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Original Article

Computational Evaluation on the Interactions of an Opaque-Phase ABC Transporter Associated with Fluconazole Resistance in *Candida albicans*, by the *Psidium guajava* Bio-Active Compounds

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Objectives: *Candida albicans* is an opportunistic pathogen that occurs as harmless commensals in the intestine, urogenital tract, and skin. It has been influenced by a variety of host conditions and has now evolved as a resistant strain. The aim of this study was thus detect the fluconazole resistant *C. albicans* from the root caries specimens and to computationally evaluate the interactions of an opaque-phase ABC transporter protein with the *Psidium guajava* bio-active compounds.

Methods: 20 carious scrapings were collected from patients with root caries and processed for the isolation of *C. albicans* and was screened for fluconazole resistance. Genomic DNA was extracted and molecular characterization of *Cdrp1* and *Cdrp2* was done by PCR amplification. *P. guajava* methanolic extract was checked for the antifungal efficacy against the resistant strain of *C. albicans*. Further *in-silico* docking involves retrieval of ABC transporter protein and ligand optimization, molinspiration assessment on drug likeness, docking simulations and visualizations.

Results: 65% of the samples showed the presence of *C.albicans* and 2 strains were fluconazole resistant. Crude methanolic extract of *P. guajava* was found to be promising against the fluconazole resistant strains of *C. albicans*. *In-silico* docking analysis showed that Myricetin was a promising candidate with a high docking score and other drug ligand interaction scores.

Conclusion: The current study emphasizes that bioactive compounds from *Psidium guajava* to be a promising candidate for treating candidiasis in fluconazole resistant strains of *C. albicans* However, further *in-vivo* studies have to be implemented for the experimental validation of the same in improving the oral health and hygiene.

Keywords: *Candida albicans*, *Psidium guajava*, fluconazole, health, environment, antifungal resistance

INTRODUCTION

Candida albicans is the best-studied and most prevalent human opportunistic fungal pathogen, which is often considered an imperfect fungus growing as a yeast. *C. albicans*, previously considered an obligate diploid, can form true filamentous hyphae in addition to the budding yeast and pseudohyphae cells found in other *Candida* species [1]. *C. albicans*, an opportunistic pathogen, is a harmless commensal in the intestine, urogenital tract, and skin. Being a part of the functional oral biome [2], it transforms into an opportunistic pathogen under various host conditions, such as a weakened immune response

or an imbalance in competing bacterial microflora. *C. albicans* is commonly reported in different mucosal infections, such as oral candidiasis and vaginitis, and is usually not life-threatening. Such infections also represent sentinel symptoms of immunosuppression, including human immunodeficiency virus [3]. *C. albicans* is also implicated in blood poisoning causing high mortality. The underlying reason for this scenario is the limited arsenal of antifungal agents and drug resistance developing through multiple mechanisms, including the natural drug resistance of biofilms that contributes to drug resistance in candidiasis [4].

Patients with candidal infections are treated with antifungal medications. The azoles are antifungal medications that work by blocking sterol 14a-demethylase (14DM) and cytochrome P-450 enzymes, which are necessary for the formation of ergosterol, the main sterol in the fungal plasma membrane. 14DM catalyzes the oxidative removal of the 14a-methyl group from lanosterol during ergosterol biosynthesis. The azoles binding to heme in the 14DM active site causes substrate competition, inhibiting its function [5]. Due to its favorable absorption and safety, fluconazole is used to treat candidal infections in healthcare settings. Fluconazole typically provides a good clinical response in patients with candidal infections, but relapses are common because fungi are only partially eradicated by azoles as they are fungistatic rather than fungicidal. Because fluconazole-resistant strains of *C. albicans* emerged due to prolonged and repeated treatment, therapy failures similar to those seen in acquired immune deficiency syndrome may occur [6].

The overexpression of multidrug transporter genes in *C. albicans* is one of the resistance mechanisms to azole antifungals [7]. The efflux of azoles increases due to the overexpression of multidrug transporter genes, causing a lower drug buildup and inhibition of the *ERG11* target. At least two families of multidrug transporters from the ABC transporter family are linked to azole resistance [8]. Additionally, two important mediators from the ABC transporter family, *CDR1P* and *CDR2P* (Candida drug resistance), are connected to transport mechanisms. The genetic determinants of these *Cdr1p* and *Cdr2p* are upregulated in distinct drug-resistant strains upon significant mutations [9].

Therefore, developing a different approach to counter the threat of fluconazole-resistant strains, which involves the drug transporter system, is urgently necessary. In a developing nation such as India, many natural fruits and herbs that contain numerous bio-compounds with therapeutic potential have already been developed. *P. guajava*, a significant food and medicinal plant in this respect, is utilized worldwide as a food and folk cure. Several metabolites, primarily those originating from phenols, flavonoids, carotenoids, terpenoids, and triterpenes, have good yields and some beneficial biological actions. The plant's extracts and metabolites, especially those from the leaves and fruits, have beneficial pharmacological effects. *P. guajava* is mostly recognized for its antibacterial, antifungal, and antispasmodic activities [10]. Numerous pharmacological studies showed that it has anti-inflammatory and antinociceptive properties, supporting its traditional use. It also has antioxidant, hepatoprotective, antibacterial, antigenotoxic, antimalarial, cytotoxic, cardioactive, antitussive, and antigenotoxic properties [11]. Thus, this study aimed to screen for fluconazole-resistant *C. albicans* strains from patients with root caries and computationally evaluate the interactions of an opaque-phase ABC transporter protein associated with fluconazole resistance in *C. albicans* by *P. guajava* bio-active compounds.

MATERIALS AND METHODS

1. Chemicals and instruments

For the microbiological processing and antifungal studies, Sabouraud Dextrose Broth (SDB), Sabouraud dextrose agar, and HiMedia Differential Chromium Agar were obtained from Hi-Media labs, Mumbai. For molecular experiments, a DNA extraction kit was obtained from Qiagen. For polymerase chain reaction (PCR) amplification and amplicon determination, the Eppendorf thermocycler (Germany) and gel documentation system from ThermoFischer were used, respectively. The solvents for extraction, such as DMSO, were obtained from Sudhakar Biologicals, Chennai.

2. Study setting and preliminary identification of *C. albicans*

This study was conducted at the Department of Microbiology, Saveetha Dental College and Hospitals from April 2022 to June 2022. Carious scrapings were excavated from 20 patients with typical root caries, as examined by an endodontist. The institutional ethical committee approved the study (SRB/SDC/UG-2061/21/MICRO/055; IHEC/SDC/UG-2061/21/MICRO/596). All patients provided informed consent. The samples were collected in sterile SDB and immediately transferred to the microbiology laboratory. Then, the samples were inoculated onto sterile Sabouraud dextrose agar and incubated

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at 37℃ for 24 h. Afterward, colonies were identified using colony morphology and Gram staining. Cultures were also inoculated into sterile HiMedia Differential Chromium Agar for rapid identification of *C. albicans*.

3. Identification of fluconazole-resistant strains

Antifungal susceptibility profiles of non-repetitive *C. albicans* strains were created to assess their fluconazole resistance by the disc diffusion method as recommended by the CLSI guidelines.

4. Genotypic characterization of *CDR1P* and *CDR2P* in *C. albicans*

On fresh Saboraud dextrose agar, fresh cultures of two fluconazole-resistant *C. albicans* strains were collected and incubated at 37℃ for 48 h. Following the directions on the Qiagen kit, genomic DNA was extracted from the strains. *CDR1P* and *CDR2P* presence was observed by PCR by mixing 7.8 μL of 2× master mix with 5.6 μL of DDW. Specific primers (F: 5'-TGTGTACTATCCATCAACCATCAGC-3' and R: 5'-CACCAAAATAAGCCGTTCTACCA-3' for *CDR1P* and 5'-TGGCAAACAATCCAACAATACA-3' 5'-AATCAAGGGAATAGATGGGTCA-3' for *CDR2P*; 0.1 μL of 100-pmol/mL concentration) were used. Then, a 15-μL reaction mixture was prepared. The German Eppendorf thermocycler was used to accomplish 35 cycles of PCR amplification at an annealing temperature of 58℃. The amplicon was then visualized in a gel documentation system and electrophoresed on a 1.5% agarose gel using ETBr, followed by calculating its size using a 100-bp DNA ladder.

5. Preparation of *P. guajava* extract

Fresh *P. guajava* fruits were obtained from local vendors, washed thoroughly, and cut into thin slices with a sterile blade. Then, these were dried in the shade and ground into a coarse powder using a mechanical grinder, followed by proper storage in a sterile container for further use. For the crude extraction, the stored powder of *P. guajava* (10 g) was mixed with 100 mL of methanol and was allowed to react for 7 days/RT with intermittent mixing. After one week, the extract was filtered using the Whatman no. 1 filter in a sterile petri plate and allowed to evaporate. The crude yield was measured and stored at 4℃ until further use.

6. Antifungal bioassay

For the final formulation before the bioassay, 20 mg of *P. guajava* extract crude yield was combined with 1 mL of DMSO and vortexed. Then, it was diluted to prepare two of 10 mg and 5 mg. The freshly isolated fluconazole-resistant strain of *C. albicans* was lawn cultured on sterile Saboraud dextrose agar, and wells were punctured with a sterile agar cutter. Then, the diluted extract (50 μL) was added to appropriate wells, and plates were incubated at 37℃ for 48 h. The clearance zone was measured after the incubation using a HiMedia antibiotic measuring scale. The test was repeated three times, and the mean value was recorded.

7. ABC transport protein retrieval and optimization

The ABC transporter protein crystal structure was retrieved from the UniProt data bank, followed by its further optimization by adding hydrogen atoms (https://swissmodel.expasy.org/interactive). The AutoDock tool, version 1.5.6, was used to assign electronic charges to protein atoms. The RASMOL tool (https://www.openrasmol.org/) was used to visualize the three-dimensional structure of the ABC transporter protein.

8. Preparation, optimization, and molinspiration assessments of the ligands

ChemSketch software (https://www.acdlabs.com/resources/free-chemistry-software-apps/chemsketch-freeware/) was used to determine the structures of *P. guajava* bioactive derivatives, followed by further optimization of the 3D structures and fine saving in PDB in.mol format through open Babel molecular converter program. Using the molinspiration assessment tool, the counts of hydrogen bond acceptors and donors related to bioavailability, membrane permeability, and molecular weight of the compounds and logP for partition coefficient were evaluated. Lipinski's rule of five was used to examine the absorption, distribution, metabolism, and elimination of the selected bio-compounds.

9. Docking and visualization of drug-ligand interactions

The Auto Dock tool (https://autodock.scripps.edu/) was used for docking analysis to interpret the affinities between *P. guajava* bio-compounds and *C. albicans* ABC transporter proteins.

The hydrogen bond interaction between the bio-compounds of *P. guajava* and the ABC transporter protein of *C. albicans* was visualized using the Discovery Studio Visualizer (https://discover.3ds.com/discovery-studio-visualizer-download). The relative stabilities were assessed using additional docking score evaluations, binding affinities, molecular dynamics, and energy simulations.

RESULTS

1. Fluconazole-resistant *C. albicans*

Thirteen strains (65%) were identified as *C. albicans* from the total 20 root caries samples. Typical green colonies with gram-positive budding yeast cells on the Hi-Chrome agar were selected for further bioassays (Fig. 1). Two out of 13 strains (15.3%) were resistant to fluconazole. One strain was positive for *CDR2P* with an amplicon size of 125 bp (Fig. 2). *CDR1P* was absent in both resistant strains.

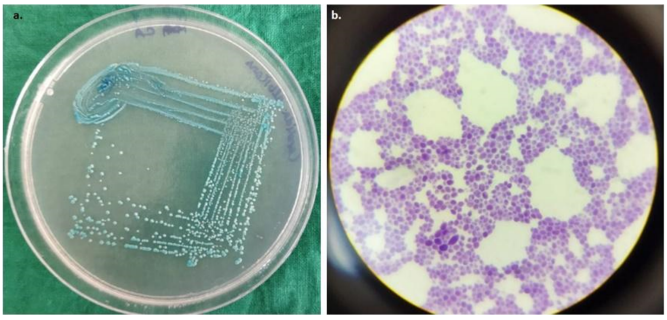

Figure 1. Identification of *C. albicans* from the root caries samples (a) Hi-Chrome agar showing greenish blue colonies of the yeast and (b) gram staining showing the gram positive budding oval cells.

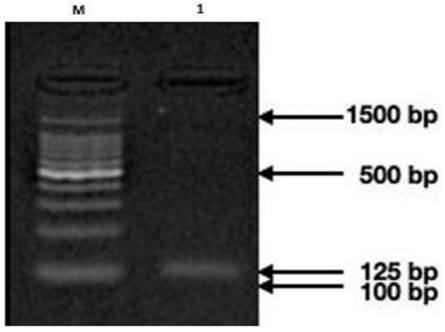

Figure 2. Showing the electrophoregram of *Cdrp1* gene product of size 125 bp in lane 1 with 1.5 Kbp marker lane (M).

2. Antifungal effect of *P. guajava* extract

The total yield of the *P. guajava* methanol extract was 27 mg/ml from 100 g of the dry powder. The extract showed a promising effect against sensitive (n = 13) and fluconazole-resistant strains with a zone of 18 mm, 15 mm, and no zone for concentrations of 20 mg, 10 mg, and 5 mg, respectively (Fig. 3). Control amphotericin B showed a zone of 32 mm.

3. ABC transporter structure retrieval from *C. albicans*

The FASTA sequence of the ABC transporter chain with the sequence ID Q5A762 was taken, followed by creating the homology model using the Swiss Model server through the template 7MPE-A from *Saccharomyces cerevisiae* chain (Fig. 4). With a 100% sequence identity of the template, the model was promising for further computational evaluation. Additionally, the Ramachandran plot revealed 90.7% of favorable regions. The 3D structure was viewed using RASMOL, where pink represented the alpha-helix, the yellow arrow represented beta sheets, and white represented the turns.

4. Structural retrieval of the ligands and their molinspiration assessment

The retrieved ligands from the ACD Chemsketch were in a compatible format. Table 1 displays the ligands from *P. guajava* that were extracted together with their 2D and 3D structures in the SMILES format. Table 2 demonstrates the predicted bioactivity scores and Lipinsky's rule of five-parameter assessments.

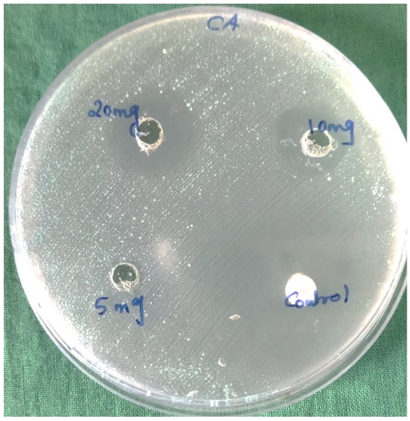

Figure 3. Antifungal effect of the crude methanolic extract of *P. guajava* against the fluconazole resistant strains of *C. albicans*.

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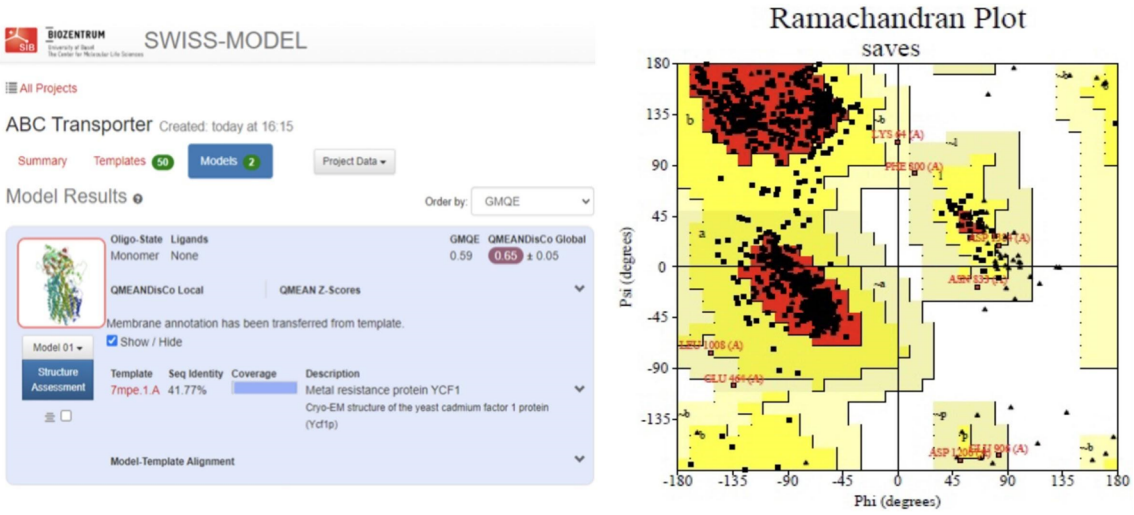


Figure 4. Prediction of ABC transporter with Swissmodel server and validation using Ramachandran plot.

Table 1. 2D and 3D structures and SMILES format of the selected bio-active compounds from *P. guajava* for the study

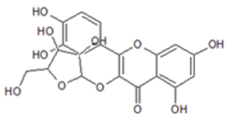
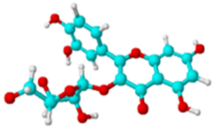
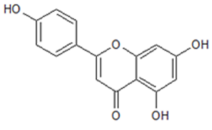
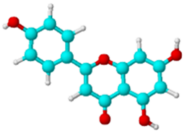
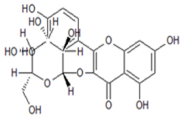
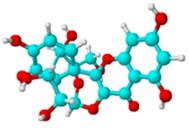
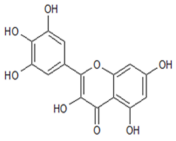
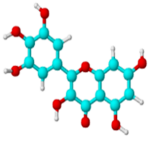
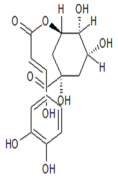
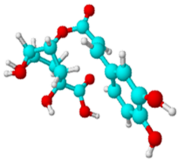
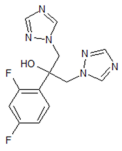
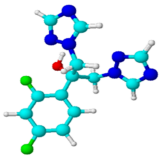
Compound name	2D	3D	SMILES	Mol formula
Avicularin			C1=CC(=C(C=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)OC4C(C(C(O4)CO)O)O)O)O	$C_{20}H_{18}O_{11}$
Apigenin			C1=CC(=CC=C1C2=CC(=O)C3=C(C=C(C=C3O2)O)O)O	$C_{15}H_{10}O_5$
Hyperin			C1=CC(=C(C=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O[C@H]4[C@@H]([C@H]([C@H]([C@H](O4)CO)O)O)O)O)O	$C_{21}H_{20}O_{12}$
Myricetin			C1=C(C=C(C(=C1O)O)O)C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O	$C_{15}H_{10}O_8$
Chlorogenic acid			C1[C@H]([C@H]([C@@H](C[C@@]1(C(=O)O)O)OC(=O)/C=C/C2=CC(=C(C=C2)O)O)O)O	$C_{16}H_{18}O_9$
Fluconazole			C1=CC(=C(C=C1F)F)C(CN2C=NC=N2)(CN3C=NC=N3)O	$C_{13}H_{12}F_2N_6O$

Table 2. Molinspiration assessments on *P. guajava* bio-compounds for drug likeness

Compound name	Molecular weight	Hydrogen bond donor	Hydrogen bond acceptor	miLogP	Rotatable bonds	nViolations	TPSA (Å)	Volume	N atoms	Binding energy
Avicularin	434.3	7	11	0.80	4	2	190.28	347.36	31	–6.58
Apigenin	270.24	3	5	2.46	1	0	90.89	224.05	20	–7.73
Hyperin	464.4	8	12	–0.36	4	2	210.50	372.21	33	–5.57
Myricetin	318.23	6	8	1.39	1	1	151.58	248.10	23	–8.71
Chlorogenic acid	354.31	6	9	–0.45	5	1	164.74	296.27	25	–6.47
Fluconazole	306.27	1	7	–0.12	5	0	81.66	248.96	22	–4.68

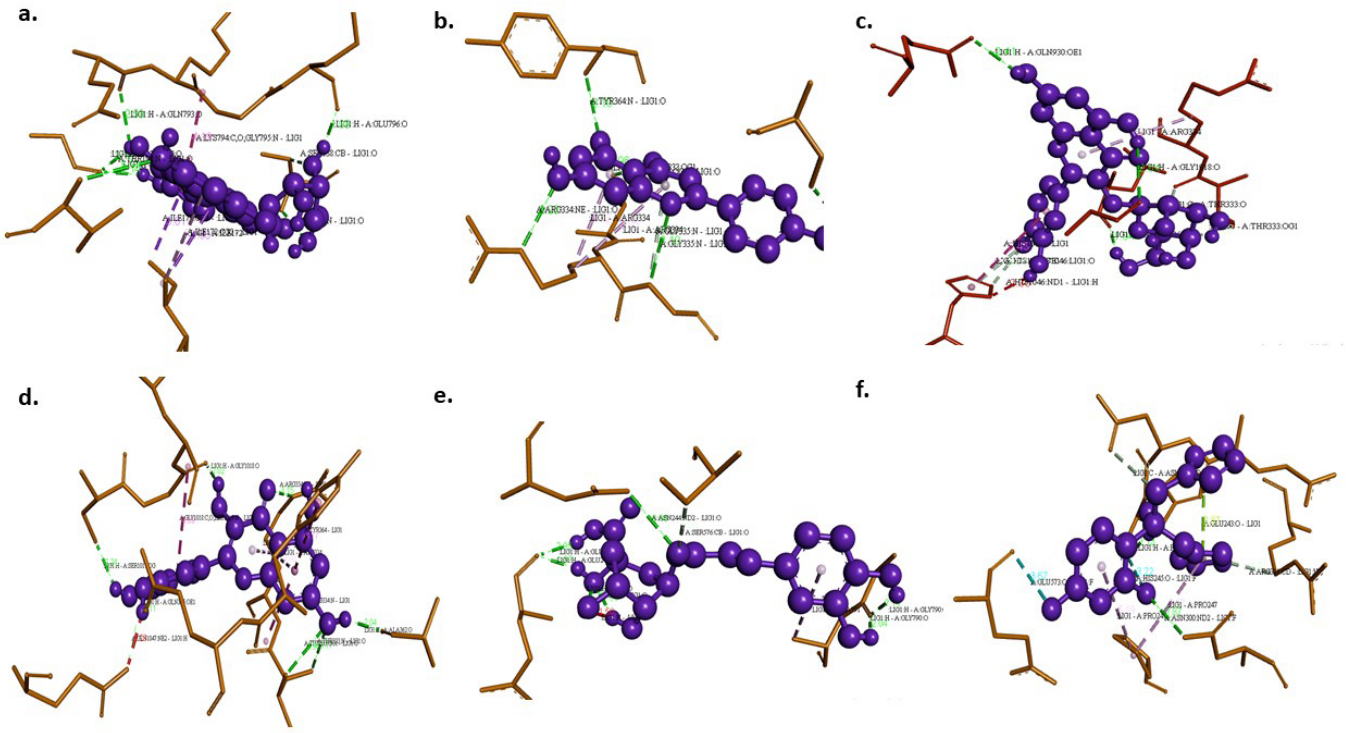


Figure 5. Visualizing hydrogen interactions between ABC transporters with (a) Avicularin, (b) Apigenin, (c) Hyperin, (d) Myricetin, (e) Chlorogenic acid, (f) Fluconazole.

5. Docking results

Fig. 5 shows the drug-ligand interactions between the essential compounds from *P. guajava* and the ABC transporter of *C. albicans* using the Autodock tool in the stick model by discovery studio visualizations. The docking scores, number of hydrogen bonds formed, torsional energy between the ligands, and the drugs were recorded (Table 3). Avicularin and ABC transporter showed a binding energy of –6.58 kcal/mol and formed 7 hydrogen bonds with SER138 (N···O), THR198 (N···O), GLU796 (O···H), GLY209 (O··H), GLY209 (O···H), GLN793 (O···H), and SER138 (CB···O); 5 hydrophobic interactions with ILE172, ILE172, LYS794, GLY795, and ILE172; and 13 Van der Waals interactions with key residues. Apigenin and ABC transporter demonstrated binding energy of –7.73 kcal/mol and formed hydrogen bonds with THR333 (OG1···O), ARG334 (NE···O), GLY335 (N···O), TYR364 (N···O), SER1017 (OG···H), THR333 (OG1···H), and GLY335 (N); 2 hydrophobic interaction with ARG334; and 6 Van der Waals interactions. Hyperin and ABC transporter complex showed binding energy of –5.57 kcal/mol and formed 7 hydrogen bonds with LEU336 (O··H), GLY1018 (O···H), GLN930 (OE1···H), HIS1046 (CE1··O), THR333 (OG1···C), THR333 (O···C), and HIS1046(O); 2 hydrophobic interactions with HIS1046 and ARG334; and 7 Van der Waals

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Table 3. Docking scores of *P. guajava* against ABC transporter protein

EfbA docking with compounds	Number of hydrogen bonds	Binding energy	Inhibition constant	Ligand efficiency	Intermolecular energy	vdW + Hbond + desolv energy	Electrostatic energy	Torsional energy	Total internal unbound
Avicularin	4	−6.58	15.05	−0.21	−9.86	−9.65	−0.21	3.28	−4.44
Apigenin	3	−7.73	2.16	−0.39	−8.92	−8.68	−0.25	1.19	−0.9
Hyperin	3	−5.57	82.83	−0.17	−8.85	−8.75	−0.1	3.28	−7.49
Myricetin	5	−8.71	413.5	−0.38	−10.8	−10.55	−0.25	2.09	−2.0
Chlorogenic acid	1	−6.47	18.06	−0.26	−9.75	−8.1	−1.65	3.28	−5.65
Fluconazole	1	−4.68	368.64	−0.21	−6.47	−6.41	−0.06	1.79	−1.43

Table 4. Overall interaction of ABC transporter with the bioactive compounds from *P. guajava*

Docking with compounds	Vander Waals	H bond	hydrophobic	Pi-pi pair	Halogen interaction
Avicularin	13	7	5	-	-
Apigenin	6	6	2	-	-
Hyperin	7	7	2	-	-
Myricetin	5	7	7	-	-
Chlorogenic acid	7	6	1	-	-
Fluconazole	3	4	2	2	1

interactions. Myricetin and ABC transporter complex exhibited the least binding energy of −8.71 and formed 7 hydrogen bonds with THR333 (N···O), THR333 (OG1···O), ARG334 (NE···O), GLN365 (OE1··H), SER1017 (OG···H), GLY1018 (O···H), and ALA362 (O···H); 7 hydrophobic interactions with TYR364, THR333 (C,O), ARG334 (N), GLY1018 (C,O), LEU1019 (N); and 5 Van der Waals interactions. Chlorogenic acid and ABC transporter showed binding energy −6.47 kcal/mol and formed 6 hydrogen bonds with ASN244 (ND2···O), GLY79 (O···H), GLY790 (O··H), GLU241 (O···H), GLU241 (O···H), and SER576 (C···O); 1 hydrophobic interaction with ALA791; and 7 van der Waals interactions. Fluconazole and ABC transporter demonstrated binding energy of −4.68 kcal/mol and formed 4 hydrogen bonds with ASN300 (ND2···F), HIS245 (O···H), ARG311 (CD··N), and ASN244 (OD1···C); 2 Halogen interactions with HIS245 (O···F) and GLU573 (O···F); 1 lone pair-π with GLU243 (O); 2 hydrophobic interactions with PRO247; and 3 Van der Waals interactions. The data also showed the least binding energy with myricetin and Van der Waals, pi-pi, alkyl/pi-alkyl, and pi-sulfur interactions. Additionally, the fluconazole compound was assessed as the promising interacting compound from *P. guajava* (Table 4).

DISCUSSION

Dental caries, especially in young children and root caries, is associated with *Candida* species [12]. Typically, root caries and *C. albicans* prevalence are strongly correlated. The present study also yielded 65% of *C. albicans* isolates from the study population with root caries. Several authors demonstrated that people with caries have a larger proportion of *Candida* species than people without caries. Additionally, *C. albicans* frequently colonizes carious lesions in the dentin rather than in biofilm or saliva [13]. A lower salivary flow rate, prevalent in older individuals, is a favorable condition for *C. albicans* in these sites [14].

The emergence of resistant strains, especially against fluconazole, is a recently sparked challenge in hospital settings. Fluconazole is fungistatic rather than fungicidal; hence, treatment can cause acquired resistance. In the US, *C. albicans* has a low incidence of fluconazole resistance, approximately 0.5%-2%. On the other hand, *C. tropicalis*, *C. parapsilosis*, and *C. glabrata* have higher rates at 4%-9%, 2%-6%, and 11%-13%, respectively [15, 16]. The emerging yeast *C. auris* can exhibit a rate of fluconazole resistance as high as 93% [17]. Alternatively, fluconazole resistance may be innate without prior exposure to antifungals, as seen in *C. krusei* [18]. In our study, we observed that 15.3% of the strains were fluconazole-resistant, although this finding

is limited by the small sample size.

Azole-susceptible isolates exhibit detectable *CDR1* gene transcription, which is amplified to higher levels in some azole-resistant isolates. Together with *CDR2*, a gene without discernible transcriptional activity in azole-susceptible isolates, *CDR1* is typically increased in these isolates. *CDR1* and *CDR2* can also be transiently activated in cells exposed to various medications, including fluphenazine and estradiol, to imitate their expression in azole-resistant cells. Significant mutations in gene loci upregulate *CDR1* and *CDR2*, a mechanism involved in fluconazole resistance [19]. Thus, we hypothesized that *CDR1* and *CDR2* are associated with the resistant strains and showed that none of the sensitive strains possessed demonstrated *CDR1* and *CDR2* transcription, while 1 resistant strain possessed *CDR2*. As many studies are documenting the mutations behind these genetic determinants in association with fluconazole resistance, the future aim is to advance gene expression studies in fluconazole-resistant strains.

Alternative medicine using herbal bio-compounds is a rapidly growing research field to address drug resistance. Many plant-based studies documented promising reports on the inhibitory effect of plant products against dental pathogens [20, 21]. Therefore, we studied the antifungal effect of the methanolic fruit extract of *P. guajava* against sensitive and resistant strains of *C. albicans*. It showed promising antifungal efficacy against both strains. This result correlates with the earlier study showing that fluconazole effectiveness against *C. tropicalis* and *C. albicans* strains was significantly modulated by *P. guajava* extracts. Furthermore, this might be related to the presence of flavonoids and phenolic components [22]. The findings showed that *P. guajava* contains many various secondary metabolites, particularly phenolics, flavonoids, squalene, and vitamin E. Thus, the plant could serve as a source of antioxidants for nutraceuticals and functional food items. Caryophyllene and several of its derivatives were found in this plant's essential oils, making *P. guajava* an anti-inflammatory agent. The striking characteristic of *P. guajava* is that all of its parts are abundant in meroterpenoids, which are primarily produced by fungi with immunosuppressive activity and are specifically derived from phloroglucinol.

Proteins seldom work tasks alone. Instead, they frequently interact with other molecules to perform particular processes. It has become one of the most actively studied research fields utilizing either experimental or bioinformatics methods since understanding how biomolecules interact with other molecules has many ramifications, such as for protein folding, drug creation, and purification strategies. Beyond predicting protein structures, molecular modeling can help select a certain conformation controlling a biomolecule's activity [23]. The *in-silico* docking-based analysis is very promising for the preliminary clue on the inhibitory activities for all microbial targets, including viruses and vectors [24]. Thus, we chose the drug-ligand interaction by autodocking to evaluate the interaction of the bio-compounds from *P. guajava* as we did not perform purification studies, which is one of the limitations of the present study.

A 3D structure of cdr1 was determined by the Ramachandran plot, showing 90.7% of the total residues in the favored region. Homology modeling is a suitable method to predict and validate target structures. Comparing the molecular weight of all the compounds, apigenin possessed the lowest molecular weight of 270.24, while hyperin possessed the highest molecular weight of 464.4. Other compounds showed a molecular weight ranging between 315 and 435. In the assessment of hydrogen bond donor and acceptor properties, chlorogenic acid had the greatest number of rotatable bonds of about 5 together with miLogP value of −0.45. The TPSA value (topological polar surface area) of a compound is important as it is attributed to the oral bioavailability of drugs and should be < 140 Å. One bioactive compound showed a TPSA value of < 140 Å. Myricetin showed the lowest binding energy of −8.71, whereas hyperin showed a binding energy of −5.57. We could also infer from the overall interaction that apigenin showed 5 hydrogen bond interactions and 5 Van der Waals interactions, indicating stabilization of the binding structures. Avicularin had the highest Van der Waals interactions, followed by hyperin with pi-alkyl interactions with both hyperin and chlorogenic acid. On the other hand, only avicularin showed pi-siga and amide-pi stacked interactions. The evaluation of the overall docking energies showed that myricetin had the greatest number of hydrogen bonds, while hyperin and chlorogenic acid had the lowest binding energies. The study required further experimental analysis for the design of novel drugs from *P. guajava* to combat the fluconazole resistance of *C. albicans* in healthcare settings.

CONCLUSION

The emergence of drug resistance can be considered an inevitable consequence of the selective pressures imposed by antifungal drugs. In the past two decades, several genes and mutations increasing resistance to fluconazole in clinical iso-

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lates, primarily *C. albicans*, were elucidated. A newer antifungal option is required to overcome fluconazole resistance. We concluded that myricetin, a bioactive compound of *P. guajava*, is a promising newer antifungal candidate for overcoming this resistance.

AUTHORS' CONTRIBUTIONS

Mithil Vora implemented the designed study: Dr.Smiline Girija contributed for the conceptualization and design, validation of the data obtained, manuscript drafting, editing and review; Dr.Shoba Gunasekaran implemented the in-silico interpretation of the study; Dr.Vijayashree contributed for the final validation and proof reading of the manuscript.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest in this work.

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