

Antifungal and antibacterial activities of *Petroselinum crispum* essential oil

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ABSTRACT. Parsley [*Petroselinum crispum* (Mill.) Fuss] is regarded as an aromatic, culinary, and medicinal plant and is used in the cosmetic, food, and pharmaceutical industries. However, few studies with conflicting results have been conducted on the antimicrobial activity of parsley essential oil. In addition, there have been no reports of essential oil obtained from parsley aerial parts, except seeds, as an alternative natural antimicrobial agent. Also, microorganism resistance is still a challenge for health and food production. Based on the demand for natural products to control microorganisms, and the re-evaluation of potential medicinal plants for controlling diseases, the objective of this study was to determine the chemical composition and antibacterial and antifungal activities of parsley essential oil against foodborne diseases and opportunistic pathogens. Seven bacteria and

eight fungi were tested. The essential oil major compounds were apiol, myristicin, and β -phellandrene. Parsley essential oil had bacteriostatic activity against all tested bacteria, mainly *Staphylococcus aureus*, *Listeria monocytogenes*, and *Salmonella enterica*, at similar or lower concentrations than at least one of the controls, and bactericidal activity against all tested bacteria, mainly *S. aureus*, at similar or lower concentrations than at least one of the controls. This essential oil also had fungistatic activity against all tested fungi, mainly, *Penicillium ochrochloron* and *Trichoderma viride*, at lower concentrations than the ketoconazole control and fungicidal activity against all tested fungi at higher concentrations than the controls. Parsley is used in cooking and medicine, and its essential oil is an effective antimicrobial agent.

Key words: Chemical composition; Antifungal activity; Parsley; Antibacterial activity; *Petroselinum sativum*; Essential oil

INTRODUCTION

Petroselinum crispum (Mill.) Fuss, also referred to as *Petroselinum sativum* Hoffm., *Petroselinum vulgare* Lagasca, *Petroselinum crispum* (Mill.) Nyman ex A.W. Hill, *Apium petroselinum* L., *Carum petroselinum* (L.) Benth., Hook. f., and *Petroselinum hortense* Hoffm., belongs to the Umbelliferae family, and is commonly known as parsley. Parsley is regarded as an aromatic, culinary, and medicinal plant and is used in the cosmetic, food, and pharmaceutical industries. However, few studies with conflicting results have been conducted on the antimicrobial activity of parsley essential oil (Gutierrez et al., 2008; Viuda-Martos et al., 2011; Teixeira et al., 2013).

In previous studies in our laboratory, parsley essential oil showed antimicrobial activity against several bacteria such as *Escherichia coli*, *Listeria monocytogenes*, *Salmonella enterica*, *Enterobacter cloacae*, and *Pseudomonas aeruginosa* among others and some fungi (Linde GA, Gazim ZC and Colauto NB, unpublished results). However, it has been reported that essential oil from parsley aerial parts does not present antibacterial activity against these bacteria (Gutierrez et al., 2008; Teixeira et al., 2013). *P. aeruginosa* is an important opportunistic pathogen that causes diseases in animals (Bjarnsholt et al., 2010) and plants (Iglewski, 1996) as well as other bacteria cited above (Addis and Sisay, 2015). In addition, the *Aspergillus* and *Penicillium* genera are common causal agents of foodborne diseases and food spoilage that increase the cost of food production and healthcare throughout the world (Mead et al., 1999). In addition, the *Trichoderma* genus is the most common contaminant in mushroom cultivation and causes enormous economic losses around the world (Burt, 2004). However, there have been no reports of essential oil obtained from parsley aerial parts, except seeds, as an alternative natural product to control most of these fungi.

Furthermore, microorganism resistance is a challenge for health and food production, and the search for alternative natural antimicrobial agents continues (Ding et al., 2010; Soković et al., 2014). Synthetic fungicides, for instance, are generally effective and inexpensive, but it has been suggested that they leave residual toxicity that may have carcinogenic and teratogenic side effects (McCarroll et al., 2002). Moreover, most of the synthetic fungicides have been restricted or banned in several countries since the early 1960s (Glamočlija et al., 2011).

Based on the demand for natural products to control microorganisms, and the re-evaluation of potential medicinal plants for controlling diseases, the objective of this study was to determine the chemical composition and antibacterial and antifungal activities of parsley essential oil against foodborne diseases and opportunistic pathogens.

MATERIAL AND METHODS

Essential oil

P. crispum was deposited at Universidade Paranaense Herbarium, voucher specimen 2215. It was cultivated in a space of 0.3 x 0.4 m located at coordinates 23°46.225'S and 53°16.730'W and an altitude of 391 m. Parsley aerial parts (leaves, stems, and flowers without seeds) were harvested in the morning, immediately after early dew evaporation. Essential oil was obtained from fresh material (1.5 x 1.5 mm cut) by hydro distillation for 2 h in a Clevenger apparatus. After distillation, the essential oil was filtered through an anhydrous Na₂SO₄ bed and stored at -20°C in a closed amber glass vial. Essential oil yield was calculated in milliliters of oil per 100 g of fresh parsley (Camilotti et al., 2015).

Chemical identification of essential oil by gas chromatographic-mass spectrometric analysis

Gas chromatographic-mass spectrometric analysis (EI) was performed using an Agilent 5973 Network chromatographer coupled to an Agilent 5973 MSD mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). The separation was achieved using an Agilent 19091S-433 HP-5MS fused silica capillary column, 30 x 0.25 mm i.d., 0.25- μ m film thickness. The gas chromatographic oven temperature was programmed from 60° to 285°C at a rate of 4.3°C/min. Helium was used as the carrier gas. The inlet pressure was 25 kPa and the linear velocity was 1 mL/min at 210°C. The injector temperature was 250°C and the injection mode was splitless. The mass spectrometric scan conditions were as follows: source temperature, 200°C; interface temperature, 250°C; energy, 70 eV; and mass scan range, 40-350 amu (Tešević et al., 2009).

Antibacterial activity

The following bacteria were used: *Bacillus cereus* Frankland and Frankland, 1887 (clinical isolate), *E. cloacae* (Jordan, 1890) Hormaeche and Edwards, 1960 (clinical isolate), *E. coli* (Migula) Castellani and Chalmers (ATCC 35218), *L. monocytogenes* (Murray et al.) Pirie (NCTC 7973), *P. aeruginosa* (Schroeter) Migula (ATCC 27853), *S. enterica* subsp *enterica* (ex Kauffmann and Edwards) Le Minor and Popoff serovar Typhimurium (ATCC 13311), and *Staphylococcus aureus* subsp *aureus* Rosenbach (ATCC 6538). All microorganisms used in this study were obtained from the Culture Collection of the Mycological Laboratory, Department of Plant Physiology, Institute for Biological Research 'Siniša Stanković', Belgrade, Serbia. The antibacterial assay was performed using the microdilution method (CLSI, 2009; Tsukatani et al., 2012). The bacterial suspensions were adjusted with sterile saline to a concentration of 1.0 x 10⁵ CFU/mL. The inocula were prepared daily and stored at 4°C. Dilutions of inocula were cultured to check the validity of the inoculum.

Essential oil was dissolved in dimethyl sulfoxide (50 mg/mL) and Tween 80 (1 mg/mL), and added to 100 μ L tryptic soy broth (TSB) culture medium with a bacterial inoculum of 1.0×10^4 CFU per well. The minimum inhibitory concentration (MIC) obtained from the susceptibility test (Espinel-Ingroff, 2001) was also determined by the colorimetric microbial viability assay based on the reduction of INT (*p*-iodonitrotetrazolium violet) [2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride; Sigma] color and compared with positive controls for each bacterial strain (Janačković et al., 2015). The minimum bactericidal concentration (MBC) was determined by 2 μ L serial sub-cultivation into microtiter plates containing 100 μ L TSB per well and further incubation for 24 h. The lowest concentration without growth was defined as the MBC, indicating 99.5% killing of the original inoculum. The optical density of each well was measured at 655 nm by a Microplate Manager 4.0 (Bio-Rad Laboratories) and was compared with a blank control and positive controls. Streptomycin (Sigma™ P7794) and ampicillin (Panpharma™) were used as positive controls (1 mg/mL in sterile physiological saline). A solution of dimethyl sulfoxide (50 mg/mL) was used as a negative control.

Antifungal activity

For the antifungal bioassays, eight fungal species were used: *Aspergillus fumigatus* Fresenius (ATCC 1022), *Aspergillus niger* van Tieghem (ATCC 6275), *Aspergillus ochraceus* Wilhelm (ATCC 12066), *Aspergillus versicolor* (Vuillemin) Tiraboschi (ATCC 11730), *Penicillium funiculosum* Thom (ATCC 8725), *Penicillium ochrochloron* Biourge (ATCC 9112), *Penicillium verrucosum* var. *cyclopium* (Westling) Samson, Stolk & Hadlok (food isolate), and *Trichoderma viride* Pers. (IAM 5061). Fungi were maintained on malt agar medium culture, stored at 4°C, and sub-cultured once a month. In order to investigate antifungal activity, a modified microdilution technique was used (Espinel-Ingroff, 2001; Janačković et al., 2015). Fungal spores were washed from the agar plate surface with sterile saline solution (8.5 mg/mL) containing Tween 80 (1 mg/mL). A spore suspension was adjusted with sterile saline to a concentration of 1.0×10^5 spores/mL and stored at 4°C as inoculum for further use. Dilutions of inocula were cultured on solid malt agar to check inoculum validity. MIC determination was performed utilizing a serial dilution technique with 96-well microtiter plates and a solution containing dimethyl sulfoxide (50 mg/mL) with Tween 80 (10 mg/mL) added to broth malt (BM) culture medium with inoculum. Microplates were incubated on a rotary shaker (160 rpm) for 72 h at 28°C. The lowest concentrations without visible microbial growth in an optical microscope were defined as the MIC. Minimum fungicidal concentration (MFC) was determined by serial sub-cultivation of 2 μ L of tested compounds dissolved in BM inoculated for 72 h into microtiter plates containing 100 μ L BM per well and incubated at 28°C for 72 h. The lowest concentration with no microbial visible growth was defined as the MFC, indicating 99.5% killing of the original inoculum. A solution of dimethyl sulfoxide (50 mg/mL) was used as a negative control and the commercial fungicides bifonazole (Srbolek™) and ketoconazole (Zorkapharma™) were used as positive controls (1-3500 μ g/mL).

Statistical analysis

Three samples from each microbial strain were used, and every assay was carried out three times. The results are reported as the average and standard deviation. A comparison

among treatments for the same microbial strain was performed by the Tukey test ($P \leq 0.05$) using the SPSS v. 18.0 program.

RESULTS

Parsley essential oil yield was 0.2% and thirty compounds were identified consisting of 100.0% of volatile constituents (Table 1). Major compounds were apiol (50.3%), myristicin (14.0%), both phenylpropanoids, and β -phellandrene (14.6%), a terpenoid (Table 1).

Table 1. Identification of the chemical composition of *Petroselinum crispum* essential oil based on retention index (RI) and on comparison of mass spectra methods.

Peak	Compound*	RI	Composition (%)
1	n.i.	845	0.2
2	α -Pinene	913	2.1
3	β -Pinene	967	1.3
4	Myrcene	984	4.4
5	α -Phellandrene	1002	0.1
6	ρ -Cymene	1017	0.4
7	β -Phellandrene	1023	14.6
8	Terpinolene	1075	1.5
9	meta-Cymenene	1077	1.9
10	Cryptone	1183	1.6
11	β -Cyclocitral	1224	0.5
12	n.i.	1383	0.4
13	β -Elemene	1389	1.4
14	n.i.	1392	1.0
15	(E-)Caryophyllene	1412	0.3
16	n.i.	1466	0.3
17	Germacrene D	1480	0.4
18	α -Selinene	1494	0.2
19	Germacrene A	1506	0.8
20	Myristicin	1518	14.0
21	Carotol	1580	0.3
22	n.i.	1638	0.1
23	Selin-11-en-4 α -ol	1651	0.2
24	Apiol	1684	50.3
25	n.i.	1707	0.3
26	n.i.	1721	0.3
27	n.i.	1722	0.1
28	n.i.	1779	0.4
29	n.i.	1836	0.4
30	n.i.	1935	0.2
Total			100.0
Compound groups (%)			
Monoterpene hydrocarbons			26.50
Oxygenated monoterpenes			0.90
Sesquiterpene hydrocarbons			4.40
Oxygenated sesquiterpenes			0.30
Phenylpropanoids			64.40

*Compound listed in order of elution from a HP-5MS column; n.i. = unidentified compound.

Parsley essential oil inhibited the growth of all bacteria with MICs ranging from 0.04 to 1.00 mg/mL (Figure 1). Parsley essential oil showed bacteriostatic activity at lower concentrations than at least one of the controls against *L. monocytogenes*, *S. enterica*, and *S. aureus* (Figure 1). The essential oil killed all bacteria with MBCs ranging from 0.15 to 10.00 mg/mL (Figure 2). The most resistant bacteria were *E. cloacae* and *E. coli* with MBC values of

10.00 mg/mL (Figure 2). The most susceptible bacteria were *P. aeruginosa*, *S. enterica*, and *S. aureus* (Figure 2). The MBC value of essential oil against *S. aureus* was similar to ampicillin and 1.7 times lower than streptomycin (Figure 2).

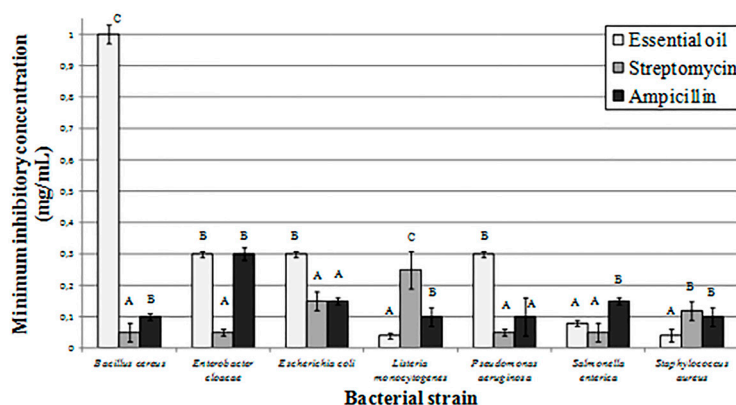


Figure 1. Minimum inhibitory concentration of *Petroselinum crispum* essential oil, streptomycin, and ampicillin on bacterial strains. Different letters above bars indicate statistically significant differences only among the treatments performed for each bacterial strain according to the Tukey test ($P \leq 0.05$).

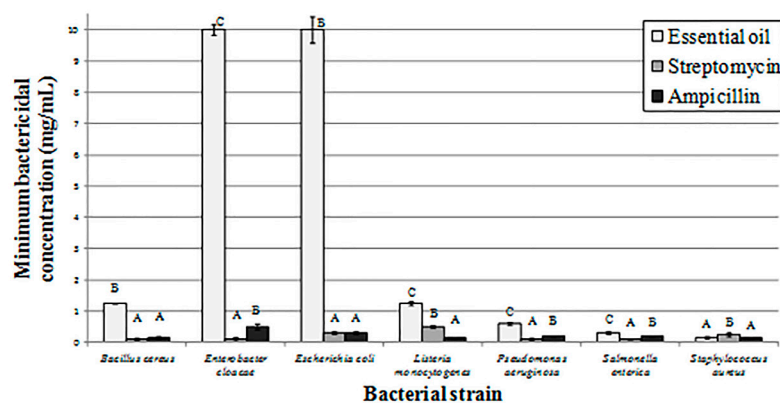


Figure 2. Minimum bactericidal concentration of *Petroselinum crispum* essential oil, streptomycin, and ampicillin of bacterial strains. Different letters above bars indicate statistically significant differences only among the treatments performed for each bacterial strain according to the Tukey test ($P \leq 0.05$).

Parsley essential oils showed fungistatic activity against all fungi, mainly *P. ochrochloron* and *T. viride* (Figure 3). *P. ochrochloron* and *T. viride* had MIC values at low concentrations of essential oil compared with the ketoconazole control (Figure 3). For the fungicidal activity, parsley essential oil was effective against all fungi, mainly *P. funiculosum* and *T. viride*, with MFCs of 1.25 and 2.50 mg/mL, respectively (Figure 4). However, in general, fungicidal concentrations of the essential oil ranged from 5 to 62.5 times higher than the controls (Figure 4).

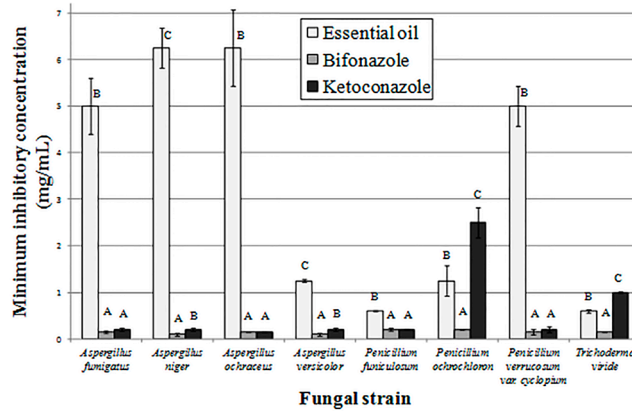


Figure 3. Minimum inhibitory concentration of *Petroselinum crispum* essential oil, bifonazole, and ketoconazole of fungal strains. Different letters above bars indicate statistically significant differences only among the treatments performed for each fungal strain according to the Tukey test ($P \leq 0.05$).

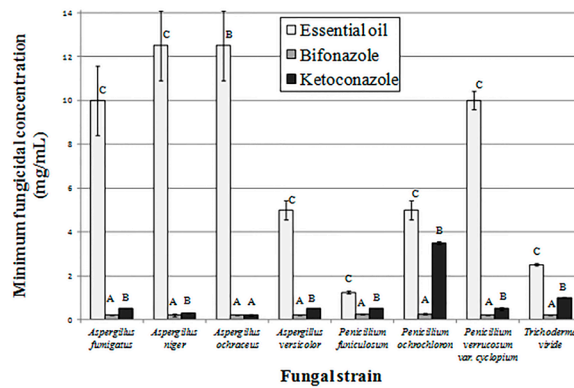


Figure 4. Minimum fungal concentration of *Petroselinum crispum* essential oil, bifonazole, and ketoconazole of fungal strains. Different letters above bars indicate statistically significant differences only among the treatments performed for each fungal strain according to the Tukey test ($P \leq 0.05$).

DISCUSSION

The yield of parsley essential oil obtained from aerial parts ranges from 0.03 to 3.2% (Petropoulos et al., 2009; Viuda-Martos et al., 2011; Vokk et al., 2011). These results indicate a great variation for this species, and that our findings were within the normal range. The major compounds reported for parsley essential oil obtained from aerial parts, except seeds, vary widely in quantity and composition worldwide (Table 2). Our results showed apiol (50.3%), myristicin (14.0%), and β -phellandrene (14.6%) in Brazil. This diverse chemical variation could be related to genetic and environmental factors affecting the secondary metabolism of this species, harvesting time of the biological material in different development stages, interaction with microorganisms and insects, and different post-harvest techniques, as suggested by Morais and Castanha (2012) and Camilotti et al. (2015). Although there are

variations in the major compounds of parsley essential oil worldwide, apiol and/or myristicin have always been among the major constituents.

Table 2. Major compounds reported for parsley essential oil obtained from aerial parts, except seeds.

Country	Major chemical compounds (%)								Reference
	Apiol	Myristicin	<i>p</i> -mentha-1,3,8-triene	Sabinene	α -pinene	β -phellandrene	β -pinene	1-allyl-2,3,4,5-tetramethoxybenzene	
Brazil	41.0	5.1	-	-	-	-	-	-	Camilotti et al. (2015)
China	17.5	32.8	-	-	16.6	-	11.5	10.0	Zhang et al. (2006)
Egypt	46.5	-	-	-	22.2	-	16.1	-	Viuda-Martos et al. (2011)
Greece	-	0.1-27.7	-	-	-	26.7-40.3	-	-	Petropoulos et al. (2009)
Lebanon	7.6	-	8.2	21.1	-	-	-	-	Knio et al. (2008)
Spain	34.3	11.2	13.3	-	-	21.9	-	-	Díaz-Maroto et al. (2002)

There are few reports on the antibacterial activity of parsley essential oil obtained from aerial parts, except seeds. Viuda-Martos et al. (2011), using the disc diffusion method, reported that essential oil of parsley aerial parts (without seeds) had no antibacterial activity against *Listeria innocua*, *Serratia marcescens*, and *Pseudomonas fluorescens*. Gutierrez et al. (2008), using a microtitre plate test, reported no antibacterial activity of commercial parsley essential oil against *Enterobacter aerogenes*, *E. cloacae*, *E. coli*, *P. aeruginosa*, *P. fluorescens*, *Pseudomonas putida*, *S. enterica*, *B. cereus*, *Lactobacillus brevis*, *Lactobacillus plantarum*, and *S. aureus*. Teixeira et al. (2013), using the disc diffusion method, reported no antibacterial activity of commercial essential oil from parsley aerial parts, without seeds, on *E. coli*, *P. putida*, and *S. enterica*, but bacteriostatic activity against *Brochothrix thermosphacta*, *L. innocua*, *L. monocytogenes*, and *Shewanella putrefaciens*. Hogg (2005) suggested that resistance of Gram-negative bacteria could be due to the complexity of their double layer cell membrane in comparison with the single membrane of Gram-positive bacteria. Our study partially agrees with Hogg as the most resistant bacteria to parsley essential oil were two Gram-negative bacteria: *E. cloacae* and *E. coli* (Figure 2). However, Gram-negative bacteria such as *P. aeruginosa* and *S. enterica* were susceptible as well as Gram-positive bacteria such as *B. cereus*, *L. monocytogenes*, and *S. aureus* (Figure 2). On the other hand, these different results regarding antibacterial activity among different authors could be related to distinct composition of the essential oil. In regards to Teixeira et al. (2013), the parsley essential oil composition was different from our results in which the main compounds were 4-methoxy-6-(2-propenyl)-1,3-benzodioxole (45.1%), 1,2,3,4-tetramethoxy-5-(2-propenyl)-benzene (16.9%), and apiol (29.9%), and this variation could have affected the antimicrobial activity. Thus, the industrial food production methods for natural plant extracts should control for biotic and abiotic factors in order to avoid variation in the essential oil composition.

The antimicrobial methods could also affect MIC and MBC values. The disc diffusion test used by Teixeira et al. (2013) and Viuda-Martos et al. (2011) is simple, convenient, and has been well-standardized but presents qualitative results and approximate MIC values (Jorgensen and Ferraro, 2009). In our study, a microdilution test, a quantitative test that allows for higher accuracy and reproducibility, was used to determine MIC and MBC values (Jorgensen and Ferraro, 2009). The parsley essential oil in our study showed high bactericidal activity mostly against *P. aeruginosa*, *S. enterica*, and *S. aureus*. These bacteria are common food pathogens and produce a wide variety of toxins (Al-Qurainy et al., 2013). *S. aureus* - for instance - is heat resistant, can live at low pH with activity in the digestive tract after ingestion,

and is a common cause of skin infection and food poisoning (Evenson et al., 1988; Al-Qurainy et al., 2013). Thus, parsley essential oil could be an alternative to control these bacteria. Furthermore, several bacteria are becoming resistant to methicillin, which is a new challenge for the food industry (Martin et al., 2014). In our study, the antibacterial activity of parsley essential oil - a plant that is widely used as fresh green sprigs to garnish and aromatize foods - could be an alternative to control bacteria during food processing, storage, and/or packing. Moreover, according to Al-Qurainy et al. (2013), the use of crude plant extracts and essential oils can be used to reduce the use of synthetic chemical compounds in the preservation of foodstuff. Synthetic food preservatives are effective and inexpensive, but they can be also toxic for animals, the environment, and increase microorganism resistance (Aarestrup, 2000).

The lowest essential oil concentration with fungistatic and fungicidal activities was against *P. funiculosum* and *T. viride*. These fungi are common causal agents of foodborne diseases, food spoilage, and opportunistic pathogens. In addition, *T. viride* is the most common contaminant in mushroom cultivation (Burt, 2004). It can grow on compost and/or the casing layer, contaminating mushroom production and result in enormous losses. In order to maintain mushroom yield, fungicides such as benomyl (Benlate®) and prochloraz (Sporgon®) are used. However, it has been suggested that they cause carcinogenic side effects (Soković and Van Griensven, 2006). Thus, parsley essential oil could be an alternative to control fungal contamination or diseases as a result of green mold in mushroom cultivation. In addition to its beneficial culinary and medicinal uses, this report demonstrates the potential use of *P. crispum* as an antimicrobial agent. Other studies on the use and application of parsley essential oil on a larger scale are encouraged.

CONCLUSIONS

A parsley essential oil yield of 0.2% has antibacterial and antifungal activities. The major compounds of the essential oil are apiol and myristicin, both phenylpropanoids, and β -phellandrene, a terpenoid. *P. crispum* essential oil had bacteriostatic activity against all tested bacteria, mainly *S. aureus*, *L. monocytogenes*, and *S. enterica*, at similar or lower concentrations than streptomycin or ampicillin. Bactericidal activity was effective against all tested bacteria, mainly *S. aureus*, at similar or lower concentrations than streptomycin or ampicillin. This essential oil also had fungistatic activity against all tested fungi, mainly *P. ochrochloron* and *T. viride*, at lower concentrations than the ketoconazole control. Fungicidal activity of the essential oil was effective for all tested fungi but requires a higher concentration than the one used for the bifonazole and ketoconazole controls. Considering the culinary and medicinal properties of parsley, this report expands its importance as an alternative antimicrobial agent.

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