PharmacologyOnLine

Archives • 2021 • vol.3 • 847-856

EVALUATION OF THE ANTIBACTERIAL ACTIVITY OF THE ESSENTIAL OIL OF *ROSMARINUS OFFICINALIS* L. FROM KHENIFRA (MIDDLE ATLAS OF MOROCCO)

Mohamed Abdoul-Latif, Fatouma[1]* ; Ainane, Ayoub[2] ; Oumaskour, Khadija[2] ; Boujaber, Nabila[2] ; Mohamed, Jalludin[1] ; Ainane, Tarik[2]

[1]Medicinal Research Institute, Center for Research and Study of Djibouti, BP 486, Djibouti.

[2]Superior School of Technology of Khenifra (EST-Khenifra), University of Sultan Moulay Slimane, BP 170, Khenifra 54000 Morocco.

*fatouma_abdoulatif@yahoo.fr

Abstract

Rosemary is a medicinal plant, it is interesting to know its therapeutic virtues, in order to replace synthetic products with bioactive molecules which are based on plants. This work consists of evaluating the antibacterial activity of the essential oil of spontaneous *Rosmarinus officinalis* L. collected in the region of Khenifra (Morocco). After extracting the essential oils from the aerial part of *Rosmarinus officinalis* L. by hydrodistillation using an " Alambic" pilot extractor, the chromatography analysis was carried out by gas chromatography coupled with mass spectrometry for the determination of chemical compositions. The results showed that the essential oil of *Rosmarinus officinalis* L. is characterized by the presence of eucalyptol (44.97%), camphor (10.79%), and caryophyllene (9.43%). these three major compounds were obtained with a percentage of (65.19%). On the other hand, the antimicrobial power of the essential oil of *Rosmarinus officinalis* L. has been studied *in vitro* against the following bacterial strains: *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus cereus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. The agar diffusion method made it possible to determine the diameters of the zones of inhibition. Antibacterial tests show that four out of five bacterial strains tested are sensitive to rosemary essential oil. In addition, a significant antibacterial activity against Gram + bacteria compared to Gram- bacteria.

Keywords: *Rosmarinus officinalis L.; essential oil; GC-MS; antimicrobial activities.*

Introduction

The Mediterranean basin is characterized by a large amount of aromatic plants, producing essential oils and low molecular weight metabolites. These plants belong to different botanical families and their presence is important in deciding the potential interference within the ecosystem **[1-4]**. Morocco benefits from a very diverse climate with heterogeneous ecological conditions which have allowed the development of a very rich and highly diversified flora **[5]**. Moroccan flora comprises around 4,200 spaces and subspecies belonging to almost all of the known botanical families, 800 of which may have medicinal use **[6]**. The Moroccan aromatic and medicinal flora is still unknown and underexploited, its valuation for its use in fields as diverse as cosmetics, pharmacy, perfumery and food industry is of paramount importance. But this valuation must inevitably go through in-depth studies and solid data **[7-9]**. The evaluation of phytotherapeutic properties remains a very interesting and useful task, especially for plants of rare or less frequent use, or even not known in medicine and folk medicinal traditions **[10]**. These plants constitute an inexhaustible source of active molecules. However, there are few plants that have undergone very extensive phytochemical and biological studies **[11]**.

Rosemary, *Rosmarinus officinalis* L. is a medicinal plant native to the Mediterranean basin that grows wild. Rosemary likes limestone soils and adapts very well to arid and rocky areas. You can easily recognize him all year round **[12]**. These are the leaves, the flowering tops, which have been taken care to dry, or the essential oil that are used in herbal medicine. Rosemary has been the subject of recent research in the pharmaceutical and food industries. It has anti-inflammatory and antispasmodic properties and an action on the nervous system. Rosemary has excellent antioxidant and antimicrobial properties. Rosemary, like all aromatic and medicinal plants, contains chemical compounds with antibacterial properties. The use of these herbal molecules can have many advantages over current synthetic products **[13-15]**.

The objective of this work is to make a chemical composition of the essential oil of *Rosmarinus officinalis L.* thus to make an evaluation of this oil in the antibacterial activities against medical strains.

Material and methods

Plant Material

In this study *Rosmarinus officinalis* L., was collected from the region of Khenifra (Morocco). These species were verified by a botanist at the Forestry Research Center of Khenifra.

Essential oil extraction

The extraction of the essential oil was carried out by hydrodistillation using a "mini Alambic" pilot extractor for 2 hours, in a laboratory at the Higher School of Technology-khenifra, Morocco. The amount of 10 kg of dry plant material was used with a volume of 50 liters of water. The temperature of the device is set at 100 ° ± 5 ° C, to promote maximum evaporation of water and essential oil without destroying it. This method is recommended by the European Pharmacopoeia. The recovered oil is dehydrated with sodium sulfate (Na_2So_4), then weighed to calculate the yield, then and stored in opaque and hermetically sealed bottles at 4 °C ± 1. This essential oil will be studied and analyzed. The yield was calculated according to the formula: R% = (MHE / MVS) x 100. MHE: mass of the essential oil and MVS: mass of the dry plant material. The two masses are expressed in the same unit **[16]**.

Chemical analysis

Chromatographic analyzes were performed on a Varian type gas chromatograph (model 3380) equipped with a fused silica capillary column: HP-5 J&W Agilent (5% phenyl 95% methyl polysiloxane; 30 m × 0.25 mm di × 0.25 µm) and a flame ionization detector (FID) set at 250 ° C. Injector temperature 200 ° C; split mode injection (leakage ratio: 1: 100) of 1 µl of a 10% solution of HE in hexane; the carrier gas used is nitrogen with a flow rate of 0.8 ml / min; oven temperature programmed from 50 to 200 ° C at a rate of 5 ° C / min, then maintained at 200 ° C for 20 minutes. In the case of ET analysis, the dichloromethane extract is analyzed without prior dilution. GC-MS analyzes were carried out on a Hewlett-Packard gas chromatograph (GC 5890, series II) equipped with a capillary column made of HP-5 fused silica (5% phenyl 95% methyl polysiloxane; 30 m × 0.25 mm id × 0.25 µm). The mass detector is

of the quadrupole type (Model 5972), the ionization energy used is equal to 70 eV. The temperature of the column is programmed from 70 to 200 ° C at a rate of 10 ° C / min. Injection in split mode 1:10; quantity injected: 1 µl of an EO solution diluted to 10% in hexane. The injector temperature is set at 220 ° C and the transfer temperature is 180 ° C. The carrier gas is helium, the flow rate of which is set at 0.6 ml / min; ionization energy 70 eV; electron multiplier 1400 eV; scanning area [35–300]; scanning speed 3 scans / s. The identification of the constituents was carried out on the basis of their retention indices calculated by comparing the retention times with those of a series of alkanes (C9 – C20) and by comparing their mass spectra with those of the NBS libraries. 75K / NIST 98, from the literature and from the laboratory library (constituted from isolates of formally identified natural raw materials). The relative percentages of the different constituents are obtained by electronic integration of the signals obtained by CG-DIF (assuming that all the constituents are characterized by the same response coefficient with respect to the detector) **[17-18]**.

Microbiological analysis

In order to evaluate the antimicrobial activity of Rosmarinus officinalis L., five bacterial strains were used. The strains tested are widely found in various pathologies in humans (Table 1). These bacterial strains were provided by the local Laboratory of the Higher School of Technology-khenifra, after being purified and identified.

To assess the antimicrobial activity of rosemary essential oil, we used the agar disc diffusion method. The sterile Mueller Hinton agar is poured into petri dishes at a rate of 15 ml per dish and then allowed to cool. The bacterial inoculum, adjusted to 0.5 Mc Farland and a concentration of 10^7 CFU / ml, is rubbed over the entire Mueller Hinton surface from top to bottom in tight streaks. Whatman # 1 paper discs 6 mm in diameter are sterilized in aluminum foil in an autoclave at 121 °C for 15 min. Using sterile forceps, the discs impregnated with a quantity of crude essential oil (approximately 50 µL) were placed on the surface of the petri dishes inoculated with the strains to be tested. The boxes are then closed and left to air at room temperature for 30 minutes and put in an oven at a temperature of 37 °C for 24 hours. The reading is taken by measuring the diameter of the inhibition zone around each disc, in mm **[19]**. The results are expressed by the diameter of the zone of inhibition and can be symbolized by signs based on the sensitivity of the strains to the essential oil (Table 2).

Statistical analysis

For each bacterial strain, the tests were repeated ten (10) times for the essential oil *Rosmarinus officinalis* L., or 100 tests in total. The statistical analysis of the results obtained was carried out using the SPSS © software (version 22). The results obtained were subjected to an analysis of variance (ANOVA) at a probability level of 5%.

Results and discussion

The average yield of essential oil of *Rosmarinus officinalis* L. was calculated according to the dry plant material obtained from the aerial parts (stems, leaves and flowers). The essential oil yield obtained gave a percentage of 1.2% (v/w). The average essential oil yield of *Rosmarinus officinalis* L. was calculated based on the dry plant matter of the aerial part of the plant. The average yield obtained from essential oils extracted from *Rosmarinus officinalis* L studied is of the order of 1.08% **[20]**. According to previous works **[21-22]** the difference between the rates of return obtained in each study may be related to the method and conditions of extraction in the laboratory. Also, the geographical origin of the plant, the ecological factors including climatic (temperature and humidity) of the study region, the conservation of the plant species, the stage of growth and the sampling period can also have an effect on the yield of essential oils **[23-25]**.

Essential oil analysis

The analysis of the results of the chemical composition carried out by gas chromatography coupled with mass spectrometry of essential oil of *Rosmarinus officinalis* L. studied is listed in Table 3. Analysis of this results showed that *Rosmarinus officinalis* L. contains 51 biologically active compounds. The main major chemical compounds of essential oil identified are: Eucalyptol (44.97%), Camphor (10.79%), and Caryophyllene (9.43%). These three major compounds were obtained with a percentage of (65.19%). According to Bekkara *et al.*,

(2007) **[20]** the chemical composition of the essential oil of Algerian *Rosmarinus officinalis* from the Tlemcen region shows some differences, the majority compound of spontaneous rosemary is α-pinene (23.1%), followed by camphor (15.3%) and β-pinene (12.2%). In cultivated rosemary, the main compound is camphor (13.8%), followed by α-pinene (12.6%), cineole (11.8%) and borneol (10.8%) **[26]**. Other works, the majority compound is 1,8-cineole (52.4%), followed by camphor (12.6%) **[27]**. Comparatively, Moroccan rosemary has a high content of one of the 3 compounds: α-pinene (37.0-40.0%, Rabat), cineole (58.7-63.7%, El Ateuf), camphor (41.7-53.8%, Taforhalt) **[28-30]**. Rosemary from Tunisia is also rich in cineole (40.1-55.1%) and also contains the usual monoterpenes **[31]**. Rosemary cultivated in the North East of Spain presents an essential oil among which camphor and α-pinene are the majority constituents **[32]**. On the other hand, in Egypt, there are two compositions, one classically dominated by camphor, α-pinene and cineole, the other rich in verbenone and camphor **[33]**. Also, rosemary from Corsica and Sardinia contains an essential oil rich in verbenone, bornyl acetate and α-pinene **[34]**. The variations, encountered in the chemical composition from a qualitative and quantitative point of view of our samples compared to some previous work, may be due to certain ecological factors, to the part of the plant used, to the age of the plant and the period of the vegetative cycle, or even genetic factors.

Antibacterial activities

The results obtained on the sensitivity of the bacterial strains to the essential oil of officinal rosemary are shown in Table 4. According to the results obtained the antimicrobial activity by the method of diffusion on agar medium of essential oil of *Rosmarinus officinalis* L., is very important against the studied strains. The diameters of the zones of inhibition are respectively 25.46 mm for *Staphylococcus aureus*, 23.91 mm for *Enterococcus faecalis*, 12.62 mm for *Bacillus cereus*, 7.35 mm for *Escherichia coli*, and 5.71 mm for *Pseudomonas aeruginosa*. On the other hand, *Staphylococcus aureus* and *Enterococcus faecalis* show very high sensitivity. *Bacillus cereus* shows medium sensitivity, although *Pseudomonas aeruginosa* represents resistance against essential oil use. When we compare the degrees of sensitivity of the gram-positive strains, *Staphylococcus aureus*, *Enterococcus faecalis* and *Bacillus cereus* with the gram-negative strains, *Escherichia coli* and *Pseudomonas aeruginosa*, we find that our results are comparable to those confirming that the gram bacteria positive are more sensitive to the essential oil than gram negative bacteria. The high resistance of gram-negative bacteria to essential oil is linked to the complexity of the cell envelope of these microorganisms, which contain a double membrane, unlike the simple membrane structure of gram-positive bacteria.

Conclusion

In short, the extraction of essential oils from plant material can be carried out by means of several processes, based on old or recent techniques. The latter, although having many advantages, in particular that of considerably reducing the extraction time, are not, for the moment, recognized by the European Pharmacopoeia. However, regardless of the process used, the final extract corresponds to a concentration of the compounds initially present in the raw material. In addition, production methods, such as geographical origin, climate, soil, harvest period or even agricultural practices, can have a direct influence on the chemical composition of distilled essential oil. The coming years will reasonably see a significant increase in the number of publications relating to the use of new innovative, reliable and rapid techniques in the field of extraction of aromatic substances. As a result of these results, it would be interesting to extend the range of antimicrobial tests on other microbial agents in order to confirm their effectiveness. As it is essential to look for new effective antibacterial substances with a broad spectrum of action. All of these results obtained only constitute a first step in the search for substances of biologically active natural origin. A chemical analysis is desirable to obtain a more in-depth view on the qualitative and quantitative composition of these extracts studied in order to shed light on the therapeutic effect of this medicinal plant *Rosmarinus officinalis* L.

References

1. Mohamed Abdoul-Latif, F., Ainane, A., Oumaskour, K., Boujaber, N., Mohamed, J., Ainane, T. (2021). chemical composition and antimicrobial activity of the essential oil of chamaemelum nobile (l.) all. PharmacologyOnLine, 2, 449-457.
2. Ouassil, M., Mohamed Abdoul-Latif, F., Am, A., Ainane, A., Ainane, T. (2021). Chemical composition of bay laurel and rosemary essential oils from Morocco and their antifungal activity against fusarium strains. *Pharmacologyonline*, 2, 426–433.
3. Eddabbeh, F.-E., Mohamed Abdoul-Latif, F., Ainane, A., Ejjabraoui, M., Ainane, T. (2021). The composition of the essential oil and the antimicrobialand antifunqal activities of tetraclinis articulata (Vahl) masters from the Moroccan central plateau (Morocco). *Pharmacologyonline*, 2, 458–464.
4. Ainane, T., Elkouali, M., Ainane, A., Talbi, M. (2014). Moroccan traditional fragrance based essential oils: Preparation, composition and chemical identification. *Der Pharma Chemica*, 6(6), 84-89.
5. Talbi, M., Ainane, T., Boriky, D., Bennani, L., Blaghen, M., & Elkouali, M. (2015). Antibacterial activity of Eudesmanolide compounds isolated from medicinal plant Artemisia herba-alba. *J. Mater. Environ. Sci*, 6, 2125-2128.
6. Ainane, A., Cherroud, S., El Kouali, M., Abba, E.H., Ainane, T. (2020). Chemical compositions, insecticidal and antimicrobial activities of two moroccan essential oils of citrus limonum and syzygium aromaticum. *Pharmacologyonline*, 2, 190–199.
7. Talbi, M., Saadali, B., Boriky, D., Bennani, L., Elkouali, M., Ainane, T. (2016). Two natural compounds–a benzofuran and a phenylpropane–from Artemisia dracunculus. *Journal of Asian natural products research*, *18*(8), 724-729.
8. Ainane, A., Abdoul-Latif, F. M., Mohamed, J., Attahar, W., Ouassil, M., Shybat, Z. L., Ainane, T. (2021). Behaviour desorption study of the essential oil of Cedrus atlantica in a porous clay versus insecticidal activity against Sitophilus granarius: explanation of the phenomenon by statistical studies. *International Journal of Metrology and Quality Engineering*, 12, 12.
9. Ainane, A., Khammour, F., M'hammed, E. L., Talbi, M., Oussaid, A., Lemhidi, A., Ainane, T. (2019). Evaluation of the toxicity of the essential oils of certain mints grown in the region of Settat (Morocco): Mentha piperita, Mentha pulegium and Mentha spicata against, Sitophilus Granarius, Sitophilus Oryzae and Sitophilus Zeamais. *Journal of Analytical Sciences and Applied Biotechnology*, *1*(1), 1-1.
10. Attahar, W., Mohamed Abdoul-Latif, F., Mohamed, J., Ainane, A., Ainane, T. (2021) Antimicrobial and antioxidant activities of trigonella foenum-graecum essential oil from the region of settat (Morocco). Pharmacologyonline, 2021, 2, 434–442.
11. Ejjabraoui, M., Mohamed Abdoul-Latif, F., Eddabbeh, F.-E., Ainane, A., Shybat, Z. L., Ainane, T. (2021). Chemical study and insecticidal activity of two species of Moroccan Pinus: Pinus halepensis mill. and Pinus pinaster sol. 2, 508-517.
12. Amjadi, S., Almasi, H., Ghorbani, M., & Ramazani, S. (2020). Reinforced ZnONPs/rosemary essential oil-incorporated zein electrospun nanofibers by κ-carrageenan. *Carbohydrate polymers*, 232, 115800.
13. Gharby, S., Asdadi, A., Ibourki, M., Hamdouch, A., Ainane, T., & Hassani, L. A. I. (2020). Chemical characterization of the essential oil from aerial parts of Lavandula rejdalii Upson & Jury, a medicinal plant endemic to Morocco. *Journal of Essential Oil Bearing Plants*, 23(6), 1422-1427.
14. Shybat, Z. L., Mohamed Abdoul-Latif, F., Mohamed, J., Ainane, A., Ainane, T. (2021). Antifungal activity of the essential oil of Morrocan myrtle (Myrtus communis l.): application in agriculture. Pharmacologyonline, 2, 485-491.
15. Ainane, A., Mohamed Abdoul-Latif, F., Mohamed, J., Boujaber, N., Oumaskour, K., Benaziz, M., Ainane, T. (2021). Chemical composition and antimicrobial activity of the

essential oil of Pistacia lentiscus L. Pharmacologyonline, 2, 518-526.
16. Ainane, A., Khammour, F., Charaf, S., Elabboubi, M., Elkouali, M., Talbi, M., Ainane, T. (2019). Chemical composition and insecticidal activity of five essential oils: Cedrus atlantica, Citrus limonum, Rosmarinus officinalis, Syzygium aromaticum and Eucalyptus globules. *Materials Today: Proceedings*, *13*, 474-485.
17. Ainane, T., Mohamed Abdoul-Latif, F., Ouassil, M., Attahar, W., Am, A., Ainane, A. (2021). Antagonistic antifungal activities of Mentha suaveolens and Artemisia absinthium essential oils from morocco. Pharmacologyonline, 2, 470-478.
18. Ainane, A., Abdoul-Latif, F. M., Abdoul-Latif, T. M., & Ainane, T. (2020). Evaluation of biological activities of two essential oils as a safe environmental bioinsecticides: case of Eucalyptus globulus and Rosmarinus officinalis. Przegląd Naukowy Inżynieria i Kształtowanie Środowiska, 29, 544-556.
19. Ainane, T., Abourriche, A., Kabbaj, M., Elkouali, M., Bennamara, A., Charrouf, M., Lemrani, M. (2014). Biological activities of extracts from seaweed Cystoseira tamariscifolia: Antibacterial activity, antileishmanial activity and cytotoxicity. *J. Chem. Pharm. Res*, *6*, 607-611.
20. Bekkara, F. A., Bousmaha, L., Bendiab, S. T., Boti, J. B., & Casanova, J. (2007). Composition chimique de l'huile essentielle de Rosmarinus officinalis L poussant à l'état spontané et cultivé de la région de Tlemcen. *Biologie & Santé*, *7*(1).
21. El-Demerdash, F. M., El-Sayed, R. A., & Abdel-Daim, M. M. (2021). Hepatoprotective potential of Rosmarinus officinalis essential oil against hexavalent chromium-induced hematotoxicity, biochemical, histological, and immunohistochemical changes in male rats. *Environmental Science and Pollution Research*, *28*(14), 17445-17456.
22. El-Demerdash, F. M., El-Sayed, R. A., & Abdel-Daim, M. M. (2021). Rosmarinus officinalis essential oil modulates renal toxicity and oxidative stress induced by potassium dichromate in rats. *Journal of Trace Elements in Medicine and Biology*, *67*, 126791.
23. Mehalaine, S., & Chenchouni, H. (2021). Quantifying how climatic factors influence essential oil yield in wild-growing plants. *Arabian Journal of Geosciences*, *14*(13), 1-12.
24. Yingngam, B., Brantner, A., Treichler, M., Brugger, N., Navabhatra, A., Nakonrat, P. (2021). Optimization of the eco-friendly solvent-free microwave extraction of Limnophila aromatica essential oil. *Industrial Crops and Products*, *165*, 113443.
25. Anwar, F., Alkharfy, K. M., Mehmood, T., Bakht, M. A. (2021). Variation in Chemical Composition and Effective Antibacterial Potential of Ocimum basilicum L. Essential Oil Harvested from Different Regions of Saudi Arabia. *Pharmaceutical Chemistry Journal*, *55*(2), 187-193.
26. Bekkara, F. A., Bousmaha, L., Bendiab, S. T., Boti, J. B., Casanova, J. (2007). Composition chimique de l'huile essentielle de Rosmarinus officinalis L poussant à l'état spontané et cultivé de la région de Tlemcen. *Biologie & Santé*, *7*(1).
27. Chahboun, N., Esmail, A., Rhaiem, N., Abed, H., Amiyare, R., Barrahi, M., Ouhssine, M. (2014). Extraction and study of the essential oil Rosmarinus officinalis cuellie in the region of Taza, Morocco. *Der Pharma Chemica*, *6*(3), 367-372.
28. Mohammed, H. A., Al-Omar, M. S., Mohammed, S. A., Aly, M. S., Alsuqub, A. N., Khan, R. A. (2020). Drying induced impact on composition and oil quality of rosemary herb, Rosmarinus officinalis Linn. *Molecules*, *25*(12), 2830.
29. Elamrani, A., Zrira, S., Benjilali, B., Berrada, M. (2000). A study of Moroccan rosemary oils. *Journal of Essential Oil Research*, *12*(4), 487-495.
30. Salido, S., Altarejos, J., Nogueras, M., Saánchez, A., Luque, P. (2003). Chemical composition and seasonal variations of rosemary oil from southern Spain. *Journal of Essential Oil Research*, *15*(1), 10-14.

31. Hcini, K., Sotomayor, J. A., Jordan, M. J., Bouzid, S. (2013). Chemical composition of the essential oil of Rosemary (Rosmarinus officinalis L.) of Tunisian origin. *Asian Journal of Chemistry*, *25*(5), 2601.
32. Guillén, M. D., Cabo, N., Burillo, A. J. (1996). Characterisation of the essential oils of some cultivated aromatic plants of industrial interest. *Journal of the Science of Food and Agriculture*, *70*(3), 359-363.
33. Sahab, A. F., Aly, S., Hathout, A. S., Ziedan, E. S. H., Sabry, B. A. (2014). Application of some plant essential oils to control Fusarium isolates associated with freshly harvested maize in Egypt. *Journal of Essential Oil Bearing Plants*, *17*(6), 1146-1155.
34. Pintore, G., Usai, M., Bradesi, P., Juliano, C., Boatto, G., Tomi, F., Casanova, J. (2002). Chemical composition and antimicrobial activity of Rosmarinus officinalis L. oils from Sardinia and Corsica. *Flavour and Fragrance Journal*, *17*(1), 15-19.

Table 1. General information on the bacterial strains used.

Bacterial strains tested	Bacteriological characters	Environments	Pathogenicity
Staphylococcus aureus	Gram +	✓ The nasal cavities. ✓ The throat. ✓ The digestive tract.	✓ Hospital infection. ✓ Responsible for abscesses, wounds, sepsis, pneumonia and food poisoning.
Enterococcus faecalis	Gram +	✓ The digestive tract.	✓ Fatal infections in humans. ✓ Chronic inflammation of the intestine. ✓ Infection of the bladder and prostate.
Bacillus cereus	Gram +	✓ Soils and waters.	✓ A food contaminant of plant origin (rice, spices). ✓ A contaminant of drugs and medications. ✓ Food poisoning
Escherichia coli	Gram -	✓ The digestive tract.	✓ Urinary tract infections. ✓ Infant meningitis septicemia, surgical wounds and gastroenteritis. ✓ Abdominal pain and bloody diarrhea.
Pseudomonas aeruginosa	Gram -	✓ Water and wet soils. ✓ Plant surface.	✓ Nosocomial infections (people weakened or immunocompromised). ✓ Urinary tract, eye and pulmonary.

Table 2. Sensitivity of microbial strains according to zones of inhibition.

Sensitivity	Inhibition zone
Not sensitive or resistant (-)	diameter <8mm
Sensitive (+)	diameter between 9 to 14 mm
Very sensitive (++)	diameter between 15 to 19 mm
Extremely sensitive (+++)	diameter> 20 mm

Table 3. Chemical composition of the essential oil of *Rosmarinus officinalis* L.

Pic	RT	Components	(%)
1	7.78	3-Carene	2.53
2	8.23	Cyclofenchene	0.99
3	8.67	Camphene	0.69
4	9.38	β-Terpinen	1.11
5	9.55	β-Pinene	2.20
6	9.83	α-Pinene	1.99
7	10.30	Santolina triene	0.18
8	10.72	(+)-4-Carene	0.22
9	11.01	trans-3-Caren-2-ol	0.94
10	11.15	D-Limonene	1.05
11	**12.20**	**Eucalyptol**	**44.97**
12	12.43	cis-Sabinenhydrate	1.41
13	13.03	α-Fenchene hydrate	0.08
14	13.29	Terpinolene	0.54
15	14.08	Linalyl anthranilate	1.20
16	14.67	Terpineol	0.06
17	15.05	Chrysanthenone	0.20
18	15.13	3,5-Heptadien-2-ol, 2,6-dimethyl-	0.03
19	15.42	3,4-Dihydroxymandelic acid, ethyl ester, tri-TMS	0.05
20	**16.17**	**Camphor**	**10.79**
21	16.49	5H-Inden-5-one, 1,2,3,3a,4,7a-hexahydro-7a-methyl-, trans-	0.13
22	16.79	endo-Borneol	2.74
23	17.02	Terpinen-4-ol	1.45
24	17.66	α-Terpineol	3.25
25	17.78	Isobornyl thiocyanoacetate	0.07
26	18.68	Verbenone	0.26
27	19.32	Pulegone	0.39
28	20.77	Borneol	0.70
29	21.52	Cyclohexasiloxane, dodecamethyl-	0.34
30	22.64	cis-muurola-3,5-diene	0.09
31	23.09	2H-Inden-2-one, 1,4,5,6,7,7a-hexahydro-7a-methyl-, (S)-	0.10
32	23.46	Ylangene	0.19
33	23.66	α-Copaene	1.17
34	24.78	Acoradiene	0.05
35	**25.50**	**Caryophyllene**	**9.43**
36	25.91	Alloaromadendrene	0.30
37	26.16	cis-α-Farnesene	0.06
38	26.45	Humulene	1.06
39	26.94	τ-Cadinol	0.12
40	27.07	γ-Muurolene	1.14
41	27.36	Cycloheptasiloxane, tetradecamethyl-	0.11
42	27.48	Isoeremophilene	0.08
43	27.73	α-Gurgujene	0.33

44	27.78	Isoledene	0.27
45	27.98	α-himachalene	0.30
46	28.31	γ-Cadinene	0.58
47	28.58	δ-Cadinene	1.66
48	28.86	Naphthalene	0.09
49	29.02	α-Muurolene	0.03
50	29.25	α-Calacorene	0.05
51	30.91	Tricyclo[5.2.2.0(1,6)]undecan-3-ol, 2-methylene-6,8,8-trimethyl-	0.11
Total			**97.88**

Table 4. Sensitivity of bacterial strains to the essential oil of *Rosmarinus officinalis* L

Bacterial strains tested	Bacteriological characters
Staphylococcus aureus	(+++)
Enterococcus faecalis	(+++)
Bacillus cereus	(+)
Escherichia coli	(-)
Pseudomonas aeruginosa	(-)