RESEARCH PAPER

# Antimicrobial Evaluation of Cassia Auriculata Extracts Against Streptococcus Mutans and Enterococcus Faecalis: An In Vitro Study

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**Abstract:**

**Background:** Dental caries and endodontic infections caused by Streptococcus mutans and Enterococcus faecalis, respectively, remain a significant public health concern. Natural products, including plant extracts, have gained attention for their potential antimicrobial properties. Cassia auriculata, a medicinal plant rich in bioactive compounds, has been investigated for its antibacterial properties. This study aimed to compare the antimicrobial efficacy of aqueous and ethanolic extracts of Cassia auriculata against Streptococcus mutans and Enterococcus faecalis.

**Aim:** To evaluate aqueous and ethanolic extract of Cassia auriculata against Streptococcus mutans and Enterococcus faecalis.

**Method:** Aqueous and ethanolic extracts of Cassia auriculata were prepared and antimicrobial activity of these extracts was evaluated against pure cultures of Streptococcus mutans and Enterococcus faecalis using well diffusion and minimum inhibitory concentration (MIC) assays.

**Result:** The present study results shows an excellent antimicrobial activity of Cassia auriculata of both aqueous and ethanolic extract at 25 to 50 µg/l against e fecalsis and streptococcus mutans. The plant derived synthetic chemical compound can be used for various dental applications like mouthwash, root canal irrigants, toothpaste and dental cements.

**Conclusion:** Both aqueous and ethanolic extracts show potent antimicrobial activity against Streptococcus mutans and Enterococcus faecalis. Thus this can be applied in the field of medicine and future research can be focussed on various dental applications for orodental care.

**Keywords:** Cassia auriculata, antimicrobial activity, Streptococcus mutans, Enterococcus faecalis, phytochemicals, medicinal plants, dental caries, endodontic pathogens, molecular docking, ADMET profiling, Good Health and Well-being.

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## Introduction:

Dental caries remains one of the most prevalent chronic diseases affecting children and adults worldwide, posing a significant public health burden despite advances in preventive and restorative dentistry.(1) The disease is primarily biofilm-mediated, driven by complex interactions between cariogenic microorganisms, dietary sugars, and host factors. Among the microbial species

implicated, Streptococcus mutans plays a central role in caries initiation due to its acidogenicity, aciduricity, and ability to synthesize extracellular polysaccharides that facilitate biofilm formation on tooth surfaces.(2) In addition, Enterococcus faecalis is frequently associated with persistent endodontic infections and secondary caries, owing to its resistance to harsh environmental conditions, ability to penetrate dentinal tubules, and survival following conventional antimicrobial treatments.(3)

Currently employed antimicrobial agents such as chlorhexidine and synthetic antibiotics have demonstrated efficacy against oral pathogens; however, their long-term use is associated with several limitations, including adverse effects, tooth staining, taste alteration, and the emerging problem of antimicrobial resistance.(4),(5) These concerns have stimulated growing interest in plant-derived natural products as alternative or adjunctive antimicrobial agents in oral healthcare. Medicinal plants offer a rich source of bioactive phytochemicals such as flavonoids, tannins, phenolic acids, and alkaloids, many of which exhibit antimicrobial, anti-inflammatory, and antioxidant properties.(6)

Cassia auriculata (family: Fabaceae), commonly known as Tanner's cassia, is a traditional medicinal plant widely used in Indian systems of medicine for the management of diabetes, skin disorders, inflammation, and infections.(7) Previous phytochemical investigations have reported the presence of flavonoids, polyphenols, anthraquinones, and tannins in C. auriculata, compounds known to interfere with microbial cell walls, enzyme systems, and virulence mechanisms.(8),(9) Several studies have demonstrated the antimicrobial activity of C. auriculata extracts against non-oral bacterial strains; however, evidence specifically targeting key oral pathogens remains limited and fragmented.(10) Moreover, variations in extraction methods, solvent systems, and test organisms across studies have resulted in inconsistent outcomes, limiting direct clinical extrapolation.

Importantly, most available studies on herbal antimicrobials in dentistry are confined to in vitro screening assays and lack mechanistic insights into how phytochemicals interact with bacterial targets at the molecular level. Molecular docking and in silico approaches have emerged as valuable tools to complement in vitro findings by predicting interactions between bioactive compounds and essential bacterial enzymes involved in adhesion, biofilm formation, and cell survival.(11).

Despite the traditional medicinal importance of Cassia auriculata, systematic studies comparing the antimicrobial efficacy of different solvent extracts of this plant against Streptococcus mutans and Enterococcus faecalis, two clinically relevant oral pathogens, remain limited. Addressing this gap is important to support the scientific basis for its potential use in preventive and therapeutic dental formulations. The present study was designed to evaluate and compare the antimicrobial activity of aqueous and ethanolic extracts of Cassia auriculata against S. mutans and E. faecalis using standard in-vitro assays.

The null hypothesis ($H_0$) states that there is no significant difference in the antimicrobial efficacy of aqueous and ethanolic extracts of Cassia auriculata against Streptococcus mutans and Enterococcus faecalis.

**Materials and Methods**

**Study design**

This experimental study employed an in vitro antimicrobial evaluation of aqueous and ethanolic extracts of Cassia auriculata against Streptococcus mutans and Enterococcus faecalis, complemented by in silico molecular docking analysis to explore potential mechanistic interactions between selected phytochemicals and bacterial target proteins.

Plant material collection and authentication

Fresh flowers of Cassia auriculata were collected from a local authenticated source in Tamil Nadu, India. Botanical identification and authentication were performed by a qualified taxonomist, and a voucher specimen was deposited in the institutional herbarium for future reference. Plant material was thoroughly washed under running water, shade-dried at room temperature, and pulverized into a coarse powder using a mechanical grinder.(12)

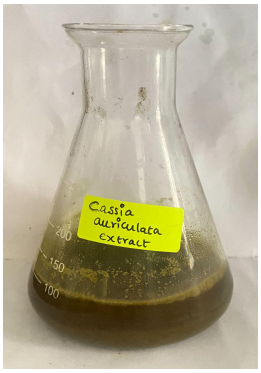
Preparation of plant extracts

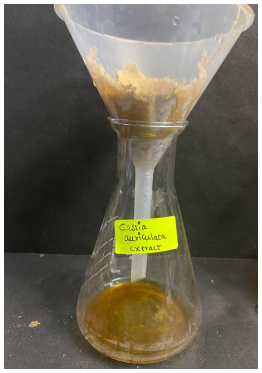
**Two solvent extracts were prepared:**

**Aqueous extract:**

Fifty grams of powdered plant material were mixed with 500 mL of distilled water and subjected to hot maceration at 60–70 °C for 6 hours with intermittent stirring. The mixture was filtered using Whatman No.1 filter paper, and the filtrate was concentrated using a rotary evaporator, followed by drying to obtain a crude aqueous extract [2]. 2g of Cassia auriculata (powdered form) was taken and mixed in 100 ml distilled water.The solution is kept for boiling under a heating mantle for 20 mins under

50 to 60 degree celsius. Then, the solution was filtered by muslin cloth or Whatman no.1 filter paper. The filtered extract was boiled under 50 to 60 degree celsius until it's been condensed till 5ml and the aqueous extract was prepared.



Powdered Sample Preparation of Extract Filtration of Extract Crude extract

**Ethanolic extract:**

Five grams of powdered material were extracted with 20ml of 95% ethanol using Soxhlet extraction for 8 hours. The extract was filtered and concentrated under reduced pressure using a rotary evaporator to obtain the crude ethanolic extract [3].5g of Cassia auriculata (powdered form) was taken and mixed in 20 ml ethanol. Kept in the orbital shaker for 24 hrs and the extract was formed.

The dried extracts were weighed to calculate percentage yield and stored at 4 °C in airtight containers until further use.

**Microbial strains and culture conditions**

Standard reference strains of Streptococcus mutans (ATCC 25175) and Enterococcus faecalis (ATCC 29212) were obtained from a certified microbial repository. The strains were revived and maintained on Brain Heart Infusion (BHI) agar. Prior to antimicrobial testing, bacterial suspensions were adjusted to 0.5 McFarland standard (≈1.5 × $10^8$ CFU/mL) to ensure uniform inoculum density.(13)

**In vitro antimicrobial assay (agar well diffusion method)**

Antimicrobial activity was assessed using the agar well diffusion technique. Sterile Mueller–Hinton agar plates (supplemented with 5% sheep blood for S. mutans) were inoculated uniformly with standardized bacterial suspensions using sterile swabs. Wells of 6 mm diameter were punched aseptically, and 50 µL of extract solutions at varying concentrations (25, 50, 100, and 200 µg/mL) were introduced into the wells.

Chlorhexidine gluconate (0.2%) served as the positive control, while the respective solvents acted as negative controls. Plates were incubated at 37 °C for 24 hours under appropriate atmospheric conditions. Zones of inhibition were measured in millimeters using a digital caliper. All experiments were performed in triplicate, and mean values were recorded.

**Time-kill assay**

The bactericidal activity of aqueous and ethanolic extracts of Cassia auriculata was evaluated using a time-kill kinetic assay. Standardized bacterial suspensions of Streptococcus mutans and Enterococcus faecalis were prepared in Brain Heart Infusion (BHI) broth and adjusted to approximately 1.5 × $10^8$ CFU/mL (0.5 McFarland standard).

Aliquots of the bacterial suspension were exposed to aqueous and ethanolic extracts at concentrations of 25, 50, and 100 µg/mL. Chlorhexidine gluconate (0.2%) served as the standard antimicrobial control, while extract-free broth inoculated with bacteria served as the growth control. All experiments were performed in triplicate.

The cultures were incubated at 37 °C under appropriate atmospheric conditions, and bacterial growth was monitored at 1, 2, 3, and 4 hours. At each time point, bacterial growth was assessed by measuring optical density (OD) at 600 nm using a UV–visible spectrophotometer. The change in optical density over time was used as an indirect indicator of bacterial viability and growth kinetics.

Time-kill curves were constructed by plotting mean optical density values against time for each concentration and extract type. A concentration- and time-dependent reduction in optical density relative to the growth control was interpreted as bactericidal activity. Results were expressed as mean ± standard deviation of three independent experiments.(14)

**Statistical analysis**

All quantitative data were expressed as mean ± standard deviation. Statistical analysis was performed using SPSS software (version 26.0). Intergroup comparisons among different extract concentrations were conducted using one-way ANOVA followed by Tukey's post-hoc test. A p-value < 0.05 was considered statistically significant. The data were entered in an Excel spreadsheet and subjected to statistical analysis in IBM SPSS Statistics software version 23.0. Independent T-test was used for intragroup and intergroup comparisons.

**Results:**

| Organism | Extract | Concentration (µg/mL) | Zone of inhibition (mm) (Mean ± SD) |
|---|---|---|---|
| Streptococcus mutans | Aqueous | 25 | 17.0 ± 1.0 |
| | | 50 | 20.0 ± 1.0 |
| | | 100 | 26.0 ± 1.5 |
| | Ethanolic | 25 | 20.0 ± 1.0 |
| | | 50 | 24.3 ± 1.5 |
| | | 100 | 26.0 ± 1.0 |
| Enterococcus faecalis | Aqueous | 25 | 20.0 ± 1.0 |
| | | 50 | 22.0 ± 1.0 |
| | | 100 | 25.0 ± 1.0 |
| | Ethanolic | 25 | 18.0 ± 1.0 |
| | | 50 | 20.0 ± 1.0 |
| | | 100 | 24.3 ± 1.5 |
| Chlorhexidine (0.2%) | — | — | 9.0 ± 0.0 |

Table 1: Zone of inhibition (mm) of aqueous and ethanolic extracts against Streptococcus mutans and Enterococcus faecalis (mean ± SD, n = 3)

Table 1 summarizes the antimicrobial activity of aqueous and ethanolic extracts of Cassia auriculata against Streptococcus mutans and Enterococcus faecalis as assessed by the agar well diffusion assay. For both organisms, a clear concentration-dependent increase in antimicrobial activity was observed with increasing extract concentration.

When comparing extract types, the ethanolic extract consistently demonstrated equal or greater inhibitory activity than the aqueous extract across most concentrations, indicating enhanced extraction of bioactive constituents in ethanol. Overall, Streptococcus mutans showed higher susceptibility to the extracts compared with Enterococcus faecalis, reflecting organism-specific differences in resistance. Chlorhexidine served as the reference standard and confirmed assay validity.

| Organism | Source of variation | df | F value | p value | Partial eta squared ($\eta p^2$) |
|---|---|---|---|---|---|
| Streptococcus mutans | Extract type | 1 | 20.35 | 0.0007 | 0.63 |
| | Concentration | 2 | 55.85 | < 0.00 | 0.9 |

| | | | | | |
|---|---|---|---|---|---|
| | | | | 1 | |
| | Extract × Concentration | 2 | 4.31 | 0.0389 | 0.42 |
| Enterococcus faecalis | Extract type | 1 | 8.91 | 0.0114 | 0.43 |
| | Concentration | 2 | 40.55 | < 0.001 | 0.87 |
| | Extract × Concentration | 2 | 0.73 | 0.503 | 0.11 |

Footnote: Partial eta squared ($\eta p^2$) was calculated as SS_effect / (SS_effect + SS_error). Effect size interpretation: small ≈ 0.01, moderate ≈ 0.06, large ≥ 0.14.

TABLE 2. Summary of two-way ANOVA for agar well diffusion assay

Table 2 summarizes the two-way ANOVA assessing the effects of extract type and concentration on antimicrobial activity, together with effect size estimation using partial eta squared. The analysis indicates that concentration was the primary contributor to variability in inhibition, confirming a strong dose-dependent response for both organisms. Extract type also accounted for a meaningful proportion of the observed variation, highlighting the influence of the extraction solvent on antimicrobial efficacy. The interaction term revealed that, for Streptococcus mutans, the effect of increasing concentration differed between aqueous and ethanolic extracts, whereas Enterococcus faecalis exhibited similar response patterns across extract types. Overall, effect size analysis complements significance testing by demonstrating the biological relevance and relative contribution of each experimental factor to antimicrobial activity.

| Organism | Comparison | Mean difference (mm) | p value |
|---|---|---|---|
| Streptococcus mutans | Aqueous 25 vs 50 µg/mL | 3 | < 0.05 |
| | Aqueous 50 vs 100 µg/mL | 6 | < 0.01 |
| | Ethanolic 25 vs 50 µg/mL | 4.3 | < 0.05 |
| | Ethanolic 50 vs 100 µg/mL | 1.7 | > 0.05 |
| | Ethanolic vs Aqueous (50 µg/mL) | 4.3 | < 0.05 |
| | Ethanolic vs Aqueous (100 µg/mL) | 0 | > 0.05 |
| Enterococcus faecalis | Aqueous 25 vs 50 µg/mL | 2 | < 0.05 |
| | Aqueous 50 vs 100 µg/mL | 3 | < 0.01 |
| | Ethanolic 25 vs 50 µg/mL | 2 | < 0.05 |
| | Ethanolic 50 vs 100 µg/mL | 4.3 | < 0.01 |
| | Ethanolic vs Aqueous (25 µg/mL) | −2.0 | < 0.05 |
| | Ethanolic vs Aqueous (100 µg/mL) | −0.7 | > 0.05 |

TABLE 3. Tukey's post-hoc multiple comparison test for Streptococcus mutans 7 Enterococcus faecalis

Tables 3 present the post-hoc multiple comparison analysis assessing how antimicrobial activity varied across concentrations and between extract types for both test organisms. The analysis demonstrates a clear concentration-dependent enhancement of antimicrobial efficacy within each extract, indicating that increasing doses consistently improved bacterial inhibition. Comparisons between extract types revealed that the ethanolic extract generally produced stronger inhibitory

effects than the aqueous extract at corresponding concentrations, supporting the role of solvent efficiency in extracting active phytoconstituents. Notably, differences between extracts became less pronounced at the highest concentration, suggesting a convergence of maximal antimicrobial activity. Overall, the post-hoc findings reinforce the dose–response relationship and clarify that the observed differences in antimicrobial performance are driven by both concentration effects and extraction solvent rather than random variability.

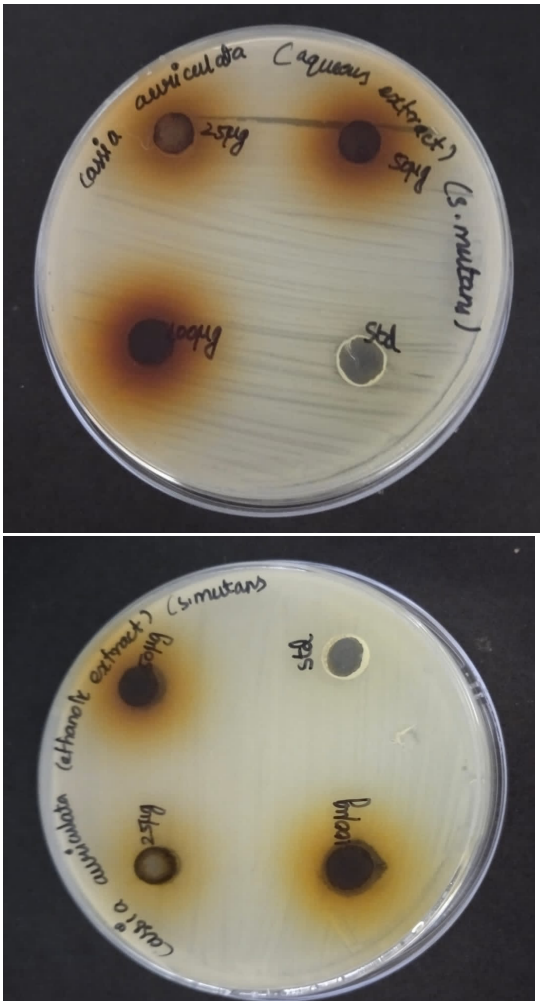



Figure 1(A) and (B) graphs depicts Antimicrobial activity of C.auriculata against st mutans by assessing aqueous and ethanolic extract comparing with the standard antibiotic.

In Streptococcus mutans, the ethanolic extract produced a progressive reduction in optical density over time, with the greatest decline observed at 100 µg/mL. The reduction was evident as early as 2 hours and became more pronounced at 4 hours, indicating a concentration- and time-dependent bactericidal effect (Figure 1). The optical density values at higher concentrations approached those observed for the standard control.

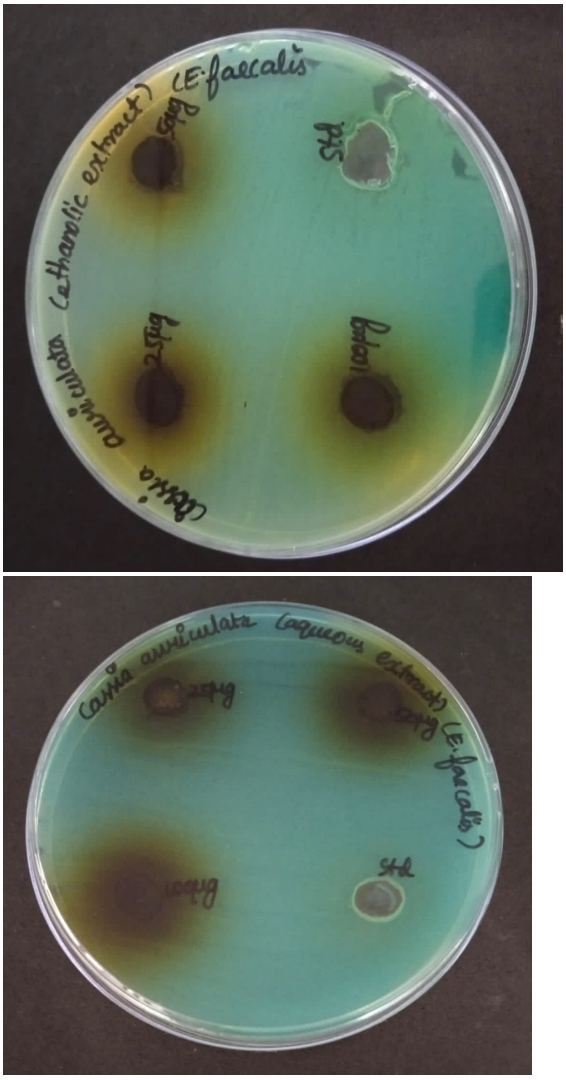



Figure 2(A) and (B) graphs depicts Antimicrobial activity of C.auriculata against Enterococcus faecalis by assessing aqueous and ethanolic extract comparing with the standard antibiotic.

Similarly, in Enterococcus faecalis, the ethanolic extract demonstrated time-dependent growth suppression. Although the magnitude of reduction was slightly lower than that observed for S. mutans, higher concentrations consistently resulted in reduced optical density compared with untreated controls, confirming antimicrobial activity against this relatively resistant organism (Figure 2).

Time-kill assay

Time-kill kinetics of Cassia auriculata extracts were assessed by monitoring changes in optical density over a

4-hour period at different concentrations (25, 50, and 100 µg/mL), with chlorhexidine as the standard and untreated cultures as controls.

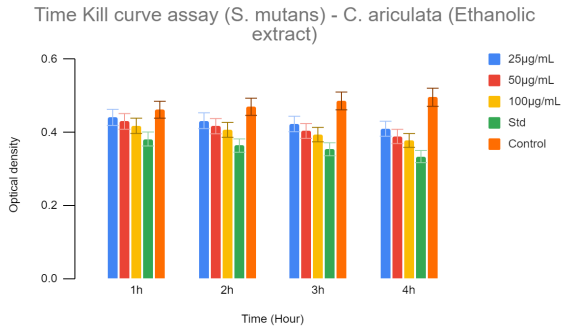



Figure 3: Time-kill curve showing the effect of ethanolic extract of Cassia auriculata on Streptococcus mutans. Optical density was measured at 1, 2, 3, and 4 hours for different extract concentrations (25, 50, and 100 µg/mL). Data represent mean ± SD (n = 3). A concentration- and time-dependent reduction in bacterial growth was observed.

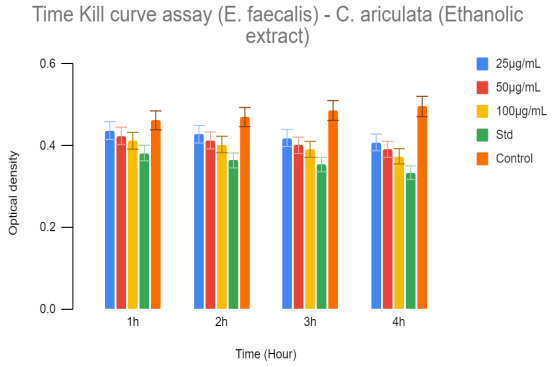



Figure 4: Time-kill curve illustrating the antimicrobial effect of ethanolic extract of Cassia auriculata against Enterococcus faecalis. Higher extract concentrations produced greater reductions in optical density over time compared with untreated controls.

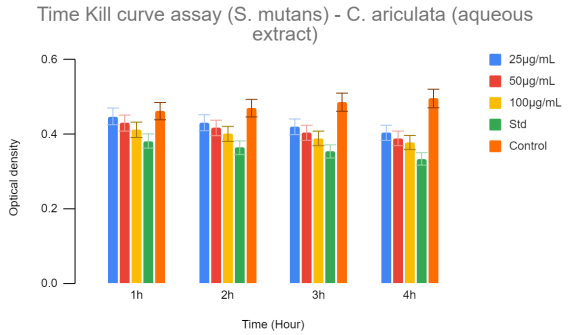



Figure 5: Time-kill kinetics of aqueous extract of Cassia auriculata against Streptococcus mutans. A gradual decline in optical density was observed with increasing concentration and exposure time.

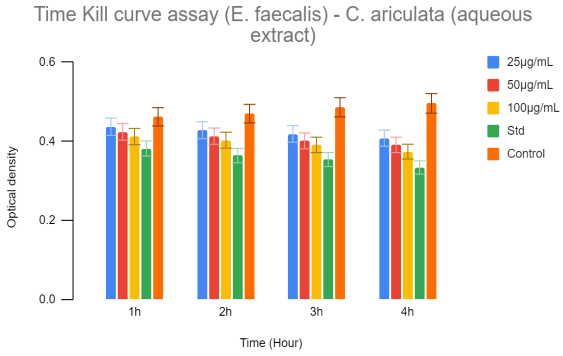



Figure 6: Time-kill curve depicting the antimicrobial activity of aqueous extract of Cassia auriculata against Enterococcus faecalis. Growth suppression was concentration-dependent but less pronounced compared with the ethanolic extract.

The aqueous extract also demonstrated time-dependent antimicrobial effects against Streptococcus mutans. A gradual decrease in optical density was observed with increasing concentration and exposure time, with the maximum reduction noted at 100 µg/mL after 4 hours. However, the overall magnitude of reduction was lower than that observed with the ethanolic extract, indicating comparatively reduced bactericidal efficiency.(Figure 3&4)

Against Enterococcus faecalis, the aqueous extract exhibited modest but consistent growth suppression over time. Although reductions in optical density were concentration-dependent, the bactericidal effect was less pronounced when compared with the ethanolic extract and the standard control. Overall, the time-kill assay confirmed that Cassia auriculata extracts exert concentration-dependent and time-dependent antimicrobial effects, with the ethanolic extract demonstrating superior bactericidal activity against both tested organisms. The observed reduction in optical density over time supports the agar diffusion findings and suggests enhanced extraction of bioactive phytochemicals in ethanol.(Figure 5&6)

**Discussion:**

Plant-derived antimicrobial agents have attracted increasing scientific interest because of their therapeutic efficacy combined with a favorable safety profile. Yun et al. (2023) emphasized that natural antimicrobials are

capable of controlling infectious diseases while reducing the adverse effects commonly associated with synthetic drugs.(15) The present study supports this concept, as both aqueous and ethanolic extracts of Cassia auriculata demonstrated significant antimicrobial activity against Streptococcus mutans and Enterococcus faecalis, two clinically relevant oral pathogens. Importantly, the observed antimicrobial action was concentration dependent, indicating a predictable and controllable biological response.

Earlier investigations by Vijayaraj et al. (2011) reported antimicrobial efficacy of Cassia auriculata extracts and suggested their potential use as affordable therapeutic alternatives.(16) Our findings are consistent with these observations, confirming that C. auriculata possesses inherent antimicrobial properties irrespective of the extraction medium. However, the present study further refines this understanding by demonstrating that the ethanolic extract exhibited superior antimicrobial activity, highlighting the importance of solvent selection in maximizing phytochemical extraction.

The role of extraction solvents has also been discussed by Purushotham et al. (2014), who suggested that ethanolic plant extracts are particularly suitable for formulation into oral healthcare products such as mouthwashes and intracanal medicaments.(17) The superior performance of the ethanolic extract in our study reinforces this proposition and supports its potential translational application in preventive and therapeutic dentistry, particularly in caries control and endodontic disinfection.

Comparative antimicrobial studies conducted by Kumar et al. demonstrated that several plant-based extracts exhibit antimicrobial efficacy comparable to synthetic agents against oral pathogens.(18) In agreement with these findings, the antimicrobial zones produced by C. auriculata extracts in the present study were substantial across all tested concentrations, suggesting that plant-derived agents can serve as effective alternatives or adjuncts to conventional antimicrobials.

The differential susceptibility observed between S. mutans and E. faecalis in this study aligns with reports by Sharma et al, who evaluated stem bark and leaf extracts of medicinal plants against Gram-positive and Gram-negative organisms.(19) They reported higher antimicrobial sensitivity among certain organisms, depending on cell wall composition and metabolic activity. In our study, S. mutans consistently exhibited greater inhibition than E. faecalis, reflecting the latter's well-documented resistance and persistence in oral environments.

Nanotechnology-based enhancements of plant antimicrobials have been explored by Chowdhury et al., who demonstrated that metal nanoparticles synthesized using plant extracts exhibit enhanced antibacterial activity in a concentration-dependent manner. While our study did not involve nanoparticle synthesis, the strong antimicrobial performance of crude C. auriculata extracts suggests that nano-formulation could further potentiate its efficacy, particularly against resistant pathogens such as E. faecalis.(20)

Studies on other medicinal plants further contextualize the relevance of our findings. Jamróz et al. (2023) highlighted the broad antimicrobial, antioxidant, and anti-inflammatory properties of lemon grass, attributing its efficacy to diverse phytocompounds. This parallels our observations, as C. auriculata likely owes its antimicrobial action to a synergistic combination of phenolics and flavonoids rather than a single active compound.(21),(22)

Senthil et al. demonstrated that plant-mediated nanoparticle synthesis significantly enhances antibacterial potential against both Gram-positive and Gram-negative bacteria. These findings suggest that C. auriculata extracts could be explored not only as standalone antimicrobials but also as biological reducing agents in green nanotechnology applications.(23)

The endodontic relevance of natural antimicrobials has been highlighted by Ngangoue et al, who observed notable antimicrobial activity of plant extracts against resistant microbial species.(24) In contrast to studies that focused on fungal pathogens or a broader microbial spectrum, our study specifically targeted E. faecalis, a key pathogen implicated in endodontic failures. The consistent inhibition observed across concentrations supports the potential role of C. auriculata extracts as adjunctive intracanal medicaments.(25),(26)

Research by this study on c auriculata extracts report significant antimicrobial and protective biological effects. These findings highlight the broader therapeutic potential of bioactive agents and support our results for the future, where nanoparticle coated zirconia crowns and stainless steel crowns against cariogenic bacteria. (27)

The present study is limited by its in vitro design and the inclusion of only two oral pathogens, which may not fully represent the complex oral microbiome. However, a major strength lies in the combined in vitro, statistical,

time-kill, and in silico evaluation, providing a robust and multidimensional assessment of antimicrobial efficacy. The use of both aqueous and ethanolic extracts enhances clinical relevance by reflecting realistic formulation options. Future research should include a broader range of oral pathogens, detailed phytochemical characterization, cytotoxicity testing, and in vivo or clinical studies. Additionally, nano-formulation and controlled-release delivery systems may further enhance the therapeutic potential of Cassia auriculata–derived antimicrobials.

**Conclusion**

This study confirms that Cassia auriculata exhibits meaningful antimicrobial activity against Streptococcus mutans and Enterococcus faecalis, with both aqueous and ethanolic extracts showing a clear concentration-dependent effect. The ethanolic extract demonstrated comparatively greater efficacy, highlighting the influence of extraction solvent on antimicrobial performance. The combined use of agar diffusion, time-kill kinetics, and molecular docking strengthens the biological relevance of these findings. Overall, the results support the potential of C. auriculata as a natural, cost-effective antimicrobial agent for preventive and therapeutic applications in dentistry, warranting further validation through in vivo and clinical studies.

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**CONFLICT OF INTEREST:** The authors would like to declare no conflict of interest in the present study.

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