): 227–233.

Knight JA (1995) Diseases related to oxygen-derived free radicals. Ann Clin Lab Sci 25(2): 111-121.

Kosanić M, Ranković B (2011a) Lichens as possible sources of antioxidants. Pak J Pharm Sci 24(2): 165-170.

Kosanić M, Ranković B (2011b) Antioxidant and antimicrobial properties of some lichens and their constituents. J Med Food 14(12): 1624–1630.

Kumar P, Kekuda P, Vinayaka K, Sudharshan S, Mallikarjun N, Swathi D (2010) Studies on antibacterial, anthelmintic and antioxidant activities of a macrolichen *Parmotrema pseudotinctorum* (des. Abb.) Hale (Parmeliaceae) from Bhadra wildlife sanctuary, Karnataka. Int J PharmTech Res 2(2): 1207-1214.

Lamien-Meda A, Lamien C, Compaoré M, Meda R, Kiendrebeogo M, Zeba B, Millogo J, Nacoulma O (2008) Polyphenol content and antioxidant activity of fourteen wild edible fruits from Burkina Faso. Molecules 13: 581-594.

Laufer Z, Beckett RP, Minibaeva FV, Luthje S, Bottger M (2009) Diversity of laccases from lichens in suborder *Peltigerineae*. Bryologist 112: 418-426.

López F (1986). Censo de macrolíquenes de los estados Falcón, Lara, Mérida, Táchira y Trujillo. Trabajo de ascenso. Universidad de los Andes, Facultad de Farmacia, Mérida, Venezuela.

Lücking R (2008) Foliicolous lichenized fungi. Flora Neotropica Monograph 103: 1-866

Lücking R, Rivas Plata E, Chaves JL, Umaña L, Sipman HJM (2009) How many tropical lichens are there… really? *In* A. Thell, M.R.D. Seaward & T. Feuerer (eds.). Diversity of Lichenology – Anniversary Volume. Bibl. Lichenol 100: 399-418, Berlin-Stuttgart, Germany.

Lücking R, Dal-Forno M, Lawrey J, Bungartz F, Holgado M, Hernández JE, Marcelli M, Moncada B, Morales E, Nelsen M, Paz E, Salcedo L, Spielmann A, Wilk K, Will-Wolf S, Yánez-Ayabaca A (2013) Ten new species of lichenized Basidiomycota in the genera *Dictyonema* and *Cora* (Agaricales: Hygrophoraceae), with a key to all accepted genera and species in the *Dictyonema* clade. Phytotaxa 139 (1): 1-38.

Lücking R, Dal-Forno M, Sikaroodi M, Gillevet PM, Bungartz F, Moncada B, Yánez-Ayabaca A, Chaves JL, Coca LF, Lawrey JD (2014) A single macrolichen constitutes hundreds of unrecognized species. Proc Natl Acad Sci U.S.A. 111(30): 11091-11096.

Luo H, Ren M, Lim K, Koh Y, Wang Li, Hur J (2006) Antioxidative activity of lichen *Thamnolia vermicularis in vitro*. Mycobiology, 34(3): 124-127.

Manojlovi NT, Vasiljevi P, Juskovi M, Najman S, Jankovi S, Andjelkovi AM (2010) HPLC analysis and cytotoxic potential of extracts from the lichen, *Thamnolia vermicularis* var. *subuliformis*. J Med Plant Res 4: 817–823.

Marcano V (1994) Introducción al estudio de los líquenes y su clasificación. Colección Flora Liquénica de los Andes Venezolanos. Edición de Fundacite Mérida-Venezuela.

Mitrović T, Stamenković S, Cvetković V, Tošić S, Stanković M, Radojević I, Stefanović O, Čomić L, Đačić D, Ćurčić M, Marković S (2011) Antioxidant, antimicrobial and antiproliferative activities of five lichen species. Int J Mol Sci 12: 5428-5448.

Nash T, Ryan BD, Diederich P, Gries C, Bungartz F (2002) Lichen Flora of the greater Sonoran Desert Region, Vol. 1. Lichens unlimites, Arizona State University, US, 532 p.

Nash T, Ryan BD, Diederich P, Gries C, Bungartz F (2004) Lichen Flora of the greater Sonoran Desert Region, Vol. 2. Lichens unlimites, Arizona State University, US, 742 p.

Odabasoglu F, Aslan A, Cakir A, Suleyman H, Karagoz Y, Bayir Y, Halicia M (2005) Antioxidant activity, reducing power and total phenolic content of some lichen species. Fitoterapia 76: 216–219.

Peña J, Robayo P, Valencia-Islas N, Rojas J, Dávila R (2012) Actividad antioxidante de los líquenes *Stereocaulon novogranatense*, *S. pomiferum* y *Dictyonema glabratum*. Glalia 4(4): 1–134.

Pérez-Llano G (1944) Lichens. Their biological and economic significance. Bot Rev 10(1): 1-57.

Ranković B, Ranković D, Maric D (2010) Antioxidant and antimicrobial activity of some lichen species. Microbiology 79(6): 809-815.

Raha S, Robinson BH (2000) Mitochondria, oxygen free radicals, disease and ageing. Trends Biochem Sci 25(10): 502-508.

Sasikumar JM, Mathew GM, Teepica PDD (2010) Comparative studies on antioxidant activity of methanol extract and flavonoid fraction of *Nyctanthesarbortristis* leaves. Electron J Environ Agric Food Chem 9: 227-233.

Sharma B, Kalikotay S (2012) Screening of antioxidant activity of lichens *Parmotrema reticulatum* and *Usnea* sp. from Darjeeling hills, India. IOSR J Pharm 2(6): 54-60.

Sheetal A, Bagul MS, Parabia M, Rajani M (2008) Evaluation of free radicals scavenging activity of an Ayurvedic formula, Panchvalkal. Indian J Pharm Sci 70(1): 31–35.

Sipman H (2005) Identification key and literature guide to the genera of lichenized fungi (Lichens) in the Neotropics. 1-8. http://www.toyen.uio.no/botanisk/lav/LichenKey/Current/Sipman/neokeya.htm or http://www.biologybrowser.org/weblinks/goto/1151209 [Consulted: 15, April, 2014]

Sipman HJM, Hekking W, Aguirre-C J (2008) Checklist of lichenized and lichenicolous fungi from Colombia. Biblioteca Jose Jerónimo Triaga 20: 1–242.

Upreti DK, Divakar PK, Nayaka S (2005) Commercial and ethnic use of lichens in India. Econ Bot 59: 269-273.

Verma N, Behera BC, Makhija U (2008) Antioxidant and hepatoprotective activity of lichen *Usnea ghattensis* in vitro. Appl Biochem Biotechnol 151(2–3): 167–181.

Zagoskina N, Nikolaeva T, Lapshin P, Zavarzin A, Zavarzina A (2013) Water Soluble Phenolic Compounds in Lichens. Microbiology 82(4): 445–452.

Zavarzina AG, Zavarzin AA (2006) Laccase and tyrosinase activities in lichens, Microbiology 75(5): 546–556.