Research Article

Antimicrobial Efficacy of the Essential Oil of *Origanum Vulgare* From Algeria

Hicham Boughendjioua[1*] and Ratiba Seridi[2]

[1]Department of Natural Sciences. High School Professors Technological Education, Skikda, 21000, Algeria.

[2]Department of Biology, Faculty of Science, University of Badji Mokhtar, Annaba, 23000, Algeria.

[*]**Corresponding Author:** Hicham Boughendjioua, Department of Natural Sciences. High School Professors Technological Education, Skikda, 21000, Algeria, E-mail: boughendjioua.hicham@yahoo.com

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Abstract

Objective: This study aimed to identify the chemical composition and the antibacterial properties of essential oil from *origanum vulgare* growing wild in Algeria.

Methods: The antibiotic activity of the essential oil was assessed on 05 strains of *Bacillus* (*B. amyloliquefaciens* FZB42, *B. amyloliquefaciens* S499, *B. subtilis* ATCC 21332, *B. licheniformis* ATCC 14580, *B. pumilus*), using the method of diffusion in a solid medium. MIC was determined by the method of integration in an agar medium.

Results: The essential oil extracted from the aerial part of *Origanum vulgare* harvested in Azzaba located at Skikda city (North-east of Algeria) gave a yield of 2.50 %. Its analysis by CG/SM allowed the identification of 25 components, principally phenols and terpenes. The main constituents are *p*-Cymene (24.01 %), Thyme (23.49 %) and Carvacrol (21.31 %). The five strains showed high sensitivity towards the essential oil with inhibition diameters ranging from 21.5 mm to 41 mm and a MIC of 0.4 mg/ml.

Conclusion: The essential oil of *Origanum vulgare* proved to be endowed with bactericidal properties against *Bacillus* strains.

Keywords: *Origanum vulgare*; Essential oil; GC/MS; Antibacterial activity.

1. Introduction

Bacillus is a genus of gram-positive, rod-shaped bacteria and a member of the phylum Firmicutes. *Bacillus* species can be obligate aerobes (oxygen reliant), or facultative anaerobes (having the ability to be aerobic or anaerobic). They will test positive for the enzyme catalase when there has been oxygen used or present [1]. Ubiquitous in nature, *Bacillus* includes both free-living (nonparasitic) and parasitic pathogenic species. Under stressful environmental conditions, the bacteria can produce oval endospores that are not true 'spores', but to which the bacteria can reduce themselves and remain in a dormant state for very long periods. These characteristics originally defined the genus, but not all such species are closely related, and many have been moved to other genera of the Firmicutes [2].

The genus Origanum L., (Lamiaceae), comprises 38 species of annual, perennial, and shrubby herbs, most of which are native to or restricted to the eastern part of the Mediterranean area, Europe, Asia, and North Africa [3, 4]. Oregano is one of the most commonly known culinary herbs worldwide for cooking purposes. The dried herbs are used in many processed foods such as alcohol beverages, meat products, snack foods, and milk products. Some of the Origanum spp. are also used as a fragrance component in soaps, detergents, perfumes, cosmetics, flavorings, and pharmaceuticals. Oregano oil has antibacterial, antifungal, antiparasitic, antimicrobial, and antioxidant properties [5].

2. Material and methods

2.1 Plant material

Our study was carried out during the 2016 growing period, in order to determine the content of essential oil, the samples of fresh herbs, aerial part of the plant. Growing wild in Azzaba located at Skikda city (North-east of Algeria), were harvested in the morning hours at the flowering stage. The taxonomic identity of the plant was confirmed by the well-known Algerian flora of Quezel and Santa, (1962) [6].

2.2 Isolation of the Essential Oil

The essential oil was isolated by hydrodistillation for 60 minutes, using the Clevenger-type apparatus: 100 g of fresh aerial part per 200 ml of water [7]. Following distillation, the essential oil was dried over anhydrous sodium sulphate and stored at 4-6 °C.

2.3 Analytical techniques

Gas chromatography-mass spectrometry (GC/MS) analysis of the essential oil was performed on a TRACE GC ULTRA equipped with non-polar VB5 (5% phenyl, 95% methylpolysiloxane) capillary column (30 m × 0.25 mm × 0.25 µM film thickness), directly coupled to a mass spectrometer (Polaris Q). The electron ionization energy was set at 70 eV. The temperature of injector and detector was set at 220 and 300 °C, respectively. The oven temperature was programmed from 40 to 180 °C at 4 °C/min, then for 180 to 300°C at 20 °C/min. The components of the oil were identified by comparison of their mass spectra with those in the Willey NIST 7th Edition Library of mass spectral data. The composition of the oil sample was calculated from GC/MS peak areas and given by percentages.

2.4 Bacterial strains

The test strains were isolated from hospitals (University Hospitals, health centers) of Annaba (Algeria). The tests were performed on five strains of *Bacillus*: (*B. amyloliquefaciens* FZB42, *B. amyloliquefaciens* S499, *B. subtilis* ATCC 21332, *B. licheniformis* ATCC 14580, *B. pumilus*).

2.5 Test organism (Microbiological study)

2.5.1 Aromatogram

The aromatogram has the same principle as the antibiogram technique. On Mueller Hinton medium we performed seeding tested strains as recommended by the Comity of the Antibiogram of French Society of Microbiology [8]. On the surface of the agar we introduced two sterile discs: one is impregnated with pure Thyme oil, the other is a witness disk devoid of any substance. After an incubation of 24 hours at 37 °C, we proceeded to the reading of the results by measuring the diameter of the inhibition zones formed around the disc.

2.5.2 Determination of MIC (minimal inhibition concentration)

MIC is the lowest concentration of essential oil to which no bacterial drive is observed. The calculation of the MIC was conducted by the method of incorporation agar following the recommendations of the Clinical Laboratory Standards Institue (CLSI) [9], the principle is to prepare dilutions of the essential oil in Dimethylsulfoxid (DMSO) from a stock solution; each dilution is incorporated into a fixed volume of medium Mueller Hinton then poured into a Petri dish. After the drying of the medium, we have placed on the surface of each dish spots, each one representing a tested strain. After an incubation of 24 hours, we distinguished susceptible strains from resistant strains for each concentration.

2.5.3 Determination of MBC (minimal bactericidal concentration)

The MBC is the lowest concentration of essential oil, which destroys 99.9 % of the bacterial inoculum, which is a bacterial count lower than an interval between 104 and 102 CFU/ml after 24 hours of incubation (the initial inoculum is between 106 and 108). Using a platinum loop, we collected a sample from each dish that showed no bacterial growth. Then these samples were plated on a nutritive agar and incubated at 37 °C for 24 hours. The minimum bactericidal concentration is the lowest concentration of the essential oil for which no growth was observed.

3. Results and discussion

3.1. Yield and chemical composition of essential oil

The essential oil yielded obtained by hydrodistillation from the aerial part of *Origanum vulgare* was 2.50 %, the same yield was noticed in the sample collected from Nechmaya region of Guelma city (Algeria) by Bouhaddouda *et al*., (2016) [10], where it was reported that essential oil *Origanum vulgare* L. *ssp. glandulosum* (Desf.) *Ietswaart* yielded from 2.52 (w/w).

As shown in **Table 1**, GC/MS analysis resulted in the identification of twenty-five compounds representing 90.19 % of the essential oil. The major constituents of the oil were *p*-Cymene (24.01 %), Thyme (23.49 %) and Carvacrol **(**21.31 %). γ-Terpinene (9.5 %) and α-Terpineol (3.4 %) were also present at significant concentrations.

Algerian *Origanum vulgare* from Jijel, Constantine [11], Setif [12, 13] and Tlemcen [14] presented a low *p*-cymene content compared to our essential oil (*p*-Cymene (24.01 %)). And all showed a thymol and/or carvacrol chemotype. However, it is important to note that the compositions of these essential oils obtained by hydrodistillation may vary from one region to another. This variability concerns particularly carvacrol for which food manufacturers have a particular interest.

To the best of our knowledge, there are many reports on the chemical composition of the essential oil isolated from different *Origanum vulgare* subspecies from different regions. Most of them indicate the presence of two main chemotypes of this essential oil, one contains as major components the phenols thymol and/or carvacrol [15, 16] and other consists mainly monoterpene alcohols [17, 18].

Amrouni *et al*., (2014) [19] reported and confirms; a different composition for this essential oil which showed a carvacrol chemotype with 33.85 % carvacrol, 23.64 % thymol and 20.85 % para-cymene.

It is known that *Origanum vulgare* species presents great variability in its essential oil composition due to the existence of different subspecies, but also to a numerous of parameters [20].

However, the chemical composition of the studied essential oil differs completely with those previously reported on the literature and displayed a different specific oil, chemical profile with para-cymene, thymol and carvacrol as dominant components.

This deviation from the common chemotypes may be attributed to the effect of the factors that specifically affect the composition and yield of the essential oil, which include seasonal and maturity variation, geographical origin, genetic variation, growth stages, postharvest drying and storage [21, 22, 23].

N°	**Compounds**	**Retention time (min)**	**Percentage %**
01	α-Pinene	6.10	0.2
02	Camphene	7.09	0.5
03	β -Pinene	7.61	1.11
04	Myrcene	8.02	0.09
05	β -Phellandrene	8.12	0.07
06	***p*-Cymene**	8.38	**24.01**
07	1,8-Cineol	9.31	0.4

08	γ -Terpinene	10.31	9.5
09	Sabinene hydrate	11.53	1.45
10	Terpinolene	11.67	0.2
11	Linalol	12.81	0.5
12	Camphor	15.91	0.2
13	Borneol	15.98	0.5
14	Terpinene-4-ol	16.24	1.3
15	α-Terpineol	17.60	3.4
16	Bornyl acetate	19.80	0.2
17	**Thymol**	20.89	**23.49**
18	**Carvacrol**	22.19	**21.31**
19	Eugenol	23.89	0.1
20	α-Copaene	24.36	0.3
21	β -Caryophyllene	24.80	0.4
22	α-Humulene	25.27	0.2
23	Germacrene D	38.24	0.06
24	α-Muurolene	48.69	0.3
25	α-Farnesene	65.20	0.4
Total			**90.19**

Table 1: Chemical composition of *Origanum vulgare* essential oil identified by GC-MS.

3.2. Antimicrobial Activity

Table 2 shows the inhibition diameters, MIC and MBC of *Bacillus* strains.

<table>
<tr><th>Strains</th><th>D (mm)</th><th>MIC (mg/ml)</th><th>MIB (mg/ml)</th></tr>
<tr><td>B. amyloliquefaciens FZB42</td><td>23</td><td rowspan="5">0,4</td><td rowspan="5">0,4</td></tr>
<tr><td>B. amyloliquefaciens S499</td><td>21,5</td></tr>
<tr><td>B. subtilis ATCC 21332</td><td>30</td></tr>
<tr><td>B. licheniformis ATCC 14580</td><td>35</td></tr>
<tr><td>B. pumilus</td><td>41</td></tr>
</table>

Table 2: Activity of the essential oil of *Origanum vulgare* on *Bacillus*

D: Inhibition diameters, MIC: Minimal inhibitrice concentration, MIB: Minimal bactericidal concentration.

Despite the resistance of gram-negative bacteria to essential oil [24], strains of *Bacillus* showed an interesting sensitivity against the essential oil of *Origanum vulgare*, with inhibition zones ranging from 21.5 mm in strain *B. amyloliquefaciens* S499 to 41 mm in *B. pumilus*. MIC obtained for all strains investigated (0.4 mg/ml) is very interesting and indicates a high activity of this oil on this bacterial species. This efficiency can be explained in terms of the high prevalence of phenolic derivatives that are the source of the antibacterial effect of essential oils according to several authors [25, 26].

The antibacterial activity of the essential oil of *Origanum vulgare* can be partly attributed to its high content level of thymol which, according to Lambert *et al*., (2001) [27] binds to membrane proteins and increases the permeability of the bacterial cell membrane. Dorman and Deans, (2000) [28] demonstrated that thymol is the compound that has the widest spectrum of antibacterial activity and that against 25 types of bacteria tested. Other studies suggest that volatile compounds are responsible of the inactivation of enzymes, including those involved in energy production and synthesis of structural components [29, 28].

Minor components are not of lesser importance since they produce synergies with others and potentiate their effects; this is what was discovered by Marino *et al*., (2001) [30] in a study on the sage. This was also highlighted by Lambert *et al*., (2001) [27] who have tested the activity of thymol and carvacrol on strains of Staphylococcus aureus and Pseudomonas aeruginosa. Ultee *et al*., (2000) [31] reported that *p*-cymene could cause swelling of the cytoplasmic membrane of Bacillus cereus and disturbances in its structure.

The presence of para-cymene, thymol and carvarol as dominant components in this essential oil and the potential synergistic phenomenon between them might be involved in this great antimicrobial activity.

Thymol is structurally very similar to carvacrol, having the hydroxyl group at a different location on the phenolic ring. Both substances appear to make the cell membrane permeable. Thymol has been previously described as able to interrupt the bacterial membrane, by affecting both the pH gradient and the electron flow across the membrane, and it may justify the highest antimicrobial activity of the essential oil [27].

It was shown that hydroxyl group gets inserted in cytoplasmic membrane, changes the membrane physical and chemical properties and affects both lipid ordering and stability of bilayer, inducing an increase of proton passive flux across the membrane [32].

Despite the p-cymene was an ineffective antimicrobial agent lonely, but combined with carvacrol has led to a synergistic activity resulted by swelling bacterial cell membranes to a greater extent than carvacrol does. By this mechanism p-cymene probably enables carvacrol to be more easily transported across the cytoplasmic membrane so that a synergistic effect is achieved when the two are used together [33].

4. Conclusion

The essential oil of *Origanum vulgare* harvested in Azzaba located at Skikda city (North-east of Algeria) is characterized by the presence of 25 components; the most important are *p*-Cymene, Thyme and Carvacrol. The essential oil was tested over five strains of *Bacillus*: (*B. amyloliquefaciens* FZB42, *B. amyloliquefaciens* S499, *B. subtilis* ATCC 21332, *B. licheniformis* ATCC 14580, *B. pumilus*).

All these strains have a profile of resistance to imipenem. The tests showed a strong antibacterial activity of essential oil of *Origanum vulgare* towards all the strains tested. This strength is mostly attributed to the high concentration of terpenes and phenolic compounds in this essential oil including *p*-Cymene, Thymol and Carvacrol which is the major component.

Acknowledgements

None.

Conflicts of Interest

The authors report no conflicts of interest in the presentation of data.

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