Antimicrobial Activity of Aqueous Extract from *P. atlantica* Roots

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Abstract

In the present study, the roots aqueous extract of *P. atlantica* was characterized for antimicrobial activity. This activity was evaluated against a panel of 7 bacteria and 5 fungal strains using agar diffusion and broth microdilution methods. Results have shown that the roots of *P. atlantica* exhibited moderate to strong antimicrobial activity against the tested species. The results suggested that the roots of *P. atlantica* possess antimicrobial properties, and is therefore a potential source of active ingredients for pharmaceutical industry.

Keywords: *P. atlantica*; Aqueous extract; Antimicrobial activity

Materials and Methods

**Chemicals**

Reagents used in this study were purchased from Sigma-Aldrich-Fluka (Saint-Quentin France).

**Plant material and preparation of extract**

The roots of *P. atlantica* were sampled from Gafsa, south western Tunisia, in January 2017 and deposited at the herbarium in the Faculty of Sciences, University of Gafsa, Tunisia. 10 g of Roots were washed with distilled water and dried at room temperature (approximately 20% relative humidity and 24% temperature) for 48 h. Then, the samples were ground to fine powder using a blender (Moulinex, France) and extracted for 24 h with water for three times at room temperature with magnetic stirring. After filtration using what man number 1 filter paper, the mixture was centrifuged for 10 min at 4500 g. The supernatant was lyophilized and stored at -20°C, until use.

**Antimicrobial activity**

**Microorganisms and growth conditions:** Pure cultures of microorganisms were obtained from international culture collections (ATCC) and the local culture collection of the Center of Biotechnology of Sfax, Tunisia. They included Gram-positive bacteria: *Bacillus subtilis* ATCC6633, *Bacillus cereus* ATCC 14579, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Enterococcus faecalis* ATCC 29212, and Gram-negative bacteria: *Salmonella enterica* (food isolate), *Klebsiella pneumoniae* ATCC 10031. The following fungal strains were also tested: *Aspergillus niger* CTM10099, *Aspergillus flavus* (food isolate), *Fusarium oxysporum* (CTM10402), *Rhizopus nigricans* (CTM10150), and *Alternaria alternata* (CTM 10230). The bacterial strains were cultivated in Muller-Hinton agar (MH) (Oxoid Ltd, UK) at the appropriate temperature for each strain at 37°C and fungi were cultured on Potatoes Dextrose Agar (PDA) medium at 28°C. Cultures were prepared by inoculating each test bacteria in 3 ml of Muller-Hinton broth (MH) (Oxoid Ltd, UK) and then incubated at 37°C for 12 hours. The final inoculums concentrations of bacteria were 106 CFU/ml. fungal spore suspensions were collected and suspended in 10 ml Potato Dextrose Broth (PDB), after those vortexes for 15 min to 20 min. The concentration of the diluted solution was 106 spores/ml.
**Results and Discussion**

**Antibacterial activity**

Antimicrobial activity of PARE against the tested microorganisms was assessed by analyzing inhibition zone diameters and MIC values. As shown in (Table 1), PARE exhibited varying degrees of antibacterial activity against all tested strains. The inhibition zones were between 14 mm to 22 mm. PARE showed an antibacterial effect against tested strains with MIC values of 0.325 mg/ml to 2.25 mg/ml, and MFC values of 0.645 mg/ml to 2.1 mg/ml. The Gram positive bacterial strains were more susceptible to the antimicrobial properties of PARE compared to Gram negative bacterial strains. The differences could be attributed to the presence of high concentration of flavonoids and other secondary metabolites that can disrupt the permeability barrier of cell membrane resulting in cell death.

**Antifungal activity**

Contamination by Aspergillus, Fusarium and Alternaria species is considered as a challenge for the pharmaceutical and food industries. The current study reports the capacity of PARE to control Aspergillus sp., Fusarium sp. and Alternaria alternata strains. Results showed a strong inhibitory effect of PARE on the growth of Aspergillus flavus, Fusarium oxysporum (CTM 10402), and Aspergillus Niger (CTM 10099) which is responsible for spoilage of many foods. The maximal inhibition zone diameters were 28 mm to 15 mm and MIC values ranged from 0.678 mg/ml to 0.645 mg/ml respectively (Tables 2).

<table>
<thead>
<tr>
<th>Strains</th>
<th>Inhibition Zones diameter (mm)</th>
<th>MIC (mg/ml)</th>
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</thead>
<tbody>
<tr>
<td>PARE</td>
<td></td>
<td></td>
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<tr>
<td>Bacillus subtilis</td>
<td>18 ± 0.8</td>
<td>0.325 ± 0.08</td>
</tr>
<tr>
<td>Bacillus cereus</td>
<td>19 ± 0.4</td>
<td>2.25 ± 0.4</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>22 ± 0.6</td>
<td>0.58 ± 0.07</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>16 ± 0.8</td>
<td>1.45 ± 0.06</td>
</tr>
<tr>
<td>Salmonella enterica</td>
<td>14 ± 0.6</td>
<td>2.1 ± 0.5</td>
</tr>
<tr>
<td>Klebsiella pneumonia</td>
<td>15 ± 0.6</td>
<td>0.4 ± 0.1</td>
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</tbody>
</table>

Values are given as mean ± SD of triplicate experiments

<table>
<thead>
<tr>
<th>Fungal stains</th>
<th>Inhibition Zones diameter (mm)</th>
<th>MFC (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PARE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>28 ± 0.8</td>
<td>0.645 ± 0.08</td>
</tr>
<tr>
<td>Aspergillus flavus</td>
<td>24 ± 0.4</td>
<td>0.512 ± 0.4</td>
</tr>
<tr>
<td>Fusarium oxysporum</td>
<td>15 ± 0.6</td>
<td>0.678 ± 0.07</td>
</tr>
<tr>
<td>Rhizopus nigricans</td>
<td>19 ± 0.8</td>
<td>0.615 ± 0.06</td>
</tr>
<tr>
<td>Alternaria alternata</td>
<td>17 ± 0.4</td>
<td>1.45 ± 0.05</td>
</tr>
</tbody>
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Values are given as mean ± SD of triplicate experiments

**Conclusion**

In the present work, we reported the antimicrobial activities of Pistacia atlantica roots extract. This extract demonstrates antibacterial activity on the studied bacterial, confirmed by a low Minimum Inhibitory Concentration (MIC). One can say that, PARE can be used as a natural alternative to control antibiotics-resistant bacteria.
antimicrobial agent for the treatment for several infection diseases. Our results are a contribution to a better valorisation of this medicinal plant. Several other biological tests will be worthwhile to search for more eventual activities of this plant to characterize active principles, and assess toxicity by laboratory assays.

References