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RESEARCH ARTICLE

Anti-fungal efficacy of Carvacrol against *Candida glabrata* clinical isolates of vulvovaginal candidiasis

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Abstract

Fungal infections affect over 1 billion people worldwide each year, including superficial infections like athlete's foot and more severe systemic infections. Fungal diseases are responsible for an estimated 1.5 million deaths annually, a figure comparable to or exceeding the mortality rate of diseases like malaria or tuberculosis. The limited arsenal of available antifungal drugs, coupled with the emergence of drug-resistant fungal strains, has increased this concern. Therefore, there is a significant need to explore alternative therapeutics to overcome fungal pathogens. Carvacrol, phenolic monoterpenoids, is present in essential oils of many plants and is known for its biological and pharmacological properties. In the present study, the efficacy of carvacrol was investigated against four *Candida glabrata* strains isolated from patients of vulvovaginal candidiasis, which have shown varying extents of susceptibility against fluconazole. Carvacrol, a phytoactive monoterpene phenol, has shown a minimum inhibitory concentration (MIC_{50}) ranging from 75 µg/mL to 125 µg/mL and minimum fungicidal concentration from 150 µg/mL and 175 µg/mL for all clinical isolates, including wild-type strains. Carvacrol, in combination with fluconazole, has shown a strong synergism against wild type *C. glabrata* with a FIC index value of 0.156. Preliminary mechanistic investigations unveiled that exposure to carvacrol significantly reduced cell surface hydrophobicity and ergosterol content in all strains. In conclusion, carvacrol holds promising potential as an effective antifungal agent against *C. glabrata,* which is categorized as high priority in the first fungal pathogen priority list of the World Health Organisation released in 2022 for highlighting priority areas for action, including the development of effective therapeutic solution.

Keywords

Antimicrobial resistance; *Candida glabrata* ; Carvacrol; Clinical isolates; Fluconazole; Vulvovaginal candidiasis; WHO FPPL.

Introduction

Fungal infections have emerged as a significant global health concern influenced by a complex interplay of individual, geographical, and global factors (1-4). In the year 2022, the World Health Organization (WHO) introduced a fungal priority pathogens catalog, WHO FPPL (fungal priority pathogen list), listing the fungal pathogens associated with a high risk of morbidity and mortality (5). In the list, *Candida albicans* has been categorized as the critical priority group, followed by *Candida glabrata* (*Nakaseomyces glabrata*) in

the high-priority group. *Candida* species are responsible for diverse kinds of fungal infections, including superficial, invasive, and systemic. One of the most common forms of candidiasis is vulvovaginal candidiasis (VVC), which results primarily from *C. albicans* along with other non-*albicans Candida* (NAC) species. VVC is a remarkably common condition, with an estimated 75 % of women experiencing at least one episode in their lifetime and approximately 40 % to 45 % enduring recurrent occurrences (6). Azoles are the mainstay antifungals in the treatment of vulvovaginal candidiasis. However, it is important to acknowledge that certain non-*albicans Candida* strains, predominantly *C. glabrata*, exhibit inherent resistance to azoles, posing challenges in disease management (7). Notably, *C. glabrata,* with other NAC species, is found in 10 % to 20 % of women experiencing recurrent VVC. This underscores the significance of recognizing and effectively managing NAC species, especially in cases of recurrent VVC, to ensure the best possible clinical outcomes and alleviate the associated healthcare costs. *Candida* pathogenesis is attributed to known virulence factors such as cell surface hydrophobicity, yeast to hyphae transition, adhesion and biofilm formation etc. (8). Sterol is another key component in maintaining cell wall integrity and virulence of *Candida* species (9). Antifungal resistance carries significant implications for human health, typically resulting in extended treatment durations and hospitalization as well as an elevated demand for costly and frequently highly toxic secondary antifungal medications. Unfortunately, these secondary treatments are often inaccessible in low- and middle-income countries, potentially contributing to a higher mortality rate (10). Natural products have served as a rich and enduring source of medicinal compounds from prehistoric times to the present era. Phenolic monoterpenes, a class of organic compounds found abundantly in various plants and their essential oils, show significant potential for medicinal use due to their diverse pharmacological properties (11). Carvacrol, a monoterpene predominantly found in essential oils of plants like oregano, thyme and other herbs, represents a potent bioactive agent renowned for its diverse biological properties and it has demonstrated promising anti-*Candida* properties in various studies (12-15). Moreover, the use of carvacrol against *Candida* sp. is appealing due to its natural origin, relatively low toxicity to human cells at effective concentrations and its potential to combat drug-resistant strains of *Candida*.

This work is an attempt to examine the efficacy of carvacrol against *C. glabrata* isolates of VVC. The antifungal properties of carvacrol have also been investigated in combination with fluconazole. Carvacrol has proven its potential as a promising antifungal agent against VVC clinical isolates, offering plant-based therapeutics against potential fungal pathogens.

Materials and Methods

Strains, Reagents and Culture Condition

The study included clinical isolates and wild type strains of *C. glabrata*, as delineated in Table 1. Wild type strain (MTCC3019) was procured from CSIR-IMTECH Chandigarh, India and clinical isolates of vulvovaginal candidiasis were obtained from Shri Guru Ram Rai Institute of Medical and Health Science (SGRRIMHS), Dehradun, Uttarakhand, India. The reference strain of *C. glabrata* (MTCC3019) is denoted as CG, while the clinical isolates (VVC) are denoted as CG1, CG2, CG3 and CG4 in figures and text. Strains were consistently maintained in YPD medium, consisting of 1 % yeast extract, 2 % Bacto-peptone and 2 % dextrose (HiMedia), at 37 °C (CLSI, M27-A2). RPMI-1640 medium (with L-glutamine and sodium bicarbonate) (HiMedia), Sabouraud dextrose broth and YPD were used for antifungal assays. YPD broth was used for the growth curve and time-kill assay. Carvacrol with a purity of over 98 % was procured from Sigma-Aldrich (Cat. no. 282197-10G). Stock solutions were prepared using 100 % dimethyl sulfoxide (DMSO) from Sigma Aldrich and then diluted in RPMI before utilization. The fluconazole susceptibility of the *C. glabrata* strains listed in Table 1 had been determined previously (16).

Antifungal susceptibility test

The minimum inhibitory concentrations (MIC_{50}) of carvacrol against wild-type strain and clinical isolates were determined through broth microdilution assay following CLSI guidelines (M27-A2) (9). Briefly, a log-phase cell suspension of 2.5×10^3 cells/ml in RPMI-1640 medium was prepared and subsequently, 100 µL of this cell suspension (2.5×10^3 CFU/ml) was added to each well of microtiter plate (MTP). Following this, 100 µL of RPMI media containing varying concentrations of carvacrol (0, 25, 50, 75, 100, 125 and 150 µg/mL) was added to the wells. The MTP was then incubated at 37 °C for 48 h and the optical density (OD) at 600 nm was measured using an ELISA plate reader (Bio-Rad). The minimum inhibitory concentration was confirmed as the lowest drug concentration that inhibited over 50 % of cell growth. The untreated control group was

Table 1. Details of the *C. glabrata* strains used in the study.

Sl. No	Strain Type	Strain Description (represented as)	Susceptibility to Fluconazole (Flz)*	Source
1	*C. glabrata* wild-type strain	MTCC3019(CG)	R	CSIR-IMTECH (INDIA)
2	Vulvovaginal *C. glabrata* Clinical isolate (VVC).	CG1	R	Shri Guru Ram Rai Institute of Medical and Health Science, (SGRRIMHS), Dehradun, Uttarakhand, India.
3		CG2	SDD	
4		CG3	R	
5		CG4	S	

R- Resistance, **SDD**- Susceptible for dose dependent, **S**- Sensitive. ***Fluconazole susceptibility was confirmed (16).

included in the assay and each concentration point of carvacrol was set up in triplicate. The average values of the triplicates were used for graph plotting and data analysis. The minimum fungicidal concentration (MFC) of carvacrol was investigated by spotting 5 µL cell suspension from each well of the above MTP plate onto the YPD agar plate, followed by incubation at 37 °C for 16 h before being photographed. The concentration point of no growth was observed as the MFC (9).

Growth curve analysis

The effect of carvacrol on the growth of the fungi was studied using growth curve analysis (17). Briefly, log phase cultures of the wild type and clinical isolate were analyzed for growth kinetics in YPD medium with and without carvacrol at MIC_{50} concentration for 7 h. The readings of the culture at OD_{600} nm were recorded at an interval of 1h. Each experiment was performed in triplicate and the average values were used to generate graphs and draw conclusions.

Time-kill assay

A time kill assay was performed to analyze the time dependent fungicidal effect of carvacrol on the *C. glabrata* wild-type strain, as previously defined (18). Briefly, log phase cultures were exposed to carvacrol at MIC_{50} (1X, 2X and 4X) for different time points (0.5, 1, 2, 3, 4, 5 and 24 h) in YPD broth at 3 places for each. Cultures were washed to remove carvacrol before resuspension in YPD broth and 5 µL culture from each tube was spotted onto YPD agar plates. The plates were incubated at 37 °C for 16 h before being photographed (18).

Checkerboard Assay

The assays were conducted in RPMI medium using 96-well round-bottom plates, as in previous studies, with slight modification (4). Carvacrol and fluconazole stock solutions were prepared in DMSO and further diluted in RPMI medium. Dilutions were made for each drug so that the final concentration ranged from 512 to 0.5 µg/mL for fluconazole (A) and from 75 to 6.25 µg/mL for carvacrol (B). 1640 medium (with L-glutamine and sodium bicarbonate) 2.5×10^3 cells/mL in RPMI-1640 medium (with L-glutamine and sodium bicarbonate) was prepared. Subsequently, 100 µL of the cell suspension was added to each well of MTP, followed by the addition of 50 µL of different concentrations of each drug in each well as per the plan of the MTP (Fig. 1.). The minimum inhibitory concentration of drug A or B was defined as the lowest drug concentration that inhibited over 50 % of cell growth, determined by measuring OD_{600} nm.

The fractional inhibitory concentration (FIC) index was calculated using the formula:

$$FIC\ index = \frac{MIC\ of\ drug\ A\ in\ combination}{MIC\ of\ drug\ A\ alone} + \frac{MIC\ of\ drug\ B\ in\ combination}{MIC\ of\ drug\ B\ alone} \quad \text{.....(Eqn.1)}$$

Effect of carvacrol on cell surface hydrophobicity

The effect of carvacrol on cell surface hydrophobicity (CSH) of wild type strains and clinical isolates was determined following a previous study (9). Overnight cultures were diluted at 0.1 OD_{600} nm after being exposed to MIC_{50} of carvacrol followed by incubation for 24 h at 37 °C. After the cells were cultured, they were collected, washed using sterile PBS and then suspended in 3 mL of 50 mM sodium phosphate buffer at a pH of 7.2, resulting in a concentration of 2×10^6 cells/mL. Subsequently, 500 µL of octane was introduced to this cell suspension and vigorously mixed for 1 min by vortexing. The CSH was calculated by comparing the % decrease in optical density to the control suspension. A larger variance in absorbance values indicated a higher hydrophobic nature of the yeast cells. Each strain was evaluated 3 times independently in triplicates. The hydrophobic index (HI) was calculated using the following equation:

Checker-board assay			Fluconazole concentration (µg/ml)											
			0	0.5	0.1	2	4	8	16	32	64	128	256	512
			1	2	3	4	5	6	7	8	9	10	11	12
carvacrol concentration µg/ml	0	A												
	6.25	B												
	12.5	C												
	25	D												
	37.5	E												
	50	F												
	62.5	G												
	75	H												

Fig. 1. Plan of MTP for setting checkerboard assay.

$$HI = \frac{A1\text{-}A2}{A1} \times 100 \quad \text{.....(Eqn. 2)}$$

Where A1 is the absorbance of the inoculum and A2 is the absorbance of the aqueous phase.

Quantification of ergosterol of planktonic cells

Ergosterol levels in C. glabrata were measured by exposing mid-log phase cells in Sabouraud dextrose broth to the MIC_{50} of carvacrol for 24 hours at 37 °C (9). After centrifugation at 6000 rpm for 5 min, the cells were collected and washed with sterile water and the wet weight of the pellet was measured before being resuspended in 3 mL of a lysing agent (25 % alcoholic KOH). Subsequently, the cell suspension underwent an hour-long incubation at 85 °C in a water bath to aid in sterol extraction. Sterols were then extracted by vigorous mixing with a 1:3 mixture of distilled water and n-heptane and the heptane layer was meticulously collected and stored at -20 °C for 24 h. Finally, the sterol extracts were analyzed by scanning a 20 µL sample mixed with 100 µL of absolute ethanol, scanning the spectrum from 230 to 300 nm using a UV-visible spectrophotometer (SHIMADZU, UV-1900).

The amount of ergosterol was quantified using the following equation:

$$\%ERG = \frac{\left[\left(\frac{A_{281}}{290}\right)\times F\right]}{pellet\ weight} - \frac{\left[\left(\frac{A_{230}}{518}\right)\times F\right]}{pellet\ weight}$$ ....(Eqn. 3)

where F is the dilution factor; 290 and 518 are the E values for crystalline ergosterol and 24 (28) dehydroergosterol respectively.

Statistical Analysis

All experiments were conducted in triplicate and the results are presented as mean ± standard deviation. The data were subjected to student's t-test analysis using the Microsoft Excel program. Statistical significance was considered when $p<0.05$ or $p<0.01$.

Results

Antifungal susceptibility test of carvacrol

Minimum inhibitory concentration of carvacrol

The MIC_{50} and MIC_{90} values of carvacrol against *C. glabrata* wild type (CG) and its clinical isolates (CG1, CG2, CG3 and CG4) have been given in Table 2. It has been observed that clinical isolates have shown higher MIC values than the wild type *C. glabrata*. Analysis of OD_{600} nm at different concentrations of carvacrol has indicated that CG3 is slightly more resistant than the other isolates used in the assay (Fig. 2).

Table 2. Minimum inhibitory and minimum fungicidal concentrations of carvacrol.

Strains	Carvacrol (µg/mL)		
	MIC_{50}	MIC_{90}	MFC
CG	75-100	100-125	150
CG1	100-125	125-150	150
CG2	100-125	125-150	150
CG3	100-125	125-150	175
CG4	100-125	125-150	150

µg - microgram, **mL**- milli liter, **MIC_{50}**- minimum inhibitory concentration for 50 % cells inhibition, **MIC_{90}**- minimum inhibitory concentration for 90 % cells inhibition, **MFC**- minimum fungicidal concentration.

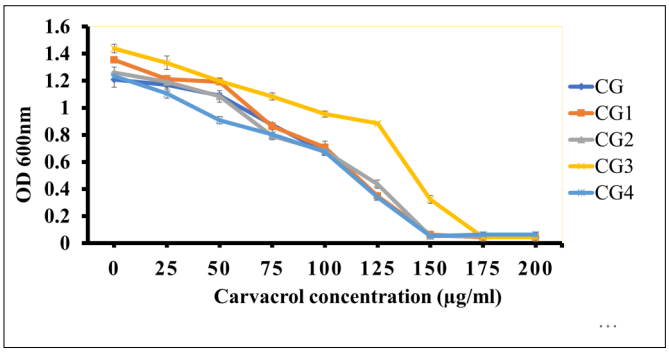

Fig. 2. Broth microdilution assay was performed on planktonic cells of *C. glabrata* and its clinical isolate in RPMI media upon exposure to carvacrol. The mean values of OD_{600} nm ± SD of three replicates is taken at the y-axis.

Minimum fungicidal concentration of carvacrol

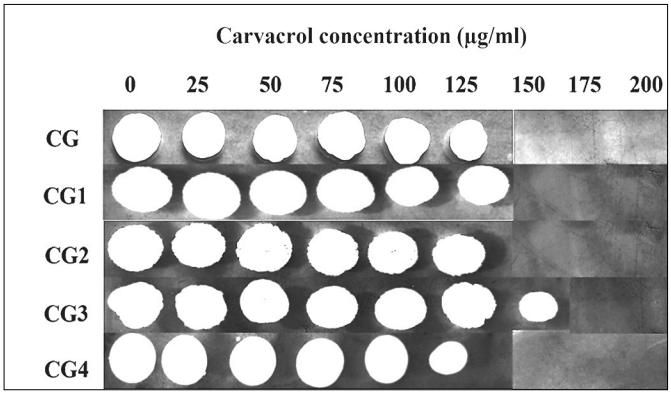

Fig. 3. Minimum fungicidal concentration of carvacrol by **s**potting assay. *C. glabrata* and the clinical isolate were exposed to different doses of carvacrol in a microtiter plate for 48 h followed by incubation on YPD agar plate for 18 h.

Further investigation into the effects of higher drug concentrations as shown in Fig. 3, allowed the determination of the minimum fungicidal concentrations (MFC) for these antifungal agents. Carvacrol, on the other hand, showed MFC values of 150 µg/mL for CG1, CG2 and CG4 and 175 µg/mL for CG3 (Table 2).

Growth curve analyses of carvacrol-treated strains

Growth curve analysis was performed to check the cellular fitness and growth rate of the wild-type and clinical isolate of *C. glabrata*. The growth kinetics of all tested strains exhibited a sigmoidal growth curve, clearly indicating the presence of distinct lag, log and stationary phases. More cellular fitness was found in CG1 and CG3 when compared to wild type strain (Fig. 4A). In general, about 1.30 to 2 h was required by the cells to adapt to the normal growth environment before they were ready to proliferate and

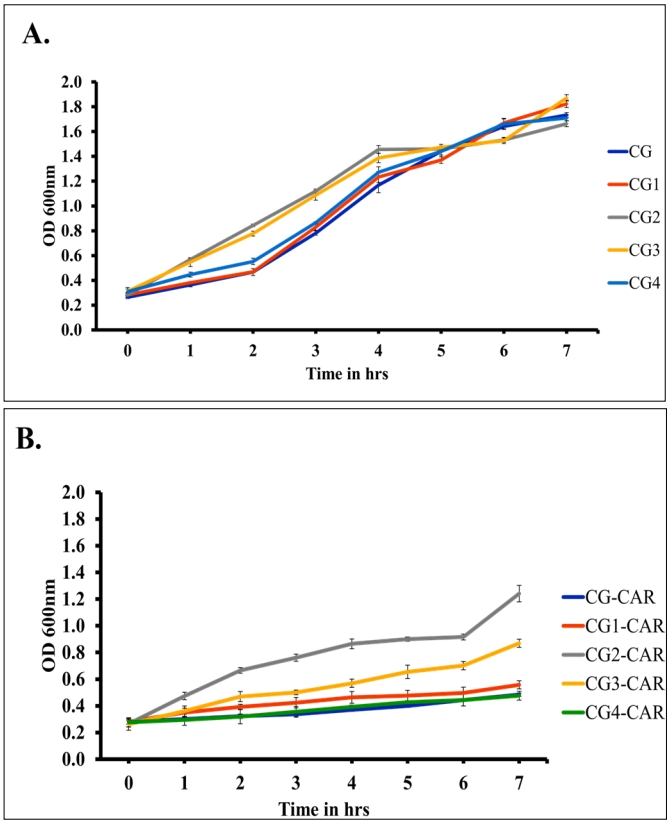

Fig. 4. The growth curve analysis of the *C. glabrata* and its clinical isolate on exposure to carvacrol for 7 h. **A**- Untreated cells. **B**- Cells treated with a subletha concentration of carvacrol. **CG**-Wild type cells **CG1**, **CG2**, **CG3** and **CG4**-clinical isolates.

enter the log phase. Upon exposure to MIC_{50} of carvacrol, the strains showed significant reduction after 7 h of exposure i.e., CG (72 %), CG1 (69 %), CG2 (25 %), CG3 (53 %) and CG4 (72 %), when compared to their untreated controls (Fig. 4). In YPD media, CG2 has shown better growth potential in presence of carvacrol than that of others including wild type strain.

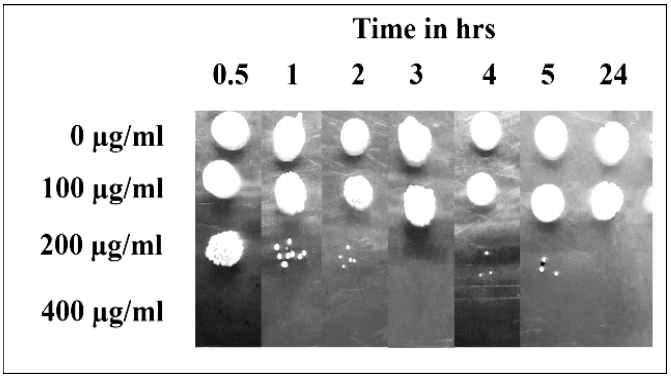

Fig. 5. Time kill assay of the carvacrol in wild type *C. glabrata* strain (CG).

Time Kills Assay

The fungicidal potential of carvacrol depends on the dosage and duration of the exposure. In YPD broth, carvacrol exhibited fungicidal activity at concentrations of 200 µg/mL and 400 µg/mL. Carvacrol has shown fungicidal activity at concentrations of 400 µg/mL for 0.5 h exposure and at 200 µg/mL for 2 h exposure (Fig. 5)

Checkerboard assay: Synergism of carvacrol and fluconazole against C. glabrata

The interaction between carvacrol and fluconazole was meticulously scrutinized through the checkerboard assay as detailed in the methodology. Effective concentrations of fluconazole (drug-A) and carvacrol (drug-B) ranged from 512 to 0.5 µg/mL and from 75 to 6.25 µg/mL respectively. The minimum inhibitory concentrations of drugs A and B were found to be 256 and 100 µg/mL respectively. Carvacrol showed a 16-fold reduction in the MIC_A when incorporated at concentrations starting from 12.5 µg/mL as shown in Table 3. This demonstrated a consistent and significant enhancement in the antifungal activity of carvacrol when combined with fluconazole.

Table 3. MIC, FIC and FIC index of carvacrol with fluconazole.

Strain	Drug	MIC_{50} (µg/mL)		FIC	FICI
		Alone	In combination		
C. glabrata Wild type	Fluconazole (A)	256	8	0.031	0.156
	Carvacrol (B)	100	12.5	0.125	

FIC- frictional inhibitory concentration, **FICI**- frictional inhibitory concentration index.

Carvacrol effectively reduced the CSH in clinical isolates

The ability of *C. glabrata* to survive and cause disease within a host is greatly influenced by the CSH, which is a crucial virulence characteristic. The major role of cell surface hydrophobicity is facilitating cell adhesion and biofilm formation, leading to drug resistance (19). The untreated clinical isolates have shown higher CSH than the wild type strain except for the CG1 isolate, as shown in Fig. 5. Upon treatment with MIC_{50} of carvacrol, all tested strains showed a significant reduction of CSH. Upon exposure to carvacrol, each one showed a reduction in the CSH values, but the reduced values of CSH for all isolates were higher than that of the reduced value of wild type strain (Fig. 6).

Carvacrol reduced ergosterol content in clinical isolates

Ergosterol is a critical component of fungal cell membranes and a primary target of azole drugs. Except CG2, all other untreated clinical isolates have shown ergosterol content lower than wild type strain of *C. glabrata* (Fig. 6). Reductions in ergosterol content when compared with that of wild type strain were 31 % in CG4, 23 % in CG3 and 16.5 % in CG1 (Fig. 6). Upon treatment with MIC_{50} of carvacrol, all tested strains showed a significant reduction in membrane ergosterol content in comparison to respective untreated control. CG4 showed the highest reduction of ergosterol content (61 %), while CG3 showed in the lowest ergosterol content (23 %), as shown in the Fig. 6.

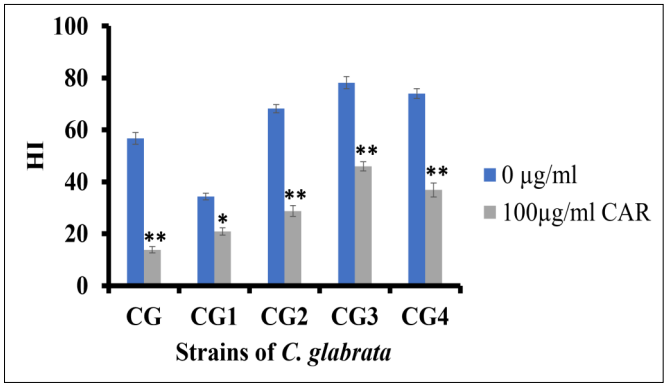

Fig. 6. Carvacrol significantly reduced the relative CSH of C. *glabrata* and their clinical isolates. The results represent mean ± standard deviation (**p<0.01 and *p<0.05).

Discussion

Carvacrol is well-documented in the scientific literature for its anti-*Candida* properties. Studies have shown that it can effectively inhibit the growth and biofilm formation of various *Candida* species, including *Candida albicans* and *Candida glabrata*. Its mechanism of action involves disrupting the fungal cell membrane and interfering with essential metabolic processes. This makes carvacrol a promising candidate for developing natural antifungal treatments, particularly in an era of increasing antifungal resistance (14, 15). Numerous studies have shown their effectiveness in inhibiting the growth of various fungal species. These compounds are found in essential oils such as oregano (*Origanum vulgare*) and thyme (*Thymus vulgaris*) and have been explored for their potential applications in both traditional and modern medicine as natural antifungal agents (20, 21). *Candida* infections can occur in different parts of the body, including the mouth, throat, gut, vagina and bloodstream. Azoles are a primary class of antifungal agents commonly used to treat most of the *Candida* infections. The development of resistance in *C. albicans* and NAC species for azole is an adaptation of many paths, such as mutations in the target enzyme, overexpression of

efflux pumps, and the genetic variability of *Candida* species (22, 23). Naturally active compounds show alternate anti-*Candida* potential in pursuit of antifungal therapeutic (24, 25). The present investigation reveals the effectiveness of carvacrol as a potent antifungal agent against VVC clinical isolates of a model NAC species, *C. glabrata*. The isolates used in the present study have shown varying levels of susceptibility to fluconazole, demonstrating the intricate nature of these fungal strains listed in Table 1 (16). The conventional treatment of VVC is occasionally restricted by limitations of emerging resistance to available drugs in NAC species, especially against *C. glabrata* (22, 26). VVC isolates have different levels of fluconazole sensitivity. CG3 is more resistant than other clinical isolates. In the present study, carvacrol is found to be more effective than fluconazole against clinical isolates of *C. glabrata* (Table 2). Also, carvacrol has shown promising synergistic potential with azole against NAC species (Table 3).

The monoterpenes have already been reported as an antifungal agent against *C. albicans* and *C. glabrata* (14, 15, 27). However, reliable evidence of their potential use in antifungal therapy is scarce. Our primary objective was to assess the efficacy of carvacrol against VVC clinical isolates of *C. glabrata*. As shown in Fig. 1, unlike fluconazole susceptibility (16), all isolates have shown similar MIC values for carvacrol, which were slightly higher than the MIC of wild type (MTCC3019). This shows multiple modes of action of the carvacrol other than targeting ergosterol synthesis. The MIC values of the carvacrol in this study are in the range of the MIC reported earlier (15). In this study, carvacrol is reported to be fungicidal in the range of 150-175 µg/mL for all isolates, including wild type (Table 2). Like fluconazole susceptibility data (Table 1), CG2 and CG3 have shown more tolerance to carvacrol in growth curve assay and fungicidal assay (CG3) (Fig. 3 and Table 2). In the rich media YPD, carvacrol exhibited fungicidal activity against *C. glabrata* in dose and time dependent manner (Fig. 5).

Furthermore, the interaction between carvacrol and fluconazole was investigated against wild strain using a checkerboard method. These findings suggest that carvacrol potentiates the activity of fluconazole remarkably in a synergistic manner with an FIC index of 0.156 (Table 3). Such an amazing synergistic activity gives a strong basis for developing combinatorial drug modules based on phytoactive and synthetic molecules. A similar kind of synergistic effect has been observed between an isomeric monoterpene (thymol) and fluconazole in a previous study (28).

There are some reports on the anti-*Candida* mode of action of carvacrol against members of *Candida* sp., which involves disrupting endoplasmic reticulum and inducing unfolded protein response inhibiting ergosterol biosynthesis and altering membrane potential, affecting membrane structure and permeability and inducing apoptosis through elevated cytoplasmic and mitochondrial Ca^{2+} levels (29-33). In most of the published reports, antifungal properties and mode of action of the carvacrol have been investigated in *C. albicans*, leaving a gap to study in non-albicans *Candida*. Therefore, we tried to analyze the mode of action of carvacrol in *C. glabrata*, a model NAC species.

The hydrophobic nature of the cell surface allows *Candida* cells to interact with host tissues and contribute to the establishment of infection (19). Carvacrol, an essential oil, significantly reduced cell surface hydrophobicity in *C. albicans* with an unknown mechanism (34, 35). We have shown in this study that carvacrol has reduced cell surface hydrophobicity in wild type as well as in clinical isolates of *C. glabrata* (Fig. 6). These findings underscore the potential of carvacrol as an antifungal agent that can effectively modulate the cell surface hydrophobicity to reduce adhesion and biofilm formation properties. Carvacrol has the potential to inhibit ergosterol biosynthesis and the disruption of membrane integrity (30). Carvacrol exposure to the wild type and VVC clinical isolates has resulted in a significant reduction of membrane ergosterol, strengthening the fact that carvacrol affects ergosterol biosynthesis (Fig. 7). This study unveils that carvacrol may be explored and developed further for topical application in case of vulvovaginal candidiasis.

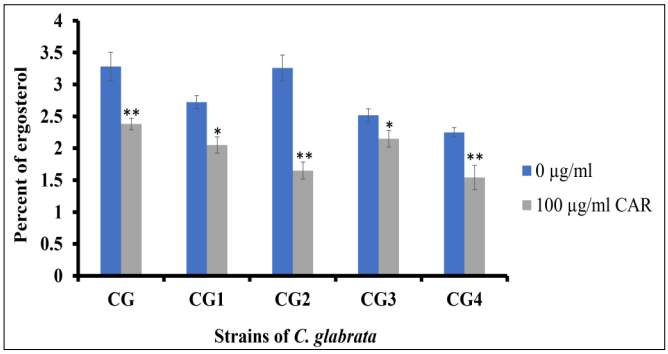

Fig. 7. Quantification of membrane ergosterol of *C. glabrata* and their clinical isolates upon exposure to 100 µg/mL carvacrol. **CG**-Wild type; **CG1**-**CG4**-Clinical isolates of VVC. The results represent mean ± standard deviation (**P<0.01 and *P<0.05).

Conclusion

Carvacrol, a phenolic monoterpene of plant origin, has been reported to have strong fungicidal potential against a model NAC species, *C. glabrata* and its clinical isolate from patients of vulvovaginal candidiasis. Carvacrol has the potential to reduce planktonic growth of the clinical isolates as well as wild type *C. glabrata* strains. The phytoactive compound behaved alike for clinical isolates irrespective of resistance or susceptibility of isolates to fluconazole. Carvacrol potentiated fluconazole in a synergistic manner to reduce its MIC 16-fold. Carvacrol exposure reduced cell surface hydrophobicity and membrane ergosterol in clinical isolates and wild type strains. This study suggests that a naturally occurring phytoactive compound, carvacrol, holds promise as a potential drug against non *albicans Candida* species.

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Authors' contributions

DK conducted all experimental work and drafted the manuscript with AA. NR and AG aided in formatting the manuscript and identifying suitable journal along with corrections in the manuscript. NK designed this study, and overall supervised experimentations and manuscript preparation. All authors have thoroughly reviewed and approved the final version of the manuscript.

Compliance with ethical standards

Conflict of interest: Authors have no conflict regarding the content and data of this manuscript.

Ethical issues: None.

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