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# FORMULATION, DEVELOPMENT, AND EVALUATION OF HERBAL TOOTHPASTE USING SUGARCANE JUICE EXTRACT

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**Abstract**

Herbal toothpaste formulated with natural ingredients is increasingly favored by public opinion over chemical-based synthetic alternatives in the current dental care landscape, primarily due to its perceived safety and effectiveness in reducing dental caries and preventing other dental conditions prevalent in today's society. This study introduces the use of Sugarcane juice extract, Neem, Babul, and Turmeric extract, which have not been previously utilized in any research. These extracts possess anti-caries, antibacterial, and wound-healing properties, along with unique characteristics such as anti-decay and antifungal properties.

The evaluation of the herbal toothpaste included key physical attributes such as pH, stability, extrudability, spreadability, foamability, and homogeneity to enhance the product's effectiveness and stability. The objective of this project is to formulate and assess herbal toothpaste.

The findings from this research indicate that our herbal-based toothpaste formulation with natural ingredients delivers excellent performance.

**Keywords**: **Sugarcane Juice Extract Toothpaste, Herbal Toothpaste.**

## I. INTRODUCTION

Herbal and herbal-based toothpastes have been an integral part of oral health care practice for centuries. Historical records suggest that the formulation and manufacturing of toothpaste can be traced back to 300-500 BC in China and India, where early formulations utilized abrasives such as crushed bone, ground eggshells, and clamshells for cleaning teeth. The development of modern toothpaste compositions emerged in the nineteenth century, incorporating ingredients like chalk and soap due to advancements in medical knowledge.[3] Following the emergence of independence, various formulations of detergents were developed, with sodium lauryl sulfate being utilized as an emulsifying agent.[16-20] In contemporary times, the emphasis has shifted towards the release of active substances during the formulation process to prevent and treat oral diseases.[16-18] Toothpaste serves not only to maintain oral hygiene but also functions as an abrasive that effectively removes dental plaque and food particles, aids in addressing halitosis, and delivers active ingredients such as fluoride to help prevent dental and gum diseases. [1] The primary cleaning action is achieved through the mechanical action of the toothbrush, supplemented by excipients present in the toothpaste. Many herbal formulations demonstrate effectiveness due to their inclusion of active chemical components such as polyphenols, gums, alkaloids, and glycosides. [21,22,24] Ongoing research has explored various biological functions of these formulations, thereby broadening the potential for developing and testing innovative herbal toothpaste products. The primary objective is to formulate and evaluate herbal toothpastes. [5]

Sugarcane (Saccharum officinarum) is a tall, perennial grass utilized for sugar production, typically growing to heights of 2–6 meters (6–20 feet) with robust, jointed, fibrous stalks high in sucrose. Sugarcane juice is a nutrient-rich substance that possesses various medicinal and pharmacological benefits. It contains essential minerals such as calcium and phosphorus, which contribute to strengthening tooth enamel and protecting against decay. Additionally, the abundance of nutrients in sugarcane juice can help combat bad breath, often resulting from nutrient deficiencies. [7]

**What if Sugar (Sucrose) in Sugarcane juice causes microbial growth?**

To overcome the problem of microbial growth due to the presence of sugar concentration we used the principle that helps to inhibit microbial growth. High sugar concentrations causes the bacterium to lose water by osmosis and it doesn't have any cellular machinery to pump it back in against the osmotic gradient. Without enough water, the bacteria can't grow or divide. [23]

The substantial amounts of nutrients in sugarcane juice help fight bad breath that can occur due to nutrient deficiency.[7]

## II. Material and Method

### 1. Collection:

Mature sugarcane stems were sourced from local vendors. Upon arrival at the laboratory, the stems were thoroughly cleaned, hand-peeled, and divided into three equal sections, each measuring approximately 40 cm, for use in the experiment. The sugarcane juice was extracted using a powered sugarcane crusher. The extracted juice was then filtered through a double sieve and muslin cloth to eliminate any impurities and subsequently dried in a water bath. To ensure the purity of the final product, commercial sugarcane juice powder was utilized, and phytochemical screening was conducted thereafter.

A total of 100 grams each of turmeric, Babul bark, and neem leaves were separately combined with 100 milliliters of distilled water. This mixture was then heated at a temperature of 60-70°C for a duration of 15 minutes using the decoction method. After heating, the mixture was filtered using muslin cloth and subsequently dried in an oven at 50°C for two days. The dried material was then passed through a sieve with a mesh size of 80. This procedure was repeated for three batches. [20]

The weight of each ingredient was established based on findings from a previous study examining the composition of herbal toothpaste. The combined weight percentage of all ingredients in this formulation equals 100%, indicating that the total quantity produced will be 20 grams of toothpaste.[5]

### 2. Qualitative phytochemical analysis of Sugarcane Juice extract:[14]

#### 2.1. Carbohydrates Test:

A small portion of the extract is dissolved in water and treated with Fehling's reagents to detect the presence of different sugars. Fehling's Reagent A & B gives the Red Precipitate which Confirms the presence of Reducing sugars.

#### 2.2. Detection of alkaloids:

The small portions of solvent-free chloroform, alcoholic and water extracts are stirred separately with a few drops of diluted hydrochloric acid and filtered. The filtration may be assessed carefully with various alkaloidal reagents.

i. Hager's reagent (yellow precipitate) and

ii. Dragendorff's reagent (Orange-brown precipitate).

#### 2.3. Detection of saponins:

About 1 ml of alcoholic and aqueous extracts are diluted separately with distilled water to 20 ml and shaken in a graduated cylinder for 15 minutes. One cm layer of foam indicates the presence of saponins. [1]

#### 2.4. Detection of Tannins:

A small portion of the extract is dissolved in water and treated with Lead Acetate; it gives the white Precipitate confirming the presence of Tannins.

#### 2.5. Flavonoids:

A small portion of the extract is dissolved in water and diluted HCL, the solution of the extract turns colorless to detect the presence of flavonoids.

**Table 1: Qualitative phytochemical analysis of Sugarcane Juice extract [26]**

| Sr No. | Phytochemicals | Tests | Observation | Inference |
|---|---|---|---|---|
| 1. | Alkaloids | Hager's reagent | Yellow precipitate | Present |
| | | Dragendorff's tests | Orange, brown ppt | Present |
| 2. | Saponins | Foam Test | A layer of foam present | Present |
| 3. | Tannins | Lead acetate | Formation of white ppt | Present |
| 4. | Carbohydrates | Fehling's test | A brick-red coloured ppt | Present |
| 5. | Flavonoids | Alkaloid reagent test | Colourless | Present |

## III. Method of Preparation of Herbal Toothpaste:

The dry gum method has been used during the formulation of this toothpaste. For the preparation of the base, solid ingredients sodium lauryl sulphate, and citric acid, were weighed accurately as mentioned in the formula and sieved with sieve no.80 to maintain the particle size. These ingredients were also mixed in a mortar and pestle, then triturated with precisely weighed glycerin until a semisolid substance was created. Addition of herbal ingredients-Accurately weighed herbal extracts of Sugarcane juice Powder, Turmeric Extract Powder, Babul Extract Powder, and Neem Extract Powder were added to the base. In the end, Clove Oil and Mentha oil were added as a flavor. [2]

**Table 2: Ingredients for 20 gm of toothpaste**

| Sr No. | Ingredients | Concentration | Uses |
|---|---|---|---|
| 1 | Sugarcane juice Extract | 3.5 gm | Anti- decay |
| 2 | SLS | 2 gm | Foaming agent |
| 3 | Turmeric Extract | 0.5 gm | Preservatives |
| 4 | Neem Extract | 2 gm | Anti- microbial |
| 5 | Babul Extract | 2 gm | Inflammatory |
| 6 | Mentha Oil | 2 ml | Cooling agent |
| 7 | Clove Oil | 2 ml | Flavour |
| 8 | Citric acid | 2 gm | Whitening agent |
| 9 | Glycerin | 2 ml | Humectant |
| 10 | Water | q.s. | Water |

## IV. Evaluation of herbal Toothpaste: [11-13]

### 4.1. Physical examination (colour, odour, taste, and smoothness):

The color of the toothpaste was evaluated through visual inspection. The presence of any odor was assessed through olfactory examination. A sensory evaluation of the formulation's taste was conducted manually. The texture of the paste formulation was confirmed by tactile assessment using finger application.

### 4.2. pH:

To prepare a 50% aqueous suspension, transfer 10 grams of toothpaste from the container into a 50 mL beaker. Then, add 10 mL of freshly boiled and cooled water at a temperature of 27°C. To achieve a uniform suspension, stir the mixture thoroughly. After allowing it to sit for 5 minutes, measure the pH of the suspension using a pH meter.

### 4.3. Homogeneity:

By applying normal force at 27°C, the toothpaste should consistently extrude a homogeneous mass from the collapsible tube or an appropriate container. Additionally, the majority of the contents should be expelled from the crimp of the container in a gradual manner.

### 4.4. Sharp and edge abrasive particles:

To ensure the absence of any sharp or abrasive particles, the contents were applied to a finger and then gently scratched against butter paper for a distance of 15-20 cm. This procedure was conducted a minimum of ten times, and no sharp or abrasive particles were detected.

### 4.5. Foamability:

The foaming capacity of the herbal toothpaste was assessed by combining 2 grams of toothpaste with 5 milliliters of water in a measuring cylinder and shaking the mixture ten times. The total volume was then recorded.

### 4.6. Determination of Spread ability:

The slip and drag characteristics of the paste are utilized to assess its spreadability. Approximately 2 grams of herbal toothpaste were measured and positioned between two glass slides, which were subsequently moved in opposite directions. After allowing the paste to settle for 2-3 minutes, the diameter of the spread was measured in centimeters. This experiment was repeated, and the average of three readings was calculated. The total circumference of the spread toothpaste was recorded as 18.84 cm.

**Table 3: Description of evaluation of formulated toothpaste**

| Sr No. | Parameters | Observation |
|---|---|---|
| 1 | Colour | Brownish |
| 2 | Odour | Characteristic |
| 3 | Spread ability | Easily spreadable |
| 4 | Stability | Stable |
| 5 | Abrasiveness | Good |
| 6 | Foam ability | Good |
| 7 | pH | Acidic |
| 8 | Viscosity | Good |
| 9 | Taste | Slightly Bitter |
| 10 | True extrudability | Good |

## V. Microbial Studies

### 5.1. Antibacterial activity against toothpaste

Antibacterial activity against Staphylococcus aureus and Escherichia coli was assessed using the well diffusion method. The inoculums were prepared from the respective bacterial cultures. A total of 15 mL of nutrient agar (HiMedia) was poured into clean, sterilized Petri dishes and allowed to cool and solidify. Subsequently, 100 µL of the bacterial broth was pipetted onto the agar surface and spread evenly using a spreading rod until it dried completely. Once the agar had solidified, sample slides were placed on the agar surface, and the plates were incubated at 37°C for 24 hours. The antibacterial activity was evaluated by measuring the diameters of the zones of inhibition (ZI). [8-9]

**Table 4: Antibacterial Activity of Samples against *E. coli.***

| Sr No. | SAMPLES | CONCENTRATION (mg/ml) | ZONE IN DIAMETER (mm) |
|---|---|---|---|
| 1 | Control | | |
| 2 | Standard(streptomycin) | 1 | 32 |
| 3 | Formulated herbal toothpaste | 5 | 08 |
| | | 10 | 15 |

**Fig 1. Antibacterial Activity Of Samples Against *E. Coli***

**Table 5: Antibacterial Activity of Samples against *Staphylococcus Aureus***

| Sr No. | SAMPLES | CONCENTRATION (mg/ml) | ZONE IN DIAMETER (mm) |
|---|---|---|---|
| 1 | Control | | |
| 2 | Standard (streptomycin) | 1 | 34 |
| 3 | Formulated herbal toothpaste | 5 | 04 |
| | | 10 | 06 |

**Fig 2. Antibacterial Activity Of Samples Against *Staphylococcus Aureus***

**5.2. Antifungal activity**

**Preparation of Stock Solution for Antifungal Activity**: For the antifungal study, transdermal patches were cut into various sizes using a punching machine and subsequently stored in a refrigerator until needed. The antifungal efficacy of the different patches was assessed through a disc diffusion assay. This assay was conducted utilizing Sabouraud dextrose agar (HiMedia) for fungal growth. The medium, prepared with an acidic pH of 5.5 to 5.6, contained a relatively high concentration of glucose (40%). To prepare the medium, Sabouraud dextrose agar (SDA) was mixed with distilled water and autoclaved at 121°C for 15 minutes. Aseptic transfer of 25 ml of molten SDA medium (at 45°C) into each 100mm×15mm sterile Petri dish was performed. For counting the fungal spores, a suspension was prepared in normal saline to a final volume of 1 ml, which was then counted using a hemocytometer (Neubauer chamber). After the agar solidified, transdermal patch discs were placed on the plates alongside a control (0.2% DMSO) and a standard (fluconazole 1 mg/ml). The plates were incubated for 72 hours at 29°C. Antifungal activity was assessed by measuring the diameter (in mm) of the clear zone of growth inhibition.[9]

**Table 6: Antifungal Activity of Samples against *C. Albicans***

| Sr. No. | SAMPLES | CONCENTRATION (mg/ml) | ZONE IN DIAMETER (mm) |
|---|---|---|---|
| 1 | Control | | |
| 2 | Standard (fluconazole) | 1 | 20 |
| 3 | Formulated herbal toothpaste | 5 | 15 |
| | | 10 | 28 |

**Fig 3. Antifungal Activity of Samples against *C. Albicans*.**

**Table 7: Antibacterial Activity of Samples against Lactobacilli.**

| Sample | Lactobacilli (1.1 * $10^6$) (Zone of inhibition in mm) | Antibacterial activity Observed/ not observed |
|---|---|---|
| **Formulated toothpaste** | **37.70mm** | **Observed** |

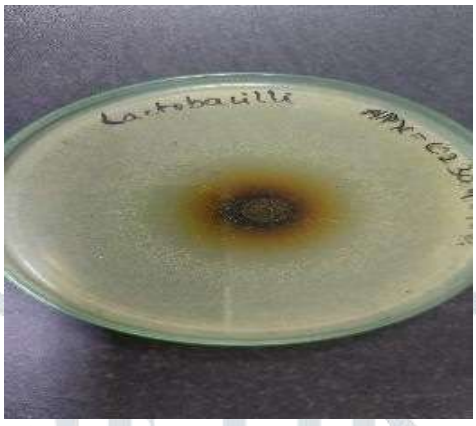


**Fig 4. Antibacterial Activity of Samples against *Lactobacilli* Culture**

| Antibiotics | Test Organisms | Inhibition at 1.8grams | Zone (mm) at 2.4grams | Average Inhibition Zone (mm) |
|---|---|---|---|---|
| Ampicillin | *Streptococcus mutans* | 23 | 26 | 24.5 |
| | *Staphylococcus epidermidis* | 21 | 25 | 23.0 |
| | *Lactobacillus acidophilus* | 23 | 24 | 23.5 |
| | *Enterobacter sp* | 20 | 22 | 21.0 |
| | *Klebsiella pneumonia* | 22 | 23 | 22.5 |
| | | | | Mean: 22.90 |
| Tetracycline | *Streptococcus mutans* | 26 | 30 | 28.0 |
| | *Staphylococcus epidermidis* | 23 | 25 | 24.0 |
| | *Lactobacillus acidophilus* | 24 | 25 | 24.5 |
| | *Enterobacter sp* | 22 | 24 | 23.0 |
| | *Klebsiella pneumonia* | 24 | 28 | 26.0 |
| | | | | Mean: 24.50 |
| Chloramphenicol | *Streptococcus mutans* | 28 | 33 | 30.5 |
| | *Staphylococcus epidermidis* | 24 | 28 | 26.0 |
| | *Lactobacillus acidophilus* | 22 | 27 | 24.5 |
| | *Enterobacter sp* | 23 | 28 | 25.5 |
| | *Klebsiella pneumonia* | 27 | 30 | 28.5 |
| | | | | Mean: 27.00 |

**Fig 5. Standard results of antibiotics against organisms**

## VI. Results and Conclusion:

The following conclusion can be derived from the results obtained in the study.

Sugarcane has been identified as an effective active ingredient in toothpaste. Comprehensive evaluation studies indicate that this herbal toothpaste demonstrates promising benefits.

The formulated herbal toothpaste was tested for antibacterial activity against bacterial strains, specifically *Staphylococcus aureus* and *E. coli*. At a concentration of 10 mg/ml, it exhibited favorable activity in comparison to the control standard.

Additionally, the herbal toothpaste was assessed for antifungal activity using the fungal strain *Candida albicans*. At the same concentration of 10 mg/ml, it also showed commendable effectiveness relative to the standard.

Furthermore, anti-decay activity tests were conducted using a *Lactobacilli* culture. The results indicated a significant zone of inhibition against this culture.

In conclusion, the formulated herbal toothpaste not only displays anti-decay properties but also exhibits antimicrobial activity, aligning with its intended cleansing effects.

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