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Full Length Research Paper

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

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The essential oil from the gum of *Pistacia atlantica* 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 *Pistacia atlantica* 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). *Streptococcus pyogenes*, like *S. aureus*, causes an array of suppurative diseases and toxinoses, in addi-tion 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 disease treatment (Reinert et al., 2001; Sundqvist and Kahlmeter, 2005). For this reason research is ongoing for new antimicrobial agents, either by the design and synthesis of new agents or through the search of natural sources for as yet undiscovered antimicrobial agents (Cock, 2008). Essential oils have been traditionally used for the treatment of infections and diseases all over the world for centuries (Rios and Recio, 2005). "Gum" mastic, an oleoresin exudate from the stem 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-Habbal, 1986). *Pistacia* species have also been reported to possess stimulant and diuretic properties (Bentleyand 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* Desf. 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.*

MATERIALS AND METHODS

Plant material and essential oil extraction

The resin of *P. atlantica* (pistachio tree of the Atlas) was collected from the Ain Fkain region, 24 km from Mascara, west of Algeria between May-June 2006, which corresponds to the period of oleoresin formation. The essential oil was extracted from the resin the resin by hydrodistillation with ethanol. The combined hydroalcoholic ex-

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tract was filtered through filter paper and evaporated to dryness under reduced pressure in a Rota-vapor and then stored in the dark at 4°C with no air contact. The extract was further used for screening purposes (Benhassaini et al., 2003).

Microbial strains

The gram positive bacteria (*S. aureus and S. pyogenes)* and the gram negative bacteria (*E. coli)* were obtained from urine and stool samples respectively from patients attending Dr Yessaâd Khaled Hospital at Mascara city, west of Algeria. The bacterial samples were identified by standard biochemical tests and morphological studies (Marcha et al., 1982; Euzéby, 1998) in the Microbiology Laboratory, Biology Institute, Mascara University, Algeria.

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

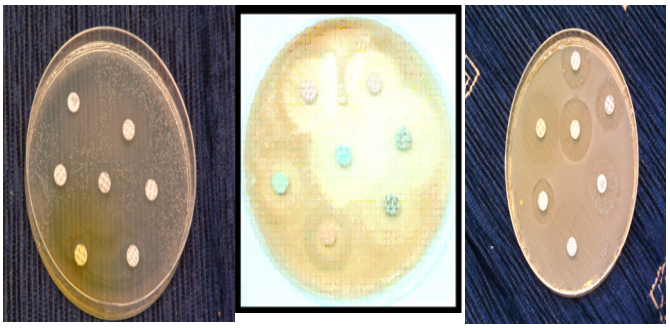
In vitro antibacterial activity of *P. atlantica* essential oil was determined by the agar disc diffusion method according to Benhassaini et al. (2003). Disc assays were found to be a simple, cheap and reproducible practical method (Maidment et al., 2006). 18 ml of sterilized Mueller Hinton agar medium was taken in each Petri dish and then spread with a suspension of the tested micro-organism (average concentration is 10^6 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 micro-organism after overnight incubation. Minimum inhibitory concentrations are important in diagnostic laboratories to confirm resistance of micro-organisms 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 µl. In the center of the Petri dish, a control disc is impregnated in parallel with 2 µl 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.



S. aureus *S. pyogenes* *E. Coli*

Plate 1. Antibiotic sensitivity testing against *S. aureus, S. pyogenes* and *E. coli*.

The MIC of penicillin G, paromomycin, gentamicin, cefazolin, amoxicillin, nitroxoline, vanomycine, oxacillin, chloramphenicol, colistin, neomycin, pristinamycin, trimethoprim-sulfamethoxazole and ampicillin were also determined in parallel experiments in order to control the sensitivity of the test micro-organisms.

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 nitroxoline, 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, neomycin 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 micro-organisms, 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 oleoresin 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 organisms were sensitive to the oil. On the other hand, at low concentrations, a very limited inhibitory effect was observed

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

Antibiotics	Micro-organisms					
	E. coli		*S. aureus*		*S. pyogenes*	
	Inhibition zone diameter (mm)	Results	Inhibition zone diameter (mm)	Results	Inhibition zone diameter (mm)	Results
Penicillin G	-	-	-	-	29	S
Paromomycin	-	-	6	R	-	-
Gentamicin	-	-	-	-	25	S
Cefazolin	17	S	10	I	-	-
Amoxicillin	-	-	-	-	20	S
Nitroxoline	09	R	20	S	-	-
Vanomycine	-	-	-	-	18	I
Oxacillin	13	I	10	I	-	-
Chloramphenicol	20	S	24	S	08	I
Colistin	-	-	-	-	04	R
Neomycin	29	S	19	S	-	-
Pristinamycin	-	-	-	-	02	R
Trimethoprim-sulfamethoxazole	2	R	24	S	-	-
Ampicillin	14	I	-	-	-	-

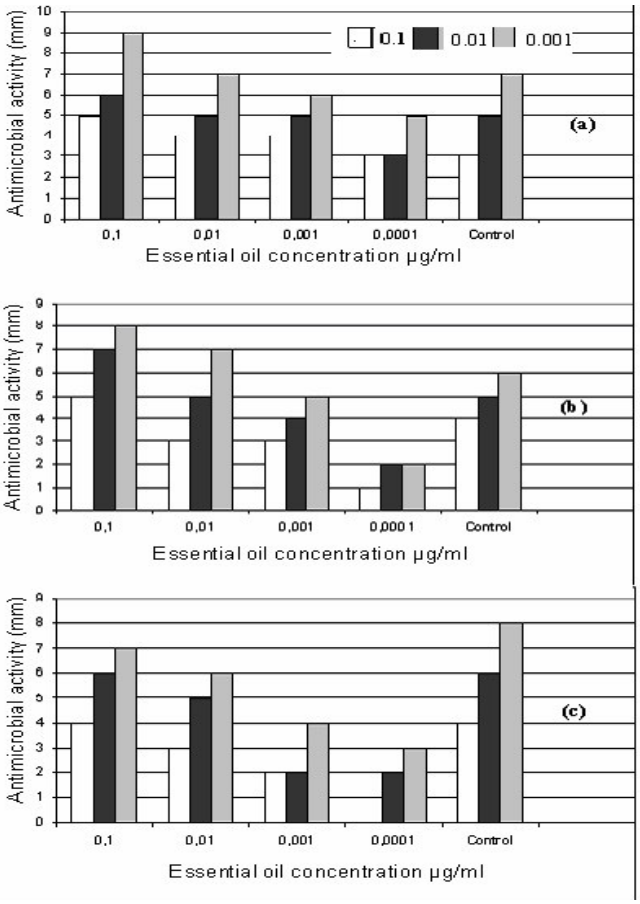


Figure 1. Antimicrobial activity evaluation of the essential oil resin of *P. atlantica* using agar disc diffusion method. Dilutions (0.1, 0.01, and 0.001); (a) *E. coli,* (b) *S. aureus,* and (c) *S. pyogenes*.

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 µg/ml against *E. coli,* 1 - 10 µg/ml against *S. aureus* and 0 - 8 µg/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

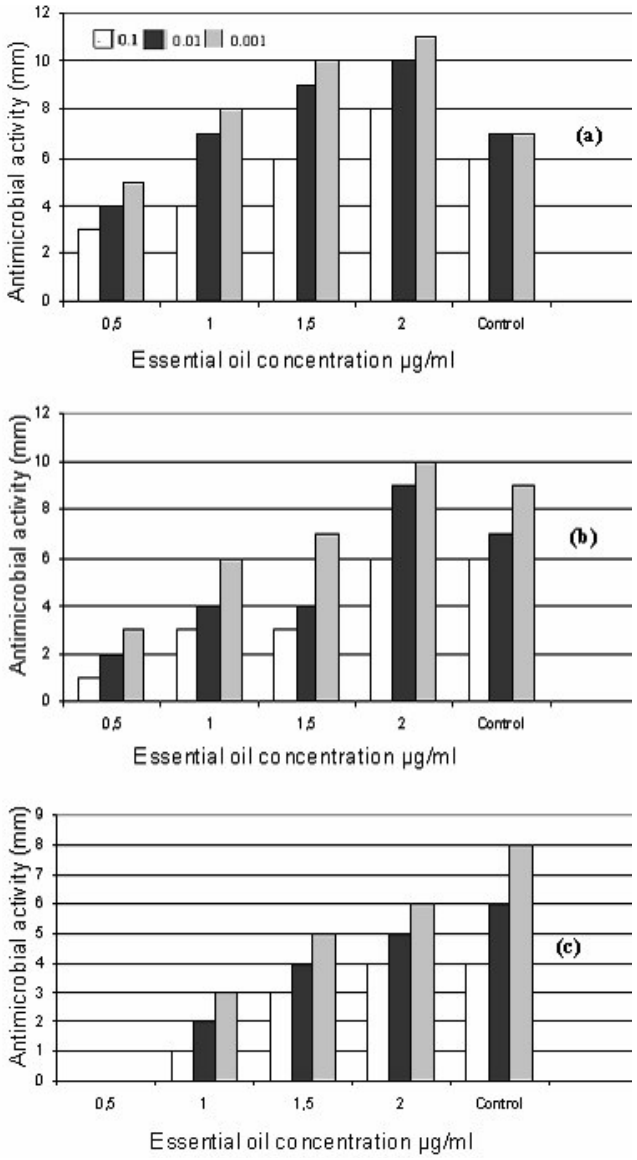


Figure 2. MIC evaluation of the essential oil resin of *P. atlantica* (0.1, 0.01 and 0.001), (a) *E. coli,* (b) *S. aureus,* and (c) *S. pyogenes.*

showed that, all bacterial strains were sensitive to 10^{-1} µg/ml (Table 2). With an increasing dose of essential oil resin of *P. atlantica*, the resulting diameter of the zone of inhibition increased for all the organisms.

The results of the study revealed that essential oil resin of *P. atlantica* have antibacterial activity against gram-positive as well as gram-negative bacteria. Some authors have reported that gram-positive micro-organisms are slightly more sensitive to essential oils when compared to gram-negative (Canillac and Mourey, 2001; Dermetzos and Perdetzoglou, 2001). This lower sensitivity of gram-negative organisms has been related to the presence of an outer membrane surrounding their cell wall, which restricts the diffusion of hydrophobic compounds through its lipopolyssacharide covering (Vaara, 1992).

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

Bacterial strain	Essential oil (µg/ml)				
	Control (0 µg/ml)	10^{-1}		10^{-2}	10^{-3}
E.coli	++	S/C	-	-	+
		D/C	-	-	+
S. aureus	++	-		+	++
S. pyogenes	++	-		+	++

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, α-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.

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