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Killing Kinetics of Carvacrol against Fluconazole-Susceptible and -Resistant Isolates of *Candida tropicalis*

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ABSTRACT

Background and objectives: Overuse and misuse of antibiotics in the agricultural and healthcare sectors have led to the emergence of antibiotic-resistant strains. Therefore, finding alternative antimicrobial compounds, such as phytochemicals, is of great importance. This study evaluated the feasibility of carvacrol as an antifungal agent in suppressing the planktonic and hyphal growth of clinical isolates of fluconazole-susceptible and -resistant *Candida tropicalis*.

Methods: Clinical isolates of fluconazole-resistant *C. tropicalis* were identified using the CLSI guidelines and the World Health Organization's WHONET software. The inhibitory effect of carvacrol on planktonic cells was assessed by determining the minimum inhibitory concentration (MIC) and time-kill profile. The inhibitory effect of carvacrol on hyphal growth was studied by using light field microscopy.

Results: The findings indicated that 50% of clinical isolates of *C. tropicalis* were resistant to fluconazole. The MIC90 and MIC50 of carvacrol against clinical isolates of fluconazole-susceptible and -resistant *C. tropicalis* were 25.00-300.00 µg/ml and 12.50-100.00 µg/ml, respectively. The time-kill analysis indicated that carvacrol exhibited fungicidal activity against the fluconazole-susceptible and -resistant *C. tropicalis* isolates 2-48 hours after exposure. Moreover, planktonic and hyphal growth of the isolates decreased significantly after exposure to carvacrol.

Conclusion: The findings revealed that carvacrol exhibits inhibitory effects on the planktonic and hyphal cells of fluconazole-susceptible and -resistant *C. tropicalis* isolates. Therefore, the antifungal potential of carvacrol as a natural antifungal could be further exploited for the treatment of resistant *C. tropicalis* infections.

Keywords: Carvacrol, Candida tropicalis, Fluconazole.

INTRODUCTION

Candida tropicalis has emerged as an important opportunistic fungal pathogen and the second most common cause of candidiasis. This *Candida* species has been also recognized as a potent biofilm producer, which is highly adherent to most human tissues and cells (1, 2). During biofilm formation, planktonic cells attach to a surface, resulting in the formation of a complex network of different layers of polymorphic cells, including hyphal cells, pseudohyphal cells, and round planktonic cells, which are enclosed in an extracellular polymeric matrix (3).

The transition from yeast to hyphal growth and involved signaling pathways are very well-established for *Candida albicans*. The environmental signals together with signaling pathway-specific transcription factors regulate the expression of target genes involved in filamentation. However, certain *C. albicans* morphological regulatory functions are evolutionarily conserved in *C. tropicalis* (4, 5). Misuse and overuse of antifungals in the clinical and industrial sectors have led to the emergence of antifungal-resistant *C. tropicalis* strains, which could be life-threatening, particularly in vulnerable groups such as immunocompromised patients (1, 3, 6). Antifungal agents such as azoles, polyenes, and echinocandins are currently considered the last resort antibiotics for treating candidiasis (7). Resistance to these antifungals has been reported in *C. tropicalis* due to the increased reliance upon these lines of antibiotics (6). Azole-resistant *Candida* species produce efflux pumps, which can reduce the amount of intracellular drug content. Other antibiotic resistance mechanisms such as the alteration of the concentration or structure of antifungal target sites as well as alteration in the sterol composition of the fungal membrane are sufficient to confer azole resistance among *Candida* species (8, 9). Resistance to fluconazole has been commonly reported amongst clinical isolates of *C. tropicalis* (6). The point mutation *Y132F* has been reported in a single fluconazole-resistant *C. tropicalis*, which impacts susceptibility to fluconazole (11).

In order to mitigate issues caused by antifungal resistance, considerable effort has been directed toward the development of novel antifungal agents. Natural products have provided key scaffolds for drug development due to their relative diversity (12). Several studies have shown that natural products possess tremendous potential as an antifungal resource (13-16). A strong body of evidence suggests that essential oils can disrupt the structural integrity of the membrane and affect cell metabolism, eventually causing cell death (17-19). Despite the great potential of carvacrol-rich essential oils against *C. tropicalis*, no study has yet investigated the inhibitory effects of such essential oils on hyphal growth. Carvacrol, also known as cymophenol (2-methyl-5-propan-2-ylphenol), is a monoterpene phenol found in several aromatic plants. This compound has strong antimicrobial, acetylcholinesterase inhibitory, anti-tumor, anti-inflammatory, anti-mutagenic, anti-genotoxic, anti-elastase, anti-spasmodic, anti-hepatotoxic, anti-platelet, analgesic, angiogenic, insecticidal, and hepatoprotective activities (19, 20). The antifungal activity of carvacrol against *C. tropicalis* has been well demonstrated (13-16). Data obtained from time-kill kinetics have provided critical information regarding the effects of antimicrobials over time. Appiah et al. analyzed the time-kill kinetics of antifungal agents against *C. albicans* (21).

This study aimed to investigate the antifungal activity of carvacrol on planktonic and hyphal cells of clinical isolates of fluconazole-susceptible and -resistant *C. tropicalis*, with a focus on the time-kill kinetics.

MATERIALS AND METHODS

The study protocol was approved by the ethics committee of the Islamic Azad University of Yasuj, Iran (ethical code: IR. IAU.YASUJ.REC.1395.11). Fluconazole and carvacrol were purchased from Sigma-Aldrich Corporation (St. Louis, MO, USA). A stock solution of carvacrol was made by dissolving carvacrol in dimethyl sulfoxide (DMSO). Ten clinical isolates of *C. tropicalis* SN-1, SN-2, IAU-1-8, and *C. tropicalis* ATCC 750 were used in this study. Clinical isolates of *C. tropicalis* SN-1 and SN-2 from the vagina of patients with recurrent vulvovaginal candidiasis were kindly provided by the Microbiology Laboratory of Cellular and Molecular Research Center, Yasuj University of Medical Sciences, Iran. Clinical isolates of *C. tropicalis* IAU-1-8 were taken from diabetics and patients with liver disease.

The isolates of *C. tropicalis* were identified based on the morphological, biochemical, and molecular characteristics such as germ tube production, colony appearance on sabouraud dextrose agar (SDA; Q-Lab, England), CHROMagar *Candida* (CHROMagar Company, France), urease test, polymerase chain reaction (PCR) using primers of ITS1 (5′-TCC GTA GGT GAA CCT GCG G-3′) and ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′), and sequencing (22).

Fluconazole-resistant *C. tropicalis* isolates were identified by the disk diffusion (M44-A2) and broth microdilution (M27-A3 and M27-S4) methods according to the Clinical and Laboratory Standards Institute recommendations. The results were interpreted by the World Health Organization's WHONET software (23).

The minimum inhibitory concentration (MIC) of fluconazole and carvacrol against the isolates was determined by broth microdilution assay in accordance with CLSI M27-A3 as described previously (22). Two-fold dilutions were performed in each microplate well to a final volume of 50 µl of carvacrol and 50 µl of clinical isolates of fluconazole-susceptible and -resistant *C. tropicalis* suspension at approximately $0.5–5 \times 10^3$ CFU/ml. The MIC50 and MIC90 were defined as the lowest concentration of the drugs that inhibited 50% and 90% of the isolates, respectively. Wells with no antifungal agents and those containing medium alone were used as the positive and negative controls, respectively.

A standard inoculum of 1×10^6 CFU/ml was treated with the MIC90 of carvacrol in the time-kill assay through viable plate counting. Samples were incubated at 35 ˚C with shaking at 200 rpm. Immediately after the inoculation, viable counting was performed at different times (i.e., 0, 2, 4, 6, 8, 12, 24, and 48 hours). Next, 100 µl of the samples were subjected to 100-fold dilution with 0.85% (w/v) saline and then plated onto SDA and incubated at 35 ˚C for 24 hours. In the experimental groups, fluconazole-treated (positive control), untreated (negative control), and DMSO without antifungal agents were also included (16).

The inhibitory effect of carvacrol on hyphal growth was assessed according to the method described by Khodavandi et al. (24). In brief, a starting cell density of 1×10^6 CFU/ml was treated with carvacrol at concentrations equal to 1/4× MIC, 1/2× MIC, 1× MIC, and 2× MIC values. The samples were dispensed into the cell culture plates and incubated at 35 ˚C for 90 minutes. Subsequently, the mixture was incubated at 35 ˚C for 16 hours with shaking at 200 rpm. Finally, the samples were washed with phosphate buffer saline and observed via a light field microscope (Nikon, Japan).

All experiments were done in triplicate. The normality of data was assessed using the Kolmogorov–Smirnov test. One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was performed to compare the normally distributed data. The nonparametric tests including the Kruskal-Wallis test followed by Bonferroni's post hoc test were used when appropriate. A *p*-value of ≤0.05 was considered statistically significant. Statistical analysis was performed in IBM SPSS Statistics (version 24, SPSS Inc., Chicago, IL, USA).

Table 1- Morphological, biochemical, and molecular characterization of *C. tropicalis* isolates

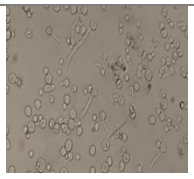
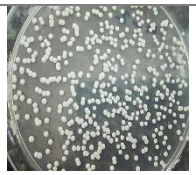
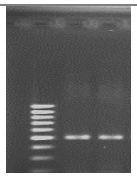
Isolate	Germ tube production (In human serum)	Colony appearance (On SDA)	Colony appearance (On CHROMagar *Candida*)	Urease test	ITS1-ITS4 PCR product
IAU-2	 A young hypha growing out of a planktonic cell	 Colonies with white to cream-colored, creamy texture, smooth and glabrous	 Metallic blue colony	 Negative	 Providing a single PCR product of 524 bp

Table 2-The fluconazole-susceptibility results in clinical isolates of *C. tropicalis* using the disk diffusion and broth microdilution assays

CLSI disk diffusion assay										
Antibiotic name	**Antibiotic class**	**Code**	**Breakpoints**	**Number**	**%R**	**%I**	**%S**	**%R 95%C.I.**		
Fluconazole	**Antifungals**	**FLU**	**15 - 18**	**10**	**50**	**0**	**50**	**20.1-79.9**		
CLSI broth microdilution assay										
Antibiotic name	**Antibiotic class**	**Code**	**Breakpoints**	**Number**	**%R**	**%I**	**%S**	**%R 95%C.I.**	**Geom. Mean**	**MIC Range**
Fluconazole	**Antifungals**	**FLU**	**S<=2 R>=8**	**10**	**50**	**0**	**50**	**20.1-79.9**	**9.849**	**2 - 64**

R: resistant; S: sensitive; I: Intermediate; C.I.: Confidence Interval; Geom. Mean: Geometric Mean

RESULTS

Morphological, biochemical, and molecular characteristics of *C. tropicalis* isolates are shown in table 1. All clinical isolates of *C. tropicalis* produced metallic blue colonies on CHROMagar *Candida*, and other identification tests confirmed the isolates as *C. tropicalis*. The PCR process using the universal primer pairs generated a single PCR product of 524 bp. The nucleotide sequence of *C. tropicalis* isolates was analyzed using the Basic Local Alignment Search Tool (BLAST, http://blast.ncbi.nlm.nih.gov/Blast.cgi). The sequence displayed 100% identity with the respective gene sequence.

Based on the results, 50% of the clinical isolates of *C. tropicalis* were resistant to fluconazole (Table 2).

The MIC of carvacrol on planktonic cells was determined using the broth microdilution assay. According to the results, the MIC90 and MIC50 values of carvacrol against planktonic cells were 25.00-300.00 µg/ml and 12.50-100.00 µg/ml, respectively. However, carvacrol showed more potency in vitro with MIC90 and MIC50 values ranging from 25.00 to 150.00 µg/ml and 12.50 to 50.00 µg/ml, respectively against fluconazole-susceptible isolates (Table 3).

The killing kinetics of carvacrol was evaluated at a concentration equal to the MIC value by performing the time-kill analysis. A more complete killing profile of fluconazole-susceptible and -resistant *C. tropicalis* isolates was observed after 24 hours of treatment with carvacrol (Figure 1).

The results of one-way ANOVA showed significant differences amongst carvacrol-treated clinical isolates of *C. tropicalis* at different times. At 0-hour, there was no significant difference between the carvacrol- and fluconazole-treated *C. tropicalis* isolates and the control groups (Figure 1). After 2 to 48 hours, the number of fluconazole-susceptible isolates decreased significantly compared with the control groups ($p<0.05$). Of note, after 8 and 12 hours, a significant difference was observed between fluconazole-susceptible and -resistant *C. tropicalis* isolates ($p<0.05$) (Figures 1A, 1B). The number of fluconazole-resistant isolates decreased only in treatment with carvacrol after 2-48 hours (Figure 1C).

Both planktonic and hyphal cells of fluconazole-susceptible and -resistant *C. tropicalis isolates* reduced at different MICs after 16 hours (Figure 2).

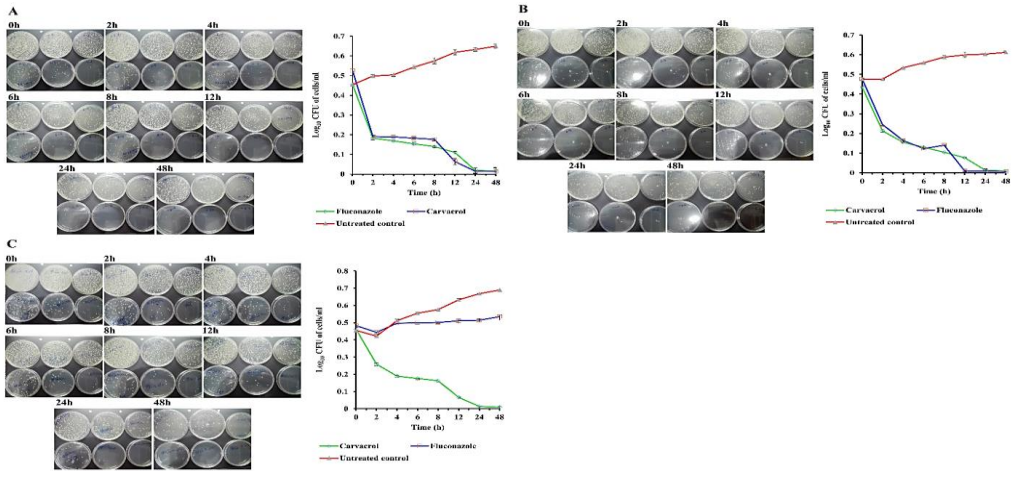


Figure 1- Time-kill assay of (A) *C. tropicalis* ATCC 750, (B) clinical isolates of fluconazole-susceptible (SN-1), and (C) fluconazole-resistant (IAU-2) *C. tropicalis* isolates treated with MIC90 of carvacrol at different times (i.e., 0, 2, 4, 6, 8, 12, 24, and 48 hours). Positive control represents *C. tropicalis* treated with MIC of fluconazole. Data are shown as the mean and standard error of the mean of three independent experiments.

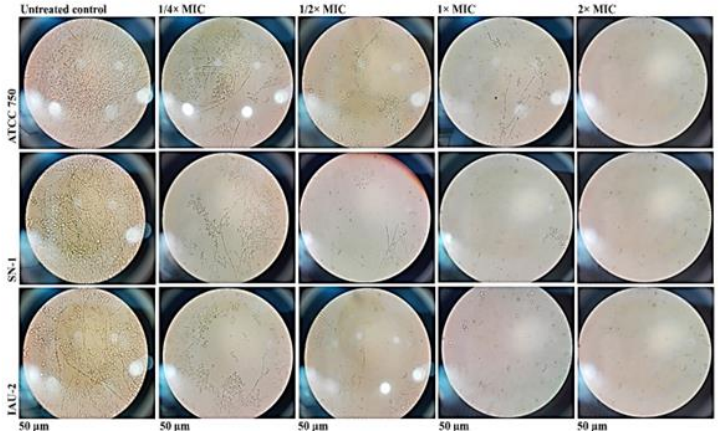


Figure 2- Light field microscopy of the inhibitory effect of different MICs of carvacrol on hyphal cells of clinical isolates of fluconazole-susceptible (SN-1) and -resistant (IAU-2) *C. tropicalis* after 16 hours. *C. tropicalis* ATCC 750 was used as a reference control. The images were taken under × 40 magnification. Bar = 50 µm.

Table 3- MIC90 and MIC50 of carvacrol and fluconazole against clinical isolates of *C. tropicalis*

Isolates /Antifungal	Carvacrol						Fluconazole					
	MIC90 rang (µg/ml)	Geom. Mean	95%C.I.	MIC50 rang (µg/ml)	Geom. Mean	95%C.I.	MIC90 rang (µg/ml)	Geom. Mean	95%C.I.	MIC50 rang (µg/ml)	Geom. Mean	95%C.I.
C. tropicalis ATCC *750*	50-75	57.23	58.3 ± 16.3 (42 to 74.6)	25	25	25 ± 0 (25 to 25)	2	2	2 ± 0 (2 to 2)	0.25	0.25	0.25 ± 0 (0.25 to 0.25)
IAU-1	100-125	107.72	108 ± 16.3 (91.7 to 124)	25-50	31.5	33.3 ± 16.3 (17 to 49.6)	2	2	2 ± 0 (2 to 2)	0.25	0.25	0.25 ± 0 (0.25 to 0.25)
IAU-2	150	150	150 ± 0 (150 to 150)	50	50	50 ± 0 (50 to 50)	64	64	64 ± 0 (64 to 64)	16	20.16	21.3 ± 10.5 (10.8 to 31.8)
IAU-3	25	25	25 ± 0 (25 to 25)	12.5-25	15.75	16.7 ± 8.17 (8.53 to 24.9)	2	2	2 ± 0 (2 to 2)	0.25	0.25	0.25 ± 0 (0.25 to 0.25)
IAU-4	100-125	107.72	108 ± 16.3 (91.7 to 124)	25	25	25 ± 0 (25 to 25)	32	32	32 ± 0 (32 to 32)	16	16	16 ± 0 (16 to 16)
IAU-5	50	50	50 ± 0 (50 to 50)	12.5	12.5	12.5 ± 0 (12.5 to 12.5)	2	2	2 ± 0 (2 to 2)	0.25	0.25	0.25 ± 0 (0.25 to 0.25)
IAU-6	150	150	150 ± 0 (150 to 150)	25	25	25 ± 0 (25 to 25)	32	32	32 ± 0 (32 to 32)	8	10.08	10.7 ± 5.23 (5.47 to 15.9)
IAU-7	300-325	316.44	308 ± 16.3 (292 to 324)	100	100	100 ± 0 (100 to 100)	64	64	64 ± 0 (64 to 64)	16	20.16	21.3 ± 10.5 (10.8 to 31.8)
IAU-8	300-325	316.44	308 ± 16.3 (292 to 324)	50	50	50 ± 0 (50 to 50)	64	64	64 ± 0 (64 to 64)	32	32	32 ± 0 (32 to 32)
SN-1	100	100	100 ± 0 (100 to 100)	25-50	31.5	33.3 ± 16.3 (17 to 49.6)	2	2	2 ± 0 (2 to 2)	0.25	0.32	0.333 ± 0.163 (0.17 to 0.496)
SN-2	150	150	150 ± 0 (150 to 150)	50	50	50 ± 0 (50 to 50)	2	2	2 ± 0 (2 to 2)	0.25	0.25	0.25 ± 0 (0.25 to 0.25)

IAU: Clinical isolates of C. tropicalis strains isolated in this study.
SN: Clinical isolates of C. tropicalis strains provided by the Yasuj University of Medical Sciences .
C.I.: Confidence Interval; Geom. Mean: Geometric Mean

DISCUSSION

The emergence and spread of antifungal resistance by opportunistic fungi have become a major challenge in healthcare settings, impeding treatment progress and causing substantial morbidity and mortality. This issue is further complicated by the emergence of azole-resistant fungal infections (25). Thus, finding plant-based, natural antifungals is of great importance for finding alternative solutions to the increasing resistance problem.

The bioactivity of carvacrol, a natural phenolic monoterpenoid, has been documented in many studies (13-16, 19, 20). Thus, this study was carried out to investigate the killing kinetics of carvacrol against hyphal growth of *C. tropicalis* isolates. We first determined the bioactivity of carvacrol against planktonic cells of fluconazole-susceptible and -resistant *C. tropicalis* isolates and found that planktonic cells of the fluconazole-susceptible isolates

were more sensitive to carvacrol. From the presented antifungal assay, small MIC values are noticed in clinical fluconazole-resistant isolates of *C. tropicalis*, indicating the antifungal potential of carvacrol. In a study conducted by Khodavandi et al. (24), carvacrol showed antifungal activity against multidrug-resistant *C. albicans*.

To further elucidate the overall effect of carvacrol on planktonic cells, the killing kinetics of both clinical isolates of fluconazole-susceptible and -resistant *C. tropicalis* isolates were determined via time-kill analysis. The time-kill profile showed a reduction in the number of planktonic cells, 2-48 hours after treatment with carvacrol, while a more complete killing profile was observed after 24 hours. This indicates the fungicidal potential of carvacrol against planktonic cells of fluconazole-susceptible and -resistant *C. tropicalis* isolates as previously proposed in other studies (24, 26).

Carvacrol exposure also caused a reduction in hyphae formation. Both clinical isolates of fluconazole-susceptible and -resistant shared a similar reduction of hyphae and planktonic cells. Studies by Manoharan et al. (27) and Raut et al. (28) also showed that essential oil components such as α-longipinene, linalool, and carvacrol can inhibit planktonic cells, hyphae, and biofilm of *C. albicans.* In line with our results, Tobaldini-Valerio et al. showed the antifungal and antibiofilm activities of propolis against *Candida* species (29). These authors also observed that the time-kill assay results were in accordance with the antifungal susceptibility results. Moreover, Lemos et al. (30) reported the reduction of planktonic and biofilm populations of *Streptococcus mutans* in different environmental conditions.

To the best of our knowledge, this study is the first to evaluate the killing kinetics of carvacrol on fluconazole-susceptible and -resistant isolates of *C. tropicalis*. The cellular and molecular mechanisms involved in the antifungal activity of carvacrol are not completely clear, but inhibition of ergosterol biosynthesis, the impairment of membrane integrity, endoplasmic reticulum stress, the unfolded protein response, and perturbing H^+ and Ca^{2+} ion homeostasis have been proposed as possible mechanisms (26, 30). Niu et al. (31) found that carvacrol could trigger *C. albicans* apoptosis associated with Ca^{2+}/calcineurin pathway.

The complex structure of the fungal cell is a limitation of the present study when assessing the antifungal activity of carvacrol.

CONCLUSION

This study investigated the antifungal effects of carvacrol against planktonic and hyphal cells of fluconazole-susceptible and -resistant *C. tropicalis* isolates. The findings revealed that carvacrol exhibits inhibitory effects on the planktonic and hyphal cells of fluconazole-susceptible and -resistant *C. tropicalis* isolates. Therefore, the antifungal potential of carvacrol as a natural antifungal could be further exploited for the treatment of resistant *C. tropicalis* infections.

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DECLARATIONS

FUNDING

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Ethics approvals and consent to participate

The study protocol was approved by the ethics committee of the Islamic Azad University of Yasuj, Iran (ethical code: IR. IAU.YASUJ.REC.1395.11).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

1. Zuza-Alves DL, Silva-Rocha WP, Chaves GM. *An Update on Candida tropicalis Based on Basic and Clinical Approaches.* Front Microbiol. 2017 13;8:1927. [DOI:10.3389/fmicb.2017.01927] [PubMed] [Google Scholar]

2. Megri Y, Arastehfar A, Boekhout T, et al. *Candida tropicalis is the most prevalent yeast species causing candidemia in Algeria: the urgent need for antifungal stewardship and infection control measures.* Antimicrob Resist Infect Control 2020;9: 50. doi: 10.1186/s13756-020-00710-z. [View at Publisher] [DOI:10.1186/s13756-020-00710-z] [PubMed] [Google Scholar]

3. Tseng YK, Chen YC, Hou CJ, Deng FS, Liang SH, Hoo SY, et al . *Evaluation of Biofilm Formation in Candida tropicalis Using a Silicone-Based Platform with Synthetic Urine Medium.* Microorganisms. 2020 1;8(5):660. [DOI:10.3390/microorganisms8050660] [PubMed] [Google Scholar]

4. Kornitzer D. *Regulation of Candida albicans Hyphal Morphogenesis by Endogenous Signals.* J Fungi (Basel). 2019 28;5(1):21. [DOI:10.3390/jof5010021] [PubMed] [Google Scholar]

5. Lackey E, Vipulanandan G, Childers DS, Kadosh D. *Comparative evolution of morphological regulatory functions in Candida species.* Eukaryot Cell. 2013 ;12(10):1356-68. [DOI:10.1128/EC.00164-13.] [PubMed] [Google Scholar]

6. Sanguinetti M, Posteraro B. *Susceptibility Testing of Fungi to Antifungal Drugs.* J Fungi (Basel). 2018. 15; 4(3): 110. [View at Publisher] [DOI:10.3390/jof4030110] [PubMed] [Google Scholar]

7. Costa-de-Oliveira S, Rodrigues AG. *Candida albicans antifungal resistance and tolerance in bloodstream infections: the triad yeast-host-antifungal.* Microorganisms. 2020; 8: 154. [View at Publisher] [DOI:10.3390/microorganisms8020154] [PubMed]

8. Cowen LE, Sanglard D, Howard S, et al. *Mechanisms of antifungal drug resistance.* Cold Spring Harb Perspect Med 2014;5: a019752. [View at Publisher] [DOI:10.1101/cshperspect.a019752] [PubMed] [Google Scholar]

9. Berkow EL, Lockhart SR. *Fluconazole resistance in Candida species: a current perspective.* Infect Drug Resist. 2017; 10: 237-45. [DOI:10.2147/IDR.S118892] [PubMed]

10. Sadeghi G, Ebrahimi-Rad M, Mousavi SF, Shams-Ghahfarokhi M, Razzaghi-Abyaneh M. *Emergence of non-Candida albicans species: epidemiology, phylogeny and fluconazole susceptibility profile.* Mycol Med. 2018; 28: 51-58. [View at Publisher] [DOI:10.1016/j.mycmed.2017.12.008] [PubMed] [Google Scholar]

11. Tan J, Zhang J, Chen W, Sun Y, Wan Z, Li R. *The A395T mutation in ERG11 gene confers fluconazole resistance in Candida tropicalis causing candidemia.* Mycopathologia. 2015; 179: 213-8. [View at Publisher] [DOI:10.1007/s11046-014-9831-8] [PubMed] [Google Scholar]

12. Katz L, Baltz RH. *Natural product discovery: past, present, and future.* J Ind Microbiol Biotechnol. 2016; 43: 155-76. [View at Publisher] [DOI:10.1007/s10295-015-1723-5] [PubMed] [Google Scholar]

13. Piras A, Cocco V, Falconieri D, Porcedda S, Marongiu B, Maxia A. *Isolation of the volatile oil from Satureja thymbra by supercritical carbon dioxide extraction: chemical composition and biological activity.* Nat Prod Commun. 2011; 6: 1523-6. [DOI:10.1177/1934578X1100601029] [PubMed] [Google Scholar]

14. Gallucci MN, Carezzano ME, Oliva MM, Demo MS, Pizzolitto RP, Zunino MP, et al. *In vitro activity of natural phenolic compounds against fluconazole-resistant Candida species: a quantitative structure-activity relationship analysis.* J Appl Microbiol. 2014; 116: 795-804. [View at Publisher] [DOI:10.1111/jam.12432] [PubMed] [Google Scholar]

15. Soliman S, Alnajdy D, El-Keblawy AA, Mosa KA, Khoder G, Noreddin AM. Plants' natural products as alternative promising anti-Candida drugs. Pharmacogn Rev. 2017; 11: 104-22. [DOI:10.4103/phrev.phrev_8_17] [PubMed] [Google Scholar]

16. Alizadeh F, Khodavandi A, Esfandyari S, Nouripour-Sisakht S. *Analysis of ergosterol and gene expression profiles of sterol Δ5,6-desaturase (ERG3) and lanosterol 14α-demethylase (ERG11) in Candida albicans treated with carvacrol.* J Herbmed Pharmacol. 2018; 7: 79-87. [View at Publisher] [DOI:10.15171/jhp.2018.14] [Google Scholar]

17. Fei Lv, Hao Liang, Qipeng Yuan, Chunfang Li. *In vitro antimicrobial effects and mechanism of action of selected plant essential oil combinations against four food related microorganisms.* Food Res Int 2011;44: 3057-64. [View at Publisher] [DOI:10.1016/j.foodres.2011.07.030] [Google Scholar]

18. Chouhan S, Sharma K, Guleria S. Antimicrobial activity of some essential oils-present status and future perspectives. Medicines (Basel) 2017;4: 58. [View at Publisher] [DOI:10.3390/medicines4030058] [Google Scholar]

19. Wijesundara NM, Lee SF, Cheng Z, et al. *Carvacrol exhibits rapid bactericidal activity against Streptococcus pyogenes through cell membrane damage.* Sci Rep 2021;11: 1487. [View at Publisher] [DOI:10.1038/s41598-020-79713-0] [PubMed] [Google Scholar]

20. Husnu Can Baser K. *Biological and pharmacological activities of carvacrol and carvacrol bearing essential oils.* Curr Pharm Des. 2008;14: 3106-19. [DOI:10.2174/138161208786404227] [PubMed] [Google Scholar]

21. Appiah T, Boakye YD, Agyare C. *Antimicrobial activities and time-kill kinetics of extracts of selected ghanaian mushrooms.* Evid Based Complement Alternat Med. 2017: 4534350. [View at Publisher] [DOI:10.1155/2017/4534350] [PubMed] [Google Scholar]

22. Alizadeh F, Khodavandi A, Zalakian S. *Quantitation of ergosterol content and gene expression profile of ERG11 gene in fluconazole-resistant Candida albicans*. Curr Med Mycol. 2017; 3: 13-19. [DOI:10.29252/cmm.3.1.13] [PubMed] [Google Scholar]

23. Zare-Khafri M, Alizadeh F, Nouripour-Sisakht S, Khodavandi A, Gerami M. *Inhibitory effect of magnetic iron oxide nanoparticles on the pattern of expression of lanosterol 14α-demethylase (ERG11) in fluconazole-resistant colonizing isolate of Candida albicans.* IET Nanobiotechnol. 2020; 14: 375-81. [View at Publisher] [DOI:10.1049/iet-nbt.2019.0354] [PubMed] [Google Scholar]

24. Khodavandi A, Alizadeh F, Sanaee T. *Antifungal activity of carvacrol on ergosterol synthesis in multidrug resistant Candida albicans.* Hormozgan Med J. 2018; 22: e87226. [View at Publisher] [DOI:10.29252/hmj.22.2.113] [Google Scholar]

25. Gao J, Wang H, Li Z, Wong AH, Wang YZ, Guo Y, et al. *Candida albicans gains azole resistance by altering sphingolipid composition.* Nat Commun. 2018; 9(1): 4495. [View at Publisher] [DOI:10.1038/s41467-018-06944-1] [PubMed] [Google Scholar]

26. Ahmad A, Khan A, Akhtar F, 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: 41-50. [View at Publisher] [DOI:10.1007/s10096-010-1050-8] [PubMed] [Google Scholar]

27. Manoharan RK, Lee JH, Kim YG, et al. *Inhibitory effects of the essential oils α-longipinene and linalool on biofilm formation and hyphal growth of Candida albicans.* Biofouling. 2017; 33: 143-55. [View at Publisher] [DOI:10.1080/08927014.2017.1280731] [PubMed] [Google Scholar]

28. Raut JS, Shinde RB, Chauhan NM, et al. *Terpenoids of plant origin inhibit morphogenesis, adhesion, and biofilm formation by Candida albicans.* Biofouling. 2013; 29: 87-96. [View at Publisher] [DOI:10.1080/08927014.2012.749398] [PubMed] [Google Scholar]

29. Tobaldini-Valerio FK, Bonfim-Mendonça PS, Rosseto HC, et al. *Propolis: a potential natural product to fight Candida species infections*. Future Microbiol. 2016; 11: 1035-46. [View at Publisher] [DOI:10.2217/fmb-2015-0016] [PubMed] [Google Scholar]

30. Lemos JAC, Brown TA, Burne RA. *Effects of RelA on key virulence properties of planktonic and biofilm populations of Streptococcus mutans.* Infect Immunity. 2004; 72: 1431-40. [View at Publisher] [DOI:10.1128/IAI.72.3.1431-1440.2004] [PubMed] [Google Scholar]

31. Niu C, Wang C, Yang Y, et al. *Carvacrol induces Candida albicans apoptosis associated with Ca2+/calcineurin pathway*. Front Cell Infect Microbiol. 2020; 10: 192. [View at Publisher] [DOI:10.3389/fcimb.2020.00192] [PubMed] [Google Scholar]

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