Journal of Herbal Medicine 39 (2023) 100651

Contents lists available at ScienceDirect

Journal of Herbal Medicine

journal homepage: www.elsevier.com/locate/hermed

Essential oil of *Origanum minutiflorum* exhibits anti-inflammatory and antioxidative effects in human bronchial cells and antimicrobial activity on lung pathogens

Katarina Veljovic [a], Vele Tesevic [b], Hristina Mitrovic [c], Marija Stankovic [c,*]

[a] *Laboratory for Molecular Microbiology, Group for Probiotics and Microbiota-Host Interaction, Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Belgrade, Republic of Serbia*
[b] *University of Belgrade-Faculty of Chemistry, Belgrade, Republic of Serbia*
[c] *Laboratory for Molecular Biology, Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Belgrade, Republic of Serbia*

ARTICLE INFO

Keywords:
Origanum minutiflorum essential oil
Bronchial epithelial cells
Anti-inflammatory
Antioxidative
Antibacterial

ABSTRACT

Introduction: Oregano essential oil (OEO) is one of the most widely used essential oils worldwide due to its huge therapeutic benefits. Nevertheless, data on the effects of the endemic species *Origanum minutiflorum*, also known as wild or Turkish oregano, is scarce. On the other hand, various chronic lung diseases, characterised by persistent inflammation, oxidative stress, and common bacterial infections, do not have effective pharmacological therapy. Hence, the aim of this study is to examine the effects of wild oregano essential oil (WOEO) on human bronchial epithelial cells and lung pathogens.

Methods: We provided a detailed chemical composition of WOEO using GC-MS and GC-FID analysis. Anti-inflammatory effects of WOEO were analysed using the qRT-PCR and ELISA methods, while antioxidative properties were examined by using the dichlorofluorescein assay in BEAS-2B cells. Antibacterial activity was tested on lung pathogens by using the agar diffusion assay.

Results: The major constituents of WOEO, analysed in this study, were carvacrol, linalool, *p*-cymene, γ-terpinene, and (*E*)-caryophyllene. We found that treatment with WOEO attenuated LPS-induced *IL8* gene expression and hydrogen peroxide-induced oxidative stress in BEAS-2B cells. Moreover, WOEO showed an inhibitory effect on pathogenic bacteria *Acinetobacter baumannii* and highly resistant *Klebsiella pneumoniae* commonly seen in healthcare-associated pneumonia.

Conclusion: Our work presents new insights into the anti-inflammatory, antioxidative, and antimicrobial properties of WOEO which may be used as a simple and local treatment in various chronic lung diseases.

1. Introduction

The genus *Origanum* encompasses important medicinal and aromatic plants which have been used over the years for their exclusive culinary and therapeutic properties. *Origanum* species is widely distributed around the Mediterranean, North African, Euro-Siberian, and Iran-Siberian regions (Ali et al., 2020; Bayramoglu et al., 2008). *Origanum* plants are extensively used in everyday life as a well-known spice for cooking, but they are also exploited in the food industry and used in alcoholic beverages and soft drink aromas (Sharifi-Rad et al., 2021).

Origanum species have been traditionally used as a medicinal plant worldwide. The most commonly studied oregano species is *Origanum vulgare* L. Numerous studies of oregano essential oils (OEO), deriving from various *Origanum* species, have shown its therapeutic effects *in vitro* and *in vivo* (Cheng et al., 2018; Zou et al., 2016; Han et al., 2017). OEO is an appreciated component in modern phytotherapy due to its antimicrobial, anti-inflammatory, antioxidant, antitumor, antimutagenic, and hepatoprotective properties (Lombrea et al., 2020; Sharifi-Rad et al., 2021). The ethnopharmacological uses of OEO comprise treatment of digestive, respiratory, and dermatological disorders (Lombrea et al., 2020). Essential oils of *Origanum* sp. have been reported to contain carvacrol, linalool, borneol, terpinen-4-ol, thymol, *p*-cymene, and γ-terpinene (Baser, 2002; Demirci et al., 2004; Ozel and Kaymaz, 2004). OEO has potent biological activities, which are primarily attributed to

* Correspondence to: Laboratory for Molecular Biology, Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Vojvode Stepe 444a, 11042 Belgrade 152, Republic of Serbia.
E-mail address: marijast@imgge.bg.ac.rs (M. Stankovic).

https://doi.org/10.1016/j.hermed.2023.100651
Received 10 January 2022; Received in revised form 3 March 2023; Accepted 23 March 2023
Available online 28 March 2023
2210-8033/© 2023 Elsevier GmbH. All rights reserved.

the presence of carvacrol, thymol, and monoterpenes (Sharifi-Rad et al., 2021). It has been suggested that OEO exerts its effects by modulating signalling pathways involved in inflammation, tissue remodelling, and cancer. Previous studies have demonstrated anticancer properties of OEO by reducing tissue remodelling biomarkers, cell proliferation, cell migration and colony formation, and by inducing apoptosis. A number of studies have reported the anti-inflammatory and antioxidative properties of OEO, including the reduction of proinflammatory cytokine and chemokine levels, inhibition of mitogen-activated protein kinases (MAPK) and reactive oxygen species (ROS) production, and an increase of superoxide dismutase and catalase activity (Sharifi-Rad et al., 2021). The antimicrobial activities of carvacrol and thymol, as the most abundant components of oregano oil, have been widely studied (Rostro-Alanis et al., 2019; Barbosa et al., 2020). Specifically, carvacrol has extensively been tested as an antimicrobial agent in food for the control of Gram-positive and Gram-negative pathogens, such as *Bacillus cereus*, *Enterococcus faecalis*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli* O157:H7, *Pseudomonas fluorescens, Salmonella typhimurium*, and so-forth (Magi et al., 2015). The antibacterial effects of carvacrol are mainly based on its ability to disrupt bacterial membrane (Magi et al., 2015). Carvacrol easily penetrates through the cell membrane of bacteria, leading to disruption of cell membrane integrity and the release of bacterial cell content (Khan et al., 2017). Chauhan and Kang (2014) showed that thymol, an isomer of carvacrol, was able to disrupt bacterial plasma membrane and cause the release of cellular content. However, the effects of OEO from various species vary due to differences in the chemical composition of the essential oil, which is influenced by numerous factors, such as environmental and climatic conditions (Alkan, 2020). Carvacrol is the major component in the essential oils of *O. onites* (57.0 %), *O. minutiflorum* (52.0 %), and *O. vulgare* (59.9 %). However, carvacrol was not the dominant compound in the essential oil of *O. syriacum* (3.2 %), and it was not detected in the essential oil of *O. majorana* (Arslan and Dervis, 2010).

O. minutiflorum O.Schwarz & P.H.Davis is an endemic species, also known as wild or Turkish oregano, with scarcely explored biological effects. Studies on *O. minutiflorum* have shown its antioxidative effects in cell-free systems, and antibacterial, insecticidal, and anticancer effects *in vitro* (Elmastas et al., 2018; Oke and Aslim, 2010; Sokmen et al., 2020; Dorman et al., 2004; Arserim et al., 2021; Evrendilek, 2015; Cetin et al., 2009; Cetin and Yanikoglu, 2006). The oil content of wild oregano has been investigated by different authors and most studies have demonstrated that carvacrol was found to be the major constituent (42–84 %). The other major constituents were *p*-cymene, *γ*-terpinene, borneol, with their quantities raging between 4.3–8.1 %, 1.7–5.1 %, 0.3–5.3 %, respectively (Kirimer et al., 1995; Dadalioğlu and Evrendilek, 2004; Spyridopoulou et al., 2019).

Various chronic lung diseases such as chronic obstructive pulmonary disease (COPD), asthma, cystic fibrosis, and pulmonary fibrosis, although different have some common features. These include activated immune and structural cells that produce pro-inflammatory mediators and oxidative stress leading to excessive and self-perpetuating airway inflammation and tissue destruction (Grondona et al., 2014; Yang et al., 2018; Roesch et al., 2018; Desai et al., 2018). Increased oxidative stress causes chronic lung inflammation, disease progression, and acute exacerbations, which are associated with significant morbidity and mortality (Barnes, 2020a, 2020b). In addition, common bacterial colonization of the lower respiratory tract in patients with chronic lung diseases is responsible for a lung inflammatory response leading to exacerbation of the disease (Grondona et al., 2014). Currently, there are no effective disease modifying therapies for many chronic lung diseases and there is an urgent need for the development of novel approaches, which concurrently target inflammation, oxidative stress, and bacterial infection in the lungs (Barnes, 2013; Cantin et al., 2015; Barnes, 2020a, 2020b).

In this study, the chemical composition of commercially available wild oregano essential oil (WOEO), *O. minutiflorum* (Probotanic), was determined by gas chromatography-mass spectrometry (GC-MS) and gas chromatography-flame ionization detector (GC-FID) analysis. Anti-inflammatory effects of WOEO were tested in human bronchial epithelial cells by measuring the expression and production of interleukin 8 (IL8)/chemokine 8 (CXCL8), as one of the primary pro-inflammatory chemokines implicated in inflammation and acute exacerbation in chronic inflammatory lung diseases (Barnes et al., 2003). Moreover, the antioxidative properties of WOEO were analysed by measuring the level of intracellular oxidative stress in human bronchial epithelial cells. Also, the antibacterial effects of WOEO were tested on two lung pathogenic bacteria, *Acinetobacter baumannii* and *Klebsiella pneumoniae*.

2. Material and methods

2.1. WOEO

The WOEO (930 μg/μL) obtained from Probotanic (Republic of Serbia) is commercially available pure organic oil intended for oral consumption. The composition of WOEO is determined by GC-MS, as shown in Table 1. The WOEO tested on BEAS-2B cells was freshly and completely solubilized in dimethyl sulfoxide (DMSO, Sigma-Aldrich).

2.2. GC-MS and GC-FID analysis

Analysis was carried out with an Agilent 7890 A apparatus equipped with a 5975 C MSD, FID, and a HP-5MS fused-silica capillary column (30 m × 0.25 mm, film thickness 0.25 μm). The carrier gas was helium (1 mL min^{-1} at 210 °C). The injector temperature was 250 °C, with an injection volume of 1 μL, split ratio, 10:1. MS detection was carried out under source temperature conditions of 230 °C and an interface temperature of 315 °C. The EI mode was set at electron energy, 70 eV with mass scan range of 40–550 amu. The temperature was programmed from 60 °C to 300 °C at a rate of 3 °C/minute. Components were identified based on their linear retention index relative to C_8-C_{32} n-alkanes and comparisons with data reported in literature (Adams4 and NIST17 databases). The relative abundance of compounds (Table 1) was

Table 1
Analysis of WOEO by GC-MS and GC-FID.

Compound	RI[a]	RI[b]	%
α-Thujene	924	924	0.2
α-Pinene	931	932	0.4
Camphene	945	946	0.3
β-Pinene	974	974	0.1
Myrcene	988	988	0.7
α-Phellandrene	1003	1002	0.1
α-Terpinene	1015	1014	0.7
p-Cymene	1022	1020	3.5
β-Phellandrene	1026	1025	0.2
1,8-Cineole	1033	1026	0.2
γ-Terpinene	1055	1054	2.4
(*Z*)-Sabinene hydrate	1069	1065	0.4
α-Terpinolene	1086	1086	0.1
Linalool	1103	1095	4.6
Borneol	1167	1165	1.2
Terpinen-4-ol	1178	1174	0.5
α-Terpineol	1193	1186	0.2
Thymol	1294	1289	1.1
Carvacrol	1308	1298	78.2
(*E*)-Caryophyllene	1419	1417	1.5
Aromadendrene	1439	1439	0.2
α-Humulene	1454	1452	0.1
β-Bisabolene	1510	1505	1.1
δ-Cadinene	1525	1522	0.1
Spathulenol	1581	1577	0.1
Caryophyllene oxide	1588	1582	0.2
Total			98.4

[a] Experimental retention index
[b] Literature-reported retention index (Adams, 2007)

calculated from signal intensities in the GC-FID traces.

2.3. BEAS-2B cell culture

A normal human bronchial epithelial cell line BEAS-2B (ATCC CRL-9609) was cultured in RPMI medium supplemented with 10 % fetal bovine serum (FBS, Gibco), 100 μg/mL streptomycin and 100 U/mL penicillin (Sigma) at 37 °C and 5 % CO_2. The cells were maintained up to 90 % confluency. All experiments were performed at least three times, independently.

2.4. Lactate dehydrogenase (LDH) release assay

Cellular cytotoxicity was determined by measuring the activity of LDH from the culture medium. The activity of LDH was measured spectrophotometrically using a CyQUANT LDH Cytotoxicity Assay Kit (Thermo Fisher), as per the manufacturer's instructions. Briefly, BEAS-2B cells were treated with WOEO 100–0.01 μg/mL, lipopolysaccharide (LPS, Sigma) 100 ng/mL, and hydrogen-peroxide (H_2O_2, Sigma) 300 μM and LDH activity was determined by measuring absorbance at 490 nm and 680 nm, using a plate reader (Infifnite Multimode Reader Tecan).

2.5. Quantitative real time-polymerase chain reaction (qRT-PCR) assay

BEAS-2B cells (1.3×10^5) were seeded and pre-treated the following day with DMSO < 0.04 % (positive control) or WOEO 10 μg/mL, for 30 min. Next, the cells were treated with LPS 100 ng/mL and DMSO < 0.04 % (positive control) or LPS 100 ng/mL and WOEO 10 μg/mL, for 23.5 h.

Total RNA was isolated from BEAS-2B cells using the RNeasy Plus Mini Kit (Qiagen) according to the manufacturer's instructions. RNA purity and concentration were determined by BioSpec-nano (Shimadzu Corporation).

cDNA was synthesized from 1 μg of total RNA using the High Capacity cDNA Reverse Transcription Kit (Thermo Fisher) as per manufacturer's instructions. TaqMan Gene Expression Assays (Thermo Fisher) for *IL8* and endogenous control, *glyceraldehyde 3-phosphate dehydrogenase* (*GAPDH*) gene, were used for RT-PCR *via* a 7500 Real-Time PCR system (Applied Biosystems) following the manufacturer's protocols. RT-PCR reactions were carried out in 10 μL, in duplicates. Relative quantification by ΔΔCT method was used to calculate *IL8* gene expression relative to a positive control (DMSO), which was used as a calibrator. Results were analysed using Applied Biosystems 7500 System Software.

2.6. Enzyme-linked immune sorbent (ELISA) assay

The supernatants of BEAS-2B cells pre-treated with DMSO < 0.04 % (positive control) or WOEO 10 μg/mL and treated with LPS 100 ng/mL and DMSO < 0.04 % (positive control) or LPS 100 ng/mL and WOEO 10 μg/mL were collected after 24 h and stored at − 20 °C until analysis. IL8 was quantified using a Human IL8/CXCL8 DuoSet ELISA kit (R&D systems), according to the manufacturer's instructions. The optical density of each sample was determined at 450 nm and 570 nm using a plate reader (Infifnite Multimode Reader Tecan).

2.7. Dichlorofluorescein (DCF) assay

Measuring the level of intracellular oxidative stress was performed in BEAS-2B cells treated with WOEO and hydrogen peroxide using a DCF assay, as previously described (Popović et al., 2019).

BEAS-2B cells, 5×10^3, were seeded in 96-well plate. The following day the cells were left non-treated (negative control), treated with DMSO < 0.04 % (positive control) or WOEO 10 μg/mL for 24 h. The cells were then incubated with 10 μM 2′,7′–dichlorofluorescin diacetate (DCFH-DA) for 30 min. After the cells were washed with PBS, a medium with 300 μM hydrogen-peroxide (Sigma) was added to the positive control sample and WOEO treated cells, whereby fluorescence was measured *via* a plate reader (Infifnite Multimode Reader Tecan) immediately after treatment administration. The fluorescence readings were performed every 5 min during 3 h, with excitation at 485 nm and emission at 530 nm.

The data deriving from the fluorescence reading was used for calculating the area under the curve (AUC). The AUC for each sample was subtracted for the AUC of the blank sample that represents cells treated with DCFH-DA and culture medium (negative control). The fluorescence was calculated relative to the positive control sample, namely the cells treated with DMSO and H_2O_2, which was taken as 100 %. Each experiment was performed in four replicates.

2.8. Agar diffusion assay (disc-variant)

Antimicrobial activity of WOEO was assessed against *Acinetobacter baumannii* ATCC 19606 and *Klebsiella pneumoniae* ATCC BAA2146 according to Valgas et al. (2007). The test was performed by pouring 7 mL of soft (0.7 %) LA agar into petri dishes with solid LA (Luria agar), approximately 10^5-10^6 cells of indicator strain per mL of medium. After spilling the indicator strains, sterile disks (Identification Discs, Abtek Biological) were arranged on the surface of the petri dishes. The antimicrobial activity of various concentrations of WOEO, from concentrated oil to 1 μg/μL (930, 500, 100, 50, 10, and 1 μg/μL), was tested. Serial dilutions were prepared with DMSO, which was also used as a negative control. A 5 μL volume of appropriate oil concentration was applied to the sterile discs. The cups were first incubated for 3 h at 4 °C to allow the discs to absorb the oil as well as possible, and then at 37 °C, aerobically, for 24 h.

2.9. Statistical analysis

The data is expressed as mean ± standard deviation. The statistical significance of the differences between treatment and control was examined using a Student's *t*-test. A *p*-value of less than 0.05 was considered significant. Statistical analysis was carried out and graphs were prepared by using GraphPad Prism 6 software.

3. Results

3.1. Analysis of WOEO by GC-MS and GC-FID

Twenty-seven constituents in the WOEO (*O. minutiflorum*) were determined, with the major components being 78.2 % carvacrol, 4.6 % linalool, 3.5 % *p*-cymene, 2.4 % γ-terpinene, and 1.5 % (*E*)-caryophyllene (Table 1).

3.2. Cytotoxicity

Treatment of BEAS-2B cells with WOEO of 10 μg/mL and lower concentrations and LPS 100 ng/mL for 24 h, as well as 300 μM hydrogen peroxide for 3 h, showed cytotoxicity of less than 5 %.

3.3. WOEO attenuated LPS-induced IL8 gene expression

The effect of WOEO on LPS induced inflammatory response was analysed in BEAS-2B cells treated with WOEO and LPS for 24 h. LPS-induced *IL8* gene expression was significantly decreased ($p = 0.0063$) by treatment with 10 μg/mL of WOEO, as shown in Fig. 1. The decrease of LPS-induced *IL8* gene expression was 40.2 % in comparison to the positive control sample (DMSO and LPS treatment).

3.4. IL8 secretion

The secretion of IL8 was analysed in a medium of BEAS-2B cells treated with WOEO and LPS for 24 h. In comparison to non-treated cells

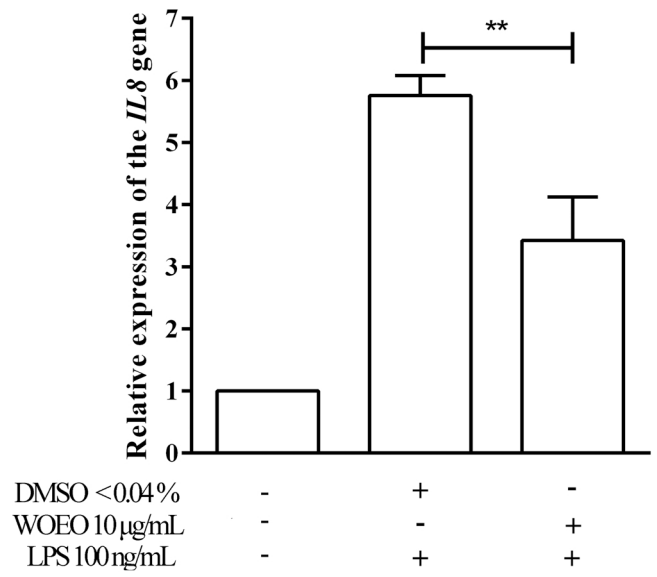

Fig. 1. WOEO decreases LPS-induced expression of the *IL8* gene in BEAS-2B cells. BEAS-2B were pre-treated with DMSO < 0.04 % or WOEO 10 µg/mL for 30 min and then treated with LPS 100 ng/mL and DMSO < 0.04 % (positive control) or LPS 100 ng/mL and WOEO 10 µg/mL respectively, for 23.5 h. Relative expression of the *IL8* gene was analysed by TaqMan gene expression assay using the qRT-PCR method and calculated by the ΔΔCT method. WOEO non-treated BEAS-2B cells were used as a calibrator. Data is expressed as mean ± standard deviation; N = 3; * * $p = 0.0063$.

(negative control), treatment of BEAS-2B cells with 100 ng/mL of LPS (positive control) showed significant induction of IL8 secretion (1046 ± 153 *versus* 1372 ± 135 pg/mL $p = 0.050$). However, there was no effect of WOEO 10 µg/mL on IL8 secretion in comparison to the positive control sample (1357 ± 59 *versus* 1372 ± 135 pg/mL), as shown in Fig. 2.

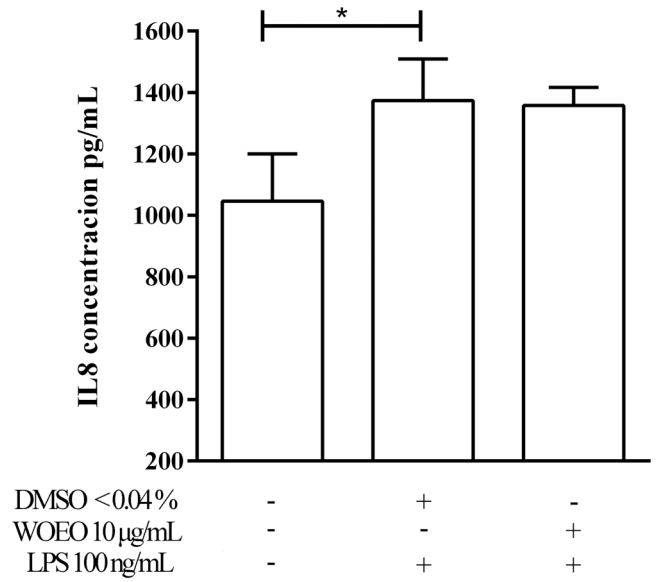

Fig. 2. WOEO did not affect LPS-induced secretion of IL8 in BEAS-2B cells. BEAS-2B were pre-treated with DMSO < 0.04 % or WOEO 10 µg/mL for 30 min and then treated with LPS 100 ng/mL and DMSO < 0.04 % (positive control) or LPS 100 ng/mL and WOEO 10 µg/mL respectively, for 23.5 h. Secretion of IL8 was measured *via* the ELISA method. Data is expressed as mean ± standard deviation; N = 3. Secretion of IL8 between non-treated BEAS-2B cells (negative control) and the positive control sample was significant; * $p = 0.050$.

3.5. WOEO decreased hydrogen peroxide-induced oxidative stress

The antioxidative property of WOEO was examined using a DCF assay that enables the detection of intracellular level of oxidative stress by measuring the kinetics of oxidized DCFH-DA fluorescence. The cells treated with WOEO had significantly lower level of oxidative stress than the control cells (DMSO) (Fig. 3). The level of oxidative stress, in cells treated with WOEO, was more than three times lower when the cells were exposed to 300 µM hydrogen peroxide (28.90 % *versus* 100.00 %, $p = 0.0002$).

3.6. Antimicrobial activity

The antimicrobial activity of WOEO was tested on two pathogenic bacteria that are capable of causing lung infections, *Acinetobacter baumannii* ATCC 19606 and *Klebsiella pneumoniae* ATCC BAA2146 (Fig. 4). The results showed that at a concentration of 930 µg/µL (1), 500 µg/µL (2), 100 µg/µL (3) and 50 µg/µL (4) WOEO had an inhibitory effect on both tested pathogenic bacteria due to the appearance of a bright inhibition around the disk where the test substance was applied. In addition, the application of WOEO at a concentration of 10 µg/µL (5) had a weak inhibitory effect on *Acinetobacter baumannii* and no effect on *Klebsiella pneumoniae*. WOEO did not show an inhibitory effect at a concentration of 1 µg/µL (6) on the tested bacteria. DMSO was used as a negative control (7) (Fig. 4).

4. Discussion

Chronic inflammatory lung diseases, such as COPD and asthma, are among the leading causes of severe illness and death, affecting more than 540 million people worldwide (Soriano et al., 2020). Chronic lung diseases are characterized by inflammation and oxidative stress associated with the destruction of lung tissue and the progression of disease, as

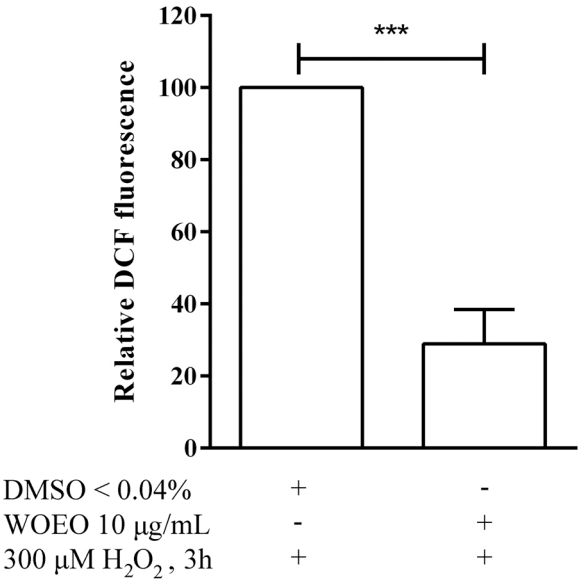

Fig. 3. WOEO decreases hydrogen peroxide-induced oxidative stress in BEAS-2B cells. BEAS-2B cells were treated with DMSO < 0.04 % (positive control) or WOEO 10 µg/mL. After 24 h the cells were incubated with 10 µM DCFH-DA for 30 min, then 300 µM H_2O_2 was added and DCF fluorescence was measured. Obtained DCF fluorescence readings were used for calculating the area under the curve (AUC). The AUC of the non-treated cells (negative control) was subtracted from the AUC of the positive control sample and WOEO treated cells. DCF fluorescence in the positive control sample was taken as 100 %. Data is expressed as mean ± standard deviation; N = 3. The difference of DCF fluorescence between the positive control sample and the cells treated with WOEO was significant; * ** $p = 0.002$.

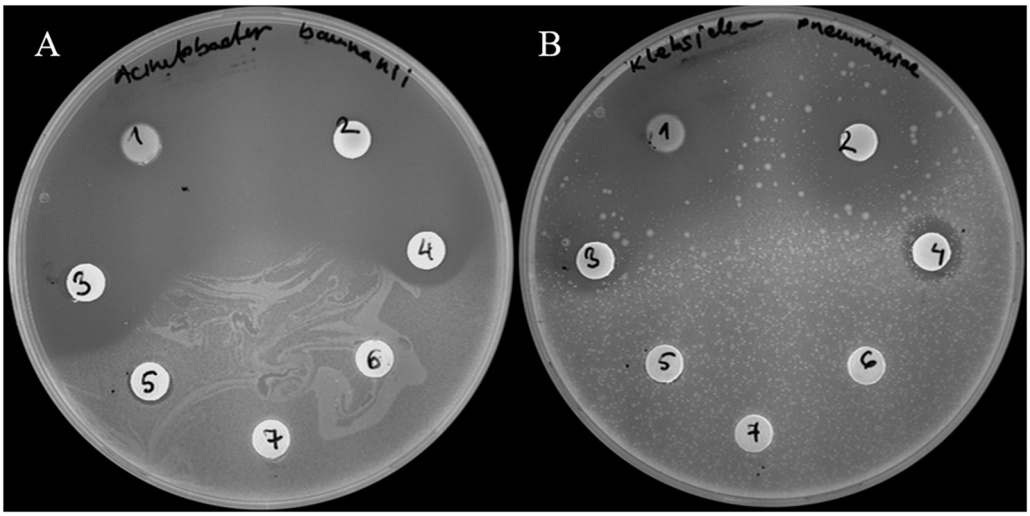

Fig. 4. Antimicrobial activity of WOEO on *Acinetobacter baumannii* ATCC 19606 (A) and *Klebsiella pneumoniae* ATCC BAA2146 (B). Numbers from 1 to 7 represent a decreasing concentration of WOEO from concentrated oil to 1 μg/μL (930, 500, 100, 50, 10 and 1 μg/μL). DMSO was used as a negative control sample.

well as with common pathogen expansion responsible for most of the acute exacerbations of the disease (Grondona et al., 2014; Yang et al., 2018; Roesch et al., 2018; Desai et al., 2018; Barnes, 2013; Barnes, 2016). In the past decades there has been no significant improvement in the therapy of chronic lung diseases, which is inefficient in controlling lung inflammation and oxidative stress (Barnes, 2013; Cantin et al., 2015; Funke and Geiser, 2015; Soriano et al., 2020; Gross and Barnes, 2017; Mitri et al., 2020). Moreover, inappropriate use of antibiotics in chronic lung diseases has been found to be associated with antimicrobial resistance, which is already considered a serious threat to public health (Bremmer et al., 2019; Llor and Bjerrum, 2014). OEO is one of the most widely used essential oils worldwide. Its beneficial biological effects have been attributed to the presence of carvacrol, thymol, and other monoterpenes (Sharifi-Rad et al., 2021; Pontes-Quero et al., 2021; Aljaafari et al., 2021). Accordingly, in the last decades, the use of various OEOs in the biomedical field, has attacked a lot of attention, due to its therapeutic potential as immunomodulatory, anti-inflammatory, antioxidant, and antibacterial mediators (Pontes-Quero et al., 2021; Aljaafari et al., 2021). Because of its volatility, WOEO can easily reach the upper and lower parts of the respiratory tract, and be an effective locally administrated treatment in chronic lung diseases (Horváth and Ács, 2015). However, there are no reports on the effects of WOEO on human respiratory epithelial cells which are also directly exposed to microbes.

Recent studies have shown anti-inflammatory and antioxidative effects of OEO in murine macrophages, porcine intestinal epithelial cells, and human dermal fibroblasts (Cheng et al., 2018; Zou et al., 2016; Han and Parker, 2017). In LPS-induced murine macrophages, OEO treatment efficiently reduced the activation of MAPK, protein kinase B, and nuclear factor κB activity, as well as the expression and secretion of pro-inflammatory mediators IL1β, IL6 and tumor necrosis factor (TNF) (Cheng et al., 2018). It has been suggested that the beneficial effects of OEO on human health are due to two phenolic derivatives, carvacrol and thymol, but also two other minor constituents such as monoterpene hydrocarbons γ-terpinene and ρ-cymene (Alkan, 2020).

In this study, we showed that carvacrol, which is thought to be responsible for most of the therapeutic effects of various oregano oils, was the major constituent (78.2 %) of analysed WOEO (*O. minutiflorum*, Probotanic), as reported by other workers in the field (Kirimer et al., 1995; Dadalioğlu and Evrendilek, 2004; Spyridopoulou et al., 2019). For the first time, we demonstrated that WOEO decreased LPS-induced *IL8* gene expression and hydrogen peroxide-induced oxidative stress in human bronchial epithelial cells. Moreover, results presented in this study showed that WOEO possesses an inhibitory effect on lung pathogenic bacteria *Acinetobacter baumannii* and *Klebsiella pneumoniae*.

As regards the WOEO analysed in this study, the content of carvacrol, which is thought to be responsible for the inhibition of expression and secretion of pro-inflammatory cytokines, was high (Lombrea et al., 2020). It was also found that treatment of BEAS-2B cells with WOEO prevented LPS-induced *IL8* gene expression, while there was no effect on IL8 secretion (Figs. 1 and 2). In the study of Han and Parker (2017), no effect of OEO on IL8 secretion was detected using dermal fibroblasts, which is consistent with results obtained in our study using bronchial epithelial cells. However, to date, there is no report on the effect of WOEO on IL8 in human bronchial epithelial cells. Inflammatory lung conditions are followed by elevated expression and release of IL8, a potent neutrophil chemoattractant and activator, which is implicated in neutrophilic inflammation of the lungs in chronic lung diseases (Mukaida, 2003). Apart from immune cells such as monocytes and neutrophils, bronchial epithelial cells represent one of the major sources of IL8 in the lungs (Barnes et al., 2003). Results presented in this study showed that treatment of human bronchial epithelial cells with WOEO significantly decreased LPS-induced *IL8* gene expression (Fig. 1), which may have a beneficial effect in many inflammatory lung conditions.

Additionally, increased oxidative stress plays an important pathophysiological role in chronic lung diseases by amplifying the inflammatory response in the lungs (Barnes et al., 2003). Results obtained in this study showed that the level of hydrogen peroxide-induced oxidative stress was significantly lower in WOEO treated human bronchial epithelial cells. This is in accordance with the study of Zou et al. (2016), which reported antioxidative effects of OEO by suppressing the formation of ROS and malondialdehyde, as well as by an increase of the nuclear factor-erythroid 2-related factor-2 in porcine intestinal epithelial cells. However, the antioxidant activity of WOEO has, so far, been confirmed only in radical scavenging and metal chelating activity assays (Elmastas et al., 2018; Oke and Aslim, 2010; Sokmen et al., 2020; Dorman et al., 2004). The antioxidant effects of oregano oil are thought to be due to carvacrol, thymol, and *p*-cymene, which have the ability to form chemical complexes with metal ions and free radicals (Lombrea et al., 2020). Since increased oxidative stress is implicated in inflammation, corticosteroid resistance, and acute exacerbations of many chronic lung diseases it is likely that supplements with antioxidative effects would be effective in the treatment of chronic inflammatory lung diseases (Barnes, 2020a, 2020b). Results of our study and others have shown that OEO and WOEO possess antioxidative activities and enhance endogenous antioxidant defense which may be valuable in the alleviation of increased oxidative stress in the lungs.

Microbial expansion is common in chronic lung diseases and associated with elevated inflammation leading to a decline in lung function (Huffnagle and Dickson, 2015). The antimicrobial activity of WOEO has been tested by a large number of authors on different pathogenic bacteria, including foodborne pathogens, plant pathogens, various ciprofloxacin-resistant *Campylobacter* spp., and so-forth (Oke and Aslim, 2010; Dadalioğlu and Evrendilek, 2004; Baydar et al., 2004; Vardar-Ünlü et al., 2007; Aslim and Yucel, 2008; Altundag et al., 2011). The antimicrobial activity of WOEO against various pathogenic bacteria that cause pneumonia was also examined (Vardar-Ünlü et al., 2007). In this study, the antimicrobial activity of WOEO on two Gram-negative opportunistic pathogens *Acinetobacter baumannii* ATCC 19606 and *Klebsiella pneumoniae* ATCC BAA2146 was demonstrated. Both pathogens are commonly connected with ventilator-associated pneumonia and different types of healthcare-associated infections, which can be lethal among patients with pneumonia and other chronic lung diseases (Horváth and Ács, 2015; Salemi et al., 1995). Moreover, *Klebsiella pneumoniae* ATCC BAA2146 is Extended-Spectrum β-Lactamase positive and New Delhi Metallo-β-Lactamase producing strain resistant to a broad spectrum of antibiotics (Li et al., 2014). Bacterial infections are usually managed by prevention and treatment with antibiotics. However, overuse of antibiotics may lead to drug resistance in bacteria, especially in nosocomial pulmonary infections. Therefore, research is currently focused on the use of natural antibacterial agents, such as oregano oil, for the effective treatment of various bacterial infections. Therefore, it is highly important that WOEO acts against these pathogens, which could be one of the ways to alleviate the problem of antibiotic resistance, especially in terms of nosocomial pulmonary infections.

This study provided data on the anti-inflammatory and antioxidative effects of WOEO (*O. minutiflorum*) by lowering the expression of the *IL8* gene and the level of intracellular oxidative stress in human bronchial epithelial cells. Because of the lack of effective treatments for neutrophilic inflammation and oxidative stress, which are amplified in many chronic lung diseases, there is a need for the development of novel drugs with anti-inflammatory and antioxidative effects. WOEO holds enormous promise for its beneficial effects on human bronchial epithelial cells, and can be used in the alleviation of symptoms in chronic lung diseases. Apart from these properties, WOEO also exhibited antibacterial activities against two common pathogens involved in hospital-associated, ventilation-associated, and healthcare-associated pneumonia, *Acinetobacter baumannii* ATCC 19606 and *Klebsiella pneumoniae* ATCC BAA2146.

Funding

This work was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia under Contract 451-03-47/2023-01/200042 and by the Innovation Fund of the Republic of Serbia under Voucher IV665.

CRediT authorship contribution statement

KV: Investigation, Formal analysis, Visualization, Writing – original draft, Validation. **VT**: Investigation, Formal analysis, Writing – original draft. **HM**: Formal analysis, Visualization. **MS**: Investigation, Formal analysis, Funding acquisition, Supervision, Writing – original draft, Writing – review & editing, Validation, Conceptualization.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Part of Voucher IV665 was financed by Probotanic (Republic of Serbia) that sells WOEO. Probotanic had no role in the study design, data collection, analyses, or interpretation, writing of the manuscript, or decision to publish the results of this study.

References

Adams, R.P., 2007. Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry, fourth ed. Allured Publ, Carol Stream, IL USA.

Ali, H., Al-Khalifa, A.R., Aouf, A., Boukhebti, H., Farouk, A., 2020. Effect of nanoencapsulation on volatile constituents, and antioxidant and anticancer activities of Algerian Origanum glandulosum Desf. essential oil. Sci. Rep. 10, 1–9. https://doi.org/10.1038/s41598-020-59686-w.

Aljaafari, M.N., AlAli, A.O., Baqais, L., Alqubaisy, M., AlAli, M., Molouki, A., Ong-Abdullah, J., Abushelaibi, A., Lai, K.S., Lim, S.H.E., 2021. An overview of the potential therapeutic applications of essential oils. Molecules 26, 628. https://doi.org/10.3390/molecules26030628.

Alkan, M., 2020. Chemical composition and insecticidal potential of different *Origanum* spp. (Lamiaceae) essential oils against four stored product pests. Turk. J. Entomol. 44, 149–163. https://doi.org/10.16970/entoted.620387.

Altundag, S., Aslim, B., Ozturk, S., 2011. *In vitro* antimicrobial activities of essential oils from *Origanum minutiflorum* and *Sideritis erytrantha* subsp. *erytrantha* on phytopathogenic bacteria. J. Ess. Oil Res. 23, 4–8. https://doi.org/10.1080/10412905.2011.9700422.

Arserim, S.K., Cetin, H., Yildirim, A., Limoncu, M.E., Cinbilgel, I., Kaya, T., Ozbel, Y., Balcioglu, I.C., 2021. The toxicity of essential oils from three origanum species against head louse, *Pediculus humanus capitis*. Acta Parasitol. 66, 1003–1011. https://doi.org/10.1007/s11686-021-00370-y.

Arslan, M., Dervis, S., 2010. Antifungal activity of essential oils against three vegetative-compatibility groups of *Verticillium dahlie*. World J. Microbiol. Biotech. 26, 1813–1821. https://doi.org/10.1007/s11274-010-0362-2.

Aslim, B., Yucel, N., 2008. *In vitro* antimicrobial activity of essential oil from endemic *Origanum minutiflorum* on ciprofloxacin-resistant *Campylobacter* spp. Food Chem. 107, 602–606. https://doi.org/10.1016/j.foodchem.2007.08.048.

Barbosa, L.N., Alves, F.C.B., Andrade, B.F.M.T., Albano, M., Rall, V.L.M., Fernandes, A.A.H., Buzalaf, M.A.R., Leite, A.L., de Pontes, L.G., Dos Santos, L.D., Fernandes Junior, A., 2020. Proteomic analysis and antibacterial resistance mechanisms of Salmonella Enteritidis submitted to the inhibitory effect of Origanum vulgare essential oil, thymol and carvacrol. J. Proteom. 214, 103625 https://doi.org/10.1016/j.jprot.2019.103625.

Barnes, P.J., 2013. New anti-inflammatory targets for chronic obstructive pulmonary disease. Nat. Rev. Drug Discov. 12, 543–559. https://doi.org/10.1038/nrd4025.

Barnes, P.J., 2016. Inflammatory mechanisms in patients with chronic obstructive pulmonary disease. J. Allergy Clin. Immunol. 138, 16–27. https://doi.org/10.1016/j.jaci.2016.05.011.

Barnes, P.J., 2020a. COPD 2020: new directions needed. Am. J. Physiol. Lung Cell Mol. Physiol. 319, L884–L886. https://doi.org/10.1152/ajplung.00473.2020.

Barnes, P.J., 2020b. Oxidative stress-based therapeutics in COPD. Redox Biol. 33, 101544 https://doi.org/10.1016/j.redox.2020.101544.

Barnes, P.J., Shapiro, S.D., Pauwels, R.A., 2003. Chronic obstructive pulmonary disease: molecular and cellular mechanisms. Eur. Respir. J. 22, 672–688. https://doi.org/10.1183/09031936.03.00040703.

Baser, K.H.C., 2002. Aromatic biodiversity among the flowering plant taxa of Turkey. Pure Appl. Chem. 74, 527–545. https://doi.org/10.1351/pac200274040527.

Baydar, H., Sağdic, O., Özkan, G., Karadoğan, T., 2004. Antibacterial activity and composition of essential oils from *Origanum*, *Thymbra* and *Satureja* species with commercial importance in Turkey. Food Control 15, 169–172. https://doi.org/10.1016/S0956-7135(03)00028-8.

Bayramoglu, B., Sahin, S., Sumnu, G., 2008. Solvent-free microwave extraction of essential oil from oregano. J. Food Eng. 88 (4), 535–540. https://doi.org/10.1016/j.jfoodeng.2008.03.015.

Bremmer, D.N., Moffa, M.A., Ma, K., Bean, H.R., Snatchko, J., Trienski, T.L., Watson, C., Walsh, T.L., 2019. Acute exacerbations of chronic obstructive pulmonary disease with a low procalcitonin concentration: impact of antibiotic therapy. Clin. Infect. Dis. 68, 725–730. https://doi.org/10.1093/cid/ciy552.

Cantin, A.M., Hartl, D., Konstan, M.W., Chmiel, J.F., 2015. Inflammation in cystic fibrosis lung disease: pathogenesis and therapy. J. Cyst. Fibros. 14, 419–430. https://doi.org/10.1016/j.jcf.2015.03.003.

Cetin, H., Yanikoglu, A., 2006. A study of the larvicidal activity of *Origanum* (Labiatae) species from southwest Turkey. J. Vector Ecol. 31, 118–122. https://doi.org/10.3376/1081-1710(2006)31[118:ASOTLA]2.0.CO;2.

Cetin, H., Cilek, J.E., Aydin, L., Yanikoglu, A., 2009. Acaricidal effects of the essential oil of *Origanum minutiflorum* (Lamiaceae) against *Rhipicephalus turanicus* (Acari: Ixodidae). Vet. Parasitol. 160, 359–361. https://doi.org/10.1016/j.vetpar.2008.11.009.

Chauhan, A.K., Kang, S.C., 2014. Thymol disrupts the membrane integrity of *Salmonella* ser. typhimurium *in vitro* and recovers infected macrophages from oxidative stress *in* an *ex vivo* model. Res. Microbiol. 165, 559–565. https://doi.org/10.1016/j.resmic.2014.07.001.

Cheng, C., Zou, Y., Peng, J., 2018. Oregano essential oil attenuates RAW264.7 cells from lipopolysaccharide-induced inflammatory response through regulating NADPH oxidase activation-driven oxidative stress. Moleculs 23, 1857. https://doi.org/10.3390/molecules23081857.

Dadalioğlu, I., Evrendilek, G.A., 2004. Chemical compositions and antibacterial effects of essential oils of Turkish oregano (*Origanum minutiflorum*), Bay Laurel (*Laurus nobilis*), spanish Lavender (*Lavandula stoechas* L.), and Fennel (*Foeniculum vulgare*) on common foodborne pathogens. J. Agric. Food Chem. 52, 8255–8826. https://doi.org/10.1021/jf049033e.

Demirci, F., Paper, D.H., Franz, G., Baser, K.H.C., 2004. Investigation of the *Origanum onites* L. essential oil using the chorioallantoic membrane (CAM) assay. J. Agric. Food Chem. 52, 251–254. https://doi.org/10.1021/jf034850k.

Desai, O., Winkler, J., Minasyan, M., Herzog, L.E., 2018. The role of immune and inflammatory cells in idiopathic pulmonary fibrosis. Front Med. 5, 43. https://doi.org/10.3389/fmed.2018.00043.

Dorman, H.J.D., Bachmayer, O., Kosar, M., Hiltunen, R., 2004. Antioxidant properties of aqueous extracts from selected *Lamiaceae* species grown in Turkey. Agric. Food Chem. 52, 762–770. https://doi.org/10.1021/jf034908v.

Elmastas, M., Celik, S.M., Genc, N., Aksit, H., Erenler, R., Gulcin, I., 2018. Antioxidant activity of an anatolian herbal tea—*Origanum minutiflorum*: isolation and characterization of its secondary metabolites. Int. J. Food Prop. 21, 374–384. https://doi.org/10.1080/10942912.2017.1416399.

Evrendilek, G.A., 2015. Empirical prediction and validation of antibacterial inhibitory effects of various plant essential oils on common pathogenic bacteria. Int. J. Food Microbiol. 202, 35–41. https://doi.org/10.1016/j.ijfoodmicro.2015.02.030.

Funke, M., Geiser, T., 2015. Idiopathic pulmonary fibrosis: the turning point is now. Swiss Med. Wkly. 145, w14139. https://doi.org/10.4414/smw.2015.14139.

Grondona, E., Gatti, G., López, A.G., Sánchez, L.R., Rivero, V., Pessah, O., Zunino, M.P., Ponce, A.A., 2014. Bio-efficacy of the essential oil of oregano (*Origanum vulgare* Lamiaceae. ssp. Hirtum). Plant Foods Hum. Nutr. 69, 351–357. https://doi.org/10.1007/s11130-014-0441-x.

Gross, N.J., Barnes, P.J., 2017. New therapies for asthma and chronic obstructive pulmonary disease. Am. J. Respir. Crit. Care Med. 195, 159–166. https://doi.org/10.1164/rccm.201610-2074PP.

Han, X., Parker, T.L., 2017. Anti-inflammatory, tissue remodeling, immunomodulatory, and anticancer activities of oregano (*Origanum vulgare*) essential oil in a human skin disease model. Biochim. Open 4, 73–77. https://doi.org/10.1016/j.biopen.2017.02.005.

Horváth, G., Ács, K., 2015. Essential oils in the treatment of respiratory tract diseases highlighting their role in bacterial infections and their anti-inflammatory action: a review. Flavour Fragr. J. 30, 331–341. https://doi.org/10.1002/ffj.3252.

Huffnagle, G.B., Dickson, R.P., 2015. The bacterial microbiota in inflammatory lung diseases. Clin. Immunol. 159, 177–182. https://doi.org/10.1016/j.clim.2015.05.022.

Khan, I., Bahuguna, A., Kumar, P., Bajpai, V.K., Kang, S.C., 2017. Antimicrobial potential of carvacrol against uropathogenic *Escherichia coli* via membrane disruption, depolarization, and reactive oxygen species generation. Front Microbiol. 8, 2421. https://doi.org/10.3389/fmicb.2017.02421.

Kirimer, N., Baser, K.H.C., Tumen, G., 1995. Carvacrol– rich plants in Turkey. Chem. Nat. Comp. 31, 37–41. https://doi.org/10.1007/BF01167568.

Li, J., Liu, F., Wang, Q., Ge, P., Woo, P.C.Y., Yan, J., Zhao, Y., Gao, G.F., Liu, C.H., Liu, C., 2014. Genomic and transcriptomic analysis of NDM-1 *Klebsiella pneumoniae* in spaceflight reveal mechanisms underlying environmental adaptability. Sci. Rep. 4, 6216. https://doi.org/10.1038/srep06216.

Llor, C., Bjerrum, L., 2014. Antimicrobial resistance: risk associated with antibiotic overuse and initiatives to reduce the problem. Ther. Adv. Drug Saf. 5 (6), 229–241. https://doi.org/10.1177/2042098614554.

Lombrea, A., Antal, D., Ardelean, F., Avram, S., Pavel, I.Z., Vlaia, L., Mut, A.M., Diaconeasa, Z., Dehelean, C.A., Soica, C., Danciu, C., 2020. A recent insight regarding the phytochemistry and bioactivity of *Origanum vulgare* L. essential oil. Int. J. Mol. Sci. 21, 9653. https://doi.org/10.3390/ijms21249653.

Magi, G., Marini, E., Facinelli, B., 2015. Antimicrobial activity of essential oils and carvacrol, and synergy of carvacrol and erythromycin, against clinical, erythromycin-resistant Group A Streptococci. Front Microbiol. 6, 165. https://doi.org/10.3389/fmicb.2015.00165.

Mitri, C., Xu, Z., Bardin, P., Corvol, H., Touqui, L., Tabary, O., 2020. Novel anti-inflammatory approaches for cystic fibrosis lung disease: identification of molecular targets and design of innovative therapies. Front Pharm. 11, 1096. https://doi.org/10.3389/fphar.2020.01096.

Mukaida, N., 2003. Pathophysiological roles of interleukin-8/CXCL8 in pumonary disease. Am. J. Physiol. 284, L566–L577. https://doi.org/10.1152/ajplung.00233.2002.

Oke, F., Aslim, B., 2010. Biological potentials and cytotoxicity of various extracts from endemic *Origanum minutiflorum* O. Schwarz & P.H. Davis. Food Che Toxicol. 48, 1728–1733. https://doi.org/10.1016/j.fct.2010.03.053.

Ozel, M.Z., Kaymaz, H., 2004. Superheated water extraction, steam distillation and Soxhlet extraction of essential oils of *Origanum onites*. Anal. Bioanal. Chem. 379, 1127–1133. https://doi.org/10.1007/s00216-004-2671-5.

Pontes-Quero, G.M., Esteban-Rubio, S., Pérez, C.J., Aguilar, M.R., Vázquez-Lasa, B., 2021. Oregano essential oil micro- and nanoencapsulation with bioactive properties for biotechnological and biomedical applications. Front Bioeng. Biotechnol. 9, 703684 https://doi.org/10.3389/fbioe.2021.703684.

Popović, J., Klajn, A., Paunesku, T., Ma, Q., Chen, S., Lai, B., Stevanović, M., Woloschak, G.E., 2019. Neuroprotective role of selected antioxidant agents in preventing cisplatin-induced damage of human neurons *in vitro*. Cell Mol. Neurobiol. 39, 619–636F. https://doi.org/10.1007/s10571-019-00667-7.

Roesch, E.A., Nichols, D.P., Chmiel, J.F., 2018. Inflammation in cystic fibrosis: an update. Pedia Pulmonol. 53 (S3), S30–S50. https://doi.org/10.1002/ppul.24129.

Rostro-Alanis, M., de, J., Báez-González, J., Torres-Alvarez, C., Parra-Saldívar, R., Rodriguez-Rodriguez, J., Castillo, S., 2019. Chemical composition and biological activities of oregano essential oil and its fractions obtained by vacuum distillation. Molecules 24, 1904. https://doi.org/10.3390/molecules24101904.

Salemi, C., Morgan, J., Padilla, S., Morrissey, R., 1995. Association between severity of illness and mortality from nosocomial infection. Am. J. Infect. Control 23, 188. https://doi.org/10.1016/0196-6553(95)90040-3.

Sharifi-Rad, M., Berkay, Y.Y., Antika, G., Salehi, B., Tumer, T.B., Kulandaisamy, V.C., Das, G., Patra, J.K., Karazhan, N., Akram, M., Iqbal, M., Imran, M., Sen, S., Acharya, K., Dey, A., Sharifi-Rad, J., 2021. Phytochemical constituents, biological activities, and health-promoting effects of the genus *Origanum*. Phytother. Res. 35 (1), 95–121. https://doi.org/10.1002/ptr.6785.

Sokmen, A., Abdel-Baki, A.A.S., Al-Malki, E.S., Al-Quraishy, S., Abdel-Haleem, H.M., 2020. Constituents of essential oil of Origanum minutiflorum and its *in vitro* antioxidant, scolicidal and anticancer activities. J. King Saud. Univ. Sci. 32, 2377–2382. https://doi.org/10.1016/j.jksus.2020.03.018.

Soriano, J.B., Kendrick, P.J., Paulson, K.R., Gupta, V., Abrams, E.M., Adedoyin, R.A., Adhikari, T.B., Advani, S.M., et al., 2020. Prevalence and attributable health burden of chronic respiratory diseases, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet Respir. Med. 8, 585–596. https://doi.org/10.1016/S2213-2600(20)30105-3.

Spyridopoulou, K., Fitsiou, E., Bouloukosta, E., Tiptiri-Kourpeti, A., Vamvakias, M., Oreopoulou, A., Papavassilopoulou, E., Pappa, A., Chlichlia, K., 2019. Extraction, chemical composition, and anticancer potential of Origanum onites L. essential oil. Molecules 24, 2612. https://doi.org/10.3390/molecules24142612.

Valgas, C., de Souza, Machado, Smânia, S., Smânia, J.A, F.A.E., 2007. Screening methods to determine antibacterial activity of natural products. Braz. J. Microbiol. 38, 369–380. https://doi.org/10.1590/S1517-83822007000200034.

Vardar-Ünlü, G., Unlü, M., Dőnmez, E., Vural, N., 2007. Chemical composition and *in vitro* antimicrobial activity of the essential oil of *Origanum minutiflorum* O Schwarz & PH Davis. J. Sci. Food Agric. 87, 255–259. https://doi.org/10.1002/jsfa.2708.

Yang, X., Li, H., Ma, Q., Zhang, Q., Wang, C., 2018. Neutrophilic asthma is associated with increased airway bacterial burden and disordered community composition. Biomed. Res. Int. 2018, 9230234. https://doi.org/10.1155/2018/9230234.

Zou, Y., Wang, J., Peng, J., Wei, H., 2016. Oregano essential oil induces SOD1 and GSH expression through Nrf2 activation and alleviates hydrogen peroxide-induced oxidative damage in IPEC-J2 cells. Oxid. Med. Cell Longev. 2016, 5987183. https://doi.org/10.1155/2016/5987183.