Essential oil from gum of *Pistacia atlantica* Desf.: Screening of antimicrobial activity

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The essential oil from the gum of *Pistaciaatlantica* Desf. grown in Algeria was obtained by the hydrodistillation method, and its antimicrobial activities against the growth of clinical isolates of *Staphylococcus aureus*, *Escherichia coli* and *Streptococcus pyogenes* were evaluated using three different methods; agar disc diffusion and dilution broth methods and minimum inhibitory concentration (MIC) which was subsequently determined. The results of the study revealed that essential oil resin of *P. atlantica* has antimicrobial activity against gram-positive and -negative bacteria which are resistant to commonly used antimicrobial agents, and they were considerably dependent on concentration.

**Key words:** Gum of *Pistaciaatlantica* Desf., essential oil, antimicrobial activities, *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pyogenes*.

**INTRODUCTION**

Infectious diseases are the leading cause of death worldwide. Antibiotic resistance has become a global concern (Westh et al., 2004). The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens (Bandow et al., 2003). *Staphylococcus aureus* is mainly responsible for post-operative wound infections, toxic shock syndrome, endocarditis, osteomyelitis and food poisoning (Mylotte, 1987). *Streptococcuspyogenes*, like *S. aureus*, causes an array of suppurative diseases and toxinoses, in addition to some autoimmune or allergic diseases (Todar, 2008). *Escherichia coli* is present in the human intestine and causes lower urinary tract infection, coleocystis or septicaemia (Singh et al., 2000). These pathogens can acquire antimicrobial resistance to prevent effective dis-ease treatment (Reinert et al., 2001; Sundqvist and Kahl-meter, 2005). *Streptococcuspyogenes*, like *S. aureus*, causes an array of suppurative diseases and toxinoses, in addition to some autoimmune or allergic diseases (Todar, 2008).

**MATERIALS AND METHODS**

Plant material and essential oil extraction

The essential oil from the gum of *Pistacia lentiscus* has a long history of use as a therapeutic agent with many reported medicinal properties (Demirci, 2001). Amongst its therapeutic properties, it has been implicated in the relief of upper abdominal discomfort, stomachaches, dyspepsia and peptic ulcer (Al-Said et al., 1986; Huwez and Al-Hab-bal, 1986). *Pistacia* species have also been reported to possess stimulant and diuretic properties (Bentley and Trimon 1980). The antimicrobial activity of *P. lentiscus* essential oils and its resin against different microorganisms has been reported by several researchers (Tassou and Nychas, 1995; Ben Douissa et al., 2005; Benhammou et al., 2008) but little is known of the bactericidal effect of *P. atlantica* extracts, precisely its oleoresin oils.

In this study we evaluate the antibacterial activity of essential oils extracted from mastic gum, a resin obtained from the *P. atlantica* tree, against clinical isolates of *S. aureus*, *S. pyogenes* and *E. coli*.

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Antimicrobial screening

Three different methods were employed for the determination of antimicrobial activities: an agar disc diffusion and dilution broth methods, followed by minimal inhibitory concentration (MIC) determination.

Agar disc diffusion method

In vitro antibacterial activity of *P. atlantica* essential oil was determined by the agar disc diffusion method according to Benhassaini et al. (2003). Disc assays were found to be a simple, cheap and reproducible practical method (Maidment et al., 2006). 18 ml of sterilized Mueller Hinton agar medium was taken in each Petri dish and then spread with a suspension of the tested micro-organism (average concentration is $10^8$ cells/ml). Sterilized Whatman’s No. 3 filter papers (6 mm diameter) were thoroughly moistened with 15 µl of the oil and placed on the seeded agar plates and then incubated at 37°C for 24 h. The diameters of the inhibition zones were measured in millimeters.

Minimal inhibitory concentration (MIC) determination

The minimal inhibitory concentration (MIC) is the lowest concentration of an antimicrobial agent that will inhibit the visible growth of a microorganism after overnight incubation. Minimum inhibitory concentrations are important in diagnostic laboratories to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents (Andrews, 2001). 3 dilutions each of *S. aureus*, *S. pyogenes* and *E. coli* strains $10^{-1}$, $10^{-2}$ and $10^{-3}$ were prepared. The diluted bacterial strains were spread over the surface of the Petri dish containing Mueller-Hinton agar medium. 4 discs of 6 mm diameter are placed on agar containing the following quantities of the resin oil dilution: 0.5, 1, 1.5 and 2 l. In the center of the Petri dish, a control disc is impregnated in para-iodo with 2 l of ethanol. The Petri dishes are then incubated at 37°C for 24 h.

Dilution broth method

Dilution broth susceptibility assay (Bouhadjera et al., 2005) was used for the antimicrobial evaluation. Stock solutions of the resin oils were prepared in ethanol by mixing 1 ml of the extracts with 9 ml of alcohol in test tubes to obtain the mother solution, followed by successive dilutions at $10^{-2}$ and $10^{-3}$. The control was prepared by mixing 1 ml of distilled water with 9 ml of alcohol. 1 ml of each dilution and 0.5 ml of bacterial strains were added to test tubes containing 8 ml of nutrient broth, maintained later in a Marie bath at 37°C under stirring for 24 h, then seeded by streaking the surface of the agar medium and incubated for 24 h at 37°C.

RESULTS AND DISCUSSIONS

The antibiotic susceptibility pattern of the gram negative and positive bacteria was determined (Table 1, Plate 1). The most effective antibiotics for *S. aureus* were neomycin, chloramphenicol, trimethoprim - sulfamethoxazole and nitrofurantoin, but for the second gram positive bacteria, *S. pyogenes*, they were penicillin G, gentamicine and amoxicillin. While, the most effective antibiotics for the gram-negative (*E. coli*) bacteria were chloramphenicol, neo-mycin and cefazolin.

Disc diffusion is one of the most common assays used in the evaluation of antibacterial activity of essential oils. Figure 1 shows the *in vitro* antimicrobial property of the essential oil resin of *P. atlantica* of three bacterial strains, with their three dilutions exposed at different concentrations to oil resin of *P. atlantica*. Antimicrobial activity by disc diffusion method showed that the oil resin of *P. atlantica* was most active against *E. coli* followed by *S. aureus* and *S. pyogenes*. The oil resin at all volumes showed potent inhibitory activity against the tested microorganisms, with the exception of $10^{-1}$ dilution of the strain *S. pyogenes* with $10^{-4}$ of essential resin where there were no reports of inhibition. The diameter of the inhibition zone of oil resin of *P. atlantica* varied from 0 to 9 mm. The largest zone of inhibition was obtained for *E. coli* ($10^{-3}$ dilution) with $10^{-1}$ µg/ml concentration of oil resin of *P. atlantica* and the lowest for *S. pyogenes* ($10^{-4}$ dilution) with $10^{-1}$ µg/ml concentration of oil resin of *P. atlantica*. A more significant inhibition was seen with a higher oil oleo-resin concentration. The oil resin at $10^{-2}$ and $10^{-3}$ µg/ml showed moderate activity. On one hand, the growths of tested bacteria in high concentrations of oil resin were highly inhibited, where it was considered that these orga-nisms were sensitive to the oil. On the other hand, at low concentrations, a very limited inhibitory effect was observed.
<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>E. coli Inhibition zone diameter (mm)</th>
<th>Results</th>
<th>S. aureus Inhibition zone diameter (mm)</th>
<th>Results</th>
<th>S. pyogenes Inhibition zone diameter (mm)</th>
<th>Results</th>
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<tbody>
<tr>
<td>Penicillin G</td>
<td>-</td>
<td>-</td>
<td></td>
<td>-</td>
<td>29</td>
<td>S</td>
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<tr>
<td>Paromomycin</td>
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<td>-</td>
<td>6</td>
<td>R</td>
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<td>Gentamicin</td>
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<td>-</td>
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<td>-</td>
<td>25</td>
<td>S</td>
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<tr>
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<td>17</td>
<td>S</td>
<td>10</td>
<td>I</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Amoxicillin</td>
<td>-</td>
<td>-</td>
<td></td>
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<td>20</td>
<td>S</td>
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<tr>
<td>Nitrooxide</td>
<td>09</td>
<td>R</td>
<td>20</td>
<td>S</td>
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<tr>
<td>Vancomycin</td>
<td>-</td>
<td>-</td>
<td></td>
<td>-</td>
<td>18</td>
<td>I</td>
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<tr>
<td>Oxacillin</td>
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<td>I</td>
<td>10</td>
<td>I</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>20</td>
<td>S</td>
<td>24</td>
<td>S</td>
<td>08</td>
<td>I</td>
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<tr>
<td>Colistin</td>
<td>-</td>
<td>-</td>
<td></td>
<td>-</td>
<td>04</td>
<td>R</td>
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<tr>
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<td>S</td>
<td>19</td>
<td>S</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
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<td>-</td>
<td>-</td>
<td></td>
<td>-</td>
<td>02</td>
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<tr>
<td>Trimethoprim-sulfamethoxazole</td>
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<td>R</td>
<td>24</td>
<td>S</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Ampicillin</td>
<td>14</td>
<td>I</td>
<td>-</td>
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</tbody>
</table>

**Table 1.** Antibiotic results of *S. aureus*, *E. coli* and *S. pyogenes* with traditional antibiotics.

**Figure 1.** Antimicrobial activity evaluation of the essential oil resin of *P. atlantica* using agar disc diffusion method. Dilutions (0.1, 0.01, and 0.001); (a) *E. coli*, (b) *S. aureus*, and (c) *S. pyogenes*.

on the growth of micro-organisms in comparison with those witnessed.

The gram-positive bacterial strains (*S. aureus* and *S. pyogenes*) were more susceptible to the extracts as compared to the gram-negative bacteria (*E. coli*). This is in agreement with previous reports that plant extracts are more active against gram-positive than gram-negative bacteria (Iauk et al., 1996; Koutsoudaki et al., 2005; Özçelik et al., 2005; Kamrani et al., 2007 and Benhammou et al., 2008).

Similarly, when the minimal inhibitory concentration was evaluated using the resin oil of *P. atlantica* against gram-negative and positive bacteria, similar results to the disc diffusion method was produced.

The minimal inhibitory concentration (MIC) was defined as the lowest concentration of the test samples where the absence of growth was recorded (Glowniak et al., 2006). The results showed a variable effect of the oils on the micro-organisms (Figure 2). The MIC of the active essential oils of the resin oil of *P. atlantica* was tested at a volume ranging from 0.5 to 2.0 µl. The minimum inhibitory concentration (MIC) for the resin oil of *P. atlantica* showed an activity with values ranging from 3 - 11 g/ml against *E. coli*, 1 - 10 g/ml against *S. aureus* and 0 - 8 g/ml against *S. pyogenes* (Figure 2). *S. aureus* and *S. pyogenes* on the one hand were susceptible at 0.5 µg/ml and *E. coli* on the other hand was tolerant to this concentration (0.5 µg/ml).

Gram-negative bacteria were more resistant to the essential oils and can be attributed in part to the great complexity of the double membrane-containing cell envelope in contrast to the single membrane structure of gram-positive bacteria (Bagamboula et al., 2004). The data...
Figure 2. MIC evaluation of the essential oil resin of P. atlantica (0.1, 0.01 and 0.001), (a) E. coli, (b) S. aureus, and (c) S. pyogenes.

Table 2. MIC evaluation of essential oil resin of P. atlantica using the dilution broth method against the three bacterial strains.

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>Essential oil (µg/ml)</th>
<th>Control (0 µg/ml)</th>
<th>10⁻¹</th>
<th>10⁻²</th>
<th>10⁻³</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>S/C</td>
<td>D/C</td>
<td>+</td>
<td>+</td>
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<tr>
<td>E. coli</td>
<td>++</td>
<td>S/C</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>S. aureus</td>
<td>++</td>
<td>D/C</td>
<td>-</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>S. pyogenes</td>
<td>++</td>
<td>S/C</td>
<td>-</td>
<td>+</td>
<td>++</td>
</tr>
</tbody>
</table>

S/C : sample concentration - D/C : double concentration ; ++ : comparable growth with control; + : slow growth; - : growth inhibition.

Some researchers reported that there is a relationship between the chemical structures of the most abundant in the tested essential oil and the antimicrobial activity. Essential oils rich in phenolic compounds such as Pistacia specie are widely reported to possess high levels of antimicrobial activity (Malekzadeh, 1974; Yalpani and Tyman, 1983; Conner and Beuchat, 1984; Marner et al., 1991; Kubo et al., 1993; Ben Douissa, 2005). On the other hand, it should be noted that the two major volatile constituents, -pinene and terpinolene contained in the Pistacia specie are compounds with interesting antibacterial activity (Tsokou et al, 2007).

Conclusion

In this study, the antimicrobial activity of the essential oils resin of P. atlantica was studied. The oil showed activity against S. aureus, S. pyogenes and E. coli which are used as gram-negative and -positive bacterial models, respectively. The 3 studied methods confirm that the resin and its essential oil have inhibiting effects according to the dilution of the strain on one hand and the concentration of resin and essential oil on the other.

The oil was found to have significant antibacterial activity and therefore can be used as a natural antimicrobial agent for the treatment of several infectious diseases caused by these germs, which have developed resistance to antibiotics.

REFERENCES


