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Research article

Anticandidal Activities of Selected Thai Plant Extracts and Essential Oils Against Oral Candidiasis *Candida* spp. Isolates

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Abstract: The antifungal properties of 13 ethanol plant extracts (PE) and 6 essential oils (EO) of Thai traditional herbs were screened for their anti-*Candida* activity against a standard strain of *Candida albicans* ATCC 10231 using agar disk diffusion and broth microdilution methods. Two PE, *Piper betle* and *Alpinia galanga* extracts, showed the lowest MIC and MFC values against *C. albicans* of 0.313 mg/mL and 0.625 mg/mL, and three EO; cinnamon bark oil exhibited the lowest MIC and MFC value of 0.039 mg/mL, followed by lemongrass oil and clove bud oil. The main compounds of these EOs and PEs were identified using gas chromatography-mass spectrometry (GC-MS). The major compounds were geranial (42.7%) and neral (22.2%) in lemongrass oil, eugenol (85.5%) in clove bud oil, cinnamaldehyde (80.6%) in cinnamon bark oil, 4-allyl-1,2-diacetoxybenzene (29.5%) and hydroxychavicol (24.8%) in *P. betle* extract, and 1'-acetoxychavical acetate (78.0%) in *A. galanga* extract. All EO and PE showed antifungal activity against three oral candidiasis isolates, including *C. albicans* R01, *C. krusei*, and *C. dubliniensis*, with MIC/MFC ranging from 0.156 – 5.000 mg/mL. The checkerboard dilution method revealed that the combination of EO and PE showed an additive effect on *C. albicans* R01. In conclusion, the combination of EO with PE lowered the MIC of each agent, which could lead to decreased side effects; hence, this combination could be a promising treatment alternative for oral candidiasis.

Keywords: Antifungal activities; Plant extracts; Essential oils; *Candida* spp.; Oral candidiasis

1. Introduction

Candida species are common colonizers of the oral cavity but can also act as opportunistic pathogens, leading to oral fungal infections, also known as oral candidiasis. This infection is prevalent in immunocompromised individuals, the elderly, and individuals who wear dentures. While *Candida albicans* is the primary cause, non-albicans *Candida* (NAC) species such as *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, *C. dubliniensis*, and *C. krusei* are also frequently identified [1-3]. Some commercially available antifungal agents are used to treat oral candidiasis; however, their applications are limited by side effects such as oral irritation, burning, and discoloration, as well as drug interactions and the development of resistance over prolonged use. [4]. Thus, these limitations lead to a search for novel antifungal agents with low side effects.

Interest in herbal medicines is growing, particularly in countries with rich ethnomedical traditions, such as Thailand. Natural products, rich in bioactive compounds, offer a promising alternative to synthetic drugs due to their diverse therapeutic properties and generally low side effect profiles. Several studies documented antifungal activities of plant extracts and essential oils. For instance, extracts from various plant parts (rhizome, root, leaves, stem, and flower) of species including *Piper betle*, *Punica granatum*, *Curcuma longa*, and *Alpinia galanga*, have been used in traditional medicine for bacterial and fungal infections and have demonstrated anticandidal activity [5-8]. The antifungal activity is attributed to phytochemicals, including flavonoids, saponins, and alkaloids, found in ethanolic extracts. Among the natural products, essential oils, such as lemongrass, clove, cinnamon, peppermint, and eucalyptus, are commonly used in complementary medicine to treat oral infections [9, 10]. However, there is limited knowledge about the activity of these plant extracts against oral candidiasis isolates of *C. albicans* and NAC, which are resistant to the major classes of antifungal drugs (polyenes and azoles) [3, 11]. Additionally, few studies have explored the combination of plant extracts and essential oils against *Candida* isolates. Therefore, effective combinations of these natural products could provide new antifungal agents for treating oral candidiasis.

This study aimed to investigate the anti-*Candida* activity of 13 ethanol extracts and 6 essential oils from Thai herbs. The most effective extracts and oils were tested against three oral candidiasis isolates, with their major compounds identified to explore potential anticandidal mechanisms. The synergistic antifungal effects of combining these extracts and oils were evaluated.

2. Materials and Methods

2.1 Preparation of plant extracts

Thirteen Thai traditional plants were collected from local areas in Phitsanulok, Thailand (Table 1). The samples were washed, dried at 60 °C for 24 hours, and then ground into a fine powder. They were extracted by maceration with 95% ethanol at room temperature for 3 days, repeated three times. The extracts were filtered and concentrated using a rotary evaporator (Buchi, Switzerland). A stock solution of plant extracts in dimethyl sulfoxide (DMSO, RCI Labscan, Thailand) was prepared and diluted with a solution of 4% DMSO and 4% Tween 80 (Phitsanu Chemical, Thailand) to a concentration of 10 mg/mL for anticandidal activity tests.

2.2 Preparation of essential oils

Essential oils, including lemongrass (product code; 2562-40003), clove bud (product code; 2562-20216), cinnamon bark (product code; 2562-20104), cinnamon leaf (product code; 2562-20086), peppermint (product code; 2562-20103), and eucalyptus (product code; 2562-20014), were purchased from Thai China Flavours & Fragrances Industry Co., Thailand. To test their antifungal activity, the essential oils were diluted in a solution of 4% DMSO and 4% Tween 80 to a concentration of 20 mg/mL.

2.3 Anticandidal activity

2.3.1 Strains and Culture Conditions

C. albicans ATCC10231 is obtained from the Faculty of Medical Sciences, Naresuan University. At the same time, clinical isolates of C. albicans R01, C. krusei, and C. dubliniensis were collected from the Faculty of Dentistry at Naresuan University. All strains were preserved on Sabouraud Dextrose Agar (SDA, Himedia, India) slants and subcultured onto SDA plates at 37 °C for 24 hours before testing.

2.3.2 Agar disk diffusion method

The plant extracts (PE) and essential oils (EO) were evaluated for their ability to inhibit *C. albicans* ATCC 10231 growth using a modified agar disk diffusion assay (Clinical and Laboratory Standards Institute (CLSI) M44-A2 protocol). A yeast suspension (1-5 × 10^6 cells/mL) was spread onto Mueller-Hinton agar containing 2% glucose and 0.5 μg/mL methylene blue dye. Paper disks, 6 mm, impregnated with the extracts or oils, were placed on agar surfaces, and their antifungal activity was assessed by measuring the size of the clear zones around the disc after incubation at 37 °C for 24 hours [12].

2.3.3 Determination of minimum inhibitory concentrations (MIC) and minimum fungicidal concentration (MFC)

The MIC against was determined by the broth dilution method following the method by Rodríguez-Tudela *et al*. (2001) [13]. The samples were serially diluted two-fold with RPMI-1640 medium (GibcoTM, Austria) in microtiter plates to concentrations ranging from 0.020 to 5.00 mg/mL. *C. albicans* ATCC 10231 suspension (1 x 10^6 cells/mL) was added to each well. Various concentrations of nystatin, ranging from 1.0 to 250 µg/mL, and Candida in RPMI-1640 medium were designated as the positive control and control, respectively. The plates were incubated at 37 °C for 24 hours. *Candida* growth was measured by optical density at 600 nm. The MIC was the lowest concentration that provided 90% growth inhibition. The percentage of growth inhibition was calculated using the following equation: %Inhibition = [1- (A_{t24} – A_{t0})/Ac_{t24} – Ac_{t0})] x 100, where A_{t24} and A_{t0} are the OD of the test well at 24 hours and 0 hours. Ac_{t24}, $A_{t0,}$ and Ac_{t0} are the OD of the control healthy growth at 24 hours and 0 hours. The MFC was evaluated by incubating turbidity-free wells on SDA plates at 37 °C for 24 hours. The lowest concentration preventing visible growth was determined. All experiments were conducted in triplicate [14]. The plant samples with large, clear zones and low MIC and MFC values were selected for further testing of anticandidal activity against clinical isolates of *C. albicans* R01 and NAC species (*C. krusei* and *C. dubliniensis*) (Table 3).

2.4 Identification of major compounds using GC-MS analysis

The component identification of PE and EO was achieved by the GC-MS analysis using HP-5MS series (Agilent, USA), with a capillary column (30 m × 250 µm, film thickness 0.25 µm). The column temperature was maintained at 50°C for 2 minutes. The column temperature was initially set at 70 °C for 5 min, then increased to 120 °C at a rate of 3 °C/min, followed by an additional 5 °C/min to 270 °C, and maintained at this temperature for 3 min. A 1 µL manual injection was performed in a split mode (1:100), with helium as the carrier gas at a flow rate of 1 mL/min. The scan range is 35–550 m/z with a scan rate of 1000 amu/s. The components were identified by comparing their mass spectra with published data and performing computer matching with the National Institute of Standards and Technology (NIST17.L) library.

2.5 Determination of synergistic activity from a combination of essential oils and plant extracts

The synergistic activities of selected EO and PE combinations were determined using the checkerboard dilution assay [15]. The respective MIC values of EO and PE were used together to define a fractional inhibitory concentration (FIC) of 1. The dilutions of each agent's FIC were prepared in a series of 0xMIC, 1.25xMIC, 0.25xMIC, 0.50xMIC, 0.75xMIC, and 1xMIC in a 96 well-plate. *C. albicans* R01 was added to the combinations and incubated at 37 °C for 24 hours. The MIC values were determined as described above. The FIC index (FICI) was determined according to the following equation [16]:

FIC_{EO} = MIC of EO in combination / MIC of EO alone

FIC_{PE} = MIC of PE in combination / MIC of PE alone

FICI = FIC_{EO} + FIC_{PE}

The results are interpreted as follows: FICI ≤ 0.5, synergistic; > 0.5 – 1.0, additive; > 1.0 – 4.0, indifference; > 4.0, antagonistic.

2.6 Statistical analysis

The extraction of plants was repeated three times, and the mean % yield values were calculated. All microbiological tests were repeated on three different occasions, with triplicate determinations on each occasion.

3. Results and Discussion

C. albicans is a common cause of oral fungal infections, especially in people with weakened immune systems. The overuse of antifungal agents has led to drug-resistant strains [17]. This study aims to identify natural, plant-based alternatives that can combat C. albicans, potentially through a synergistic combination. The selection of plant extracts for this study was based on their established antifungal potential and traditional use in treating fungal infections. Numerous studies have demonstrated the anticandidal activity of plants against various *Candida* species [17-19]. Ethanol was chosen as the extraction solvent due to its efficacy in extracting bioactive compounds, coupled with its non-toxic and biodegradable nature [20]. Commercial

essential oils were employed in this study to assess their feasibility for the large-scale production of potential products. The company provided certificates of analysis for each essential oil, confirming that each essential oil meets its specified characteristics.

Table 1. Anticandidal activity of plant extracts against *C. albicans* ATCC10231

Family	Scientific name	Part used	% yield (dry weight)	Inhibition zone diameter (mm)*	MIC (mg/mL)	MFC (mg/mL)
Euphorbiaceae	*Euphorbia hirta* L.	Leaf	17.30 ± 2.13	15.33 ± 2.52	5.000	> 5.000
	Baliospermum solanifolium (Burm.) Suresh	Leaf	14.62 ± 0.66	nz	> 5.000	> 5.000
	Acalypha indica L.	Leaf	15.74 ± 1.32	nz	> 5.000	> 5.000
Piperaceae	*Piper betle* L.	Leaf	20.65 ± 2.06	24.67 ± 0.58	0.313	0.625
	Piper sarmentosum Roxb	Leaf	11.63 ± 0.91	8.67 ± 0.58	0.625	> 5.000
Fabaceae	*Leucaena leucocephala* (Lam.) de Wit	Leaf	16.23 ± 0.50	nz	> 5.000	> 5.000
	Tamarindus indica L.	Seed coat	32.57 ± 2.67	nz	> 5.000	> 5.000
Zingiberaceae	*Curcuma longa* L.	Rhizome	27.20 ± 3.99	8.67 ± 1.15	> 5.000	> 5.000
	Curcuma manga Val.& Zijp	Rhizome	10.68 ± 0.50	Nz	> 5.000	> 5.000
	Alpinia galanga (L.) Willd	Rhizome	18.81 ± 1.98	9.33 ± 1.15	1.250	1.250
	Boesenbergia rotunda (L.) Mansf.	Rhizome	8.24 ± 0.26	8.33 ± 0.58	> 5.000	> 5.000
	Curcuma zedoaria (Christm.) Roscoe	Rhizome	11.35 ± 1.19	8.33 ± 0.58	> 5.000	> 5.000
Bignoniaceae	*Dolichandrone serrulata* (Wall. ex DC.) Seem.	Leaf	26.25 ± 1.03	nz	> 5.000	> 5.000
	Nystatin			17.00 ± 2.65	0.003	0.003

Data are reported as Mean ± SD; nz = No inhibition zone; * = 6 mm paper disk; MIC = Minimum inhibitory concentration; MFC = Minimum fungicidal concentration

3.1 Anticandidal activity of plant extracts and essential oils

Table 1 shows that ethanolic extracts from seven of thirteen plants had significant anticandidal activity. *Piper betle* exhibited the largest inhibition zone (~25 mm), followed by *Euphorbia hirta*, *Alpinia galanga*, *Curcuma longa*, *Boesenbergia rotunda*, and *Curcuma zedoaria*. *P. betle* was most active against *C. albicans* ATCC10231, with MIC and MFC values of 0.313 mg/mL and 0.625 mg/mL, respectively.

Table 2 shows the inhibitory zone, MIC, and MFC of six EOs against *C. albicans* ATCC10231. All EOs demonstrated antifungal activity, with MIC and MFC values ranging from 0.078 to 2.50 mg/mL and 0.078 to 5.00 mg/mL, respectively. Cinnamon bark oil had the lowest MIC and MFC (0.039 mg/mL), followed by lemongrass, clove bud, cinnamon leaf, peppermint, and eucalyptus oils.

Table 2. Anticandidal activity of essential oils against *C. albicans* ATCC10231

Essential oils	Inhibition zone diameter (mm)*	MIC (mg/mL)	MFC (mg/mL)
Lemongrass oil	17.67 ± 1.53	0.156	0.313
Clove bud oil	20.33 ± 0.58	0.313	0.625
Cinnamon bark oil	> 50	0.078	0.078
Cinnamon leaf oil	24.00 ± 1.00	0.156	1.250
Peppermint oil	Nz	2.500	5.000
Eucalyptus oil	Nz	>10	>10
Nystatin	17.00 ± 2.65	0.003	0.003

Data are reported as Mean ± SD; nz = No inhibition zone; * = 6 mm paper disk; MIC = Minimum inhibitory concentration; MFC = Minimum fungicidal concentration

P. betle and *A. galanga* extracts showed potential anticandidal activity with the lowest MIC and MFC values against *C. albicans* ATCC10231. However, the MIC and MFC values of these PE against *C. albicans* are inconsistent with those of previous studies. In another study using the same fungus and extract solvent, *P. betle* showed antifungal activity against *C. albicans*, with a much higher MIC value of 3.13 mg/mL and an MFC value of 4.17 mg/mL. On the other hand, their studies showed a slight antifungal activity of *A. galanga* against *C. albicans* [18]. In the study by Khodavandi *et al.* [19], the methanolic extract of A. galanga exhibited no antifungal activity against *C. albicans* and *C. krusei*, but showed activity against *C. glabrata* and *C. tropicalis*, with the same MIC value of 0.064 mg/mL. The different antifungal effects observed in previous studies compared to the present study may be attributed to variations in the main ingredients of the tested substance, the types of fungi used, or the method of extraction. Furthermore, among the tested essential oils, 3 EOs —cinnamon bark oil, lemongrass oil, and clove bud oil — exhibited the most effective antifungal activity, with cinnamon bark oil possessing the strongest inhibitory effect against *C. albicans* ATCC 10231. Similarly, Satthanakul *et al*. (2019) demonstrated that cinnamon oil exhibited the lowest MIC, followed by lemongrass oil and clove oil against *C. albicans* [15].

3.2 Chemical composition of plant extracts and essential oils

Two PE (*P. betle* and *A. galanga* extract) and 3 EO (lemongrass oil, clove oil, and cinnamon bark oil) with low MIC and MFC values were chosen for further studies on their chemical composition and the activity against clinical isolates. The major compounds of PE and EO are shown in Table 3. Four major compounds of *P. betle* extract were 4-allyl-1,2-diacetoxybenzene (29.5%), hydroxychavicol (24.8%), eugenol (24.7%), and eugenol acetate (21.0%). The major compound of *A. galanga* extract was 1'-acetoxychavical acetate (78.0%). Furthermore, the major compounds of all EO were geranial (42.7%) and neral (22.2%) in lemongrass oil, eugenol (85.5%) in clove bud oil, and cinnamaldehyde (80.6%) in cinnamon bark oil. The major compounds of the most effective 2 PE and 3 EO were further elucidated using GC-MS. The compounds identified in the tested samples are similar to those previously reported for *P. betle* extract (hydroxychavicol 69.46%, 4-chromanol 24% and eugenol 4.86%) [20]; and *A. galanga* extract (1,8-cineol, α-fenchyl acetate, β-farnesene, β-bisabolene, α-bergamotene, β-pinene, and 1'acetoxychavicol acetate) [21]. In the study by Nordin *et al*. (2014), *P. betle* extract treatment led to physical damage and morphological alterations in *Candida* cells [22]. Hydroxychavicol, isolated from P. betle leaves, was also reported to be effective against various fungal species [23]. The antifungal activity of *A. galanga* extract against *Candida* species has been reported [19]. The *A. galanga* extract is reported to cause damage to the outer and inner membranes, as well as coagulation of the cytoplasm, in *Staphylococcus aureus* cells [24].

Citral, eugenol, and cinnamaldehyde, the major compounds in lemongrass, clove bud, and cinnamon bark oils, have been shown to have antifungal activity against *Candida* species [15, 21]. Citral disrupts cell membrane integrity [25], while eugenol and cinnamaldehyde deactivate hydrolytic enzymes, generate reactive oxygen species, induce apoptosis, and modulate ergosterol content [26].

Table 3. The major compounds of plant extracts and essential oils were determined by GC-MS

Plant extracts/ Essential oils	Main compounds	Retention time (min)	Relative area (%)
P. betle extract	Eugenol	22.9	24.7
	Hydroxychavicol	26.5	24.8
	Eugenol acetate	28.2	21.0
	4-allyl-1,2-diacetoxybenzene	31.3	29.5
A. galanga extract	1′-acetoxychavical acetate	31.2	78.0
Lemongrass oil	6-methyl-5-hepten-2-one	6.6	3.6
	Beta-myrcene	6.7	12.5
	Isoneral	14.8	5.3
	Beta-citral (Neral)	17.8	22.2
	Geraniol	17.9	6.1
	Alpha-citral (Geranial)	18.7	42.7
Clove bud oil	Eugenol	22.4	85.5
	Caryophyllene	24.7	10.4
	Eugenol acetate	28.2	4.2
Cinnamon bark oil	Cinnamaldehyde	18.6	80.6
	Eugenol	22.4	19.4

Relative area = compound percentages were obtained electronically from the GC-MS percent area data.

3.3 Anticandidal activity of plant extracts and essential oils against clinical isolates of *Candida* species

P. betle extract, *A. galanga* extract, lemongrass oil, clove oil, and cinnamon bark oil demonstrated anticandidal activity against clinical isolates of *C. albicans* R01, *C. krusei*, and *C. dubliniensis*. All extracts had MIC/MFC values ranging from 0.156 to 5.000 mg/mL. Cinnamon bark oil had the lowest MIC among all *Candida* species. *A. galanga* extract was particularly effective against *C. krusei* (Table 4). Some *in vitro* studies have reported low susceptibility of antifungal agents or mouthwashes in oral *C. albicans* isolates [27, 28]. Thus, utilizing clinical isolates to test the effectiveness of antifungal agents is necessary for research on anticandidal activity. To this end, all EO and PE were tested against 3 clinical isolates of *Candida* species. Although they showed antifungal activity against all 3 *Candida* species, it was worth noting that *C. krusei* was more susceptible to *A. galanga* extract than *C. dubliniensis* and *C. albicans*. These results were possibly related to its ability to alter the cell surface hydrophobicity (CSH) of several *Candida* species. Harun and Razak (2013) demonstrated that *C. krusei* showed the highest degree of CSH, followed by C. dubliniensis and C. albicans, at 30.23%, 26.19%, and less than 10%, respectively [29]. The major antimicrobial phytochemicals in *A. galanga* extract are hydrophobic compounds, particularly 1′- acetoxychavicol acetate (1′-ACA) [6], which may alter the CSH of *Candida* cells and could penetrate the CSH in the cell wall matrix of *C. krusei* more easily than *C. dubliniensis* and *C. albicans*. However, this postulate requires more studies to confirm the mode of action of 1′-ACA in *A. galanga* extract against different *Candida* species.

Table 4. Minimum inhibitory concentrations (MIC) and minimum fungicidal concentration (MFC) of plant extracts and essential oils against clinical isolates of *Candida* species*

Essential oils/ plant extracts	MIC/MFC (mg/mL)		
	***C. albicans* R01**	***C. krusei***	***C. dubliniensis***
P. betle extract	2.500/2.500	1.250/2.500	2.500/2.500
A. galanga extract	2.500/5.000	0.313/0.313	1.250/1.250
Lemongrass oil	0.313/0.625	0.313/0.313	0.313/0.313
Clove bud oil	0.625/1.250	0.625/2.500	0.625/1.250
Cinnamon bark oil	0.156/0.156	0.156/0.156	0.156/0.156
Chlorhexidine	0.031/0.031	0.004/0.008	0.008/0.016

* Representative data from three duplicate experiments

3.4 Synergistic activity from a combination of essential oils and plant extracts

Table 5 shows that combining EOs and PEs reduced MIC values against *C. albicans* R01. Interestingly, adding all EO decreased the MIC values of P. *betle* 8-fold *and A. galanga* 4-fold, while two PE reduced the MIC values of all EO by 2-fold. All combinations had FICI values between 0.625 and 0.875, indicating an additive effect. Plant-based products, like *P. betle* and *A. galanga* extracts, offer potential alternatives to antifungal drugs. Therefore, the synergistic interaction between PE and EO may be a novel strategy for treating infections. The present study found that combining EOs with *P. betle* or *A. galanga* extracts effectively reduced MIC values against *C. albicans*, a common drug-resistant oral pathogen [30]. Recent studies have shown synergism between essential oils and antifungal drugs. The combination of CHX with either clove oil, cinnamon oil, or lemongrass oil exhibited synergistic effects (FICI ≤ 0.5) against *C. albicans* ATCC10231 and additive effects against clinical isolates of *C. tropicalis* and *C. krusei* [15]. Combining three essential oils (*Thymus leptobotrys, Origanum compactum, and Artemisia herba alba*) with two common antifungal drugs (fluconazole or amphotericin B) was more effective against four *Candida* strains. The addition of all tested EO at sub-inhibitory concentrations reduced the fluconazole and amphotericin B MICs of the tested *Candida* strains by 16 to 512-fold and 1 to 4-fold [31]. Previous studies have demonstrated that the synergistic effect of plants, when combined with other antimicrobials with different modes of action, may inhibit multiple targets.

Three common EO constituents (citral, eugenol, and cinnamaldehyde) disrupt membrane integrity by inhibiting ergosterol biosynthesis [30, 32]. Therefore, combining EO with PE might enhance PE penetration and reduce EO toxicity, offering a promising strategy for treating *Candida* infections. Recent studies have shown that *Candida* species, particularly *C. albicans*, tend to form complex biofilms, which are frequently detected on denture surfaces and oral tissues [3]. Biofilm formation represents a major virulence factor contributing to the pathogenesis of oral candidiasis, also known as denture stomatitis [33]. These biofilms exhibit increased resistance to most antifungal drugs, leading to treatment failure. This resistance is likely due to a combination of factors: high cell density, extracellular matrix, persister cells, drug efflux pumps, and phenotypic alterations in sessile cells [27, 33]. Consequently, the potential use of these combinations to inhibit and control biofilm formation associated with *Candida* infection is under investigation. These extracts and essential oils show high potential as healthcare product ingredients due to their biological activities and favorable toxicity profiles. For example, *Piper betle* leaf extract exhibited moderate toxicity to *Artemia salina* (LC_{50}: 0.58–0.61 mg/mL), and it was deemed safe in rats concerning hematotoxicity, hepatotoxicity, genotoxicity, organ weights, gross morphology, and behavior [34, 35]. Similarly, *Alpinia galanga* extract is safe in a subchronic rat toxicity study [36]. Lemongrass oil and citral exhibit low toxicity (LD_{50} > 2000 mg/kg) in albino rats and are classified as category 5/unclassified under the Globally Harmonized System for chemical hazards [37]. Clove buds and their extracts are FDA-approved food additives. A phenolic-rich clove fraction demonstrated no adverse effects in Wistar rats at 1000 mg/kg body weight/day [38]. Furthermore, cinnamon oil and its constituents offer a promising natural alternative with diverse therapeutic potential. Studies indicate that doses of cinnamon extract below 0.5 g/kg are generally safe for rats [39, 40].

Table 5. MIC and FICI of essential oils combinations with plant extracts against clinically isolated strains of *C. albicans* R01*

Combinations PE/EO	MIC**in combination PE/EO	FIC_{PE} /FIC_{EO}	FICI	Outcome
P. betle extract / Lemongrass oil	0.313/0.156	0.125/0.500	0.625	Additive
P. betle extract / Clove bud oil	0.313/0.313	0.125/0.500	0.625	Additive
P. betle extract / Cinnamon bark oil	0.313/0.078	0.125/0.500	0.625	Additive
A. galanga extract/ Lemongrass oil	0.625/0.156	0.250/0.500	0.750	Additive
A. galanga extract/ Clove bud oil	0.313/0.469	0.125/0.750	0.875	Additive
A. galanga extract/ Cinnamon bark oil	0.313/0.078	0.125/0.500	0.625	Additive

* Representative data from three duplicate experiments

** MIC of essential oil and plant extract expressed in mg/mL.

4. Conclusions

This study demonstrates that lemongrass oil, clove oil, cinnamon bark oil, and the extracts of *P. betle* and *A. galanga* possessed potent antifungal effects on oral candidiasis isolates, including *C. albicans* R01, *C. krusei*, and *C. dubliniensis*. The combination of these EOs, either *P. betle* extract or *A. galanga* extract, was observed to decrease the individual MIC value of all tested agents, indicating an additive effect against clinical isolates of *C. albicans*. Moreover, the presence of bioactive compounds in these EO and PE indicates the potential of their future application as plant-based antifungal agents for the treatment of oral candidiasis.

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