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Antimicrobial Potential of Selected Natural Compounds: A Comparative Study on *Enterococcus faecalis*

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Abstract

*The present study aimed to assess and compare the antimicrobial effectiveness of three natural plants - Neem (*Azadirachta indica*), Turmeric (*Curcuma longa*) and Guava (*Psidium guajava*) against one of the endodontic pathogens, that is, *Enterococcus faecalis*. Broth-based assays were used to determine minimum inhibitory concentrations (MICs) by measuring optical density.*

*All the extracts used in the study showed antimicrobial potential, with *Curcuma longa* (turmeric) having the best inhibitory effect. *Psidium guajava* (guava), on the other hand, was relatively least active. The observed antimicrobial effects in the extracts are attributed to various bioactive compounds present in these plants. Neem leaves (*Azadirachta indica*) have azadirachtin, nimbin and nimbidin; turmeric rhizome has curcumin, while guava leaves are known to contain flavonoids such as quercetin and tannins. The phytochemicals may have demonstrated antimicrobial effects against bacteria by disrupting cell wall integrity, inhibiting essential enzymes, and interfering with microbial metabolic pathways.*

These findings suggest that turmeric extract has significant antibacterial potential and can serve as a natural alternative for managing endodontic infections. The article highlights the promise of plant extracts in developing new therapeutic protocols for dental procedures.

Keywords: Antimicrobial activity, Endodontic pathogens, Natural compounds, Turmeric, Minimum inhibitory concentration (MIC)

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1.0 Introduction

Natural antimicrobial agents derived from plants, including neem, turmeric, and guava, have traditionally been used in medical systems and are progressively being studied in contemporary studies due to their broad-spectrum effect, safety, and minimal side effects (Tripathi et al., 2016; 2017; Abdallah et al., 2023; Matei & Visan, 2025).

With the rising problem of antibiotic resistance, plant-based compounds are being investigated as viable substitutes for traditional antimicrobial medications (Singh et al., 2016; Surana et al., 2024). The antimicrobial action of medicinal plants is mainly attributed to active phytochemical constituents like alkaloids, flavonoids, tannins, terpenoids, and phenolic compounds (Akkalwar et al., 2026). Among these, neem, turmeric, and guava are extensively studied for their effectiveness against pathogenic microorganisms, including those involved in oral infections. One of the most common medicinal plants in Ayurveda is neem (*Azadirachta indica*), which has antibacterial, antifungal, antiviral, and anti-inflammatory effects (Wylie and Merrell, 2022). It contains key active compounds, such as azadirachtin, nimbin, and nimbolide, that make it antimicrobial. Neem acts by destabilising bacterial cell wall and membrane integrity, preventing the enzyme activity and microbial growth, and by preventing biofilm formation. It has demonstrated strong inhibitory effects on gram-positive and gram-negative bacterial species, as well as the oral pathogens (Wylie and Merrell, 2022). Moreover, neem has many dental uses as it is commonly used as neem sticks to clean teeth, inhibits the formation of plaque and gingivitis, and is a natural mouth disinfectant. (Megha et al., 2023).

Turmeric (*Curcuma longa*) is another important plant-derived antimicrobial agent, widely recognised for its therapeutic potential due to the presence of its principal active compound, curcumin. Curcumin is a potent antibacterial, anti-inflammatory, and antioxidant compound that makes it effective specifically in the treatment of infections and related inflammation (Adamczak et al., 2020; Hussain et al., 2022; Umaphathy et al., 2022; Odo et al., 2023). The action of curcumin is associated with damaging bacterial cell membranes, hindering the synthesis of protein and the activity of

enzymes, and causing a decrease in inflammation at the site of infection (Odo et al., 2023). It has been demonstrated to prevent the proliferation of various pathogenic bacteria and is particularly effective in curbing inflammation that is related to infections. The use of turmeric is also important in dentistry, and it has been used to treat gingivitis and periodontitis, enhance wound healing of oral tissues, and decrease microbial load, hence improving the overall oral health. (Umaphathy et al., 2022).

Another plant of interest that has been studied and recorded to have antimicrobial effects is Guava (*Psidium guajava*) and its bioactive compounds, especially in the leaf, have been used traditionally to treat infections and to care for oral health (Biswas et al., 2013; Gutierrez-Montiel et al., 2025). Tannins like guavins A-D, flavonoids, like guajaverin and other phenolic compounds are the active compounds found in guava and makes it effective against microbes (Pereira et al., 2023). The action of these compounds takes place through precipitation of microbial proteins, cell membrane disruption, prevention of microbial adhesion, and biofilm formation (Biswas et al., 2013). The extracts of the guava leaf have been shown to possess significant antimicrobial effects against a broad spectrum of bacteria and particularly those that cause oral infections (Pereira et al., 2023). In dental practice, guava is prevalently employed in mouth rinses to keep the mouth hygienic, prevent toothache and gum infections, and is useful in preventing the formation of plaque and bacterial proliferation. All in all, neem, turmeric, and guava demonstrate high antimicrobial potential, which can be explained by their various bioactive compounds, thus providing effective agents of oral pathogenic microorganisms with a relatively safe profile and a promising non-dental treatment option.

1.1 Oral Pathogens and Need for Alternative Therapies

Oral health constitutes a vital component of overall health, and the human oral cavity harbours a rich diversity of microorganisms, many of which exist as normal flora but may become pathogenic under favourable conditions, leading to dental caries, root canal infections, and periodontal diseases. Among these, *Enterococcus faecalis* is a Gram-positive, coccal-shaped bacterium that is known to be a major opportunistic pathogen in relation to oral infections, especially persistent and secondary endodontic infections. It is very

tough and is usually present in infected root canals, dental plaque and saliva, particularly where root canal treatment has failed (Stuart et al., 2006). It can endure unfavourable environmental conditions such as a lack of nutrients, elevated pH, and exposure to antimicrobial agents, which are attributed to its remarkable survival abilities when subjected to conventional treatment methods (Alghamdi & Shakir, 2020).

Its capacity to enter deep into dentinal tubules and build complicated biofilms is another feature that contributes to the persistence of *E. faecalis* in endodontic infections as the protective barriers against antimicrobial agents (Neelakantan et al., 2013). This property allows it to survive in the root canal system in the long run and plays a major role in the failure of treatment and apical periodontitis. Also, the bacterium has many virulence factors, such as collagen- and dentin-binding adhesins, aggregation substances that facilitate cell-to-cell contact, and enzymes, such as gelatinase and cytolysin, that aid in the destruction of tissues and the evasion of the host immune system (Halkai et al., 2016). Its ability to resist any of the most common disinfectants and antibiotics further makes it hard to eliminate and contributes to chronic oral infections.

Increased resistance of oral bacteria to traditional antimicrobial agents is also another significant problem in dental treatment. Widespread use of chemical disinfectants and antibiotics is rather ineffective in *E. faecalis* eradication, especially with its ability to form biofilm, which restricts drug infiltration (Neelakantan et al., 2013; Surana et al., 2024; Jain et al., 2024). In addition, the long-term use of synthetic antimicrobial agents can cause unwanted adverse effects (irritation of tissues and disruption of the normal microbial balance in the mouth cavity) and diminish their clinical performance and safety.

In this respect, increased attention is being given to the application of natural products as alternative therapeutic agents in the field of dentistry (Trivedi et al., 2013). The natural extracts of neem, turmeric, and guava have proven to have significant potential in preventing biofilm formation, reducing bacterial load, and being effective alternatives to chemical disinfectants in root canal therapy (Chaitanya et al., 2016; Joy Sinha et al., 2017; Hussain et al., 2022). Their wide-spectrum antimicrobial effect, combined with moderate toxicity and enhanced biocompatibility, precondition their potential application in dentistry (Jain et al., 2024; Surana et al., 2024). Thus,

to develop safe, effective, and sustainable treatment strategies in contemporary dentistry, it is necessary to assess the antimicrobial activity of these natural products against oral pathogens, especially *E. faecalis*.

2.0 Methodology

2.1 Preparation of Extracts

The extracts of neem, guava and turmeric were prepared using two different methods to evaluate their antimicrobial efficiency. In the aqueous extraction method, fresh neem and guava leaves and turmeric rhizome (2 gm each) were weighed and washed properly using tap water, then using sterile distilled water to eliminate dust and other contaminants. The purified leaves were crushed in a sterile mortar and pestle with sterile distilled water to have a homogenised product. This was then filtered using filter paper to get a clear extract, and the final volume was brought to 10 mL using sterile distilled water (Sasidharan et al., 2011). The ethanol extraction was also made for all three test natural substances. In this technique too, 2 gm each of fresh leaves of neem and guava and turmeric rhizome were initially thoroughly washed and shade-dried over a period of two days to extract the extra moisture. The dried leaves and rhizome were then soaked overnight in 95% ethanol to release the active compounds. The leaves and rhizome were then crushed to a fine paste in a mortar and pestle, followed by filtration of the paste through filter paper to get the extract. Lastly, the volume of the extract was made up to 10 ml with the help of dimethyl sulfoxide (DMSO) to obtain the required concentration (Azwanida, 2015).

2.2 Test Organism

The endodontic bacterium *Enterococcus faecalis*, used as the test organism in this study, was prepared as a stock culture by inoculating 100 µL of the bacterial suspension into 25 mL of fresh Luria broth. The culture was then incubated overnight to allow sufficient bacterial growth. To maintain the viability and purity of the organism throughout the study, regular subculturing was carried out on fresh nutrient broth at appropriate intervals. The actively growing cultures obtained through this process were used consistently for all experimental procedures conducted during the study.

2.3 Broth Dilution Method MIC Determination

The minimum inhibitory concentration (MIC) of neem, turmeric, and guava extracts was determined using the broth dilution method. In this procedure, 5 mL sterile Luria broth was dispensed into 6 test tubes, followed by the addition of varying concentrations of the plant extracts to each tube. A standardised bacterial culture (10 µL) was then inoculated into all the test tubes. Two controls were maintained to ensure the reliability of the results: a positive control consisting of broth, bacterial culture, and chlorhexidine, and a negative control containing only broth and bacterial culture. All the inoculated tubes were incubated at 37°C for 18–24 hours. After incubation, the tubes were examined for turbidity, which indicates bacterial growth. The lowest concentration of the extract that showed no visible growth, as evidenced by a clear broth, was recorded as the MIC value (Wiegand et al., 2008). The percentage inhibition of bacterial growth was calculated as described above, using the formula:

$$\text{Inhibition \%} = \frac{\text{Negative Control value} - \text{Test Sample value}}{\text{Negative Control Value}} \times 100$$

2.4 In vitro Analysis

Extracted human teeth were used to evaluate the antimicrobial properties of the natural extracts under simulated oral conditions, as they serve as reliable models for ex vivo dental research and experimental procedures (Nawrocka & Łukomska-Szymańska, 2019). The experimental setup consisted of normal extracted teeth, in which cavity was artificially created with the

help of a root canal borer. The teeth were divided into different test groups for each plant extract, including one positive control, one negative control each. The positive control tooth cavity was inoculated with bacterial culture along with chlorhexidine, while the negative control contained only the bacterial culture. The remaining three groups of teeth served as test samples, each treated with different concentrations of neem, guava, and turmeric extracts, respectively, with total volume 10 µL in all tests set up. Following the overnight incubation period with bacterial active culture and plant extract, the samples were collected with sterile paper points and further incubated for 24h in 5 mL media. The results were recorded next day by measuring the OD at 600 nm and compared to assess the antimicrobial effectiveness of each extract in inhibiting the bacterial load (Gogineni et al., 2016). The percentage inhibition of bacterial growth was calculated as described above.

3.0 Results and Discussion

The antimicrobial activity of the selected plant extracts, turmeric, neem, and guava against *Enterococcus faecalis* was evaluated using minimum inhibitory concentration (MIC) assays through optical density (OD) measurements. This approach enabled a comparative assessment of the effectiveness of each extract relative to the untreated control.

The findings as presented in Figure 1 revealed that all three plant extracts exhibited antibacterial activity; however, their efficacy varied considerably.

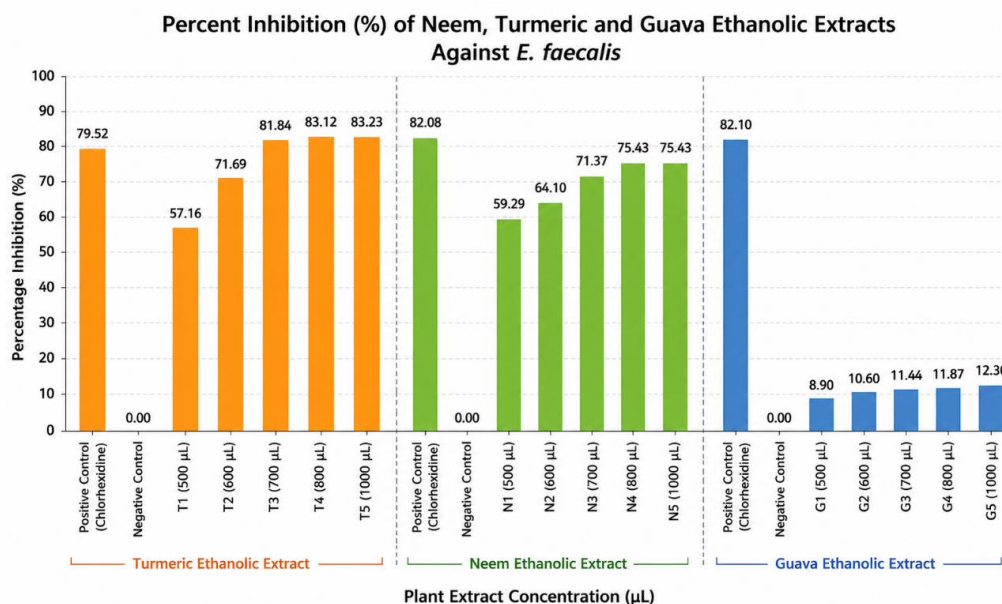


Figure 1: Inhibition of *E. faecalis* by different plant extracts

As shown below in Table 1, turmeric crude extract demonstrated the highest antimicrobial potential, exhibiting approximately 83% inhibition of bacterial growth at a concentration of 800 µL. Below 500 µL, the inhibition was not significant, so the results are not considered for further evaluation. Ethanolic extract of

Neem also showed significant activity, with around 75% inhibition at the same concentration (Table 1), whereas guava extract exhibited comparatively weaker antibacterial effects under identical conditions. These results were consistent across all concentrations tested for three natural extracts.

Table 1: Percent Inhibition values of neem, turmeric, and guava extracts against *E. faecalis*

Ex. Set No.	Ethanolic Conc. (µL)	*O.D. at 600 nm	Percentage Inhibition (%)
Turmeric Extract			
Positive Control (Chlorhexidine)	-	0.204	79.52
Negative Control (Bacteria only)	-	1.008	0.00
T1	500	0.401	57.16
T2	600	0.265	71.69
T3	700	0.170	81.84
T4	800	0.158	83.12
T5	1000	0.157	83.23
Neem Extract			

Positive Control (Chlorhexidine)	-	0.205	82.08
Negative Control (Bacteria only)	-	1.138	0.00
N1	500	0.381	59.29
N2	600	0.336	64.10
N3	700	0.268	71.37
N4	800	0.230	75.43
N5	1000	0.230	75.43
Guava Extract			
Positive Control (Chlorhexidine)	-	0.205	82.1
Negative Control (Bacteria only)	-	1.145	0.00
G1	500	1.042	8.9
G2	600	1.023	10.6
G3	700	1.014	11.44
G4	800	1.009	11.87
G5	1000	1.004	12.3

* Average values of three replicates. T1 – T5: Bacteria + Turmeric extract; N1 – N5: Bacteria + Neem extract; G1 – G5: Bacteria + Guava extract

The in vitro study using extracted human teeth further supported these observations. Teeth treated with turmeric extract showed the lowest optical density values, indicating a greater reduction in bacterial load compared to neem and guava-treated samples (Table 2). However, the percent inhibition cannot be compared with MIC method, as much larger volume was tested in MIC

method as compared to in vitro method. This consistency across different experimental models strengthens the reliability of the findings and highlights turmeric as the most effective antimicrobial agent among the tested extracts, consistent with findings reported in previous studies (Joy Sinha et al., 2017; Umapathy et al., 2022; Odo et al., 2023).

Table 2: Percent inhibition of test extracts against *Enterococcus faecalis* done in vitro in extracted tooth cavity

Tooth Experiment Set	Description	OD at 600 nm	Percentage Inhibition (%)
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Tooth Set 1	Positive Control (Bacteria + Chlorhexidine)	0.012	98.91
Tooth Set 2	Negative Control (Bacteria only)	1.104	0.00
Tooth Set 5	Bacteria + Turmeric Extract	0.691	58.17
Tooth Set 3	Bacteria + Neem Extract	0.598	45.83
Tooth Set 4	Bacteria + Guava Extract	1.175	-6.43

A significant finding of the study was how extraction methods affected the antimicrobial efficacy. Extracts of all plant samples in aqueous solution were relatively inactive in terms of antibacterial activity, but ethanolic extracts were much more active. This indicates that ethanol would be more effective in extracting bioactive compounds that cause antimicrobial action, which will enhance the overall activity of the plant extracts. This difference can be attributed to the differential solubility of phytochemicals in various solvents. Ethanol, being a moderately polar organic solvent, can extract many bioactive compounds, including phenolics, flavonoids, tannins, alkaloids, terpenoids, and essential oils, many of which are well-documented antimicrobials (Altemimi et al., 2017).

This high antimicrobial effect of turmeric can be explained by the presence of curcumin, a popular bioactive substance with potent antimicrobial and anti-inflammatory effects (Adamczak et al., 2020; Hussain et al., 2022). Neem was also notably active, which is presumably due to compounds like azadirachtin and nimbidin, but with a lower effectiveness compared to turmeric (Wylie and Merrell, 2022; Megha et al., 2023). The relative decreased activity of guava could be caused by the lower levels or poor bioavailability of the active constituents in the test conditions (Huynh et al., 2025).

These results present significant implications for dental research and practice. The high effectiveness of turmeric suggests its potential use as a natural antimicrobial in dentistry, especially against root canal infection and other oral diseases (Sinha et al., 2017; Jain et al., 2024). The research justifies the feasibility of creating plant-based preparations like mouthwash, gels, toothpastes, or root canal irrigants as healthier and more sustainable

substitutes for traditional synthetic products (Surana et al., 2024; Anwar et al., 2025).

However, there are some limitations that should be noted. The research was done in a controlled laboratory setting and with a small sample size, which might not entirely reflect the complexity of oral environments in vivo. Additionally, crude plant extracts were used, which may contain impurities along with active compounds, potentially influencing the results. The study also did not isolate or quantify specific bioactive constituents responsible for the observed antimicrobial effects. Future research should focus on the use of purified compounds, exploration of additional medicinal plants, and clinical trials to validate these findings and enhance their applicability in real-world dental treatments.

4.0 Conclusion

The present study focuses on a comparative evaluation of antimicrobial properties of the selected natural compounds - Neem, Turmeric, and Guava to compare the efficacy of extracts of these plants on endodontic bacteria. The aim is to identify the ability of each extract to prevent the growth of these dangerous microorganisms (Surana et al., 2024; Jain et al., 2024).

To establish the antimicrobial properties of the identified natural compounds, a series of tests was performed with the inoculated and incubated extracted human teeth. As was experimentally observed, each of the three extracts was antimicrobial. Turmeric was better since it was indicated by the low optical density values, which are due to the enhanced inhibitory action on bacterial growth. Neem was moderately active, and Guava was comparatively less active in terms of antimicrobial activity.

The results of the experiment using the teeth models also supported the results; the samples to which turmeric was added had lower OD values against both the bacteria than the neem and the guava-treated samples. Hence, the conclusions of the comparative analysis refer to the fact that turmeric demonstrated a comparatively high antimicrobial potential in comparison to the selected natural compounds in the given conditions, similar to previously reported studies (Adamczak et al., 2020; Hussain et al., 2022; Jain et al., 2024).

5.0 Future Perspectives

The findings of the present study serve as a foundation for future research, especially in light of the necessity to validate the results in an in vivo setting and ensure the validity of the results in a real oral setting. Different factors can affect the activity of antimicrobials in such settings, such as saliva, host immune responses, and intricate microbial interactions, which can contribute to the establishment of the clinical significance of the findings (Matsuoka et al., 2025).

The use of purified bioactive compounds, including curcumin of turmeric and azadirachtin of neem, should be the subject of future research, too, as purified bioactive compounds provide more precision, consistency, and a better understanding of the mechanism-based action in comparison with crude extracts (Vaou et al., 2022; Mungwari et al., 2025; Akkalwar et al., 2026). Moreover, the possibilities of creating herbal dental care products, such as mouthwashes, toothpastes, and root canal irrigants, that could be safer and cheaper alternatives to synthetic chemical agents are significant (Anwar et al., 2025).

Further studies are also required to compare against a wider array of oral microorganisms, considering that oral infections are polymicrobial and cover several bacterial species. Additionally, extraction methods and solvent systems, as well as concentration parameters, need to be optimised to improve the antimicrobial effects and give reproducible and reliable results (Anwar et al., 2025).

Author Declaration Statements

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- Any potential conflicts of interest, whether financial or non-financial, have been fully disclosed. – Yes / Not Applicable[√]
- All sources of funding and financial support received for the conduct of the study have been appropriately acknowledged. – Yes / Not Applicable[√]
- Necessary ethical approvals have been obtained from the relevant institutional or regulatory bodies for studies involving human participants, animals, or sensitive data, wherever applicable. – Yes / Not Applicable[√]

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