

Essential Oils of Rosemary as Antimicrobial Agent against Three Types of Bacteria

Ali Mohammed Jawad, Asmahan K. Allawi¹, Hind Mufeed Ewadh²

Food Pollution Research Center, Ministry of Science and Technology, ¹Department of Gardening, College of Agriculture, Baghdad University, Baghdad, ²Environmental Research Center, University of Babylon, Hilla, Iraq

Abstract

Background: Interest in Rosemary has increased due to the importance of being wide antimicrobial, anti-inflammatory, and antioxidant. **Objective:** This study aimed to evaluate the biological affectivities of essential oils (EOs) of rosemary against three types of bacteria. **Materials and Methods:** Chemical analyses were conducted using gas chromatography technique on the volatile oils of rosemary, which were extracted by Clevenger. **Results:** The major contents of these oils were camphor (22.35%), camphene (1.85%), β -pinene (3.75%), sabinene (10.25%), limonene (7.64%), linalool (11.58%), and myrcene (2.14%). The biological affectivities of these oils were examined on three types of bacteria: *Bacillus cereus*, *Escherichia coli*, and *Pseudomonas*. The results proved that the EOs of rosemary were influential against bacteria and gave minimum inhibitory concentration (MIC). The MIC values were 37 μ g/ml for *E. coli*, 69 μ g/ml for *Pseudomonas*, and 20 μ g/ml for *B. cereus*. Results found that *Pseudomonas* was less sensitive for these oils. **Conclusion:** The EOs of Rosemary can be used in the pharmaceutical industry for the production of new synthetic agents in the treatment of bacterial disease caused by these three types of bacteria.

Keywords: Clevenger, essential oil, gas chromatography, minimum inhibitory concentration, rosemary

INTRODUCTION

Rosemary (*Rosmarinus officinalis* L.) is known as a kind of spices and medicinal herbs that belongs to the Lamiaceae family. Recently, interest in rosemary has been increased due to its importance of being wide antimicrobial, anti-inflammatory, and antioxidant agent.^[1] Rosemary leaves contain between 1.0%–2.5% essential oil (EO). The essential oil in the rosemary has a very distinctive and refreshing aroma that is light yellow to pale in most cases.^[2] Plant material in rosemary is useful medically and commercially as it contains antioxidants and anti-inflammatory substances.^[3]

Chemically rosemary contains many phytochemicals such as camphor, rosemarynic acid, Caffeic acid, Ursolic acid and botulinum. And a number of antioxidants such as carnosic acid and Carnosol. Rosemary oils are used to a large extent in the perfume industry in the manufacture of liquor and in traditional medicine. Rosemary contains a number of phytochemicals, including rosmarinic acid, camphor, caffeic acid, ursolic acid, betulinic acid, carnosic acid and carnosol. In traditional medicine, extracts and essential oil from flowers and leaves are used in the belief they may be useful to treat a

variety of disorders. Rosemary essential oil contains 10–20% camphor, though the chemical composition can vary greatly between different samples, according to in vitro studies.^[4] Several methods are used to obtain essential oils, including the use of direct distillation with water or steam or the use of organic solvents such as hexane or ether, or the use of Soxhlet extraction technology insulation distillation to remove the oil from the dried or fresh plants.^[5] The aim of this study is to identify the active compounds in rosemary using gas chromatography (GC) techniques and investigate the antimicrobial activity against some bacterial isolate oils.

MATERIALS AND METHODS

Sample collection and preparation

This study was conducted in the laboratories of the Ministry

Address for correspondence: Dr. Ali Mohammed Jawad,
Food contamination Research Center, Ministry of Science and Technology,
Baghdad, Iraq.
E-mail: hotbird812000@yahoo.com

Access this article online

Quick Response Code:



Website:
www.medjbabylon.org

DOI:
10.4103/MJBL.MJBL_14_18

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

How to cite this article: Jawad AM, Allawi AK, Ewadh HM. Essential oils of rosemary as antimicrobial agent against three types of bacteria. Med J Babylon 2018;15:53-6.

of Science and Technology of Baghdad from October to December 2016. Rosemary leaves were collected from the gardens of College of Agriculture/Baghdad University. Volatile oils were extracted by Clevenger. Two hundred and fifty millimeters of distilled water was added to 50 g of sample and placed in conical flask for 24 h and then transferred to the Clevenger. This process was repeated five times to get a sufficient amount of oil.^[6,7]

Analyses' conditions

Analyses of the EOs were performed using GC (Shimadzu 2010 Japan), equipped with Flame Ionized Detector temperature = 320°C and (DB-5) capillary column (30 m × 0.25 mm, film thickness 0.25 µm) with program temperature (60–120°C) (7 C / min) - (120–230°C) (10 C/min) and injector temperature = 280°C. Nitrogen was the carrier gas, at a flow rate of 1 ml/min. Samples of 1 µl were injected automatically in the split mode (split ratio 1:10).

Antimicrobial assay

A test was conducted to determine the biological efficacy of essential oils extracted from the rosemary as a potential antimicrobial activity against microorganisms. Reported minimum inhibitory concentration (MIC) was conducted by diffusion wells and ways to mitigate the agar, using Mueller–Hinton agar.^[8] Dimethyl sulfoxide is used to facilitate blending with broth.^[9] Various concentrations of employers' organizations (32,64,128,256 and 512 µl) were added to sterile petri dishes. Then, the agar melts had been poured into the plates of bacterial isolates and darted to mix the ingredients well.

They were left to solidify, and then, 100 µl of comments containing the spread of tested bacteria using a sterile glass rod were then incubated at 37° C for 24 h to grow bacterial isolates, and then, counting the colonies and counting the multiplying multiplied by sample dilutions representing a number of microorganisms. Mick has also taken a lower percentage of the oil in which the test object has not shown visible growth.

On the other hand, the wells are created in an intermediate sclerosis tool using a cork borer. Applied alicots of 100 µl of pollen into wells and then incubated as mentioned above. The diameters of inhibition zones were measured in millimeters. All tests were conducted in triplicate. Statistical analysis was performed and data provided are the average of three replicates. Calculated the Least significant difference level (LSD).

The collected data were calculated and analyzed by using the Statistical Package for Social Sciences (SPSS) 20th version (IBM SPSS Software, Chicago, Illinois, U.S.A.) for windows. Data were expressed as mean ± standard deviation (mean ± standard deviation) and statistically analyzed to verify the accuracy and sensitivity of the measurements.^[11]

RESULTS AND DISCUSSION

Gas chromatography analyses of essential oils

Fundamental oils extracted from rosemary leaves were examined and compared with standard oils by using GC, as is

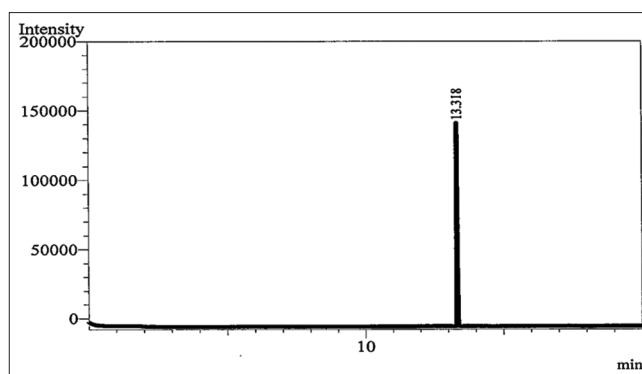


Figure 1: Complete baseline separation of limonene standard by gas chromatography

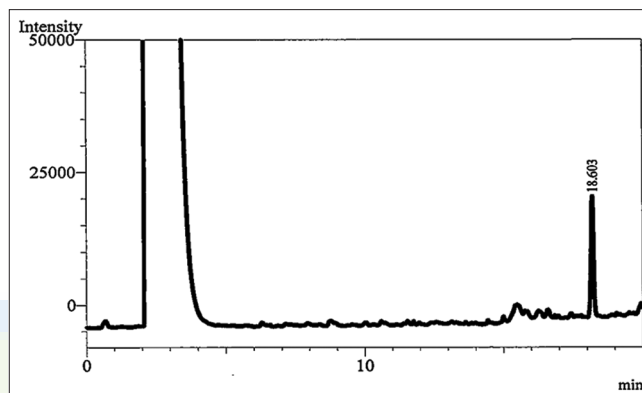


Figure 2: Complete baseline separation of linalool standard by gas chromatography

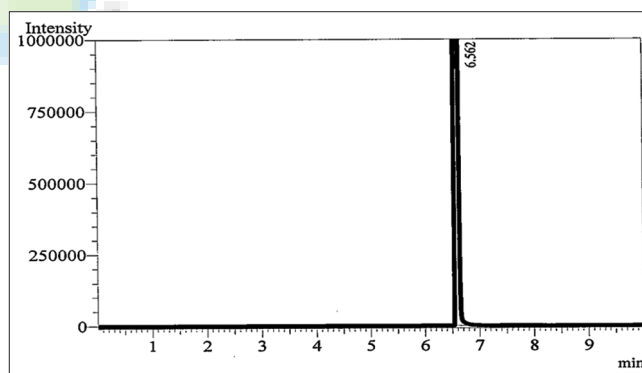


Figure 3: Complete baseline separation of camphene standard by gas chromatography

Table 1: Retention time (min) of different compounds found in rosemary essential oil analyzed by gas chromatography

Compound	Retention time (min)	Area	Concentration (%)
Camphene	6.5	3790	1.85
Camphor	9.1	7145	22.35
Limonene	13.3	2604	7.64
Linalool	18.6	1327	11.58
Myrcene	7.3	6193	2.14
Sabinene	12.8	2085	10.25
β-pinene	4.3	1146	3.75

shown in Figures 1-7 and Table 1 which reported the retention time and concentration of the oil.

Figure 8 Chromatographic analysis of rosemary sample by using gas chromatography.

Results of GC analysis showed that rosemary leaf contains high concentrations of several EOs in comparison with the remaining ingredients.^[12,13]

The investigation of antibacterial activity was conducted against negative bacteria (*Escherichia coli* and *Pseudomonas*) and one positive bacteria (*Bacillus cereus*). The data are listed in Table 2 and Figure 9 which pointed out that the EOs of rosemary have a wide range of effectiveness against both the positive and negative bacteria used in this study with the MIC in the range of 20–69 µg/ml. *B. cereus* showed highest sensitivity to the oil of rosemary (MIC = 20 µg/ml).^[14,15]

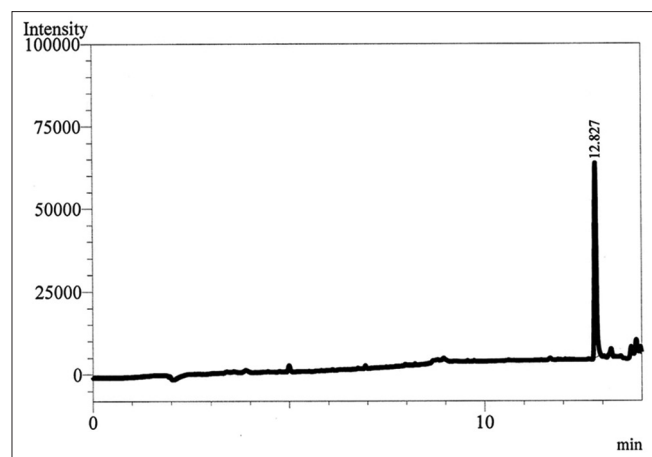


Figure 4: Complete baseline separation of sabinene standard by gas chromatography

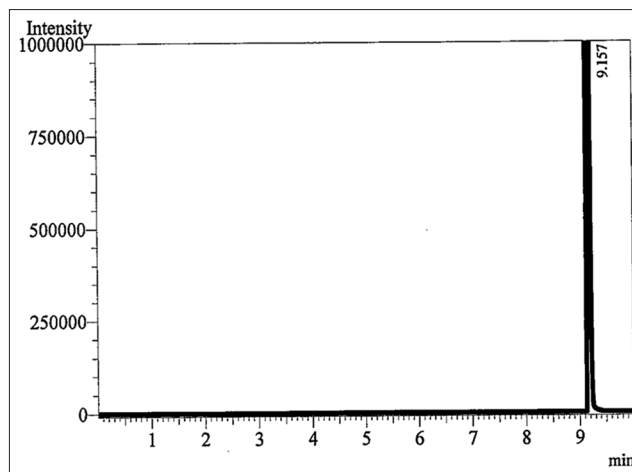


Figure 5: Complete baseline separation of camphor standard by gas chromatography

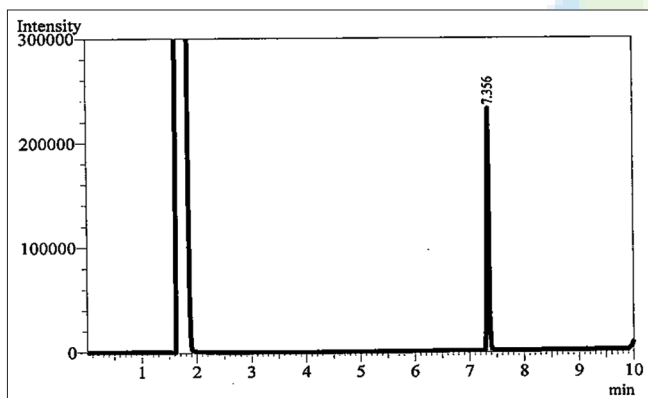


Figure 6: Complete baseline separation of myrcene standard by gas chromatography

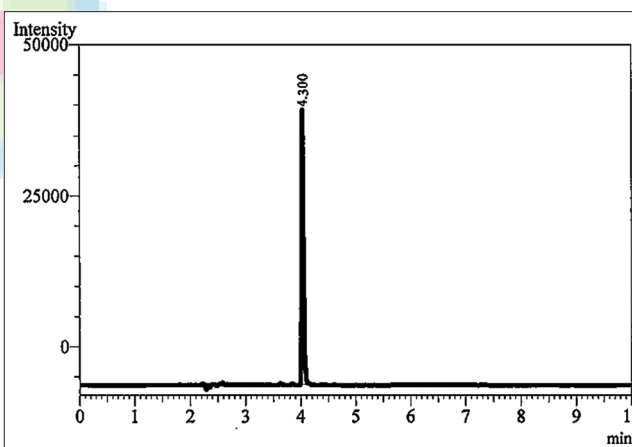


Figure 7: Complete baseline separation β -pinene standard of by gas chromatography

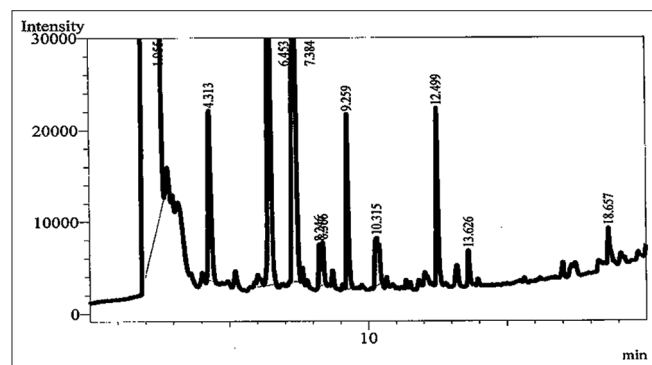


Figure 8: Chromatographic analysis of rosemary sample by using gas chromatography

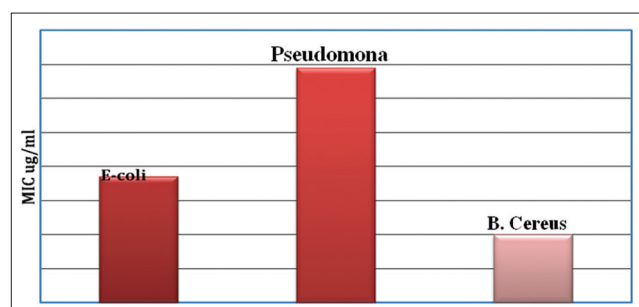


Figure 9: Minimum inhibitory concentration of rosemary essential oils (µg/ml) against selected bacteria

Table 2: Effect of different concentrations of rosemary essential oils on bacterial growth

Concentration (µl/ml)	Inhibition zone diameter (mm)		
	Bacterial isolates		
	<i>Escherichia coli</i>	<i>Pseudomonas</i>	<i>Bacillus cereus</i>
32	17	0	18
64	19	18	19
128	22	20	21
256	25	24	25
512	26	25	26
Control	0	0	0
LSD	3.352	2.596	2.802

LSD: Least significant differences

E. coli showed moderate sensitivity (MIC = 37 µg/ml), while *Pseudomonas* showed weak oil sensitivity (MIC = 69 µg/ml). However, periodic inspections of these oils are performed to be safer when used in the medical field

The other importance of essential oils added to its wide activity against bacteria, these oils gave a large inhibitory against microbes about 14-26 mm activity against pathogens known food diseases (*B. cereus*, *Pseudomonas*), which was the cause of food spoilage and poisoning, thereby causing human diseases. The results obtained may be different from previous reports, due to the differences in the composition of the oil, which can probably be attributed to seasonal changes, and the method of extraction and environmental factors, or because of the nutritional status of the plant.

Results are consistent with Marzouk *et al.*^[18] who reported that the antimicrobial activity of EOs of rosemary is not only linked to the major compounds but also on the simple components of oil. The results were similar to those previously documented.^[5] *E. coli* that is resistant to many antibiotics are susceptible basic oils of rosemary. Therefore, the EOs of this plant can be used in the pharmaceutical industry for the production of new synthetic agents in the treatment of this disease infection.

Acknowledgments

The authors are grateful and extend sincere thanks with all respect to headmaster of food contamination research center and other researchers at Ministry of Science and Technology, Iraq, for their help and continued support.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Eva SB, Maria HT, Attila H, Csilla R, Szollosi V. Antioxidant effect of various rosemary (*Rosmarinus officinalis* L.) clones. *Acta Biol Szeged* 2003;47:111-3.
2. Bauer K, Garbe D, Surburg H. Common Fragrance and Flavor Materials. 3rd ed. Germany: Wiley-VCH; 1997.
3. Munné-Bosch S, Alegre L. Subcellular compartmentation of the diterpene carnosic acid and its derivatives in the leaves of rosemary. *Plant Physiol* 2001;125:1094-102.
4. Burt S. Essential oils: Their antibacterial properties and potential applications in foods – A Review. *Int J Food Microbiol* 2004;94:223-53.
5. Mounchid K, Bourjilat F, Dersi N, Aboussaairia T, Rachidai A, Tantaoul-Elaraki A, *et al.* The susceptibility of *Escherichia coli* strains to essential oils of *Rosmarinus officinalis* and *Eucalyptus globules*. *Afr J Biotechnol* 2005;4:1175-85.
6. Bos R. Analytical and phytochemical studies on valerian and valerian based preparations. Department of Pharmaceutical Biology. Groningen: Rijks Universiteit Groningen; 1997. p. 184-93.
7. Mounchid K, Bourjilat F, Dersi N, Aboussaairia T, Rachidai A, Tantaoul-Elaraki A, *et al.* Toxicity of South Morocco *Rosmarinus officinalis* essential oil: Antibacterial and histopathological effects. *Les Actes Del'institut Agronomique Vet* 2004;144:139-44.
8. Donald RS, Clyde T. Broth-dilution method for determining the antibiotic susceptibility of bacteria. *Antimicrobial Agent and Chemtherapy. Am Soc Microbiol* 1974;7:15-21.
9. Donald CS, Ann VB. Antimicrobial Susceptibility Testing. Wisconsin Veterinary Diagnostic Laboratory; 2006.
10. Josep G, Isabel P, Carmen A, Montserrat O. *In vitro* Antifungal Susceptibility of Nondermatophytic Keratinophilic Fungi. Virgili, Tarragona, Spain: Unitat de Microbiologia, Facultat de Medicina, Universitat Rovira; 2000.
11. SAS. SAS/STAT User's Guide for Personal Computers. Release 6.12. USA: SAS Inst. Inc. NC; 2001.
12. Toth J, Mrlanova M, Tekelora D, Korenova M. Rosmarinic acid – An important phenolic active compound of lemon balm (*Melissa officinalis* L.). *Acta Facultatis Pharmaceuticae. Unversitatis Comenianae. Tomus*; 2003.
13. Tomei PE, Cioni PL, Flamini G, Stefani A. Essential oil. *J Essent Oil Res* 1995;7:279-82.
14. Chalchate JC, Garry RP, Michet A, Benjilali B, Chabart JJ. Essential oils of rosemary (*Rosmarinus officinalis* L.). The chemical composition of oils of various origins (Morocco, Spain, France). *J Essent Oil Rosmarinus* 1993;5:613-8.
15. Porte A, Godoy RL, Koketsu M, Goncalves SL, Torquillo HS. Essential oil. *J Essent Oil Res* 2000;12:577-80.
16. Martos MV, Navajas YR, Fernandez-Lopez J, Perez Alvarez JA. Chemical composition of the essential oils obtained from me spices widely used in Mediterranean region. *Acta Chim Slov* 2007;54:921-6.
17. Magenga A. Influence of variety and organic cultural practices on yield and essential oil content of lavender and rosemary in Interior BC. *Ecorational Technol Kamloops* 2004;1-23:1-23.
18. Marzouk Z, Neffati A, Marzouk B, Chraief I, Fathia K, Chekir G, *et al.* Chemical composition and antibacterial and antimutagenic activity of Tunisian *Rosmarinus officinalis* L. oil from Kasrine. *J Food Agric Environ* 2006;4:61-5.