International Journal of Science and Technology (IJST)
ISSN: 3049-1118, Volume- 2, Issue- 3 (Jul – Sep 2025)

Development and Formulation of Probiotic Using Fermentation Method

Aakansha Ravindra Patil[1*], Rutik Asaram Hajare[2], Abhay Bajrang Jadhav[3], Kalpita Manik Patil[4], Kumudini Kisan Pandharkar[5]

[1]Doctorate Of Pharmacy, Bharati Vidyapeeth Poona College of Pharmacy
ap826893@gmail.com
[2]Bachelor Of Pharmacy, Indala Institute of Pharmacy
rutikhajare26522@gmail.com
[3]Bachelor Of Pharmacy, Indala Institute of Pharmacy
abhayjadhav6540@gmail.com
[4]MSc. Medical Genetic, MGM School of Biomedical Sciences
kalpitapatil0l@gmail.com
[5]MSc. Medical Genetic, MGM School of Biomedical Sciences
pandharkarkumudini@gmail.com

Abstract

The present study focuses on the development and formulation of a probiotic using a fermentation-based approach with blueberry (*Vaccinium corymbosum*) and black rice (*Oryza sativa L. indica*). Plant extracts were prepared by Soxhlet extraction using 50% ethanol, followed by concentration with rotary evaporation and lyophilization. The extracts were combined in a 7:3 ratio (BB:BR) and fermented with *Lactobacillus plantarum* 2656 under controlled conditions to obtain a fermented product (FBBBR) with 60% yield. Antimicrobial activity of the formulation was assessed against *Shigella boydii* ATC 9207 using agar cup diffusion, resazurin-based minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) assays, confirming its inhibitory potential. Additionally, an orally disintegrating film (ODF) was formulated using carboxymethyl cellulose, gelatine, starch, and sorbitol as film-forming agents for the incorporation of the probiotic. The ODF was evaluated for swelling index, disintegration time in water and simulated saliva, pH, and probiotic viability during storage. Results demonstrated that the fermented extracts enhanced antimicrobial activity and maintained probiotic viability within the ODF matrix. Overall, this study highlights a novel formulation strategy that integrates natural bioactive extracts with probiotics, offering a promising functional nutraceutical delivery system with potential therapeutic benefits.

Keywords: Probiotics, fermentation, blueberry, black rice, *Lactobacillus plantarum*, oral disintegrating film, antimicrobial activity.

Introduction

Probiotics are defined as *"live microorganisms which, when administered in adequate*

*Corresponding Author Email: ap826893@gmail.com
Published: 30/09/2025
DOI: https://doi.org/10.70558/IJST.2025.v2.i3.241103

amounts, confer a health benefit on the host." Their role in promoting gut health, preventing gastrointestinal disorders, and providing therapeutic benefits against infections has been widely studied. Probiotic strains exert their beneficial effects through multiple mechanisms, including competitive exclusion of pathogens, production of antimicrobial metabolites, modulation of the host immune system, and inhibition of toxin expression.

Traditionally, probiotics are consumed through fermented foods such as yogurt, cheese, kimchi, sauerkraut, kombucha, and miso, as well as dietary supplements in capsule or powder form. However, many commercially available probiotics face challenges such as poor survival during gastrointestinal transit and reduced viability during storage. To overcome these limitations, advanced formulation techniques such as spray-drying, freeze-drying, fluidized bed drying, and extrusion have been developed to enhance stability and targeted delivery of probiotic strains.

The therapeutic applications of probiotics are diverse, ranging from the prevention of antibiotic-associated and traveller's diarrhoea to reduction of respiratory infections, mitigation of irritable bowel syndrome, alleviation of atopic dermatitis, and support in eradicating *Helicobacter pylori*. They have also shown potential in managing inflammatory bowel diseases and other systemic conditions, underscoring their significance in both preventive and therapeutic healthcare.

In recent years, functional foods combining probiotics with natural bioactive compounds have gained increasing attention. Black rice (*Oryza sativa L. indica*), rich in anthocyanins, fibbers, and micronutrients, has demonstrated antioxidant, anti-inflammatory, and anti-diabetic properties. Similarly, blueberries (*Vaccinium corymbosum*), abundant in flavonoids, phenolic acids, and vitamins, are known for their prebiotic, antimicrobial, antioxidant, and cardioprotective activities. Together, these natural sources provide an excellent nutritional base to enhance the growth and efficacy of probiotic strains.

Among probiotics, *Lactobacillus plantarum* is one of the most versatile species, naturally found in fermented foods and the human gastrointestinal tract. It produces antimicrobial substances effective against both Gram-positive and Gram-negative pathogens, thereby contributing to gut microbiota balance and protection against infections. Notably, pathogens such as *Shigella boydii*, a causative agent of bacillary dysentery, remain a major concern in developing regions, highlighting the need for novel probiotic-based strategies for prevention and treatment.

The present study aims to develop and formulate a probiotic using a fermentation-based approach, incorporating black rice and blueberry extracts as natural substrates. The study further evaluates the antimicrobial activity of the formulation and explores the potential of incorporating the probiotic into an orally disintegrating film (ODF) for enhanced delivery and stability. This innovative strategy integrates natural bioactive compounds with probiotic technology to create a functional nutraceutical system with significant therapeutic potential.

Materials and Methods

2.1 Extraction and Purification of Plant Materials

Black rice (*Oryza sativa L. indica*) and blueberry (*Vaccinium corymbosum*) were procured from local certified suppliers. The raw materials were dried, powdered, and subjected to Soxhlet extraction using 50% ethanol as solvent. Extraction was carried out for 24 h at 150 °C, followed by concentration using a rotary vacuum evaporator (BR: 90–120 rpm; BB: 80–140 rpm). The dried extracts were freeze-dried at –80 °C and stored in airtight containers. The extraction yield was 8% for black rice and 12% for blueberry.

2.2 Microorganisms

The probiotic strain *Lactobacillus plantarum* 2656 was obtained from NCIM, CSIR-NCL, Pune, India. For antibacterial assays, *Shigella boydii* ATC 9207 was used as the test pathogen. Additional probiotics (*L. acidophilus* Ki, *L. rhamnosus* R11, *Bifidobacterium animalis* Bb12, and *B. animalis* Bo) and pathogens (*Escherichia coli* NCTC 9001, *Salmonella enteritidis* ATCC 13076, *Listeria monocytogenes* ESB 3562, and *Bacillus cereus* NCTC 2599) were included for comparative evaluation as per standard culture collection protocols.

2.3 Fermentation of Extracts

The blueberry and black rice extracts were mixed in a 7:3 ratio (BB:BR) and inoculated with *L. plantarum* 2656. Fermentation was carried out in a shaking incubator at 37 °C and 120 rpm for 24 h. The fermented product (FBBBR) was freeze-dried and stored at –20 °C. The overall yield of the fermented extract was approximately 60%.

2.4 Antibacterial Activity

2.4.1 Agar Cup Diffusion Assay

The antimicrobial activity of FBBBR against *S. boydii* was assessed using the agar cup diffusion method. Test cultures were grown in Mueller–Hinton broth for 24 h, adjusted to 0.5 McFarland standard (~1 × 10^8 CFU/mL), and further diluted to ~1 × 10^6 CFU/mL. Wells containing 100 µL of the fermented extract were loaded, with chloramphenicol and sterile distilled water serving as controls. Plates were incubated at 37 °C for 24 h, and zones of inhibition were recorded.

2.4.2 Minimum Inhibitory Concentration (MIC)

The MIC of the fermented extracts was determined using the Resazurin Microtiter Assay (REMA). Serial two-fold dilutions of samples were prepared in 96-well plates containing Mueller–Hinton broth. Each well was inoculated with 1 × 10^4 CFU/mL of *S. boydii* and incubated at 37 °C for 24 h. Resazurin (10 µL of 2 mg/mL) was added to each well, and color change was monitored after 30 min. The MIC was defined as the lowest concentration showing no color change.

2.4.3 Minimum Bactericidal Concentration (MBC)

Aliquots from wells corresponding to the MIC and higher concentrations were sub-cultured onto Mueller–Hinton agar plates. Plates were incubated at 37 °C for 24 h. The MBC was defined as the lowest concentration resulting in ≥99.9% reduction in viable counts compared to the initial inoculum.

2.5 Development of Oral Disintegrating Films (ODF)

ODFs were prepared by the solvent casting method using carboxymethyl cellulose (CMC), gelatin, starch (0.5:0.5:0.5), and sorbitol as plasticizer. Solutions were prepared separately, hydrated, heated, and combined, followed by addition of probiotic culture. The homogenized solution was degassed by ultrasonication and poured into plates (8 × 9 cm). After drying for 24 h at room temperature, the films were cut into uniform pieces and stored under vacuum and non-vacuum conditions.

2.6 Evaluation of ODFs

- **Swelling Index:** Measured by immersing the film in 7 mL of distilled water and recording weight gain at 30 s intervals.
- **Disintegration Time:** Determined in distilled water and simulated saliva medium at 37 °C until complete breakdown of the film.
- **pH Measurement:** The film was dissolved in phosphate buffer (pH 7.45), and changes in pH were noted.
- **Probiotic Viability:** Films were stored at 20 °C under vacuum and non-vacuum conditions. Viability was assessed periodically by plating on MRS agar and determining colony-forming units (CFU).

Results

- Extraction of blueberry (BB) and black rice (BR) yielded total residues of 1,232 ml and 1,190 ml, respectively. After rotary evaporation, the concentrated extracts provided 12% residue for BB and 8% for BR. The final fermentation product volume obtained was 20 ml.
- The antibacterial activity of the BB:BR (7:3) formulation was assessed against *Shigella boydii* ATCC 9207. Zone of inhibition measurements revealed activity proportional to the concentration used, with 22 nm at 50 µl and 26 nm at 100 µl, compared to the positive control (40 nm). Distilled water showed no inhibitory activity.

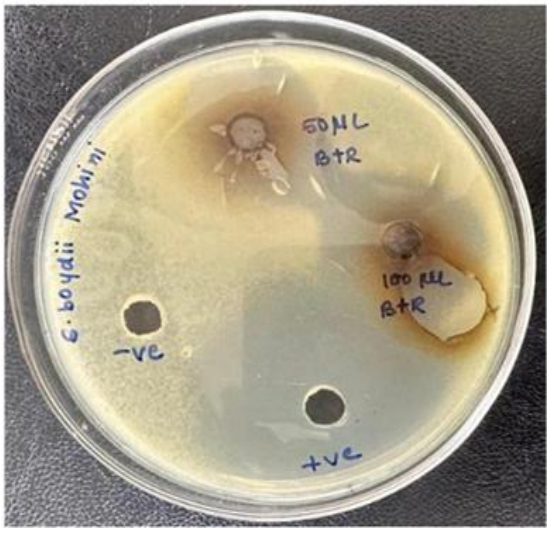

Figure No 1: Sample against Shigella boydii

Table No1. Zone of Inhibition against Shigella boydii

Sr. no.	Organism	Sample & Concentration	Zone of Inhibition (nm)
1	Shigella boydii ATC 9207	B+R 50 µl	22
		B+R 100 µl	26
		PC	40
		DW	-

- The minimum inhibitory concentration (MIC) of the formulation against *S. boydii* was consistently observed at a 1:6 dilution, while the minimum bactericidal concentration (MBC) was confirmed within the range of 1:4 to 1:7 dilutions.

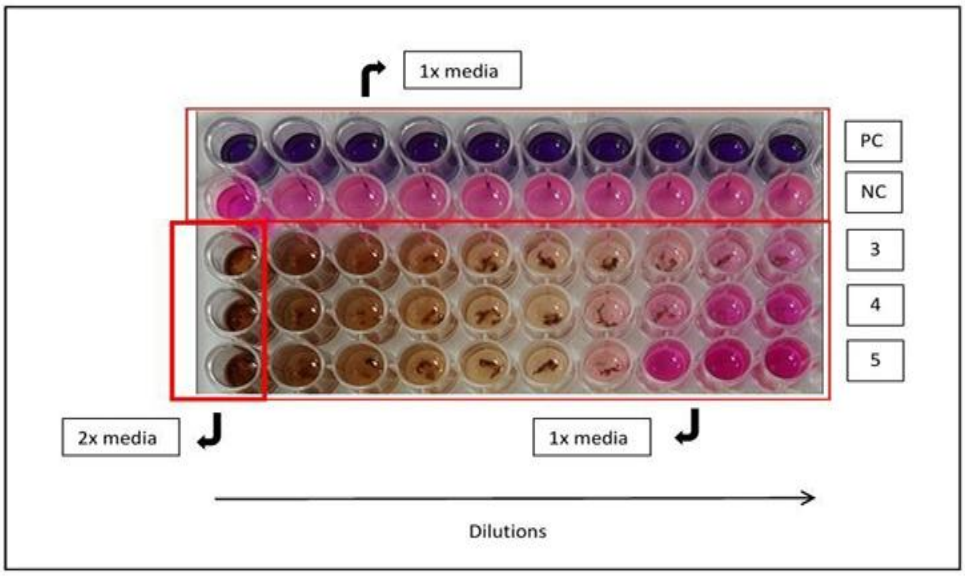

Figure No 2: Resazurin microtiter plate minimum inhibitory concentrations

Table no 2: Dilutions of the Samples

Sr. no.	Culture	Conc. 1	Conc. 2	Conc. 3	Conc. 4	Conc. 5	Conc. 6	Conc. 7	Conc. 8	Conc. 9
1	PC	PC	PC	PC	PC	PC	PC	PC	PC	PC
2	DW	DW	DW	DW	DW	DW	DW	DW	DW	DW
3	BB and BR	1:1	1:2	1:3	1:4	1:5	1:6	1:7	1:8	1:9
4	BB and BR	1:1	1:2	1:3	1:4	1:5	1:6	1:7	1:8	1:10

5	BB and BR	1:1	1:2	1:3	1:4	1:5	1:6	1:7	1:8	1:10

Table No 3: Observed Minimum Inhibitory Concentrations

Sr. no.	Organism	Minimum Inhibitory Concentration (Dilution)
1	Shigella boydii	1:6

Minimum bactericidal concentrations

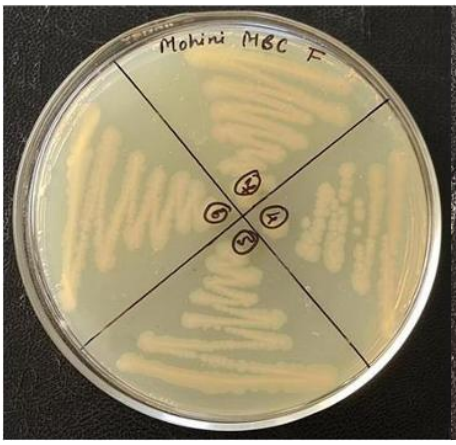

Figure no 3 Plate 1: MBC results for 1:4, 1:5, 1:6& 1;7 dilustion of the 3th row

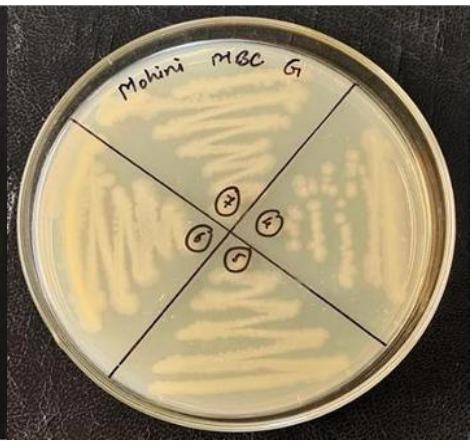

Figure no4 Plate 2 : MBC results for 1:4,1:5, 1:6, & 1:7 dilutions of the 4th row

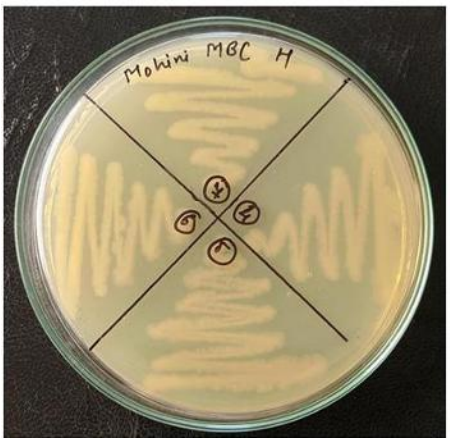

Figure no 5 Plate 3 : MBC results for 1:4, 1:5 , 1:6 & 1:7 dilutions of the 5th row

- Physicochemical characterization of the oral dispersible film (ODF) formulation showed promising results. The swelling test indicated a gradual increase in weight from 0.242 g initially to 0.274 g after 90 seconds, confirming hydration capacity. The

disintegration test (DT) demonstrated dissolution of the ODF within 3 minutes in distilled water and 6 minutes in simulated saliva, both within the pharmacopeial standards (2.5–6.5 min).

- The pH of the product was measured at 7.2, aligning with the physiological salivary pH range (6.9–7.4).

Discussion

This study demonstrates the successful development of a probiotic formulation combining blueberry (Vaccinium corymbosum) and black rice (Oryza sativa L. indica) extracts fermented with *Lactobacillus plantarum* 2656. The fermentation process yielded a stable product with enhanced antimicrobial potential, supporting the hypothesis that polyphenol-rich plant substrates can improve probiotic activity.

The antimicrobial assays confirmed the inhibitory effect of the formulation against *Shigella boydii*, an enteric pathogen of global health concern. The observed inhibition zones (22–26 nm) were smaller than the positive control but indicate a measurable antimicrobial effect attributable to synergistic action between phytochemicals and probiotic metabolites. The MIC (1:6 dilution) and MBC (1:4–1:7 dilutions) values reinforce the bacteriostatic and bactericidal efficacy of the formulation at relatively low concentrations, suggesting practical therapeutic potential. Previous studies have similarly reported that anthocyanin- and flavonoid-rich substrates enhance lactic acid bacteria activity by providing growth-promoting phenolic compounds and generating antimicrobial metabolites during fermentation.

The oral dispersible film (ODF) developed as a delivery system exhibited desirable pharmaceutical characteristics, including rapid disintegration (3 min in water, 6 min in saliva) and acceptable pH (7.2), which ensures compatibility with oral physiology. The swelling index confirmed hydration capacity, essential for rapid release and bioavailability. Such properties make ODFs a patient-friendly dosage form, particularly for pediatric and geriatric populations, overcoming limitations of conventional probiotic supplements such as capsules and powders.

Collectively, these findings suggest that the integration of natural extracts with probiotic technology enhances both functionality and stability. While the study focused primarily on *S. boydii*, broader antimicrobial screening against other clinically relevant pathogens would provide a more comprehensive evaluation. Additionally, in vivo studies and stability assessments over extended storage are needed to validate clinical applicability.

Conclusion

The present study successfully formulated a probiotic product by fermenting blueberry and black rice extracts with *Lactobacillus plantarum* 2656 and incorporating it into an oral dispersible film. The formulation demonstrated notable antimicrobial activity against *Shigella boydii*, with favourable MIC and MBC values, along with optimal physicochemical properties such as rapid disintegration and physiological pH compatibility. These results highlight the potential of combining plant bio actives with probiotics in innovative delivery systems to enhance therapeutic efficacy and consumer compliance.

This work contributes to the growing field of functional nutraceuticals, offering a novel approach to probiotic delivery. Future investigations should focus on long-term stability, large-scale production feasibility, and clinical trials to establish efficacy and safety in human populations.

References

1. Denkova, R., Goranov, B., Teneva, D., Denkova, Z., & Kostov, G. July 2017 Antimicrobial activity of probiotic microorganisms: Mechanisms of interaction and methods of examination. In Antimicrobial research: Novel bioknowledge and educational programs (Vol. 1, pp. 201-212).
2. https://ods.od.nih.gov/factsheets/Probiotics-HealthProfessional/ National Institutes of Health November 3, 2023
3. https://en.m.wikipedia.org/wiki/Lactiplantibacillus_plantarum Lactiplanti bacillus plantarum. Wikipedia
4. Aysha Aslam, Chika N Orator. 2022 Shigella. StatPearls [Internet]
5. B Chandramouli, M Madhavi Latha, K Narendra, K Mallikarjuna. 15 Jan. 2018 Phytochemical and Antimicrobial Investigations of Methanolic Seed Extract of Black Rice (Oryza Sativa L.) Mentioned in An Ancient Palm Leaf Manuscript (Talapatra). World Journal of Pharmaceutical Research.
6. Hyuk-Ju Kwon, Hyunwoo Ahn, Bong Sun Kim, Seok-Seong Kang, Kwang-Geun Lee. 2022.Anti-bacterial and anti-inflammatory activities of lactic acid bacteria-bioconversioned indica rice (Oryza sativa L.) extract. Chemical and Biological Technologies in Agriculture 9 (1), 44
7. Young Min Lee, In Sook Kim, Beong Ou Lim. 2019 Black Rice (Oryza sativa L.) Fermented with Lactobacillus casei Attenuates Osteoclastogenesis and Ovariectomy-Induced Osteoporosis. BioMed Research International 2019
8. Sariya Mapoung, Warathit Semmarath, Punnida Arjsri, Pilaiporn Thippraphan, Kamonwan Srisawad, Sonthaya Umsumarng, Kanokkarn Phromnoi, Sansanee Jamjod, Chanakan Prom-u-Thai, Pornngarm Dejkriengkraikul.2023. Comparative analysis of bioactive-phytochemical characteristics, antioxidants activities, and anti-inflammatory properties of selected black rice germ and bran (Oryza sativa L.) varieties. European Food Research and Technology 249 (2), 451-464.
9. Irini Lazou Ahrén, Jie Xu, Gunilla Önning, Crister Olsson, Siv Ahrné, Göran Molin. 2015.Antihypertensive activity of blueberries fermented by Lactobacillus plantarum DSM 15313 and effects on the gut microbiota in healthy rats. Clinical Nutrition 34 (4), 719726.
10. Jie Xu, Irini Lazou Ahrén, Olena Prykhodko, Crister Olsson, Siv Ahrné, Göran Molin. 2013.Intake of Blueberry Fermented by Lactobacillus plantarum Affects the Gut

Microbiota of L-NAME Treated Rats. Evidence-based complementary and alternative medicine 2013.

11. Ji-Yeon Ryu, Hye Rim Kang, Somi Kim Cho. 2019.Changes Over the Fermentation Period in Phenolic Compounds and Antioxidant and Anticancer Activities of Blueberries Fermented by Lactobacillus plantarum.Journal of food science 84 (8), 2347-2356

12. Xianming Su, Jian Zhang, Hongqing Wang, Jing Xu, Jiuming He, Liying Liu, Ting Zhang, Ruoyun Chen, Jie Kang. 2017. Phenolic acid profiling, antioxidant, and antiinflammatory activities, and miRNA regulation in the polyphenols of 16 blueberry samples from China. Molecules 22 (2), 312.

13. Yu Zhang, Weipeng Liu, Zehua Wei, Boxing Yin, Chaoxin Man, Yujun Jiang.2021 Enhancement of functional characteristics of blueberry juice fermented by Lactobacillus plantarum. Lwt 139, 110590.

14. Mi Jin Cho, Luke R Howard, Ronald L Prior, John R Clark.2004. Flavonoid glycosides and antioxidant capacity of various blackberry, blueberry and red grape genotypes determined by high-performance liquid chromatography/mass spectrometry. Journal of the Science of Food and Agriculture 84 (13), 1771-1782.

15. Yue Wu, Sujin Li, Yang Tao, Dandan Li, Yongbin Han, Pau Loke Show, Guangzhong Wen, Jianzhong Zhou. 2021.Fermentation of blueberry and blackberry juices using Lactobacillus plantarum, Streptococcus thermophilus and Bifidobacterium bifidum: Growth of probiotics, metabolism of phenolics, antioxidant capacity in vitro and sensory evaluation. Food Chemistry 348, 129083.

16. Seo Hyun Park, Jae Gon Kim, Young Ah Jang, Al Borhan Bayazid, Beong Ou Lim.2021.Fermented black rice and blueberry with Lactobacillus plantarum MG4221 improve UVB-induced skin injury. Food and Agricultural Immunology 32 (1), 499-515.

17. Seong Min Hong, Min Cheol Kang, Mirim Jin, Taek Hwan Lee, Beong Ou Lim, Sun Yeou Kim.2021 Fermented blueberry and black rice containing Lactobacillus plantarum MG4221: a novel functional food for particulate matter (PM2.5)/dinitrochlorobenzene (DNCB)-induced atopic dermatitis. Food & Function 12 (8), 3611-3623.

18. Nilushni Sivapragasam, Nilanivetha Neelakandan, HP Vasantha Rupasinghe.2023. Potential health benefits of fermented blueberry: A review of current scientific evidence. Trends in Food Science & Technology 132, 103-120.

19. . Bipul Biswas, Kimberly Rogers, Fredrick McLaughlin, Dwayne Daniels, Anand Yadav.2013. Antimicrobial Activities of Leaf Extracts of Guava (Psidium guajava L.) on Two GramNegative and Gram-Positive Bacteria. International journal of microbiology 2013.

20. Amin Chowdhury, Shofiul Azam, Mohammed Abdullah Jainul, Kazi Omar Faruq, Atiqul Islam.2014. Antibacterial Activities and In Vitro Anti-Inflammatory (Membrane Stability) Properties of Methanolic Extracts of Gardenia coronaria Leaves. International journal of microbiology 2014.

21. Carlos L Céspedes, J Guillermo Avila, Andrés Martínez, Blanca Serrato, José C Calderón-Mugica, Rafael Salgado-Garciglia.2006. Antifungal and Antibacterial Activities of Mexican Tarragon (Tagetes lucida). Journal of agricultural and food chemistry 54 (10), 3521-3527.

22. Riana JB Heinemann, Rosemary A Carvalho, Carmen S Favaro-Trindade. 2013.Orally disintegrating film (ODF) for delivery of probiotics in the oral cavity—development of a novel product for oral health. Innovative food science & emerging technologies 19, 227-232.

23. . K Harini, Krishnamachari Janani, Kavalipurapu Venkata Teja, Chandra Mohan, M Sukumar. 2022.Formulation and evaluation of oral disintegrating films using a natural ingredient against Streptococcus mutans. Journal of Conservative Dentistry and Endodontics 25 (2), 128-134.

24. Neelesh Kumar Maurya, Latika Yadav, Shweta Chaudhary.2022. Nutraceutical potentials of black rice. Research & Reviews: Journal of Food Science & Technology 11 (3), 27-35.

25. Naseem Zahra, Muhammad Khalid Saeed, Hufsah Hamid, Abdullah Qamar, Asma Saeed. Nutritional Composition, Health Benefits and Potential Applications of Blueberry: A Comprehensive Review.25 July 2023. Food and Biotechnology Research Centre, PCSIR Laboratories Complex, Ferozepur Road, Lahore - 54600, Pakistan.

26. Yingchun Zhang, Lanwei Zhang, Ming Du, Huaxi Yi, Chunfeng Guo, Yanfeng Tuo, Xue Han, Jingyan Li, Lili Zhang, Lin Yang.2011. Antimicrobial activity against Shigella sonnei and probiotic properties of wild lactobacilli from fermented food. Microbiological research 167 (1), 27-31.

27. Elnaze Zare Mirzaei, Elahe Lashani, Abolfazl Davoodabadi. Antimicrobial properties of lactic acid bacteria isolated from traditional yogurt and milk against Shigella strains. GMS Hygiene and Infection Control 2018, Vol. 13