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Research Article

ANTI-BACTERIAL ACTIVITY OF ESSENTIAL OIL OF THE FLOWERING TOPS OF *Rosmarinus officinalis* L., FROM TÉBESSA (ALGERIA)

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ABSTRACT. The objective assigned to the present study is the evaluation of the antibacterial activity of essential oil of the flowering tops of *Rosmarinus officinalis* L., from Tébessa (Algeria). The antibacterial activity of the essential oil is carried out by the diffusion method on agar medium vis-à-vis eighteen bacterial strains, chosen according to the traditional use of this species in Algeria: *Escherichia coli* (ATCC22), *Escherichia coli* (BLSE), *Escherichia coli* (ciproR), *Escherichia coli* (mcr1), *Klebsiella pneumoniae* (C+), *Klebsiella pneumoniae* (C-), *Salmonella sp*, *Enterobacter cloacae* (FOSR1), *Bacillus cereus*, *Pseudomonas aeruginosa* (ATCC53), *Pseudomonas aeruginosa* (VIM21), *Acinetobacter* (OXA23), *Acinetobacter* (NDM1), *Staphylococcus aureus* (13), *Staphylococcus aureus* (23), *Staphylococcus aureus* (23), *Enterococcus faecalis* (ATCC12), *Sphingomonas sp*. *Staphylococcus aureus* (23) was the most sensitive species followed by *Pseudomonas aeruginosa* (ATCC53) and *Acinetobacter* (NDM1). Significant sensitivity was observed for the *Escherichia coli* (BLSE), *Salmonella sp* and *Sphingomonas sp*. However, *Bacillus cereus* was the most resistant to the essential oil tested.

Keywords: *Rosmarinus officinalis L., Tébessa, flowering tops, essential oil, antibacterial effect*

INTRODUCTION

The Lamiaceae family consists of herbs, bushes and occasionally trees with a cosmopolitan distribution, including approximately 300 genera and 7500 species [1].

Essential oils also called volatile odoriferous are aromatic and natural complex mixture of compounds obtained from different parts of vegetables, such as flowers, peels, barks, leaves, roots, stems and seeds [2].

The antimicrobial properties of essential oils have been known for many years, and oils from popular, commercially available aromatic plants, have been used extensively to treat bacterial and fungal infections. The therapeutic limits of classical antibiotics have pushed scientists to direct research towards new avenues and especially the use of active plant ingredients (phenolic compounds, alkaloids, essential oils...) as antibacterial agents.

The Algerian flora is characterized by its floral diversity: Mediterranean, Saharan and a Paleo Tropical flora, estimated at more than 3000 species belonging to several botanical families, of which 15% are endemic [3]. This has given the traditional pharmacopoeia an inestimable richness. Among these, *Rosmarinus officinalis* L., subject of this article, this species is widely used in traditional medicine such as: antirheumatic. It is placed in the category of purifying plants for its action on the digestive and urinary systems. In gastronomy it is used also as a spice for food preparation and preservation of food (i.e. meat). Commonly called in Eastern Algeria "Klil, Iklil", in order to confirm or deny these therapeutic virtues, we evaluated in vitro its inhibitory power against eighteen pathogenic and multi-resistant strains.

MATERIALS AND METHODS

Plant Material

The species *Rosmarinus officinalis* L. was harvested in the region of Tébessa (north-eastern Algeria) in February 2021. The identification of the plant was done with the key to determining the flora of Quezel and Santa [4].

Specimens were kept at the Laboratory of Cryptogamy and Medical Botany, Department of Pharmacy, Faculty of Medicine Annaba-Algeria.

Essential oil

Essential oil of flowering tops of *Rosmarinus officinalis* L. has been procured from production unit of essential and vegetable oils “Elexir Est” Algeria.

The yield of the essential oil was calculated by the following formula: R (%) = (M/M0) ×100. With: R (%): yield expressed in%; M: mass in grams of the resulting essential oil; M0: mass in grams of the plant material to be extracted.

Antibacterial test

The test of the sensitivity of the bacteria is carried out by the diffusion method in agar medium (the disk method). It is a method similar to that of the antibiogram which consists in determining the sensitivity of a bacterial strain vis-à-vis one or more substances [5]. The antibacterial activity of the essential oil of the flowering tops of *Rosmarinus officinalis* L. is evaluated vis-à-vis vis à-vis eighteen bacterial strains, chosen according to the traditional use of this species in Algeria: *Escherichia coli* (ATCC22), *Escherichia coli* (BLSE), *Escherichia coli* (ciproR), *Escherichia coli* (mcr1), *Klebsiella pneumoniae* (C+), *Klebsiella pneumoniae* (C-), *Salmonella sp, Enterobacter cloacae* (FOSR1), *Bacillus cereus, Pseudomonas aeruginosa* (ATCC53), *Pseudomonas aeruginosa* (VIM21), *Acinetobacter* (OXA23), *Acinetobacter* (NDM1), *Staphylococcus aureus* (13), *Staphylococcus aureus* (23), *Staphylococcus aureus* (23), *Enterococcus faecalis* (ATCC12), *Sphingomonas sp.* These strains were kindly provided by the Microbiology Laboratory Manager at Annaba Medical School, Algeria.

Preparation of The Inoculum

By taking eighteen tubes that each contains 5 ml of sterile physiological saline. Using a platinum loop, some well isolated colonies are scraped from each of the bacteria, each of which will be discharged into a tube. The five dilutions (1/2, 1/4, 1/8, 1/16, 1/32) of the essential oil were prepared in dimethylsulfoxide (DMSO) solvent without any antibacterial effect. The tubes are vortexed until complete dissolution of the essential oil. Seeding should be done within 15 minutes after the preparation of the inoculum. In 18 sterile Petri® dishes, 20 ml of agar are poured. After solidification of the medium, the latter is inoculated with 1 ml of bacteria to be studied. Then, it is spread on the surface using a glass rake. Sterile 5 mm diameter disks prepared in

Whatman® n°1 papers are impregnated with a sterile metal forceps in each dilution and placed on the surface of the solidified medium (Mueller-Hinton). The dishes were incubated for half an hour at room temperature, then for 24 to 48 hours in an oven at 37 °C. The reading is carried out by measuring the diameter of the inhibition zone (Ø), which translates into a translucent halo around each disc; the presence or absence of a halo would explain the sensitivity or the resistance of the germs vis-a-vis extracts tested; according to a symbolic notation scale from - to +++ [6, 7].

Table 1. *Sensitivity of microbial strains according to zones of inhibition*

Sensitivity	**Inhibition zone**
Not sensitive or resistant (-)	Diameter <10 mm
Sensitive (+)	Diameter between 10 to 16 mm
Very sensitive (++)	Diameter between 16 to 25 mm
Extremely sensitive (+++)	Diameter > 25 mm

RESULTS AND DISCUSSION

Extraction Yield

The yield of the essential oil obtained from the hydro-distillation of the flowering tops of *Rosmarinus officinalis* L., was 0.35%.

The essential oils are influenced by diverse factors, several of them have been studied such as seasonal and maturity variation, geographical origin, genetic variation, growth stages, part of plant utilized and postharvest drying and storage [8,9,10].

Reading Antibiograms

The results of the antibacterial activity are shown in Table 2.

Table 2. *Inhibition Diameter (mm) of raw essential oil of Rosmarinus officinalis L. and these dilutions*

	Raw	**1/2**	**1/4**	**1/8**	**1/16**	**1/32**
Escherichia coli **(ATCC22)**	13.55	16.78	14.29	13.75	12.02	10.05
Escherichia coli **(BLSE)**	17.66	6.1	5.2	5.1	5.4	5.33
Escherichia coli **(ciproR)**	8.58	7.26	5.71	5.40	5.30	5.20
Escherichia coli **(mcr1)**	5.20	8.95	8.14	7.07	7.04	6.14
Klebsiella pneumoniae **(C+)**	9.07	9.91	9.17	7.89	6.43	5.68
Klebsiella pneumoniae **(C-)**	5.34	14.56	12.28	10.74	8.69	9.94
Salmonella sp	9.28	17.7	15.1	13.71	9.65	7.94
Enterobacter cloacae **(FOSR1)**	9.49	10.86	7.19	7	6.26	5.79
Bacillus cereus	6.6	8.16	7.23	5.29	6.23	4.65
Pseudomonas aeruginosa **(ATCC53)**	20	5.34	5.2	5.1	5.22	5.1
Pseudomonas aeruginosa **(VIM21)**	10.46	8.91	8.19	7.09	8.72	11.04
Acinetobacter **(OXA23)**	14.25	5.48	5.06	10.74	10.36	10.45
Acinetobacter **(NDM1)**	16.38	19.66	15.2	14.22	9.8	9.13
Staphylococcus aureus **(13)**	13.01	<6	<6	<6	<6	<6
Staphylococcus aureus **(23)**	22.15	<6	<6	<6	<6	<6
Staphylococcus aureus **(23)**	14.88	<6	<6	<6	<6	<6
Enterococcus faecalis **(ATCC12)**	12.46	6.16	7.35	8.44	8.45	9.54
Sphingomonas sp	16.07	11.43	10.66	10.08	9.2	9.17

The antibacterial activity of *Rosmarinus officinalis* L., essential oil was qualitatively assessed by the presence or absence of inhibitions zones and zone diameters. Table 2 reports the inhibition zones of the essential oil determined for Gram negative and Gram-positive bacteria using the diffusion technique on solid media. The results show that the essential oil of this species possesses good antibacterial activity. *Staphylococcus aureus* (23) was the most sensitive species followed by *Pseudomonas aeruginosa* (ATCC53) and *Acinetobacter* (NDM1). Significant sensitivity was observed for the *Escherichia coli* (BLSE), *Salmonella sp* and *Sphingomonas sp*. However, *Bacillus cereus* was the most resistant to the essential oil tested.

However, many studies reported in the literature show that the activity of the oils varies according to the method of extraction, the volume of inoculum deposited around the discs, and the incubation time [11].

Previous studies on the essential oil obtained from *Rosmarinus officinalis* revealed that it displays antimicrobial activity [12,13]. Some major compounds identified were previously reported to have antimicrobial activity, including 1,8-cineole, verbenone, α-pinene, camphor, myrcene and β-caryophyllene.

According to the literature, as typical lipophiles, the essential oil and its constituents pass through the cell wall and cytoplasmic membrane, disrupting the structure of the different layers of polysaccharides, fatty acids, and phospholipids in the cell, thereby leading to cell permeabilization. Cytotoxicity appears to include such membrane damage, but the mode of action depends largely on the chemical composition of the essential oil as well as on the bacterial strain [14].

The antibacterial activity of essential oils, in general, has been explained mainly by the presence of terpenes which possess aromatic rings and groups capable of forming hydrogen bonds with the active sites of the enzymes. Alcohols, aldehydes and esters can contribute to the overall antimicrobial effect of essential oils [15].

The phenol compounds such as thymol and carvacrol appear to be able to increase membrane permeability [16] by destroying the outer membrane of Gram-negative bacteria, they would increase the permeability of the plasma membrane to cellular metabolites [17].

In fact Gram-negative *Escherichia coli* (ATCC22) *Escherichia coli* (BLSE) *Pseudomonas aeruginosa* (ATCC53) *Pseudomonas aeruginosa* (VIM21) were inhibited by the essential oil of *Rosmarinus officinalis.* These bacteria are very sensitive to camphor. It is cited that camphor is readily absorbed through the skin and produces a feeling of cooling similar to that of menthol and acts as a slight local anesthetic and antimicrobial substance.

Some previous studies have observed that essential oils have more activity against Gram positive than Gram-negative isolates [18]. This is thought to be due to the more complex, rigid outer membrane of Gram-negative bacteria with lipopolysaccharide that limits the diffusion of hydrophobic compounds. The complex outer membrane is not present in Gram-positive bacteria, and the peptidoglycan cell wall provides less resistance against the hydrophobic compounds. [19]

CONCLUSION

The obtained results have demonstrated that the essential oil from *Rosmarinus officinalis* have an inhibitory effect on the majority of the strains tested. Nevertheless, further studies are in progress to identify promising compounds that could be used as lead to develop natural antimicrobials agents.

We conclude that the results of this study suggest the possibility of using either the essential oil or some of its components as natural food preservatives as the oil possesses strong antimicrobial activity. These properties are much needed by the food industry in order to find possible alternatives to synthetic preservatives.

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