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Assessment of homeopathic ingredients based dentifrice on caries causing bacteria: *In-vitro*

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Abstract

Objective: The aim of this study was to perform *In vitro* assessment of Homeopathic ingredient based toothpaste against disease causing oral pathogens. To determine the antimicrobial susceptibility of disease causing periodontal pathogens to homeopathic ingredient based toothpaste through zone of inhibition and time kill assay.

Herbal toothpastes are more efficacious and safe due to containing natural actives as compared with the synthetic & chemical toothpastes. The present study aimed to evaluate the poly herbal toothpaste prepared using homeopathic medicinal plants and evaluate its efficiency in the protection of oral hygiene and prevention of dental caries by inhibition of disease causing microorganism.

Methods: The toothpaste Dabur homeopathic ingredient based toothpaste was made of 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 has tested against pathogenic bacteria Streptococcus mutans 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 Streptococcus mutans.

Conclusions: *In vitro* assessment of Homeopathic ingredient based toothpaste against disease causing oral pathogens revealed the 99.9% effectiveness of toothpaste against major cavity causing oral pathogen such as *S. mutans*.

Keywords: Hekla lawa, Anti-bacterial activity, *S. mutans*, Natural alternative

1. Introduction

One of the most unvaried chronic oral infections across the world is dental caries ^[1]. Oral pathogenic microorganisms have been the reason for dental caries, dental plaques as well as gingival and periodontal diseases ^[2]. Streptococcus mutans is one of the main opportunistic pathogens of dental caries, which is responsible for dental plaque and caries development ^[3]. 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.

In recent years, the use of natural products has gained more attention to attenuate the action of oral 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 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.

The test product contains herbal extracts like Calendula which is an annual plant of the family Asteracea. The active components are found in calendula sesquiterpenes, triterpene saponins, flavonoid glycosides triterpene alcohols, carotenoids, flavonoids, xanthophylls, phenolic acids, steroids, mucilage, tocopherol, and calenduline ^[6, 7]. Thus the extract from this flower is used widely as an antimicrobial agent ^[8, 9].

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 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.

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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 cavity causing *Streptococcus mutans*.

Hekla Lawa, is a fine ash from mount Hekla, an Iceland volcano. It helps in treatment of dental sensitivity, gum abscess, caries of the bone, tooth decay. It can be used as intra canal medicament in root canal treatment, since it constituents large amount of sulphur, silica, lime, magnesia, ferrous oxide and fluoride. Hekla Lawa powder has anti-inflammatory effect which helps in repair the loose sockets, gingivitis, chronic periodontitis etc. [10].

2. Material and Methods

Table 1: Toothpaste Composition

Ingredients
Hekla Lawa Extract, Hamamelis Virginica Extract, Plantago Major Extract, <i>Phytolacca decandra</i> Extract, Calendula Officinalis Extract, Kreosotum Extract, Zinc Gluconate in Calcium Carbonate Base

Media and Reagents

Soyabean Casein Digest Agar (SCDA), Sabouraud Dextrose Agar (SDA), Brain Heart Infusion agar (BHIA), Dey-Engley Neutralizing Broth, Sodium chloride, Sterile water

Preparation of test organisms

Working culture of test organism

For first subculture, test microorganism was inoculated from working stock culture onto fresh sterile slant of Brain heart infusion agar (BHIA) for *Streptococcus mutans* ATCC 25175 incubated at 35 °C±1 °C for 48 hr. For second subculture, growth obtained in the first subculture slants was inoculated onto the surface of slants as indicated above.

Test suspension and Neutralization challenge microbial suspension

From second subculture, a loopful of test cultures were

suspended in sterile saline & dislodged using mechanical vortex. The test culture suspension was adjusted at 0.5 McF for bacteria and 1.0 McF for fungus such that the initial test suspension contained 1.5 x 10⁸ to 5 x 10⁸ cfu/ml. The neutralization challenge microbial suspension was prepared to achieve 30 to 100 colonies on plate when inoculated in the neutralizer. Cell strength of all the suspensions was confirmed by carrying out tenfold serial dilution and plating 1 ml of the appropriate dilutions in duplicates using respective culture media by pour plate technique. After solidification, plates were incubated as per section 2.1. Cell strength of the test suspension was reconfirmed after completion of the test.

Preparation of test sample

50% concentration of sample was prepared using sterile distilled water and mixed.

Neutralization Assay

Neutralization assay was performed as per ASTM 1054-08 (Reapproved 2013) based on neutralization validation with recovery on semisolid medium. Following four tests were performed in a timely manner such that significant proliferation of the test organism did not occur.

- Neutralization effectiveness Test (Test A): 0.1ml of the neutralization challenge microbial suspension was added to sterile vial containing 8.9 ml of neutralizer further, 1.0 ml of test sample was added and mixed.
- Neutralizer toxicity (Test B): 0.1ml of the neutralization challenge microbial suspension was added to sterile vial containing 8.9 ml of neutralizer further, 1.0 ml of sterile 8.5% saline was added and mixed.
- Test organism viability (Test C): 0.1ml of the neutralization challenge microbial suspension was added to sterile vial containing 9.9 ml of sterile 8.5% saline and mixed.
- At 0 time (within 1 min of execution of test) and 30 minutes, 1 ml from the reaction mixture of each test was plated in duplicate onto respective culture media by pour plate technique. After solidification, plates were incubated as per section 2.1.

Test Procedure: Determination of antimicrobial activity by zone of inhibition by cup plate method:

Sterile culture media was inoculated with 100 µl of microbial culture and petri plates were prepared for SDA-BHIA- inoculated with *Streptococcus mutans*. After solidification wells were made using sterile borer. In each well 100 µl of test sample was loaded using micropipette. All the plates were incubated as per section 2.1. After incubation, the zone of inhibition around the well was measured and antimicrobial activity was determined.

Control (Number Control-Spiked water control)

- 10 ml of sterile glass distilled water was transferred in a sterile vial and maintained at specified test temperature to equilibrate.
- 0.1 ml of test suspension was added and kept for highest contact time under constant stirring.
- At the end of contact time, 1 ml aliquot was transferred to a test tube containing 9.0 ml of neutralizer (10⁻¹). This mixture was serially 10-fold diluted up to 10⁻⁵. 1ml of all the dilutions was plated in duplicates in recommended culture media as per section 2.1.
- After solidification plates were incubated as per section 2.1.

Evaluation of Antimicrobial activity of the test sample

- A. After equilibration a 0.1 ml aliquot of test suspension was transferred to the vial containing test sample and mixed.
- B. The test suspension was exposed to the test sample for contact time 2 minutes using a calibrated minute and second timer.
- C. At the end of contact time, 1 ml aliquot was transferred to a fresh test tube containing 9.0 ml of neutralizer (10^{-1}). This mixture was serially 10-fold diluted up to 10^{-5} and 1ml of each dilution was plated in duplicates in recommended culture media as per section 2.1.
- D. After solidification plates were incubated as per section 2.1.

Calculation: Post incubation, plates having colonies in range of 30 to 300 were counted & considered for calculation; enumeration was done using following formula:

1. Average counts per ml = $c / (n1+0.1n2) d$
2. Where, c = sum of all colonies taken into account
3. n1 = number of plates taken in account in the lower dilution

4. n^2 = number of plates taken in account in the higher dilution
5. d = dilution factor corresponding to the lower dilution
6. Average counts per ml were converted to Log₁₀.
7. Log₁₀ reduction (LR) = Log₁₀ of water control - Log₁₀ of test sample
8. % reduction = Water control – test sample/test sample x 100

3. Result and Discussion

Antimicrobial activity by zone of inhibition by cup plate method was tested and the zone of inhibition was reported as indicated in Table 1.

Table 2a: Zone of inhibition (in mm)

Test organism	Zone of inhibition (in mm)
<i>Streptococcus mutans</i>	24.99

Neutralization validation assay was tested and reported as indicated in Table 2b.

Table 2b: Results for Neutralization Validation Assay based on recovery

Test organism	Exposure time	Neutralization challenge microbial suspension		Neutralization effectiveness Test A		Neutralization toxicity Test B		Test Organism Viability Test C	
		Count (cfu/ml)	Log ₁₀	Count (cfu/ml)	Log ₁₀	Count (cfu/ml)	Log ₁₀	Count (cfu/ml)	Log ₁₀
<i>Streptococcus mutans</i>	0 min	4.5×10^3	3.65	40	1.60	39	1.59	42	1.62
	30 min	4.3×10^3	3.63	38	1.58	36	1.56	40	1.60

Evaluation of Antimicrobial activity of the test sample and control was test and reported as indicated in Table 3 and Table 4.

Table 3: Number Control (Spiked water control)

Test Organism	Initial count (cfu/ml)	Log (initial count)
<i>Streptococcus mutans</i>	1.4×10^6	6.15

Table 4: Results for Evaluation of Antimicrobial activity of the test sample

Test Organism	Contact Time	Conc. of product	Test count (cfu/ml)	Test Log	Log Reduction	% Reduction
<i>Streptococcus mutans</i>	2 minutes	50%	7.0×10^2	2.85	3.3	99.95

Antimicrobial activity of test sample by zone of inhibition was reported as 24.99mm for *Streptococcus mutans*. The quantitative assessment of activity in percentage reduction was calculated as 99.95% against *Streptococcus mutans*.

4. Discussion

Maintenance of good oral hygiene is the key to the prevention of oral & dental diseases. The biofilms by the oral microbiome being the centre of caries and periodontal disease, it requires to control these biofilms by mechanical debridement and use of adjunctive antimicrobials in toothpastes in prevention of plaque aggravated dental diseases [11]. Several clinical studies have demonstrated the inhibitory effect of toothpaste on oral pathogens and gingival [12]. Concerns regarding the increase in antibiotic resistance in microorganisms against chemical based dentifrices [13-15] has promoted interest in the therapeutic use of non-conventional or alternative dentifrices and thus this study.

“Homeopathy” system has been used successfully for treating various systemic ailments in Indian medicine. Using natural medicines to cure various diseases has become an increasing trend [16]. In recent years, a number of toothpaste preparations containing herbal ingredients have made significant contribution to dental prophylaxis in improving oral health. The popularity of herbs is due the antimicrobial

and anti-inflammatory effects of their ingredients known as Phytochemicals [17].

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 Porphyromonas gingivalis and cavity causing Streptococcus Mutans. Hence, study was conducted to investigate the effects of a toothpaste containing homeopathic ingredients on cavity causing Streptococcus mutans.

The uniqueness of the herbomineral toothpaste in the current study owes to its natural homeopathic compounds. Therefore, it can be claimed that this homeopathic ingredients based toothpaste can be used as a completely natural product without the complications of the marketed products. The results showed that homeopathic ingredients based toothpaste had different degrees of effectiveness against the tested microorganism. In this regard, the homeopathic ingredients based toothpaste exerted a highly significant antimicrobial effect against Streptococcus

mutans.

In the present study, Dabur homeopathic ingredient based toothpaste formulations was found to have antimicrobial activities against cavity causing bacteria *Streptococcus mutans*. This may be attributed to the polyherbal combined interactions between the ingredients present in their formulations, which, however, need to be established [16]. The principle components of homeopathic ingredients based toothpaste include Hekla Lava extract fine ash from mount Hekla Lava, Hamamelis Virginica Extract, Plantago Major Extract, *Phytolacca decandra* Extract and Calendula Officinalis Extract. The presence of actives such as alkaloids, flavonoids and polyphenols in these ingredients are considered to be the main cause of their antimicrobial efficacy [18]. Some of these ingredients were previously demonstrated and known to have antimicrobial activity (6-8). Against *Streptococcus mutans* and *Porphyromonas Gingivalis* herbal formulations showed significant antimicrobial activity ($p < 0.05$). Many studies on anti-plaque activity of herbal base toothpaste have been reported [19, 20].

5. Conclusions

In vitro assessment of Homeopathic ingredient based toothpaste against cavity causing oral pathogens revealed the 99.9% effectiveness of toothpaste against *S. mutans*. Hence, could be utilized in the treatment of variety of dental diseases. Nevertheless, *In vitro* method is commonly used in screening the antimicrobial agents before *In vivo* testing. Thus, dental professionals may recommend a dentifrice based on patients clinical conditions and possible susceptibilities.

6. Conflict of Interest

Not available

7. Financial Support

Not available

8. References

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