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In vitro antibacterial, antioxidant and cytotoxic activity of methanol-acetone extracts from Antarctic lichens (*Usnea antarctica* and *Usnea aurantiaco-atra*)

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ABSTRACT

The lichens are symbiotic organisms, synthesize a great variety of chemically complex. Natural and potential source of bioactive compounds. *U. antarctica* and *U. aurantiaco-atra* were collected during 23rd Peruvian Scientific Expedition (ANTAR XXIII–2015) from the Antarctic Scientific Station "Machu Picchu" and were transferred to the ITP for processing. The samples were dried and grounded, after that, an extraction with acetone (1:10 w/v) also with methanol (1:10 w/v) was performed. Both extracts were mixed and vacuumed dried (30 °C). A methanol-acetone extract (MAE) from each lichen was obtained. MAE from *U. antarctica* showed a major concentration of total phenols (22.80 ± 0.08 mg GA/g MAE) than *U. aurantiaco-atra* (19.42 ± 0.32 mg GA/g MAE). Besides, *U. antarctica* exhibited a superior value of inhibition of $ABTS^{\bullet+}$ radical (89.05 ± 0.01 µmol TE/g MAE) than *U. aurantiaco-atra* (79.84 ± 0.09 µmol TE/g MAE). The antibacterial activity of MAEs against *Staphylococcus aureus* ATCC 14775, *Pseudomonas aeruginosa* ATCC 27853 and *Vibrio alginolyticus* ATCC 17749 was performed however was only demonstrated against *S. aureus.* The minimum inhibitory concentration (MIC) was evaluated, *U. antarctica* and *U. aurantiaco-atra* exhibited 94.76% and 98.43%, respectively of inhibition bacterial growth at 31.25 µg/mL of MIC value. MAE of *U. antarctica* (IC_{50} = 169.64 µg/mL) and *U. aurantiaco-atra* (IC_{50} = 270.82 µg/mL).

1. Introduction

Lichens are symbiotic organisms consisting of fungi, algae and/or cyanobacteria, they have great adaptability to the environment, getting to colonise many terrestrial habitats, distributing from the poles to tropical regions and from the plains to the highest mountains (Oksanen, 2006). These associations synthesise a great variety of secondary metabolites; some of them have been successfully identified through the development of analytical techniques and experimental methods (Molnar and Farkas, 2010). The phytochemistry of lichens is abundant and diverse; they biosynthesise a large number of compounds: aliphatics, pulvinic acid derivatives, hydroxybenzoic acid derivatives, depsids, depsidones, dibenzofuran derivatives, anthraquinones, naphthoquinones, phenolic compounds, among others (Nash, 2008). The secondary metabolites present in lichens are chemically complex compounds, and most of them are different from those that can be found in plants (Müller, 2001). The lichen metabolites have several biological activities: antibacterial, antimycobacterial, antiviral, antitumor, antiprotozoal, antimutagenic, anti-inflammatory, antioxidant, analgesic and cytotoxic (Oksanen, 2006; Molnar and Farkas, 2010).

Previous studies suggested that Antarctic lichens contain a more significant amount of antioxidants to scavenge the free radicals caused by the extreme habit such as severe desiccation and intense solar radiation (Kranner et al., 2005; Paudel et al., 2008). Besides, Luo et al., 2009 reported that Antarctic lichens showed extraordinary stronger antioxidant activity than tropical or temperate lichens. The natural products derived from Polar Regions appear to have a high hit rate regarding biological activity, varying from cytotoxic, enzyme inhibitory, antioxidant, antiparasitic, antiviral, antibacterial, and so on. Secondary metabolism in Polar habitats is driven mainly by environmental requirements of the producing organism. Successful organisms will often have specific metabolic pathways that produce unique functional natural products that bestow environmental advantage, increasing the possibility of finding pharmaceutical lead molecules (Tian et al., 2017). Li et al. (2010) determined that the extract of *Astrothelium*

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sp. presented antibacterial activity against Gram-positive bacteria, as did Ivanovic et al. (2013) in *Usnea barbata* against *S. aureus*. Acetone extracts of *Umbilicaria crustulosa*, *Umbilicaria cylindrica* and *Umbilicaria polyphylla* presented antimicrobial activity (Kosanić et al., 2012). Similar results reported with methanol extracts from *Usnea intermedia*, *Usnea filipendula* and *Usnea fulvoreagens* exhibiting antibacterial activity against *Escherichia coli* and *S. aureus* (Oran et al., 2016). Bhattarai et al. (2013) reported antibacterial activity against *Bacillus subtilis* and *S. aureus* isolating a compound of *Stereocaulon alpinum* collected from Antarctica. Because of this, industries from different sectors have shown interest in Antarctic studies as a source for new applications (Walton, 2013).

The study of new drugs or active ingredients with therapeutic properties obtained from natural sources has gained great importance, achieving significant progress in biomedicine (Ravelo and Braun, 2009). The frequent excessive use of antibiotics in the treatment of bacterial infections causes an increase of pathogenic microorganisms resistant to conventional treatments. Consequently, it is necessary to increase the administered dose or provide new products. In this sense, it is convenient to look for new sources of compounds with antibacterial activity to threat nosocomial bacterial and foodborne disease such as *P. aeruginosa,* which is a pathogenic bacteria with nosocomial prevalence and common cause of pneumonia, urinary tract infection, infection of surgical wounds and bacteremia in hospitalized patients (Mandell et al., 2006). *S. aureus* is present in patients with acquired infections in intensive care units (ICU), mainly in pneumonias related to mechanical ventilation. Álvarez Lerma et al., 2006 did not identify differences in the evolution of patients with methicillin-resistant or susceptible *S. aureus* infections. *Vibrio alginolyticus* is most commonly associated with wound infections, otitis media, and otitis extern (Newton et al., 2012; Hlady and Klontz, 1996), it is increasingly recognized as an important intestinal pathogen in humans. Hiratsuka et al. (1980) isolated the first *V. alginolyticus* from a patient with acute enterocolitis. It mainly affects persons who have had direct contact with seawater or those who have handled shellfish (Caccamese and Rastegar, 1999). Moreover, there is a significant risk in the use of different antibiotics such as aminoglycosides, cephalosporins, amphotericin B and erythromycin (Miyahira, 2003) due to considerable damage suffered by kidneys in its structure and function.

There are investigations that not consider the cytotoxic assay; however, this evaluation detecting adverse effects of interference with structure and/or essential properties for cell survival, proliferation and/or functions (Arencibia et al., 2003), is essential to continue the purification of crude extract. Besides, there is only a report where the antibacterial activity of crude extracts from *U. antarctica* and *U. aurantiaco-atra* was determinated against bacteria isolated from dental caries different from *S. aureus* (Eun-Mi and Min-Jeong, 2012). For this reason, the crude extracts from *U. antarctica* and *U. aurantiaco-atra* were evaluated as a potential antibacterial and antioxidant source against *S. aureus*, *V. alginolyticus* and *P. aeruginosa*; and its cytotoxic effect, because it will be the first report evaluates those activities. In addition, this study is the base for isolating the bioactive compound and in the early future to produce it through heterologous expression (Stocker-Wörgötter, 2008; Gagunashvili et al., 2009; Abdel-Hameed et al., 2016).

2. Methods

2.1. Collection and identification of lichen species

U. antarctica and *U. aurantiaco-atra* were collected from the Machu Picchu Antarctic Scientific Station (ECAMP) on King George Island (62°05′27″S and 58°28′12″W), from January to March 2015 (ANTAR XXIII – Peruvian Scientific Expedition). The sampling was authorised for Directorate of Antarctic Affairs of the Ministry of Foreign Affairs of Perú. The species were identified morphologically, and the voucher specimens have been deposited in the Laboratory of Biotechnology from the Instituto Tecnológico de la Producción (ITP).

2.2. Preparation of extracts

Lichens (250 g) (cortices, medullae, photobionts in the thalli and apothecia) were sampled. After the lichens were dried, grounded, homogenised, and vacuum packed to avoid any oxidation. We used 10 g of each lichen processed to extract with acetone (1:10 w/v) and a subsequent one with methanol (1:10 w/v). They were mixed and vacuum dried (30 °C) in a rotary evaporator. Finally, crude extract from each lichen was obtained, which were called methanol-acetone extract (MAE).

2.3. Determination of total phenolic content

The amount of total phenols was determined according to the methodology described by Singleton et al. (1999) with some modifications, using Folin-Ciocalteu reagent in addition to gallic acid (GA) in 100% methanol solution as a standard. MAEs from each lichen were dissolved in methanol at a concentration of 10 mg/mL, and 125 µL of each MAE solution (or calibration standard or the solvent mixture that corresponds to the blank) was added to 125 µL of Folin-Ciocalteu reagent. It was shaken and allowed to stand for 3–8 min at room temperature. Then 2.5 mL Na_2CO_3 (7.5% w/v) and 3.5 mL of distilled water were added. The mix was incubated for 60 min at room temperature. Subsequently, the absorbance readings were taken at 750 nm front a blank in a UV-VIS spectrophotometer (PerkinElmer, USA) and the absorbance were interspersed in a GA calibration curve ($y = 0.0019x-0.0147$, $R^2 = 0.9965$). The total phenolic compound content of the extract was expressed in milligrams GA equivalent per gram of MAE [mg GAE/g MAE].

2.4. Evaluation of antibacterial activity

Both MAE (1 mg/mL) were dissolved in Müller-Hinton Broth (MHB) with 0.5% of dimethyl sulfoxide (DMSO), then 9 serial dilutions with MHB were made at different concentrations (250–0.49 µg/mL) to evaluate the antibacterial activity against *S. aureus* ATCC 14775, *V. alginolyticus* ATCC 17749 and *P. aeruginosa* ATCC 27853, according to the plate microdilution method (CLSI, 2012). MHB and Müller-Hinton Agar (MHA) were used at pH (7.2 ± 0.2). Bacterial inoculum was prepared according to Sarker et al. (2007). The number of colony-forming units (CFU) was determined by plate count, calculating the dilution factor. Finally, an inoculum of 1×10^7 CFU/mL was obtained. The 96-well plates were incubated at 37 °C for 24 h. The positive growth controls were MHB and MHB with 0.125% DMSO (this concentration represents the percentage of DMSO used in the highest concentration evaluated in the plate microdilution [250 µg/mL]). The negative growth control were 10% DMSO and Oxacillin (1 µg/ml). The assay was performed in triplicate. To determine the minimum bactericidal concentration (MBC), the qualitative analysis of culture by extension from 100 µL was used. For the quantitative analysis, the optical density was measured at 600 nm, and the percentage of bacterial inhibition was determined, according to Banjara et al. (2012).

2.5. Evaluation of antioxidant capacity by the ABTS method

The antioxidant capacity of MAE from each lichen was evaluated according to the decrease of the $ABTS^{\bullet+}$ radicals according to Marfil (2008) with some modifications. $ABTS^{\bullet+}$ radical was formed from 7 mM ABTS stock solution and 2.45 mM of potassium persulfate, mixed in equal proportions in the dark for 12–16 h before his use at room temperature. 600 µL of $ABTS^{\bullet+}$ radical was diluted with 30 mL of methanol until 0.70 ± 0.02 absorbance in UV-VIS spectrophotometer (PerkinElmer, USA) at a wavelength of 734 nm. After that, 30 µL MAE

in methanol solution (10 mg/mL) and 3 mL $ABTS^{\bullet+}$ radical solution was mixed and reacted at room temperature, in dark during 1 h, then the absorbance was read compared to the reagent reference ($ABTS^{\bullet+}$ radical solution with MAE solvent) at the wavelength above mentioned. A methanol solution standard curve of the synthetic antioxidant Trolox® (0.2–2.0 mM) was used as a control at the same volumes as to the MAE. The results were expressed in µmol Trolox equivalent (TE) per g of MAE [µmol TE/g MAE].

2.6. Evaluation of cytotoxic activity

African green monkey kidney cell line (VERO–ATCC CCL-81) obtained from the American Type Culture Collection (ATCC, USA) were cultured at a concentration of 1×10^5 cells in 96-well plates with the Eagle's Minimum Essential Medium (EMEM) supplemented with 10% foetal bovine serum (FBS) in humid atmosphere at 37 °C and 5% CO_2 for 24 h. To evaluate the cytotoxic effect, the colourimetric tetrazolium salt method (MTT) (3-(4.5-dimethylthiazol-2-yl)-2.5-diphenyltetrazolium bromide) was used. Both MAE (1 mg/mL) was dissolved in EMEM with 10% FBS and 0.5% DMSO. Dilutions were prepared for the cytotoxic assay (250–7.81 µg/mL) at a final volume of 100 µL. After 24 h of treatment, 10 µL MTT was added and incubated for 3.5 h in a 5% CO_2 atmosphere at 37 °C. The medium was decanted, and 100 µL/well of acid isopropanol (0.04 mM HCl) was added; and the optical density was read on a PowerWave HT plate spectrophotometer (BioTek, USA) at 570 nm. The dose-response effect of toxicity was determined with concentration effect curves (% cell proliferation) by exponential regression analysis.

2.7. Statistical analysis

Results were expressed as mean value ± standard error of the mean. One-way analysis of variance (ANOVA) was performed in STATA 13. *P values* lower than 0.05 ($P < 0.05$) were considered significant.

3. Results

3.1. Organisms collected

The fruticose lichens collected were identified as *U. aurantiaco-atra* and *U. antarctica* (Fig. 1). Crude extracts from *U. antarctica* (3.03 g) and *U. aurantiaco-atra* (3.23 g) was obtained.

3.2. Total phenols concentration

It was performed according to Singleton et al. (1999) with some modifications, MAE from *U. antarctica* showed a higher concentration of mg GAE than MAE from *U. aurantiaco-atra* (Table 1).

Table 1
Total phenols concentration ($P < 0.05$ ANOVA).

MAE	mg de GAE/g
U. aurantiaco-atra	19.42 ± 0.32
U. antarctica	22.80 ± 0.08

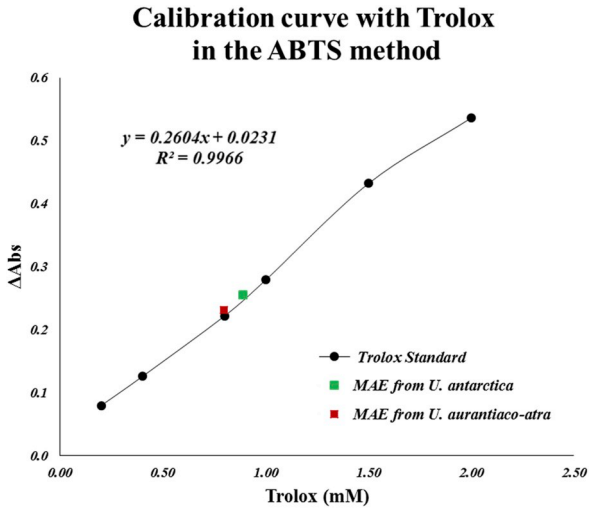

Fig. 2. Calibration curve (Trolox, in mM) and samples in the ABTS method.

3.3. Total antioxidant capacity

The ABTS method was performed, and the antioxidant capacity was based on decolourisation of $ABTS^{\bullet+}$ radical. Trolox was used as a standard for the ABTS method (Re et al., 1999). The measurement of $ABTS^{\bullet+}$ reduction was expressed as TE.

Changes of absorbance expressed as percentage of inhibition for individual concentrations of Trolox are shown in Fig. 2. Base on the calibration by Trolox, $ABTS^{\bullet+}$ radical can be used for determination of the antioxidant activity up to 200 µmol/g. The calibration curve equation related to Trolox standard was $y = 0.2604x + 0.0231$ with a confidence coefficient $R^2 = 0.996$, within a concentration range from 20 to 200 µmol/g. The calibration curve was plotted with methanol as solvent. Total antioxidant capacity of MAE from *U. antarctica* and *U. aurantiaco-atra* were expressed in µmol TE per g MAE. MAE from *U. antarctica* showed $ABTS^{\bullet+}$ radical inhibition values of 89.05 ± 0.01 µmol TE/g, that was slightly similar to *U. aurantiaco-atra* of 79.84 ± 0.09 µmol TE/g (Table 2 and Fig. 2). The samples P value < 0.05 was considered as statistically significant ($P < 0.05$ ANOVA).

Fig. 1. Antarctic lichens collected. (a) *U. aurantiaco-atra*, (b) *U. antarctica*.

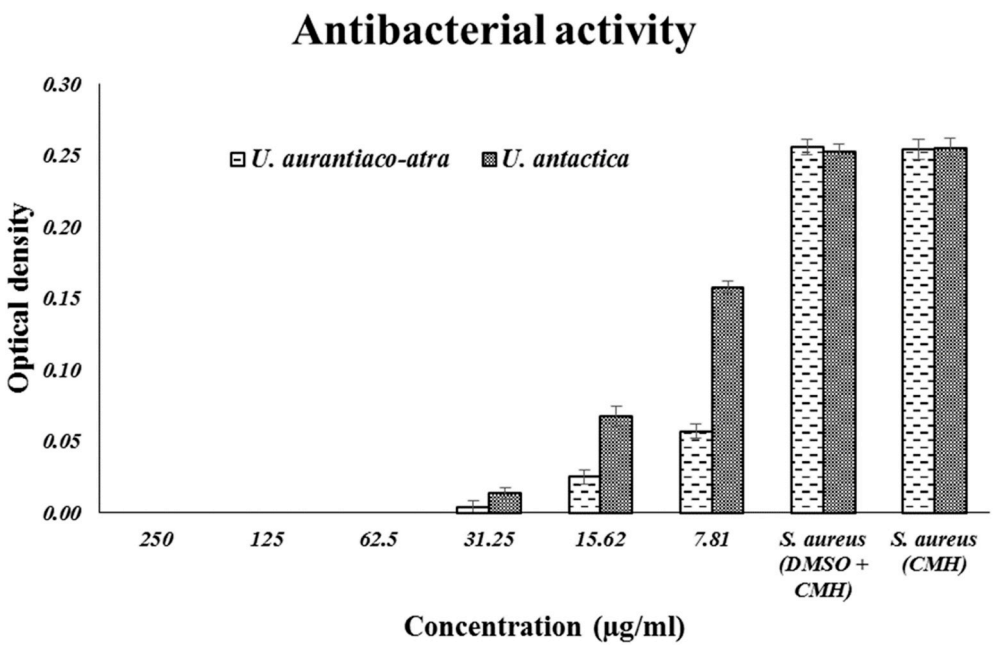

Fig. 3. Evaluation of antibacterial activity of MAE from *U. auriantiaco-atra* and *U. antarctica* against *S. aureus* ($P < 0.05$ ANOVA).

Table 2
Antioxidant activity expressed in µmol TE/g extract and mg TE/100 g dried lichen.

Lichen	µmol TE/g extract	mg TE/100 g dried lichen
U. antarctica	89.05	675.84
U. aurantiaco-atra	79.84	645.04

3.4. Antibacterial activity

Antibacterial activity of MAE was evaluated against *S. aureus, V. alginolyticus* and *P. aeruginosa.* However, this activity only was demonstrated against *S. aureus.* The MIC of MAE from *U. aurantiaco-atra* was100% inhibition at a concentration of 62.5 µg/ml and at 31.25 µg/ml inhibited 98.43% of bacterial growth, in contrast to MAE from *U. antarctica* was MIC 94.76% at 31.25 µg/ml. Both growth controls were very similar: MHB (0.254 ± 0.01 and 0.255 ± 0.01) and MHB with 0.125% DMSO (0.256 ± 0.01 and 0.253 ± 0.01) for the antibacterial assays of MAE from *U. aurantiaco-atra* and *U. antarctica,* respectively. 10% DMSO and Oxacillin (1 µg/ml) not showed bacterial growth as we expected (see Fig. 3).

3.5. Cytotoxic activity

The cytotoxic effect from MAE on Vero cells was evaluated *in vitro* MTT assay. Different concentrations of MAE from *U. antarctica* and *U. aurantiaco-atra* were used, and effective doses were calculated from the dose-response curve. The cytotoxicity evaluation on Vero cell line of lichens extracts is shown in Fig. 4. The MAE of *U. antarctica* and *U. aurantiaco-atra* on the Vero cell line achieving an IC_{50} value of 169.64 µg/mL and 270.82 µg/mL, respectively.

For the statistical analysis of results, the mean values and the standard deviation of triplicates were taken; all the results were significantly different from each other at a confidence level of 95.0% ($P < 0.05$ ANOVA).

4. Discussion

In this study, it was demonstrated that MAE from *U. antarctica* and *U. aurantiaco-atra* showed antibacterial activity against *S. aureus*, our results confirm that both lichens have antibacterial activity. Eun-Mi and Min-Jeong (2012) obtained two different crude extracts (acetonic and methanolic) from the same lichens, they also demonstrated antibacterial activity against different Gram-positive bacteria, isolated from dental caries (*Actinomyces oris, Corynebacterium durum, Rothia dentocariosa, Lactobacillus casei, Lactobacillus acidophilus, Lactobacillus rhamnosus, Streptococcus mutans, Streptococcus oralis, Streptococcus salivarius, Streptococcus sanguinis* and *Neisseria* sp.). We also evaluated MAE against *P. aeruginosa* and *V. alginolyticus*, although they did not exhibited an antibacterial effect. Moreover, these results were similar to those of Paudel et al. (2008), who also did not demonstrate antibacterial activity of water-methanol extract from the Antarctic lichens (*S. alpinum, Ramalia terebrata, Caloplaca* sp. and *Lecanora* sp.) against *E. coli* and *P. aeruginosa.* The effect of MAE probably was associated of the sensitivity and differences in the permeability of the cell wall. The single cell membrane of Gram-positive bacteria is made up of peptidoglycan and teichoic acids that offers less protection against antibiotic substances in comparison to Gram-negative bacteria which is composed of peptidoglycans, lipopolysaccharides and lipoproteins (Nostro et al., 2000).

Mostly investigations on bioactive compounds with antibacterial properties of lichens are mainly focused on usnic acid (UA) (Moura et al., 2017; Segatore et al., 2012), some of them employee UA purchased from a commercial business (Sigma Aldrich) (Dos Santos et al., 2018; Maciąg-Dorszyńska et al., 2014; Nithyanand et al., 2015), this UA was isolated from *Cladonia* spp. Cansaran et al. (2006) quantified the UA from lichens of Usnea genus (*U. subflorida, U. florida, U. barbata, U. longissima, U. hirta* and *U. rigida*) and demonstrated the antibacterial activity of acetone extracts against *B. subtilis* and *Bacillus megaterium*, no lichen assessed antibacterial activity against *S. aureus*, even *U. subflorida*, which had the highest amount of UA. According to available literature, secondary metabolites of Antarctic lichen with antibacterial properties against *S. aureus,* different of UA, have been evaluated such as vicanicin of *Psoroma pallidum* Malme (Piovano et al., 1985); sphaerophorin of *Sphaerophorus globosus* (Huds.) Vain (Quilhot et al., 1989), atranorin of *Acarospora macrocyclos* Vain, fumarprotocetraric acid of *Cladonia cornuta* (L.) Hoffm, psoromic acid of *Rhizocarpon geographicum* L. DC and variolaric acid of *Ochrolechia deceptionis* (Hue) Darb. (Piovano et al., 1991); ramalin, usimines A, B and C from *Ramalina terebrata* (Paudel et al., 2010) and pseudodepsidone of *S. alpinum*

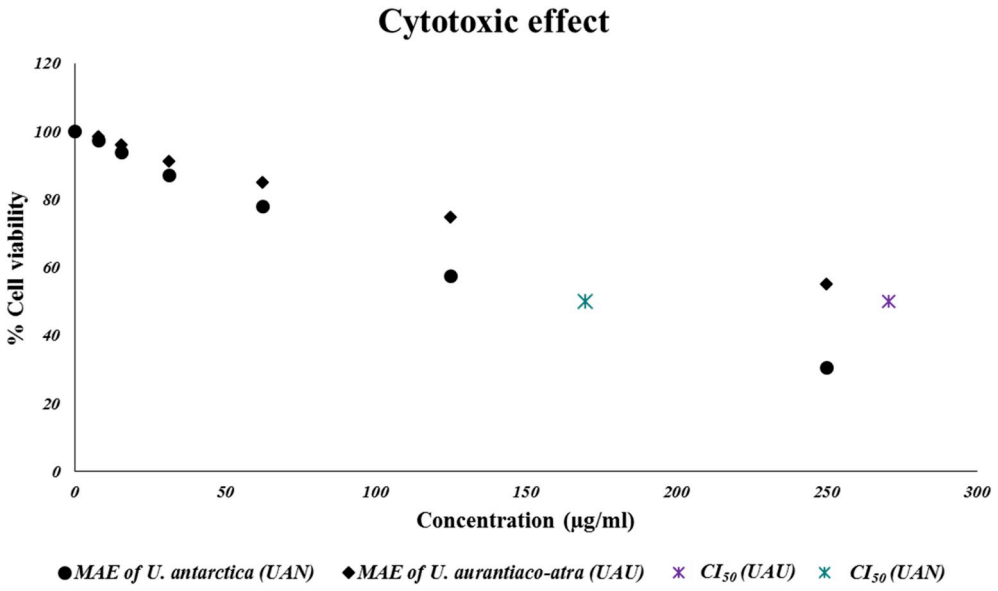

Fig. 4. Cytotoxic effect from MAEs on Vero cells. ($P < 0.05$ ANOVA).

(Bhattarai et al., 2013). Furthermore, Bellio et al. (2015) showed the antibacterial activity of secondary metabolites of *Lecanora atra* (α-collatolic acid), *S. alpinum* (lobaric acid), and *Cornicularia aculeata* ((+)-protolichesterinic acid) against *S. aureus*.

In the present study, *U. aurantiaco-atra* showed more percentage of inhibition bacterial growth (98.43%) than *U. antarctica* (94.76%), the MIC of MAE from *U. aurantiaco-atra* and *U. antarctica* against *S. aureus* was 31.25 µg/mL, this value is lower than reported by Madamombe and Afolayan (2003), where indicated a significant antibacterial property of *U. barbata* against *B. subtilis, Enterococcus faecalis, Micrococcus viradans* and *S. aureus* with a MIC as low as 0.1 mg/mL. Celenza et al. (2013) found antibacterial activity in *C. cornuta, R. geographicum, O. deceptionis* and *S. globosus* (MIC of 1024 µg/mL, 1024 µg/mL, 256 µg/mL, 32 µg/mL, respectively). Bhattarai et al. (2013) reported that *S. alpinum* shows activity against *B. subtilis* and *S. aureus*, with a MIC of 44 µg/mL and 35.2 µg/mL, respectively. Ranković et al. (2012) demonstrated that the acetone extract from *U. barbata* inhibited *S. aureus* with MIC of 0.5 mg/mL, and the MIC of its UA was 0.125 mg/mL. Schmeda-Hirschmann et al. (2008) determined that extracts from *U. florida* showed antibacterial effect against methicillin-resistant and methicillin-sensitive strains of *S. aureus* with MICs of 100 and 850 µg/mL, respectively. Moreover, the UA from *U. florida* exhibited activity against the same bacteria with a MIC of 100 and 750 µg/mL, respectively. It is possible that antibacterial activity exhibit from *U. aurantiaco-atra* and *U. antarctica* are associated to concentration of bioactive compounds, the type of extraction and the use of the complete lichens that were different from the studies described above.

Total phenol content of MAE from *U. aurantiaco-atra* and *U. antarctic* was 19.42 and 22.80 mg of GA/g of extract. However, Fernández-Moriano et al. (2016) reported a total phenol content of 22.4 ± 0.3 µg GA/mg dry extract from *U. aurantiaco-atra.* This difference can be explained considering that the Folin method may produce a slight overestimation due to the fact that other components different from phenols can react with the Folin-Ciocalteu reagent (García et al., 2011; Fernández-Moriano et al., 2016). Also, we performed the MAE using the whole lichens. Furthermore, other species such as *Usnea contexta* showed 20.7 ± 0.2 µg GA/mg dry extract (Fernández-Moriano et al., 2016) and *Usnea pictoides* 25 mg GA/g of extract (Pavithra et al., 2013) similarly to our results probably because them belonging from the same genus. Phenolic compounds can donate hydrogen to reactive radicals and break the chain reaction of lipid oxidation at the initiation step (Gülçin et al., 2004). Then, the intense antioxidant activity shown by some lichen extracts or metabolites, and assessed by different systems, can be attributed to their high total polyphenolic contents (especially depsides, depsidones, dibenzofurans, etc.). Since a positive correlation between phenolic composition and antioxidant activity has been proved for most of them (Kosanić et al., 2011; Manojlovic et al., 2012; Plaza et al., 2014); at least, it suggests that polyphenols might be the major antioxidant compounds in studied lichens.

In our study, MAE from *U. antarctica* showed $ABTS^{\bullet+}$ radical inhibition values of 89.05 µmol TE/g, that was slightly similar to *U. aurantiaco-atra* of 79.84 µmol TE/g. We used the ABTS radical assay, because it was employed in several polar lichen species (Paudel et al., 2008; Singh et al., 2011), as well as in *Usnea ghattensis* (Verma et al., 2008) and ramalin compound from *R. terebrata* (Paudel et al., 2010). Oran et al. (2016) performed TE with ABTS in three types of extracts from lichens with different solvents. The highest antioxidant activity was the methanol extract of *U. fulvoreagens* exhibited 125.2 ± 6.4 mg TE/100 g of dried lichen. The Usnea species evaluated: *U. filipendula*, *U. fulvoreagens* and *U. intermedia* showed a lower antioxidant capacity in contrast with our results (*U. antarctica* [675.84 mg TE/100 g of dried lichen] and *U. aurantiaco-atra* [645.04 mg TE/100 g of dried lichen]), even though our values were more than twice that the reported in this study, Singh et al. (2011) reported that Polar lichens different from Usnea genus (three foliose: *Pseudophebe pubescens, Xanthoria elegans* and *Umbilicaria hyperborean*; and six fruticose: *Cladonia amaurocraea, Cladonea mediterranea, Physcia caesia, Flavocetraria nivalis* and *Cetraria fastigata*) showed more antioxidant activity than our study. However, they performed the antioxidant activity with the enzymatic method (myoglobin, H_2O_2) and we used the chemical method (potassium persulfate). Re et al. (1999) mentioned that the faster reaction antioxidants could also contribute to the reduction of the ferril myoglobin radical. The most appropriate test is a decolourisation technique in which the radical is generated directly in a stable form before the reaction with putative antioxidants such as the formation of ABTS that comes through the reaction between $ABTS^{\bullet+}$ and potassium persulfate. Furthermore, Marfil (2008) reported that the enzymatic method has several drawbacks, such as interferences with substances with peroxidase activity and the possible overestimation of the results. MAE from the Antarctic lichens assessed, demonstrated a positive correlation between total phenolic compounds and antioxidant activity in Trolox with ABTS, this relation is maintained between both samples.

MAE of *U. antarctica* and *U. aurantiaco-atra* on the Vero cell line

demonstrated an IC_{50} value of 169.64 μg/mL and 270.82 μg/mL, respectively. Moreover, MAE showed a lower IC_{50} on the Vero cell line than ethanol extract of other lichens (*Cetrelia olivetorum, Lecanora muralis* and *Ramalina farinácea*), which showed a 20–25 μg/ml of concentration of extract, which reduced the viability of the Vero cell by 50% (Karagöz et al., 2009).

5. Conclusions

It is concluded that the MAE from Antarctic lichen *U. aurantiaco-atra* at low concentration shows antibacterial activity against *S. aureus*, there is at least one bioactive compound that confers this activity. This work determined that *U. aurantiaco-atra* has lower cytotoxicity and more significant antibacterial activity compared to *U. antarctica*, so it is considered that lichen *U. aurantiaco-atra*, is a potential source for the isolation of bioactive compound(s) with antibacterial property. Even though both lichens showed antioxidant activity, we consider more relevant to emphasize in the elucidation of bioactive compound(s), related to antibacterial activity from *U. aurantiaco-atra*. However, we do not suggest exploiting the Antarctic lichen resources on an industrial scale because of their slow growth. On the other hand, new tools developed recently in the fields of bioinformatics (Harvey et al., 2015), analytics (Bouslimani et al., 2014), and molecular biology (Weissman, 2015), in combination with rapid improvement in sequencing technology, might herald a new era of research into this specific source. Moreover, the heterologous expression is way to apply the natural resources of bioactive compounds like Antarctic lichens in a sustainable way and not invasive (Stocker-Wörgötter, 2008; Gagunashvili et al., 2009; Abdel-Hameed et al., 2016).

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