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

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Background: Interest in Rosemary has increased due to the importance of being a wide antimicrobial, anti-inflammatory, and antioxidant. Objective: This study aimed to evaluate the biological activities 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 activities 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 μg/ml for E. coli, 69 μg/ml for Pseudomonas, and 20 μg/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 a 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 botulin. 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 of Science and Technology, Baghdad, Iraq. A total of 100 mL of essential oil was extracted from 100 g of fresh rosemary leaves using a Clevenger apparatus. The extracted oils were then analyzed using gas chromatography (GC) techniques.

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 activities 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 μg/ml for E. coli, 69 μg/ml for Pseudomonas, and 20 μg/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

Abstract

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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.

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. Dimethyl sulfoxide is used to facilitate blending with broth. 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.

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
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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).\(^{[14,15]}\)
E. coli showed moderate sensitivity (MIC = 37 \mu g/ml), while Pseudomonas showed weak oil sensitivity (MIC = 69 \mu g/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.\cite{11} 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.\cite{11} 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.

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

| Concentration (\mu l/ml) | Escherichia coli | Pseudomonas | Bacillus cereus |
|--------------------------|-----------------|-------------|----------------|
| 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

**Conflicts of interest**

There are no conflicts of interest.

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