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# *In-vitro* assessment of anti-gingivitis effect of homeopathic ingredients based toothpaste

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**Abstract**
**Aim:** The aim of this study was to perform *In vitro* assessment of Homeopathic ingredient based toothpaste against oral disease causing pathogens *Porphyromonas gingivalis* (*P. gingivalis*).
**Objective:** The present study was designed to evaluate in efficacy of the herbo-mineral toothpaste composition prepared using certain homeopathic medicinal plant extracts & Zinc Salt for the protection of oral hygiene and prevention of gum diseases by inhibition of disease causing microorganism *P. gingivalis.*
**Methods:** The homeopathic ingredient based toothpaste was made of Zinc Salt & Hekla Lawa Extract fine ash from mount Hekla Lawa, *Hamamelis virginica* Extract, Plantago Major Extract, *Phytolacca decandra* Extract and *Calendula officinalis* Extract combined in Toothpaste formulation. 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 homeopathic ingredient based toothpaste against disease causing oral pathogens revealed the efficacy of toothpaste against major gingivitis causing oral pathogen *P. gingivalis*.

**Keywords:** Homeopathy, Hekla lawa, *P. gigivalis*, oral hygiene, toothpaste, dental diseases

## 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.
Homeopathic herbo-mineral containing Toothpastes with Hekla Lawa extract fine ash from mount Hekla Lawa, *Hamamelis virginica* Extract, Plantago Major Extract, *Phytolacca decandra* Extract and *Calendula officinalis* Extract have been commercially sold based on the claim that it prevents gum diseases possess, helps to relief sensitivity pain caused extreme cold and hot, help to cure toothache in difficult dentition and help to heal mouth ulcer.
The use of natural herbs and mineral 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 compounds from plants have been used since ages in oral care and appear to be safe.
Herbomineral ingredients of Dabur Homeopathic ingredient based toothpaste are known to strengthen the teeth and prevent bleeding gum. They are also known to fight plaque and teeth stains. It has Anise flavour that helps fight the oral malodour and has magic to bring fresh breath.

Hekla Lawa, is obtained as a fine ash from mount Hekla, an Iceland volcano. It offers support in issues of dental caries, sensitivity, tooth decay and gum abscess. It can be used as intra canal medicament in root canal treatment, since it contains large amount of sulphur, silica, fluoride, lime, magnesia and ferrous oxide. HeklaLawa powder has anti-inflammatory property which benefits in repair of the loose sockets, chronic periodontitis, gingivitis etc. [10]. Hekla's ash is not really an ash, but tephra, "a lightweight solid containing silica, like a very small grain of sand containing bubble cavities which have dissolved acidic salts attached to the surface as a precipitation". This tephra is known to have great healing properties [10].

Calendula is an annual plant belonging to the family Asteracea. The following chemical components are found in calendula; triterpene saponins, sesquiterpenes, carotenoids, flavonoid glycosides, triterpene alcohols, flavonoids, xanthophylls, steroids, phenolic acids, tocopherol, calenduline and mucilage, [6, 7]. Thus the extract from flower is used widely as an antimicrobial agent [8, 9].

With this background in mind, the present study aimed to evaluate antimicrobial properties of homeopathic ingredient based toothpaste containing Hekla Lawa, *Hamamelis virginica* Extract, Plantago Major Extract, *Phytolacca decandra* Extract, *Calendula officinalis* Extract and without any fluoride or whitening agent on Gingivitis causing oral bacteria *P. gingivalis*.

## Material and Methods

**Toothpaste Composition**

| Ingredient |
|---|
| Hekla Lawa Extract, *Hamamelis virginica* Extract, Plantago Major Extract, *Phytolacca decandra* Extract, *Calendula officinalis* Extract, Kreosotum Extract, Zinc Gluconate in Calcium Carbonate Base |

## Materials

**Test sample details**

| Name of the test sample | Active INCI | Batch No. | Storage conditions |
|---|---|---|---|
| Homeopathic ingredient containing toothpaste (Hekla Lawa toothpaste) | Hekla Lawa extract and essential Oil in calcium carbonate base q.s. | BS00001 | RT |

**Microbial strain**

| S. No. | Tester Strain | ATCC No | Source |
|---|---|---|---|
| 1 | *P. gingivalis* | 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 5days.The growth was scrapped and transferred to sterile and the turbidity was adjusted to $10^7$CFU/ml.

**Test procedure**

A. 1 ml of 50% test sample and 1ml of *P. gingivalis* and to this add 8 ml 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 1:** Percentage reduction of test organisms tested by homeopathic ingredient based toothpaste Hekla Lawa 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 |
|---|---|---|---|---|---|---|
| Toothpaste (Hekla Lawa toothpaste) | *P. gingivalis* (ATCC 33277) | 2min | 4 x 107 | 3 x 104 | 3.125 | 99.9250 |

## 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 [11]. Several clinical studies have established the inhibitory effect of dentifrice on gingival and oral bacteria [12]. Heightened concerns regarding the upsurge in antibiotic resistance in microbes against chemical based dentifrices [13-15] has stimulated interest in the therapeutic use of alternative or non-conventional dentifrices and thus this study.

*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.

Homeopathy is a form of alternative medicine that uses highly diluted substances to stimulate the body's natural healing processes. “Homeopathy” system has been used effectively for treating various systemic ailments in Indian medicine. Using herbal medicines to cure various ailments has become an increasing trend [16]. 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 [17].

Homeopathic toothpaste typically contains ingredients such as herbs, minerals, and essential oils. These ingredients are believed to have anti-inflammatory and antibacterial properties that can help to prevent tooth decay, gum disease, and bad breath. Homeopathic herb containing Toothpastes with Hekla Lawa extract fine ash from mount Hekla Lawa, *Hamamelis virginica* Extract, Plantago Major Extract, *Phytolacca decandra* Extract and *Calendula officinalis* Extract have been commercially sold based on the claim that it prevents gum diseases possess, helps to relief sensitivity pain caused extreme cold and hot, help to cure toothache in difficult dentition and help to heal mouth ulcer. 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 homeopathic ingredients on Gingivitis causing oral bacteria *P. gingivalis*.

These toothpastes are based on the traditional use of homeopathic remedies, which are based on the principle of “like cures like”. This means that substances that cause certain symptoms in a healthy person can be used in small doses to treat people with similar symptoms. Hekla Lawa extract is thought to have anti-inflammatory and antiseptic properties, which can help reduce inflammation in the gums and help prevent gum diseases. *Hamamelis virginica* Extract is thought to have antiseptic and astringent properties, which can help reduce the pain and discomfort associated with tooth sensitivity. Plantago Major Extract is thought to have anti-bacterial and anti-inflammatory properties, which can help reduce gum inflammation and prevent plaque formation. *Phytolacca decandra* Extract is thought to have antiseptic and analgesic properties, which can help reduce the pain and discomfort associated with toothache. Finally, *Calendula officinalis* Extract is thought to have anti-inflammatory and antiseptic properties, which can help reduce inflammation in the gums and help heal mouth ulcers.

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 Homeopathy toothpaste formulations was found to have antimicrobial activities against Gingivitis causing oral bacteria *P. gingivalis*. This may be attributed to the ingredients present in the formulations, which, however, need to be established [16]. The principle components of this toothpaste include Hekla Lawa extract fine ash from mount Hekla Lawa, *Hamamelis*

*virginica* Extract, Plantago Major Extract, *Phytolacca decandra* Extract and *Calendula officinalis* Extract.

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 [19, 20].

## Conclusions

*In vitro* assessment of Homeopathic ingredient based toothpaste against disease causing periodontal pathogen revealed its effectiveness against major disease causing pathogen such as *P. gingivalis*. Hence, the toothpaste have potential to be utilized in the treatment of variety of dental diseases. However, *In vitro* method is commonly used in screening the antimicrobial agents before *In vivo* testing. Thus, homeopathy experts may endorse a dentifrice based on patient's clinical conditions and possible benefits.

## Conflict of Interest

Not available

## Financial Support

Not available

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