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In-vitro assessment of 32 Ayurvedic herbs containing toothpaste on gingivitis causing bacteria

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Abstract

Objective: The aim of this study was to perform *in vitro* assessment of 32 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 Dant Rakhak toothpaste containing 32 Ayurvedic herbs like Neem, Elaichi, Badam, Dalchini, Lavang and Dalchini for the protection of oral hygiene and prevention of gum diseases by inhibition of disease causing micro-organism *P. gingivalis*.

Methods: Dabur Dantrakhak toothpaste containing 32 Ayurvedic herbs like Neem, Elaichi, Badam, Lavang and Dalchini. 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 against disease causing oral pathogens revealed the efficacy of Dabur Dant Rakhak toothpaste containing 32 Ayurvedic herbs like Neem, Elaichi, Badam, Lavang and Dalchini against major gingivitis causing oral pathogen *P. gingivalis*.

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

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. 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.

This black-pigmented bacterium is known for its ability to produce various virulence factors that contribute to the destruction of periodontal tissues, either through direct damage or by indirectly triggering an immune response in the host. It's important to note that none of these microbial species are individually capable of causing the destructive events associated with the progression of periodontal disease. Instead, the development of periodontal diseases involves a complex interplay and cooperation among these microorganisms to establish their niches in the oral cavity [3, 4].

The presence of *P. gingivalis*, whether acting alone or as part of a mixed infection with other oral pathogens, combined with potential deficiencies in certain host immunological factors, can lead to the development of periodontal diseases. Consequently, addressing the growth and virulence of *P. gingivalis* holds significant promise in preventing and managing gingivitis and related oral health issues.

Traditional medicinal systems, such as Ayurveda, have long recognized the potential of herbal remedies in maintaining oral hygiene and preventing various oral diseases. Dabur Dant Rakshak toothpaste, enriched with a blend of 32 Ayurvedic herbs, represents a holistic approach to oral care by harnessing the synergistic effects of multiple phytoactive compounds.

The following Ayurvedic herbs have been found to be effective against *P. gingivalis*

- **Neem (*Azadirachta indica*):** The active components in Neem, such as nimbidin and azadirachtin, have shown significant inhibitory effects against various pathogenic microorganisms, including *P. gingivalis*. These compounds disrupt the bacterial cell membrane, impairing its integrity and leading to bacterial death [15].
- **Elaichi (Cardamom):** Elaichi possesses potent antioxidant and anti-inflammatory properties, attributed to its high content of essential oils and phytochemicals. These properties may contribute to its ability to counteract the inflammatory response triggered by *P. gingivalis* infection. Additionally, certain compounds in elaichi have demonstrated antimicrobial activity, which could aid in controlling the growth of *P. gingivalis*.
- **Badam (Almond):** Badam is a rich source of nutrients, including vitamins and minerals that contribute to overall health. Its antioxidant content, particularly vitamin E, can help neutralize oxidative stress induced by *P. gingivalis*. Furthermore, Badam's anti-inflammatory effects may help mitigate the inflammation associated with gingivitis [16].
- **Dalchini (Cinnamon):** Cinnamon is renowned for its antimicrobial and anti-inflammatory properties. Cinnamaldehyde, a primary component of cinnamon oil, has demonstrated inhibitory effects against *P.*

gingivalis growth. Additionally, cinnamon's anti-inflammatory actions could aid in attenuating the tissue inflammation caused by *P. gingivalis*.

- **Lavang (Clove):** Eugenol, a major compound in clove oil, has exhibited strong antibacterial activity against *P. gingivalis*. Moreover, its analgesic effects could provide relief from discomfort associated with gingivitis.

The combined efficacy of these Ayurvedic herbs has the potential to hinder the growth of *P. gingivalis*, suppress its virulence factors, and mitigate the inflammatory response triggered by the bacterium. By addressing multiple aspects of the pathogenic process, these herbs could provide a holistic solution for both preventing and managing gingivitis.

Given this context, our current study aims to assess the antimicrobial properties of a toothpaste formulated with 32 Ayurvedic herbs, free from fluoride or whitening agents, against the oral bacteria *P. gingivalis*, which is known to be a causative agent of gingivitis.

Material and Methods Toothpaste Composition

Ingredient
Compounded herbal extract and essential oil in calcium carbonate blend

Materials

Test sample details

RR number	Name of the test sample	Active INCI	Batch No.	Storage conditions
RR230073	Dabur Dant Rakshak Paste	Compounded herbal extract and essential oil in calcium carbonate base	BD0125	RT

Microbial strain

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

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

- 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 5 days. The growth was scrapped and transferred to sterile and the turbidity was adjusted to 10^7 CFU/ml.

Test procedure

- 1 ml 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 Dabur Dant Rakshak 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 Dant Rakshak Paste	<i>P. gingivalis</i> (ATCC 33277)	2 min	4 x 10 ⁷	2 x 10 ⁴	3.301	99.9500

Ensuring proper oral hygiene is essential for preventing dental diseases. Biofilms generated by the oral micro-flora play a significant role in the development of both caries and periodontal diseases. Therefore, it is crucial to control these biofilms through mechanical removal and the use of antimicrobial agents in toothpaste to prevent diseases caused by plaque build-up [9]. Numerous clinical studies have demonstrated the inhibitory effects of toothpaste on oral and gingival bacteria [10]. Growing concerns about the rise in antibiotic resistance among microbes due to chemical-based toothpaste [11-12] have led to increased interest in exploring alternative or non-conventional toothpaste options, prompting the current study.

Porphyromonas gingivalis, often referred to as *P. gingivalis*, is a type of bacteria commonly associated with periodontal disease a chronic inflammatory condition affecting the tissues surrounding the teeth. *P. gingivalis* is a gram-negative anaerobic bacterium that resides within the oral microbiome, and it stands as one of the primary pathogens contributing to the onset and progression of periodontal disease [3].

Moreover, *P. gingivalis* has the ability to interact with other oral bacteria, forming complex biofilms. This phenomenon enhances its resistance to antimicrobial agents and complicates treatment strategies. Apart from its role in periodontal disease, *P. gingivalis* has also been implicated in various 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 procedures like scaling and root planing, in addition to antimicrobial therapy, such as topical or systemic antibiotics. However, the emergence of antibiotic-resistant strains of *P. gingivalis* has rekindled interest in exploring alternative treatments, including natural products like clove and other botanical extracts.

Numerous toothpaste preparations containing herbal ingredients have made significant contributions to dental

prophylaxis by enhancing oral health. The popularity of herbs in oral care is attributed to the anti-inflammatory and antimicrobial effects of phytochemicals [8]. However, there have been no reports on the effects of such herbal toothpaste formulations on periodontitis-causing oral bacteria like *P. gingivalis* and cavity-causing *P. gingivalis*. The novelty of the herbal toothpaste in this study lies in its natural compounds.

The present study aimed to evaluate the efficacy of Dabur Dant Rakshak toothpaste, 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 findings of this study offer valuable insights into the potential benefits of incorporating traditional herbal ingredients into oral care products.

The results of this study indicated that Dabur Dant Rakshak toothpaste demonstrated promising effects in maintaining oral hygiene. The presence of Ayurvedic herbs like Neem, known for its antimicrobial properties, and Dalchini (cinnamon), which has demonstrated anti-inflammatory and antimicrobial activities, could contribute to the toothpaste's effectiveness in inhibiting the growth of *P. gingivalis*. This is particularly significant as *P. gingivalis* is closely associated with the development of periodontal diseases.

Furthermore, the inclusion of Elaichi (cardamom) and Lavang (clove) in the toothpaste's formulation is noteworthy due to their potential antibacterial and anti-inflammatory properties [13-14]. These properties may play a role in reducing the microbial load in the oral cavity and subsequently preventing gum diseases. Badam (almond) is another key ingredient that may contribute to oral health, given its reported antimicrobial and antioxidant properties, which could help maintain oral hygiene and combat the detrimental effects of oxidative stress in the oral environment.

While the results of this study are promising, it is essential to acknowledge some limitations. The study primarily focused on assessing the inhibitory effects of Dabur Dant

Rakhak toothpaste on *P. gingivalis*. Further investigations are warranted to explore its impact on other oral microorganisms and its long-term effects on oral health. Additionally, conducting clinical trials involving human subjects would provide valuable insights into the real-world efficacy and safety of this toothpaste.

In conclusion, the present study suggests that Dabur Dant Rakhak toothpaste, formulated with a blend of Ayurvedic herbs, holds promise in promoting oral hygiene and preventing gum diseases by inhibiting *P. gingivalis*. The antimicrobial, anti-inflammatory, and antioxidant properties of the herbal ingredients in the toothpaste could collectively contribute to its effectiveness.

Conclusions

In vitro studies have shown that Ayurvedic herbs containing toothpaste like Neem, Elaichi, Badam, Dalchini, Lavang and Dalchini can be effective in controlling plaque and gingivitis, particularly against major disease-causing pathogens such as *P. gingivalis*. The use of herbal toothpaste formulations instead of non-herbal ones should be considered for promoting oral health care. However, practitioners should endorse a dentifrice based on the patient's clinical conditions and possible benefits.

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