https://doi.org/10.31925/farmacia.2022.6.15 **ORIGINAL ARTICLE**

EVALUATING *IN VITRO* ANTIBACTERIAL AND ANTIOXIDANT PROPERTIES OF *ORIGANUM VULGARE* VOLATILE OIL

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Abstract

There is a growing interest in complementary and effective compounds in healthcare today, with the goal of reducing the consumption of antibiotics and finding alternative natural preservatives with both antibacterial and antioxidant properties, as well as finding natural antiproliferative agents. The antibacterial potency of volatile oregano oil was tested using bacterial cultures *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 25668). *P. aeruginosa* was the most immune microorganism studied, while *S. aureus* was the most susceptible pathogen to oregano oil, with an average inhibition varying between 19.67 mm for the 1:1 diluted oregano oil samples to 35.67 mm for the concentrated oregano oil samples. The two samples containing oregano volatile oil have demonstrated antioxidant efficacy, as indicated by the stable values of the free radical scavenging factor DPPH of 80.80 ± 0.11 µg/mL for the O1sample and 68.47 ± 0.10 µg/mL for the O2 sample. Obtained results showed that oregano volatile oil may be used as a potential natural antibacterial and a good source of natural antioxidants.

Rezumat

Există un interes tot mai mare pentru compuși complementari eficienți și actuali în ceea ce privește îngrijirea sănătății, cu scopul de a reduce consumul de antibiotice sintetice și de a utiliza conservanți naturali ca alternativă, cu proprietăți antibacteriene și antioxidante. Potențialul antibacterian al uleiului volatil de oregano a fost testat folosind culturile bacteriene *S.aureus, E.coli* și *P. Aeruginosa*. *P. aeruginosa* a prezentat cea mai mare rezistență, în timp ce *S. aureus* a fost patogenul cel mai mult inhibat de uleiul de oregano, cu medii de inhibiție care variază de la 19,67 mm în cazul probelor diluate, până la 35,67 mm în cazul probelor concentrate comparativ cu probele de antibiotice standard. Cele două probe conținând ulei volatil extras din specia *Origanum vulgare* au prezentat, de asemenea, activitate antioxidantă, fapt evidențiat de valorile stabile ale factorului de inhibiție a eliminării radicalilor liberi DPPH 80,80 ± 0,11 µg/mL pentru proba O1, respectiv 68.47 ± 0.10 µg/mL pentru proba O2. Rezultatele obținute au arătat că uleiul volatil de oregano poate fi folosit ca un potențial antibacterian natural și o sursă bună de antioxidanți naturali.

Keywords: Oregano, volatile oil, GC/MS, antibacterial, antioxidant, inoculated bacterial culture, DPPH, FRAP

Introduction

The *Lamiaceae/Labiatae* family is found in temperate regions all over the world. It contains approximately 4000 species and roughly 220 genera [10].

Volatile aromatic and medicinal plant oils have a strong potential for use as antibacterial and antioxidant agents [9, 12].

Various research has already shown effective antibacterial effects of oregano [5, 31, 32]. A number of sources for antioxidants from medicinal plants have been reviewed by researchers [2, 14]. The antioxidant attributes of many herbal extracts were shown to be beneficial in slowing the cycle of lipid peroxidation in oils and fatty environments, the antioxidant activity of which was due to the involvement of hydroxyl groups in their molecular structure [24, 38].

The chemical composition of *Origanum vulgare* include monoterpenic phenols (carvacrol, thymol), bitter principles, tannins, anthocyanins (peonidol, marvidol), polyphenolcarboxylic acids (caffeic, rosemarinic, chlorogenic), flavones (apigenol, kempferol, luteol, along with their glocosides), terpene compounds (ursolic acid, oleanolic acid) and minerals [21].

Carvacrol and thymol in the composition are believed to be responsible for the antibacterial activity, which works by destabilizing bacterial membranes [30].

The antimicrobial action of the aforementioned volatile oil will facilitate its use as effective preservative and conservation agent in cosmetics and pharmaceutical

formulations, but also as a phytotherapeutic remedy in mild bacterial infection treatments, potentially reducing overuse of antibiotics and multi-resistance [15].

Antioxidant activity can be determined using a variety of free radicals. The chosen strategy used the 2,2-Diphenyl-1-picrylhydrazile (DPPH) radical, which converts its' purple colour to yellow [7, 34]. The reduction reaction is achieved with antioxidants compounds [3, 4]. DPPH radical shift is an indicator of the antioxidants concentration required to reduce a certain amount of radicals. Spectrophotometric measurements must be used to assess the colour intensity [12, 26].

The aim of this study was to explore the antibacterial and antioxidant properties of the *Origanum vulgare* volatile oil. The antibacterial activity was evaluated against three bacteria strains, using a standardized seed-diffusion (Kirby-Bauer) process [13, 16]. For the antioxidant properties, the assessment included using a spectral method related to 3 standard substances: ascorbic acid, gallic acid and caffeic acid.

Materials and Methods

Plants origin

We chose to prepare two main volatile oil samples for this study, from two different culture environment. We harvested the oregano plant from two different places in Bihor County, Romania, in June 2020 right before blooming, when the concentration in volatile oil is optimum. The oregano leaves for the first sample were harvested from a private culture in Oradea, Romania, and for the second sample from a private culture in Ștei region, Romania. The fresh leaves were cleaned and washed with purified water, sliced in tiny fragments and left to dry in the shade. The resulting dried leaves were crushed in powder form.

Extraction procedure

Both samples were prepared individually, using three hundred grams of dry powder for every sample, these were hydro-distilled using Neo-Clevenger device for 3 h in a dark environment. We determined the refractive index of both samples using the Abbe refractometer. The determinations for each sample were made in triplicate followed by a ± value represented by the standard deviation.

The two main volatile oil samples were kept in dark-coloured glass bottles and held at 4ºC until analysis was carried out.

GC-MS analysis

The O1 and O2 concentrated *Origanum vulgare* volatile oil (corresponding to the S1 sample and S3 sample, respectively) was GC-MS analysed using a Thermo GC system 5975C inert XL EI/CI MSD with Triple-Axis Detector. The following were the operating conditions: the GC was outfitted with a capillary column TG-5MS (30 m * 0.25 mm), with a film thickness of 0.25 m. The flow rate of the carrier gas was 1 mL He/min, and the injected volume was 10 µL. The split ratio was one-twentieth. The column temperature was kept constant at 60ºC while programming 4ºC/min to 180ºC and then 10ºC/min to 260ºC. The detector and evaporator temperatures were both 260ºC.

Antioxidant properties

The antioxidant capacity of the oregano oil was highlighted through its DPPH radical scavenging efficiency and Fe^{3+} ion reducing power. The spectrophotometric method with DPPH in visible light at 517 nm was used to analyse the antioxidant properties of the two concentrated *Origanum vulgare* volatile oil, respectively S1 and S2 [8, 27]. Spectrophotometric tests were carried using the UV-VIS Spectrophotometer (PG Instruments Ltd., Leicestershire, United Kingdom) using an UVWIN software. We used ethanol 96% p.a. (Chempur), ascorbic acid (A1300000), gallic acid (G7384) and caffeic acid (C0625), substances from Sigma-Aldrich/ Merck and DPPH from Cayman Chemical. The subsequent antioxidant standard substance solutions have been prepared in order to develop the calibration lines [19, 38]. All data analysis was obtained using Origin 2019 for the calibration lines and ANOVA analysis of variance software in Excel 2019.

Ascorbic acid (AA). A 1 mM DPPH solution in ethanol that is stored in a cold and dark space until it was used for analysis that same day was prepared. A stock solution of ascorbic acid 20 µg/mL was prepared in the same solvent from which 5 other solutions with the following concentrations were prepared by successive dilutions: 10 µg/mL; 5 µg/mL; 2.5 µg/mL; 1.25 µg/mL and 0.625 µg/mL. We combined 1 mL of ascorbic acid solution with 3 mL of 1 mM of DPPH solution, placing it in the dark and also at room temperature for 30 minutes. The absorbance of the solutions was assessed at 517 nm.

The standard solution was represented by alcohol and the control solution was obtained by mixing 1 mL of alcohol with 3 mL of 1 mM of DPPH solution [22].

Gallic and caffeic acid (GA/CA). Two distinct 0.5 mM DPPH solution in ethanol were obtained, which were maintained in a cold and dark area and used for analysis on the same day. Two stock solutions of gallic/caffeic acid 100 µg/mL were prepared in the same solvent from which 5 other solutions for each standard substance containing the following concentrations were prepared by successive dilutions: 80 µg/mL, 60 µg/mL, 40 µg/mL, 20 µg/mL and 10 µg/mL. We combined 50 µL of gallic/caffeic acid solution with 3 mL of 0.5 mM DPPH solution and placed it in a dark environment at room temperature for 30 minutes. The absorbance of the solutions was measured at 517 nm. Alcohol was used as standard solution and the control one was obtained by combining 50 µL of alcohol with 3 mL of 0.5 mM DPPH solution [23, 39]. The rate of inhibition was determined as follows:

$$Inhibition\ \% = \frac{A_{control} - A_{sample}}{A_{control}}\ x\ 100$$

The obtained results utilizing standards had been used to define the calibration line for the 50% inhibition index (IC_{50}) [23, 31, 39].

The calibration curves were used to express the main correlations between absorbance and inhibition index of oregano oil, followed by the results of anti-oxidant capacity for the two samples of oregano oil.

We chose different DPPH concentrations based on the bibliographic sources we used to formulate our working theory, on the one hand, and the different molecular formulations of ascorbic acid than of gallic and caffeic acid, on the other.

Caffeic and gallic acids are polyphenolic acids with hydroxyl groups in the meta position of the benzene nucleus, which increases their antioxidant activity due to the electromer conjugation. Ascorbic acid, on the other hand, is a pentatomic heterocycle with lowered antioxidant activity and stability than the other two [22, 39].

In addition of varying the DPPH concentrations, we adjusted the concentrations of the standard compounds in order to obtain absorbance values as close to the 0.3 - 0.8 range specified by the Romanian Pharmacopoeia, 10^{th} edition [41].

Ferric reducing antioxidant power (FRAP) assay

The antioxidant potential in the samples is determined by the reduction of ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) by antioxidants present in the samples. A blue hue develops after the ferric iron is reduced, which can be colourimetrically measured at 700 nm. The FRAP assay was carried out using the Oyaizu method [35]. Briefly, 2.5 mL of potassium hexa-cyanoferrate III (1%), 1 mL of the essential oils (3000 ppm), and 2.5 mL of phosphate buffer were combined. 15 minutes were spent heating the mixture to 50ºC. The mixture was then mixed with 2.5 mL of a 10% (w/v) trichloroacetic acid solution, and centrifuged for 15 minutes at 3000 rpm. A 2.5 mL aliquot of the top layer was combined with 0.5 mL of a 0.1% $FeCl_3$ solution and 2.5 mL of deionized water. A UV-visible spectrophotometer was used to detect absorbance at 700 nm.

Enhanced reducing power was demonstrated by the reaction mixture's increased absorbance. The final results were represented as ppm of ascorbic acid equivalents (AAE), gallic acid equivalents (GAE), and caffeic acid equivalents (CAE) using ascorbic acid (AA), gallic acid (GA), and caffeic acid (CA) as reference materials. Each test was performed in triplicate.

Antibacterial properties

To evaluate the antibacterial effects of oregano oil, we prepared for each main sample obtained previously three different samples:

First main oregano oil sample: (i) S1 – sample 1 – containing 40 µL volatile oil; (ii) S3 – sample 3 – containing 20 µL volatile oil diluted with 20 µL olive oil (1:1); (iii) S5 – sample 5 – containing 30 µL volatile oil diluted with 10 µL olive oil (3:1).

Second main oregano oil sample: (i) S2 – sample 2 – containing 40 µL volatile oil; (ii) S4 – sample 4 – containing 20 µL volatile oil diluted with 20 µL olive oil (1:1); (iii) S6 – sample 6 – containing 30 µL volatile oil diluted with 10 µL olive oil (3:1).

Bacterial strains

Antibacterial activity of the *Origanum vulgare* volatile oil was evaluated against three Gram-positive and negative bacteria strains: *Staphylococcus aureus* (+) – ATCC 25923, *Escherichia coli* (-) – ATCC 25922 and *Pseudomonas aeruginosa* (-) – ATCC 25668.

The inoculation medium was represented by agar. After 15 minutes of calibration, the Mueller Hinton containers were seeded using fresh, sterile swabs. The inoculation of the dried surface of a MH agar plate was done by streaking the swab three times over the entire agar surface; rotating the plate approximately 60 degrees each time to ensure an even distribution of the inoculum. The antibacterial activity of the oil was investigated using a standardized seed-diffusion (Kirby-Bauer) process.

The bacterial suspensions were prepared using a sterile inoculating loop or needle by touching four or five isolated colonies of the organism to be tested. Then the organism was suspended in 2 mL of sterile 0.9% saline. Next step was to vortex the saline tube to create a smooth suspension. Afterwards we adjusted the turbidity of this suspension to a 0.5 McFarland standard by adding more organism if the suspension was too light or diluting with sterile saline if the suspension was too heavy. We used the suspension in within 15 minutes of preparation.

We used 6 mm diameter paper disk. Each disk was infused with 1000 µg of the sample solutions (S1 - S6) and then manually applied to the surface of the containers with agar inoculated with microorganisms.

As positive reference levels for the susceptibility of Gram-positive and negative bacterial species, ciprofloxacin (for all three bacterial species), azithromycin, cefoxitin, doxycycline, clindamycin (*S. aureus*), gentamicin, nitrofurantoin, meropenem and ceftriaxone 30 µg/disk were used (*E. coli* and *P. aeruginosa* strains). The placement of the appropriate antimicrobial-impregnated disks and of the S1 - S6 disks on the surface of the agar, was done using a forceps to dispense each disk one at a time which was sterilized using a Bunsen torch after each use. The containers were incubated at 37ºC for 24 h before reading the results. Antibacterial activity was measured by calculating the diameter of the inhibition areas, including the dimension of the disk (6 mm). All tests were conducted in triplicate [18].

Statistical analysis

Data were presented as mean ± SD. Results were statistically processed using Microsoft Excel 2007. Multiple comparisons were performed using one-way analysis of variance ANOVA. All results were considered statistically significant when $p < 0.05$.

Results and Discussion

From the extraction we obtained the following samples

From the first main sample, we obtained 3 mL of yellowish-brown oregano volatile oil with a refractive index (RI) of 1.4802 ± 0.0024.

We collected 4 mL of yellowish-brown volatile oil from the second main sample, with RI of 1.4602 ± 0.0024.

The essential oils of *Origanum vulgare* were analysed by GC-MS using a mobile phase consisting of 2-aziridinil-ethyl-amine, trimethylethylen, dichloro-methane, chloroform and a TraceGOLD TG-5SilMS column. By comparing the relative retention times and mass spectra of oil components with authentic samples and mass spectra from the data library, the compounds were identified. In both *O. vulgare* essential oils, 18 compounds were identified, accounting for 99.87% of the total composition [20, 37]. The essential oil's major components (Table I), which are also responsible for its antioxidant [29] and antimicrobial activities, were: o-Cimen (11.17; 13.17), Terpinen-4-ol (10.57; 12.71), γ-Terpinen (22.31; 16.45), Thymol (3.77; 4.26), Carvacrol (41.84; 40.52). Other researchers discovered that oregano essential oil has a similar chemical composition with minor variations in component concentration [2].

Antibacterial properties

Oregano volatile oil was shown to be efficient against pathogenic strains of *P. aeruginosa* and *E. coli* [33].

This research assessed that all 6 volatile oil samples showed antibacterial activity comparable to that of the standard antibiotics used against the strains of the bacteria tested, the only exception observed was in the case of P. *aeruginosa*, in which case our diluted oregano volatile oil samples did not have the ability to suppress the Gram-negative bacteria growth. Following incubation, bacterial growth was found to be significantly diminished in proportion to the increase in the concentration of oregano volatile oil. The suppression of bacterial growth was influenced by the concentration of the volatile oil (Figure 1 and Figure 2).

Our concentrated oregano-volatile oil sample collected from Oradea, Romania cultivated oregano (S1) was more effective against *S. aureus* and *P. aeruginosa* and almost remarkably similar to *E. coli* with S2, while our concentrated oregano-volatile oil sample extracted from Ștei region, Romania, cultivated oregano (S2) had more impact against *E. coli*.

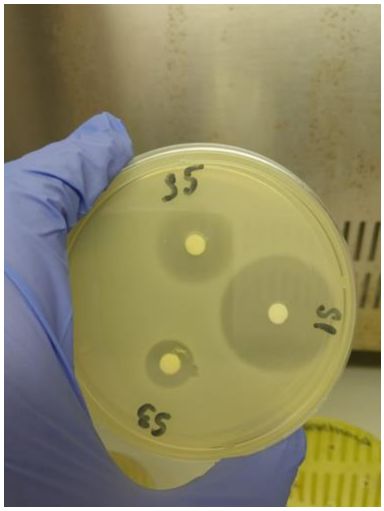

Figure 1.
The influence of oil concentration on the bacterial growth inhibition capacity – samples S1, S3 and S5

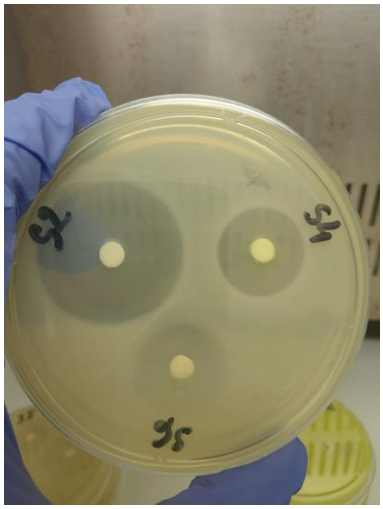

Figure 2.
The influence of oil concentration on the bacterial growth inhibition capacity – samples S2, S4 and S6

Of the examined strains, S. aureus was the most sensitive to oregano volatile oil

For *S. aureus*, samples S1 – (35.67 mm ± 0.58, p = 0.0167), S2 (34.33 mm ± 0.58, p = 0.00196) containing undiluted volatile oil, respectively sample S5 – (3:1) (33 mm ± 1.00, p = 0.0244) displayed an inhibitory potential in a higher manner than the antibiotic standards [6]. Sample S6 – (3:1) (24 mm ± 1.00, p = 0.0810) had a statistically significant inhibitory potential and samples S3 – (1:1) (19.67 mm ± 0.58, p = 0.0987) and S4 – (1:1) (18.64 mm ± 0.58, p = 0.1196) had a medium significant capacity according to the standards (Figure 3).

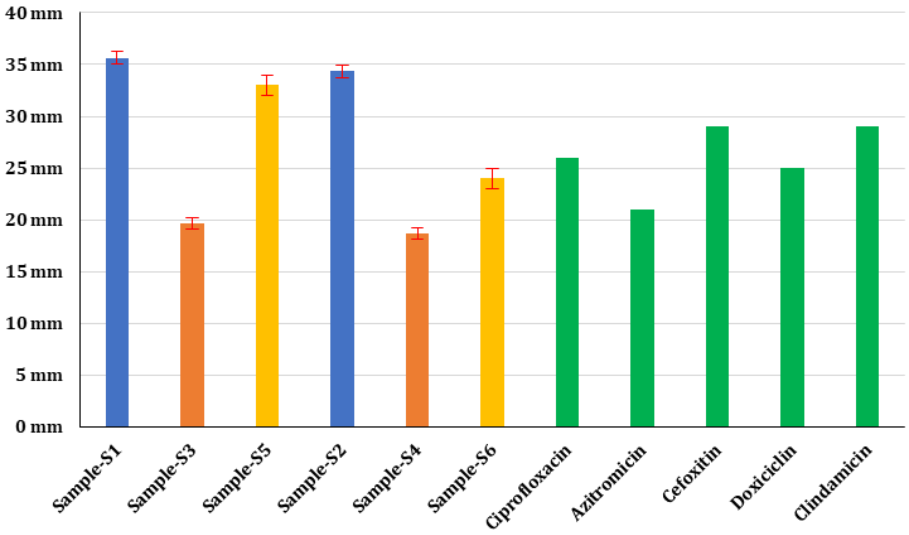

Figure 3.
Oregano volatile oil antibacterial activity on *S. aureus* (S1, S2, S5 $p < 0.05$)

For the assessment of the inhibition ability of oregano oil samples on *E. coli*, there is a statistical relevance of the inhibition average comparable to that of the antibiotic standards in the specialty literature [6] for samples S1 – (32 mm ± 1.00, p = 0.0372), S2 – (32.67 mm ± 0.58, p = 0.0362) containing concentrated volatile oregano oil. The diluted samples S4 – (1:1) (20.67 mm ± 0.58, p = 0.1032), S5 – (3:1) (20.33 mm ± 0.58, p = 0.1029) and S6 – (3:1) (21.67 mm ± 0.58, p = 0.0898) had a moderately significant inhibitory potential and the diluted oil sample collected from Oradea's oregano leaves S3 – (1:1) had a weakly relevant capacity according to the criteria adopted (13.67 mm ± 1.15, p = 0.1407) (Figure 4) [6].

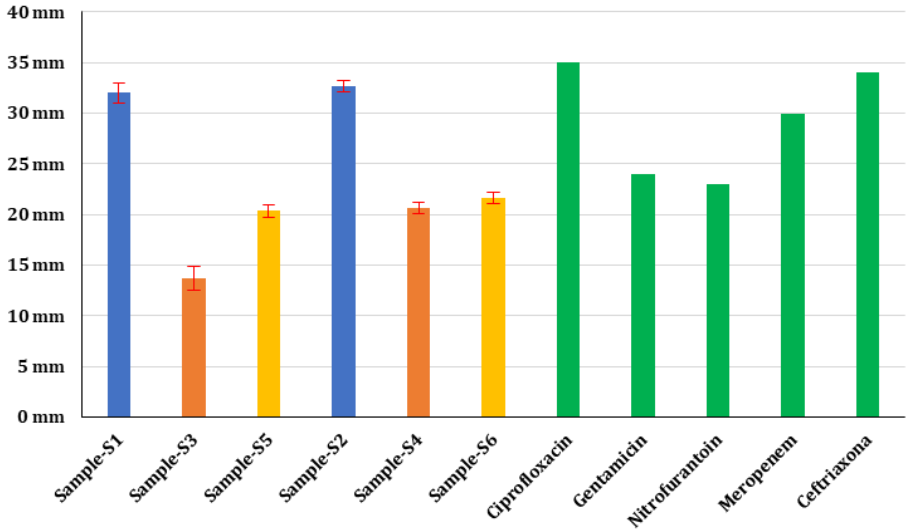

Figure 4.
Oregano volatile oil antibacterial activity on *E. coli* (S1, S2 $p < 0.05$)

The resistance exhibited by *P. aeruginosa* can be observed both from our research findings and in contrast to antibiotic standards [6]. Samples S1 had an inhibition average of 16.33 mm ± 1.15, p = 0.0170 and S2 15.67 mm ± 0.58, p = 0.0190 respectively. The diluted samples S3, S4, S5 and S6 had no inhibitory ability compared to the standards (Figure 5).

We acknowledge that the concentrations of thymol and carvacrol differ with temperature and soil in oregano volatile oil. *P. aeruginosa* can form biofilms to ensure virulence, longevity and antibiotic resistance [1]. The formed biofilm inhibited oregano oil from adhering to the bacterial surface, thus preventing the expression of antibacterial activity [17]. This, we believe, is why the concentration of oregano volatile oil affects the inhibitory effect on *P. aeruginosa*.

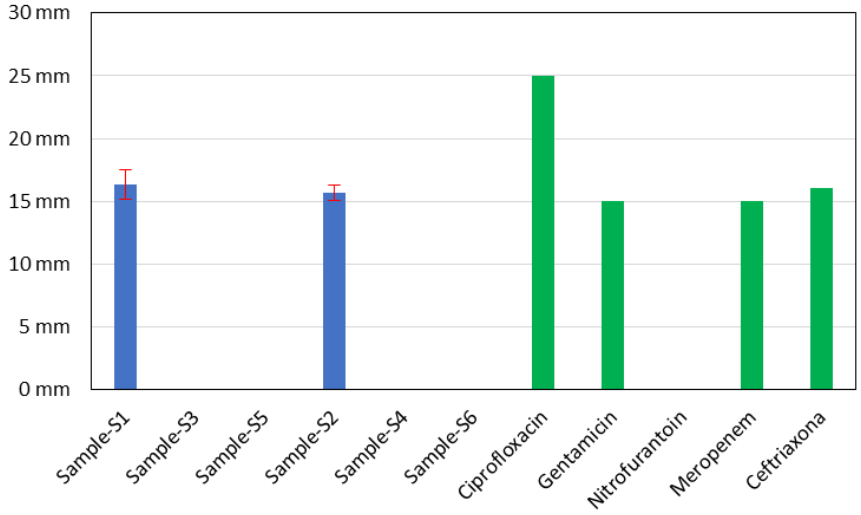

Figure 5.
Oregano volatile oil antibacterial activity on *P. aeruginosa* (S1, S2 $p < 0.05$)

Antioxidant activity

There was no need for varying concentrations of S1 and S2 oregano oil samples to calculate DPPH antioxidant activity because it was measured compared to a regression curve for each standard substance used. Calibration curves (absorbance and inhibition), and the results are presented in Table I.

The correlation coefficients outlined in Table II suggest a clear connection between the absorbance value and the standard substances concentration.

Table I
Standard substances calibration curves (absorbance and inhibition)

Standard substance	Equation of the standard curve		Correlation coefficient (R^2)	
	Absorbance	% Inhibition	Absorbance	% Inhibition
Ascorbic acid	A = 0.84824 - 0.0681 x C (µg/mL)	I (%) = -0.83184 + 4.39625 x C (µg/mL)	0.99525	0.99930
Gallic acid	A = 0.56479 - 0.00522 x C (µg/mL)	I (%) = -5.71885 + 0.98994 x C (µg/mL)	0.98690	0.99575
Caffeic acid	A = 0.90087 - 0.00456 x C (µg/mL)	I (%) = -4.16291 + 0.55585 x C (µg/mL)	0.96730	0.98758

Table II
Analysis of oregano essential oils (O1 and O2) by GC-MS

No	Compounds	RT	Area% O1	Area% O2
2614	α-Pinen	7.69	1.89	2.16
2615	Camfen	8.06	0.41	0.54
2616	α-Terpinen	8.48	0.72	1.70
2617	β-Pinen	8.76	0.48	0.21
2618	p-Cimen	8.92	1.28	1.94
2619	α-Humulen	9.04	0.04	0.11
2620	β-Mircen	9.09	1.02	0.87
2621	4-Caren	9.75	1.29	2.11
2622	o-Cimen	9.94	11.17	13.17
2623	Terpinen-4-ol	10.82	10.57	12.71
2624	Borneol	11.37	0.34	0.29
2625	γ-Terpinen	11.52	22.31	16.45
2626	Linalol	11.82	1.02	0.82
2627	Thymol	16.42	3.77	4.26
2628	Carvacrol	16.70	41.84	40.52
2629	Cariofilen	19.41	1.02	1.48
2630	β-Bisabolen	21.15	0.39	0.31
2631	Cariofilen oxid	22.77	0.31	0.35

The antioxidant capacity of the two oregano oil samples is presented in Table III. O1 sample results were superior to O2 in terms of antioxidant capacity in all three assessments with the standard substances (Table IV).

The IC_{50} value was determined to assess the concentration of the sample needed to inhibit 50%

of the radical. The lower the value of IC_{50}, the higher the antioxidant function of the samples. IC_{50} is inversely proportional to the anti-radical activity, expressed in ARP (anti-radical power activity, ARP = $1/IC_{50}$) [28].

The calculated IC_{50} results revealed that our second oregano oil sample had the highest antioxidant effect (68.47 ± 0.10 μg/mL) proceeded by our first oregano sample (80.80 ± 0.11 μg/mL) compared to the standard ascorbic acid (11.56 ± 0.05 μg/mL), gallic acid (56.29 ± 0.08 μg/mL) and respectively caffeic acid (97.44 ± 0.07 μg/mL). Data is expressed as an average value of ± SD of triplicate samples. Values of distinct rows are meaningful ($p < 0.05$). The values obtained are revealed by Table IV.

Table III

Antioxidant capacity of O1 and O2 oil samples compared to standard substances

Sample	DPPH			FRAP		
	μmol AAE/g dry plant material	**μmol GAE/g dry plant material**	**μmol CAE/g dry plant material**	**μmol AAE/g dry plant material**	**μmol GAE/g dry plant material**	**μmol CAE/g dry plant material**
Oregano 1	40.54 ± 0.02	27.41 ± 0.11	37.16 ± 0.12	35.47 ± 0.15	25.04 ± 0.27	33.31 ± 0.20
Oregano 2	24.15 ± 0.03	15.91 ± 0.08	28.13 ± 0.12	18.28 ± 0.21	14.83 ± 0.14	21.99 ± 0.18

*AAE – ascorbic acid equivalents; GAE – gallic acid equivalents; CAE – caffeic acid equivalents;
Significant difference between all components $p < 0.05$

Table IV

DPPH radical scavenging activity (IC_{50} in μg/mL) of standard substances and two oregano oil samples

Extracts and standard	DPPH test (IC_{50} in μg/mL)	ARP*
Ascorbic acid	11.56 ± 0.05	0.0865
Gallic acid	56.29 ± 0.08	0.0177
Caffeic acid	97.44 ± 0.07	0.0002
O1	80.80 ± 0.11	0.0123
O2	68.47 ± 0.10	0.0146

According to other research [34], extracts with IC_{50} values varying from 50 to 100 mg/mL are known to exhibit intermediate antioxidant function. In the meanwhile, extracts with an IC_{50} value ranging from 10 to 50 mg/mL are found to have a high anti-oxidant activity. In our research, both samples of oregano oil showed intermediate antioxidant activity. Terpenoids are all classified as compounds that are distinguished as isoprene building blocks. Terpenoid-derived substances have been referred to as possible bioactive compounds. In addition, terpenoids perform a significant function in human wellbeing. These results concur that oregano volatile oil has notable antioxidant qualities. These findings suggest that oregano volatile oil is a valuable natural antioxidant source for the healthcare industry.

The oregano volatile oil contains carvacrol and thymol, which seem to be responsible for the antibacterial effect that acts by destabilizing the bacterial membrane. The presence of polyphenolic acids, especially rosmarinic acid, as well as carvacrol in the product is associated with the antioxidant activity [4, 19, 30]. Since several commonly used synthetic antioxidants have been linked to harmful consequences in human health, herbs and spices may be the most appropriate safe candidates for natural antioxidants [40].

Numerous researches on the antioxidant and anti-microbial effects of oregano oil have been conducted. A similar study attempted to demonstrate the presence of antioxidant and antibacterial activity in volatile oils of *Origanum vulgare* and *Thymus vulgaris* at various concentrations. Antibacterial activity was assessed using *E. coli*, *P. aeruginosa* and *B. cereus*. *P. aeruginosa* was the pathogen with the highest resistance to the volatile oil of *Origanum vulgare* and *E. coli* was the pathogen that was strongly inhibited. In terms of antiproliferative activity, oregano oil was highlighted using DPPH scavenging activity method, which used as standards ascorbic acid and BTH, while in our research we used the same method, but with three standard compounds, ascorbic, gallic and caffeic acid. According to the findings of both studies, the presence of thymol and carvacrol in volatile oregano oil results in significant antioxidant and antibacterial activity [25, 26, 32].

Another research sought to assess the antimicrobial, antifungal and antioxidant properties of volatile oils on the Portuguese island of Madeira. In the case of *Origanum vulgare*, the results demonstrated a moderate inhibition of bacterial activity compared to antibiotics used as a control for all bacterial and fungal strains studied, with the exception of P. *aeruginosa*, as observed in our current research. In terms of anti-oxidant efficacy, the research used DPPH to demonstrate the antioxidant role of thymol, but they used n-hexane and polar solvents for extraction [11].

Conclusions

Our research has shown that oregano oil has both antioxidant and antibacterial activities due to their

chemical composition. These antibacterial agents permeate the cell membrane due to their impregnation in the hydrophobic domains; this effect is stronger against gram positive bacteria. Furthermore, oregano essential oil has antioxidant properties that are effective in slowing the process of lipid peroxidation in fatty foods and scavenging free radicals.

Following incubation, bacterial growth was found to be significantly diminished in proportion to the increase in the concentration of oregano volatile oil. The suppression of bacterial growth was influenced by the concentration of the volatile oil.

Our concentrated oregano-volatile oil sample collected from Oradea cultivated oregano (S1) was more effective against *S. aureus* and *P. aeruginosa* and almost remarkably similar to *E. coli* with S2, while our concentrated oregano-volatile oil sample extracted from Ștei region cultivated oregano (S2) had more statistical impact against *E. coli*.

Of the strains examined, *S. aureus* was the most sensitive to the oregano volatile oil while *P. aeruginosa* was the most resistant strain of bacteria to oregano oil as well as antibiotic standards used for determination of inhibition capacity due to its known capacity of forming a biofilm that limits the adherence of antibacterial agents.

The results obtained for the antioxidant capacity showed approximately equal values by the two methods, with DPPH and FRAP respectively. Overall, both samples showed intermediate antioxidant activity, however, a lower one was observed for the Oregano 2 sample. These findings conclude that oregano oil is a valuable natural antioxidant source for the health-care industry. The exhibited antioxidant properties were dependent on the concentration in terpenoids.

Conflict of interest

The authors declare no conflict of interest.

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