

Short Communication

Bactericidal activity of *Pistacia atlantica*. Desf mastic gum against certain pathogens

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The hydro-distilled essential oils from the exudates of *Pistacia atlantica*. Desf stems have been tested against three bacteria (*Escherichia coli*, *Staphylococcus aureus* and *Streptococcus pyogenes*) using three different methods (agar disc diffusion method, minimal inhibitory concentration and Maruzella method, and showed antimicrobial activity against all these microbes.

Key words: Antibacterial activity, essential oil, gum of *Pistacia atlantica*.

INTRODUCTION

The genus *Pistacia* includes many species widely distributed in the Mediterranean and Middle Eastern areas (Ben Douissa et al., 2005). Four species of *Pistacia* namely; *Pistacia lentiscus*, *Pistacia terebinthus*, *Pistacia atlantica* and *Pistacia vera* are reported in Algeria (Belhadj, 1999). "Gum" mastic, an oleoresin exudates from the stem of this plant (Dogan et al., 2003) is a source of traditional medicinal agent for the relief of upper abdominal discomfort, stomach aches, dyspepsia and peptic ulcer (Al-Said et al., 1986; Huwez and Al-Habbal, 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 micro-organisms has been reported by several researchers (Tassou and Nychas, 1995; Ben Douissa et al., 2005; Benhammou et al., 2008) but little is known on the bactericidal effect of *P. atlantica*. Desf extracts, precisely its oleoresin oils.

The objective of this work is to evaluate the antibacterial activity of the essential oil of mastic gum extracted from *P. atlantica*. Desf against the growth of clinical isolates of *Staphylococcus aureus*, *Escherichia coli* and *Streptococcus pyogenes*.

MATERIALS AND METHODS

Plant material and essential oil extraction

The resin of *P. atlantica* (pistachio tree of the Atlas) was collected in

May-June, 2006 from the Ain Fkain region, 24 km far from Mascara west of Algeria. The essential oil was extracted from the resin by hydrodistillation with ethanol using a Clevenger apparatus. The combined hydro-alcoholic extract was filtered through filter paper and evaporated to dryness under reduced pressure in a Rota-vapor (Heidolph Laborota 4000) and then stored in the dark at 4°C with an air tight container. The extract is further used for screening purpose.

Microbial strains

This study involves two clinical strains of *S. aureus* and *S. pyogenes*, gram positive bacteria, isolated from urine sample of a patient and a gram negative bacteria, *Escherichia coli* from a patient's stool culture at the Bacteriology Laboratory, Yessaâd Khaled Hospital, Mascara West Algeria, and identified in the Microbiology Laboratory, Biology Institute, Mascara University using standard biochemical and morphological methods (Edwards and Ewing, 1972; Marchal et al., 1982).

Antimicrobial screening

Three different methods were employed for the determination of antimicrobial activities; an agar disc diffusion method, determination of minimal inhibitory quantity (MIQ) and in the liquid phase by Maruzella method.

Agar disc diffusion method: *In vitro* antibacterial activity of the *P. atlantica* essential oil was determined by the agar disk diffusion method according to Rubio et al. (2003). A suspension of each tested micro organism (average concentration is 10^5 cells per ml) was mixed with 18 ml of Mueller Hinton Agar (MHA), then poured on Petri plates with sterilized Whatman No.3 filter paper discs (diameter 6 mm) impregnated with 15 µl of the oil and were incubated at 37°C for 24 h. The diameters of the inhibition zones were measured (Rubio et al., 2003).

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Table 1. Antimicrobial activity evaluation of the essential oil resin of *P. atlantica* with Agar disc diffusion method

| Bacterial strain | Resin concentration (µg/ml) Dilution | 10 ⁻¹ | 10 ⁻² | 10 ⁻³ | 10 ⁻⁴ | Witness |
|--------------------|--------------------------------------|------------------|------------------|------------------|------------------|---------|
| <i>E. coli</i> | 10 ⁻¹ | 5 | 4 | 4 | 3 | 3 |
| | 10 ⁻² | 6 | 5 | 5 | 3 | 5 |
| | 10 ⁻³ | 9 | 7 | 6 | 5 | 7 |
| <i>S. aureus</i> | 10 ⁻¹ | 5 | 3 | 3 | 1 | 4 |
| | 10 ⁻² | 7 | 5 | 4 | 2 | 5 |
| | 10 ⁻³ | 8 | 7 | 5 | 2 | 6 |
| <i>S. pyogenes</i> | 10 ⁻¹ | 4 | 3 | 2 | 0 | 4 |
| | 10 ⁻² | 6 | 5 | 2 | 2 | 6 |
| | 10 ⁻³ | 7 | 6 | 4 | 3 | 8 |

Table 2. MIQ evaluation essential oil resin of *P. atlantica* with the three bacterial strains

| Bacterial strain | Resin concentration (µg/ml) Dilution | 0.5 | 1 | 1.5 | 2 | Witness |
|--------------------|--------------------------------------|-----|---|-----|----|---------|
| <i>E. coli</i> | 10 ⁻¹ | 3 | 4 | 6 | 8 | 6 |
| | 10 ⁻² | 4 | 7 | 9 | 10 | 7 |
| | 10 ⁻³ | 5 | 8 | 10 | 11 | 7 |
| <i>S. aureus</i> | 10 ⁻¹ | 1 | 3 | 3 | 6 | 6 |
| | 10 ⁻² | 2 | 4 | 4 | 9 | 7 |
| | 10 ⁻³ | 3 | 6 | 7 | 10 | 9 |
| <i>S. pyogenes</i> | 10 ⁻¹ | 0 | 1 | 3 | 4 | 4 |
| | 10 ⁻² | 0 | 2 | 4 | 5 | 6 |
| | 10 ⁻³ | 0.5 | 3 | 5 | 6 | 8 |

Minimal inhibitory quantity (MIQ) determination: The minimal inhibitory quantity (MIQ) is defined as the smallest amount of product for which no growth is visible compared to the witness without the product. (De Billerbeck et al., 2002).

Three dilutions each of *S. aureus* and *E. coli* strain 10⁻¹, 10⁻² and 10⁻³ were prepared. The diluted microbes were spread over the surface of the Petri dish containing Mueller-Hinton agar medium.

Four discs of 6 mm diameter are placed on agar containing the following quantities of the resin dilution: 0.5, 1, 1.5 and 2 µl. In the centre of Petri dish, a witness disc is impregnated in parallel with 2 µl of ethanol. The Petri dishes are incubated then at 37°C for 24 h.

The liquid phase by Maruzella method: Principal of this technique is to act in the liquid phase of increasing concentrations of essential oil, after adding an emulsifier (Benhassaini et al., 2003).

Dilution series (10⁻¹, 10⁻² and 10⁻³) were prepared from the essential oil solution. 1 ml on each dilution and 0.5 ml of tested culture strains were added to 8 ml of Maruzella's nutrient broth, maintained in a Bain Marie at 37°C under constant agitation for 24 h, then seeded by streaking the surface of agar medium with tested culture strains and thereafter incubated at 37°C for 24 h (Benhassaini et al., 2003).

RESULTS AND DISCUSSION

Table 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 volumes of oil resin of *P. atlantica*. Antimicrobial activity by disc diffu-

sion 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 are no reports of inhibition. The gram (+) bacterium *S. aureus* and *S. pyogenes* were found to be more sensitive to the oil than the gram (-) bacterium *E. coli*. The oil resin at 10⁻² and 10⁻³ µg/ml showed moderate activity. The growths of tested bacteria in high concentrations of oil resin were highly inhibited, where it was considered that these organisms were sensitive to the oil.

The MIQ values varied from 0 to 11 µg/ml of oil for the microbes tested (Table 2).

S. aureus and *S. pyogenes* on the one hand were susceptible to 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 with the single membrane structures of gram-positive bacteria (Bagamboula et al., 2004). The data showed that, all bacterial strains were sensitive to 10⁻¹ µg/ml (Table 3). With an increasing dose of essential oil resin

Table 3. MIC evaluation of essential oil resin of *P.atlantica* with the three bacterial strains

| Bacterial strain | Essential oil (µg/ml) | | | | |
|--------------------|-----------------------|------------------|------------------|------------------|---|
| | witness« 0 µg/ml » | 10 ⁻¹ | 10 ⁻² | 10 ⁻³ | |
| <i>E. coli</i> | ++ | S/C | - | - | + |
| | | D/C | - | - | + |
| <i>S. aureus</i> | ++ | - | + | ++ | |
| <i>S. pyogenes</i> | ++ | - | + | ++ | |

S/C: Simple concentration

D/C: Double concentration

++: Comparable growth with that witness

+: Slow growth

- : Growth inhibition

of *P. atlantica*, the resulting diameter of the zone of inhibition increased for all the organisms.

Conclusion

The results of the study revealed that essential oil resin of *P. atlantica* has antibacterial activity against gram-positive *S. aureus* as well as gram-negative bacteria *E. coli* which are resistant to commonly used antimicrobial agents.

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