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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 Laminaceae 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 10^6 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 fugal 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	*p-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	*E,E-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)			
	4th	5th	7th	9th
Control	30 ± 1	43 ± 1.7	63 ± 2.6	all over the plate
50	8 ± 0.6	11.5 ± 2.1	20 ± 1.5	32 ± 1.5
75	Inhibition	8 ± 0.6	12 ± 1.5	28 ± 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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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.

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