Signed Details of Excellence in work for which the research award is claimed including references and illustrations (24/08/2023)

In-vitro bio-relevant media and time simulation probiotic proliferation methodology to determine prebiotic potentials of flaxseed powder.

https://doi.org/10.1016/j.bcdf.2022.100335

https://drive.google.com/file/d/1tvGYjwMAoiSfW9OOu671__uOchM96raW/view?usp=sharing

https://docs.google.com/document/d/1vTFH1ShWGnEZm742iGVMthvfR1j8kJ2j/edit?usp=sharing&ouid=106962687213151518134&rtpof=true&sd=true

The research work claimed for the award is part of the core research area of Dr. Gitanjali Deokar.

Dr. Gitanjali has stepwise plans to reach the target. She believes in value additions related to the field and preparations to reach the ultimate target of her research.

Dr. Gitanjali is a researcher heading the Department of Quality Assurance, MET's Institute of Pharmacy, Bhujbal Knowledge City, Nashik, Maharashtra, India. Her research contributes, Human well-being (SDG-3, Target 3.4). She works on plant seeds for Prebiotics, Probiotics, Pectin, Bioactive Peptides, Natural Excipients etc. Work on Microbiome regulations through Food, for management of autoimmune disorders. she also deals with Agricultural waste and its prebiotic potentials to explore its utilization as value added products (SDG-12, Target 12.2, 12.4, 12.5)

Presently she deals with basic research to prove the concepts. Currently, there are no established regulatory frameworks and guidelines for products like prebiotics, probiotics, post-biotics and synbiotics etc

In present research work it is observed that various physiological factors play very important role in therapeutic efficacy and functionality of these components.

The most important aspect focused on here in the present research work is how a marketed probiotic act when it is taken orally.

Questions hypothesized were ---------

Do they really work when consumed orally?

Do they work in the presence of antibiotics?

How marketed probiotics act in simulated gastric acidic conditions?

How marketed Probiotics act in simulated intestinal basic conditions?

What if they are consumed without any prebiotic food?

What is the impact of gastric enzyme Pepsin and intestinal enzyme Alpha amylase on the sustenance and viability and growth of probiotics once consumed?

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Marketed probiotic proliferation aspects have been studied considering above mentioned questions.

Prebiotic potentials of flaxseeds have been evaluated under simulated conditions.

Fermented flaxseed meal was prepared and the impact of this post biotic formulation was also evaluated on the performance of marketed probiotics in simulated conditions

The most important aspect of the present research work is that it is a simple cost-effective microbiological assay methodology designed to check and validate the performance of marketed probiotics under simulated conditions and mimic the real-time body conditions

Research Findings of the present work can stand as helpful guidance to Physicians treating patients with probiotics, the concept emerging day by day.

The research findings could contribute in the design of guidelines for products like Prebiotics, probiotics, post-biotics, and Synbiotics.

One more important aspect we touched on and continued the work: Flaxseed endophytes have been found to get activated in simulated conditions. In our further studies which is unpublished work we have performed various tests and proved the endophytic activation of lactobacillus genera from the flaxseed prebiotics. We further took trials on onion waste peels, Ragi seeds, and Garden cress seeds for endophytic activation studies using our own microbiological assay methodology models. (Publications are in communication). Further, we are planning to send the samples for 16 S PCR sequencing which we could not do due to the unavailability of funds.

Endophytes: Plants and various plant parts especially seeds, which are used in vegetal diet, provide not only fibers, vitamins, minerals, essential amino acids, and metabolites but also the important microbes maintaining animal gut flora. Endophytes are an endosymbiotic group of microorganisms that colonize in plants and microbes that can be readily isolated from any microbial or plant growth medium.

Note: The study methodology is validated and found to be reproducible. And the studies were performed under strict sterile conditions maintaining both positive and negative environmental growth controls

For the said work she received excellent comments through the reviewers when the manuscript was under consideration for publication in the journal. One of the reviewers commented that Reviewer #2: "Originality: this is an original study that was designed to investigate whether supplementation of ground flaxseed could stimulate probiotic proliferation during antibiotic treatment in vitro. The implications of this study could have relevance for recommendations on how probiotics are prescribed during or after antibiotic therapy. Also, this work demonstrated that the inclusion of flaxseed may efficiently optimize the growth potential of probiotics to a higher degree than without synbiotics. Scientific quality: This study carried out careful enzymatic assays, sub-culturing of probiotic bacteria, and proliferation assays." https://drive.google.com/drive/folders/1MiIlpYK1Xl7_yfnIAXjRsfXBM-gzPIHD?usp=sharing

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Highlights and important findings of Present research work makes it more important and I sincerely feel it should reach to maximum readers: These findings would definitely guide Physicians prescribing marketed probiotics to patients https://doi.org/10.1016/j.bcdf.2022.100335

- Gastric enzyme pepsin has an inhibitory effect on growth of probiotics as observed in acidic condition
- One very important observation is that simulated intestinal pH as well as simulated acidic pH with or without enzymes does not support the growth of spores *(Bacillus coagulans SNZ 1969)* as well as culture *(Bacillus coagulans SNZ 1969* may be indicative of growth media requirement for the incubation and growth of the spores and culture in human body.
- The real time bio-relevant media digestion study shows that presence of antibiotic (Azithromycin) does not support the growth of probiotic spores *(Bacillus coagulans SNZ 1969)*and probiotic cultures *Bacillus coagulans SNZ 1969*) without the presence of growth media components.
- In case of fermented flaxseed powder in the presence of citric acid even acidic condition with enzyme pepsin supports the growth of probiotic spores.*(Bacillus coagulans SNZ 1969).*
- In case of real time bio-relevant media simulation, fermented product shows the supportive nature for the growth of probiotics. *(Bacillus coagulans SNZ 1969)* as well as antibiotic (Azithromycin) effect has been found to be diminished as the scanty growth of the species is being observed.
- Fermented flaxseed powder could be effective post biotic supplement which could be explored further in post biotic supplement development.
- It has been observed in present study that factors like gastrointestinal pH, transit time, gastrointestinal fluid composition and presence of prebiotics, fermentation etc play very important role in proliferation and gastrointestinal colonization of orally supplied probiotics as part of the treatment strategy
- The research findings justify the importance of synbiotic and postbiotic supplements as the part of treatment and prescription strategies in gut flora microbial dysbiosis caused by antibiotics rather than probiotic supplements alone.
- Or probiotics should be prescribed after completion of antibiotic therapy with proper instruction to consume probiotics with prebiotic dietary ingredients.

Based on the concept further works are prebiotic and probiotic potentials of garden cress seeds, Onion peel agro waste to explore the prebiotic and probiotic potentials wherein excellent results are obtained. Publications of the research is in process.

Dr,Gitanjali has also developed Prebiotic and probiotic base feed for fish using agricultural and municipal vegetable waste. Wherein it is observed that the said feed is developed by unique calculation methodology. It is more digestible and cost effective in comparison to marketed

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feeds which are prepared by specially grown grains. Whereas the developed feed has excellent prebiotic and probiotic potentials. (Patent filing in process) The said project was also considered and reached the grand finale of Smart India Hackathon 2022. https://drive.google.com/drive/folders/1QVxMftxlHT_qZ9IDgGJXdDplowNXz_GI?usp=sharing

Ongoing projects applications are (Projects ongoing)

1. In -vitro Human Bio- simulation studies to evaluate, characterize, and understand the role of plant seed endophytes as probiotics to explore the potentials of seeds as natural sources of synbiotics in gut flora management. The project has been forwarded to SERB Power Fellowship award. It is under evaluation

2. Nanotechnology-based approach to modulate and evaluate the bioactive peptides from selected plant seeds for their anticancer and antimicrobial potentials along with dosage form design, and optimization with targeted drug delivery aspects. Project submitted to CSIR-Special call for research grants for women scientists.

3. Preclinical Studies to Understand and Design Functional Treatment Options for PCOS through Gut Flora Management. Project submitted to DST-SEED

Any findings, supplementary materials, and photographs of any work related to present research findings as well as the core research area except some formula compositions and process of preparation which are in process of patent filing, could be made available or could be discussed as the part of evaluation of the present application. Research work is conceptualized by Dr.Gitanjali Deokar. She acknowledge the contribution of research students working under guidance on her core research area as the part of M.Pharm research work.

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