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DEVELOPMENT OF PROBIOTIC HERBAL ORAL CARE FORMULATION

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ABSTRACT

This formulation is an herbal probiotic powdered mouthwash designed to enhance oral health through natural ingredients and probiotics. Addressing the strong link between poor oral hygiene and systemic health issues such as diabetes and cardiovascular diseases, which provides a safe, eco-friendly alternative to conventional mouthwashes. Formulated with Lactobacillus and Bifidobacterium strains, sage, mint, clove, coconut oil powder, and xylitol, it targets plaque reduction, gum health, and microbiome balance. Analytical results revealed an average moisture content of 5.85%, ensuring probiotic viability below the 6% threshold. The ash content measured 28.37%, reflecting notable mineral content, while the pH of 6 and 0.1% total sugars supports a mild, balanced oral environment. Phytochemical screening confirmed the presence of phenolics, gums & mucilage, and terpenoids contributing to antioxidant and anti-inflammatory benefits. Microbial analysis demonstrated viable probiotic counts (~11.68 × 10^8 CFU/g) and strong antibacterial activity, indicated by a 15mm zone of inhibition against oral pathogens, outperforming select commercial samples. Sensory evaluation yielded a favorable overall acceptance score of 7.5, reflecting high consumer appeal. These findings underscore formulation efficacy in promoting oral health and suggest its potential to fill the market gap for chemical-free, multifunctional oral care products.

KEYWORDS: Herbal mouthwash, probiotics, oral health, sage powder.

INTRODUCTION

The mouth serves as both the entry point to the digestive system and a gateway to overall health. Oral health significantly influences an individual's well-being and quality of life. Poor oral health can hinder essential functions like eating, speaking, and smiling, profoundly affecting personal and social interactions. However, in today's fast-paced lifestyle, oral care is often overlooked. Irregular brushing habits, skipped dental checkups, and the excessive consumption of sugary or acidic foods have led to a surge in dental issues such as cavities, gum disease, and bad breath.

To address these concerns, many individuals turn to chemical-based toothpastes and mouthwashes, which often contain fluoride and alcohol. While these products may provide temporary relief, their excessive use can result in adverse effects, including dental fluorosis, dry mouth, and irritation of oral tissues.[1] Growing concerns about the long-term safety and health impacts of these chemicals have highlighted the need for safer, more natural alternatives. This has driven the increasing interest in herbal oral care products as a holistic and sustainable solution for maintaining oral health.

By rebuilding and preserving a balanced oral microbiome, probiotics offer health advantages to the host when given in sufficient quantities. Dental caries, periodontal disease, and other oral health problems can result from an imbalance in the diverse population of microorganisms that live in the mouth cavity. Probiotics work to reduce inflammation and improve oral health by creating antimicrobial compounds, competing with harmful bacteria for adhesion sites, and modifying the immune system.[2]

Herbal components including mint and sage (Salvia officinalis) have also been identified as having medicinal qualities, in addition to probiotics. Bioactive substances including flavonoids and phenolic acids, which have antibacterial, antioxidant, and anti- inflammatory properties, are abundant in sage.[3] Mint has been demonstrated to have antibacterial qualities and a pleasant taste, which improves oral hygiene and the sensory experience.[4] This formulation gains additional benefits from the addition of coconut oil powder, which is well-known for its antibacterial qualities and capacity to moisten the oral mucosa.[5]

In order to give a comprehensive approach to oral care, this study focuses on creating a powdered mouthwash with herbal probiotics. The composition attempts to provide a natural, efficient way to enhance oral health by utilizing the complementary benefits of probiotics and

herbal ingredients. The study presents this innovative product as a potential game-changer in the dental care sector by analyzing its antibacterial efficacy, plaque- reduction potential, and overall impact on gum health.

MATERIALS AND METHODS

Table 1: Formulation.

Ingredients	Composition
Probiotics	46%
Sage Powder	15%
Lemon Powder	10%
Coconut Oil Powder	10%
Clove Powder	6%
Mint Powder	6%
Xylitol	4%
Menthol Crystal	2%

1. **Ingredient Preparation**

a. Weigh and sieve all herbal powders (sage, clove, mint, etc.) to ensure uniform particle size.

2. **Initial Blending**

a. Combine base powders (e.g., sage, mint, coconut oil powder) in a mixing vessel for even distribution.

3. **Addition of Menthol**

a. Incorporate menthol crystals carefully, ensuring they disperse uniformly throughout the powder.

4. **Probiotic Integration**

a. Introduce probiotic strains (e.g., *Lactobacillus* spp.) under controlled conditions to preserve viability.

5. **Packaging and Storage**

a. Transfer the final blend into moisture-resistant containers (glass bottles).

b. Seal tightly to maintain low moisture content and store in a cool, dry environment to preserve probiotic activity.

Proximate Analysis

Determination of Moisture Content

A moisture analyzer calculates how much weight a sample loses when heated to a predetermined temperature, usually 105°C. The device measures the sample's initial weight after it is set on a weighing pan. The sample loses bulk as a result of water evaporating when heated. The device determines the proportion of weight loss, which correlates to the sample's moisture content, once the weight stabilizes, signifying that moisture has been forced out.

Determination of Ash Content

When a known weight sample is ignited to prepare ash, the weight of ash thus obtained is expressed in terms of percentage. The sample is commonly ignited at 550°c to oxidize all organic matter without flaming. Water and other volatile compounds are vaporized and the organic substances are burned in the presence of oxygen with CO_2, H_2O and N_2. Minerals are converted into oxides, sulfates, phosphates, chlorides or silicates.

Extraction of Fat by Soxhlet Extraction Method

Soxhlet extraction is a continuous solid-liquid extraction method. Lipid is soluble in organic solvent and insoluble in water hence organic solvent like hexane, petroleum ether are used to solubilize the fat. Thus, further a simple filtration can be used to separate the desired compound from the insoluble impurity.

Determination of pH using pH paper

pH is the scientific scale for measuring how acidic or basic a substance is when it is dissolved in water. pH scale runs from 0 to 14. A measurement of 0 means the substance is very acidic; 7 means it is neither acidic nor basic but right in the middle like plain water (neutral); and 14 means it is very basic. pH paper contains a chemical indicator that changes color in response to the pH of the solution it is exposed to. The color change corresponds to a specific pH range, which can be compared against a color chart provided with the pH paper to determine the pH of the food product.

Determination of Total Soluble Solids (BRIX)

Refractometer measures total soluble solids (TSS) concentration based on the principle of refraction of light. When a ray of light travels obliquely from one medium to another, It is bent or refracted. The refraction occurs because light travels at slightly different velocities in different media, the extent being proportional to the density of the solution or the soluble solids

concentration. The refractive index of a medium is defined as the ratio of, the sine of the angle of incidence to the sine of the angle of refraction when a ray of monochromatic light is refracted from a vacuum (or, to a very close approximation, from Air) into the medium. In a Brix refractometer, the refractive index is calibrated into Brix readings. As the refractive index is dependent on the density of the solution, the measurements Have to be made at a specific temperature (20° C) or suitable corrections have to be applied.

Qualitative Analysis of Phytochemicals

Table 2: Phytochemical Estimation.

Tests	Observation	Inference
1. Alkaloids Test **Mayer's Test** 0.5ml sample extract + 2 drops of Mayer's reagent along the sides of test tube,	A creamy white precipitate.	Alkaloids present
2. Glycosides Test **Libermann's Test** 0.5ml sample extract + 2 ml Chloroform +2 ml Acetic Acid(mixture was kept in ice water bath)Add Conc.H2SO4	Color changes from violet to blue to green	Glycosides present
3. Flavonoids Test **Shinoda Test** 0.5ml sample extract dissolved in alcohol + few fragments of magnesium ribbon + few drops of conc. HCL.	Pink to crimson coloured solution.	Flavonoids present
4.Saponins Test **Foam Test** 0.5ml sample extract + 2ml D/W [Shake vigorously]	Persistent foam for 10 minutes	Saponins present
5. Phenolic Compounds **Ferric Chloride Test** 0.5ml sample extract + 5 ml D/W+few drops of 10% ferric chloride solution.	Dark green precipitate or bluish black colour.	Phenolic compound present
Gelatin Test 0.5ml sample extract + 5 ml D/W+ 2 ml of 1% Gelatin solution.	A white precipitate.	Phenolic compound present
Lead Acetate Test 0.5ml sample extract + 2ml DW+ 3ml lead acetate solution.	A white precipitate.	Phenolic compound present
6. Gums and Mucilage **Alcohol Test** 0.5ml sample extract + 10ml D/V+ 2ml absolute solution. [Constant stirring]	White or Cloudy precipitate.	Gums and Mucilage present
7. Terpenoids Test: Salkowski's Test 0.5ml sample extract + 2 ml chloroform + 3ml sulphuric acid.	Reddish Brown ring at interface.	Terpenoids present

Microbial Analysis

Determination of total microbial load of Herbal Sample by TPC Method

Total Plate Count (TPC) gives a quantitative idea about the presence of microorganisms such as bacteria, yeast and mold in a sample. To be specific, the count actually represents the number of colonies forming units (CFU) per gram or (per ml) of the sample. A TPC is achieved by plating dilutions of the culture until 30-300 colonies exist on a single plate.

Determination of Probiotic Bacteria present in sample by TPC Method

Total Plate Count (TPC) is a method used to determine the viable count of probiotic bacteria present in a sample. It provides quantitative data in terms of colony-forming units per gram (CFU/g) or per milliliter (CFU/mL) of the sample. MRS (de Man, Rogosa, and Sharpe) agar is a selective medium specifically designed for the cultivation and enumeration of lactic acid bacteria, including probiotic strains such as Lactobacillus, Bifidobacterium, and other beneficial microbes. The process involves serial dilution of the probiotic sample, followed by plating on MRS agar to achieve a countable range of 30-300 colonies per plate. A positive control probiotic sample with a known viable count is used for comparison.

Sensory Evaluation

Sensory Analysis examines the properties (texture, flavor, taste, appearance, smell, etc.) of a productor food through the senses (sight, smell, taste, touch and hearing) of the panelists. This type of analysis has been used for centuries for the purpose of accepting or rejecting food products. Historically, it was considered a methodology that complements technological and microbiological safety when assessing the quality of food. However, its important evolution and impact in recent decades has placed it as one of the most important methodologies.

Scale Description

1 - Extremely dislike, 2 - Dislike very much, 3 - Dislike moderately, 4 - Dislike slightly, 5 - Neither like nor dislike, 6 - Like slightly, 7 - Like moderately, 8 - Like very much, 9 - Extremely like.

Observation (For Powder)

Colour: Light- or pale-yellow shade. Appearance: Uniform powder, no visible clumps.

Odour: Mild herbal aroma with a refreshing minty note.

Observation (After Dissolving)

1. Colour: Olive green, Light ochre/ Pale golden, Brown.
2. Appearance: Clear or slightly translucent liquid, absence of sediment or floating particles.
3. Odour: Mild herbal aroma with refreshing minty notes, Earthy, Spiced.
4. Taste: Balanced profile with mild sweetness, herbal undertones, and refreshing citrus/mint flavor.
5. Consistency: Smooth liquid, no granules remaining after dissolution.
6. Mouthfeel: Clean, smooth, and refreshing sensation.
7. Aftertaste: Light, refreshing herbal and minty finish.

EVALUATION OF ANTIMICROBIAL ACTIVITY

Agar Cup Well Diffusion Method

The Agar Cup Well Diffusion Method is a qualitative antimicrobial testing technique used to evaluate the effectiveness of an antimicrobial agent against microbial strains. A well is cut into an agar plate inoculated with the test microorganism, and the antimicrobial agent is introduced into the well. During incubation, the agent diffuses into the agar, and its effectiveness is measured by the zone of inhibition (clear area around the well), indicating microbial growth suppression.

Sample Preparation

In an aseptic environment, collect saliva using a sterile cotton swab. Transfer the swab into a tube containing sterile saline to create an oral flora suspension. Next, pipette 0.1 mL of this suspension onto a nutrient agar plate and inoculate it either by pour plate or streaking. Finally, incubate the plate under the appropriate conditions for microbial growth.

Result Interpretation

Larger zones of inhibition indicate higher antimicrobial activity.

No clear zone suggests the microorganism is resistant to the tested antimicrobial agent.

RESULTS AND OBSERVATION

Determination of Moisture Content

Table 3: Moisture Content.

Weight of sample (g)	Moisture (%)
1.020	6.29%
1.003	6.27%
1.00	5%

Calculation

$$= \frac{6.29+6.27+5}{3}$$

= 5.85 %

Result

The average moisture content of the given samples is 5.85%, indicating a moderate level of moisture in the samples.

Conclusion

The average moisture content of 5.85% is below the 6% maximum typically required to maintain probiotic viability. Therefore, the product's moisture level meets the recommended specification for ensuring the stability and efficacy of the probiotic strains included in the formulation.

Determination of Ash Content

Observation

Table 4: Ash Content.

Sample	Initial weight of crucible	Weight of sample	Weight of crucible + sample weight (before drying)	Weight of crucible + sample weight (after drying)	Ash Content
1	18.563g	2.013g	20.575 g	19.134 g	28.37%

Average Ash Content =28.37%

Result: The total ash content of the sample was found to be 28.37%

Conclusion: Determination of Ash content was performed by Muffle furnace. The ash content of the product was measured at 28.3%, indicating a good mineral content, which

contributes to its overall nutritional value

Determination of Fat Content

Weight of beaker (W1) = 95.370g

Weight of sample (W) = 4.5g

Weight after evaporation (W2) = 96.099g

$$\text{Fat content} = \frac{W2-W1}{W}$$

$$\text{Fat content} = \frac{96.099-95.370}{4.5}$$

= 16.2 %

Result: The percent fat obtained from our product was 0.0924%.

Conclusion: The Fat content in product was determined using Soxhlet fat extraction method and was found to be 16.25%.

Determination of pH using pH Paper.

Observation

Fig. no. 1: pH Determination.

Result: pH of the product is 6.

Conclusion: pH of the product was determined using pH paper. The pH was found to be 6.

Determination of Sugar Content

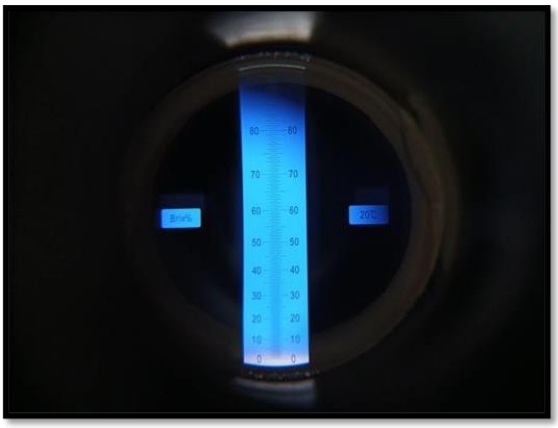

Fig. no. 2: Brix Refractometer.

Result: Sugar content was identified using a brix refractometer and was found to be 0.1%.

Conclusion: The Sugar content of the product was determined by Brix Refractometer. The Sugar content was found to be 0.1%.

Phytochemical Analysis

Table 5: Observation for Phytochemical Analysis.

Tests	Observation	Inference
1. Phenolic Compounds: Ferric Chloride Test 0.5ml sample extract + 5 ml D/W+few drops of 10% ferric chloride solution.	Dark green precipitate or bluish black colour.	Phenolic compound present
Gelatin Test 0.5ml sample extract + 5 ml D/W+ 2 ml of 1% Gelatin solution.	A white precipitate.	Phenolic compound present
Lead Acetate Test 0.5ml sample extract + 2ml DW+ 3ml lead acetate solution.	A white precipitate.	Phenolic compound present
2. Gums and Mucilage: Alcohol Test 0.5ml sample extract + 10ml D/V+ 2ml absolute solution. [Constant stirring]	White or Cloudy precipitate.	Gums and Mucilage present
3. Terpenoids Test: Salkowski's Test 0.5ml sample extract + 2 ml chloroform + 3ml sulphuric acid.	Reddish Brown ring at interface.	Terpenoids present

Fig. no. 3: Phytochemical Analysis.

Result

Qualitative Estimation of Phytochemicals was performed for the product. Phytochemicals such as Phenolic compounds, Gums and mucilage and Terpenoids were present in the product. The phytochemical analysis of the product showed the presence of the following bioactive compounds: Phenolics– known for their antioxidant properties, which help in reducing oxidative stress and promoting overall health. Gums and Mucilage– Contribute to improved texture, potential prebiotic benefits, and dietary fiber content. Terpenoids – Recognized for their anti-inflammatory, antimicrobial, and functional properties.

Conclusion

The phytochemical analysis of the product was performed, and the presence of phenolics, gums & mucilage, and terpenoids was confirmed. These bioactive compounds contribute to its antioxidant, anti-inflammatory, and textural properties, making this formulation a potential product.

Microbial Analysis

Determination of total microbial load of Herbal Sample by TPC Method.

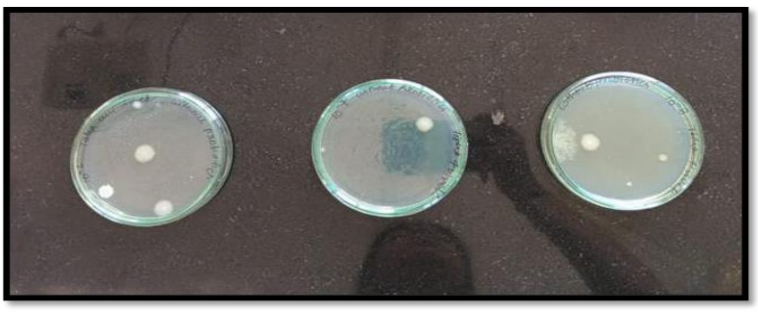

Fig. no. 4: TPC of Herbal Sample.

Observation

Table 6: Observation Table for TPC.

Sr. no	Dilution	No.of colonies	No.of colonies cfu/ml	Average
1	10^6	6	0.06 x 10^8	
2	10^7	4	0.4×10^8	1.153 x 10^8
3	10^8	3	3×10^8	

Determination of Probiotic Bacteria present in sample by TPC Method in MRS Agar

Fig. no. 5: Probiotic Sample on MRS Agar (Positive Control).

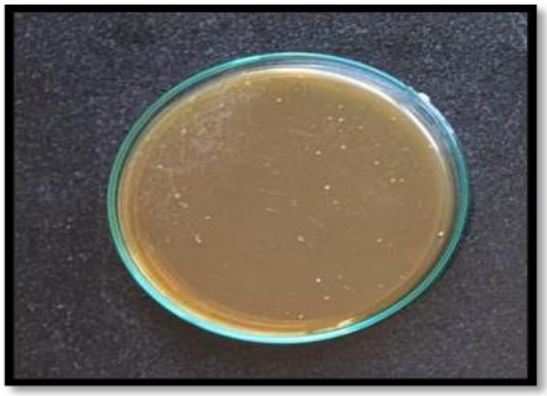

Fig no 6. Pour Plate of 10^7 Plate.

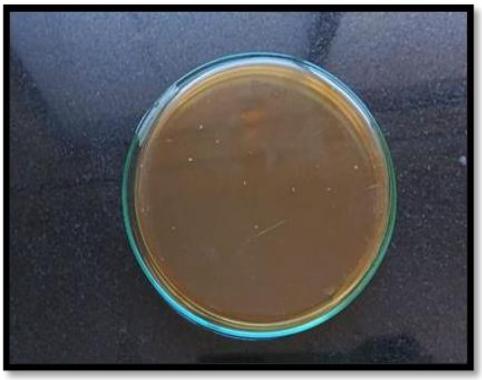

Fig no 7. Pour Plate of 10^8 Plate.

Observation

Table 7: Observation Table for Probiotic Count.

Sr. no	Dilution	No. of colonies	No.of colonies cfu/ml	Average
1	10^6	55	0.55×10^8	
2	10^7	45	4.5×10^8	11.68 x 10^8
3	10^8	30	30×10^8	

Conclusion

The probiotic count in the sample was found to be 11.68 x 10^8 CFU/ml, which is well above the minimum required 10^8 CFU/ml for the effective probiotic activity. This confirms that

the sample contains a sufficient number of viable probiotics to provide potential health benefits.

Sensory Analysis

Result of Sensory Analysis for the Powder

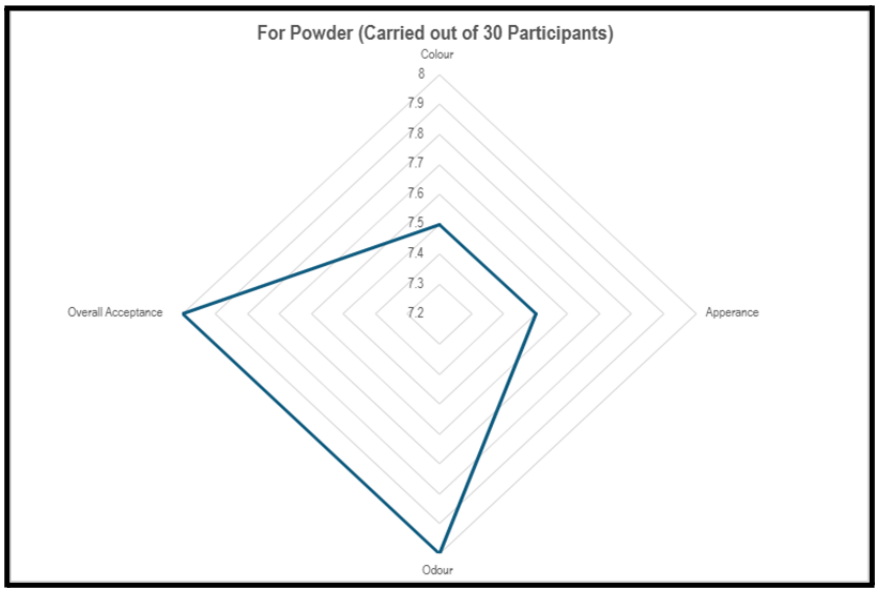

Fig. No. 8: Sensory Analysis for Powder.

Observation

Table 8: Sensory Analysis of Powder.

Parameters	Score (Out of 9)
Colour	7.5
Appearance	7.5
Odour	8
Overall Acceptance	8

Results

It was observed that half of the panelists gave an 8 to the product, hence it can be said that people liked the product, since there were no negative score attributes from the population towards the product. Also, it was observed that the odor, color, and appearance of the product was greatly liked by many panelists.

Conclusion

The probiotic mouthwash powder was well-received by the panelists, with half of them rating the product an 8, indicating a positive overall perception. The absence of negative feedback highlights general acceptance among the participants. Additionally, the product's odor, color, and appearance were particularly appreciated, suggesting that its sensory attributes

significantly contributed to its appeal. These findings indicate that the product has strong consumer acceptance potential.

Result of Sensory Analysis for the powder after dissolving.

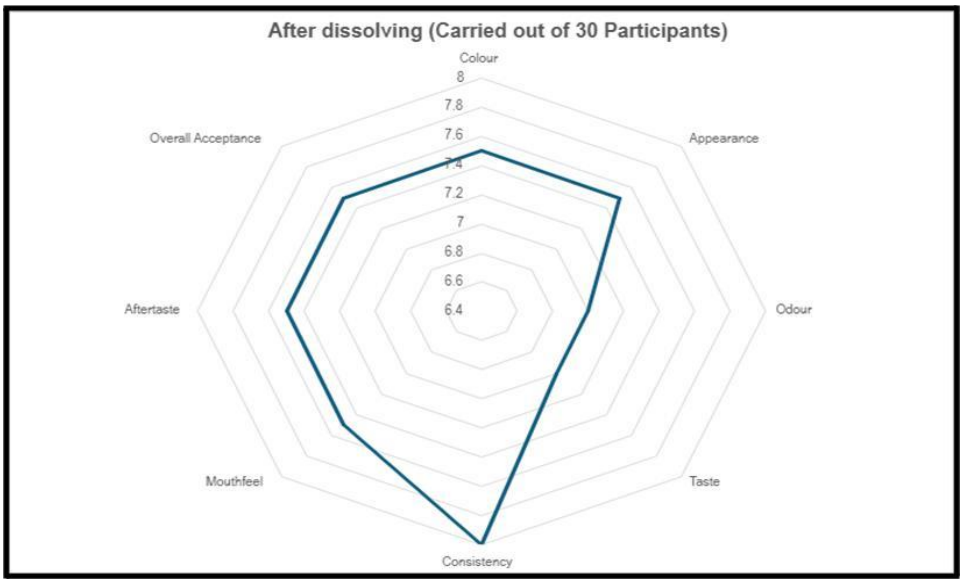

Fig. No. 9: Sensory Analysis for Powder after dissolution.

Observation

Table 9: Sensory Analysis of Powder after Dissolution.

Parameters	Score (Out of 9)
Colour	7.5
Appearance	7.5
Odour	7
Taste	7
Consistency	8
Mouthfeel	7.5
Aftertaste	7.5
Overall Acceptance	7.5

Results

After dissolution the product showed positive results, with the highest score in consistency (8.0) and consistent ratings of 7.5 for colour, appearance, taste, mouthfeel, and aftertaste. Odour scored slightly lower at 7.0. An overall acceptance score of 7.5 indicates good consumer appeal with minor scope for improvement.

Conclusion

The product demonstrated positive acceptance, achieving an Overall Acceptance score of

7.5. The highest rating was observed for Consistency (8.0), indicating a favorable texture and ease of use. Parameters like Colour, Appearance, Taste, Mouthfeel, and Aftertaste consistently scored 7.5, reflecting a balanced and well-formulated product. However, Odour (7.0) scored slightly lower, suggesting a minor area for improvement to enhance user satisfaction. Overall, the product shows strong potential for market acceptance, with favorable sensory attributes contributing to its appea Evaluation of Antimicrobial Activity.

Fig. no. 10: Antimicrobial Activity.

Results

The formulated sample showed a zone of inhibition of 1.5 cm, indicating significant antimicrobial activity. Marketed Sample 1 exhibited a zone of inhibition of 1.0 cm, suggesting moderate antimicrobial activity. Marketed Sample 2 showed no zone of inhibition, indicating an absence of antimicrobial activity.

Conclusion

The results of the Agar Cup Well Diffusion Method indicate that the formulated sample exhibited a zone of inhibition of 1.5 cm, demonstrating significant antimicrobial activity. In comparison, Marketed Sample 1 showed a 1.0 cm zone of inhibition, suggesting moderate antimicrobial effects, while Marketed Sample 2 did not produce any zone of inhibition, indicating the absence of antimicrobial activity. The larger zone observed for formulation suggests that it possesses a stronger inhibitory effect against the tested microorganism compared to the marketed products. This enhanced antimicrobial activity could be attributed to its unique probiotic and herbal formulation, which may provide additional benefits for oral health. The results highlight the potential of formulation as an effective antimicrobial agent in comparison to commercially available alternatives.

Shelf- Life Studies

The shelf-life investigation began on Day 2 of production. The product was monitored at regular intervals for any changes in color, odor, taste, texture, and probiotic viability.

Observation

Table 10: Observation Table for Shelf-Life Studies.

Week	Days	Observations
Week 1	Day 1- Day 7.	No change in color, odor, or taste. Probiotic viability remained within acceptable limits
Week 2	Day 8 – Day 14	No change in color or odor. Taste remained stable; moisture content stayed below 6%, ensuring probiotic viability.

Results

No significant alterations in the organoleptic properties (color, odor, taste, and texture) were noted during the first two weeks. Probiotic viability remained above the functional threshold, indicating that formulation retained its efficacy and stability under recommended storage conditions.

DISCUSSION

Probiotics can be delivered in a powdered form for dental care, as demonstrated by the successful integration of Lactobacillus and Bifidobacterium strains in a low-moisture herbal matrix. Probiotics and herbal extracts work in concert to improve gum health, breath freshness, and the microbial load, among other elements of oral health. The product's competitive advantage over chemical-based mouthwashes, which frequently contain alcohol and artificial additives, is demonstrated by the results of microbiological and sensory analyses. Additionally, the use of coconut oil powder and xylitol improves oral mucosal hydration and plaque reduction. These results demonstrate that formulation can successfully satisfy the growing demand from customers for sustainable, natural, and microbiome-friendly dental care products.

CONCLUSION

A multipurpose herbal mouthwash powder with probiotics, this formulation was created to address common oral health issues such gum irritation, dental cavities, and microbial imbalance. It provides antibacterial, anti-inflammatory, and antioxidant effects in a single, environmentally friendly solution by combining sage, clove, mint, coconut oil powder, and

probiotics. Probiotic viability is maintained by the moisture content (5.85%), and phytochemical tests show the presence of helpful substances such phenolics, gums & mucilage, and terpenoids. Its capacity to inhibit oral infections with a 1.5 cm zone of inhibition is confirmed by in vitro antimicrobial testing, outperforming several commercial options. Consumer acceptability is positive, according to sensory evaluations, showing that it has the potential to be a safe and efficient substitute for conventional mouthwashes.

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