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Jyoti Singh
Dabur Research and
Development Centre,
Ghaziabad, Uttar Pradesh,
India

Mahesh Katariya
Dabur Research and
Development Centre,
Ghaziabad, Uttar Pradesh,
India

Amit Sirdesai
Dabur Research and
Development Centre,
Ghaziabad, Uttar Pradesh,
India

Prasun Bandyopadhyay
Dabur Research and
Development Centre,
Ghaziabad, Uttar Pradesh,
India

Corresponding Author:
Jyoti Singh
Dabur Research and
Development Centre,
Ghaziabad, Uttar Pradesh,
India

In vitro efficacy of an ayurvedic dentifrice against gingivitis causing pathogen

Jyoti Singh, Mahesh Katariya, Amit Sirdesai and Prasun Bandyopadhyay

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Abstract

Objective: The objective of this study was to study *in vitro* efficacy of Ayurvedic herbs containing toothpaste against oral disease causing pathogens *Porphyromonas gingivalis* (*P. gingivalis*). The present study was designed to evaluate in efficacy of the Dabur Red paste containing Ayurvedic herbs like, Kalimirch, Pippali, Tomar, Sunthi, Lavang, Karpura & Pudina for prevention of gum diseases by inhibition of disease causing micro-organism *P. gingivalis*.

Material and Methods: The product was tested against microorganism's *P. gingivalis* using agar well diffusion method. The agar-well diffusion method was used to test the antimicrobial effect. 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 ayurvedic paste containing actives like, Kalimirch, Pippali, Tomar, Sunthi, Lavang, Karpura, Pudina and Gairica against gum disease causing oral pathogens revealed the 99.95 % germ kill efficacy of toothpaste against major gingivitis causing oral pathogen *P. gingivalis* in 2 min contact time.

Keywords: *P. gingivalis*, ayurvedic paste, oral hygiene, toothpaste, dental diseases, clove oil and gingivitis

Introduction

Porphyromonas gingivalis (*P. gingivalis*) is a type of bacteria commonly found in the human oral cavity. It is considered a keystone pathogen in the development of periodontitis, a chronic inflammatory disease that affects the tissues surrounding and supporting the teeth.

P. gingivalis is a gram-negative, anaerobic bacterium that is able to survive in low-oxygen environments. It produces a number of virulence factors, including proteases, lipopolysaccharides, and fimbriae, which allow it to attach to and invade host cells, evade the immune system, and promote tissue destruction. *P. gingivalis* is thought to contribute to the development and progression of periodontitis by inducing chronic inflammation and bone loss in the gums and supporting structures of the teeth. It has also been linked to other systemic diseases, including cardiovascular disease, diabetes, and Alzheimer's disease. Effective oral hygiene practices, including regular brushing and flossing, can help to reduce the levels of *P. gingivalis* in the mouth and prevent the development of periodontitis [1].

Oral pathogenic microorganisms have been the major cause for dental plaques, dental caries as well as periodontal and gingival disease [2]. While periodontal disease is considered a polymicrobial infection, *Porphyromonas gingivalis* (*P. gingivalis*) 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.

The Present study was conducted to evaluate the efficacy of an oral care product Ayurvedic Paste contains herbal ingredients such as Pippali (*Piper longum*), Marich (*Piper nigrum*), Sunthi (*Zingiber officinale*), Tumburu (*Zanthoxylum alatum*), Pudina satva, as active ingredients which are used from the ancient times in oral care and are said to be proven effective in combating oral malodour & gingivitis.

The herbal dentifrice containing Lavang, Tomar, Pippali, Kalimirch, Sunthi, Karpura which are known to reduce inflammation and maintain gum health in Ayurvedic text.

Lavang is known to cure gingivitis. Piper longum is ayurvedic rasayanas (Rejuvenators) which commonly used for antiaging and cell rejuvenation benefits in ayurveda [8].

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.

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 [1].

Tomar (*Zanthoxylum armatum*) present in ayurvedic toothpaste helps remove bad odor and has an antiseptic and antibacterial property. Tomar is one of the best remedies for toothache. Eugenol or clove oil has analgesic, antibacterial, antiviral, anti-inflammatory, and antioxidant properties. It has been used to relieve toothache, in periodontitis, as an anesthetic, and to treat bleeding gums [1].

Karpura (*Cinnamomum camphora*) is another component in herbal toothpastes with antioxidant, antibacterial, and anti-inflammatory properties. The leaves of Pudina or Mint and possess several biological effects as antiseptic in oral preparations, antibacterial, antifungal, antiviral antioxidant effects [9].

The collective effectiveness of these Ayurvedic herbs can potentially contribute to the inhibition of *P. gingivalis* growth, suppression of its virulence factors, and attenuation of the inflammatory response triggered by the bacterium. By targeting multiple facets of the pathogenic process, these herbs may offer a comprehensive solution for gingivitis prevention and management.

Material and Methods

Table 1: Product Details

Groups	Active Ingredients
Test Group - Ayurvedic Paste (Dabur Red Paste)	Herbal Extracts And Essential Oils- Pipali, Tomar, Maricha, Sunthi, Kapur, Pudina, Clove Oil, Gairic Powder. Excipients (Calcium Carbonate Base): Q.S

Table 2: Microbial strain

S. No.	Tester Strain	ATCC No	Source
1	<i>P. gingivalis</i>	ATCC 33277	American Type culture collection, USA

Table 3: Chemicals and Media

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

- Dilution fluid or Diluent: 0.9% saline
- Neutralizer: Lecithin soya and Tween-80
- Growth media: Supplemented tryptic soya agar
- Sterile deionized water or Equivalent
- Anaero gas Pack
- Anaero Indicator Tablet
- 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 5 days. The growth was scrapped and transferred to sterile and the turbidity was adjusted to 10^7 CFU/ml.

Test procedure

- 1ml of 50% test sample and 1 ml of *P. gingivalis* and to this add 8 ml of neutralizer and mix well and allow it for 2 minutes of contact time.
- Repeat above steps in a duplicate test concentration and each tested sample is plated in a duplicate.
- Take 1 ml of above treated sample and serially dilution with dilution fluid (saline) before and after contact time.
- A positive control is run to verify that to determine the number of surviving microorganism in inoculum.
- 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.
- 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 and Discussion

Table 1: Percentage reduction of test organisms tested by 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
Ayurvedic Paste	<i>P. gingivalis</i> (ATCC 33277)	2 min	4 x 10 ⁷	4 x 10 ⁴	3.301	99.9500

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 [10]. Several clinical studies have established the inhibitory effect of dentifrice on gingival and oral bacteria [11].

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 [12].

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 [13].

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

The present study aimed to evaluate the efficacy of Dabur Red Paste, formulated with a blend of Ayurvedic herbs, in promoting oral hygiene and preventing gum diseases by inhibiting the growth of *Porphyromonas gingivalis* (*P. gingivalis*). The results of this study provide valuable insights into the potential benefits of incorporating traditional herbal ingredients into oral care products.

Conclusions

In vitro assessment of Ayurvedic Paste against periodontal disease causing pathogen revealed its 99.95 % effectiveness at 2 min contact time. Hence, the Ayurvedic Paste have potential to be utilized in the treatment of variety of dental diseases. Further research is needed to explore the effectiveness of herbal toothpastes against other oral microorganisms and their long-term effects on oral health. Clinical trials involving human subjects would provide valuable insights into the real-world efficacy and safety of Ayurvedic toothpastes.

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