Expansão do conhecimento e inovação tecnológica no campo das ciências farmacêuticas

Débora Luana Ribeiro Pessoa
(Organizadora)

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Atena Editora
Ano 2021

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Dados Internacionais de Catalogação na Publicação (CIP)

E96 Expansão do conhecimento e inovação tecnológica no campo das ciências farmacêuticas 2 / Organizadora Débora Luana Ribeiro Pessoa. – Ponta Grossa - PR: Atena, 2021.

Formato: PDF
Requisitos de sistema: Adobe Acrobat Reader
Modo de acesso: World Wide Web
Inclui bibliografia
ISBN 978-65-5983-454-9
DOI: https://doi.org/10.22533/at.ed.549212709

1. Farmácia. 2. Medicamentos. I. Pessoa, Débora Luana Ribeiro (Organizadora). II. Título.
CDD 615

Elaborado por Bibliotecária Janaina Ramos – CRB-8/9166

Atena Editora
Ponta Grossa – Paraná – Brasil
Telefone: +55 (42) 3323-5493
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APRESENTAÇÃO

A coleção “Expansão do conhecimento e inovação tecnológica no campo das ciências farmacêuticas” é uma obra organizada em dois volumes que tem como foco principal a apresentação de trabalhos científicos diversos que compõe seus 31 capítulos, relacionados às Ciências Farmacêuticas e Ciências da Saúde. A obra abordará de forma interdisciplinar trabalhos originais, relatos de caso ou de experiência e revisões com temáticas nas diversas áreas de atuação do profissional Farmacêutico nos diferentes níveis de atenção à saúde.

O objetivo central foi apresentar de forma sistematizada e objetivo estudos desenvolvidos em diversas instituições de ensino e pesquisa do país. Em todos esses trabalhos a linha condutora foi o aspecto relacionado à atenção e assistência farmacêutica, farmacologia, saúde pública, controle de qualidade, produtos naturais e fitoterápicos, práticas integrativas e complementares, entre outras áreas. Estudos com este perfil podem nortear novas pesquisas na grande área das Ciências Farmacêuticas.

Temas diversos e interessantes são, deste modo, discutidos aqui com a proposta de fundamentar o conhecimento de acadêmicos, mestres e todos aqueles que de alguma forma se interessam pela Farmácia, pois apresenta material que apresenta estratégias, abordagens e experiências com dados de regiões específicas do país, o que é muito relevante, assim como abordar temas atuais e de interesse direto da sociedade.

Deste modo a obra “Expansão do conhecimento e inovação tecnológica no campo das ciências farmacêuticas” apresenta resultados obtidos pelos pesquisadores que, de forma qualificada desenvolveram seus trabalhos que aqui serão apresentados de maneira concisa e didática. Sabemos o quão importante é a divulgação científica, por isso evidenciamos também a estrutura da Atena Editora capaz de oferecer uma plataforma consolidada e confiável para estes pesquisadores exporem e divulguem seus resultados. Boa leitura!

Débora Luana Ribeiro Pessoa

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Data de aceite: 01/09/2021
Data de submissão: 30/06/2021

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ABSTRACT: Lichens produce several classes of phenolic compounds, including depsides, depsidones, usnic acids, dibenzofuranes,

xanthones, anthraquinones, pulvinic acid derivatives and aliphatic acids. Chagas disease is caused by *Trypanosoma cruzi* and affect millions of people becaming a public health and economic problems and it is considered as an endemic disease in 21 american countrys such as Brazil, Argentina, Bolivia, Colombia and Mexico and a neglected tropical disease by the mainly health authorities of the world. The lichen sample of *Parmotrema dilatum* (Vainio) Hale was collected in Piraputanga, municipality of Aquidauna, Mato Grosso do Sul (Brazil). The sample was cleaned under a magnifying glass, stereomicroscope, using brush and forceps to remove foreign materials and pieces of substrate. After cleaning, the samples were fragmented using scissors and extracted using acetone, previously dried in sodium sulfate (Na_2SO_4). The extract composition was evaluated by LC-DAD-MS/MS and through UV, MS, and MS/MS. Exponential *T. cruzi* culture epimastigotes were used for the evaluation of the antiproliferative activity on epimastigote forms by MTS (3-(4,5-dimetilthyazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium). The results obtained were compared with prior published data as their trypanocidal activity not only of the extract but also of isolated major compounds.
KEYWORDS: lichen, *Parmotrema dilatatum, Trypanosoma cruzi,* trypanocidal activity, biological activity.

ISOLAMENTO DOS PRINCIPAIS METABÓLITOS SECUNDÁRIOS E AVALIAÇÃO TRIPANOCIDA DE ESPÉCIES DO GÊNERO *PARMOTREMA*

RESUMO: Os liquens produzem várias classes de compostos fenólicos, incluindo depsídeos, depsidonas, ácidos úsnicos, dibenzofuranos, xantonas, antraquinonas, derivados do ácido pulvínico e ácidos alifáticos. A doença de Chagas é causada pelo *Trypanosoma cruzi* e afeta milhões de pessoas se tornando um problema de saúde pública e econômica, sendo considerada uma doença parasitária endêmica em 21 países das Américas, com destaque ao Brasil, Argentina, Bolívia, Colômbia e México e tropical negligenciada pelas principais autoridades de sáude mundiais. A amostra do *líquen Parmotrema dilatum* (Vainio) Hale oi coletada em Piraputanga, município de Aquidauana, Mato Grosso do Sul (Brasil). A amostra foi limpa sob lupa, estereomicroscópio, utilizando escova e pinça para remoção de materiais estranhos e pedaços de substrato. Após a limpeza, a amostra foi fragmentada com tesoura e extraída com acetona, previamente seca em sulfato de sódio (Na2SO4). A composição do *extrato* foi avaliada por LC-DAD-MS / MS e através de UV, MS e MS / MS. Epimastigotas exponenciais de cultura de *T.* cruzi foram utilizadas para avaliação da atividade antiproliferativa em formas epimastigotas por MTS (3- (4,5-dimetiltiaazol-2-il) -5- (3-carboximetoxifenil) -2- (4-sulfofenil) -2H-tetrazólio). Os resultados obtidos foram comparados com dados publicados anteriormente quanto à atividade tripanocida não apenas do extrato, mas também de compostos principais isolados.
PALAVRAS - CHAVE: líquen*, Parmotrema dilatatum*, *Trypanosoma cruzi*, atividade tripanocida, atividade biológica.

1 | INTRODUCTION

Lichens are organisms that present a unique metabolism, producing exclusive secondary metabolites (Carvalho et al., 2005). These organisms are associations between fungi (Kingdom Fungi) and green algae (Kingdom Protista) or cyanobacteria (Kingdom Eubacteria), and this relationship is a symbiosis in which the fungus provides an adequate environment for algae, which through photosynthesis provide essential organic compounds to lichens (Raven, Evert, Eichhorn, 2007). However, this vision is sometimes contested by specialists, and recently a new definition of lichens was proposed (Hawksworth & Grube, 2020): "A lichen is a self-sustaining ecosystem formed by the interaction of an exhabitant fungus and an extracellular arrangement of one or more photosynthetic partners and an indeterminate number of other microscopic organisms". Several biological activities have been described by lichens, such as antiviral activity against hepatitis C vírus (Vu et al., 2015), anti-inflammatory and analgesic properties (Bugni et al., 2009).

The primary aromatic compounds produced by lichens are generated through polyketide route. Lichens have been used with medicinal purposes since the ancient times. For instance, *Usnea barbata* (L.) Weber ex F.H. Wigg (Parmeliaceae) and other *Usnea* species were used to treat hair-related diseases, *Lobaria pulmonaria* (L.) Hoffm. (Lobariaceae) and *Parmelia sulcata* Taylor (Parmeliaceae) for pulmonary and cranial diseases, respectively, yellow-orange colored *Xanthoria parietina* (L.) Th. Fr. (Teloschistaceae) for jaundice, *Peltigera aphthosa* (L.) Willd. (Peltigeraceae) for aphta, and *Parmelia saxatilis* (L.) Ach. (Parmeliaceae) for epilepsy (Brodo et al., 2001; Malhotra et al., 2008; Fernandez-Moriano et al., 2015).

Despite the potencial use of lichens extracts and compounds as chemotherapic treatments, few studies have been performed on their activity against parasitic protozoa (Carvalho et al., 2005). Chagas disease is caused by *Trypanosoma cruzi* and affect millions of people living in developing countries, causing great disruption of quality of life. There is more than a century of Chagas disease discovery and it is still a serious health and economic problem, being considered as an endemic disease in more than 21 countries. About 6 to 8 million people worldwide are infected with these protozoa, causing about 12,000 deaths per year (Lee et al., 2013).

The morbidity and mortality associated with this disease is superior to other neglected ones (malaria, schistosomiasis, and leishmaniasis) (Martins-Melo et al., 2016). Benznidazole is the first-line drug for the treatment of Chagas disease (Davanço et al., 2016) and this medicine shows good efficacy in the acute phase of the disease (80–90% cure), however it has limited cure efficacy in the chronic phase (8–20% cure) (Bern, 2015). Other problems are associated with benznidazol treatment, such as high administered doses, long term treatment and high incidence of adverse reactions (Palmeiro-Roldan et al., 2014; Bermudez et al., 2016). Thus, the discovery of new treatments to this disease is

of great pharmaceutical interest.

Due to the urgent need for new treatments for chagas disease, and considering that lichens are organisms that present a wide spectrum of biological activities, the aim of this study was to evaluate the chemical composition of lichen *Parmotrema dilatatum (Vainio) Hale* extract by LC-DAD-MS/MS, as well as evaluating the trypanocidal activity of the extract and its isolated major compounds (protocetraric acid, usnic acid and atranorin) by MTS method (3- (4.5-dimethyl-2-thiazolyl) -5- (3-carboxymethoxyphenyl) -2- (4-sulfophenyl) -2H-tetrazolio).

2 | MATERIALS AND METHODS

2.1 Sample, extraction and isolation of lichen compounds

The lichen sample of *P. dilatum* was collected in Piraputanga, Municipality of Aquidauana, Mato Grosso do Sul (Brazil) and identified by professor Dr. Adriano Afonso Spielmann. A voucher specimen was deposited in CGMS herbarium at the Federal University of Mato Grosso do Sul under number 49840. This specie is registered at SisGen platform (entry A4CE261). The samples were cleaned with a magnifying glass, stereomicroscope, brush and forceps to remove foreign materials and pieces of substrate. After cleaning, the samples were fragmented using scissors. After cleaning, the samples were fragmented using scissors and extracted using acetone, previously dried in sodium sulfate (Na_2SO_4). Extractions were performed 3 times of 30 minutes in ultrasound and after were dried in an exhaust chapel and stored in a desiccator until the analysis.

The atranorin, usnic acid and protocetraric acid compounds were isolated according to the procedure described by Honda et al. (2010) from the acetonic extract of *P. dilatatum*. The compounds were analyzed by NMR (^{1}H and ^{13}C) (Bruker DRX300 spectrometer with the deuterated solvent).

2.2 Identification of costituents by LC-DAD-MS/MS

The analysis of the chemical profile of *P. dilatatum* extract was performed by a Shimadzu Prominence UFLC Shimadzu device coupled to a diode array detector (DAD) and mass spectrometer MicrOTOF-Q III (Bruker Daltonics) was used. The column was a Kinetex C18 column (2.6 μm, 150 ´ 2.1 mm, Phenomenex). The samples were prepared at 300 μg/mL, filtered in PTFE filters (Millex 0.22 mm X 13 mm, Millipore) and 1 μL was injected into the chromatographic system at a temperature of 50 ºC and a flow of 0.3 mL/minute. The mobile phase used was acetonitrile (B) and water (A), both added with 0.1% formic acid (v/v). Elution was performed in gradient mode at the following concentrations: 0 to 8 minutes 3% of B, 8 to 30 minutes 3 to 25% of B, 30 to 60 minutes 25 to 80% of B, 60 to 63 minutes 80% of B, 63 to 68 minutes 80 to 3% of B. Nitrogen was used as a nebulizer gas (4 bar) and drying gas (9 L/min), applying a capillary voltage of 2500 Kv. The analyses were performed

in negative ion mode. The compounds were identified based on UV spectra, the accurate masses and fragments obtained by MS/MS, which were compared with published data and isolated standards.

2.3 Antitrypanosomal activity

The protozoan used in the biological assays, belonging to the species *Trypanosoma cruzi*, was isolated from *Didelphis marsupialis*, a skunk species. The isolation was performed at the University of Carobobo, Venezuela. Axenic culture was established *in vitro* with LIT (Liver Infusion Tryptose) medium and a clonal population was obtained. The *T. cruzi* clone Dm28c was biologically characterized and an aliquot was kindly provided for use in research by the Carlos Chagas-Fiocruz Institute/PR.

Epimastigote forms of *T. cruzi*, clone Dm28c, were kept at 28 °C in LIT medium supplemented with 10% inactivated fetal bovine serum, with passages at every three days. Exponential *T. cruzi* culture epimastigotes were used for the experiments. The antiproliferative activity on epimastigote forms was evaluated by MTS (3-(4,5-dimetilthyazol-2-yl)-5-(3-carboxymethoxyphenyl)-2- (4 sulfophenyl)-2H-tetrazolium) assay. The cells were seeded in 96-well tissue plates at a density of $1x10^6$ cell/mL and incubated with several concentrations of extract or isolated compounds at 28 °C for 72h. As a control, the parasites were kept in LIT medium without addition of compounds. After adding the MTS/PMS the plate was incubated at 28 °C for 4h. The absorbance was evaluated in a microplate reader (µQuant) at 490 nm. The assays were performed in triplicate.

3 I RESULTS AND DISCUSSION

Lichens are able to synthesize several metabolites, often called lichen acids, most of which are unique and exclusive to lichens, while some of the structures may also be found in other living organisms (i.e. carotenoids, anthraquinones in vegetals). These metabolites comprise aliphatic, cycloaliphatic, aromatic and terpenic compounds (Studzińska-Sroka, 2017; Huneck, 1996). In this study the lichen *P. dilatatum* extract was evaluated by LC-DAD-MS/MS and was possible to identify three compounds (Table 1) based on UV, MS, and MS/MS data compared with published data (Huneck; Yoshimura, 1996; Kumar et al., 2018).

Peak	RT (min)	Compound	UV (nm)	Class	MF	Negative mode (*m/z*)	
						MS $[M-H]^-$	MS/MS
1	35.8	Protocetraric acid[st]	243, 315, 317	Depsidone	$C_{18}H_{14}O_3$	373.0582	177,197, 213, 229, 239, 255, 267, 285, 311
2	51.8	Usnic acid[st]	282	Dibenzofuran	$C_{18}H_{16}O_7$	343.0832	231, 259, 286, 299, 313

3	52.7	Atranorin[st]	282, 323	Depside	$C_{18}H_{18}O_8$	373.0906	163,177, 181

Table 1. Constituents identified from the *P. dilatum* extract by LC-DAD-MS/MS.

RT: retention time; MF: molecular formula; [st]: confirmed by authentic standard.

The Peak 1 (rt 35.8 min) was identified as protocetraric acid. The λ_{max} were 243, 315 and 317 nm and *m/z* 373.0582 $[M-H]^-$ was compatible with molecular formula $C_{18}H_{16}O_7$. The main fragment ions were *m/z* 177, *m/z* 197, *m/z* 213, *m/z* 229, *m/z* 239, *m/z* 255 $[M-H-2CO_2-OCH_2]^-$, *m/z* 267 $[M-H-2CO_2-H_2O]^-$, *m/z* 285 $[M-H-2CO_2]^-$ and *m/z* 311 $[M-H-CO_2-H_2O]^-$. The injection of authentic standard allowed the confirmation of protocetraric acid structure.

The peak 2 (rt 51.8 min) showed λ_{max} 282 nm and *m/z* 343.0832 $[M-H]^-$ that was compatible with molecular formula $C_{18}H_{16}O_7$. This ion yelded the fragment ions *m/z* 231, *m/z* 259, *m/z* 286, *m/z* 299 and 313 $[M-H-CH_3-CH_3]^-$. The compound was identified as usnic acid (Kumar et al., 2018) and confirmed by injection of authentic standard.

The peak 3 (rt 52.6 min) with λ_{max} 282 and 323 nm presented *m/z* 373.0906 $[M-H]^-$ compatible with $C_{19}H_{18}O_8$. The fragment ions obtained *m/z* 163 $[M-H-CH_3]^-$, *m/z* 177 $[M-H-H_2O]^-$ and *m/z* 181 $[M-H-CH_3]^-$ were compatible with ester cleavage of the depside atranorin. This compound was confirmed by injection of authentic standard.

The isolated compounds (Figure 1) were also analyzed by RMN ^1H and ^{13}C and the structures were confirmed by literature data (König & Wright 1999; Sundholm & Huneck, 1981; Huneck & Yoshimura, 1996).

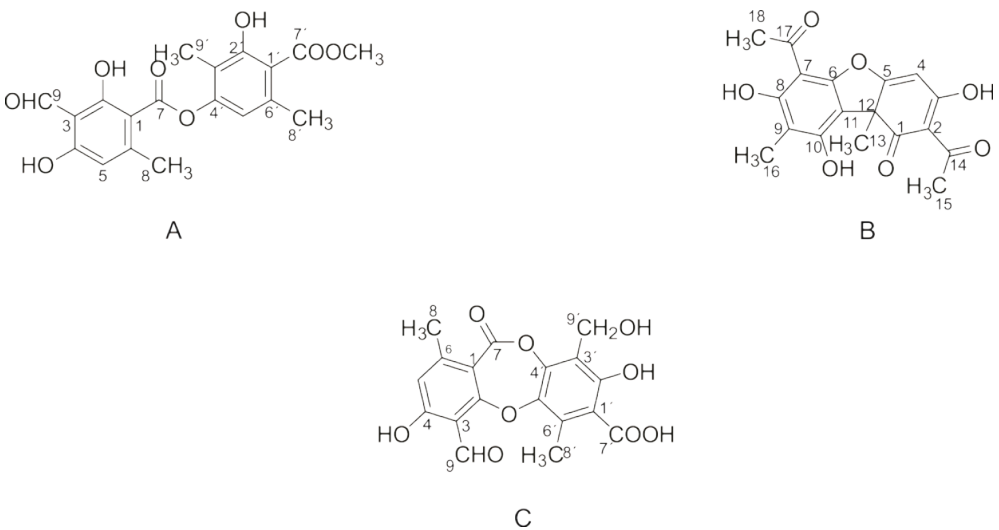

Figure 1. Structure of (A) atranorin, (B) usnic acid and (C) protocetraric acid.

Atranorin (Figure 1A): **^{1}H-RMN** (300 MHz, $CDCl_3$) δ 2,07 (s, 3H, CH_3-8'), 2,52 (s, 3H, CH_3-9'), 2,67 (s, 3H, CH_3-8), 3,96 (s, 3H, $COOCH_3$), 6,38 (s, 1H, H-5), 6,49 (s, 1H, H-5'), 10,33 (s, 1H, CHO), 11,95 (s, 1H, OH-2'), 12,50 (s, 1H, OH-4), 12,55 (s, 1H, OH-2). **^{13}C-RMN** (75 MHz, CDCl3) δ 9,34 (C-8'), 24,0 (C-9'), 25,5 (C-8), 52,3 ($COOCH_3$), 102,7 (C-1), 108,4 (C-3), 110,2 (C-1'), 112,8 (C-5), 116,0 (C-5'), 116,7 (C-3'), 139,8 (C-6'), 151,9 (C-4'), 152,4 (C-6), 162,8 (C-2'), 167,4 (C-4), 169,0 (C-2), 169,6 (C-7), 172,1 (C-7'), 193,8 (C-9) (König and Wright 1999).

Usnic acid (Figure 1B): **^{1}H-RMN** (300 MHz, $CDCl_3$) δ 1,73 (s, 3H, CH_3-13), 2,08 (s,3H, CH_3-16), 2,65 (s, 6H, CH_3-15, CH_3-18), 6,0 (s, 1H, H-4), 11,0 (s, 1H, OH-10), 13,29 (s, 1H, OH-8), 18,82 (s, 1H, OH-3). **^{13}C- RMN** (75 MHz, $CDCl_3$) δ 8,2 (C-16), 28,6 (C-13), 31,9 (C-15), 32,8 (C- 18), 59,7 (C-12), 99,0 (C-4', C-6), 101,6 (C-5), 104,0 (C-9), 105,7 (C-11), 109,9 (C-7), 155,8(C-3), 158,1 (C-8), 163,7 (C-10), 180,0 (C-2), 198,7 (C-1), 201,0 (C- 17), 202,5 (C-14) (König and Wright, 1999). [α]D + 495.5° a 23°C ($CHCl_3$).

Protocetraric acid (Figure 1C): **^{1}H-RMN** (300 MHz, DMSO-d_6,) δ 2,38 (s, 3H, CH_3-8'), 2,40 (s, 3H, CH_3-8), 4,61 (s, 2H, CH_2OH-9'), 6,83 (s, 1H, H-5), 10,57 (s, 1H, CHO), 11,94 (sl, 1H, OH-4). **^{13}C-RMN** (75 MHz, DMSO-d_6) δ 14,4 (C- 8'), 21,4 (C-8), 52,8 (C-9'), 111,8 (C-3), 112,4 (C-1), 116,7 (C-1'), 117,1 (C-5), 118,7 (C-3'), 129,3 (C-6'), 141,8 (C-5'), 144,5 (C-4'), 152,0 (C-6), 154,4 (C-2'), 161,1 (C-7), 163,8 (C-2), 163,9 (C-4), 170,1 (C-7'), 191,7 (C-9) (Sundholm and Huneck, 1981; Huneck and Yoshimura, 1996).

The effects of *Parmotrema dilatum* extract and its isolated compounds (atranorin, usnic acid and protocetraric acid) on epimastigote forms of *T. cruzi* are shown in table 2.

Sample	IC_{50} (μg/mL)
Parmotrema dilatatum (Vainio) Hale	36.04
Atranorin	NA*
Usnic acid	NA*
Protocetraric acid	NA**
Benznidazol	4.20

Table 2. Evaluation of antitrypanosomal activity of *Parmotrema dilatum* extract and its isolated compounds.

IC_{50}: concentration that inhibited 50% of epimastigote forms of *T. cruzi*; determined by MTS assay. *Not active at 150 μg/mL. **Not active at 100 μg/mL.

The *Parmotrema dilatum* extract showed an IC_{50} of 36.04 μg/mL against epimastigote forms of *T. cruzi*, while the standard benznidazol presented an IC_{50} of 4.20 μg/mL. In order to know the compounds that are responsible for antitrypanosomal activity in lichen extract,

we isolated its compounds and tested against *T. cruzi*.

Atranorin is one of the major depside metabolites from lichens, and exhibits a versatile biological roles, such as in scavenging free radical (Khader et al., 2019), antiinflammatory, analgesic, antibacterial, antifungal, antioxidant, antiviral (Studzinska-Sroka et al., 2017), as well as suppressive activity in several solid tumors and leukaemia, for example on colorectal carcinoma cells (HCT-116, DLD-1 and HT-29) (Paluszczak et al., 2018; Backorova et al., 2012), on A549 human lung cancer cells (Zhou et al., 2017), on hepatocellular carcinoma cells (SKHep1, Huh-7 and SNU-182) (Jeon et al., 2019), and on P388 murine leukaemia cell line (Dias and Urban, 2009).

Atranorin has been also shown antiparasitic activity. Zofou et al. (2011) showed that atranorin was able to inhibit three strains (W-2, CAM10 and SHF4) of *Plasmodim falciparum,* and Zofou et al. (2012) observed a good antimalarial activity of atranorin, with IC_{50} lower than 5 μM against W2mef *Plasmodium falciparum* strain. Despite the antimalarial potential presented by atranorin, in this study this substance showed no activity against epimastigote forms of *T. cruzi* of 50 μg/mL.

Protocetraric acid has been shown a wide antimicrobial spectrum (Manojlovic et al. 2012; Prateeksha et al. 2016; Dieu et al., 2019), with significant MIC against *Salmonella typhi* (0.5 μg/mL), *Klebsiella pneumoniae* (1 μg/mL), and *Trychophyton rubrum* (1 μg/mL), demonstrating a result better than ciprofloxacin and amphotericin B, the standards clinically used drug (Nishanth et al., 2015). Protocetraric acid has been also demonstrated cytotoxic properties on UACC-62 (human melanoma), B16-F10 (murine melanoma) (Brandão et al., 2013), FemX (human melanoma) and LS174 (human colon carcinoma) cells.

In addition, protocetraric acid isoalted from *Parmotrema dilatatum* influenced the development of *Lactuca sativa* seedings (Tigre et al., 2012). Hauck and Huneck (2007) tested the hypothesis that lichen substances can control the uptake of toxic metals by adsorbing metal ions at cation exchange sites on cell walls, and observed that protocetraric acid was able to reduce the adsorption of Mn^{2+}, Na^{+} and Fe^{3+}. In our study protocetraric acid was not active against *T. cruzi* parasite, as well as was found by Igoli et al. (2014) on *Trypanosoma brucei brucei*.

Usnic acid exists naturally as both (+) D-usnic acid and (–) L-usnic acid enantiomers, with R or S projection of the stereogenic. Enantiomer compounds do not substantially differ in terms of certain physical properties, like solubility, melting point or spectroscopic characteristics (O¨ zek et al. 2010), but may have a different aroma or flavour (Silva et al. 2012) and also may reveal differences in their biological and pharmacological activity (Nguyen et al. 2006).

Usnic acid and its derivatives have been shown a large spectrum of biological activities, such as antimicrobial, including antiviral activity against influenza viruses, antiproliferative, antiinflammatory, antioxidant, and analgesic activities (Okuyama et al., 1995; Lauterwein et al., 1995; Ingolfsdottir et al., 1998; Ogmundsdottir et al., 1998; Shtro et

al., 2015; Oran et al., 2016; Vanga et al., 2017). Many of the biological activities have not been attributed to one or the other enantiomeric of usnic acid.

However, some studies show that the enantiomers exhibit different potency in biological activities. Cetin et al. (2008) examined the inseticidal activity of usnic acid enantiomers against *Culex pipiens* larvae and observed that although both enantiomers provoked strong mortality of the larvae, the left-handed enantiomer was more active than right-handed form, with LD_{50} value more than 10 times lower. In addition, Wu et al. (1995) verified that (-) usnic acid exhibited a strong effect against *Trichomonas vaginalis in vitro*.

For anti-*T.cruzi* activity, Carvalho et al. (2005) verified that usnic acid isolated from lichen *Cladonia substellata* inhibited the growth of epimastigote forms of *T.cruzi in vitro* in a dosis dependent manner, causing damage to mitochondria in concentrations of 5-30 μg/mL. However, in the present study, the usnic acid isolated from lichen *P. dilatatum* did not show anti-*T. cruzi* activity until 150 μg/mL, suggesting that the difference in results may be due to different enantiomerical forms of usnic acid.

As with other chiral substances that are produced in living organisms, usnic acid is present in lichen species as one enantiomer, usually with the predominance of one single isomer form, or as a scalemic mixture of enantiomers (Galanty et al., 2019).

In this way, as the isolated lichen compounds (atranorin, usnic acid and protocetraric acid) did not show activity against *T. cruzi*, it is suggested that the activity presented by the extract may be due to synergism between the compounds. Synergistic effects can be produced if the constituents of an extract or a drug combination affect different targets or interact with one another in order to improve the solubility and thereby enhance the bioavailability of one or more substances in the mixture (Wagner and Ulrich-Merzenich 2009).

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