Formulation and Evaluation of Oral Gel Incorporating Tephrosia and Piper Betle Plant Extracts: an in Vitro Study

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**Abstract:** Oral health significantly impacts systemic health, with poor oral hygiene linked to cardiovascular disease, diabetes, and respiratory infections. Traditional oral care products often contain synthetic chemicals that may have side effects with prolonged use. Herbal alternatives, such as Tephrosia calophylla (known for anti-inflammatory properties) and Piper betle (known for antibacterial properties), offer promising natural options for maintaining oral health. To formulate and evaluate the effectiveness of an oral gel combining Tephrosia calophylla and Piper betle extracts, using antimicrobial, anti-inflammatory, and antioxidant properties for potential use in oral hygiene. The study formulated an herbal oral gel with extracts from Tephrosia calophylla and Piper betle. In vitro assays were conducted to evaluate its antimicrobial activity against E. coli and S. aureus, anti-inflammatory effects, antioxidant potential, and cytotoxicity. Standardized laboratory protocols were followed to ensure the reproducibility of results. Antimicrobial Activity: The gel showed significant antibacterial effects, particularly with Piper betle extract, effectively inhibiting E. coli and S. aureus. Anti-inflammatory Activity: Tephrosia calophylla exhibited high anti-inflammatory effects, surpassing the control at various concentrations. Antioxidant Activity: Both extracts demonstrated notable antioxidant properties, with no significant difference between them. Cytotoxicity: Initial cytotoxic effects were observed with Piper betle, which diminished over time, while Tephrosia calophylla displayed consistent, non-toxic effects. The combined properties of Tephrosia calophylla and Piper betle in this formulation suggest that the gel can serve as a natural, multi-functional oral care product, aiding in bacterial control, reducing inflammation, and protecting against oxidative stress. Further in vivo studies and clinical trials are recommended to validate these findings and assess the gel’s stability and efficacy under real-world conditions.With further research and formulation optimization, it could serve as a sustainable and safe option for daily oral care.

**Keywords**: Tephrosia calophylla, Piper betle, oral gel, antimicrobial, anti-inflammatory, antioxidant, oral hygiene.

# Introduction

Oral health is a critical aspect of overall systemic health, with links to cardiovascular disease, diabetes, and respiratory infections[(National Research Council et al., 2012)](https://paperpile.com/c/JB1097/z1Tr). Oral diseases such as dental caries and periodontal disease are primarily driven by the accumulation of bacterial plaque and the subsequent inflammation[(Miller, 1890)](https://paperpile.com/c/JB1097/M5CE). Current oral health products, though effective, often contain synthetic agents that may lead to adverse effects, contributing to an increasing interest in plant-based alternatives[(DK, 2021)](https://paperpile.com/c/JB1097/HKHN) [(Harsha & Subramanian, 2022)](https://paperpile.com/c/JB1097/xeyzw)[(Deepika et al., 2022)](https://paperpile.com/c/JB1097/F07Sf)[(Solanki et al., 2022)](https://paperpile.com/c/JB1097/o4YdG)

Tephrosia calophylla is a plant species belonging to the genus Tephrosia, which is part of the family Fabaceae. This genus comprises over 350 species distributed in tropical and subtropical regions worldwide[(Reddy et al., 2009)](https://paperpile.com/c/JB1097/TUjB)[(Chidambaram et al., 2022)](https://paperpile.com/c/JB1097/45Z7b).[(Ajay, Sasikala, et al., 2022)](https://paperpile.com/c/JB1097/Yls3D). Tephrosia calophylla has been studied for its pharmacological activities, particularly its hepatoprotective and anti-diabetic properties[(Parine et al., 2015)](https://paperpile.com/c/JB1097/IwMC)[(Ajay, Rakshagan, et al., 2022)](https://paperpile.com/c/JB1097/DtqDh). The plant is known for containing various phytoconstituents, with flavonoids being the most commonly identified compounds. These phytoconstituents contribute to the plant's diverse biological activities, including anti-inflammatory, anti-nociceptive, antidiabetic and hepatoprotective effects[(Ganapaty et al., 2014)](https://paperpile.com/c/JB1097/vwat)[(Ajay, Suma, et al., 2022)](https://paperpile.com/c/JB1097/mJivD) [(Katyal et al., 2021)](https://paperpile.com/c/JB1097/70Qbt). Additionally, Tephrosia calophylla has been found to inhibit carbohydrate digestive enzymes, scavenge free radicals, and enhance glucose uptake rates. The plant's bioactive compounds have shown potential in treating various conditions, making Tephrosia calophylla a valuable resource in herbal medicine.[(Reddy et al., 2009)](https://paperpile.com/c/JB1097/TUjB)[(Jabin et al., 2021)](https://paperpile.com/c/JB1097/ps61c)[(Balaji Ganesh S & Sugumar, 2021)](https://paperpile.com/c/JB1097/SzEa4) [(Govindaraj & Dinesh, 2021)](https://paperpile.com/c/JB1097/QNY8i)

Piper betle, commonly known as betel leaf, is a perennial plant indigenous to Asian and Southeast Asian regions, deeply rooted within the cultures of these regions for generations.Recent studies have showcasing its antibacterial, anti-biofilm, and antioxidative properties against Bacillus gaemokensis, a bacterium implicated in dental caries[(Raikwar et al., 2025)](https://paperpile.com/c/JB1097/aeVY) [(Tiwari & Jain, 2023)](https://paperpile.com/c/JB1097/05p2L)[(Graf et al., 2023)](https://paperpile.com/c/JB1097/cOGTQ). Phytochemical analysis of Piper betle leaf extract revealed the presence of bioactive compounds such as tannins, steroids, phenolic compounds, and alkaloids. Notably, the chloroform extract exhibited robust antibacterial activity, particularly against B. gaemokensis biofilms, an oxosteroid compound, as a potent antibacterial agent. Moreover, the extract effectively inhibited biofilm formation and disrupted established biofilms, leading to significant changes in bacterial morphology observed through scanning electron microscopy[(Sikdar et al., 2024)](https://paperpile.com/c/JB1097/4W84)[(Sabarathinam & Madhulaxmi, 2021)](https://paperpile.com/c/JB1097/PNmZY)[(Sushanthi et al., 2021)](https://paperpile.com/c/JB1097/sXWDQ)[(Harsha et al., 2022)](https://paperpile.com/c/JB1097/0Cd4f). Molecular docking studies suggested that Piper betle may exert its protective effects against dental caries by interacting with proteins involved in relevant biological pathways[(Latipudin et al., 2024)](https://paperpile.com/c/JB1097/LMDI)[(Neha et al., 2021)](https://paperpile.com/c/JB1097/iGC3H)[(Maliael et al., 2021)](https://paperpile.com/c/JB1097/3SIXh)[(Lakshmi, 2021)](https://paperpile.com/c/JB1097/mewHG).

The integration of these plant extracts into a dental gel formulation presents a novel strategy for targeting oral infections. By utilizing the antimicrobial properties of Tephrosia calophylla and Piper betle, this mouth gel aims to address plaque formation, dental caries, and periodontitis effectively. The mucoadhesive nature of the gel enhances its ability to deliver these plant-derived compounds precisely to the affected areas, maximizing their therapeutic impact[(Shaikh et al., 2011)](https://paperpile.com/c/JB1097/eR5v)[(Dharman 2021)](https://paperpile.com/c/JB1097/r2BvA). Such targeted drug delivery systems hold promise in improving treatment outcomes while minimizing side effects associated with systemic drug administration.[(*Website*, n.d.)](https://paperpile.com/c/JB1097/XjVn)

This study aims to utilize the bioactive constituents of Tephrosia calophylla and Piper betle extracts to develop a novel mouth gel formulation aimed at addressing oral infections. The primary objective is to evaluate the cytotoxic effects of this herbal mouth gel, paving the way for its potential application in treating conditions such as thrush and candidiasis.

# Methods

## Plant Material and Extraction

Plant material comprising Tephrosia calophylla and Piper betle was collected and authenticated prior to extraction. Each plant material, weighing 2.5g, underwent grinding and subsequent boiling in 20ml of distilled water to extract bioactive compounds. Following filtration, the resulting extracts were combined with 2.5g of carbopol to formulate the mouth gel. The mixture was vigorously stirred until homogenized and facilitated for gel formation.

## Gel Formulation

The gel base was prepared using a carbopol polymer as the gelling agent, combined with glycerin as a humectant and methylparaben as a preservative. Sodium hydroxide was used to adjust the pH of the gel to match the physiological pH of the oral cavity (around 6.8-7.0). The extracts of Tephrosia and Piper betle were then incorporated into the gel base at concentrations suitable for antimicrobial, anti-inflammatory, and antioxidant testing. The final formulation was homogenized and stored at room temperature(Chehelgerdi et al., 2023).

Comprehensive bioactivity assessments were conducted to evaluate the antioxidant, anti-inflammatory, antimicrobial, and cytotoxic properties of the mouth gel extract.

## Antibacterial Assay

The antibacterial activity of the gel was evaluated against oral pathogens, including Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa, using the disk diffusion method. The bacterial cultures were grown on nutrient agar plates and standardized to a 0.5 McFarland standard. Sterile filter paper disks impregnated with the gel formulation were placed on the inoculated agar plates, and zones of inhibition were measured after 24 hours of incubation at 37°C. The activity of the gel was compared with that of standard antibiotics to assess its efficacy.

## Anti-inflammatory Assay

To assess the anti-inflammatory potential of the gel, an in vitro protein denaturation inhibition assay was performed. Various concentrations of the gel formulation were mixed with egg albumin solution and incubated (Saadh et al., 2024). The reaction mixture was heated to induce denaturation, and the absorbance was measured at 660 nm. Additionally, a red blood cell (RBC) membrane stabilization test was conducted. Human RBCs were mixed with different concentrations of the gel and subjected to hypotonic stress. The extent of hemolysis was recorded as an indicator of membrane stabilization and anti-inflammatory activity.

## Antioxidant Assay

The antioxidant potential of the gel was measured using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. Various concentrations of the gel were added to a DPPH solution, and the absorbance was measured at 517 nm after 30 minutes. The percentage of DPPH radical scavenging was calculated and compared with ascorbic acid, a standard antioxidant.

## Cytotoxicity Testing

Cytotoxicity was evaluated using mammalian cell cultures. The cells were exposed to the gel formulation, and cell viability was assessed using the MTT assay. The assay measured mitochondrial activity as an indicator of cell health, providing insights into the potential toxicity of the gel. The cytotoxic effects of Piper betle and Tephrosia extracts were analyzed individually, with repeated measurements over a 24-hour period.

# Results

## Antibacterial Activity

The gel formulation demonstrated significant antibacterial activity, particularly against E. coli and S. aureus, with a greater zone of inhibition observed with the Piper betle component. This result supports the potential of Piper betle in managing oral pathogens commonly associated with dental plaque and periodontal infections.

Figure A and Figure B in the results section illustrate the zones of inhibition for both bacteria, indicating that the gel has effective antimicrobial activity suitable for oral hygiene applications.

## Anti-inflammatory Activity

The anti-inflammatory effects of the gel were quantified through protein denaturation and RBC membrane stabilization assays. The gel formulation showed dose-dependent inhibition of protein denaturation, with higher concentrations displaying greater inhibitory activity. Tephrosia calophylla showed a more pronounced anti-inflammatory effect compared to Piper betle. This is beneficial for conditions such as gingivitis and periodontitis, where inflammation plays a key role in disease progression(Figure 2)

## Antioxidant Activity

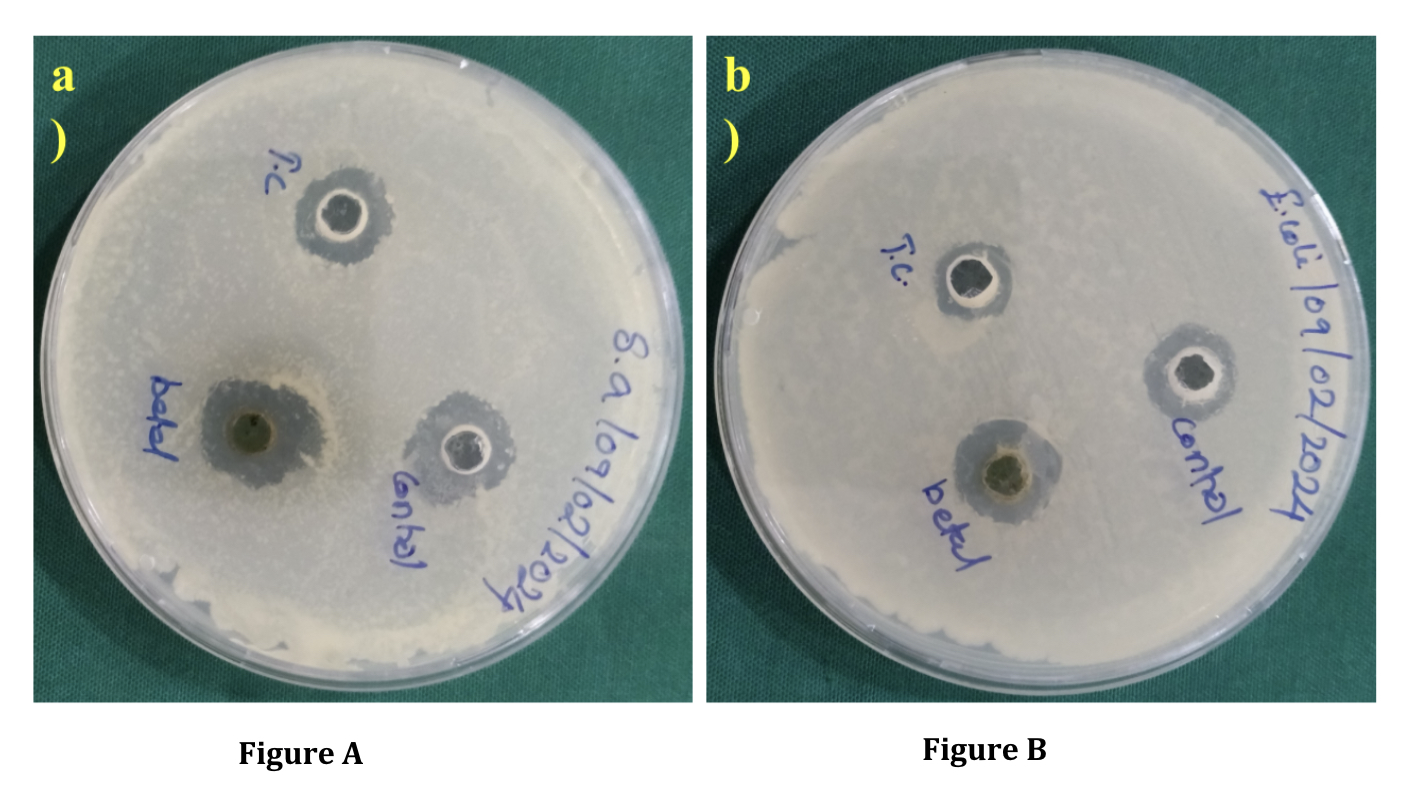
The gel formulation exhibited significant antioxidant activity in the DPPH radical scavenging assay. Both Tephrosia and Piper betle extracts demonstrated comparable antioxidant effects, indicating their role in reducing oxidative stress in the oral environment. This antioxidant property could be beneficial in neutralizing free radicals and preventing oxidative damage in oral tissues(Figure 3)

## Cytotoxicity

The cytotoxicity testing indicated that Piper betle exhibited initial high cytotoxicity, which decreased over time, whereas Tephrosia calophylla maintained a consistent, low level of cytotoxicity. This suggests that Tephrosia may be more suitable for sustained use in oral formulations, while Piper betle may require further concentration optimization for long-term applications.(Figure 4)

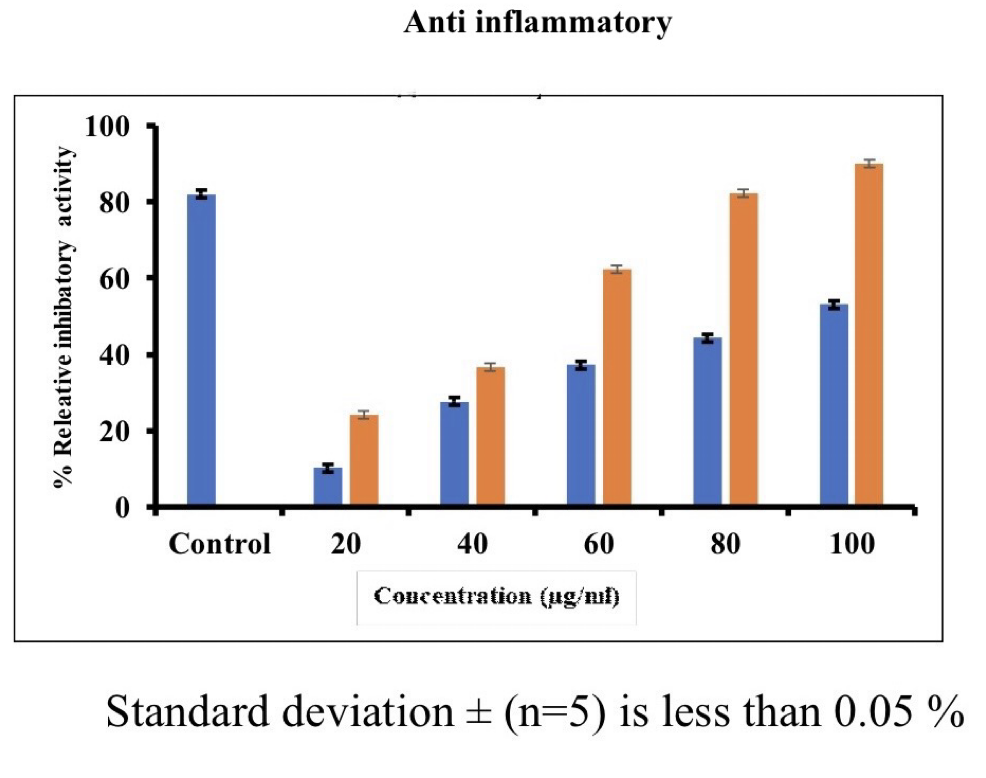
# Discussion

The formulation of an oral gel incorporating Tephrosia calophylla and Piper betle extracts shows promising therapeutic potential for maintaining oral hygiene. The in vitro results demonstrate that this herbal gel possesses a combination of antimicrobial, anti-inflammatory, and antioxidant properties, essential for addressing common oral health issues. The significant antibacterial activity observed against E. coli and S. aureus underscores the gel’s potential to combat pathogenic bacteria responsible for dental caries and periodontal diseases[(Vijayashree Priyadharsini, 2019)](https://paperpile.com/c/JB1097/8qtO). This is particularly notable, as Piper betle extract displayed a more pronounced antibacterial effect, which aligns with existing literature indicating its potent activity against various bacterial strains[(Singh et al., 2018)](https://paperpile.com/c/JB1097/2NEA). Additionally, the anti-inflammatory properties, especially from Tephrosia calophylla, suggest that this gel may alleviate gum inflammation, reducing the risk of conditions such as gingivitis and periodontitis. The antioxidant activity observed in both extracts further enhances the formulation’s appeal, as it can help neutralize free radicals, thereby protecting oral tissues from oxidative damage that can exacerbate oral diseases. Taken together, these properties support the potential efficacy of the gel as a multi-functional, natural oral care product, adding to the growing body of evidence advocating for plant-based alternatives in dental care.

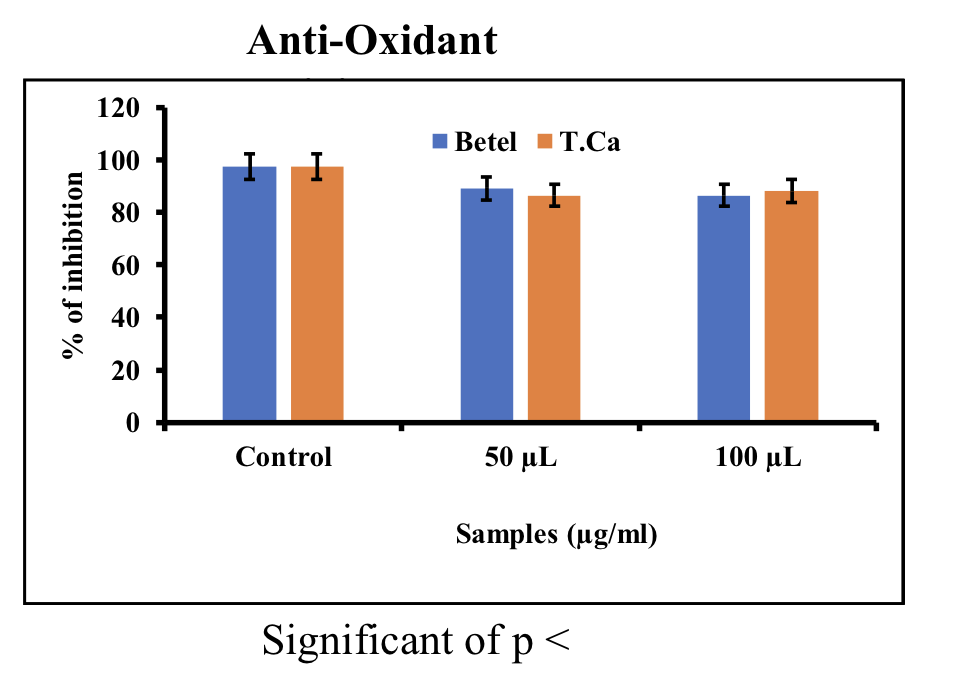


1. (b)

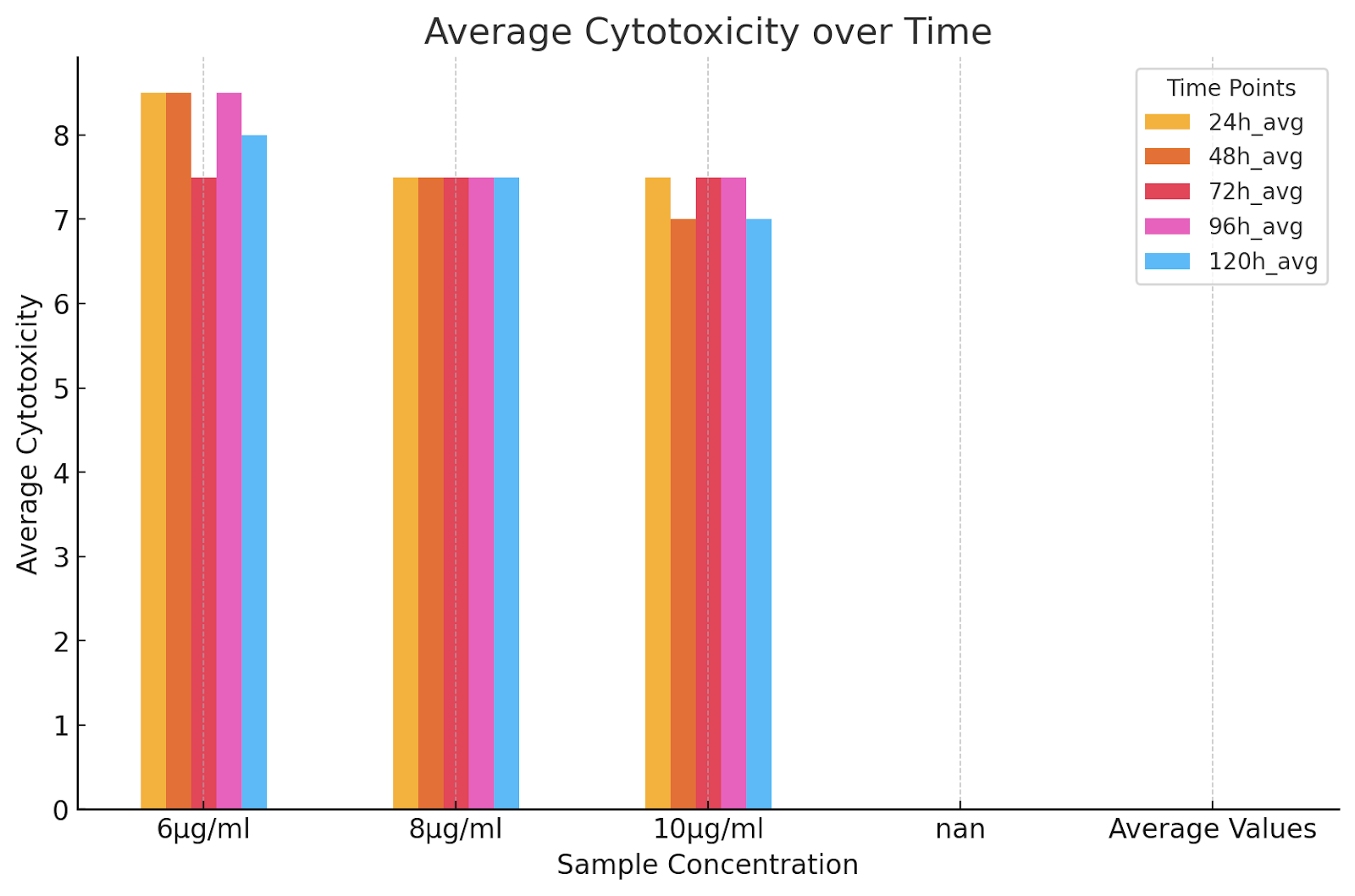
**Figure 1- (a) (b)** Antibacterial activity of the formulated oral gel against E. coli and S. aureus using the disk diffusion method. Zones of inhibition indicate the antimicrobial efficacy of the Tephrosia calophylla and Piper betle extracts.



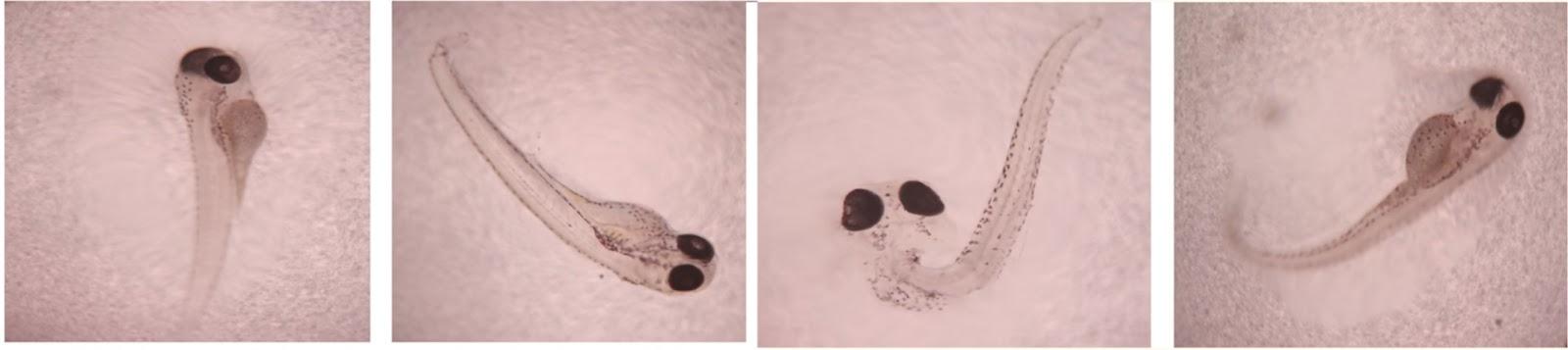
**Figure 2-** Anti-inflammatory activity of the oral gel assessed via the protein denaturation inhibition assay and RBC membrane stabilization test. Tephrosia calophylla demonstrated a more pronounced anti-inflammatory effect.



**Figure 3-**Antioxidant activity of the gel formulation measured using the DPPH radical scavenging assay. Both Tephrosia calophylla and Piper betle extracts exhibited significant antioxidant properties.



**Figure 4-** Cytotoxicity evaluation of the oral gel on zebrafish embryos. The survival rate, morphological abnormalities, and behavioral changes were monitored to assess potential toxic effects. Piper betle exhibited initial cytotoxic effects, which diminished over time, whereas Tephrosia calophylla maintained stable biocompatibility.



**Figure 5-** Images of zebrafish larvae exposed to Tephrosia calophylla and Piper betle gel formulation for cytotoxicity assessment. Morphological changes, including body curvature and yolk sac edema, were observed to evaluate biocompatibility and potential toxic effects.

Despite the promising results, certain factors may affect the generalizability of these findings to broader populations and settings. In vitro assays, though useful for preliminary assessments, may not fully replicate the complexities of the human oral cavity, The presence of saliva, varying pH levels, and a diverse microbial community can influence the stability and efficacy of the gel formulation[(Wong, 2009)](https://paperpile.com/c/JB1097/e5yL). For instance, saliva may dilute the concentration of active compounds, potentially diminishing their effectiveness over time[(Eick, 2020; Wong, 2009)](https://paperpile.com/c/JB1097/e5yL+4SHV). Therefore, conducting in vivo studies is crucial to verify the gel’s efficacy and stability in real-world scenarios. Clinical trials on human participants with varying oral health conditions would provide a more accurate assessment of the formulation’s effectiveness, tolerability, and potential side effects. Such studies could also investigate the ideal dosage and frequency of application, ensuring that the gel maintains its bioactivity within the dynamic oral environment.

Additionally, the application of this herbal gel could cater to diverse age groups and oral health needs, further enhancing its practical significance. With an increasing global demand for safer, natural oral care products, this formulation offers a potential alternative to synthetic agents, which may have adverse side effects, especially with long-term use[(Chauhan et al., 2020)](https://paperpile.com/c/JB1097/rYo4). The gel’s combination of Tephrosia and Piper betle extracts, both well-documented for their therapeutic properties, supports its application across various demographics, including populations sensitive to synthetic products, such as children and elderly individuals. However, optimizing the concentration of Piper betle in the formulation is necessary to minimize its initial cytotoxic effects, especially for younger and more vulnerable users. By refining the formulation to achieve a controlled, sustained release of active compounds, the gel could provide prolonged protection against bacterial growth and inflammation, catering to a wider range of oral health needs.

Overall, this study underscores the potential of herbal formulations as a viable alternative in oral care. The unique combination of Tephrosia calophylla and Piper betle extracts may not only enhance oral hygiene but also offer a safer, eco-friendly solution compared to conventional products.[(Chauhan & Shah, 2021)](https://paperpile.com/c/JB1097/5EKZ) Further research should explore its long-term effects, optimal formulation adjustments, and real-world efficacy across diverse populations to fully harness its benefits for public health[(Chauhan & Shah, 2021; Mehta et al., 2024)](https://paperpile.com/c/JB1097/5EKZ+WTNb). This gel could pave the way for developing more plant-based oral care products, contributing to sustainable and preventive healthcare practices globally.

## Limitations and future scope

Further research is needed to be done using advanced technologies and clinical trials is necessary to fully understand and explore clinical applications of Tephrosia and Piper betle herbal formulation

# Conclusion

The study highlights the potential of an oral gel formulation incorporating Tephrosia calophylla and Piper betle extracts as a natural alternative for synthetic oral gels[(Thongmuang et al., 2024)](https://paperpile.com/c/JB1097/Ka7b). The in vitro results demonstrate that the gel possesses significant antimicrobial, anti-inflammatory, and antioxidant properties, which are essential for managing oral pathogens, reducing inflammation, and mitigating oxidative stress in the oral cavity[(Grey, 2016; Thongmuang et al., 2024)](https://paperpile.com/c/JB1097/Ka7b+gRZ5).

The antibacterial activity of the formulation, particularly against E. coli and S. aureus, suggests its efficacy in controlling microbial populations responsible for dental caries and periodontal diseases. The anti-inflammatory effect, particularly from Tephrosia calophylla, offers a potential therapeutic benefit for conditions associated with gum inflammation, such as gingivitis and periodontitis[(Zamanifard et al., 2024)](https://paperpile.com/c/JB1097/GE0f). Furthermore, the antioxidant activity observed in both extracts provides an additional layer of defense, protecting oral tissues from oxidative damage that could exacerbate oral health conditions.

The initial cytotoxicity of Piper betle, which decreased over time, points to the need for further optimization, particularly in dosage and application frequency, to ensure user safety. The results also underscore the potential advantages of combining these plant extracts in a formulation, leveraging the synergistic effects of their individual properties.

In conclusion, the herbal gel formulation with Tephrosia calophylla and Piper betle represents a promising candidate for an alternative oral care product aimed at preventing and managing oral diseases. With further research, clinical validation, and formulation refinement, this plant-based gel could serve as an effective and safer alternative to conventional synthetic oral care products, offering benefits in plaque control, inflammation reduction, and overall oral hygiene maintenance.

# References

1. [Ajay, R., Rakshagan, V., Queenalice, A., Vinothkumar, S., Ravivarman, C., & Saravanadinesh, P. (2022). Effect of triazine comonomer substitution on the structure and glass transition temperature of monomethacrylate-based resin polymer: An in vitro study. *The Journal of Contemporary Dental Practice*, *23*(2), 202–207.](http://paperpile.com/b/JB1097/DtqDh)
2. [Ajay, R., Sasikala, R., Rakshagan, V., Raghunathan, J., LalithaManohari, V., & Baburajan, K. (2022). Evaluation of cytocompatibility of thermopolymerized denture base copolymer containing a novel ring-opening oxaspiro comonomer. *World Journal of Dentistry*, *13*(2), 127–132.](http://paperpile.com/b/JB1097/Yls3D)
3. [Ajay, R., Suma, K., Sasikala, R., Rakshagan, V., Baburajan, K., & Kalarani, G. (2022). Evaluation of linear dimensional stability of monomethacrylate-based dental polymer containing a novel tricyclic diacrylate cross-linker using a novel surface-level index technique. *World Journal of Dentistry*, *13*(6), 568–573.](http://paperpile.com/b/JB1097/mJivD)
4. [Balaji Ganesh S, & Sugumar, K. (2021). Internet of Things—A novel innovation in dentistry. *Journal of Advanced Oral Research*, *12*(1), 42–48.](http://paperpile.com/b/JB1097/SzEa4)
5. [Chauhan, D. N., & Shah, K. (2021). *Phytopharmaceuticals: Potential Therapeutic Applications*. John Wiley & Sons.](http://paperpile.com/b/JB1097/5EKZ)
6. [Chauhan, D. N., Singh, P. R., Shah, K., & Chauhan, N. S. (2020). *Natural Oral Care in Dental Therapy*. John Wiley & Sons.](http://paperpile.com/b/JB1097/rYo4)
7. Chehelgerdi M., Chehelgerdi, M., Allela, O. Q. B., Pecho, R. D. C., Jayasankar, N., Rao, D. P. & Akhavan-Sigari, R. (2023). Progressing nanotechnology to improve targeted cancer treatment: overcoming hurdles in its clinical implementation. Molecular cancer, 22(1), 169.
8. [Chidambaram, S. R., George, A. M., Muralidharan, N. P., Prasanna Arvind, T. R., Subramanian, A., & Rahaman, F. (2022). Current overview for chemical disinfection of dental impressions and models based on its criteria of usage: A microbiological study. *Indian Journal of Dental Research : Official Publication of Indian Society for Dental Research*, *33*(1), 30–36.](http://paperpile.com/b/JB1097/45Z7b)
9. [Deepika, B. A., Ramamurthy, J., Girija, S., & Jayakumar, N. D. (2022). Evaluation of the antimicrobial effect of Ocimum sanctum L. oral gel against anaerobic oral microbes: An in vitro study. *World Journal of Dentistry*, *13*(S1), S23–S27.](http://paperpile.com/b/JB1097/F07Sf)
10. [Dharman, S (2021). Ecofriendly Synthesis, Characterisation and Antibacterial Activity Of Curcumin Mediated Silver Nanoparticles. *International Journal of Dentistry and Oral Science*, 2314–2318.](http://paperpile.com/b/JB1097/r2BvA)
11. [DK. (2021). *New Guide to Medicine and Drugs: The Complete Home Reference to Over 3,000 Medicines*. Dorling Kindersley Ltd.](http://paperpile.com/b/JB1097/HKHN)
12. [Eick, S. (2020). *Oral Biofilms*. Karger Medical and Scientific Publishers.](http://paperpile.com/b/JB1097/4SHV)
13. [Ganapaty, S., Nair, V., Devi, D. R., Pannakal, S. T., Laatsch, H., & Dittrich, B. (2014). Rare prenylated isoflavones from Tephrosia calophylla. *Natural Product Communications*, *9*(7), 937–940.](http://paperpile.com/b/JB1097/vwat)
14. [Govindaraj, A., & Dinesh, S. P. S. (2021). Effect of chlorhexidine varnish and fluoride varnish on White Spot Lesions in orthodontic patients- a systematic review. *The Open Dentistry Journal*, *15*(1), 151–159.](http://paperpile.com/b/JB1097/QNY8i)
15. [Graf, S., Thakkar, D., Hansa, I., Pandian, S. M., & Adel, S. M. (2023). 3D metal printing in orthodontics current trends, biomaterials, workflows and clinical implications. *Seminars in Orthodontics*. https://doi.org/](http://paperpile.com/b/JB1097/cOGTQ)[10.1053/j.sodo.2023.01.001](http://dx.doi.org/10.1053/j.sodo.2023.01.001)
16. [Grey, J. (2016). *Homemade Oral Care: 40 Natural Herbal Recipes to Take Care of Your Teeth and Oral Cavity: (20 Bonus Anti-Cold Lip Balm and Kids Lip Balm Recipes Included!)*. Createspace Independent Publishing Platform.](http://paperpile.com/b/JB1097/gRZ5)
17. [Harsha, L., Navaneethan, R., Acid, T., & Acid, C. A.-A. (2022). CITRIC ACID-AN VITRO STUDY. *International Journal Clinical Dentistry*, *15*(3), 413–419.](http://paperpile.com/b/JB1097/0Cd4f)
18. [Harsha, L., & Subramanian, A. K. (2022). Comparative assessment of pH and degree of surface roughness of enamel when etched with five commercially available etchants: An in vitro study. *The Journal of Contemporary Dental Practice*, *23*(2), 181–185.](http://paperpile.com/b/JB1097/xeyzw)
19. [Jabin, Z., Nasim, I., Vishnu Priya, V., & Agarwal, N. (2021). Quantitative Analysis and Effect of SDF, APF, NaF on Demineralized Human Primary Enamel Using SEM, XRD, and FTIR. *International Journal of Clinical Pediatric Dentistry*, *14*(4), 537–541.](http://paperpile.com/b/JB1097/ps61c)
20. [Katyal, D., Subramanian, A. K., Venugopal, A., & Marya, A. (2021). Assessment of Wettability and Contact Angle of Bonding Agent with Enamel Surface Etched by Five Commercially Available Etchants: An In Vitro Study. *International Journal of Dentistry*, *2021*, 9457553.](http://paperpile.com/b/JB1097/70Qbt)
21. [Lakshmi, T. (2021). Medicinal value oral health aspects acacia catechu-an update. *International Journal Dentistry Oral ScienceVolume*, *8*, 1399–1401J.](http://paperpile.com/b/JB1097/mewHG)
22. [Latipudin, D., Tumilaar, S. G., Ramdani, Y., Dudi, D., & Kurnia, D. (2024). Potential Piperolactam A Isolated From Piper betle as Natural Inhibitors of Brucella Species Aminoacyl-tRNA Synthetase for Livestock Infections: In Silico Approach. *Veterinary Medicine and Science*, *10*(6), e70042.](http://paperpile.com/b/JB1097/LMDI)
23. [Maliael, M. T., Subramanian, A. K., & Srirengalakshmi. (2021). Effectiveness of a fluoride-releasing orthodontic primer in reducing demineralization around brackets – a systematic review. *Orthodontic Waves (English Ed.)*, *80*(4), 218–223.](http://paperpile.com/b/JB1097/3SIXh)
24. [Mehta, V., Mathur, A., Tripathy, S., Sa, R., & Sharma, T. (2024). Effectiveness of herbal oral care products in reducing dental plaque and gingivitis: an overview of systematic reviews. *Canadian Journal of Dental Hygiene : CJDH = Journal Canadien de L’hygiene Dentaire : JCHD*, *58*(2), 120–134.](http://paperpile.com/b/JB1097/WTNb)
25. [Miller, W. D. (1890). *The Micro-organisms of the Human Mouth: The Local and General Diseases which are Caused by Them*.](http://paperpile.com/b/JB1097/M5CE)
26. [National Research Council, Institute of Medicine, Board on Health Care Services, Board on Children, Youth, and Families, & Committee on Oral Health Access to Services. (2012). *Improving Access to Oral Health Care for Vulnerable and Underserved Populations*. National Academies Press.](http://paperpile.com/b/JB1097/z1Tr)
27. [Neha, N., Maiti, S., & Jessy, P. (2021). Adhesion microflora role denitrifies colour stability provisional crowns: in-vitro study. *Int J Dentistry Oral Sci*, *8*(8), 3805–3809.](http://paperpile.com/b/JB1097/iGC3H)
28. [Parine, N. R., Lakshmi, P., Kumar, D., Shaik, J. P., Alanazi, M., & Pathan, A. A. K. (2015). Development and characterisation of nine polymorphic microsatellite markers for Tephrosia calophylla Bedd. (Fabaceae). *Saudi Journal of Biological Sciences*, *22*(2), 164–167.](http://paperpile.com/b/JB1097/IwMC)
29. [Raikwar, G., Mohan, S., & Dahiya, P. (2025). Chemical composition, antibacterial and antioxidant activities of Piper betle and Anethum graveolens essential oils against Methicillin-resistant Staphylococcus aureus clinical isolates. *Brazilian Journal of Microbiology : [publication of the Brazilian Society for Microbiology]*. https://doi.org/](http://paperpile.com/b/JB1097/aeVY)[10.1007/s42770-024-01567-9](http://dx.doi.org/10.1007/s42770-024-01567-9)
30. [Reddy, R. V. N., Khalivulla, S. I., Reddy, B. A. K., Reddy, M. V. B., Gunasekar, D., Deville, A., & Bodo, B. (2009). Flavonoids from Tephrosia calophylla. *Natural Product Communications*, *4*(1), 59–62.](http://paperpile.com/b/JB1097/TUjB)
31. Saadh, M. J., Rasulova, I., Almoyad, M. A. A., Kiasari, B. A., Ali, R. T., Rasheed, T. & Ciongradi, C. I. (2024). Recent progress and the emerging role of lncRNAs in cancer drug resistance; focusing on signaling pathways. Pathology-Research and Practice, 253, 154999.
32. [Sabarathinam, J., & Madhulaxmi, R. (2021). Development anti inflammatory antimicrobial silver nanoparticles coated suture materials. *Int J Dentistry Oral Sci*, *8*(3), 2006–2013.](http://paperpile.com/b/JB1097/PNmZY)
33. [Shaikh, R., Raj Singh, T. R., Garland, M. J., Woolfson, A. D., & Donnelly, R. F. (2011). Mucoadhesive drug delivery systems. *Journal of Pharmacy & Bioallied Sciences*, *3*(1), 89–100.](http://paperpile.com/b/JB1097/eR5v)
34. [Sikdar, B., Mukherjee, S., Bhattacharya, R., Raj, A., Roy, A., Banerjee, D., Gangopadhyay, G., & Roy, S. (2024). The anti-quorum sensing and biofilm inhibitory potential of Piper betle L. leaf extract and prediction of the roles of the potent phytocompounds. *Microbial Pathogenesis*, *195*, 106864.](http://paperpile.com/b/JB1097/4W84)
35. [Singh, D., Narayanamoorthy, S., Gamre, S., Majumdar, A. G., Goswami, M., Gami, U., Cherian, S., & Subramanian, M. (2018). Hydroxychavicol, a key ingredient of Piper betle induces bacterial cell death by DNA damage and inhibition of cell division. *Free Radical Biology & Medicine*, *120*, 62–71.](http://paperpile.com/b/JB1097/2NEA)
36. [Solanki, L., Shantha Sundari, K. K., Muralidharan, N. P., & Jain, R. (2022). Antimicrobial effect of novel gold nanoparticle oral rinse in subjects undergoing orthodontic treatment: An ex-vivo study. *Journal of International Oral Health: JIOH*, *14*(1), 47.](http://paperpile.com/b/JB1097/o4YdG)
37. [Sushanthi, S., Doraikannan, S., Indiran, M., & Rathinavelu, P. (2021). *Rajeshkumar S. Vernonia Amygdalina*. 3330–3334.](http://paperpile.com/b/JB1097/sXWDQ)
38. [Thongmuang, P., Thongkao, K., Owen, R. W., & Sudjaroen, Y. (2024). Efficacy of Thai Herbal Extract Oral Care Products in Promoting Antimicrobial Health Among Healthy Volunteers. *Journal of Pharmacy & Bioallied Sciences*, *16*(Suppl 3), S2718–S2720.](http://paperpile.com/b/JB1097/Ka7b)
39. [Tiwari, A., & Jain, R. K. (2023). Comparative evaluation of White Spot lesion incidence between NovaMin, probiotic, and fluoride containing dentifrices during orthodontic treatment using laser fluorescence - A prospective randomized controlled clinical trial. *Clinical and Investigative Orthodontics*, 1–8.](http://paperpile.com/b/JB1097/05p2L)
40. [Vijayashree Priyadharsini, J. (2019). In silico validation of the non-antibiotic drugs acetaminophen and ibuprofen as antibacterial agents against red complex pathogens. *Journal of Periodontology*, *90*(12), 1441–1448.](http://paperpile.com/b/JB1097/8qtO)
41. [*Website*. (n.d.).](http://paperpile.com/b/JB1097/XjVn) [Hardeep Singh, Gauri, Recent development of novel drug delivery of herbal drugs, RPS Pharmacy and Pharmacology Reports, Volume 2, Issue 4, October 2023, rqad028, https://doi.org/10.1093/rpsppr/rqad028](about:blank)
42. [Wong, D. T. (2009). *Salivary Diagnostics*. John Wiley & Sons.](http://paperpile.com/b/JB1097/e5yL)
43. [Zamanifard, M., Nasiri, M., Yarahmadi, F., Zonoori, S., Razani, O., Salajegheh, Z., Imanipour, M., Mohammadi, S. M., Jomehzadeh, N., & Asadi, M. (2024). Healing of diabetic foot ulcer with topical and oral administrations of herbal products: A systematic review and meta-analysis of randomized controlled trials. *International Wound Journal*, *21*(2), e14760.](http://paperpile.com/b/JB1097/GE0f)