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ANTIFUNGAL POTENTIAL OF ESSENTIAL OILS FROM LEAVES OF *SYZYGIUM CUMINI* AND *PSIDIUM GUAJAVA* AGAINST *CANDIDA ALBICANS*

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ABSTRACT

Candida albicans, inhabits the urogenital and gastrointestinal tract as a commensal yeast and its growth is limited by the action of immune system. Plant-derived natural products play a vital role in pharmaceutical field, since substantial quantity of contemporary medicines are obtained either from naturally occurring molecules or their byproducts. With this background, *Syzygium cumini* and *Psidium guajava* have been selected for the present investigation. We explored whether essential oils (EOs) from leaves of *S. cumini* and *P. guajava* can hinder the harmful behaviors of *C. albicans*, a pathogen that often takes advantage of weakened immune systems. The EOs derived from the leaves of *S. cumini* and *P. guajava* were extracted by hydro-distillation method and main components were analyzed using GC–MS. Sub-MIC concentrations of EOs were used to evaluate the effectiveness of the EOs against biofilm formation and hyphal transition of *C. albicans*. The most dominant components in *S. cumini* were identified as α-Cadinol (24.33%), Naphthalene (13.878%), Spathulenol (12.47%) and in case of. *P. guajava* oil, Caryophyllene oxide (36.09%), β-Nerolidol (23.40%), Epoxy farnesol (4.12%), Humulene epoxide (3.89%) and α-Cadinol (3.65%) were identified as major components. In a sub-lethal concentration (4% and 8% v/V), essential oil blocked the hyphal transition, a major virulence factor of *C. albicans*. The results demonstrated that EOs of the selected plants were able to reduce biofilm formation by as much as 50%, with the effect increasing in a dose-dependent fashion. The molecular docking studies reveal that the dominant compounds had considerable binding affinities against adhesin. These findings indicate that both EOs might possess the capacity to inhibit the growth and colonization of *C. albicans,* highlighting potential usage for a range of antifungal applications.

Keywords: Essential oil, *Candida albicans*, Hyphal inhibition, Antifungal and Anti-biofilm

INTRODUCTION

In healthy individuals, *Candida albicans* inhabit the urogenital and gastrointestinal tract as a commensal and its growth is limited by the action of immune system. However, when the immune system is compromised, the commensal *C. albicans* can become highly pathogenic and lead to the systemic as well as superficial infection (**Lopes & Lionakis, 2022; Bojang *et al*., 2021**). According to a recent survey, *C. albicans* is responsible for 88% of all nosocomial fungal infections in the United States, 75% of them were invasive fungal infections that cost the US health-care system roughly $3 billion per year (**Bongomin *et al*., 2017**). *C. albicans* can switch between morphological states such as yeast, pseudo-hypha, and hypha. This switching from yeast to hypha form is very critical for pathogenesis and host tissue invasion of the *Candida* species. The hypha forms make the fungus more resistant against phagocytosis and enhance adherence to host surfaces which help to invade epithelial cell layers, and finally results in damage to the tissue (**Williams & Lewis, 2011**). Studies revealed that the *Candida* species which are mutant to hypha formation have reduced virulence (**Staniszewska, 2020; Bohner *et al*., 2022**). Generally, *Candida* forms a three-dimensional multicellular structure attached to surfaces commonly referred to as biofilms. The biofilm structure is generally found as an increasing source of infection by *C. albicans*. In hospitals the biomaterials such as stents, catheters and orthopedic joints are the best sources for the adhesion of *Candida* and subsequently to form biofilm. The removal of biofilm is very difficult by the use of conventional antibiotics because of its architecture which prevents the antibiotics to reach the target cells. Several studies showed the correlation between the biofilm and hyphal formation in *C. albicans*. It was observed that bridges of hyphae help in the formation and stability of biofilms by microcolonies of *candida* species **(Rodriguez-Cerdeira *et al*., 2020**). Other workers also elaborated that the hyphae play an important role in biofilm maturation (**Garcia-Sanchez *et al*., 2004; Blankenship & Mitchell, 2006**). Due to the eukaryotic cell structure, *C. albicans* share some similarity in the biological process with human cells, which is a major side effect of the use of antifungal drugs (**Coleman *et al*., 2010**). Emergence of antibiotic resistance in microbes is a serious threat which is a result of extensive use of antibiotics (**Baruah *et al*., 2025; Baruah *et al*., 2024; Salvia *et al*., 2023; Rai *et al*., 2025**). These complications led researchers to focus their endeavors on finding compounds that could block the virulence pattern rather than directly killing microbes. *Psidium guajava* is used traditionally for medicinal purposes, mainly for antioxidant, antimicrobial, antispasmodic, antiallergy, anti-inflammatory, and hepato-protective properties (**Chechani *et al*., 2024**). The leave extracts of *P. guajava* have been reported to exhibit potential antifungal activity against different strains of *C. albicans* and *Cryptococcus neoformans* (**Ally-Charles *et al*., 2024**). *The Syzygium cumini leaf* extract has been reported to inhibit the growth of fungi such as *Penicillium chrysogenum and C. albicans* (**Pareek *et al*., 2015**). Essential oils (EOs), derived from different parts of plants, have historically been utilized for their antimicrobial activity (**Laishram *et al*., 2006**). Over time, their bioactive potential has been widely explored against a variety of bacterial pathogens (**Meenu *et al*., 2023**). However, there are limited reports which suggest the use of EOs to inhibit the fungal growth and its virulence. *Psidium guajava* and *Syzygium cumini* belongs to the Myrtaceae family. These plants are considered as phytotherapic plant which is utilized in traditional medicinal practices that helps in the treatment of various diseases like malaria, gastroenteritis, diarrhea, dysentery, wounds, ulcers (**Bakkali *et al*., 2008; Wang *et al*., 2017; Oyeyemi *et al*., 2019; Lutterodt, 1992**). There are few reports which suggest the antimicrobial activity of EOs from these plants against common pathogens. But there is a lack of extensive research which evaluates the biofilm and virulence inhibition by the *P. guajava* and *S. cumini* EOs.

The aim of the study was to extract EOs from the leaves of *P. guajava* and *S. cumini* and study the anti-virulent properties. Furthermore, in silico studies were performed to examine the interactions between the primary compounds of the EOs and the target protein (adhesin), with the goal to understand the possible mechanisms behind the inhibition of *C. albicans*. For *C. albicans*, adhesin proteins are very important in biofilm development, colonization and infection **(Mba & Nweze, 2020)**. As the oils have very low antifungal activity hence there is a limited chance of emergence of resistance and hence it could be a novel antifungal prototype.

MATERIALS AND METHODS

Plant Sample Collection and Identification

The plant sample was collected between July and August 2024 from Birkuchi area, Guwahati, Assam. The plant herbarium file was created, and the plant was recognized as *Psidium guajava* and *Syzygium cumini* by the Botanical Survey of India, Shillong. Fresh plant leaves were harvested, washed with sterile water, and subjected to surface sterilization using 70% ethanol and 5% sodium hypochlorite solution. Finally, the leaves were rinsed with sterile double-distilled water to ensure removal of any remaining contaminants.

Fungal Strain and Culture Condition

Candida albicans reference strains MTCC 3017 and MTCC 227 were obtained from the Microbial Type Culture Collection (MTCC) at the CSIR-Institute of Microbial Technology, Chandigarh, India. Before each series of experiments, *C. albicans* was grown in potato dextrose broth (PDB). For the uniform dispersion of EOs, 0.2% Tween-80 (HiMedia Laboratories Pvt. Ltd, Mumbai, India) was utilized as a surfactant.

Isolation of EOs

Fresh leaves of the plant samples were hydro-distilled for 4h in a Clevenger apparatus to separate the essential oil using a previously described method (**Mohamed *et al*., 2013**). Following extraction, the essential oil was dehydrated using anhydrous sodium sulfate and subsequently stored at −20 °C until required for experimentation.

GC-MS Analysis of EOs

Chemical composition of *S. cumini* and *P. guajava* leaves essential oils were analyzed using a GC-MS system (Agilent 7890 A, Agilent Technologies, Palo Alto, CA, USA) equipped with an DB -5MS capillary column (30 m × 0.25 mm id., 0.25 µm film thickness; Agilent Technologies, USA). The mass spectrometer was operated in the electron ionization mode with an electron energy value 70 eV. A volume of 1 µl of essential oil was injected into the GC-MS coupled to a Mass Selective Detector (MSD) (Model: 5975C, Agilent, USA). The oven temperature was initially held at 60 °C and then increased by 5 °C/min to 280 °C and then subsequently kept at 280 ºC for 5 min. Helium was used as the carrier gas at a flow rate of 2 ml/min. The mass spectra or peaks were interpreted by computer searching in a commercial mass spectral reference library (Wiley NIST)

Minimum Inhibitory Concentration (MIC) of EOs

The Minimum Inhibitory Concentration (MIC) of the EOs was determined following the principles outlined in the Clinical and Laboratory Standards Institute (CLSI, 2017) guidelines for yeast as well as methods described by **Singh *et al*., (2014).** Due to the volatile nature of EOs, agar incorporation assay as described by Singh *et al*. was also taken into consideration along with the CLSI guidelines. In brief, various concentrations of essential oil were combined with potato dextrose agar (PDA) and poured into the petri plates. 100 µl of the fungal suspension (3×10^5 CFU/ml in sterile normal saline) of the test *C. albicans* strains was inoculated by spreading on the PDA plate separately and incubated at 30°C for 48 h. Following the incubation period, the plates were examined for growth inhibition of the test *C. albicans* strains. The MIC of EOs was determined as the lowest concentration that completely inhibited growth of *C. albicans.* Only experiments below the MIC concentration were carried out.

Effects of Essential Oils on the Growth Pattern of C. albicans

The growth curve for *C. albicans*, cultivated in the presence and absence of essential oils at sub-MIC value was used to ensure if there is any effect of the EOs on growth of the *C. albicans*. Test inoculum, 100 µl of *C. albicans* (3×10^5 CFU/ml) according to (**Rai *et al*., 2025**) was added to PDB medium containing EOs at MIC value (2% to 8% v/V). Test tubes were incubated at 30 ºC and the optical density (OD) were recorded at 600 nm at 2 h intervals up to 28 h.

Inhibition of yeast to hyphal transition

Effect of EOs on *C. albicans* yeast to hyphal transition was estimated by the method described in CLSI, USA guidelines (CLSI, 2017) with slight modifications. In brief, *C. albicans* (3×10^5 CFU/ml) was grown on Roswell Park Memorial Institute (RPMI) medium supplemented with fetal bovine serum (FBS) (10%) containing EOs at the sub-MIC value for 24 h at 30 ºC. After incubation period the hyphal transition of the *Candida* cells were visualized under inverted microscope.

Effect on Cell Surface Hydrophobicity (CSH)

The hydrophobicity of *C. albicans* after the treatment with EOs was determined using a protocol described elsewhere with some modifications. Cells were grown in media containing different concentrations of essential oils (2, 4, 6 and 8% v/v) and incubated for 48 h. Cells grown in PDB were used as control. After 48 h cells were harvested by centrifugation and resuspended in Phosphate-buffered saline (PBS) (pH 7.4) and OD was measured at 600 nm. Equal volume of cell suspension was mixed with xylene and vortexed for 2 min and left undisturbed. After 1h the aqueous layer was carefully pipetted out and OD was measured at 600 nm. CSH was calculated using the formula: All assays are representatives of three independent experiments, performed in triplicates.

$$\text{Hydrophobicity}(\%) = \frac{\text{A0} - \text{A1}}{\text{A0}} \times 100$$

Where, A0 = OD_{600} at 0 h and A1 = OD_{600} at 1 h.

Biofilm Inhibition Assay: Tissue Culture Plate Method

For biofilm inhibition assay, Tissue Culture Plate method was used as described elsewhere (**Noble *et al*., 2010**). Briefly, 100 µl of $3x10^5$ CFU/ml of fungal culture was added in 1 ml of RPMI medium supplemented with 10% FBS in 24 well plate. To the culture, Essential oil at different concentration (2% - 8% v/V) was added in corresponding wells. The plate was incubated at 30 ºC for 48 h. After incubation the content of each well was gently removed and the wells were washed three times with PBS (pH 7.4) to remove free-floating planktonic cells. Adherent biofilms on the surface of the well were stained with crystal violet (0.1%, w/v) followed by removal of excess stain and thoroughly rinsed by deionised water. The plate was kept for drying and after drying, 100% ethanol was added to the wells to solubilise the bound stain and the OD of stained adherent cells were determined with a microplate reader (Thermo Scientific Multiskan GO) at 570 nm. The biofilm inhibition was calculated based on the solubility of the retained dye in wells by using the formula-

$$\%\ \text{Biofilm inhibition} = \frac{\text{ODcontrol} - \text{ODtest}}{\text{ODcontrol}} \times 100$$

Where control OD is the absorbance which is obtained without addition of essential oil.

Molecular docking

A protein molecule relevant to the research objectives and accessible in the Protein Data Bank (PDB) was chosen (**Behzadi & Gajdacs, 2022**). The protein molecule selected for docking was the Als3 adhesin (PDB ID: 4LEE), chosen for its critical role in *C. albicans* biofilm development, colonization, and infection. The selected protein underwent preprocessing steps to optimize its structure, including removing water molecules, hetero atoms and co-crystallized ligands (**Karthikeyan & Vyas, 2014**). Additionally, any missing atoms or residues were addressed, and hydrogen atoms were added to facilitate accurate docking simulations. These ligand structures were downloaded from PubChem and prepared for docking studies, which involved energy minimization, determining protonation states and assigning appropriate atom types and charges (**Keiser *et al*., 2007**). Docking simulations were conducted using PyRx and BIOVIA Discovery Studio software. The prepared protein and ligand structures were loaded into the software with the protein acting as the receptor and ligands as the docking ligands (**Sekar *et al*., 2022**). Docking parameters were carefully selected to enhance the accuracy and reliability of predictions.

Statistical Analysis

All experiments were carried out in triplicate, and the results are given as mean values and standard deviations. For unpaired samples, differences between controls and tests were examined using the student's t-test, with only findings at $p< 0.05$ considered significant.

RESULTS

Chemical Composition of the Essential Oils

As shown in the Table 1, total 19 and 14 compounds have been identified in *S. cumini* and *P. guajava* leaf EOs respectively. The major components identified by GC–MS in EO of *P. guajava* were Caryophyllene oxide (36.09%), β-Nerolidol (23.40%), Epoxyfarnesol (4.12%), Humulene epoxide (3.89%) and α-Cadinol (3.65%) along with some other minor components presented in trace amounts. The main components identified in *S. cumini* EO were α-Cadinol (24.33%), Naphthalene (13.78%), Spathulenol (12.47%) and Caryophyllene (3.46%).

Table 1 Chemical Composition of the Essential Oils Extracted from *S. cumini and P. guajava*

Compounds	Retention Time	*S. cumini* (% Area)	*P. guajava* (% Area)
Copaene	21.221	0.54	1.573
α-Cubebene	20.427	0.260	
Caryophyllene	22.398	3.460	4.74
α-Caryophyllene	23.326	1.040	1.21
β-Bourbonene	21.44	0.379	
Cadinene	23.79		0.63
α-Curcumene	23.924		0.53
cis-α-Bisabolene	24.397		1.1
γ-Muurolene	24.745		0.6
α-Calacorene	25.44	0.669	
β-Nerolidol	25.878		23.4
Caryophyllenyl alcohol	26.315		0.89
Caryophyllene oxide	26.375		36.09
Humulene epoxide I	27.109		3.89
α-Cadinol	27.921	24.334	3.65
Cubenol	27.22	0.986	
Epoxyfarnesol	28.412		4.12
Bisabolene epoxide	30.535		1.57
Pentacosane	43.88	0.575	
Hexacosane	45.417	1.000	
Heptacosane	46.89	0.955	
Octacosane	48.32	1.032	
Oleic acid	30.23	2.165	
Nerolidol	25.878	1.878	
Globulol	26.53	2.041	
Spathulenol	27.22	12.468	
Isospathulenol	27.61	2.74	
Naphthalene	24.86	13.878	

Antimicrobial Assays and Minimum Inhibitory Concentration

Since EOs have been reported to possess antimicrobial activity, attempts were made to assess the antifungal activity of *S. cumini* and *P. guajava* leaf EOs. The antimicrobial activity was determined against *Candida* strains by well diffusion assay as well as through MIC determination (Fig. 1). The MIC value against *C. albicans* was found to be more than 10% v/V as shown in Table 2.

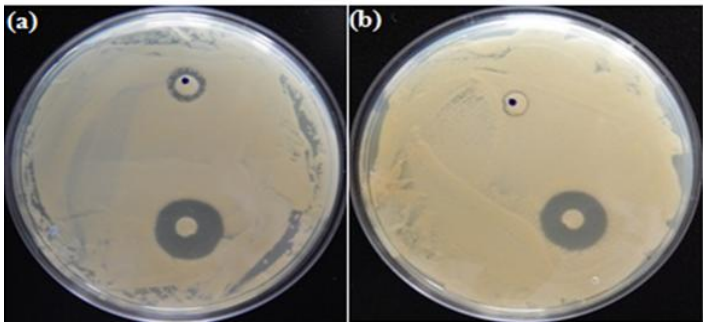


Figure 1 Antifungal activity of essential oil of (a) *S. cumini* and (b) *P. guajava* essential oil against *C. albicans.*

Table 2 Antifungal Activity and MIC Value of essential oils of *S. Cumini* and *P. guajava*

Microorganisms name	Diameter of mean zone of inhibition(mm)*		Minimum Inhibitory concentration (MIC) (% v/V)	
	S. cumini	*P. guajava*	*S. cumini*	*P. guajava*
Candida albicans MTCC 3017	8 ± 0.05	6 ± 0.05	11.67± 0.76	12.17±0.58
Candida albicans MTCC 227	10 ± 1	8 ± 0.05	10.83±0.76	11.83±0.58

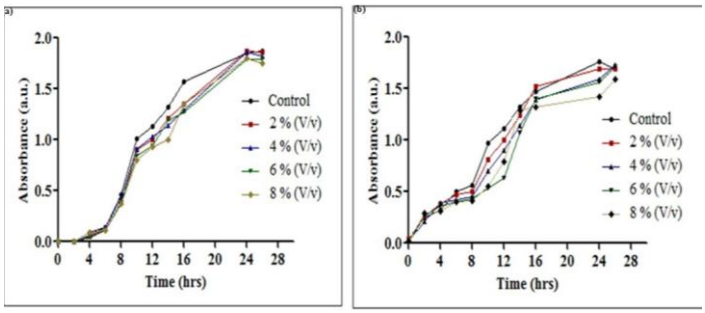


Figure 2 Influence on *Candida albicans* growth by the essential oils of (a) *S. cumini* and (b) *P. guajava*

Effect of EOs on C. albicans Growth

The effect of EOs was evaluated by monitoring the growth of *C. albicans* at 600 nm in presence and absence of essential oil (Fig. 2a) *S. cumini* and (Fig 2b) *P. guajava* at sub-MIC value. The result suggested that the EOs at sub-MIC value (2%, 4%, 6% and 8% v/V) did not affect the growth pattern of the fungi.

Inhibition of Yeast to Hyphal Transition

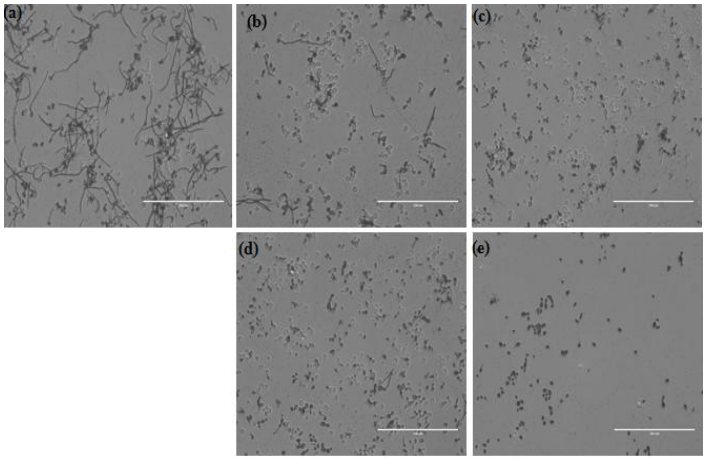


Figure 3 Influence on *Candida albicans* hyphal transition by different concentration of essential oil of *S. cumini* (b) 2 %; (c) 8% and *P. guajava* (d) 2%; (e) 8% in comparison of control (a).

The hyphal transition was induced by supplementing the RPMI medium with 10% FBS at 30 ºC. After 6 h of incubation period the cells were visualized under microscope (Fig. 3). The control cells (0% v/V EOs) shown to have developed a mass of hyphal transition (Fig. 3a). However, the cells incubated with EOs with lower concentration (2% v/V) showed some hyphae like structure (Fig. 3b and d) while at higher concentration (8% v/V) the hyphal growth was absent (Fig. 3c and e). The results indicated the concentration dependent inhibition of the hyphal growth in the *C. albicans*.

Effect on Cell Surface Hydrophobicity

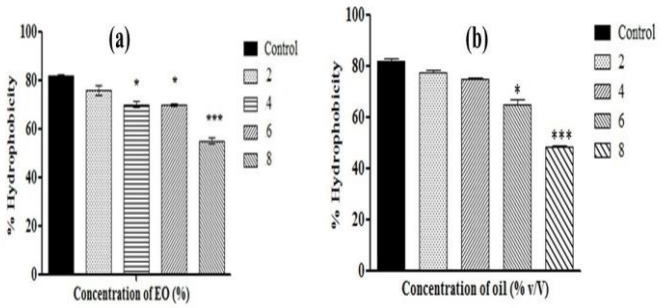


Figure 4 Hydrophobicity assessed by Microbial Adhesion to Hydrocarbons (MATH) assay. Data represent the mean ± SD of three independent experiments. All experiments were performed in triplicates. Statistical significance compared to the untreated control is indicated as *p < 0.05 and ***p < 0.001.

The hydrophobicity of untreated and oil treated *C. albicans* planktonic cells were determined by the biphasic hydrocarbon/aqueous method. It was found that there was a significant change in the hydrophobicity of the cells treated with higher concentration of essential oil (Fig. 4). The control cells without treatment of essential oil showed cell surface hydrophobicity 81.75 % ± 0.742. On treatment with EOs of *S. cumini* and *P. guajava* at higher concentration (8% v/V) it reduced to 52% ±1.621 (Fig.4a) and 48% ± 1.27 (Fig.4b) respectively.

The influence of EOs on biofilm development was assessed *in-vitro* by monitoring the binding of crystal violet to adherent cells on the tissue culture plate, which directly quantifies the total biofilm biomass. With increasing essential oil concentration, treatment for 24h resulted significant reduction in biofilm biomass (Fig.5). It was discovered that essential oils had no influence on biofilm formation at lower concentrations. Yet, when the concentration grew, biofilm formation was dramatically suppressed.

Molecular docking

The structural integrity and quality of the protein 4LEE (adhesin) obtained from the Protein Data Bank (PDB) was rigorously assessed using online simulations where it was found that in ERRAT - Protein Quality Factor is 87%. In VERIFY 3D, 85.54% of the residues have averaged 3D-1D score >= 0.1 and in Ramachandran plot, residue in favourable region is above 90% ensures the protein to be of good quality. After conducting docking studies utilizing essential oils from *S. cumini* and *P. guajava* with Als3 adhesin (4LEE), it was observed that several compounds, including Spathulenol (-6.8 kcal/mol) and Humulene epoxide (-6.6 kcal/mol), exhibited strong binding affinities. These interactions provide a structural basis for how the EOs may interfere with Als3-mediated adhesion (Table 3).

Effect of Essential Oils on Biofilm Formation

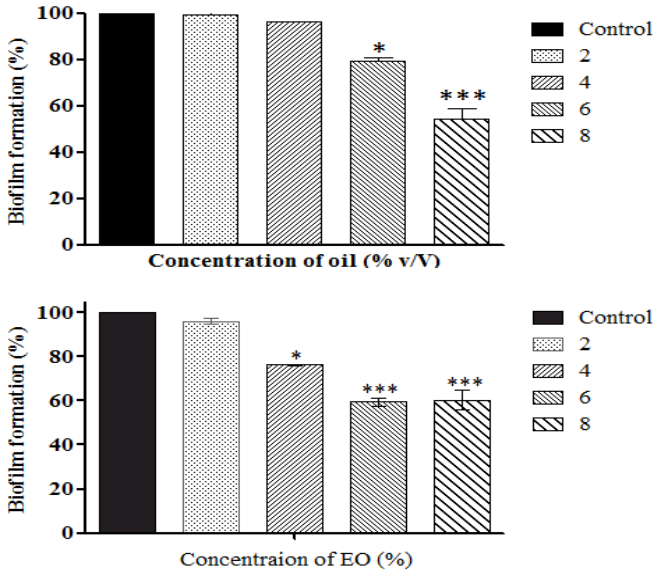
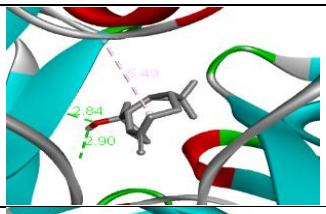
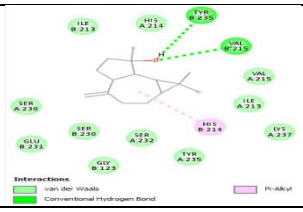
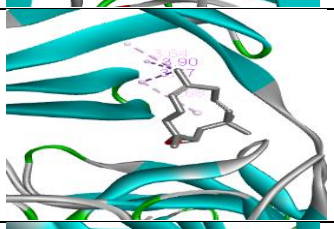
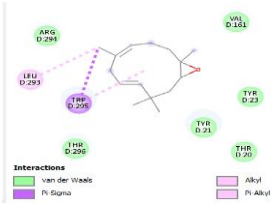
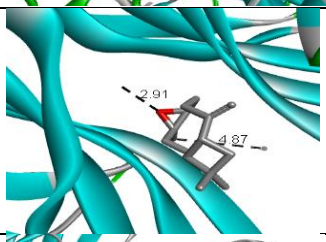
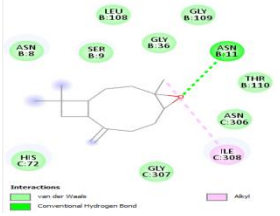
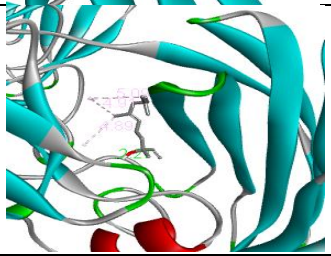
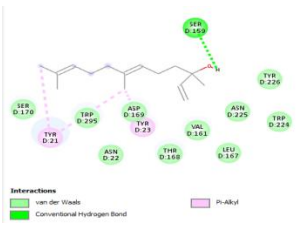
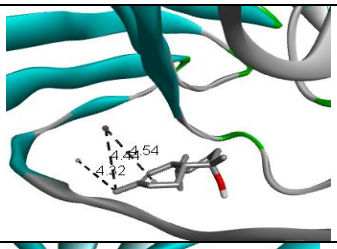
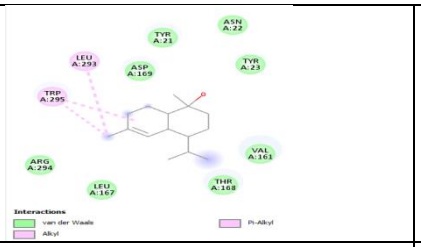
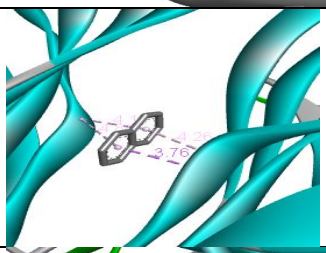
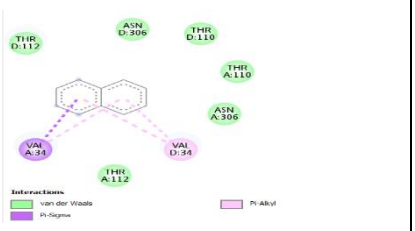
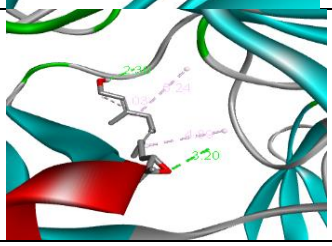
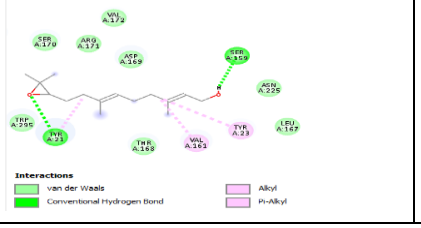


Figure 5 Biofilm formation assessed by spectrophotometric technique using crystal violet. The results are expressed as percentage biofilm formation of *Candia albicans* with or without EOs of (a) *S. cumini* (a) and (b) *P. guajava*. All experiments were performed in triplicates. Statistical significance compared to the untreated control is indicated as *p < 0.05 and ***p < 0.001.

Table 3 Docking simulations of the selected phytoconstituents

S. No.	Constituents	Docking	2D Interactions	Binding Affinity
1	Spathulenol			-6.8
2	Humulene epoxide			-6.6
3	Caryophyllene oxide			-6.4
4	Nerolidol			-5.9

5	Cadinol			-5.9
6	Napthalene			-5.6
7	Epoxy Farnesol			-5.4

DISCUSSION

As the emergence of new fungal infections and increasing drug-resistance in the *Candida albicans* there is an urgent need for the development of new antifungal agents. Plant secondary metabolites have been well known to possess a broad spectrum of antimicrobial activity. Among the metabolites essential oils are well known for their biological properties. The present study lies on the determination of anti-virulence capability rather than direct antifungal activity. As our results indicate, the relatively high MIC values (>10% v/V) suggest that the therapeutic promise of *S. cumini* and *P. guajava* EOs does not reside in directly killing *C. albicans*. Instead, their value aligns with the emerging 'path blocker' paradigm. We also investigated their EO compositions. As reported by previous researchers, the EOs of both plants contained many abundant compounds primarily found in the Myrtaceae family. Interestingly, naphthalene was found as a major constituent, which is not common in the EO profile of Myrtaceae family. Although some researchers report trace amount of naphthalene in certain plants of the aforementioned family, there is a probability that this represents an environmental contaminant or an analytical artifact rather than a true endogenous secondary metabolite (**Holanda *et al*., 2024**). Further purification and targeted validation would be required to confirm whether this compound is endogenously produced by the plant. While antimicrobial activities of essential oils of *S. cumini* from other geographical origin have been shown (**Gupta *et al*., 2014**), most of the studies are limited with the antibacterial activity. Interestingly there is no report citing the antimicrobial activity of the *P. guajava* leaf essential oil till now. However, there are few reports on antimicrobial activity of plant extracts of *P. guajava* (**Sharifzadeh *et al*., 2016**).

The morphological switch of *C. albicans* from single yeast cell to filamentous structure is one of the most important and well-known virulence factors. This alteration also leads to other virulence enhancer like adhesion and biofilm formation. One recent approach is to search for the novel therapeutics which attenuates the virulence rather than targeting its killing, so called path blockers. In this case the possibility of resistance development against the anti-virulence compounds should be rare, since avirulent sub-populations can survive. This hyphal transition is now a target to minimize the pathogenicity of *C. albicans.* There are numerous small molecules with potential to modulate the yeast-to-hypha transition in *C. albicans* (**Mohareb *et al*., 2013**). In the present study we evaluated the inhibition of yeast to hyphal transition in two *C. albicans* strains by the plant essential oil at sub-MIC value. We have found that the essential oil can reduce the yeast to hyphal transition in concentration dependent manner without altering the growth of the fungi. The results are in accordance with other findings in which the compounds are used to inhibit the hyphal transition without acting on its normal growth. Lee *et al* showed that phorbasin H isolated from *Phorba* sp. can inhibit the yeast-to-hypha transition in *C. albicans* (**Lee *et al*., 2013**). Other natural resources like Gymnemic acid (**Vediyappan *et al*., 2013**), purpurin (**Tsang *et al*., 2012**) and *Solidago virgaurea* water extracts (**Chevalier *et al*., 2012**) are also reported to have yeast-to-hypha inhibition activity.

Surface-attached self-produced matrix enclosed microbial communities known as biofilm is a major virulence factors in microbial world. This matrix enclosed biofilm increases the resistance to antimicrobial drugs by jamming or preventing the entry of drugs to the cells inside the biofilm structure. So, there is a need to search for alternatives which can prevent the formation of this matrix associated biofilm. Plant secondary metabolites can be an alternative to prevent this biofilm like structure. Our study reports the antibiofilm activity of essential oils of the selected plants at sub-MIC value, and concluded that the essential oil at very low concentration is able to reduce the biofilm biomass in *C. albicans*. Previous findings suggest that the essential oils from plants such as *Satureja montana* L., *Thymus vulgaris* L. and *Rosmarinus officinalis* L. can be an alternative strategy to inhibit the biofilm in *Salmonella* strains (**Miladi *et al*., 2016**). Essential oils from *Satureja hortensis* L. have been found to inhibit the biofilm formation of *C. albicans* isolated from the HIV patient (**Sharifzadeh *et al*., 2016**). Thus, the present study also supports the previous reports that essential oils can be a prominent agent to inhibit the biofilm formation and also to combat the resistance problem.

The initial event of biofilm development is microbial adhesion to the surface of substrates which is mediated by the cell envelope to create a bridge between the microorganism and the environment. The hydrophobicity of the cell surface is thought to be a significant factor in *Candida* spp. adhesion to various surfaces (**Silva-Dias *et al*., 2015**). It has been shown that Biofilm formation ability on polystyrene plate positively correlates with cell surface hydrophobicity of *C. albicans* planktonic cells (**Sharifzadeh *et al*., 2016**). The present study evaluated that in presence of essential oils at sub-minimum inhibitory concentration the cell surface hydrophobicity is reduced significantly which corroborates with the previous finding report that eugenol can alter the cell surface hydrophobicity which finally leads to the reduction in biofilm formation (**Jafri & Ahmad, 2020**). It was also reported that some essential oils can alter the cell surface hydrophobicity of the *Candida* cells (**Bona *et al*., 2016**). So, the results suggest that essential oil may interfere with the adhesion properties *of C. albicans*.

The findings from the *in-silico* analysis support the idea that the biofilm formation and inhibition of colonization may be due to the interaction of exogenous EO compounds with Als3. This is specifically indicated by the moderate binding affinities between Spathulenol and Caryophyllene oxide to Als3. This result corroborates with our experimental findings of deceased cell surface hydrophobicity and biofilm biomass. Our computational results give a convincing, hypothesis-generating model that matches the *in-vitro* observations of decreased cell surface hydrophobicity and lower biofilm growth, warranting further focused biophysical validation.

The limitations of the present study include reliance of the antifungal evaluation mainly on agar diffusion assay and anti-biofilm activities at high essential oil concentrations.

CONCLUSIONS

Essential oils isolated from the plants *S. cumini* and *P. guajava* show a mild antifungal activity against *C. albicans*. The yeast to hyphal transition was inhibited by these oils at low concentration. Apart from reducing cell surface hydrophobicity significantly these oils are also proficient to inhibit the biofilm formation. Thus, the isolated essential oils are found to inhibit the main virulence factors of *C. albicans* without affecting its normal growth.

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