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Antifungal Effects of Essential Oils of *Zataria multiflora*, *Mentha pulegium*, and *Mentha piperita*

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HIGHLIGHTS

- All Essential Oils (EOs) of *Zataria multiflora*, *Mentha pulegium*, and *Mentha piperita* showed antifungal activities.
- Among three studied plant EOs, *Z. multiflora* EO showed the strongest antifungal activity.
- Inhibition zone of *Z. multiflora* EO for *Aspergillus* spp., *Penicillium* spp., and *Geotrichum candidum* were 11.6, 5.7, and 7.1 mm, respectively.

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Acronyms and abbreviations
EO=Essential Oil
MFC=Minimum Fungicidal Concentration
MIC=Minimum Inhibitory Concentration

ABSTRACT

Background: Among important fungi associated with foods are *Aspergillus* spp., *Penicillium* spp., and *Geotrichum* spp. In this study, we evaluated antifungal effects of Essential Oils (EOs) of *Zataria multiflora*, *Mentha pulegium*, and *Mentha piperita*.

Methods: Antifungal properties of EOs of *M. piperita*, *M. pulegium*, and *Z. multiflora* against *Aspergillus* spp., *Penicillium* spp., and *Geotrichum candidum* were determined by agar well diffusion and broth macrodilution method. Data were analyzed by SPSS 20.

Results: Among three studied plant EOs, *Z. multiflora* EO had the strongest antifungal activity ($p<0.05$) on tested fungi; so that the Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) were 0.01 and 0.3% for *G. candidum*, 0.005 and 0.3% for *Penicillium* spp., and 0.1 and 0.3% for *Aspergillus* spp.

Conclusion: All three studied plant EOs showed antifungal activities. However, as *Z. multiflora* EO showed the most antifungal effect, it could be specially suggested as natural powerful antifungal preservatives in the food industry.

Introduction

Some fungi associated with foods, such as *Aspergillus* spp., *Penicillium* spp., and *Geotrichum* spp., may cause food spoilage and diseases (Derek and Gary, 2014). Control of fungi in foods is usually done using synthetic chemical additives, but some synthetic preservatives may have mutagenic, carcinogenic, and allergic effects. Thus, in recent years, researches on using natural compounds, especially Essential Oils (EOs) of the plants have been focused to prevent fungal growth and spoilage in foods (Khatibi et al., 2015). EOs are natural plant compounds obtained from the plants by different methods such as steam distillation (Burt, 2004; Moradi et al., 2014).

Mentha piperita (peppermint) and *Mentha pulegium* (pennyroyal) are the members of Lamiaceae family, produce high quality plant EOs (Singh and Pandey, 2018). *Zataria multiflora* Bioss (Shirazi thyme) belongs to the Lamiaceae family and grows mainly in Iran, Pakistan and Afghanistan (Zarei Mahmoudabadi et al., 2007). In this

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in vitro study, we evaluated antifungal effects of EOs of *Z. multiflora*, *M. pulegium*, and *M. piperita.*

Materials and methods

EOs

EOs of *M. piperita*, *M. pulegium*, and *Z. multiflora* used in this study were obtained from Barij® Essence Pharmaceutical Company (Kashan, Iran). The dilutions of EOs and itraconazole were prepared using dimethyl sulfoxide (Sigma-Aldrich Chemie GmbH, Steinheim, Germany).

Organisms

The tested fungi included *Aspergillus* spp., *Penicillium* spp., *Geotrichum candidum* that were isolated from cheese culture. Ten g of each cheese sample was homogenized using a stomacher 400 with 90 ml of sterile physiological saline for at least 2 min. Decimal dilutions of the homogenized samples were prepared in sterile physiological saline and were plated in duplicates onto Potato Dextrose Agar (PDA; Sigma-Aldrich Chemie GmbH, Steinheim, Germany) plates and were incubated at 30 ˚C for 3-7 days. After that, the plates were observed for the presence of mold colonies. The fungal isolates were identified and purified using a combination of macroscopic observation and microscopic Tease mount test (Reiss et al., 2012). Isolated strains were cultured on plates and incubated at 30 ˚C for 7 days. The 7 day old culture slants were flooded with 5 ml of 0.01% tween 80® (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) with a sterile Pasteur pipette and shaken vigorously to give inoculums; then, the conidia was counted with a haemocytometer and diluted in sterilized distilled water to correspond to a final inoculums concentration of 2.4×10^6 spores/ml.

Determination of antifungal effects

-Agar well diffusion method

After mixing a solution of fungi, 0.1 ml of fungal suspension at a concentration of 2.4×10^4 Colony Forming Unit (CFU)/ml was transferred to PDA plates and was spread using a sterile bent glass rod. At the center of plates, 6 mm wells by sterile cork borer were made and 50 µl of different concentrations of EOs were poured into each well. The plates were incubated at 30 ˚C for 3-7 days. The inhibition zone diameter (mm) was measured and as the control experiments were used itraconazole and dimethyl sulfoxide 5% into wells instead of EOs. The experiment was repeated in triplicate and an average value was reported (Fontenelle et al., 2007).

-Broth macrodilution method

A completely synthetic medium of RPMI-1640® containing glutamine and pH indicator (Sigma, Germany) was provided and buffered with MOPS® (Sigma, Germany). The various concentrations of each EO were induced in broth medium, and then fungal inoculums (2.4×10^6 CFU/ml) were added to all tubes. After shaking, for determination of Minimum Inhibitory Concentration (MIC), all tubes were incubated at 30 ˚C for 3-7 days and readings were taken when the turbidity of the growth control tube was evident. For determining Minimum Fungicidal Concentration (MFC), a bent glass rod was used to spread 0.1 ml of each MIC tube showed complete inhibition on the plates. The plates were incubated at 30 ˚C for 3-7 days and were observed daily for the presence of fungal colonies.

Statistical analyses

The data were analyzed by SPSS 20 software using descriptively for MIC and MFC and, one way ANOVA for comparing the inhibition diameter zones. $P<0.05$ was considered as a significant differences among groups.

Results and discussion

Among three studied plant EOs, *Z. multiflora* EO showed the strongest antifungal activity ($p<0.05$) on tested fungi by macrodilution method (Table 1); so that the MIC and MFC were 0.01 and 0.3% for *G. candidum*, 0.005 and 0.3% for *Penicillium* spp., and 0.1 and 0.3% for *Aspergillus* spp.

By increasing the concentration of EOs, the diameter of the inhibitory zone was also increased in agar well diffusion method (Tables 2-4). *Z. multiflora* EOs had the most antifungal activity ($p<0.05$) against tested fungi; and the inhibition zone for *Aspergillus* spp., *Penicillium* spp., and *G. candidum* were 11.6, 5.7, and 7.1 mm, respectively.

Fungi are of the important agents of food spoilage due to the production of different enzymes and break down the nutrients (Ledenbach and Marshall, 2009). In this study, the antifungal effect of *M. piperita*, *M. pulegium*, and *Z. multiflora* on the growth of *Aspergillus* spp., *Penicillium* spp., and *G. candidum* were determined. The results of the present study showed that all the tested plants EOs had the acceptable antifungal activities on tested fungi. However, the most antifungal activity was recorded for *Z. multiflora* EOs.

The findings of this study are close to that of Gandomi et al. (2009) who found inhibitory effect of *Z. multiflora* EOs against *A. flavus* in culture media and white cheese. In their study, the value of MIC and MFC were reported 400 and 1000 ppm, respectively on PDA. Zomorodian

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Table 1: Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) levels of three studied plant essential oils against *Aspergillus* spp., *Penicillium* spp., and *Geotrichum candidum*

Plant essential oils	*Geotrichum candidum*		*Penicillium* spp.		*Aspergillus* spp.	
	MIC (%)	MFC (%)	MIC (%)	MFC (%)	MIC (%)	MFC (%)
Mentha piperita	0.3	1	0.05	1	0.5	1
Mentha pulegium	0.1	0.5	0.01	0.5	0.3	1
Zataria multiflora	0.01	0.3	0.005	0.3	0.1	0.3

Table 2: Mean inhibition zone (mm) of mycelial growth in media treated by *Mentha pulegium* essential oil

Concentrations (%)	*Aspergillus* spp.	*Penicillium* spp.	*Geotrichum candidum*
1	0 [a]	2.00 [a]	0.25 [a]
2	3.0 [b]	3.14 [a]	2.14 [b]
3	3.5 [b]	4.42 [b]	2.28 [b]
5	5.0 [b]	5.36 [b]	7.55 [c]
7	6.5 [c]	5.42 [b]	7.66 [c]

The different letters in each column indicate the significant difference (p<0.05).

Table 3: Mean inhibition zone (mm) of mycelial growth in media treated by *Mentha piperita* essential oil

Concentrations (%)	*Aspergillus* spp.	*Penicillium* spp.	*Geotrichum candidum*
1	0 [a]	1 [a]	0 [a]
2	1 [b]	1.16 [a]	0.8 [b]
3	2.5 [c]	3.37 [c]	4.4 [c]
5	3 [c]	4.28 [c]	4.5 [c]
7	5 [d]	4.62 [c]	5.87 [c]

The different letters in each column indicate the significant difference (p<0.05).

Table 4: Mean inhibition zone (mm) of mycelial growth in media treated by *Zataria multiflora* essential oil

Concentrations (%)	*Aspergillus* spp.	*Penicillium* spp.	*Geotrichum candidum*
1	0.5 [a]	0.83 [a]	0.66 [a]
2	3.5 [b]	2 [b]	0.71 [a]
3	4 [b]	2.12 [b]	1.66 [a]
5	9 [c]	4.42 [c]	3.28 [b]
7	11.6 [c]	5.66 [c]	7.1 [c]

The different letters in each column indicate the significant difference (p<0.05).

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et al. (2011) stated that the MIC and MFC ranges of *Z. multiflora* EOs against *Candida* sp. were 0.003-0.6 and 0.007-4 µl/ml, respectively. In another study, Effatpanah et al. (2010) examined the antifungal effects of the *Z. multiflora* EO on five different saprophytes and dermatophytes. These researchers showed that a >10 mg/ml concentration of *Z. multiflora* EO prevented the growth of *Aspergillus fumigatus* and *Aspergillus flavus*. The variation between the results may be related to the geographical condition and the time of harvesting the plants.

The differences in antifungal activities of EOs relate mainly to their chemical components. The main components of *Z. multiflora*, *M. pulegium*, and *M. piperita* EOs are thymol, pulegone, and menthol, respectively which may show different antimicrobial properties (Khosravi

et al., 2009; Saharkhiz et al. 2012; Sokovic et al. 2009; Verma et al. 2011). Hammer et al. (1999) reported that the antifungal activity of thyme (*Thymus vulgaris*) EO was stronger than the peppermint oil which is in agreement with the results of the present study. Similar findings were also reported by Ameziane et al. (2007) who showed perfect antifungal activity of *M. pulegium* against three fungal species, including *Penicillium digitatum*, *Penicillium italicum*, and *G. candidum*.

Conclusion

All EOs of *Z. multiflora*, *M. pulegium*, and *M. piperita* showed antifungal activities. However, as *Z. multiflora* EO showed the most antifungal effect, it could be specially suggested as natural powerful antifungal preservatives in food industry.

Author contributions

M.B. and H.M. designed the study; Z.Z.C. and A.E. conducted the experimental work; M.B. and A.E. analyzed the data; Z.Z.C. and M.B. wrote the manuscript. All authors revised and approved the final manuscript.

Conflicts of interest

There is no conflict of interest in the study.

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References

Ameziane N., Boubaker H., Boudyach H., Msanda F., Jilal A., Ait Benaoumar A. (2007). Antifungal activity of Moroccan plants against citrus fruit pathogens. *Agronomy for Sustainable Development*. 27: 273-277. [DOI: 10.1051/agro: 2007022]

Burt S. (2004). Essential oils: their antibacterial properties and potential applications in foods-a review. *International Journal of Food Microbiology*. 94: 223-253. [DOI:10.1016/j. ijfoodmicro.2004.03.022]

Derek J.S., Gary P.M. (2014). Human pathogenic fungi: molecular biology and pathogenic mechanisms. Caister Academic Press, UK.

Effatpanah H., Sabokbar A., Kordbacheh P., Bahonar A.R., Bayat M., Saeednejad L. (2010). Antifungal effect of *Zataria multiflora*: an *in vitro* evaluation. *Journal of Global Veterinaria*. 4: 140-143.

Fontenelle R.O.S., Morais S.M., Brito E.H.S., Kerntopf M.R., Brilhante R.S.N., Cordeiro R.A., Tomé A.R., Queiroz M.G.R., Nascimento N.R.F., Sidrim J.J.C., Rocha M.F.G. (2007). Chemical composition, toxicological aspects and antifungal activity of essential oil from *Lippia sidoides* Cham. *Journal of Antimicrobial Chemotherapy*. 59: 934-940. [DOI: 10.1093/jac/ dkm066]

Gandomi H., Misaghi A., Akhondzadeh Basti A., Bokaei S., Khosravi A., Abbasifar A., Jebelli Javan A. (2009). Effect of *Zataria multiflora* Boiss. essential oil on growth and aflatoxin formation by *Aspergillus flavus* in culture media and cheese. *Food and Chemical Toxicology*. 47: 2397-2400. [DOI:10. 1016/j.fct.2009.05.024]

Hammer K.A., Carson C.F., Riley T.V. (1999). Antimicrobial activity of essential oils and other plant extracts. *Journal of Applied Microbiology*. 86: 985-990. [DOI:10.1046/j.1365-2672.1999.00780.x]

Khatibi S.A., Misaghi A., Moosavy M.H., Amoabediny G., Akhondzadeh Basti A. (2015). Effect of preparation methods on the properties of *Zataria multiflora* Boiss. essential oil loaded nanoliposomes: characterization of size, encapsulation efficiency and stability. *Pharmaceutical Sciences*. 20: 141-148.

Khosravi A.R., Shokri H., Tootian Z., Alizadeh M., Yahyaraeyat R. (2009). Comparative efficacies of *Zataria multiflora* essential oil and itraconazole against disseminated *Candida albicans* infection in BALB/c mice. *Brazilian Journal of Microbiology*. 40: 439-445. [DOI: 10.1590/S1517-83822009000300003]

Ledenbach L.H., Marshall R.T. (2009). Microbiological spoilage of dairy products. In: Sperber W., Doyle M. (Editors). Compendium of the microbiological spoilage of foods and beverages. Springer-Verlag, New York.

Moradi M., Hassani A., Ehsani A., Hashemi M., Raeisi M., Naghibi S. (2014). Phytochemical and antibacterial properties of *Origanum vulgare* ssp. *gracile* growing wild in Kurdistan Province of Iran. *Journal of Food Quality and Hazards Control*. 1 :120-124.

Reiss E., Jean Shadomy H., Marshall Lyon G. (2012). Fundamental medical mycology. Wiley-Blackwell, New Jersey.

Saharkhiz M.J., Motamedi M., Zomorodian K., Pakshir K., Miri R., Hemyari K. (2012). Chemical composition, antifungal and antibiofilm activities of the essential oil of *Mentha piperita* L. *International Scholarly Research Network Pharmaceutics*. 2012: 718645. [DOI: 10.5402/2012/718645]

Singh P., Pandey A.K. (2018). Prospective of essential oils of the genus *Mentha* as biopesticides: a review. *Frontiers in Plant Science*. 9: 1295. [DOI: 10.3389/fpls.2018.01295]

Sokovic M.D., Vukojević J., Marin P.D., Brkic D.D., Vajs V., Van Griensven L.J.L.D. (2009). Chemical composition of essential oils of *Thymus* and *Mentha* species and their antifungal activities. *Molecules*. 14: 238-249. [DOI: 10.3390/molecules 14010238]

Verma R.K., Chaurasia L., Kumar M. (2011). Antifungal activity of essential oils against selected building fungi. *Indian Journal of Natural Products and Resources*. 2: 448-451.

Zarei Mahmoudabadi A., Dabbagh M.A., Fouladi Z. (2007). *In vitro* anti-Candida activity of *Zataria multiflora* Boiss. *Evidence-Based Complementary and Alternative Medicine*. 4: 351-353. [DOI: 10.1093/ecam/nel099]

Zomorodian K., Saharkhiz M.J., Rahimi M.J., Bandegi A., Shekarkhar G., Bandegani A., Pakshir K., Bazargani A. (2011). Chemical composition and antimicrobial activities of the essential oils from three ecotypes of *Zataria multiflora*. *Pharmacognosy Magazine*. 7: 53-59. [DOI: 10.4103/0973-1296.75902]

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