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Mahesh Katariya
Topical Scientist Assistant Manager, Integrated Product Development, Bachupally, Qutubullapur, Hyderabad, Telangana, India

Jyoti Singh
Dabur Research & Development Centre, Maharajpur, Site 4, Sahibabad, Ghaziabad, Uttar Pradesh, India

Amit Sirdesai
Dabur Research & Development Centre, Maharajpur, Site 4, Sahibabad, Ghaziabad, Uttar Pradesh, India

Prasun Bandyopadhyay
Dabur Research & Development Centre, Maharajpur, Site 4, Sahibabad, Ghaziabad, Uttar Pradesh, India

Correspondence
Mahesh Katariya
Topical Scientist Assistant Manager, Integrated Product Development, Bachupally, Qutubullapur, Hyderabad, Telangana, India

In vitro assessment of babool extract, mint and clove oil containing toothpaste on gingivitis causing bacteria

Mahesh Katariya, Jyoti Singh, Amit Sirdesai and Prasun Bandyopadhyay

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Abstract
Aim: The aim of this study was to perform *in vitro* assessment of Babool extract, Mint and Clove containing toothpaste against oral disease-causing pathogens *Porphyromonas gingivalis* (*P. gingivalis*).
Objective: The present study was designed to evaluate in efficacy of the Dabur Babool toothpaste containing certain medicinal plant extracts like Babool extract and clove oil for the protection of oral hygiene and prevention of gum diseases by inhibition of disease-causing micro-organism *P. gingivalis*.
Methods: The herbal ingredient like Babool, Mint and Clove is combined in Toothpaste formulation. The product was tested against microorganism's *P. gingivalis* using agar well diffusion method. Inhibition zones formed around toothpastes after 24 hours of incubation were measured and the data collected were statistically analysed. The time-dependent killing assay was carried out on *P. gingivalis*.
Conclusions: *In vitro* assessment of herbal toothpaste with Babool, Mint and Clove toothpaste against disease causing oral pathogens revealed the efficacy of toothpaste against major gingivitis causing oral pathogen *P. gingivalis.*

Keywords: Herbal, babool, *P. gigivalis*, oral hygiene, toothpaste, dental diseases, clove oil and gigivitis

Introduction
Oral pathogenic microorganisms have been the major cause for dental plaques, dental caries as well as periodontal and gingival disease [1, 2]. While periodontal disease is considered a polymicrobial infection, *Porphyromonas gingivalis* (*P. gigivalis*) is suspected to be one of the most important causative agents of the chronic form of periodontitis [3, 4]. This bacterial species induces the transition from a symbiotic microbial community to a dysbiotic microbiota [5].
Pathogenesis of periodontitis is contributed through the expression of wide variety of virulent factors, including but not limited to cysteine proteinases, also known as gingipains that perturbs host defence mechanisms, modulates inflammatory response and degrades tissue proteins [6, 7]. *P. gingivalis* is one of the leading opportunistic pathogen responsible for gingivitis.
Dabur Babool toothpaste is believed to have a number of benefits for oral health. The antibacterial properties of Babool can help to fight against harmful bacteria in the mouth, while the anti-inflammatory properties can help to soothe inflamed gums [8]. Clove, on the other hand, is believed to have a numbing effect on the teeth and gums, making it useful for reducing tooth pain and sensitivity. In addition to these benefits, Babool and clove toothpaste may also help to freshen breath and promote overall oral hygiene (ref).
Babool (Acacia arabica) has been traditionally used in oral care for centuries. The bark, leaves, and pods of the Babool tree contain various compounds that have antibacterial, antifungal, and anti-inflammatory properties [9].
Babool has been found to be effective in reducing plaque formation, which is a major cause of tooth decay and gum disease. Its antibacterial properties help to kill harmful bacteria in the mouth, reducing the risk of oral infections. Babool is also believed to have astringent properties that can help to tighten and tone the gums, reducing the risk of gum disease [10].
Babool extract has been used in toothpaste formulations, mouthwashes, and other oral care products. Some studies have found that toothpaste containing Babool extract is more effective in reducing plaque formation and gingivitis compared to toothpaste without Babool extract [11, 12].
In addition to its oral care benefits, Babool has also been used in traditional medicine for its analgesic and anti-inflammatory properties.

It is believed to be effective in reducing pain and inflammation in the mouth, which can be useful for treating conditions like mouth ulcers, sore throat and gum infections [11, 12].

Overall, Babool has shown promising results in improving oral health and is a safe and natural alternative to synthetic ingredients commonly used in oral care products.

Babool (Acacia arabica) contains a variety of chemical compounds that contribute to its medicinal properties. The chemical composition of Babool varies depending on the part of the plant and the extraction method used, but some of the main compounds found in Babool include:

- **Tannins:** Babool is a rich source of tannins, which are water-soluble polyphenolic compounds. Tannins have astringent properties that can help to tighten and tone the gums, reducing the risk of gum disease.
- **Flavonoids:** Babool contains various flavonoids, which are plant pigments that have antioxidant and anti-inflammatory properties. Flavonoids are believed to be responsible for the antibacterial and antifungal properties of Babool.
- **Alkaloids:** Babool contains alkaloids, which are nitrogen-containing compounds that have pharmacological effects. Alkaloids in Babool include catechin, epicatechin, and procyanidins, which have been found to have antioxidant and anti-inflammatory properties.
- **Saponins:** Babool contains saponins, which are glycosides that have detergent-like properties. Saponins in Babool have been found to have antimicrobial properties and can help to reduce the risk of oral infections.
- **Gum:** Babool contains a type of gum called gum arabic, which is a complex polysaccharide that has emulsifying and thickening properties. Gum arabic is commonly used as a binder in toothpaste and other oral care products.

In nut shell, the chemical composition of Babool contributes to its various medicinal properties, including antibacterial, antifungal, anti-inflammatory and astringent properties [13].

Clove (Syzygium aromaticum) has been used for centuries in oral care for its antimicrobial, antifungal, and analgesic properties. Clove oil, which is derived from the flower buds of the clove tree, contains a compound called eugenol, which is responsible for its medicinal properties.

Clove oil has been found to be effective in reducing toothache and other forms of oral pain. Its analgesic properties help to numb the affected area, reducing the sensation of pain. Clove oil also has antimicrobial properties, which make it effective in fighting oral infections. It can help to kill harmful bacteria in the mouth, reducing the risk of tooth decay, gum disease and bad breath [14].

- Clove (Syzygium aromaticum) contains various chemical compounds that contribute to its medicinal properties. The chemical composition of clove varies depending on the part of the plant and the extraction method used, but some of the main compounds found in clove include:
- **Eugenol:** Clove oil is rich in eugenol, which is responsible for most of its medicinal properties. Eugenol has antimicrobial, antifungal, anti-inflammatory, and analgesic properties.
- **Acetyleugenol:** Acetyleugenol is a derivative of eugenol found in clove oil. It has similar properties to eugenol and contributes to the therapeutic effects of clove oil.
- **Caryophyllene:** Clove contains caryophyllene, a terpene that has anti-inflammatory and antioxidant properties.
- **Tannins:** Clove contains tannins, which are water-soluble polyphenolic compounds that have astringent properties. Tannins can help to tighten and tone the gums, reducing the risk of gum disease.
- **Flavonoids:** Clove contains various flavonoids, which are plant pigments that have antioxidant and anti-inflammatory properties. Flavonoids are believed to be responsible for some of the therapeutic effects of clove.
- **Beta-caryophyllene oxide:** Clove oil also contains beta-caryophyllene oxide, which has anti-inflammatory properties and may help to reduce pain.

The chemical composition of clove contributes to its various medicinal properties, including antimicrobial, antifungal, anti-inflammatory, and analgesic properties. Clove oil and other preparations of clove have been used for centuries in traditional medicine for their therapeutic effects [15].

Herbal toothpastes containing Babool and Clove have been commercially sold with various claims based on the therapeutic properties of these ingredients. Some of the common claims made by these products include:

- **Promoting healthy gums:** Both Babool and Clove are believed to have astringent properties that can help to tighten and tone the gums, reducing the risk of gum disease.
- **Reducing plaque and tartar:** Babool and Clove are believed to have antimicrobial properties that can help to kill harmful bacteria in the mouth, reducing the risk of plaque formation and tartar build-up.
- **Freshening breath:** Clove has a strong, pleasant aroma that can help to freshen the breath and reduce bad breath.
- **Relieving toothache and oral pain:** Clove oil has analgesic properties that can help to reduce toothache and other forms of oral pain.
- **Fighting oral infections:** Both Babool and Clove have antimicrobial properties that can help to fight oral infections, reducing the risk of tooth decay, gum disease, and other oral infections.

The use of natural herbs has gained more traction in oral care to attenuate the oral disease-causing pathogens. Plant-derived natural products have been widely explored as the therapeutic roles in regulating interactions between microorganisms. One of the appealing therapeutic feature is bioactive.

Mint is a popular flavour in oral care products such as toothpaste, mouthwash, and dental floss due to its natural refreshing and cooling effect. Mint has antibacterial properties that can reduce bacteria growth in the mouth and help to fight cavities, and it can also soothe sore gums. Additionally, some toothpaste with mint can whiten teeth. Therefore, mint is beneficial for maintaining good oral hygiene and promoting overall dental health.

With this background in mind, the present study aimed to

evaluate antimicrobial properties of herbal ingredient-based toothpaste containing Babool extract, clove oil, mint and without any fluoride or whitening agent on Gingivitis causing oral bacteria *P. gingivalis*.

Material and Methods

Toothpaste Composition

Table 1: Show Ingredient

Ingredient
Clove, Babool and Mint in Calcium Carbonate Base.

Materials

Test sample details

Table 2: Show RR number, Name of the test sample, Active INCI, Batch No and Storage

RR number	Name of the test sample	Active INCI	Batch No.	Storage conditions
RR230074	Dabur Babool Toothpaste	Babool, Clove and Mint in calcium carbonate base Q.S.	BM0479	RT
RR230076	Dabur Clove Toothpaste	Clove, Mint and Babool in calcium carbonate base Q.S.	CI0046	RT

Microbial strain

Table 3: Show Tester Strain, ATCC No and Source

S. No.	Tester Strain	ATCC No	Source
1.	*Porphyromonas gingivalis*	ATCC 33277	American Type culture collection, USA

Chemicals and Media

Table 4: Show Chemical, Lot No and Manufacturer

Chemical	Lot No.	Manufacturer
Sodium Chloride	MB023-1KG	HiMedia, India
Demineralized water	NA	Spectrum reagents and chemicals, India
Tween-80	GRM159-500G	HiMedia, India
Soya Lecithin	GRM637-100G	HiMedia, India
Tryptic soya broth	211825	Difco
Tryptic soya agar	M1968-500g	HiMedia, India
Hemin	RM237-250MG	HiMedia, India
Vitamin K	FD115-5VL	HiMedia, India
L-Cysteine hydrochloride	CH038-100G	HiMedia, India
Yeast extract	RM027-500G	HiMedia, India
Diphosphate hydrogen phosphate	TC596-100G	HiMedia, India
Anaerogas Pack	LE002F-5NO	HiMedia, India
Anaero Indicator Tablet	LE065	HiMedia, India

Material and Reagents

a) **Dilution fluid or Diluent:** 0.9% saline.
b) **Neutralizer:** Lecithin soya and Tween-80.
c) **Growth media:** Supplemented tryptic soya agar.
d) Sterile deionized water or Equivalent.
e) Anaero gas Pack.
f) Anaero Indicator Tablet.
g) Petri plates and conical bottom centrifuge tubes.

Method

Preparation of 50% w/v test sample

50 gm of test sample is dissolved in 100 mL of distilled water and vortexed thoroughly, used for further procedure.

Preparation and Standardization of Stock cultures

A loopful culture of *P. Gingivalis* was grown on Supplemented tryptic soya agar and incubated at 37±2 °C for 5days.The growth was scrapped and transferred to sterile and the turbidity was adjusted to 10^7CFU/ml.

Test procedure

A. 1 ml of 50% test sample and 1ml of *P. gingivalis* and to this add 8ml of neutralizer and mix well and allow it for 2 minutes of contact time.
B. Repeat above steps in a duplicate test concentration and each tested sample is plated in a duplicate.
C. Take 1ml of above treated sample and serially dilution with dilution fluid (saline) before and after contact time.
D. A positive control is run to verify that to determine the number of surviving microorganism in inoculum.
E. The sampling solution was enumerated using pour plating technique. Supplemented tryptic soya agar was used as growth medium for *P. gingivalis*, incubated for 5 days at 37±2 °C.
F. Plate counting procedures were used to count the colonies of test cultures under digital colony counter.

Determination of reduction

To determine the surviving organisms, count colonies and record raw data as CFU/plate. Average duplicate plate counts and multiply by the dilution factor to arrive a cfu/mL of test suspension. Average plate count was multiplied by dilution factor to arrive at cfu/ml of test suspension the microbial count were then converted to log 10 scale.

Log_{10} Reduction (LR) = Mean Log_{10} (Microbial population)-Mean Log_{10} (surviving test population).

Results

Table 5: Percentage reduction of test organisms tested by Dabur Babool and Dabur Clove Toothpaste against *P. gingivalis* at 2 min contact time

Sample Name	Test Organisms	Contact Time (min)	Initial Inoculum (cfu/ml)	Number of cells per mL at the end of contact time	Log Reduction	% Reduction
Dabur Babool Toothpaste	*P. gingivalis* (ATCC 33277)	2 min	4 x 107	3 x 104	3.125	99.9250
Dabur Clove Toothpaste	*P. gingivalis* (ATCC 33277)	2 min	4 x 107	1 X 10^4	3.602	99.9750

Discussion

Maintenance of good oral hygiene is the vital to the avoidance of dental diseases. The biofilms produced by the oral microflora plays pivotal role in producing caries and periodontal disease, it is of extreme importance to control these biofilms by mechanical removal and use of supportive antimicrobials in dentifrices in prevention of plaque-mediated diseases [16]. Several clinical studies have established the inhibitory effect of dentifrice on gingival and oral bacteria [17]. Heightened concerns regarding the upsurge in antibiotic resistance in microbes against chemical-based dentifrices [18-20] has stimulated interest in the therapeutic use of alternative or non-conventional dentifrices and thus this study.

P. gingivalis, or *Porphyromonas gingivalis*, is a type of bacteria that is commonly associated with periodontal disease, a chronic inflammatory condition that affects the tissues surrounding the teeth. *P. gingivalis* is a gram-negative anaerobic bacterium that is found in the oral microbiome, and it is one of the primary pathogens involved in the development and progression of periodontal disease [3].

P. gingivalis can produce several virulence factors that contribute to its pathogenicity, including gingipains, fimbriae, and lipopolysaccharides. Gingipains are proteolytic enzymes that can degrade host proteins and evade the host immune system, while fimbriae and lipopolysaccharides can trigger inflammatory responses and tissue destruction.

P. gingivalis can also interact with other oral bacteria to form complex biofilms, which can make it more resistant to antimicrobial agents and more difficult to treat. In addition to its role in periodontal disease, *P. gingivalis* has also been implicated in other systemic diseases, such as cardiovascular disease, rheumatoid arthritis, and Alzheimer's disease [4].

Treatment of *P. gingivalis* infections typically involves mechanical removal of the bacterial biofilm through scaling and root planing, as well as antimicrobial therapy, such as topical or systemic antibiotics. However, the emergence of antibiotic-resistant strains of *P. gingivalis* has led to a renewed interest in the development of alternative treatments, such as natural products like clove or other botanical extracts.

There is limited scientific evidence available on the specific effect of Babool (*Acacia arabica*) toothpaste on the oral bacteria *Porphyromonas gingivalis* (*P. gingivalis*). However, some studies have investigated the antimicrobial properties of Babool extracts against a range of oral bacteria.

One study published in the Journal of Phyto-therapy research in 2003 investigated the antibacterial activity of Babool extracts against a range of oral bacteria, including *P. gingivalis*. The study found that the Babool extracts exhibited significant antibacterial activity against all of the tested oral bacteria, including *P. gingivalis* [25].

However, it is important to note that these studies were conducted using Babool extracts, and not specifically Babool toothpaste. The specific antimicrobial properties of Babool toothpaste against *P. gingivalis* may require further investigation.

Overall, while the scientific evidence on the effect of Babool toothpaste on *P. gingivalis* is limited, the antimicrobial properties of Babool extracts suggest that it may have potential in promoting oral health and reducing the risk of gum disease caused by *P. gingivalis*.

Clove (*Syzygium aromaticum*) has been reported to have antimicrobial properties, including against the oral bacteria *Porphyromonas gingivalis* (*P. gingivalis*). *P. gingivalis* is a key bacterial species involved in the development of periodontal disease, which is a common oral health problem.

Several studies have investigated the effect of clove extracts or essential oil on *P. gingivalis*. One study published in the Microbial Pathogenesis in 2017 investigated the antimicrobial activity of clove essential oil against *P. gingivalis*. The study found that clove essential oil exhibited strong antimicrobial activity against *P. gingivalis* [26]. These studies suggest that clove may have potential in promoting oral health and reducing the risk of gum disease caused by *P. gingivalis*. However, more research is needed to fully understand the mechanisms by which clove exerts its antimicrobial effects and to determine the optimal concentrations and formulations of clove-containing dentifrices for use in oral hygiene.

A number of dentifrices preparations containing herbal ingredients have made substantial contribution to dental prophylaxis in boosting oral health. The popularity of herbs is due the anti-inflammatory and antimicrobial effects of Phytochemicals [22]. However, there have been no reports on the effects of such toothpastes on periodontitis causing oral bacteria *P. gingivalis* and cavity causing *P. gingivalis*. Hence, study was conducted to investigate the effects of a toothpaste containing herbal ingredients like Babool extract, mint and Clove oil on Gingivitis causing oral bacteria *P. gingivalis*.

The novelty of the herbal toothpaste in the current study owes to its natural compounds. In addition to being a natural alternative to traditional toothpaste, herbal toothpaste is also considered to be more environmentally friendly since it is typically made from natural and renewable resources that too without complication of chemical based commercial toothpastes. The results revealed that our developed toothpaste had different degrees of effectiveness against the tested microorganism. In this regard, the formulated toothpaste exerted a highly significant effect against *P. gingivalis*.

In the present study, Dabur Babool toothpaste and Dabur Clove formulations were found to have antimicrobial activities against Gingivitis causing oral bacteria *P. gingivalis*. This may be attributed to the synergistic interactions between the ingredients present in the formulations, which, however, need to be established [21]. The principle components of this toothpaste include Babool, Mint and Clove. The presence of secondary metabolites such as flavonoids, alkaloids and polyphenols in these

constituents are considered to be the sole reason of their antimicrobial efficacy [18]. Some of these ingredients were previously demonstrated and known to have antimicrobial activity. Against *P. gingivalis* herbal formulations showed significant antimicrobial activity ($p<0.05$). Many studies on anti-gingivitis activity of herbal base toothpaste have been reported [23, 24].

Conclusions

In vitro assessment of herbal toothpastes against disease causing periodontal pathogen revealed its effectiveness against major disease-causing pathogen such as *P. gingivalis*. Hence, the toothpastes have potential to be utilized in the treatment of variety of dental diseases. The alternative use of an herbal toothpaste instead of a non-herbal toothpaste formulation should be considered for promoting oral health care. Practitioner may endorse a dentifrice based on patient's clinical conditions and possible benefits.

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