ORIGINAL ARTICLE

# Herbomineral Toothpaste: A Novel Formulation for Dental Caries – An *In Vitro* Study

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## Abstract

**Background:** The demand for a broad spectrum antimicrobial toothpaste is highly desirable to reduce, control, and prevent various types of dental diseases. Therefore, research into the production of herbal toothpaste to suppress the incidence of dental diseases is pertinent. Plant-based tooth paste has proved to be best at treating the gingivitis and other oral problems. **Aim:** In the present study, the herbomineral tooth paste was prepared using 18 different herbal ingredients which possess different uses both internally and externally and also four mineral salts that help to avoid tooth decay and gingival inflammation. The formulated toothpaste was evaluated for physicochemical properties and anti-bacterial activity. **Methodology:** Three different formulations were prepared and evaluated for various physicochemical properties such as stability, pH, homogeneity, compatibility with toothpaste packaging material, spreadability, abrasiveness, foaming ability, cleaning ability, and anti-bacterial activity that was tested on two bacteria, *Staphylococcus aureus* strain (MTCC 96) and *Streptococcus mutans* strain (MTCC 890) in comparison with commercial formulation, Vicco Vajradanth. **Results:** All the results of evaluated parameters showed that experimental formulation (F3) was comparably equal and better when compared with commercial formulation in terms of standards specified by the Bureau of Indian Standards 6356–1993 and found to be of good quality. The results were analyzed using two-way ANOVA. The results of the formulations are promising when compared to a commercial tooth paste. **Conclusion:** This formulation prevented the growth of *S. mutans* and *S. aureus,* organisms causing dental caries. This formulation maintains oral hygiene thereby preventing gingivitis and dental caries.

**Key words:** Herbal extracts, minerals, physicochemical properties

## INTRODUCTION

Toothpaste is a paste or gel dentifrice which is used with a tooth brush, to clean, maintain the aesthetics and health of teeth. It is an irreplaceable agent in effective home care system. There are different problems which are associated with teeth. They are bad breath, tooth decay, gum disease, tooth sensitivity, calculus, dental caries, and dental plaque. As we all know that dental caries is the most common multifactorial oral disease which is due to the demineralization of inorganic portion and destruction of organic substance of tooth that leads to cavitations. Dental caries problem is still a major problem in most industrialized countries as it affects 60–90% school aged children and great number of adults. This is because increase in consumption of sugar and inadequate exposure to fluorides.[1] Hence, in the health sector across the world, dental caries is becoming a major concern.

During the past few decades, the incidence of microbial diseases has amplified drastically. Microorganisms are the major agents responsible for causing dental caries. Many facultative and obligatory anaerobic bacteria dominate the microbial community of dental caries. However, the most important etiological agent of dental caries is *Streptococcus*

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*mutans*.[2] It is an acid producing bacteria and causes fermentation of carbohydrates which results in tooth decay.[3] *Staphylococcus aureus*, next to the *S. mutans,* is a reputed bacteria of many oral diseases, such as oral mucositis, periodontitis, peri implantitis, endodontic infections, and even dental caries.[4-7]

The primary function of the tooth paste is to clean the surface of the teeth and also helps in removal of the food particles, removal of the superficial plaque or stain, and refreshing mouth breath which was formulated with different anti-microbial agents that can prevent degradation of tooth enamel. Now, the advent of modern technology has bought in many branded tooth pastes and mouth washes with the incorporation of synthetic chemicals with antimicrobial activity but they have major drawbacks, such as pigmentation of teeth, weakening of enamel, irritation and oral dryness, scaling of gingival, and negative systemic effects in ingestion.[8-10] Although the modern chemical based tooth paste and mouthwashes have been effective in combating cariogenic microbes, the resistance of these cariogenic microorganisms to few commonly used antibiotics such as penicillin, chloramphenicol, clindamycin, ampicillin, and other antimicrobial chemicals.[11,12] Moreover, thus these factors act as driving force for coming with the herbal based tooth paste with herbal ingredients containing natural antimicrobial agents. Since time immemorial nature is endowed with herbs for human use but the world which is desired for modern development has overlooked the potency of the herbs. The rationale behind preparing the herbomineral tooth paste is to fight against the bacteria that cause various dental problems. The different herbal ingredients used in preparing this tooth paste have reported to have beneficial effects in treating the problems mentioned above.[13-30] Minerals which are used in this formulation are having different properties like citric acid has cleansing effect and whitening of teeth,[31] alum is for whitening and gum disease,[32] salt is for anti-bacterial effect and for cleansing,[33] and zinc chloride is to reduce enamel solubility.[34] Hence, herbomineral tooth paste possess many benefits, being natural and maintaining individual oral health, when compared to the non herbomineral toothpaste. The most non-herbal tooth paste contains trace amounts of chemicals that may be toxic when ingested; tooth paste is not allowed to be swallowed.

## MATERIALS AND METHODS

### Chemicals and plant materials

Calcium carbonate, CMC, hydroxy ethyl cellulose, SLS, glycerin, saccharin, sorbitol, methyl papaben, titanium dioxide, propylene glycol, citric acid, rock salt, alum, and zinc chloride were of analytical grade (SD Fine chemicals) purchased from Sai Krishna Enterprises, Hyderabad.

Plant materials were collected from our college campus and some were purchased in local market. All the plant materials were authenticated by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupati, A.P., India. Authentication numbers are Neem (*Azadiracta indica* – 0447), Jatropha (*Jatropha curcas* – 0919), Kantakari (*Solanum xanthocarpum* – 0697), Myrobalan (*Terminalia chebula* – 0674), Cinnamon (*Cinnamomum zeylanicum* – 0909), Black Pepper (*Piper nigrum* – 0972), Ginger (*Zingiber officinale* – 0980), Clove (*Syzygium aromaticum* – 0547), Ajowan (*Tachyspermum ammi* – 0679), Liquorice (*Glycyrrhiza glabra* – 0675), Black Catechu (*Acacia catechu* – 0599), Ashoka (*Polyalthia longifolia* – 0311), Betel nut (*Piper betle* – 0778), Amla (*Phyllanthus emblica* – 0889), and Tulsi (*Ocimum sanctum* – 0805).

### Extraction of plant materials

Aqueous extracts of Neem leaves, Liquorice roots, Jatropha leaves, Myrobalan fruits, Kantakari aerial parts, Betel nut seed, and Amla fruit were prepared by cold extraction (maceration) process for 3 days followed by filtration and concentration. The extracts were dried in vacuum oven at 60°C. Volatile oil was extracted from Cinnamon bark, Tulsi leaves, Clove flower bud, Ajowan fruits, and Black pepper fruits using Clevenger's apparatus.[35]

### Formulation of toothpaste

Required quantity of calcium carbonate was weighed and mixed with little quantity of water prior to addition of glycerol and made into a paste in a mortar and pestle. The polymers were soaked in small amount of water and added to the above mixture. The ingredients which are present in solid form (particle size <15 μ) except SLS were weighed accurately and mixed with above mixture. The weighed quantities of volatile oils were added. Then, all the plant extracts were dissolved in small amount of water and added to the above mixture till semisolid toothpaste with good consistency was formed. The detergent (SLS) was added last under slow speed agitation to minimize foaming. It is generally in solid form to avoid adding water to the formulation at this stage. All the components were blended until the mixture had approximately the same consistency as commercial toothpaste.[36] Three different formulations were prepared and evaluated. The formula taken for toothpaste preparation is given in Table 1.

### Evaluation of toothpaste

#### *Determination of physicochemical properties*

##### *pH*

The pH of the formulations was measured by the methods[37] described in ISO3996 (Grade 3).

**Table 1:** Composition of herbomineral toothpaste

| S. No. | Name of drug | Category | F1 | F2 | F3 |
|---|---|---|---|---|---|
| 1 | Calcium carbonate | Polishing agent | 45 | 40 | 36.5 |
| 2 | CMC | Binding agent | 1 | 1 | 1.5 |
| 3 | Hydroxy ethyl cellulose | Binding agent | - | - | 1 |
| 4 | SLS | surfactant | 1 | 1 | 1.5 |
| 5 | Glycerin | Humectant | 38 | 30 | 20 |
| 6 | Saccharin | Sweetner | 0.2 | 0.2 | 0.25 |
| 7 | Sorbital | Sweetner | 10 | 10 | 10 |
| 8 | Methyl paraben | Preservative | 0.2 | 0.2 | - |
| 9 | Titanium dioxide | Colour additive | 0.1 | 0.1 | - |
| 10 | Propylene glycol | Texture smoothner | - | - | 5 |
| 11 | Citric acid | Crystals | - | - | 0.5 |
| 12 | Rock salt | Crystals | 2 | 2 | 5 |
| 13 | Alum | Powder | 1 | 1 | 1 |
| 14 | Zinc Chloride | Crystals | 0.6 | 0.6 | 0.6 |
| 15 | Cinnamon | Oil | 0.1 | 0.1 | 0.1 |
| 16 | Clove | Oil | 0.4 | 0.2 | 0.2 |
| 17 | Ajowan | Oil | 0.2 | 0.2 | 0.1 |
| 18 | Black pepper | Oil | 0.2 | 0.2 | 0.1 |
| 19 | Tulsi | Oil | 0.2 | 0.2 | 0.2 |
| 20 | Camphor | Crystals | 0.5 | 0.3 | 0.5 |
| 21 | Menthol | Crystals | 0.1 | 0.1 | 0.3 |
| 22 | Thymol | Crystals | 0.1 | 0.1 | 0.2 |
| 23 | Neem | Extract | 1 | 1 | 1 |
| 24 | Myrobalan | Extract | 0.5 | 0.5 | 0.5 |
| 25 | Kantakari | Extract | 0.5 | 0.5 | 0.5 |
| 26 | Betel leaf | extract | 0.5 | 0.5 | 0.5 |
| 27 | Amla | Extract | 0.5 | 0.5 | 0.5 |
| 28 | Black catechu | Extract | 1 | 1 | 1 |
| 29 | Jatropha | Extract | 0.5ml | 0.5ml | 0.5ml |
| 30 | Liquorice | Extract | 0.25 | - | - |
| 31 | Ashoka | Extract | 0.5 | 0.5 | 0.5 |
| 32 | Ginger | Extract | 0.5 | 0.5 | 0.5 |
| 33 | Acacia | Powder | 1 | 0.5 | 0.5 |
| 34 | Water | - | q.s. | q.s. | q.s. |

### *Homogeneity*

Homogeneity of the formulations was studied by applying normal force to containers at 27 ± 2°C.[38]

### *Tube inertness*

Tube inertness was determined by cutting the tubes and checking internal surface for any signs of deterioration in different storage conditions including normal and heating temperature at 45 ± 2°C for 180 days.[38]

### *Hard and sharp edged particles*

This test was conducted considering the extradition of the content of the tubes and investigating the presence of sharp and hard edged abrasive particles.[38]

### *Stability*

The stability test was performed by storing formulations at 45 ± 2°C for a period of 28 days shall meet the requirements of the standards and which was done according

to the guidelines given by Bureau of Indian Standards (BIS).[39]

### Foaming power

Foaming power was measured to know the foam produced after adding water to the weighed amount of toothpaste at 30°C after 12 complete shakes.[38]

#### *Spreadability*

The spreadability is the term used to express or denote the extent of area to which the paste readily spreads on application area. Spreadability is one of the criteria for the paste to have an ideal quality. 1 g of each sample of experimental and commercial formulations were weighed and placed at the center of the glass plate (10 × 10 cm) and another glass plate was placed over it carefully. Above the glass plates, 2 kg weight was placed at the center of the plate to avoid sliding of the plate. The diameter of the paste was measured in cm, after 30 min for all samples. The averages were reported for all samples after the experiment was repeated for 3 times.[40]

#### *Determination of viscosity*

The viscosities of the formulations were measured at 20, 50, and 100 rpm at 25°C using Brookfield viscometer with spindle number 4.[38]

### Cleaning ability

The cleaning ability of toothpaste was tested on the shells of the eggs which are colored earlier with food colors. Moisten a toothbrush with water and shake off any excess water. One side of an egg with the damp toothbrush is brushed for 5–10 strokes. Inspect the egg to see if any color is removed. It is recommended to use 5–10 brush strokes for each toothpaste.[40]

#### *Determination of antibacterial activity*

*In vitro* anti-bacterial study of formulated and commercial formulations was performed by disc diffusion method in triplicate manner using Mukker Hinton Agar medium against a pathogenic bacterial strains *Staphyloccus aureus* (MTCC 96) and *S. mutans* (MTCC 890). Both organisms were initially cultured cells that were tend to multiple in the Muller Hington agar plates. Then, the formulated and commercial toothpaste in 1:1 dilution were prepared with DMSO solvent and placed over the bacterial plates and incubated at 37°C for 24 h. After incubation, the diameter of zones of inhibition (locations where no growth of bacteria was present) was measured. The mean diameter of the well's measurements (in mm) represented the inhibition value of the tested product.[41]

### Statistical analysis

The difference in the anti-bacterial activity between experimental formulations and commercial toothpaste was analyzed using two-way ANOVA. The differences were considered significant if $P < 0.002$.

## RESULTS

In the present work, we focused on designing of herbomineral therapeutic medicated dental toothpaste for treatment of gingivitis and dental caries. We prepared three formulations by using three different formulas. These pastes were tested for various physicochemical properties such as pH, spreadability, homogeneity, tube inertness, hard and sharp edged abrasive particles, stability, foaming power, and cleaning ability. The formulations were also tested for anti-bacterial activity by standard diffusion method using two organisms, that is, *S. mutans* and *S. aureus.* All the results were compared with commercial formulation, vico vajradanti toothpaste.

Significant results were obtained in physicochemical parameters and found to be within the limits of official standards. Interestingly, there was no significant difference in the physicochemical parameters before and after stability study in all the formulations developed. The results are given in Table 2. pH of the formulations ranged from 7.6 to 8.1. The spreadability and foaming ability of all the formulations ranged from 5.5 cm to 7.5 cm and 45 mL to 55 mL. Abrasiveness was absent in all the formulations. All the formulations showed good cleaning ability.

The viscosity of the prepared formulations and the standard was measured by different spindle speeds at 25°C as shown in Figure 1. The results shown that viscosity significantly decreased as the spindle speed increased.

The antibacterial activity of the prepared formulations and the standard was presented in Figures 2 and 3. Formulation

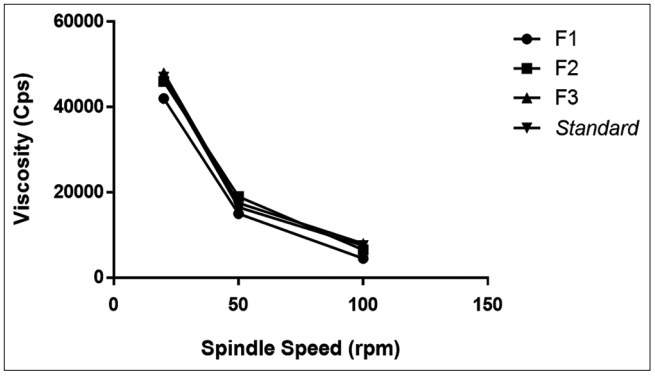


**Figure 1:** Viscosity of experimental and commercial toothpastes. Reduction of viscosity with increase in the spindle speed in experimental and commercial toothpastes

**Table 2:** Measurement of physicochemical parameters

| Evaluation parameter | F1 | F2 | F3 | Standard |
|---|---|---|---|---|
| pH | 8.1 | 7.6 | 7.9 | 7.2 |
| Spreadability (cm) | 5.5 | 6.2 | 7.5 | 4.2 |
| Foaming power (ml) | 45.8 | 50 | 55 | 50 |
| Loss on drying (%) | 7.5 | 6.1 | 5.8 | 3.7 |
| Abrasiveness | Absent | Absent | Absent | Absent |
| Cleaning ability | Good | Good | Good | Good |

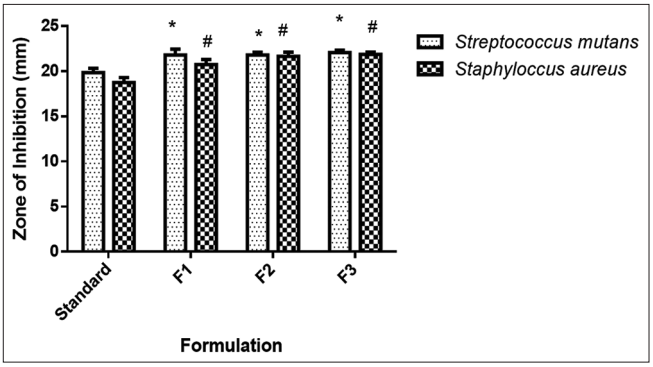


**Figure 2:** Anti-bacterial efficacy of experimental and commercial toothpastes. Effect of anti-bacterial activity of experimental formulations and commercial toothpaste on zone of inhibition. The results are mean ± SE of three parallel measurements ($P < 000.2$). The asterisk (*) denotes that the results are significantly differ from standard in case of *Staphyloccus mutans* and the hash (#) denotes that the results are significantly differ from standard in case of *Staphyloccus aureus*

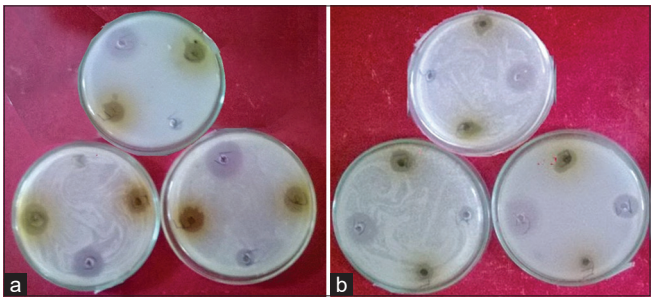


**Figure 3:** Anti-bacterial activity of experimental and commercial formulations against. (a) *Streptococcus mutans* and (b) *Staphyloccus aureus*

F3 shown significant antibacterial activity when compared with standard, vico vajradanti, against both *S. aureus* and *S. mutans* when compared with standard in terms of zone of inhibition.

## DISCUSSION

Herbal extracts have very special attention because of being non-chemical and non-synthetic in nature and are being used in traditional medicine. The craze in using the natural products compared to the synthetic products has being more now a days. Over time, there have been a great number of dentifrices, many aiming at marketing strategies to sell products. Considering that major number of dentifrices available in the market are chemical based where they might have side effects but the herbal dentifrices being non-chemical and non-synthetic have proved to be more effective than the synthetic ones.[42] Dental diseases are most prevalent chronic disorders affecting mankind. Untreated gingivitis can lead to periodontitis. With the progression of time, plaque can spread and grow below the gum line, toxins produced by the bacteria in plaque irritates the gums. The toxins which stimulate a chronic inflammatory response in which the body essence turns on itself and the tissues and bones that support the teeth are damaged which leads to broken down. Gums separate from the teeth, forming pockets that turns into infection. As the disease prolong the spaces deepen and more gum tissue and bones are destroyed. Often, this destructive process has mild symptoms. Eventually, teeth may become loose and result in removal of teeth.[43] The use of herbal dentifrices led to a notable reduction in dental plaque accumulation both on smooth and proximal tooth surfaces. Probably, the active ingredients of the herbal dentifrices go in to the biofilm and prevent plaque accumulation, thereby potentially preventing the colonization of the oral bacteria on the tooth surfaces.

In Ayurveda, Rock salt is the healthiest form of salt and is known since centuries as a wonderful solution for whitening of teeth. It has antiseptic properties that help to fight against tooth decay. This further prevents cavities and strengthens the gums.[33] For teeth and gums related problems, alum is an effective remedy. It helps to avoid tooth decay and also control bleeding gums because of its astringent property.[44] Zinc is one of the essential trace element. Naturally, it is present in the mouth in plaque, saliva, and enamel. It is formulated into oral health products to control plaque and to reduce calculus formation. It has good oral substantivity, and high concentrations can persist for many hours in plaque and saliva following delivery from mouth rinses and toothpastes.[45] Lemon extracts containing the higher amount of citric acid helps to bleach some extents of yellow teeth naturally.

The oral microbial community functions as a host defense by acting as a barrier, for example, by competition for essential nutrients and creation of unfavorable conditions to exogenous organisms that may be infective to the host.

Over 700 bacterial taxa have been found in the oral cavity, but they are not all present in the same mouth region.[46] The composition varies in different sites in the oral cavity, with a large and more diverse bacterial load on the dorsum of the tongue. The most of these microbes are harmless, but under certain conditions, some can cause oral infections such as caries or periodontal disease.[47] Most commonly encountered pathogens are *S. mutans, S. sanguis, A. viscosus*, *S. aureus*, *S.pyogenes, and C. albicans.* Oral streptococci, such as *S. mutans,* are associated with pyogenic and other infections in various sites such as mouth, heart, joints, skin, muscle, and central nervous system.[48] *S. mutans,* acid producing bacteria, aids in fermentation of carbohydrates which results in tooth decay and dental caries.

All the results of the evaluated parameters showed that experimental formulation (F3) is comparably equal and better in terms of results than commercial formulation. The demonstration of antibacterial activity against both Gram-positive bacteria is an indication that the plants are a potential source for production of drugs with a broad spectrum of activities. The study has also shown that these plant extracts are potentially rich source of antibacterial agents. Moreover, this demonstrates their importance in traditional remedies in the rural populations. This study revealed that experimental formulations possess significant antibacterial activity which may reduce the development of dental plaque and dental caries, thereby influencing the adhesion of bacteria to the tooth surfaces.

## CONCLUSION

Following conclusion can be drawn through the results of the present work of investigation. Herbal formulations have progressing demand in the global market. Natural remedies are more acceptable in the belief that they are safer than synthetic ones with fewer side effects. The formulated toothpaste was successfully evaluated using different standard physicochemical parameters and anti-bacterial activity. All the parameters of formulated toothpaste are within the limits specified by BIS and gave better results when compared with commercial formulation. Therefore, the present piece of work is applicable from industrial point of view to formulate localized drug delivery medicated toothpaste formulations. Further research on these dentifrices *in vivo* could provide an insight into the antibacterial properties of the commercially available herbal dentifrices.

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