

Antibiofilm activity and molecular docking studies of bioactive secondary metabolites from endophytic fungus *Aspergillus nidulans* on oral *Candida albicans*

S. S. Meenambiga¹*, K. Rajagopal²

¹Department of Bio-Engineering, School of Engineering, Vels University, Chennai- 600 117, Tamil Nadu, India.

²Department of Plant Biology and Biotechnology, Ramakrishna Mission Vivekananda College, Chennai- 600 004, Tamil Nadu, India.

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ABSTRACT

Aspergillus nidulans, an endophytic fungus isolated from the medicinal plant *Acacia nilotica* showed potent antifungal activity against oral pathogenic strains of *Candida albicans*. Biofilm forming *C. albicans* was isolated and tested for its susceptibility to various solvent extracts of *A. nidulans* using biofilm inhibition assay. Gas chromatography-mass spectrometry (GC-MS) analysis was performed to identify the potential compounds in the endophytic fungal extract and mode of interaction of these compounds with the target enzyme of *C. albicans* was revealed using molecular docking analysis. All the solvent extracts showed good inhibitory activity on the biofilm forming *C. albicans* strain with maximum activity in the chloroform extract. The percentage reduction in biofilm inhibition for chloroform, ethyl acetate, hexane and methanol extracts were 74.86%, 72.53%, 60.61% and 52.60% respectively. GC-MS analysis of chloroform extract revealed the presence of flavonoids, fatty acids, amides etc. of which the flavonoids-saltillin, taxifolin and 6-methoxyflavone showed good binding interaction with the *C. albicans* growth regulator N-myristoyltransferase (NMT). The bioactive compounds present in *A. nidulans* not only inhibit *C. albicans* growth but also controls the biofilm formation thereby reducing the virulence of *C. albicans*.

INTRODUCTION

The oral microflora comprises of more than 700 microbial species which makes it one of the most complex microbial flora of the human body. Over representation of pathogenic species contribute to the onset and progression of many oral diseases such as oral candidiasis, caries and periodontal diseases (Metwalli *et al.*, 2013). *C. albicans* is a commensal fungal species present in the human mucosal surfaces but they become opportunistic pathogens under conditions of immune dysfunction such as HIV infection and in malnourished individuals (Calderone and Gow, 2002). Pathogenicity of *C. albicans* is attributed to its ability to switch from its filamentous to hyphal forms which

adhere to tissue surfaces and leads to inflammation and infection by *C. albicans* is one of the leading causes of death in immune compromised patients. Available options for antifungal therapy are very limited when compared to bacterial antibiotics. However, prolonged antifungal therapy is linked with a significant risk of hematologic, hepatic, and/or renal toxicity. *C. albicans* causes both superficial and systemic disease. Further, with the use of commercial drugs for current antifungal therapy, invasive candidiasis has a mortality rate of about 40%. Candidiasis is usually associated with the attached medical devices (e.g., dental implants, catheters, heart valves etc.) which can act as substrates for biofilm growth. Biofilm formation is also critical in the development of denture stomatitis affecting 65% of toothless individuals. Infection is re-established with the use of antifungal drugs. These clinical observations reflect the biofilm formation to both superficial and systemic circulation and the inability of current antifungal therapy to cure such diseases (Vanden Abbeele *et al.*, 2008).

*Corresponding Author

S. S. Meenambiga, Department of Bio-Engineering, School of Engineering, Vels University, Chennai- 600 117, Tamil Nadu, India.
E-mail: meena.bt@gmail.com

Recent studies suggest that caries development is due to the interaction between fungal pathogen *C. albicans* and oral bacteria as there is high prevalence of *Streptococci* where *Candida* resides (Metwalli *et al.*, 2013). It has become very difficult to control these organisms because of their tolerance towards various antimicrobial agents in routine use during therapy. Natural products make excellent leads for new drug development which are safer and biodegradable. In addition to conventional and pharmacologically proven therapeutic measures, medicinal plants constitute an important source of new biologically active compounds.

Various medicinal plants have been traditionally used for oral ailments as chewing sticks since many years in developing countries and acquiring popularity in developed countries. The traditional use of *A. nilotica* for oral problems implies its antimicrobial activity against potential oral pathogens. Dried fruits of *A. nilotica* are active against *C. albicans* and used to treat oral candidiasis. Endophytic fungi which harbour almost all plant species have the characters similar to that of the host plants in novel secondary metabolite production. It was proposed that plants with immense medicinal values and ethnobotanical uses house endophytes which produce novel bioactive products (Strobel and Daisy, 2003).

Zhao *et al.* (2013) reviewed that the potential of endophytic fungi producing novel secondary metabolites like their host plants. Some of the plant secondary metabolites such as paclitaxel, camptothecin, hypericin and vinblastine are produced by the endophytic fungi colonizing them. Endophytic fungi studies on *A. nilotica* is relatively less explored and research on their diversity will ultimately result in novel taxa with antifungal activity against oral *C. albicans*. More targeted research is likely to be undertaken as *C. albicans* form stronger biofilms with the possibility of deriving active compounds from endophytic fungi that specifically inhibits biofilm formation.

For oral science, it is vital to understand the mechanism of biofilm formation, drug resistance and virulence of biofilms to develop compounds that can target dental biofilms to destabilize them (Guido and Andricopulo, 2008; Andricopulo *et al.*, 2009). N-myristoyltransferase (NMT) enzyme has been reported as a potential drug target which is involved in signaling networks and required for the growth of human pathogen *C. albicans* (Chen *et al.*, 2007).

The current study was aimed to study the endophytic fungi organic extracts isolated from *A. nilotica* against the oral biofilm forming *C. albicans*. As there are limited reports available on the biofilm inhibition efficiency of endophytic fungi and particularly from *A. nilotica* plant, this study would be an initiative of exploring novel compounds of endophytic fungi against oral *C. albicans*.

MATERIALS AND METHODS

Biofilm formation ability of collected oral *Candida albicans*

Pure cultures of *C. albicans* were identified and authenticated by Dr. Kayalvizhi, Microbiologist, Billroth Hospitals, Chennai. The yeast *C. albicans* was cultured on Sabouraud Dextrose Broth (SDB) (HiMedia Labs.) and the cultures were maintained on the agar slants at 4°C for further use.

Biofilm formation of 10 oral pathogenic strains of *C. albicans* was assessed by crystal violet assay and only the broth without cell suspension served as control (Feldman *et al.*, 2012). The value of optical density at 570 nm was considered as an index of forming biofilms. The mean OD value of the number of experiments performed was considered. The difference between the OD values of control and their respective test organisms was calculated (Kadurugamuwa *et al.*, 2003). If the difference is less than 0.1, it indicates weak biofilm formation and for values between 0.1 to 0.3, it indicates moderate biofilm formation. Isolates with OD values greater than 0.3, were considered as forming stronger biofilms (Dhanasekaran *et al.*, 2013).

Isolation and identification of endophytic fungi

The endophytic fungus used in this present work was isolated from the leaves of *A. nilotica* collected from reserved forest near Madurai, Tamilnadu, India. The segments measuring about 0.5 × 0.5 cm² were cut from the plant samples using sterile scalpel. Leaflets were collected and washed thoroughly with sterile distilled water and surface sterilized by immersing sequentially in 70% ethanol for 5 seconds and 4% sodium hypochlorite for 1 min and rinsed with sterile distilled water 2-3 times (Schulz *et al.*, 1993). The surface sterilized segments were inoculated on to petridishes containing Potato Dextrose Agar (PDA) media supplemented with chloramphenicol (50 µg/ml) to suppress bacterial growth. The plates were incubated at 25°C in a light chamber with 12 hours of light followed by 12 hours of dark cycles for 3-6 days (Bills *et al.*, 1992). Fungal hyphae tips growing out from sterile segments were sub cultured and the pure fungal isolates were transferred to PDA slants and stored at 4°C for further use. The endophytic fungi were identified using standard keys (Ellis, 1971).

Secondary metabolite extraction from the endophytic fungus

The endophyte isolated was grown on 500 ml of Potato Dextrose broth inoculated with a mycelial agar block taken from an actively growing colony on Potato Dextrose Agar plate. The flask cultures were incubated at 25°C for 2 weeks under stationary conditions. The fungal broth culture was filtered and the cell free supernatant was extracted using solvents such as ethyl acetate, chloroform, methanol and hexane. To the filtrate equal volume of solvent was added and mixed vigorously for 10 minutes. The organic layer was separated using separating funnel and the sample is dried. The dried residue was dissolved in dimethyl sulfoxide (DMSO) at a concentration of 1 mg/ml and stored at 4°C for further use.

Anticandidal assay of endophytic fungal extract by Agar well Diffusion method

Antimicrobial assay was carried out using agar well diffusion method in Mueller Hinton agar (Hi Media Labs.) as described by NCCLS 172056 (Barry *et al.*, 1999). Inoculum was standardized by picking six colonies of *C. albicans* in to the media and incubated at 37°C for 18-24 hours. Inoculum of the test microorganism was adjusted to 0.5 McFarland standard (10⁸ Cfu/ml) and 100 µl of the standardized inoculum was swabbed on to Mueller-Hinton agar for antimicrobial assay by agar well diffusion method. The wells were punched using sterile cork borer and 100 µl of 1 mg/ml concentration of fungal extracts were loaded on

to the agar wells. 100 µl of 0.12% chlorhexidine was used as positive control. The plates were incubated at 37°C for 24 hours. The diameter of inhibition zone around the well was measured in millimeter (Antibiotic zone scale, Hi Media Labs., Mumbai). The values of antimicrobial activity were expressed as mean ± standard error mean (n = 3) for each sample. Minimum inhibitory concentration of the extracts was then determined by the method described by Wariso and Ebong at a concentration range between 62.5 µg/ml and 500 µg/ml (Wariso and Ebong, 1996).

Biofilm inhibition assay using different solvent extracts of the endophyte

Biofilm inhibition assay of *C. albicans* was performed using various solvent extracts of *A. nidulans*. 100 µL of standardized cell suspension of *C. albicans* cells (1×10^6 cells/ml) were seeded in 96 well plate and 100 µL of solvent extracts at the sub-MIC concentration were added. The microtitre plate was incubated at 37°C for 24 hrs under stationary conditions. Culture medium was removed from the 96 well plate and the wells were washed with 200 µL of Phosphate Buffer saline (PBS). PBS was removed and the wells were stained with 1% Crystal violet solution to stain the polysaccharides of the biofilm. It was incubated for 10 minutes at room temperature. The plate was washed and drained upside down on paper towels and 1% SDS was added to solubilize the stain. The plate was agitated on orbital shaker until colour is uniform with no areas of dense coloration in bottom of wells. Absorbance of each well was measured at 570 nm. Chlorhexidine (0.12%) was used as a positive control which inhibits biofilm formation. Culture without adding endophytic fungal extract with the media served as negative control (Pitts *et al.*, 2003). The % of biofilm inhibition was calculated using the following formula.

$$\% \text{ of biofilm inhibition} = 100 \times \left(\frac{[\text{Control OD}_{570 \text{ nm}} - \text{Test OD}_{570 \text{ nm}}]}{\text{Control OD}_{570 \text{ nm}}} \right)$$

Gas Chromatography-Mass Spectrometry (GC-MS) analysis of the extract

The endophytic fungal extracts were analysed by GC MS which was done at SAIF, Indian Institute of Technology (IIT), Madras. The chloroform extract of endophyte was subjected to GC and MS JOEL GC mate equipped with secondary electron multiplier (Agilent Technologies 6890N Network GC system for gas chromatography). Hp5-MS column was used. Helium was the carrier gas. Temperature at the front inlet was 220°C and at the oven was 50°C to 250°C, gradually raised at 10°C/min. Ion chamber and GC interface temperature was maintained at 250°C. 1 µl of the sample was loaded at a temperature of about 300°C with a run time of 22 min. The mass spectrum of GC-MS was analyzed using the database of NIST (The National Institute of Standards and Technology). The name, structure and molecular weight of the compounds were recorded using NIST search.

Molecular docking studies

Molecular docking studies were performed for the active endophytic fungal components against the target enzyme of *C. albicans* by AutoDock 4.2. It is an automated docking tool which works by Lamarckian Genetic Algorithm. N-myristoyltransferase

(NMT) has been reported as a potential drug target which is involved in signalling networks and required for the growth of human pathogen *C. albicans* (Chen *et al.*, 2007). The three-dimensional structure of NMT (PDB id: 1nmt) which has 451 amino acids with 3 chains A, B and C was retrieved from Protein Data Bank (PDB) (Figure 2). The active site residues of NMT enzyme was retrieved from PDBsum software. To prepare for the docking experiment, the original protein coordinates extracted from the pdb file and the ligand pdb files from PRODRG server were submitted to AutoDock 4.2. The binding energy is obtained for each ligand and the contact analysis of the docked complexes was done using Discovery studio Visualizer 3.1.

Table 1: Screening of *Candida albicans* for biofilm formation.

S.No.	Codes of <i>Candida albicans</i> isolates	Quantitative assay (OD at 570 nm)	Biofilm formation
1	CA 01	0.421	Strong
2	CA 02	0.245	Moderate
3	CA 03	0.132	Moderate
4	CA 04	0.012	Weak
5	CA 05	0.142	Moderate
6	CA 06	0.210	Moderate
7	CA 07	0.462	Strong
8	CA 08	0.162	Moderate
9	CA 09	0.530	Strong
10	CA 10	0.228	Moderate

Table 2: Growth and biofilm inhibition efficiency of various solvent extracts of *Aspergillus nidulans* on oral isolate of *Candida albicans* (CA 09).

Extract	Growth inhibition (Zone of inhibition/ diameter in mm)	MIC value of the endophytic fungal extract (µg/ml)	Percentage of biofilm reduction
Ethyl acetate	18.4 ± 0.29	250	72.53 ± 0.51
Chloroform	20.6 ± 0.36	125	74.86 ± 0.57
Hexane	16.5 ± 0.5	>500	60.61 ± 0.22
Methanol	12.3 ± 0.43	>500	52.60 ± 0.28

Results are expressed as mean ± standard deviation (n = 3).

All the docked compounds were subjected to further selection for ADMET property analysis based on Lipinski's rule of five (Ro5) and compounds with any Ro5 violations were eliminated. Ro5 includes properties such as molecular weight, lipophilicity, molar refractivity, number of hydrogen bond donors and acceptors. The Molinspiration server (<http://www.molinspiration.com/cgi-bin/properties>) was used for calculating the physicochemical properties of compounds.

RESULTS AND DISCUSSION

Detection of biofilm formation of oral *Candida albicans*

Oral pathogens form biofilms to protect themselves from various stress conditions. Biofilm is a complex association of microorganisms formed on a solid surface. *Streptococcus mutans* and *C. albicans* are found to be the major organisms residing in the oral biofilms and both the pathogens are benefitted mutually because of their associations (Broden *et al.*, 2005). In this study,

the oral *C. albicans* collected were tested for their biofilm forming ability using the microtitre plate method. The isolates of *C. albicans* collected were given codes from CA 01 to CA 10. Out of the 10 isolates, 6 isolates showed moderate biofilm forming ability ($OD < 0.3$), 3 isolates showed strong biofilm activity ($OD >$

0.3), and 1 isolate was biofilm negative ($OD < 0.1$) (Table 1). The biofilm formation of the strain CA 09 was stronger with optical density value of 0.53 assessed by crystal violet staining and the strain CA 09 was selected for further study.

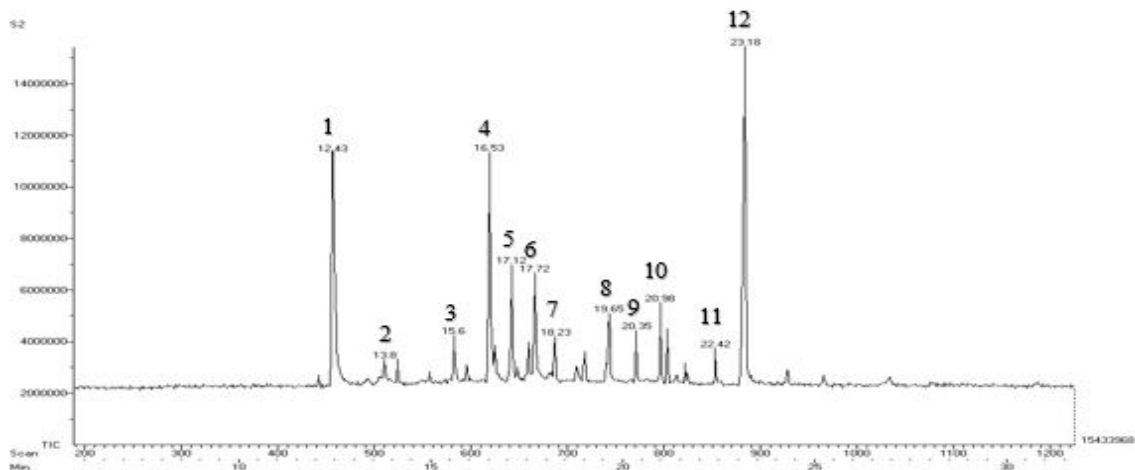


Fig. 1: GC-MS chromatogram of chloroform extract of *Aspergillus nidulans* showing the compounds and their retention time (within parentheses). (1) Phenol,2,4-bis(1,1-dimethylethyl) (12.43); (2) Eudesma-5,11(13)-dien-8,12-olide (13.8); (3) 6-methoxyflavone (15.6); (4) 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester (16.53); (5) Carbetamide (17.12); (6) 1-Eicosanol (17.72); (7) Flavone, 5-hydroxy-4'-methoxy-7-methyl (Saltillin) (18.23); (8) Acetamide, N-[2-(1,2-dihydro-1-hydroxy-2-oxo-3-quinolinyl) phenyl]- (19.65); (9) 4H-1-Benzopyran-4-one, 2-(3,4-dihydroxyphenyl)-2,3-dihydro-3,5,7-trihydroxy (Taxifolin) (20.35); (10) Oxamide, N,N'-bis(2'-acetylphenyl)- (20.98); (11) 3,4-Dihydroxy-1,6-bis-(3-methoxy-phenyl)-hexa-2,4-diene-1,6-dione (22.42) and (12) Bis-2-ethylhexyl phthalate (23.18).

Identification and optimization of endophytic fungus isolated from *Acacia nilotica*

In general, medicinal plants growing in extreme environmental conditions such as dry habitats harbour endophytes with bioactive secondary metabolites. The endophytes can synthesize secondary metabolite similar to their host plant (Strobel and Daisy; 2003). *A. nilotica* is traditionally used in treating oral diseases and have remarkable activity against the oral pathogens. In this study, the endophyte isolated from *A. nilotica* showed remarkable activity against the oral pathogen *C. albicans*. Interestingly, the extracts of endophytic fungi from various other medicinal plants have shown remarkable activity against *C. albicans* (Pranay Jain and Tarun Kumar, 2014; Elaasser *et al.*, 2011). In this study, the endophytic fungi isolated from *A. nilotica* was identified as *Aspergillus nidulans* (Eidam) G. Winter and authenticated by National Fungal Culture Collection of India (NFCCI), Agharkar Research Institute, Pune, India. This fungus was selected for the present study as it was among the 20 endophytes isolated that showed good antifungal activity against oral *C. albicans* (Meenambiga and Rajagopal, 2016). Conventional and statistical method of optimization of the endophytic fungi for enhanced antifungal activity against *C. albicans* was done by one factor at a time method and response surface method. Various process parameters such as growth media, different carbon and nitrogen sources, pH, temperature, incubation days were optimized for the extraction of bioactive compounds from the endophytic fungi (data not shown).

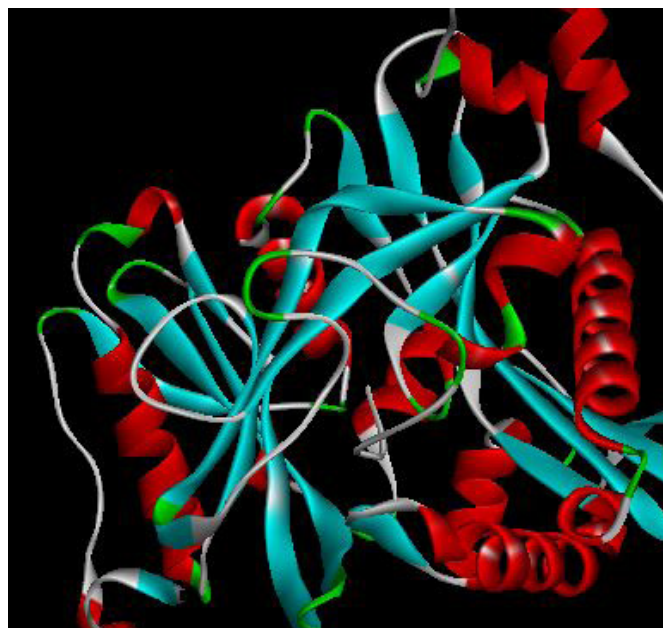


Fig. 2: Structure of N-myristoyltransferase enzyme of *Candida albicans* (PDB id: 1nmt) used for docking experiments with bioactive compounds of *Aspergillus nidulans*.

Anticandidal and antibiofilm activity of the endophyte *Aspergillus nidulans*

The anticandidal and antibiofilm activity of different extracts of the endophyte *Aspergillus nidulans* showed that all the

four different extracts have significant anticandidal effect and biofilm inhibition activity against oral *C. albicans* isolate (CA 09) (Table 2). The inhibition of *C. albicans* by different extracts depends upon the solvent used for extraction and the method of extraction procedure. The MIC value of the chloroform extract that inhibited oral *C. albicans* was found to be 125 µg/ml. The MIC value of chloroform extract was minimum when compared to other extracts. The biofilm inhibition efficiency of different solvent extracts of *A. nidulans* were tested by microtitre plate method. When the anti-biofilm activity of extracts such as ethyl acetate, chloroform, hexane and methanol were tested on the

selected biofilm forming strain CA 09, the percentage reduction of chloroform and ethyl acetate extracts were found to be 74.86% and 72.53% respectively. The growth inhibition of *C. albicans* by different solvents extracts of *A. nidulans* varied considerably due to the presence of one or more bioactive compounds in the extracts. Interestingly, the chloroform extract of *A. nidulans* was more potent against *C. albicans* growth and biofilm formation. Similar studies were done by [Martin-Rodriguez et al. \(2014\)](#) in which the extracts of marine endophytes such as *Fusarium* sp., *Khuskia* sp., *Epicoccum* sp. and *Sarocladium* sp. have good biofilm inhibition activity.

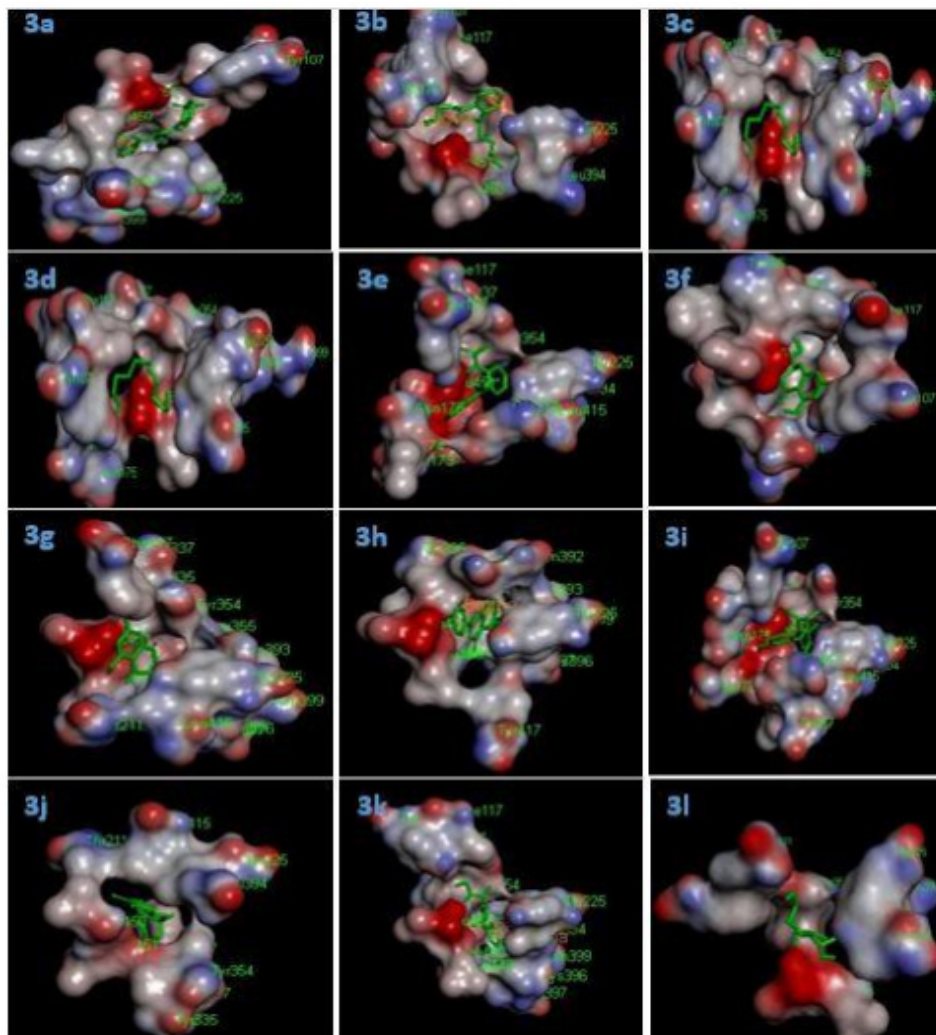


Fig. 3: *Candida albicans* N-myristoyltransferase enzyme (PDB id: 1nmt) docked confirmations with the compounds of the endophyte *Aspergillus nidulans* (a) Bis-2-ethylhexyl phthalate; (b) 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester; (c) 1-Eicosanol; (d) Oxamide, N,N'-bis(2'-acetylphenyl); (e) Acetamide, N-[2-(1,2-dihydro-1-hydroxy-2-oxo-3-quinolinyl) phenyl]-; (f) Taxifolin; (g) Saltillin; (h) 6-Methoxyflavone; (i) 3,4-Dihydroxy-1,6-bis-(3-methoxy-phenyl)-hexa-2,4-diene-1,6-dione; (j) Eudesma-5,11(13)-dien-8,12-olide and (k) Carbetamide.

GC-MS analysis of the chloroform extract of *Aspergillus nidulans*

In the discovery of lead compounds for use as therapeutic drugs, the active principals need to be identified. Gas Chromatography-Mass Spectrometry method is as an interesting tool for studying the presence of active components. The chloroform extract of *A. nidulans* which showed maximum

growth inhibition of *C. albicans* was analysed for its active compounds using GC-MS analysis. [Qiu et al. \(2010\)](#) reported the presence of flavonoids in the endophytes *A. nidulans* and *Aspergillus oryzae* isolated from *Ginkgo biloba*. Similarly, in our present study flavonoid compounds such as taxifolin, saltillin and 6-methoxyflavone were identified in the chloroform extract. 1-Eicosanol, an arachidyl alcohol and the sesquiterpene compound

eudesma-5,11(13)-dien-8,12-olide were also present in the extract of *A. nidulans*. The GC-MS analysis of *A. nidulans* chloroform extract showed the presence of fatty acids, amide compounds, arachidyl alcohol, flavonoids and sesquiterpene class of compound (Figure 1). About 12 major peaks have been identified by NIST library search (Table 3). Bis-2-ethylhexyl phthalate is the major compound present in the chloroform extract with a retention time of 23.18 followed by 1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester and phenol,2,4-bis(1,1-dimethylethyl) with

retention times of 16.53 and 12.43 respectively. The amides oxamide, acetamide and carbetamide were also present in the extract. Taxifolin, a flavanonol compound was produced by *A. nidulans* with a retention time of 20.35. Table 3 shows the compounds present in the chloroform extract of *A. nidulans* and their retention time. The present study confirmed the presence of potentially rich antimicrobial compounds in the chloroform extract of *A. nidulans* isolated from *A. nilotica*.

Table 3: Compounds identified in the chloroform extract of *Aspergillus nidulans*.

S.No.	Name of the compound	Retention time
1	Phenol,2,4-bis(1,1-dimethylethyl)	12.43
2	Eudesma-5,11(13)-dien-8,12-olide	13.8
3	6-Methoxyflavone	15.6
4	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	16.53
5	Carbetamide	17.12
6	1-Eicosanol	17.72
7	Flavone, 5-hydroxy-4'-methoxy-7-methyl (Saltillin)	18.23
8	Acetamide, N-[2-(1,2-dihydro-1-hydroxy-2-oxo-3-quinolinyl) phenyl]-	19.65
9	4H-1-Benzopyran-4-one, 2-(3,4-dihydroxyphenyl)-2,3-dihydro-3,5,7-trihydroxy (Taxifolin)	20.35
10	Oxamide, N,N'-bis(2'-acetylphenyl)-	20.98
11	3,4-Dihydroxy-1,6-bis-(3-methoxy-phenyl)-hexa-2,4-diene-1,6-dione	22.42
12	Bis-2-ethylhexyl phthalate	23.18

Table 4: Molecular docking analysis of the compounds of *Aspergillus nidulans* against NMT enzyme.

No	Compound name	Binding energy (kCal/mol)	Vanderwaal's interacting residues	No. of H bonds	H bond interaction residues	No. of direct contacts (all polar, non-polar interactions)
1	Bis-2-ethylhexyl phthalate	-6.20	Tyr 107, Phe 117, Glu 173, Ile 174, Asn 175, Phe 176, Tyr 210, Leu 337, Tyr 354, Leu 355, Cys 393, Leu 394, Cys 396, Gln 397, Asn 399, Val 449, Leu 450.	1	Tyr 225	21
2	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	-6.20	Phe 117, Phe 123, Phe 176, Tyr 225, Leu 337, Leu 394, Leu 450.	1	Tyr 335	12
3	Phenol,2,4-bis(1,1-dimethylethyl)	-4.94	Thr 211, Tyr 225, Tyr 354, Leu 355, Leu 394, Leu 415, Val 449, Leu 450.	1	Leu 451	10
4	1-Eicosanol	-5.41	Tyr 107, Phe 117, Tyr 119, Asn 175, Phe 176, Tyr 225, Tyr 335, Leu 337, Leu 451, Cys 393, Leu 415, Val 449, Leu 450.	2	Leu 355, Leu 394	15
5	Oxamide, N,N'-bis(2'-acetylphenyl)-	-6.20	Tyr 107, Phe 117, Leu 337, Tyr 354, Leu 355, Cys 393, Leu 394, Cys 396, Gln 397, Asn 399, Leu 415, Val 448, Val 449.	3	Leu 450, Tyr 335	17
6	Acetamide, N-[2-(1,2-dihydro-1-hydroxy-2-oxo-3-quinolinyl)phenyl]-	-7.26	Tyr 107, Phe 117, Glu 173, Ile 174, Asn 175, Gly 212, Leu 337, Tyr 354, Leu 394, Leu 415, Leu 450.	2	Tyr 225, Leu 451	15
7	Taxifolin	-7.69	Phe 117, Leu 415, Val 449.	4	Leu 335, Thr 211	16
8	Saltillin	-8.23	Phe 117, Leu 337, Cys 393, Cys 396, Gln 397, Leu 415, Val 449.	1	Leu 394	15
9	6-methoxyflavone	-7.58	Tyr 225, Tyr 354, Phe 356, Asn 392, Cys 393, Cys 396, Val 449, Leu 451.	1	Leu 450	15
10	3,4-Dihydroxy-1,6-bis-(3-methoxy-phenyl)-hexa-2,4-diene-1,6-dione	-6.08	Leu 433, Leu 334, Phe 907, Tyr 916.	4	Tyr430, Asp 477, Glu 515, Asn 862, Asp 909	16
11	Eudesma-5,11(13)-dien-8,12-olide	-6.59	Phe 117, Thr 211, Tyr 335, Leu 337, Tyr 354, Leu 415, Leu 451.	1	Leu 450	10
12	Carbetamide	-6.21	Phe 117, Tyr 119, Leu 337, Leu 355, Asn 392, Cys 393, Leu 394, Cys 396, Gln 397, Val 449	3	Tyr 335, Leu 451	15
13	Chlorhexidine	-4.22	Ile 111, Phe 117, Tyr 225, Tyr 335, Tyr 354, Leu 394, Leu 415, Leu 450, Leu 451.	0	-	9

Molecular Docking Analysis

Failure of new drugs in the market is due to the wrong selection of targets in the preclinical research stages. Bioinformatics approach provides a cost effective and quick result in identifying the potential drug targets (da Silva *et al.*, 2014). N-myristoyltransferase (NMT) enzyme was the potential drug target of *C. albicans* as it is involved in various biological processes such as signal transduction pathways and apoptosis (Boutin, 1997; Gelb *et al.*, 2003). Molecular interaction between the active compounds identified in *A. nidulans* and the NMT enzyme of *C. albicans* provided a possible mechanism of *C. albicans* growth inhibition (Figure 3 and Table 4). The active site residues of NMT enzyme comprised of the following amino acid residues: Tyr 107, Phe 117, Phe 176, Tyr 225, Phe 240, Phe 339, Tyr 354, Val 390 and Leu 394. The docking of compounds present in the chloroform extract of *A. nidulans* against the NMT enzyme of *C. albicans* showed that the flavonoid compound saltillin bound with good binding energy of -8.23 kCal/mol forming one hydrogen bond with Leu 394 whereas, the antibiotic chlorhexidine binds with NMT at a dock score of -4.2 kCal/mol (Figure 4b).

Taxifolin formed four hydrogen bond interactions at the active site with a dock score of -7.69 kCal/mol. (Figure 4a).

6-methoxyflavone formed pi and sigma bond with Tyr 354 residue at the active site region (Figure 4c). The compounds oxamide, N,N'-bis(2'-acetylphenyl) and acetamide, N-[2-(1,2-dihydro-1-hydroxy-2-oxo-3-quinolinyl) phenyl] formed sigma and pi bond with Leu 394 amino acid residue (Figure 3d and Figure 3e). The sesquiterpenoid compound eudesma-5,11(13)-dien-8,12-olide docked with a dock score of -6.49 kcal/mol forming one hydrogen bond at the residue Leu 450 of NMT (Figure 3j). Compounds such as 6-methoxyflavone, saltillin, taxifolin, oxamide, N,N'-bis(2'-acetylphenyl)- and eudesma-5, 11(13)-dien-8,12-olide have good binding energies at the active site region with a dock score greater than -6.5 kCal/mol (Table 4). Hence, the molecular docking studies of compounds with NMT of *C. albicans* indicated that the compounds identified in the chloroform extract of *A. nidulans* helps in effective prevention of oral *C. albicans* from expressing its virulence. Similarly, Guerrero-Perilla *et al.* (2015) performed docking studies on 32 natural compounds against NMT enzyme of *C. albicans* which showed that flavonoids, xanthenes, quinones, alkaloids have better interaction with NMT than terpenoids, phenolics and coumarins when compared to previously reported synthetic inhibitor.

Table 5: Lipinski's properties of the compounds of *Aspergillus nidulans*.

Compound name	MW (<500 Da)	Log P (<5)	Molar refractivity (40-130)	HBA (<10)	HBD (<5)	No. of violations
3,4-Dihydroxy-1,6-bis-(3-methoxy-phenyl)-hexa-2,4diene-1,6-dione	328	3.27	86.39	6	4	0
Bis-2-ethylhexyl phthalate	390	6.43	113.62	4	0	1
1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	278	3.312	76.68	4	0	0
Phenol,2,4-bis(1,1-dimethylethyl)	206	3.98	65.51	1	1	0
1-Eicosanol	298	7.02	95.87	1	1	1
Oxamide, N,N'-bis(2'-acetylphenyl)-	324	2.66	90.15	6	2	0
Acetamide, N-[2-(1,2-dihydro-1-hydroxy-2-oxo-3-quinolinyl)phenyl]-	294	2.81	84.48	5	2	0
Taxifolin	304	1.19	73.25	7	5	0
Saltillin	282	3.325	78.77	4	1	0
6-methoxyflavone	252	3.31	72.37	3	0	0
Eudesma-5,11(13)-dien-8,12-olide	232	3.24	66.33	2	0	0
Carbetamide	236	1.76	64.69	5	2	0

MW: Molecular Weight; Log P: Lipophilicity; HBD: Hydrogen bond donor; HBA: Hydrogen bond acceptor.

Drug likeness behaviour of active compounds present in *Aspergillus nidulans*

The compounds identified by GC-MS analysis of the endophyte *A. nidulans* extract were further analyzed for drug likeness by Lipinski's rule of five. Most of the compounds satisfied Lipinski's rule and the values of molecular properties are tabulated in Table 5. Most of the compounds identified in *A. nidulans* could penetrate biological membranes and compounds with NMT of *C. albicans* high binding energies has no violations of Lipinski's rule of five. Hence, the bioactive compounds of *A. nidulans* provided acceptable scores for the Lipinski's properties and their docking scores indicate their potential use for specifically targeting growth regulator of *C. albicans*.

CONCLUSION

The current study indicated that the endophyte *A. nidulans* isolated from the traditionally used oral aliments preventive plant *Acacia nilotica* was effective in treating oral *C. albicans*. Based on the results obtained, it could be concluded that the compounds of *A. nidulans* particularly flavonoids could be used for the prevention of oral Candida infections and their biofilm-mediated diseases. This study provides a scope for exploration of the active compounds of the endophyte to be used as a lead drug against *C. albicans*.

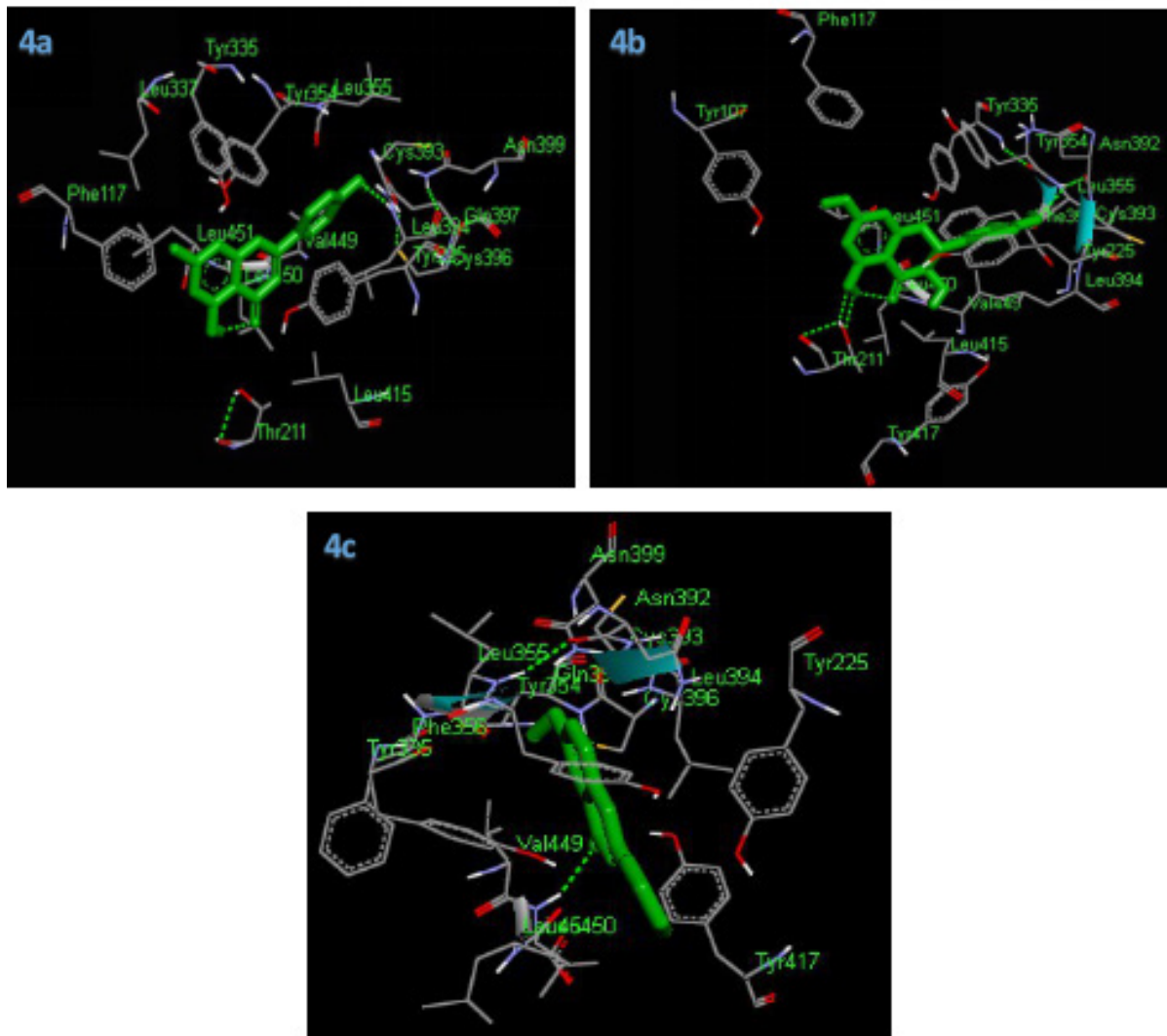


Fig. 4: Residual interaction maps of *Canthida albicans* N-myristoyltransferase enzyme with (a) Taxifolin; (b) Saltillin and (c) 6-methoscyflavone. Hydrogen bonds are indicated by green colour dotted lines.

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CONFLICTS OF INTEREST

There are no conflicts of interest.

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