ISSN Print: 2394-7489
ISSN Online: 2394-7497
Impact Factor (RJIF): 7.85
IJADS 2025; 11(3): 185-190

www.oraljournal.com
Received: 11-07-2025
Accepted: 16-08-2025

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Comparative evaluation of antimicrobial efficacy of aqueous and ethanolic extracts of *Eucalyptus globulus* against periodontal pathogens: An *in-vitro* study

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DOI: https://www.doi.org/10.22271/oral.2025.v11.i4c.2275

Abstract
Introduction: The oral cavity harbors a complex microbiota, some of which are directly implicated in periodontal disease. Conventional chemotherapeutic agents and mechanical approaches are widely used for periodontal therapy; however, issues such as microbial resistance and systemic toxicity limit their long-term application. In this context, *Eucalyptus globulus*, a natural phytochemical agent, has demonstrated promising antimicrobial properties against oral pathogens, including *Porphyromonas gingivalis*.
Objective: To comparatively evaluate the antibacterial efficacy of aqueous and ethanolic extracts of *Eucalyptus globulus* leaves against *P. gingivalis*.
Methodology: Leaves of *E. globulus* were washed, dried, and powdered. Ethanolic and aqueous extracts were prepared using a Soxhlet apparatus. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by serial broth dilution. Extract concentrations ranged from 100 mg/mL to 0.2 mg/mL, with tetracycline serving as the control. Following 48 hours of incubation, turbidity was used as an indicator of bacterial growth. Comparative analysis of MIC and MBC values was performed for both extracts and tetracycline against *P. gingivalis*.
Results: *Aqueous extract* of *E. globulus* inhibited the growth of *P. gingivalis* at a MIC of 0.8 mg/mL, whereas the *ethanolic extract* demonstrated inhibition at 1.6 mg/mL. Tetracycline exhibited antibacterial activity at 0.2 mg/mL. Bactericidal activity was observed at 6.25 mg/mL for the aqueous extract and 12.5 mg/mL for the ethanolic extract. Statistical analysis showed significant differences ($p < 0.05$) in antimicrobial activity between extracts and the negative control.
Conclusion: Both aqueous and ethanolic extracts of *E. globulus* displayed antibacterial activity against *P. gingivalis*. The aqueous extract demonstrated superior antimicrobial efficacy compared to the ethanolic extract, suggesting its potential as a natural adjunct in periodontal therapy.

Keywords: *Eucalyptus globulus*, aqueous extract, ethanolic extract, *Porphyromonas gingivalis*

Introduction
Periodontal diseases constitute a major public health concern worldwide, contributing significantly to tooth loss and diminished quality of life. These chronic inflammatory conditions, ranging from gingivitis to advanced periodontitis, are predominantly initiated by anaerobic gram-negative bacteria within the subgingival biofilm, including *Porphyromonas gingivalis*, *Prevotella intermedia*, *Tannerella forsythia*, and *Treponema denticola* [1].
Standard periodontal therapy combines mechanical debridement with adjunctive chemotherapeutic agents [2]. However, the increasing prevalence of antibiotic-resistant strains and side effects of systemic antibiotics necessitate the search for safer, plant-derived alternatives [3, 4].
Natural phytochemicals have shown considerable promise as antimicrobial and anti-inflammatory agents [5]. Among these, *Eucalyptus globulus* commonly known as Tasmanian blue gum—has attracted attention for its broad pharmacological properties. Its leaves are rich in bioactive components, particularly monoterpenes (e.g., 1, 8-cineole) and sesquiterpenes, known for their antibacterial, antioxidant, and anti-inflammatory actions [6, 7].

Compounds such as 1, 8-cineole (eucalyptol) inhibit pro-inflammatory cytokines and reduce neutrophil infiltration, while other constituents exhibit antioxidant and analgesic effects [8, 9]. Takarada *et al*. demonstrated that eucalyptus oil exhibits antibacterial activity against oral pathogens, including *Streptococcus mutans* and *Candida albicans* [10].

Despite such findings, limited studies have explored the antimicrobial potential of *E. globulus* against key periodontal pathogens such as *P. gingivalis*. Therefore, this study aims to evaluate and compare the antimicrobial efficacy of aqueous and ethanolic extracts of *E. globulus* against *P. gingivalis*, an important etiologic agent in periodontitis.

Aim

To evaluate the antimicrobial activity of aqueous and ethanolic extracts of *Eucalyptus globulus* leaves against key periodontal pathogens, particularly *Porphyromonas gingivalis*.

Objectives

1. To assess the antibacterial activity of aqueous and ethanolic extracts of *Eucalyptus globulus* and tetracycline against *P. gingivalis.*
2. To compare the antimicrobial efficacy of aqueous and ethanolic extracts.
3. To determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of both extracts and tetracycline against *P. gingivalis.*

Materials and Methods

Preparation of Plant Extracts

Commercially available fine leaf powder of *Eucalyptus globulus* was used. Aqueous and ethanolic extracts were prepared by the Soxhlet extraction method.

- **Aqueous extract:** 50 g of powdered leaves were extracted with 500 mL of distilled water at 100 °C for 2 hours. The filtrate was concentrated using a rotary evaporator at 40 °C and stored at 4 °C.
- **Ethanolic extract:** 50 g of powdered leaves were extracted with 500 mL of 70% ethanol by maceration at room temperature for 48 hours, filtered, and concentrated at 40 °C. The dried extracts were stored at 4°C in airtight containers until use.

Fig 1: Extraction method. A - Eucalyptus powder, B - Water and ethanol tubes, C - Mixed with water and ethanol (99.9%), D - Poured into test tube, E- Centrifuged, F - Supernant collected into plates, G - Evaporated, H - Aqueous and ethanolic extracts.

Serial Broth Dilution Method

Serial broth dilution was used to determine the MIC of *E. globulus* extracts and tetracycline.

Stock solutions (200 mg/mL) of both aqueous and ethanolic extracts were prepared. Twelve sterile tubes were arranged serially, each containing 200 µL of thioglycolate broth. Serial dilutions were performed to achieve final extract concentrations of 100, 50, 25, 12.5, 6.25, 3.1, 1.6, 0.8, 0.4, and 0.2 mg/mL.

Each tube received 10 µL of *P. gingivalis* suspension, except the broth control (tube 11) and organism control (tube 12). After incubation at 37°C for 48 hours, turbidity was visually assessed. The lowest concentration with no turbidity was recorded as the MIC. The corresponding tubes were sub-cultured on agar plates to determine MBC values after 24 hours of incubation.

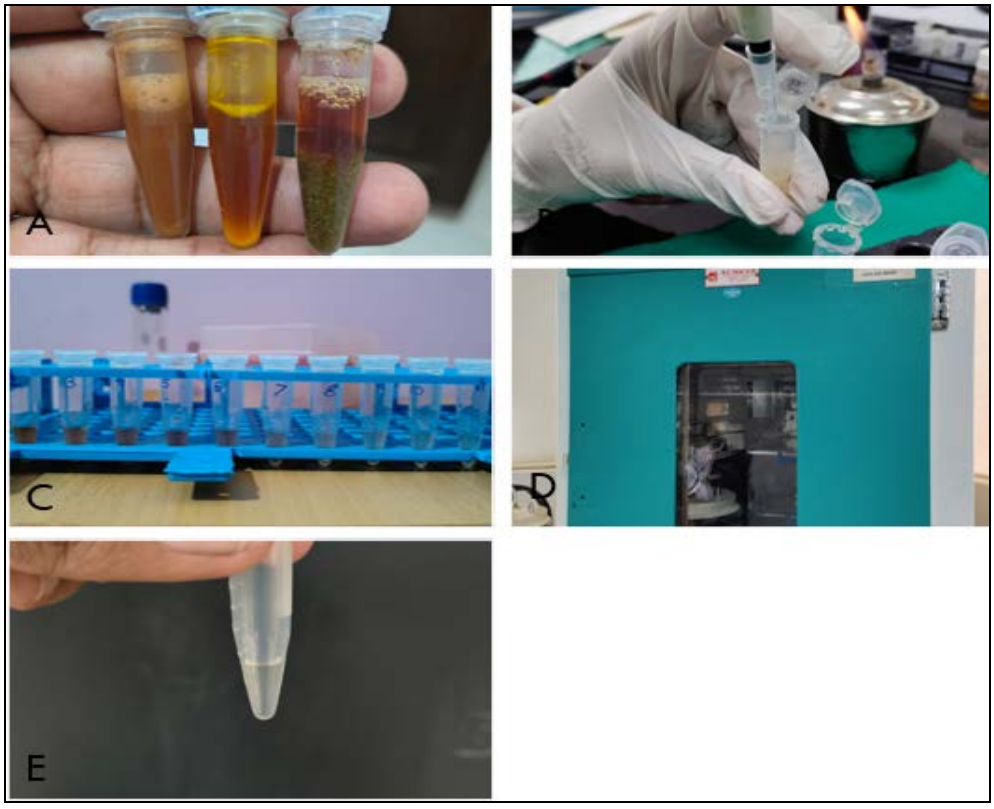


Fig 2: A - Stock solutions, B - Dilution with thioglycolate broth, C - Serial broth dilutions, D - Incubation for 48 hrs, E - MIC

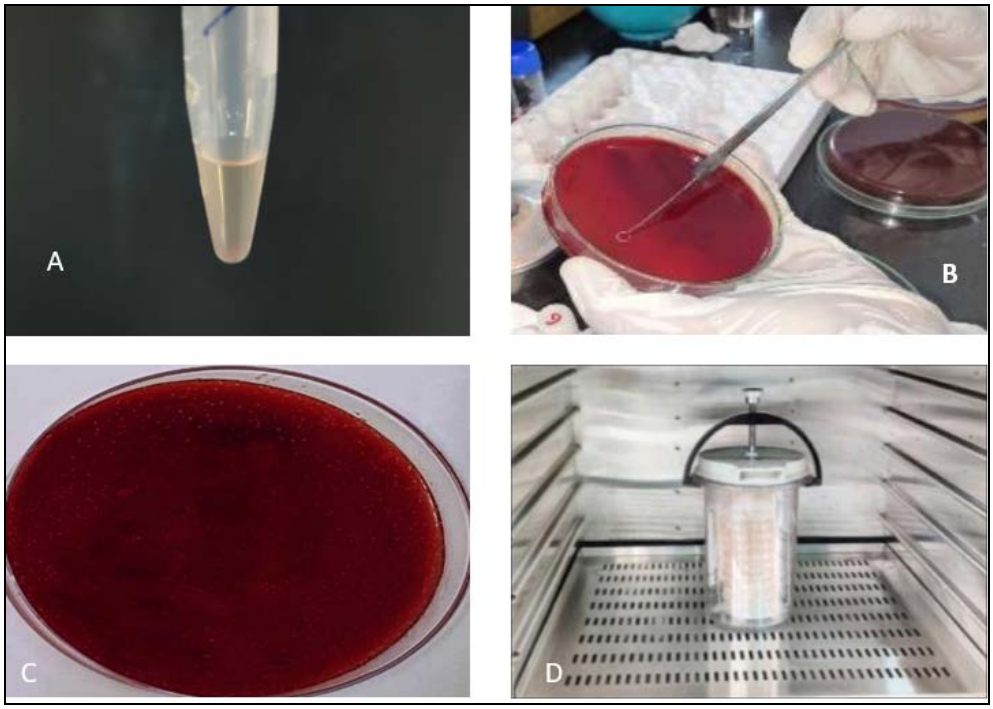


Fig 3: A - Tube sensitive to MIC, B - Nutrient medium for isolation of p.gingivalis, C - Plated on nutrient medium, D - Incubation for 48 hrs.

Results

The antimicrobial activity of both extracts was assessed using MIC and MBC methods.

- The aqueous extract inhibited *P. gingivalis* at 0.8 mg/mL, while the ethanolic extract showed inhibition at 1.6 mg/mL.
- Tetracycline demonstrated inhibition at 0.2 mg/mL.
- The bactericidal effect was observed at 6.25 mg/mL for the aqueous extract, 12.5 mg/mL for the ethanolic extract, and 0.2 mg/mL for tetracycline.

The aqueous extract exhibited bacteriostatic activity at 0.8 mg/mL, whereas the ethanolic extract showed bacteriostatic activity at 1.6 mg/mL. Tetracycline showed bactericidal activity at all concentrations tested. Statistical analysis ($p < 0.05$) confirmed that both extracts demonstrated significant antimicrobial effects compared to the negative control.

Table 1: Minimum inhibitory concentration of *Eucalyptus globulus* extracts (aqueous and ethanolic) and tetracycline in mg/ml against *Porphyromonas gingivalis*.

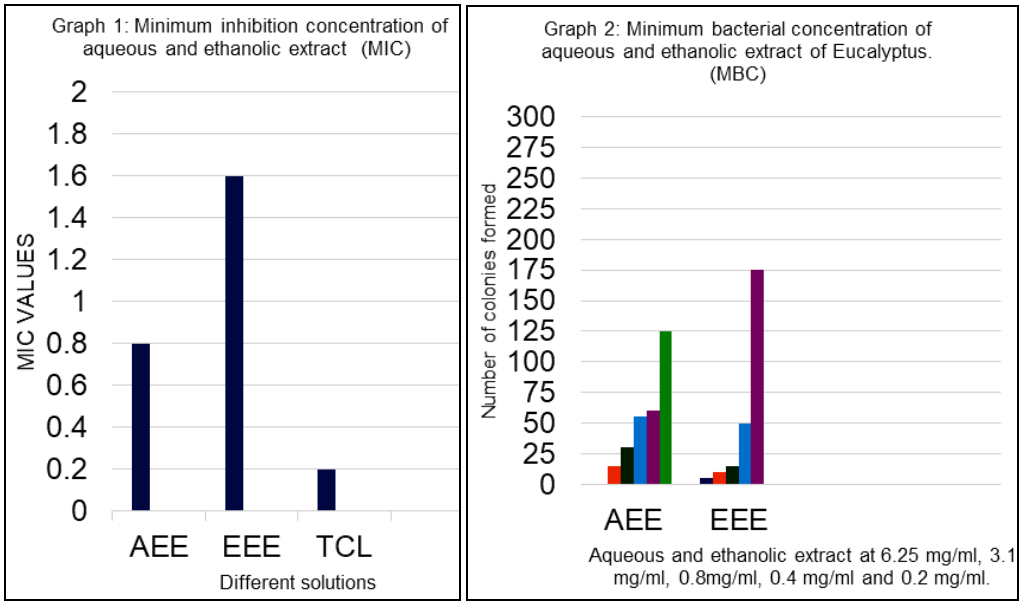
Concentration (mg/ml)	100	50	25	12.5	6.25	3.1	1.6	0.8	0.4	0.2
AEE (Aqueous extract of *Eucalyptus*)	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Resistant	Resistant	Resistant
EEE (Ethanolic extract of *Eucalyptus*)	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Resistant	Resistant	Resistant	Resistant
TCL (Tetracycline)	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Resistant

AEE - Aqueous extract of *Eucalyptus*; EEE - Ethanolic extract of *Eucalyptus*; TCL - Tetracycline

Table 2: Minimum bactericidal concentration of *Eucalyptus globulus* extracts (aqueous and ethanolic) and tetracycline in mg/ml against *Porphyromonas gingivalis*.

Concentration (mg/ml)	100	50	25	12.5	6.25	3.1	1.6	0.8	0.4	0.2
Group A (AEE)	NG	NG	NG	NG	5*	15*	30*	55*	60*	125*
Group B (EEE)	NG	NG	NG	NG	5*	10*	15*	50*	175*	200*
Group C (TCL)	NG	NG	NG	NG	NG	NG	NG	NG	NG	10*

NG - No Growth; G - Growth; * indicated number of colonies formed; AEE - Aqueous extract of *Eucalyptus*; EEE - Ethanolic extract of *Eucalyptus*; TCL - Tetracycline.



Graph 1: MIC & MBC of aqueous and ethanolic extract against P. gingivalis.

The antimicrobial activity of the extracts was statistically significant ($p<0.05$) compared to the negative control, and no significant differences were observed between the extract activities and the positive control (tetracycline) for most pathogens.

Discussion

Dental plaque biofilm plays a central role in the etiology of periodontal disease. Long-standing plaque accumulation leads to gingivitis, which may progress to periodontitis. Mechanical debridement and chemotherapeutic agents remain the mainstay of therapy; however, antibiotic resistance and adverse drug reactions limit their efficacy [11].

In this study, both aqueous and ethanolic extracts of *Eucalyptus globulus* demonstrated substantial antimicrobial activity against *P. gingivalis.* The MIC and MBC findings suggest that the aqueous extract was more effective, indicating higher solubility and bioavailability of active compounds in water. The MBC values were approximately twice those of MICs, indicating a typical relationship between inhibitory and bactericidal concentrations [12].

The observed antimicrobial activity can be attributed to the presence of bioactive monoterpenes and sesquiterpenes particularly 1, 8-cineole, α-pinene, and limonene which possess strong antibacterial effects by disrupting bacterial membranes and interfering with enzyme function [13].

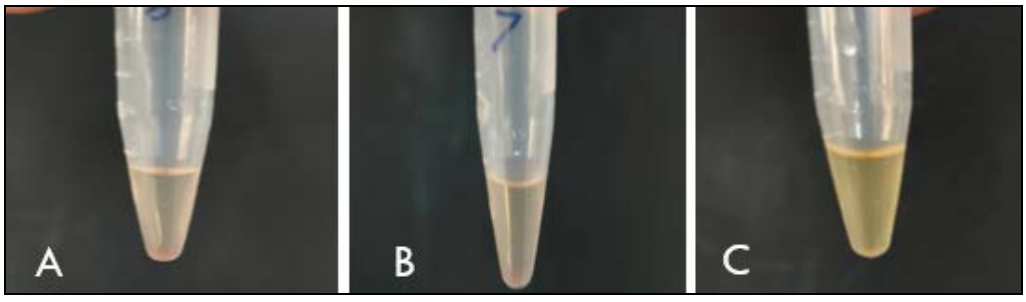


Fig 4: Minimum inhibitory concentration of Eukalyptus globulus extracts (aqueous and ethanolic) and tetracycline hydrochloride in mg/ml against Porphyromonas gingivalis.

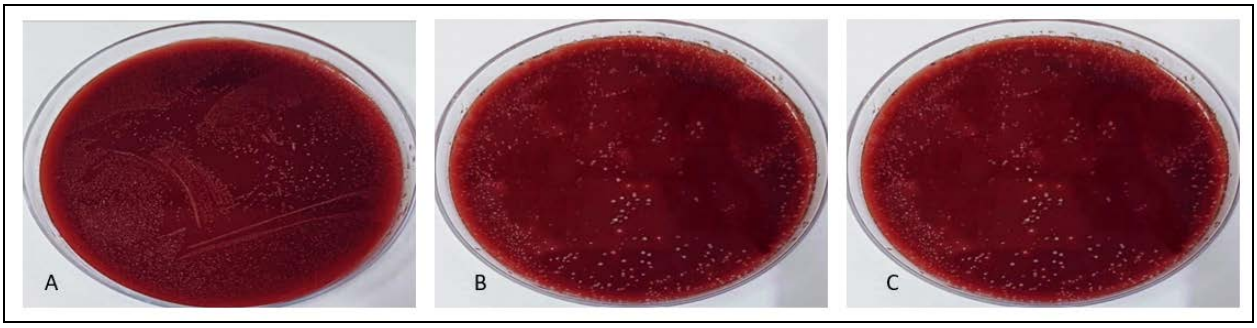


Fig 5: In Minimum Bactericidal Concentration (MBC) of Eukalyptus globulus (aqueous and ethanolic extract) and tetracycline hydrochloride exhibited bacteriostatic activity against P.gingivalis.

These findings align with prior studies. Takarada *et al.* [10] reported eucalyptus oil's antibacterial effect against *S. mutans*, while Balouiri *et al.* demonstrated its antifungal potential against *Candida albicans*. Similarly, Gontiya and Galgali [14] confirmed the antimicrobial activity of *E. globulus* extracts against oral pathogens.

Although the ethanolic extract contained more terpenes, its slightly reduced efficacy compared to the aqueous extract may result from solvent-dependent extraction variability or lower solubility of certain active components.

The comparable antimicrobial activity between *E. globulus* extracts and tetracycline is particularly significant, highlighting the potential of plant-derived agents as natural alternatives or adjuncts to conventional antibiotics [15].

Furthermore, the biofilm-disrupting potential of Eucalyptus globulus extracts is worth emphasising. While our study focused on planktonic growth of Porphyromonas gingivalis, it is well-established that periodontopathogens exist within structured biofilms which demonstrate greater resistance to antimicrobials. Recent work has shown that E. globulus leaf extracts exert significant antibiofilm activity, meaning they reduce bacterial adherence and extracellular matrix formation (e.g., MIC/MBIC values reported). [13] This suggests that its application in periodontal settings may not only inhibit free-floating bacteria but also target the more resilient biofilm communities underlying disease progression.

In addition, the phytochemical profile of E. globulus provides mechanistic insight into its antimicrobial efficacy. GC-MS analyses reveal that over 60 % of its essential oil consists of monoterpenes such as 1, 8-cineole, α-pinene, and limonene, which possess membrane-disruptive, enzyme-inhibitory and oxidative-stress inducing activities against bacteria. [14] In periodontopathogens, such as P. gingivalis, disruption of the outer membrane or inhibition of virulence factor secretion may reduce colonization and tissue destruction. Thus, our findings of lower MIC/MBC values for the aqueous extract may reflect optimal extraction of these active compounds in this solvent system, resulting in better bioavailability and antimicrobial action.

Finally, integration of E. globulus extracts into periodontal treatment protocols offers prospective benefits beyond direct antimicrobial action. Their documented anti-inflammatory and antioxidant activities imply a dual role: suppressing microbial load while moderating host inflammatory response and oxidative tissue damage characteristic of periodontitis. [16] combination of actions could help reduce tissue breakdown and promote healing, unlike conventional antimicrobials which target bacteria alone. However, translation from in-vitro to clinical practice requires careful formulation, dose-optimisation, and rigorous safety evaluation.

Additional References (to add to your reference list):

Nevertheless, as this was an in-vitro study, results may vary under in-vivo conditions due to factors like host immune responses, bioavailability, and metabolic alterations. Future research should include cytotoxicity assessments, in-vivo studies, and formulation development (e.g., gels, mouthwashes) to establish clinical relevance and safety.

Conclusion

This study demonstrated that both aqueous and ethanolic extracts of *Eucalyptus globulus* possess significant antibacterial activity against *P. gingivalis*. Among the two, the aqueous extract exhibited superior inhibitory and bactericidal effects. These findings support the potential use of *E. globulus* as a natural, safe, and effective alternative antimicrobial agent in periodontal therapy.

However, further in-vivo and clinical studies are warranted to validate its efficacy, safety, and applicability in long-term periodontal disease management.

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How to Cite This Article
Gaikwad PD, Moolya NN, Rajhans NS, Landge NM, Lawande AS. Comparative evaluation of antimicrobial efficacy of aqueous and ethanolic extracts of *Eucalyptus globulus* against periodontal pathogens: An in-vitro study. International Journal of Applied Dental Sciences 2025; 11(4): 185-190.