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Anti-dermatophyte activity of *Pelargonium graveolens* essential oils against dermatophytes

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Abstract

Background: *Pelargonium graveolens* as valuable aromatic plant is used for extracting the essential oil, which their biological effects such as antioxidant, antimicrobial, anti-inflammatory and wound healing effects have been confirmed.

Methods: In this study, the anti-dermatophyte effects of nine *P. graveolens* essential oils were confirmed against *Microsporum canis*, *M. gypseum*, *Trichophyton rubrum*, *T. mentagrophytes*, and *T. schoenleinii* by mycelium growth inhibition percent and micro-broth dilution assays. The chemical composition of essential oils was determined by GC and GC-MS.

Results: The main components of *P. graveolens* essential oils and their percentages changed from one sample to others. The highest growth inhibition percent and the lowest MIC values of *P. graveolens* essential oils for dermatophytes were for E_{20} with 95% geraniol content, followed by E_4 and E_{14} with highest amounts of geraniol (21%) and citronellol (30–35%) contents.

Conclusion: Therefore, the use of *P. graveolens* essential oils with high geraniol and citronellol is recommended for dermatophytes. Animal models studies of dermatophytes are required to confirm the efficacy and application of *P. graveolens* essential oils in human studies.

Keywords: *P. graveolens*, Essential oil, Geraniol, Citronellol, Dermatophytes

Introduction

Pelargonium genus as perennial small shrubs compromises more than 250 species [1], *P. graveolens* (*Pelargonium roseum*) is highly valued by industries for producing geranium essential oil [2]. According to BS ISO 4371-2012, *P. graveolens* essential oil from different geographical origins should have geraniol (5–20%), citronellol (18–43%), citronellyl formate (4–12%), geranyl formate (1–8%), and linalool (2–11%) as the main components [3]. The chemical profile of *P. graveolens* essential oil is affected from its geographical origin. The pharmacological effects of *P. graveolens* essential oil such as antioxidant [4, 5], antimicrobial [6–9], anti-inflammatory [10, 11] and wound healings [12] activities is well documented. Furthermore, evaluation the antifungal activities of *P. graveolens* essential oil have been the subject of different experimental studies [13–16]. The antifungal activities of *P. graveolens* essential oil were confirmed against *Trichophyton* spp. [15], and *Candida albicans* [13, 14, 16]. Although, there are one report on anti-dermatophyte effects of *P. graveolens* essential oil against *Trichophyton* spp. [15], but there is no investigation to compare the anti-dermatophyte effects of *P. graveolens* essential oils against different kinds of dermatophytes (*Microsporum canis*, *M. gypseum*, *Trichophyton rubrum*, *T. mentagrophytes*, and *T. schoenleinii*).

Dermatophytes (*Trichophyton*, *Microsperum*, *Epidermophyton*) are one of major cause of superficial fungal infections with an estimate lifetime risk of 20–25% [17]. The use of topical and systemic drugs (Azoles/allylamines, terbinafine) is used for treatment of dermatophytes that are associated with adverse effects and

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appearance of resistant microbial strains [18]. Different kinds of essential oils have been evaluated against dermatophytes. *Prunus armeniaca*, *Prunus dulcis var. amara*, *Olea europaea*, *Mentha piperita* [19], *Artemisia sieberi* [20], *Juniperus communis ssp. alpina*, *J. oxycedrus* ssp. *oxycedrus* and *J. turbinate* [21] were effective against dermatophytes.

Therefore, the subject of this study was to evaluate the anti-dermatophyte effects of nine different *P. graveolens* essential oils from Iran, Germany and India against dermatophytes.

Materials and methods

P. graveolens essential oils

Nine different *P. graveolens* essential oils with identified chemical profiles as Table 1 were used in this study. Essential oils were prepared by Barijessence Pharmaceutical Company (Kashan, Iran). The chemical compositions of essential oil were determined by Quality Control of BarijEssence Pharmaceutical Company, Kashan, Iran.

Dermatophytes strains

Microsporum canis PTCC (Persian Type Culture Collection) 5069, *Microsporum gypseum* PTCC 5070, *Trichophyton rubrum* PTCC 5143, *Trichophyton mentagrophytes* PTCC 5054, and *Trichophyton schoenleinii* PTCC 5221 were used in this study. For preparing the dermatophytes, they were cultured on Sabauraud dextrose agar and the cultures were incubated at 22.5 ± 2.5 °C for 14 days. After growth of dermatophytes, suspensions of dermatophytes were prepared in normal saline with 0.05% polysorbate-80. This suspension was used for further antifungal evaluations.

Anti-dermatophyte activity of *P. graveolens* essential oils by mycelium growth inhibition method

P. graveolens essential oils (200 ppm) were incorporated into Sabauraud dextrose agar. Sabauraud dextrose agar without essential oils was used as controls. Then, the fungal patches were prepared by spreading the dermatophyte suspensions on the surface of Sabauraud dextrose agar and were incubated for 7 days at 25 °C in aerobic condition. The surface of cultured media (6 mm) were punched and put on the surface of Sabauraud dextrose agar with or without *P. graveolens* essential oils. The plates were incubated at the same conditions for 7 days. The mycelium growth inhibitions were measured for each plate and the growth inhibition percent were calculated from comparison between the growth in Sabauraud dextrose agar with or without essential oils. The experiment were replicated five times and the means of replicates were used in calculations [22].

Anti-dermatophytes evaluations by micro-broth dilution assay

For determining the Minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC) of *P. graveolens* essential oils, each essential oil were dissolved in dimethyl sulfoxide (DMSO) and serially diluted in RPMI broth media (8–0.015 μl/ml) in 96 micro-titer plates. Then, the adjusted dermatophyte suspensions (10^3–10^4 CFU/ml) in RPMI by spectrophotometric method was added to the wells of plates containing essential oil's serial dilutions, and was incubated at 22.5 ± 2.5 °C for 5 days. The lowest concentration of essential oil with visible fungal growth inhibition was defined as Minimal Inhibitory Concentration (MIC) and the first dilution with

Table 1 The chemical composition of *P. graveolens* essential oils

P. graveolens Essential oil	Main components	Source
E3	Citronellol (29.5%), linalool (13.4%), geraniol (10.6%), citronellyl formate (7.9%), geranyl formate (10.8%), geranyl acetate (7.2%), limonene (5%)	Germany
E4	Citronellol (30.2%), Geraniol (21.9%), linalool (11.04%), citronellyl formate (5%), geranyl formate (3.4%)	India
E20	Geraniol (95.6%), Trans-caryophyllene (3.5%), geranyl formate (1.6%), linalool (0.7%), citronellyl formate (0.3%), citronellol (0.2%)	India
E6	Citronellol (41%), citronellyl formate (12.7%), geraniol (8%), geranyl formate (2.1%), linalool (1%)	Zanjan, Iran
E9	Citronellol (48%), citronellyl formate (16.2%), geraniol (5.1%), geranyl formate (3%), linalool (1.1%)	Delijan, Markazi Province, Iran
E11	Citronellol (45.6%), citronellyl formate (14.1%), geraniol (3.4%), geranyl formate (1.6%), linalool (0.8%)	Shahrood, Iran
E14	Citronellol (34.7%), geraniol (21.3%), citronellyl formate (7.4%), linalool (6.3%), geranyl formate (4.4%), geranyl acetate (1.5%)	Mashhad-E-Ardehal, Isfahan Province, Iran
E21	Citronellol (48.3%), citronellyl formate (10.9%), geraniol (8.4%), linalool (2.2%), geranyl formate (1.7%)	Noorabad, Fars Province, Iran
E22	Citronellol (39.9%), citronellyl formate (13.4%), geraniol (4.2%), geranyl formate (2.8%), linalool (1.3%)	Dezfool, Iran

no growth after culturing on Sabauraud dextrose Agar was Minimal Fungicidal Concentration (MFC).The experiments were replicated three times [22].

Results and discussion

Essential oils are favorable natural compounds that can be used as natural alternative treatment for management of dermatophytosis. Citronellol (0.2–48.3%), geraniol (3.4–95.6%), citronellyl formate (0.3–16.2%), geranyl formate (1.6–10.8%), and linalool (0.7–13.4%) were the main components of 9 samples of *P. graveolens* essential oils (Table 1). The highest and lowest amounts of geraniol (95.6%) and citronellol (0.2%) were found in *P. graveolens* essential oil E_{20} from India. Trans-caryophyllene was the second main component of this essential oil (3.5%), followed by geranyl formate (1.6%), and linalool (0.7%). The highest amounts of citronellol were in *P. graveolens* essential oil E_{21} (48.3%), E_9 (48.0%), E_{11} (45.6%), and E_6 (41%).

Citronellol was the first main component of 8 samples of *P. graveolens* essential oils, while geraniol was the second main component of 2 samples of *P. graveolens* essential oils (E_4 and E_{14}). Linalool was the second main component of one sample *P. graveolens* essential oil. Citronellyl formate was found in 5 samples of *P. graveolens* essential oil as the second main component.

The chemical profiles and main components of *P. graveolens* essential oil were changed from one sample to others. The chemical profile of *P. graveolens* essential oils were not according to standard *P. graveolens* essential oil (BS ISO 4371-2012) [3].

The anti-dermatophyte effects of *P. graveolens* essential oils against dermatophytes by mycelium growth inhibition test exhibited that *P. graveolens* essential oil E_{20} had the highest mycelium growth inhibition percent against dermatophytes (30.9–53.3%), followed by E_4 (17.8–43.6%) and E_{14} (19.9–43.6%), respectively. *T. rubrum* had the higher mycelium inhibition percent (22.5–53.3%) by *P. graveolens* essential oils, followed by *M. gypseum* (17–45.4%), and *T. mentagrophytes* (16.5–43.6%). *M. canis* and *T. schoenleinii* had the less mycelium inhibition percent by *P. graveolens* essential oils (Table 2).

In micro-broth dilution assay, the MIC and MFC values for E_{20} were in the ranges of 0.03–0.125 and 0.06–0.25 μl/ml, respectively. E_{14} (MIC, MFC values of 0.06–0.125, 0.125–0.25 μl/ml), E_4 and E_{21} (MIC, MFC values of 0.06–0.25, 0.125–0.5 μl/ml) showed anti-dermatophyte activity, followed by E_{11}, E_9 (MIC, MFC values of 0.125–0.25, 0.125–0.5 μl/ml), E_3 (MIC, MFC values of 0.125–0.25, 0.25–0.5) μl/ml), E_6 (MIC, MFC values of 0.125–0.5, 0.25–0.5 μl/ml). E_{22} (MIC, MFC values of 0.125–0.5, 0.25–1 μl/ml) had the higher MIC and MFC values against dermatophytes than the others (Table 3).

As the results of anti-dermatophyte screening exhibited that E_{20} with high amount of geraniol had the highest activity against dermatophytes, followed by E_4, E_{14}. Indeed the *P. graveolens* essential oils with high amount of citronellol and geraniol as the first and second main components had the higher anti-dermatophyte effects. The essential oil with high amounts of citronellol or citronellyl formate showed anti-dermatophytes effects lower than the essential oils with citronellol and geraniol as the main components.

The results of anti-dermatophyte effects of *P. graveolens* essential oils have not been in according to their anti-candidal effects. There was no significant difference in anti-candidal activities of six samples of *P. graveolens* essential oils with citronellol (7.7–43.7%) and geraniol (19.3–48.5%) against clinical isolates of *C. albicans* in regard to inhibition zone diameters and the MIC and MFC values [23].

The results of this study confirmed that corresponding components responsible for anti-dermatophyte activity of *P. graveolens* essential oils were geraniol and citronellol. The mechanism of action for geraniol and citronellol is interfering with cell membrane of dermatophytes and decreasing the ergosterol content of cell by inhibition of ergosterol biosynthesis [24, 25], while the anti-candidal

Table 2 Mycelium growth inhibition of dermatophytes by 200 ppm *P. graveolens* essential oils

P. graveolens Essential oil	*T. schoenleinii*	*M. gypseum*	*T. mentagrophytes*	*T. rubrum*	*M. canis*
E21	15.9	17.0	16.5	22.5	17.2
E6	14.5	23.1	20.4	31.8	13.9
E22	9.8	18.7	22.6	28.5	10.3
E14	19.9	25	22.8	35.0	16.0
E20	30.9	45.4	36.3	53.3	41.4
E3	14.0	20.7	15.8	28.5	11.3
E11	20.5	20.0	19.5	28.8	8.8
E4	17.8	23.3	43.6	26.8	16.1
E9	14.9	18.0	31.0	27.6	17.2

Table 3 The antifungal activity of *P. graveolens* essential oils against dermatophytes

P. graveolens essential oil	*T. schoenleinii*		*M. canis*		*M. gypseum*		*T. mentagrophytes*		*T. rubrum*	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
E3	0.25	0.25	0.25	0.5	0.125	0.25	0.125	0.25	0.25	0.5
E4	0.125	0.125	0.125	0.25	0.125	0.25	0.06	0.125	0.25	0.5
E6	0.125	0.25	0.125	0.25	0.125	0.25	0.125	0.25	0.25	0.5
E9	0.125	0.25	0.125	0.25	0.25	0.25	0.125	0.25	0.25	0.5
E11	0.125	0.125	0.25	0.25	0.125	0.25	0.125	0.25	0.25	0.5
E14	0.125	0.125	0.125	0.25	0.06	0.125	0.125	0.25	0.125	0.25
E20	0.06	0.125	0.125	0.25	0.03	0.06	0.06	0.125	0.125	0.25
E21	0.125	0.125	0.125	0.5	0.125	0.25	0.06	0.125	0.25	0.25
E22	0.125	0.125	0.25	0.5	0.25	0.5	0.25	0.5	0.5	0.5
Amphotericin	0.25	0.25	0.25	0.25	0.25	0.25	0.5	1	0.5	0.5

MIC Minimal Inhibitory Concentration (μl/ml), *MFC* Minimal Fungicidal Concentration (μl/ml)

activity against *C. albicans* is not mediated by ergosterol biosynthesis [26].

Conclusions

Therefore, for preparing anti-dermatophyte products containing *P. graveolens* essential oils, the use of essential oil with main components of geraniol (30–35%) and citronellol (21%) are recommended. The third main component of essential oil should be linalool or citronellyl formate in higher percentage of 5%. More studies are required for comparing the efficacy of *P. graveolens* essential oils in animal studies.

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Authors' contributions
Mehdi Valian prepared and analyzed the essential oils; Mohaddese Mahboubi supervised the study and wrote the manuscript.

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The authors declare no conflict of Interest.

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