Antifungal activity and detailed chemical characterization of *Cistus ladanifer* phenolic extracts

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**A B S T R A C T**

The life-threatening mycoses caused by opportunistic fungal pathogens (mainly species from the genus *Candida*) associated with nosocomial infections, are one of the major health problems in our days. Therefore, it is crucial to identify new compounds, especially natural ones, that are active against the most broad spectrum of Candida species. Herein, a screening of the antifungal potential of a phenolic extract of *Cistus ladanifer* from Northeastern Portugal, against *Candida* species was performed. Furthermore, the extract was characterized by HPLC–DAD-ESI/MS. Phenolic acids and derivatives (3.96 mg/g extract), ellagic acid derivatives (30.34 mg/g extract), and flavonoids (4.15 mg/g extract), such as catechins, flavonols and flavones, were found in the sample. The most abundant group was ellagic acid derivatives in which punicalagin gallate, a derivative of punicalagin attached to gallic acid, was found in highest amount (15.99 ± 0.02 mg/g extract). These compounds (i.e., ellagitannins) could be related to the strong inhibition of *Candida albicans*, *C. glabrata* and *C. parapsilosis* growth (MIC < 0.05 mg/mL). Moreover, the best antifungal activity was against *C. glabrata*, where the studied extract was able to cause at least 3 log of reduction at concentrations below 0.05 mg/mL and a total growth inhibition at concentrations above 0.625 mg/mL.

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1. Introduction

Nosocomial infections are one of the major health problems in our days, being the life-threatening mycoses caused by opportunistic fungal pathogens associated with those infections, and the most prevalent species from the genus *Candida* (Calderone, 2002; Lass-Flör, 2009). In clinical practice, the most commonly encountered member of the genus is *Candida albicans*. However, several non-*Candida albicans* Candida (NCAC) spp., such as *C. tropicalis*, *C. parapsilosis*, and *C. glabrata*, have been increasingly implicated in human disease (Costa et al., 2010; Martins et al., 2010). This increased incidence can be attributed to improved identification methods but can also be a reflection of the high level of resistance often exhibited by NCAC species to antifungal agents (Gonzalez et al., 2008; Negri et al., 2009).

It has been reported the antimicrobial activity of several plants, related to their phenolic compounds (Rauha et al., 2000; Erasto et al., 2004; Tepe et al., 2004), including the antibacterial activity of *Cistus ladanifer* L. aqueous extracts containing ellagitannins (Barrajón-Catalán et al., 2010). This aromatic plant is a Mediterranean shrub from the Cistaceae family, having white flowers and viscid stems and leaves producing a fragrant oleoresin used in perfumes especially as a fixative. It is widely distributed in Portugal, being one of the most abundant species in the southern part of the country, occurring in large areas as pure dense stands (Teixeira et al., 2007). *C. ladanifer* and other species of Cistaceae are used as general remedies in folk medicine for treatment of various skin diseases, as antidiarrheics, and as anti-inflammatory agents (Attaguile et al., 2000).

As far as we know there are no reports on antifungal activity of the mentioned plant and the reports on phenolic composition refers to samples from Spain (Fernández-Arroyo et al., 2010; Barrajón-Catalán et al., 2010, 2011). Herein, a screening of the antifungal potential of phenolic extracts of *C. ladanifer* against *Candida* species was performed, and the extracts were further characterized by HPLC–DAD-ESI/MS.
2. Materials and methods

2.1. Samples

_C. ladanifer_ fresh leaves from flowering steams were collected randomly, from wild plants growing in the Natural Park of Montesinho (Northeastern Portugal) in July 2008.

Morphological key characters from the Flora Iberica were used for plant identification: _C. ladanifer_ (Castroviejo, 2005). Voucher specimens from each plant were numbered and deposited in the Herbarium of the ESA. The vegetal material was lyophilized (Ly-8-FM-ULE, Snijders, Netherlands) and stored in the deep-freezer at −20 °C for subsequent analysis.

2.2. Standards and reagents

HPLC-grade acetonitrile was obtained from Merck KgaA (Darmstadt, Germany). Formic and acetic acids were purchased from Prolabo (VWR International, France). The phenolic compounds standards (apigenin-6-C-glucoside, catechin, ellagic acid, gallic acid, kaempferol-7-O-neohesperidoside, quercetin-3-O-glucoside, vanillic acid) were from Extrasynthese (Genay, France). RPMI 1640 medium was from Sigma (St. Louis, MO). Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, USA).

2.3. Preparation of the phenolic extracts

The sample (1 g) was extracted with 30 mL of methanol:water 80:20 (v/v) at room temperature, 150 rpm, for 1 h. The extract was filtered through Whatman n° 4 paper, and the residue was re-extracted twice. The combined extracts were evaporated at 35 °C (rotary evaporator Büchi R-210) to remove methanol. The aqueous phase was lyophilized and re-dissolved in (a) 20% aqueous methanol at 5 mg/mL and filtered through a 0.22-μm disposable LC filter disk for High Performance Liquid Chromatography (HPLC) analysis, or (b) distilled water at 200 mg/mL for antifungal assays.

2.4. Phenolic compounds identification and quantification

The extracts were analysed using a Hewlett-Packard 1100 chromatograph (Agilent Technologies) with a quaternary pump and a diode array detector (DAD) coupled to an HP Chem Station (rev. A.05.04) data-processing station. A Waters Spherisorb S3 ODS-2 C18, 3 μm (4.6 mm × 150 mm) column thermostatted at 25 °C was used. The solvents used were: (A) 2.5% acetic acid in water, (B) 2.5% acetic acid/acetonitrile (90:10, v/v) and (C) HPLC-grade acetonitrile. The elution gradient established was 0–100% B for 5 min, from 0 to 5% C for 35 min, from 5 to 50% C for 5 min, isocratic 50% C for 5 min, and re-equilibration of the column, using a flow rate of 0.5 mL/min. Double online detection was carried out in the DAD using 280 nm and 370 nm as preferred wavelengths and in a mass spectrometer (MS) connected to HPLC system via the DAD cell outlet.

MS detection was performed in an API 3200 Qtrap (Applied Biosystems, Darmstadt, Germany) equipped with an ESI source and a triple quadrupole-ion trap mass analyzer that was controlled by the Analyst 5.1 software. Zero grade air served as the nebulizer gas (30 psi) and turbo gas for solvent drying (400 °C, 40 psi). Nitrogen served as the curtain (10 psi) and collision gas (medium). The quadrupoles were set at unit resolution. The ion spray voltage was set at −4000 V in the negative mode. The MS detector was programmed for recording in two consecutive modes: Enhanced MS (EMS) and enhanced product ion (EPI) analysis. EMS was employed to achieve a full scan spectra, allowing an overview of all the ions present in sample. Settings used were: declustering potential (DP) −40 V, entrance potential (EP) −7 V, collision energy (CE) −20 V. EPI mode was performed in order to obtain the fragmentation pattern of the parent ion(s) in the previous scan using the following parameters: DP −40 V, EP −10 V, CE −25 V, and collision energy spread (CES) 0 V. Spectra were recorded in negative ion mode between m/z 100 and 1400.

The phenolic compounds present in the samples were characterised according to their UV and mass spectra and retention times compared with commercial standards when available. For the quantitative analysis of phenolic compounds, a calibration curve was obtained by injection of known concentrations (1–100 μg/mL) of different standards compounds: apigenin-6-C-glucoside (y = 517.4x + 268.26; R² = 0.9921); catechin (y = 161.23x + 177.26; R² = 0.9992); ellagic acid (y = 36.81x + 257.13; R² = 0.9979); gallic acid (y = 298.26x − 634.14; R² = 0.9949); kaempferol-7-O-neohesperidoside (y = 279.99x + 404.53; R² = 0.9902); quercetin-3-O-glucoside (y = 363.45x + 117.86; R² = 0.9994); vanillic acid (y = 364.98x + 603.85; R² = 0.9985). The results were expressed in mg per g of extract, as mean ± standard deviation of three independent analyses.

2.5. Antifungal activity

Four reference strains from the American Type Culture Collection (ATCC), namely _C. albicans_ (ATCC 90028), _Candida tropicalis_ (ATCC 750), _Candida glabrata_ (ATCC 2001) and _Candida parapsilosis_ (ATCC 22019) were used. Before each experiment, all reference species were grown on Sabouraud Dextrose Agar (SDA; Merck, Germany) for 24 h at 37 °C. Yeast cells from at least five colonies (1 mm diameter) were suspended in 5 mL of sterile saline solution (0.85%) and the resulting yeast suspension was mixed for 15 s in a vortex. Then, the suspensions were adjusted by spectrophotometric method, adding saline solution to reach the value of 0.5 in the McFarland scale corresponding to a final concentration of 3.0 ± 0.2 × 10⁵ cells/mL. The minimal inhibitory concentration (MIC) was determined according with the guidelines from the National Committee for Clinical Laboratory Standards (NCCLS, M27-A2 document (NCCLS, 2002) with some modifications. Briefly, serial dilutions of plant extract stock solutions were prepared in RPMI 1640 medium (Sigma, St. Louis, MO), buffered to pH 7 and ranging from 0.05 to 5 mg/mL. Aliquots of the extract (100 μL), at a twofold final concentration, and _Candida_ species suspensions (100 μL) were mixed in the 96-well plates (Orange Scientific, Braine-l’Alleud, Belgium). The MIC values and the colony forming units (CFUs) were determined after 48 h of incubation at 37 °C. Drug-free and yeast controls were also included.

3. Results and discussion

The difficulties associated with the management of _Candida_ infections have been increasing during the last decades due to their low susceptibility to the available antifungal therapies (Ruhnke, 2000; Naeni et al., 2009). Thus, in order to overcome this problem, it is of major importance to identify new compounds, especially natural ones, that are active against the most broad spectrum of _Candida_ species. Herein, phenolic extracts from _C. ladanifer_ were explored as a source of antifungal agents. The phenolic composition of the sample was characterized as containing phenolic acids and derivatives, ellagic acid derivatives and flavonoids (Fig. 1 and Table 1).

Among phenolic acids and derivatives, peaks 1, 2 and 6 were determined as galloyl glucose isomers, presenting a pseudomolecular ion [M−H]⁻ at m/z 331 that released an MS² fragment at m/z 169 ([M−162]⁻, loss of a hexose moiety) corresponding to gallic acid. Similarly, peak 7 was characterized as digalloyl-glucose. Peak 5 presented a pseudomolecular ion [M−H]⁻ at m/z 315 releasing a unique MS² fragment at m/z 153 ([M−162]⁻) and was
assigned to gentisoyl glucoside, based on a previous identification by Fernández-Arroyo et al. (2010). Peaks 9 and 24 corresponded to vanillic and ellagic acids, respectively, identified by comparison of their UV and mass spectra and retention time with commercial standards.

Various peaks were identified as ellagitannins. Peaks 10 and 12 were assigned to punicalagin isomers according to their pseudomolecular ion ([M−H]− at m/z 1083) and fragmentation pattern as described by Seeram et al. (2005) and Fernández-Arroyo et al. (2010). Similarly, peaks 3 and 4 ([M−H]− at m/z 781) were identified as punicalin isomers (Seeram et al., 2005), and peak 14 ([M−H]− at m/z 1085) as cornusin B, already described in C. ladanifer by Fernández-Arroyo et al. (2010). Peaks 11 and 15 ([M−H]− at m/z 1251) were tentatively identified as derivatives

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**Table 1**
Retention time (Rt), wavelengths of maximum absorption in the UV–vis region (\(\lambda_{max}\)), pseudomolecular and MS² fragment ions (in brackets, relative abundances), tentative identification and quantification of the phenolic compounds in the studied C. ladanifer extract.

<table>
<thead>
<tr>
<th>Peak</th>
<th>Rt (min)</th>
<th>(\lambda_{max}) (nm)</th>
<th>Pseudomolecular ion [M−H]− (m/z)</th>
<th>MS² (m/z)</th>
<th>Tentative identification</th>
<th>Quantificationa (mg/g extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.74</td>
<td>280</td>
<td>331</td>
<td>331(100), 169(14), 125(33)</td>
<td>Galloyl glucose</td>
<td>0.76 ± 0.02</td>
</tr>
<tr>
<td>2</td>
<td>8.80</td>
<td>270</td>
<td>331</td>
<td>331(33), 169(100), 125(37)</td>
<td>Galloyl glucose</td>
<td>1.35 ± 0.09</td>
</tr>
<tr>
<td>3</td>
<td>9.4</td>
<td>260, 380</td>
<td>313</td>
<td>781(100), 721(7), 601(23), 299(10)</td>
<td>Punicalin isomer 1</td>
<td>0.07 ± 0.01</td>
</tr>
<tr>
<td>4</td>
<td>9.78</td>
<td>260, 381</td>
<td>315</td>
<td>781(100), 721(7), 601(35), 299(15)</td>
<td>Punicalin isomer 2</td>
<td>0.32 ± 0.07</td>
</tr>
<tr>
<td>5</td>
<td>10.29</td>
<td>260, 312</td>
<td>351</td>
<td>153(91), 109(27)</td>
<td>Gentisoyl hexoside</td>
<td>nq</td>
</tr>
<tr>
<td>6</td>
<td>10.75</td>
<td>278</td>
<td>331</td>
<td>169(100), 125(63)</td>
<td>Galloyl glucose</td>
<td>0.87 ± 0.05</td>
</tr>
<tr>
<td>7</td>
<td>11.21</td>
<td>243, sh297, 339</td>
<td>483</td>
<td>331(40), 169(100), 125(23)</td>
<td>Digalloyl glucose</td>
<td>0.78 ± 0.05</td>
</tr>
<tr>
<td>8</td>
<td>11.63</td>
<td>273</td>
<td>305</td>
<td>219(45), 179(41), 125(100)</td>
<td>Galloacatechin</td>
<td>0.48 ± 0.05</td>
</tr>
<tr>
<td>9</td>
<td>11.90</td>
<td>252, 290</td>
<td>167</td>
<td>152(100), 123(15)</td>
<td>Vanillic acid</td>
<td>0.05 ± 0.01</td>
</tr>
<tr>
<td>10</td>
<td>12.61</td>
<td>258, 380</td>
<td>1083</td>
<td>781(17), 601(16), 301(100)</td>
<td>Punicalagin isomer 1</td>
<td>5.90 ± 0.15</td>
</tr>
<tr>
<td>11</td>
<td>14.65</td>
<td>259, 378</td>
<td>1251</td>
<td>1083(4), 781(13), 601(4), 301(13)</td>
<td>Punicalagin gallate 1</td>
<td>7.89 ± 0.29</td>
</tr>
<tr>
<td>12</td>
<td>16.17</td>
<td>260, 379</td>
<td>1083</td>
<td>781(12), 601(25), 301(93)</td>
<td>Punicalagin gallate 2</td>
<td>7.90 ± 0.19</td>
</tr>
<tr>
<td>13</td>
<td>17.42</td>
<td>274</td>
<td>305</td>
<td>219(33), 179(33), 125(100)</td>
<td>Epigallocatechin</td>
<td>1.59 ± 0.21</td>
</tr>
<tr>
<td>14</td>
<td>19.65</td>
<td>240, 378</td>
<td>1085</td>
<td>783(10), 542(44), 451(46), 301(100)</td>
<td>Cornusin B</td>
<td>0.15 ± 0.02</td>
</tr>
<tr>
<td>15</td>
<td>21.34</td>
<td>258, 380</td>
<td>1251</td>
<td>1083(6), 781(4), 601(19), 301(33)</td>
<td>Punicalagin gallate 2</td>
<td>8.10 ± 0.31</td>
</tr>
<tr>
<td>16</td>
<td>29.38</td>
<td>276</td>
<td>327</td>
<td>327(59), 165(100)</td>
<td>3,4-Dihydroxypropophenone-3-β-o-glucoside</td>
<td>nq</td>
</tr>
<tr>
<td>17</td>
<td>34.53</td>
<td>338</td>
<td>593</td>
<td>473(30), 383(31), 353(66)</td>
<td>Apigenin-6-C-glucose-8-C-glucose</td>
<td>0.53 ± 0.01</td>
</tr>
<tr>
<td>18</td>
<td>40.24</td>
<td>348</td>
<td>595</td>
<td>301(100)</td>
<td>Quercetin-O-hexoside-O-pentoside</td>
<td>0.26 ± 0.00</td>
</tr>
<tr>
<td>19</td>
<td>45.01</td>
<td>348</td>
<td>625</td>
<td>463(84), 301(100)</td>
<td>Quercetin-O-hexoside-O-hexoside</td>
<td>0.40 ± 0.02</td>
</tr>
<tr>
<td>20</td>
<td>47.83</td>
<td>334</td>
<td>609</td>
<td>447(29), 285(58)</td>
<td>Kaempferol-O-hexoside-O-hexoside</td>
<td>0.06 ± 0.00</td>
</tr>
<tr>
<td>21</td>
<td>51.51</td>
<td>356</td>
<td>595</td>
<td>301(100)</td>
<td>Quercetin-O-hexoside-O-pentoside</td>
<td>0.08 ± 0.01</td>
</tr>
<tr>
<td>22</td>
<td>52.29</td>
<td>360</td>
<td>463</td>
<td>301(100)</td>
<td>Quercetin-3-O-glucoside</td>
<td>0.14 ± 0.00</td>
</tr>
<tr>
<td>23</td>
<td>52.38</td>
<td>354</td>
<td>433</td>
<td>301(100)</td>
<td>Quercetin-O-pentoside</td>
<td>0.16 ± 0.00</td>
</tr>
<tr>
<td>24</td>
<td>53.19</td>
<td>294, 358</td>
<td>301</td>
<td>256(6), 185(15)</td>
<td>Ellagic acid</td>
<td>0.15 ± 0.02</td>
</tr>
<tr>
<td>25</td>
<td>53.28</td>
<td>334</td>
<td>475</td>
<td>313(100)</td>
<td>Kaempferol dimethylether</td>
<td>0.06 ± 0.00</td>
</tr>
<tr>
<td>26</td>
<td>54.24</td>
<td>334</td>
<td>593</td>
<td>447(8), 285(100)</td>
<td>Kaempferol-O-rutinoside</td>
<td>0.06 ± 0.00</td>
</tr>
<tr>
<td>27</td>
<td>56.45</td>
<td>350</td>
<td>299</td>
<td>285(100), 255(57), 227(29)</td>
<td>Kaempferol methylether</td>
<td>0.32 ± 0.02</td>
</tr>
</tbody>
</table>

nq – not quantified.

a The results were expressed in mg per g of extract, as mean ± standard deviation of three independent analyses.
of punicalagin attached to gallic acid, previously reported in Cistus species by Saracini et al. (2005). In this case the gallic acid would not be bound to punicalagin by the carboxyl group, as denoted by the fragment at m/z 1083 corresponding to the loss of gallic acid itself. These compounds were not identified in the sample of C. ladanifer analyzed by Fernández-ARroyo et al. (2010).

Considering flavonoids, peaks 8 and 13 showed \( \lambda_{\text{max}} \) at 274 nm characteristic of prodelphinidins with a pseudomolecular ion \([M-H]^-\) at m/z 305, were identified as gallocatechin and epigallocatechin (flavan-3-ols) by comparison with our data library. Peaks 18, 19, 21, 22 and 23 were identified as quercetin derivatives (\( \lambda_{\text{max}} \) around 350–360 nm, and MS\(^2\) fragment at m/z 301). Peak 22 ([M–H]– at m/z 463) was positively identified as quercetin-3-O-glucoside by comparison with a commercial standard. Peak 23 ([M–H]– at m/z 433) was associated to a quercetin-3-O-pentoside, and peaks 18 and 21 ([M–H]– at m/z 595) to quercetin-3-hexoside-pentosides, in which the sugar moieties constituted a disaccharide as deduced from the release of a unique MS\(^2\) fragment. Peak 19 presented a pseudomolecular ion \([M-H]^-\) at m/z 625, showing two fragments at m/z 463 and 301 corresponding to the losses of one and two a hexosyl residue, respectively, which allowed identifying it as a quercetin-3-O-hexoside-O-hexoside (sugars located at different positions on quercetin).

Peaks 20 and 26 corresponded to kaempferol derivatives (\( \lambda_{\text{max}} \) around 334 nm, and MS\(^2\) fragment at m/z 285). Peak 20 ([M–H]– at m/z 609) was characterised as kaempferol-3-O-hexoside-O-hexoside based on its fragmentation pattern similar to peak 19. Peak 26 presented a pseudomolecular ion \([M-H]^-\) at m/z 593 that released fragments at m/z 447 ([M–H]–146), loss of a rhamnosyl moiety) and 285 ([M–H]–146–162), loss of a hexosyl moiety). The fact that no fragment resulting from the exclusive loss of hexosyl residue pointed to that the rhamnose was not directly to the aglycone but the two glycosyl moieties are constituting a disaccharide. Indeed, this fragmentation pattern is characteristic of flavonoid rutinosides, in which the 1→6 linkage between rhamnose and glucose allows for free rotation being more accessible to fragmentation than other disaccharides (Giusti et al., 1999). Therefore, the peak was tentatively identified as a kaempferol-3-rutinoside.

Peak 25 presented a pseudomolecular ion \([M-H]^-\) at m/z 475 releasing a fragment at m/z 313 ([M–H]–162) co-homogenous with a kaempferol dimethylether aglycone, which was identified by Fernández-ARroyo et al. (2010) in a sample of C. ladanifer. Thus, the compound was assigned to a kaempferol-dimethylether hexoside. A peak with a pseudomolecular ion \([M-H]^-\) at m/z 475 was also detected by those authors in C. ladanifer although they did not offer any tentative identity. Peak 27 ([M–H]– at m/z 299) was identified as a methylether of kaempferol based on the production of a major MS\(^2\) fragment at m/z 285 ([M–H]–14), loss of a methyl residue).

Peak 17 showed a pseudomolecular ion \([M-H]^-\) at m/z 593 that released three MS\(^2\) fragments ions at m/z 473 and 383, corresponding to the loss of 120 and 90 amu, characteristic of C-hexosyl flavones, and at m/z 253 that might correspond to an agpigenin aglycone bearing some sugar residues [apigenin+83 nu] that remained linked to it (Ferreres et al., 2003). A compound with similar mass characteristics was found by Barrajón-Catalán et al. (2011) in C. ladanifer and associated to a diglycosylated apigenin. Since the two hexosyl residues are C-attached, it might be tentatively identified as apigenin-6-C-glucose-8-C-glucose.

Finally, peak 16 ([M–H]– at m/z 327) was assigned to 3,4-dihydroxypropophenone-3–β-D-glucoside as previously reported in C. ladanifer by Fernández-ARroyo et al. (2010).

Herein, it was found a similar profile to the ones reported by Fernández-ARroyo et al. (2010) and Barrajón-Catalán et al. (2010, 2011) in aqueous extracts of Spanish C. ladanifer samples. Nevertheless, some compounds reported by those authors (digalloyl-β-D-glucopiranose, pedunculagin, urane neoise, strictinin, mirciphenoine B, ellagic acid–7-xyloside, ducheside A, apigenin and apigenin methylether) were not detected in our sample, which in turns contained others (punicalagin gallate, quercetin-3-O-hexoside-O-pentoside, quercetin-3-O-pentoside and kaempferol-3-rhamnoside-O-hexoside) that were not cited in the Spanish samples.

Fernández-ARroyo et al. (2010) and Barrajón-Catalán et al. (2011) did not perform the quantification of individual compounds, so it is not possible to compare quantities found in both samples. Barrajón-Catalán et al. (2011) performed only the quantification of four compounds and expressed the results as percentages (0.242±0.004 (w/w) of gallic acid, and 3.50%±0.02 for the sum of three ellagitanins including punicalin and punicalagin), being also different the comparison of the results. The sample studied herein presented a total amount of phenolic compounds of 38.44±g/g extract, distributed in phenolic acids and derivatives (3.96 mg/g), ellagic acid derivatives (30.34 mg/g) and flavonoids (4.15 mg/g). The most abundant group was ellagic acid derivatives in which punicalagin gallates (peaks 11 and 15, 7.89±0.29 and 8.10±0.31 mg/g extract, respectively) were the compounds found in highest amount.

The in vitro activity of the studied C. ladanifer extract against four Candida species was evaluated. Aligiannis et al. (2001) proposed a classification for plant extracts effect against Candida species based on MIC values obtained in: strong inhibitors (MIC up to 0.5 mg/mL), moderate inhibitors (MIC between 0.6 and 1.5 mg/mL), and weak inhibitors (MIC above 1.6 mg/mL). According to that distribution, the phenolic extract of C. ladanifer would act as a strong inhibitor of C. albicans, C. glabrata and C. parapsilosis growth (MIC<0.05 mg/mL), and as a moderate inhibitor of C. tropicalis growth (MIC=0.625 mg/mL). Bruni et al. (2003) also demonstrated that C. albicans ATCC 48274 was markedly inhibited by oils rich in phenolics, aldehydes and alcohols. The results of chemical characterization (Table 1) shows that the studied C. ladanifer extract was mostly constituted by phenolic compounds, which would thus explain its antifungal activity caused by C. ladanifer extract against the Candida species under study. The number of viable Candida, determined by CFU enumeration, was determined using different concentration of C. ladanifer extract (Fig. 2), showing that the activity of this latter varied with the species. Specifically, the highest antifungal activity was against C. glabrata, where this extract was able to cause at least 3 log of reduction at concentrations below 0.05 mg/mL, and a total growth inhibition at concentrations above 0.625 mg/mL. In the case of C. albicans and C. tropicalis, complete growth inhibition was only observed for the highest assayed concentration (5 mg/mL), indicating less susceptible of these species.
to *C. ladanifer* extract than *C. glabrata*. Nonetheless, the extract was able to cause a reduction of approximately 3 log in *C. albicans* and *C. tropicalis* at concentrations above 0.155 mg/mL. None of the extract concentrations tested was able to reduce completely the *C. parapsilosis* growth. This was very interesting considering the low MIC value (0.05 mg/mL) obtained for this species, indicating that even though its growth was impaired at 0.05 mg/mL, in fact, cells were still viable.

No results concerning antifungal activity have been found in the literature for phenolic extracts of *C. ladanifer*. Barrajón-Catalán et al. (2010) reported antimicrobial activity against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria of *C. ladanifer* aqueous extract that was more active against Gram-positive bacteria.

Overall, the phenolic extract of *C. ladanifer* affected the growth of different Candida sp., suggesting that the compounds present in the extract could play an active role in the protection against fungi related to several diseases. Future work will include the evaluation of antifungal activity of those compounds (isolated from the plant or commercial compounds when available).

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