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ORIGINAL ARTICLE

In Vitro and In Vivo Antibiofilm Potential of Eicosane Against *Candida albicans*

Raja Mohamed Beema Shafreen[1,2] · Siddharthan Seema[1] · Selvaraj Alagu Lakshmi[2] · Adimoolam Srivathsan[2] · Kannapiran Tamilmuhilan[2] · Anupama Shrestha[3] · Balamuralikrishnan Balasubramanian[4] · Ranjithkumar Dhandapani[5] · Ragul Paramasivam[5] · Sami Al Obaid[6] · Saleh H. Salmen[6] · Mohamad Faiz Mohd Amin[7] · Saravanan Muthupandian[8]

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Abstract

Candida albicans is the most prevalent fungus in humans, producing infections ranging from mucosal to systemic. *C. albicans* colonizes mucosal surfaces asymptomatically as commensal, but, if the host environment is disrupted, or if the host immune system is compromised, *C. albicans* can multiply and infect almost all places in the host. The present study was aimed to identify a promising antibiofilm agent against *Candida albicans* biofilm. Through the molecular docking approach, it was identified that Eicosane was the top hit among the alkanes screened. Furthermore, in vitro analysis revealed that Eicosane at 100 µg/mL was able to inhibit 60% of *C. albicans* biofilm without inhibiting the growth. Moreover, light microscopic investigation unveiled the significant reduction in the adhesion and colonization of yeast cells to the matrix on Eicosane-treated samples. The CLSM images showing a reduction in biomass and thickness of *C. albicans* biofilm in the presence of Eicosane were validated using COMSTAT. The results were well corroborated with SEM micrograph in which a pellucid gap between the cells was observed and colonization was considerably reduced. Further from qPCR analysis, the genes responsible for biofilm formation and hyphal growth were found to be downregulated in the presence of Eicosane. Similarly, Eicosane at BIC was able to significantly inhibit the adhesion and colonization of yeast cells on the chorion of the zebrafish embryos. Moreover, the binding ability of Eicosane to ALS3 was revealed through docking and molecular dynamics (MD) simulation studies.

Keywords Eicosane · Antibiofilm · CLSM · Molecular docking · Zebrafish

Raja Mohamed Beema Shafreen and Siddharthan Seema share equal contributions.

✉ Saravanan Muthupandian
bioinfosaran@gmail.com

Extended author information available on the last page of the article

Introduction

Candida albicans is a commensal that survives on different parts of the human such as the gastrointestinal tract, genitourinary tract, and oral cavity [1–3]. It is an opportunistic pathogen that can cause superficial and systemic infection [4]. *Candida albicans* are mostly associated with nosocomial infections in immunocompromised individuals and cause recurrent infections [5]. In general, *Candida albicans* exist in different morphological forms such as yeast, pseudohyphae, chlamydospores, and hyphae [6]. The pathogenicity of *C. albicans* in humans is controlled by the virulence mechanism such as adhesion, yeast to hyphal transition, biofilm formation, thigmotropism, and phenotypic switching [7–9]. The biofilms caused by *C. albicans* are responsible for a wide-range of infections in humans [10]. Biofilms are microbial communities containing a mixture of yeast and filaments surrounded by a self-produced exopolymeric matrix which makes them resistant against chemotherapeutic agents [11]. *C. albicans* forms a heterogeneous structured biofilm that contains four different stages such as initial adhesion, proliferation, growth of pseudohyphae (in which hyphae are associated with the production of extracellular matrix), and sluggish dispersion of yeast cells from biofilm to seed on new sites [12]. The persister cells in *C. albicans* biofilm mainly contribute to the tolerance of antifungals agents. However, planktonic cells are completely devoid of these cells. Indeed, persister cells from biofilm are the memory cells that act as a major factor for recurrent infections [13–15].

Currently, three classes of antifungal drugs are available for the treatment of *Candida* infections namely azole, echinocandins, and amphotericin B. Azole (fluconazole, imidazole, and ketoconazole)-based molecules are commonly used as antifungal agents to target the sterol biosynthesis [16]. Echinocandins are highly active antifungal drugs that target the 1,3-β-glucan synthase essential for the synthesis of β-glucan in the cell wall. Amphotericin B targets the fungal lipid bilayers and creates membrane pores. Though these antifungal drugs are used for treatment against *C. albicans* there are several limitations; azoles are fungistatic and show a deprived effect on *C. albicans* biofilm. Echinocandins, even at higher doses, are not clinically useful to treat *C. albicans*-mediated urinary tract infections [17]. Similarly, amphotericin B used for systemic infection has been reported toxic due to reduced solubility [18]. These limitations have increased the morbidity and mortality rate of *Candida*-mediated infections. Alarmingly, the increase in drug resistance has provided strong impulsion to understand the mechanism of enhanced tolerance of biofilm-associated infections to antimicrobial therapy [19–21]. Therefore, a search for alternative drugs for treatment and prevention of *C. albicans* infections is warranted [22]. Biofilm is the important virulence factor that increases the rate of drug resistance in *C. albicans*.

To overcome the current scenario and to protract less risk of drug resistance, antivirulence therapy is recommended as an alternative approach. Anti-virulent compounds can impede biofilm without altering the viability and existence. Several natural products from marine environment have been revealed to hamper the biofilm formation in *C. albicans*. *Streptomyces* are important microorganisms in the environment, producing ample new structurally diverse class of bioactive compounds that have potential against different human pathogens. *Streptomyces* are renowned for antibiotic and novel secondary metabolite productions. Due to the promising effect of bioactive from *Streptomyces* species, it is considered as pharmacologically most valuable organism. Interestingly, bioactive compounds identified from *S. chrestomyceticus* and *S. olivaceus* were studied for antibiofilm, anti-hyphal, and antivirulence properties against *C. albicans* [23, 24]. We have previously shown that ethyl acetate extract of *S. diastaticus* isolated from the marine crustacean

Portunus sanguinolentus has antibiofilm potential against *C. albicans* [25]. Thus, the present study has gathered interest to screen the chemical entities identified from the bioactive extracts of *S. diastaticus* using a computational approach. The top hit was further evaluated using in vitro and in vivo investigation for their antibiofilm potential against *Candida albicans.*

Materials and Methods

Bacterial Cultures

A highly potential biofilm-forming *Candida albicans* (ATCC:90,028) was selected and the cultures were maintained in Sabouraud dextrose agar (SDA) slants. For all the assays performed in this study, culture suspension of 10^8 cells/mL (100µL) was used [26].

Molecular Docking

The 3D structures of 10 compounds were obtained from the PubChem database in.sdf format. The crystal structures of agglutinin-like sequence ASL3 wild type Protein Data Bank (PDB ID: 4LEE) with a resolution of 1.4 Å and 3.0 Å was obtained from Research Collaboratory for Structural Bioinformatics PDB (RCSB PDB) database (https://www.rcsb.org/pdb). The compound structures were minimized by applying CHARMM force field protocol by Discovery studio (DS 2.5). The non-bonded interactions were set to a spherical cut-off radius of 13.0 Å. The define and edit binding site approach was used to identify the possible binding location area of the target proteins. The ligands were docked into the binding domain using the LigandFit module in DS 2.5 [27]. All the parameters were set to their default and molecular docking was performed. The resulting best pose with maximum dock score was extracted and imaged through BIOVIA Discovery studio 4.5 software. All the runs were performed in triplicates.

Molecular Dynamics (MD) Simulation

MD simulations and analyses were performed for each protein–ligand system using the GROMACS 4.5.5 program. The force field parameters such as GROMOS96 and PRODRG tool were used to clean the receptor and ligands structures. The simulation system was applied with SPC explicit solvation model, inside the dodecahedron box (1 nm). The protein and protein–ligand complex were imported into the box. Energy minimization of the system was carried out using Maxwell Boltzmann distribution at 300 K with a periodic scaling of 0.1 ps. The initial pre-simulation run of 20 ps was applied and the geometric restraints were removed. Using the hybrid water system, the steepest descent energy minimization and conjugant gradient were carried out each for 1000 steps. Initially, the temperature was adjusted with a simple simulation run of 1200 ps. Later, constant conditions of 200 ps were adjusted for the number of particles, system volume, and temperature (NVT). The density of the system was adjusted with the initial run of 500 ps of the number of particles, pressure, and temperature (NPT). Finally, the equilibrated structure was further subjected to 20 ns production dynamic at the temperature of 310 K and 1 bar of pressure. The trajectory files were saved for every 100 ps

time step. The root-mean-square deviation (RMSD) of the backbone was calculated with the first frame as a reference obtained through simulation run [28].

Impact of Eicosane on *Candida albicans* Biofilm Formation

Biofilm Inhibition Assay

Eicosane was introduced to the Sabouraud dextrose broth (SDB) containing 10^8 cells/mL of *Candida albicans* at doses ranging from 12.5 to 200 µg/mL. The microtitre plate (24 well) was incubated for 48 h at 37 °C. The planktonic cells were removed after incubation and the biofilm-formed wells were stained with 0.4 percent crystal violet and Milli-Q was used to eliminate excess staining in the wells, which was then air-dried. After the addition of 1 mL of ethanol to the well plate, the optical density (OD) value was measured using a multimode reader (Spectramax M3, USA) at 570 nm. The following calculation was used to calculate the percentage of biofilm inhibition (BI):

$$\% \text{ BI} = ((\text{OD of Control} - \text{OD of Treated})/(\text{OD of Control})) \times 100$$

The minimal concentration, at which maximal reduction in biofilm formation was observed, was considered as Biofilm Inhibitory Concentration (BIC) [29].

Light and Confocal Laser Scanning Microscopic (CLSM) Observation

C. albicans were cultured on glass slides (1 × 1 cm) and tested for the antibiofilm activity of Eicosane. The slides were placed in the microtitre plate wells which contained SD broth enriched with BIC of Eicosane. The wells that did not contain Eicosane were used as a control. The well plate was incubated for 48 h at 37 °C. The slides were removed after incubation, washed with Milli-Q, stained with 0.4% CV, and then dried to remove any remaining stain. After that, the biofilm formation on the slides was examined and pictures were taken under 400 × magnification using Nikon Eclipse 80i, USA [30]. For CLSM, the glass slides were stained with 0.1% acridine orange. The excess stain was removed and the glass plates were dried and imaged under CLSM integrated with Zen 2009 software (LSM 710, Carl Zeiss, Germany) at 200×. The complete examination of significant characteristics such as maximum thickness and biomass of control and treated images were evaluated using the statistical tool COMSTAT2 to determine the efficacy of Eicosane in biofilm reduction [31].

Scanning Electron Microscope (SEM)

The formation and development of *C. albicans* developed biofilm was investigated using SEM. The successfully formed *C. albicans* biofilms were preserved in 2.5% of glutaraldehyde solution for 1 h. The slides were dried using 25%, 50%, and 100% ethanol for 3 min. Then slides were gold sputtered and examined in SEM (Vega 2 Tescan, Czech Republic) [30].

Quantitative Polymerase Chain Reaction (PCR) Analysis for Biofilm Genes

Triazol (Sigma-Aldrich) method was used to isolate the total RNA from control and Eicosane (at BIC) treated *C. albicans*. The resultant RNA was measured using a Shimadzu nano spectrophotometer and reverse transcribed into cDNA with high-capacity

cDNA reverse transcription kit (Applied Biosystems, USA). The attachment and hyphal development genes (*ALS3*, *EAP1*, *NRG1*, *HWP1*) were investigated. The expression pattern of genes was standardized using the reference gene (*ITS* gene). Table 1 shows the list of potential genes as well as the primer sequence. According to the manufacturer's guidelines, the produced cDNA was combined with 2 × QuantiNova SYBR Green RT-PCR Master Mix (Qiagen Germany) and qPCR was done in the 7500-sequence detection system (Applied Bio systems Inc. Foster, CA, USA). The $2^{-\Delta\Delta CT}$ approach was used to find the percentage fold change in gene expression. All the experiments were performed in triplicate.

Effect of Eicosane on Zebrafish Infected with *Candida albicans*

Wild-type zebrafish were kept at room temperature and nourished on a 10-h light/14-h dark cycle. The eggs were obtained by spontaneous pair-wise breeding, as described by Westerfield 1993 [32]. The trials were performed in accordance with the general criteria of the Institutional Animal Ethics Committee, Alagappa University (Reg NO: 2007/GO/ReBi/S/18/CPCSEA dt 14/03/2018). The investigations were performed on healthy embryos. Mature zebrafish eggs were harvested, rinsed with deionized water, and divided into three groups namely control, infection, and treated. Ten embryos were used in each well in each phase of the study. In a 12-well microtiter plate, positive control embryos, embryos with *C. albicans* were maintained as an experimental model. BIC of Eicosane was administrated to the co-incubated embryos to examine the antivirulence impact of the Eicosane. This plate was incubated at 30 °C for 48 h and their activity was periodically checked during various time intervals (0, 24, 48 h). Using a Leica M165FC stereo microscope, the images were observed [33].

Retrieval of SMILES and Compound Target Network (C-T-N) Analysis

The respective canonical SMILES of Eicosane was retrieved from the PubChem database. The SMILE was searched against the Homo sapiens database using the Swiss target prediction database (www.swisstargetprediction.ch/) tool. The human targets showing interaction with Eicosane were retrieved for further analysis. The molecular interactome represents the interaction of the target protein with the bioactive molecule (Eicosane). From the interactome, the compounds were denoted as nodes and edges are Eicosane. The network was visualized by Cytoscape v3.8.2.

Table 1 The list of genes with primer sequence

S. No	Gene	Forward primer	Reverse primer
1	*ITS*	ATTGTAGGTGAACCTGCGG	TCCTCCGCTTATTGATATGC
2	*EAP1*	TGTGATGGCGGTTCTTGTTC	GGTAGTGACGGTGATGATAGTGACA
3	ALS3	ACCTTACCATTCGATCCT AACC	GATGGGGATTGTGAAGTGG
4	*NRG1*	CCAAGTACCTCCACCAGCAT	GGGAGTTGGCCAGTAAATCA
5	*HWP1*	GCTCCTGCTCCTGAAATGAC	CTGGAGCAATTGGTGAGGTT

Results and Discussion

Molecular Docking with Wild-Type and Mutant-Type *ALS3*

Ligand ALS3 is a member of the agglutinin-like sequence from *C. albicans*, and the mature protein sequence contains 299 amino acids. *ALS3* mediates the entry of the organism through attachment to the host epithelial and endothelial cells, as well as through host cell receptors such as E-cadherin and N-cadherin. *ALS3* is essential for biofilm formation on abiotic and biotic surfaces. The other functions associated with *ALS3* include hyphal formation and binding of ferritin protein from the host cell and utilize iron as a functional source. Thus, *ALS3* with a multifaceted role is determined as the promising target for antivirulence therapy. In the present study, *ALS3* (both wild and mutant type) was docked with 10 ligands identified from ethyl acetate extract of *S. diastaticus* [25]. Among them, Eicosane achieved the top hit with the highest docking score of 20.71 and 19.03 on interaction with wild and mutant type, respectively (Table 2).

Mutant type of *ALS3* used in the current investigation is a triple mutant (*K59M*, *A116V*, and *Y301F*), and it is present in the binding cavity. Eicosane with the wild type has shown one alkyl interaction with *VAL161* and two van der Waals interactions. However, docking of Eicosane with mutant type showed Pi-alkyl (*TRP295*) and alkyl (*LEU182*) interactions. Thus, docking studies with wild and mutant type *ALS3* divulged that Eicosane can interact with binding pocket residues of both wild and mutant types regardless of mutations in the binding region (Fig. 1). Based on the results from docking analysis, it was identified that Eicosane has the ability to interact more efficiently with *ALS3* of *C. albicans*. Therefore, for further in vitro and in vivo investigations, Eicosane procured from Sigma-Aldrich was employed to study their impact on *C. albicans* biofilm formation.

Molecular Dynamics Simulation

To understand the stable interaction of the ligand with the receptor (wild-type and mutant-type), molecular dynamics simulation was performed for 20 ns (Fig. 1C). All the trajectories for the protein and the ligand interaction complex, with the information about the coordinates of all atoms, were analyzed. RMSD analysis of *ALS* mutant-type (with the evolution with time) showed the fluctuation around 4 Å to 4.5 Å. However, the interaction of eicosane with wild-type and mutant-type showed the RMSD fluctuation around 5 to 5.5 Å. Thus, from the RMSD plot, it was observed that the introduction of mutation into the wild-type protein resulted in fluctuation in RMSD. However, the interaction of the eicosane with the backbone of the mutant-type *ALS r*esulted with a stable equilibrium of the protein-drug

Table 2 The top five ligands showing interaction with both wild and mutant type of ALS3 is represented with scoring functions

Compound name	PubChem ID	Dock score	
		Wild-type	Mutant-type
2-bromotetradecane	12798926	19.641	9.38
Dodecane	8182	11.962	8.65
Eicosane	8222	20.701	19.033
3-Methyl heneicosane	522120	13.572	15.81
Hexadecane	11006	16.695	11.66

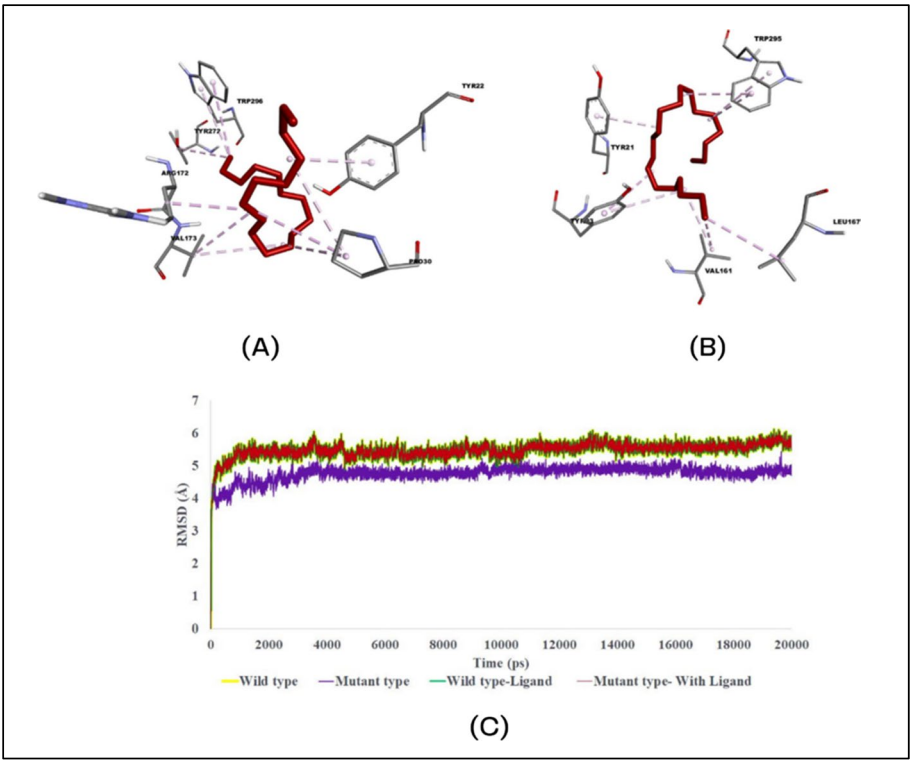

Fig. 1 2D micrograph of Eicosane showing interaction with the *ALS3*. **A** Wild-type *ALS3*. **B** Triple mutant-type *ALS3*. **C** Molecular dynamic simulation represented with RMSD plot for the proteins (wild-type and mutant-type) showing interaction with the ligands

complexes. Thus, the average RMSD of C-alpha backbone for wild-type, wild-type with ligand, and mutant with the ligand were similar. Altogether, these results are suggestive that ligands form a stable complex with *ALS3* regardless of wildtype or mutant phenotype.

Impact of Eicosane on *C. albicans* Biofilm Formation In Vitro Condition

Biofilm Inhibition Assay

Static biofilm assay revealed the inhibitory effect of Eicosane against *C. albicans* biofilm formation. At all tested concentrations ranging from 12.5 to 200 µg/mL, Eicosane exhibited a considerable antibiofilm effect, in a concentration-dependent manner without exhibiting a negative impact on the growth of *C. albicans*. Eicosane at 100 µg/mL of was observed to be the least concentration that showed > 50% reduction of biofilm formation of *C. albicans* (60%) and above this, there was no significant decrease in antibiofilm effect and there was no considerable change in the growth of the *C. albicans* with the increasing concentration (Fig. 2A, B). In accordance with these results, 100 µg/mL was determined as BIC of Eicosane against *C. albicans* and considered for the succeeding experimentations.

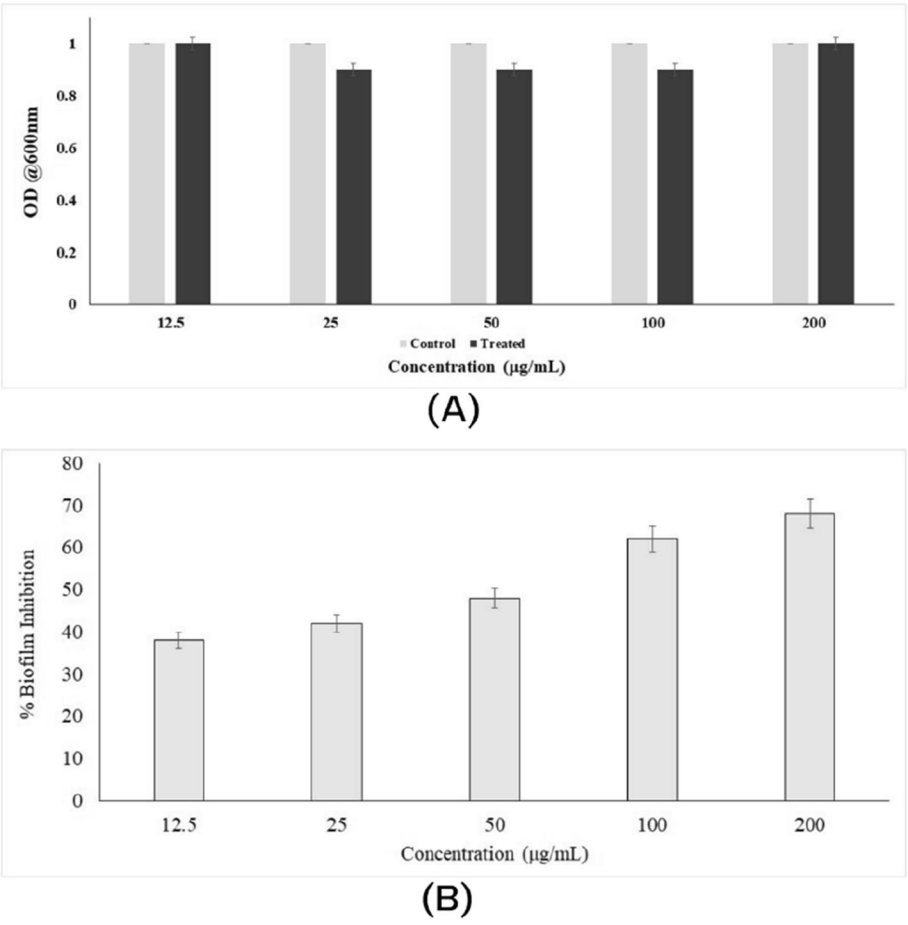

Fig. 2 **A** Antibiofilm activity of Eicosane determined at different concentrations ranging from 12.5 to 200 µg/mL against *C. albicans*. **B** Growth of *C. albicans* at different concentrations of Eicosane ranging from 12.5 to 200 µg/mL

Light and CLSM Microscopic Observation

C. albicans visualized under light and CLSM microscopy divulged the yeast cells' adherence and colonization to the matrix surface in the case of control samples. However, Eicosane-treated slides showed reduced microbial adherence, and only scanty cells were observed on the matrix surface (Fig. 3A, B). Moreover, an increase in Eicosane concentration has shown a dose-dependent significant reduction in the adhesion of the yeast cells to the matrix surface and subsequently prevented the colonization. Statistical analysis using COMSTAT2 highlighted the decrease in biomass and thickness of biofilm in the presence of Eicosane (Table 3).

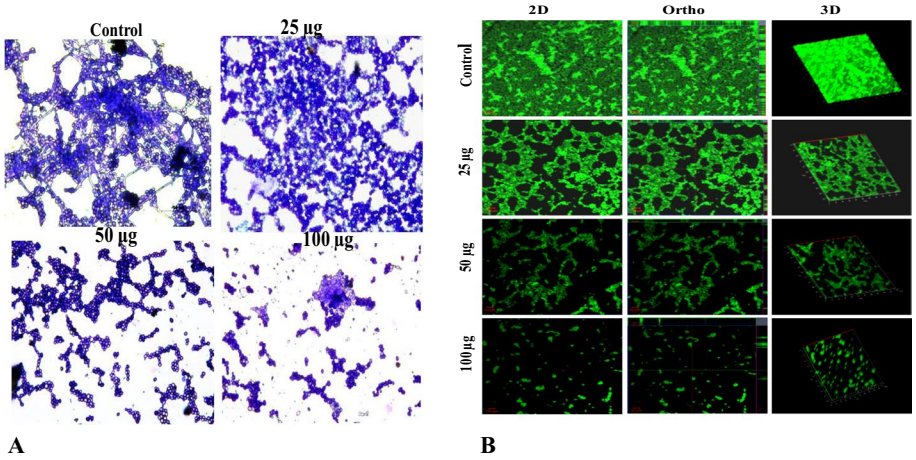

Fig. 3 **A** Microscopic analysis of *C. albicans* biofilm under a light microscope at (400×). The untreated and Eicosane-treated represent reduced hyphal development and microbial adherence to the matrix surface. **B** CLSM images representing control and treated biofilm of *C. albicans* in a different view

Table 3 COMSTAT analysis for control and treated (Eicosane at BIC concentration (100 µg/mL)) biofilms of *C. albicans*

S. No	Statistical parameters	*C. albicans*	
		Control	Eicosane-treated
1	Biomass	26.5	17.9
2	Maximum thickness	24.5	16.1

SEM Microscopic Observation

The SEM analysis revealed the complex cluster of biofilm formation in control glass slides whereas remarkable inhibition of biofilm was observed in Eicosane-treated glass slides. The Eicosane-treated SEM micrographs show only fewer cells adhering to the matrix and aggregation of cells were considerably reduced at 100 µg/mL. The significant reduction in the biofilm formation observed in SEM analysis is depicted in Fig. 4. From the overall analysis of light, CLSM, and SEM microscopy, it was evident that the Eicosane at BIC concentration (100 µg/mL) possesses the antibiofilm activity against *Candida albicans*.

Quantitative PCR Analysis for Biofilm Genes

Treatment of *C. albicans* biofilm with Eicosane at BIC resulted in a threefold decrease in *EAP1* and *ALS3* transcript levels. *EAP1* is the cell wall protein of *C. albicans*, which mediates cell–cell, cell-substrate adhesion, and a key player of biofilm formation. Interestingly, there was a tenfold decrease in the expression of *HWP1* transcript level in the presence of Eicosane. Apparently, there was no significant difference in the transcript level of *NRG1* in

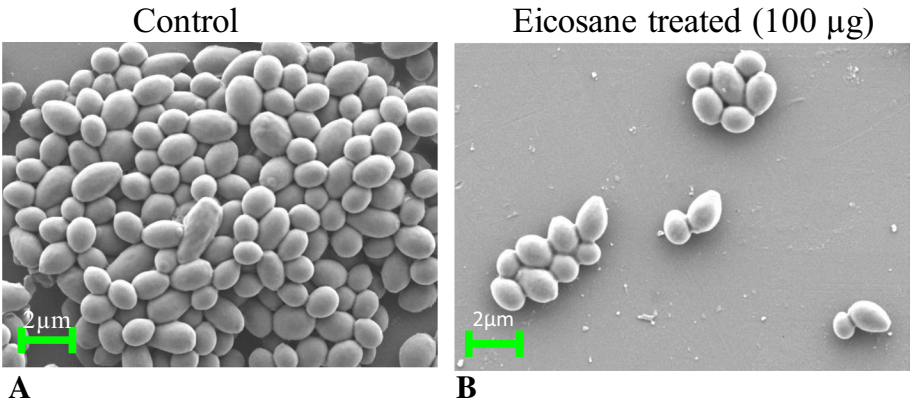

Fig. 4 SEM images of *C. albicans* biofilms representing reduced cell–cell adhesion and interaction upon Eicosane treatment (at BIC)

C. albicans treated with Eicosane. *NRG1* is the antagonist for hyphal growth, thus from the results, it was observed that Eicosane does not alter the *NRG1* expression level. Thus, from the comparative analysis with control and treated mRNA transcripts it was observed that Eicosane has significantly decreased the expression of cell-to-cell adhesive genes (*EAP1*, *ALS3*, and *HWP1*) required for biofilm integrity, attachment to host, and virulence (Fig. 5). Moreover, the biofilm slide assay correlates well with the qPCR results where it showed decreased expression of genes such as *EAP1*, *HWP1*, and *ALS3*, responsible for biofilm formation in *C. albicans.*

Effect of Eicosane on Zebrafish Infected with *Candida albicans*

Through the infection model, adherence and colonization of yeast cells on chorion of zebrafish embryos were evaluated. The infected embryos were witnessed with the colonization of the yeast cells on the chorion. This further mitigated the chorion to enter the next larval stage. Chorion is the protective sac for the embryo from external influences and it

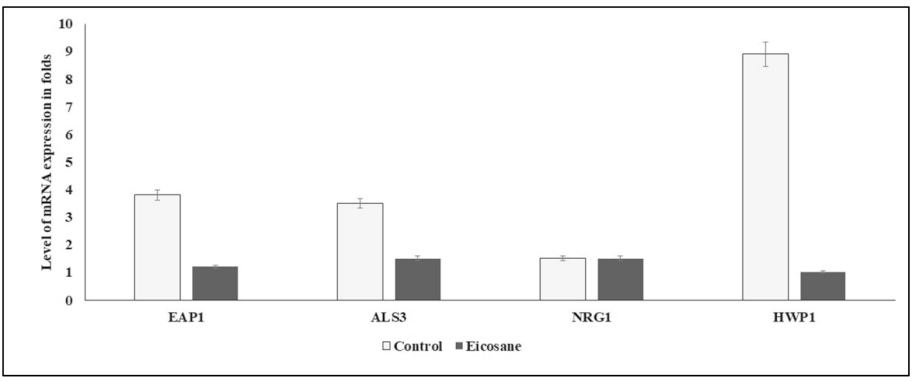

Fig. 5 Transcription analysis of biofilm genes from control and Eicosane treated *C. albicans*

mediates the absorption of the nutrient to the embryo [34]. Here, yeast cell colonization in the infection groups has prevented the absorption of nutrients. This resulted in the death of the embryos due to a lack of nutrients. Strikingly, Eicosane treated embryos showed decreased adhesion of the yeast cells on the chorion and allowed the development of the embryo to larvae. Adhesion and colonization are the important stage for the yeast cell to form biofilm. Thus, mitigating at the initial stage would prevent the formation of biofilm. Moreover, Eicosane has been evidenced to exert substantial inhibition of adhesion on chorion (Fig. 6).

Retrieval of SMILES and Compound Target Network (C-T-N) Analysis

Figure 7 represents the compound-target-network cross-talk between eicosane and 75 genes from H. sapiens. The interactome is represented in blue color (Fig. 8). Further subnetwork analysis revealed 18 target classes including family AG protein-coupled receptor, enzyme, electrochemical transporter, kinase, voltage-gated ion channel, ligand-gated ion channel,

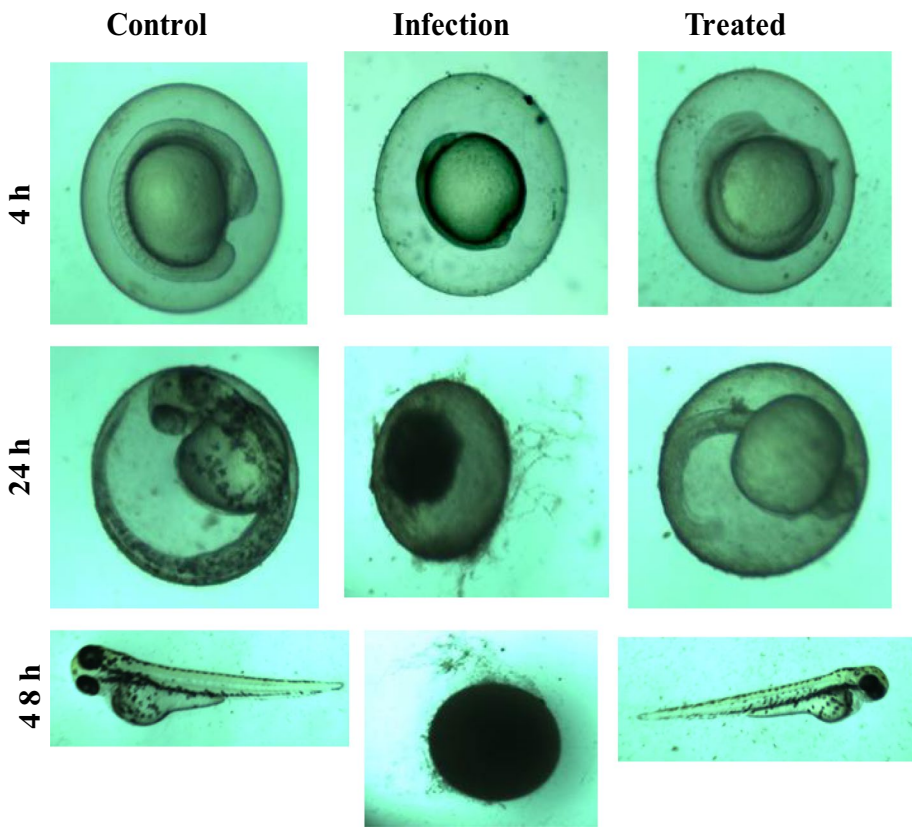

Fig. 6 Zebrafish embryo infection model. Infection groups represent the attachment of yeast cells to chorion of the embryo and well-developed hyphae at 24 h and 48 h. Hyphal formation eventually resulted in the mortality of the embryo. Embryos treated with Eicosane showed substantial reduction of *C. albicans* on the chorion and the embryos were hatched to larvae at 48 h

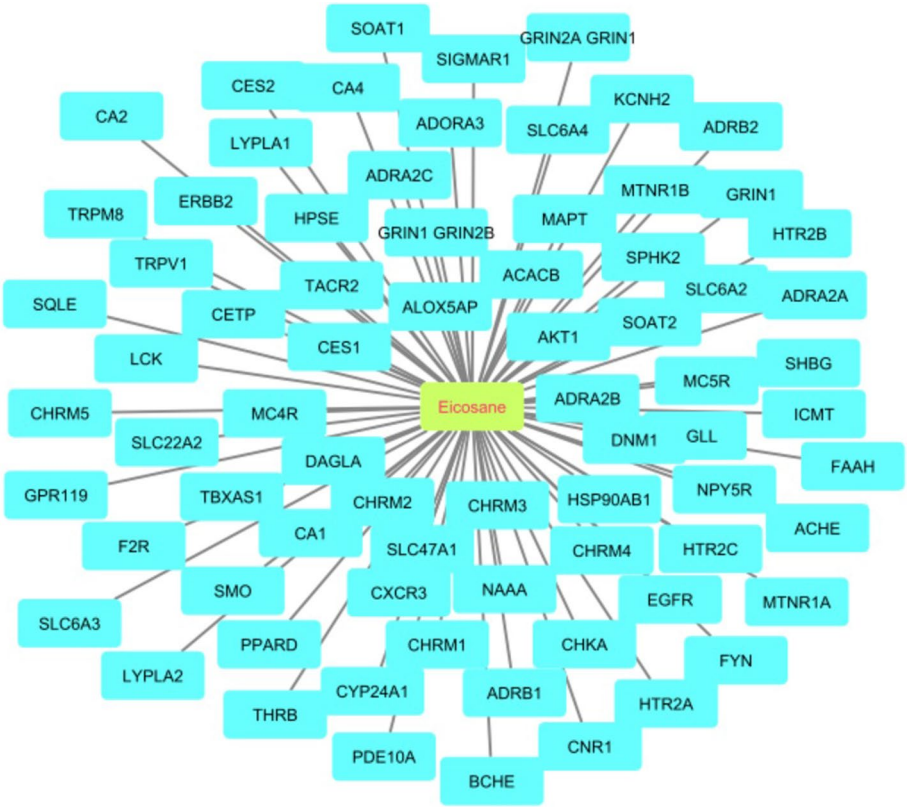

Fig. 7 The compound-target-network representing the cross-talk between eicosane and other human proteins

lyase, hydrolase, other cytosolic protein, nuclear receptor, secreted proteins, membrane receptors, ligase, unclassified protein, phosphodiesterase, and other ion channel genes from the human. The important genes interacting with these classes are shown in Fig. 8. Overall, the network analysis revealed the interacting potential of eicosane with human genes.

Discussion

Adhesions of *C. albicans* to the host tissues are indispensable for *C. albicans* infection. Attachment of the yeast cells to the abiotic surfaces or receptors of host is usually achieved by the expression of Agglutinin-like sequence (*ALS*) [35]. The members of the *ALS* family include eight genes (*ALS1-ALS8*), among them, *ALS3* is the most important gene requisite for host infection. *ALS3* is one among the *ALS* family members involved in adherence to host epithelial cells, endothelial cells, and extracellular matrix proteins (E-cadherin and N-cadherin) [36]. Binding of the *ALS3* to the E-cadherin and N-cadherin mediates the host cells to endocytose the yeast cells and thereby promotes the hyphal formation inside the phagocytotic cells. The hyphal penetrates the host membrane and evades the phagocytosis effects [37, 38]. Additionally, *ALS3* also interacts with the microbial pathogens in a mixed

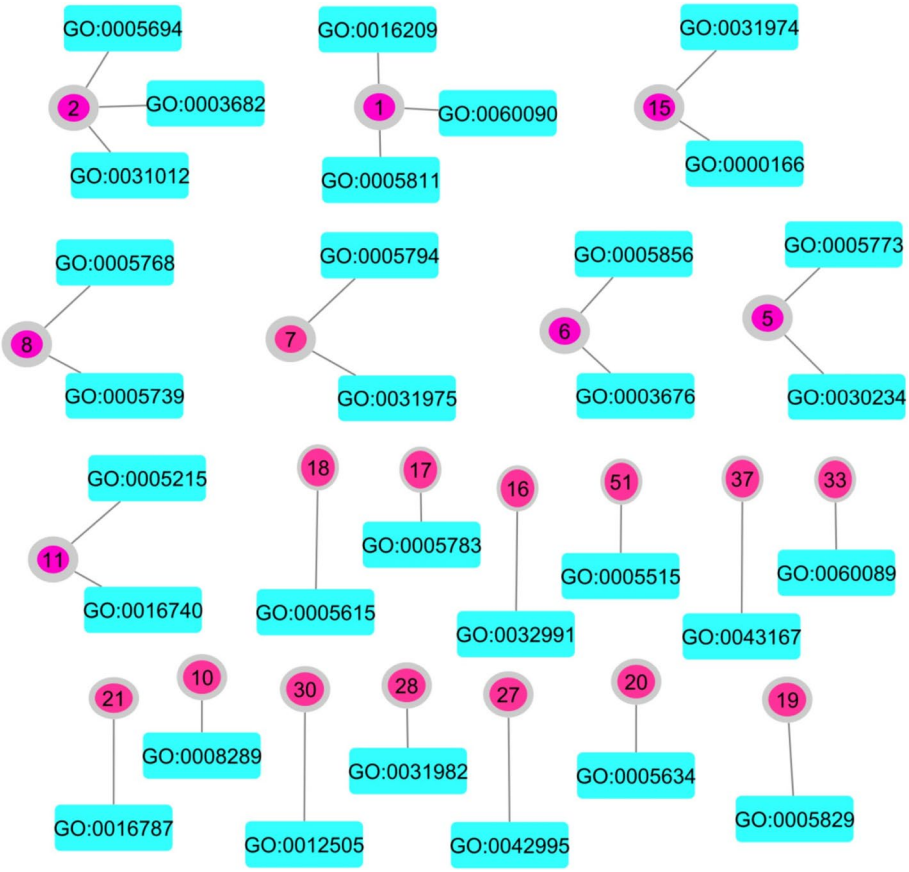

Fig. 8 Different classes of target proteins involved in several biological processes based on their GO classification. Color codes: cyan represents the gene ontology number and pink represents the number of proteins involved from the target class of GO

biofilm environment, which exaggerate the morbidity and mortality rate [39]. The hyphal wall protein (*HWP1*) is obligatory for fungal growth and pathogenesis. Hyphal forms of yeast cells are crucial for causing asymptomatic infections [40, 41]. Interestingly, one more key adhesion gene known as extracellular adherence protein (*EAP1*) mediates the cell-to-cell adhesion and colonization of *C. albicans*. The colonization process appears at the point of contact with the indwelling devices and host cells. *EAP1* can potentially restore haploid invasive growth and diploid pseudohyphal formation [42]. *NRG1*, a DNA binding protein represses the filamentous growth of *C. albicans*. Downregulation of *NRG1* is reported to promote the hyphal growth of *C. albicans*. Thus, *NRG1* is an indicator and natural antagonist for hyphal forms of *C. albicans* [43]. However, several extracellular enzymes with functional redundancy are produced by *C. albicans* such as secreted aspartyl proteinases (Saps), phospholipases, DNase, and hemolysin [44]. These external factors aid in host invasion, increased adherence, degradation of host defense proteins, and mainly nutrient acquisition.

From the overall results, it was observed that Eicosane has significant antibiofilm activity against *C. albicans*. *ALS3* of *C. albicans* is considered as a promising target for antifungal therapy due to its multiple functions such as adhesion, hyphal formation, and nutrient acquisition. In the present study, Eicosane, identified from ethyl acetate extract of *S. diastaticus* was docked into the binding pocket of *ALS3* (both wild type and mutant-type). Eicosane has the ability to interact with the binding pocket residues of both wild and mutant-type. Thus, Eicosane has been considered as the top hit and further in vitro and in vivo investigations were carried out. Both in vitro and in vivo results revealed the efficacy of the antibiofilm potential of Eicosane against *C. albicans*. Eicosane has already been used as a food additive, food contact products (such as coating of paper plates, cups), in cosmetics, and plasticizer. As a future perspective, Eicosane inhibiting *C. albicans* biofilm can be used as an active ingredient for preparing ointment and toothpaste as well as for topical applications to treat biofilm-mediated infections caused by *C. albicans*.

In conclusion, *Candida*, an opportunistic pathogen, can cause severe invasive fungal bloodstream infections in immunocompromised patients. Candidemia and Candidiasis are the most common infections among nosocomial fungal infections. Besides the appearance and spreading of multi-drug resistance, *Candida* species are detected all over the globe. At present, there is no successful antibiotic treatment to treat *Candida* infections [45]. Therefore, the present study was aimed to identify a promising antibiofilm agent against *C. albicans* from the economically important bacteria *Streptomyces*. Although Eicosane has been previously identified as a plant metabolite and reported for pharmacological activity including antifungal [46], wound healing [47], and antiviral activities [48] there is no report on antibiofilm activity. The current study for the first time unveiled the antibiofilm activity of the bioactive molecule "Eicosane" against *C. albicans* through in vitro and in vivo analysis. The concentration at which maximal biofilm inhibition was observed was also revealed. Besides, the study also describes the binding effect of eicosane on mutant and wildtype *ALS3*, a member of agglutinin-like sequence from *C. albicans*, required for entry of the organism.

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Author Contribution Conceptualization, Writing Original draft, Validation: Raja Mohamed Beema Shafreen, Siddharthan Seema; Methodology: Selvaraj Alagu Lakshmi; Project Administration: Adimoolam Srivathsan; Formal analysis: Kannapiran Tamilmuhilan; Investigation: Anupama Shrestha; Visualization: Balamuralikrishnan Balasubramanian; Writing review and Editing, Data curation, Software: Ranjithkumar Dhandapani, Ragul Paramasivam; Funding: Sami Al Obaid, Saleh H. Salmen; Supervision: Saravanan Muthupandian

Data Availability All the data are available within the manuscript and its supplementary document

Declarations

Ethical Approval This ethical clearance for this study was approved by Institutional Animal Ethics Committee, Alagappa University (Reg NO: 2007/GO/ReBi/S/18/CPCSEA dt 14/03/2018).

Consent to Participate Not applicable

Consent for Publication Not applicable

Competing Interests The authors declare that they have no known competing financial interest or personal relationships that could have appeared to influence the work reported in this paper

References

1. Gagne, J. J., & Goldfarb, N. I. (2007). Candidemia in the in-patient setting: Treatment options and economics. *Expert Opinion on Pharmacotherapy, 8*, 1643–1650.
2. Ramage, G., Jose, A., Sherry, L., Lappin, D. F., Jones, B., & Williams, C. (2013). Liposomal amphotericin B displays rapid dose-dependent activity against Candida albicans biofilms. *Antimicrobial Agents and Chemotherapy, 57*, 2369–2371.
3. Bandele, D. A., Nnenna, U. U., Victoria, A. O., & Solomon, B. A. (2021). Isolation and evaluation of Candida species and associated microbes causing gastrointestinal tract infections among patients accessing care at a tertiary health facility in Lagos, Southwest Nigeria. *Biomedical Journal of Scientific & Technical Research, 36*, 28423–28432.
4. Ganguly, S., & Mitchell, A. P. (2011). Mucosal biofilms of Candida albicans. *Current Opinion in Microbiology, 14*, 380–385.
5. Kabir, M. A., Hussain, M. A., & Ahmad, Z. (2012). Candida albicans: A model organism for studying fungal pathogens. *ISRN Microbiol, 2012*, 538694.
6. Villa, S., Hamideh, M., Weinstock, A., Qasim, M. N., Hazbun, T. R., Sellam, A., ... & Thangamani, S. (2020). Transcriptional control of hyphal morphogenesis in Candida albicans. *FEMS yeast research, 20*(1), foaa005.
7. Priya, A., & Pandian, S. K. (2020). Piperine impedes biofilm formation and hyphal morphogenesis of Candida albicans. *Frontiers in Microbiology, 11*, 756.
8. Pereira, R., dos Santos Fontenelle, R., de Brito, E., & de Morais, S. (2021). Biofilm of Candida albicans: Formation, regulation and resistance. *Journal of Applied Microbiology, 131*, 11–22.
9. Atriwal, T., Azeem, K., Husain, F. M., Hussain, A., Khan, M. N., Alajmi, M. F., & Abid, M. (2021). Mechanistic understanding of Candida albicans biofilm formation and approaches for its inhibition. *Frontiers in Microbiology, 12*, 932.
10. Denega, I., d'Enfert, C., & Bachellier-Bassi, S. (2019). Candida albicans biofilms are generally devoid of persister cells. *Antimicrobial Agents and Chemotherapy, 63*, e01979-e1918.
11. Sudbery, P., Gow, N., & Berman, J. (2004). The distinct morphogenic states of Candida albicans. *Trends in Microbiology, 12*, 317–324.
12. Alim, D., Sircaik, S., & Panwar, S. L. (2018). The significance of lipids to biofilm formation in Candida albicans: an emerging perspective. *Journal of Fungi, 4*(4), 140.
13. Denega, I., d'Enfert, C., & Bachellier-Bassi, S. (2019). Candida albicans biofilms are generally devoid of persister cells. *Antimicrobial Agents and Chemotherapy, 63*(5), e01979–18.
14. Ponde, N. O., Lortal, L., Ramage, G., Naglik, J. R., & Richardson, J. P. (2021). Candida albicans biofilms and polymicrobial interactions. *Critical Reviews in Microbiology, 47*, 91–111.
15. Mourer, T., El Ghalid, M., d'Enfert, C., & Bachellier-Bassi, S. (2021). Involvement of amyloid proteins in the formation of biofilms in the pathogenic yeast Candida albicans. *Research in Microbiology, 172*, 103813.
16. Johnson, M. D., & Perfect, J. R. (2010). Use of antifungal combination therapy: Agents, order, and timing. *Current Fungal Infection Reports, 4*, 87–95.
17. Malani, A. N., & Kauffman, C. A. (2007). Candida urinary tract infections: Treatment options. *Expert Review of Anti-Infective Therapy, 5*, 277–284.
18. Klepser, M. (2010). The value of amphotericin B in the treatment of invasive fungal infections. *Journal of Critical Care, 26*(2), 225-e1.
19. de Barros, P. P., Rossoni, R. D., de Souza, C. M., Scorzoni, L., Fenley, J. C., & Junqueira, J. C. (2020). Candida biofilms: An update on developmental mechanisms and therapeutic challenges. *Mycopathologia, 185*, 415–424.
20. Bharadwaj, K. K., Rabha, B., Choudhury, B. K., Rosalin, R., Sarkar, T., Baishya, D., Chanu, N. B., Singh, Y. D., Panda, M. K., & Pati, S. (2021). Current strategies in inhibiting biofilm formation for combating urinary tract infections: Special focus on peptides, nano-particles and phytochemicals. *Biocatalysis and Agricultural Biotechnology, 38*, 102209.
21. Ghosh, S., Lahiri, D., Nag, M., Dey, A., Sarkar, T., Biswas, R., Dutta, B., Mukherjee, D., Pati, S., Pattanaik, S. (2022). Analysis of antibiofilm activities of bioactive compounds from honeyweed

(Leonurus sibiricus) against P. aeruginosa: An in vitro and in silico approach. *Applied Biochemistry and Biotechnology*, 1–17.
22. Ghosh, S., Saha, I., Dey, A., Lahiri, D., Nag, M., Sarkar, T., Pati, S., Rebezov, M., Shariati, M. A., Thiruvengadam, M. (2021). Natural compounds underpinning the genetic regulation of biofilm formation: An overview. *South African Journal of Botany*.
23. Meng, L., Sun, C., Zhang, C., Song, S., Sun, X., Ju, J., & Deng, Y. (2019). Efficacy of Compounds Isolated from Streptomyces olivaceus against the Morphogenesis and Virulence of Candida albicans. *Marine Drugs, 17*(8), 442.
24. Srivastava, V., & Dubey, A. K. (2016). Anti-biofilm activity of the metabolites of Streptomyces chrestomyceticus strain ADP4 against Candida albicans. *Journal of Bioscience and Bioengineering, 122*, 434–440.
25. Siddharthan, S., Rajamohamed, B. S., & Gopal, V. (2020). Streptomyces diastaticus isolated from the marine crustacean Portunus sanguinolentus with potential antibiofilm activity against Candida albicans. *Archives of Microbiology*. https://doi.org/10.1007/s00203-020-01918-8
26. Shafreen, R. M., Muthamil, S., & Pandian, S. K. (2014). Inhibition of Candida albicans virulence factors by novel levofloxacin derivatives. *Applied Microbiology and Biotechnology, 98*, 6775–6785.
27. Venkatachalam, C. M., Jiang, X., Oldfield, T., & Waldman, M. (2003). LigandFit: A novel method for the shape-directed rapid docking of ligands to protein active sites. *Journal of Molecular Graphics and Modelling, 21*, 289–307.
28. Shafreen, R. M. B., Selvaraj, C., Singh, S. K., & Pandian, S. K. (2013). Exploration of fluoroquinolone resistance in Streptococcus pyogenes: Comparative structure analysis of wild-type and mutant DNA gyrase. *Journal of Molecular Recognition, 26*, 276–285.
29. Subramenium, G. A., Swetha, T. K., Iyer, P. M., Balamurugan, K., & Pandian, S. K. (2018). 5-hydroxymethyl-2-furaldehyde from marine bacterium Bacillus subtilis inhibits biofilm and virulence of Candida albicans. *Microbiological Research, 207*, 19–32.
30. Nithyanand, P., Beema Shafreen, R. M., Muthamil, S., & Karutha Pandian, S. (2015). Usnic acid inhibits biofilm formation and virulent morphological traits of Candida albicans. *Microbiological Research, 179*, 20–28.
31. Vorregaard, M. (2008). *Comstat2 - a modern 3D image analysis environment for biofilms, in Informatics and Mathematical Modelling*. Technical University of Denmark.
32. Westerfield, M. (1993). *The zebrafish: A guide for the laboratory use of zebrafish (Brachydanio reriro)*. University of Oregon.
33. Beema Shafreen, R., Seema, S., Martinez-Ayala, A. L., Lozano-Grande, M. A., Robles-Sanchez, M., Szterk, A., Grishko, M., Hanuka, E., Katrich, E., & Gorinstein, S. (2019). Binding and potential antibiofilm activities of Amaranthus proteins against Candida albicans. *Colloids and Surfaces. B, Biointerfaces, 183*, 110479.
34. Chen, Z.-Y., Li, N.-J., Cheng, F.-Y., Hsueh, J.-F., Huang, C.-C., Lu, F.-I., Fu, T.-F., Yan, S.-J., Lee, Y.-H., & Wang, Y.-J. (2020). The effect of the chorion on size-dependent acute toxicity and underlying mechanisms of amine-modified silver nanoparticles in zebrafish embryos. *International Journal of Molecular Sciences, 21*, 2864.
35. Oh, S.-H., Schliep, K., Isenhower, A., Rodriguez-Bobadilla, R., Vuong, V. M., Fields, C. J., Hernandez, A. G., Hoyer, L. L. (2021). Using genomics to shape the definition of the agglutinin-like sequence (ALS) family in the Saccharomycetales. *Frontiers in Cellular and Infection Microbiology*, *11*, 794529–794529.
36. Williams, D., & Lewis, M. (2011). Pathogenesis and treatment of oral candidosis. *Journal of Oral Microbiology, 3*(1), 5771.
37. Liu, Y., & Filler, S. G. (2011). Candida albicans Als3, a multifunctional adhesin and invasin. *Eukaryotic Cell, 10*, 168–173.
38. Hirschfeld, J. (2014). Dynamic interactions of neutrophils and biofilms. *Journal of Oral Microbiology, 6*, 26102.
39. Peters, B. M., Ovchinnikova, E. S., Krom, B. P., Schlecht, L. M., Zhou, H., Hoyer, L. L., Busscher, H. J., van der Mei, H. C., Jabra-Rizk, M. A., & Shirtliff, M. E. (2012). Staphylococcus aureus adherence to Candida albicans hyphae is mediated by the hyphal adhesin Als3p. *Microbiology, 158*, 2975.
40. Naglik, J. R., Fostira, F., Ruprai, J., Staab, J. F., Challacombe, S. J., & Sundstrom, P. (2006). Candida albicans HWP1 gene expression and host antibody responses in colonization and disease. *Journal of Medical Microbiology, 55*, 1323.
41. Hollomon, J. M., Liu, Z., Rusin, S. F., Jenkins, N. P., Smith, A. K., Koeppen, K., Kettenbach, A. N., Myers, L. C., & Hogan, D. A. (2022). The Candida albicans Cdk8-dependent phosphoproteome reveals repression of hyphal growth through a Flo8-dependent pathway. *PLoS Genetics, 18*, e1009622.

42. Li, F., & Palecek, S. P. (2003). EAP1, a Candida albicans gene involved in binding human epithelial cells. *Eukaryotic Cell, 2*, 1266–1273.
43. Braun, B. R., Kadosh, D., & Johnson, A. D. (2001). NRG1, a repressor of filamentous growth in C. albicans, is down-regulated during filament induction. *The EMBO Journal, 20*, 4753–4761.
44. Ahmad Khan, M. S., Alshehrei, F., Al-Ghamdi, S. B., Bamaga, M. A., Al-Thubiani, A. S., & Alam, M. Z. (2020). Virulence and biofilms as promising targets in developing antipathogenic drugs against candidiasis. *Future Science OA, 6*, FSO440.
45. Fürnkranz, U., & Walochnik, J. (2021). Nosocomial infections: Do not forget the parasites! *Pathogens, 10*, 238.
46. Ahsan, T., Chen, J., Zhao, X., Irfan, M., & Wu, Y. (2017). Extraction and identification of bioactive compounds (eicosane and dibutyl phthalate) produced by Streptomyces strain KX852460 for the biological control of Rhizoctonia solani AG-3 strain KX852461 to control target spot disease in tobacco leaf. *AMB Express, 7*, 1–9.
47. Chuah, X. Q., Okechukwu, P. N., Amini, F., & Teo, S. S. (2018). Eicosane, pentadecane and palmitic acid: The effects in in vitro wound healing studies. *Asian Pacific Journal of Tropical Biomedicine, 8*, 490.
48. Chathuranga, K., Weerawardhana, A., Dodantenna, N., Ranathunga, L., Cho, W.-K., Ma, J. Y., & Lee, J.-S. (2021). Inhibitory effect of Sargassum fusiforme and its components on replication of respiratory syncytial virus in vitro and in vivo. *Viruses, 13*, 548.

Authors and Affiliations

Raja Mohamed Beema Shafreen[1,2] · Siddharthan Seema[1] · Selvaraj Alagu Lakshmi[2] · Adimoolam Srivathsan[2] · Kannapiran Tamilmuhilan[2] · Anupama Shrestha[3] · Balamuralikrishnan Balasubramanian[4] · Ranjithkumar Dhandapani[5] · Ragul Paramasivam[5] · Sami Al Obaid[6] · Saleh H. Salmen[6] · Mohamad Faiz Mohd Amin[7] · Saravanan Muthupandian[8]

1 Sathyabama Institute of Science and Technology, Chennai, Tamil Nadu, India

2 Department of Biotechnology, Alagappa University, Science Campus, Karaikudi, Tamil Nadu, India

3 Department of Biotechnology, School of Science, Kathmandu University, P.O Box: 6250, Dhulikhel, Nepal

4 Department of Food Science and Biotechnology, Sejong University, Seoul, South Korea

5 Chimertech Private Limited, Chennai 600082, India

6 Department of Botany and Microbiology, College of Science, King Saud University, PO Box-2455, Riyadh 11451, Saudi Arabia

7 Faculty of Earth Science, Universiti Malaysia Kelantan, Jeli Campus, Jeli, Kelantan, Malaysia

8 AMR and Nanotherapeutics Lab, Centre for Transdisciplinary Research (CFTR), Department of Pharmacology, Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai, India