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ORIGINAL ARTICLE/ARTICLE ORIGINAL

The anti-dermatophyte activity of *Zataria multiflora* essential oils

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Summary
Objective. — Dermtophytes are a group of pathogenic fungi and the major cause of dermatophytosis in humans and animals. Fighting dermatophytes by natural essential oils is one important issue in new researches.
Materials and methods. — In this investigation, we evaluated the anti-dermatophyte activities of three samples of *Z. multiflora* essential oils against dermatophytes along with analysis of chemical compositions of the essential oils and their anti-elastase activities on elastase production in dermatophytes.
Results. — Carvacrol (1.5—34.4%), thymol (25.8—41.2%), carvacrol methyl ether (1.9—28.3%) and p-cymene (2.3—8.3%) were the main components of *Z. multiflora* essential oils. *Z. multiflora* essential oils (100 ppm) inhibited the mycelium growth of dermatophytes (6 ± 1.7—47.0 ± 1.4%) and had the minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC) values of 0.03—0.25 μl/ml against dermatophytes. Essential oils inhibited elastase produced in dermatophytes and pure porcine elastase.
Conclusion. — *Z. multiflora* essential oils can be used as natural anti-dermatophyte agent for fighting dermatophytes in further preclinical and clinical studies.
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Introduction

Dermatophytosis, as a common contagious disease, is caused by pathogenic keratin digesting fungi known as dermatophytes. The members of this infectious fungi cause illness in humans and animals [1]. In fact, this infection affect significantly children, adolescents and adults [2]. There are a limited number of antifungal agents to overcome fungal infections that are associated to poor efficacy and severe adverse effects [3]. Furthermore, the resistance to

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dermatophytes has increased worldwide, recently [4]. Therefore, finding the new antifungal agent by screening the natural or synthetic compounds with high efficacy and low side effects is essentially important to overcome these problems [3].

Medicinal plants are one of the natural sources for discovering new antifungal agents. In Iranian traditional medicine, *Zataria multiflora*, with traditional name of ''Satar'' or ''Zatar'', is popular as a strong efficient plant for treatment of many infectious diseases [5]. Up to now, there are many investigations on antibacterial and antifungal activities of *Z. multiflora* essential oils [6], but its antifungal activity have been limited to *Candida* and *Malassezia* species [7–10].

In this investigation, we evaluated the anti-dermatophyte activity of three commercial samples of *Z. multiflora* essential oils against dermatophytes with consideration to their chemical compositions. In addition, we examined the potency of these essential oils against the elastase production by dermatophytes and porcine pancreatic elastase *in vitro* condition.

Materials and methods

Z. multiflora essential oils and chemical composition analysis by GC and gas chromatography mass spectra (GC-MS)

Three commercial samples of *Zataria multiflora* essential oils (ZM921, ZM875, ZM8710) were prepared from Barij Essence Pharmaceutical Company, Kashan, Iran. The chemical compositions of essential oils were determined by GC-MS analysis using a Thermofinnigan Trace GC/MS single quadruple mass spectrometer with AS 800 auto sampler. The separations were achieved by capillary column, DB-5 (30 m × 0.25 mm, film thickness 0.25 μm). The column temperature was kept at 60 °C for 5 min and then at 250 °C for 10 min. The injection volume was 0.2 μl with split ratio 1/100. The flow rate of helium as carrier gas was 1.1 ml/min. MS was taken at 70 eV electron ionization, trap current 150 μA and source temperature 200 °C. The essential oil compounds were identified by comparison of their retention indices (RI), mass spectra fragmentation with those on the stored Wiley 7n.1 mass computer library and National Institute of Standards and Technology (NIST) [11].

Fungal strains and antifungal evaluations

Trichophyton rubrum PTCC 5143, *Trichophyton mentagrophytes* PTCC 5054, *Microsporum canis* PTCC 5069, *Microsporum gypseum* PTCC 5070 and *Trichophyton schoenleinii* PTCC 5221 were used in this investigation. The fungi were cultured on Sabouraud dextrose agar medium (SDA) and were incubated at 20–25 °C for 7–14 days in aerobic condition.

The antifungal activities of *Z. multiflora* essential oils were determined by two methods including microbroth dilution assay and inhibitory effects of *Z. multiflora* essential oils against mycelium growth of dermatophytes.

Z. multiflora essential oils were mixed with SDA at final concentrations of 100, 150, 200 ppm, separately. The plates containing SDA without essential oils were used as controls. For preparing the fungal patches, fungal suspensions were prepared by inoculating the fungal colonies in normal saline containing 0.05% Tween-80 and its turbidities were adjusted to 10^5–10^6 CFU/ml. One milliliter of this suspension was spread on the surface of SDA and was incubated for 7 days. From the surface of this culture, patches in the size of 6 mm were cut and inoculated on the center of the plates. The plates were incubated at 20–25 °C for 7 days in aerobic condition. The inhibition of mycelium growth was estimated as the results of mycelium growth of each intervention in comparison to control plates [12].

Minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC) of *Z. multiflora* essential oils were determined by microbroth dilution assay. The essential oils were serially diluted (64–0.015 μl/ml). After shaking, 100 μl of the solutions were added to the wells of 96-microtiter plates. The turbidity of each organism suspension was adjusted to 10^3–10^4 CFU/ml in Roswell Park Memorial Institute (RPMI) 1640 (Sigma) and was added to each well, and was incubated at 20–25 °C for 5 days. The MIC was defined as the lowest concentration of essential oil that completely inhibits visible fungal growth in the well after 5 days of incubation. MFC was the first dilution with no growth after culturing on SDA [13]. The experiments were replicated three times.

Anti-elastase activity of *Z. multiflora* essential oils

Different concentrations of *Z. multiflora* essential oils (1–0.125 μl/ml) were dissolved in 0.2 M Tris-HCl buffer (pH 8.0) (800 μl) and were mixed with 0.2 U porcine pancreatic elastase type IV (Sigma Chemical Co. St-Louis, 160 USA) (100 μl) and were incubated for 10 min at 25 °C. Then, 0.8 mM Suc-Ala-Ala-Ala-pNA (100 μl) was inserted to the above reaction. The controls were the reaction mixture without enzyme and the reaction without essential oils. After incubation at 25 °C for 20 min, the optical density (OD) was measured at 405 nm and the inhibition percent (I%) was calculated as $(1 - B/A) \times 100$; where A is the enzyme activity without essential oil, and B is the enzyme activity in the presence of essential oil [14]. The experiments were replicated in three times.

Effect of essential oils on elastase produced by dermatophytes

In order to evaluate the effects of essential oil on elastase produced by dermatophytes, it is essential to measure their elastase production.

Elastase production in dermatophytes was determined by colorimetric assay by employing elastin congo red (Sigma) as substrate. The fungi were cultured in RPMI supplemented with 0.8% glucose and 0.01% yeast extract for 7 days. The cultures were centrifuged and the supernatants were used as the test samples. Briefly, 650 μl of 50 mM Tris-HCl buffer (pH 8.5) and elastin congo red in buffer were mixed at room temperature for 5 min. Next, 250 μl of culture supernatant was added and incubated at 37 °C for 24 h at 180 rpm. Culture supernatant was replaced by 250 μl of Tris-HCl buffer as control. After incubation, insoluble material was removed by centrifugation and the absorbance was read at 495 nm [15].

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