RESEARCH ARTICLE

Formulation and Evaluation of *Aegle marmelos* Infused Anti-ulcerative Toothpaste

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Received: 30th Apr, 2025; Revised: 25th May, 2025; Accepted: 9th Jun, 2025; Available Online: 25th Jun, 2025

ABSTRACT
Aegle marmelos, commonly referred to as Bael, is renowned for its therapeutic potential, largely attributed to its rich content of bioactive flavonoids such as anthocyanins and betacyanins. These phytoconstituents are associated with notable antioxidant and anti-ulcer effects, primarily due to their capacity to neutralize free radicals. This investigation focuses on formulating a herbal toothpaste incorporating *Aegle marmelos* fruit extract, with the aim of aiding in the prevention and treatment of oral ulcers. The research encompassed both the development and comprehensive evaluation of the toothpaste formulation. Key quality parameters assessed included physical appearance, pH, consistency, foaming ability, sharpness of abrasive particles, spreadability, moisture level, and volatile matter content; all essential for product performance and consumer acceptability. The antimicrobial efficacy of the herbal toothpaste was examined through zone of inhibition studies against oral pathogens such as *Bacillus subtilis* and *Staphylococcus aureus*. Furthermore, the antioxidant activity was assessed through the DPPH free radical scavenging method, a well-established and commonly employed technique. Quantitative analysis of flavonoids was also performed, given their crucial role in therapeutic effectiveness. Overall, the results demonstrated that the toothpaste enriched with *Aegle marmelos* extract possesses significant antioxidant and antimicrobial activity, supporting its potential use as a natural remedy for managing and preventing mouth ulcers.
Keywords: *Aegle marmelos*; Anti-microbial activity; Anti-oxidant properties; DPPH; Toothpaste
How to cite this article: Vijaykumar T Pawar, Sanjivani D Mulik, Arya A Musale, Firoj A Tamboli, Dipak P Mali, Dinanath T Gaikwad. Formulation and Evaluation of *Aegle marmelos* Infused Anti-ulcerative Toothpaste. International Journal of Drug Delivery Technology. 2025;15(2):433-38. doi: 10.25258/ijddt.15.2.8
Source of support: Nil.
Conflict of interest: None

INTRODUCTION

In recent times, herbal medicine has gained global momentum as a complementary approach to conventional therapies. These plant-derived formulations are being increasingly recognized for their potential to yield novel bioactive compounds with significant therapeutic promise. Ongoing scientific exploration in this domain emphasizes the importance of identifying phytoconstituents from medicinal plants as foundational elements for new drug discovery. The appeal of herbal remedies stems from their widespread cultural acceptance, relative affordability, and a general perception of safety, thereby positioning them as vital components of modern healthcare systems[1]. Among such botanicals, *Aegle marmelos* (commonly known as Bael) holds a distinguished place. Belonging to the Rutaceae family and the Clauseneae tribe, this flowering plant has long been integral to traditional healing systems such as Ayurveda and Unani. Indigenous to the Indian subcontinent, *Aegle marmelos* is pharmacologically important due to its diverse array of constituents—ranging from alkaloids and coumarins to essential oils. These compounds contribute to its utility in addressing gastrointestinal ailments, inflammatory responses, and microbial infections[2]. Traditional medicinal applications of *Aegle marmelos* span the treatment of chronic diarrhea, dysentery, and peptic ulcers, and it is also recognized for its laxative, astringent, and carminative properties. Additionally, the plant has shown therapeutic potential in managing jaundice and demonstrates broad-spectrum pharmacological activities such as antifungal, antimicrobial, antidiabetic, cardioprotective, anti-ulcer, and antihyperlipidemic effects[3,4].

Phytochemical screening of *Aegle marmelos* has revealed a rich composition, including alkaloids, tannins, flavonoids, saponins, steroids, terpenoids, phlobatannins, and anthraquinones. The presence of carotenoids contributes to the characteristic pale-yellow hue of its fruit. Other minor constituents include ascorbic acid, sitosterol, crude fibers, tannins, α-amylase, and proteins. To date, over 100 phytoconstituents have been isolated from different plant

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parts. The leaves are particularly rich in biologically active molecules such as skimmianine, aeglin, rutin, γ- and β-sitosterol, flavones, lupeol, cineole, citral, glycosides, and various essential oil derivatives including citronellal and eugenol. These molecules are associated with therapeutic benefits across a spectrum of diseases. From the fruit, key components such as psoralen, marmelide, marmelosin, tannins, phenolic acids, luvangetin, and auraptene have been identified.

Nutritional profiling of the Bael fruit indicates that per 100 grams of edible material, it contains 61.5% moisture, 1.7% minerals, 2.9% fiber, 0.3% fat, 1.8% protein, and 31.8% carbohydrates, yielding an energy value of approximately 137 kcal. Moreover, it provides a rich array of essential micronutrients such as calcium, iron, phosphorus, carotene, thiamine, riboflavin, niacin, and vitamin C. Its anti-ulcer potential is especially notable, with anthocyanins and betacyanins believed to be primary contributors to this effect, acting through mechanisms that involve the scavenging of reactive oxygen species[1-5].

The wide-ranging pharmacological profile of *Aegle marmelos* supports its suitability for applications such as anti-ulcerative toothpaste formulation. Its efficacy in oral care stems from its antioxidant, antimicrobial, antifungal, analgesic, and wound-healing activities. Antioxidant potential has been extensively investigated using ethanolic and methanolic extracts of the fruit through various *in vitro* assays, including DPPH, reducing power, nitric oxide, and superoxide radical scavenging methods. Antimicrobial actions have been demonstrated through agar well diffusion methods, showing effectiveness against pathogens including *Escherichia coli*, *Bacillus subtilis*, and *Staphylococcus aureus*. Antifungal efficacy has been demonstrated against dermatophytic fungi, including *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Microsporum canis*, and *Epidermophyton floccosum*.

Furthermore, its wound-healing properties have been validated *in vivo* using excision models in Wistar rats, where both ointment-based and injectable formulations of *Aegle marmelos* extract enhanced tissue regeneration. The medicinal properties of this plant are primarily linked to the presence of diverse secondary metabolites, including flavonoids, tannins, and alkaloids. Notably, the presence of anthocyanins and betacyanins is thought to play a central role in ulcer protection, likely mediated through antioxidant pathways[6–7]. Given this pharmacological profile, incorporating *Aegle marmelos* into an oral care product, such as a herbal toothpaste, holds promise for the prevention and management of mouth ulcers—an increasingly common oral health concern.

MATERIAL AND METHODS

Plant Material

Aegle marmelos fruits were collected from Kolhapur district, the plant samples were dried under shade and ground to coarse powder.

Chemicals

The formulation was prepared using a combination of sorbitol, titanium dioxide, calcium carbonate, sodium saccharin, sodium lauryl sulfate, methylparaben, propylparaben, sodium carboxymethyl cellulose, potassium phosphate, and clove oil.

Table 1: Formulation of toothpaste

Drug and excipient	Quantity	Property
Aegle marmelos extract	0.6 g	Therapeutic agent
Potassium phosphate	1 g	pH stabilizer
Calcium carbonate	25 g	Mild abrasive
Sodium lauryl sulfate	1.5 g	Foaming agent
Sorbitol	20 g	Moisture retention agent
Sodium carboxymethyl cellulose	20 g	Thickening/binding agent
Sodium saccharin	0.3 g	Sweetening agent
Methylparaben	0.1 g	Antimicrobial preservative
Propylparaben	0.02 g	Antifungal preservative
Titanium dioxide	0.5 g	Whitening/opacity agent
Clove oil	0.5 g	Flavoring and antiseptic
Distilled water	20 ml	Base/solvent

Extraction

A quantity of 25 g of *Aegle marmelos* fruit powder was subjected to successive extraction using 250 mL of 70% ethanol in a Soxhlet apparatus, maintained 8 hours at 30 °C. The resulting extract was then passed through Whatman No. 1 filter paper to remove any particulate matter. The extract was then concentrated with a rotary evaporator maintained at 40 °C until the solvent was entirely removed.

Formulation of toothpaste

A total of 0.6 g of *Aegle marmelos* fruit extract was placed in Beaker No. 1, followed by the addition of 1 g of potassium phosphate. To this, 20 g of sorbitol, 0.3 g of sodium saccharin, and 20 mL of H_20 were added. Subsequently, the mixture was stirred continuously and heated at 40 °C for 15 minutes to facilitate complete dissolution.

In a separate container (Beaker No. 2), 25 g of calcium carbonate and 20 g of sodium carboxymethyl cellulose were accurately weighed. Preservatives including 0.1 g of methylparaben and 0.02 g of propylparaben were also added to this mixture[7].

The contents of Beaker No. 2 were transferred to a mortar and blended thoroughly using a pestle. The warm solution from Beaker No. 1 was gradually incorporated into this dry mixture with continuous trituration until a smooth and uniform paste was formed. Subsequently, 1.5 g of sodium lauryl sulfate, 0.5 g of titanium dioxide, and an appropriate quantity of clove oil were added. The final mixture was homogenized to obtain the desired consistency and stored in a suitable container for further use[8].

The detailed composition of the formulated toothpaste is depicted in Table 1.

Evaluation of anti-ulcerative toothpaste

Physical Evaluation: The formulated herbal toothpaste was evaluated for key physical attributes including color, odor, taste, texture, relative density, and viscosity parameters to

ensure consistency and consumer acceptability[8].

pH determination: To assess the pH, 10 g of toothpaste was transferred into a 150 ml beaker and combined with 10 ml of previously boiled and cooled distilled water. The mixture was stirred vigorously to obtain a uniform suspension. The pH was taken by a calibrated pH meter[2].

Homogeneity: Homogeneity was examined by extruding the paste from a collapsible tube or similar container at 27±2 °C. A consistent and continuous flow, along with uniform bulk extrusion upon gentle pressure from the crimp end, indicated good formulation uniformity[8].

Foamability: Foamability was assessed by mixing a small quantity of toothpaste with water in a 50 ml measuring cylinder. The initial volume was recorded, followed by vigorous shaking for 10 cycles. The final foam volume was measured to determine foam expansion[8].

Sharp-edge abrasive particles: To test for the presence of sharp or abrasive particles, a portion of the formulation was placed on the fingertip and gently rubbed across a butter paper strip of 15–20 cm. Any scratch marks indicated the presence of undesired coarse particles.

Spreadability: The spreadability was evaluated using a slip and drag method. About 2 g of the paste was sandwiched between two glass slides, compressed for 5 minutes, and then the upper slide was pulled using an 80 g weight. Time (T) required to cover 6.2 cm was recorded. Spreadability (S) was calculated using:

$$S = M \times \frac{L}{T} \quad \ldots. (1)$$

Where, S = Spreadability (g·cm/sec), M = Weight applied (g), L = Distance moved by upper slide (cm), T = Time taken (sec). A separate test involved placing 1 g of the paste between two 10 × 10 cm glass slides with a 250 g weight placed on top. After 3 minutes, the spread diameter was measured and averaged over three trials[8].

Moisture content and volatile matter: About 5 g of the formulation was weighed into a porcelain dish and dried at 105 °C in a hot air oven. Loss in mass was used to compute moisture and volatile matter content:

$$\text{Percent (\%) by mass} = \frac{M1}{M2} \times 100 \quad \ldots\ldots (2)$$

Where, M1 is loss of mass (in grams) on drying and M is mass (g) of the material taken for the test[9].

Anti-microbial Activity

The growth of bacterial colonies can be significantly suppressed when exposed to antimicrobial agents. Nevertheless, the increasing resistance of bacteria to conventional antibiotics presents a challenge. To assess the effectiveness of specific antimicrobial agents, including newly formulated products, the Kirby-Bauer disc diffusion assay is widely utilized for antimicrobial susceptibility testing[9–10].

Table 2: Chemical test of extract

Active constituents	Test Name	Observations
Carbohydrate	Molisch's test	+
Reducing sugar	Fehling test	+
Alkaloids	Mayers test	+
Tannins	Ferricchloride test	+
Proteins	Folin Ciocalteau test	++
Flavonoids	Shinoda's test	++
Aminoacids	Ninhydrin test	+
Saponins	Foam test	+
Phenols	Ferricchloride test	++
Glycosides	Sodium nitroprusside test	--
Sodium		

Table 3: Antimicrobial activity

Microorganism	Amount of culture	Zone of inhibition
Staphylococcus aureus	100µl	5 mm
	200µl	8 mm
Bacillus subtilis	100µl	4 mm
	200µl	7 mm

Preparation of Standard: A standard antibiotic solution was prepared by dissolving a 250 mg amoxicillin tablet in 3 ml of distilled water, followed by sonication for 15 minutes to ensure uniform dispersion.

Preparation of culture: Nutrient agar medium was prepared using beef extract, peptone, sodium chloride, and agar, and the mixture was sterilized by autoclaving at 121 °C for 20 minutes along with all required glassware and petri dishes. After autoclaving, the working area was sterilized using ethanol and flame to maintain aseptic conditions. Approximately 20 ml of sterile molten agar was poured into each petri plate and allowed to solidify for 5–10 minutes. Once solidified, microbial strains were spread across the surface using a sterile rod. Separate petri dishes were inoculated with *Bacillus subtilis* and *Staphylococcus aureus*, respectively. Using a sterile borer or vial, four uniform wells were created in each plate. One well was filled with the standard amoxicillin solution, one served as control, and the remaining two were filled with different concentrations of the herbal toothpaste formulation. The plates were then incubated at 37 °C for 24 hours. After

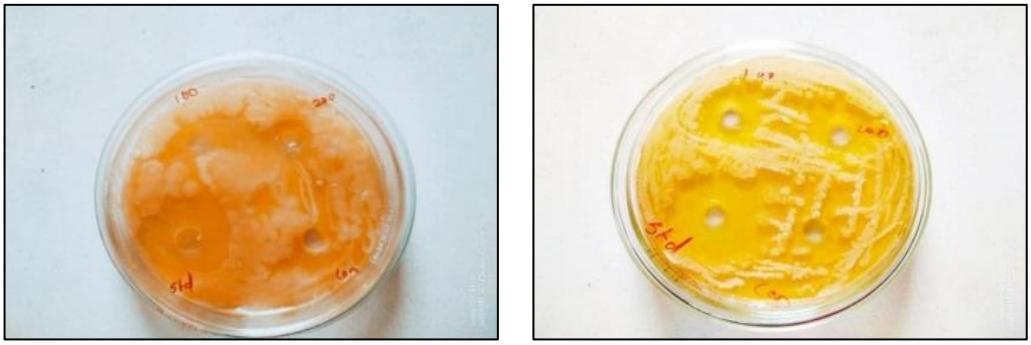

Figure 1: Zone of inhibition of *Bacillus subtilis* and *Staphylococcus aureus*

incubation, zones of inhibition were measured and documented to determine the antibacterial efficacy of the formulation[11].

Antioxidant property

The antioxidant activity of the herbal extract was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. DPPH is a stable free radical that exhibits a deep violet color due to its absorption band in the 515–528 nm range. Upon accepting an electron or hydrogen atom from an antioxidant compound, this radical is reduced, resulting in a color change from violet to yellow. This transition is spectrophotometrically monitored, and the extent of discoloration reflects the radical scavenging potential of the sample[6].

One gram of extract was accurately weighed and dissolved in 25 ml of ethanol. This solution was sonicated for two hours to facilitate extraction and then filtered. Five test tubes were prepared, each containing 1, 2, 3, 4, and 5 ml of the prepared extract solution, respectively. The volume in each tube was adjusted to 10 ml.

From each tube, 1 ml of solution was transferred to a new test tube, followed by the addition of 3 ml of DPPH reagent solution. The final volume was adjusted to 6 ml using ethanol. Spectra recorded at 517 nm with UV-visible spectrophotometer[6].

Flavonoid content

Flavonoids are secondary plant metabolites known for their pharmacological properties, including antimicrobial and antioxidant activities. In the case of *Aegle marmelos*, anthocyanins and leucocyanins are the key flavonoid constituents. The ethanolic extract of *Aegle marmelos* fruit has demonstrated high flavonoid content, which significantly contributes to its antimicrobial efficacy against *Escherichia coli*, *Bacillus subtilis*, and *Staphylococcus aureus*[12].

Preparation of sample: To quantify flavonoids, 1 ml of extract (0.1 mg/ml) was mixed with 0.5 ml of sodium nitrite solution, followed by the addition of 5 ml of 10% aluminum chloride and 2 ml of 1 M sodium hydroxide. The volume was made up to 10 ml with methanol.

Preparation of blank: A blank solution was prepared using the same reagents and volumes as above, except that the extract was omitted.

Table 4: Anti-oxidant activity

Concentrations (microgram)	Control	Sample	%RSA	IC50
0	0.693	0	-	-
10	0.693	0.582	16.01732	0.50652
20	0.693	0.51	26.40693	1.326663
30	0.693	0.399	42.42424	2.146806
40	0.693	0.319	53.96825	2.966948
50	0.693	0.255	63.20346	3.787091

Table 5: Flavonoid content analysis

Concentration (microgram)	Absorbance
10	0.28
20	0.43
30	0.56
40	0.7
50	0.8

The absorbance of both sample and blank was measured at 510 nm using a UV spectrophotometer. The total flavonoid content (C) was determined using the following formula:

$$C = \frac{c \times V}{M} \quad \ldots\ldots (3)$$

Where: C = Total flavonoid content (mg/g quercetin equivalent), c = Concentration of quercetin from the standard calibration curve (mg/ml), V = Volume of extract (ml), M = Weight of extract used (g).

RESULTS AND DISCUSSION

The anti-ulcerative toothpaste formulated with *Aegle marmelos* extract was subjected to a series of evaluations to determine its physicochemical features, phytochemical profile, and biological activity. The comprehensive results offer strong evidence for its potential therapeutic application in oral care.

Physical Evaluation

The formulation was assessed for its organoleptic and physicochemical parameters. It exhibited acceptable values in terms of color, odor, taste, texture, and relative density. The light yellow appearance is attributed to the natural carotenoid pigments present in *Aegle marmelos*, known for imparting such pigmentation. The pleasant aroma and subtle sweetness are likely derived from the inherent volatile constituents of the herbal extract, contributing to

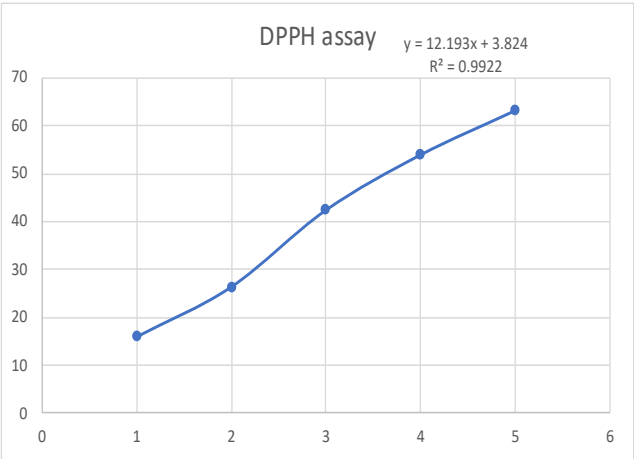

Figure 2: Graph of DPPH assay

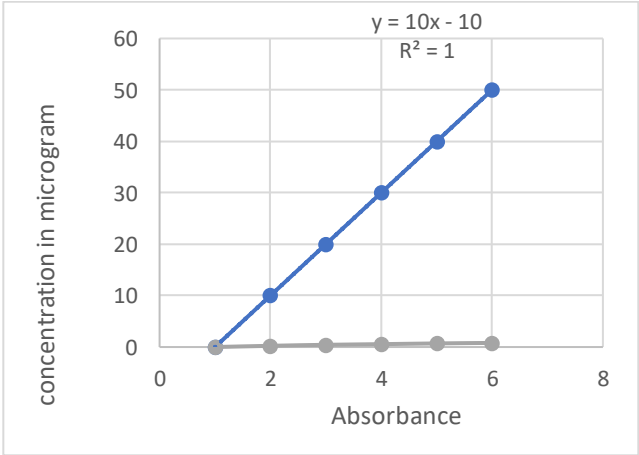

Figure 3: Graph of flavonoid content

better consumer acceptance. The smooth consistency and a relative density of 10.2 confirm a homogenous blend, indicative of efficient formulation and uniform distribution of active ingredients[8].

Evaluation test of toothpaste:

The toothpaste showed excellent pH stability, maintaining a neutral value of 7, which is ideal for maintaining oral mucosal integrity and preventing irritation. Tests for homogeneity, foamability, and spreadability yielded favorable outcomes, showing that the product is easy to apply, generates sufficient foam, and spreads uniformly[8]. The absence of sharp or gritty particles indicates a non-abrasive formulation, crucial for protecting enamel while ensuring proper plaque removal. Moisture content was found to be 10%, while the viscosity measured 11191 cP, signifying suitable consistency and long-term stability. The flavonoid content, determined to be 130 (mg quercetin equivalents/g), confirms the presence of antioxidant bioactives, which play a key role in oral tissue protection and ulcer prevention[8].

Phytochemical Analysis

Preliminary phytochemical screening revealed that the formulation is rich in active plant constituents such as tannins, flavonoids, saponins, phenols, coumarins, sterols, and triterpenoids. These phytoconstituents are recognized for their pharmacological actions, including antioxidative, antimicrobial, and anti-inflammatory properties, which contribute to the prevention and treatment of oral ulceration. The confirmed presence of such compounds aligns with the therapeutic objective of the herbal toothpaste in enhancing oral health[9].

Chemical test of extract

Further chemical analysis of *Aegle marmelos* extract used in the toothpaste showed the presence of a variety of phytochemicals, including carbohydrates, reducing sugars, alkaloids, tannins, proteins, flavonoids, amino acids, saponins, and phenols. Glycosides were notably absent. Strong positive reactions were observed particularly for proteins, flavonoids, and phenols, suggesting a significant antioxidant and healing potential. The lack of glycosides may indicate a reduced risk of mucosal irritation, enhancing the safety profile of the formulation. These results are detailed in Table 2.

Antimicrobial Activity

The antimicrobial assessment of the *Aegle marmelos*-enriched toothpaste revealed its notable inhibitory action against common oral pathogens such as *Staphylococcus aureus* and *Bacillus subtilis*. Larger zones of inhibition were recorded with increased concentrations of the formulation, indicating a dose-dependent antimicrobial effect[13]. These findings highlight the potential of the herbal formulation to curb microbial growth and thus contribute significantly to oral health by minimizing the risk of infections. The inhibition zones recorded—5 and 8 mm for *Staphylococcus aureus*, also 4 mm and 7 mm for *Bacillus subtilis*—validate its efficacy across varying dosages, suggesting its usability for both mild and severe microbial load scenarios. The detailed outcomes of the antimicrobial study are presented in Table 3 and illustrated in Figure 1.

Antioxidant property

The results of the DPPH radical scavenging assay confirmed that the formulated toothpaste exhibits potent antioxidant potential, as evidenced by the increase in percentage radical scavenging activity (%RSA) with rising sample concentrations. The IC_{50} value, calculated at 3.787091, indicates the concentration required to inhibit half of DPPH radicals, demonstrating the formulation's strong free radical neutralization capacity[6]. The presence of *Aegle marmelos* extract contributes significantly to this activity, owing to its abundance of phenolic and flavonoid compounds. Such antioxidant action plays a crucial role in mitigating oxidative stress within oral tissues, a known factor in the development of ulcers and other inflammatory oral conditions. The findings of the antioxidant evaluation are depicted in Table 4 and represented graphically in Figure 2.

Flavonoid Content

The assessment of flavonoid content revealed a concentration-dependent increase in absorbance, confirming the substantial presence of flavonoid compounds in the formulated toothpaste. These bioactive constituents are well-documented for their antioxidant and anti-inflammatory effects, both of which are beneficial in preventing and managing oral ulcerations[12]. The quantified flavonoid content was determined to be 130, corresponding to a concentration of 1.87 mg/ml. This high flavonoid level reinforces the therapeutic potential of the formulation. The outcomes of the flavonoid analysis are depicted in Table 5 and presented in Figure 3.

CONCLUSION

The formulated anti-ulcerative toothpaste containing *Aegle marmelos* extract demonstrated promising performance across multiple evaluation criteria. Its favorable physicochemical attributes, presence of diverse phytochemicals, robust antimicrobial and antioxidant activities, and substantial flavonoid content collectively highlight its potential as a natural remedy for managing and preventing oral ulcers. These synergistic properties not only contribute to maintaining oral hygiene but also provide a plant-based alternative to conventional treatments, combining therapeutic efficacy with safety. Further clinical investigations are warranted to validate these preliminary findings and to explore its applicability in routine oral healthcare.

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