



Chemical Composition and Antifungal Activity of Essential Oil of *Zataria Multiflora*

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ABSTRACT

Background: *Aspergillus flavus* is a toxic contaminant in foods, which can induce mutagenic, teratogenic, and carcinogenic effects. In last decades, synthetic fungicides have been used for inhibition of fungal growth in plants. The public attention was also attracted to contamination of food chain by these chemicals. Therefore, in the current study, we decided to use *Zataria multiflora* (ZM) essential oil to inhibit the *Aspergillus flavus* growth. **Method:** The essential oil from ZM was obtained by hydro-distillation and analyzed by GC/MS. The minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC) of ZM essential oil were determined at different concentrations (0-1000 ppm). **Results:** In this study, Carvacrol (33.45%) and Thymol (34.44%) were the most frequent compounds of the ZM essential oil. The minimum inhibitory and fungicidal concentrations were 100 and 400 ppm, respectively. **Conclusions:** The presence of phenolic compounds such as Thymol and Carvacrol, as the major components of ZM essential oil inhibits the *Aspergillus flavus* growth. Furthermore, this essential oil has a very strong anti-bacterial effect. Considering these results, it seems that ZM essential oil is potentially an appropriate natural alternative for controlling *Aspergillus flavus* fungus.

Keywords: *Aspergillus flavus*; Chemical composition; *Zataria multiflora*; Antifungal activity

Introduction

Aflatoxins are produced by three strains of *Aspergillus flavus*, *Aspergillus parasiticus*, and *Aspergillus nomius* (Allameh *et al.*, 2011). The *Aspergillus flavus* can contaminate crops such as cotton seed, corn, pistachio, and peanuts. Pre- and

post-harvest as well as the biological and environmental factors are important in mycotoxin contaminations (Nikan and Ghamari, 2015). The secondary metabolism of *Aspergillus flavus*, aflatoxin consists of the toxic contaminants in

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foods that can induce mutagenic, teratogenic, and carcinogenic effects. Despite of health problems, contamination of crops with aflatoxin has direct and indirect economics effects. The regulatory guide lines of Food and Drug Administration (FDA) recommend that the standard level of aflatoxin in food products is 20 ppb (Bhatnagar-Mathur *et al.*, 2015). Moreover, the International Agency for Research on Cancer (IARC) has classified aflatoxin as a class I carcinogenic agents. In last decades, synthetic fungicides have been produced for inhibition of fungal growth in plants. However, the development of resistant strain and adverse environmental effects of these chemicals have drawn the attentions to substitute them by bioactive compounds such as essential oils (Ferreira *et al.*, 2013). Many researchers investigated the anti-fungal activity of herbal extracts to inhibit mycotoxin production (Ferreira *et al.*, 2013).

Zataria multiflora (ZM) is a thyme-like plant in Lamiaceae family, which grows in Iran, Pakistan, and Afghanistan. ZM has been used in traditional medicine as a herbal drug for treatment of different diseases (Jafari *et al.*, 2015). ZM essential oil is rich in phenolic compounds including carvacrol, thymol, and gamma-terpinene (Azizkhani *et al.*, 2013). The antibacterial, antifungal, and antiviral activities of ZM essential oil were proved in many studies (Jafari *et al.*, 2015, Mohammadi *et al.*, 2015). In the present study the effects of ZM essential oil as the anti-fungal plants were investigated.

Materials and Methods

The ZM was prepared from the local market and the herbarium confirmation process was performed by the Academic Center for Education, Culture, and Research (ACECR) of the University of Tehran. We dried the purchased ZM and obtained its essential oil by hydro-distillation method using a Clevenger. Then, the extracted oil was dried over anhydrous sodium sulfate followed by filtration. Later, it was stored in 4°C until injection to GC/MS apparatus (Saei-Dehkordi *et al.*, 2010)

The components of the essential oils were

estimated by Gas Chromatography-Mass Spectrometry device model (GC Hewlett-Packard 6890N) equipped with a HP-5MS (0.25x30) column and a Hewlett-Packard 5973N mass spectrophotometer. For GC-MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium gas was used as a carrier gas at a constant flow rate of 1 ml min⁻¹. Injector and mass transfer line temperatures were set at 250 °C and 300 °C, respectively. The oven temperature was programmed from 50 °C to 200 °C at 8 °C min⁻¹ and then held isothermally for 20 min and finally raised to 300 °C at 10 °C min⁻¹. Diluted samples (1/100 v/v, in methanol) of 0.2 µl were manually injected in the split less mode. Identification of compounds of the essential oil was based on GC retention time on VF-5 capillary column, computer matching of mass spectra with standards. The type of components entering the mass spectrometry was determined by the library study of Wiley and the ISIRI No 5192 (Saei-Dehkordi *et al.*, 2010)

Evaluation of the anti-fungal effects of essential oil of ZM: The antifungal activity of oil was determined by disc diffusion method. The sterile potato dextrose agar (PDA) containing 0, 50, 75, 100, 200, 300, and 400 ppm essential oil of ZM was prepared. A 5mm Whatman No.1 disk paper was inoculated with 10µl of a suspension of 10⁶ spore/mL and placed in the center of the plate. The plates were incubated for 9 days under 26°C. The diameter of the colony was measured daily in control group (lacking essential oils). The plate was completely covered with the fungi. In order to determine the minimal inhibitory concentration (MIC) or minimal fungicidal concentration (MFC), the disk of the plates with no fungal growth was transferred to a medium without essential oil. After that, discs with no growth were transferred to PDA plates without essential oil and incubated for 48 hours to determine the fungi static or fungicidal effect. The lowest concentration that inhibited the growth of the fungus was considered as MIC and the lowest concentration of essential oil with no observed growth in plate (which killed the test fungus) was taken as the minimum fungicidal

concentration (MFC). All treatments were carried out in triplicate (Akrami *et al.*, 2015).

Data Analysis: The tests were performed in randomized design with three repetitions and SPSS version 18 was used for statistical analysis of data. In addition, the inhibition of radial mycelia growth was examined using analysis of variance (ANOVA) and the mean scores were compared. It should be noted that the statistical difference was significant at the level of $P < 0.05$.

Results

The yield of *ZM* essential oil used in the current study was measured as 1.51% (v/w). The chemical compositions of the essential oil of *ZM* were identified using GC-MS analysis (Table 1).

Thymol (34.44%), Carvacrol (33.45%), and Parasymn (15.62%) were identified as the principle components in essential oil.

In order to analyze the effect of *ZM* essential oil on the growth of *Aspergillus flavus*, the fungus was cultured on a sterile PDA medium. In this experiment, the media containing 0, 50, 75, 100, 200, 300, and 400 ppm of the *ZM* essential oil were cultured for 9 days using a 5mm disk. The results are presented in Table 2. As shown, the plates containing ≤ 100 ppm essential oil did not show any fungal growth after 9 days. The disks without any growth were transferred to the PDA medium to determine the MIC and the MFC. The results are shown in Table 2. The concentration of MIC and MFC were 100 and 400 ppm, respectively.

Table 1. Chemical compounds of the essential oils of *Zataria multiflora*

Type of compound	RT ^a	KI ^b	Percentage	Type of compound	RT	KI	Percentage
Terpinen-4-ol	99.13	1200	1.11	γ -Pyronene	7.62	915	0.33
α - Terpineol	44.14	1222	0.93	o-Propylphenol	8.57	954	0.25
Thymyl methyl ether	14.93	1248	0.50	1,3-Cyclopentadiene	8.69	959	0.18
Carvacrol methyl ether	15.13	1258	0.85	α -Terpinolene	8.98	971	0.28
Bornyl acetate	15.93	1299	0/18	1,3-Cyclohexadiene	9.33	986	0.52
Thymol	16.76	1345	34.44	Myrcene	9.55	995	1.65
Carvacrol	16.98	1357	33.45	α -Phellandrene	9.89	1010	0.45
Thymol acetate	17.27	1373	0.86	α -Terpinene	10.17	1022	1.12
Carvacryl acetate	17.65	1393	0.95	<i>p</i> -Cymene	10.46	1035	15.62
Isocaryophyllene	18.20	1426	0.64	γ -Terpinene	11.17	1067	1.80
Alloaromadendrene	19.74	1516	1.23	α -Terpinolene	12.29	1118	1.85
Acetophenone	21.34	1615	0.53	<i>E,E</i> -Alloocimene	13.02	1153	0.20

^a: Retention Time; ^b: Kovats Index

Table 2. The effect of *Zataria multiflora* Essential oil on radial growth by *Aspergillus flavus* on PDA

Concentration (ppm)	Duration (day)			
	4 th	5 th	7 th	9 th
Control	30 \pm 1	43 \pm 1.7	63 \pm 2.6	all over the plate
50	8 \pm 0.6	11.5 \pm 2.1	20 \pm 1.5	32 \pm 1.5
75	Inhibition	8 \pm 0.6	12 \pm 1.5	28 \pm 1.7
100	NG	NG	NG	NG
200	NG	NG	NG	NG
300	NG	NG	NG	NG
400	NG	NG	NG	NG

Discussion

ZM is a popular and medicinal plant in Iran. During the recent years, due to its remarkable

antimicrobial activity in food industry, the plant has received high attention. The current study determined the chemical composition and

antifungal properties of *ZM* essential oil. *ZM* essential oil is a yellow liquid with a favorable odor and hot flavor. Thymol is a phenolic compound and the most effective component in the essential oil. Another important component of the essential oil is Carvacrol, which can be solved easily in the alcohol and organic solvents. These materials are generally stored in young leaves during the growth of the plant (Nabavi *et al.*, 2015). According to the literature, frequencies of the essential oils range from 0.8 to 2.6 percent in different parts of the *ZM*. This is consistent with the results of the present study (1.51%) (Nabavi *et al.*, 2015). The most frequent components of the *ZM* essential oil are Thymol and Carvacrol. In accordance with these results, (Hudaib *et al.*, 2002) Thymol, Carvacrol, and Parasymn were introduced as the major compounds of the essential oil of *ZM* with 5.08, 14.4, and 10.6 percent. In another study (Imelouane *et al.*, 2009), it was found that Carvacrol (71.12%) was the most frequent component in the *ZM*. Moreover, a study (Safari *et al.*, 2015) reported a similar result and measured the percentage of the Carvacrol as 60.3 percent. This confirms the results obtained by the present study. In another study, Goodarzi *et al.* reported Carvacrol as the most effective component of *ZM*. Most compounds found in the essential oils of *ZM* include anti-oxidant, anti-bacterial, anti-inflammatory, and analgesic (Goudarzi *et al.*, 2006). The anti-bacterial activity of *ZM* essential oils is attributed to existence of Thymol and Carvacrol. In this regard, Lambert *et al.* stated that a proper combination of Thymol and Carvacrol may prevent the growth of microorganisms more effectively than other herbal essential oils (Lambert *et al.*, 2001). This inhibitory effect occurs due to the damage to the cellular membrane resulted from influences on homeostatic pH and inorganic ion balance. Viuda-Martos *et al.* found that the essential oil of *ZM* inhibits the growth of mycelium of *Aspergillus flavus* fungus by denaturing the enzyme proteins (Viuda Martos *et al.*, 2007). This inhibitory effect is attributed to the compounds such as Thymol, Carvacrol, and Eeugenol. Furthermore, Centeno *et al.* showed that the Thymol, Carvacrol,

and Borneol compounds prevented the mycelium of *Aspergillus flavus* fungus from germinating by granulation of the cytoplasm contents and destroying the cytoplasm membrane (Centeno *et al.*, 2010).

The MIC is defined as a dose of an anti-microbial agent that may inhibit the in vitro growth of fungi or bacteria. Moreover, the MFC means the minimum anti-microbial agent that destroys the fungi or bacteria. In the present study, the MIC and MFC concentrations were 100 ppm and 400 ppm for the *Aspergillus flavus* fungus, respectively. Our results are consistent with the results of Fani-Makki *et al.* which reported the MIC and MFC of the *Zataria* for *Aspergillus flavus* as 200 and 400ppm, respectively (Fani-Makki *et al.*, 2015). In another study, Omid-Beigi *et al.* indicated that the *ZM* essential oil with the concentration of 350 µL/mL in a liquid medium prevented the growth of *Aspergillus flavus* with the inhibition rate of 100 percent (Omidbeygi *et al.*, 2007). Nikan *et al.* revealed that the concentrations of 0.06, 0.1, and 0.14 mg/mL of *ZM* essential oils were effective in prevention of the growth of *Aspergillus flavus* (Nikan and Ghamari, 2015). In addition, regarding the effects of the *ZM* essential oil on other microorganisms, Mohajerfar *et al.* reported the MIC of the *ZM* essential oils for *Listeria monocytogenes* as 400 ppm (Mohajerfar *et al.*, 2012). However, the lysozyme enzyme could not inhibit the growth of the bacteria even at 1000 ppm. In another study, Akhond-Zadeh Basti *et al.* found that the MIC of the *ZM* essential oils for the *Vibrio parahaemolyticus* bacteria was 100 ppm. (Mohajerfar *et al.*, 2012).

Conclusions

The existence of phenolic compounds in the *ZM* such as Thymol and Carvacrol as the major components may control the *Aspergillus flavus* effectively. In the current study, the Carvacrol and Thymol were found to be the most prevalent components of the *ZM* essential oils with frequencies of 33.45 and 34.44 percent, respectively. We found strong anti-bacterial effects for the essential oils of *ZM*. Therefore, use of essential oils is very advantageous. So, the

essential oils may be used as natural preservatives for most foods.

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References

- Akrami F, et al.** 2015. Antioxidant and antimicrobial active paper based on Zataria (Zataria multiflora) and two cumin cultivars (Cuminum cyminum). *Food science and technology*. **60** (2): 929-933.
- Allameh A, Ziglari T & Rasooli I** 2011. Phytoinhibition of growth and aflatoxin biosynthesis in toxigenic fungi. In *Aflatoxins-Detection, measurement and control*. InTech.
- Azizkhani M, Misaghi A, Basti AA, Gandomi H & Hosseini H** 2013. Effects of Zataria multiflora Boiss. essential oil on growth and gene expression of enterotoxins A, C and E in Staphylococcus aureus ATCC 29213. *International journal of food microbiology*. **163** (2): 159-165.
- Bhatnagar-Mathur P, Sunkara S, Bhatnagar-Panwar M, Waliyar F & Sharma KK** 2015. Biotechnological advances for combating Aspergillus flavus and aflatoxin contamination in crops. *Plant science*. **234**: 119-132.
- Centeno S, Calvo M, Adelantado C & Figueroa S** 2010. Antifungal activity of extracts of Rosmarinus officinalis and Thymus vulgaris against Aspergillus flavus and A. ochraceus. *Pakistan journal of biological sciences: PJBS*. **13** (9): 452-455.
- Fani-Makki O, Omidi A & Hasheminejad SA** 2015. Comparison of thyme, milk thistle and cape aloe essences on the growth of Aspergillus flavus and aflatoxin B1 production. *Journal of Shahrekord Uuniversity of medical sciences*. **17** (2): 92-98.
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- Authors' Contributions**
Akrami, Hekmatimoghaddam, Jebali and Khalili conceived and designed the experiments. Rahimi and heydari carried out the experiment. All authors contributed to the final version of the manuscript.
- Conflict of interest**
The authors declare no conflict of interests.
- Ferreira FD, et al.** 2013. Inhibitory effect of the essential oil of Curcuma longa L. and curcumin on aflatoxin production by Aspergillus flavus Link. *Food chemistry*. **136** (2): 789-793.
- Goudarzi M, Sattari M, Najar piraieh S, Goudarzi G & Bigdeli M** 2006. Antibacterial effects of aqueous and alcoholic extracts of Thyme on enterohemorrhagic Escherichia coli. *Yafteh*. **8** (3): 63-69.
- Hudaib M, Speroni E, Di Pietra AM & Cavrini V** 2002. GC/MS evaluation of thyme (Thymus vulgaris L.) oil composition and variations during the vegetative cycle. *Journal of pharmaceutical and biomedical analysis*. **29** (4): 691-700.
- Imelouane B, et al.** 2009. Chemical composition and antimicrobial activity of essential oil of thyme (Thymus vulgaris) from Eastern Morocco. *International journal of agriculture and biology*. **11** (2): 205-208.
- Jafari AA, Falah Tafti A, Hoseiny SM & Kazemi A** 2015. Antifungal Effect of Zataria multiflora Essence on Experimentally Contaminated Acryl Resin Plates With Candida albicans. *Iranian red crescent medical journal*. **17** (1): e16552.
- Lambert R, Skandamis PN, Coote PJ & Nychas GJ** 2001. A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. *Journal of applied microbiology*. **91** (3): 453-462.
- Mohajerfar T, et al.** 2012. Determination of minimum inhibitory concentration (MIC) of Zataria Multiflora boiss. essential oil and

- lysozim on *L. monocytogenes*. *Journal of medicinal plants*. **11 (44)**: 70-77.
- Mohammadi A, Hashemi M & Hosseini SM** 2015. Nanoencapsulation of *Zataria multiflora* essential oil preparation and characterization with enhanced antifungal activity for controlling *Botrytis cinerea*, the causal agent of gray mould disease. *Innovative food science & emerging technologies*. **28**: 73-80.
- Nabavi SM, et al.** 2015. Plants belonging to the genus *Thymus* as antibacterial agents: From farm to pharmacy. *Food chemistry*. **173**: 339-347.
- Nikan J & Ghamari S** 2015. In vitro growth inhibition of *Aspergillus flavus*, a major aflatoxin producing fungus, using *Thymus eriocalyx* essential oil. *Scientia*. **10 (3)**: 110-114.
- Omidbeygi M, Barzegar M, Hamidi Z & Naghdibadi H** 2007. Antifungal activity of thyme, summer savory and clove essential oils against *Aspergillus flavus* in liquid medium and tomato paste. *Food control*. **18 (12)**: 1518-1523.
- Saei-Dehkordi SS, Tajik H, Moradi M & Khalighi-Sigaroodi F** 2010. Chemical composition of essential oils in *Zataria multiflora* Boiss. from different parts of Iran and their radical scavenging and antimicrobial activity. *Food and chemical toxicology*. **48 (6)**: 1562-1567.
- Safari R, Adel M, Monji H, Riyahi Cholicheh H & Nematollahi A** 2015. Evaluation of antibacterial effect of some of the endemic herbal essential oils on *Streptococcus iniae* in vitro. *Journal of aquatic ecology*. **4 (4)**: 33-40.
- Viuda Martos M, Ruiz Navajas Y, Fernández López J & Pérez Álvarez J** 2007. Antifungal activities of thyme, clove and oregano essential oils. *Journal of food safety*. **27 (1)**: 91-101.