

**PHARMACEUTICAL AND BIOLOGICAL EVALUATION OF A POLYHERBAL
TOOTHPASTE**Bhagyasri Y.*¹, Ramaiah Maddi², Siva Subramanian N.¹, Pavan Kumar Sara¹ and Suman Vadithya¹¹Department of Pharmacognosy, Gland Institute of Pharmaceutical Sciences, Kothapet - 502220, Telangana, India.²Department of Pharmacognosy, Hindu College of Pharmacy, Amaravathi Road, Guntur – 522002, Andhra Pradesh, India.

*Corresponding Author: Bhagyasri Y.

Department of Pharmacognosy, Gland Institute of Pharmaceutical Sciences, Kothapet - 502220, Telangana, India

Article Received on 05/06/2017

Article Revised on 26/06/2017

Article Accepted on 17/07/2017

ABSTRACT

The demand for herbal based product such as toothpaste is high in these days. Consumers believed by using herbal based toothpaste are safe, effective and less toxicity because less and only safe chemical used as compared to the synthetically produced toothpaste. Therefore, this study was aimed to formulate and evaluate new polyherbal toothpaste for antibacterial activity. In the present study, we are prepared two different formulations for making toothpaste. In formulation-1, three ingredients (Neem leaf, Amla seeds and Tulsi leaves) and in formulation-2 (Clove bud and Neem bark) compared with standard Meswak toothpaste. The formulations were subjected to various pharmaceutical evaluation tests like homogeneity, pH, spreadability, abrasiveness, foaming ability, tube inertness, and stability studies. All the results are within the range of pharmaceutical grade and the formulation is comparatively equal and rarely better than marketed formulation. Hence the selected formulation of toothpaste was found to be ideal for large scale manufacturing.

KEYWORDS: Meswak Herbal Toothpaste, Pharmaceutical Evaluation, Antibacterial Activity.**INTRODUCTION**

Toothpastes have been used since the ancient past^[1] and are one of main irreplaceable components of oral health care.^[2] The design of toothpaste formulations began in China and India, as 300-500 BC. During that period, squashed bone, pulverized egg and clam shells were utilized as abrasives as a part of tooth cleaning.^[3] Modern toothpaste formulations were developed in the 19th century. Later on, chalk and soap were incorporated to those formulations. After 1945, several formulation advancements of different detergents had begun; sodium lauryl sulfate had been used as emulsifying agent.^[2, 3] In recent years, the focus has shifted towards the release of active ingredients during formulation developments to prevent and /or treat oral illness.^[1,3] Toothpaste is a dentifrice used to clean, maintain and improve the health of teeth. Toothpaste is mainly used to promote oral cleanliness and also acts as an abrasive that helps to prevent the dental plaque and food particles from the teeth, aids in the removing and/or veiling of halitosis, and releases active ingredients such as fluoride to aid in preventing tooth and gum disease (eg. Gingivitis).^[4] The majority of the cleaning is performed by the mechanical utilization of the toothbrush with the help of excipients used in toothpaste. The main aim of this investigation is to evaluate the Herbal toothpaste formulations and comparing with commercial toothpastes.

MATERIALS AND METHODS**Materials**

The weight of every each ingredient was decided by review previous study formulation of Herbal toothpaste. The combination of percentage by weight of all the ingredients of this is 100%, which means the sum of quantity of toothpaste will formulate 100gm of toothpaste formulation. The ingredients of all toothpaste formulations are given in table 1, 2 and the marketed herbal tooth paste Meswak was used as a standard.

Preparation of an extract

Neem leaves, Tulsi leaves and Amla fruits (F-1 ingredients)/ Neem bark and clove buds (F-2 ingredients) were taken and washed thoroughly to remove sand and earthy materials. They were shade dried for about 4 days and after proper drying, they were grounded to a fine powder passed through sieve no-6. The powder was packed in soxhlet apparatus and continuously extraction process was done for about 6 hours at 50^o C with ethanol. After the extraction process, the extract was collected and powdered.

Preparation of Toothpaste Base

Caco₃, sodium lauryl sulphate, methyl cellulose in mortar and triturate with a pestle to form a homogenous mixture to that add methyl paraben and propyl paraben and mix continuously. To that mixture add glycerine and mix

properly to form a homogenous mixture and finally make up with a purified H₂O.

Preparation of Polyherbal Toothpaste Formulation-1

(F-1): The standard toothpaste base was formulated. Neem leaves, Tulsi leaves and amla fruits powders were incorporated in the base in various concentrations as given in table 1. All the formulations were filled in regular metal tubes used for toothpastes. The storage in tubes was done to correct the problem of Crusting and drying of extruded toothpaste during evaluation and stability studies.^[6]

Table 1: Polyherbal Toothpaste F-1 Ingredients

S. No.	Ingredients	Quantity used (%)
1	Neem leaf extract	15
2	Tulsi leaf extract	15
3	Amla fruit extract	15
4	Glycerine	30
5	Methyl cellulose	1.00
6	Calcium carbonate	40
7	Sodium lauryl sulphate	1.50
8	Methyl paraben	0.10
9	Propyl paraben	0.02
10	Purified water	q.s

Preparation of Toothpaste F-2

The standard toothpaste base was formulated. Neem bark and clove bud powders were incorporated in the base in various concentrations as given in table 2. All the formulations were filled in regular metal tubes used for toothpastes. The storage in tubes was done to correct the problem of Crusting and drying of extruded toothpaste during evaluation and stability studies.^[6]

Table 2: Polyherbal Toothpaste F-2 Ingredients

S. No.	Ingredients	Quantity used (%)
1	Neem bark extract	15
2	Clove buds extract	15
3	Glycerine	30
4	Methyl cellulose	1.00
5	Calcium carbonate	40
6	Sodium lauryl sulphate	1.50
7	Methyl paraben	0.10
8	Propyl paraben	0.02
9	Purified water	q.s

Evaluation of Formulated Toothpaste^[8]

According to the guidelines, the standards were prescribed for each evaluation test of non-fluorinated (type I) or fluorinated (type II) toothpastes. Composition Toothpaste is not composed of mono or disaccharides such as sucrose or fermentable carbohydrates. All ingredients should comply with the Indian standards.

Homogeneity: The toothpaste shall extrude a homogenous mass from the collapsible tube or any

suitable container by applying of normal force at $27\pm 2^{\circ}\text{C}$. In addition, bulk of contents shall extrude from the crimp of container and then rolled it gradually.

Tube inertness

The toothpaste container shall not produce any corrosion or deterioration in normal storage conditions like heating temperature at $45\pm 20^{\circ}\text{C}$ for ten days. Tube inertness can be examined by cutting the internal surface, open and observing whether any sign of deterioration or chemical attack occurred in the container.

Determination of sharp and edge abrasive particles

Extrude the contents 15-20 cm long on the butter paper, repeat the same process for at least ten collapsible tubes. Press with the contents of the entire length with fingertip for the presence of sharp and hard edged abrasive particles. Toothpaste shall not contain such particles.



Figure 1: Determination of Sharp and Edge abrasive Particles

Determination of spreadability: One gram of toothpaste placed on a glass slide (10 x 10 cm), cover with another glass slide. Then carefully place two kg weight of on covered glass slide (sliding, shall not take place). Measure the spreading (in cm) of the toothpaste after 3 minutes. Repeating the experiment and note the average value of three readings.

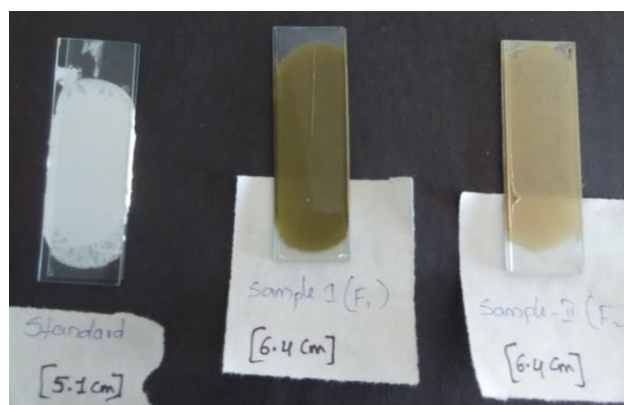


Figure 2: Determination of Spreadability of Standard (5.1 cm), F-1 (6.4 cm) and F-2 (6.4 cm).

Determination of fineness: Weigh accurately about 10 g of toothpaste placed in a 100 ml beaker. Allow 50 ml of

water; stand for 30 min with stirring until the paste gets completely dispersed. Transfer the solution to 150 micron IS sieve and washes with a slow stream of tap water. Allow running tap water drained the on sieve and dry (at $105\pm 20^{\circ}\text{C}$) the sieve by place it in an oven. Transfer any residue particles are present on the sieve to a watch glass and weigh it.

Calculation

i. Material on the sieve = % by (Retained mass / Material taken) x 100.

ii. Weigh accurately about 10 g of toothpaste placed in a 100 ml beaker. Allow 50 ml of water, stand for 30 min with Allow 10 ml of boiled and then cooled water. Stir vigorously to make a suspension. Measure the pH of the suspension using pH meter.

Foaming power

Take a suspension of the material in measuring cylinder and shake the suspension for 12 times. And measure the volume of the foam produced after shaking for 5 minutes. Procedure: weigh 5 g of toothpaste in a 100 ml glass beaker. Add 10 ml of water cover the glass beaker with a watch glass and stand for 30 minutes. Heat the suspension gently to dissolve the detergent if present in it. Stir the suspension with glass rods and transfer it to 250 ml measuring cylinder. Examine if no foam is produced (more than 2 ml). Transfer the residue retained in the beaker to measuring cylinder by adding of 56 ml of water. Then make up the cylinder with 50ml of water. Stir the contents with up-down movements to get uniform suspension at 30°C . After shaking, keep the cylinder stand for 5 minutes and finally note the volume obtained with foam and water.

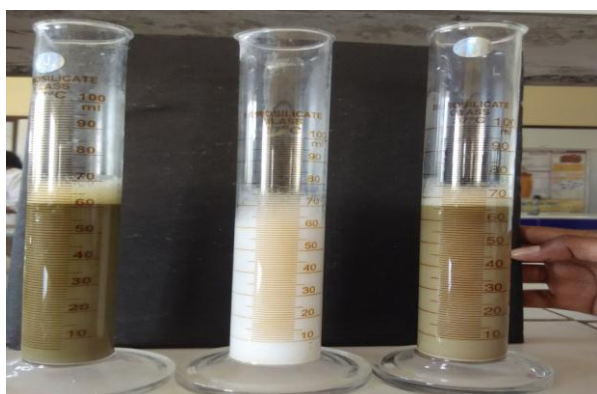


Figure 3: Foaming power of F-1, Standard and F-2.

Stability

The toothpaste shall be stable, but not to be deteriorating, ferment and segregate during normal storage conditions and usage. Stability of toothpaste can be tested when it exposes to $45\pm 20^{\circ}\text{C}$ for a period of 28 days. After storage, no phase separation, fermentation and gassing can be observed. Also exposed to cool conditions such as 5°C for 1 hour, no obstruction of extrudable form from the container is observed.

ANTIBACTERIAL ACTIVITY^[6,7]

Table 3: Preparation of agar medium

Ingredients	For 250ml
Beef extract	0.75g
Peptone	1.25g
Agar	0.75g
PH	7 ± 0.2
Dist.H ₂ O	250ml

Procedure

Weigh accurate amount of beef extract and peptone in a beaker and add water 50 ml to dissolve it and prepare a solution .To the above solution adjusts the PH to 7 ± 0.2 . After that make up the solution with water upto 250ml and add agar medium to above solution and heat it to dissolve agar medium in a solution. Transfer the solution in a conical flask and it is tightly packed with cotton and kept it for auto clave for 3hrs. Take Petri plates, to that petri plates agar medium is poured in that after that bacteria's are inoculated .samples are placed on that agar medium by disk diffusion method [Disk size 5mm]. Inoculated by swab method.

RESULTS AND DISCUSSION

Many commercial types of toothpaste claim to have abrasive, spreadability, foaming ability and have caries counteractive action, very little research has been conducted to compare these properties in lab made dental product. Hence the purpose of this study was to evaluate and compare the efficacy of commercial toothpastes with lab made toothpaste. Evaluation tests of toothpastes were carried out according to the standards specified by the Bureau of Indian standards IS 6356-1993 (table.3) for herbal tooth pastes samples (Meswak) and lab made toothpaste sample. All the samples were complied with BIS and they found to be of good quality. Evaluation tests were carried out to view the different properties of lab made and commercial toothpastes. All the results of evaluating parameters were given in table 4 in the present study, comparatively equal and rarely better results have been observed with lab made formulation than marketed formulations. Both preparations have shown equal efficacy in terms of foaming ability and pH. But increased activity in terms of abrasiveness and spreadability was appeared in lab made formulation (figure 1). Comparison of the abrasiveness of marketing pastes with lab made formulation suggests that lab made formulation has more abrasiveness than marketed pastes. And also, given significant result for cleaning ability which is similar to the results obtained in the commercial formulations. All the toothpastes were having good consistency and smooth texture. Also shown no symptoms for deterioration such as phase separation, gassing, fermentation when all the samples were placed at a temperature of $45\pm 2^{\circ}\text{C}$ for a period of 28 days. We found extrudable from the all tubes after placing it in cool temperatures (5°C) for 1 hour. So it confirmed that all toothpastes have good stability. The internal part of all collapsible tubes has given no sign of corrosion or damage during normal storage conditions at a

temperature of $45\pm 2^{\circ}\text{C}$ for 10 days. So it was confirmed that the containers of lab made, Meswak have shown good tube inertness. The preferable amount of residue has retained on sieve for lab made formulation which is better than the residue obtained by Himalaya Herbal and Meswak toothpastes but little less to the residue of Dant kanti toothpaste. So it was found that lab made preparation has shown reasonably good % of fineness (figure 2). The moisture and volatile matter present in Himalaya Herbal was significantly more than the rest of the formulations. The % of moisture and volatile content in lab made formulation is given the same value as that of Dant kanti and Meswak got the least value. These results explaining all the formulations have good

moisture and volatile content (figure 3). The colour produced with hydrogen sulfide in test solution is less than obtained with standard solution that indicates all the samples have limited amount of lead impurities. Stain produced by Himalaya Herbal sample is more than standard stain that indicates Himalaya Herbal toothpaste is having a little more amount of arsenic impurities. All the remaining formulations passed the limit test of arsenic. Fluoride ions present in the sample were potentiometrically determined by fluoride ion sensitive electrodes. The concentration (ppm) of fluoride ion in lab made formulations is less than the standard values mentioned in table.

Table 4: Evaluation Tests for Lab made and Commercial Herbal Toothpastes

S. No.	Properties	Standard (Meswak toothpaste)	F-1	F-2
1	Hard and sharp edged abrasive particles	Absent	Absent	Absent
2	Spreadability (cm)	5.1cm	6.4cm	6.4cm
3	pH of 10% aq. suspension	7.4	8.6	8.8
4	Foaming ability	74	76	78
5	Tube inertness (at $45\pm 20^{\circ}\text{C}$ for 10 days)	No corrosion	Slightly corrosion	No corrosion
6	Arsenic	Within limits	Exceeding limit	Within limit
7	Stability	Good	Good	Good

Table 5: Antibacterial Activity (Zone of Inhibition) of Standard, F-1 and F-2

Organism	F-1	F-2	Standard
<i>Bacillus subtilis</i>	26mm	25mm	80mm
<i>Pseudomonas aeruginosa</i>	27mm	28mm	40mm

CONCLUSION

Eventually Herbal toothpastes having an emphasized role in the maintaining the oral hygienic nature as well as preventing dental caries. Based on this pattern, lab made Herbal toothpaste was formulated by selecting suitable ingredients to get the formulation more stable. Evaluation and comparison of results with commercial Herbal toothpaste are demonstrated that lab made toothpaste is having equal patronizing and engrossing passion over the marketed formulations (Meswak). The marketed Herbal tooth pastes and lab made Herbal toothpaste which had been evaluated compared with the standards specified by Bureau of Indian standards. This preliminary in vitro study demonstrated that lab made Herbal toothpaste was equally efficacious as commercially popular toothpaste in terms of all evaluation properties of toothpaste. Hence, by the evidence of in vitro studies, it is concluded that lab made Herbal toothpaste formulated in a laboratory was found to be of good quality.

REFERENCES

- Davies R, Scully C, Preston A.J. Dentifrices- an update. *Medicina Oral Patologia Oral y Cirugia Bucal*, 2010; 15(6): 976-82.
- Ersoy M; Tanalp J; Ozel E; Cengizlier R; Soyman M. The allergy of toothpaste: a case report. *Allergologia et Immunopathologia*, 2008; 36(6): 368-70.
- Jardim J, Alves L, Maltz M. The history and global market of oral home-care products. *Brazilian Oral Research*, 2009; 23(1): 17-22.
- The History of Toothpaste and Toothbrushes. *Bbc.co.uk*. Retrieved on April 4, 2013.
- Abirami CP, Venugopal PV. In vitro evaluation of antifungal activity of toothpastes. *Journal of Medical Mycology*, 2005; 15: 247-249.
- Vidya Namdeo Dange, Magdum CS, Mohite SK; Nitlikar M. Review On Oral Care Product: 45.Sachin patil, formulation of toothpaste from various and extracts of tender twigs of neem, *Journal of Pharmaceutical Research* 2008; 1(2): 148-152.
- Bipul Biswas, Kimberly Rogers, Fredrick Mc Laughlin, Dwayne Daniels and Anand Yadav. Antimicrobial Activities of Leaf Extracts of Guava (*Psidium guajava* L.) of Two Gram-Negative and Gram-Positive Bacteria, *International Journal of Microbiology* 2013; 1: 1-7.
- Abirami CP, Venugopal PV. In vitro evaluation of antifungal activity of toothpastes. *Medical Mycology Journal* 2005; 15: 247-249.