International Journal of Research in Medical Sciences
Devika PS et al. Int J Res Med Sci. 2026 Mar;14(3):1021-1028
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012
DOI: https://dx.doi.org/10.18203/2320-6012.ijrms20260620

Original Research Article

Evaluation of the synergistic antifungal activity of *Elettaria cardamomum* extract in combination with Amphotericin B, Clotrimazole, and Ketoconazole against oral *Candida* species: an *in vitro* study

Devika P. S., Ierene Anna Sajan, S. Athulya, Harish Kumar K. S.*

Department of Medical Microbiology, School of Medical Education, Centre for Professional and Advanced Studies, Kottayam, Kerala, India

Received: 28 November 2025
Revised: 05 January 2026
Accepted: 13 January 2026

***Correspondence:**
Dr. Harish Kumar K. S.,
E-mail: drharishkumarks@gmail.com

ABSTRACT

Background: The growing incidence of antifungal resistance in *Candida* species presents a major clinical concern, highlighting the need for alternative therapeutic strategies. This study aimed to evaluate the synergistic potential of *Elettaria cardamomum* extract (ECE) in combination with conventional antifungals-amphotericin B, clotrimazole, and ketoconazole - against oral *Candida* isolates, to enhance antifungal efficacy.
Methods: A cross-sectional study was conducted from June 2024 to June 2025, including 161 clinical isolates of *Candida* species obtained from oral cavity. Identification was performed using standard mycological techniques. Antifungal susceptibility testing of ECE alone and in combination with standard drugs was carried out using the checkerboard microbroth dilution method. Fractional Inhibitory Concentration Index (FICI) were calculated to interpret the type of interaction - synergy, additive, indifference, or antagonism and statistical analyses compared susceptibility profiles between clinical isolates and control strain.
Results: ECE significantly potentiated the activity of azole antifungals, particularly ketoconazole, across all *Candida* species. The ketoconazole and ECE combination showed the highest synergy in *C. albicans*, with additive or synergistic interactions also observed in *C. tropicalis* and *P. Kudriavzevii*. Clotrimazole and ECE exhibited additive to moderate synergy, while amphotericin B and ECE combinations were largely indifferent or antagonistic. Clinical isolates exhibited higher MICs and lower synergy compared to control strains, indicating resistance trends.
Conclusions: ECE combined with imidazole antifungals demonstrated promising synergistic or additive activity, suggesting potential benefits such as reduced dosage, minimized toxicity, and delayed resistance. Further studies on phytochemical profiling, optimized formulations, and in vivo validation are warranted to support clinical application.

Keywords: Amphotericin B, Clotrimazole, Ketoconazole, *Elettaria cardamomum* extract, *Candida* species, Checkerboard microbroth dilution assay, FICI, Synergy, Additive, Indifference, Antagonism

INTRODUCTION

Candida species are unicellular, yeast like fungi and one of the most common commensals colonizing mucosal surfaces of human beings, that can transform into pathogenic forms under certain predisposing conditions, leading to infections collectively known as candidiasis. Among various species of this yeast, *Candida albicans* are more frequently isolated. Other species such as *Nakaseomyces glabratus* (formerly known as *Candida*

glabrata), *Candida tropicalis*, *Candida parapsilosis* complex, *Pichia kudriavzevii* (formerly *C. krusei*) and *Meyerozyma guilliermondii* (formerly *Candida guilliermondii*) are also being frequently isolated mainly from HIV infected individuals.[1] The treatment of candidiasis largely depends on a limited number of antifungal drugs such as polyenes, azoles, fluoropyrimidines and echinocandins. Among these, amphotericin B (polyene antifungal), has been considered as "gold standard" for severe invasive fungal infections, that act by binding to ergosterol in the fungal cell membrane, resulting in cell lysis and death.[2,3] Azoles particularly imidazoles such as clotrimazole and ketoconazole, act by inhibiting fungal enzyme lanosterol 14-α- demethylase, which is essential for the synthesis of ergosterol.[4] However, the use of these conventional antifungals are being limited due to its high toxicities and emerging drug resistance especially by Non-albicans *Candida* (NAC) isolates.

Developing novel antifungal agent is a resource-intensive process, hence combination therapy, involving the simultaneous administration of multiple antimicrobial agents, has emerged as a promising approach to enhance treatment efficacy.[5] Lately, there has been a surge in attention towards natural products as potential sources of new antimicrobial compounds or as addition to existing therapies.[6] Many plant derived compounds possess intrinsic antifungal properties and may show synergistic effects when combined with conventional antifungal thus overcoming the various resistance mechanism shown by the organism. Specifically, *Eletteria cardamomum* (small cardamom) extract, have demonstrated potential in augmenting antifungal activity against *Candida* species, due to their rich composition of bioactive compounds such α-terpinyl acetate.[7] The extract has anti-inflammatory, anti-cancer, anti-oxidant, gastroprotective activities along with antifungal activity because of which this extract was chosen to evaluate the synergistic potential in combination with antifungal agents – amphotericin B , clotrimazole and ketoconazole by checkerboard microbroth dilution assay.[8]

METHODS

The present cross-sectional study was conducted between June 2024 and June 2025, performed at School of Medical Education (SME), Kerala, India. During this period, a total of 161 oral *Candida* isolates retrieved from the culture collection of Department of Medical Microbiology, Gandhinagar, Kottayam, Kerala, India was included in the present study, while other *Candida* isolates were excluded. To ensure the accuracy of identification, gram staining was performed which revealed Gram positive budding yeast cells. This was then sub-cultured onto HiCromeTM Candida Differential Agar, for further confirmation and species level identification. *Elettaria cardamomum* extract (ECE) was supplied by Zum Heilen Health Care Private limited. Amphotericin B, ketoconazole and RPMI-1640 broth were purchased from HiMedia Laboratories, Mumbai, India. Clotrimazole was purchased from Tokyo Chemical Industry (India) Pvt Ltd. For standardisation of the test, *C. albicans* MTCC227, purchased from Institute of Microbial Technology (IMTECH), Chandigarh, India, was used.

Determination of antifungal activity

Dimethyl sulfoxide (DMSO) was used as the solvent for amphotericin B and ethanol for azoles due to their poor water solubility and RPMI-1640 broth as the diluent for subsequent dilutions ranging from 16µg/ml–0.016 µg/ml (amphotericin B), 256µg/ml–0.25µg/ml (clotrimazole), 128 µg/ml –0.125 µg/ml (ketoconazole), according to CLSI M27-A3 guidelines.9 Two-fold serial dilutions of ECE was prepared in RPMI-1640, with concentration ranging from 0.5µl/ml to 0.008 µl/ml The antifungal activity of amphotericin B, clotrimazole, ketoconazole and ECE were evaluated against the control strain *C. albicans* MTCC 227, using the microbroth dilution method. Inoculum was prepared following CLSI guidelines.[9] Equal amount of organism was added to each well and incubated at 35°C for 24-48 hours. After incubation the Minimum Inhibitory Concentration (MIC) was read visually as the lowest drug concentration that prevents any detectable growth.

Checkerboard assay

It is a two-dimensional broth microdilution method performed in a sterile 96 well U bottom microtitre plate. The procedure was done according to protocol described by Schwalbe et al with minor modifications.[10] Briefly, dilutions of ECE was made along the Y axis of the microtitre plate. Initially, 50 µl of RPMI-1640 broth was dispensed into all wells of rows B-H. Subsequently, 50 µl of ECE was added to wells B1–B12. Then two-fold serial dilutions of ECE were done vertically from wells B1–H1, B2–H2, and so on, up to B12–G12, to achieve a concentration range from 0.5 µl/ml to 0.008 µl/ml. Dilution of antifungal was done along the X-axis of the microtitre plate. 50 µl from the prepared two-fold dilution series, ranging from highest to lowest concentration were added to column 2–12, respectively, to make a horizontal two-fold dilution. 50 µl of inoculum was added to all wells except A1, which served as sterility control. The plate was then incubated for 24 -48 hours at 35°C. Now the wells in row A (A2–A12) contain dilution series of antifungal alone. And in the wells of column 1 (B1–H1) contain the dilution series of ECE alone. The wells B2–B12, C2–C12, so on up to H2–H11, contain the combination series. H12 served as growth control containing 50 µl of RPMI–1640 broth and 50 µl of organisms. Each well was visually examined for growth by comparing it to the growth control and confirming the absence of growth with the sterility control. MIC values of both antifungal and ECE alone and in combination was determined by identifying the well with least concentration of drug that showed no visible growth.

For each drug combination, the Fractional Inhibitory Concentration (FIC) of the agent was determined. The FIC for an agent in a given well was calculated by:

$$\text{FIC of Antifungal} = \frac{(\text{MIC of Antifungal in Combination})}{(\text{MIC of Antifungal Alone})}$$

$$\text{FIC of Extract} = \frac{(\text{MIC of Extract in Combination})}{(\text{MIC of Extract Alone})}$$

Fractional Inhibitory Concentration Index (FICI) of each well was calculated by adding the FIC values of both agents in that particular combination.

$$\text{FICI} = \text{FIC of Antifungal} + \text{FIC of Extract}$$

Based on the calculated FICI values, the results were interpreted as follows: synergy when FICI ≤0.5, additive when 0.5<FICI≤1, indifference: 1<FICI≤4, and antagonisim when FICI >4.

Statistical analysis

All data and graphs were processed using Microsoft Excel and appropriate statistical analysis were performed. The Study was approved by the Institutional Ethical Committee (IEC) at the School of Medical Education, Kerala, India.

RESULTS

A total of 161 oral Candida isolates belonging to C. albicans, comprising 53% isolates (n=85) followed by C. tropicalis with 30% isolates (n=48) and *P. kudriavzevii* with 14% isolates (n=22). Less frequently encountered species included *C. parapsilosis*, *C. lusitaniae*, and *M. guilliermondii*, each represented by 1% isolates, contributing (n=2 each) individually to the total distribution, as shown in Figure 1. As these three species were lesser in number, they were not included in the statistical analysis but included in the experimental study.

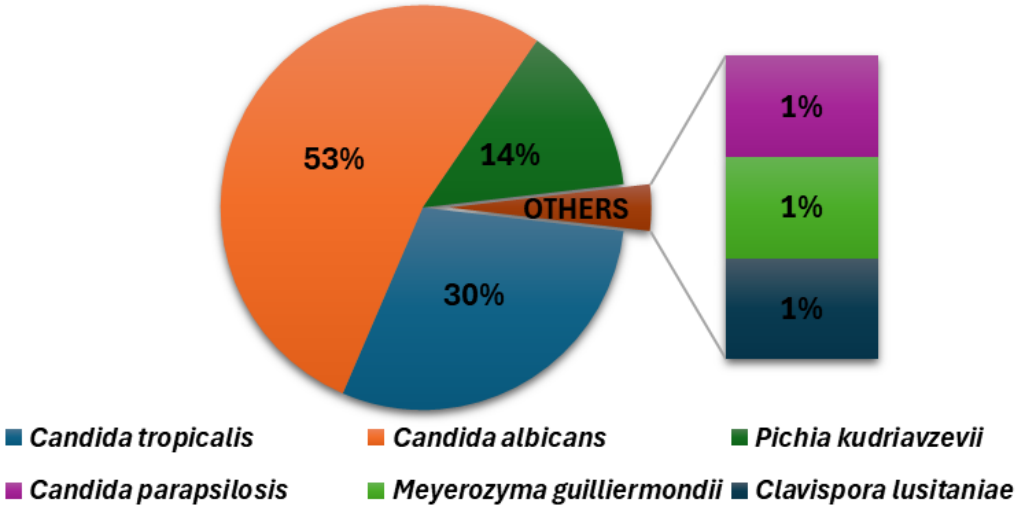

Figure 1: Percentage distribution of *Candida* species.

The MICs of selected drugs and ECE were evaluated against the control strain C. albicans MTCC 227. Amphotericin B (0.125 µg/ml) with ECE (0.125 µl/ml) produced an indifferent interaction (FICI=1.0625). Clotrimazole (16 µg/ml) and the ECE (0.125 µl/ml) showed an additive effect in combination (FICI=0.625). In contrast, ketoconazole (16 µg/ml) combined with the ECE (0.125 µl/ml) showed synergism (FICI=0.33), reducing. Overall, ECE enhanced azole activity, particularly that of ketoconazole against control strain.

Clinical isolates of *Candida* uniformly showed reduced susceptibility to all tested antifungals compared to the control strain, assessed by one sample t test indicating varying levels of resistance. While comparing activity of ketoconazole MIC against clinical isolates (mean 47.25 µg/ml) and control strain (16 µg/ml) showed an increased significant MIC (p<0.001), while ECE alone exhibited a lower MIC value of 0.1103 µl/ml and 0.125 µl/ml respectively. In combination, ECE's MIC (0.0253 µl/ml) was significantly higher than the test value 0.015 µl/ml (p=0.000), and the mean FICI (0.5) significantly greater than the benchmark value of 0.33 (p<0.001) indicated synergism, though weaker in clinical isolates. Similarly, clotrimazole showed elevated MICs (mean 42.9 µg/ml) compared to the control (16 µg/ml, p<0.001), which decreased significantly in combination (16.58 vs. 8 µg/ml, p<0.001). Although the ECE showed comparable activity across all strains, its mean MIC in combination against clinical isolates (0.03 µl/ml) was significantly higher than the control strain value of 0.016 µl/ml (p=0.002), reflecting that clinical isolates required nearly double the concentration compared to the control strain for equivalent inhibition. The mean FICI was 0.68 in clinical isolates, significantly higher than the control strain value of 0.625 (p=0.015) which reflected an additive interaction. In contrast, amphotericin B demonstrated reduced efficacy with the mean MIC of clinical isolates (0.44 µg/ml) being

statistically and significantly higher than the test value of 0.125 µg/ml ($p<0.001$). In combination therapy, the mean MIC of amphotericin B in clinical isolates (0.4 µg/ml) remained significantly higher than the test value of 0.125 µg/ml ($p<0.001$). The mean MIC of the extract against clinical isolates (0.07 µl/ml) was significantly lower than that of the control strain value of 0.125 µl/ml ($p<0.001$), suggesting that the extract maintained potent inhibitory activity against isolates without evidence of resistance. However, when tested in combination, the mean MIC in clinical isolates (0.05 µl/ml) was significantly higher than the test value of 0.0078 µl/ml ($p<0.001$), reflecting that clinical isolate required considerably more extract compared to the control strain for equivalent inhibition. The overall mean FICI of amphotericin B for clinical isolates was 2.15, significantly higher than the control strain value of 1.0625 ($p<0.001$), suggesting a shift from an indifferent profile in control strain to an antagonistic interaction in clinical isolates.

When the combined activity was evaluated using the χ^2 square test, significant differences were observed in the interaction profile between a primary antifungal (amphotericin B, clotrimazole or ketoconazole) and extract among the three species studied. For *C. albicans*, ketoconazole produced the most favourable outcome (62.4% synergy, 32.9% additive, 4.7% indifference, 0% antagonism with 95% net positive), making ketoconazole the most promising agent for enhancing activity, while clotrimazole combinations yielded a strong positive profile (60.0% additive, 21.2% synergy, 18.8% indifference, 0% antagonism with 81% net positive interactions) ;in contrast amphotericin B combinations were dominated by indifference (55.3%) with a substantial antagonism (18.8%), indicating little benefit and negative effect from combination with the extract; A similar pattern holds for *C. tropicalis*: ketoconazole again showed substantial synergy (58.3%) with further additive effects (29.2%), clotrimazole gave a very high additive benefit (79.2%) with additional synergy (12.5%), amphotericin B combinations were mostly indifference (66.7%) with minor antagonism (4.2%) therefore, clotrimazole and ketoconazole combinations both provide meaningful gains while amphotericin B does not. For *P. kudriavzevii*, ketoconazole dominated with a high true synergy rate (72.7%) and minimal antagonism, and clotrimazole produced a balanced response (36% additive, 27% synergy, 36% indifference; 63.7% net positive), whereas amphotericin B was again largely ineffective in combination (72.7% indifference), making it clearly the most effective drug against this species (Table 1) (Figure 2).

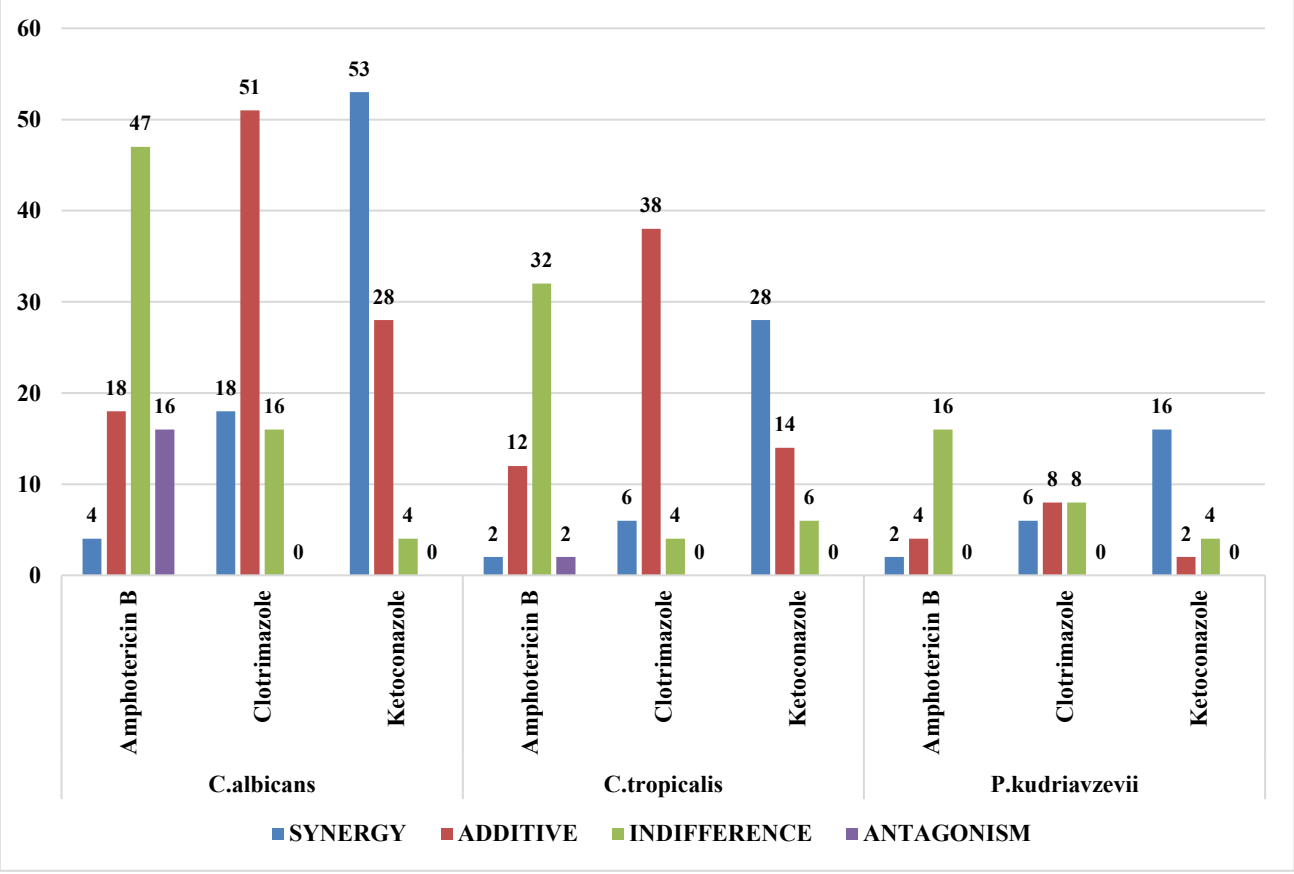

Figure 2: Comparison of antifungals/ECE activity against three yeast species.

Table 1: Checkerboard assay for the determination of combined activity for antifungals with ECE.

Organisms	Antifungals	FICI interpretation			
		Synergy (%)	Additive (%)	Indifference (%)	Antagonism (%)
C. albicans	Amphotericin B	4 (4.70)	18 (21.20)	47 (55.30)	16 (18.80%)
	Clotrimazole	18 (21.20)	51 (60)	16 (18.80)	0
	Ketoconazole	53 (62.40)	28 (32.90)	4 (4.70)	0
C. tropicalis	Amphotericin B	2 (4.20)	12 (25.00)	32 (66.70)	2 (4.20%)
	Clotrimazole	6 (12.50)	38 (79.20)	4 (8.30)	0
	Ketoconazole	28 (58.30)	14 (29.20)	6 (12.50)	0
P. kudriavzevii	Amphotericin B	2 (9.10)	4 (18.20)	16 (72.70)	0
	Clotrimazole	6 (27.30)	8 (36.40)	8 (36.40)	0
	Ketoconazole	16 (72.70)	2 (9.10)	4 (18.20)	0

In other species, *C. lusitaniae* (n=2) and *M. guilliermondii* (n=2) demonstrated synergistic effects while *C. parapsilosis* (n=2) showed additive effects for both tested strains with ketoconazole. In case of clotrimazole, additive interactions were consistently observed across all species, while amphotericin B combinations yielded indifferent results.

Overall, ketoconazole-based combinations consistently produced the highest proportions of true synergy across species, clotrimazole yielded substantial additive/synergistic benefit (particularly for *C. tropicalis* and *C. albicans*), and amphotericin B combinations were the least advantageous and in some cases antagonistic; these findings support prioritizing azole (especially ketoconazole) combination strategies over amphotericin B for enhanced antifungal effect, while cautioning against combinations with amphotericin B without further targeted evaluation.

DISCUSSION

The increasing problem of antifungal resistance presents a major challenge in managing candidiasis, particularly affecting immuno-compromised patients, where invasive candidiasis often leads to severe complications or death. Treatment options remain limited, with azoles such as ketoconazole, clotrimazole and polyenes like amphotericin B being widely used. However, increasing resistance to these antifungals has raised concerns about treatment failures and long-term efficacy. These limitations highlight the need for an alternative therapy that enhance antifungal activity, reduce drug dosage, and delay resistance development. Plant-derived phytochemicals have gained interest because of their broad bioactivity, accessibility, and lower toxicity. ECE, known for its antimicrobial and medicinal properties, was evaluated in this study for its antifungal potential in combination with antifungals against clinical candida isolates.

A study undertaken by Prabhakaran et al, reported that ethanolic and acetonic extract of *E. cardamomum* completely inhibited biofilm formation by multidrug resistant *C. albicans*, at concentrations of 200 µl and 125 µl respectively.[11] Similarly, Noumi et al showed that *E. cardamomum* essential oil exhibited stronger antifungal property than itraconazole, with MIC values ranging from 0.097 to 0.78 mg/ml and produced significant antibiofilm and anti-exoenzyme effects, achieving up to 99% biofilm inhibition in *C. albicans*.[12] These studies collectively emphasize that cardamom-derived phytochemicals, particularly α-terpinyl acetate and 1,8-cineole, possess intrinsic antifungal and anti-virulence activities, with their potential extending beyond simple growth inhibition to interference with virulence mechanisms, thereby supporting the applicability of *E. cardamomum* in combination therapies. The present investigation confirmed this activity, with ECE exhibiting consistent inhibitory effects across different *Candida* species, suggesting consistent inhibitory potential without evidence of resistance. Furthermore, Tiwari et al confirmed the antifungal activity of cardamom oil against *C. albicans*, reporting MIC values between 64–128 µg/ml, thus providing additional evidence of its direct inhibitory effect on fungal growth.[7] In contrast, the present study additionally demonstrated that ECE exhibited clear synergistic activity when combined with ketoconazole, reinforcing recent reports that emphasize the enhanced antifungal efficacy of ECE when used in combination with azole antifungal agents.

The present study is a novel investigation to best of our knowledge; hence we have compared similar studies using other extract in traditional medicine. An Investigation by Feng et al evaluated the antifungal potential of four Traditional Chinese Medicine (TCM) extracts against *C. albicans* and explored their synergistic interactions with the conventional antifungal agent clotrimazole.[13] Clotrimazole showed a potent individual MIC of 1.25 µg/ml among the extracts tested, *Coptis chinensis* emerged as the most potent, with MIC and MFC values of 30 mg/ml and 60 mg/ml respectively and achieving an inhibition rate of 99.6% at its highest concentration. Notably, *C. chinensis* exhibited strong synergy with clotrimazole, with a fractional inhibitory concentration index (FICI) of 0.25. In addition to this synergy, several additive interactions were reported. For example, both *Oroxylum indicum* and *Verbena officinalis* demonstrated additive effects when combined with *C. chinensis* (FICI=0.56 for both). Additive interactions were also observed in specific triple

combinations, including *Verbena officinalis*, *C. chinensis* and clotrimazole (FICI=0.56) and *C. chinensis*, eugenol and clotrimazole (FIC=0.53). Even the positive control combination of clotrimazole and eugenol yielded only a weak additive effect (FICI=1.0). These findings underscore that while strong synergy is achievable, additive interactions remain valuable, particularly considering *V. officinalis*'s longer-lasting antifungal activity against *C. albicans*, highlighting the potential of these combinations as promising adjuvant therapies. The present study similarly demonstrated that the combination of clotrimazole and ECE exerted a significant additive antifungal effect against a broad spectrum of *Candida* species isolated from oral samples. For the control strain, *C. albicans* MTCC 227, the combination produced a FICI of 0.625, indicating an additive effect. Across all clinical isolates, the overall mean FICI was 0.68, also within the additive range (0.5-1.0). Of these, 18.6% exhibited synergy, 64% showed additivity, and 17.4% demonstrated indifference, with *C. tropicalis* isolates showing a pronounced skew toward additivity (79.2%). Importantly, clinical isolates presented significantly higher MICs for clotrimazole alone (e.g., *C. albicans* 42.92 µg/ml vs. 16 µg/ml in the control strain), indicating reduced susceptibility or potential resistance in clinical settings. Furthermore, the mean FICI in clinical isolates (0.68) was significantly higher than that observed in the control strain (p=0.015), suggesting diminished but still additive responsiveness to the combination in real-world strains.

The study done by Wagle et al on-areca nut extract combined with ketoconazole demonstrated a synergistic antifungal effect against *C. albicans*, where the bioactive compounds in areca nut, such as phenolics, saponins, and alkaloids, enhanced the fungicidal activity of ketoconazole, improving its effectiveness *in vitro*.[14] In comparison, the present study similarly demonstrated synergistic antifungal activity between ketoconazole and ECE against *Candida* species, indicating that phytochemicals derived from diverse plant sources can potentiate the action of azole antifungals, possibly through complementary mechanisms involving disruption of fungal cell integrity or interference with metabolic pathways. These synergistic interactions substantiated by checkerboard analysis (FICI≤0.5), which revealed a marked reduction in ketoconazole MIC values when used in combination with ECE. Ketoconazole alone exhibited higher mean MIC values in clinical isolates (47.25 µg/ml) compared with the control strain (16 µg/ml), reflecting reduced susceptibility. However, upon combination with cardamom extract, the mean MIC of ketoconazole decreased significantly in both clinical isolates (14.05 µg/ml) and the control strain (4 µg/ml), with the most pronounced effect observed in *C. albicans*, where MIC values were reduced nearly fourfold. These findings reinforce the potential of diverse plant extracts, including areca nut and cardamom, as effective adjuvants to azole antifungals in combating *Candida* infections via synergistic interactions that might lower required drug doses.

Previous research has demonstrated synergistic effects between amphotericin B, and other plant derived compounds. For example, Wan Himratul-Aznita et al.,15 reported strong synergistic effects when combined amphotericin B with hydroxychavicol derived from Piper betle. The combination, especially at 1:1 or1:2 ratio produced a pronounced synergistic effect against *C. albicans*, with FICI as low as 0.07. In contrast to this report of strong synergy, our study revealed a more complex interaction profile. The interaction between amphotericin B and ECE generally showed indifference, with a mean FICI of 2.15 in clinical isolates, while the control strain demonstrated indifference bordering on additivity (FICI 1.06). This highlights the fact that not all plant extract-drug combination may lead to enhanced antifungal effects This specificity of interaction is further illustrated in the study conducted by El-Ahmady et al where thyme oil and cinnamon oil markedly potentiated the activity of amphotericin B against *C. albicans*, leading to a four-fold reduction in the MIC, while other oils such as clove and eucalyptus demonstrated indifferent interaction.[16] These observations reinforce that the synergistic potential of plant-derived compounds with amphotericin B is selective rather than universal, depending on the phytochemical composition and the tested organism. Despite this overall trend of indifference, a deeper analysis of species-specific outcomes revealed clinically meaningful benefits that strengthen the present study. In *C. albicans*, 21.2% of isolates exhibited additivity and 4.7% synergy, and in *P. kudriavzevii* the combination improved antifungal activity, with 18.2% additivity and 9.1% synergy. For *C. tropicalis*, although indifference predominated (66.7%), a statistically significant reduction in MIC of amphotericin B (0.63 to 0.46 µg/ml) was observed in combination with ECE. These outcomes align with reports of Soulaimani et al on other essential oils combined with amphotericin B, where EOs of *Thymus leptobotrys*, *Origanum compactum*, and *Artemisia herba alba* produced clear synergy against *C. krusei* and additive effects against *C. albicans*, *C. glabrata*, and *C. parapsilosis*, while other oils were largely indifferent.[17] Collectively, the evidence highlights that the success of natural product–amphotericin B combinations is highly species-dependent and determined by phytochemical composition, underscoring the need for targeted evaluation of plant derived compounds alongside conventional antifungals. Our finding of species-specific activity reinforces this perspective, marking an important step forward by shifting the focus from a broad-spectrum approach to a more precise and clinically relevant strategy, where tailored combinations can be designed for particular pathogens to enhance therapeutic outcomes in resistant *Candida* infections.

Limitations of the present study included constraints associated with the in vitro checkerboard microdilution assay design, which could not assess fungicidal activity, assumed a linear dose–response relationship, and provided only a static view of antimicrobial interactions, potentially limiting its relevance to in vivo outcomes. In addition, formulation related issues such as reduced solubility or

suboptimal dispersion of amphotericin B within the extract matrix could further limit the effective interaction between the two agents. Moreover, the absence of established CLSI or EUCAST breakpoints for clotrimazole complicates the direct clinical interpretation of MIC values. The variability observed among different Candida species and the reduced responsiveness of clinical isolates compared with the reference strain further suggest that the combination's effectiveness may not be uniform across all species. Furthermore, the use of crude extract represents another limitation, as it is a complex mixture of phytochemicals some of which may have antifungal properties while others may be inert or slightly antagonistic, making the observed FICI as a net outcome of competing interactions, these complexity highlights the need for bioassay-guided fractionation to isolate and identify the specific phytochemicals responsible for the extract's antifungal activity.

CONCLUSION

The combination of ECE with conventional antifungal agents demonstrated promising yet variable enhancement of antifungal efficacy. Notably, the amphotericin B and ECE combinations were generally indifferent, exhibiting only species-specific additive or synergistic effects. In contrast, combinations with azole antifungals produced more consistent and pronounced improvements. Clotrimazole in conjunction with the extract resulted in significant reductions in MIC values, exhibiting additive to synergistic interactions, while ketoconazole combined with the extract consistently restored and enhanced antifungal activity (FICI≤0.5), including against clinical isolates with reduced susceptibility, with *C. albicans* showing the strongest response. These findings suggest that the phytochemical constituents of *E. cardamomum*, such as terpenes and flavonoids, may potentiate the action of membrane and ergosterol targeting antifungal agents, enabling dose minimization, reduced toxicity, and delayed resistance development in specific fungal species. However, the observed variability underscores that the effectiveness of such combinations is dependent on both the fungal species and the specific drug herbal interaction. Consequently, future investigations should employ bioassay guided fractionation to isolate and characterize the active principles, optimize formulation strategies to enhance drug herbal compatibility, and validate these outcomes through biofilm studies, dynamic in vivo experiments, and clinical evaluations to develop standardized, efficacious, and safe integrative antifungal therapies.

ACKNOWLEDGEMENTS

Authors would like to thank Dr. Prashanth Varkey of Zum Heilen Health Care Private limited for providing Elettaria cardamomum extract and Mrs. Rajumol B. Zacharia for her technical assistance.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. de Oliveira Santos GC, Vasconcelos CC, Lopes AJO, de Sousa Cartágenes MDS, Filho AKDB, do Nascimento FRF, et al. Candida infections and therapeutic strategies: Mechanisms of action for traditional and alternative agents. Front Microbiol. 2018;9:1351.
2. Grela E, Zdybicka-Barabas A, Pawlikowska-Pawlega B, Cytryńska M. Modes of the antibiotic activity of amphotericin B against Candida albicans. Sci Rep. 2019;9:17029.
3. Hamill RJ. Amphotericin B formulations: A comparative review of efficacy and toxicity. Drugs. 2013;73(9):919–34.
4. Van Tyle JH. Ketoconazole. Mechanism of action, spectrum of activity, pharmacokinetics, drug interactions, adverse reactions and therapeutic use. Pharmacotherapy. 1984;4(6):343-73.
5. Li Y, Jiao P, Li Y, Gong Y, Chen X, Sun S. The synergistic antifungal effect and potential mechanism of D-penicillamine combined with fluconazole against Candida albicans. Front Microbiol. 2019;10:2853.
6. Newman DJ, Cragg GM. Natural Products as Sources of New Drugs over the Nearly Four Decades from 01/1981 to 09/2019. J Nat Prod. 2020;83(3):770-803.
7. Tiwari N, Sivakumar A. Synergistic antifungal potential of fluconazole-based cardamom oil oral microemulsion formulation against C. albicans. J Res Pharm. 2022;26(5):1065-83.
8. Ashokkumar K, Murugan M, Dhanya MK, Warkentin TD. Botany, traditional uses, phytochemistry and biological activities of cardamom [Elettaria cardamomum (L.) Maton] - A critical review. J Ethnopharmacol. 2020;246:112244.
9. Clinical and Laboratory Standards Institute (CLSI). Reference method for broth dilution antifungal susceptibility testing of yeasts. 4th Ed. CLSI standard M27. Wayne, PA: CLSI; 2017.
10. Schwalbe, R., Steele-Moore, L., & Goodwin, A.C. (Eds). Antimicrobial Susceptibility Testing Protocols (1st ed.). CRC Press; 2007.
11. Vijayalakshmi P, Thenmozhi S, Rajeswari P. The Evaluation of the virulence factors of clinical Candida isolates and the anti-biofilm activity of Elettaria cardamomum against multi-drug resistant Candida albicans. Curr Med Mycol. 2016;2(2):8-15.
12. Noumi E, Snoussi M, Alreshidi MM, Rekha PD, Saptami K, Caputo L, et al. Chemical and Biological Evaluation of Essential Oils from Cardamom Species. Molecules. 2018;23(11):2818.
13. Feng WY, Cheang UI, Wong KI, Cheong HI, Meng LR, Fong P. Synergistic activity of Coptis Chinensis and clotrimazole against Candida Albicans.

Pharmacological Research - Modern Chinese Medicine. 2023;8:100287.
14. Wagle MW, Lobo KN, Rajath KR, Chandrashekar A, Subrahmanyam VM. A Combination approach using Areca nut and Ketoconazole for Treating Cutaneous Candidiasis. Res J Pharm Tech. 2021;14(6):3074-6.
15. Himratul-Aznita WH, Nor-Zulaila CO, Nurul-Fatihah K. Antifungal activity of dual combination of hydroxychavicol with commercialized agents against oral Candida species. Springerplus. 2016;5(1):1696.
16. Sherweit El-Ahmady, Mohamed El-Shazly, Rola Milad. The Synergetic Efficacy of the Combination of Amphotericin B and Certain Essential Oils against Selected Fungal Clinical Isolates. J App Pharm Sci. 2013;3(04):26-30.
17. Soulaimani B, Varoni E, Iriti M, Mezrioui NE, Hassani L, Abbad A. Synergistic Anticandidal Effects of Six Essential Oils in Combination with Fluconazole or Amphotericin B against Four Clinically Isolated Candida Strains. Antibiotics. 2021;10:1049.

Cite this article as: Devika PS, Sajan IA, Athulya S, Harish Kumar KS. Evaluation of the synergistic antifungal activity of *Elettaria cardamomum* extract in combination with Amphotericin B, Clotrimazole, and Ketoconazole against oral *Candida* species: an *in vitro* study. Int J Res Med Sci 2026;14:1021-8