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An *in vitro* study of *the origanum minutiflorum* O. Schwarz & P. H. Davis and *Coriandrum sativum* L. essential oils as chronic tonsillitis therapeutics: antibacterial, antibiofilm, antioxidant, and cytotoxic activities

Marina Kostić[a], Marija Ivanov[a], Tatjana Markovic[b], Snežana Sanković Babić[c], Lillian Barros[d], Ricardo Calhelha[d], Marina Sokovic[a] and Ana Ciric[a]

[a]Department of Plant Physiology, University of Belgrade, Institute for Biological Research "Siniša Stanković" - National Institute of Republic of Serbia, Belgrade, Serbia; [b]Institute for Medicinal Plant Research "dr Josif Pančić" Belgrade, Belgrade, Serbia; [c]ENT Clinic, Clinical Hospital Centre Zvezdara, Belgrade, Serbia; [d]Centro de Investigação de Montanha (CIMO), Instituto Politécnico de BragançaCampus de Santa Apolónia, Bragança, Portugal

ABSTRACT

The chemical composition and biological activity of essential oils (EOs) from leaves of *Origanum minutiflorum* O. Schwarz & P.H.Davis, and seeds of *Coriandrum sativum* L. were investigated. Chemical analyses of EOs were performed and the major components were carvacrol (81.5%) and linalool (69.6%), respectively. The antimicrobial activity was assessed against several bacteria originating from the tonsillar tissue. Activities of EOs against *Staphylococcus aureus* biofilm were investigated, as well as the effect of the mixture of these EOs and antibiotics against the pathogen. The antioxidant activity of both EOs was determined by TBARS assay, and examined wild oregano EO showed better activity. Also, cytotoxicity of EOs was evaluated *in vitro* and both EOs showed potential to inhibit further proliferation of tumor cells. This study reported for the first time the effects of EOs on chronic tonsillitis causative pathogens, supporting their role as a natural bioactive therapeutic agent with possible antimicrobial applications.

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Wild oregano leaf oil; coriander seed oil; infections; *Staphylococcus aureus*; cytotoxicity

1. Introduction

In recent decades, due to a large amount of research on phytochemistry and pharmacognosy, natural plant products have gained particular importance in treating different diseases. It's estimated that almost 80% world population uses products from natural origin (teas, extracts, essential oils (EOs)) for prevention and treatment of various diseases, including infections, cardiovascular disease, diabetes, Alzheimer's, cancer, etc. Biological activity (antimicrobial, synergistic, antioxidant) of EOs and their chemical components against various human and food pathogens have been recognized by several researchers in the past (1 – 4). Likewise, excessive use of antibiotics and their reduced effectiveness has led to the recent comeback of natural compounds application in medicine, associated also with increased interest in EOs application (5).

Origanum minutiflorum O. Schwarz & P. H. Davis (Lamiaceae) is an aromatic plant, endemic in Turkey. The local Turkish population use wild oregano in form of spices and tea to treat colds, rheumatism and as analgesic (6). Coriander (*Coriandrum sativum* L., Apiaceae) is aromatic plant native to the eastern Mediterranean, however today it's a ubiquitous cultivated plant. In traditional medicine coriander is used in the form of powder or tincture against appetite loss and for the treatment of anxiety and respiratory problems such as cough and bronchitis (7).

Tonsillitis is an infection of palatine tonsils and/or adenoid. Various etiological factors (use of antibiotics, diet and multiple viral and/or bacterial infections) lead to appearance of the chronic tonsillitis (CT). In practice, it has been shown that CT is almost always associated with infection caused with beta hemolytic group A streptococci, *Staphylococcus aureus* and *Haemophilus influenzae* (8). The aforementioned species produce various virulence factors (toxins, immune-modulatory factors, exoenzymes) which have an impact on the pathophysiology of the disease (9). Diverse network of virulence factors of *S. aureus* along with its ability to form biofilms, presents a challenge to the host immune system to fully eradicate bacteria and significantly complicates the treatment of the CT (as antibiotics have less effect on cells embedded in biofilm than on planktonic cells) leading to persistent chronic infection (9).

CONTACT Ana Ciric rancic@ibiss.bg.ac.rs

Additionally, patients with removed tonsils may be more susceptible to other upper respiratory tract conditions such as chronic rhinosinusitis and inflammatory bowel diseases (10), which raises new health concerns and increases demand for natural products that can be used as adjuvants in therapy of the CT. The oxidation products produced during inflammation are involved in the tissue injury. The antioxidants play role in neutralizing the destruction by these oxidation products. Since CT is a chronic inflammatory disease in the oro- and nasopharynx, the balance between oxidation products and antioxidants is involved in the appearance and the chronicity of this disease in the pharynx (11). Chronic inflammation is induced by biological, chemical, and physical factors and is associated with an increased risk of several human cancers (12). The link between inflammation and cancer has been suggested by epidemiological and experimental data and confirmed by anti-inflammatory therapies that show efficacy in cancer prevention and treatment (13). Combinations of antimicrobial agents provide many benefits, including stronger activity and reduced toxic effects of the combined components. Many studies have been devoted to combining antibiotics both within and between groups (12), (13), but studies on combinations of antibiotics and EOs, are rather scarce.

In this study, inhibitory activities of *O. minutiflorum* leaf EO and *C. sativum* seed EO towards the range of pathogens linked with CT were estimated along with their impact on inhibition of *S. aureus* biofilm. In order to determine bioactive constituents in EOs chemical analysis was performed. Antioxidant and citotoxic activities of both EOs were also examined. Given the above-mentioned findings, there is a lack of studies on biological activity of EOs against CT pathogens. In addition, there is scarce information about antibiofilm and cytotoxic activity of wild oregano and coriander EOs. Therefore, the significance of this study is high, because it provides information on new activities of already researched EOs.

2. Materials and methods

2.1. Essential oils

The *Origanum minutiflorum* O. Schwarz & P.H. Davis leaf EO and *Coriander sativum* L seed EO were purchased as commercial samples from Probotanic Ltd., Belgrade, Serbia.

2.2. Chemical analysis of the essential oils

Gas chromatographic method was used to determine the chemical composition of commercial EOs. The samples were dissolved in 10 µL of 75% ethanol and accelerated to split mode (1:30). The chemical profile of the samples was analyzed using a gas chromatograph (Agilent Technologies 7890A, Santa Clara, USA) with a split/splitless injector connected to an HP-5 column (30 m, 0.32 mm, film thickness 0.25 µL) and a flame ionizing detector (FID). The carrier gas flow (hydrogen) was 1 mL/min at 210°C. The temperature of the injector was 250°C and 280°C, while the temperature of the column was changed in the linear mode of temperature programming from 40°C to 260°C and 4°C/min. The oils compositions was calculated based on the area of the obtained peaks and were expressed as a percentage.

The GC/MS method was used to identify individual oil components. Identification was determined on an HP G 1800C Series II GCD analytical system, with HP-5 MS column. The carrier gas flow (helium) was 1 mL/min at 260°C. Mass spectra were recorded in EI mode (70 eV) in the range m/z 40–400. The identification of individual constituents was accomplished by comparing their MS spectra to those available in MS libraries (Adams, NIST, and Wiley) and by comparing their experimentally determined linear retention indices against a mixture of *n*-alkanes (C8-C31) on the HP-5 column (14).

2.3. Specimen collection, identification, and culture conditions

The following Gram positive (*Micrococcus luteus* (dT_9/2), *Rothia mucilagenosa* (oT_22/2), *Streptococcus agalactiae* (oT_20/1), *Streptococcus anginosus* (oT_26), *Streptococcus constellatus* (dT_22/1), *Streptococcus dysgalactiae* (oT_21/2), *Streptococcus oralis* (oT_5), *Streptococcus parasanquinis* (oT_3), *Streptococcus pseudopneumoniae* (dT_22/2), *Streptococcus pyogenes* (dT_14), *Streptococcus salivarius* (dT_12), *Staphylococcus aureus* (oT_4), *Staphylococcus hominis* (oT_14/2), and *Staphylococcus warnerii* (oT_7/1)) and Gram negative bacteria (*Enterobacter cloacae* (oT_18) and *Stenotrophomonas maltophilia* (A_12)), were used for the evaluation of antibacterial potential of the selected EOs. These bacteria were maintained on Blood Agar (Torlak, Serbia). They were obtained from tonsillar tissue of patients after obtaining informed written consent, at Otorhinolaryngology clinic at Clinical Hospital Center Zvezdara, Belgrade, Serbia. All tested isolates were identified by Matrix Assisted Laser Desorption

and Ionization Time-Of-Flight (MALDI-TOF) mass spectrometry (VITEK MS bioMerieux, France). Tested microorganisms were deposited at the Mycological Laboratory, Department of Plant Physiology, Institute for Biological Research 'Siniša Stankovic', National Institute of Republic of Serbia, University of Belgrade.

2.4. Evaluation of antimicrobial activity

Antimicrobial activity of wild oregano and coriander EOs, were determined by modified microdilution method (15,16). The results were presented as minimum inhibitory/bactericidal concentrations (MIC/MBC). Amoxicillin with clavulanic acid (Hemofarm, Serbia) and cefixime (Alkaloid, North Macedonia) were used as positive controls.

2.5. Evaluation of the synergistic activity

The two-agent broth microdilution checkerboard method (17) was used to investigate the synergistic effect of the mixture of EOs and antibiotics on the growth of *S. aureus*. The obtained MICs were used as initial for further tests. A series of mixture dilutions was created in a microplate (96 system), where a vertical gradient was the first substance, and subsequently dilutions of second substance were introduces in horizontal grade. Dilutions were made in Tryptic soy broth (TSB) medium in the range of 1/128 MIC to MIC. An untreated inoculum was used as control. The plates were incubated at 37°C for 24 h. After incubation, *p*-iodonitrotetrazolium (INT) dye was added and plate was reincubated for 30 min. Combinations of EOs, EOs and antibiotics that not cause colorations were recorded and fractional inhibitory concentration index (FICI) values were calculated according to the following formula: FICI = (FIC1°/MIC1°) + (FIC2°/MIC2°). The results were interpreted as follows: FICI <0.5 synergistic, 0.5 ≤1 additive, 1 < FICI ≤4 indifferent and FICI >4 antagonistic.

2.6. Evaluation of selected EOs activity against Staphylococcus aureus biofilm

S. aureus was selected among the others tested bacteria because the high MIC/MBC and due to its ability to form biofilm.

2.6.1. Inhibition of biofilm formation

The ability of EOs to inhibit biofilm formation was determined as previously described by Stepanović et al. (18) with some modification. In short, *S. aureus* cells were incubated with TSB medium with 2% glucose (Torlak, Belgrade, Serbia) in 96-well microtiter plates with adhesive bottom (Spektar, Čačak) at 37°C with MIC and subMIC EO concentrations, for 24 h. After incubation and fixation of the cells, the plates were dried and stained using 0.1% crystal violet dye (Bio-Merieux, France). Wells were washed again, then air dried after and the remaining cells were fixed with 96% ethanol (Zorka, Serbia). The absorbance was read and percentage of inhibition of biofilm formation was calculated, according to Kostić et al. (19).

2.6.2. Eradication of S. aureus biofilm

S. aureus was grown in TSB enriched with 2% glucose in microtiter plates with adhesive bottom at 37°C for 24 h. Wells were washed with sterile PBS and the remaining biofilm was treated for 30 s with EOs at MBC values. The wells were washed; the biofilm was fixed with methanol, air dried and stained with crystal violet (0.1%). After dissolving the crystal violet in ethanol, the absorbance was read and the percentage of biofilm diminishing was calculated according to Smiljković et al. (20).

2.7. Antioxidant activity of the EOs

The lipid peroxidation inhibition was evaluated through the thiobarbituric acid reactive substances (TBARS) assay. The antioxidant potential of each EO was measured by the decrease in TBARS as previously described by Kostić et al. (19). The results were expressed in mg/mL corresponding to the sample concentration providing 50% of antioxidant activity (IC_{50} value).

2.8. Evaluation of cytotoxicity of the selected EOs

The cytotoxicity of EOs was evaluated towards human tumor cell lines (MCF-7, NCI-H460, HCT-15, HeLa and HepG2) and towards a non-tumor liver cell primary culture (PLP2). The EOs were dissolved in water (8 mg/mL). The assay of sulforhodamine B (Extra synthèse, Genay, France) was conducted according to the procedure described by Vichai and Kirtikara (21). Ellipticine was used as positive control and the results were expressed as GI_{50} (concentration that causes 50% growth inhibition). The results were expressed in µg/mL (21). The criterion used to categorize the cytotoxic activity of extract to cancer cell lines was as follows: GI_{50} ≤20 µg/mL = highly cytotoxic, GI_{50} ranged between 21 and 200 µg/mL = moderately cytotoxic, GI_{50} ranged between 201 and 400 µg/mL = weakly cytotoxic, and GI_{50} >400 µg/mL = no cytotoxicity.

2.9. Statistical analysis

The statistical analysis was performed through analysis of variance (ANOVA) and Tukey's HSD test ($p = 0.05$), and applied to assess the statistical differences ($p = 0.05$). Analyses were performed GraphPad software.

3. Results and discussions

3.1. Chemical composition

Chemical analysis of EOs revealed 25 constituents of wild oregano leaf EO and 17 constituents of coriander fruit EO; they are comparatively presented in Table 1. The most dominant groups of compounds in both EOs were oxygenated monoterpenes (Table 1). The major wild oregano EO component was carvacrol (81.5%), while in coriander EO it was linalool (69.6%). The components presented in amounts above 1% in the wild oregano EO sample were *p*–cymene > borneol > γ-terpinene > terpinen-4-ol > *cis*–caryophyllene > β-bisabolene > thymol, while in coriander EO they were *p*–cymene >α-pinene > γ-terpinene > limonene > camphor > geraniol > geranyl acetate.

Based on available literature data, EO of *O. minutiflorum* is poorly examined as compared with other *Origanum* EOs. Baser et al. (22) analyzed the chemical composition of *O. minutiflorum* EO originating from several localities in Turkey and showed that the dominant compounds were carvacrol (75.4–83.6%) and *p*-cymene (5.9%), although the prevalence of compounds depended on the locality from which the plants were harvested. In several other studies, similar results for wild oregano EO were recorded through a wider range of its major component carvacrol, 50.04–90.87% (23,24).

Unlike the wild oregano EO, coriander fruit EO is one of the most studied worldwide. According to standard ISO 3516 (1997) (25) that prescribes its quality, the most dominant component of this EO is linalool (65–78%), while the other few characteristic compounds range as follows: camphor (4–6%) > α-pinene (3–7%) > γ-terpinene (2–7%) > limonene (2–5%). In the literature, data presenting the chemical profile of this EO are quite variable.

This EO originating from different regions confirms its richness in oxygenated monoterpenes, namely linalool, but the other major components differ. Thus, in Tunisian coriander fruit EO the content of linalool was 87.54% while *cis*–dihydrocarvon (2.36%) was the second dominant compound (26). In Algerian EO linalool was 73.1% but there were also high portions of *p*–mentha-1,4-dien-7-ol (6.51%) > α-pinene (3.41%) > neryl acetate (3.22%) (27). In Iran EO, linalool ranged 40.9–79.9%, while other major components were γ-terpinene (0.1–13.6%) > α-pinene (1.2–7.1%) > neryl acetate (2.3–14. 2%), while in EO from Bangladesh linalool was only 37.65% and the other major compounds were geranyl acetate (17.57%) > γ-terpinene (14.42%) (27,28). The sample from Brazil apart to linalool 77.4 %, had γ-terpinene (4.6%) > α-pinene (3.9%) (27) while in EO from Canada, linalool ranged 64–84.6% and camphor was next major compound that ranged 3,4–6.2% (27) which was similar to EO from Serbia, which had linalool 78.2% and camphor 11% (29).

Table 1. Chemical composition of essential oils obtained from leaves of wild oregano (*Origanum minutiflorum*) and seeds of coriander (*Coriandrum sativum*).

				Contribution to essential oil (%, w/w)	
No	RI *lit*	RI *exp*	Components	Wild oregano	Coriander
1	924	922	α-Thujene		0.92
2	932	927	α–Pinene	0.46	4.62
3	946	941	Camphene	0.33	0.10
5	969	967	Sabinene		0.03
4	974	972	β–Pinene	0.13	.
6	988	987	Myrcene	0.59	0.35
7	1002	998	α–Phellandrene	0.11	
8	1014	1011	α–Terpinene	0.68	
9	1020	1019	*para*-Cymene	3.73	4.77
10	1025	1023	β–Phellandrene	0.32	
11	1024	1025	Limonene		4.42
12	1026	1025	1,8-Cineol	0.21	
13	1054	1053	α–Terpinene	1.70	4.71
14	1067	1066	*cis*-Linalool oxide		0.46
15	1085	1082	*para*-Mentha-2,4(8)-diene	0.09	
16	1084	1085	*trans*-Linalool oxide		0.42
17	1095	1092	Linalool	0.17	69.60
18	1131	1133	*dihydro*-Linalool		0.56
19	1141	1137	Camphor	.	4.47
20	1165	1162	Borneol	2.32	
21	1174	1174	Terpinen-4-ol	1.53	
22	1178	1180	*2-methyl*-Isoborneol		0.39
23	1186	1190	γ-Terpineol	0.93	
24	1249	1250	Geraniol		1.95
25	1289	1286	Thymol	1.22	
26	1298	1294	Carvacrol	81.53	
27	1359	1360	Neryl acetate		0.58
28	1349	1351	Thymol acetate	0.18	
29	1370	1367	Carvacrol acetate	0.12	
30	1379	1380	Geranyl acetate		1.64
31	1408	1407	*cis*-Caryophyllene	1.36	
32	1430	1426	β-Copaene	0.23	
33	1484	1483	Germarene-D	0.15	
34	1505	1499	β-Bisabolene	1.29	
35	1513	1507	γ-Cadinene	0.43	
36	1582	1579	Caryophyllene oxide	0.13	
Monoterpene hydrocarbons (comp.1–11,13,15)				*8.14*	*19.92*
Oxygenated monoterpenes (comp.12,14,16–30)				*88.21*	*80.08*
Sesquiterpene hydrocarbons (comp.31–35)				*3.46*	*0.00*
Oxygenated sesquiterpenes (comp.27–29,36)				*0.13*	*0.00*
Total identified				***99.94***	***100.00***
Number of identified components				*25*	*17*

RI *lit* – Retention Index from literature (Adams, NIST, Wiley); RI *exp* - Retention Index reported in the investigation.

In terms of the contribution to the pleasantness of the EO aroma, monoterpene hydrocarbons are less important in comparison to oxygenated compounds, which are highly fragrant which is important for oral use because they are more pleasant to use due to the aroma. Nevertheless, the monoterpenes are considered generally more useful for their therapeutic purposes as antimicrobial agents, while sesquiterpenes more for their anti-inflammatory and anticarcinogenic properties (30).

3.2. Antimicrobial activity

Antibacterial activity was determined by the microdilution method and the susceptibility of 16 tested bacterial species to selected EOs is shown in Table 2. *O. minutiflorum* EO MICs were in range from 0.03 to 1.38 mg/mL (Table 2). The most sensitive species were as follows: *S. dysgalactiae* (MIC 0.05 mg/mL, MBC 0.11 mg/mL) and *S. hominis* (MIC 0.03 mg/mL, MBC 0.05 mg/mL), while the most resistant bacterium was *S. aureus* (MIC 1.38 mg/mL, MBC 2.77 mg/mL). *C. sativum* EO had slightly lower antibacterial activity compared to *O. minutiflorum* EO (Table 2). Coriander EO exhibited the most promising inhibitory potential towards *Streptococcus* spp. (MICs range 0.24–2.41 mg/mL). *S. agalactiae* and *S. dysgalactiae* (MIC 0.24 mg/mL, MBC 0.56 mg/mL) were the most sensitive to the action of coriander EO, while *M. luteus* (MIC 4.82 mg/mL, MBC 9.65 mg/mL) and *S. hominis* (MIC 5.07 mg/mL, MBC 9.65 mg/mL) were the most resistant among the examined bacterial species. In general, G (+) bacteria showed greater sensitivity to the tested oils compared to G (-) bacteria. Compared to the tested essential oils, commercial antibiotics amoxicillin with clavulanic acid and cefixime whose MIC values are in the range 0.20–28.0 µg/mL and 0.20–13.0 µg/mL, respectively, exhibited better antibacterial activity towards all tested bacteria. Despite the fact that antibiotics proved to be more effective against tested bacteria, this data are very important because antibiotics have proven to be very toxic. Therefore, it's important that the obtained MIC values indicate the possible effective use of tested EOs.

Currently, there is no data on the antibacterial activity of *O. minutiflorum* EO on pathogens isolated from infected tonsils. However, current data on *O. vulgare* EO indicate very good antimicrobial activity. Lombrea et al. (31) showed that *O. vulgare* EO has good inhibitory activity against *S. aureus* (MIC 0.08–1.193 mg/mL), *S. pyogenes* (MIC 0.5 mg/mL), and *S. pneumoniae* (MIC 2.5–10 mg/mL). Coriander seed EO has good antibacterial activity against *K. pneumoniae* (MIC 2.65 mg/mL), *P. aeruginosa* (MIC 3.2 mg/mL) and *S. aureus* (MIC 1.3

Table 2. Antibacterial activity of tested essential oils and antibiotics (Mg/ml/µg/ml).

		Essential oil (mg/mL)		Antibiotic (µg/mL)	
Bacteria		*Origanum minutiflorum*	*Coriandrum sativum*	Amoxicillin with clavulanic acid	Cefixime
Micrococcus luteus	MIC	0.23	4.82	0.20	2.00
	MBC	0.46	9.65	0.40	3.00
Rothia mucilagenosa	MIC	0.69	0.60	7.00	2.00
	MBC	1.38	1.21	14.0	3.00
Streptococcus agalactiae	MIC	0.35	0.24	7.00	2.00
	MBC	0.69	0.56	14.0	4.00
Streptococcus anginosus	MIC	0.35	0.60	28.0	0.20
	MBC	0.69	1.21	56.0	0.30
Streptococcus constellatus	MIC	0.69	0.60	0.20	0.20
	MBC	1.38	1.21	0.40	0.40
Streptococcus dysgalactiae	MIC	0.05	0.24	7.00	0.20
	MBC	0.11	0.56	14.0	0.40
Streptococcus oralis	MIC	0.35	0.60	0.40	2.00
	MBC	0.69	1.21	1.00	3.00
Streptococcus parasanquinis	MIC	0.69	1.21	4.00	3.00
	MBC	1.38	2.41	7.00	6.00
Streptococcus pseudopneumoniae	MIC	0.69	1.21	1.00	13.0
	MBC	1.38	2.41	2.00	27.0
Streptococcus pyogenes	MIC	0.23	2.41	0.40	0.80
	MBC	0.46	4.82	0.80	2.00
Streptococcus salivarius	MIC	0.69	1.20	10.0	13.0
	MBC	1.38	2.41	14.0	27.0
Staphylococcus aureus	MIC	1.38	2.49	1.00	3.00
	MBC	2.77	5.03	2.00	6.00
Staphylococcus hominis	MIC	0.03	5.07	4.00	2.00
	MBC	0.05	9.65	7.00	3.00
Staphylococcus warnerii	MIC	0.46	2.41	0.10	3.00
	MBC	0.92	4.82	2.00	6.00
Enterobacter cloacae	MIC	0.69	1.20	28.00	3.00
	MBC	1.38	2.41	56.00	6.00
Stenotrophomonas maltophilia	MIC	0.69	1.20	3.00	3.00
	MBC	1.38	2.41	7.00	6.00

mg/mL) (32). Also, Pawar et al. (33), using the disc diffusion and microdilution methods, showed that coriander essential oil has moderate antibacterial activity against oral pathogens (*Lactobacillus acidophilus*, *S. salivarius*, *S. sanguinis*). Aelenei et al. (34) examined the effect of coriander oil on the growth inhibition of *S. aureus* isolates sensitive to methicillin and isolates resistant to methicillin, with the obtained MICs differing significantly (5.44–11.1 μg/mL). Deviations in MIC values obtained in our study and others research that are observed, may be consequence to different ratio of represented components in essential oil, different experimental protocols, and/or different strains of bacteria used.

The most common mechanisms of essential oils antimicrobial action are membrane damage and inhibition of the activity of certain enzymes. The exact sites of action are known for some essential oil compounds, while the detailed mechanism of action is still unknown (35).

Previous research on oregano and coriander EOs has been largely based on testing their antibacterial activity on food pathogens, but there are numerous studies that have examined the antimicrobial activity of various essential oils towards oral pathogens and have shown that essential oils inhibit the growth of different types of oral pathogens (species of the genera *Streptococcus* and *Staphylococcus*), which indicates that various essential oils can be used not only in the food industry, but also in otorhinolaryngology (16,36).

3.3. Antibacterial activity of a mixture of essential oils and antibiotics

In order to reduce the use of antibiotics as well as the occurrence of antibiotic resistance, the antibacterial effect of various combinations of essential oils with antibiotics, as well as the combination of two essential oils, was investigated. The interaction of the two tested substances (in this case essential oil + antibiotic; essential oil + essential oil) was determined by the FICI index describing synergistic (FICI <0.5), additive (0.5 < FICI ≤1), indifferent (1 < FICI ≤4) and antagonistic relationship (FICI >4). *O. minutiflorum* in combination with the antibiotic amoxicillin and clavulanic acid has an indifferent effect, as well as in combination with cefimixin (FICI = 1.008 and 2, respectively) (Table 3). Also, coriander EO in combination with commercial antibiotics exhibits an indifferent effect (FICI = 1.009 and 2), while essential oils in combination with each other exhibit additive activity (FICI = 0.65) (Table 3).

Based on literature data, oregano oil in combination with gentamicin has synergistic effect towards *B. cereus*, *B. subtilis* and *S. aureus* (37). Also, the same authors point out that the dominant component of oregano – carvacrol in combination with ampicillin, penicillin and bacitracin has a synergistic antibacterial effect against *S. aureus*, while in combination with erythromycin was effective against *S. pyogenes*. Since essential oils and their individual compounds generally damage cell membranes, and most antibiotics have specific sites of action (inhibition of DNA synthesis or specific proteins), synergism is thought to be due to their action at multiple sites in the cell (37). Aelenei et al. (34) showed that coriander essential oil in combination with amoxicillin and gentamicin acts synergistically on MRSA isolates. They also determined the synergistic effect of coriander and oxacillin and tetracycline oils on growth inhibition of *S. aureus* ATCC 33,591. Namely, the same authors examined the interaction of linalool, as the most common component of essential oil and various antibiotics, and concluded that linalool and oxacillin/amoxicillin/gentamicin/tetracycline or ciprofloxacin act synergistically in inhibiting the growth of MRSA isolates. Also, as shown in the interaction of coriander essential oil and gentamicin, and in the combination of linalool and gentamicin, linalool increases the sensitivity of *S. aureus* (i.e. reduces MIC), in contrast to the data obtained in this manuscript where synergism between essential oil and antibiotics was not detected. To the authors' best knowledge, this is the first report regarding wild oregano EO effect in the interaction with antibiotics.

Table 3. Synergistic potential of different combination of Eos and antibiotics.

Mixture of EOs antibiotics	FICI	Interaction effect
O. minutiflorum EO + Amoxicillin with clavulanic acid	1.008	Indifferent
O. minutiflorum EO + cefixime	2	Indifferent
C. sativum EO + Amoxicillin with clavulanic acid	1.009	Indifferent
C. sativum EO + cefixime	2	Indifferent
O. minutiflorum EO + *C. sativum* EO	0.65	Additive

3.4. Antibiofilm activity of essential oils

The potential of commercial essential oils to inhibit the formation of *S. aureus* biofilm, as well as their influence on the already formed *S. aureus* biofilm, was also investigated. Inhibitory effect of tested commercial essential oils in concentrations: MIC, 0.5 MIC and 0.25 MIC, is shown in Figure 1). The obtained results have shown that the essential oils inhibit formation of biofilm in concentration-dependent manner. Both essential oils applied in MIC values showed similar inhibition capacity towards the formation of *S. aureus* biofilm. *O. minutiflorum* essential oil tested at MIC concentrations; 0.5 MIC and 0.25 MIC inhibited the formation of

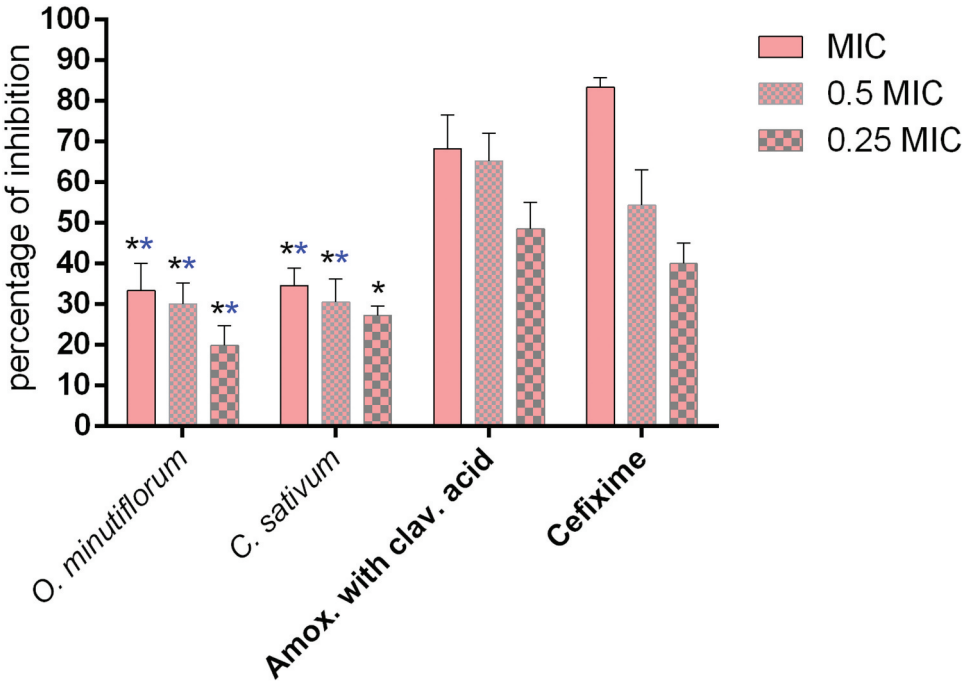

Figure 1. Percentage of inhibition of *S. Aureus* biofilm formation after treatment with Eos and antibiotics (0.25 MIC - MIC). The results are presented as Mean ± SD ($N = 3$). The asterisks represent statistical significance, *$p \leq 0.05$ compared with Amoxicillin with Clavulanic Acid; *$p \leq 0.05$ compared with cefixime.

S. aureus biofilm for 33.46%, 30.16%, 19.86%, respectively. Likewise, the antibiofilm activity of *C. sativum* EO was 34.63%, 30.64%, 27.25% at MIC concentrations, 0.5 MIC and 0.25 MIC, respectively (Figure 1). Essential oils showed weaker activity compared to commercial antibiotics whose inhibitory effect at MIC values was 68.30% for amoxicillin and clavulanic acid and 83.35% for cefixime (Figure 1).

The effect of short treatment (30 s) with EOs on 24 h old *S. aureus* biofilm is shown in Figure 2. Wild oregano essential oil compared to coriander essential oil, as well as compared to tested antibiotics showed better destructive effect in applied concentration (MBC). Namely, after 30 s of treatment with wild oregano essential oil, the destruction of the formed *S. aureus* biofilm was 61.96%. Antibiotics (amoxicillin with clavulanic acid and cefixime) had a similar inhibitory effect (61.14% and 59.05%, respectively), while coriander essential oil exhibited lower inhibitory potential towards *S. aureus* 24 old biofilm (49.28%) compared to wild oregano essential oil and antibiotics (Figure 2).

Currently, literature data on the inhibitory activity of *O. minutiflorum* EO against *S. aureus* biofilm is scarce. However, the activity of *O. marjorama* EO on the prevention of biofilm formation as well as on the inhibition of the already formed biofilm of MRSA isolates was investigated. *O. marjorama* oil has been shown to exhibit excellent effect on inhibition of formation (10.29–95.9%) and on destruction of already formed biofilm (98.01%) (38). Bazargani & Rohloff (39) examined the effect of *C. sativum* EO on the inhibition of *S. aureus* biofilm formation and on the metabolic activity of the biofilm. The authors found that coriander essential oil has good antibiofilm activity (inhibition percentage 91%), but also that it is very effective in weakening the metabolic activity of *S. aureus* cells adherent in the biofilm (38.3–72.6% inhibition).

3.5. Antioxidant activity of essential oils

The results of lipid peroxidation showed that only wild oregano EO has antioxidant activity (Table 4). The antioxidant activity of wild oregano EO has been insufficiently investigated in contrast to *O. vulgare* EO, for which there are abundant data on its antioxidant activity. Elgndi et al. (29) examined the antioxidant activity of *C. sativum* EO from Serbia by DPPH method and concluded that coriander seed essential oil has very low antioxidant activity ($IC_{50} = 39.15$ µg/mL), while the sample from India showed even lower activity ($IC_{50} = 47.2$ µg/mL) (40). By comparing the antioxidant activity of coriander EO obtained from seeds and essential oil obtained from leaves, it was found that essential oil from seeds has better activity (ability to capture radicals). It is believed that the reason for better activity is the higher percentage of linalool in the essential oil obtained from

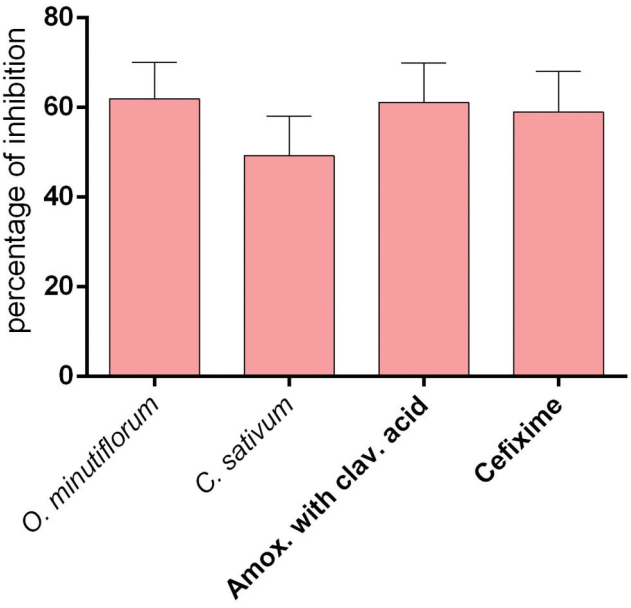

Figure 2. Percentage of destruction of *S. Aureus* pre-formed biofilms after 30 S treatment with Eos and antibiotics MBC. The results are presented as Mean ± SD ($N = 3$).

Table 4. Antioxidant activity of tested essential oils (Mean ± SD).

Essential oil	TBARS (IC_{50}mg/mL)
Origanum minutiflorum	0.250 ± 0.004
Coriandrum sativum	>400
Trolox	0.020 ± 0.001

coriander seeds (41). Namely, in addition to linalool, limonene, camphor and α-pinene are also responsible for the strong antioxidant activity of coriander EO (41).

3.6. Cytotoxic activity of tested essential oils

Given that bronchitis, one of the common complications of HT, is a risk factor for lung cancer (42), the effect of EOs on human tumor cell line NCI-H460 (cause of non-small cell lung cancer) was examined. Primary porcine liver cells (PLP2) were used as non-tumor primary cells to evaluate the cytotoxicity of the EOs. Essential oils of wild oregano and coriander showed moderate cytotoxic activity on the tested tumor cell line and PLP2 cell culture compared to the ellipticin used as a positive control (Table 5). By comparing the values of both tested oils it can be concluded that coriander essential oil has stronger cytotoxic activity (GI_{50} 74.46 µg/mL) on the tested NCI – H460 cell line than wild oregano oil (GI_{50} 81.44 µg/mL). In addition to the NCI-H460 cell line, cytotoxic activity was tested on other tumor cell lines with the aim of possible use of essential oils as adjuvants in the treatment of various malignant diseases. Coriander EO has shown

Table 5. Cytotoxic activity of tested essential oils (GI_{50} (µg/mL) ± SD).

	O. minutiflorum EO	*C. sativum* EO
Cytotoxicity to non-tumor cell line		
PLP2 (porcine liver primary culture)	151±4	140±7
Cytotoxicity to tumor cell lines		
HeLa (cervical carcinoma)	77±2	67±3
HepG2 (hepatocellular carcinoma)	85±1	73±3
MCF-7 (breast carcinoma)	81±4	71±3
NCI-H460 (non-small cell lung cancer)	81±4	74±2

GI_{50} values correspond to the sample concentration responsible for 50% inhibition of growth in a primary culture of liver cells-PLP2 or in human tumor cell lines. GI50 values for ellipticine (positive control): 3 µg/mL (PLP2), 1.0 µg/mL (MCF-7), 1.0 µg/mL (NCI-H460), 2.0 µg/mL (HeLa) and 1.0 µg/mL (HepG2).

slightly better cytotoxic potential against HeLa, MCF-7, and HepG2 tumor cell lines than wild oregano EO (Table 5).

O. minutiflorum oil has been shown to possess strong cytotoxic activity on HepG2, A549 and MCF-7 tumor lines (24). Koparal & Zeytinoglu (43) demonstrated that carvacrol inhibits the growth of A549 cells of the lung cancer cell line. However, it is important to point out that carvacrol in certain doses can be toxic to healthy cells. Elgndi et al. (29) investigated the cytotoxicity of *Satureja montana*, *Coriandrum sativum* and *Ocimum basilicum* oils and pointed out that coriander oil has the lowest toxicity towards HeLa cells (292.85 µg/mL), while it showed no activity on other tumor cell lines (MRC-5, K562). Since the tested essential oils showed a moderate cytotoxicity (GI_{50} 151.18 µg/ml and 139.72 µg/ml) on healthy liver cells, but in lower concentrations compared to the moderate cytotoxic activity (GI_{50} 67.32–85.43 µg/ml) on the tumor cell line, this indicates that the tested oils could find application as additional agents in antitumor therapy but additional care should be taken to avoid side effects.

4. Conclusion

The present study demonstrated biological potential of the *O. minutiflorum* and *C. sativum* essential oils. Both EOs represent a rich source of oxygenated monoterpenes that are known for many beneficial properties, including strong antibacterial activity. This is the first study reporting antibacterial and antibiofilm activities of the tested EOs against bacterial isolated from the infected tonsils. The EOs proved to be toxic to tumor cell lines, which makes them good candidates for further anticancer drug development. The findings of benefits suggest that the EOs may be considered as adjuncts to conventional

management of CT and that more research is warranted to determine the mechanism of action of the tested oils.

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Disclosure statement

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