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Formulation and Evaluation of Herbal Toothpaste to Counteract *Streptococcus mutans*

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Abstract

The current work was proposed to develop a traditional Indian toothpaste accompanying its physical and chemical characteristics and screening of antibacterial action activity counteract to *Streptococcus mutans* (S. *Mutans* ATCC 10449) causes periodontal disease through *in vitro* screening. Pharmacopoeial guidelines were followed to prepare the plant extract paste and as per the Indian Government Toothpaste Descriptions its pharmaceutical evaluation had been conducted. *In vitro* testing was examined the antibacterial action of S. *Mutans* through applying Kirby–Bauer test. Chief ingredients were neem, catechu, liquorice, rock salt, tulsi, cinnamon, clove and amla. Inhibition zone radii was considered as the final result against S. *Mutans*. Dimethyl sulphoxide (DMSO) and Gentamicin were applied as negative and positive control respectively. Herbal toothpaste was prepared and possessing the compliance standards as reported in Bureau of Indian Standard (BIS). *In vitro* screening revealed the promising antibacterial action against S. *Mutans* with specific sensitivity with satisfied zone of inhibition towards gram positive bacterial infection. The maximum inhibition zone in agar plate for the test sample and gentamicin was computed 1.2 cm and 1.6 cm respectively which proved its promising significance. The envisioned traditional toothpaste could be accounts for the better patient compliance. However, *in vivo* screening against the S. *Mutans* should be investigated in controlling pathogenic oral microflora relevant to the positive control. Beyond the restriction of clinical trial, obtained data suggests that herbal toothpowders have the potential to be a valuable addition to oral care practices, especially for those who are looking for natural and effective alternatives to commercial toothpaste.

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Keywords

Toothpaste, *Streptococcus mutans*, Dental caries.

Introduction

Currently periodontal disorders like tooth caries, gum inflammation, and gum disease periodontitis have been inclining occurrence which is not restricted to the certain age group. Such kind of problems could be arisen because of life living alterations (1, 2).

Youth get more get more concern about constituent of the tooth cleaning agents and harmful effects of fluoride; therefore, herbal toothpowder withdraws the attention of dentist due to their accountability in improving hygienic

condition of oral cavity (3). In the previous investigation, numerous herbal toothpastes have been reported their effectiveness and promising safe as herbal toothpaste.

Individual ingredients of herbal toothpaste have been reported their antibacterial action individually. Neem (4), catechu (5), liquorice (6), tulsi (7), black pepper, clove, cinnamon (8), amla (9) and arjuna (10) have been investigated for the antimicrobial action. S. *Mutans* is the common gram-positive bacteria causes for periodontitis disorder (11). Therefore, current investigation is intended to produce the tooth paste against the S. *Mutans*.

Materials and Methods

Composition of proposed herbal toothpaste was modified, mentioned in Hamdard Pharmacopoeia, and as shown in Table-1. The component of herbal toothpaste was procured from the local market of Dehradun, Uttarakhand, India. The crude part of the plant is converted into powered form after cleaning (12).

Firstly, Indian neem powder, tulsi, black pepper, arjuna, liquorice, clove, cinnamon, catechu, amla, mentha, and rock salt were ground properly. Table 2 provides a summary of the formulation's ingredients. The required weight of each of the paste ingredients of herbal tooth powder was completely extracted by using absolute ethanol in Soxhlet assembly. Resultant extracts were subjected to the rotary evaporator.

Charcoal dissolved into distilled water and added to the resultant mixture. The concentrated extract was washed separately and dried. Toothpaste was formulated by taking respective powder of all the plant drugs separately except charcoal solution, in which extract powder was added. Heating liquid phase process trituration method was opted to prepare herbal toothpaste as per the guidelines (13).

Required herbal extract powder and 50ml solution of charcoal was mixed with continuous stirring for 15min at 50°C. A warm solution of humectant, distilled water and sweetener was prepared particularly which gradually incorporated with the soothing stirring to the herbal powder. Finally add mentha as preservative.

***In vitro* S. Mutans inhibition action of toothpaste against dental disorder**

Pathogenic bacterial strains of *S. Mutans* were selected for periodontal ailments. Nutrient Agar No.2 (Batch no. 3171042-500G, Sisco Research Laboratories Pvt. Ltd., India) was employed as the solid medium to develop nutrient plates. The antimicrobial potential was studied by Kirby-Bauer test or agar well disc method. Autoclaved agar medium was kept into different disc and cultured the bacterial colony of *S. Mutans*, incubated for 15 min at 37°C to allow proper diffusion and proliferation of the bacteria. Test sample of toothpaste (0.1% DMSO) into the pits of 6 mm diameter of the seeded agar plates. All the plates were incubated at 37°C for one day. A zone of inhibition was calculated after the conduction of antibacterial activity against *S. Mutans* (14).

Result and Discussion

Organoleptic behavior of toothpaste

Antibacterial susceptibility testing

Toothpaste was examined for antibacterial activity using Kirby-bauer's disk diffusion procedure method as per the CLSI Guideline. 50 µg of the test sample was examined and assessed with Gentamicin. Figures -1.

The results are presented in Table No. 4 and are stated as mean ± standard error of standard deviation.

Tooth decaying is the painful ailments associated with the many prevalent disorders like COVID-19 (15, 16), Monkeypox (17, 18) etc. More than half Indian population are victims of common dental ailments like gingivitis and periodontitis because major food consumption include sugar. To the till date, numerous dosage form either single or combination which are prescribed as traditional healers to suppress the pain & spoiling periodontal disorder in the growing child and aged people. Dabur Red Paste, Dabur Meswak, Dabur Babool, Dabur Lal Dant Manjan, Dant Kanti examples of ayurvedic formulations have been accounted for their potential to reduce inflammation, analgesic and antibacterial action. Due to unwanted adverse effects of antibiotics like disturbance of microbial pleora in oral and gut flora, antibiotics are not the reliable option for the treatment of dental ailments (19, 20). However, bacterial flora streptococci viz. *S. mutans*, and most descriptive human cariogenic oral bacteria are described towards the antibiotic's resistance. These barriers have arisen in research and development for effective antimicrobial dental products. Established ayurvedic formulation has been employed to cure various dental ailments. It is ironic that to the till date could not develop at the present parameter for credit of better compliance along with its authentication and examination.

Depict of Table 4 showed that test when compared with the gentamicin as positive control and DMSO as negative control against gram positive *S. Mutans*. As the concentration increases, zone of inhibition inclines parallel to the positive control and DMSO showed the less zone of inhibition in compared to the test sample. The maximum zone of inhibition in agar plate for the test sample and gentamicin was computed 1.2cm and 1.6cm respectively which proved its significance.

Table.1 List of herbal toothpaste ingredients

Particulars	Part	Qty. (%w/w)	Characteristics
Neem	Leaf	20gm	Active Ingredients
Catechu	Bark	14gm	Digestive and astringent
Charcoal	Crystals	12gm	Binding agents
Liquorice	Root	10gm	Sweetener
Rock salt	Crystals	10gm	Salting agents
Tulsi	Leaf	7gm	Digestive and astringent
Black Paper	Fruit	7gm	Abrasive
Arjuna	Bark	6gm	Humectant
Cinnamon	Bark	5gm	Stimulant
Amla	Fruit	4.5gm	Humectant
Clove	Bud	3gm	Flavouring agent
Mentha	Leaf	1.5gm	Preservative
Distilled water	-	100ml	Vehicle

Table.2 Appearance of toothpaste

S. No.	Characteristics	Appearance
1	Colour	Black
2	Taste	Aromatic
3	Odour	Salty
4	Homogeneity	Uniform
5	Smoothness	Smooth
6	Elegance	Aesthetic
7	Spreadability	6.31±0.112cm
8	Extrudability	69.98± 1.35 (%) by weigh
9	pH	7.5
10	Foaming potential	8.92±0.112ml
11	Dispersion time in water	28± 1.345 min.
12	Volatile matters and moisture	30.72±1.456

Table.3 Assessment of toothpaste characteristics to the standard value reported in BIS.

S. No.	Characteristics	Standard Value	Observation
1	Hard- & Sharp-edged abrasive particle	Nil	Nil
2	Spreadability	8.5	6.31±0.112cm
3	pH	5.5-10.5	7.5
4	Fineness by weight (%)	0.5	0.35
5	Foaming Forming (lowest)	50ml/min.	115ml/min
6	Dispersion time in water (min)	10-30	28

Table.4 Inhibition Zone herbal tooth powder against *S. mutans*

Conc.(%w/v)	Zone of Inhibition(mm)		
	Test	DMSO	Gentamicin
20	0.9±0.88	0.3±0.54	1± 0.55
40	1 ±0.84	0.4±0.55	1.3 ±0.54
60	1.1±0.89	0.5±0.49	1.4 ±0.53
80	1.2±0.87	0.6±0.48	1.6 ±0.58

Figure.1 Antibacterial Activity of Toothpaste, Gentamicin and DMSO against *S. mutans*

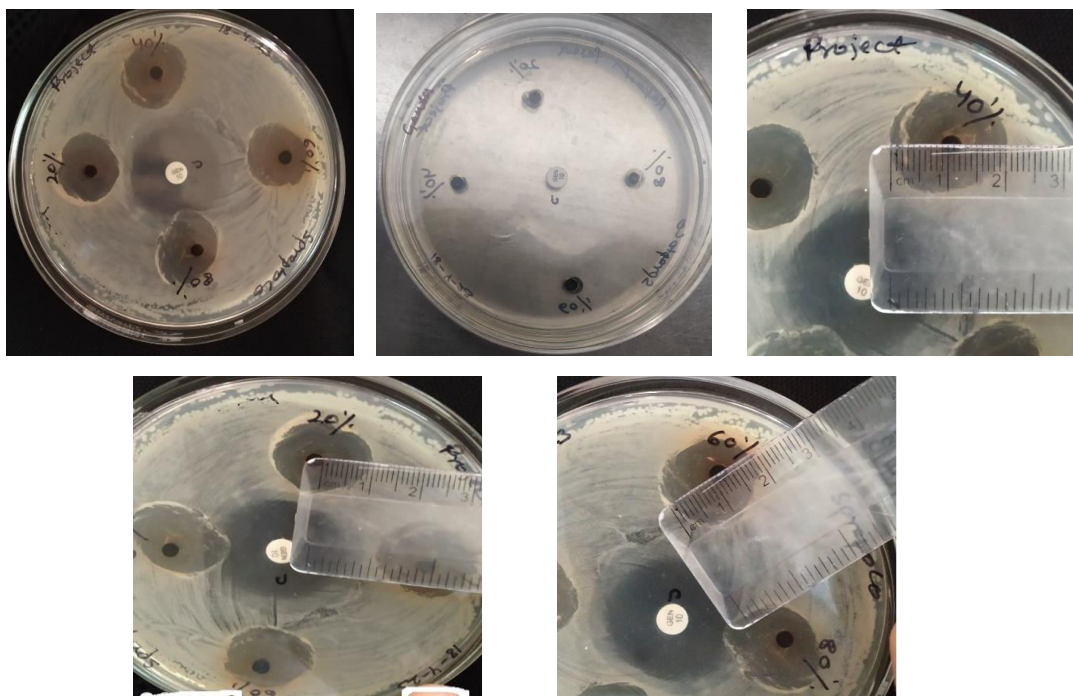


Figure.2 Comparative evaluation of Toothpaste, Gentamicin and DMSO against *S. mutans* (Gram Positive bacteria)

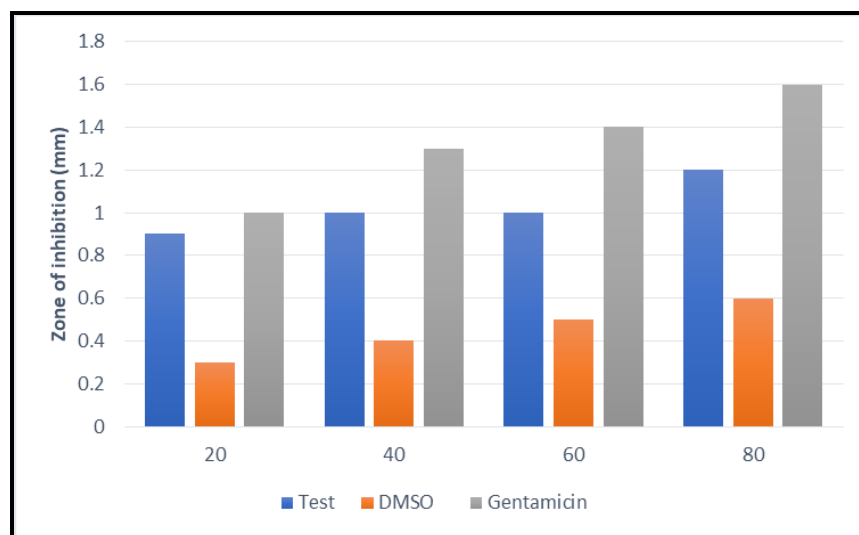


Figure -2 demonstrates the promising antibacterial action that could belong to the occurrence of various phytoconstituents viz. tannins and terpenes. Current study employed the agar disk diffusion techniques for the antibacterial screening of herbal toothpaste. Though, Lowest Inhibition Intensity observed could be used for the development of effective dental products in the forthcoming era.

Conclusion

The prepared toothpaste from the renowned components would proof its better amenability to the patients. It is needed to explore the utilization of the traditional treasure for offering new dental products. Thus, prepared toothpaste could be subjected for future investigation and clinical screening and promising its potency in the aspects of reliable safety with minimum adverse effects.

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