Bilge International Journal of Science and Technology Research
ISSN: 2651-401X
e-ISSN: 2651-4028
Research Article
2023, Volume: 7, Issue: 1, 1-8
Received: 22.11.2022; Accepted: 06.03.2023
DOI: 10.30516/bilgesci.1208483

Antifungal Activities of Essential Oil Obtained from *Mentha spicata* var. *Crispa* against Selected *Penicillium* Species

Hana Ďúranová[1*], Veronika Valková[1,2], Lucia Galovičová[2], Nenad L. Vukovic[3], Milena Vukic[3], Miroslava Kačániová[2,4]

Abstract: Attention of the scientific community has still focused on application of essential oils (EOs) as natural antifungal agents in the food industry to prolong the shelf-life of food products. In this regard, the current study was designed to evaluate chemical composition, antioxidant and both antifungal (*in vitro*, *in situ*) activities of spearmint (*Mentha spicata* var. *crispa*) essential oil (SEO) commercially obtained from Slovak company against selected *Penicillium* species. The EO was used in four concentrations (62.5, 125, 250, and 500 µl/l) chosen, and gas chromatography–mass spectrometry, DPPH, agar disc diffusion and vapor phase methods were employed for such analyses. Results revealed that carvone (57.5%) and α-limonene (17.6%) were the principal constituents in the EO chemical composition. Although only a weak antioxidant activity (20.40 ± 0.80% free radical-scavenging inhibition) was displayed by SEO, the highest EO concentration (500 µl/l) was shown to be a moderate growth inhibitor of *P. expansum* (inhibition zone of 11.46 ± 0.63 mm) and *P. crustosum* (inhibition zone of 12.93 ± 0.46 mm). The growth of *P. citrinum* was only weakly inhibited by the SEO (≥ 250 µl/l). Most importantly, the ability of the SEO to inhibit the mycelial growth of three *Penicillium* spp. tested was pronounced ($p < 0.05$) for all applied concentrations. Accordingly, the results from the current study complement our previous ones dealing with the possibility of utilizing diverse EOs commercially achieved from the same company in the food sector.

Keywords: Antifungal activity, DPPH assay, essential oil, Mentha spicata, vegetable, volatile substances

[1]**Address:** AgroBioTech Research Centre, Slovak University of Agriculture, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia
[2]**Address:** Institute of Horticulture, Faculty of Horticulture and Landscape Engineering, Slovak University of Agriculture, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia
[3]**Address:** Department of Chemistry, Faculty of Science, University of Kragujevac, 34000 Kragujevac, Serbia
[4]**Address:** Department of Bioenergy, Food Technology and Microbiology, Institute of Food Technology and Nutrition, University of Rzeszow, 4 Zelwerowicza Str., 35-601 Rzeszow, Poland

***Corresponding author**: hana.duranova@uniag.sk

Citation: Ďúranová, H., Valková, V., Galovičová, L., Vukovic, NL., Vukic, M., Kačániová, M. (2023). Antifungal Activities of Essential Oil Obtained from *Mentha spicata* var. *Crispa* against Selected *Penicillium* Species. Bilge International Journal of Science and Technology Research, 7(1): 1-8.

1. INTRODUCTION

Due to microbial contamination of food products, the food sector is facing a great challenge to find a promising alternative to synthetic preservatives reducing food spoilage while being bio-incompatible, non-biodegradable, and environmentally unsustainable (Maurya et al., 2021). One of the major emerging technologies to extend the shelf-life of foods seems to be the extraction of essential oils (EOs) from various plant organs and their application to food systems (Fernández-López and Viuda-Martos, 2018; Ribeiro-Santos et al., 2018; LaLonde et al., 2019; Rao et al., 2019; Santos et al., 2022). Indeed, as a valuable source of diverse biologically active compounds, EOs possess antioxidant and antimicrobial properties which participate in food shelf-life enhancement, thus making them an ideal

natural, eco-friendly, renewable, and cost-effective replacement of synthetic food additives (Fernández-López and Viuda-Martos, 2018; Basavegowda and Baek, 2021; Saeed et al., 2022).

Spearmint [*Mentha* (*M*.) *spicata*, equivalent to *M*. *viridis*] is a perennial herbaceous medicinal plant of the Lamiaceae family with a pungent smell, commercially cultivated in many regions of the world (Ounoki et al., 2021; El Menyiy et al., 2022). Leaves of the plant are traditionally used as tea (Dhifi et al., 2013) and an ingredient in a variety of mixed drinks including the mojito and the mint julep (Ounoki et al., 2021). Spearmint EO (SEO), belonging among the 10 most commercialized EOs (Delfine et al., 2022), is produced and stored in the glandular trichomes of the leaves. The EO is characterized by the high presence of monoterpenes (Chrysargyris et al., 2017), mainly of carvone as the major component responsible for its aroma (Dionísio et al., 2012). Hence, it is especially applied in the flavoring of chewing gums, toothpastes, and other oral products (Kokkini et al., 2003). There are many *in vitro* and *in vivo* experimental reports demonstrating M. spicata extracts and EOs as agents with remarkable antimicrobial, antiparasitic, antidiabetic, anti-inflammatory, and anticancer biological activities (El Menyiy et al., 2022). Additionally, SEO is reputed for its carminative, antispasmodic, and diuretic properties (Dhifi et al., 2013). Regarding its antimicrobial efficacy, common food-borne pathogenic bacteria, such as *Staphylococcus aureus*, *Bacillus* (*B*.) *subtilis*, *B. cereus*, *Listeria monocytogenes*, *Escherichia coli* O157:H7, *Pseudomonas aeruginosa*, *Shigella flexneri* (Ullah et al., 2012; Shahbazi, 2015; Horváth and Koščová, 2017) were shown to be sensitive to SEO actions. Also, antifungal activity of the EO is extensively tested (Şarer et al., 2011; Houicher et al., 2016; Bardaweel et al., 2018). However, it is well-known that chemical profile and relative proportions of organic compounds of any EO extracted from a selected single plant species depend on a plethora factors such as agricultural aspect (e.g., environment, climate, soil conditions, time of harvesting and postharvest handling prior to isolation) (Sankarikutty and Narayanan, 2003), extraction methods used (Berka-Zougali et al., 2012; Pintatum et al., 2020; Messaoudi et al., 2021), plant parts being used for extraction (Pintatum et al., 2020), and many others, which are principal causes of serious discrepancies identified among the studies concerning this field of research area. Taking into account this fact, the current study evaluated *in vitro* and *in situ* antifungal activities of the EO obtained from *M*. *spicata* var. *crispa* against selected *Penicillium* spp. inoculated on potato slices as a model of food substrate. In such a way, application of SEO in active food packaging to prolong shelf-life of foods can be considered. Furthermore, the report also adds another piece to our comprehensive view creation of various antifungal actions exerted by diverse commercial EOs achieved from the same company.

2. MATERIAL AND METHOD

2.1. Essential oil

Spearmint (*M*. *spicata* var. *crispa*) essential oil (SEO) was purchased from Hanus s.r.o Company (Nitra, Slovakia) to complement our previous studies (Galovičová et al., 2021a; Galovičová et al., 2021b; Kačániová et al., 2021a; Valková et al., 2021a; Valková et al., 2021b; Galovičová et al., 2022; Kačániová et al., 2022a; Valková et al., 2022a; Valková et al., 2022b). The EO was prepared by steam distillation of flowering stems.

2.2. Fungal strains

Three *Penicillium* (*P*.) strains (*P*. *crustosum*, *P*. *citrinum*, and *P*. expansum) isolated from *Vitis vinifera* berries were employed to assess *in vitro* and *in situ* antifungal activities of the SEO. The strains were classified using a reference-based MALDI-TOF MS Biotyper followed by comparison with the taxonomic identification obtained by 16S rDNA sequences analysis.

2.3. Chemical characterization of SEO

Gas chromatography/mass spectrometry (GC/MS) analysis of the SEO was performed using an Agilent 6890N gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) coupled to a quadrupole mass spectrometer 5975B (Agilent Technologies, Santa Clara, CA, USA) according to the methodology described by Valková et al. (2021a). The individual volatile constituents of the injected EO samples were identified based on their retention indices (Adams, 2007), and a comparison with reference spectra (Wiley and NIST databases). The retention indices were experimentally determined using the standard method which included retention times of n-alkanes (C6–C34), injected under the same chromatographic conditions. The percentages of the identified compounds (amounts higher than 0.1%) were derived from their GC peak areas.

2.4. Antioxidant activity of SEO

Antioxidant activity (AA) of the SEO was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay as it was carried out in our previous experiments (Galovičová et al., 2021a; Valková et al., 2021a; Valková et al., 2021b; Kačániová et al., 2021b; Kačániová et al., 2022b). The AA was expressed as the percentage of DPPH inhibition, and calculated according to the formula: (A0 – A1)/A0 × 100; where A0 and A1 were absorbances of the DPPH and the samples, respectively. The power of AA was assessed based on the following scheme: weak (0 – 29%) < medium-strong (30 – 59%) < strong (60% and more). Moreover, the value for total AA was expressed as Trolox equivalent antioxidant capacity (TEAC) according to the calibration curve as 1 μg of standard reference Trolox to 1 ml of the SEO sample. All analyses were performed in triplicate.

2.5. *In vitro* antifungal activity of SEO

In vitro antifungal activity of the SEO was performed using the agar disc diffusion method (Valková et al., 2021a; Valková et al., 2021b; Valková et al., 2022b). To prepare fungal media, the strains were inoculated in Sabouraud Dextrose Agar (SDA; Oxoid, Basingstoke, UK) and incubated for 5 days at 25 °C. Subsequently, small aliquots of the fungi were transferred to test tubes, each containing 3 ml of distilled water, and the inoculum concentration was standardized by comparison with the 0.5 McFarland scale (1.5 × 108 CFU/ml). For the analysis, an aliquot of 100 µl of the culture media was firstly inoculated on the SDA, and the discs of filter paper (6 mm) impregnated with 10 µl of the SEO sample (in four concentrations: 62.5, 125, 250, and 500 µl/l; diluted in 0.1% dimethyl sulfoxide, DMSO) were applied on the SDA surfaces. Fungi were consequently incubated aerobically at 25 ± 1 °C for 5 days. After the incubation, diameters of the inhibition zones were measured in mm. The values for inhibitory activity were expressed in the following manner: weak antifungal activity (5 - 10 mm) < moderate antifungal activity (10 - 15 mm) < very strong antifungal activity (zone > 15 mm) (Valková et al., 2022a).

2.6. Moisture content and water activity of potato food model

Potato slices as a substrate for fungal growth were applied. The vegetable was purchased at the local market (Nitra, Slovakia). Its moisture content (MC) and water activity (aw) were measured using the Kern DBS 60-3 moisture analyzer (Kern and Sohn GmbH, Balingen, Germany) and the Lab Master aw Standard (Novasina AG; Lachen, Switzerland), respectively (Kačániová et al., 2020a; 2020b). All analyses were performed in triplicate.

2.7. *In situ* antifungal analysis

The vapor phase (contact) method was employed to assess *in situ* antifungal activity of the SEO (Valková et al., 2022a; 2022b). Firstly, sliced potato (5 mm) was placed on the bottom of Petri dishes, and the inoculum was applied by stabbing one time with an injection pin on the vegetable surface. Next, 10 µl of the SEO in the same four concentrations was applied on the sterile filter paper disc (60 mm) which was consequently placed at the top of Petri dishes. The dishes were hermetically closed by parafilm and cultivated at 25 °C for 14 days. All analyses were performed in triplicate.

After the cultivation, the size of the fungal colonies with visible mycelial growth and visible sporulation (Kačániová et al., 2020a; 2020b) was evaluated using stereological methods. In this concept, the volume density of the colonies was firstly assessed using ImageJ software (National Institutes of Health, Bethesda, MD, USA), counting the points of the stereological grid hitting the colonies and those falling to the reference space (growth substrate used, potato). The antifungal activity of the SEO was expressed as the percentage of mycelial growth inhibition (MGI), which was calculated using the formula: MGI = [(C − T)/C] × 100 (Sempere-Ferre et al., 2021), where C and T were volume fractions of fungal colonies in the control (untreated) and treated samples, respectively.

2.8. Statistical analysis

Statistical analysis of obtained data was performed using Prism 8.0.1 (GraphPad Software, San Diego, California, USA). Significant differences between the analyzed groups of samples were assessed using one-way analysis of variance (ANOVA) followed by Tukey's test. A value for $p < 0.05$ denoted the level of statistical significance.

3. RESULTS AND DISCUSSION

Using GC/MS analysis, a total of 35 organic components were identified in our SEO, completely accounting for 99.7% of the EO chemical composition (Table 1). The dominant constituents were monoterpenes (98.5%), followed by sesquiterpenes (1%), and non-terpenic compounds (alcohols; 0.2%). Out of monoterpenes, oxygenated ones (77.9%), especially monoterpene ketones (63.8%) with carvone (57.5%) and monoterpene alcohols (8.1%) with dihydrocarveol (4.3%) have been detected in the highest amounts. Among monoterpene hydrocarbons (20.6%) as the second major class of compounds identified, α-limonene (17.6%) was the most prominent.

Table 1. Chemical composition of essential oil obtained from *Mentha spicata* var. *crispa*

No	RI [a]	Compound [b]	% [c]
1	938	α-pinene	0.6
2	977	sabinene	0.1
3	980	β-pinene	1.0
4	992	β-myrcene	0.4
5	993	3-octanol	0.2
6	1023	*p*-cimene	0.9
7	1028	α-limonene	17.6
8	1033	1,8-cineole	0.8
9	1060	γ-terpinene	tr
10	1068	cis-sabinene hydrate	tr
11	1148	isopulegol	tr
12	1151	menthone	1.1
13	1162	iso-menthone	0.4
14	1164	neo-menthol	0.3
15	1173	menthol	1.8
16	1178	4-terpinenol	tr
17	1192	cis-dihydrocarvone	4.1
18	1199	dihydrocarveol	4.3
19	1200	trans-dihydrocarvone	0.7
20	1217	trans-carveol	0.7
21	1229	cis-carveol	1.0
22	1241	carvone	57.5
26	1253	3-carvomenthenone	tr
27	1254	(*Z*)-anethole	tr
28	1297	menthyl acetate	0.9
29	1306	iso-menthyl acetate	0.5
30	1311	dihydrocarveol acetate	3.8
31	1379	α-copaene	tr
32	1385	β-bourbonene	0.2
33	1388	β-elemene	tr

34	1422	(*E*)-caryophyllene	0.6
35	1583	caryophyllene oxide	0.2
Total		**99.7**	

[a] Values of retention indices on HP-5MS column; [b] Identified compounds; [c] tr - compounds identified in amounts less than 0.1 %

In general, biological activities of EOs being interested in the food and cosmetic industries, as well as in the field of human health are strongly dependent on their chemical composition (Dhifi et al., 2016). Diverse hydrocarbon and oxygenated monoterpenes compounds have been intensively studied in terms of their antimicrobial and antifungal activities against various foodborne pathogens (Badawy et al., 2019). Similarly to our results, carvone (65.33%), limonene (18.19%), and dihydrocarvone (2.97%) as the major compounds of *M. spicata* EO were reported by Liu et al. (2012). Additionally, the carvone and limonene being the predominant components of SEO have also been identified in the research by Bardaweel et al. (2018) and Snoussi et al. (2015). However, while first authors determined values for carvone (49.5%) and limonene (16.1%) to be close to our ones, the latter authors showed that both compounds participate in different proportions (40.8 ± 1.23% of carvone, 20.8 ± 1.12% of limonene) in the EO chemical profile in comparison with our study. Carvone (56.6%; 41.1%; 78.76%; 62.9%; 62–65%; 41.1%) and limonene (27.3%; 20.1%; 11.50%; 8.5%; 11–13%; 14.1%) as the major constituents of *M. spicata* EO have also been detected by other researchers (Aggarwal et al., 2002; Martins et al., 2012; Shahbazi, 2015; Ounoki et al., 2021; Piras et al., 2021; Giménez-Santamarina et al., 2022), respectively. Generally, the concentrations of monoterpenes (such as carvone) being extracted from the same species is strongly influenced by the plant parts and the method itself selected for such procedure (Bouyahya et al., 2021). Also, already above-mentioned (in the part "Introduction") other factors must be kept in mind, all of them contributing to different findings identified between studies employed for data comparison as it was shown in our report.

Data from DPPH free radical-scavenging activity analysis has revealed a weak (20.40 ± 0.80% free radical-scavenging inhibition) AA of our SEO with a value of 107.66 ± 3.0 µg TEAC.ml^{-1}. This assay is commonly used for measurement of AA of diverse EOs (Bag and Chattopadhyay, 2015; Inaam et al., 2015; Anggraeni et al., 2018; Olmedo et al., 2018; Chambre et al., 2020; Galovičová et al., 2021a; Valková et al., 2021a; Valková et al., 2021b; Kačániová et al., 2022a). According to many authors (Amiri, 2012; Bag and Chattopadhyay, 2015), the DPPH has been largely used as an easy, quick, reliable, and reproducible assay for screening *in vitro* antioxidant activity of EOs or plant extracts. Using the method, we have found that the SEO was able to scavenge the radical; however, only to a lesser extent indicating its weak AA. The same finding was also displayed by EO from *M. spicata* growing in Portugal (Martins et al., 2012) and Poland (Grzeszczuk and Jadczak, 2009). By contrast, moderate inhibition of DPPH radicals (54.68%) of *M. spicata* EO from Oman have been observed by Alsaraf et al. (2021). Furthermore, Ahmad et al. (2012) have detected even higher values for AA (61-71%) of *M. spicata* methanolic extract. As compared to our previous study, the EO from *M. spicata* exhibited lower AA than that from *M. piperita* (36.85 ± 0.49%) recognizing menthol, menthone, and menthyl acetate to be the major constituents (Valková et al., 2021a). The same fact was also reported by other researchers (Dorman et al., 2003; Nikavar et al., 2008).

In spite of only a weak AA displayed by our SEO, an *in vitro* antifungal activity of the EO against the growth of *Penicillium* spp. selected (Table 2) was observed. In effect, the disc diffusion method revealed a moderate inhibitory efficiency of the highest SEO concentration (500 µl/l) against the growth of *P. expansum* and *P. crustosum*, with inhibition zones of 11.46 ± 0.63 mm and 12.93 ± 0.46 mm, respectively. Against *P. citrinum* mycelial growth, the highest concentration of the EO induced only a weak inhibitory action; the same impact was also noticed for 250 µl/l of the EO against all three fungal species.

Table 2. *In vitro* antifungal activity of spearmint essential oil expressed as the diameter of the inhibition zone (in mm)

Fungi	SEO (µl/l)			
	62.5	125	250	500
P. expansum	1.73 ± 0.55 [a]	4.55 ± 1.03 [b]	9.05 ± 0.83 [c]	11.46 ± 0.63 [d]
P. crustosum	2.76 ± 0.57 [a]	5.14 ± 0.74 [b]	8.36 ± 1.09 [c]	12.93 ± 0.46 [d]
P. citrinum	2.88 ± 0.36 [a]	4.86 ± 0.77 [b]	6.56 ± 0.34 [c]	9.47 ± 0.59 [d]

Note: Mean ± standard deviation. SEO: spearmint essential oil. Values in the same line with different superscripts are significantly different ($p < 0.05$).

In addition, all concentrations of the SEO were able to considerably ($p < 0.05$) inhibit the mycelial growth of *P. expansum*, *P. crustosum*, and *P. citrinum* inoculated on potato slices as a food model substrate. The data from *in situ* analysis is summarized in Table 3. Moreover, there was a more pronounced inhibitory action of higher concentrations of SEO (≥ 125 µl/l; ≥ 250 µl/l) on the growth of *P. expansum* and *P. citrinum*, respectively, as compared to the lower concentrations. On the other hand, *P. crustosum* was equally sensitive to all the EO concentrations.

Table 3. *In situ* antifungal activity of spearmint essential oil expressed as mycelial growth inhibition

Fungi	MGI (%)			
	SEO (µl/l)			
	62.5	125	250	500
P. expansum	55.07 ± 5.15 [a]	91.35 ± 9.39 [b]	100.00 ± 0.00 [b]	91.67 ± 9.24 [b]
P. crustosum	100.00 ± 0.00 [a]	93.30 ± 10.17 [a]	97.03 ± 5.84 [a]	95.12 ± 5.99 [a]
P. citrinum	78.43 ± 3.69 [a]	92.80 ± 12.92 [ab]	95.96 ± 7.58 [b]	96.74 ± 9.15 [b]

Note: Mean ± standard deviation. MGI: mycelial growth inhibition; SEO: spearmint essential oil. Values in the same line with different superscripts are significantly different ($p < 0.05$).

The characterization of our EO from *M. spicata* from both *in vitro* and *in situ* antifungal activities has shown that the SEO is an effective inhibitor of the *Penicillium* spp. growth. Similarly to our study, low to moderate antimicrobial activity of SEO against pathogenic microorganisms including gram positive, gram negative bacteria, and fungi was also reported by Bardaweel et al. (2018). Spearmint EO has also been found to be active against *P. citrinum* (Liu et al., 2012), *Candida albicans*, *Aspergillus niger*, *Fusarium oxysporum* (Martins et al., 2012), and *Vibrio* spp. strains (Snoussi et al., 2015). Aggarwal et al. (2002) have tested the effect of SEO, as well as their main isolated components, carvone and limonene, on a wide spectrum of human pathogenic fungi and bacteria revealing their high *in vitro* bioactivity. The use of carvone as an antifungal agent against various fungal strains is suggested by many reports (Morcia et al., 2012; Boni et al., 2016; Hassan et al., 2017; Moro et al., 2017). From this aspect it can be assumed that the low to moderate antifungal activity of our SEO demonstrated by the disc diffusion method can be attributed mainly to the carvone abundance in its chemical composition.

Food spoilage is a very common phenomena in which genus *Penicillium* plays an important concern because of its ubiquity and mycotoxin production (Pitt, 2014). In the current study, potato slices as a food model substrate for *Penicillium* spp. growth were employed. Moisture content and aw of the potato substrate were estimated to be 75.18 ± 1.23% and 0.969 ± 0.002, respectively; the data of these physical parameters demonstrates its suitability for microbial (fungal) spoilage. Indeed, food substrates with values for MC and aw being around 80% and higher than 0.60, respectively, are well suited for the *in situ* antifungal activity analysis of diverse EOs (Valková et al., 2022b). Moreover, our data of both physical parameters have been found to be in accordance with the study of the mentioned authors (Valková et al., 2022b). A strong antifungal potential of our SEO against the mycelial growth of all three *Penicillium* species could be explained by the presence of oxygenated monoterpenes, such as carvone that has emerged as a promising antifungal compound (Bouyahya et al., 2021) due to its disruptive impact on the cell membrane and fungal mitochondria (Zhang et al., 2022). Also, limonene (another major component of SEO) is able to inhibit the growth of *C. albicans* by generation of oxidative stress in the cell envelope and induction of oxidative DNA damage, leading to cell-cycle modulation and apoptosis (Sales et al., 2022). Thus, the *in situ* antifungal activity of SEO demonstrated in our study can be associated mainly with the two major compounds and their mutual interactions. However, the biological activities of EOs as a multicomponent mixture cannot be easily ascribed to only one or two specific components but they are rather a result of additive, synergistic or antagonistic actions of different constituents present in their chemical profile (Bardaweel et al., 2018).

5. CONCLUSION

The results from the present study showed carvone (57.5%) and α-limonene (17.6%) to be the principal constituents in chemical composition of the SEO which displayed only a weak AA (20.40 ± 0.80% free radical-scavenging inhibition). On the other hand, the highest concentration (500 µl/l) of the EO exhibited a moderate inhibition efficacy against the growth of *P. expansum* (inhibition zone of 11.46 ± 0.63 mm) and *P. crustosum* (inhibition zone of 12.93 ± 0.46 mm), whilst the growth of *P. citrinum* was inhibited by the SEO (≥ 250 µl/l) only in a weak manner. Essentially, the ability of the SEO (in all four concentrations used) to act as an inhibitor against the mycelial growth of three *Penicillium* spp. tested was evident ($p < 0.05$). In conclusion, the findings from all our analyses suggest the SEO to be a promising natural agent for extending the shelf life of vegetables (including potato) which can be a very helpful aspect for the food sector in terms of active food packaging.

Ethics Committee Approval
N/A

Peer-review
Externally peer-reviewed.

Author Contributions
Conceptualization: H.Ď., V.V., M.K.; Investigation: H.Ď.; V.V., L.G., N.L.V., M.V., M.K.; Material and Methodology: H.Ď., V.V., L.G., N.L.V., M.V., M.K.; Supervision: M.K.; Visualization: H.Ď., V.V.; Writing-Original Draft: H.Ď..; Writing-review & Editing: H.D., V.V., M.K.; All authors have read and agreed to the published version of manuscript.

Conflict of Interest
The authors have no conflicts of interest to declare.

Funding
This research was funded by the grant of the Slovak Research and Development Agency, APVV-20-0058 "The potential of the essential oils from aromatic plants for medical use and food preservation".

REFERENCES

Adams, R.P. (2007). Identification of essential oil components by gas chromatography/mass spectrometry; Allured Publishing Corporation: Carol Stream, IL, USA, 2007; Volume 456.

Aggarwal, K. K., Khanuja, S. P. S., Ahmad, A., Santha Kumar, T. R., Gupta, V. K., Kumar, S. (2002). Antimicrobial activity profiles of the two enantiomers of limonene and carvone isolated from the oils of *Mentha spicata* and *Anethum sowa*. Flavour and Fragrance Journal, 17(1), 59-63.

Ahmad, N., Fazal, H., Ahmad, I., Abbasi, B. H. (2012). Free radical scavenging (DPPH) potential in nine *Mentha* species. Toxicology and Industrial Health, 28(1), 83-89.

Alsaraf, S., Hadi, Z., Akhtar, M. J., Khan, S. A. (2021). Chemical profiling, cytotoxic and antioxidant activity of volatile oil isolated from the mint (*Mentha spicata* L.,) grown in Oman. Biocatalysis and Agricultural Biotechnology, 34, 102034.

Amiri, H. (2012). Essential oils composition and antioxidant properties of three thymus species. Evidence-Based Complementary and Alternative Medicine, 2012, 728065.

Anggraeni, N. I., Hidayat, I. W., Rachman, S. D., Ersanda. (2018). Bioactivity of essential oil from lemongrass (*Cymbopogon citratus* Stapf) as antioxidant agent. In AIP Conference Proceedings (Vol. 1927, No. 1, p. 030007). AIP Publishing LLC.

Badawy, M. E., Marei, G. I. K., Rabea, E. I., Taktak, N. E. (2019). Antimicrobial and antioxidant activities of hydrocarbon and oxygenated monoterpenes against some foodborne pathogens through *in vitro* and *in silico* studies. Pesticide Biochemistry and Physiology, 158, 185-200.

Bag, A., Chattopadhyay, R. R. (2015). Evaluation of synergistic antibacterial and antioxidant efficacy of essential oils of spices and herbs in combination. PloS One, 10(7), e0131321.

Bardaweel, S. K., Bakchiche, B., ALSalamat, H. A., Rezzoug, M., Gherib, A., Flamini, G., (2018). Chemical composition, antioxidant, antimicrobial and antiproliferative activities of essential oil of *Mentha spicata* L. (*Lamiaceae*) from Algerian Saharan atlas. BMC Complementary and Alternative Medicine, 18(1), 1-7.

Basavegowda, N., Baek, K. H. (2021). Synergistic antioxidant and antibacterial advantages of essential oils for food packaging applications. Biomolecules, 11(9), 1267.

Berka-Zougali, B., Ferhat, M. A., Hassani, A., Chemat, F., Allaf, K. S. (2012). Comparative study of essential oils extracted from Algerian *Myrtus communis* L. leaves using microwaves and hydrodistillation. International Journal of Molecular Sciences, 13(4), 4673-4695.

Boni, G. C., de Feiria, S. N. B., de Leat Santana, P., Anibal, P. C., Boriollo, M. F. G., Buso-Ramos, M. M., Barbosa, J. P., de Oliveira, T. R., Hofling, J. F. (2016). Antifungal and cytotoxic activity of purified biocomponents as carvone, menthone, menthofuran and pulegone from *Mentha* spp. African Journal of Plant Science, 10(10), 203-210.

Bouyahya, A., Mechchate, H., Benali, T., Ghchime, R., Charfi, S., Balahbib, A., Burkov, P., Shariati, M. A., Lorenzo, J. M., Omari, N. E. (2021). Health benefits and pharmacological properties of carvone. Biomolecules, 11(12), 1803.

Chambre, D. R., Moisa, C., Lupitu, A., Copolovici, L., Pop, G., Copolovici, D. M. (2020). Chemical composition, antioxidant capacity, and thermal behavior of *Satureja hortensis* essential oil. Scientific Reports, 10(1), 1-12.

Chrysargyris, A., Xylia, P., Botsaris, G., & Tzortzakis, N. (2017). Antioxidant and antibacterial activities, mineral and essential oil composition of spearmint (*Mentha spicata* L.) affected by the potassium levels. Industrial Crops and Products, 103, 202-212.

Delfine, S., Velikova, V. B., Mastrodonato, F. (2022). Soil-mulching influence on spearmint oil yield, ecophysiological activities and essential-oil content in rainfed environment of Southern Italy. Agronomy, 12(7), 1521.

Dhifi, W., Jelali, N., Mnif, W., Litaiem, M., Hamdi, N. (2013). Chemical composition of the essential oil of *Mentha spicata* L. from Tunisia and its biological activities. Journal of Food Biochemistry, 37(3), 362-368.

Dhifi, W., Bellili, S., Jazi, S., Bahloul, N., Mnif, W. (2016). Essential oils' chemical characterization and investigation of some biological activities: A critical review. Medicines, 3(4), 25.

Dionísio, A. P., Molina, G., de Carvalho, D. S., Dos Santos, R., Bicas, J. L., Pastore, G. M. (2012). Natural flavourings from biotechnology for foods and beverages. In Natural food additives, ingredients and flavourings (pp. 231-259). Woodhead Publishing.

Dorman, H. D., Koşar, M., Kahlos, K., Holm, Y., Hiltunen, R. (2003). Antioxidant properties and composition of aqueous extracts from *Mentha* species, hybrids, varieties, and cultivars. Journal of Agricultural and Food Chemistry, 51(16), 4563-4569.

El Menyiy, N., Mrabti, H. N., El Omari, N., Bakili, A. E., Bakrim, S., Mekkaoui, M., Balahbib, A., Amiri-Ardekani, E., Ullah, R., Alqahtani, A. S., Shahat, A. A., Bouyahya, A. (2022). Medicinal uses, phytochemistry, pharmacology, and toxicology of *Mentha spicata*. Evidence-Based Complementary and Alternative Medicine, 2022, 7990508.

Fernández-López, J., Viuda-Martos, M. (2018). Introduction to the special issue: application of essential oils in food systems. Foods, 7(4), 56.

Galovičová, L., Borotová, P., Valková, V., Vukovic, N. L., Vukic, M., Štefániková, J., Ďúranová, H., Kowalczewski, P. Ł., Čmiková, N., Kačániová, M. (2021a). *Thymus vulgaris* essential oil and its biological activity. Plants, 10(9), 1959.

Galovičová, L., Borotová, P., Valková, V., Vukovic, N. L., Vukic, M., Terentjeva, M., Štefániková, J., Ďúranová, H., Kowalczewski, P. Ł., Kačániová, M. (2021b). *Thymus serpyllum* essential oil and its biological activity as a modern food preserver. Plants, 10(7), 1416.

Galovičová, L., Borotová, P., Valková, V., Ďúranová, H., Štefániková, J., Vukovic, N. L., Vukic, N., Kačániová, M. (2022). Biological activity of Pogostemon cablin essential oil and its potential use for food preservation. Agronomy, 12(2), 387.

Giménez-Santamarina, S., Llorens-Molina, J. A., Sempere-Ferre, F., Santamarina, C., Roselló, J., Santamarina, M. P. (2022). Chemical composition of essential oils of three *Mentha* species and their antifungal activity against selected phytopathogenic and post-harvest fungi. All Life, 15(1), 64-73.

Grzeszczuk, M., Jadczak, D. (2009). Estimation of biological value of some species of mint (*Mentha* L.). Herba Polonica, 55(3), 194-199.

Hassan, B., Soumya, E., Amal, A. E., Sanae, G., Saad, K. I. (2017). Antifungal activities of B-ionone, carvone and 1, 8-cineole essential oil components against *Aspergillus niger* spores. Journal of Chemical and Pharmaceutical Research, 9, 52-56.

Horváth, P., Koščová, J. (2017). *In vitro* antibacterial activity of *Mentha* essential oils against Staphylococcus aureus. Folia Veterinaria, 61(3), 71-77.

Houicher, A., Hechachna, H., Teldji, H., Ozogul, F. (2016). *In vitro* study of the antifungal activity of essential oils obtained from *Mentha spicata*, *Thymus vulgaris*, and *Laurus nobilis*. Recent Patents on Food, Nutrition & Agriculture, 8(2), 99-106.

Inaam, E. M., Sara, H., Saadia, L., Mohamed, E., Abdeslam, L. (2015). Study of antioxidant activity of essential oils extracted from Moroccan medicinal and aromatic plants. European Journal of Medicinal Plants, 10, 1-12.

Kačániová, M., Galovičová, L., Ivanišová, E., Vukovic, N. L., Štefániková, J., Valková, V., Borotová, P., Žiarovská, J., Terentjeva, M., Felšöciová, S., Tvrdá, E. (2020a). Antioxidant, antimicrobial and antibiofilm activity of coriander (*Coriandrum sativum* L.) essential oil for its application in foods. Foods, 9, 282.

Kačániová, M., Terentjeva, M., Galovičová, L., Ivanišová, E., Štefániková, J., Valková, V., Borotová, P., Kowalczewski, P. Ł., Kunová, S., Felšöciová, S., Tvrdá, E., Žiarovská, J., Prokeinová R. B., Vukovic, N. (2020b). Biological activity and antibiofilm molecular profile of *Citrus aurantium* essential oil and its application in a food model. Molecules, 25, 3956.

Kačániová, M., Galovičová, L., Borotová, P., Valková, V., Ďúranová, H., Kowalczewski, P. Ł., Hussein A. H. Said-Al Ahl, H. A. H., Hikal, W. M., Vukic, M., Savitskaya, T., Grinshpan, D., Vukovic, N. L. (2021a). Chemical composition, *in vitro* and *in situ* antimicrobial and antibiofilm activities of *Syzygium aromaticum* (Clove) essential oil. Plants, 10(10), 2185.

Kačániová, M., Galovičová, L., Valková, V., Tvrdá, E., Terentjeva, M., Žiarovská, J., Kunová, S., Savitskaya, T., Grinshpan, D., Štefániková, J., Felsöciová, S., Vukovic, N., Kowalczewski, P. Ł., (2021b). Antimicrobial and antioxidant activities of *Cinnamomum cassia* essential oil and its application in food preservation. Open Chemistry, 19(1), 214-227.

Kačániová, M., Galovičová, L., Valková, V., Ďuranová, H., Štefániková, J., Čmiková, N., Vukic, M., Vukovic, N. L., Kowalczewski, P. Ł. (2022a). Chemical composition, antioxidant, *in vitro* and *in situ* antimicrobial, antibiofilm, and anti-ınsect activity of *Cedar atlantica* essential oil. Plants, 11(3), 358.

Kačániová, M., Galovičová, L., Borotová, P., Vukovic, N. L., Vukic, M., Kunová, S., Hanus, P., Bakay, L., Zagrobelna, E., Kluz, M., Kowalczewski, P. Ł. (2022b). Assessment of *Ocimum basilicum* essential oil anti-insect activity and antimicrobial protection in fruit and vegetable quality. Plants, 11(8), 1030.

Kokkini, S., Karousou, R., Hanlidou, E. (2003). Herbs of the *Labiatae*. Encyclopedia of Food Sciences and Nutrition (Second Edition), 2003, Pages 3082-3090

LaLonde, T., Bowser, T., Jadeja, R. (2019). Essential oils as antimicrobials. Madridge Journal of Food Technology, 4(1), 163-169.

Liu, K. H., Zhu, Q., Zhang, J. J., Xu, J. F., Wang, X. C. (2012). Chemical composition and biological activities of the essential oil of *Mentha spicata Lamiaceae*. In Advanced Materials Research (Vol. 524, pp. 2269-2272). Trans Tech Publications Ltd.

Martins, M. R., Tinoco, M. T., Almeida, A. S., Cruz-Morais, J. (2012). Chemical composition, antioxidant and antimicrobial properties of three essential oils from Portuguese flora. Journal of Pharmacognosy, 3(1), 39-44.

Maurya, A., Prasad, J., Das, S., Dwivedy, A. K. (2021). Essential oils and their application in food safety. Frontiers in Sustainable Food Systems, 5, 653420.

Messaoudi, M., Rebiai, A., Sawicka, B., Atanassova, M., Ouakouak, H., Larkem, I., Egbuna, C., Awuchi, C. G., Boubekeur, S., Ferhat, M. A., Begaa, S., Benchikha, N. (2021). Effect of extraction methods on polyphenols, flavonoids, mineral elements, and biological activities of essential oil and extracts of *Mentha pulegium* L. Molecules, 27(1), 11.

Morcia, C., Malnati, M., Terzi, V. (2012). *In vitro* antifungal activity of terpinen-4-ol, eugenol, carvone, 1, 8-cineole (eucalyptol) and thymol against mycotoxigenic plant pathogens. Food Additives & Contaminants: Part A, 29(3), 415-422.

Moro, I. J., Gondo, G. D. G. A., Pierri, E. G., Pietro, R. C. L. R., Soares, C. P., Sousa, D. P. D., Santos, A. G. D. (2017). Evaluation of antimicrobial, cytotoxic and chemopreventive activities of carvone and its derivatives. Brazilian Journal of Pharmaceutical Sciences, 53(04).

Nikavar, B., Ali, N. A., Kamalnezhad, M. (2008). Evaluation of the antioxidant properties of five *Mentha* species. Iranian Journal of Pharmaceutical Research, 7(3), 203-209.

Olmedo, R., Ribotta, P., Grosso, N. R. (2018). Antioxidant activity of essential oils extracted from *Aloysia triphylla* and Minthostachys mollis that improve the oxidative stability of sunflower oil under accelerated storage conditions. European Journal of Lipid Science and Technology, 120(8), 1700374.

Ounoki, R., Ágh, F., Hembrom, R., Ünnep, R., Szögi-Tatár, B., Böszörményi, A., Solymosi, K. (2021). Salt stress affects plastid ultrastructure and photosynthetic activity but not the essential oil composition in spearmint (*Mentha spicata* L. var. *crispa* "Moroccan"). Frontiers in Plant Science, 12, 739467.

Pintatum, A., Laphookhieo, S., Logie, E., Berghe, W. V., Maneerat, W. (2020). Chemical composition of essential oils from different parts of *Zingiber kerrii* Craib and their antibacterial, antioxidant, and tyrosinase inhibitory activities. Biomolecules, 10(2), 228.

Piras, A., Porcedda, S., Falconieri, D., Maxia, A., Gonçalves, M. J., Cavaleiro, C., Salgueiro, L. (2021). Antifungal activity of essential oil from *Mentha spicata* L. and *Mentha pulegium* L. growing wild in Sardinia island (Italy). Natural Product Research, 35(6), 993-999.

Pitt, J. I. (2014). *Penicillium* and *Talaromyces*: Introduction. Encyclopedia of Food Microbiology, 3, 6-13.

Rao, J., Chen, B., McClements, D. J. (2019). Improving the efficacy of essential oils as antimicrobials in foods: Mechanisms of action. Annual Review of Food Science and Technology, 10, 365-387.

Ribeiro-Santos, R., Andrade, M., Sanches-Silva, A., de Melo, N. R. (2018). Essential oils for food application: Natural substances with established biological activities. Food and Bioprocess Technology, 11(1), 43-71.

Saeed, K., Pasha, I., Jahangir Chughtai, M. F., Ali, Z., Bukhari, H., Zuhair, M. (2022). Application of essential oils in food industry: challenges and innovation. Journal of Essential Oil Research, 34(2), 97-110.

Sales, G., Medeiros, S., Soares, I., Sampaio, T., Bandeira, M., Nogueira, N., Queiroz, M. (2022). Antifungal and modulatory activity of lemon balm (*Lippia alba* (MILL.) NE BROWN) essential oil. Scientia Pharmaceutica, 90(2), 31.

Sankarikutty, B., Narayanan, C. S. (2003). Essential oils: Isolation and production. In: Encyclopedia of Food Sciences and Nutrition (Second Edition), 2003, Pages 2185-2189.

Santos, M. I. S., Marques, C., Mota, J., Pedroso, L., Lima, A. (2022). Applications of essential oils as antibacterial agents in minimally processed fruits and vegetables—A Review. Microorganisms, 10(4), 760.

Şarer, E., Toprak, S. Y., Otlu, B., Durmaz, R. (2011). Composition and antimicrobial activity of the essential oil from *Mentha spicata* L. subsp. *spicata*. Journal of Essential Oil Research, 23(1), 105-108.

Sempere-Ferre, F., Asamar, J., Castell, V., Roselló, J., Santamarina, M. P. (2021). Evaluating the antifungal potential of botanical compounds to control *Botryotinia fuckeliana* and *Rhizoctonia solani*. Molecules, 26, 2472.

Shahbazi, Y. (2015). Chemical composition and *in vitro* antibacterial activity of *Mentha spicata* essential oil against common food-borne pathogenic bacteria. Journal of Pathogens, 2015, 916305.

Snoussi, M., Noumi, E., Trabelsi, N., Flamini, G., Papetti, A., De Feo, V. (2015). *Mentha spicata* essential oil: chemical composition, antioxidant and antibacterial activities against planktonic and biofilm cultures of Vibrio spp. strains. Molecules, 20(8), 14402-14424.

Ullah, N., Muhammad, K., Muhammad, U. A., Taj, A. K., Sahibzada, U. K., Farhat, A. K., Umberin, N., Saleem, U. (2012). Impact of geographical locations on *Mentha spicata* antibacterial activities. Journal of Medicinal Plants Research, 6(7), 1201-1206.

Valková, V., Ďúranová, H., Galovičová, L., Vukovic, N. L., Vukic, M., Kačániová, M. (2021a). *In vitro* antimicrobial activity of lavender, mint, and rosemary essential oils and the effect of their vapours on growth of *Penicillium* spp. in a bread model system. Molecules, 26(13), 3859.

Valková, V., Ďúranová, H., Galovičová, L., Štefániková, J., Vukovic, N., Kačániová, M. (2021b). The Citrus reticulate essential oil: Evaluation of antifungal activity against *Penicillium* species related to bakery products spoilage. Potravinarstvo Slovak Journal of Food Sciences, 15, 1112–1119.

Valková, V., Ďúranová, H., Galovičová, L., Borotová, P., Vukovic, N. L., Vukic, M., Kačániová, M. (2022a). *Cymbopogon citratus* essential oil: Its application as an antimicrobial agent in food preservation. Agronomy, 12(1), 155.

Valková, V., Ďúranová, H., Vukovic, N. L., Vukic, M., Kluz, M., Kačániová, M. (2022b). Assessment of chemical composition and anti-penicillium activity of vapours of essential oils from *Abies Alba* and two *Melaleuca* species in food model systems. Molecules, 27(10), 3101.

Zhang, L., Chen, Y., Li, Z., Li, X., Fan, G. (2022). Bioactive properties of the aromatic molecules of spearmint (*Mentha spicata* L.) essential oil: A review. Food & Function, 13, 3110-3132.