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Original Article

Formulation and Evaluation of *Azadirachta indica* Taste Masked Oral Pellets for Gut Microbiome Modulation

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ABSTRACT

Introduction: *Azadirachta indica* has long been known for its therapeutic benefits, which include immunomodulatory and antibacterial activities. Human health is greatly influenced by the gut microbiota, and disturbances with this composition have been connected to a number of illnesses. Using natural items like *Azadirachta indica* to alter the gut flora provides a fresh strategy for preserving gut health and averting illness. **Aim and Objectives:** The purpose of the present research was to create and assess *Azadirachta indica* taste masked oral pellets for their capacity to alter the gut microbiota and support a balanced microbial population. **Materials and Methods:** The extrusion-spheronization technique was used to combine *Azadirachta indica* extract into oral pellets. An evaluation of the physicochemical properties of *Azadirachta indica* extract was carried out. The pellets were assessed for flow properties, loss on drying, friability, disintegration time to predict taste masking, microbial limit test and *in vitro* gut microbiome modulation using rat fecal samples. **Results:** Acceptable physicochemical properties were displayed by the prepared pellets. A well-balanced formulation for quick disintegrating and effective flavor masking is shown by disintegration period, which makes oral pellets highly palatable and suitable for oral administration. Potential for gut microbiome modification was indicated by *in vitro* study, which showed that prepared pellets specifically suppressed harmful bacteria while encouraging the growth of healthy gut microbiota. **Conclusion:** The Taste masked oral pellets made from *Azadirachta indica* demonstrated encouraging outcomes in regulating the microbes in the gut by promoting the development of beneficial bacteria. Potential applications in gut health treatments and as a prophylactic against illnesses linked to the microbiota are suggested by this.

Keywords: *Azadirachta indica*, Extrusion-spheronization, *Glycyrrhiza glabra*, Taste masked oral pellets etc.

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INTRODUCTION

Many physiological functions, including digestion, metabolism, immunological control, and pathogen defense, depend on the gut microbiome, a complicated community of billions of microorganisms that live in human gastrointestinal tract.[1] While dysbiosis, or an imbalance in the microbial community, has been connected to a number of illnesses, such as immunological dysfunction, metabolic diseases, and gastrointestinal problems, a balanced and healthy gut microbiome is crucial for preserving overall health.[2] In order to improve health and avoid disease, recent studies have concentrated on investigating natural solutions for modifying the gut microbiota. *Azadirachta indica*, or neem, is one such potential natural remedy. Ayurvedic medicine has long utilized *Azadirachta indica* because of its strong antibacterial,[3] anti-inflammatory[4,5] and immunomodulatory qualities.[6] According to these bioactivities, neem may improve gut health, especially by influencing the makeup and activity of the gut microbiota.[7]

Azadirachta indica's extremely bitter taste, however, presents a barrier when using it for therapeutic purposes. This can lower patient compliance, especially when using oral preparations. The creation of neem-containing oral pellets has addressed this issue by using taste-masking strategies to improve the formulation's palatability.[8] A number of techniques, including polymer coatings,[9] encapsulation[10] and the use of sweeteners[11] can be used to mask taste so that the bioactive ingredients of neem are supplied without its disagreeable flavor. This Process is Important for Increasing the acceptability and usability of neem-based oral therapies.

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A controlled-release medicine delivery method called oral pellets is intended to overcome issues with taste and dosage while maximizing the bioavailability and efficacy of active ingredients.[12] Pellets improve the therapeutic potential of neem for gut microbiota modification by providing a focused and extended release of bioactive substances in the gastrointestinal system.

Investigating the advantages of *Azadirachta indica* oral pellets for gut microbiota modulation is the goal of this study. These pellets may improve gut health, assist to restore microbial balance, and offer a natural way to prevent and treat gut-related disorders by administering the bioactive components in a controllable and taste-masked manner. Through microbiome manipulation, this study aims to investigate the potential benefits of neem oral pellets as an innovative approach for enhancing gut health.

MATERIALS AND METHODS

Development of *Azadirachta indica* taste masked pellets

Powder of *Azadirachta indica* leaves was purchased from Shatayu Vanaushadhalay medical nigdi, Pune. he most often used process for creating pellets is the extrusion–spheronization technique, which consists of four steps: 4 (Figure 1).[13,14]

Magnesium stearate was chosen as an antiadherent, starch as a disintegrant, methyl and propyl parabens as preservatives, and coloring additives to provide color. Glycyrrhiza glabra was used as a taste masker to mask the bitter taste of *Azadirachta indica*. Formulation used for development of oral pellets is provided in Table 1. Steps involved in formulation of *Azadirachta indica* is discussed in Figure 2. Oral Pellets Formulation of taste mask *Azadirachta indica* oral pellets using the extrusion-spheronization pelletization process is provided in Figure 3.

Powdered *Azadirachta indica* was purchased and utilized for the extraction procedure. To obtain the crude extract, 10 g of the powder was heated for 48 hr using 100 mL of distilled water, and then the mixture was filtered. Using common qualitative tests, a preliminary screening for phytochemicals was then carried out. The presence of several phytochemicals in water-based extract was satisfactorily determined by these methods.[15,16]

Analysis of *Azadirachta indica* taste masked oral Pellets

Flow properties Azadirachta indica taste masked oral Pellets

Particle size and Size distribution

Sieve analysis was used to assess the physical properties of the *Azadirachta indica* taste masked oral Pellets in order to ascertain their uniformity and size distribution. The pellets were sorted using a set of common sieves with different mesh sizes (e.g., 0.5 mm, 1 mm, and 2 mm). After placing about 50 g of the prepared pellets on the upper sieve, the stack was shaken mechanically for 10 min. To determine the percentage distribution over the various sieve sizes, the pellets that were retained on each sieve were gathered and weighed and assed for the required data.[17,18]

Angle of Repose

To evaluate the flowability and stability of *Azadirachta indica* taste masked oral pellets during handling and storage, the angle of repose was calculated. The pellets were passed via a funnel onto a level surface to create a conical pile using the funnel method. Using the formula angle of repose (θ)=arctan(height/diameter), the height and diameter of the resultant pile were measured in order to determine the angle of repose. With an average angle of repose of 33º, the flow characteristics were determined to be favorable. The pellets' comparatively low angle indicates that they are cohesive enough and manageable without posing a serious risk of caking or clumping, which is crucial for reliable dosing and efficient production procedures.[19,20]

Bulk density, Tapped Density, Hausner ratio and Carr's index

The bulk density was determined using the ratio of the powder's total mass to its bulk volume. After 50 tapings of the same quantity of powder, the taped density was determined. The ratio of tapped density to bulk density is known as the Hausner ratio. The percentage difference between the tapped and bulk densities is known as Carr's index.[21]

Loss on drying

At conduct this test, the product was dried in an oven at 105ºC until a constant weight was achieved.[22]

Friability

Friabilator running for 10 min at 25 rpm was used to assess friability of *Azadirachta indica* taste masked oral pellets.[23]

In vitro taste-masking evaluation

Taste masking of *Azadirachta indica* taste masked oral pellets was accessed by placing in 150 mL simulates saliva at 37ºC with 50 rpm stirring. Pellets were allowed to stay in the solution for 3 considering the residence time in the mouth. After 3 min' percentage of the active ingredient released was determined using UV spectroscopy. Minimal drug release (less than 10%) during the disintegration stage indicates successful taste masking, indicating that the bitter taste of the active ingredient is successfully covered up before swallowing.[24]

In vitro Gut Microbiome Test Using Rat Fecal Samples

In vitro gut microbiome test for prepared *Azadirachta indica* taste masked oral pellets was carried out using rat fecal samples using MacConkey and Mann Rogosa Sharpe (MRS) broth to study the effect of prepared *Azadirachta indica* taste masked oral pellets on gut microbiota, specifically focusing on Lactobacilli. This

setup will help investigate how *Azadirachta indica* taste masked oral pellets affects the growth and activity of certain microbial populations in the gut, particularly those that MRS, MacConkey broth can support (Figures 4 and 5).

MRS Broth supports the growth of Lactic Acid Bacteria (LAB), including species such as *Lactobacillus*. As these bacteria ferment sugars, they produce lactic acid, causing the pH of the medium to decrease.

Formed CFU further used for Gram Staining: Perform Gram staining to confirm the presence of lactic acid bacteria (Gram-positive, rod-shaped).[25,26]

RESULTS AND DISCUSSION

Qualitative phytochemical screening of *Azadirachta indica* extract

Phytochemicals in water-based extract was satisfactorily determined and tabulated in Table 2.

Evaluation *Azadirachta indica* taste masked oral Pellets

Flow properties Azadirachta indica taste masked oral Pellets

Particle size and Size distribution

The oral pellets of *Azadirachta indica* showed a homogeneous size distribution in the sieve analysis, which is necessary for steady therapeutic activity. The Table 3 provides a summary of the findings.

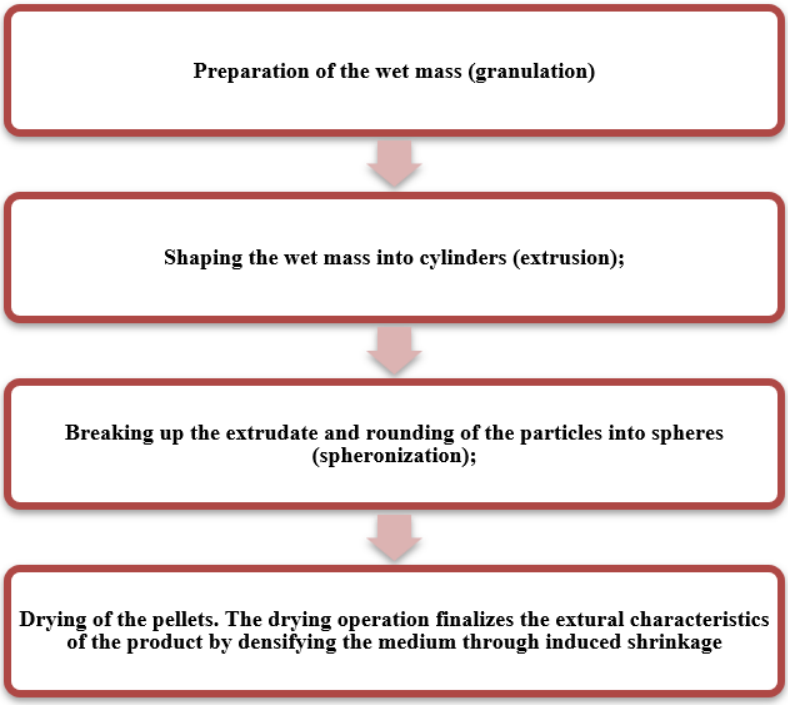

Figure 1: Pellet preparation process.

Table 1: ***Azadirachta indica*** **oral Pellets Formulation.**

Sl. No.	Ingredients	Quantity (%)	Category
1	*Azadirachta indica*	25	Antimicrobial, anti-inflammatory, antioxidant, and immunomodulator.
2	Starch	25	Disintegrant
3	Magnesium stearate	2	Antiadherant
4	Methyl paraben	0.4	Preservative
5	Propyl paraben	0.1	Preservative
6	*Glycyrrhiza glabra* extract	8.84	Sweetening agent
7	Distilled Water	q.s	

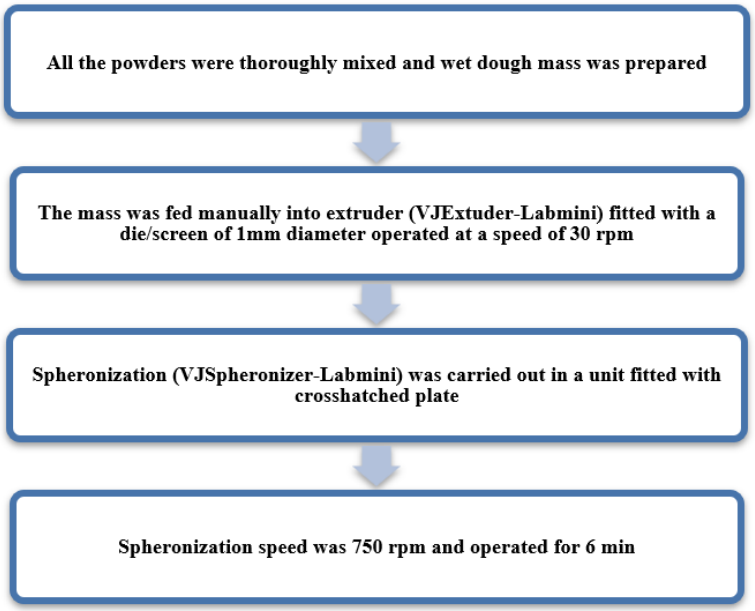

Figure 2: Formulation of oral Pellet.

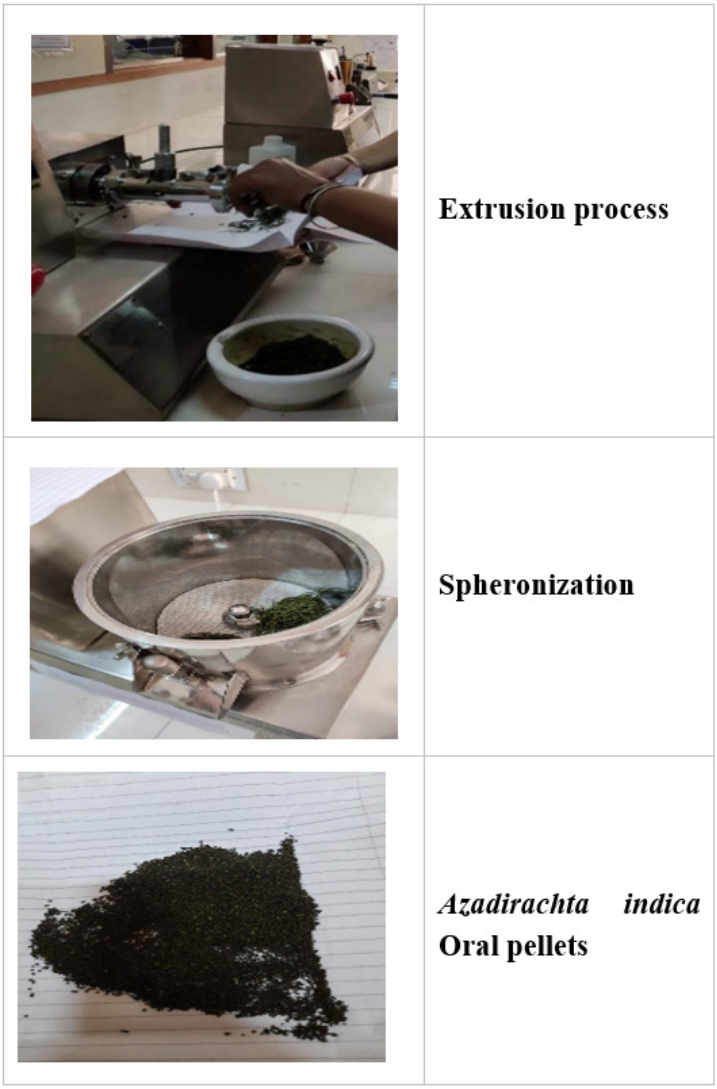

Figure 3: Formulation of taste mask *Azadirachta indica* oral pellets using the extrusion-spheronization pelletization process.

The majority of the pellets (80.0%) fell within the ideal size range for oral administration, which is between 1.0 and 1.5 mm. Just 1.0% of the pellets were larger than 1.5 mm, while a very tiny percentage (6.0%) were smaller than 0.5 mm. Good homogeneity in pellet size was shown by the fact that no pellets larger than 2 mm were kept on the sieve. These findings support the potential therapeutic use of *Azadirachta indica* oral pellets by showing that they have an appropriate size distribution for efficient administration and gastrointestinal transit.

Angle of Repose

To assess the flow characteristics of *Azadirachta indica* taste masked oral pellets, the angle of repose was evaluated. The Table 4 provides a summary of the findings:

Good flowability was shown by the *Azadirachta indica* taste masked oral pellets average angle of repose, which was found to be 18.91°. The uniformity in pellet production and handling characteristics is further supported by the range of angles from the experiments (18.34° to 19.40°). A low angle of repose indicates that there is less chance of caking or clumping during processing, which is essential for efficient production and precise dose. These findings show that the oral pellets of *Azadirachta indica* have appropriate flow characteristics, which increases its potential for useful use in pharmaceutical formulations.

Bulk density, Tapped Density, Hausner ratio and Carr's index

It was discovered that the *Azadirachta indica* taste masked oral pellets has a bulk density of 0.368 g/cm^3 and a tapped density of 0.459 g/cm^3. The Hausner ratio and Carr's index, two crucial measures of flowability, were computed using these numbers. The powder has good flowability, as indicated by the Hausner ratio of 1.24±0.21, which is the ratio of tapped density to bulk density. Likewise, the powder's strong flowability is indicated by Carr's index, which is determined by calculating the percentage difference between the tapped and bulk densities and came out to be 19.83±0.29%. These findings offer crucial information about the material's flow characteristics, which are crucial for the powdered extract's formulation and processing. The bulk density and tapped density of the powdered material used in the formulation offer information about handling and processing properties in the context of oral pellets. The powder's good flowability, as demonstrated by the Hausner ratio and Carr's index, indicates that it is appropriate for manufacturing processes like spheronization, extrusion, and pelletization, which call for uniform flow and little bridging or clogging during processing.

Figure 4: Test Using Rat Fecal Samples.

Loss on drying

The proportion of moisture content preserved in the formulation was indicated by the 1.15% loss on drying of the manufactured oral pellets. A low loss on drying value indicates that the pellets have been well dried, which lowers the possibility of microbial development and improves the product's stability over time. The oral pellets' comparatively low moisture content is indicated by their loss on drying value of 1.15%. Given that moisture can affect the stability and shelf life of medicinal formulations, this is a good outcome. In this case, the 1.15% moisture content suggests that the pellets are adequately dried, reducing the risk of microbial growth, degradation, or changes in physical properties

Table 2: **Results of Preliminary phytochemical screening of *Azadirachta indica*.**

Sl. No.	Phytochemical	Results
1	Alkaloids	Positive
2	Flavonoids	Positive
3	Tannins	Positive
4	Saponins	Positive
5	Terpenoids	Positive
6	Glycosides	Negative
7	Phenolic Compounds	Positive
8	Essential Oils	Negative
9	Reducing Sugars	Positive

Table 3: **Results of Particle size and Size distribution.**

Sieve Size (mm)	Weight Retained (g)	Percentage Retained (%)
<0.5	3	6
0.5-1	7	14
1-1.5	40	80
1.5-2	0.5	1
>2.0	0	0
Total	50	100

Table 4: **Result of *in vitro* Gut Microbiome Test Using Rat Fecal Samples.**

Bacterial Group	Control Group (CFUs/g)	*Azadirachta indica* Group (CFUs/g)
Lactobacillus	1.5×10^6	4.2×10^7
Escherichia coli	3.4×10^5	8.7×10^4

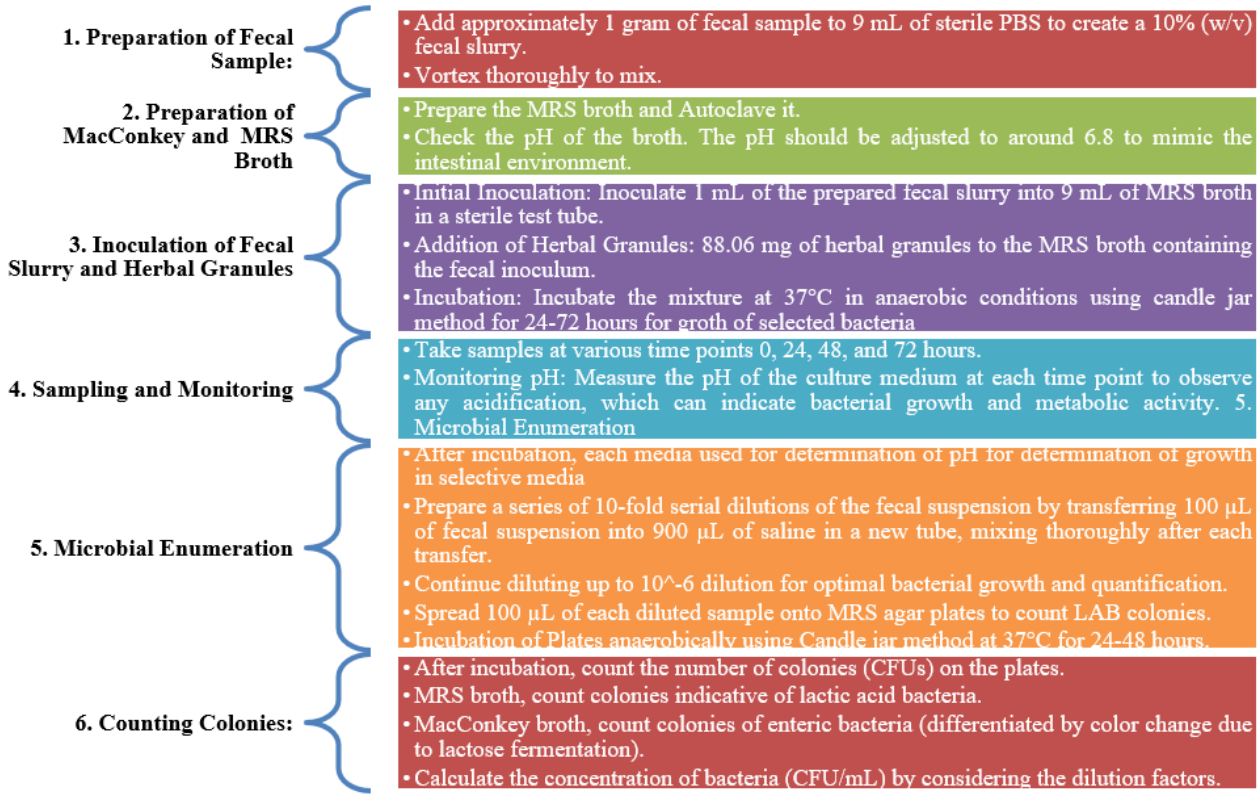

Figure 5: *In vitro* Gut Microbiome Test Using Rat Fecal Samples.

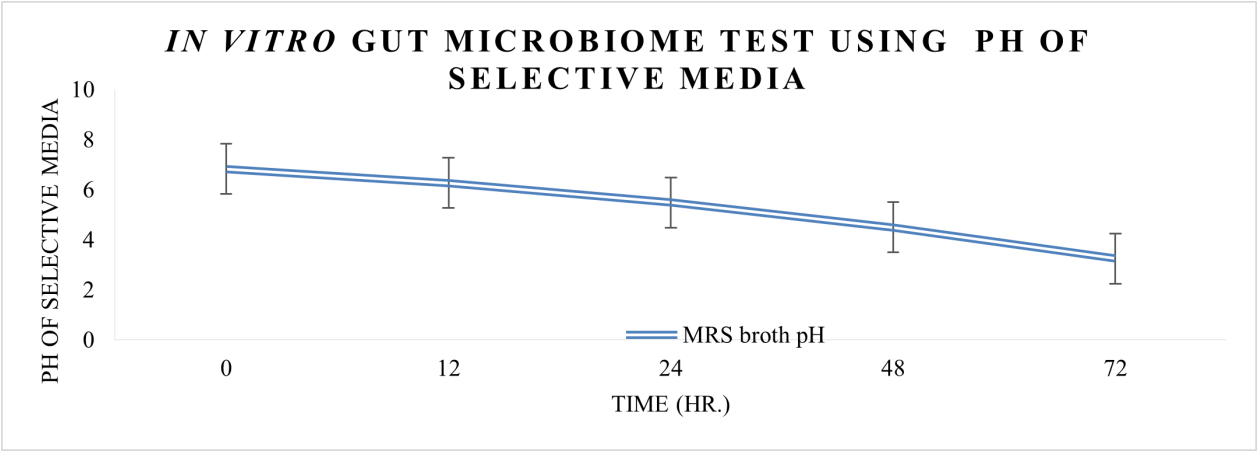

Figure 6: *In vitro* Gut Microbiome Test Using pH of selective media.

like hardness or dissolution rates. It ensures the product remains stable during storage and maintains its integrity throughout its shelf life.

Friability

The formulation's physical resilience was demonstrated by the measured friability of the pellets, which was 0.35±0.2%. Pellets with low friability ratings (usually less than 1%) are thought to have strong mechanical strength and are therefore unlikely to crumble or break apart when handled, packed, or transported.

In vitro taste-masking evaluation

As per results obtained, in the first 3 min of simulated saliva, the taste-masked oral pellets showed less than 10% medication release. This implies that by keeping the active ingredient hidden from taste buds prior to ingesting; the flavor-masking formulation was successful. This suggests that the oral pellets are well-suited for oral administration and have been engineered for quick breakdown while retaining efficient flavor masking.

In vitro Gut Microbiome Test Using Rat Fecal Samples

The administration of *Azadirachta indica* oral pellets resulted in significant changes in the gut microbiome composition of the treated rats compared to the control group. In the *In vitro* Gut Microbiome Test using rat fecal samples and the media Man Rogosa Sharpe (MRS) broth and MacConkey broth, the observed changes in pH reflect the bacterial activity. Over time, a significant drop in pH which produces more acidic environment (pH around 3.2) observed in the Man Rogosa Sharpe (MRS) broth, indicating active fermentation by LAB. A drop in pH is a positive indicator of the herbal granules' potential to support beneficial gut microbiota, such as lactobacilli, which are associated with gut health (Figure 6).

The total viable bacterial count (measured as Colony-Forming Units, CFUs) increased in the treated group, indicating enhanced overall microbial activity. Specific bacterial populations, such as *Lactobacillus* exhibited a substantial increase in the treated group, with CFUs rising from an average of 1.5×10^6 CFUs/g in the control group to 4.2×10^7 CFUs/g in the *Azadirachta indica* group. Conversely, pathogenic bacteria such as *Escherichia coli* showed a marked decrease, with counts declining from 3.4×10^5 CFUs/g to 8.7×10^4 CFUs/g. These findings suggest that *Azadirachta indica* oral pellets effectively modulate the gut microbiome by promoting beneficial bacterial growth while inhibiting pathogenic populations (Table 4).

CONCLUSION

In order to improve patient compliance, the study effectively produced taste-masked oral pellets of *Azadirachta indica*, and investigated their capacity to modify the gut microbiota. The optimised pellets successfully covered up the bitter flavour of *Azadirachta indica* while exhibiting good physical characteristics, stability, and controlled release. Rat faecal samples used in *in vitro* experiments demonstrated that the pellets had a positive effect on gut microbiota, encouraging good lactic acid bacteria while possibly suppressing harmful enteric bacteria. According to these results, *Azadirachta indica* pellets may be used as a natural remedy to enhance gut health by modulating the gut flora. To define dosage guidelines for clinical use and prove efficacy, more *in vivo* research is required.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

CFU: Colony Forming Units; **GIT:** Gastrointestinal Tract; **PBS:** Phosphate Buffered Saline; **MRS:** Man Rogosa Sharpe; **g:** Gram; **pH:** Potential of hydrogen; **LOD:** Loss on Drying; **LAB:** Lactic acid bacteria.

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE HUMAN AND ANIMAL RIGHTS

Approved College IAEC 1554/PO/Re/S/11/CCSEA.

SUMMARY

Azadirachta indica is well-known for its antimicrobial and immunomodulatory qualities. A variety of disorders are associated with an imbalance in the gut microbiota, which is vital to human health. The purpose of this study was to create and assess taste-masked oral pellets of *Azadirachta indica* for the regulation of gut microbiota. The extrusion-spheronization process was used to create the pellets, and their physicochemical characteristics were evaluated. Flow characteristics, loss upon drying, friability, disintegration time, microbiological limit test, and *in vitro* gut microbiota manipulation were among the evaluations. The produced pellets demonstrated good taste masking and acceptable physicochemical properties. Studies conducted *in vitro* showed that good gut microbiota was promoted while harmful bacteria were selectively suppressed. Palatability and ease of administration are ensured by the rapid disintegration time. The results point to possible uses in treatments for intestinal health. These pellets may be used as a preventative measure against conditions linked to the microbiota.

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