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Bio-efficacy of the Essential Oil of Oregano (*Origanum vulgare* Lamiaceae. Ssp. *Hirtum*)

Ezequiel Grondona · Gerardo Gatti · Abel G. López · Leonardo Rodolfo Sánchez · Virginia Rivero · Oscar Pessah · María P. Zunino · Andrés A. Ponce

Abstract The aim of this study was to investigate the bioactivity of the essential oil isolated from *Origanum vulgare* L. (EOv). We analyzed the *in vivo* anti-inflammatory properties in a mouse-airway inflammation model and the *in vitro* antimicrobial activity, genotoxicity over the anaphase-telophase with the *Allium cepa* strain and its cytotoxicity/viability in A549 culture cells. *In vivo*, EOv modified the levels of tumor necrosis factor -α and viable activated macrophages and was capable to mitigate the effects of degradation of conjugated dienes. *In vitro*, EOv reduced the viability of cultured A549 cells as well as the mitotic index and a number of chromosomal aberrations; however, it did not change the number of phases. We found that EOv presents antimicrobial activity against different Gram (−) and (+) strains, measured by disc-diffusion test and confirmed with a more accurate method, the AutoCad software. We postulate that EOv presents antibacterial, antioxidant and chemopreventive properties and could be play an important role as bioprotector agent.

Keywords Antibacterial · Cancer · Chemopreventive · Lung inflammation · *Origanum vulgare* · Terpenes

Abbreviations

AMs	Alveolar macrophages
BALF	Bronchoalveolar lavage fluid
CDs	Conjugated dienes
EOs	Essential oils
EOv	Essential oil of *Origanum vulgare*
PMNs	Polymorphonuclear neutrophils
TNF-α	Tumor necrosis factor-α

Introduction

During the last decade, the essential oils (EOs) have received special attention as source of potentially useful bioactive

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E. Grondona · A. A. Ponce
Cátedra de Fisiología Humana, Facultad de Ciencias Médicas, Universidad Nacional de Córdoba, Córdoba, Argentina

G. Gatti · L. R. Sánchez · V. Rivero
Centro de Investigaciones en Bioquímica Clínica e Inmunología, Dpto. de Bioquímica Clínica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Córdoba, Argentina

G. Gatti
Fundación para el Progreso de la Medicina, Córdoba, Argentina

A. G. López
Cátedra de Microbiología General y de los Alimentos, (IMBIV-CONICET), Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, Córdoba, Argentina

O. Pessah
Cátedra de Microbiología y Virología Médicas. Facultad de Ciencias Médicas, Universidad Nacional de Córdoba, Córdoba, Argentina

M. P. Zunino
Cátedra de Química Orgánica y Productos Naturales (IMBIV-CONICET), Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, Córdoba, Argentina

A. A. Ponce (✉)
Cátedra de Fisiología Humana, Dpto. Académico de Ciencias de la Salud y Educación, Universidad Nacional de la Rioja, La Rioja, Argentina
e-mail: andresaponce@daad-alumni.de

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compounds in food manufacturing (as flavoring and preservatives), the pharmaceutical industry (due to their therapeutic action), and in human therapy, due to their antioxidants and anti-inflammatory properties [1]. *Origanum vulgare* L. species, known as "oregano", belongs to the Lamiaceae family and is distributed in Europe, Mediterranean Basin and Asia. We consider that the importance of the oil of *Origanum vulgare* (EOv) is based on its economic profit [2]. In addition to its antimicrobial ability, *Origanum* has demonstrated important antioxidant, anti-inflammatory, antifungal, phytotoxic and insecticidal properties in *in vivo* and *in vitro* models [3–5].

This study was designed to elucidate the effects of different concentrations of EOv on lung inflammation previously induced by intranasal administration of lipopolysaccharide (LPS), in addition to the local expression of TNF-α, lipid-conjugated dienes (CDs) and cellular migration in bronchoalveolar lavage fluid (BALF). Furthermore, we analyzed the effects of EOv on the chromosome morphology of *Allium* cell test, its antimicrobial activity and the *in vitro* viability on A549 human lung adenocarcinoma ephithelial cell line.

Material and Methods

Collection of Plant Material and Essential Oil Extraction The plant material consisted of flowered tops and stalks (15–20 cm) of *Origanum vulgare* L. ssp. *hirtum* collected in Córdoba, Argentina. Samples of at least 200 g dried leaves were steam distilled in triplicate for 1 h using a Clevenger-type apparatus. The EOv was dried over anhydrous sodium sulfate and stored at −20 °C until further analysis. The components of the essential oils were identified by comparison of their mass spectra and retention indices with those published in the literature and presented in the MS computer library (Online Resource 1, Appendix 1) [6, 7].

Animal Preparation Adult male Swiss albino mice (25–30 g) were randomly distributed in 10 groups and placed in standard polycarbonate cages (30×20×15 cm, six animals per group). The animals were housed in appropriate facilities and kept at 24–26 °C with 55–75 % humidity and a 12/12 h light/dark cycle with continuous access to standard food and water *ad libitum*.

Anatomical and Histological Analyses The relative organ weight [(organ weight/body weight) 100] was calculated for the lung disease; animals were sacrificed and lungs were dissected and fixed in Bouin's fluid for 12 h. Haematoxylin and eosin were used as standard staining method. BALF was obtained for PMN and macrophage quantification as described by Bigliani *et al.* and Roque *et al.* [7, 8].

Study protocol and mice instillation BALF samples were subjected to the following treatments: Vehicle: instilled only with phosphate buffered saline solution (PBS: 140 mM NaCl; 70 mM NaH_2PO_4; 3 mM KCl; 1.5 mM KH_2PO_4 at 37 °C) or only with one concentration of the EOv group during 3 h (EOv groups: EOv 1, EOv 50 and EOv 300 mg/kg; IP). Remaining procedures started with the administration of LPS followed by the different treatments: LPS (lipopolysaccharide of *Pseudomonas aeruginosa,* 100 μg LPS/kg of mouse body mass; serotype 10, ATCC27316, N L9147, Sigma Aldrich, USA) during 2 h and afterwards, a single dose of a specific EOv concentration or Dexamethasone (DX, 2.5 mg/kg; IP) for 3 h. Glucocorticoids similar to DX, widely used as anti-inflammatory agents for several inflammatory lung diseases, as well as DX were used as positive controls, dissolved in PBS [9]. Animals were anesthetized according to the guidelines of Bigliani *et al.* [7].

Detection of Lipid Peroxidation Products and TNF-α in BALF TNF-α in BALF was quantified by ELISA protocol according to the manufacturer's instructions (BD Biosciences). The detection and quantification of CDs (early oxidation products) from lipid oxidation was performed following the procedure by Ogura *et al.* [10].

Cytogenetic Parameters We analyzed the effects of EOv on the mitotic index (MI). MI was defined as the number of cells in mitosis compared to the total counted cells (1,000 cells/treatment). Healthy and equal-sized bulbs of *Allium cepa* L. (onion) were used for cytogenetic experiments. Sprouted onion bulbs were exposed to distilled water for 48 h. The bulbs were exposed to the highest concentration tested of EOv oil (300 mg/kg); water was used as negative control and methanol (40 %) as positive control [11, 12].

Bacterial Strains and Screening of EOv Antimicrobial Activity Using Disk Diffusion Technique

Disk diffusion method was used to assay the antibacterial effects of EOv on Muller-Hinton agar plates. Susceptibility testing for eight bacterial strains was appraised as inhibition zones surrounding the wells. The bacterial strains were American Type Culture Collection Maryland, USA: Gram-positive: *Staphylococcus aureus* (ATCC25923), *Streptococcus faecalis* (ATCC 29212), *Streptococcus pyogenes, Listeria monocytogenes*; Gram-negative: *Escherichia coli* (ATCC 25922), *Salmonella* spp. and *Shigella* spp. The zones of inhibition around each disc were obtained with a digital camera and the pictures were copied on the software AutoCAD® 2013 (Autodesk, Inc., USA). The diameter of the halos of the inhibition zones were measured in the photographs with different 20 aligned points in scale 1:1.

The strains that developed inhibition zone were considered susceptible to EOv and those without such a zone were considered resistant. Each inoculum was prepared in the same medium with density values adjusted to a 0.5 McFarland turbidity standard [10^8 colony-forming units (CFU)/ml]. All experiments were performed in duplicate. According to Valeriano *et al.* [13], the antibacterial activity of the EOv was expressed in arbitrary units per mL (AU mL^{-1}). Values were calculated using the following formula: AU mL^{-1}=mean diameter (mm)×dilution factor×50.

Statistical Analysis

The normal distribution of the data was confirmed with Kolmogorov-Smirnov test. The statistical significance of the differences between treatment subjects and vehicle was determined by factorial analysis of variance (ANOVA) followed by Duncan's multiple-range test. Differences were considered statistically significant when p values were lower than 0.05. Calculations were performed with Info-Stat software (Córdoba, Argentina, 2014).

Results and Discussion

The EOv has more than 200 components grouped into two main fractions: a volatile fraction, which constitutes 90–95 % of the total oil and is responsible for its typical aroma and a non-volatile fraction (5–10 %). EOv is constituted by substances of different chemical nature: aliphatic hydrocarbons and their oxygenated analogs of low molecular weight (alkanes, alcohols, aldehydes, ketones, esters and acids), monoterpenes and sesquiterpenes, among others.

The terpenes of *O. vulgare* found in our profile were different than those described by other authors, which designate carvacrol and thymol as the predominant compounds, supporting that their main attributes are the bio-efficacy properties (Online Resource 1, Appendix 1) [19]. The composition of the oil of *Origanum* presents great variability, not only because of the existence of different subspecies, but also because of many other parameters that can vary mainly due to environmental and climatic conditions. Among these, several factors like altitude, climatic conditions, stage of growth, influence of fertilization, time of collection, packaging processes and environmental situations can in turn affect the biological properties of the oils [6].

Macroscopic Evaluation, Relative Organ Weight and Clinical Observations Non-macroscopic changes were observed in lung tissues of the animals that received a single dose of EOv. Moreover, we did not detect any changes in the ambulation or clinical status of these animals while they were under the effects of LPS, EOv alone or a combination of both. Also, Bukovska *et al.* [14] reported that mice treated with *O. vulgare* recovered their corporal weight after treatment with oregano, which suggests that this species can have direct effects on the adipocytes. Arcila-Lozano *et al.* [15] presented a clinical study demonstrating that *Oregano* spp. induces allergenicity and that the excessive consumption of *O. vulgare* and *O. majorama* during pregnancy is not advisable because of its abortive properties.

O. vulgare did not modify the relative organ weight of the lungs of mice that received different treatments when compared to vehicle group or between groups (Table 1).

Effects of EOv on BALF-Cells The administration of LPS induced a marked inflammatory response and triggered the release of the granulocyte-macrophage colony-stimulating factor (GM-CSF) in monocytes, endothelial cells, AMs and PMNs. In agreement with this idea, LPS obtained from BALF exhibited strong chemotactic activity (Table 2). PMNs are recruited into the lungs, releasing proinflammatory cytokines, including interleukins and TNF-α, which are present in the airways and lung compartments. EOv of *O. vulgare* can change the resident cells in mice airways after a 3 h-treatment, depending on the degree of cellular activation.

According to data previously described, the viability of the total cells and the percentage of PMNs from BALF rose after a 3 h-treatment with any EOv concentration in a dose-dependent manner. In this study, LPS was used as reference

Table 1 Effects of different doses (i.p.) of essential oil of *Origanum vulgare* (EOv) on LW/BW (lung weight/body weight) in adult male Albino Swiss mice

Groups	n	LW/BW
Basal	6	1.04±0.042
Vehicle	5	1.16±0.071
EOv 1	6	1.11±0.034
EOv 50	6	1.11±0.034
EOv 300	6	1.07±0.041
LPS	6	1.07±0.024
LPS+DX	6	0.98±0.06
LPS+EOv 1	6	1.12±0.053
LPS+EOv 50	6	1.17±0.035
LPS+EOv 300	6	1.07±0.032

The total body weight of all animals was recorded before slaughter and later on, at the same that lung weight was measured. Basal: untreated animals; Control: animals instilled (in.) with PBS; LPS: instilled (in.) with *Pseudomonas aeruginosa* lipopolysaccharide (LPS, 60 μl solution of 1.67 μg/ ml); LPS+DX: instilled (in.) and subsequently treated with LPS (i.p.) DX (2.5 mg/kg); LPS+EOv: instilled animals (in.) with LPS (60 μl solution of 1.67 mg/ml) and then treated (i.p.) with EOv (1, 50 and 300 mg/kg); EOv: treated animals (i.p.) EOv with different concentrations (1, 50 and 300 mg/Kg). n: number of animals. Data are expressed as mean±SD

Table 2 Percentage of total viable cells (TVCs), polymorphonuclear neutrophils (PMNs) and alveolar macrophages (AMs) in BALF from LPS-treated mice

Treatment	% of cells in BALF		
	TVCs	PMNs	AMs
Control	83.94±2.14 ^{a,b,c,d}	0.28±0.28 ^{a}	99.72±0.28 ^{a,b}
Vehicle	89.15±4.38^{d}	3.42±1.22 ^{a,b}	96.58±1.22 ^{a}
EOv 1	66.74±8.76 ^{a}	83.74±2.45 ^{c,d,e}	16.26±2.45 ^{c,d,e}
EOv 50	69.17±7.55 ^{a}	87.22±1.16^{d,e}	12.78±1.16 ^{d,e}
EOv 300	84.55±2.63 ^{b,c,d}	89.70±1.42^{d,e}	10.30±1.42 ^{d,e}
LPS	79.29±4.19^{a,b,c}	78.80±4.81^{c,d}	21.20±4.8 ^{c,d}
LPS+DX	87.90±3.15 ^{c,d}	24.03±4.68 ^{a,b,c}	75.97±4.6 ^{a,b,c}
LPS+EOv 1	78.00±3.37 ^{a,b,d}	57.05±17.81 ^{b,c,d}	42.95±3.53 ^{b,c,d}
LPS+EOv 50	86.41±1.66 ^{b,c,d}	91.91±1.09 ^{e}	8.09±1.09^{e}
LPS+EOv 300	79.35±6.59 ^{a,b,c,d}	82.81±5.68^{d,e}	17.19±5.68 ^{d,e}

EOv: Essential oil of *Origanum vulgare*. Means followed by the different letters within the same column are significantly different, or means with the same letters within the same column indicate no significant differences. Mean±SEM; number of animals 5–6 animals per group

agent for the release of TNF-α, and PMN were recruited by the lungs in a concentration-dependent manner, as previously described by our team [7, 8]. However, to our surprise, in the group treated with LPS and subsequent EOv, the total number of cells did not show the same profile that the EOv group previously described.

In the analysis of stimulation by LPS, its action after 3 h-treatments with EOv at different concentrations lacked the ability to sustain AMs levels, compared to Control subjects, and in accordance with Stelter *et al.* [16], it did not modulate the acute inflammatory response induced by LPS stimulation. This occurs because these cells are present in the alveoli in high proportions, which probably reflects the activation of the AMs into foam cells as response to acute inflammation. This phenomenon can be attributed to the spread of the essential oils throughout the body; these, after reaching the alveolar capillaries, induce a non-specific response, probably in a dose-dependent manner. All of this is provoked by the EOv components, which in turn, produce an increase of PMNs and other cells.

In accordance with Koparal and Zeytinoglu [17] in our experiments, the essential oils of *O. vulgare* caused degeneration of cell morphology. Moreover, Ocaña-Fuentes *et al.* [18] reported that *in vitro* concentrations of 30 μg/mL of oregano are toxic for the macrophages.

Effects of EOv on TNF-α In agreement with other authors, the level of the proinflammatory cytokine TNF-α decreased with the administration of EOv after LPS induction, but the only dose that yielded significant values was 300 mg/Kg or DX upon stimulation by endotoxin LPS. In addition, the EOv groups showed a non-significant increase of TNF-α; however, this could be associated with a response to the increase of PMNs, since these phagocytic cells are crucial in specific and non-specific immune responses.

The terpenes found in our profile of *O. vulgare* were different than data described by other authors, which designate carvacrol and thymol as predominant compounds, supporting that their main attributes are their anti-inflammatory properties [19, 18, 20] (Online Resource 1, Appendix 1). However, in another study, the monoterpene hydrocarbons γ-terpinene and terpinen-ol showed a very high level of biological activity [21].

Effects of EOv Over Oxidative Stress Oxidative stress by free radicals is an important event that can produce cellular aging and in consequence, human degenerative diseases, such as arthritis, asthma, chronic obstructive pulmonary disease (COPD), Parkinson's disease, Alzheimer's disease, viral, bacterial and protozoan infections, in addition to cancer. Numerous studies have demonstrated the antioxidant properties of the natural products [22]. This ability confers a therapeutic potential based on its antinociceptive and anti-inflammatory properties, as well as other less characterized attributes, like their anti-cancer activity. In addition, it is important to remark their nutraceuticals properties, which make them attractive to the food industry, prompting their use as replacement for synthetic antioxidants and playing a role in preventing several diseases [23]. The balance between the ratio of oxidants/antioxidants in the pathogenesis of lung diseases has achieved special relevance [24]. It should be noticed that in mice lungs, the ratio oxidation-antioxidation is lower than in other tissues, but in other small animals with faster metabolism and rate of inflammation progress, the susceptibility to oxidative stress is greater [25].

The EOv could prevent oxidative damage by physiological mechanisms similar to the endogenous antioxidant system, inhibiting or preventing the generation of free radicals, enhancing the activity of antioxidant enzymes or by blocking the amplification of the oxidative damage [26]. However, free radicals and other reactive species produce oxidation of biomolecules (*e.g.*, protein, amino acids, lipids, and DNA), which in turn leads to cell injury and death. When analyzing the CDs of BALF from mice lungs, LPS demonstrated to significantly decrease the obligatory passage of conjugated dienes lipid hydroperoxides to peroxyl radicals (isoprostanes and decomposition products: *e.g.*, malondialdehyde) but not to the CDs pathway [27]. Shokrzadeh *et al.* [28] showed that *O. vulgare* protects lung tissues from cyclophosphamide -induced pulmonary damage and suggest a role for oxidative stress. In this context, the administration of EOv 1, 50 or 300 of *O. vulgare* after LPS induction, attenuated the progress to malondialdehyde that occurs during oxidative stress in a dose-dependent manner (Table 3). It is suggested that these

Table 3 Conjugated dienes (CDs) and TNF-α concentration in BALF from LPS-treated mice

Treatment	BALF	
	CDs (μM/mL)	TNF-α (pg/mL)
Control	0.13±0.06 [a,b]	ND [a]
Vehicle	0.05±0.01[a]	159.01±26.51[a,b]
EOv 1	0.08±0.02[a,b]	86.04±61.71[a, b]
EOv 50	0.13±0.03[b]	152.67±30.41[a,b]
EOv 300	0.15±0.03 [b]	307.28±73.30[a,c]
LPS	0.05±0.006 [a,b]	1368.28±300.16 [c,d]
LPS+DX	0.04±0.005[a]	25.53±11.99 [a]
LPS+EOv1	0.08±0.006 [b,c,d]	1512.33±62.93[d]
LPS+EOv 50	0.12±0.01 [c,d]	1129.00±204.10[c,d]
LPS+EOv 300	0.24±0.12 [c]	454.33±100.41[a,b,e]

EOv: Essential oil of *Origanum vulgare*. ND: Not detected. Means followed by the different letters within the same column are significantly different or means with the same letters within the same column indicate no significant differences. Mean±SEM; number of animals 5–6 animals per group

antioxidant effects could be related to the functional anti-inflammatory activities of the EOv. However, the precise mechanism of the EOv on the oxidative stress in BALF remains unknown.

Allium cepa Root-tip Cell Test and Viability of in vitro Cultured A549 Cells The *Allium cepa* test is a good direct method to quantify the damage of systems exposed to potential mutagens or carcinogens. As shown in Table 4, a decrease of the MI induces an increase of the cells in prophase compared to other mitotic phases. Ipek *et al.* [29] have reported that the EOv have the property of inhibiting the separation of sister chromatids and DNA synthesis in cultured cells. According to our results and data from Savini *et al.* [30], EOv decreased the MI and significantly reduced the number of aberrations but did not modify the number of phases (Table 4).

These results suggest that the EOv modifies the onset of mitosis, possibly prior to the G2 phase and prophase. Overall prophase arrest is associated with errors in DNA repair or inhibition of the arrangement of the mitotic spindle [31]. It has been demonstrated that the extract of Caco-2 tumor cells EOv promotes G2 arrest and induction of apoptosis [30]. EOv showed potential cytotoxic effects at the analyzed concentrations. Non-viable cells were observed when cancer cell lines were incubated (Online Resource 2, Appendix 2) demonstrating that EOv significantly decreased the proliferation of A549 cells after 24 h incubation, compared with untreated control cells ($p<0.05$). This result is in agreement with findings in cultures of human melanoma cells exposed to EOv, in which a strong apoptotic activity was noted when we assessed cellular viability with MTT (Online Resource 2, Appendix 2). The carvacrol, present in low percentages in EOv, has been described as the major responsible for the important *in vitro* cytotoxic activity against tumor cells [32]. Also, Mezzoug *et al.* [33] have described the *Origanum compactum* essential oil and its antimutagenic effect. Moreover, *Origanum rotundifolium* essential oil suppressed the mutagenic effects of Aflatoxin B1 [34]. However, further investigations should be performed to clarify its cytotoxic and genotoxic effect, as well as its role in apoptosis.

Antibacterial Activity of EOv The drugs with a natural origin should be more accurately analyzed during the research process; therefore, new methods are necessary to verify the activity of natural compounds, as well as their antibacterial action.

The EOv have demonstrated to possess a broad and strong spectrum of antimicrobial activity when studied with disc-diffusion method (containing mainly 53.2 % of hydrocarbonated compounds) (Online Resource 1, Appendix 1); therefore, we concluded that the bioactivity effects of the EOs may be performed through the cytoplasmic membrane, by disorganizing the structure of different proteins, fatty acids, phospholipids and polysaccharides, and sealing them [35]. Furthermore, the antimicrobial mechanism of EOv may not

Table 4 Mitotic index and chromosome aberrations in the root tip meristem cells of *Allium cepa* after treatment with essential oils of *Origanum vulgare* (EOv)

Test substance	Mitotic index	Chromosomal aberrations*	Phase index (PI) **			
			PI-Prophase	PI-Metaphase	PI-Anaphase	PI-Telophase
H_2O	3.4±0.84[b]	0.45	0.21	0.21	0.31	0.28
EOv	2.25±1.28 [a,b]	0.49	0.33	0.29	0.22	0.16
Metanol	1.7±0.82 [a]	2.45	0.76	0.00	0.13	0.11

*Chromosomal aberrations (number of aberrations/number of mitotic cells)

**Phase ratio (number of cells in each phase/number of cells in mitosis)

[a,b] Values for each parameter in the same row, followed by the same letter, are not significantly different ($p<0.05$)

only be attributable to one signal and/or more than one terpene, but rather produced by several signals produced by different terpenes found in the EOv.

All the bacterial strains demonstrated to have some degree of sensitivity to EOv, except the uropathogenic *Escherichia coli* (1:100–1000). In general, Gram-positive bacteria demonstrated to be more susceptible to EOv than Gram-negative strains. Maximum activity was observed against Gram-positive *S. aureus* and Gram-negative *Shigella*.

In our study, we used AutoCad software to measure the inhibition halos, by integrating AutoCAD images with digital photos obtained in the diffusion test experiments. This new procedure allowed a more precise and easy measurement of the inhibition halos, especially in cases of heterogeneous growth, defined as the occurrence of small colonies in the circular growth inhibition area. This process could improve the interpretation of the results with the addition of the antimicrobial activity of these agents and the test results should be standardized to allow a better classification of the isolates as susceptible, intermediate (or exhibiting decreased susceptibility), or resistant to antimicrobial drugs (Table 5).

Conclusion

It is estimated that between 30 and 50 % of the manufactured drugs have a natural origin; therefore, there is an improved interest in finding new natural agents for several chronic illnesses using new bioactive compounds, like terpenes. This occurs because current medicinal therapies for prolonged diseases have undesirable side effects. In addition, the major compounds found in our essential oil do not necessarily reflect their biological actions, since it is possible that the activity of the main components could be modulated by other minor components with putative synergism, antagonism or zero-interaction with the terpenes present in the EOv.

Plant essential oils continue to be the focus of attention in terms of their nutritional bioactivity and therefore, on human health. In our study, oregano oil was evaluated for its bio-efficacy over different types of cells. *Origanum vulgare* oil has strong anti-bacterial, antioxidant and chemopreventive properties activity. Its bioprotector effect might be related to the presence of some major components such as γ-terpinene, terpinen-4-ol, and trans-sabinene hydrate, which are responsible for its biological efficacy. The mechanism of action of EOv is supported by the fact that its terpenes can interact with the cells and microorganisms by different mechanisms, such as increasing the rate of membrane permeability for ions and protons, generating a loss of the structural integrity of the lipid bilayer of the cell membrane and interfering with the lipophilic compounds [35]. Moreover, the EOv could have synergistic effects when associated with other compounds, through the cellular membranes in an easy and efficient manner, and eventually induce biological response.

Table 5 Antimicrobial activity of the essential oil of *Origanum vulgare* (EOv) against bacterial strains, determined by disc diffusion method, measured with AutoCad® 13 software

Bacterial strains	Antimicrobial activity (AU ml^{-1})		
	Dilution of the EOv		
	1/10	1/100	1/1000
Gram-positive			
Staphylococcus aureus	91.20 ± 1.31^{a}	10.47 ± 1.28^{b}	1.29 ± 0.15^{c}
Enterococcus faecalis	69.18 ± 1.20^{a}	7.76 ± 1.74^{b}	2.24 ± 1.4^{b}
Listeria monocytogenes	69.18 ± 1.32^{a}	4.68 ± 1.66^{b}	2.57 ± 1.5^{c}
Streptococcus pyogenes	19.49 ± 1.78^{a}	1.86 ± 1.4^{b}	0.37 ± 0.04^{c}
Gram-negative			
Shigella spp.	81.28 ± 1.62^{a}	12.58 ± 1.23^{b}	1.12 ± 0.03^{c}
Escherichia coli	64.56 ± 1.26^{a}	1.12 ± 0.02^{b}	0.12 ± 0.02^{c}
Salmonella spp.	13.80 ± 1.44^{a}	0.67 ± 0.07^{b}	$0,07\pm0.03^{c}$
uropathogenic *E. coli*	12.02 ± 1.26^{a}	-	-

Values in a different column with different superscript letters are significantly different ($p\leq0.05$). Mean values±standard deviation. The antibacterial activity of the EOv expressed in arbitrary units per ml (AU ml^{-1}) was calculated using the following formula: activity (AU ml^{-1})=mean diameter (mm) of the minimal inhibition zone x dilution factor×50

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Conflict of Interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could constitute as a potential conflict of interest.

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