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Original Article

Microbiology Section

Targeting the Enterococcal Fibronectin Binding Protein- A of *Enterococcus faecalis* with Bioactive Compounds from Aegle Marmelos: An In-vitro and In-silico Pilot Study

VISHAKA KOTHARI[1], AS SMILINE GIRIJA[2], SHOBA GUNASEKARAN[3], J VIJAYASHREE PRIYADHARSINI[4]

ABSTRACT

Introduction: *Enterococcus faecalis (E. faecalis)* is a significant endodontic pathogen, and its virulence has been shown to be significantly influenced by Enterococcal fibronectin binding protein-A (EfbA). Targeting this virulent protein using alternative strategies would be a novel idea to combat the complications of *E. faecalis* in dental healthcare settings. Thus, the rationale of this investigation is to identify potent bioactive compounds from *Aegle marmelos* for their antimicrobial properties against *EfbA* of *E. faecalis*.

Aim: To assess the frequency of *EfbA* among clinical isolates of *E. faecalis* and evaluate the antibacterial activity of essential bioactive compounds from *Aegle marmelos*.

Materials and Methods: An in-vitro and in-silico pilot study was conducted from April 2022 to June 2022 in the Department of Microbiology, Saveetha Dental College and Hospitals, Chennai, Tamil Nadu, India to detect the frequency of EfbA in *E. faecalis*. Patients with typical root caries were included. Microbiological processing of 20 root caries samples was performed to characterise *E. faecalis* and detect the EfbA gene using Polymerase Chain Reaction (PCR) amplification. Crude methanol extract was obtained from *A. marmelos* and assessed for its antimicrobial effect against the isolated strains of *E. faecalis*. Furthermore, several biomolecules from *A. marmelos* were evaluated for their inhibitory properties through in-silico docking analysis.

Results: *E. faecalis* was identified in 12 (60%) out of 20 root caries samples, and three isolates (25%) were identified as Multidrug-Resistant (MDR) strains based on the antibiogram susceptibility profile, showing resistance to more than three different tested antibiotic groups. *EfbA* was detected in two of the three MDR strains (66.7%). The crude extract of *A. marmelos* exhibited promising antibacterial activity. In-silico analysis of the essential oil compounds from *A. marmelos* revealed that Aegeline had a high interaction with low docking energy and a high number of hydrogen bonds.

Conclusion: The current study highlights the potential of aegeline from *A. marmelos* as an antibacterial agent against resistant strains of *E. faecalis*. However, additional in-vivo research must be conducted to experimentally validate these findings.

Keywords: Aegeline, Health, Virulence

INTRODUCTION

Enterococcus faecalis, a Gram-positive Group D *Streptococcus*, has become an opportunistic pathogen and has contributed significantly to healthcare-associated infections since the late 1970s [1]. *E. faecalis* is considered the most prevalent species in endodontic infections, with associated complications such as endocarditis [2]. Due to its propensity for MDR against routine antibiotics like aminoglycosides, treating enterococcal endocarditis has been clinically challenging, with a mortality rate of up to 20% [3]. In addition to its drug-resistant properties, many virulent factors are known to contribute to its pathogenesis and the development of systemic infections and oral mucosal diseases [4].

Among various pathogenic mechanisms in *E. faecalis*, bacterial adhesion is a critical stage in disease development as it facilitates colonisation and penetration of the mucosal barrier, ultimately leading to subcellular spread within the host. Similar to other Gram-positive bacteria such as *Staphylococcus aureus*, *E. faecalis* is highly virulent and possesses specific adhesins called Microbial Surface Component-Recognising Adhesive Matrix Molecules (MSCRAMMs), which aid in attachment to human receptors or various Extracellular Matrix (ECM) components [5]. Numerous adhesins have been documented in *E. faecalis*, with the first characterised adhesin being EfbA, a PavA-like fibronectin-binding protein encoded by the EF1249 gene, which was initially reported in the JH2-2 strain of *E. faecalis* [6]. Its role was demonstrated through mutation-related studies, where an isogenic deletion mutant for EfbA showed severe impairment in its ability to bind to immobilised human fibronectin. EfbA plays a role in the pathogenesis of enterococcal Urinary Tract Infections (UTIs), and experimental evidence suggests that immunisation against EfbA can prevent infective endocarditis [7]. Targeting EfbA in *E. faecalis* could be a novel approach to overcome the complications caused by the organism in all healthcare settings.

In this context, the identification of potential novel bioactive compounds from natural sources to combat the pathogenic mechanisms in virulent, and resistance traits of *E. faecalis* has gained significant interest in recent years. The Rutaceae plant *Aegle marmelos* (L.) *Correa*, commonly known as Bael, has been extensively used in traditional Indian medical practices. *A. marmelos* is indigenous to Northern India but is also grown in other parts of the Indian subcontinent, including Ceylon, Burma, Bangladesh, Thailand, and Indo-China. Reports suggest that *A. marmelos* contains various phytoconstituents, primarily aegelin and marmelin, along with small amounts of tannin and riboflavin. Many compounds from *A. marmelos* are known for their bioactive properties, providing protection against various systemic ailments [8]. Therefore, the hypothesis of this study aims to evaluate the antimicrobial properties of crude extracts from *A. marmelos* against clinical strains of *E. faecalis.*

E. faecalis is associated with endocarditis, and novel alternative therapeutic strategies utilising potent bioactive compounds from natural sources offer promising methods to control these infections. In this context, the selection of active compounds can be effectively achieved using computational tools and databases. Numerous studies have documented the evaluation of various parameters of bioactive compounds derived from plants to observe promising drug-ligand interactions [9,10]. This study is unique as it implements the investigation for the first time to identify potent compounds from *A. marmelos* against EfbA of *E. faecalis*. As a pilot study, the aim of this investigation was to target the EfbA protein using bioactive compounds from *A. marmelos* through in-vitro assays to assess antimicrobial efficacy and a computational approach to analyse drug-ligand interactions.

MATERIALS AND METHODS

This in-vitro and in-silico pilot study was conducted from April 2022 to June 2022, spanning a three-month period, in the Department of Microbiology at Saveetha Dental College and Hospitals, Chennai, Tamil Nadu, India. Prior to the start of the investigation, Institutional Ethical clearance was obtained (Ref No: SRB/SDC/UG-2077/21/MICRO/056; IHEC/SDC/UG-2077/21/MICRO/597). Since this was a pilot study, statistical analysis for power calculation was not performed.

Twenty individuals who reported having root caries, as examined by an endodontist, had their carious scrapings collected. Sample collection was conducted with the informed consent of the patients and approval from the institutional review board. The carious dentine was immediately transferred to the microbiology lab after collection and placed in sterile trypticase soy broth. The samples were then plated onto clean Brain Heart Infusion (BHI) Agar and incubated for 24 hours at 37°C. Following incubation, the colonies were identified through routine biochemical testing, colony morphology, and gram staining.

Antimicrobial susceptibility tests: The clinical strains isolated from the samples were subjected to antibiotic susceptibility profiling to evaluate the antibiogram pattern. Briefly, a lawn culture of the fresh broth suspension of *E. faecalis* was prepared on Mueller Hinton agar, and antibiotics as per the Clinical and Laboratory Standards Institute (CLSI) guidelines for 2021 [11] were placed on the surface of the lawn using sterile forceps. The included antibiotics were amoxyclav (30 µg), ceftriaxone (30 µg), cefoperazone-sulbactam (75/30 µg), clindamycin (2 µg), cefixime (5 µg), levofloxacin (5 µg), linezolid (30 µg), vancomycin (30 µg), azithromycin (15 µg), amikacin, tetracycline (30 µg), ciprofloxacin (5 µg), cefoperazone (2 µg), and gentamicin (10 µg). The plates were then incubated at 37°C for 24 hours, and the zones of inhibition on the plates were measured to determine the patterns of susceptibility.

Genotypic characterisation of EfbA gene in *E. faecalis*: The clinical strains that were resistant to more than three groups of drugs tested were isolated on MacConkey agar after 24 hours of incubation at 37°C. Genomic DNA was extracted from the *E. faecalis* strains following the manufacturer's instructions using a Qiagen kit. PCR was performed to detect the presence of EfbA. For the PCR reaction, 7.8 µL of 2 µL master mix from (Takara, Japan) was combined with 5.6 µL of DDW. A 15 µL reaction mixture was prepared using specific primers for *EfbA* (F: GCACAAGTCCCAAAAGGAGC and R: AAGTGCGGCTTCAGTAAGGG) at a concentration of 0.31 µL of 100 pmol/mL (Eurofins Genomic India Pvt., Ltd., Bangalore). The amplification was carried out using Germany's Eppendorf thermocycler with 35 cycles at an annealing temperature of 58°C. The resulting amplicon was visualised using a gel documentation system and examined on a 1% agarose gel electrophoresis with Ethidium bromide. The size of the amplicon was determined using a 100 bp DNA ladder from New England Biolabs.

Preparation of the *A. marmelos* extract: Fresh *A. marmelos* fruits were purchased from the neighborhood markets. The fruits were externally cleaned by washing them three times in sterile distilled water. Then, they were chopped into small pieces using sterilised knives and dried in the shade. After drying, the fruits were mechanically milled into a coarse powder. The powder was then stored in sterile containers for further antibacterial bioassay [12].

Next, 10 grams of the dried *A. marmelos* leaf powder was mixed with 100 mL of methanol and incubated at room temperature for a week with intermittent shaking. After the incubation period, the extract was filtered using a Whatman No. 1 filter. The filtered extract was placed onto sterile petri dishes and evaporated to obtain the crude extracts. These extracts were preserved at 4°C for additional in-vitro assays.

Antimicrobial bioassay: For the final formulation, 20 mg of the *A. marmelos* crude extract was combined with 1 mL of Dimethyl Sulphoxide (DMSO) and vortexed. The clinical strains of *E. faecalis* were cultured as a lawn on sterile Saboraud's dextrose agar, and the wells were punctured using a sterile agar cutter [13]. Then, 50 µL of the diluted extract was added to each well, and the plates were incubated for 48 hours at 37°C. After incubation, the size of the zones was measured and recorded using a HiMedia antibiotic measuring scale. The test was repeated three times with different concentrations of 20, 10, and 5 mg/mL, and the mean value was recorded.

Retrieval of EfbA and protein optimisation: The crystal structure of the EfbA protein was provided by the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (PDB) (http://www.rcsb.org/pdb) [14]. By adding hydrogen atoms, the crystal structure of EfbA was optimised. The AutoDock program, version 1.5.6, was used to assign electrical charges to the protein atoms, and the RASMOL tool was used to visualise the three-dimensional structure of the EfbA protein.

Ligand preparation and optimisation: ChemSketch software was used to obtain the chemical structures of *A. marmelos* bioactive derivatives, as done in earlier studies [15,16]. Afterward, the 3D structures were optimised. The open-label molecular converter program underwent additional conversions on the chosen ligands. After that, they were stored in PDB format. The chosen ligands were subsequently saved in molecular structural formats.

Molinspiration assessment of the selected ligands: The Molinspiration evaluation software was used to determine the counts of hydrogen bond acceptors and donors in connection with the membrane permeability and bioavailability of the compounds, as well as the partition coefficient (logP) and molecular weight of the compounds as basic molecular descriptors. The Lipinski's Rule of Five was employed to further examine the characteristics of the selected bio compounds' absorption, distribution, metabolism, and elimination.

Docking interactions: To assess the affinity between bio-compounds from *A. marmelos* and the *E. faecalis* EfbA protein, docking analysis was performed using the AutoDock tool, and the interaction scores were further evaluated.

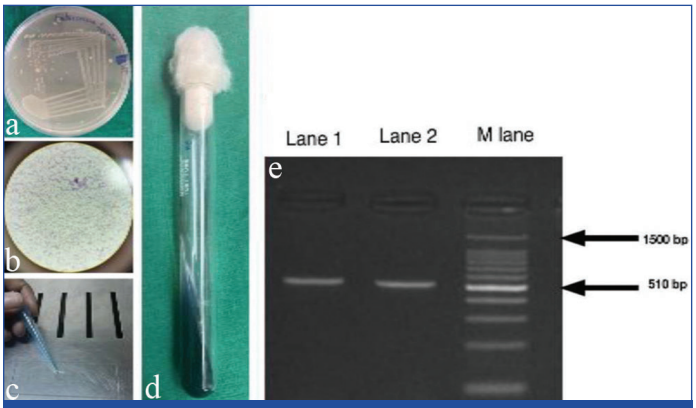
Docking visualisation: The hydrogen bond interactions between the bio-compounds of *A. marmelos* and EfbA of *E. faecalis* were visualised using discovery studio visualiser. The relative stabilities were assessed through additional docking score evaluations, binding affinities, molecular dynamics, and energy simulations.

STATISTICAL ANALYSIS

The statistical significance of the results was evaluated using SPSS version 21.0 (Chicago, IL).

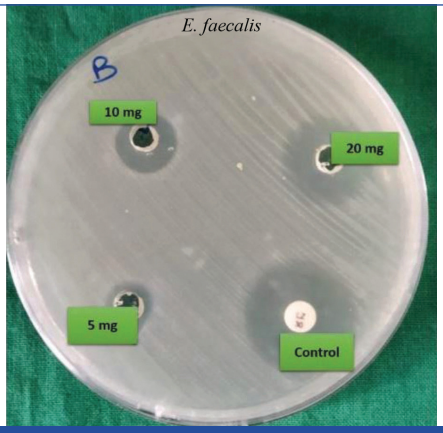
RESULTS

Characterisation of *E. faecalis*: From a total of 20 samples processed, 12 strains (60%) of *E. faecalis* were identified. The *E. faecalis* colonies were identified as pinpoint colonies on the BHI agar plates. Gram staining revealed typical diplococci that are gram-positive and have pairs of oval cocci. Phenotypic characterisation studies revealed colonies with positive bile esculin hydrolysis but negative catalase [Table/Fig-1].

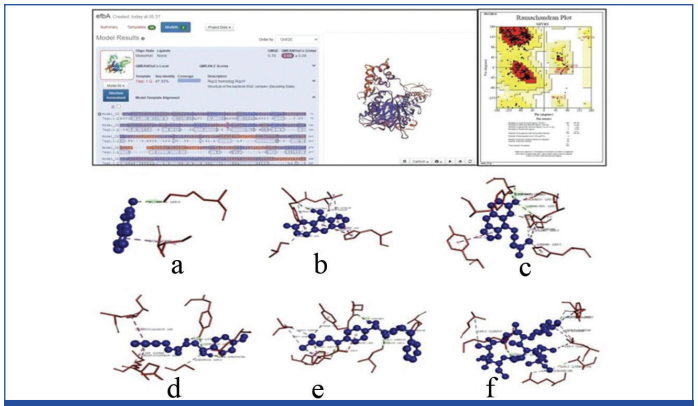


[Table/Fig-1]: Phenotypic characterisation of *E. faecalis* from the clinical samples: a) *E. faecalis* growth on Brain heart infusion agar; b) Gram staining showing gram positive diplococcic; c) Catalase tested negative; d) Positive bile esculin test; e) Electropherogram of EfbA gene product of size 510 bp in lanes 1 and 2 with 1.5 k bp marker lane (M).

Three isolates (25%) were identified as MDR strains based on the antibiogram susceptibility profile, showing resistance to more than three different tested antibiotic groups. Two out of the three MDR strains (66.7%) tested positive for EfbA, with an amplicon size of 510 bp [Table/Fig-2].



[Table/Fig-2]: Antimicrobial effect of the crude methanolic extracts at varying concentrations (20 mg, 10 mg and 5 mg) of *A. marmelos* against the clinical strains of *E. faecalis*.



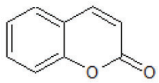
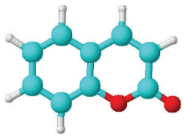
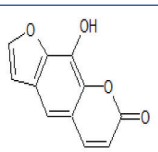
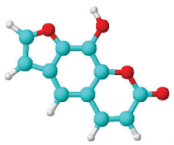
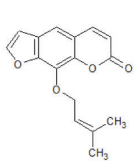
[Table/Fig-3]: Prediction of EfbA structure and homology modelling in Swiss model server and validation of the predicted structure using Ramachandran plot and visualising hydrogen interactions between EfbA with: a) Coumarin; b) Xanthotoxol; c) Imperatorin; d) Aegeline; e) Marmeline; f) Erythromycin.

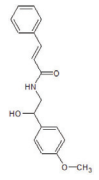
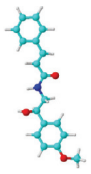
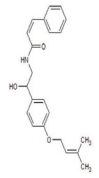
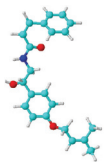
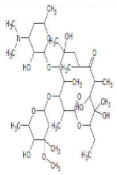
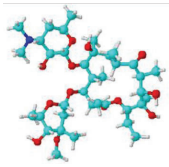
Antifungal effect of *A. marmelos* extract: The total yield of the *A. marmelos* methanol extract from 100 g of the crude powder was 23 mg (w/v). The extract exhibited a promising effect against all the sensitive strains (n=9) and the clinical strains (n=3) with zone sizes of 18 mm, 13 mm, and 10 mm for 20 mg, 10 mg, and 5 mg, respectively.

Structural retrieval of the EfbA protein from *E. faecalis*: The UNIPROT database was searched for the FASTA sequence of EfbA from *E. faecalis*, and the sequence ID was A0A335NTF8. A homology model was created using the Swissmodel server, utilising the template 5WQO-A chain. With a 100% sequence identity to the template, the model appeared quite plausible. Moreover, the Ramachandran plot demonstrated that 88.1% of residues were located in the most favorable regions, with none falling within the restricted zones [Table/Fig-3]. The 3D structure of EfbA was visualised using RASMOL, where the alpha-helices were represented in pink, the beta sheets in yellow arrows, and the turns in white colour.

Structural retrieval of the ligands from compounds in *A. marmelos* essential oils: To perform ligand optimisation, ACD Chemsketch was utilised, and the Open Babel molecular converter tool was used to obtain a compatible format. [Table/Fig-4] presents the 2D and 3D structures, as well as the Simplified Molecular Input Line Entry System (SMILES) format, of the ligands extracted from *A. marmelos*.

Molinspiration assessment towards drug likeliness: Based on the calculation of ion channel modulation, GPCR ligand, nuclear receptor ligand, kinase inhibitor, enzyme inhibition, and protease inhibition, the bioactivity score prediction of essential compounds of *A. marmelos* against EfbA of *E. faecalis* was assessed for drug likeliness, and the results were tabulated in [Table/Fig-5].

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			2D	3D	10212

Aegeline	COC1=CC=C(C=C1)C(CNC(=O)/C=C/C2=CC=CC=C2)O			$C_{18}H1_9NO_3$	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

[Table/Fig-4]: A 2D and 3D structures and SMILES format of the selected biocompounds from *A. marmelos* for the study.

Compound name	Hydrogen bond donor	Hydrogen bond acceptor	miLogP	Rotatable bonds	nViolations	TPSA (Å)	Vol	N 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
Erythromycin	5	14	2.28	7	2	193.92	709.28	51

[Table/Fig-5]: Molinspiration assessments for the properties of the selected bio-compounds from *A. marmelos*.
*Note: Drug likeliness score: >0.2 above. The larger the value of the score is, the higher is also probability that the particular molecule will be active

Docking analysis of the *A. marmelos* derivatives against EfbA of *E. faecalis*: LGA was employed to select the finest conformers. [Table/Fig-6] presents the interactions of the EfbA protein with bioactive substances from *A. marmelos*. Docking scores, hydrogen bond counts, and torsional energy between the ligands and the drugs were recorded as data [Table/Fig-7]. The overall docking energies and interactions between EfbA and the *A. marmelos* biocompounds were assessed using ligand efficiency, intermolecular energy, electrostatic energy, vdW + Hbond + desolv energy, internal energy, and torsional energy in kcal/mol [Table/Fig-8]. The results indicate that the imidazole molecule from *A. marmelos* exhibited the

Compound name	EfbA		Ligand atoms	Distance (A)	Docking energy (Kcal/Mol)
	Residue	Atom			
Coumarin	LYS460	NZ	O	2.99376	-5.1
	ILE410	-	-	4.99246	
Xanthotoxol	GLY540	O	H	1.66947	-6.14
	TYR541	CA	O	3.78258	
	SER403	OG	-	3.88468	
	HIS481	-	-	4.57133	
	HIS481	-	-	3.66454	
	TYR541	-	-	4.88118	
	VAL542	-	-	5.25507	
	VAL542	-	-	4.57073	
Imperatorin	HIS481	ND1	O	2.95645	-6.34
	ASP484	N	O	3.22782	
	TYR544	OH	O	2.75498	
	HIS481	CE1	O	2.79937	
	TYR541	-	-	5.03023	
	PRO486	-	C	4.84007	
	HIS481	-	C	4.68674	
	HIS489	-	C	5.08422	
Aegeline	TYR511	OH	O	3.13652	-6.75
	GLU556	OE1	H	1.99379	
	TYR511	OH	H	2.07972	
	GLY278	CA	O	3.46947	
	GLU281	C, O	-	3.89781	
	LYS282	N	-	3.89781	
	ILE410	C,O	-	4.59117	
	ALA411	N	-	4.59117	
	LYS282	-	-	4.9018	
Marmeline	SER2	OG	O	2.91057	-6.64
	TYR255	OH	O	3.24475	
	TYR276	OH	H	2.55528	
	GLU248	O	H	2.69493	
	SER2	OG	C	2.85103	
	ASP206	OD2	-	3.60533	
	TYR255	-	C	3.73462	
	LEU258	-	C	4.7028	
	LEU258	-	-	4.12144	
	TYR249	-	C	4.16517	
	TYR255	-	C	4.18284	
Erythromycin	LYS246	N	O	3.19683	-5.19
	ALA274	O	C	2.97298	
	GLU261	OE1	C	3.2314	
	THR263	OG1	C	3.21368	
	LEU517	-	C	5.21554	
	LEU241	-	C	5.12951	
	LEU241	-		4.18894	
	LEU241	-	C	5.21633	

[Table/Fig-6]: EfbA protein interactions with the bioactive compounds from *A. marmelos*.

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
Coumarin	2	-5.1	182.56	-0.46	-5.1	-4.98	-0.12	0.0	0.0
Xanthotoxol	3	-6.14	31.4	-0.41	-6.44	-6.33	-0.11	0.3	-0.44
Imperatorin	4	-6.34	22.52	-0.32	-7.24	-7.16	-0.08	0.89	-0.76
Aegeline	4	-6.75	11.32	-0.31	-8.84	-8.56	-0.28	2.09	-0.76
Marmeline	1	-6.64	13.57	-0.26	-9.33	-9.25	-0.07	2.68	-1.19
Erythromycin	1	-5.19	156.73	-0.1	-8.77	-8.08	-0.69	3.58	-7.39

[Table/Fig-7]: Docking scores of *A. marmelos* against EfbA protein of *E. faecalis*.

EfbA docking with compounds	Hydrogen bonds	van der Waals interactions	pi-sigma interaction,amide-pi stacked interactions, pi-cation interactions	alkyl, pi-alkyl interactions	pi-pi T shaped, pi- pi stacked interaction	Carbon hydrogen bond	Docking energy (Kcal/mol)
Coumarin	2	1	-	1	-	-	-5.1
Xanthotoxol	3	3	-	1	1	2	-6.14
Imperatorin	4	7	-	2	1	-	-6.34
Aegeline	4	6	1	1	-	1	-6.75
Marmeline	1	8	1	2	-	-	-6.64
Erythromycin	1	6	-	3	-	3	-5.19

[Table/Fig-8]: Overall interactions of EfbA with the bioactive compounds from *A. marmelos*.

most promising interaction due to its low binding energy. Additionally, alkyl/p-alkyl interactions, van der Waals, p-sulfur interactions, and p-r interactions were observed.

DISCUSSION

Enterococci are considered transient members of the oral microbiome and can contribute to various systemic and oral-dental disorders. The presence of MDR and virulent traits makes Enterococci a challenging nosocomial pathogen for healthcare professionals. Enterococci are also part of the functional biomes of the gut and oral cavity [17]. Limited studies have been conducted on the prevalence of *E. faecalis* strains carrying EfbA genetic determinants in healthcare settings. Therefore, this study aimed to assess the prevalence of these determinants among clinical isolates from patients with endodontic infections. Resistance among *E. faecalis* strains has been frequently reported, and present study observed a prevalence of 60% for *E. faecalis* among patients with root caries, with 25% of the strains exhibiting MDR. Among the resistant strains, 66.7% were found to possess the EfbA gene. This finding aligns with a previous study that associated the virulent EfbA gene with MDR, where the presence of EfbA was documented in 97.1% of resistant strains [18].

Plant-based biocompounds have been recognised for their potent bioactive properties and their effectiveness against microbial pathogens. In recent years, researchers have focused on identifying and validating plant-derived compounds for the treatment of various ailments [19]. It is worth noting that Indian medicinal plants are considered a valuable source of pharmacologically active compounds, which are frequently used to address a wide range of health conditions. Many pharmacologically active chemicals have already been extracted and identified from essential medicinal herbs such as neem, turmeric, and various other herbs [20]. In line with this, the Indian plant known as Bael *(Aegle marmelos (L.) Cor.),* which has a long history of traditional use in the treatment of various ailments, was selected for preliminary evaluation of its antimicrobial activity against *E. faecalis* in this study.

In this context, the present study demonstrated a promising antimicrobial effect against *E. faecalis*, which was consistent with earlier studies where a 5% concentration of *A. marmelos* extract showed significant antimicrobial activity [21]. However, in the present study, antibacterial activity was observed within the concentration range of 5 mg to 20 mg. In contrast, a study conducted by Subhashini A et al., did not observe any antimicrobial activity against *E. faecalis* [13]. These correlating and contrasting results could be attributed to variations in techniques, solvents used, and the strains employed in the studies. Nevertheless, the promising results of the present study are noteworthy as the activity was evaluated against drug-resistant strains of *E. faecalis*.

To further evaluate the potential, an in-silico approach was employed to assess the drug-ligand interactions between five different compounds of *A. marmelos* and the EfbA protein of *E. faecalis*. The drug-likeness assessment based on the molinspiration results appeared promising, with no violations observed for any of the chosen biocompounds. However, two violations were identified for the control compound, erythromycin. Coumarin exhibited the lowest molecular weight among the chemicals, while marmeline had the highest molecular weight of 351.4. Coumarin and its analogs are known for their antimicrobial activity [22]. The molecular weights of the other substances ranged from 200 to 370. In terms of hydrogen bond donor and acceptor properties, marmeline had the highest number of rotatable bonds, approximately 8, and the highest miLogP value of 4.32. An important evaluation parameter is the Topological Polar Surface Area (TPSA) value, which indicates the oral bioavailability of a drug and should be >140. Encouragingly, all five chosen bioactive compounds had TPSA values exceeding 140. When comparing overall docking energies, coumarin exhibited the least hydrogen bonds, followed by aegeline, marmeline, imperatorin, and xanthotoxol. Aegeline displayed a binding energy of approximately -6.75, while coumarin had the lowest binding energy of -5.1. Aegeline also exhibited the lowest inhibition constant, whereas coumarin had the highest. Marmeline demonstrated higher levels of torsional, electrostatic, and ligand efficiency.

Purified compounds like imperatorin are known for their antimicrobial activity, as reported from various other plants [23]. In the present study, imperatorin exhibited four hydrogen bond contacts, seven van der Waals interactions, two pi-alkyl interactions, and one pi-pi stacking interaction, indicating the stabilisation of binding structures. Aegeline showed four hydrogen bonds, followed by xanthotoxol with three hydrogen bonds, coumarin with two hydrogen bonds, and marmeline with one hydrogen bond interaction. Marmeline displayed the highest van der Waals interaction, followed by imperatorin. Aegeline and marmeline showed the highest pi-sigma interaction. Marmeline and imperatorin exhibited stronger pi-alkyl interactions. Only xanthotoxol and imperatorin displayed pi-pi stacked interactions. Considering all these interactions, aegeline is considered the most promising candidate for further analysis.

In dental and other healthcare settings, where *E. faecalis* is prevalent with resistant and virulent traits, the implementation of novel drugs can serve as an excellent alternative treatment strategy. From a clinical perspective, periodic surveillance activities on the prevalence of these strains can be conducted. Additionally, computational analysis can be utilised to design novel drugs from various natural sources, screening for potent bioactive compounds to address complications caused by these pathogens.

Limitation(s)

With a promising antibacterial activity of the crude extract of *A. marmelos* against *E. faecalis*, authors did not proceed with further purification analysis for the biocompounds. Additionally, the values for Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) were not determined. Further experimental evaluation of the purified compounds is needed to elucidate their toxicity and inhibitory activity.

CONCLUSION(S)

The current investigation has shown the presence of EfbA among MDR strains of *E. faecalis*, which may require frequent monitoring in dental and hospital settings. *A. marmelos* demonstrated a good antimicrobial effect against EfbA-producing strains of *E. faecalis*. Computational studies revealed a stronger interaction between aegeline and EfbA. However, further experimental validation must be performed for in-vivo inhibitory and toxicity studies to develop aegeline from *A. marmelos* as a promising drug.

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PARTICULARS OF CONTRIBUTORS:

1. Undergraduate Student, Department of Microbiology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai, Tamil Nadu, India.
2. Professor, Department of Microbiology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai, Tamil Nadu, India.
3. Assistant Professor, Department of Biotechnology, Dwaraka Doss Goverdhan Doss Vaishnav College, Chennai, Tamil Nadu, India.
4. Associate Professor, Department of Microbiology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai, Tamil Nadu, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. AS Smiline Girija,
Professor, Department of Microbiology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai-600077, Tamil Nadu, India.
E-mail: smilinejames25@gmail.com

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