Jundishapur J Nat Pharm Prod. 2021 February; 16(1):e65005. doi: 10.5812/jjnpp.65005.

Published online 2021 February 1. **Research Article**

Anti-adherence and Anti-fungal Abilities of Thymol and Carvacrol Against *Candida* Species Isolated From Patients with Oral Candidiasis in Comparison with Fluconazole and Voriconazole

Aghil Sharifzadeh [1,*], Hojjatollah Shokri [2] and Farzad Katiraee [3]

[1]Mycology Research Center, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
[2]Department of Pathobiology, Faculty of Veterinary Medicine, Amol University of Special Modern Technologies, Amol, Iran
[3]Department of Pathobiology, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran

[*]*Corresponding author*: Mycology Research Center, Faculty of Veterinary Medicine, University of Tehran, Azadi St., Tehran, Iran. Tel: +98-2161117151, Fax: +98-2166933222, Email: asharifzadeh@ut.ac.ir

Received 2018 January 03; **Revised** 2019 March 06; **Accepted** 2019 March 16.

Abstract

Background: Natural isopropyl cresols, such as thymol and carvacrol, have been known to have antifungal activities.
Objectives: The current study aimed to investigate the anti-adherence and antifungal activities of thymol, carvacrol, fluconazole, and voriconazole against oral isolates of *Candida albicans* (*C. albicans*), *C. glabrata*, and *C. krusei*.
Methods: The susceptibility assay for the test compounds was performed using the disk diffusion method against all *Candida* isolates. Also, anti-adherence activity was examined using a rapid and highly reproducible 96 well microtiter-based method.
Results: Both natural phenols and antifungal drugs revealed various efficacies against studied *Candida* species. The susceptibility to fluconazole and voriconazole were 100% for *C. albicans*, 50% and 90% for *C. glabrata*, and 0% and 100% for *C. krusei* isolates, respectively. The mean diameter of the inhibition zone was greater for thymol than carvacrol in *C. albicans* (19.89 $\pm$ 0.80 mm versus 17.05 $\pm$ 0.61 mm), *C. glabrata* (18.87 $\pm$ 0.71 mm versus 15.77 $\pm$ 0.57 mm), and *C. krusei* (15.11 $\pm$ 0.91 mm versus 13.91 $\pm$ 1.04 mm) isolates tested. Thymol showed more effective inhibition on adherence of all *Candida* species than other treatments. The mean relative adherence ratios for *C. albicans*, *C. glabrata*, and *C. krusei* were 0.50, 0.60, and 0.64, respectively.
Conclusions: This study demonstrated significant inhibitory properties of thymol and carvacrol on the adherence and growth of azole susceptible- and -resistant *Candida* isolates. Also, thymol was more effective for preventing the adherence of yeast cells to polystyrene in comparison to carvacrol.

Keywords: Anti-fungal, Anti-adherence, *Candida* spp, Oral Candidiasis, Thymol, Carvacrol, Voriconazole

1. Background

Opportunistic fungal infections are common in immunocompromised patients, which causes resistant fungi to antifungal agents (1). Oral candidiasis is a frequent infection in the oral cavity caused by different *Candida* species. According to the studies performed during the past two decades, *Candida albicans*, *C. glabrata*, and *C. krusei* are the most frequent pathogenic *Candida* species in the development of oral infections (2).

Azole drugs, as an important class of antifungals, affect the lanosterol 14α-demethylase, resulting in the inhibition of biosynthesis of the ergosterol in the fungal plasma membrane. These drugs contain fungistatic properties, and some of them, in particular fluconazole, can cause side effects in patients receiving azoles for a long time (3). In this regard, *C. glabrata* and *C. krusei* become quickly resistant to fluconazole. For this reason, finding novel and natural antifungal substances for clinical applications is necessary (4, 5). Thymol and carvacrol are phytocomponents classified as monoterpenes. They are the major components found in thyme (*Thymus vulgaris*) essential oil (6).

2. Objectives

The current study aimed to assess the anti-adherence and antifungal activities of thymol and carvacrol against *C. albicans*, *C. glabrata*, and *C. krusei* isolates obtained from patients with oral candidiasis concerning growth inhibition and fungal death as compared to the synthetic antifungals such as fluconazole and voriconazole.

3. Methods

3.1. Chemicals

The 1 μg disks of voriconazole and 25 μg fluconazole were purchased from Neo-sensitabs, Rosco Diagnostica, Denmark.

3.2. Isolation of Thymol and Carvacrol

Thymol and carvacrol were isolated from essential oils of *Trachyspermum ammi* and *Zataria multiflora*, respectively (7, 8). Thymol and carvacrol isolation was performed based on the column chromatography method. Briefly, 4 mL of essential oil was used for a silica gel column (1.5 cm i.d. and 22 cm in length). N-Hexane and ethyl acetate were used as elution solvents. The eluted fractions consisted of hydrocarbon constituents of essential oil in n-hexane (fraction A) and oxygenated constituents in ethyl acetate (fraction B). Carvacrol and thymol were purified from fraction B by preparative thin-layer chromatography (TLC). Toluene/ethyl acetate was used as a solvent system in preparative TLC. The purity of isolated carvacrol and thymol was validated by Gas chromatography-mass spectrometry (GC-MS). Stock solutions of thymol and carvacrol were prepared by 70°GL ethanol diluent.

3.3. Organism Identification

Clinical isolates of *C. albicans* ($Ca_{1\text{-}10}$), *C. glabrata* ($Cg_{1\text{-}10}$), and *C. krusei* ($Ck_{1\text{-}10}$) were obtained from patients with oral candidiasis. Written informed consent was obtained from all participants. The identification of *Candida* strains was performed at the Mycology Research Center affiliated to the University of Tehran. The yeasts were grown on Sabouraud glucose agar (Merck Co., Darmstadt, Germany) and CHROM agar (CHROMagar Company, Paris, France) at 35°C for 48 h. The identification of isolated *Candida* strains was confirmed using the RAPID yeast plus system (Remel Inc., USA), as recommended by the manufacturer.

3.4. Disk Diffusion Method

Assessment of antifungal activity was performed using the disk diffusion method based on the CLSI-M44-A2 standard for yeasts (9). In brief, agar plates (90-mm diameter) containing Mueller-Hinton agar (Merck Co., Darmstadt, Germany) accompanied with glucose (2%), and methylene blue (0.5 mg) were used. Sterile cotton swabs dipped in yeast suspensions adjusted to 1×10^6 cells/mL were inoculated on the agar surface. Thymol, carvacrol (20 μL of stock solutions with 80 mg/mL concentration), fluconazole, and voriconazole disks were placed on the agar surfaces. Subsequently, the media was kept at 35°C and read at 24 h. After the colonies grew, the zones of inhibition around the disks were measured and recorded. All experiments were performed in duplicate. The interpretation of antifungal tests of standard drugs was done based on the CLSI protocol: zone diameters of $\geq$ 19 mm for fluconazole and $\geq$ 17 mm for voriconazole as susceptible (S); zone diameters of 15 to 18 mm for fluconazole and 14 to 16 mm for voriconazole as susceptible dose-dependent (SDD); and zone diameters of $\leq$ 14 mm for fluconazole and $\leq$ 13 mm for voriconazole as resistant (R).

3.5. Anti-adherence Assay

The yeasts inocula were adjusted in normal saline to 2×10^6 cells/mL. Stock solutions of treatments were prepared using 70°GL ethanol. The working solutions were prepared by diluting the stock solutions to two-fold sub-MIC concentrations of treatments using RPMI 1640 medium. Twofold dilutions of thymol, carvacrol, fluconazole, and voriconazole were dispensed into the 96-well flat-bottomed polystyrene microtiter plates in 100 μl volumes for each well. The inoculation was done using 100 μL of *Candida* suspension with the final concentration of 1×10^6 cells/mL. Wells without treatments (thymol, carvacrol, and antifungal drugs) were considered as positive controls. The inoculated media was kept at 35°C for 90 min with a shaking rate of 75 rpm. Then, the wells were washed three times with PBS to remove any residual planktonic cells, and stained with 1% solution (w/v) of crystal violet for 5 min. The optical densities (OD) for each well were determined at 540 nm wavelength using an ELISA reader. The relative adherence was calculated by dividing the OD value of a treated well by the positive control. To count the yeasts, the medium content was aspirated and air-dried. Then, it was fixed and gram stained (10).

3.6. Statistical Analysis

Data were subjected to analysis of variance (ONE-WAY ANOVA) and Tukey's tests (SPSS software package, V. 17.0 for Windows, USA). Statistical significance was considered when P-value < 0.05.

4. Results

The results of inhibition zones of fluconazole and voriconazole against different *Candida* isolates, based on disk diffusion test, are shown in Table 1. Fluconazole susceptibilities were calculated as 100% for *C. albicans*, 50% for

C. glabrata, and 0% for *C. krusei* strains. *C. glabrata* (20% SDD and 30% R) and *C. krusei* (50% SDD and 50% R) strains presented low susceptibilities to fluconazole. For voriconazole, 100% of *C. albicans* and *C. krusei* isolates and 90% of *C. glabrata* isolates were susceptible. As shown in Table 2, the mean diameter of the inhibition zone was greater for thymol than carvacrol in *C. albicans* (19.89 $\pm$ 0.80 mm versus 17.05 $\pm$ 0.61 mm), *C. glabrata* (18.87 $\pm$ 0.71 mm versus 15.77 $\pm$ 0.57 mm), and *C. krusei* (15.11 $\pm$ 0.91 mm versus 13.91 $\pm$ 1.04 mm) isolates tested. No significant difference was found between antifungal activities of thymol and carvacrol against various *Candida* species ($P > 0.05$).

As shown in Table 3, the results revealed that the lowest relative adherence ratio was among *C. albicans* isolates in comparison to other species. The mean relative adherence ratios (mean of adherence values of all treatments) for *C. albicans*, *C. glabrata*, and *C. krusei* were 0.50, 0.60, and 0.64, respectively. However, considering the absolute number of yeasts adhered to the polystyrene field, *C. krusei* was found to be adhered to the polystyrene surface with the lowest number of cells in comparison to the other two species. The mean absolute number of adherent *C. albicans*, *C. glabrata*, and *C. krusei* cells on the surface of the non-treated wells were 112.6 $\pm$ 16, 337 $\pm$ 45.9, and 73 $\pm$ 10.8 yeasts/field, respectively. Significantly higher numbers of *C. glabrata* cells could adhere to the polystyrene in comparison to *C. albicans* cells ($P < 0.01$, Tukey's HSD test), but the difference between *C. krusei* and *C. albicans* was not significant ($P > 0.05$, Tukey's HSD test), although a markedly lower number of *C. krusei* cells exhibited the ability to adhere. The most effective treatment to inhibit adherence among all species was thymol at which with the doses of 24.5, 25.5, and 35 μg/mL, it could inhibit the adherence of 72%, 67%, and 55% of *C. albicans*, *C. glabrata*, and *C. krusei* cells, respectively. Fluconazole was the least effective agent in preventing adherence for all species. However, voriconazole could inhibit the adherence of all three species more effectively than fluconazole. Also, thymol was more effective in preventing the adherence of yeast cells to polystyrene in comparison to carvacrol.

5. Discussion

The high occurrence of oral candidiasis in patients with underlying disorders has encouraged scientists to search for novel and natural antifungal agents (4). In this study, 50% of *C. krusei* strains were resistant to fluconazole, according to the results of the disc diffusion susceptibility testing method. This finding is not consistent with some studies that reported resistance rates higher than 80% (2). In a worldwide study by Pfaller et al. (11), the resistance of *C. glabrata* strains to fluconazole is reported as 5.9% in Brazil, 36% in Venezuela, 21.7% in Ecuador, 18.2% in Colombia, and 14.6% in Argentina. In our experiments, although voriconazole was highly active against *C. glabrata*, susceptible dose-dependent isolates to voriconazole were also detected, as confirmed by the worldwide data reported (2). The discrepancy of *C. glabrata* susceptibility to azoles could be related to long-term use of fluconazole and/or itraconazole in order to treat infections caused by *Candida* species (12).

In this study, both thymol and carvacrol revealed remarkable effects against *Candida* species, in particular the resistant strains. Disk diffusion assay has been performed on fluconazole-resistant *Candida* species, such as *C. glabrata* and *C. krusei*. Moreover, these species also demonstrated susceptibility to 80 mg/mL concentration of thymol and carvacrol. Previous studies reported the potent anti-*Candida* activity of thymol and carvacrol (13, 14). In agreement with our results, other scholars reported higher antifungal activity of thymol than carvacrol (15). The presence of a phenolic hydroxyl at different locations on the phenolic ring of thymol (C3) and carvacrol (C2) influences the degree of antifungal activity (16-18). Consistent with the findings of the present study, Ahmad et al. (16) demonstrated that thymol (100 μg/mL) and carvacrol (75 μg/mL) had fungicidal activity against fluconazole-susceptible and -resistant *Candida* species. In fact, the monoterpenes, such as thymol and carvacrol, were associated with decreased ergosterol content of the fluconazole-susceptible and -resistant *Candida* strains (16).

In this study, the most effective treatment to inhibit adherence among all species was thymol. So that with the doses of 24.5, 25.5, and 35 μg/mL, it could inhibit the adherence of 72%, 67%, and 55% of *C. albicans*, *C. glabrata*, and *C. krusei* cells, respectively. Fluconazole was the least effective treatment in preventing adherence for all species. However, voriconazole could inhibit the adherence of all three species more effectively than fluconazole. Samaranayake et al. (19) indicated an association between hydrophobicity and adherence of *C. albicans* and *C. krusei* to the HeLa cells. In another research, *C. glabrata* showed a four-fold higher relative cell surface hydrophobicity (CSH) and a two-fold higher tendency to attach on the acrylic surfaces compared to *C. albicans*, at which highly a significant correlation was found between hydrophobicity and adherence (20). However, some *Candida* species with high adherence profiles also showed good biofilm mass formation (21). There are frequent reports on the superior adherence abil-

Table 1. Antifungal Susceptibility of Fluconazole and Voriconazole Against *Candida albicans*, *Candida glabrata*, and *Candida krusei* Isolated From Oral Candidiasis

	Fluconazole, Disk Diffusion		Voriconazole, Disk Diffusion	
Candida albicans	**Mean ± SD, mm**	**Interpretation**	**Mean ± SD, mm**	**Interpretation**
Ca_1	34.5 ± 0.7	S	37.5 ± 0.7	S
Ca_2	36 ± 1.4	S	38.5 ± 0.7	S
Ca_3	39.5 ± 0.7	S	41.5 ± 0.7	S
Ca_4	41.5 ± 0.7	S	49 ± 1.4	S
Ca_5	36 ± 1.4	S	39 ± 1.4	S
Ca_6	37.5 ± 0.7	S	39.5 ± 0.7	S
Ca_7	38.5 ± 0.7	S	45.5 ± 0.7	S
Ca_8	40 ± 1.4	S	49 ± 1.4	S
Ca_9	34 ± 1.4	S	39 ± 1.4	S
Ca_{10}	36.5 ± 2.1	S	39 ± 1.4	S
Cg_1	33.5 ± 0.7	S	34.5 ± 0.7	S
Cg_2	24 ± 0	S	27.5 ± 0.7	S
Cg_3	15.75 ± 1.1	SDD	20.5 ± 2.1	S
Cg_4	22.5 ± 0.7	S	24.5 ± 0.7	S
Cg_5	11 ± 1.4	R	17 ± 1.4	S
Cg_6	24 ± 1.4	S	25.5 ± 0.7	S
Cg_7	16.5 ± 2.1	SDD	16 ± 2.8	SDD
Cg_8	12.5 ± 0.7	R	18 ± 0	S
Cg_9	18.5 ± 0.7	S	19 ± 1.4	S
Cg_{10}	13.5 ± 0.7	R	21.5 ± 0.7	S
Ck_1	33.5 ± 0.7	S	29 ± 1.4	S
Ck_2	24 ± 0	S	26 ± 1.4	S
Ck_3	15.75 ± 1.1	SDD	26 ± 1.4	S
Ck_4	22.5 ± 0.7	S	27 ± 1.4	S
Ck_5	11 ± 1.4	R	26.5 ± 0.7	S
Ck_6	24 ± 1.4	S	28 ± 0	S
Ck_7	16.5 ± 2.1	SDD	27.5 ± 0.7	S
Ck_8	12.5 ± 0.7	R	28.5 ± 2.1	S
Ck_9	18.5 ± 0.7	S	30.5 ± 0.7	S
Ck_{10}	13.5 ± 0.7	R	27.5 ± 0.7	S

Abbreviations: Ca, *Candida albicans*; Cg, *Candida glabrata*; Ck, *Candida krusei*; SD, standard deviation.

ity of non-albicans *Candida* species to host cells and synthetic surfaces in comparison to *C. albicans* (19). Voriconazole (at the doses of 0.06 - 16 μg/L) showed significant anti-biofilm activity against *C. albicans*, *C. glabrat*a, and some other *Candida* spp. The reduction rates for *C. albicans* and *C. glabrat*a were 64.5 and 23.8%, respectively, indicating more resistance of *C. glabrat*a. The authors explained that the inhibition may be due to yeast cell surface modifications and interruption of the adhesion process in biofilm formation (22). In the present study, although voriconazole showed promising anti-adhesion activity against all evaluated species but fluconazole was not as effective as voriconazole. Our results are consistent with other studies indicating more resistance of non-*C. albicans* species against the anti-adhesion effect of azole drugs, particularly fluconazole. Thymol and carvacrol have been shown to be potent biofilm inhibitors, at which thymol showed significant anti-biofilm activity at half of the dose required

Table 2. Antifungal Susceptibility of Thymol and Carvacrol Against Different Pathogenic *Candida* Strains Isolated From Oral Candidiasis

Candida Species	Thymol, Disk Diffusion, Mean ± SD, mm	Carvacrol, Disk Diffusion, Mean ± SD, mm
Ca_1	19.85 ± 0.4	16.45 ± 0.4
Ca_2	18.75 ± 0.4	17.15 ± 0.4
Ca_3	20.15 ± 1.2	16.35 ± 0.4
Ca_4	20.25 ± 1.1	16.6 ± 0.4
Ca_5	19.4 ± 0.3	17.9 ± 0.1
Ca_6	20.6 ± 0.8	17.8 ± 0.4
Ca_7	19.05 ± 0.2	17.7 ± 0.1
Ca_8	19.8 ± 0.3	16.75 ± 0.4
Ca_9	20.9 ± 0.1	17.2 ± 0.3
Ca_{10}	20.2 ± 0.6	16.6 ± 0.3
Cg_1	19 ± 0.3	16.2 ± 0.3
Cg_2	18.5 ± 0.4	16.05 ± 0.2
Cg_3	19 ± 0.8	16.8 ± 0.3
Cg_4	20.05 ± 0.4	15.3 ± 0.3
Cg_5	19.35 ± 0.5	15.8 ± 0.1
Cg_6	18.75 ± 0.5	15.15 ± 0.2
Cg_7	17.8 ± 0.3	15.35 ± 0.5
Cg_8	18.5 ± 0.3	15.05 ± 0.2
Cg_9	19.4 ± 0.6	16.05 ± 0.1
Cg_{10}	18.35 ± 0.4	15.95 ± 0.1
Ck_1	16.2 ± 0.3	13.6 ± 0.3
Ck_2	16.05 ± 0.2	14.9 ± 0.4
Ck_3	16.8 ± 0.3	15.25 ± 0.4
Ck_4	15.3 ± 0.3	13 ± 0.1
Ck_5	15.8 ± 0.1	13.25 ± 0.2
Ck_6	15.15 ± 0.2	14.4 ± 0.4
Ck_7	15.35 ± 0.5	14.75 ± 0.1
Ck_8	15.05 ± 0.2	13.25 ± 0.2
Ck_9	16.05 ± 0.1	14.7 ± 0.4
$Ck1_0$	15.95 ± 0.1	12 ± 0.1

Table 3. The Relative Adherence (Mean ± Standard Deviation) of *C. albicans*, *C. glabrat*a, and *C. krusei* After Treatment with Azole Drugs, Thymol, and Carvacrol Quantified by Crystal Violet Staining[a]

Species	Antifungal Agents							
	Fluconazole		Voriconazole		Thymol		Carvacrol	
	1/2 MIC	1/4 MIC	1/2 MIC	1/4 MIC	1/2 MIC	1/4 MIC	1/2 MIC	1/4 MIC
C. albicans	0.67 ± 0.09*	0.78 ± 0.12	0.46 ± 0.09†	0.55 ± 0.08†	0.28 ± 0.07†	0.4 ± 0.06†	0.41 ± 0.06†	0.5 ± 0.09†
C. glabrata	0.75 ± 0.12	0.83 ± 0.12	0.64 ± 0.06†	0.76 ± 0.1	0.33 ± 0.04†	0.42 ± 0.07†	0.45 ± 0.06†	0.52 ± 0.07†
C. krusei	0.86 ± 0.14	0.93 ± 0.07	0.5 ± 0.05†	0.58 ± 0.07†	0.45 ± 0.07†	0.52 ± 0.07†	0.6 ± 0.07†	0.65 ± 0.06†

[a] Significantly higher numbers of *C. glabrat*a cells could adhere to the polystyrene in comparison to *C. albicans* cells ($P < 0.01$, Tukey's HSD test).

by carvacrol (23). Moreover, thymol demonstrated greater anti-biofilm and anti-cell surface hydrophobicity activity than that by fluconazole against *C. albicans* (24, 25). Interestingly, the anti-adhesion activity of these natural compounds has been established at sub-MIC doses indicating a possible specific anti-adherence activity regardless of their fungicidal effect. This assumption is in accordance with the results of other studies indicating the potent specific anti-biofilm/anti-adherence effect of some phenolic terpenoids (23).

5.1. Conclusions

In summary, this study demonstrated that voriconazole had higher activity than fluconazole against various *Candida* spp tested, in particular resistant strains. Thymol and carvacrol exhibited fungicidal activity against clinical isolates of *C. albicans*, *C. glabrata*, and *C. krusei* strains. Also, thymol was more effective in the case of preventing the adherence of yeast cells to polystyrene in comparison to carvacrol.

Footnotes

Authors' Contribution: All authors contributed to the study. They conducted the experiment, analyzed and discussed results, wrote the conclusion, and prepared the manuscript for publication.

Conflict of interests: The authors declare no conflict of interest.

Funding/Support: The current study was supported by the Research Council of the University of Tehran, Tehran, Iran.

References

1. Cornet M, Gaillardin C. pH signaling in human fungal pathogens: a new target for antifungal strategies. *Eukaryot Cell*. 2014;**13**(3):342–52. doi: 10.1128/EC.00313-13. [PubMed: 24442891]. [PubMed Central: PMC3957587].
2. Pfaller MA, Boyken L, Hollis RJ, Kroeger J, Messer SA, Tendolkar S, et al. Comparison of results of fluconazole and voriconazole disk diffusion testing for Candida spp. with results from a central reference laboratory in the ARTEMIS DISK Global Antifungal Surveillance Program. *Diagn Microbiol Infect Dis*. 2009;**65**(1):27–34. doi: 10.1016/j.diagmicrobio.2009.05.007. [PubMed: 19679232].
3. Monge RA, Roman E, Nombela C, Pla J. The MAP kinase signal transduction network in Candida albicans. *Microbiology (Reading)*. 2006;**152**(Pt 4):905–12. doi: 10.1099/mic.0.28616-0. [PubMed: 16549655].
4. Bondaryk M, Kurzatkowski W, Staniszewska M. Antifungal agents commonly used in the superficial and mucosal candidiasis treatment: mode of action and resistance development. *Postepy Dermatol Alergol*. 2013;**30**(5):293–301. doi: 10.5114/pdia.2013.38358. [PubMed: 24353489]. [PubMed Central: PMC3858657].
5. Newman DJ, Cragg GM. Natural products as sources of new drugs over the 30 years from 1981 to 2010. *J Nat Prod*. 2012;**75**(3):311–35. doi: 10.1021/np200906s. [PubMed: 22316239]. [PubMed Central: PMC3721181].
6. de Lira Mota KS, de Oliveira Pereira F, de Oliveira WA, Lima IO, de Oliveira Lima E. Antifungal activity of Thymus vulgaris L. essential oil and its constituent phytochemicals against Rhizopus oryzae: interaction with ergosterol. *Molecules*. 2012;**17**(12):14418–33. doi: 10.3390/molecules171214418. [PubMed: 23519243]. [PubMed Central: PMC6268362].
7. Sharifzadeh A, Khosravi AR, Shokri H, Sharafi G. Antifungal effect of Trachyspermum ammi against susceptible and fluconazole-resistant strains of Candida albicans. *J Mycol Med*. 2015;**25**(2):143–50. doi: 10.1016/j.mycmed.2015.03.008. [PubMed: 25982599].
8. Khosravi AR, Shokri H, Tootian Z, Alizadeh M, Yahyaraeyat R. Comparative efficacies of Zataria multiflora essential oil and itraconazole against disseminated Candida albicans infection in BALB/c mice. *Braz J Microbiol*. 2009;**40**(3):439–45. doi: 10.1590/S1517-83822009000300003. [PubMed: 24031384]. [PubMed Central: PMC3768526].
9. Clinical and Laboratory Standards Institute (CLSI). *Method for antifungal disk diffusion susceptibility testing of yeasts; approved guideline*. Wayne, PA: Clinical and Laboratory Standards Institute; 2009. Report No.: M44-A2.
10. Imbert C, Rodier MH, Daniault G, Jacquemin JL. Influence of sub-inhibitory concentrations of conventional antifungals on metabolism of Candida albicans and on its adherence to polystyrene and extracellular matrix proteins. *Med Mycol*. 2002;**40**(2):123–9. doi: 10.1080/mmy.40.2.123.129. [PubMed: 12058724].
11. Pfaller MA, Diekema DJ, Gibbs DL, Newell VA, Barton R, Bijie H, et al. Geographic variation in the frequency of isolation and fluconazole and voriconazole susceptibilities of Candida glabrata: an assessment from the ARTEMIS DISK Global Antifungal Surveillance Program. *Diagn Microbiol Infect Dis*. 2010;**67**(2):162–71. doi: 10.1016/j.diagmicrobio.2010.01.002. [PubMed: 20338711].
12. Whaley SG, Berkow EL, Rybak JM, Nishimoto AT, Barker KS, Rogers PD. Azole Antifungal Resistance in Candida albicans and Emerging Non-albicans Candida Species. *Front Microbiol*. 2016;**7**:2173. doi: 10.3389/fmicb.2016.02173. [PubMed: 28127295]. [PubMed Central: PMC5226953].
13. Braga PC, Culici M, Alfieri M, Dal Sasso M. Thymol inhibits Candida albicans biofilm formation and mature biofilm. *Int J Antimicrob Agents*. 2008;**31**(5):472–7. doi: 10.1016/j.ijantimicag.2007.12.013. [PubMed: 18329858].
14. Sharifzadeh A, Khosravi AR, Shokri H, Shirzadi H. Potential effect of 2-isopropyl-5-methylphenol (thymol) alone and in combination with fluconazole against clinical isolates of Candida albicans, C. glabrata and C. krusei. *J Mycol Med*. 2018;**28**(2):294–9. doi: 10.1016/j.mycmed.2018.04.002. [PubMed: 29661606].
15. Abbaszadeh S, Sharifzadeh A, Shokri H, Khosravi 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**(2):e51–6. doi: 10.1016/j.mycmed.2014.01.063. [PubMed: 24582134].
16. Ahmad A, Khan A, Akhtar F, Yousuf S, Xess I, Khan LA, et al. Fungicidal activity of thymol and carvacrol by disrupting ergosterol biosynthesis and membrane integrity against Candida. *Eur J Clin Microbiol Infect Dis*. 2011;**30**(1):41–50. doi: 10.1007/s10096-010-1050-8. [PubMed: 20835742].
17. Morcia C, Malnati M, Terzi V. In vitro antifungal activity of terpinen-4-ol, eugenol, carvone, 1,8-cineole (eucalyptol) and thymol against mycotoxigenic plant pathogens. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess*. 2012;**29**(3):415–22. doi: 10.1080/19440049.2011.643458. [PubMed: 22257275].
18. Perez-Alfonso CO, Martinez-Romero D, Zapata PJ, Serrano M, Valero D, Castillo S. The effects of essential oils carvacrol and thymol on growth of Penicillium digitatum and P. italicum involved in lemon decay. *Int J Food Microbiol*. 2012;**158**(2):101–6. doi: 10.1016/j.ijfoodmicro.2012.07.002. [PubMed: 22831817].
19. Samaranayake YH, Wu PC, Samaranayake LP, So M. Relationship between the cell surface hydrophobicity and adherence of Candida krusei and Candida albicans to epithelial and denture acrylic surfaces. *APMIS*. 1995;**103**(10):707–13. [PubMed: 8534429].

20. Luo G, Samaranayake LP. Candida glabrata, an emerging fungal pathogen, exhibits superior relative cell surface hydrophobicity and adhesion to denture acrylic surfaces compared with Candida albicans. *APMIS*. 2002;**110**(9):601–10. doi: 10.1034/j.1600-0463.2002.1100902.x. [PubMed: 12529012].
21. Silva-Dias A, Miranda IM, Branco J, Monteiro-Soares M, Pina-Vaz C, Rodrigues AG. Adhesion, biofilm formation, cell surface hydrophobicity, and antifungal planktonic susceptibility: relationship among Candida spp. *Front Microbiol*. 2015;**6**:205. doi: 10.3389/fmicb.2015.00205. [PubMed: 25814989]. [PubMed Central: PMC4357307].
22. Valentin A, Canton E, Peman J, Martinez JP. Voriconazole inhibits biofilm formation in different species of the genus Candida. *J Antimicrob Chemother*. 2012;**67**(10):2418–23. doi: 10.1093/jac/dks242. [PubMed: 22733651].
23. Raut JS, Shinde RB, Chauhan NM, Karuppayil SM. Terpenoids of plant origin inhibit morphogenesis, adhesion, and biofilm formation by Candida albicans. *Biofouling*. 2013;**29**(1):87–96. doi: 10.1080/08927014.2012.749398. [PubMed: 23216018].
24. Khan MS, Ahmad I, Cameotra SS, Botha F. Sub-MICs of Carum copticum and Thymus vulgaris influence virulence factors and biofilm formation in Candida spp. *BMC Complement Altern Med*. 2014;**14**:337. doi: 10.1186/1472-6882-14-337. [PubMed: 25220750]. [PubMed Central: PMC4177179].
25. Pemmaraju SC, Pruthi PA, Prasad R, Pruthi V. Candida albicans biofilm inhibition by synergistic action of terpenes and fluconazole. *Indian J Exp Biol*. 2013;**51**(11):1032–7. [PubMed: 24416942].