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# The anti-candidal activity of *Pelargonium graveolens* essential oils against clinical isolates of *Candida albicans*

**Mohaddese Mahboubi*, Elaheh Mahdizadeh, Rezvan HeidaryTabar**

## Abstract

***Objectives:*** *Candida albicans* as important opportunistic dimorphic fungi can cause the life threatening infections in humans. In this study, we evaluated the anti-candidal activities of six samples of *Pelargonium graveolens* essential oils against 31 clinical isolates of *C. albicans*.
***Materials and methods:*** The anti-candidal activity was performed by disc diffusion and micro-broth dilution assays. The chemical compositions of essential oils were analyzed by Gas Chromatography (GC) apparatus.
***Results:*** *P. graveolens* essential oil samples with citronellol (7.7-43.7%) and geraniol (19.3-48.5%) showed the same anti-candidal activity in two different methods. There is no significant difference between the inhibition zone diameters (19.3-24.1 mm), and the MIC and MFC values (1.06-1.48 and 1.5-1.72 µl/ml) of essential oil samples with different percent of citronellol and geraniol.
***Conclusion:*** Therefore, *P. graveolens* essential oils can be used as anti-candidal agent for further studies.

***Key Words:*** *Pelargonium graveolens*, Citronellol, geraniol, Clinical isolates, *Candida albicans*

**La actividad anti-Candida *Pelargonium graveolens* de aceites esenciales contra aislados clínicos de *Candida albicans***

## Resumen

***Objetivos:*** *Candida albicans* es un importante hongo dimórfico oportunista que puede llegar a amenazar la vida de pacientes con inmunosupresión. En este estudio se evaluaron las actividades anti-*Candida* de seis muestras de aceites esenciales de *Pelargonium graveolens* contra 31 aislamientos clínicos de *C. albicans*.
***Materiales y métodos:*** La actividad anti-Candida se realizó por difusión en disco y ensayos de dilución micro-caldo. La composición química de los aceites esenciales se analizó mediante cromatografía de gases.
***Resultados:*** Las muestras de aceite esencial de *P. graveolens* con citronelol (7,7 a 43,7%) y geraniol (19,3 a 48,5%) mostraron la actividad anti-*Candida* en dos métodos diferentes. No hubo ninguna diferencia significativa entre los diámetros de la zona de inhibición (19,3-24,1 mm), y valores de MFC (1,06 a 1,48 y de 1,5 a 1,72 l / ml) de muestras de aceites esenciales con diferentes porcentajes de citronelol y geraniol.
***Conclusión:*** Los aceites esenciales de *P. graveolens* se pueden utilizar como agentes anti-*Candida* para estudios adicionales.

***Palabras clave:*** *Pelargonium graveolens*, citronelol, geraniol, aislamientos clínicos de *Candida albicans*

## Introduction

*C. albicans* is the major cause of superficial and life-threatening systemic infections like oral candidiasis, vulvovaginal candidiasis and so on in humans[1]. *C. albicans* is able to cause infection in different host niches[2] and forms microbial biofilms on catheters, dentures and mucosal cell surfaces that makes *C. albicans* resistance to antimicrobial agents and host immune factors[3].

Essential oils as secondary metabolites of plants have been the subject of many investigations in recent years[4-6].

Geranium essential oil is extracted from *Pelargonium graveolens* leaves which grow in dry rocky slopes. Geranium essential oils have many applications in cosmetic and food industries[7]. Citronellol and geraniol are two main components of these essential oils that are criteria for standardization of geranium essential oil[8]. Geranium essential oil has been generally recognized as safe for use at 1.6 to 200 ppm in food industry by American Food and Drug Administration.

The biological activities of geranium essential oils such as antifungal activity against *Malassezia* sp[9] and *Candida* sp[10,11],

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anti-inflammatory[12], hypoglycemic[13], and spasmolytic effects have been confirmed[14].

Although, there are some investigations on geranium essential oil, but for the first time, we compared the anti-candidal activities of six different samples of geranium essential oils with different content of geraniol and citronellol against 31 isolates of *C. albicans*.

## Materials and method

### *Essential oils and chemical analysis*

Six samples of geranium essential oils were prepared from Barij Essence Pharmaceutical Comopany, Kashan, Iran. The essential oils were analyzed by GC-FID. The GC apparatus was Agilent technology (HP) 6890 system, capillary column of HP-5MS (60 m × 0.25 mm, film thickness 0.25 µm). The oven temperature program was initiated at 40 °C, held for 1 min then raised up to 230 °C at a rate of 3 °C /min held for 10 min. Helium was used as the carrier gas at a flow rate of 1.0 ml/min. The detector and injector temperatures were 250 and 230 °C, respectively[15].

### *Candida albicans strains*

This study was conducted on 29 clinical isolates of *C. albicans* isolated from vaginal or oral cavities of infected patients. *C. albicans* strains were cultured on Sabauraud dextrose agar with chloramphenicol and incubated at 25 ºC. The isolates were identified on the basis of positive germ tube test, chlamydospore formation, glucose, galactose, maltose, and sucrose absorption, glucose, galactose fermentation and negative absorption of urea, nitrate. *Candida albicans* ATCC 10231 and ATCC 66506 were used as control strains.

### *Antifungal activity of geranium essential oils against clinical isolates of C. albicans*

Micro-broth dilution and disc diffusion assays were used for antifungal screenings.

The turbidity of each clinical isolate was adjusted to $10^6$ CFU/ml (0.5 McFarland) by Spectrophotometer instrument after inoculating one colony of yeast in normal saline. The microbial suspensions were cultured on Sabouraud dextrose agar. Subsequently, paper discs (Padtan Teb Co, Tehran, Iran) were saturated with 15 µl of diluted geranium oils in DMSO. The concentration of stock solution was 256 µl/ml (4 µl/disc). Amphotericin B (10 U) and clotrimazole (10 µg) (Rosco, Diagnostica 140 A/S, Taastrupgaardsvej 30 DK-2630 Taastrup) were used as controls. *C. parapsilosis* ATCC 22019 were used as quality control strain in disc diffusion method. The plates were incubated at 20-25 ºC for 48 h. The inhibition zone diameters were measured by dial caliper and were recorded in millimeter ±Standard Deviation (mm±SD)[16].

The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) values of geranium essential oils were determined by micro-broth dilution assay. 100 µL of diluted essential oil (16-0.125 µl/ml) was prepared in two-fold serially pattern in 96-well micro-titer plates. MOPS-buffered RPMI 1640 was the broth media. The above microbial suspensions was diluted to $10^4$ CFU/mL, then 100 µL of microbial suspension was added to each well and incubated at 20-25 ºC. MICs were defined as the lowest concentration of essential dilution that inhibits yeast growth after 48 h. MFC values were the first well showing no growth on Sabouraud dextrose agar[17].

### *Statistical analysis*

All experiments were performed in triplicate and means of them were used for analysis by SPSS software (version 17, Chicago, IL, USA). ONE-Way ANOVA test was used to compare the difference between compounds and the p values were calculated. The results were significant at level 0.05.

## Results

Analysis of geranium essential oils by GC exhibited the amount of citronellol and geraniol in the ranges of 7.7-67.6% and 19.3-42.2%, respectively. Low citronellol was in geranium PG5 (7.7%), followed by geranium PG6 (20.9%), PG1 (35.5%) and PG3 (38.3%). Geranium PG4 (40.6%), PG2 (43.7%) had the high amounts of citronellol.

The amount of geraniol was between 19.3-48.5% in geranium essential oils. Geranium PG5 had the high amount of geraniol (48.5%), followed by PG6 (42.2%). Other geranium essential oils (PG1, PG2, PG3, and PG4) had the same amounts of geraniol (19.3, 23.9, 23.8 and 22.2%). Therefore, citronellol and geraniol constituted 67.6% of total oil composition of Geranium essential oil PG2, followed by PG3, PG4 and PG6 (~62-63%), and PG1 and PG5 (54.8-56.2%) (Figure 1).

Anti-candidal activity evaluations of geranium essential oils against clinical isolates of *C. albicans* by disc diffusion assay showed, geranium essential oils had inhibition zone diameters between 19.3-24.1 mm. The means of inhibition zone diameters of Geranium PG6 against clinical isolates were 24.1 mm, followed by PG4 (23 mm) and PG2 (20.7 mm). Geranium PG3, PG5 and PG1 had the low inhibition zone diameter 19.3, 19.6 and 19.7 mm, respectively. Statistical analysis the inhibition zone diameters exhibited there is no significant difference between the inhibition zone diameters of geranium essential oils against clinical isolates of *C. albicans* ($P>0.05$) (Table 1). On the base of criteria for susceptibility testing of amphotericin B (Resistant≥15, intermediate 10-14, resistant<9) and clotrimazole (Resistant≥20, intermediate 12-19, resistant≤11), All strain were sensitive to clotrimazole and amphotericin B.

The MIC and MFC values of geranium essential oils against *C. albicans* were 1.06-1.48 and 1.5-1.72 µl/ml, respectively. Geranium PG5 had the lowest MIC and MFC values 1.06 and 1.5 µl/ml. There is no significant statistically difference between the MIC and MFC values of geranium essential oils ($P>0.05$) (Table 1).

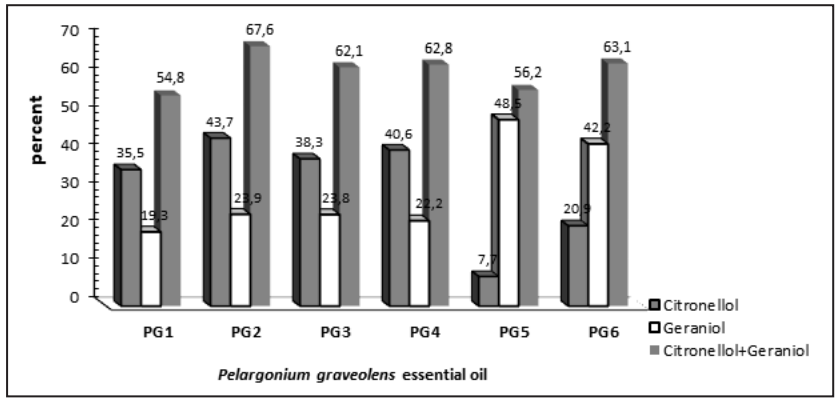


**Figure 1.** The percent of geraniol and citronellol in six samples of geranium essential oils

## Discussion

The emergence of antibiotic resistant *C. albicans*[18,19] along with the essential role of *C. albicans* in high incidence of infections[20,21] have been persuaded the scientist to find the new antifungal agents especially among the medicinal plants.

Geranium essential oil is a popular essential oil for fighting against *C. albicans* infections[11,22,23] but the chemical composition of essential oil takes effects by environmental factors and many other condition[24] and changing in chemical composition of essential oil may affect on its antimicrobial activities[25].

There is this concern about the variation in chemical composition of geranium essential oils and its anti-candidal activity. In this study we replied to this question whether the variation in chemical composition of geranium essential oils affects on anti-candidal activities or no?

As our results have shown geraniol and citronellol as the main components of geranium essential oils had varied amounts in different essential oil samples. It has been confirmed the antifungal activity of geranium is related to geraniol and citronellol as the main components[26,27].

It has been reported, geraniol and citronellol act on cell wall and cell membrane via interfering with ergosterol biosynthesis[26] while Leite et al (2014) stated geraniol does not inhibit ergostrol biosynthesis, while it alters the bilayer properties of fungal membranes[27]. In despite of the machanism of action, geraniol and citronellol participate in anticandidal activity of geranium essential oil. The geranium essential oil with low amount of citronellol (7.7%) and high amount of geraniol (48.5%) acts like geranium with 40.5% geraniol and 22.5% citronellol. Therefore, the total composition of citronellol and geraniol play essential role in anticandidal activity of geranium essential oils. If total composition of citronellol and geraniol had critical role in anticandidal activity, so, geranium essential oil PG2 with 67.6% geraniol and citronellol should have the higher anticandidal activity, while our results showed that there is no significant difference between the essential oil with high amounts of citronellol and geraniol (PG2) and geranium essential oils with lower amounts of them (PG1).

Therefore, geraniol, citronellol and other minor components of geranium essential oil play essential role in anticandidal activity of it.

**Table 1.** The anti-candidal activity of *P. graveolens* essential oils

| | Clinical isolates of *C. albicans* | | |
|---|---|---|---|
| | IZ (mm) | MIC (µl/ml) | MFC (µl/ml) |
| Geranium PG1 | 19.7±0.61 | 1.27±0.79 | 1.68±1.1 |
| Geranium PG2 | 20.7±0.61 | 1.27±0.94 | 1.72±1.07 |
| Geranium PG3 | 19.3±0.49 | 1.48±1.1 | 1.68±1.06 |
| Geranium PG4 | 23.0±0.8 | 1.32±0.9 | 1.71±1.07 |
| Geranium PG5 | 19.6±0.73 | 1.06±0.76 | 1.5±1.05 |
| Geranium PG6 | 24.1±0.79 | 1.22±0.78 | 1.5±1.01 |
| Clotrimazole | 38.3±0.23 | - | - |
| Amphotericin B | 24.1±0.54 | - | - |

IZ= inhibition Zone, MIC=Minimal Inhibitory Concentration; MFC= Minimal Fungicidal Concentration. The inhibition Zone diameters for *C. parapsilosis* ATCC 22019 were 30, 19 mm for clotrimazole and amphotericin B, respectively

## Conclusion

Six samples of commercial geranium essential oils were analyzed. The results of chemical investigation showed the presence of geraniol and citronellol in different amounts. Although geranium essential oils had varied geraniol and citronellol but they exhibited the same anti-candidal activity against clinical isolates of *C. albicans*. Therefore, whole components in geranium essential oil acts as anti-candidal agents. In conclusion, the use of geranium essential oil with the amounts of geraniol and citronellol higher than that of 50% is recommended for further clinical studies as anticandidal agent.

## Ethical responsibilities

***Protection of human and animal subjects.*** This research do not use animal nor human material or data.

***Confidentiality of data:*** Not applicable

***Right to privacy and informed consent:*** No applicable

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## Conflicts of interest

The authors declare no conflict of interest.

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