# Chemical Composition and Antimicrobial Activity of Essential Oil of Romanian *Origanum vulgare*

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*The aim of this study was to determine the chemical characterization and antimicrobial properties of the essential oil of Romanian Origanum vulgare. The oil was isolated by hydro distillation. The chemical composition was characterized by Gas chromatography-Mass spectroscopy. Antibacterial activity was evaluated by disk diffusion method and determination of the minimum inhibitory concentration (MIC).Thirty-two volatile constituents were identified in the oil studied and the major compounds were thymol (35.51%), γ-terpinen (19.19%), durenol (17.99%), durene (11.40) and carvacrol (2.69%). It showed a bactericidal activity towards all tested reference strains: Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pyogenes and Candida albicans.*

*Key words: Origanum vulgare L, essential oil, antibacterial activity*

The essential oil extracted by hydrodistillation from *Origanum vulgare* has two great qualities: antioxidant effect and antibacterial action due to the content of phenols and thymol. According to some studies, oregano oil has also been effective on multidrug-resistant bacteria such as methicillin-resistant Staphylococcus aureus (MRSA) [1].

## Experimental part

*Plant material*

Leaves of *O. vulgare* were purchased from south-western part of Romania: Marghitas (45.126 latitude, 21.894 longitude). Botanical identification of this species was performed with the help of *The Illustrated Flora of Romania* [2]. The plant material was dried at room temperature and stored at 4°C until distillation. One voucher specimen was deposited in the Herbarium of the Victor Babes University of Medicine and Pharmacy Timisoara, Romania.

*Extraction of essential oil*

Extraction of essential oil was carried out by hydrodistillation, using a Clevenger-type apparatus [3]. Several distillations were carried out by boiling 100 g of dried leaves of *O. vulgare* in 1liter of distilled water during 3h, the yield of essential oil was determined in relation to the dry matter. The obtained essential oil was stored at 4°C in amber glass tubes and the dark [4].

*Gas chromatography-Mass spectrometry analysis*

The chemical composition analysis of essential oil of *O. vulgare* was performed with a chromatographer in gas phase (Hewlett Packard Agilent 6890) equipped with an HP-5MS capillary column (30 m / 0.25 mm and film thickness 0.25 µm). The steady state temperature was programmed from 60°C for two minutes and then gradually increased (4°C/min) to 220°C for ten minutes. The temperature of the injector was set at 250°C and the volume of injection was 1.5 µL. Helium was used as the carrier gas, in a flow rate of 1mL/min. This chromatographer in gas phase was coupled to a mass spectrometry (Hewlett Packard Agilent 5973) with ionization energy of 70 eV, ion source at 230°C and interface temperature at 280°C.

*In vitro antimicrobial activity*

The determination of antibacterial activity was performed on reference strains (table 1) both by the diffusimetric method on standardized subculture and by the method of dilutions with the MIC (minimum inhibitory concentration) determination, as previously described by other authors [5-8].

**Table 1**
REFERENCE STRAINS

| Bacterial species | ATCC | Producer |
|---|---|---|
| *Klebsiella pneumoniae* | 700603 | ThermoScientific |
| *Pseudomonas aeruginosa* | 27853 | ThermoScientific |
| *Staphylococcus aureus* | 25923 | ThermoScientific |
| *Streptococcus pyogenes* | 19615 | ThermoScientific |
| *Candida albicans* | 10231 | ThermoScientific |

*Disk diffusion method*

On the Mueller-Hinton (Sanimed, Bucharest, Romania) agar plate, bacterial suspensions were deposited in physiological saline (0.5 Mac Farland). After about 10 min, a blank paper disk (BioMaxima, Lublin, Poland) was placed, and 10µL of each of the undiluted test oil was dispensed. The plates were then incubated for 24 h at 37°C. The reading of the inhibition areas was performed with a ruler, and the diameters were expressed in mm. All tests were performed in duplicate. As blind control Gentamicin and Fluconazole (BioMaxima, Lublin, Poland) were used.

*Determination of the minimum inhibitory concentration (MIC)*

The oil was tested by the macrodilution method in Mueller-Hinton broth (Sanimed, Bucuresti, Romania). From the bacterial suspension of 0.5 Mc Farland an inoculum of about 500.000 germs/mL was prepared. Five vials were pipetted with 0.5 mL of the bacterial suspension, then 0.5 mL of the oil dilution obtained in DMSO (80, 40, 20, 10, 5 mg/mL) making a volume of 1mL, which was homogenized. MIC represents the lowest concentration at which the growth of germs did not occur.

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## Results and discussions

*Chemical composition*

**Table 2**
CHEMICAL COMPOSITION OF ESSENTIAL OIL OF *ORIGANUM VULGARE*

| No. | Constituents | % Content |
|---|---|---|
| 1 | Thymol | 35.51 |
| 2 | Y-Terpinen | 19.19 |
| 3 | Durenol | 17.99 |
| 4 | Durene | 11.40 |
| 5 | Carvacrol | 2.69 |
| 6 | Beta-Thujene | 1.92 |
| 7 | Caryophyllene | 1.82 |
| 8 | Beta-Sesquiphellandren | 1.31 |
| 9 | Linalool | 1.30 |
| 10 | Alpha-Thujene | 1.20 |
| 11 | Alpha-Pinene | 0.69 |
| 12 | Vinylamylcarbinol | 0.60 |
| 13 | Alpha-Phellandrene | 0.58 |
| 14 | Alpha-Terpineol | 0.51 |
| 15 | Cyclohexene | 0.49 |
| 16 | Terpinolene | 0.41 |
| 17 | Isothymolmethylether | 0.39 |
| 18 | Alpha-Caryophyllene | 0.23 |
| 19 | L-4-Terpineneol | 0.22 |
| 20 | 4-Terpineneol | 0.21 |
| 21 | Epoxycaryophyllene | 0.21 |
| 22 | Alpha-Humulen | 0.20 |
| 23 | Sorbic acid | 0.20 |
| 24 | Beta-Pinene | 0.18 |
| 25 | Camphene | 0.12 |
| 26 | p-Cymenene | 0.12 |
| 27 | 2.5-Diethylphenol | 0.11 |
| 28 | 3.4-Xylenol | 0.10 |
| 29 | Thymol-TMS | 0.10 |
| 30 | Pinane | 0.06 |
| 31 | Carvone | 0.04 |
| 32 | Borneol | 0.04 |
| | Total | 99.07 |

The yield of essential oil of *O. vulgare* was 1.16%. The chemical composition of this oil is presented in table 2.

Thirty-two volatile constituents were identified in the oil studied, representing 99.07% of the composition. Tymol was the most represented component (35.51%), other major components were: γ-terpinen (19.19%), durenol (17.99%), durene (11.40) and carvacrol (2.69%).

The composition of the essential oils varies depending on the geographical area, climatic conditions, cultivation, drying, storage and processing [9].

Phenols were present in all *O. vulgare* oils from Algeria, France, Italy and Morocco, of which the most isolated was carvacrol. Also, timol was significantly reported in all of these studies [10,11].

*Antibacterial activity*

The antibacterial activity of O. vulgare oil determined by diffusion method is shown in table 3, while the minimum inhibitory concentrations are included in table 4.

The results obtained in this study, following the testing of antimicrobial activity, demonstrate that essential O. vulgare oil inhibits both Gram-positive bladders and Gram-negative bacilli and fungi. Similar data has been reported by other authors who have been monitoring the activity of O. vulgare oil, in which Gram-negative bacilli, such as Escherichia coli, were demonstrated [12,13]. Mourad M.H. and collaborators have demonstrated the antibacterial activity of essential oils on bacterial strains involved in the etiology of urinary infections [14]. The antibacterial activity of compounds in essential oils (carvacrol, linalool and a-terpineol) has been demonstrated on *Bacillus cereus* strains, periodontogen and cariogenic bacteria [15-18].

**Table 3**
DIAMETERS OF INHIBITION ZONES OBTAINED BY DIFFUSION METHOD

| *Klebsiella pneumoniae* | *Pseudomonas aeruginosa* | *Staphylococcus aureus* | *Streptococcus pyogenes* | *Candida albicans* |
|---|---|---|---|---|
| 19 mm | 17 mm | 21 mm | 25 mm | 22 mm |

**Table 4**
MIC VALUES (mg/mL) OBTAINED BY MACRODILUTION METHOD

| *Klebsiella pneumoniae* | *Pseudomonas aeruginosa* | *Staphylococcus aureus* | *Streptococcus pyogenes* | *Candida albicans* |
|---|---|---|---|---|
| 40 mg/mL | 80 mg/mL | 20 mg/mL | 10 mg/mL | 20 mg/mL |

## Conclusions

*O. vulgare* oil, originated in the southwestern part of Romania, due to its rich content of antibacterial compounds, has a bactericidal and fungicidal effect. Further studies on a numerous clinical bacterial or fungal isolates are necessary to investigate and standardize the inhibitory effect of *O. vulgare* oil against these pathogens. On the other hand, studies would be needed to estimate the potential toxicity of these oil. The results of this study may open a way for new antibacterial agents, such as essential oils.

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