The Antimicrobial activityof Essential Oil of Zataria multiflora Boiss and Satureja khuzestanica and their Stability

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Abstract

Introduction: Some plants show antimicrobial activity against wide range of microorganism.The aim of this study was to investigate the antimicrobial activity and stability of Zataria multiflora Boiss and Satureja khuzestanica essential oil (EO).

Materials and Methods: First, these plants (Zataria multiflora Boiss and Satureja khuzestanica) were collected, verified by a botanist, dried, and grinded. Then, hydro-distallation method was used, to obtain EO from each one. Their antimicrobial activity of each EO was evaluated by micro dilution broth method. For stability test, the surfaces (stone, MDF, and steel) were cleaned by 70% ethanol, and then separately incubated with each EO. Then, all were dried, and incubated for 12, 24 and 36 hours at room temperature. After incubation, sampling was done, inoculated on nutrient agar. Finally, the number of colonies grown on each plate was counted.

Results: The MIC50 of both EO was 3.1%. But, the MIC90 of Zataria multiflora Boiss EO against all strains was 12.5%, except Escherichia coli which was 6.2%. Also, the MIC90 of Satureja khuzestanica EO against all strains was 12.5%, except Aspergillus niger which was 6.2%. Both EOs had high stability until 36 hours on the stone and steel. In case of MDF, EOs were stable until 24 hours. No significant difference was observed between stability of Zataria multiflora Boiss and Satureja khuzestanica EO.

Conclusion: It can be concluded that Zataria multiflora Boiss and Satureja khuzestanica EO have approximately same antimicrobial activity and stability. Since both EOs have high antimicrobial property, they can be applied to remove microbes from different sources.

Keywords: Essential oil, Zataria multiflora Boiss, Satureja khuzestanica, Antimicrobial activity

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Introduction

The presence of pathogens in environment is a big problem in environmental health. It has prompted scientists to cope with the invisible enemies. Disinfection is one way to fight. Disinfection means the remove of vegetative forms of pathogens on the inanimate objects (such as surface houses, clothes, utensils, foods, etc.) or minimization of infection risk. Generally, when sterilization is impossible or unnecessary, disinfection is used. Disinfection is mostly done by chemicals, and leads to reduce of infected microbes. Today, there are a large number of chemical disinfectants against infectious agents. Moreover, disinfectants can be adsorbed by inhalation, ingestion, or skin. Some of them lead to lung disease, allergy, and hypersensitivity. [1, 2]

Genetic diversity of microbial pathogens, the emergence of resistant strains, and the adverse effects of disinfectants are main issues. These problems can be covered by natural disinfectant, such as essential oil (EO) of herbal plant. Some plants show a wide range of antimicrobial activities. In the present study, Zataria multiflora Boiss and Satureja khuzestanica which are native plants of Iran were selected. Lamiaceae family is one of the largest plant families that have a global distribution, and many investigations have been done on their antimicrobial property. The genus Thymus is one of the most important genera of this family. Approximately, it has 350 species in worldwide and 14 species in Iran. Moreover, Satureja is another important genus. Satureja khuzestanica is native herbal plant, and has branched stems and leaves. Both of them (Zataria multiflora Boiss and Satureja khuzestanica), have active chemicals such as EOs, tannins, saponins, thymol, carvacrol, alcohols, etc. [3, 4]

It has been established that certain EOs which extracted from herbal plant, have antiseptic and antimicrobial effects against many microorganisms. The aim of this study was to investigate the antimicrobial activity and stability of Zataria multiflora Boiss and Satureja khuzestanica EO.

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Materials and Methods

Extraction of EO from Zataria multiflora Boiss and Satureja khuzestanica

In the first step, these plants were collected, and verified by a botanist. Next, these plantswere dried, and grinded. To obtain EO, hydro-distillation was used as following: 200 mL of distilled water was added to 50 gram of each grinded plant in clevenger apparatus, and it was shaken for 2 hours at 100 ºC. Finally, EO of each plant was collected, and used in this study [5].Then, one mL of EO was diluted with 9 mL of diethyl ether to achieve 10% EO.

The evaluation of antimicrobial activity

At first, standard isolates of Candida albicans, Aspergillusniger, Escherichia coli, and Staphylococcus aureus were provided from Iranian Research Organization for Science and Technology (Table 1).

Table 1. Standard microbial strains which used in this study

Species	**Error! No text of specified style in document.**ATCC
E.coli	25922
S.aureus	25923
C.albicans	10231
A.niger	16888

Bacterial strains were inoculated on nutrient agar (Invitrogen, UK), and incubated for 24 hours at 35 °C. Also, fungal strains were inoculated on Sabouraud dextrose agar (Invitrogen, UK), and incubated for 24 hours at 25 °C. Next, microbial suspensions were prepared in Mueller Hilton broth (Invitrogen, UK) to adjust to ½ McFarland. In the next step, serial concentrations (10%, 5%, 2.5%, 1.25%, and 0.6%) of each EO were prepared in DMSO.

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Then, 100 µL of bacterial and fungal suspension was separately added to 100 µL of serial concentrations of each one, and incubated for 24 hours at 35 °C. After incubation, the optical density (OD) of each well was read by spectrophotometer (Novin Gostar, Iran) at 405nm. To calculate minimum inhibitory concentration (MIC), the difference of OD (ODt0-ODt24) in negative control was considered as 100% growth or 0% death, and the difference of OD (ODt0-ODt24) in each well was measured. Both MIC50 and MIC90 were obtained from Formula 1.[6]. In this study, controls were: a) Microbial suspensions which separately incubated with solvents, b) Bacterial isolates which incubated with 5 µg/mL ciprofloxacin, c) Fungal isolates which incubated with 3 µg/mL nystatin. In negative control, microbial suspensions were exposed to normal saline.

Here, each experiment was done three times, and the mean of MIC was calculated. As noted, both MIC50 and MIC90 of all isolates were reported.

Formula 1.

The inhibition percentage = (ODt0-ODt24) test×100/ (ODt0-ODt24) negativecontrol

Stability test

For this study, three types of surface were used, including stone, steel, and MDF. These surfaces were 5 cm×5 cm, and provided from shops of Yazd. At first, all surfaces were cleaned by 70% ethanol. In the next step, 100 µL of 10% EO obtained from each plant was separately spread to all surfaces by sterile swabs. Then, all of them were dried at room temperature, and incubated for 12, 24 and 36 hours at room temperature. After incubation, by a sterile swab, sampling was done from each surface, inoculated on nutrient agar (Invitrogen, UK) under sterile condition, and incubated for 48 hours at 37 °C. At end, the number of colonies grown on each plate was counted [7].

The surfaces which were not treated with EO, considered as negative control. Also, the surfaces which were treated with silver nanoparticles (ZFS Co., Iran) considered as positive control.

Results and Discussion

The MIC50 and MIC90 of Zataria multiflora Boiss and Satureja khuzestanica EO are shown in Table 2 and Table 3, respectively. As seen,

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the MIC50 of both EO was 3.1%. But, MIC90 of each EO was different, i.e. the MIC90 of Zataria multiflora Boiss EO against all strains was 12.5%, except Escherichia coli which its MIC90 was 6.2%. Also, the MIC90 of Saturejakhuzestanica EO against all strains was 12.5%, except Aspergillus niger which was 6.2%. It seems that both EOs have same antimicrobial activity. In case of Escherichia coli, there was significant difference between MIC90 of Zataria multiflora BoissEO and MIC90 of Saturejakhuzestanica EO ($P<0.05$). Also, significant difference was observed between MIC90 of Zataria multiflora Boiss EO and MIC90 of Satureja khuzestanica EO against Aspergillus niger ($P<0.05$).

Table 1. The MIC50 and MIC90 of Zataria multiflora Boiss EO.

	MIC_{50} (v/v%)	MIC_{90} (v/v%)
Aspergillus niger	3.1	12.5
Candida albicans	3.1	12.5
Escherichia coli	3.1	6.2
Staphylococcus aureus	3.1	12.5

Table 2. The MIC50 and MIC90 of Satureja khuzestanica EO.

	MIC_{50} (v/v%)	MIC_{90} (v/v%)
Aspergillus niger	3.1	6.2
Candida albicans	3.1	12.5
Escherichia coli	3.1	12.5
Staphylococcus aureus	3.1	12.5

The results of colony count after different incubation times when exposed to EO of Zataria multiflora Boiss and Satureja khuzestanica on stone, MDF, and steel surfaces are shown in Table 4, Table 5, and Table 6, respectively. As seen, the stone surfaces which were treated with EO of Zataria multiflora Boiss and EO of Satureja khuzestanica and incubated for 12 and 24 hours had approximately same colony count. But, after 36 hours, the stone surface which

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treated with EO Satureja khuzestanica had more colony count than surfaces which treated with EO of Zataria multiflora Boiss .There were significant differences between colony count of all treated stones and colony count of control surfaces ($P<0.05$). In case of MDF surfaces, same pattern was seen. But, no significant differences were seen between colony count of treated surfaces and control surfaces ($P>0.05$) after 36 hours. In case of steel surfaces, the colony count was decreased after 12, 24, and 36 hours when it treated with EO of Zataria multiflora Boiss and Satureja khuzestanica. Significant differences were seen between colony count of treated steel surfaces and control at all incubation times ($P<0.05$). As an important finding, the colony count of all treated surfaces was increased with increasing of incubation time. This phenomenon showed that the efficacy of both EOs was decreased along with time. Overall, both EOs had high stability until 36 hours on the stone and steel. These materials (plant EOs) may be having higher stability, and must be evaluated in future studies. In case of MDF, EOs were stable until 24 hours. The authors hypothesized that the coating materials of MDF have some components that interact with both EOs, and decrease their stability. As important finding, no significant difference was observed between stability of Zataria multiflora Boiss EO and Satureja khuzestanica EO.

Table 4. The colony count after different incubation times when exposed to EO of Zataria multiflora Boiss and Satureja khuzestanica on stone surfaces

	Control	EO of Zataria multiflora Boiss	EO of Satureja khuzestanica
12 h	63±5	2±2*	2±1*
24 h	92±2	23±8*	25±2*
36 h	183±3	48±17*	67±10*

*Compared with control which was not exposed to EO

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Table 5. The colony count after different incubation times when exposed to EO of Zataria multiflora Boiss and Satureja khuzestanica on MDF surfaces

	Control	EO of Zataria multiflora Boiss	EO of Satureja khuzestanica
12 h	65±2	5±3*	7±1*
24 h	70±3	51±3*	42±15*
36 h	76±2	69±5	76±3

*Compared with control which was not exposed to EO

Table 6. The colony count after different incubation times when exposed to EO of Zataria multiflora Boiss and Satureja khuzestanica on steel surfaces

	Control	EO of Zataria multiflora Boiss	EO of Satureja khuzestanica
12 h	42±2	24±12*	10±4*
24 h	105±2	76±30*	89±16*
36 h	227±1	107±10*	119±30*

*Compared with control which was not exposed to EO

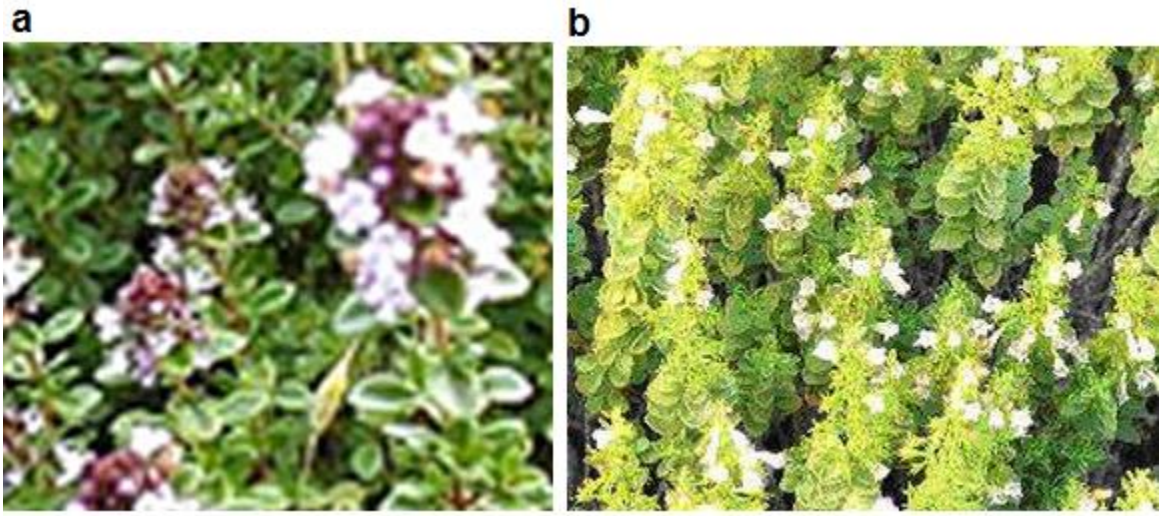

Figure 1. The illustration ofZataria multiflora Boiss(a) and Satureja khuzestanica(b) (3, 4).

Previously, although the antimicrobial property of Zataria multiflora Boiss and Satureja khuzestanica (Figure 1) have been approved, their stability has not been studied. In this section, some related researches are reported. Sharififar et al evaluated antibacterial and

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antioxidant properties of EO and methanol extracts of Zataria multiflora Boiss. The antibacterial test showed that the EO of the plant strongly inhibited the growth of all microorganisms. Its polar fraction of methanol extract has been effective against Gram-positive strains, while the non-polar fraction has shown activity similar to EO[3]. Ebrahimzadeha et al analyzed the EO and extract of Zataria multiflora Boiss by capillary gas chromatography. The results showed that the major components of the plant was thymol (44.6%), λ-terpinene (21.5%) and ρ-cymene (13.7%)[8]. Mahmoudabadiet al evaluated anti-Candida activity of Zataria multiflora Boiss against different species of Candida in vitro. Minimal inhibitory concentration of the methanolic and ethanolic extracts was 70.7 and 127 mg/L, respectively. Aqueous extract had no remarkable activity against Candida species [9]. Fazelia et al investigated antimicrobial effects of Zataria multiflora Boiss against some pathogenic food-borne bacteria. This study showed the OE had considerable antibacterial effects[10]. Misaghi et al worked on the effects of Zataria multiflora Boiss EO and nisin on Bacillus cereus. The antimicrobial efficacy of EO was significantly affected by temperature. The strong inhibitory action was observed by increasing EO concentration[11]. Mansour et al evaluated the antibacterial effect and physicochemical properties of EO of Zataria multiflora Boiss. They showed that the EO was effective on pathogenic bacteria particularly Staphylococcus aureus. The MIC values were from 0.39 mg/mL to 1.56 mg/mL [12].Abdollahi et al approved antioxidant, antidiabetic, antihyperlipidemic, reproduction stimulatory properties and safety of essential oil of Satureja Khuzestanica in rat. This study indicated the safety and interesting stimulatory effect of the plant on reproduction [4]. Sadeghi-Nejadet al assessed in vitro antifungal activity of the ethanolic extract of Saturejakhuzestanicaleaves. They showed that the ethanolic extract of the plant leaves exhibited antifungal activity against all tested saprophytic fungi with MIC values (625-5000µg/ml) [13]. Kheirandish et al studied the effect of Satureja khuzestanica essential oil on the lesions induced by Leishmania major in BALB/c mice. The mortality rate in treated groups was clearly less than the control [14].

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Conclusion

It can be concluded that Zataria multiflora Boiss and Satureja khuzestanica EO have approximately same antimicrobial activity and stability. At the end, since the EOs have high antimicrobial property and good stability, they can be used as two antimicrobial agent to remove microbes from different sources.

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