Efficacy Of Ayurvedic Gel Against Oral Pathogenic Bacteria Porphyromonas Gingivalis: In-Vitro Study

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Abstract

Objective: The aim of this study was to perform In Vitro assessment of Dabur ayurvedic Freshness Gel against oral pathogens Porphyromonas gingivalis (P. gingivalis). The present study was designed to evaluate the efficacy of an ayurvedic Freshness Gel toothpaste comprising of Zinc Salt (Gandhakamleeya Yasada), Herbal extract and blend of essential oils for the protection of oral cavity and prevention of gum diseases by inhibition of gingival disease causing microorganism P. gingivalis.

Material & 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 Freshness Gel against disease causing oral pathogens revealed the 99.9% germ kill efficacy of gel toothpaste against major gingivitis causing oral pathogen P. gingivalis.

Keywords: Gingivitis, Toothpaste, P. gingivalis, Zinc Salt, Gum Health, Periodontitis.

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.

In the ancient Indian traditional medicine system concept to stay healthy is described in various scriptures. From the use of products to seasonal impacts, on specific human based on “Prakriti”, ailments; Acharya Charka has mentioned that human should use such specific herbs for “Danta Dhavana” as a daily regime to maintain the oral hygiene including bad breath. Still today people are suffering from a lot of oral problems like malodour, gingivitis, plaque etc. and people are spending a lot on treating these problems once aggravated.

The present study was conducted to evaluate the efficacy of an oral care product Ayurvedic Freshness Gel contains herbal ingredients such as Pippali (Piper longum), Marich (Piper nigrum), Sunthi (Zingiber officinale), Tumbura (Zanthoxylum alatum), Padina satva, Gandhapura oil (oil of Gaultheria fragrantissima), Mentha piperata oil, Mentha Spicata oil, Takkola oil (oil of Illicium verum)) and Gandhakamleeya Yasada (Zinc Sulphate) 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.

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.

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.

- 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.

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.

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, antiviral, and antiviral antioxidant effects.

Two clinical studies independently support the efficacy of the test toothpaste with 0.2% zinc sulphate in reducing oral malodour after a single brushing, and after four weeks for 12 hours after brushing compared to a toothpaste without zinc ions.

The effect of zinc ions on the growth of oral bacteria was strain-dependent. F. nucleatum, P. intermedia, and P. gingivalis have been recognized as VSC-producing organisms that are important to oral malodor.

Takkola oil and Clove oil in gel are mentioned for the treatment of halitosis. Pudina (Peppermint) is mentioned in ayurveda for the Mukhasodhana (Mouth Cleanser) and Durgandhinashak (Bad odour removal).

Mints (Mentha spp.) are medicinal herbs that are valued worldwide in traditional medicines for their antimicrobial and antioxidant properties. They are also odor-masking agents, as menthol and menthyl acetate are responsible for a pungent and refreshing odor. Therefore, Mentha extracts are extensively used in oral hygiene products, mouth fresheners, toothpastes, and chewing gums.

### Material and Method

#### Product Details

<table>
<thead>
<tr>
<th>Groups</th>
<th>Active Ingredients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test Group – Ayurvedic Freshness Gel</td>
<td>Zinc Sulphate 0.25% (Gandhakamliya Yasada) Herbal Extracts and essential Oils Excipients (Gel Base): q.s</td>
</tr>
</tbody>
</table>

#### Microbial strain

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Tester Strain</th>
<th>ATCC No</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>P. gingivalis</td>
<td>ATCC 33277</td>
<td>American Type culture collection, USA</td>
</tr>
</tbody>
</table>

#### Chemicals and Media

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

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

**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**
A. 1ml of 50% test sample and 1ml of *P. gingivalis* 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 serial dilution with dilution fluid (saline) before and after contact time.
D. A positive control is run to verify that 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 at 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.

\[ \text{Log}_{10} \text{Reduction (LR)} = \frac{\text{Mean Log}_{10} \text{(Microbial population)}}{\text{Mean Log}_{10} \text{(surviving test population)}} \]

**RESULTS**
Table 1: Percentage reduction of test organisms tested by homeopathic ingredient based toothpaste Hekla Lawa toothpaste against *P. gingivalis* at 2 min contact time.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Test Organisms</th>
<th>Contact Time (min)</th>
<th>Initial Inoculum (cfu/ml)</th>
<th>Number of cells per mL at the end of contact time</th>
<th>Log Reduction</th>
<th>% Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ayurvedic Gel</td>
<td><em>P. gingivalis</em> (ATCC 33277)</td>
<td>2 min</td>
<td>4 x 10^7</td>
<td>4 x 10^4</td>
<td>3.00</td>
<td>99.900</td>
</tr>
</tbody>
</table>

**Discussion**

*P. gingivalis* is a gram-negative bacterium that is commonly found in the oral cavity. It has been identified as a major etiologic agent in the development of periodontal diseases, such as gingivitis and periodontitis. Gingivitis is a mild form of periodontal disease, which is characterized by inflammation of the gums and is caused by bacteria that accumulate in the gingival sulcus. *P. gingivalis* is one of the major bacteria involved in the development of gingivitis. The accumulation of this particular species of bacteria, along with other related bacteria, leads to an infection of the gums that can cause inflammation and other signs of gingivitis.

Periodontitis is an advanced form of periodontal disease that is characterized by an infection of the gingival tissue and the bone that supports the teeth. *P. gingivalis* is also a major contributor to the development of periodontitis. The bacteria form a biofilm that adheres to the gingival tissue, which leads to an infection and inflammation of the gums and the surrounding tissue. This inflammation can cause the gums to pull away from the teeth, resulting in gum recession and destruction of the underlying bone.

*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.²

*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.³

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 herbal extracts.

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 \textit{P. gingivalis} \(^9\). These studies suggest that clove may have potential in promoting oral health and reducing the risk of gum disease caused by \textit{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 \(^10\). However, there have been no reports on the effects of such toothpastes on periodontitis causing oral bacteria \textit{P. gingivalis} and cavity causing \textit{P. gingivalis}. Hence, study was conducted to investigate the effects of a gel toothpaste containing herbal ingredients like such as \textit{Pippali (Piper longum)}, \textit{Marich (Piper nigrum)}, \textit{Santhi (Zingiber officinale)}, \textit{Tamburu (Zanthoxylum alatum)}, \textit{Padina satva}, \textit{Gandhapura} oil (oil of Gaultheria fragrantissima), \textit{Mentha piperata} oil, \textit{Mentha Spicata} oil, \textit{Takkola} oil (oil of \textit{Illicium verum}) and \textit{Gandhakamleeya Yasada} (Zinc Sulphate) on gingivitis causing oral bacteria \textit{P. gingivalis}.

The novelty of the herbal gel 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 gel toothpaste different degrees of effectiveness against the tested microorganism. In this regard, the formulated toothpaste exerted a highly significant effect against \textit{P. gingivalis}.

In the present study, Dabur Gel formulation was found to have antimicrobial activities against gingivitis causing oral bacteria \textit{P. gingivalis}. This may be attributed to the synergistic interactions between the ingredients present in the formulations, which, however, need to be established. The principle components of this toothpaste include such as \textit{Pippali (Piper longum)}, \textit{Marich (Piper nigrum)}, \textit{Santhi (Zingiber officinale)}, \textit{Tamburu (Zanthoxylum alatum)}, \textit{Padina satva}, \textit{Gandhapura} oil (oil of \textit{Gaultheria fragrantissima}), \textit{Mentha piperata} oil, \textit{Mentha Spicata} oil, \textit{Takkola} oil (oil of \textit{Illicium verum}) and \textit{Gandhakamleeya Yasada} (Zinc Sulphate). 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 \(^11\). Against \textit{P. gingivalis} herbal formulations showed significant antimicrobial activity (p<0.05). Many studies on anti-gingivitis activity of herbal base toothpaste have been reported \(^12\). \(^13\).

Conclusions

\textit{In Vitro} assessment of Ayurvedic Freshness Gel against periodontal disease causing pathogen revealed its 99.9\% effectiveness. Hence, the Ayurvedic Freshness Gel have potential to be utilized in the treatment of variety of dental diseases.

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