Efficacy of *Syzygium aromaticum* essential oil on the growth and enzymatic activity of pathogenic *Candida albicans* strains

Ashkan Hekmatpanah1, Aghil Sharifzadeh1, Hojjatollah Shokri2, Sepideh abbaszadeh3,4, Donya Nikaein1

1Department of Microbiology and Immunology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
2Department of Pathobiology, Faculty of Veterinary Medicine, Amol University of Special Modern Technologies, Amol, Iran
3Health Research Center, Lifestyle Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran
4Department of Nutrition and Food Hygiene, Faculty of Health, Baqiyatallah University of Medical Sciences, Tehran, Iran

**Article Info**

**Article type:** Original article

**Article History:**
Received: 16 August 2021
Revised: 21 November 2021
Accepted: 15 February 2022

***Corresponding author:**
Aghil Sharifzadeh
Myology Research Center, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
Email: asharifzadeh@ut.ac.ir

**How to cite this paper**
Hekmatpanah A, Sharifzadeh A, Shokri H, abbaszadeh S, Nikaein D. Efficacy of *Syzygium aromaticum* essential oil on the growth and enzymatic activity of pathogenic *Candida albicans* strains. Curr Med Mycol. 2022; 8(1): 12-19. DOI: 10.18502/cmm.8.1.9209

**Introduction**

Recently, the increase in the immunocompromised patient population has increased the occurrence of both systemic and local infections caused by *Candida* spp. In these patients, the yeasts passing through the mucosa can attack various tissues and cause considerable damage and even mortality [1]. Therefore, various hydrolytic enzymes are secreted by *Candida albicans* (*C. albicans*) to ensure its penetration into the host cells. Various studies have concentrated on assessing fungal lipases and their contribution to fungal pathogenicity [2-5]. Moreover, *C. albicans* adherence is facilitated by the secretion of aspartyl proteinases (SAPs) to numerous host tissues. Assessments of vaginal and oral clinical isolates of *C. albicans* revealed a positive association between the virulence of *C. albicans* and the level of Sap production in vitro [6,7]. Moreover, *Candida* can supply the iron needed for its growth through the production of hemolysin, so the production of hemolysin is essential in the pathogenesis of *Candida* and its ability to survive [8].

Regardless of the discovery of potent antifungals, most of these infections still present a serious medical problem owing to increasing fungal resistance [9]. This highlights the need for developing novel therapeutic approaches through searching for agents with new mechanisms of action independent of or together with conventional medicines. Different bioactive molecules...
have been reported to be obtained from various natural resources as potent antifungal compounds. In our search for natural antifungal compounds from plants, we conducted a phytochemical screening on Syzygium aromaticum (L.) flowers (S. aromaticum) (carnation), which is one of the flower crops extensively cultivated in Iran [10]. According to some microbial studies, the S. aromaticum essential oil has considerable inhibitory effects against bacterial, fungal, and viral strains [11,12]. Therefore, the present study aimed to determine the chemical composition and inhibitory effects of S. aromaticum essential oil on the growth of pathogenic C. albicans isolates and the secretion of their hydrolytic enzymes including phospholipase proteinase and hemolysin that are in charge of invasive features.

Materials and Methods

Fungal strains and growth circumstances

The fungal isolates were obtained from the archival collection of the Laboratory of Mycology Research Center, Faculty of Veterinary, University of Tehran, Iran, and included one isolate from the American Type Culture Collection (ATCC 10231) and 15 clinical C. albicans isolates. The study protocol was approved by the University Ethics Committee (Code of Ethics: 300511/6/4). All C. albicans isolates were identified in previous studies using CHROM agar Candida (CHROM agar Candida Company, Paris, France), RapID™ yeast identification system (Remel, USA), and molecular analysis [13].

Extraction and chemical analysis of S. aromaticum essential oil

S. aromaticum flower buds prepared from Pars Iman Daru Company (Tehran, Iran) were exposed to water distillation for 3 h utilizing a Clevenger-type apparatus, according to the procedure reported by Divband et al. (2017) (Table 1) [14]. The oil was then stored at 4°C in the dark before chemical analysis and mycological tests.

Gas chromatography-mass spectrometry (GC–MS) and gas chromatography (GC) were utilized for essential oil analysis. Subsequently, GC was conducted via two fused silica capillary columns with various stationary phases including SPB-1 (film thickness of 0.20 μm; polydimethylsiloxane 30 m × 0.20 mm i.d.), and SupelcoWax 10 (film thickness of 0.20 μm; polyethylene glycol 30 mx0.20 mm i.d.); injector temperature: 250°C; detector carrier gas: helium, adjusted to a linear velocity of 30 m s⁻¹; oven temperature program: 70–220°C (3°C min⁻¹), 220°C (15 min); splitting ratio 1: 50; temperatures: 250°C. To perform GC–MS, an HP1 fused silica column was used (film thickness of 0.25 μm; polydimethylsiloxane 30 m × 0.25 mm i.d.), interfaced with a mass selective detector. GC parameters included interface temperature: 250°C; MS quadrupole temperature: 150°C; MS source temperature: 230°C; ionization current: 60 μA; ionization energy: 70 eV; the scan range: 35–350 μm; and scans: s⁻¹:4.51. Ingredients were recognized by their retention indices that were calculated by linear interpolation relative to retention times of some n-alkanes, as well as their mass spectra, and were compared with those in published studies [2]. Relative quantities of individual components were determined based on GC peak areas without FID response factor correction.

Antifungal activity assay

The susceptibility of C. albicans isolates to fluconazole and S. aromaticum was determined using Roswell Park Memorial Institute 1640 medium (Sigma, St Louis, MO, USA) buffered to pH 7.0 with 0.165 M 3-(N-morpholino) propanesulphonic acid (MOPS) buffer. A minimum inhibitory concentration (MIC) assay was conducted utilizing the standardized broth microdilution practice (M27-A3-S4) of the Clinical and Laboratory Standards Institute [15]. Briefly, dilution of fluconazole was performed in the RPMI-1640 in the above-described medium in 96-well microtitre plates to ultimate concentrations (128 μg/ml– 0.125 μg/ml). Moreover, different dilutions of S. aromaticum essential oil were prepared (39, 78.1, 156.2, 312.5, 500, 625, 1000, 1250, 2000, 2500, and 5000 μg/ml) in 96-well microtiter plates through MOPS-buffered RPMI-1640 media (Sigma, St. Louis, MO, USA). Isolates were added to achieve an ultimate concentration of 0.5-2.5×10⁶ cfu/ml. Positive and negative controls consisted of wells without antifungals and microorganisms, respectively. The incubation of the microtitre plates was performed for 48 h at 30°C. The MIC was interpreted as the lowest concentration of antifungal agent that totally inhibited the growth of the tested Candida strains compared with the control, after the incubation time. Each experiment was performed thrice. Moreover, the media from wells with yeasts presenting no visible growth was further cultured on Sabouraud dextrose agar (SDA) (Merck, Darmstadt, Germany) to determine the minimum fungicidal concentration (MFC). Following the determination of the MFC value as the lowest concentration, only four colonies were yielded, corresponding to 98% mortality of the yeasts in the primary inocula.

Table 1. Ethnobotanical data and active constituents of the studied plant.

| Scientific name     | English name           | Family        | Used part            | Common Medicinal uses                                                                 | Active Constituents                        |
|---------------------|------------------------|---------------|----------------------|---------------------------------------------------------------------------------------|-------------------------------------------|
| Dianthus caryophyllus| Carnation, clove pink  | Caryophyllaceae| Flower               | Skin toner, antifungal, relief of acute dermatitis, tooth pain, vomiting and gastritis, digestive function stimulant, antispasmodic | Flavonoids, anthocyanins, Dianthramides, Antiretroviral proteins and phenols |
For each isolate, fluconazole breakpoints were allocated as susceptible dose-dependent (SDD), MIC=4 μg/ml; susceptible (S), MIC=2 μg/ml; resistant (R), MIC ≥ 8 μg/ml, utilizing the M27-A3 protocol for all isolates [15]. C. parapsilosis ATCC 22019 and C. krusei ATCC 6258 were used as reference strains.

**Pre-treating C. albicans cells with S. aromaticum essential oil**

The *S. aromaticum* essential oil at MIC was applied on fresh suspensions of *C. albicans* isolates for 1 h, at 35°C (in RPMI-1640 liquid medium). They were then rinsed three times with phosphate-buffered saline (PBS) to avoid carryover effects [16]. The prepared *Candida* suspensions were utilized twice in the assays on three separate occasions described in the following.

**Measurement of hemolysin, phospholipase, and proteinase activities of C. albicans treated with oil**

An egg yolk agar assay was used to examine phospholipase activity (Phz). The test medium comprised SDA supplemented with 1M CaCl₂, 1M NaCl, and egg yolk (10%) [17]. Bovine serum albumin (BSA) assay was used to evaluate proteinase activity (Prz). The medium included BSA (0.2%), yeast extract (0.01%), glucose (1.17%), and agar (2%) at pH 5.0 [18]. The assay of Hemolytic activity (Hz) was performed on SDA plates supplemented with 7% horse blood [19]. Incubation of an aliquot (5 μl) of the yeast suspension preincubated with essential oil was performed on the plates comprising the above substrates for 3 days (phospholipase, proteinase) or 2 days (hemolysin) at 37°C. Staining BSA plates with amido black - 0.25% (w/v) was performed in glacial acetic acid 49.75% (v/v) before measuring the proteinase activity. The plates were then washed immediately with distilled water. Phz, Hz, and Prz were presented as the proportion of *C. albicans* colony diameter in comparison with the mean precipitation/hemolysis zone’s diameter±standard deviation (SD). Based on the applied contractual scale, Phz, Hz, and Prz values of 1 revealed negative reaction, while the values of 0.9-0.99, 0.8-0.89, 0.7-0.79, 0.6-0.69, and <0.59 showed very weak, weak, moderate, strong, and very strong secretory activity, respectively [2].

**Statistical analysis**

Utilizing the t-Student test (Sigma Stat, version 3.5), all results were statistically analyzed, and the level of statistical significance was set at 0.05.

**Results**

Based on the GC-MS analysis, it was indicated that the scent compounds of *S. aromaticum* comprised of 14 volatile compounds (Table 2), of which one benzenoid, including eugenol (84.64%), was the major component in essential oil after one terpenoid including β-caryophyllene (12.76%).

Table 2 presents the anti-*Candida* activity of the *S. aromaticum* essential oil based on MIC and MFC. It was found that *S. aromaticum* oil was active against the examined yeasts at concentrations <1250 μg/ml. The MICs of *C. albicans* were within the ranges of 0.25-32 μg/ml and 625-1250 μg/ml for fluconazole (mean±SD: 3.04±0.21 μg/ml) and *S. aromaticum*, respectively (mean±SD value: 953.13±218.30 μg/ml). Moreover, MFCs of *C. albicans* were within the range of 1-64 μg/ml (mean±SD value: 9.37±1.46 μg/ml for fluconazole and 1000-2500 μg/ml (mean±SD value: 1921.90±582.50 μg/ml) for *S. aromaticum*.

An auspicious method was adopted in some studies on *C. albicans* virulence factors for creating novel treatment targets. The examination of agar plates comprising substrates for particular enzymes, such as horse blood, egg yolk, or BSA, showed that *C. albicans* isolates examined in our work had different quantities of produced enzymes. Moreover, *C. krusei* ATCC 6258 was positive for the strong production of proteinase, phospholipase, and hemolysin (Prz=0.64±0.04, Phz=0.61± 0.02, and Hz=0.78±0.01).

It was found that 68.7% and 31.2% of *C. albicans* isolates were very strong and had strong proteinase activities, before treating *C. albicans* with *S. aromaticum* essential oil. Moreover,
Table 3. Minimal fungicidal concentration (MFC) and minimal inhibitory concentration (MIC) values of fluconazole and Syzygium aromaticum essential oil in Candida albicans

| Isolate (Source) | Fluconazole (MIC, μg/ml) | Fluconazole (MFC, μg/ml) | Syzygium aromaticum essential oil (MIC, μg/ml) | Syzygium aromaticum essential oil (MFC, μg/ml) |
|------------------|--------------------------|--------------------------|---------------------------------------------|---------------------------------------------|
| 1 Animal 0.25    | 1                        | 1000                     | 2000                                        | 2000                                        |
| 2 Animal 0.25    | 2                        | 1000                     | 2500                                        | 2500                                        |
| 3 Animal 0.5     | 2                        | 625                      | 1250                                        | 1250                                        |
| 4 Animal 0.5     | 2                        | 1000                     | 2000                                        | 2000                                        |
| 5 Animal 1       | 4                        | 625                      | 1250                                        | 1250                                        |
| 6 Animal 2       | 16                       | 1250                     | 2500                                        | 2500                                        |
| 7 Animal 0.25    | 1                        | 1250                     | 2500                                        | 2500                                        |
| 8 Animal 0.25    | 1                        | 1000                     | 2000                                        | 2000                                        |
| 9 Animal 4       | 16                       | 625                      | 1250                                        | 1250                                        |
| 10 Animal 2      | 16                       | 1000                     | 2500                                        | 2500                                        |
| 11 Human 0.5     | 4                        | 1000                     | 2500                                        | 2500                                        |
| 12 Human 0.5     | 2                        | 1250                     | 2500                                        | 2500                                        |
| 13 Human 0.25    | 1                        | 1000                     | 2000                                        | 2000                                        |
| 14 Human 4       | 16                       | 625                      | 1250                                        | 1250                                        |
| 15 Human 32      | 64                       | 1000                     | 2500                                        | 2500                                        |
| C. krusei ATCC 6258 | 0.5                  | 2                       | 1000                                        | 2000                                        |

25% and 56.2% of C. albicans isolates were very strong and had strong phospholipase activities, respectively. However, strong hemolytic activity was found in 18.7% of C. albicans isolates. The mean±SD phospholipase, proteinase, and hemolytic activities of all the isolates were obtained at 0.61±0.05, 0.55±0.03, and 0.73±0.04, respectively (Figure 1).

A statistically significant reduction was found in the release of tested enzymes through analysis of the enzymatic activity of S. aromaticum oil-treated C. albicans isolates. In total, 62.5%, 56.2%, and 56.2% of C. albicans isolates showed significantly weak proteinase, hemolytic, and phospholipase activities, respectively (P<0.05). Moreover, the mean±SD phospholipase, proteinase, and hemolytic activities of the isolates were reported to be 0.33±0.06, 0.40±0.04, and 0.16±0.03, respectively (Figure 2).

Figure 1. Proteinase, phospholipase, and hemolytic activities of each C. albicans isolate before treatment with Syzygium aromaticum essential oil (C. albicans isolates 1-16: C1-C16)
**Syzygium aromaticum** EO against *C. albicans* enzymes

Hekmatpanah A et al.

**Figure 2.** Proteinase, phospholipase, and hemolytic activities of each *C. albicans* isolate after treatment with *Syzygium aromaticum* essential oil (*C. albicans* isolates 1-16: C1-C16)

**Discussion**

In Iran, the same as many other countries, the plants with health benefits were picked up and utilized for treating different diseases. The chemical compounds, as well as anti-enzymatic and antifungal properties of *S. aromaticum* essential oil against pathogenic *C. albicans* was determined in the present study. Moreover, the major components of *S. aromaticum* included eugenol and β-caryophyllene. According to a former study in Turkey [20], the chemical composition of *S. aromaticum* oil approximately contained eugenol (87%), β-caryophyllene (3.56%), and eugenyl acetate (8.01%). In other studies performed by Porta et al. (1998), Lee and Shibamoto (2001), and Tomaino et al. (2005), the results obtained by GC-MS analysis indicated that eugenol (82.60%), eugenol (89.20%), and eugenol acetate (77.40%) were the major components of *S. aromaticum* oils, respectively. Our results were consistent with the reported values [21-23].

In this study, most *C. albicans* isolates (93.5%) were susceptible to fluconazole. According to the results of the study conducted by Duarte et al. (2007), MIC of 500 µg/ml, within 501-1000 µg/ml, and ≥1001 µg/ml indicated strong, moderate, and weak inhibitory essential oil, respectively. The *S. aromaticum* essential oil presented a moderate inhibitory effect on 81.25% of pathogenic *C. albicans* [24]. Our results regarding the antifungal activity of *S. aromaticum* were consistent with those reported by Shahidi Bonjar (2004) and Dababneh (2008) [11, 25]. They utilized *S. aromaticum* taken from commonly used medicinal plants against pathogenic *C. albicans* with MIC values of 1250 µg/ml and 800 µg/ml, respectively. Erturk (2006) found that the ethanolic extract obtained from *S. aromaticum* presented weak inhibitory activity against the fungus *Aspergillus niger* (*A. niger*) (MIC value of 25000 µg/ml) and the yeast *C. albicans* (MIC value of 20000 µg/ml), compared to the standard antifungal fluconazole [26]. In another study, it was revealed that methanolic, ethanolic, and water extracts taken from *S. aromaticum* had antifungal activities compared to hexane extract [27]. They showed that *A. niger* isolates were inhibited by *S. aromaticum* extracts at concentrations between 62.5-250 µg/ml, using the broth microdilution method. Furthermore, the methanolic extract had strong antifungal activity against *A. niger* using the disc diffusion method. It represented the diameter of the zone of inhibition (24 mm) while water and ethanolic extracts revealed the diameter of the zone of inhibition (21 and 22 mm), respectively. Hexane extracts of *S. aromaticum* showed very low antifungal activity against *A. niger*
ATTC16404 (diameter of inhibition zone 14 mm) [27]. Picman et al. (1995) and Galeotti et al. (2008) also reported fungitoxic properties of S. aromaticum against Fusarium oxysporum and Verticillium alboatrum, respectively [12, 28].

The existence of several components, mainly eugenyl acetate, eugenol, and β-caryophyllene reveals the inhibitory activity of S. aromaticum [29]. Yang et al. (2003) mentioned the inhibitory activity of S. aromaticum oil/extracts in the presence of acetyl eugenol, methyl salicylate, α-humulene, isoeugenol, and methyl-eugenol. Such phenolic structure components as eugenol have higher activity against the fungi [30]. Consistently, the results in this study showed a high percentage (84.64%) of eugenol in S. aromaticum oil. This group is known as either fungicide or fungistatic agents, based on the concentration reported by Abbaszadeh et al. (2014) and Morcia et al. (2012) [31, 32]. These compounds had strong activity regardless of relatively low dissolvability in water. This result is consistent with the published data since the phospholipid bilayer of the fungal cytoplasmic membrane is sensitized by these compounds, leading to the incremented permeability, impairment of fungal enzyme systems, or unavailability of vital intracellular constituents [33].

To the best of our knowledge, this is the first study in which S. aromaticum oil has impaired the formation of phospholipase, proteinase, and hemolysin of pathogenic C. albicans. On the other hand, relatively little research was performed on the effects of essential oils and other natural substances on producing hydrolytic C. albicans enzymes. Budzyńska et al. (2014) showed that both C. albicans ATCC 90028 and C. albicans ATCC 10231 were positive for producing extracellular proteinase (Prz=0.27±0.01; Prz=0.34±0.01, respectively), phospholipase (Phz=0.44±0.02), and hemolysin. However, the former’s activity was evaluated as medium (Hz=0.57±0.03; Hz=0.62±0.04). It was shown that geranium oil, clove oil, citronella oil, and lemon balm, even at a sublethal concentration (½ MIC), exhibited considerable biological activities that decrease the production of these enzymes [16]. Silva-Rocha et al. (2015) added the crude extract of Eugenia uniflora to proteinase and phospholipase media. They found that the activity zone of the enzymes was either completely inhibited or highly reduced [34]. Rajkowska et al. (2014) in another study demonstrated alterations in cell morphology and C. albicans colony. Moreover, they reported the reduction of enzymatic activity in thyme and tea tree oils in most cases [35]. Pootong et al. (2017) also reported that the mean±SD Prz and Phz for C. albicans not exposed to cinnamaldehyde was obtained at 2.17±2.17 and 1.98±0.46, respectively. The activities of these enzymes were reduced significantly (P<0.01) when the yeast was exposed to cinnamaldehyde at 62.5 μg/ml and 31.25 μg/ml, reaching 1.45±0.27 and 1.92±0.17 for the proteinase and 1.40±0.38 and 1.65±0.49 for the phospholipase, respectively [36]. Yordanov et al. (2008) found that the cultivation of C. albicans while existing four natural substances at various concentrations led to a dose-based reduction of phospholipase activity. Ibogaine (75.0%±6.2% inhibition) had the best inhibition effect at 250 mg/ml concentration after berberine (up to 60.0%±4.5% inhibition) and kaempferol (up to 40.0%±2.0% inhibition) [37]. Well-known and important virulence factors secreted from C. albicans cells include hydrolytic enzymes, such as phospholipases and proteinases. These enzymes have a key role in adhesion to host cells, nutrition, and tissue destruction leading to the pathogen spread. Among these virulence factors, Saps are the most important. Candida blastospores secrete Saps1-3, while Saps 4-6 are mainly released by filamentous forms, and Sap 9 and 10 are strongly associated with the cell wall of both morphotypes. The phospholipase activity complements the protease activity as enzymes that are in charge of hydrolyzing one or more ester bonds in the cell membrane glycerophospholipids. Another vital virulence feature of C. albicans is the effective acquisition of iron presented by the action of some proteins with mann/mannoproteins and hemolytic activity-Rbt5, released from the cell wall [38, 39]. A comparison of the susceptibility to fluconazole and enzyme production revealed that the susceptible C. albicans strains to antifungal agent utilized in this study represented a robust positive enzymatic activity to phospholipase, proteinase, and hemolysin. This finding is vital since the growth of pathogenic C. albicans isolates is inhibited by herbal essential oils, such as S. aromaticum, which possibly reduce the enzymatic production. The contribution of such enzymes in disease progression and initiation is still largely unrecognized; however, it seems that the disease severity can be affected by their expression level in Candida infections [12]. In this regard, a correlation was observed between the existence of oral [8], vulvovaginal [7], and pulmonary [40] candidiasis and the secretion of extracellular enzymes.

Conclusion

Based on the obtained results in this study, most of the pathogenic C. albicans isolates were susceptible to S. aromaticum essential oil and fluconazole and showed high phospholipase, proteinase, and hemolytic activities. These enzymes'activities were reduced significantly by exposing the yeasts to S. aromaticum oil. Eventually, it can be assumed that S. aromaticum can significantly affect C. albicans pathogenicity through these enzymatic changes and loss of growth capability.

Acknowledgments

This research work was supported/funded by the University of Tehran, Tehran, Iran.
Authors’ contribution

Study conception and design were conducted by A.S., D.N., and H.S. Data were collected by A.H. and D.N. Analysis and interpretation of results was performed by A.H., A.S., and S.A. Manuscript draft was prepared by H.S., A.S., and S.A. All authors reviewed the results and approved the final version of the manuscript.

Conflicts of interest

No conflict of interest was declared by the authors.

Financial disclosure

The authors have no financial interests regarding the material in the manuscript.

References

1. Khalili V, Shokri H, Khoosravi AR, Akim A, Amri Saroukolaei S. Purification and comparison of heat shock protein 90 (Hsp90) in Candida albicans isolates from Malaysian and Iranian patients and infected mice. J Mycol Med. 2016; 26: 94-102.
2. Sharifzadeh A, Khoosravi AR, Shokri H, Sharafi G. Antifungal effect of Thymuspermum ammi against susceptible and fluconazole resistant strains of Candida albicans. J Mycol Med. 2015; 25:143-150.
3. Sharifzadeh A, Soltani M, Shokri H. Evaluation of virulence factors and antifungal susceptibility patterns of different Candida species isolated from the female camel (Camelus dromedarius) genital tract. Mycoses. 2015; 58:478-484.
4. Sharifzadeh A, Javan AJ, Shokri H, Abbaszadeh S, Keykhoosravy K. Evaluation of antioxidant and antifungal properties of the traditional plants against foodborne fungal pathogens. J Mycol Med. 2016; 26:61-7.
5. Tsang CSP, Chu FCS, Leung WK, Jin LJ, Samaranyakey LP, Sui SC. Phospholipase, proteolase and haemolytic activities of Candida albicans isolated from oral cavities of patients with type 2 diabetes mellitus. J Med Microbiol. 2007; 56: 1393-8.
6. Menezes EA, Monteiro MNR, Parente TMA, Cunha FA, Augusto KL, Freire CCF. Frequency and enzymatic activity of Candida albicans isolated from the oral cavity of HIV-positive patients at Fortaleza, Ceará. J Bras Patol Med Lab. 2006; 42(4):253-256.
7. Fatahinia M, Valaveezadeh M, Rezaei-Matehkolaei A. Comparison of enzymatic activities in different Candida species isolated from women with vulvovaginitis. J Mycol Med. 2017; 27(2):188-194.
8. Rossom RD, Barbosa JO, Vilela SFG, Jorge AOC, Junqueira JC. Comparison of the hemolytic activity between C. albicans and non-albicans Candida species. Braz Oral Res. 2013; 27:484-9.
9. Papon N, Courdavault V, Clastre M, Bennett RJ. Emerging and emerging pathogenic Candida species: beyond the Candida albicans paradigm. PLos Pathog. 2013; 9: e1003350.
10. Chandra S, Rawat DS, Chandra D, Rastogi J. Nativity, phytochemistry, ethnobotany and pharmacology of Dianthus caryophyllus. Res J Med Plant. 2016; 10(1):1-9.
11. Dababneh BF. Antimicrobial activity of selected Jordanian medicinal plant extracts against pathogenic microorganisms. J Food Agric Environ. 2008; 6(2):134-139.
12. Galeotti F, Barile E, Curri P, Dolci M, Lanzotti V. Flavonoids from carnation (Dianthus caryophyllus) and their antifungal activity. Phytochem Lett. 2008; 1:44-48.
13. Tamai IA, Salehi TZ, Sharifzadeh A, Shokri H, Khoosravi AR. Repetitive sequences based on genotyping of Candida albicans isolates obtained from Iranian patients with human immunodeficiency virus. Iran J Basic Med Sci. 2014; 17(11): 831-5.
14. Dibvand K, Shokri H, Khoosravi A. Down-regulatory effect of Thymus vulgaris L. on growth and Tri4 gene expression in Fusarium oxysporum strains. Microb Pathog. 2017; 104:1-5.
15. CLSI. Reference method for broth dilution antifungal susceptibility testing of yeasts. 3rd ed. Wayne, PA: Clinical and Laboratory Standards Institute; 2017.
16. Budzyska A, Sadowska B, Węcowska-Szakiel M, Rożalska B. Enzymatic profile, adhesive and invasive properties of Candida albicans under the influence of selected plant essential oils. Acta Biochim Pol. 2014; 61(1):115-12.
17. Oksu S, Sahin I, Yildirim M, Gulcan A, Yavuz T, Kaya D, Koc AN. Phospholipase and proteinase activities in different Candida species isolated from anatomically distinct sites of healthy adults. Jpn J Infect Dis. 2007; 60(5):280-3.
18. Patel M, Gulube Z, Dutton M. The effect of Dondanae viscosa var. angustifolia on Candida albicans proteinase and phospholipase production and adherence to oral epithelial cells. J Ethnopharmacol. 2009;124:562-565.
19. Manns JM, Mosser DM, Buckley HR. Production of a hemolytic factor by Candida albicans. Infect Immun. 1994; 62(11):5154-5156.
20. Alna HK, Ertas M, Nitz S, Kollmannsbarger H. Chemical composition and content of essential oil from the bud of cultivated Turkish clove (Syzygium aromaticum L.). BioResources. 2007; 2(2):265-269.
21. Porto GD, Taddeo RD, Usoro E, Reverchon E. Isolation of clove bud and star anise essential oil by supercritical CO2 extraction. LWT-Food Sci Technol. 1998; 31:454-460.
22. Lee KG, Shubamoto T. Antioxidant property of aroma extract isolated from clove buds (Syzygium aromaticum Lam). Food Chem. 2001; 74:443-448.
23. Tomiano A, Ciminelli F, Zimbballati V, Venuiti V, Sulfaro V, De Pasquale A, Saija A. Influence of heating on antioxidant activity and the chemical composition of some spice essential oils. Food Chem. 2005; 89:549-554.
24. Daunte MCT, Leme EE, Delarmelina C, Soares AA, Figueira GM. Sartorato A. Activity of essential oils from Brazilian medicinal plants on Escherichia coli. J Ethnopharmacol. 2007; 111(2):197-201.
25. Shahidi Bonjar GH. Inhibition of clortimazole-resistant Candida albicans by plants used in Iranian folkloric medicine. Fitoterapia. 2004; 75:74-76.
26. Erturk O. Antibacterial and antifungal activity of ethanolic extracts from eleven spice plants. Biolegia. 2006;61(3):275-278.
27. Abdelkader HS, Halawani EM. GC-MS analysis and antimicrobial activity of Dianthus caryophyllus extracts from Tai, Saudi Arabia. Int J Pharm Bio Sci. 2014; 5(3):389-401.
28. Picman AK, Schneider EF, Pieman J. Effect of flavonoids on mycelial growth of Verticillium albo-atrum. Biochem Syst Ecol. 1995; 23:683-693.
29. Chaebe K, Hajlaovi H, Znaant Z. The chemical composition and biological activity of clove essential oil Eugenia caryophyllata (Syzygium aromaticum L. Myrtaceae): a short review. Phytother Res. 2007; 21:501-506.
30. Yang YC, Lee SH, Lee WJ, Choi DH, Ahn YJ. Ovicidal and adul ticidal effects of Eugenia caryophyllata buds and leaf oil compounds on Pediculus capitis. J Agric Food Chem. 2003; 51(17):4884-4888.
31. Abbaszadeh S, Sharifzadeh A, Shokri H, Khoosravi AR, Abbaszadeh A. Antifungal efficacy of thymol, carvacrol, eugenol and menthol as alternative agents to control the growth of food relevant fungi. J Mycol Med. 2014; 24:151-156.
32. Morcia C, Malanati M, Terzi V. In vitro activity of terpinen-4-ol, eugenol, carvone, 1,8-cineole (eucalyptol) and thymol against mycotoxicogenic plant pathogens. Food Addit Contam. 2012; 29:415-22.
33. Campaniello D, Corbo MR, Sinigaglia M. Antifungal activity of eugenol against Penicillium Aspergillus, and Fusarium species. J Food Protect. 2010; 73: 1124-8.
34. Silva-Rocha WP, Lemos VLB, Ferreira MRA, Soares LAL, Svidzinski TIE, Milan EP, Chaves GM. Effect of the crude extract of Eugenia uniflora morphogenesis and secretion of hydrolytic enzymes in Candida albicans from the oral cavity of kidney transplant recipients. BMC Complement Altern Med. 2015; 15:6.
35. Rąjkowska K, Kunicka-Styczynska A, Maroszyńska M, Dąbrowski M. The effect of thyme and tea tree oils on morphology and metabolism of Candida albicans. Acta Biochim Pol. 2014; 61(2):305-310.
36. Pootong A, Norrapong B, Cowawintaweesat S. Antifungal
activity of cinnamaldehyde against *Candida albicans*. Southeast Asian J Trop Med Public Health. 2017; 48(1):150-158.

37. Yordanov M, Dimitrova P, Patkar S, Saslo L, Ivanovska N. Inhibition of *Candida albicans* extracellular enzyme activity by selected natural substances and their application in *Candida* infection. Can J Microbiol. 2008; 54:435–440.

38. Deorukhkar S, Saini S. Evaluation of phospholipase activity in biofilm forming *Candida* species isolated from intensive care unit patients. Br Microbiol Res J. 2013; 3:440–447.

39. Sorgo AG, Heilmann CJ, Brul S, de Koster CG, Klis FM. Beyond the wall: *Candida albicans* secret(e)s to survive. FEMS Microbiol Lett. 2013; 338:10–17.

40. Kumar VG, Latha R, Vedhagiri K, Suthiamoorthy T, Jayaram G, Sasikala R, Selvin J, Natarajaseenivasan K. Phospholipase C, proteinase and hemolytic activities of *Candida* spp. isolated from pulmonary tuberculosis patients. J Mycol Med. 2009; 19:3-10.