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Parsley (*Petroselinum crispum*): chemical composition and antibacterial activity of essential oil from organic against foodborne pathogens

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Abstract

Microbial contamination is a serious problem for food industries, potentially leading to foodborne diseases that affect human health. *Petroselinum crispum*, popularly known as parsley, can be used for the production of biologically active essential oil. Considering the demand for novel natural products to control microorganisms, this study aimed to analyze the chemical composition and antibacterial activity of *P. crispum* essential oil from organic cultivation. Essential oil was extracted by hydrodistillation from the aerial part of *P. crispum* cv. plain (plain leaf type) at 70 days of age and analyzed for chemical composition by gas chromatography–mass spectrometry (GC-MS). Antibacterial activity was evaluated against five bacterial pathogens by the broth microdilution method. The essential oil yield was 0.02% and the major compounds were apiol (61.94%) and myristicin (9.33%). The minimum inhibitory concentration (MIC) of essential oil ranged from 1.70 to 10.00 mg mL^{-1}, with the best activities against *Staphylococcus epidermidis* and *Staphylococcus aureus*. These results demonstrate that apiol-rich essential oil from organic *P. crispum* shows promise as an antibacterial agent in food and pharmaceutical industries.

Keywords: Apiol, Foodborne diseases, Myristicin, Parsley, Phenylpropanoid.
Abbreviations: GC-MS, gas chromatography–mass spectrometry; MIC, minimum inhibitory concentration.

Introduction

Foodborne illnesses caused by ingestion of pathogenic microorganisms are a major global public health problem (Eleftheriadou et al., 2017; Pisoschi et al., 2018). In developing countries, such illnesses cause an estimated 2.2 million deaths per year, 86% of which are of children (Singh and Mondal, 2019). These alarming numbers highlight the need for disease monitoring and prevention (Schirone et al., 2019). Furthermore, microbial deterioration reduces the shelf life of food products and results in the waste of 30 to 50% of food supply (Krepker et al., 2017; Fung et al., 2018; Eleftheriadou et al., 2017). The impact generated by unsafe foods amounts to about US$95 billion annually in low- and middle-income countries (Jaffee et al., 2019). One of the Sustainable Development Goals proposed by the United Nations is to reduce worldwide per capita food waste by half in all sectors of the food chain by 2030, representing a sustainable manner of decreasing loss of natural resources (Santos et al., 2020).

Emergence of antimicrobial resistance in foodborne pathogens stemming from inadequate use of antibiotics constitutes a worldwide problem and represents a challenge for food security (Prestinaci et al., 2015; Chouhan et al., 2017) and a risk to human quality of life (Costa and Silva Junior, 2017). Food preservatives are used to inhibit or delay chemical and biological deterioration of food, thereby extending shelf life (Davidson et al., 2004). Synthetic antimicrobial preservatives are commonly used, but they are known to exert toxic effects (Bensid et al., 2020). Nitrates and nitrites are present in drinking water, natural and processed foods, air, and soil. Increased application of inorganic fertilizers and animal manure on agricultural land are the main factors contributing to the increase in nitrate concentration in rivers and soils (Parvizishad et al., 2017). Nitrite, when present in drinking water, may cause adverse health effects, leading to an increase in the occurrence of diseases in humans, such as colorectal cancer, thyroid disease, neural tube defects (Ward et al., 2018), and methemoglobinemia (Parvizishad et al., 2017).

New strategies are being explored by food industries to select effective natural antimicrobials that contribute to product safety and prolong shelf life (Shen et al., 2017; Pisoschi et al., 2018; Frederico et al., 2021). Thus, it is necessary to investigate natural resources, such as plants, to potentially identify novel antimicrobial compounds.

Petroselinum crispum (Mill.) Fuss, a medicinal aromatic plant popularly known as parsley and belonging to the family Apiaceae, is a herb native to the Mediterranean region and cultivated around the world (Mahmood et al., 2014). The herb is widely used as a culinary spice and flavoring agent

(Eddouks et al., 2017). *P. crispum* is rich in vitamin B, vitamin C, β-carotene, and zinc; the plant represents an important dietary component for bone strength because of its high content of boron and fluorine and presence of iron and calcium (Daradkeh and Essa, 2016). Some of its biological properties include cytoprotective, cardioprotective, hepatoprotective, nephroprotective, neuroprotective, spasmolytic, immunomodulating, antidiabetic (Farzaei et al., 2013), diuretic, antibacterial, and antifungal effects (Cardoso et al., 2005; Lorenzi and Matos, 2008; Abdellatief et al., 2017). Few studies, however, have assessed the antibacterial activity and inhibitory potential of *P. crispum* essential oil (Viuda-Martos et al., 2011; Teixeira et al., 2013; Linde et al., 2016). This study aimed to analyze the chemical composition and antibacterial activity of essential oil from organically grown *P. crispum*.

Results

Essential oil yield and chemical composition

The essential oil yield from aerial parts of *P. crispum* was 0.02% (w/w). Gas chromatographic analysis revealed 24 compounds. The major identified were apiol (61.94%) and myristicin (9.33%) (Table 1).

Antibacterial activity of essential oil

The minimum inhibitory concentration (MIC) values for the essential oil of *P. crispum* ranged from 1.70 to 10.00 mg mL^{-1} (Table 2), whereas those of streptomycin and sodium nitrite were 0.003 - 0.50 mg mL^{-1} and 5 mg mL^{-1}, respectively. The lowest MIC values of essential oil were observed against *Staphylococcus epidermidis* (1.70 mg mL^{-1}) and *Staphylococcus aureus* (3.33 mg mL^{-1}), being 3 and 1.5 times lower than that of sodium nitrite, respectively. *Salmonella* Typhi was the most resistant bacterium with MIC of 10.00 mg mL^{-1}.

Discussion

Essential oil yields can vary depending on plant genetics, environmental characteristics, climatic conditions (e.g., temperature and luminosity) seasonality, plant developmental stage, harvest time, soil, and plant nutrition (Baser and Buchbauer, 2010; Morais, 2009). The yield of essential oil from *P. crispum* aerial parts has been reported to range from 0.03% to 3.2% (Petropoulos 2010; Viuda-Martos et al., 2011; Borges et al., 2016; Linde et al., 2016; Ascrizzi et al., 2018; Pineda et al., 2018). Such findings indicate variations in essential oil yield from this species. The yield obtained in this study was slightly lower than the normal range, probably because of the above-mentioned factors.

Petropoulos et al. (2010) investigated the effect of nitrogen on essential oil concentration and observed that yield also depends on plant type (subspecies) and tissues used for extraction. Petropoulos et al. (2008) found that the essential oil yield of flat and curly leaf cultivars increased from 0.04 to 0.07% and from 0.05 to 0.11%, respectively, under water stress conditions. The essential oil yield of *P. crispum* grown in winter was lower (0.24%) than that of plants grown in summer (0.29%) (Vokk et al., 2011).

The chemical composition of aromatic species is influenced by location, seasonality, stage of development, climate, time of day, nutrients, and other factors (Oliveira et al., 2012; Fonseca et al., 2007). Major compounds may differ depending on the response of plants to environmental conditions, which influence secondary metabolism (Morais and Castanha, 2012). In the study of Camilotti et al. (2015), the major compounds of *P. crispum* essential oil were apiol (41.05%) and myristicin (5.08%), accounting for 52.07% of the total composition. In the current study, the major compounds of *P. crispum* essential oil were also apiol (61.94%) and myristicin (9.33%). Although literature data show that *P. crispum* oil composition can vary greatly, apiol and myristicin generally appear as the major compounds, in agreement with our results (Table 3).

Apiol is a phenylpropanoid found in the roots, seeds, and leaves of *P. crispum* (Punoševac et al., 2021), as well as in plants of the families Lauraceae (Xavier et al., 2020) and Piperaceae (Silva et al., 2017). The compound exerts antiproliferative (Wu et al., 2019), antioxidant, antibacterial, antihyperlipidemic, antihypercholesterolemic, antimycobacterial, chemopreventive, antidiabetic, anti-inflammatory, and antifungal effects (Pineda et al., 2018), being applied in the treatment of uterus diseases and cervical ectropion (Prinsloo et al., 2018). The mechanism of action of phenylpropenes in bacteria consists in the destabilization of cell membranes (Gharib et al., 2017), bacterial efflux pumps (Gill and Holley, 2004), and the GTPase cell division protein FtsZ (Hemaiswarya et al., 2011).

To the best of our knowledge, few studies have used the microdilution method to assess the antibacterial activity of *P. crispum* essential oil. Such a method was applied by Linde et al. (2016), who found that essential oil from the aerial parts of *P. crispum* (without seeds) inhibited the growth of all tested bacteria, with MIC values ranging from 0.04 to 1.00 mg mL^{-1}. The most susceptible bacteria were *Listeria monocytogenes*, *Salmonella enterica*, and *Staphylococcus aureus;* and the most resistant were *Enterobacter cloacae* and *Escherichia coli*.

Other researchers used the disc diffusion method, which is simple and convenient (Jorgensen and Ferraro, 2009) but provides only qualitative results, with approximate MIC values (Balouiri et al., 2016). Teixeira et al. (2013) reported that commercial essential oil from *P. crispum* aerial parts had no antibacterial activity against *E. coli* or *Salmonella* Typhimurium. In the study of Viuda-Martos et al. (2011), *P. crispum* essential oil showed no activity against *Listeria innocua*, *Serratia marcescens*, or *Pseudomonas fluorescens*. Marín et al. (2016) reported that commercial essential oil from organic *P. crispum* had low activity against *L. innocua* but showed no inhibitory effect on *P. fluorescens*. Nawel et al. (2014) found that *P. crispum* essential oil showed high antimicrobial action against *Bacillus cereus*, average effectiveness against *Clostridium perfringens*, *S. aureus*, and *Enterococcus faecalis*, and no activity against *E. coli*.

Gram-negative bacteria are generally more resistant to essential oils than Gram-positive bacteria (Trombetta et al., 2005; Nazzaro et al., 2013), as also observed in our study. Such differences are found to occur because of the complexity of the cell wall of Gram-negative bacteria, which hinders the action of essential oils (Nazzaro et al., 2013; Oussalah et al, 2007). The structure of Gram-positive bacterial cell walls allows hydrophobic molecules to easily penetrate cells and act on the cell wall and within the cytoplasm (Nazzaro et al., 2013). Furthermore, variations in essential oil composition may affect biological activity. Abiotic and biotic factors should be considered in the

Table 1. Chemical composition from the essential oil of *Petroselinum crispum* aerial parts.

Peak	Compounds	Molecular weight	Chemical formula	RI calculated	RI theoretical	Area (%)
1	n.i			748		0.35
2	Camphene	136.23	$C_{10}H_{16}$	889		0.45
3	β-pinene	136.23	$C_{10}H_{16}$	946	974	1.70
4	Myrcene	136.23	$C_{10}H_{16}$	980	988	0.71
5	p-Cymene	134.22	$C_{10}H_{14}$	1019	1020	2.68
6	β-Phellandrene	136.23	$C_{10}H_{16}$	1022	1025	0.95
7	γ-Terpinene	136.23	$C_{10}H_{16}$	1048	1054	0.45
8	m-Cymenene	132.20	$C_{10}H_{12}$	1081	1082	0.59
9	Terpinolene	136.23	$C_{10}H_{16}$	1089	1086	1.34
10	Camphor	152.23	$C_{10}H_{16}O$	1141	1141	0.69
11	Camphene hydrate	154.25	$C_{10}H_{18}O$	1152	1145	2.83
12	Terpinen-4-ol	154.25	$C_{10}H_{18}O$	1161	1174	3.15
13	Myrtenal	150.22	$C_{10}H_{14}O$	1166	1195	1.06
14	Germacrene d	204.35	$C_{15}H_{24}$	1498	1480	0.98
15	α-Muurolene	204.35	$C_{15}H_{24}$	1501	1500	2.67
16	γ-Cadinene	204.35	$C_{15}H_{24}$	1511	1513	1.15
17	Cubebol	222.37	$C_{15}H_{26}O$	1516	1514	1.47
18	Myristicin	192.08	$C_{11}H_{12}O_3$	1524	1518	9.33
19	Elemicin	208.25	$C_{12}H_{16}O_3$	1540	1548	1.01
20	Apiole	222.09	$C_{12}H_{14}O_4$	1610	1620	61.94
21	n.i			1780		1.61
22	n.i			1880		1.20
23	n.i			1887		0.92
24	n.i			1898		0.70
Total identified						100
Monoterpenes hydrocarbons						8.87
Monoterpenes oxygenated						7.73
Sesquiterpenes hydrocarbons						4.80
Sesquiterpenes oxygenated						1.47
Phenylpropanoids						72.28

RI calculated = identification based on the calculated retention index (RI) utilizing a standard homologous series of n-alkanes C7-C28 in Agilent HP-5MS UI column. RI theoretical= identification based on the comparison of mass spectra found in NIST 11.0 libraries (Adams, 2017). Area (%) = percentage of the area occupied by the compounds in the chromatogram; n.i. = non-identified.

Table 2. Minimum inhibitory concentration (MIC) from *Petroselinum crispum* aerial parts essential oil and positive control streptomycin and sodium nitrite.

Bacterium	Essential oil (mg mL^{-1})	Streptomycin (mg mL^{-1})	Sodium nitrite (mg mL^{-1})
Staphylococcus aureus	3.33 ± 0.44^{b}	0.01 ± 0.001^{a}	5.00 ± 0.01^{c}
Escherichia coli	5.00 ± 0.00^{b}	0.003 ± 0.001^{a}	5.00 ± 0.00^{b}
Bacillus cereus	6.66 ± 0.88^{c}	0.01 ± 0.004^{a}	5.00 ± 0.02^{b}
Salmonella Typhi	10.00 ± 0.00^{c}	0.12 ± 0.10^{a}	5.00 ± 0.01^{b}
Staphylococcus epidermidis	1.70 ± 0.25^{b}	$>0.50 \pm 0.00^{a}$	5.00 ± 0.01^{c}

*Averages followed by different letters in the same row for MIC differ by Tukey's HSD (honestly significant difference) teste ($p \leq 0.05$).

Table 3. Major compounds from *Petroselinum crispum* essential oil aerial parts obtained by hydrodistillation.

Major chemical compounds	Country	Source
myristicin (32.75%), apiol (17.54%), α-pinene (16.64%), β-pinene (11.54%) and 1-allyl-2,3,4,5-tetrameth-oxy-benzene (10.00%).	China	Zhang et al. (2006)
apiol (50.3%), myristicin (14.0%), and β-phellandrene (14.6%)	Brazil	Linde et al. (2016)
1,3,8-p-menthatriene (24.2%), β-phellandrene (22.8%), apiol (13.2%), myristicin (12.6%), terpinolene (10.3%) and β-pinene (2.2%).	Tunísia	Snoussi et al. (2016)
α-Pinene (3.1-13.9%), β-Pinene (1.6-30.6%), β-Myrcene (27.2–4.6%), β-phellandrene (39.0–22.0%), α-p-dimethylstyrene (11.6-12.7%)	Grécia	Petropoulos (2004)
myrcene (23.8%), myristicin (39.7%), α-pinene (6.94%), β-pinene (4.57%), α-phellandrene (1.11%), 1.3.8 p-menthatriene (17.1%), dillapiole (1.03%), bisabolole (0.71%) and camphor (0.11%)	Egito	Nawel et al. (2014)
α-pinene (32%) and β-pinene (19%) myristicin (18%), apiole (10%) and 1-allyl-2,3,4,5, -tetramethoxybenzene (13%)	Brasil	Kurowska (2006)
β-pinene (3.35%), β-myrcene (6.76%), β-phellandrene (25.07%), ρ-1,3,8-menthatriene (5.49%), myristicin (28.63%) and apiol (2.91%)	Brasil	Filho et al. (2018)
Parsley- or dill-apiole (43.25%) Myristicin or sarisan (30.8%) p-cymene (4.4%) Melilotal (3.8%)	Colombia	Pineda (2018)
myristicin (30.7–42.7%), β-phellandrene (21.8–35.9%), p-1,3,8-menthatriene (5.4–10.0%), and β-myrcene (4.5–8.7%).	Estonia	Vokk et al., (2011)

production of natural compounds for use in industrial food processes (Linde et al., 2016). Our results revealed that *P. crispum* essential oil shows antibacterial activity mainly against *S. epidermidis* and *S. aureus*, two food pathogens. Given that their natural habitat is the skin and mucous membrane of animals, these microorganisms are often found in raw meat and milk, multiplying during fermentation processes (Gazzola and Cocconceli, 2008). *S. epidermidis* is an environmental microorganism part of the normal flora of the skin (Kannappan et al., 2020). The pathogen is known to cause medical device-related infections (Dobinsky et al., 2003) because of its ability to form biofilms (Otto, 2019). It has emerged as one of the most important opportunistic pathogens, owing to its capacity to attach to industrial equipment surfaces (Gomes, et al., 2011; Zou and Liu, 2018). Thus, the ability of *S. epidermidis* to form biofilms plays a key role in food contamination (Zou et al., 2019). *S. aureus* is one of the main causes of foodborne illness outbreaks associated with foods contaminated with enterotoxins, such as meat products, poultry, eggs, dairy products, and bakery products (Greig et al. 2007). In the current study, the MIC values of essential oil against *S. aureus* and *S. epidermidis* were 1.50 and 3 times lower, respectively, than those of sodium nitrite. Nitrite can oxidize hemoglobin to methemoglobin, reducing the amount of oxygen in the blood, possibly leading to coronary ischemia and stroke (Katabami et al., 2016). Furthermore, nitrite can be converted into nitrosating agents and form *N*-nitrous compounds, especially *N*-nitrosamines in processed meat, which may exert carcinogenic and mutagenic effects (Chetty et al., 2019). Thus, *P. crispum* essential oil could be a natural alternative to control these bacteria.

Materials and methods

Plant material

Aerial parts (leaves and stems) of *P. crispum* cv. plain (plain leaf type) at 70 days of age were acquired from an organic system located at coordinates 23°43'35.1"S and 53°34'43.6"W. The plant material was harvested in the morning, immediately after dew evaporation, in July 2020.

Essential oil extraction

For essential oil extraction, aerial parts were dried at room temperature. Then, 200 g of dry material was ground in an industrial blender with 2.5 L of reverse osmosis-deionized water and hydrodistilled for 2 h in a Clevenger apparatus (Linde et al., 2016). The oil was withdrawn from the apparatus with *n*-hexane, filtered through anhydrous sodium sulfate (Na_2SO_4), stored in an amber flask, and kept at -4 °C until complete solvent evaporation. Essential oil yield (%) was calculated according to Eq. 1:

$$Yield\ (\%) = \frac{Essential\ oil\ weight\ (g)}{Plant\ weight\ (g)} x100 \quad (1)$$

Chemical composition of *Petroselinum crispum* essential oil

Chemical identification of essential oil components was carried out using a gas chromatograph (Agilent 7890B) coupled to a mass spectrometer (Agilent 5977A MSD) (GC-MS). Separation was achieved using an HP5-MS UI 5% capillary column (30 m × 250 µm × 0.25 µm; Agilent Technologies). The oven temperature was set at 60 °C, increased to 280 °C at 3 °C min^{-1}, and maintained at this temperature for 1 min. Helium was used as carrier gas at 300 °C and a linear speed of 1 mL min^{-1} with a pressure release of 8.23 psi. The injector temperature was 220 °C. Sample injection (1 µL) was performed in split mode (20:1), and the injector temperature was kept at 220 °C. The temperatures of the transfer line, ion source, and quadrupole were 260, 230, and 150 °C, respectively. Mass detection was performed in scan mode in the range of 40 to 500 *m/z* with a solvent delay of 3 min (Linde et al., 2016). Compounds were identified by comparison of their mass spectra with data from the NIST 11.0 library and comparison of their retention indices with those of a standard series of homologous *n*-alkanes (C7–C28) (Adams, 2017).

Antibacterial activity

Microorganisms

The antibacterial activity of essential oil was tested against five bacterial strains: *Staphylococcus aureus* NEWP 0023, *Escherichia coli* ATCC 1284, *Bacillus cereus* ATCC 14579, *Salmonella* Typhi NEWP 0028, and *S. epidermidis* ATCC 12228. For the assays, bacterial cells were cultured for 8 h and the cell pellet was collected. The concentration of bacterial cells was adjusted to 0.5 on the McFarland scale (1.5×10^8 colony-forming units, CFU mL^{-1}) with sterile saline by measuring the absorbance at 625 nm on a spectrophotometer (Spectra Max Plus). Then, the suspension was diluted 1:10 in Mueller–Hinton broth to obtain a cell density of 1.5×10^5 CFU mL^{-1}, and the inoculum was used in the assays.

Determination of antibacterial activity by the broth microdilution method

The MIC of *P. crispum* essential oil against the above-mentioned microorganisms was determined by serial microdilution in 96-well microplates. For each microorganism, a standard suspension was prepared in saline solution as previously described. The MIC was determined according to the broth microdilution method (CLSI, 2018) modified for natural products. The essential oil was dissolved in 2% (v/v) Tween 80 and tested at final concentrations ranging from 0.078 to 10 mg mL^{-1} in a total volume of 100 µL (culture medium and sample). After serial dilution, 50 µL of inoculum was added to each well, and plates were incubated at 35 °C for 24 h. Then, 10 µL of 1.0% 2,3,5-triphenyltetrazolium chloride (Reatec®) indicator was added to each well, and microplates were further incubated for 10 min at 37 °C. The MIC was defined as the lowest concentration that inhibited bacterial growth as assessed visually. The antibiotics streptomycin (Sigma) (0.0039 to 0.50 mg mL^{-1}) and sodium nitrite (5 to 50 mg mL^{-1}) were used as positive controls.

Statistical analysis

Antibacterial assays were performed in triplicate. Results are expressed as arithmetical mean and standard deviation. Data were subjected to one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference test at the 5% level. Statistical analyses were carried out using Statistica® software version 8.0.

Conclusion

P. crispum contained 0.02% essential oil. Chromatographic analysis identified 24 compounds, of which the major were apiol and myristicin. The essential oil showed antibacterial

activity, mainly against *S. epidermidis* and *S. aureus*. This study demonstrated the potential of essential oil from *P. crispum* as an alternative antimicrobial agent for food, agricultural, and pharmaceutical applications.

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