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STANDARDIZATION OF PROTOCOLS FOR THE DEVELOPMENT OF GUAVA LEAF TOOTH POWDER FOR A RADIANT SMILE

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ABSTRACT

Guava leaf toothpowder is a natural dental care product that harnesses the power of guava leaves for oral hygiene. Enriched with the goodness of guava leaves, this toothpowder offers a holistic approach for maintaining dental health. Known for their anti-microbial and anti-inflammatory properties, guava leaves help combat bacteria, plaque, and gum issues. The herbal formulation aims to provide a refreshing and effective alternative to conventional toothpaste, promoting a clean and healthy oral environment. Incorporating guava leaf toothpowder into your dental routine can contribute to a naturally vibrant and confident smile. The present study was carried out to study the effect of variation in concentration of guava leaf powder on toothpowder formulations. Study included the development of toothpowder formulations (T₁–Control, T₂–5% guava leaf powder, T₃–10% guava leaf powder, T₄–15% guava leaf powder, T₅–20% guava leaf powder) and evaluation of parameters. T₅ (20% guava leaf powder) was analyzed as the best formulation with pH (6.9), angle of repose (31.62°), bulk untapped density (37.16 g ml⁻¹) and bulk tapped density (27.6 g ml⁻¹) along with maximum shelf life period of two years. T₁ (Control) was considered as the best formulation with regard to loss on drying (7.0%) and foaming power (1.0 ml). Among the five formulations, T₅ with 20% guava leaf powder secured the maximum sensory scores for colour (9.0), odour (9.0), taste (8.9) and overall performance (9.0) followed by T₄ (15% guava leaf powder) (colour – 8.7, odour - 8.6, taste – 8.8 and overall performance -8.8). Microbial analysis shows that, maximum zone of inhibition was reported in methanol solvent for *Proteus vulgaris* (11.0 mm) in the sample. Cost of production of 1kg of guava leaf toothpowder was estimated with the net profit of Rs. 257.6 and the benefit cost ratio for developing toothpowder was 1.75. The formulated toothpowder was evaluated for its physicochemical parameters like pH, bulk density, flow property, per cent loss on drying, foaming ability and was found to be suitable for maintaining oral hygiene.

Key words: Bacteria, Oral health, Toothpowder, Bulk density.

Introduction

In the pursuit of holistic well-being, individuals are increasingly turning to nature's bounty to address their health concerns, and oral care is no exception. One such natural remedy gaining popularity is guava leaf toothpowder, harnessing the powerful properties of guava leaves for dental hygiene. As societies gravitate towards sustainable and organic alternatives, this herbal

toothpowder emerges as a promising solution, providing an amalgamation of traditional wisdom and modern science. Guava leaves, scientifically known as *Psidium guajava*, have long been celebrated for their therapeutic benefits in various traditional medicinal practices.

Rich in antioxidants, anti-inflammatory compounds, and antimicrobial agents, guava leaves possess unique qualities that make them an ideal candidate for promoting

oral health. As a testament to their effectiveness, the infusion of guava leaves into dental care has given rise to innovative products like guava leaf toothpowder, offering a natural alternative to conventional toothpaste. This article delves into the intriguing world of guava leaf toothpowder, exploring the science behind its efficacy, and the potential benefits it brings to oral care routines.

Broetz-Oesterhelt *et al.*, (2005) reported the anti-bacterial effect of guaijaverin from guava leaves as an inhibitor against *Staphylococcus aureus* and *Staphylococcus mutans*. Since ancient times, tender guava leaf paste has been used traditionally to maintain good oral hygiene. The null hypothesis of this study states that, the additional use of guava leaf mouth rinse may be equally effective as 0.2% CHX mouth rinse in the reduction of clinical parameters (Gingival index, Plaque index). Mittal *et al.*, (2010) found that guava leaf is rich in two major flavonoids namely, quercetin and guaijaverin, well known for its anti-bacterial actions. In Ayurveda, several natural herbs like aloe vera, turmeric, calendula, etc. are most effectively used to cure a range of oral diseases. (Somu *et al.*, 2012).

The superior activity against plaque and gingivitis of toothpowder than with toothpaste has been reported by Khan (2017). Shaheena *et al.*, (2019) reported that three formulations of guava toothpaste (F_1 , F_2 and F_3) were prepared by changing the concentration of the ingredients and was reported that ideal toothpaste should always have a pH between 5.5 and 10.5 (Price *et al.*, 2000), which was exactly found in F_2 and F_3 . Moreover, the tooth paste formulation (F_3) was found to be stable up to 12 months without change in its physicochemical properties.

Profound effect on treating gingivitis was reported in mouth rinse of guava leaf extract (Kraivaphan *et al.*, 1991). The activity of *Acacia arabica* to remove the toxins and at the same time with prebiotic activity to promote good bacteria was reported by Kister (1994). A combination of guava along with piper extract possess bacteriostatic effect against the major colonizers of teeth during plaque formation namely Actinomycetes,

Streptococcus mitis and *Streptococcus sanguinis* (Fathilah *et al.*, 2009). The procedure for making herbal toothpowder using various ingredients like *Acacia arabica*, *Zanthoxylum alatum*, *Mentha spicata*, *Azadirachta indica*, *Quercus infectria*, *Piper longum*, etc. was formulated (Devi *et al.*, 2019). From its historical roots to its contemporary applications, readers will discover the reasons behind the growing interest in this herbal toothpowder and how it stands out in the ever-expanding landscape of natural oral care products. There is no report on the formulation of guava leaf toothpowder obtained from varied concentrations of guava leaf powder yet. Hence, in the present study, five different formulations (T_1 -Control, T_2 -5% guava leaf powder, T_3 -10% guava leaf powder, T_4 -15% guava leaf powder, T_5 -20% guava leaf powder) were prepared and analysed for its proximate analysis, anti-microbial activity, shelf life, sensory scoring and cost economics were computed and presented in this article.

Materials and Methods

Guava leaves are taken for the preparation of guava leaf toothpowder. Five formulations were prepared with varying concentrations of guava leaf powder (T_1 – Control, T_2 - 5% guava leaf powder, T_3 - 10% guava leaf powder, T_4 - 15% guava leaf powder and T_5 – 20% guava leaf powder). The mature, disease-free guava leaves were handpicked, and the petiole and midrib were removed.

The leaves were washed to eliminate adhering dust and were shade-dried for about 7 days until completely dry. The dried leaves were powdered and sieved through a fine mesh. Other ingredients including cinnamon powder, clove powder, oak gall powder, gum acacia powder, Himalayan rock salt, alum, tailed pepper powder and triphala powder were used for the preparation of tooth powder. All the contents were appropriately weighed and sieved. These ingredients along with guava leaf powder were mixed at varying concentrations based on the fixed treatments. The contents were filled into an airtight container and stored in a cool, dry place.



Fig.1: Ingredient's guava leaf powder and triphala powder (left), clove powder, cinnamon powder and gum acacia powder (middle) and alum, tailed pepper and Himalayan rock salt (right).



Fig. 2: Sieving the ingredients (left) and mixing the ingredients (right).

Table 1: Phytochemical analysis of guava leaf extract.

Phytochemicals	<i>Psidium guajava</i> leaves
Phenols	+
Tannins	+
Saponins	+
Terpenoids	+
Flavanoids	+

Phytochemical screening

Preliminary phytochemical screening was carried out to determine the presence of various bioactive compounds like flavonoids, terpenoids, steroids, quinones and alkaloids in the aqueous leaf extract using standard methods.

Analysis of toothpowder

After the preparation of various treatments, we have studied different properties of the formulated toothpowder for pH, bulk density, flow property, foaming ability, per cent loss on drying, etc.

Determination of pH

The electrode assembly of the pH meter was dipped into the standard pH buffer solution and was taken in a clean and dry beaker. After washing with distilled water the electrode assembly was dipped into a solution of standard pH-4 and adjusted to the required pH by fine asymmetry potential knob. The electrode assembly was raised and washed twice with distilled water then rinsed with test sample and finally it was dipped into the test sample. Then pH was recorded from the meter (AOAC, 2004).

Determination of bulk density (g ml⁻¹)

The bulk density of the powder is the ratio of the mass of an untapped powder sample and its volume including the contribution of the inter-particulate void volume. It is expressed in g ml⁻¹ (Mohanta and Manna, 2008).

Bulk density = Untapped density – tapped density

Foaming ability (ml)

The foamability of the product was evaluated by

**Fig. 3:** Formulated guava leaf toothpowder.

taking 2g of tooth powder with water in a measuring cylinder initial volume was noted as V_1 and then shaken for ten times. Final volume of foam was noted V_2 (Bharathi *et al.*, 2020).

Flow property

A funnel was taken and fixed with a clamp to the sand. A graph paper was kept below the funnel and the height between graph paper and bottom of the funnel was measured.

Then 50g of powder was weighed and poured into funnel by blocking the orifice of the funnel by thumb, the thumb was removed. The powder started flowing down onto the graph paper and formed a cone shaped pile until the peak of pile become touched to the bottom of the funnel stem. Then, the angle of repose was calculated by following formula (Subrahmanyam and Shetty, 2002).

$$\tan \theta = H/R$$

H = Height of powder,

R = Radius of graph paper

Determination of loss on drying (%)

2g of sample was taken in the oven at 105°C, then cooled. The loss of weight is recorded as percentage loss on drying and calculated by the given formula (Bharathi *et al.*, 2020).

$$\% \text{ Loss on drying} = \frac{\text{Weight of sample after drying}}{\text{Sample weight}} \times 100$$

Anti-microbial activity

Agar well diffusion method was used for the presence of anti-microbial activity. Petriplate containing 20 ml of Muller Hinton agar were seeded with 24 hour culture of *Proteus vulgaris*, *Staphylococcus aureus* and *Bacillus subtilis*. Five wells were created among them one serves as a control and in the remaining 4 wells, different concentrations of the extract were added. The plates are incubated at a temperature of 37°C for 24 hours.

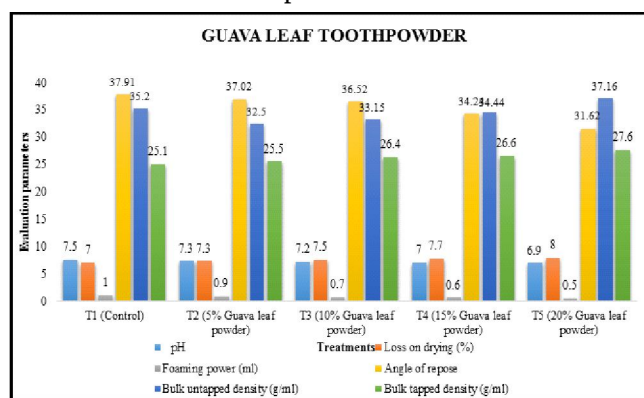
**Fig. 4:** Effect of variation in guava leaf powder on the proximate analysis of guava leaf toothpowder.

Table 2: Effect of guava leaf powder on the proximate analysis of the guava leaf toothpowder.

Treatments	pH	Loss on drying (%)	Foaming ability (ml)	Angle of repose (°)	Bulk untapped density (g ml ⁻¹)	Bulk tapped density (g ml ⁻¹)
T ₁ (Control)	7.50	7.00	1.00	37.91	35.20	25.10
T ₂ (5% guava leaf powder)	7.30	7.30	0.90	37.02	32.50	25.50
T ₃ (10% guava leaf powder)	7.20	7.50	0.70	36.52	33.15	26.40
T ₄ (15% guava leaf powder)	7.00	7.70	0.60	34.24	34.44	26.60
T ₅ (20% guava leaf powder)	6.90	8.00	0.50	31.62	37.16	27.60
S. Ed.	0.04	0.06	0.03	0.21	0.26	0.06
C.D.(P=0.05)	0.07	0.12	0.06	0.41	0.52	0.11

Each value is the mean of five replications. These values do not differ significantly (P<0.05)

The maximum and minimum zone of inhibition was measured.

Sensory evaluation

The organoleptic quality of guava soap were evaluated by the panelist for sensory attributes such as colour, odour, taste and overall performance. All evaluation session were conducted. A nine point hedonic scale (Wichchukit and Mahony, 2014) was used ranging from like extremely (9) to dislike extremely (1). All samples were presented before the panelists at room temperature under normal lighting conditions.

Statistical analysis

All data were subjected to analysis of variance (ANOVA) using CRD with five formulations and five replications each and the standard deviation and coefficient of variation has been arrived.

Results and Discussion

Phytochemical analysis

Phytochemical analysis of *Psidium guajava* leaves revealed the presence of bioactive compounds such as tannins, flavonoids, phenolic compounds, steroids, saponins and alkaloids.

Physicochemical analysis

The importance of pH in toothpowder lies in maintaining a balanced oral environment. A slightly basic pH can help neutralize acids produced by bacteria, reducing the risk of tooth decay. This helps create an environment less favourable for the growth of acid-loving bacteria in the mouth. pH value of the formulated guava

leaf toothpowder ranges from 6.9 to 7.5, which was neutral and did not show any irritation in oral cavity. The results are in line with the findings of Mamatha *et al.*, (2022). The formulated herbal toothpowder had 8% loss on drying and with good stability. While some moisture reduction is often necessary for product stability and shelf life, an excessively high LOD can have several potential consequences.

Herbal toothpowders may have lower foaming ability compared to conventional toothpaste because they often contain fewer or no synthetic surfactants. Instead they may rely on natural ingredients for cleansing and oral health benefits. While foaming can contribute to the perception of cleaning, it's not necessarily a measure of a product's effectiveness. The presence of herbal ingredients along with guava leaves might contribute to anti-microbial and anti-inflammatory properties beneficial for oral health. Results shows that the formulated herbal toothpowder had a lower foaming ability (1.0 ml) and it is line with the findings of Mamatha *et al.*, (2022). The flow property was estimated using angle of repose (31.62° to 37.91°) and it demonstrates good flow property. This range allows for good flow properties and easy dispensing while maintaining stability in the powdered form. It influences factors like powder flow and packing density affecting the product's texture and ease of use during application. The findings are similar to those reported by Rajendran *et al.*, (2020).

Bulk untapped density and bulk tapped density were 37.16 g ml⁻¹ and 27.6 g ml⁻¹ respectively and the difference between the two values was 5 which shows

Table 3: Anti-microbial activity of guava leaf toothpowder against certain pathogenic bacteria.

	Solvents	Zone of inhibition (mm)											
		<i>Proteus vulgaris</i>				<i>Bacillus subtilis</i>				<i>Staphylococcus aureus</i>			
		R1	R2	R3	Mean	R1	R2	R3	Mean	R1	R2	R3	Mean
1	Methanol	11	10.98	11.01	11.0	8.01	8.0	8.1	8.04	5.02	5.0	4.98	5.0
2	Water	-	-	-	Nil	-	-	-	Nil	-	-	-	Nil
3	n-Hexane	-	-	-	Nil	-	-	-	Nil	-	-	-	Nil

good porosity value. The results are similar to Mamatha *et al.*, (2022). Higher untapped density often correlates with improved flow, which was crucial for easy dispensing during application. Tapped density reflects the maximum packing achieved through tapping or vibration. This parameter was vital for ensuring that the powder settles efficiently, avoiding clumping and facilitating smooth dispensing.

Microbial analysis shows that, maximum zone of inhibition was reported in methanol solvent for *Proteus vulgaris* (11.0 mm) in the sample. It might be attributed due to the presence of bioactive compounds like tannins, flavonoids, phenolic compounds and alkaloids. The results of Biswas *et al.*, (2013) indicates that ethanol and methanol are better than n-hexane and water for the extraction of antimicrobial properties of guava.

Benefit cost ratio obtained through production of guava leaf toothpowder was found to be 1.75. Since this BCR provides the person who engages in value addition with considerable returns, processing of guava leaf into guava leaf toothpowder can be used for commercial exploitation. Among the five formulations, T₅ with 20% guava leaf powder secured the maximum sensory scores for colour (9.0), odour (9.0), taste (8.9) and overall performance (9.0) followed by T₄ (15% guava leaf powder) (colour – 8.7, odour - 8.6, taste – 8.8 and overall performance -8.8) with shelf life of one year. The prolonged shelf life might be contributed to care taken during drug preparation, storage and properties of herbal drugs used in the formulation.

Conclusion

In conclusion, guava leaf toothpowder encapsulates the essence of nature's healing prowess, offering a natural and effective alternative to conventional oral care. With its rich history, backed by scientific evidence, guava leaf toothpowder stands as a testament to the power of traditional wisdom in addressing modern health concerns. As individuals increasingly prioritize sustainability and holistic well-being, this herbal toothpowder emerges as a compelling choice, providing a refreshing approach to dental hygiene that harmonizes with nature. Embracing guava leaf toothpowder not only nurtures healthier teeth and gums but also contributes to a more environmentally conscious and mindful oral care routine.

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Conflicts of Interest

The author declares no conflict of interest relevant to this article.

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