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# Antibacterial activity of *Pinus halepensis* essential oil from Algeria(Tlemcen)

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### ABSTRACT

The antimicrobial activity of essential oil of Pinus halepensis tree from Ghazaouet (Tlemcen) against bacteria (Staphylococcus aureus ATCC25 923, Pseudomonas aeruginosa ATCC 27853, Escherichia coli ATCC25 922 et ATCC25 921, Bacillus cereus ATCC 11778) was determined. It was evaluated by two methods, disc diffusion and broth dilution. Essential oil of Pinus halepensis showed moderate for the whole strains tested expect P.aeruginosa et E.coli who were revelated very resistants.

Keys words: Aleppo pine (Pinus halepensis), CG/SM, antibacterial activity .

#### INTRODUCTION

Recent years have witnessed a substantial increase in bacterial and fungal infections in the hospital, this must be added to the problem of bacterial resistance to antibiotics[1-5]. Moreover, the food has not been saving from the different contamination causing economic losses and severe health consequences.

Again, and facing of the suspicion aroused by the increased use of chemicals products in the therapeutic domain and the food sector, there is a renewed interest for natural antimicrobials agents [4]. All these reasons enhance the necessity of finding new antimicrobial agents. Currently, the research turns to the operation of a natural product. Medicinal plants and plant secretions such as essential oils are interesting for their antimicrobial potency and may be a solution to these preoccupants problems. Antimicrobial activity of EO has been demonstrated by several authors. Now, 3000 EO are known and 300 of them are marketed primarily in the pharmaceutical, food and cosmetics field.[5]

The chemical complexity of essential oils prevents the decoding of the part of pathogens and reduces the risk of developing resistance. In addition, the EO also acts on the grounds of the ill,

they increase the defensive capacity of the body, rebalancing the intestinal flora and impaired functions [1].

In this context, we are interested in searching for antimicrobial activity of the essential oil of Aleppo pine (*Pinus halepensis*) in the area of Ghazaouet (Tlemcen west Algeria).

### MATERIALS AND METHODS

Microorganisms:

The evaluation of the antibacterial activity of the EO of *Pinus halepensis* was performed against four strains, including two Gram positive bacteria, *Staphylococcus aureus* ATCC 25 923, *Bacillus cereus* ATCC 11778 and two Gram negative bacteria *Escherichia coli* ATCC 25 922, *Pseudomonas aeruginosa* ATCC 27 853. Two techniques were used : disc diffusion method and the dilution agar method. The minimum inhibitory concentration(MIC) was determined by the latter method.

Disc diffusion method: Briefly, the agar plate containing the appropriate medium was spread with the inoculum containing  $10^8$  colony forming units(UFC / ml)[6].Discs of sterile Whatman paper [7] of 6 mm in diameter are impregnated with a quantity of essential oil (10µl) and deposited on the plates (one disc per box). After incubation at 37C for 24h, the diameters of inhibition zones were measured in mm for the test organisms and comparing to the controls.[8]

Dilution agar method: Because of the immiscibility of essential oils to the water, the emulsification was carried out with a solution of 0.2% agar [9, 10]. 150,200,250,300 and 350µl of EO were added to 1650,1700,1750,1800 and 1850µl of 0.2% agar respectively. Total volume(2ml)of each dilution was added aseptically to 18ml of culture medium. The tubes were sterilized in an autoclave for 20 min at 120 ° C and were stirred by a vortex tube to disperse the EO. Finally seeding is done by the filing of the inoculums containing 1µl of  $10^8$ germes/ml. The results are seen after 12 to 18h incubation at 37 °. We obtain final concentrations of 17.5, 15, 12.5, 10, 7.5 µl/ml. All the experiments were performed in triplicate.

(MIC: the lowest concentration of the product for which no growth is visible compared to the control without products

## **RESULTS AND DISCUSSION**

The antimicrobial activity of *Pinus halepensis* against foor pathogenic microorganisms is shown in table 01,our EO exhibited strong antimicrobial activity against the strains tested.

Bacterial strains	Dis diffusion method Ø(mm)	Dilution agar method MIC(µl/ml)
Staphylococcus aureus(S) ATCC 25 923	4.5 (S)	$7.5 \leq MIC \leq 10$
Bacillus cereus (B) ATCC 11778	3 (S)	7.5≤ MIC≤10
Escherichia coli (E) ATCC25 922	0.5 (R)	≥17.5
Pseudomonas aeruginosa (P)ATCC 27 853	0 (R)	>17.5

Resistant strain R:  $\emptyset < 0.6$  cm; Intermediate I strain: 1.3 cm < diameter < 0.6 cm; Sensitive strain S:  $\emptyset > 1.3$  cm [8].

Results obtained from the disc diffusion method ,followed by measurement of minimum inhibitory concentration(MIC),indicated that S.aureus ATCC 25923 was the most sensitive

bacteria showing largest inhibition zones(45mm\_, 7.5 <MIC <10  $\mu$ l) followed by *B.Cereus*, ATCC11778 (30mm\_, 7.5 <MIC <10  $\mu$ l). Less activity was observed against *E. coli ATCC 25922* and *P. aeruginosa ATCC 27853* with the smallest inhibition zones(5mm\_0mm)and highest MIC value MIC> 17 $\mu$ l/ml

These results are in good agreement with those found in other studies on the Pinus species. *Pinus scots* and *Pinus pinaster* EO show active against *S.aureus* (inhibition diameter: 30 mm / 18 mm) and inactive on *P. aeruginosa* (inhibition diameter: 6 mm / 10 mm) [8]. In fact, it has been suggested that the antibacterial activity of tea tree oil against S.aureus might be attribuated to terpinen\_4\_ol.(11) Other authors [12,13,14] argue that the presence of small amounts of phellandrene ,terpineol, and more terpinene-4-ol in EO is responsible for the activities against S.aureus. The absence of some molecules ( $\alpha$ -terpinene, terpinolene and terpinene  $\delta$ ) of the EO may be responsible for his part in activity against S. aureus. [12,13,15].

On the other hand, the study of Deba in 2008 [16] showed that EO of needles Bidens pilosa have antibacterial action against *B.cereus* and *B.subtilis*, According to the authors this action is due to its high capacity  $\beta$ -caryophyllene, which represents the major component with 10.9%. It has been demonstrated that the monoterpene hydrocarbon and oxygenated monoterpenes in flowers of Bidens pilosa EO are able to destroy cellular integrity, thus inhibiting respiration and ion transport. Bekkali et al (2008) [7] reported that  $\beta$ -caryophyllene has a moderate role in the bacterial action [5]

Among the nine fir EO from Turkey, E. coli was inhibited by any ONE [15]. In the same, the Picea EO proved inactive on E. coli strain [18] In fact, E. coli is known for its resistance to HE of conifers [12]. Several studies confirm that coliforms are sensitive to EO-containing phenolic compounds. The molecule of thymol have an inhibitory and lethal effect on various strains of E. Coli on which it causing leakage of K + ions.

Concerning the *P. aeruginosa*, this strain is knowning for its resistance to any kind of antimicrobial agents and antibiotics. [8] A large majority of selected EO showed no activity [20,21]. In reality, this behavior is not surprising as the strains of *P. aeruginosa* possess intrinsic resistance to a wide range of biocides, associated the nature of its outer membrane, the barrier "hydrophilic permeability barrier" protects against toxic agents. In addition, this bacterium has an ability to form a biofilm (a complex organization composed of different strata in which bacteria are found in physiological conditions specific to their situation).

Finally, Pinus halepensis EO was more active against Gram positive. It seems that typical Gramnegative bacteria are more resistant to EO. These results are confirmed by many studies [23.35]. However, it is important to mention the full name, the Gram micro-organisms ,the botanical species and chemotype of EO[8]. Other factors can influence, the method used, method of EO extraction and the season récolte.In addition, there is no standard method for evaluating antibacterial activity, these findings show the difficulty of comparing the results published.

Conclusion Evaluation of antibacterial activity of *Pinus halepensis* essential oil by disc diffusion and dilution agar method, showed moderate activity on *S. aureus* ATCC 25923 and *B. cereus ATCC* 11778, it is attribuated to the presence of some molecules knowning by its antibacterial activity(terpinen-4-ol for *S. aureus*,  $\beta$ -caryophyllene and caryophyllene\_oxide for *B. cereus*). *E. coli* ATCC25 922 and *P. aeruginosa* ATCC 27853 strains were revelated very resistants. it may be explained by the absence of phenol groups(thymol, carvacrol) active on E. coli and cinnamic acid and soufres compounds response for the action against P. aeruginosa

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