Research Article

Formulation and Evaluation of Prebiotic Herbal Granules

P J Prasuna Sundari*, Jadhav Soundarya[1]

*Professor, Department of Pharmaceutics, Sri Venkateswara College of Pharmacy, Madhapur, Hyderabad-500081, TS, India.

[1]M.Pharmacy Pharmaceutics 2nd year, Sri Venkateswara College of Pharmacy, Madhapur, Hyderabad-500081, TS, India.

***Corresponding author's E-mail:** jpspingali2014@gmail.com

Received: 14-12-2023; **Revised:** 26-01-2024; **Accepted:** 05-02-2024; **Published on:** 15-02-2024.

ABSTRACT

Gut flora represents a diverse community of live microorganisms inhabiting the digestive tract, like bacteria, fungi, and viruses. They play a vital role in digestion, nutrient absorption, and host immunity. Lactobacillus and Bifidobacterium species are prominent among them. Prebiotics are non-digestible foods that beneficially affect the host by altering the gut flora. Probiotics are live bacteria found in fermented foods and vegetables. Prebiotics and probiotics can exert a synergistic effect to maintain a balanced gut microbiome. Herbs rich in insoluble saccharides, fiber, polyphenols, and pigment materials serve as prebiotics, and literature review indicated many such herbs possess remarkable probiotic activity. The present work aims to develop prebiotic polyherbal granules. Herbs like onion, kalmegh, amla, fennel, ashwagandha, and garlic possess both prebiotic and antimicrobial activity and were selected for the study. Extracts were prepared and screened for probiotic and antimicrobial activity. MRS broth was used for the former and agar well diffusion method for the latter. The selected extracts were developed into formulation F1 and F2 coated granules. The formulations were evaluated for flow properties, probiotic, and antimicrobial activity. Fructo-oligosaccharide was the standard prebiotic and amoxicillin was an antibiotic. F1 and F2 yielded 550 and 580 CFUs respectively and the zones of inhibition were (12&11mm), (13&14mm), (15&13mm), and (16&15mm) against four organisms S. *aureus, E. coli, B. subtilis*, and *P. aeruginosa* respectively. Herbal prebiotic granules were successfully formulated. However, in vivo, studies are necessary to demonstrate the probiotic response and also its efficacy in reducing infections.

Keywords: Prebiotics, Lactobacillus, onion, kalmegh, ashwagandha, amla, fennel.

INTRODUCTION

Gut flora or microbiota refers to the diverse community of microorganisms including bacteria, viruses, and fungi, residing in the digestive tract. It plays a crucial role in digestion, nutrient absorption, immune system regulation, and overall health. A balanced and diverse gut flora is essential for various physiological functions in the body. The microbiome of the human gut includes firmicutes such as *Lactobacillus, Clostridium, and Bifidobacterium which* are involved in the fermentation of complex carbohydrates and the production of short-chain fatty acids vital for human health[1]. A Prebiotic was described as "a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health"[2]. Probiotics are live bacteria found in fermented foods. The health benefits of prebiotics and probiotics include digestive health, gut health barrier function, modulating function, increased nutrient absorption, reducing the risk of chronic diseases, supporting mental health, and supporting weight management[3]. Herbs like kalmegh, fennel, ashwagandha, amla, garlic, and onion possess good probiotic activity due to the presence of phytochemicals like andrographolides, tannins, anethole, limonene, quercetin, etc. These herbs apart from demonstrating probiotic effect also have considerable antimicrobial activity. When a formulation comprising, such herbs is developed it would be useful for the recovery of gut flora while simultaneously minimizing GI infections. Granules in sachets can serve as a suitable single-dose delivery system for herbs. The aim & and objective of the present study was to formulate and evaluate polyherbal granules.

MATERIALS AND METHODS

Materials

Fennel and Ashwagandha kalmegh were purchased from the local market. Fructo-oligosaccharide was a gift sample from Revelations biotech, *Lactobacillus acidophilus* was procured from Nagarjuna University, MRS broth (TM Media) Nutrient broth (HI media), HPMC, Ethyl alcohol, Span-80, and all other chemicals were purchased from Sd fine chemicals Pvt Ltd.

Preparation of extracts: Juices of onion, garlic, and amla were prepared by centrifugation and the juice was filtered and dried. for the study. Extracts of fennel, kalmegh, and ashwagandha were prepared by the soxhlation method.

Demonstration of probiotic activity

MRS broth was used as media to culture *Lactobacillus acidophilus*. Serial dilutions of stock culture ranging from 10^1 to 10^7 were prepared and inoculated into MRS broth and incubated at 24 hours at 37 ±2°C. The resulting colonies were counted. Suitable dilution was selected for the study to demonstrate probiotic activity. Agarated MRS was mixed with 1ml (50mg/ml) of the extract, inoculated with culture, and incubated for 24 hours at 37 ±2°C [4].

Determination of antimicrobial activity

The agar well diffusion method was used to demonstrate the antimicrobial activity of extracts against *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, and Pseudomonas aeruginosa.* Amoxicillin was used as a standard. Nutrient agar media was prepared and into the bored wells extracts (50mg/ml) were placed. Amoxycillin was used as standard. The plates were incubated at 37°±2°C for 24hrs and the zones of inhibition were measured[5].

Preparation of prebiotic granules

The wet granulation method was used to prepare the granules.

Table 1: Composition of prebiotic granules (20gms)

Composition	F1(gms)	F2(gms)
Kalmegh	2.5	2.5
Fennel	3.5	-
Ashwagandha	-	3.5
Starch	4.4	4.4
Magnesium stearate	0.6	0.6
Citric acid	0.8	0.8
Methyl paraben	0.04	0.04
Stevia	0.8	0.8
Mannitol	q. s up to 20gms	q. s up to 20gms

Coating of prebiotic granules

A coating solution containing HPMC-2% was prepared and sprayed. The granules were then air-dried[7].

Evaluation of granules

The prepared granules were evaluated for the following parameters.

The angle of repose: The angle of repose of powder was determined by the funnel method. The powder was passed through a funnel fixed to a burette stand at a height of 2.5 cm. A graph paper was placed below the funnel on the table. The height and radius of the pile was measured.

The angle of repose of the powder was calculated using the formula:

$$\text{Angle of repose } (\theta) = \text{Tan}^{-1} h / r$$

Where,

h = Height of the pile

r = Radius of the pile

Bulk density: It is the ratio of the total mass of powder and the bulk volume of powder. It was measured by pouring the weighed powder into a measuring cylinder and initial weight was noted. From this, the bulk density was calculated according to the formula mentioned below.

Bulk density (Db) = Mass (M) / Bulk volume (Vb)

Where,

M is the mass of powder,

Vb is the bulk volume of the powder

Tapped density: It is the ratio of the total mass of the powder to the tapped volume of the powder. Volume was measured by tapping the powder 100 times and the tapped volume was noted. It is expressed in gm/ml and is given by

Tapped density (Dt) = Mass (M) / Tapped volume (Vt)

Where,

M is the mass of powder;

Vt is the tapped volume of the powder.

Hausner's ratio:

The ratio of tapped density and bulk density is called as Hausner's ratio. It is an indirect index of ease of powder flow. It was calculated by the following formula.

Hausner ratio = Tapped density (Dt) / Bulk density (Db)

Disintegration Time:

Accurately weighed 2 grams of formulation were placed in a basket rack assembling. disintegration time of the granules were demonstrated in a phosphate buffer having pH 6.8 And temperature maintained at 37º C[8].

Screening of formulations for probiotic activity

0.02 g/ml of test sample was prepared and used for the study. The procedure was similar to that adopted for extracts. Fructo-oligosaccharide was used as a standard for comparison[4].

Screening of formulations for antimicrobial activity

0.02 g/ml of test sample was prepared and used for the study. The procedure was similar to that adopted for extracts[5].

RESULTS AND DISCUSSIONS

Physical characteristics of herbal extracts: all herbs yielded between 1.7-4% of extracts and were semi-solid in consistency.

Probiotic activity of extracts

Fig 1 contains images of colonies grown during the incubation period. Kalmegh, onion, amla, ashwagandha, fennel, and garlic yielded 450, 329, 255, 439, 338, and 288 CFUs respectively.

Kalmegh, ashwagandha, and Fennel demonstrated better probiotic activity as the CFU was found high for these herbs due to the presence of phytochemicals and dietary fiber.

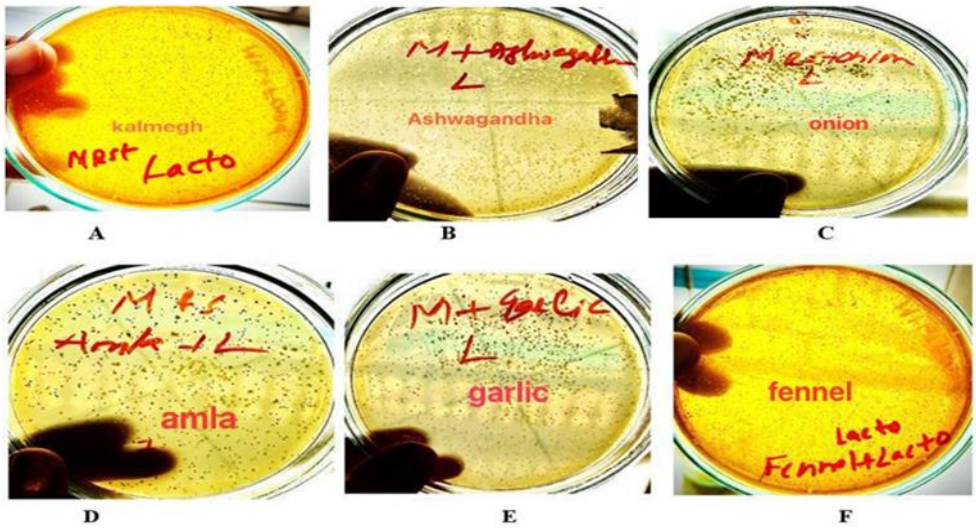
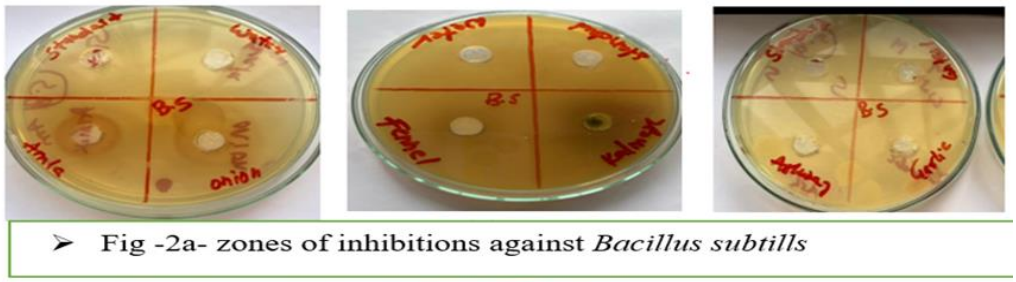


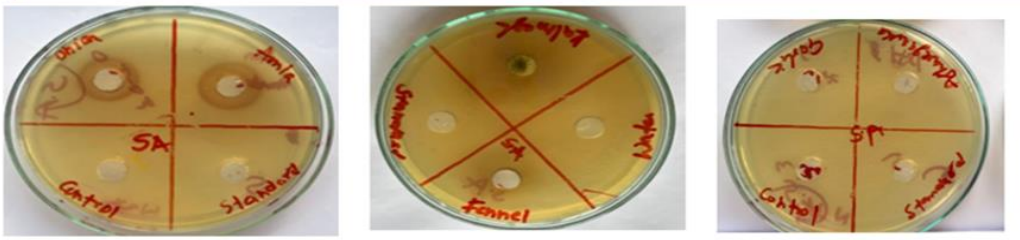
Figure 1: Images showing *lactobacillus acidophilus* colonies produced with the addition of herbs

Antimicrobial activity of extracts

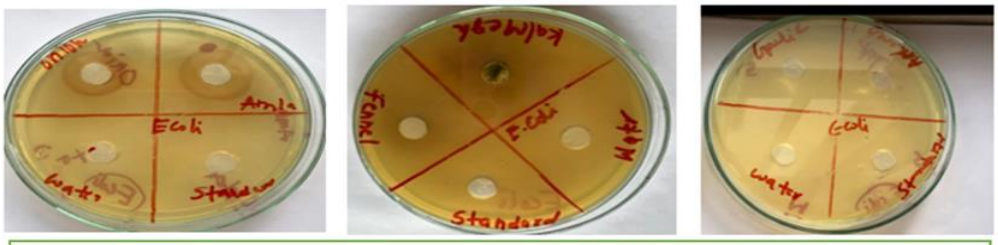
Fig-2 contains images depicting the zones of inhibition of various herbal extracts. The values are indicated in Table No-2.



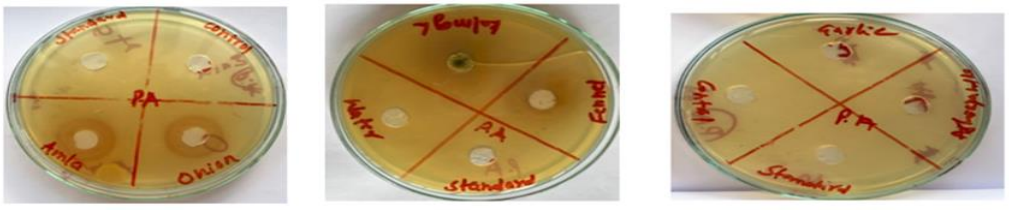
➢ Fig -2a- zones of inhibitions against *Bacillus subtills*



➢ Fig -2b- zones of inhibitions against *Staphylococcus aureus*



➢ Fig -2c- zones of inhibitions against against *E. coli*



➢ Fig -2d- zones of inhibitions against Pseudomonas *aeruginosa*

Table 2: Zones of inhibition(mm) of different extracts against selected microorganisms

Name of herb	Antimicrobial activity			
	E. coli	***Staphylococcus aureus***	***Bacillus subtilis***	***Pseudomonas aeruginosa***
Onion	12mm	15mm	23mm	18mm
Garlic	Non-consistent and minimum			
Ashwagandha	Minimum activity			
Amla	18mm	15mm	15mm	17mm
Fennel	Minimum activity			
Kalmegh	22mm	20mm	23mm	24mm

Table 3: Evaluation data of flow properties and disintegration time of coated herbal granules

S.no	Bulk density (g/m)	Tapped density (g/m)	Angle of repose (θ)	Hausner's ratio	Disintegration time (pH 6.8 buffer)
F1	0.79	0.9	26	1.13	18.22min
F2	0.80	0.95	28	1.18	18min

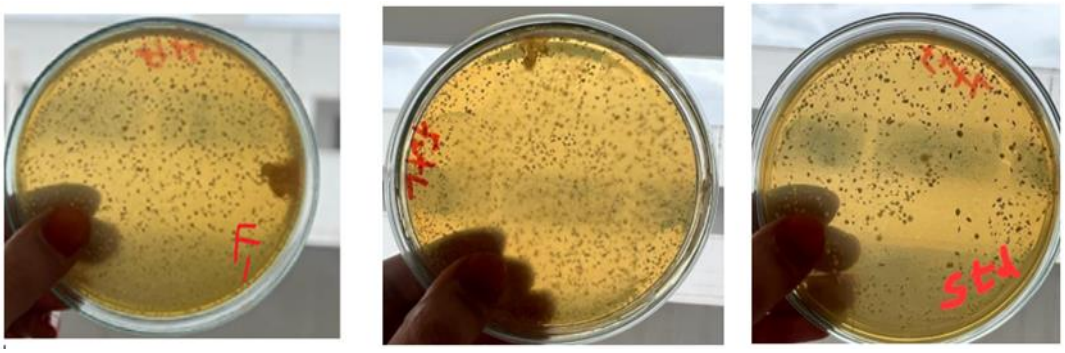


Figure 3: Images of CFU of F1 & F2 herbal formulations

The data obtained clearly indicated that amla, kalmegh, and onion have good anti-microbial activity. Amla and kalmegh are very rich in polyphenols, and onion rich in sulfur compounds might be a reason for their being better candidates.

Preparation of prebiotic granules

The prepared granules were evaluated for flow properties and disintegration time. The results obtained are depicted in table-3.

Probiotic activity and antimicrobial activity of herbal granules F1&F2

The prepared herbal granules were assessed for probiotic activity and antimicrobial activity. the results obtained are depicted in Fig no-3&4

F1 comprises of kalmegh and fennel. F2 comprises kalmegh and Ashwagandha. Kalmegh is common in both formulations because of its high probiotic and antimicrobial activity. Fennel and ashwagandha for minimum antimicrobial activity.

When kalmegh and Fennel were evaluated individually for probiotic activity they showed 450 CFU and 338 CFU respectively. When both kalmegh and fennel are used in combination F1 showed (780 CFU) a slight synergic effect. When kalmegh and Ashwagandha were evaluated individually for probiotic activity they showed 450 CFU and 439 CFU respectively. When both kalmegh and ashwagandha are used in combination F2 showed (850 CFU) a slight synergic effect. FOS (standard) CFU was found to be 332. Therefore, F1 and F2 showed probiotic activity. The CFU was obtained less when evaluated individually. However, when formulated in combination showed slight synergistic effect of herbs was observed from the results compared to standard fructo-oligosaccharide.

From Table 4 it was concluded that there was a slight decrease in the anti-microbial activity for F1 and F2 formulations. This is due to the minimal anti-microbial activity of fennel in F1 and ashwagandha in F2 apart from kalmegh.

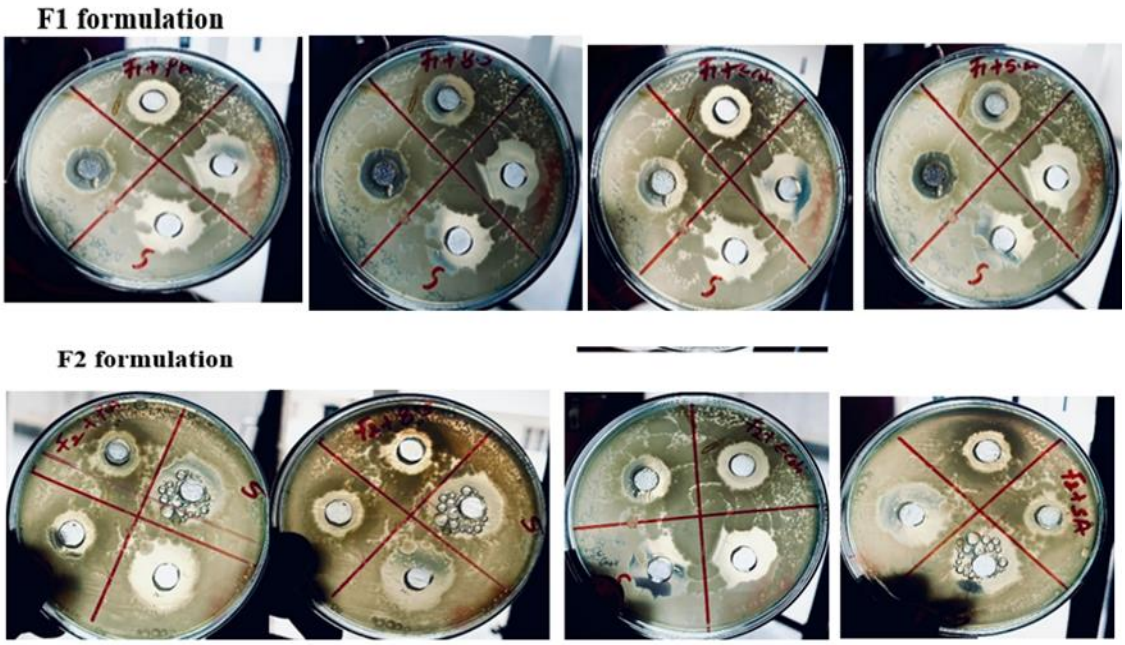


Figure 4: Zones of inhibition of F1&F2 against *staphylococcus aureus, E. coli, Bacillus subtilis, and Pseudomonas aeruginosa.*

Table 4: Evaluation data of antimicrobial activity of herbal granules result

Name of herb	Antimicrobial activity (mm)			
	E. coli	***Staphylococcus aureus***	***Bacillus subtilis***	***Pseudomonas aeruginosa***
F1	13	12	15	16
Kalmegh	22	20	23	24
Fennel	Minimum activity			
F2	14	11	13	15
Kalmegh	22	20	23	24
Ashwagandha	Minimum activity			

CONCLUSION

Prebiotics, probiotics and gut health are interrelated. The ability of this nutraceutical class to maintain a good gut microbiome, thereby promoting digestive health, supporting the immune system, and potentially influencing overall well-being is remarkable. Amla, onion, ashwagandha, kalmegh, garlic, and fennel exhibited good probiotic and antimicrobial activity. Therefore, they were selected to develop new formulations. Formulations were evaluated and demonstrated beneficial effects. This formulation can be useful in patients who suffer illnesses due to disturbed gut biome. However, be put to regular use, the *in vivo* effects of the formulations need to be demonstrated.

Acknowledgment

The authors are thankful to the management of Sri Venkateshwara College of Pharmacy for providing the necessary facilities to carry out the research work and also to Revelations Biotech Pvt. Ltd- Secunderabad, Hyderabad for providing fructo-oligosaccharide as a gift sample.

Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

REFERENCES

1. Thursby E, and Juge N. Introduction to the human gut microbiota. *Biochemical journal*, 2017;474(11):1823-1836, DOI:10.1042/BCJ20160510.
2. Davani-Davari D, Nagahdaripour M, Karimzadeh I, Seifan M, Mohkam M, Jalil Masoumi, Aydin Berenjian S, Ghasemi Y. Prebiotics: Definition, types, sources, mechanisms, and clinical applications. *Foods journal,* 2019; 8(3):25-32. PMID:3085716.
3. Reid G. Probiotics: definition, scope, and mechanisms of action. *Science Direct Journal,* 2016; 30(1): 17-25.

DOI: https://doi.org/10.1016/j.bpg.2015.12.001

4. Paramita Acharya P, Sahu A, Kar B, Ranjan Meher S. Isolation and characterization of probiotics bacteria from curd pickle and fermented rice and screening of antimicrobial activity *Asian Journal of Pharmaceutical Research and Development*, 2019; 7(2):23-29.

DOI: http://dx.doi.org/10.22270/ajprd.v7i2.481.

5. Balouiri M, Sadiki M, Koraichi Ibnsouda S. Methods for in-vitro evaluating antimicrobial activity: a review. *Science Direct journal,* 2016; 6(2):71-79;

DOI: https://doi.org/10.1016/j.jpha.2015.11.005.

6. Dighe P, Kardile G, Khilari V, Shinde R. Formulation and evaluation of polyherbal granules as a dietary supplement. *International journal of creative research thoughts*; 2021; 9(9):1-13.

7. Ramchander, Middha A, Jalwal P. Formulation, and evaluation of enteric coated tablet of senna for the treatment of constipation. *The pharma innovation journal,* 2017;6(11):263-267.

8. Ofori-Kwakye K, Osei Yeboah F, Lugrie Kipo S. Formulation and quality evaluation of two conventional release tablet formulations. *International Journal of Pharmaceutical Sciences Review and research,* 2010;4(1):94-99.