Effects of the hydroalcoholic extract of leaves and fruits of *Physalis pubescens* L. on antioxidative and microbiological parameters

Efeitos do extrato hidroalcoólico de folhas e frutos de *Physalis pubescens* L. sobre parâmetros antioxidantes e microbiológicos

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ABSTRACT
Species from Physalis genus (Solanaceae family) have various therapeutic activities, such as anti-inflammatory, antitumor and antihyperglycemic. However, there are few studies about microbiological effects of this Physalis pubescens specie. The aim of this work was to investigate on antioxidative and microbiological parameters of the hydroalcoholic extract of the leaves and fruits of Physalis pubescens L. The phytochemical analysis was realized by High Performance Liquid Chromatography - Diode Array Detector and Evaluation of antioxidant potential in vitro was with classical methodologies. The antimicrobial activity of the extracts of was to investigate against Candida spp., Sporothrix spp. and Staphylococcus aureus and determine Minimum Inhibitory Concentration (MIC), Minimum Fungicidal Concentration (MFC) and Minimum Bactericidal Concentration (MBC). Leaves and fruits extracts inhibited Candida albicans (ATCC 10231, ATCC 10234, ATCC 18804, ATCC 28367, clinical isolates 7B and 3C) and Candida parapsilosis (ATCC 22019), leaves extract also inhibited Candida krusei (ATCC 6258). Leaves and fruits extracts inhibited Sporothrix schenckii SN, leaves extract also inhibited Sporothrix brasiliensis 8309 and Sporothrix schenckii 67 MRV. Leaves and fruits extracts inhibited Staphylococcus aureus (clinical isolates MRSA-2 and MSSA-4), leaves extract also inhibited Staphylococcus aureus (ATCC 25923, clinical isolates MRSA-3, MRSA-4, MRSA-5, MRSA-6, MRSA-7, MRSA-8, MRSA-9 and MSSA-3). In the phytochemical analysis, the presence of catechin was observed as the major component both in the extract of leaves and fruits and elevated antioxidant potential in vitro. Therefore, are needs for further studies for this species and constituents in order to elucidate the promising effects.

Keywords: Physalis pubescens L., antioxidant parameters, microbiological assays, Candida spp., Sporothrix spp., Staphylococcus aureus.

RESUMO
As espécies do gênero Physalis (família Solanaceae) têm várias atividades terapêuticas, como anti-inflamatória, antitumoral e anti-hiperglicêmica. Entretanto, há poucos estudos sobre os efeitos microbiológicos dessa espécie Physalis pubescens. O objetivo deste trabalho foi investigar os parâmetros antioxidantes e microbiológicos do extrato hidroalcoólico das folhas e dos frutos de Physalis pubescens L. A análise fitoquímica foi realizada por Cromatografia Líquida de AltaEficiência - Detector de Arranjo de Diodos e a avaliação do potencial antioxidante in vitro foi feita com metodologias clássicas. A atividade antimicrobiana dos extratos de foi investigada contra Candida spp., Sporothrix spp. e Staphylococcus aureus e determinada a Concentração Inibitória Mínima (MIC), a Concentração Fungicida Mínima (MFC) e a Concentração Bactericida Mínima (MBC). Os extratos das folhas e dos frutos inibiram a Candida albicans (ATCC 10231, ATCC 10234, ATCC 18804, ATCC 28367, isolados clínicos 7B e 3C) e a Candida parapsilosis (ATCC 22019); o extrato das folhas também
inhibited the Candida krusei (ATCC 6258). The extracts of the leaves and fruits inhibited Sporothrix schenckii SN, the extract of the leaves also inhibited Sporothrix brasiliensis 8309 and Sporothrix schenckii 67 MRV. The extracts of the leaves and fruits inhibited Staphylococcus aureus (isolation clinicals MRSA-2 and MSSA-4), and the extract of the leaves also inhibited Staphylococcus aureus (ATCC 25923, isolation clinicals MRSA-3, MRSA-4, MRSA-5, MRSA-6, MRSA-7, MRSA-8, MRSA-9 and MSSA-3). In the phytochemical analysis, catechin was observed as the main component in the extract of the leaves and fruits, as well as a high potential antioxidant in vitro. Therefore, more studies are necessary for this species and its constituents to elucidate the promising effects.

Keywords: Physalis pubescens L., antioxidant parameters, microbiological tests, Candida spp., Sporothrix spp. and Staphylococcus aureus.

1 INTRODUCTION

A medical popular use of the medical plants with therapeutic resource, a millenarian trend widely diffused associated with the growing demand not only for natural products, also to phytotherapics medicines, functional foods and their constituents has contributed significantly to the consumption of certain foods and/or plants (WHO, 2012, 2013). In Brazil, the use of functional food is expanding, contributing to the effectiveness of health promotion. Functional foods produce metabolic and/or physiological effects that are beneficial to health and, in the majority, present compounds with antioxidant activity (ANWAR & PRZYBYLSKI, 2012).

In this context, Physalis pubescens L. appears widely used as functional food and in traditional medicine, as well as decorative element in culinary and ornamentation. The genus Physalis belongs to the family Solanaceae and has approximately 12 species in South America (SOARES et al., 2009). The species of the genus Physalis are popularly used as hypoglycemic, hypcholesterolemic, anti-inflammatory, antitumor and antimicrobial agents (MONIRUZZAMAN et al., 2016; YANG et al., 2016).

The demands for plants with antimicrobial and antifungal properties have been growing increasingly, as a result of the resistance of microorganisms to clinical therapy. Plants of the genus Physalis are popularly used in the treatment of diseases, including those caused by fungi and bacteria (PIETRO et al., 2000; YANG et al., 2014).

The aim of this work was to investigate on antioxidative and microbiological parameters of the hydroalcoholic extract of the leaves and fruits of Physalis pubescens L.
2 MATERIALS AND METHODS

2.1 PLANT MATERIAL

The leaves and fruits of Physalis pubescens L., Solanaceae family, were collected in the southern region of Brazil, Giruá, Rio Grande do Sul (Latitude 28°01’42”S; Longitude 54°20’59”W), in December, 2018. A voucher specimen of the plant material was deposited in Herbarium by Universidade Federal do Pampa (UNIPAMPA), Uruguaiana, Rio Grande do Sul, Brazil and identified by reference number 140.

2.1.1 Preparation of the Extracts of Physalis Pubescens L.

The dried leaves at room temperature and lowest light and fresh fruits were triturated and macerated at room temperature in hydroalcoholic solution (30 H₂O:70 Ethanol v/v) at concentration of 10 g per 100 mL of solvent for a week under daily shaking. The maceration process was repeated for two more weeks to exhaustion of the plant material. In the end of three weeks, the filtrates were pooled and evaporated under reduced pressure (80 mbar) in a rotary evaporator (70 rpm, temperature at 40°C ± 1°C) in order to remove ethanol. After the extract was taken to freeze-drying remained where the temperature at -56°C to completely dry powder, which resulted in a yield of 20.1% leaves extract and 14.7% fruits extract. The dry powder of leaves and fruits of Physalis pubescens L. were used in the following tests.

2.2 PHYTOCHEMICAL ANALYSIS

The phytochemical analysis was realized by High Performance Liquid Chromatography - Diode Array Detector (HPLC-DAD) was performed with a Shimadzu Prominence Auto Sampler (SIL-20A) HPLC system (Shimadzu, Kyoto, Japan), equipped with Shimadzu LC-20AT reciprocating pumps connected to a DGU 20A5 degasser with a CBM 20A integrator, SPD-M20A diode array detector and LC solution 1.22 SP1 software.

Physalis pubescens at a concentration of 10 mg/mL was injected by means of a model SIL-20A Shimadzu Auto sampler. Separations were carried out using Phenomenex C₁₈ column (4.6 mm x 250 mm x 5 μm particle size). The mobile phase was water with 1% phosphoric acid (v/v) (solvent A) and HPLC grade methanol (solvent B) at a flow rate of 0.6 mL/min and injection volume 40 μL. The composition gradient was: 5% solvent B reaching 15% at 10 min; 30% solvent B at 25 min, 65% solvent B at 40 min and 98% solvent B at 60 min, followed by 70 min at isocratic elution until 75 min. At 80 min the gradient reached the initial conditions again, following the method described by Carvalho et al. (2016) with slight modifications. The sample and mobile phase were filtered through 0.45 μm membrane filter (Millipore) and then
degassed by ultrasonic bath prior to use. Stock solutions of standards references were prepared in the acetonitrile: water (1:1, v/v) at a concentration range of 0.025 – 0.500 mg/mL. Quantifications were carried out by integration of the peaks using the external standard method, at 254 nm for gallic acid and ellagic acid; 280 nm for catechin; 327 nm for p-coumaric acid and chlorogenic acid; and 366 for rutin and kaempferol. The chromatography peaks were confirmed by comparing its retention time with those of reference standards and by DAD spectra (200 to 600 nm). Calibration curve for gallic acid: \( Y = 13047x + 1195.6 \) (\( r = 0.9999 \)); for chlorogenic acid: \( Y = 11953x + 10278.4 \) (\( r = 0.9998 \)); \( p \)-coumaric acid: \( Y = 12459x + 1405.1 \) (\( r = 0.9996 \)); ellagic acid: \( Y = 13283x + 11093.7 \) (\( r = 0.9997 \)); catechin: \( Y = 10986x + 1309.5 \) (\( r = 0.9999 \)); rutin: \( Y = 12384x + 10926.2 \) (\( r = 0.9998 \)) and kaempferol: \( Y = 11835x + 1548.7 \) (\( r = 0.9995 \)). All chromatography operations were carried out at ambient temperature and in triplicate.

### 2.2.1 Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ were calculated based on the standard deviation of the responses and the slope using three independent analytical curves, as defined by da Silva et al. (2016). LOD and LOQ were calculated as 3.3 and 10 \( \sigma \)/S, respectively, where \( \sigma \) is the standard deviation of the response and S is the slope of the calibration curve.

### 2.3 EVALUATION OF ANTIOXIDANT POTENTIAL IN VITRO

The antioxidant potential of the leaves (LEP) and fruits (FEP) extracts of *Physalis pubescens* L. performed by 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sharma & Bhat, 2009), 2,2′-azino-bis-3-ethylbenzthiazoline-6-carboxylic acid (ABTS) (Erel, 2004). and total polyphenol content were measured in six different concentrations (0.025 mg/mL, 0.05 mg/mL, 0.1 mg/mL, 0.2 mg/mL, 0.25 mg/mL, 0.50 mg/mL) of the extracts (Singleton et al. 1999).

### 2.4 MICROBIOLOGICAL ASSAY

#### 2.4.1 Extract Preparation

The powder were heavy and 0.1 g of dry powder of leaves and fruits of *Physalis pubescens* L. were solubilized in 1.0 mL of hydroalcoholic solution (30 H₂O:70 Ethanol v/v), final concentration of 100,000 µg/mL. Subsequently, dilutions in culture media were held to obtain final concentrations ranges of 15.6 to 8000 µg/mL, the concentrations were determined by previous screening.
2.4.2 Microorganisms

A total of 33 strains were used, all strains used were from microorganisms library of the Microbiology Laboratory of the UNIPAMPA and were chosen because of their availability access.

Eleven belonged to Candida spp.: Candida albicans (American Type Culture Collection - ATCC 10231, ATCC 10234, ATCC 18804, ATCC 28367, clinical isolates 7B and 3C); Candida dubliniensis (ATCC 7987), Candida krusei (ATCC 6258 and ATCC 20298); Candida parapsilosis (ATCC 22019) and Candida tropicalis (ATCC 750).

Eleven belonged to Sporothrix spp.: Sporothrix brasiliensis (13 and 8309); Sporothrix globosa (16490) and Sporothrix schenckii (424, 853, 364595, clinical isolates 08 UFSM, 67 MRV, STT RJ, Santa Casa II and SN).


2.4.3 Determination of the MIC for Yeasts and Filamentous Fungi

2.4.3.1 Evaluation of the Antifungal Activity Against Candida spp.

The MIC of hydroalcoholic extracts of leaves and fruits of Physalis pubescens L. and ethanol 70% were determined by using broth microdilution techniques as described by the Clinical and Laboratory Standards Institute for yeasts (M27-A2) (NCCLS, 2002b). The strains were subcultured onto Sabouraud dextrose agar at 35°C for 24 h. The inoculum was suspended in saline solution and adjusted to a final concentration of 0.5 x 10² in RPMI 1640 buffered to pH 7.0 with 165 mmol/L Morpholinopropanosulfonic Acid (MOPS). Fluconazole (Jansen-Cilag®) was used as drug control, in the final concentrations ranged from 0.06 to 32 µg/mL, as described by M27-A2 protocol. Ethanol 70% was used as control of the solvent, in the final concentrations ranged from 0.01 to 5.6%. was added the volume of ethanol equivalent concentrations of the extract ranges from 15.6 to 8000 µg/mL. Sterilized round-bottomed 96-well microtiter plates (Cral Plast) were used, with addition of 100 µL of each extract or drug to columns 1 to 10 and 100 µL of RPMI 1640-MOPS. Aliquots of 100 µL of the standardized inoculums were added to the wells and the microtiter plates were incubated at 35°C for 24 h. After incubation, the MIC was determined visually. The MIC was defined as the lowest concentration of the antifungal agent preventing visible fungal growth and this concentration was defined as fungistatic action. The experiments were conducted in triplicate for the
hydroalcoholic extracts of leaves and fruits of *Physalis pubescens* L., control of the solvent and drug control.

2.4.3.2 Evaluation of the Antifungal Activity Against Sporothrix spp.

The MIC of hydroalcoholic extracts of leaves and fruits of *Physalis pubescens* L. and ethanol 70% were determined by using broth microdilution techniques as described by the Clinical and Laboratory Standards Institute for filamentous fungi (M38-A2) (NCCLS, 2002a). Strains were subcultured onto potato dextrose agar at 35°C for 7 days. The surface was gently scraped with a sterile bent glass after flooding with sterile saline solution. The standard suspensions were adjusted by UV-visible spectrophotometry (Spectrum Instruments Co., Shanghai, China) to show transmittance at 530 nm of 80 - 82%. Adjusted suspensions were diluted in RPMI 1640-MOPS (1:50) to obtain a final inoculum of 10^4 CFU/mL, and 100 µL of the fungal suspensions were added to each microdilution well containing 100 µL of the extracts.

Itraconazole (Jansen-Cilag®) was used as drug control, in the final concentrations ranged from 0.06 to 32 µg/mL, as described by M38-A2 protocol. Ethanol 70% was used as control of the solvent, in the final concentrations ranged from 0.01 to 5.6%, was added the volume of ethanol equivalent concentrations of the extract ranges from 15.6 to 8000 μg/mL.

Plates were incubated at 35°C for 4 days. The determination of the MIC was performed visually and the MIC was defined as the lowest concentration of the antifungal agent that produced no visible fungal growth and this concentration was defined as fungistatic action. The experiments were conducted in triplicate for the hydroalcoholic extracts of leaves and fruits of *Physalis pubescens* L., control of the solvent and drug control.

2.4.4 Determination of the MFC for Yeasts and Filamentous Fungi

The determination of MFC was performed for all tested microorganisms, conform protocols described by M27-A2 and M38-A2. In order to determine the MFC, 100 µL of all wells with 100% of growth inhibition were seeded into culture tubes with 1 mL of Sabouraud dextrose broth, the tubes were incubated at 35°C for 1 day for *Candida* spp. and for 7 days for *Sporothrix* spp.

The tubes with 100% of growth inhibition were subcultured onto Sabouraud dextrose agar, the plates were incubated at 35°C for 1 day for *Candida* spp. and for 7 days for *Sporothrix* spp. The MFC was defined as the lowest concentration of the antifungal agent preventing visible fungal growth and this concentration was defined as fungicidal action.
2.4.5 Determination of the MIC and MBC: Evaluation of the Antibacterial Activity Against Staphylococcus Aureus

The MIC of hydroalcoholic extracts of leaves and fruits of Physalis pubescens L. and ethanol 70% were determined by using broth microdilution techniques as described by the Clinical and Laboratory Standards Institute for aerobic bacteria (M7-A6) (NCCLS, 2003).

Strains were subcultured onto brain heart infusion agar at 35°C for 1 day. The standard suspensions were adjusted by UV-visible spectrophotometry (Spectrum Instruments Co., Shanghai, China). Adjusted suspensions were diluted in Müller-Hinton broth medium to obtain a final inoculum McFarland de 0.5.

Chloramphenicol (Jansen-Cilag®) was used as drug control, in the final concentrations ranged from 0.03 to 16 µg/mL, as described by M7-A6. Ethanol 70% was used as control of the solvent, the final concentrations ranged from 0.01 to 5.6%, was added the volume of ethanol equivalent concentrations of the extract ranges from 15.6 to 8000 μg/mL.

Plates were incubated at 35°C for 1 day, the determination of the MIC was performed visually. The MIC was defined as the lowest concentration of the bactericidal agent that produced no visible bacterial growth and this concentration was defined as bacteriostatic action. The experiments were conducted in triplicate for the hydroalcoholic extracts of leaves and fruits of Physalis pubescens L., control of the solvent and drug control.

In order to determine the MBC conform protocol described by M7-A6, 100 µL of all wells with 100% of growth inhibition were seeded into culture tubes with 1 mL of Müller-Hinton broth, the tubes were incubated at 35°C for 1 day to determine bacterial growth. The tubes with 100% of growth inhibition were subcultured onto brain heart infusion agar and the plates were incubated at 35°C for 1 day to determine bacterial growth. The MBC was defined as the lowest concentration of the bactericidal agent preventing visible fungal growth and this concentration was defined as bactericidal action.

2.5 STATISTICAL ANALYSIS

Data were expressed as mean ± standard deviation (SD). Comparisons between groups were performed using one-way analysis of variance (ANOVA), followed by post hoc of Bonferroni for multiple comparison tests. Results were considered statistically significant when $p<0.05$. 
3 RESULTS

3.1 PHYTOCHEMICAL ANALYSIS

The HPLC profile of *Physalis pubescens* was also acquired, HPLC analysis was showed in Figure 1 and in Table 1 were expressed the components present in the *Physalis pubescens* extracts. The samples contains other minor compounds in addition to gallic acid (retention time $t_R = 10.95$ min, peak 1), catechin ($t_R = 15.07$ min, peak 2), chlorogenic acid ($t_R = 25.16$ min, peak 3), ellagic acid ($t_R = 32.04$ min, peak 4), $p$-coumaric acid ($t_R = 36.17$ min, peak 5), rutin ($t_R = 46.51$ min, peak 6) and kaempferol ($t_R = 64.09$ min, peak 7).

![Figure 1](image)

**Figure 1** – Representative high performance liquid chromatography profile of *Physalis pubescens*. Gallic acid (peak 1), catechin (peak 2), chlorogenic acid (peak 3), ellagic acid (peak 4), $p$-coumaric acid (peak 5), rutin (peak 6) and kaempferol (peak 7).

<table>
<thead>
<tr>
<th>Compounds</th>
<th><em>P. pubescens</em> Leaves</th>
<th><em>P. pubescens</em> Fruits</th>
<th>LOD</th>
<th>LOQ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/g</td>
<td>mg/g</td>
<td>µg/mL</td>
<td>µg/mL</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>0.19 ± 0.05</td>
<td>-</td>
<td>0.027</td>
<td>0.089</td>
</tr>
<tr>
<td>Catechin</td>
<td>4.73 ± 0.01</td>
<td>5.23 ± 0.01</td>
<td>0.015</td>
<td>0.051</td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>1.68 ± 0.03</td>
<td>1.75 ± 0.02</td>
<td>0.024</td>
<td>0.079</td>
</tr>
<tr>
<td>Ellagic acid</td>
<td>0.21 ± 0.02</td>
<td>0.69 ± 0.04</td>
<td>0.011</td>
<td>0.035</td>
</tr>
<tr>
<td>$p$-Coumaric acid</td>
<td>2.35 ± 0.01</td>
<td>4.32 ± 0.01</td>
<td>0.008</td>
<td>0.026</td>
</tr>
<tr>
<td>Rutin</td>
<td>0.26 ± 0.01</td>
<td>-</td>
<td>0.016</td>
<td>0.052</td>
</tr>
<tr>
<td>Kaempferol</td>
<td>1.59 ± 0.03</td>
<td>4.28 ± 0.05</td>
<td>0.023</td>
<td>0.076</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± standard deviations (SD) of three determinations. Averages followed by different letters differ by Tukey test at $p < 0.05$.

Source: Authors
3.2 EVALUATION OF ANTIOXIDANT POTENTIAL IN VITRO

The in vitro antioxidant activity of the *Physalis pubescens* extracts showed inhibition percentages ranging from 42.1% to 97.9% for ABTS in leaves extracts and 0.2% to 35.8% in fruits extracts. Already, in the leaves extracts 53% to 92.2% for DPPH and 35.4% to 48.1% in fruits extracts. Polyphenol content ranged from 3.02 to 39.09 mg GAE mL⁻¹ for leaves extracts and 0.8% to 13.19% in fruits extracts showing dose-dependent behavior (Figure 2). The leaves extracts showed higher antioxidant activity compared to fruits extracts.

![Figure 2 – Antioxidant activity in vitro of the Physalis pubescens. In A: ABTS; B: DPPH; C: Polyphenol content. In LEP: Leaves Extract Physalis pubescens; FEP: Fruits Extract Physalis pubescens; Ascorbic acid: positive control.](image)

Source: Authors

3.3 MICROBIOLOGICAL ASSAY

3.3.1 Antifungal Activity Against Candida spp.

The results for antifungal activity of the hydroalcoholic extracts of leaves and fruits from *Physalis pubescens* L. against *Candida* spp. were showed in the Table 2. The results showed that most of the yeasts tested were inhibited by different extracts in the highest concentrations tested.
Table 2. Antifungal activity of the hydroalcoholic extract of leaves and fruits from Physalis pubescens against Candida spp.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Leaves Extract (µg/mL)</th>
<th>Fruits Extract (µg/mL)</th>
<th>Fluconazole (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC</td>
<td>MFC</td>
<td>MIC</td>
</tr>
<tr>
<td>Candida albicans</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATCC 10231</td>
<td>8000</td>
<td>&gt;8000</td>
<td>8000</td>
</tr>
<tr>
<td>ATCC 10234</td>
<td>4000</td>
<td>&gt;8000</td>
<td>8000</td>
</tr>
<tr>
<td>ATCC 18804</td>
<td>2000</td>
<td>&gt;8000</td>
<td>4000</td>
</tr>
<tr>
<td>ATCC 28367</td>
<td>2000</td>
<td>&gt;8000</td>
<td>4000</td>
</tr>
<tr>
<td>7B</td>
<td>8000</td>
<td>&gt;8000</td>
<td>4000</td>
</tr>
<tr>
<td>3C</td>
<td>8000</td>
<td>&gt;8000</td>
<td>8000</td>
</tr>
<tr>
<td>Candida dubliniensis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATCC 7987</td>
<td>&gt;8000</td>
<td>&gt;8000</td>
<td>&gt;8000</td>
</tr>
<tr>
<td>Candida krusei</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATCC 6258</td>
<td>8000</td>
<td>&gt;8000</td>
<td>&gt;8000</td>
</tr>
<tr>
<td>ATCC 20298</td>
<td>&gt;8000</td>
<td>&gt;8000</td>
<td>&gt;8000</td>
</tr>
<tr>
<td>Candida parapsilosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATCC 22019</td>
<td>2000</td>
<td>2000</td>
<td>4000</td>
</tr>
<tr>
<td>Candida tropicalis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATCC 750</td>
<td>&gt;8000</td>
<td>&gt;8000</td>
<td>&gt;8000</td>
</tr>
</tbody>
</table>

Source: Authors

Leaves and fruits extracts demonstrated fungistatic action and were able of inhibit Candida albicans (ATCC 10231, ATCC 10234, ATCC 18804, ATCC 28367, clinical isolates 7B and 3C) and Candida parapsilosis (ATCC 22019), leaves extract also inhibited Candida krusei (ATCC 6258). Leaves extract demonstrated MIC values between 8000 and 2000 µg/mL, while the fruits extract demonstrated MIC values of 8000 and 4000 µg/mL. The data for MFC, leaves extract demonstrated fungicidal activity for Candida parapsilosis (ATCC 22019) with value of 2000 µg/mL.

The leaves extract was not effective only for three strains Candida dubliniensis (ATCC 7987), Candida krusei (ATCC 20298) and Candida tropicalis (ATCC 750), which are isolates resistant to fluconazole, the same result was observed for the fruits extract from the same strains.

3.3.2 Antifungal Activity Against Sporothrix spp.

The results for antifungal activity of the hydroalcoholic extracts of leaves and fruits from Physalis pubescens L. against Sporothrix spp. were showed in the Table 3. The leaves and fruits extracts revealed a small antifungal activity against Sporothrix spp. in the susceptibility test in vitro.
Table 3. Antifungal activity of the hydroalcoholic extract of leaves and fruits from *Physalis pubescens* against *Sporothrix* spp.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Leaves Extract (µg/mL)</th>
<th>Fruits Extract (µg/mL)</th>
<th>Itraconazole (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC</td>
<td>MFC</td>
<td>MIC</td>
</tr>
<tr>
<td><em>Sporothrix brasiliensis</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>&gt;8000</td>
<td>&gt;8000</td>
<td>&gt;8000</td>
</tr>
<tr>
<td>8309</td>
<td>8000</td>
<td>&gt;8000</td>
<td>&gt;8000</td>
</tr>
<tr>
<td><em>Sporothrix globosa</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16490</td>
<td>&gt;8000</td>
<td>&gt;8000</td>
<td>&gt;8000</td>
</tr>
<tr>
<td><em>Sporothrix schenckii</em></td>
<td></td>
<td></td>
<td></td>
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<tr>
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<tr>
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<tr>
<td>Santa Casa II</td>
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<td>&gt;8000</td>
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</tr>
<tr>
<td>SN</td>
<td>4000</td>
<td>&gt;8000</td>
<td>4000</td>
</tr>
</tbody>
</table>

Source: Authors

Leaves and fruits extracts demonstrated fungistatic action and were able of inhibit *Sporothrix schenckii* SN, leaves extract also inhibited *Sporothrix brasiliensis* 8309 and *Sporothrix schenckii* 67 MRV. The leaves extract demonstrated MIC values of 8000 and 4000 µg/mL, while the fruits extract obtained MIC value of 4000 µg/mL.

The leaves and fruits extracts demonstrated were effective for *Sporothrix schenckii* SN, which is an isolate resistant to itraconazole demonstrated by high MIC of itraconazole with value of 16 µg/mL.

3.3.3 Antibacterial Activity Against *Staphylococcus Aureus*

The results for antibacterial activity of the hydroalcoholic extracts of leaves and fruits from *Physalis pubescens* L. against *Staphylococcus aureus* were showed in the Table 4. The results showed that most of the *Staphylococcus aureus* strains tested were inhibited by different extracts in the highest concentrations tested.

Table 4. Antibacterial activity of the hydroalcoholic extract of leaves and fruits from *Physalis pubescens* against *Staphylococcus aureus*

<table>
<thead>
<tr>
<th>Strain</th>
<th>Leaves Extract (µg/mL)</th>
<th>Fruits Extract (µg/mL)</th>
<th>Chloramphenicol (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC</td>
<td>MFC</td>
<td>MIC</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td></td>
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<tr>
<td>ATCC 25923</td>
<td>4000</td>
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<tr>
<td>MRSA-2</td>
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<td>MRSA-9</td>
<td>8000</td>
<td>&gt;8000</td>
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</table>
Leaves and fruits extracts demonstrated bacteriostatic action and were able to inhibit *Staphylococcus aureus* (clinical isolates MRSA-2 and MSSA-4), leaves extract also inhibited *Staphylococcus aureus* (ATCC 25923, clinical isolates MRSA-3, MRSA-4, MRSA-5, MRSA-6, MRSA-7(2), MRSA-8, MRSA-9 and MSSA-3(3)). Leaves extract inhibited the bacterial growth of whole strains tested that was demonstrated MIC values of 8000 and 4000 µg/mL. The fruits extract obtained MIC values of 8000 µg/mL.

The data for MBC, leaves extract demonstrated bactericidal activity for *Staphylococcus aureus* (clinical isolates MRSA-2 and MRSA-3), which chloramphenicol has bacteriostatic action, as well as demonstrated bactericidal activity for *Staphylococcus aureus* (clinical isolate MRSA-4), which is clinical isolate resistant to chloramphenicol and demonstrated bactericidal activity for *Staphylococcus aureus* (clinical isolate MSSA-3(3)), MBC with values of 8000 µg/mL.

The leaves and fruits extracts demonstrated were effective for clinical isolate resistant to chloramphenicol *Staphylococcus aureus* (clinical isolate MRSA-4), leaves extract also demonstrated were effective for clinical isolates resistant to chloramphenicol *Staphylococcus aureus* (clinical isolates MRSA-5 and MRSA-7(2)).

### 4 DISCUSSION

The data revealed a difference in the phytochemical composition of the plant parts, although the LEP had more components identified, FEP presented its constituents in higher concentration, as catechin, *p*-coumaric acid and kaempferol, which in parts may explain our findings that demonstrated better performance and lower toxicity in fruit extracts. The benefits associated of the fruit of *Physalis peruviana* L., are mainly due to their composition because, besides having good biologically active components that provide health benefits and reduce risk for certain diseases (PUENTE et al., 2011; MARY-LUZ et al., 2017).

Phytotherapy is constantly advancing and developing, normally using preparations of leaves, roots and other parts of plants for medicinal purposes (SILVA et al., 2020). Interest in the antioxidant properties of fruits is relatively recent, our findings have shown that LEP has higher antioxidant activity than FEP, these results are in agreement with findings of some authors have reported values of the antioxidant capacity of the fruit of *Physalis peruviana* L. determined in terms of activity DPPH and concentration of total phenols, of the medicinal
properties of the fruit of *P. peruviana* L. were associated with the antioxidant capacity of polyphenols present in the fruit (BOTERO, 2008; RESTREPO, 2008).

Besides their strong antioxidant activities, their low toxicities, wide distributions and medicinal functions all make them promising sources of natural antioxidants and other bioactive compounds in food and pharmaceutical industries. In the future, the specific compounds with high antioxidant capacities should be isolated, purified and identified from these plants to further develop natural antioxidants, which will be employed to treat these diseases in association with reactive oxygen species, such as cancer, atherosclerosis, coronary heart diseases and diabetes (SHA et al., 2011).

The antimicrobial activity of plant extracts is evaluated by determining the small amount of the substance necessary to inhibit the growth of the microorganism test, this value is known as the Minimum Inhibitory Concentration (MIC). The use of this study as the first screening in the discovery of pharmacological activity of new agents is extremely important, such research can contribute significantly in the development of more effective and less toxic substances (OSTROSKY et al., 2008). In our study we developed a screening to define the concentrations to be used.

Studies ethnopharmacologicals are useful in guiding the discovery of antifungal plants, the probability of detecting antifungal activity in plants is higher when are reported the traditional use. Phenolic acids and flavonoids naturally protect plants against phytopathogenic fungi and, therefore, plant extracts containing phenolic compounds are considered a natural alternative to conventional fungicides. Considering the development of resistance and the adverse effects of the available antibacterial drugs, there is a need for new pharmacological agents. The use of plants by traditional medicine corroborates with the importance of ethnopharmacological surveys and opens the possibility for finding new clinically effective antimicrobial agents (SVETAZ et al., 2010; FILIPPI et al., 2020).

Our data revealed that most of the yeasts tested were inhibited by different extracts and that were not effective for three strains *Candida dubliniensis* (ATCC 7987), *Candida krusei* (ATCC 20298) and *Candida tropicalis* (ATCC 750), which are isolates resistant to fluconazole suggesting that extracts may have similar mechanism of action to fluconazole. The results revealed that leaves and fruit extracts of *Physalis pubescens* has antifungal activity against *Candida* spp. and the sensitivity to these extracts varies across the different species.

The sporotrichosis is a disease of difficult treatment, because various *Sporothrix* species demonstrate resistance to antifungal agents. The itraconazole is the standard drug to treatment of sporotrichosis. In general, studies have demonstrated a good antifungal activity of the
itraconazole against *Sporothrix* spp. with the lowest mean MIC and MFC values (STOPIGLIA et al., 2012; 2014). However, other investigations have reported a high MIC and MFC for itraconazole against the *Sporothrix schenckii* complex as found in our study for different extracts (NCCLS, 2002; GUTIERREZ-GALHARDO et al., 2010). The M38-A suggests that filamentous fungi with a MIC ≥ 4.0 μg/mL for itraconazole may be considered resistant (NCCLS, 2002). The leaves and fruits extracts demonstrated were effective for *Sporothrix schenckii* SN, which is an isolate resistant to itraconazole demonstrated by high MIC of itraconazole with value of 16 μg/mL. In the present study, we noted that the most clinical isolates were resistant in vitro to itraconazole demonstrated by MIC ≥ 4.0 μg/mL. Nevertheless, the clinical isolates evaluated of *Sporothrix* spp. from different Brazilian states (Rio de Janeiro, São Paulo and Rio Grande do Sul), therefore, the difference in the susceptibility profiles to antifungal activity could be related to the geographic origin of the isolates (STOPIGLIA et al., 2014). *Physalis pubescens* presented antifungal activity in some concentrations analyzed for filamentous fungi, which could suggest a potential usefulness of this natural resource for the treatment of sporotrichosis.

Further antibacterial assay indicated that leaves extract has antibacterial activity inhibiting growth of *Staphylococcus aureus* strains tested, besides bacteriostatic or bactericidal action conform strain tested. The leaves and fruits extract demonstrated were effective for clinical isolate resistant to chloramphenicol *Staphylococcus aureus* (clinical isolate MRSA-4), leaves extract also demonstrated were effective for clinical isolates resistant to chloramphenicol *Staphylococcus aureus* (clinical isolates MRSA-5 and MRSA-7(2)). Our data corroborated with study of the ethanolic extract of fruits from *Physalis angulata* L. demonstrated bacteriostatic activity against *Staphylococcus aureus* ATCC 6538 in the 25% concentration (LOPES et al., 2006). Other study reported the antibacterial activity of ethanolic and aqueous extracts of *Physalis angulata* L. were demonstrated when forward tested strains of *Staphylococcus aureus* and *Escherichia coli* (TOMASSINI et al., 1997). The chloroform fraction of *Physalis peruviana* present at low values with MIC ≤ 0.256 mg/mL against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, which is a starting point for the search for new compounds with antibacterial activity (FRANCO-OSPINA et al., 2013). Recent study reported that physalin steroids isolated from calyces of *Physalis alkekengi* var. frachetti showed high antibacterial activities against *Bacillus subtilis* (ACCC 11060) and *Escherichia coli* (ATCC 25922), as well as possible inhibitors of *Staphylococcus aureus* (ATCC 25923) and *Staphylococcus epidermidis* (CMCC 26069) (YANG et al., 2016). The physalin pool at 200 μg/mL yielded 100% inhibition for *Staphylococcus aureus* (ATCC 29213, ATCC 25923 and
ATCC 6538P) and Neisseria gonorrhoeae (ATCC 49226) at a concentration of 200 µg/mL, using agar dilution assays and physalin B (200 µg/mL) by the agar diffusion assay inhibited Staphylococcus aureus (ATCC 6538P) by ± 85% and may be considered responsible for the antimicrobial activity (SILVA et al., 2005).

It is important to salient may occur possible variations in the determination of the MIC plant extracts. This owing to factors such as the applied extraction technique, micro-organism and strain used in the test, plant origin, time of collect, plant material prepared extract (fresh or dried), concentration and solvent extract (FENNEL et al., 2004). As mentioned the extraction method has a fundamental role in the preparation of the extracts, define constituents to be extracted. These, in turn, define pharmacological activity and are therefore essential for determination of MIC. Because of that, a wide variety of data is found in ethnopharmacological studies.

In this study, evaluation of the extracts of leaves and fruits Physalis pubescens was performed with hydroalcoholic because the presence of ethanol increases the extraction of substances of intermediate polarity. While the use of organic solvents with low polarity extract more apolar constituents, as steroidal nucleus (MEDEIROS & KANIS, 2010).

The Physalis genus are pointed to the increment for research on steroidal substances and show a huge field for further studies and search new and effective drugs, as is the case of complex structures as withasteroids, which are classified in eight groups: withanolides, withanolides modified, withaphysalins, acnistinas, ixocarpalactonas, perulactonas and physalins (TOMASSINI et al. 2000). Studies report that withasteroids and derivates are responsible for biological activities, and physalins are reported by potencial antimicrobial activity (SILVA et al., 2005; LOPES et al., 2006; FRANCO-OSPINA et al., 2013; YANG et al., 2016). Physalis pubescens presented antifungal and antibacterial activity in some concentrations analyzed, these activities can be assigned to these constituents.

Leaves extract of P. pubescens showed higher antifungal and antibacterial activity compared with fruits extract probably owing the difference in the phytochemical composition of plant parts, these results could suggest a potential usefulness of this natural resource for the treatment of diseases. Our data demonstrated high MIC values that can be considered hereafter in studies with preparations for topical use to justify the use of these concentrations, as well as studies with different fractions isolated of the extract in order to decrease the MIC values.
5 CONCLUSION

Based on the results of this study, we can suggest that physalins are reported by potential antimicrobial activity, therefore, are needs for further studies for this species and constituents in order to elucidate the promising effects.
REFERENCES


