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# Antifungal effects of *Ziziphora tenuior*, *Lavandula angustifolia, Cuminum cyminum* essential oils against clinical isolates of *Candida albicans* from women suffering from vulvovaginal candidiasis

**Samaneh Dolatabadi[1], Zahra Salari[1], Mohaddese Mahboubi[2,*]**

**Abstract**

*Candida* sp. especially *C. albicans* is the main cause of candidiasis in women in reproductive ages. The prevalence of drug resistant *C. albicans* along with adverse effects of current treatments have encouraged the scientists to research on medicinal plant's essential oils. The aim of this study was to evaluate the potential effects of *Ziziphora tenuior*, *Lavandula angustifolia*, *Cuminum cyminum* essential oils against clinical isolates of *C. albicans*, which were isolated from women with vulvovaginal candidiasis. The anti-candidal effects of these commercial essential oils were screened against these clinical isolates (n=80) by disc diffusion and micro-broth dilution assays. The means of inhibition zone diameters, MIC and MFCs were reported for each essential oil. Also, the capability of fungal strain for biofilm formation in comparison with *C. albicans* ATCC 10231 were determined. The anti-biofilm effects of essential oils against 11 biofilm producing strains of *C. albicans* were determined. The results showed *C. cyminum* and *Z. tenuior* essential oils had the best anti-candidal effects, while the best biofilm killing effects was for *L. angustifolia* essential oil. *C. cyminum* and *Z. tenuior* essential oils can be formulated for more evaluations in preclinical and clinical studies.

***Key Words:*** biofilm, *Candida albicans*, *Ziziphora tenuior*, *Lavandula angustifolia*, *Cuminum cyminum*, essential oils

**Efecto antifungico de los aceites esenciales de *Ziziphora tenuior*, *Lavandula angustifolia* y *Cuminum cyminum* contra aislados clínicos de *Candida albicans* de mujeres que sufren de candidiasis vulvovaginal**

**Resumen**

*Candida* sp., especialmente *C. albicans,* es la principal causa de candidiasis en mujeres en edad reproductiva. La aparición de resistencia a los antifungicos de *C. albicans,* junto con el riesgo de efectos adversos de los tratamientos actuales, ha llevado a los científicos a buscar alternativas en los aceites esenciales derivados de plantas. Los objetivos del estudio fueron evaluar los efectos potenciales de los aceites esenciales de *Ziziphora tenuior*, *Lavandula angustifolia*, *Cuminum cyminum,* contra aislados clínicos de *C. albicans*, obtenidos de mujeres con candidiasis vulvovaginal. Los efectos anti-*Candida* de estos aceites esenciales comerciales fueron probados contra estos aislados clínicos (n=80) por difusión en disco y ensayos de microdilución. Se obtuvo el promedio de diámetro de inhibición, MIC y MFCs para cada aceite esencial. También se comparó la capacidad de formación de biopelículas de 11 cepas de *C. albicans* de cada aislado frente a la cepa de referencia *C. albicans* ATCC 10231 y la capacidad de cada aceite esencial para evitar la formación de biopelículas. Los resultados muestran que los aceites esenciales de *C. cyminum* y *Z. tenuior* tuvieron la mejor actividad anti-*Candida,* mientras que los mejores efectos para destruir biopelículas se obtuvieron con los aceites esenciales de *L. angustifolia*. Los aceites esenciales de *C. cyminum* y *Z. tenuior* ameritan tener mayores evaluaciones preclínicas y clínicas.

***Palabras claves:*** biopeliculas, *Candida albicans*, *Ziziphora tenuior*, *Lavandula angustifolia*, *Cuminum cyminum*, aceites esenciales

## Introduction

The infections related to opportunistic yeasts have increased in recent years. The main reason is the high prevalence of diseases related to opportunistic yeasts among the nosocomial infections and immune-compromised patients[1]. Candidiasis is containing a broad spectrum of opportunistic diseases that cause superficial skin infections to systemic ones in susceptible patients. Candidiasis is one of important infectious diseases in the world, which compromise high percent of nosocomial infections in intensive care units and total nosocomial infections[2]. *Candida* sp. and especially *C. albicans* can colonize the vagina and cause vulvovaginal candidiasis. Vulvovaginal candidiasis is the second prevalent infection of female reproductive systems after bacterial vaginitis by *Gardnerella vaginalis*. Seventy five percent of women are infected by vul-

1 Department of Microbiology, Neyshabur Branch, Islamic Azad University, Neyshabur, Iran
2 Medicinal Plants Research Department, Research and Development, Tabib-Daru Pharmaceutical Company, Kashan, Iran
* Autor para correspondencia.
Correo electrónico: mahboubi1357@yahoo.com
Tel: +98 3155541000

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vovaginal candidiasis, which can manifest as acute, chronic or recurrent ones[3,4]. Local treatments with Nizarol, Clotrimazole, Miconazole as the first line treatments are used for management of acute ones. The prolonged local/systematic therapy for at least six month is recommended for chronic, recurrent and resistant vulvovaginal candidiasis[5]. 138 million women worldwide annually were affected by recurrent vulvovaginal candidiasis, which 492 million of women are affected during their lifetimes. The patients with recurrent vulvovaginal candidiasis experience repeated episodes of vaginitis[6]. The resistant *C. albicans* strains[7], and the adverse effects related to these treatments[8] have encouraged the scientists to search among the essential oils as secondary metabolites of medicinal plants[9,10]. *Ziziphora tenuior*, *Lavandula angustifolia*, *Cuminum cyminum* are valuable plants for extracting the essential oils. In this investigation, we isolated 80 clinical isolates of *C. albicans* form women with vulvovaginal candidiasis. Then, the antifungal and anti-biofilm activities of *Z. tenuior*, *L. angustifolia*, *C. cyminum* essential oils were compared on these clinical isolates.

## Materials and methods

### *Essential oils and their specifications*

The essential oils were prepared by hydro-distillation method[11] in Clevenger type apparatus according to producer's certificate of analysis. *Z. tenuior* essential oil with main components of pulegone (37.9%), carvacrol (16%) and thymol (5.9%), *C. cyminum* essential oil containing cumin aldehyde (25.77%) as the main compounds, and *L. angustifolia* essential oil with main components of 1,8-cineol (36.6%), limonene (12.43%), linalool (9.24%), and terpinen-4-ol (0.72%) were prepared from TabibDaru Pharmaceutical Company, Kashan, Iran.

### Clinical isolates of *Candida albicans* strains

The fungal strains were isolated from vaginal discharges of 237 women with vulvovaginal candidiasis (22-45 years old). The samples were observed directly by light microscope. The samples with conical cells, mycelium or buds were cultured on Sabauraud dextrose agar with gentamicin and chloramphenicol (Conda Media culture). The plates were incubated at 25 °C for 48-72 h. The plates with creamy colony were screened by biochemical tests (Gram staining, germ tube and chlamydospore formation)[12] and molecular analysis by PCR-RFLP with ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) primers. *C. albicans* ATCC 10231 was used as control strain[13].

### Anti-Candidal activity of essential oils against clinical isolates of *C. albicans*

The antimicrobial evaluations of essential oils against clinical isolates of *C. albicans* strains were evaluated by disc diffusion and micro-broth dilution assays.

The confirmed clinical isolates of *C. albicans* were cultured on Sabouraud dextrose at 37 °C for 72 h. One or two colonies of each strain were suspended in sterilized normal saline and their turbidities were adjusted to 0.5 McFarland by spectrophotometric method ($1\times10^6$ CFU/ml=transmittance 85% at 590 nm). The suspension was cultured on Sabauraud dextrose agar by sterile cotton swab and discs containing different concentrations of essential oils in DMSO as solvent (2.5, 5, 10 µl/disc) were put on cultured media. DMSO and amphotericin B discs were used as controls. The plates were incubated at 35±2 °C for 72 h. After incubations, the inhibition zone diameters (IZ) were measured and the results were reported as means ±SD (Standard deviations). The experiments were performed in triplicates.

The micro-broth dilution assay was performed by diluting the dissolved essential oil in RPMI 1640 at concentrations of 32-0.125 µl/ml. The Candidal suspension were diluted to $1\times10^5$ CFU/ml. 100 µl diluted essential oil and Candidal strain were mixed in wells of 96 microtiter plates. The plates were incubated at above conditions and the first well with inhibitory effect on growth of *C. albicans* was reported as MIC (minimal Inhibitory Concentration) and the first well with no growth on solid media is defined as MFC (Minimal Fungicidal effect). The means of MIC and MFC values were determined and reported[14]. Amphotericin B 10 µg/disc (Rosco Diagnostica) and powder (Sigma) was used as positive control.

### *Determining the capability of C. albicans strains in biofilm formation*

100 µl of suspension containing $10^6$ CFU/ml *C. albicans* in RPMI were poured in the wells of 96 micro titer plates. The wells containing culture media with fungal strain and *C. albicans* ATCC 10231 were used as negative and positive wells, respectively. The plates were incubated at 25 °C for 48 h. After incubation, the plates were washed by 200 µl PBS, and dried. The plates were dyed by Crystal violet and washed by acetic acid 30% and read at 550 nm[15]. The percent of biofilm formation was determined in comparison with *C. albicans* ATCC 10231 and negative wells.

### *Anti-biofilm activity of essential oils against the strains with high potency in biofilm formation*

After determining the best strains in producing the biofilm, the biofilms were established by inoculating the fungal suspension ($10^6$ CFU/mL) into the wells and incubating at 37 ºC for 24 h as above. After that, the culture media were removed and the wells were washed with distilled water to remove the planktonic cells. The diluted essential oils (16-0.125 µl/mL) were added to wells and they were incubated at 37 ºC for 24 h, again. The biofilm staining with crystal violet and estimating the biofilm killing effects was performed and the biofilm killing effects of each compounds were estimated by determining the $OD_{550}$ of each well in comparison with control wells (fungal wells without essential oil)[16].

### *Statistical analysis*

The difference between the antimicrobial activities of essential oils by disc diffusion and micro-broth dilution assays were determined by SPSS software (version 21.0). One-way

analysis of variance (ANOVA) is used to determine the statistically significant differences between the means of independent (unrelated) groups at level of 0.05 ($p_{value}$<0.05).

### *Results*

237 vaginal discharges were gathered from the infected women, which 109 samples showed conical yeast cells with buds in direct microscopically evaluations. After culturing these samples on Sabouraud dextrose agars and identification tests, 80 samples showed the presence of *C. albicans*. *C. albicans* is the most prevalent etiological agent of acute vulvovaginal infections[17]. As our results showed 73.3% of isolated *Candida* strains was belonged to *C. albicans* strains. Rather than vulvovaginal candidiasis, *C. albicans* as opportunistic yeast is the major cause of oral candidiasis in immunocompromised individuals, or superficial skin infections or systemic candidiasis[18]. Therefore, the essential oils with antifungal activity against *C. albicans* strains can be a suitable alternative or complementary treatments in these patients. Evaluating the anti-Candidal effects of essential oils against 80 clinical isolates of *C. albicans* showed the dose response for anti-Candidal activity of each evaluated essential oil. Regardless the essential oil concentration, the inhibition zone diameters of *C. cyminum* essential oil (21.9 mm) statistically was higher than that of *Z. tenuior* essential oil (21.05 mm), while *L. angustifolia* oil (8.48 mm) had the lowest inhibition zone diameter against clinical isolates of *C. albicans*. 10 µl/disc *Z. tenuior* essential oil had the higher inhibition zone diameter (29.5±5.74 mm) than that of *C. cyminum* essential oil (27.5±7.7 mm) at the same concentration (Table 1). The inhibition zone diameter for 10 µg amphotericin B was 22.0±3.3 mm.

**Table 1-** The anti-Candidal activity of essential oils against clinical strains of *C. albicans* (n=80) by disc diffusion method (diameter in millimeter)

| Essential oils | Inhibition Zone (µl/disc) | | | |
|---|---|---|---|---|
| | 2.5 | 5 | 10 | Total |
| ***Cuminum cyminum*** | 15.6±5.4 | 20.1±±7.5 | 27.5±7.7 | 21.9[a] |
| ***Ziziphora tenuir*** | 13.6±5.27 | 20.5±6.5 | 29.5±5.47 | 21.05[b] |
| ***Lavandula angustifolia*** | 7.1±0.63 | 8.4±1.97 | 10.2±2.28 | 8.48[c] |
| **Amphotericin B (10 µg/disc)** | - | - | - | 22.0±3.3 |

The significance at the level 0.05 ($p_{value}$<0.05)

As table 2 is shown, determining the means of MIC and MFC values for clinical isolates of *C. albicans* in micro-broth dilution assay, showed that three samples of essential oils were belonged to two subsets. *C. cyminum* and *Z. tenuior* essential oils showed non-significant anti-Candidal effects against 80 clinical isolates of *C. albicans* (p>0.05). The MIC values for *C. cyminum* and *Z. tenuior* essential oils were 6.0±3.58 and 6.3±2.75 µl/ml, respectively. The corresponding MFC values were 9.58±6.51 and 10.0±3.48 µl/ml, respectively. *L. angustifolia* essential oil showed less anti-Candidal activity with MIC and MFC values of 10.3±3.64, 18.4±5.8 µl/ml, respectively. The MIC and MFC values for amphotericin B were 4.29±3.27 and 4.94±3.01 µg/ml, respectively. The higher anti-Candidal activity of *C. cyminum* and *Z. tenuior* essential oils were related to their main components. *C. cyminum* essential oil with main components of cumin aldehyde and *Z. tenuior* essential oil with main components of pulegone (37.9%), carvacrol (16%) and thymol (5.9%) are belonged to the essential oils with high antimicrobial activities[19]. The antimicrobial activity of *C. cyminum* essential oils was evaluated in different studies[20-24]. The anti-Candidal effects of *C. cyminum* essential oils with α-pinene (30%), limonene (21%), and 1,8-cineole (18.5%) against *C. albicans* ATCC 10231 with MIC value of 289 µg/ml[25] was confirmed, also *C. cyminum* essential oil with cuminic alcohol (30.3%), γ-terpinene (25.3%) and cuminic aldehyde (11.2%) showed the less activity against the *Candida albicans* ATCC 10231[25]. There is one report on anti-Candidal effect of *C. cyminum* essential oil with γ-terpinene (21.1%) against clinical isolates of *C. albicans* from recurrent vulvovaginal candidiasis with MIC value of 8.0±1.89 µg/ml[20]. The difference between our results and the last investigation is about the main components of *C. cyminum* essential oil and the MIC values (5.4 mg/ml vs. 8.0 µg/ml). According to the density of *C. cyminum* essential oil (0.904 g/mL), the means of MIC and MFC values of our essential oils were 5.4±3.2 and 8.6±5.8 mg/ml against clinical isolates of *C. albicans* from vulvovaginal Candidiasis. *Z. tenuior* essential oil with main components of pulegone (46.8%), p-menth-3-en-8-ol (12.5%), isomenthone (6.6%) had 80% germ tube inhibitory effects against *C. albicans* at concentration of 0.16 µl/ml of *Z. tenuior* essential oil[26].

**Table 2**- The anti-Candidal evaluations of essential oils against clinical isolates of *C. albicans* (n=80) by micro broth dilution assay

| Essential oil | Subset for α=0.05 | | | |
|---|---|---|---|---|
| | MIC (µl/ml) | | MFC (µl/ml) | |
| | Means±SD | Min-Max | Means±SD | Min-Max |
| ***Cuminum cyminum*** | 6±3.58[a] | 2-16 | 9.85±6.51[a] | 4-32 |
| ***Ziziphora tenuior*** | 6.3±2.75[a] | 4-16 | 10±3.48[a] | 8-16 |
| ***Lavandula angustifolia*** | 10.3±3.64[b] | 8-16 | 18.4±5.8[b] | 16-32 |
| **Amphotericin B (µg/ml)** | 4.29±3.27 | 1-8 | 4.94±3.01 | 2-8 |

Means for groups in homogenous subsets are displayed as a, b, c, which a is the smallest inhibition zone diameter.

## Discussion

Although, the anti-Candidal effect of *Z. tenuior* essential oil against *C. albicans* has been compared with clotrimazole[27], our investigation is the first study which compared the anti-Candidal effects of three essential oils with main components belonged to different groups of chemical structures against clinical isolates of *C. albicans*.

*C. albicans* can cause life threatening biofilm-based-infections in many areas of the body, particularly in gastrointestinal and genitourinary organs of human body. These biofilms form on implanted medical devices and intrinsically are resistant to treatment more than planktonic ones[28].

In this regard, evaluating the potency of strain in biofilm formation in comparison with *C. albicans* ATCC 10231 showed the strains had the less potency in formation of biofilms (Table 3). Therefore, 12 strains of *C. albicans* with higher potency in biofilm formation were chosen for further studies.

Evaluating the biofilm killing effects of essential oils against clinical isolates of *C. albicans* showed the biofilm killing effects of essential oil were dose dependent. The higher concentrations of essential oils showed the higher biofilm killing effects. Among the screened essential oil, *C. cyminum* and *L. angustifolia* essential oils had the highest biofilm killing effects, followed by *Z. tenuior* essential oils (Table 4). Although, *L. angustifolia* showed the less antifungal activity than that of *C. cyminum* and *Z. tenuior* essential oil, but screening the biofilm killing effects of *L. angustifolia* essential oil was a little higher than *C. cyminum* essential oil, followed by *Z. tenuior* essential oil. The anti-biofilm effect of *L. angustifolia* essential oil with main components of linalool was confirmed against *Staphylococcus aureus* and *Escherichia coli* biofilms[29]. Understanding the precise mechanism related to anti-Candidal effects of essential oils on biofilms need more investigations and one hypothesis related to it, can be the nature of main component(s) or interactions of components and biofilm.

In conclusion, our report is the first *in vitro* study, which compared the anti-Candidal effects of *C. cyminum*, *Z. tenuior* and *L. angustifolia* essential oils against 80 clinical isolates of *C. albicans* from women with vulvovaginal candidiasis. 73% of *Candida* isolated from vaginal discharge were belonged to *C. albicans*. 22.5% of these isolates produced biofilm between 10-22.5% in comparison with *C. albicans* ATCC 10231. The *C. cyminum*, *Z. tenuior* essential oils had the higher anti-candidal effects than that of *L. angustifolia* essential oil, while the biofilm killing effects of *L. angustifolia* essential oils was a less higher than *C. cyminum* essential oil, followed by *Z. tenuior* essential oil.

**Table 3-** The capability of *C. albicans* strains in biofilm formation in comparison with *C. albicans* ATCC 10231

| Biofilm formation (%) | Strain number |
|---|---|
| ≤5 | 28 |
| 5-10 | 34 |
| 10-15 | 2 |
| 15-20 | 14 |
| 20-25 | 2 |

**Table 4-** Biofilm killing effects of essential oils (%) against clinical isolates of *C. albicans* (n=12)

| Essential oil's concentration (µl/ml) | 16 | 8 | 4 | 2 | 1 |
|---|---|---|---|---|---|
| ***C. cyminum*** | 48.8% | 22.5% | 12.7% | 6.2% | 2.5% |
| ***Z. tenuir*** | 42.5% | 18.5% | 10.5% | 5.6% | 1.7% |
| ***L. angustifolia*** | 49.3% | 22.7% | 14.89% | 8.8% | 4.8% |

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## Ethical disclosures

**Protection of human and animal subjects.** This research do not used animal. Institutional review board approved the study.

**Confidentiality of data.** The authors declare to have followed the reccomendations of its institution to keep the confidenciality of patient´s data.

**Right to privacy and informed consent.** No data that permit to identify identity of patients is published, the authors have obtained the informed consent from patients

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**Conflict of Interest.** The author declare no conflict of interest.

## References

1. Al Mubarak S, Robert AA, Baskaradoss JK, Al-Zoman K, Al Sohail A, Alsuwyed A, et al. The prevalence of oral Candida infections in periodontitis patients with type 2 diabetes mellitus. Journal of infection and public health. 2013;6(4):296-301.
2. Garnacho-Montero J, Diaz-Martin A, Garcia-Cabrera E, Ruiz Perez de Pipaon M, Hernandez-Caballero C, Lepe-Jimenez JA. Impact on hospital mortality of catheter removal and adequate antifungal therapy in *Candida* spp. bloodstream infections. The Journal of antimicrobial chemotherapy. 2013;68(1):206-13.
3. Rathod SD, Klausner JD, Krupp K, Reingold AL, Madhivanan P. Epidemiologic features of Vulvovaginal Candidiasis among reproductive-age women in India. Infectious diseases in obstetrics and gynecology. 2012;2012:859071.
4. White DJ, Vanthuyne A. Vulvovaginal candidiasis. Sexually transmitted infections. 2006;82 Suppl 4:iv28-30.
5. Mazneikova V. Vaginal candidiasis--treatment protocols using miconazole and fluconazole. Akush Ginekol 2003;42 Suppl 2:30-4.
6. Sobel JD. Recurrent vulvovaginal candidiasis. American Journal of Obstetrics & Gynecology. 2016;214(1):15-21.
7. Cernicka J, Subik J. Resistance mechanisms in fluconazole-resistant *Candida albicans* isolates from vaginal candidiasis. International journal of antimicrobial agents. 2006;27(5):403-8.
8. Spence D. Candidiasis (vulvovaginal). BMJ clinical evidence. 2010;2010.
9. Swamy MK, Akhtar MS. Antimicrobial properties of plant essential oils against human pathogens and their mode of action: An updated review. Evidence-Based Complementary and Alternative Medicine. 2016;2016:3012462.
10. Mith H, Dure R, Delcenserie V, Zhiri A, Daube G, Clinquart A. Antimicrobial activities of commercial essential oils and their components against food-borne pathogens and food spoilage bacteria. Food science & nutrition.

2014;2(4):403-16.
11. Rassem HHA, Nour AH. Techniques for extraction of essential oils from plants: a review. Australian Journal of Basic and Applied Sciences. 2016;10(16):117-27.
12. Mahboubi M, Kazempour N. The anti-candidal activity of Satureja khuzistanica ethanol extract against clinical isolates of C. albicans. Journal de Mycologie Médicale. 2016;26(1):e6-e10.
13. Roshan R, Sabokbar A, Badali H. Identification of *Candida s*pecies isolated from vulvovaginal candidiasis using PCR-RFLP. International Journal of Molecular and Clinical Microbiology. 2014;4(2):406-10.
14. Mahboubi M, Kazempour N. The anti-candidal activity of *Satureja khuzistanica* ethanol extract against clinical isolates of *Candida albicans*. Journal de Mycologie Médicale. 2016;26(1):e6-e10.
15. Turan H, Demirbilek M. Biofilm-forming capacity of blood-borne Candida albicans strains and effects of antifungal agents. Revista Argentina de microbiologia. 2018;50(1):62-9.
16. Coffey BM, Anderson GG. Biofilm formation in the 96-well microtiter plate. Methods in molecular biology (Clifton, NJ). 2014;1149:631-41.
17. Cassone A. Vulvovaginal *Candida albicans* infections: pathogenesis, immunity and vaccine prospects. An International Journal of Obstetrics & Gynaecology. 2015;122(6):785-94.
18. Mayer FL, Wilson D, Hube B. *Candida albicans* pathogenicity mechanisms. Virulence. 2013;4(2):119-28.
19. Mahboubi M, Kazempour N, Mahboubi A. The efficacy of essential oils as natural preservatives in vegetable oil. Journal of dietary supplements. 2014;11(4):334-46.
20. Minooeianhaghighi MH, Sepehrian L, Shokri H. Antifungal effects of *Lavandula binaludensis* and *Cuminum cyminum* essential oils against *Candida albicans* strains isolated from patients with recurrent vulvovaginal candidiasis. J Mycol Med. 2017;27(1):65-71.
21. Tavakoli HR, Mashak Z, Moradi B, Sodagari HR. Antimicrobial activities of the combined use of *Cuminum cyminum* l. essential oil, nisin and storage temperature against *Salmonella typhimurium* and *Staphylococcus aureus* in vitro. Jundishapur journal of microbiology. 2015;8(4):e24838.
22. Khosravi AR, Shokri H, Minooeianhaghighi M. Inhibition of aflatoxin production and growth of *Aspergillus parasiticus* by *Cuminum cyminum*, *Ziziphora clinopodioides*, and *Nigella sativa* essential oils. Foodborne pathogens and disease. 2011;8(12):1275-80.
23. Pajohi MR, Tajik H, Farshid AA, Hadian M. Synergistic antibacterial activity of the essential oil of *Cuminum cyminum* L. seed and nisin in a food model. Journal of applied microbiology. 2011;110(4):943-51.
24. Iacobellis NS, Lo Cantore P, Capasso F, Senatore F. Antibacterial activity of *Cuminum cyminum* L. and Carum carvi L. essential oils. Journal of agricultural and food chemistry. 2005;53(1):57-61.
25. Naeini A, Naderi NJ, Shokri H. Analysis and in vitro anti-*Candida* antifungal activity of *Cuminum cyminum* and *Salvadora persica* herbs extracts against pathogenic *Candida strains*. J Mycol Med. 2014;24(1):13-8.
26. Abu-Darwish MS, Cabral C, Gonçalves MJ, Cavaleiro C, Cruz MT, Paoli M, et al. *Ziziphora tenuior* L. essential oil from Dana Biosphere Reserve (Southern Jordan); Chemical characterization and assessment of biological activities. J Ethnopharmacol. 2016;194:963-70.
27. Behmanesh F, Pasha H, Sefidgar AA, Taghizadeh M, Moghadamnia AA, Adib Rad H, et al. Antifungal effect of lavender essential oil (*lavandula angustifolia*) and clotrimazole on *Candida albicans*: An in vitro study. Scientifica. 2015;2015:261397.
28. Nobile CJ, Johnson AD. *Candida albicans* biofilms and human disease. Annual review of microbiology. 2015;69:71-92.
29. Budzynska A, Wieckowska-Szakiel M, Sadowska B, Kalemba D, Rozalska B. Antibiofilm activity of selected plant essential oils and their major components. Polish journal of microbiology. 2011;60(1):35-41.