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Full Length Research Paper

Antimicrobial activity determination of the gum of *Pistacia atlantica* Desf. oil

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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 the agar disc diffusion, the minimal inhibitory quantity (MIQ) determination method and the liquid phase by Maruzella method. The results of the study revealed that essential oil resin of *P. atlantica* has antimicrobial activity against Gram-positive as well as Gram negative bacteria resistant to commonly used antimicrobial agents, and they were considerably dependent upon concentration.

Key words: Gum, *Pistacia atlantica* Desf. oil, antimicrobial activities, clinical isolates.

INTRODUCTION

The spread of drug resistant pathogens is one of the most serious threats to successful treatment of microbial diseases. Back to the ages, essential oils and other extracts of plants evoked interest as sources of natural products. They have been screened for their potential uses as alternative remedies for the treatment of many infectious diseases (Tepe et al., 2004).

Escherichia coli and Staphylococcus aureus are two of the most important pathogens causing serious infections (Lestari, 2004) and can require antimicrobial resistance to prevent disease treatment effect (Sundqvist and Kahlmeter, 2005). *P. atlantica* Desf. is an evergreen shrub of the Anacardiaceae family, very common in the North Africa (Bailey, 1985).

In Algeria, it is a tree reaching 25 m in height and grows in arid and semi-arid areas (Benabid, 2000). *P. atlantica* is valued because it is the source of mastic gum, an exudate which strengthens gums, deodorizes breath, combats coughs, chills and stomach diseases (Bellakhder, 1997).

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* and *E. coli.*

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MATERIALS AND METHODS

Plant material and essential oil extraction

The resin of *P. atlantica* (pistachio tree of the Atlas) was collected from the Sfisef region, 40 km far from Sidi Bel Abbès west of Algeria. The essential oil extracted from resin by hydrodistillation with ethanol. The combined hydroalcoholic extract filtered through filter paper and evaporated to dryness under reduced pressure in a Rotavapor, 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

This study involves two clinical strains, where it is *S. aureus and S. pyogenes* a gram positive bacteria isolated from urine sample of a patient and the second is *E. coli* gram negative bacteria was diagnosed by patient's stool culture. The two bacterial strains are isolated in bacteriology laboratory located in Dr Yessaâd Khaled hospital at Mascara city, town situated in the west of Algeria, then confirmed by biochemical tests and morphological studies in microbiology laboratory located in biology institute to the Mascara University (Euzéby, 1998; Marcha et al., 1982).

Antimicrobial screening

Three different methods were employed for the determination of

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}	Control
E. coli	10^{-1}	5	4	4	3	3
	10^{-2}	6	5	5	3	5
	10^{-3}	9	7	6	5	7
S. aureus	10^{-1}	5	3	3	1	4
	10^{-2}	7	5	4	2	5
	10^{-3}	8	7	5	2	6
S. pyogne	10^{-1}	4	3	2	0	4
	10^{-2}	6	5	2	2	6
	10^{-3}	7	6	4	3	8

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). Briefly, a suspension of each tested microorganism (average concentration is 10^6 cells per ml) was carefully mixed in a tube with 18 ml of Mueller Hinton Agar (MHA), and then poured on Petri plates. Sterile filter-paper discs (Whatman) were impregnated with 15 µl of the oil and placed on the inoculated plates. These plates were incubated at 37°C for 24 h. The diameters of the inhibition zones were measured in millimeters and their means were calculated (Benhassaini et al., 2003; Rubio et al., 2003).

Minimal inhibitory quantity (MIQ) determination

The minimal inhibitory quantity (MIQ) is defined as the smallest amount product for which no growth is visible compared to the control without the product (De Billerbeck et al., 2002; Pauli and Kubeczka, 1996).

Three dilutions of each *S. aureus* and *E. coli* strain (10^{-1}, 10^{-2} and 10^{-3}) and control dilutions, 10^{-1} in ethanol of the *P. atlantica* resin are prepared.

From the each dilution of strain are spread slicks over the surface of the Petri dish containing Mueller-Hinton agar medium liquid which must then be solidifying.

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 l. In the centre of Petri dish, a control disc is impregnated in parallel with 2 l 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; Benjilali et al., 1986).

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

RESULTS AND DISCUSSION

Antimicrobial activity by disc diffusion method showed that the oil resin of *P. atlantica* was active against *E. coli* and *S. aureus* than *S. pyogenes.* Table 1 show 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.*

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 were highly inhibited, where it was considered that these organisms were sensitive to the oil.

From Table 2, it can be observed that all micro-organisms were susceptible to the action of essential oil resin of *P. atlantica*, with a variation in the MIC values from 0 to 11 µg/µl.

The generally verified greatest resistance of Gram-negative bacteria to essential oils has been attributed in part to the great complexity of the double membrane-containing cell envelope of these microorganisms in contrast with the single membrane structures of Gram-positive bacteria (Bagamboula et al., 2004; Hammer et al., 1999; Helander et al., 1998; Janssen et al., 1987; Shapiro et al., 1994; Tepe et al., 2004; Velickovic et al., 2002) . This idea is confirmed in our work where it was noted an important difference between *S, aureus and S. pyogenes* on the one hand and *E. coli* on the other. The dilutions 10^{-1} of *S. aureus and E .coli* have shown the

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

Bacterial strain	Resin concentration (µl) Dilution	0.5	1	1.5	2	Control
E. coli	10^{-1}	3	4	6	8	6
	10^{-2}	4	7	9	10	7
	10^{-3}	5	8	10	11	7
S .aureus	10^{-1}	1	3	3	6	6
	10^{-2}	2	4	4	9	7
	10^{-3}	3	6	7	10	9
S. pyogenes	10^{-1}	0	1	3	4	4
	10^{-2}	0	2	4	5	6
	10^{-3}	0.5	3	5	6	8

Table 3. Antimicrobial activity evaluation of essential oil resin of *P. atlantica* using the liquid phase by Maruzella method against the bacterial strains.

Bacterial strain	Essential oil (µg/ml)				
	witness« 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 that Witness; +: Slow growth ; -: Croissance inhibition.

resistance of these strains to a quantity of 0.5 µl which corresponds to the MIQ, where as for other dilutions, MIQ is 1 µl, but it is 1.5 for *S. pyogenes.*

The data show that all bacterial strain were sensitive to 10^{-1} µg/ml (Table 3). 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 has antibacterial activity against Gram-positive as well as Gram negative bacteria resistant to commonly used antimicrobial agents.

Conclusion

In this study, the antimicrobial activity of the essential oils resin of *P. atlantica* was studied. The oil showed activity against *E. coli* and *S. aureus* which are used as Gram negative and Gram positive bacterial models, respectively. The three studied methods confirm that the resin and its essential oil has an inhibiting effect according to the dilution of the strain on the 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, who have developed resistance to antibiotics and in food preservation

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