Research

Antibacterial and Plasmid-Curing Potential of Two Medicinal Plant Extracts in Dental Infections

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Abstract

The current study investigates the antibacterial properties and plasmid-curing effects of *Anacyclus pyrethrum* extract in conjunction with *Ginkgo biloba* extract on *Streptococcus mutans* and other *Streptococcus* species in patients with dental caries and periodontitis. The antibacterial evaluation was performed utilising Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). While *Ginkgo biloba* extract necessitated 9 mg/mL to attain comparable results, *Anacyclus pyrethrum* extract achieved this at 8 mg/mL with *S. mutans*. The extract activation rate exceeded 81%. The experiment revealed that *G. biloba* required a dosage of 24 mg/mL, but *A. pyrethrum* necessitated 20 mg/mL to eradicate all the germs. Among all *Streptococcus* species, *S. mutans* demonstrated the most significant resistance to tetracycline and the least resistance to ciprofloxacin, while other *Streptococcus* species showed varying antibiotic resistances. The plasmid elimination assays revealed that the herbal extracts effectively eradicated plasmids in *S. mutans*, hence inhibiting plasmid replication and resulting in a diminished band intensity on plasmid staining compared to the ampicillin-treated samples. *A. pyrethrum* and *G. biloba* have shown their efficacy as anti-resistance therapeutic agents against oral infections owing to their antibacterial properties and plasmid-curing capabilities. The study demonstrates how these herbal extracts may serve as an alternative to conventional antibiotics in the natural treatment of tooth infections. They must possess clinical relevance, necessitating additional research involving actual individuals.

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1. Introduction

Oral health is essential as it directly influences overall health and quality of life. Dental caries and periodontal disease are among the most prevalent oral illnesses. Dental caries is a chronic condition resulting from the chemical degradation of tooth surfaces caused by oral bacteria. Periodontitis is classified as a chronic inflammatory illness, as bacterial plaque triggers the host immune response, creating harmful circumstances in the structures that support the teeth. The microorganisms in the oral cavity are significant factors in the progression and exacerbation of several disorders **(Fu *et al.* 2025)**. *Streptococcus mutans* is the principal bacterium associated with dental diseases, particularly as a primary contributor to caries. Conversely, periodontal pathology arises from inflammatory responses triggered by plaque bacteria, leading to tissue damage and gum separation. The early formation of dental plaque is influenced by the interaction of various *Streptococcus* species, which have the capacity to induce cardiovascular infections. The specific species involved are *S. mutans, S. sanguinis, S. mitis, S. gordonii*, and *S. oralis*. In Japanese investigations, *S. mutans* DNA was identified in almost 70 percent of cardiovascular specimens, including heart valves and atheromatous plaques, alongside a significant quantity of dental and periodontitis-associated bacteria **(Fang *et al.* 2024)**.

Over the years, medical practitioners have created various preventative dental agents to combat periodontal diseases and dental caries. Currently, a diverse array of pharmacological agents for the prevention of dental diseases is being examined in medical research, including antibiotics, botanical extracts, oral rinses, toothpaste, gels, varnishes, and vaccines. *Anacyclus pyrethrum* and *Ginkgo biloba* garner interest for their antibacterial properties, owing to their comparatively less adverse effects relative to manufactured treatments **(Gloria-Garza *et al.* 2025)**. Management of periodontal disorders, *Anacyclus pyrethrum*, often known as Akarkara, is utilised in traditional ayurvedic medicine as a treatment for toothache and dental caries. Researchers have determined that the plant's root contains pyrethrine, a potent bioactive alkaloid, exhibiting notable antibacterial and anti-inflammatory properties. Traditional Iranian medicine utilizes pyrethrum root powder as a principal therapeutic agent to treat gingival disorders and to help prevent the progression of dental caries. This plant possesses immunomodulatory, antioxidant, antidepressant, and neuroprotective effects **(Li *et al.* 2021)**.

However, a detailed toxicity and safety evaluation of these extracts for human mucosal use prior to their incorporation into formulations such as mouthwashes, gels, and toothpaste. *Ginkgo biloba* is a medicinal herb indigenous to China. Gingkolic acid exhibits antimicrobial effects in the leaves of *Ginkgo biloba*. Interesting research demonstrates that bioactive compounds exhibit a potent antimicrobial action against *S. mutans* and other cariogenic bacteria in the oral cavity. Research indicates that *Ginkgo biloba* extracts surpass conventional dental treatments, such as periocline, in eradicating periodontal disorders. The issues arise from the persistent administration of antibiotics in the management of dental disorders, leading to the emergence of multidrug-resistant (MDR) bacterial strains as a consequence of this practice **(Mei *et al.* 2017).** The transmission of multidrug-resistant bacteria in periodontal infections occurs because resistance genes are encoded on plasmids, significantly contributing to the establishment of resistance. Prolonged use of antibiotic medications disrupts the balance of the oral microbiome, creating conditions conducive to the proliferation of resistant bacteria. Contemporary medical research is exploring herbal and natural products as alternative therapeutic agents for managing dental diseases **(Kulis *et al.* 2025)**. These compounds are more secure, cost-effective, and less prone to inducing resistance compared to conventional antibiotic medications. The present study investigates the efficacy of *Anacyclus pyrethrum* extract and *Ginkgo biloba* extract in inhibiting *S. mutans* and other *Streptococcus* strains isolated from individuals with periodontitis and dental caries. The presentation will elucidate the possible applicability of these plant extracts as alternative medicinal interventions for the treatment and prevention of oral infections **(Palombo 2011)**.

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2. Materials and Method

2.1. Study Design

The study involved five patients with chronic periodontitis and five patients with dental caries, all of whom were treated at the Department of Periodontics at a private Hospital in Malaysia. All participants completed consent forms to participate in the study, which the Institutional Review Board approved for Ethical Committee Review. Only participants in this study were individuals aged 18 to 30 with chronic periodontitis and a minimum of twelve individually countable teeth. One criterion included the occurrence of dental caries in participants aged 25 to 60 years with a DMFT level of 5 or higher **(Dhami *et al.* 2021)**. The researchers excluded any study participant having a history of drunkenness, present or prior smoking behaviour, and those who consume pan, gutka, or tobacco products. The excluded patients from the trial comprised those on phenytoin therapy, calcium channel blockers, cyclosporin therapy, or those who had experienced aggressive periodontitis within the prior 12 months **(Kumar 2020)**. The study excluded participants who had received antibiotics or immunosuppressants during the past six months, undergone dental scaling or polishing within the past thirty days, or were pregnant or lactating. Whole saliva and subgingival plaque specimens were obtained and utilised in the study involving all patients. The researchers extracted subgingival plaque from the deepest periodontal pocket in each quadrant. The scientists pooled the samples in phosphate-buffered saline (PBS) and kept them at -20 °C before initiating the testing **(Sayeed and Varghese 2024)**.

2.2. Preparation of Herbal Extracts

The researchers collected *Anacyclus pyrethrum* at Selangor, Malaysia and it was verified by the Department of Medicinal Botany at the National Institute of Ayush, Malaysia, under Certificate No: NISMB3322018. The scientists promptly harvested and subsequently collected the roots of *A. pyrethrum* plants, which they then cleaned and shade-dried. Prepared an ethanol solution and immersed the powdered dry root material, allowing it to soak for 48 hours **(Jawhari *et al.* 2020)**. The mixture was filtered and subsequently evaporated at 50 °C to produce a powdered extract of herbal origin. According to the methodology outlined in the literature, the yield of the ethanolic extract was 28.5%. The aqueous extract of *Ginkgo biloba* was prepared using standard procedures. The botanist provided *G. biloba* leaves during specimen collection, which were subsequently washed and air-dried for several weeks until fully desiccated. The desiccated leaves were placed in hermetically sealed storage containers, which were maintained at ambient temperature **(Biernacka *et al.* 2023)**. The leaf powder was extracted by boiling 10 grams of the sample in 100 millilitres of distilled water for 6 hours, until the extract volume had reduced to one-quarter of the original amount. The pulp extraction utilised Whatman filter paper no. 42 and was completed within two hours, thereafter including 10 grammes of pulp with 25 mL of distilled water stored at 4 degrees Celsius **(Shafiq *et al.* 2024).**

2.3. Isolation of *Streptococcus* Species

The subgingival plaque samples suspended in PBS were segregated into two bacterial groups using a one-minute vortex. One hundred ninety-five microlitres of the suspension was deposited on Mutans Sanguis (MS) agar and incubated in a candle jar at 37°C under an atmospheric condition of roughly 5 percent CO2 for 48 hours **(Ge *et al.* 2008)**. After subculturing *Streptococcus* colonies from MS agar onto blood agar plates, anaerobic incubation at 37 °C was conducted to yield pure Streptococci isolates. BHI broth (HiMedia) was inoculated into the pure subcultures and incubated for 18 hours at 37 °C. Preliminary phenotypic assessments were conducted using conventional morphological identification techniques and Gram stain, as outlined in the Bergey Manual of Determinative Bacteriology **(Fang *et al.* 2025)**.

2.4. Molecular Identification

The researchers utilised 16S rDNA amplification using boiling lysis to identify *Streptococcus* species under diverse conditions. One Streptococcal sample was diluted with 100 µL of nuclease-free water and subsequently boiled at 100 °C for 15 minutes. The boiling sample was initially stored at -20°C for five minutes and then centrifuged at 13,000 rpm for ten minutes. The template employed for the amplification of 16S rDNA via PCR was 4 µL of the supernatant from the lysate solution **(Lal *et al.* 2011)**. The PCR reaction result comprised 42 µL of lysate combined with 12 µL of a primer pair (16S forward primer: 27F, 5' AGAGTTGATCMTGGCTCAG 3' and reverse primer: 1492R, 5' GGTTACCTTGTTACGACTT 3') and 10 µL of Thermo Scientific PCR master mix by volume. The initial step involved denaturation at 94 °C for 2 minutes, followed by 34 cycles consisting of denaturation at 94 °C for 1 minute, annealing at 58 °C for 2 minutes, and extension at 72 °C for 1 minute, culminating in a final extension phase of 15 minutes. The reaction was conducted using the Veriti thermal cycler gradient PCR apparatus from Synergy Scientific in Chennai. The 16S rDNA products, following amplification, were sequenced at the Eurofins Genomics facility via the Sanger method **(Pearce *et al.* 2005)**.

2.5. Minimum Inhibitory Concentration Determination

The MIC of *A. pyrethrum* and *G. biloba* extracts, along with ampicillin, was determined using the macrodilution method. Stock solutions of *A. pyrethrum* and *G. biloba* (100 mg/mL) and Ampicillin (10 mg/mL) were prepared. Serial dilutions of each extract and antibiotic were added to test tubes containing 5 mL of BHI broth. A single colony of *Streptococcus* from the cultured agar plates was inoculated into the tubes, which were then incubated at 37°C for 24 hours. The experiment was conducted in triplicate to ensure consistency and reliability **(Hossain 2024)**.

2.6. Minimum Bactericidal Concentration

A 100 µL aliquot of the culture sample obtained from the MIC experiment was evenly spread onto Brain Heart Infusion (BHI) agar plates and incubated at 37°C for 24 hours. After incubation, the plates were examined for bacterial growth. The minimum bactericidal concentration (MBC) was defined as the lowest concentration of the extract that completely inhibited visible bacterial colony formation on the agar surface. To ensure accuracy, reliability, and reproducibility of the results, the entire experiment was performed in triplicate **(Parvekar *et al.* 2020)**.

2.7. Plasmid Curing and Isolation

Plasmid curing was observed in *S. mutans* isolates following exposure to *A. pyrethrum*, *G. biloba*, and ampicillin during the minimum inhibitory concentration (MIC) assay. To evaluate this effect, plasmids were isolated from the bacterial cultures using the standard alkaline lysis method. After the curing treatment, the extracted samples were subjected to 0.8% agarose gel electrophoresis to determine the presence or absence of plasmid DNA. The disappearance or reduction of plasmid bands in treated isolates, compared with untreated controls, confirmed successful plasmid curing **(Smalla *et al.* 2000)**.

2.8. Antibiotic Resistance Determination

The antibiotic resistance evaluation involved testing this bacterium against Tetracycline, Erythromycin, Ampicillin, Neomycin, Levofloxacin, Amoxicillin, Norfloxacin, Azithromycin, Rifampicin, Ofloxacin, Gentamicin, Cephalosporin, Trimethoprim, Amikacin, Streptomycin, Doxycycline, Cefixime, and Ciprofloxacin **(Urban-Chmiel *et al.* 2022)**. The researchers employed a preparation method to produce antibiotic solutions at a concentration of 10 mg/mL. The procedure added five µL of antibiotic solution to Muller-Hinton Broth test tubes that contained five µL of *Streptococcus* isolates from overnight cultures. The tubes were incubated at 37°C for 24 hours. Experimental measurements monitored

microbial growth to establish antibiotic resistance behaviors **(Cooper *et al.* 2025)**.

3. Results

3.1. Morphological Identification

MS agar successfully isolated *Streptococcus* species by inhibiting the growth of other bacterial species, allowing for confirmation of their development. The bacterial isolates that conformed to the morphological criteria of *Streptococcus mutans* aligned with the descriptions provided in the literature. The colonies of *Streptococcus mutans* exhibited distinctive morphological characteristics, including a raised, convex form and undulating borders, with an opaque, granular surface reminiscent of frosted glass, in contrast to the colonies of other *Streptococcus* species. The remaining streptococcal colonies exhibited a glossy appearance, a grayish-white hue, and a diminutive size. The microbial cells, arranged in clustered conditions beneath the microscope, exhibited a configuration resembling twisted spherical cells, akin to berries. Gram stain demonstrated a Gram-positive morphology with spherical to ovoid cells arranged in pairs or chains, as illustrated in Figure 1.

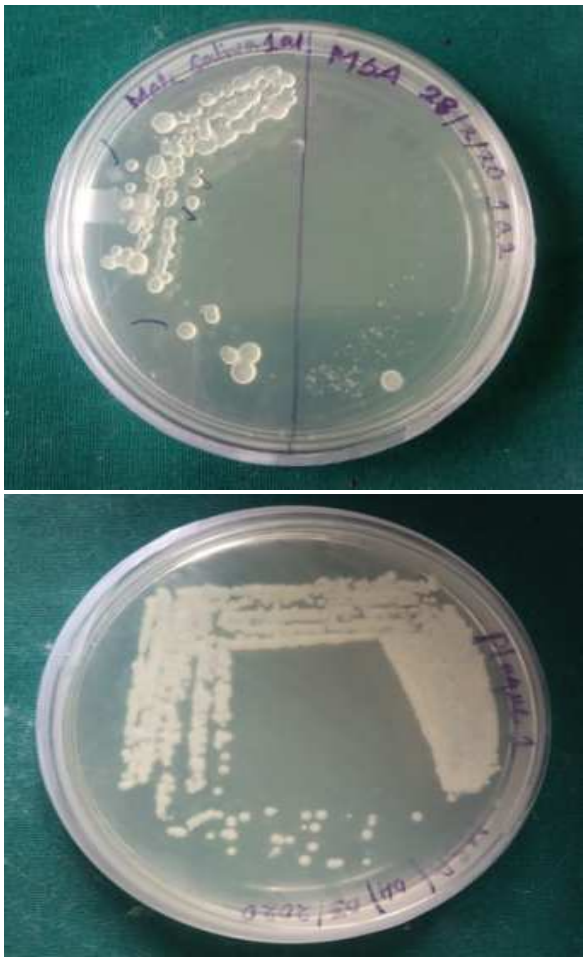

Figure 1. a) MSA agar plate with *S. mutans* from dental caries saliva sample. **b)** MSA agar plate with Streptococci sp. isolated from periodontitis subgingival plaque sample.

3.2. Molecular Identification

The laboratory used 16S rDNA sequencing to identify the isolated microorganisms at the molecular level. The ten isolates included *S. mutans* as one species, along with seven other *Streptococcus* species, which were identified as *S. anginosus* (2 isolates), *S. pyogenes* (3 isolates), *S. salivarius* (1 isolate), and *S. sobrinus* (1 isolate). The 16S rDNA sequence analysis, conducted using the BLAST tool, matched reference sequences with an identity of greater than 99%, which verified the identifications at the species level.

Table 1. Percentage of inhibition for *Streptococcus sp*. (7) [*S. anginous* (2), *S. pyogenes* (3), *S. salivarius*, *S. sobrinus*] at MIC concentration of *Anacyclus pyrethrum* extract, *Ginkgo biloba* extract and Ampicillin.

Organism		*Anacyclus pyrethrum* extract			*Ginkgo biloba* extract			With ampicillin
		8mg/ml	9mg/ml	10mg/ml	8mg/ml	9mg/ml	10mg/ml	10mg/ml
S. anginous	OD	0.345	0.301	0.253	0.430	0.397	0.360	0.07
	% of inhibition	72.4	81.53	84.48	73.61	75.64	77.91	95.70
S. anginous	OD	0.378	0.299	0.245	0.465	0.401	0.392	0.07
	% of inhibition	76.80	81.656	84.96	71.47	75.4	75.95	95.70
S. pyogenes	OD	0.403	0.397	0.311	0.456	0.389	0.309	0.06
	% of inhibition	75.28	75.64	80.92	72.02	76.13	81.04	96.32
S. pyogenes	OD	0.356	0.293	0.256	0.415	0.360	0.317	0.06
	% of inhibition	78.16	82.02	84.29	74.54	81.6	80.67	96.32
S. pyogenes	OD	0.326	0.285	0.239	0.423	0.391	0.370	0.08
	% of inhibition	80	82.51	85.34	74.05	76.01	77.3	95.09
S. sobrinus	OD	0.321	0.292	0.246	0.450	0.354	0.299	0.07
	% of inhibition	80.30	82.08	84.90	72.39	78.28	81.65	95.70
S. salivarius	OD	0.355	0.263	0.211	0.411	0.312	0.309	0.06
	% of inhibition	78.22	83.86	87.05	74.78	80.85	81.04	96.32

Table 2. Antibiotic resistance of *Streptococcus* species on different antibiotics at 10mg/ml concentration with OD values is listed.

Antibiotics	*S. mutans*	*S. anginous*	*S. pyogenes*	*S. salivarius*	*S. sobrinus*
Tetracycline	0.5	0.12	0.48	0.4	0.38
Erythromycin	0.48	0.43	0.5	0.31	1
Ampicillin	0.06	0.06	0.06	0.25	0.06
Neomycin	0.35	0.21	0.46	0.3	0.46
Levofloxacin	0.29	0.14	0.12	0.25	1
Amoxicillin	0.04	0.04	0.09	0.07	0.15
Norflox	0.25	0.16	0.2	0.2	0.32
Azithromycin	0.14	0.2	0.18	0.1	0.12
Rifampicin	0.36	0.5	0.2	0.12	0.25
Ofloxacin	0.12	0.43	0.32	0.65	0.06
Gentamicin	0.42	0.45	0.5	0.38	0.1
Trimethoprim	0.1	0. 25	0.37	0.25	0.5
Amikacin	0.46	0.47	0.38	0.36	0.1
Cefixime	0.32	0.06	0.12	0.12	0.25
Ciprofloxacin	0.02	0.12	0.06	0.08	0.12

3.3. MIC and MBC Analysis

The antibacterial efficacy of *Anacyclus pyrethrum* and *Ginkgo biloba* extracts against *Streptococcus mutans* and other *Streptococcus* species was assessed in comparison to ampicillin, utilising Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) tests. The MIC of Ampicillin was determined to be 10 µg/mL, resulting in a 96.32 percent inhibition of bacterial growth (Table 1). The minimum inhibitory concentration (MIC) of *A. pyrethrum* extract was determined to be 8 mg/mL, leading to an 82.55% suppression of *S. mutans* growth. *G. biloba* exhibited a minimum inhibitory concentration (MIC) of 9 mg/mL and an average inhibition rate of 81.73. The inhibition of seven distinct strains of *Streptococcus* and *G. biloba* consistently yielded inhibition rates of 77% and 74%, respectively. The MBC assessment indicated that *A. pyrethrum* exhibited a minimum bactericidal concentration of 20 mg/mL against *S. mutans*. At the same time, the other *Streptococcus* species required 22 mg/mL of the extract for eradication. The minimum bactericidal concentration of *G. biloba* against *S. mutans* was determined to be 24 mg/mL. Still, it was 26 mg/mL for other species. *A. pyrethrum* and *G. biloba* demonstrated superior efficacy in eradicating *S. mutans* cells relative to other *Streptococcus* species, as indicated by MIC and MBC assays; however, *S. pyogenes* and *S. sobrinus* exhibited the highest susceptibility to *A. pyrethrum* among the non-mutagenic *Streptococcus* species.

3.4. Antibiotic Resistance Determination

The *Streptococcus* isolates demonstrated differing reaction rates when exposed to 10 mg/mL of antibiotics (Table 2). Antibacterial assays revealed that *S. mutans* exhibited the lowest resistance to ciprofloxacin, yielding an OD value of 0.02, while tetracycline demonstrated the highest resistance with an OD value of 0.5. The sequencing of bacterial resistance to the studied antibiotics against *Streptococcus mutans* was as follows: amoxicillin, ampicillin, trimethoprim, ofloxacin, neomycin, rifampicin, gentamicin, amikacin, erythromycin, and tetracycline. The *Streptococcus* species S. anginosus exhibited the highest resistance to rifampicin and the lowest resistance to amoxicillin. *S. pyogenes* exhibited significant resistance to the antibiotic's erythromycin and gentamicin relative to other antimicrobial agents. The resistance profile revealed that *S. sobrinus* exhibited minimal resistance to ampicillin and ofloxacin, whereas S. salivarius showed the highest sensitivity to amoxicillin.

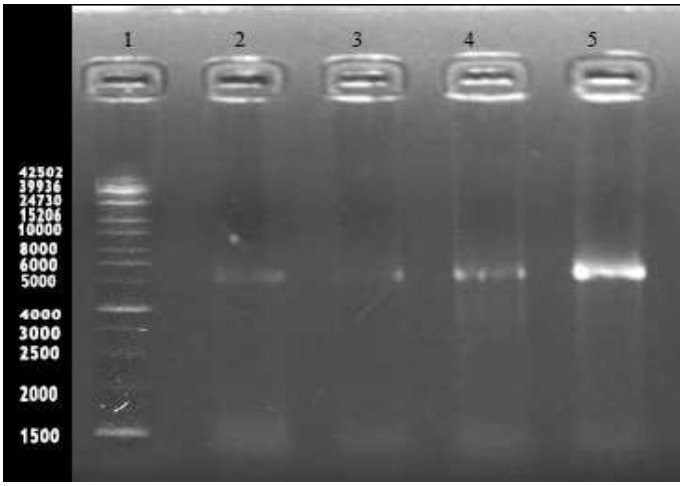

Figure 2. Plasmid curing profile analysis for *S. mutans.* Lane 1: 50kb DNA marker, lane 2: *S. mutans* with *A. pyrethrum*, lane 3: *S. mutans* with *G.biloba*, lane 4: *S. mutans* with Ampicillin, lane 5: *S. mutans* plasmid.

3.5. Plasmid Elimination and Isolation

The plasmid DNA bands of *S. mutans* extracted from caries and periodontitis samples had sizes above 5 kb (Figure 2). The plasmid DNA bands of *S. pyogenes* and S. salivarius were indistinct, as demonstrated by the findings. Figure 3 illustrates the configuration of the plasmid DNA of *S. mutans*. To evaluate the survivability of plasmid DNA, researchers performed ampicillin and herbal extract tests on plasmid-cured samples. The partial cure of plasmid DNA was attributed to treatments using *A. pyrethrum* and *G. biloba*, which resulted in the faintness of the plasmid DNA bands. The analytical results of *S. mutans* plasmid bands following Ampicillin administration exhibited reduced intensity relative to herbal extract treatment, indicating differing efficacy in plasmid DNA remediation.

4. Discussion

This investigation revealed that the antibacterial efficacy of *Anacyclus pyrethrum and Ginkgo biloba* extracts was remarkably potent against *Streptococcus mutans* and other *Streptococcus* species isolated from individuals with dental caries and periodontitis. The herbal extracts significantly inhibited the growth of *S. mutans* bacteria; however, *A. pyrethrum* exhibited more potent antibacterial properties than *G. biloba*. The antimicrobial tests revealed that the *A. pyrethrum* extract exhibited a MIC value of 8 mg/mL. In comparison, the *G. biloba* extract demonstrated a MIC value of 9 mg/mL, both achieving an inhibition rate exceeding 81%. The study's results indicate that the two extracts possess potent antibacterial growth capabilities. The MBC results demonstrated the bactericidal efficacy of the extracts, as they eradicated bacterial growth at concentrations ranging from 20 to 26 mg/mL. The remarkable antimicrobial efficacy of *A. pyrethrum* is ascribed to its bioactive phytochemicals, which include pyrethrine, exhibiting both antimicrobial and anti-inflammatory activities. Previous research indicates that *A. pyrethrum* is efficacious in periodontal therapy owing to its capacity to disrupt bacterial cell walls and impede essential enzymatic processes. *G. biloba* is associated with the antimicrobial and anti-inflammatory properties of some ginkgolic acids, which are thought to be the origin of the plant's antibacterial efficacy. The powerful antibacterial properties of the two plant extracts suggest their potential as alternative therapeutic agents for managing dental infections, which are primarily caused by *S. mutans*, the principal bacterium responsible for tooth decay. The profile analysis revealed varying levels of antibiotic resistance among the different species of Streptococci. The resistance of ciprofloxacin was negligible towards *S. mutans*; however, tetracycline resistance was pronounced against this bacterial species.

The oral and prolonged antimicrobial treatments administered by dentists have enabled *S. mutans* to develop resistance to several commonly used antibiotics. Antibiotic resistance is increasingly problematic in clinical settings as two species of Streptococcal bacteria, *S. anginosus and S. pyogenes*, demonstrate multi-drug resistance (MDR). The increased resistance of rifampicin in S. anginosus and erythromycin in *S. pyogenes* indicates the presence of horizontal gene transfer mechanisms in these strains, necessitating the development of novel treatment alternatives. The plasmid-curing efficacy of extracts was superior in the plasmid removal trial compared to ampicillin.

The *S. mutans* isolates subjected to herbal extracts exhibited a significantly diminished plasmid band, indicating either a complete loss of plasmids or a partial reduction, thus demonstrating their ability to undermine the antibiotic resistance mechanism. The plasmid-curing efficacy of the herbal compounds may result from their interference with plasmid replication or the destabilization of plasmid-associated proteins that inhibit the transmission of resistance genes. The diminished efficacy of ampicillin in plasmid curing compared to herbal extracts underscores the benefits of natural medicines in addressing antibiotic resistance. The herbal extracts possess potential as adjunctive treatments in traditional periodontal and caries therapies. Due to their antibacterial qualities, plasmid-curing abilities, and capacity to reduce resistance, such chemicals

should be incorporated into oral care products, including mouthwash, gels, and toothpaste. The extensive antiviral efficacy of these natural extracts against various *Streptococcus* species suggests their potential to prevent polymicrobial dental disorders.

5. Limitations and Future Directions

The research findings in this study were favourable, although certain limitations. The study sample comprises a limited number of bacterial isolates, hence constraining the potential for generalizing the findings. Future research must utilise extensive samples of clinical isolates to evaluate both the antibacterial efficacy and the resistance-modulating properties of these herbal extracts. The in vitro experimental setting is less complex than the oral environment, as it fails to incorporate essential elements such as biofilm formation, salivary fluid interaction, and immune system processes in patients with dental illnesses. Additional research should be conducted in vivo trials in conjunction with biofilm simulation models to assess the safety and efficacy of these herbal extracts under authentic biological settings. The researchers in this work have not investigated the molecular mechanisms that elucidate how *A. pyrethrum and G. biloba* affect plasmid curing. Future research should perform transcriptome and proteomic analyses to elucidate the precise cellular mechanisms that contribute to resistance against plasmid instability and affect the regulation of resistance genes. Researchers are expected to investigate the efficacy of herbal extracts in comparison to conventional medicines in addressing antibiotic resistance, with a focus on potential synergistic interactions between the herbal extracts and antibiotics.

6. Conclusion

The research demonstrates that the antibacterial efficacy of herbal extracts effectively inhibits and eradicates all species of Streptococci, including *S. mutans*. Their extracts demonstrated more efficacy in eliminating plasmids compared to ampicillin, indicating their potential in addressing antibiotic resistance. This study confirms that the tested plant extracts had significant promise as natural therapeutic agents that may either replace or enhance existing treatments for tooth infections. Further investigations, utilizing extensive clinical trials and live animal models, are necessary to develop thorough insights into the efficacy of substances, alongside safety concerns, and their potential use in oral healthcare practices.

7. Disclosure Statements

7.1. Author Contribution

HB: Data collection, manuscript drafting, Data curation, literature review, manuscript preparation along with Conceptualization, study design, supervision, data interpretation, manuscript writing and revision. The corresponding author have read and approved the final manuscript.

7.2. Declaration of Generative AI

The authors declare that no generative AI tools were used in the drafting, writing, or editing of the manuscript. All scientific interpretations and conclusions are the author's own.

7.3. Ethics approval (for clinical/animal studies)

This study is entirely based on computational analyses using publicly available datasets and in silico methods. No experiments involving human participants, human samples, or animal subjects were conducted. Therefore, ethical approval from an institutional review board or ethics committee was not required. All data utilized in this study were obtained from established public databases and used in accordance with their respective guidelines and policies.

7.4. Informed Consent Statement

Not applicable.

7.5. Data Availability Statement

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

7.6. Acknowledgment

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7.8. Conflicts of Interest

The authors declare that they have no known financial, personal, academic, or other relationships that could inappropriately influence, or be perceived to influence, the work reported in this manuscript. All authors confirm that there are no competing interests to declare.

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7.10. Supplementary Information

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