Antibacterial properties of the essential oil of *Pelargonium graveolens* L'Hér

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ABSTRACT

Antibiotic resistance is a problem facing health professionals around the world. Faced with these resistances, a major interest is taken to aromatic and medicinal plants whose antibacterial properties are due essentially to the essential oil fraction. In the perspective of using the essential oil of *Pelargonium graveolens* in medical practice, we proposed to research, *in vitro*, its activity against bacterial strains from clinical infections. The antibacterial test was evaluated against five microorganisms using two different methods: the disc diffusion method and the macrodilution method which aims to determine the minimum inhibitory concentration (MIC). The essential oil exhibited promising antibacterial power against the tested strains.

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1. INTRODUCTION

The natural products are becoming more important in the pharmaceutical and agricultural industries. Indeed, a number of chemical products from organic synthesis require pharmacovigilance because of their adverse effects. Research strategies for natural active substances from plants are often based on ethnobotanical approaches, highlighting the use of these plants in traditional medicine by local populations.

With the emergence of the phenomenon of resistance to current treatments, whether in human health or in the agricultural sector, strategies are now put in place to renew synthetic biomolecules.
The establishment of research strategies for new molecules has become a necessity. Given the wide variety of resistance mechanisms adopted by bacteria, fields of investigation are undoubtedly larger. However, the strategies can be based on the identification of antibacterial agents, capable of acting by new mechanisms of action, or on the identification of new bacterial targets, in particular with a view to developing agents capable of inhibiting resistance mechanisms [1-3]. Like some microorganisms such as Penicillium or Streptomyces that have served as a source of antimicrobials, medicinal and aromatic plants are integrated into the new research strategies.

In this context, essential oils constitute the major part of the natural aromatic compounds which are now increasingly used in various fields and possess a wide range of biological activities (antioxidant, antimicrobial, anti-carcinogenic, anti-inflammatory,...). Several studies have shown the antibacterial activity of essential oils on various pathogenic bacteria [4-11]. All these works, as well as others, has introduced essential oils as promising sources facing the development of bacterial resistance to antibiotics.

*Pelargonium graveolens* L’Hér, or roast Geranium (Geraniaceae family), belongs to the category of perennials with fragrant foliage. The evergreen leaves are lobed and opposite, covered with microscopic glandular hairs that release their fragrance to the touch or heat [12]. The plant is cultivated in many Mediterranean and subtropical regions, and its location strongly determines its chemical composition. Volatile research on this species has been done, Citronellol, geraniol, citronellyl formate, linalool, isomenthone and guai-6,9-diene were the most abundant constituents reported [13-22]. In addition, *P. graveolens* is consumed mainly for the treatment of dermatological conditions, against neurological disorders, for the treatment of diseases of the digestive system, the treatment of osteo-articular diseases, the treatment of respiratory and cardiovascular diseases [16]. The bio-activity of this plant has been reported in several studies [23-25].

The purpose of this study is to determine the antibacterial activity of the essential oil of the aerial part of *Pelargonium graveolens* against five bacterial strains in order to promote this species for application as an antimicrobial agent.

2. **RESEARCH METHOD**

2.1. **Plant material**

The harvest of the *Pelargonium graveolens* samples was carried out in April (2015) in the village of Ain Cheggag (33°52'59.99" N - 5°01'60.00" W), located in the region of Fes-Meknes. The samples were then dried in the shade for 8 days before extraction.

2.2. **Extraction of essential oils**

The aerial parts of the plant were subjected to hydrodistillation for 3 h using a Clevenger type apparatus [26]. The obtained collected essential oil was kept in the dark at 4 °C until further use.

2.3. **Microorganisms studied**

The essential oil was tested against five clinical bacteria: *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Streptocoque spp* and *Staphylococcus aureus*. These strains were chosen for their pathogenicity, and they were obtained from the microbiology laboratory of Military Hospital Moulay Ismail in Meknes.

2.4. **Tests of antibacterial activity**

2.4.1. **Disc diffusion method**

A bacterial suspension was diluted and adjusted to turbidity equal to that of the McFarland standard 0.5 (10^8 CFU/mL). The Mueller Hinton Agar was poured into Petri dishes 90 mm in diameter. The
agar surface was seeded with the microbial suspension. Paper dishes, of 6 mm diameter impregnated with 2 µL, 5 µL, 10 µL and 15 µL of the essential oil, were deposited on the surface of the agar. The dishes were then incubated at 37 °C for 24 hours. After incubation, an area or clear halo is present around a disc if the essential oil inhibits bacterial growth [27]. All tests were repeated three times.

2.4.2. Broth dilution method (Determination of MIC and MBC)
The minimal inhibition concentration (MIC) was determined by the method of macro-broth dilution [28]. A cascade dilution was performed in Mueller Hinton Broth-Tween 80 (0.01%, v/v), so as to obtain a concentration range between 80 µL.mL\(^{-1}\) and 0.3 µL.mL\(^{-1}\). 13 µL of a bacterial inoculum, equivalent to the standard density of 0.5 McFarland (10\(^8\) UFC.mL\(^{-1}\)), was deposited in each of the tubes of the range. A control of the bacterial growth, for which 13 µL of the standardized inoculum was deposited in MHB-Tween 80 medium (0.01 %, v/v), was also carried out. The tubes were incubated at 37 °C for 24 hours. The MIC is the lowest concentration of essential oil giving a growth inhibition.

The minimum bactericidal concentration (MBC) was determined from the MIC [28]. The tubes showing no visible growth with the naked eye after incubation and the control tube were streaked on MHA. The inoculated dishes were incubated for 24 hours at 37 °C. The MBC is the lowest concentration that shows no bacterial growth.

3. RESULTS AND ANALYSIS

The antimicrobial essential oil power of the aerial part of *Pelargonium graveolens*, from the region of Fez, has been studied against five pathogenic strains causing cases of food poisoning. The results of the zones of inhibition at the different concentrations of the essential oil studied are shown in Table 1. The results obtained from the aromatogram showed that the essential oil of the aerial part of studied plant has an antibacterial activity against the microorganisms tested. Furthermore, the zone of inhibition increased with increasing oil concentration.

The minimum inhibitory concentrations MIC are around 1.25-10 µL/mL (Table 2). Indeed, *Escherichia coli* was the most sensitive bacteria to the essential oil with a minimum inhibitory concentration of 1.25 µl / mL.

The MBC/MIC ratio defines the bacteriostatic or bactericidal character of an essential oil; if this ratio is less than 4, the oil is considered bactericidal [28]. The MBC/MIC ratio of the essential oil of the aerial parts of *P. Graveolens* is between 1 and 2, which confirms that this oil exerts a bactericidal effect (table 3).

Our results are in agreement with the investigations of several authors who have shown that the essential oil of the aerial parts of *P. Graveolens* has a significant antibacterial effect. Boukhatem et al. tested the in vitro antimicrobial activity of the essential oil from the aerial parts of *Pelargonium graveolens* grown in Blida city (Algeria), against 23 food-related microorganisms. The results obtained from the disc diffusion method, indicated that the oil exhibited antimicrobial activity against all Gram-positive bacteria and 3 Gram-negative bacteria at the concentration of 10 µL per disc [20].

Indeed, the MIC values obtained from the evaluation of the antibacterial activity of the essential oil of *Pelargonium graveolens* from Taounate (Morocco) against *Staphylococcus epidermidis* and *Acinetobacter baumannii* range from 0.0625 to 0.25% (v / v) [16].

The mechanism of action of essential oils is mainly related to the structure of the wall and the membrane permeability of bacteria. The essential oil exerts its antimicrobial capacity by its interference with the lipid bilayer of the bacterium by virtue of its hydrophobic property, which
entails: the increase of the permeability then the loss of the cellular constituents; acidification of the interior of the bacterium, blocking the production of cellular energy and synthesis of structural components; the destruction of the genetic material, leading to the death of the bacteria [29]. In contrast, Gram- bacteria are more resistant than Gram +, this is due to the structural differences of their outer membranes [30].

The exact mechanisms of the antibacterial effect of essential oils are still unclear. Indeed, it is extremely difficult to propose a general mechanism of action for all essential oils on all bacteria, since each essential oil has a particular chemical composition and specific biological properties, and each type of microorganism has a distinct sensitivity.

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<th>Table 1. Diameter of inhibition zones (mm)</th>
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<th>Table 2. MICs of the essential oil of Pelargonium graveolens in Mueller Hinton broth</th>
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(+) Growth, (-): Inhibition, C: Control, [C]: Concentration

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<th>Table 3. Antibacterial parameters (MIC and MBC) of the essential oil of the Pelargonium graveolens</th>
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4. CONCLUSION

Currently, the field of infectious diseases facing serious antibiotic resistance problems. This problem is further compounded by the effect of antibiotics on human health (alteration of the commensal flora of the intestine) and on biodiversity when they are found in the environment. Essential oils are the best candidates to participate in the search for alternatives. Currently, no one can deny the action of essential oils on resistant bacterial strains or multiresistant to antibiotics, and the literature is full of studies on the antimicrobial power of essential oils. The essential oil of Pelargonium graveolens is a potential candidate for application in the treatment of infections.
These data are important for any application of this oil as antibacterial; they allow proposing possible associations with other agents or even with antibiotics.

REFERENCES


