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Detection of *sap* in *Candida albicans* and Targeting with Bioactive Compounds from *Aegle marmelos*: An *in vitro* and *in silico* Analysis

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Background: Secreted aspartyl proteinases (SAPs) contribute to the pathogenicity of *Candida albicans* infections by aiding in tissue invasion and immune evasion.

Objective: This study aimed to identify SAP genes in clinical isolates of *C. albicans* and assess the antifungal potential of *Aegle marmelos* (also known as Bengal quince, golden apple, Japanese bitter orange, stone apple, or wood apple) using *in vitro* and *in silico* methods.

Methods: Carious scrapings were collected from patients with deep root caries. Standard microbiological techniques were employed for the phenotypic identification of *C. albicans*, and the presence of SAP genes was confirmed by PCR. A methanolic extract of *A. marmelos* was prepared and evaluated for antifungal activity against *C. albicans* isolates. In addition, *in silico* docking studies were conducted on six known bioactive compounds from *A. marmelos* to predict inhibitory effects on *C. albicans* SAPs.

Results: In total, 13 of 20 clinical samples (65.0%) tested positive for *C. albicans*, all of which were susceptible to growth suppression by the methanolic extract of *A. marmelos*. The furocourmarin imperatorin exhibited the highest binding affinity to SAP protein *in silico* (docking energy of -7.45 kcal/mol), likely by forming multiple hydrogen bonds, and showed no violations in molecular interactions.

Conclusion: Imperatonin may inhibit pathological *C. albicans* growth by inhibiting SAPs. Further experimental studies are warranted to confirm imperatorin toxicity, antifungal efficacy, and therapeutic potential.

Key Words: *Aegle marmelos*, *Candida albicans*, Health, Imperatorin, SAP protein

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INTRODUCTION

Candida albicans is a common opportunistic fungal pathogen that resides in the oral and gastrointestinal tracts of 40~60% of healthy adults[1]. While generally a harmless commensal organism, *C. albicans* can transition to a pathogenic state in individuals with weakened immune systems, resulting in a fungal infection termed candidiasis[2]. It is often referred to as a yeast-like fungus because of its ability to form pseudohyphae[3], so these infections are commonly referred to as "yeast infections".

Among *Candida* species, *C. albicans* is frequently used as a model organism to study fungal pathogens and to screen compounds for antifungal activity. Numerous virulence factors have been identified that promote candidiasis, including a family of at least 10 secreted aspartic proteases (SAPs)[4]. Furthermore, SAPs 1-3 were reported to exacerbate *C. albicans*-induced damage of reconstituted human epithelium in a model of human oral candidiasis and oropharyngeal candidiasis in individuals with HIV infection[5,6]. The genes encoding SAP1 and SAP2 are also frequently detected in vaginal candidiasis[7]. The cell surface-associated form SAP9 was demonstrated to efficiently and rapidly degrade the antimicrobial peptide histatin 5 produced by oral epithelial cells, thereby reducing endogenous resistance against *C. albicans*. In addition, SAP10 and SAP9 have been characterized as glycosylphosphatidylinositol (GPI)-anchored proteases that target proteins essential for cellular processes and host-pathogen interactions[8]. Thus, SAPs are considered essential virulence factors for *C. albicans* adhesion and invasion of epithelial cells and thus ultimately for tissue damage, but the precise contributions of individual SAP variants are not well described. Further, the host conditions conferring vulnerability and resistance to *C. albicans* infection are not well understood, although it has been shown that SAP variant activities are differentially sensitive to host tissue pH.

Numerous antifungal drugs are available to treated candidiasis, but drug resistance among candidal strains has emerged as a major clinical problem in recent decades, necessitating the development or identification of alternative therapies. Many plants and herbs demonstrate antimicrobial and antifungal bioactivities. For instance, *Aegle marmelos* (commonly known as Bael) is a deciduous shrub or small to medium-sized tree with multiple documented bioactive properties[9], which have been attributed to phytoconstituents such as marmenol, marmin, marmelosin, marmelide, psoralen, alloimperatorin, rutaretin, scopoletin, aegelin, marmelin, fagarine, anhydromarmelin, and limonene[10]. Extensive experimental and clinical research has shown that *Aegle marmelos* processes antidiarrheal, antimicrobial, antiviral, radioprotective, anticancer, chemopreventive, antipyretic, ulcer-healing, antigenotoxic, diuretic, antifertility, and anti-inflammatory effects[11]. The present study evaluated the expression of SAP genes in *C. albicans* samples from patients with root caries and the inhibitory effects of methanolic crude extracts from *A. marmelos* on *C. albicans* growth. In addition, an *in silico* analysis was conducted to investigate the drug-ligand interactions between SAP and six known bioactive compounds derived from *A. marmelos*, coumarin, xanthotoxol, imperatorin, aegeline, marmeline, and fluconazole.

MATERIALS AND METHODS

1. Study Setting and preliminary identification of *Candida albicans*

This study was conducted at the Department of Microbiology of Saveetha Dental College and Hospitals from April to June 2022. All protocols were approved by the institutional ethics review board (SRB/SDC/UG-2044/21/MICRO/063; IHEC/SDC/UG-2044/21/MICRO/604) and the study was conducted according to the tenets of the Declaration of Helsinki (1975 and subsequent amendments). Participants provided informed written consent prior to testing. Carious scrapings were collected from 20 patients with root caries as assessed by an endodontist. The samples were collected in sterile Sabouraud dextrose broth and promptly transported to the microbiology laboratory for processing. Samples were then inoculated on sterile Sabouraud dextrose agar (SDA) and incubated at 37℃ for 24 h. After incubation, colonies were identified based on their morphology and Gram staining. In addition, cultures were transferred to sterile Himedia Differential Hicrome Agar for the rapid identification of *C. albicans* by.

2. Genotypic characterization of the *sap* gene in *C. albicans*

Fresh *C. albicans* cultures were cultured on Sabouraud dextrose agar (SDA) and incubated at 37℃ for 48 h. Genomic DNA was then extracted from isolates using the Qiagen kit according to the manufacturer's instructions. The SAP genes *sap4* to *sap7* were detected by PCR using a reaction mixture composed of 7.8 µl of 2× master mix (Taraka, Japan), 5.6 µl of double-distilled water, and 1.6 µl of the target primers (Table 1) [Eurofins Genomic India Pvt Ltd, Bangalore], and 35 amplification cycles with an annealing temperature of

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Table 1. Primers used to detect sap genes from clinical isolates of *Candida albicans*

Gene target	Primers	Annealing temperature	Amplicon size
sap4	5'-CATTCATTCCTTTAATACCGACTATC-3' 5'-GGTAACAAACCCTGTAGATCTTTTAAC-3'	58℃	156
sap5	5'-CATTCATTCCTTTAATACCGACTATC-3' 5'-GGTAACAAACCCTGTAGATCTTTTAAC-3'	58℃	181
sap6	5'-CATTCATTCCTTTAATACCGACTATC-3' 5'-GGTAACAAACCCTGTAGATCTTTTAAC-3'	58℃	206
sap7	5'-GAAATGCAAAGAGTATTAGAGTTATTAC-3' 5'-GAATGATTTGGTTTACATCATCTTCAACTG-3'	58℃	196

58℃. The amplicons were then visualized by electrophoresis on a 1.5% agarose gel containing ethidium bromide and captured using a gel documentation system. Amplicon size was estimated using a 1.5-Kbp DNA ladder[12].

3. Preparation of the *A. marmelos* extract

Fresh *A. marmelos* leaves were thoroughly washed three times with sterile distilled water to eliminate any impurities and external contaminants, dried in the shade, and ground into a coarse powder using a mechanical grinder. The powder was stored in a sterile container for later extraction. To prepare the crude extract, 10 g of the powdered leaves was mixed with 100 ml of methanol and incubated at room temperature with occasional agitation for one week. The crude extract was filtered through Whatman No. 1 filter paper and concentrated by evaporation in a sterile Petri dish. The final extract was stored at 4℃ for subsequent *in vitro* studies.

4. Antifungal bioassay

A 20-mg sample of *A. marmelos* extract was mixed with 1 ml of dimethyl sulfoxide (DMSO) and vortexed. Diluted samples of 5 and 10 mg/ml were prepared by adding more DMSO. The antifungal bioassay was conducted using the disc diffusion method. Briefly, *C. albicans* suspensions were prepared in sterile saline and adjusted to match the turbidity of a 0.5 McFarland standard (1.5×10^8 CFU/ml) for reproducibility. Lawn cultures were prepared by evenly spreading the adjusted suspension over the surface of sterile Sabouraud dextrose agar plates, and wells were created using an agar cutter. A 50 µl volume of extract (20, 10, and 5 mg/ml) or 50 µl DMSO (negative control to account for any potential antifungal properties of DMSO) was added to the respective wells. The plates were incubated for 48 h at 37℃, followed by measures of the zone of inhibition to assess the antifungal activity of the applied extract. The assay was repeated three times at each dose with independently seeded cultures, and the mean zone diameter calculated.

5. Retrieval of SAP protein structure

The structure of *C. albicans* SAP was retrieved from the Universal Protein Resource (UNIPROT) Database (ID 2QZW-A-Chain, https://www.uniprot.org/). Water molecules were removed and hydrogen atoms added using AutoDock, and the final three-dimensional structure was visualized using the Raster of a Molecule server[13].

6. Preparation and optimization of the ligands

ChemSketch software was employed to determine the structures of the bioactive *A. marmelos* derivatives, coumarin, xanthotoxol, imperatorin, aegeline, marmeline, and fluconazole[14]. Structures were then optimized using an open-source molecular converter program and results saved in Protein Data Bank (PDB) format (.mol).

7. Drug likeness assessment by Molinspiration molecular parameters

The drug-likeness of the compounds was assessed by Lipinski's rule of five and by determining the number of hydrogen bond acceptors and donors (both of which are linked to bioavailability and membrane permeability), the partition coefficient (logP), and molecular weight using the

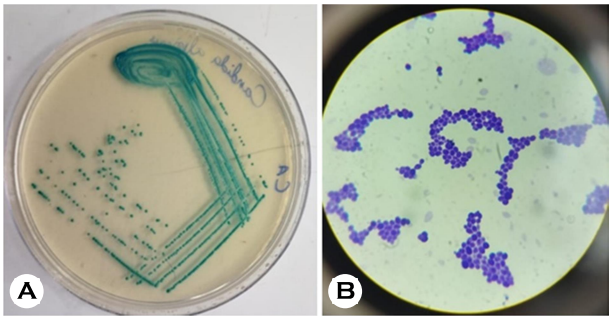

Fig. 1. Isolation and identification of *Candida albicans* from human root caries samples. (A) Hicrome agar showing greenish-blue colonies, (B) Gram staining showing the gram-positive budding of oval cells

Molinspiration tool (molinspiration.com). In addition, the absorption, distribution, metabolism, and elimination (ADME) properties of each bioactive compound were analyzed using the ADME tool[15].

8. Docking analysis and visualization of the drug ligand interactions

The interactions between SAP and the selected bioactive compounds derived from *A. marmelos* were simulated by docking analysis using the AutoDock tool and Discovery Studio Visualizer[16]. Docking score evaluations, affinities, molecular dynamics, and simulation energies were calculated to compare the relative stabilities of drug-ligand interactions.

RESULTS

1. Characterization of *C. albicans* in root caries scrapings

Of the 20 root caries scraping samples, *C. albicans* was detected in 13 (65.0%) as evidenced by the characteristic greenish-blue colonies on Hicrome Candida Differentiation Agar (Fig. 1A) and the Gram-positive staining, which revealed budding, oval-shaped cells (Fig. 1B). Among these 13 samples, three (23.07%) tested positive for the *sap4* gene by PCR amplification, which yielded the predicted amplicon size of 156 bp (Fig. 2A).

2. Antifungal effect of the *A. marmelos* extract

The methanolic extraction procedure yielded 34 mg/ml

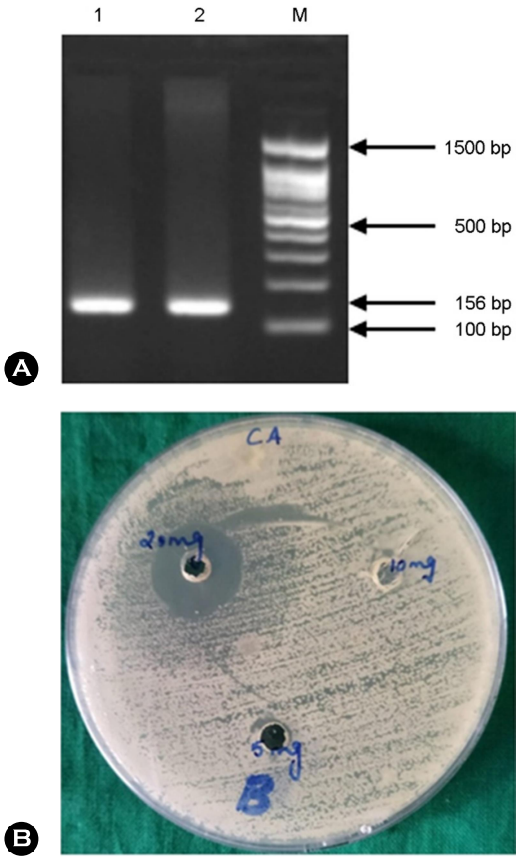

Fig. 2. Detection of the *sap4* gene product and evaluation of SAP antifungal efficacy. (A) Electrophoregram of the *sap4* gene product (156 bp) in lanes 1 and 2, and the 1.5-Kbp marker in lane M, (B) Antifungal efficacy of the *Aegle marmelos* methanolic extract against *Candida albicans* clinical isolates from root caries

from 100 g of dried *A. marmelos* leaf powder. Disk diffusion assays demonstrated substantial antifungal activity against all tested isolates at the 20 mg dose, with a zone of inhibition measuring 19±1.5 mm, while no antifungal activity was observed at 10 and 5 mg doses (Fig. 2B). No zone of inhibition was observed around the wells containing 50 µl DMSO, confirming that the solvent did not substantially interfere with or promote the antifungal activity of the extract.

3. *C. albicans* SAP structure retrieval

The Fast-All sequence of the *C. albicans sap* gene was retrieved from the Universal Protein Resource (UNIPROT) database using the sequence ID 2QZW-A-Chain. The three-dimensional structure of SAP was visualized using the RAMSOL tool with default visualization parameters, including wire-

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frame view and coloring by atom type. The structure was manipulated to highlight the key residues involved in ligand-binding interactions.

4. Retrieval of *A. marmelos* bioactive ligand structures

Structures of the selected *A. marmelos* bioactive ligands coumarin, xanthotoxol, imperatorin, aegeline, marmeline, and fluconazole were drawn and optimized using ACD Chem-Sketch. After the initial 2D structure construction, geometric optimization was conducted using the MMFF94 (Merck Molecular Force Field) application to minimize the energy of the molecules. The optimized structures were then exported in PDB format, and an OpenBabel molecular converter was used to convert these structures into a compatible format for docking simulations. Table 2 displays the ligands from *A. marmelos* together with the simplified molecular input line entry system (SMILES) format.

5. Molinspiration assessment toward drug likeness

The drug-likeness of the selected compounds was assessed based on key molecular properties using the Molinspiration tool. Lipinski's rule of five was then applied to ensure that all compounds adhered to the guidelines without violation. Molecular weight, partition coefficient (logP), and numbers of hydrogen bond acceptors, hydrogen bond donors, and rotatable bonds were also assessed. Ion channel modulation,

Table 2. Two- and three-dimensional structures, SMILES formats, molecular formulae, molecular weights, and PubChem IDs of the selected bioactive compounds derived from *Aegle marmelos*

Compound name	SMILES	Mol. formula	Mol wt.	Pubchem ID
Coumarin	C1=CC=C2C(=C1)C=CC(=O)O2	$C_9H_6O_2$	146.14	323
Xanthotoxol	C1=CC(=O)OC2=C(C3=C(C=CO3)C=C21)O	$C_{11}H_6O_4$	202.16	65090
Imperatorin	CC(=CCOC1=C2C(=CC3=C1OC=C3)C=CC(=O)O2)C	$C_{16}H_{14}O_4$	270.28	10212
Aegeline	COC1=CC=C(C=C1)C(CNC(=O)/C=C/C2=CC=CC=C2)O	$C_{18}H_{19}NO_3$	297.3	15558419
Marmeline	CC(=CCOC1=CC=C(C=C1)C(CNC(=O)/C=C₩C2=CC=CC=C2)O)C	$C_{22}H_{25}NO_3$	351.4	131750977
Erythromycin	CC[C@@H]1[C@@]([C@@H]([C@H](C(=O)[C@@H](C[C@@]([C@@H]([C@H]([C@@H]([C@H](C(=O)O1)C)O[C@H]2C[C@@]([C@H]([C@@H](O2)C)O)(C)OC)C)O[C@H]3[C@@H]([C@H](C[C@H](O3)C)N(C)C)O)(C)O)C)C)O)(C)O	$C_{37}H_{67}NO_{13}$	733.9	12560

SMILES: Simplified Molecular Input Line Entry System

Table 3. Molinspiration-based drug-likeness evaluation of *Aegle marmelos* bioactive compounds

Compound name	Hydrogen bond donors	Hydrogen bond acceptors	miLogP	Rotatable bonds	Violations (number)	TPSA (Å)	Mol. Vol.	Atoms
Coumarin	0	2	2.01	0	0	30.21	128.59	11
Xanthotoxol	1	4	2.00	0	0	63.58	162.16	15
Imperatorin	0	4	3.95	3	0	52.59	240.47	20
Aegeline	2	4	2.64	6	0	58.56	281.45	22
Marmeline	2	4	4.32	8	0	58.56	342.23	26
Fluconazole	1	7	-0.12	5	0	81.66	248.96	22

Note: Drug likeliness scores > 0.3.
TPSA: topological polar surface area

Table 4. Docking interaction scores of *Aegle marmelos* bioactive compounds with *Candida albicans* SAP

EfbA docking with compounds	H-Bonds	Binding energy (kJ)	Ki	Ligand efficiency	Inter-molecular energy	vdW + Hbond + desolv. energy	Electro-static energy	Torsional energy	Total internal unbound
Coumarin	1	-5.9	47.36	-0.54	-5.9	-5.81	-0.09	0.0	0.0
Xanthotoxol	1	-6.27	25.16	-0.42	-6.57	-6.57	-0.07	0.3	-0.39
Imperatorin	2	-7.45	3.44	-0.37	-8.35	-8.23	-0.12	0.89	-0.54
Aegeline	2	-6.82	9.94	-0.31	-8.91	-8.42	-0.49	2.09	-0.68
Marmeline	2	-6.36	21.91	-0.24	-9.04	-8.77	-0.27	2.68	-1.87
Fluconazole	2	-4.86	274.92	-0.22	-6.65	-6.55	-0.09	1.79	-1.5

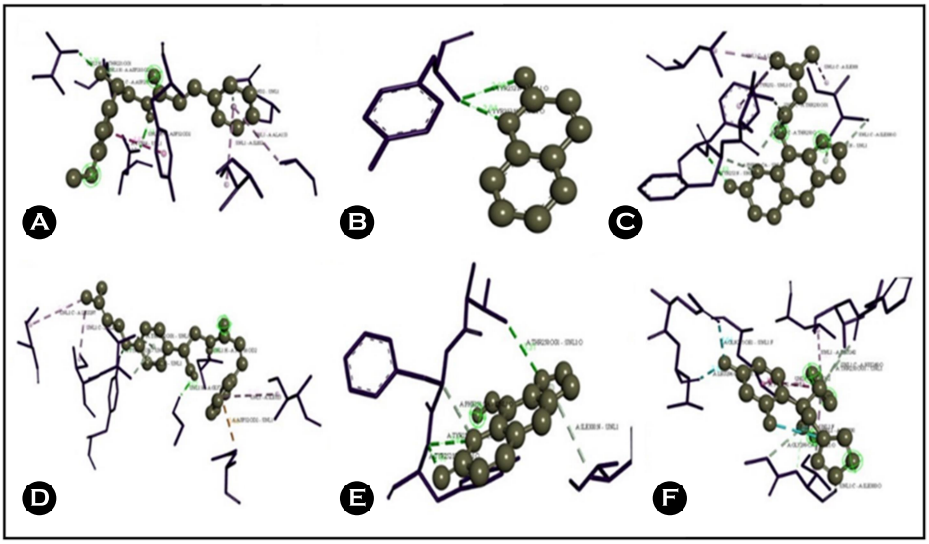

Fig. 3. Drug-ligand interactions between *Candida albicans* SAP protein (ligand) and the bioactive compounds from *Aegle marmelos* (A) Aegeline, (B) Coumarin, (C) Imperatorin, (D) Marmeline, (E) Xanthotoxol, (F) Fluconazole (reference)

G protein coupled receptor ligand, nuclear receptor ligand, kinase inhibitor, enzyme inhibition, and protease inhibition calculations were used to derive bioactivity scores for each selected compound against the SAP protein of *C. albicans* (Table 3). All compounds showed zero violations. The bioactivity scores and properties of the selected compounds are shown in Table 4.

6. Docking results of the interactions between *sap* and bio-compounds from *A. marmelos*

The interactions between SAP and the aforementioned bioactive compounds from *A. marmelos* were then examined *in silico* using Discovery Studio Visualizer (Fig. 3). The SAP protein structure was prepared by removing water molecules and adding hydrogen atoms according to the AutoDock preparation protocol. Ligand structures were imported in compatible formats (.mol) for the docking simulations. Using AutoDock Vina, the best-docked pose with the lowest binding energy was selected for further analysis. Atomic interactions, including hydrogen bonds, van der Waals interactions, π-π stacking, and alkyl/π-alkyl interactions, were visualized using Discovery Studio, and the binding energy for each ligand-protein interaction was calculated. Among the compounds tested, imperatorin exhibited the most favorable binding energy and additional π-sulfur interactions (Table 5), suggest-

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Table 5. Overall interaction scores of SAP with *Aegle marmelos* compounds

EfbA docking with the compounds	H-bonds	Van der Waals int.	pi-sigma, amide-pi stacked, and pi-cation interactions	Alkyl and pi-alkyl interactions	pi-pi T-shaped and pi-pi stacked interactions	Carbon-hydrogen bonds
Coumarin	1	–	–	–	–	7
Xanthotoxol	3	3	–	1	1	2
Imperatorin	4	4	–	1	–	3
Aegeline	3	10	–	2	–	1
Marmeline	3	5	1	2	–	1
Fluconazole	2	5	–	–	–	1

ing that imperatorin is a major contributor to SAP inhibition and *C. albicans* growth suppression.

DISCUSSION

The polymorphic fungus *C. albicans* is a part of the normal human microbiome in most individuals[17]. However, *C. albicans* is an opportunistic pathogen capable of causing a variety of infections under certain conditions, ranging from mild skin conditions to severe systemic diseases. Several virulence factors have been identified that contribute to the pathogenesis of this yeast-like fungus and greatly enhance its ability to cause infection[18]. Most of these virulent factors assist in infection by adhering to host cells, enhancing host cell invasion through hydrolase activity, aiding in the transition of the yeast-to-hyphal stage, acting as biosensors, promoting the formation of biofilms, and (or) by facilitating phenotype switching[19]. Although *C. albicans* overgrowth is commonly associated with oro-dental infections such as oral candidiasis, it can also contribute to the development of root caries, particularly in immunocompromised individuals[20]. Moreover, oral candidiasis in immunocompromised individuals can progress to a recalcitrant infection throughout the oropharynx[21]. The overgrowth of *C. albicans* in the oral cavity may also promote the formation of biofilms that increase the severity of other infections and conditions, such as root caries caused by *Streptococcus mutans* and other bacteria[22], especially under conditions of dysbiosis from immunosuppression or poor oral hygiene[23]. The capacity of *C. albicans* to exacerbate root caries stems from its ability to adhere to tooth surfaces and produce degradative hydrolytic enzymes[24]. Additionally, *C. albicans* can shift from a commensal to a pathogenic state, further enhancing candidiasis risk and caries progression[25]. Understanding the interplay between *C. albicans* and other microorganisms in the oral cavity is thus essential for developing targeted therapeutic strategies against candidiasis and root caries.

In the current study, *C. albicans* was identified in the majority of scrapping samples from root caries (65%), consistent with contributions to caries development, and *sap4* was detected in a fraction of these samples. Recombinant *sap1*, *2*, *3*, and *6* can induce the release of proinflammatory cytokines from human monocytes, and *sap4* to *sap6* (but not *sap1* to *sap3*) also contribute to the survival and escape from macrophage-induced destruction[8]. In addition to the *sap*-mediated virulence mechanisms, strains resistant to common antifungal agents have emerged, presenting a challenge to treatment using conventional therapies. For instance, several studies have reported strains of *C. albicans* resistant to amphotericin B and fluconazole[26]. In the current study, we selected *sap4* to *sap7* for PCR detection based on documented involvement in virulence and pathogenicity. Indeed, previous studies have highlighted the significant roles of these gene products in mediating host-pathogen interactions, tissue invasion, and survival during infection. Thus, these variants are likely among the predominant virulence factors in the pathogenesis of *C. albicans* infections[27].

Therefore, this study tested the efficacy of a methanolic extract derived from *A. marmelos*, a medicinal plant with a variety of documented bioactivities and a well-characterized bioactive compound profile. Many such studies have emphasized the therapeutic potential of medicinal plants due to well-documented safety profiles and global availability[28,29], and *A. marmelos* leaf has shown notable efficacy against other dermatophytes[30] and pathogens[31]. In this study as well, assessment using the disc diffusion method demonstrated substantial *C. albicans* growth inhibition at a dose of 20 mg,

suggesting therapeutic utility against oral infections.

In silico analysis further verified that individual phytochemicals contained in *A. marmelos* extract bound with high affinity to SAPs. Of the compounds investigated, imperatorin demonstrated the highest affinity (binding energy of -7.45 kcal/mol), followed by angelin (-6.82) and marmeline (-6.36). The highest number of hydrogen bonds was observed with imperatorin when compared to the fluconazole, which had only two hydrogen bonds. These findings are consistent with previous *in silico*-based assessments[32,33] and reports of promising bioactivities of imperatorin and the other assayed compounds against systemic infections[34]. For instance, coumarins are widely utilized in the treatment of prostate cancer, renal cell carcinoma, and leukemia, and also help mitigate the side effects of radiotherapy. Xanthotoxol, another furanocoumarin, is a key active component of *Cnidium monnieri*[35]. The extract of *A. dahurica* also contains imperatorin, which has sedative properties in addition to efficacy against headaches, toothaches, colds, and rheumatism. Aegeline is often marketed for enhancing weight loss, athletic performance, and vitality, although there is inadequate scientific evidence to support these claims[36]. Marmeline, an organic phenol ether, also has potent bioactive properties[37]. Further investigation is warranted to assess the contributions of these individual compounds to *C. albicans* growth suppression as well as other antifungal activities.

This study demonstrated the antifungal potential of *A. marmelos* against *C. albicans* using a combination of *in vitro* and *in silico* approaches. However, the study has limitations. First, we did not test the relative efficacy of *A. marmelos* extract against clinical antifungal agents such as fluconazole. The small sample size *(N = 20)* also limits the statistical power of the results. Third, we detected only *sap4* to *sap7* by PCR. While the *in silico* docking analysis identified promising interactions between *A. marmelos* bioactive compounds and SAP proteins, we did not assess the direct antifungal effects of these individual phytochemicals. Furthermore, the potential cytotoxic effects of *A. marmelos* extracts on human cells were not evaluated, which is critical for safe therapeutic applications. To establish the clinical relevance of *A. marmelos*, future studies should include comparative analyses with standard antifungal agents, larger sample sizes, *in vivo* validations, and cytotoxicity assessments.

CONCLUSION

The prevalence of *C. albicans* in oro-dental infections is rising in both healthy and immunosuppressed individuals. The emergence of antifungal resistance and the frequent expression of SAP-like virulence factors by *Candida* are serious threats complicating treatment, necessitating alternative therapies. The present study supports the efficacy of imperatorin from *A. marmelos* as a suitable drug candidate for further preclinical and possible clinical evaluation.

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CONFLICT OF INTEREST

In relation to this article, we declare that there is no conflict of interest.

DATA SHARING STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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ETHICAL APPROVAL STATEMENT

The study was approved by the Institutional Review Board of (IRB No. SRB/SDC/UG-2044/21/MICRO/063). This study was conducted in accordance with the principles of the Declaration of Helsinki.

AUTHOR CONTRIBUTIONS

Mitthun implemented the designed study: Dr. Smiline Girija contributed to the conceptualization and design, validation of the data obtained, manuscript drafting, editing and review;

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Shoba Gunasekaran contributed to the *in silico* studies. Dr. Vijayashree contributed to the final validation and proof reading of the manuscript; Kannika KP contributed to the formatting of the manuscript.

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