SRI VENKATESWARA INTERNSHIP PROGRAM FOR RESEARCH IN ACADEMICS (SRI-VIPRA)

SRI-VIPRA

Project Report of 2025: SVPG-2506

"Assessment Of Biological potency of Polyherbal Formulation Vs Single Herb Extract of Peppermint, Cumin, Turmeric, and Carom on growth kinetics of probiotic microorganisms as a possible therapy for IBS."

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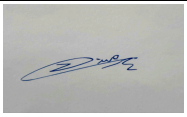
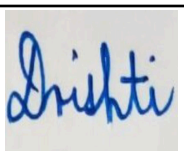
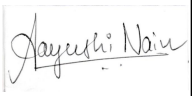
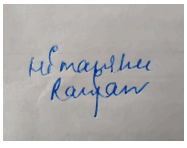
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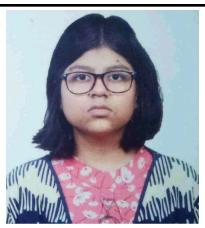
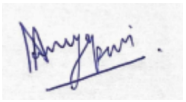
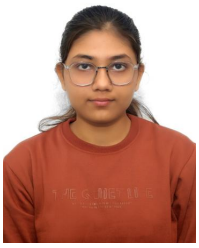
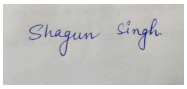
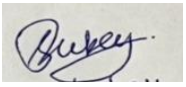
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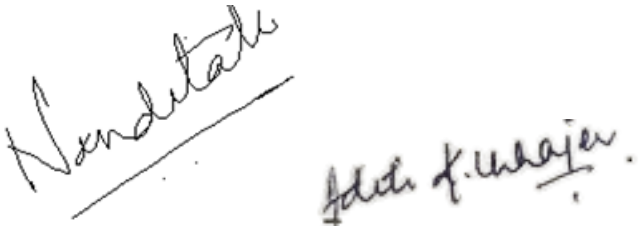
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Name of Mentor: Dr. Aditi Kothari Chhajer **Name of Department:** BOTANY **Designation:** Assistant Professor	

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9		SUKRITI DUBEY	1423052	BSc.(H) Botany	



Signature of Mentors

Certificate of Originality

This is to certify that the aforementioned students from Sri Venkateswara College have participated in the summer project SVPG2506 titled “Assessment Of Biological Potency of Polyherbal Formulation Vs Single Herb Extract of Peppermint, Cumin, Turmeric, and Carom on growth kinetics of probiotic microorganisms as a possible therapy for IBS.**”**.The participants have carried out the research project work under my guidance and supervision from 1st July, 2024 to 30th September 2025. The work carried out is original and carried out in an online/offline/hybrid mode.



Signature of Mentors

Acknowledgements

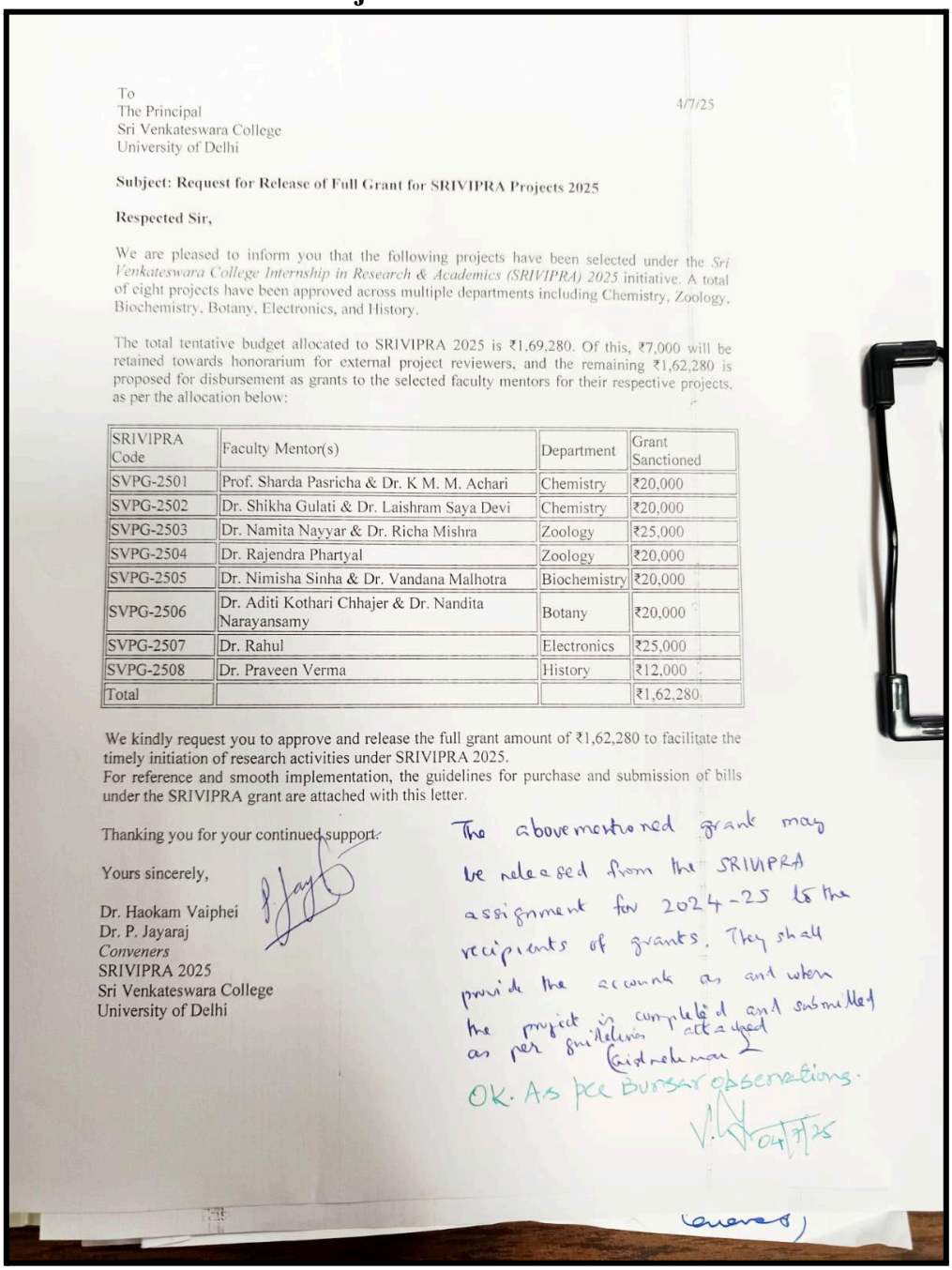
We express our sincere gratitude to our college for initiating the Sri Venkateswara Internship Program for Research in Academics (SRI-VIPRA), which provided us with a valuable platform to gain hands-on research experience and knowledge. We extend our heartfelt thanks to the Principal, Prof. Vajala Ravi, and the IQAC Coordinator, Prof.V Mathur, for their vision and initiative in launching this program.

We are also deeply thankful to the conveners of SRI-VIPRA, Dr P. Jayaraj and Dr. Haokam Vaiphei for their continuous support and guidance throughout the internship period.

We express our profound gratitude to our mentors, Dr. Nandita Narayanasamy and Dr. Aditi Kothari Chhajer, for giving us the opportunity to work on this project under their guidance. Their unwavering enthusiasm, invaluable knowledge, and constant encouragement served as a source of inspiration and greatly contributed to the successful completion of this project work.

We also extend our sincere thanks to the Biochemistry Department laboratory staff for their technical assistance and cooperation throughout the research process. This project would not have been possible without their support.

Project Sanction Letter



To
The Principal
Sri Venkateswara College
University of Delhi

4/7/25

Subject: Request for Release of Full Grant for SRIVIPRA Projects 2025

Respected Sir,

We are pleased to inform you that the following projects have been selected under the *Sri Venkateswara College Internship in Research & Academics (SRIVIPRA) 2025* initiative. A total of eight projects have been approved across multiple departments including Chemistry, Zoology, Biochemistry, Botany, Electronics, and History.

The total tentative budget allocated to SRIVIPRA 2025 is ₹1,69,280. Of this, ₹7,000 will be retained towards honorarium for external project reviewers, and the remaining ₹1,62,280 is proposed for disbursement as grants to the selected faculty mentors for their respective projects, as per the allocation below:

SRIVIPRA Code	Faculty Mentor(s)	Department	Grant Sanctioned
SVPG-2501	Prof. Sharda Pasricha & Dr. K M. M. Achari	Chemistry	₹20,000
SVPG-2502	Dr. Shikha Gulati & Dr. Laishram Saya Devi	Chemistry	₹20,000
SVPG-2503	Dr. Namita Nayyar & Dr. Richa Mishra	Zoology	₹25,000
SVPG-2504	Dr. Rajendra Phartyal	Zoology	₹20,000
SVPG-2505	Dr. Nimisha Sinha & Dr. Vandana Malhotra	Biochemistry	₹20,000
SVPG-2506	Dr. Aditi Kothari Chhajer & Dr. Nandita Narayansamy	Botany	₹20,000
SVPG-2507	Dr. Rahul	Electronics	₹25,000
SVPG-2508	Dr. Praveen Verma	History	₹12,000
Total			₹1,62,280

We kindly request you to approve and release the full grant amount of ₹1,62,280 to facilitate the timely initiation of research activities under SRIVIPRA 2025.
For reference and smooth implementation, the guidelines for purchase and submission of bills under the SRIVIPRA grant are attached with this letter.

Thanking you for your continued support.

Yours sincerely,

Dr. Haokam Vaiphei
Dr. P. Jayaraj
Conveners
SRIVIPRA 2025
Sri Venkateswara College
University of Delhi

The above mentioned grant may be released from the SRIVIPRA assignment for 2024-25 to the recipients of grants. They shall provide the accounts as and when the project is completed and submitted as per guidelines. act ≈ ject
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V.N 04/7/25

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ABSTRACT

Irritable bowel syndrome (IBS) is a multifaceted functional gastrointestinal disorder typically characterised by ongoing gastrointestinal pain or discomfort and abnormal gut microflora. As medicinal plants have long been used to manage various aspects of gastrointestinal health, researchers are increasingly investigating the potential therapeutic effectiveness of these plant products. In this study, a variety of the most commonly used herbs believed to support the relief of symptoms associated with digestive ailments were chosen for investigation, such as turmeric, peppermint, ajwain, and jeera, focusing primarily on the interplay between these herbs and cultured probiotic microorganisms.

As an initial component of this study, aqueous phytochemical extracts were prepared from dried plant materials of *Curcuma longa* (turmeric root), *Cuminum cyminum* (cumin seed), *Trachyspermum ammi* (carom seed), and *Mentha piperita* (peppermint leaves). However, during the preliminary phase of experimentation, the presence of unexpectedly high numbers of growing microorganisms that may have resulted from endophytic contamination caused difficulties in interpreting experimental data. To address this, the methodology was modified to use commercially sourced steam-based herbal extracts instead of aqueous preparations, reducing the potential risk of biological variability from contamination. Despite this revision, the observed inconsistency in results presents the difficulties of investigating complex herbal extracts.

The findings of this study indicate that standardisation of the extracts, control of contaminants/endophytes within the extract, and refining of research methods are necessary to achieve a conclusive understanding of the efficacy of these medicinal plant therapies. Although definitive conclusions regarding efficacy cannot be drawn from this study, the findings clearly illustrate limitations in the investigation and provide a guide for future research to be conducted with improved methodology.

1.INTRODUCTION

IRRITABLE BOWEL SYNDROME (IBS)

IBS is a functional gastrointestinal disorder (FGID), indicating it primarily affects how the digestive system works rather than causing any visible structural damage. IBS affects 9%-23% of the population across the globe (World Gastroenterology Organisation, 2009). [1] According to many epidemiological studies, IBS is found to be a common disorder in India. It is also observed that two-thirds of Indian patients are males, which is in contrast to the West, where IBS is prevalent in females. Indian patients with IBS also have other overlapping FGIDs. [2] All of this increases the cost of healthcare significantly and also affects the quality of life of the patient. The following symptoms are observed during IBS: stomach ache, diarrhoea, constipation or a combination of both, changes in bowel habits, mucus in the stool, and differences in the stool shape or consistency. [3]. Abdominal pain and altered bowel habits are caused by visceral hypersensitivity and motor disturbances of the gastrointestinal tract. Increased levels of inflammatory cells, like mast cells, in the mucosal lining caused by intestinal infection is recognised as a potential risk factor for IBS. Altered levels of serotonin secreted from enterochromaffin cells cause both diarrhoea and constipation; higher levels decrease gastric motility, and lower levels increase motility. Anxiety and depression is common in individuals with IBS due to impaired gut-brain interactions. Small Intestinal Bacterial Overgrowth (SIBO) causes higher faecal levels of lactobacilli, coliform, and bifidobacteria due to gut microbiome dysbiosis. [4]

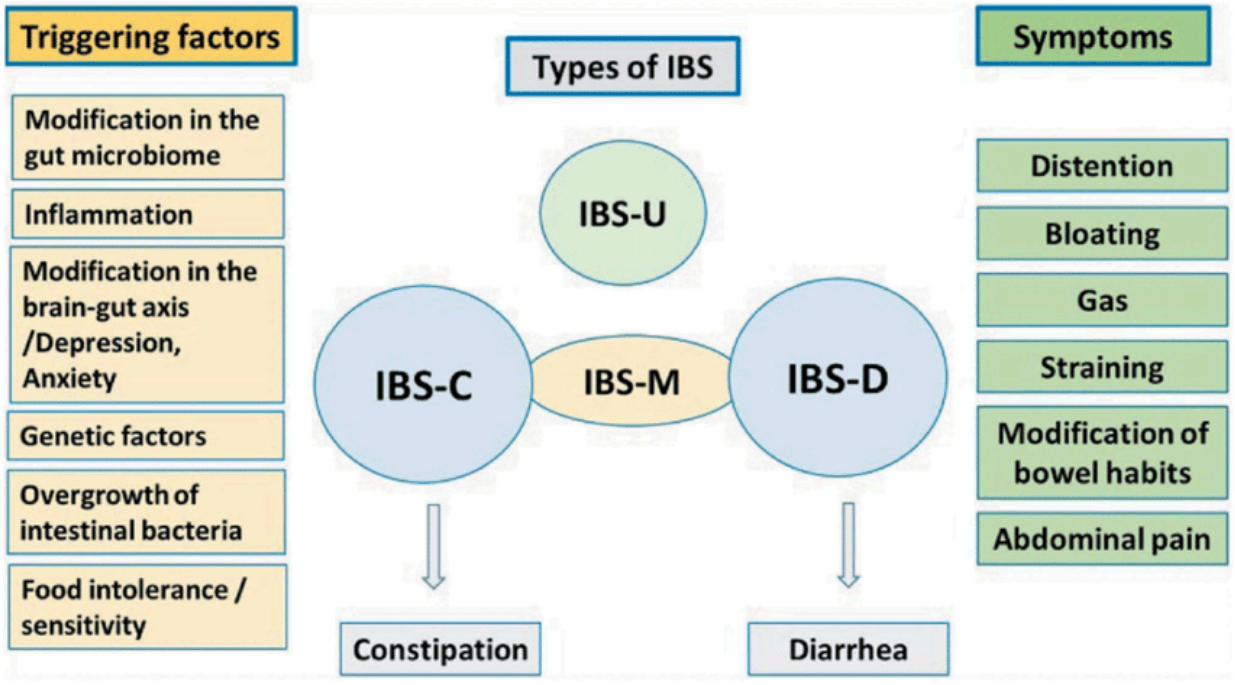


Figure 1. Triggering factors, distribution, and consequences of Irritable Bowel Syndrome (IBS). IBS-D: Irritable Bowel Syndrome-Diarrhoea-predominant; IBS-C: Constipation-predominant; IBS-M: Mixed pattern alternating diarrhea and constipation; IBS-U: Unclassifiable type (IBS-U). [5]

Diagnosis

Diagnosis of IBS can be difficult due to many reasons: i) Evolution of new symptoms overtime leads to increased complexity of disorders; ii) Other conditions, such as lactose ,and fructose intolerance, are often similar to IBS; iii) There is a lack of biomarkers to identify IBS accurately and specifically; iv) there is a lack of awareness of the latest guidelines and definition. [6]

Over the years, several sets of guidelines have been developed to diagnose IBS, including the Manning, Kruis, and Rome criteria. The first widely accepted guidelines for diagnosis of IBS were the Manning criteria, which was introduced in 1978 [6]. A specific set of symptoms was later given by Kruis and colleagues in 1984 to identify the conditions [7] . The Rome I criteria was proposed by an international group of experts in 1988, which was then modified into Rome II in 1999 and Rome III in 2006. Classification of IBS into multiple subtypes was the major highlight of Rome III; these subtypes are IBS-C (constipation), IBS-D (diarrhoea), IBS-M (mixed), and IBS-U (unclassified). The Rome IV is the most recent version that is used, and it was published in 2016. [8]

Table 1: Recurrent abdominal pain on average at least 1 day/week in the last 3 months, associated with two or more of the following criteria:
1. Related to defecation
2. Associated with a change in the frequency of stool
3. Associated with a change in the form (appearance) of stool
(These criteria should be fulfilled for the last 3 months with symptom onset at least 6 months prior to diagnosis.

Table 1: The Rome IV Criteria [8].

Management

No definitive cure for IBS has been identified as of now; only symptomatic treatment is offered. Adequate knowledge and general awareness of this condition are necessary for the patient to benefit from the therapy prescribed. Symptoms can be managed by diet changes, physical activity, and certain medications. Patients are advised to avoid consumption of inflammatory substances like allergens and chemicals, especially alcohol, methylxanthines, and caffeine; avoid food that causes symptoms to appear,

limiting intake of high-fat foods; and incorporate whole grains, fibers, and vitamins into meals. Patients are advised to have a low FODMAP (fermentable oligosaccharides, disaccharides, monosaccharides, and polyols) diet, avoiding vinegar, ketchup, pickled items, and mustard [9]. Hypnosis, biofeedback, and psychotherapy can assist with anxiety reduction associated with IBS. Massage therapy and acupuncture could also be beneficial. [6]

Various Medications Used for IBS:

- Alverine, scopolamine, trimebutine, dicyclomine, pinaverium, otilonium, and hyoscyamine are antispasmodics that help in relieving pains and cramps by relaxing intestine smooth muscles. The main mode of action is blocking calcium channels, also known as anticholinergic action.
- Antidepressant drugs like selective serotonin reuptake inhibitors (SSRIs), tricyclic antidepressants (TCAs), and serotonin-norepinephrine reuptake inhibitors reduce abdominal pain by acting upon the pain-control pathways in the CNS.
- Antidiarrheal agents like loperamide reduce muscle contraction and slow down the intestinal movement that helps in controlling diarrhoea.
- Psyllium, calcium polycarbophil, bran, and ispaghula husk are bulking agents that act as fiber supplements (both natural and synthetic). They are usually used to improve stool consistency, frequency, and movement through the gut.
- Osmotic laxatives like polyethylene glycol (PEG) 3350 and lactulose work by increasing the content of water in the intestines, which makes stools softer and easier to pass; hence, these are frequently prescribed for IBS with constipation (IBS-C).
- Lubiprostone activates the chloride channels in the intestine, which increases fluid secretion and helps improve bowel movements and stool passage.
- Linaclotide stimulates the release of guanylin and uroguanylin, which increases fluid and electrolyte secretion in the colon, thereby enhancing bowel function. [10]

GUT MICROBIOTA AND IBS

There are a plethora of microbes present in the human gut, referred to as the gut microbiota. The gut microbiome serves many functions that include modulation of nutrient absorption and drug metabolism, ensuring the structural stability of the gut mucosal barrier, modulating immune function, and preventing pathogenic invasion. The microbiota of the gastrointestinal tract comprises primarily Firmicutes (64%), Bacteroidetes (23%), Proteobacteria (8%), and Actinobacteria (3%).

Overgrowth or loss of some gut microbial communities may cause a disbalance called ‘dysbiosis,’ which leads to a reduction in microbiome diversity. Dysbiosis is regarded as the cause of inflammatory responses associated with oxidative stress, disruption of intestinal barrier integrity, and translocation of bacteria across the mucosa, a pathogenesis of IBS.

One form of dysbiosis known as Small Intestinal Bacterial Overgrowth (SIBO) is described as the excess of bacteria in the small intestines. These overgrown bacteria ferment the ingested sugar before it can be absorbed, which causes gas production, causing stomach aches, bloating, flatulence, and altered bowel movements. Its symptoms overlap with IBS-D, as diarrhoea is more common than constipation caused by immune activation and inflammation, enterotoxin bacterial strains, deconjugation of bile salts and

increased membrane permeability. [11]

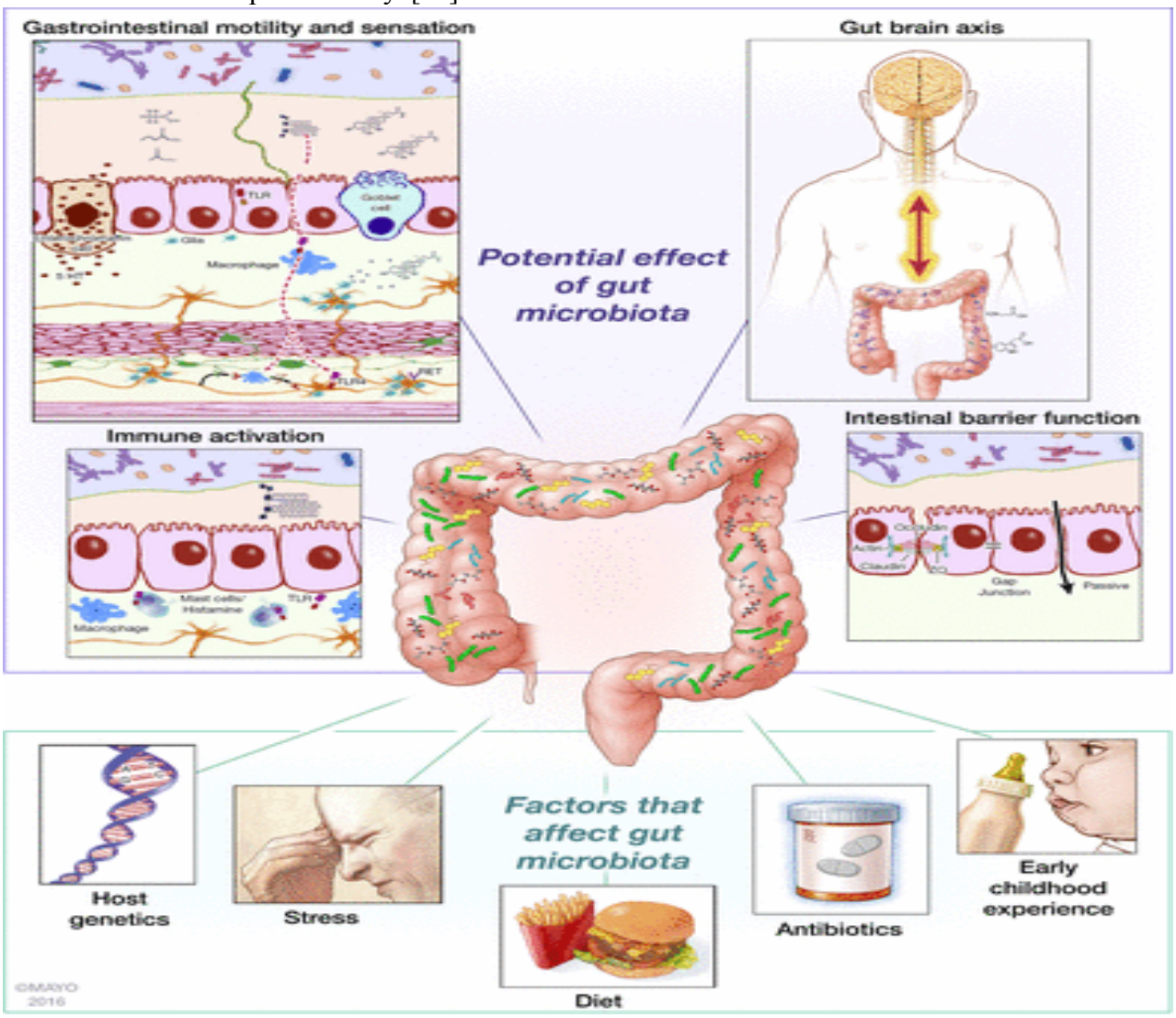


Figure 2: [5] Gut microbiota modulates pathophysiological mechanisms underlying IBS, such as gastrointestinal motility and sensation, the gut-brain axis, immune activation, and intestinal barrier function. Gut microbiota composition is affected by risk factors underlying IBS, such as host genetics, stress, diet, antibiotic usage, and early childhood experience. 5-HT, 5-hydroxytryptamine (serotonin); TLR, Toll-like receptor; ZO, zonula occludens.

Since the microbiome describes the pathogenesis of IBS, we can use microbiome-targeted therapies to treat the conditions. Some of these therapies include:

1. Prebiotics—these are bacterial growth stimulants in the form of indigestible carbohydrate compounds. These commonly known fructo-oligosaccharides (FOS) and galacto-oligosaccharides (GOS), which help in the growth of *Bifidobacteria* and *Lactobacilli* , have anti-inflammatory and antioxidative effects, reducing the gas production and bloating. [11,12] While certain other Prebiotics may exacerbate IBS symptoms because they are classified as fermentable

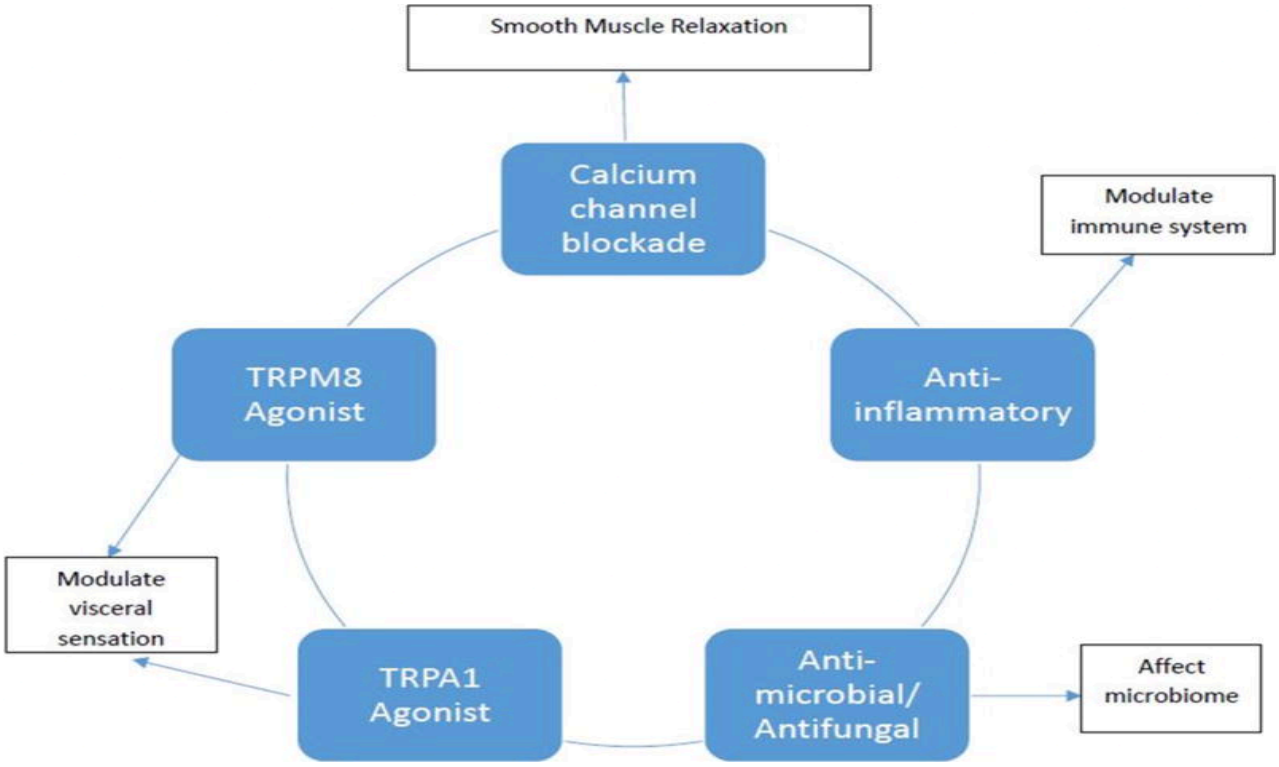
oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAP), and thus patients should refrain from consuming them.

2. Probiotics—living or attenuated microorganisms having beneficial properties specific to gut microbiota [11]. Probiotics, such as *Bifidobacterium* and *Lactobacillus* strains, may relieve IBS symptoms by improving beneficial bacteria and SCFA production. Their mechanism involves reducing intestinal inflammation, enhancing barrier function, balancing the immune response (modulating cytokines), and influencing intestinal sensitivity, pathogen damage, and gut-brain interactions. Efficacy is strain- and patient-dependent. [12]
3. Synbiotics and postbiotics—synbiotics are a combination of both probiotics and prebiotics, aimed at improving the survival and activity of beneficial bacteria in guts. It has reported improvement in bowel movement and frequency and reduction in bloating, but long-term effects and best formulations remain an area for research. Postbiotics, the metabolites formed by beneficial bacteria, are emerging as potential therapies for IBS. Early findings include reduction of inflammation and IBS-D symptom improvement. This innovative approach warrants further investigation and research [12].
4. Antibiotics—these are used to combat bacterial infections in humans and animals. These may be bactericidal and bacteriostatic, causing the death of bacteria and/or preventing their growth. Although there are few broad-spectrum, non-absorbable antibiotics that have been effective in lowering bacterial overgrowth in IBS, their administration is often restricted due to their potential for causing systemic adverse reactions. To counter these adverse reactions, non-absorbable antibiotics with activity confined mostly to the lumen of the gut have been designed. Among these, rifaximin has been found to be effective with low resistance and few adverse reactions. Neomycin has also been used in the treatment of IBS, but with the property of rapidly inducing bacteriological resistance, neomycin is rarely utilized in clinical practice.
5. Fecal Microbiota Transplantation (FMT)—It is the process in which stool from a healthy donor is transferred to the colon of a different individual with the intention of altering their gut microbiome. FMT has been studied as a safe and efficacious treatment for Clostridium difficile infection; however, only recently has it been examined as a potential treatment for IBS, as well as several other gastrointestinal conditions. [11]

HERBAL MEDICINE THERAPY FOR IBS

Utilising medicinal plants and herbs to prevent or cure clinical conditions is called herbal medicinal therapy, and it's a part of Complementary and Alternative Medicine (CAM).

1. Peppermint (*Mentha piperita*)—Peppermint is a perennial flowering plant that grows throughout Europe and North America. Peppermint (Mentha × piperita) is a (usually) sterile hybrid mint, a cross between water mint (Mentha aquatica) and spearmint (Mentha spicata) that is believed to have arisen naturally. Mint plants have a long history of medicinal use, dating to ancient Egypt, Greece, and Rome, where they were used as stomach soothers. Peppermint oil is obtained by steam distillation from the fresh leaves of peppermint [13]. It is a natural carminative herbal remedy. It has L-menthol, a monoterpene, that blocks the calcium channels in the intestinal smooth muscles, thus causing an antispasmodic effect. Peppermint oil possesses antimicrobial, anti-inflammatory, antioxidant, immunomodulating, and anesthetic activities, all of which may be relevant for the treatment of IBS [14].



TRPM8 = transient receptor potential cation channel subfamily M member 8; TRPA1 = transient receptor potential cation channel, subfamily A, member 1

Figure 3: Potential peppermint oil mechanisms of action within the gastrointestinal tract [13]

2. Carom (*Trachyspermum ammi*)—Trachyspermum *ammi*, a member of the Umbelliferae (Apiaceae) family, is an aromatic herb bearing white flowers and small brownish seeds. The seeds are a rich source of volatile essential oil, with thymol as the predominant constituent (approximately 49%), along with γ-terpinene (30.8%), p-cymene (15.7%), and smaller amounts of β-pinene, myrcene, and limonene. Traditionally, *T. ammi* has been employed in the treatment of various gastrointestinal ailments, including indigestion, colic, and diarrhea. Experimental studies have reported that extracts derived from the seeds, commonly known as *Carum copticum*, exhibit a range of pharmacological properties, such as anticholinergic, analgesic, anti-asthmatic, and antitussive activities, thereby providing scientific support for its therapeutic applications. [15]
3. Turmeric (*Curcuma longa*)—Turmeric is derived from the rhizomes of Curcuma longa, with curcumin being the major active compound responsible for the characteristic yellow color. Curcumin is endowed with versatile biological actions, which include antioxidant, anti-inflammatory (mediated by the inhibition of cytokine secretion), hepatoprotective, and antiallergic properties. It also has these properties in the gastrointestinal tract, where curcumin plays a role in the regulation of intestinal permeability, control of the gut microbiota, and protection against bacterial, parasitic, and fungal infections. In addition, curcumin demonstrates GSH-like activity [16]. However, due to its low bioavailability, poor absorption, and rapid elimination, it is suggested to take curcumin with enhancing agents like other antioxidants or

herbal products. Since curcumin reaches the gut almost unaltered, it could exert potentially beneficial effects on the gut microbiota [17].

4. Cumin (*Cuminum cyminum*)—also known as Jeera—is an aromatic herb, spice, and natural food preservative that has various pharmacological activities, including excellent antioxidant, antibacterial, antifungal, and analgesic properties, and thus, has been used to treat mild digestive disorders. The major compounds occurring in cumin are cuminaldehyde, limonene, α- and β-pinene, 1,8-cineole, o- and p-cymene, α- and γ-terpinene, safranal, and linalool. The high antioxidant activity of cumin has been attributed to the presence of polyphonic compounds like monoterpene alcohols and flavonoids. Its antimicrobial activity is attributed to cumin aldehyde. When administered, cumin increases gastric acid secretion by a cholinergic mechanism and also increases the activity of digestive enzymes. An aqueous extract derived from cumin seeds shows bioenhancer activity [18].

POLYHERBAL FORMULATION

Ayurveda is an ancient system of medicine classified under the Traditional Systems of Medicine (TSMs). The term *"Ayurveda"* is derived from the Sanskrit words *"ayur"* (life) and *"veda"* (knowledge or science), and it is commonly interpreted as the "science of life." Therapeutic approaches in Ayurveda predominantly rely on natural substances, including herbs and minerals, to restore and maintain physiological harmony [19]. Ayurvedic drug formulations are based on two fundamental approaches: the use of a single medicinal herb or the combination of multiple herbs, a practice known as polyherbal formulation (PHF), also referred to as polypharmacy or polyherbalism. This approach involves blending several herbs in specific proportions to achieve enhanced or synergistic therapeutic effects. Broadly, Ayurvedic herbal formulations are classified into two main categories: Kasthoushadhies, which consist solely of herbal ingredients, and Rasaushadhies, which are herbo-bio-mineral formulations that incorporate metals and minerals to augment therapeutic efficacy [20]. In polyherbal formulations, the constituent herbs may interact with one another in three primary ways: synergistically, summatively (additively), or antagonistically. Summative interactions involve the simple addition of the individual effects of each herb. In contrast, synergistic interactions occur when the combined therapeutic effect of two or more herbs exceeds the sum of their individual actions, whereas antagonistic interactions result in a reduced overall effect compared with the expected additive outcome. Understanding and evaluating these herb-herb interactions enables the rational design of formulations tailored for the effective management of various diseases. [21]. Polyherbal formulations (PHFs) are now not only an integral part. of traditional healing systems but also some contemporary medical practices. These plant-based therapeutic combinations are typically known by various terms, including polyherbal formulations, herbal recipes, herbal preparations, and classical prescriptions. [22]

Polyherbal Formulations Used in the Treatment of IBS

Several polyherbal formulations have been investigated for their efficacy in managing irritable bowel syndrome (IBS) due to their multi-targeted actions on gastrointestinal function.

STW 5 (Iberogast) is a well-characterized herbal formulation comprising alcoholic extracts of *Iberis amara* (whole plant), *Chelidonium majus* (root), *Silybum marianum* (fruit), *Melissa officinalis* (leaf), *Carum carvi* (fruit), *Glycyrrhiza glabra* (root), *Angelica sinensis* (root), *Matricaria recutita* (flower), and *Mentha piperita* (leaf). These components exert diverse pharmacological effects across different regions of the gastrointestinal tract, collectively targeting multiple IBS symptoms such as abdominal pain,

bloating, and altered bowel habits. Carmint is another polyherbal preparation containing extracts of *Melissa officinalis* (leaf), *Mentha spicata* (leaf), and *Coriandrum sativum* (root). This formulation exhibits antispasmodic, carminative, and mild sedative properties, which contribute to the relief of gastrointestinal discomfort and abdominal cramping in IBS patients.
Padma Lax is a complex polyherbal formulation made up of extracts from *Aloe barbadensis*, *Jateorhiza palmata* (root), *Marsdenia condurango* (bark), *Rhamnus frangula* (bark), *Gentiana lutea* (root), *Inula helenium* (rhizome), *Terminalia chebula* (fruit), *Piper longum* (fruit), *Rhamnus purshiana* (bark), *Rheum palmatum* (root), *Strychnos nux-vomica* (seed), and *Zingiber officinale* (root). This formulation is predominantly used to regulate bowel movements and mitigate constipation-associated IBS symptoms by combined laxative, digestive, and motility-modulating effects [23].

ENDOPHYTES

Endophytes are microorganisms that live in plant tissues and exhibit varied species diversity along with a broad host range, making them an important and likely source of natural products. Studies have shown that each examined plant so far harbors one or more types of endophytes. These microbial endophytes sustain a symbiotic association with their host plants, abiding internally without causing any visible symptoms of plant diseases. Bacterial endophytes have been isolated from nearly all plant parts, including roots, stems, leaves, flowers, and seeds. They produce a variety of primary and secondary metabolites that considerably affect plant metabolism and stimulate diverse biological responses. Due to these properties, bacterial endophytes provide plants with improved plant health, trigger growth, and improve yield in different plants; they also enhance pathogen resistance and stress tolerance. [24]

Recent research shows that involved endophytes have moved away from conventional physiological and biochemical studies at a cellular and molecular biological level. The application of current advances in genomic technology has also helped in increasing our knowledge with regard to plant and endophyte interactions. The result is that endophytes have been found to be a source of a variety of secondary bioactive compounds such as alkaloids, quinones, benzopyrones, flavonoids, xanthones, tetralones, terpenoids, phenolic compounds, steroids, and tannins. Endophytes can emerge as a reservoir for many naturally occurring compounds, some of which have been found to possess biological properties such as antimicrobial, anticancer, antioxidant, antiviral, and immunosuppressant properties. Furthermore, endophytic antimicrobial compounds also hold great potential for applications as a natural source for preservatives of foods and biological materials. Progress in chromatographic and spectrographic analysis has also helped in rapidly identifying known and unknown secondary compounds. [24]

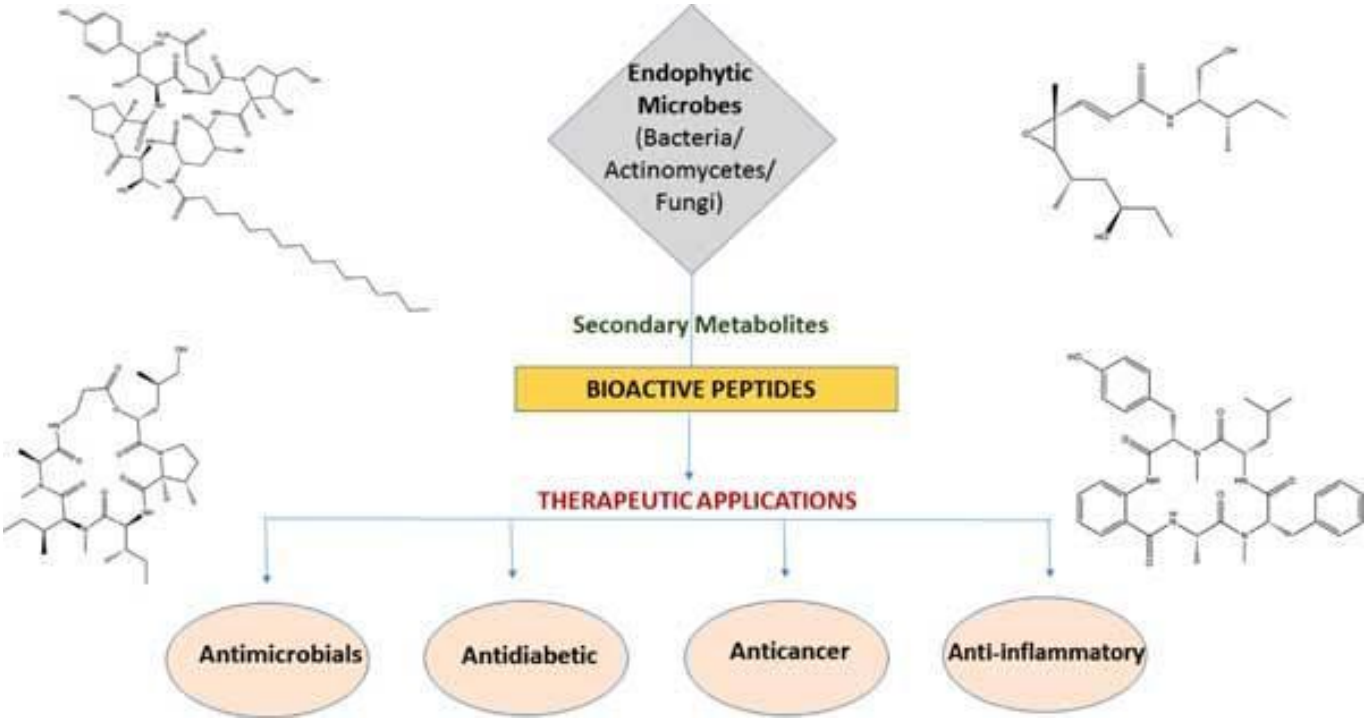


Figure 4: Secondary metabolites (peptides) in therapeutic drugs [25]

Nigella sativa (black cumin) is a well-known medicinal plant valued for its rich bioactive profile and broad therapeutic applications. In addition to its inherent antimicrobial compounds, more attention is being paid to endophytes associated as potential sources of new therapeutic agents. Endophytic fungi isolated from *N. sativa* have demonstrated notable antibacterial activity, highlighting their role as contributors to the plant's medicinal properties. Given the rising challenge of antibiotic resistance, these endophytes represent a promising reservoir of bioactive metabolites that could be developed into new antimicrobial and therapeutic drugs. Further exploration of *N. sativa*–derived endophytes may therefore expand their application in pharmaceutical and biotechnological fields. [26]

2. RATIONALE OF THE STUDY

Irritable Bowel Syndrome is a chronic functional gastrointestinal disorder characterized by abdominal pain, disturbed bowel habits, bloating, and discomfort. Accumulating evidence suggests that gut microbiota imbalance contributes to the pathophysiology of IBS [27, 28]. Probiotics have been regarded as a non-invasive therapeutic agent in IBS because they help restore the microbial balance, modulate immune responses, and improve intestinal barrier function [29, 30]. On the other hand, the efficacy of probiotics depends mostly on the survival, growth, and metabolic activity in the gastrointestinal environment [31].

Herbs like peppermint, cumin, carom, and turmeric have been traditionally used in digestive disorders, including IBS, in systems of medicine like Ayurveda and Unani. These herbs contain various bioactive compounds such as menthol, cumin aldehyde, thymol, and curcumin that exhibit antimicrobial, anti-inflammatory, carminative, and antioxidant properties [32–35]. While these properties are well documented, their direct influence on beneficial probiotic microorganisms is poorly explored.

In the majority of the existing studies, researchers have mainly focused on the antimicrobial properties of herbal extracts against pathogenic microorganisms and herbal combinations. However, the utilization of single herbal extracts allows an easy analysis of individual biological activity and an assessment of which herbs may be beneficial to the probiotic bacteria.

The impact evaluation of these herbal extracts with respect to the growth patterns and metabolic secretion levels of probiotics can provide useful insight into their compatibility and synergistic effects. This could result in better gut homeostasis and form the scientific basis for developing an adjuvant herbal probiotic therapy for the management of IBS symptoms. [36]

3. OBJECTIVES OF THE STUDY

General Objective

To evaluate the biological potency of individual plant extracts of peppermint (Mentha × piperita), cumin (Cuminum cyminum), carom (Trachyspermum ammi), and turmeric (Curcuma longa) on the growth rate and microbial secretion activity of the chosen probiotic microorganisms (Culture A & B), with the purpose of understanding their auxiliary potential in treating irritable bowel syndrome (IBS).

Specific Objectives

1. To obtain probiotic bacterial cultures from Yakult by means of colony differentiation with regard to distinctive morphological characteristics, referred to as Culture A and Culture B.
2. To determine the growth kinetics of Culture A and Culture B both aerobically and anaerobically.
3. To evaluate the influence of the 10% aqueous extract of peppermint, cumin, carom, and turmeric on the growth rate of Culture A and Culture B.
4. To test the effect of 1% steam-distilled extracts of peppermint, cumin, carom, and turmeric on the growth kinetics of Culture A and Culture B.

4. METHOD AND MATERIALS

1. Preparation of probiotics

(The entire isolation of probiotic was done under a laminar flow hood under sterility, unless stated otherwise.)

1. The probiotic was prepared by serially diluting Yakult probiotic drink into autoclaved LB media. 2. Cultures were inoculated to final concentrations of 70, 50, and 10 µL/mL, corresponding to inoculation volumes of 350, 250, and 50 µL, respectively, in 5 mL of broth.
3. This was streaked in autoclaved LB agar that was poured into autoclaved petri dishes, which helped to isolate bacteria.
4. The petri dishes were incubated at 37°C for 24 hrs.
5. Colonies were identified and further used as inoculum from the petri dishes.

2. Preparation of aqueous extract

1. Sample Preparation
a) Four fresh falcons were taken.
b) They were opened under sterile conditions inside a laminar air flow hood.
2. Preparation of Aqueous Herbal Extracts (10% w/v)
a) Four different herbs were used: jeera, ajwain, turmeric, and peppermint.
b) For each herb, 4 g was added separately to 40 ml of water containing Falcon.
c) This produced a 10% (w/v) aqueous extract of each herb.
3. Centrifugation
a) Each tube was centrifuged at 3000 rpm for 6 minutes.
b) The temperature was maintained at 14°C. This step ensured proper mixing of the settled extract.

3. Methylene Blue Test

Methylene blue acts as a redox indicator due to its ability to undergo reversible oxidation and reduction reactions. Its colour changes depending on its oxidation state; in the presence of oxygen, methylene blue remains in its oxidized form, which is blue in colour. Under anaerobic conditions, it gets reduced and becomes colourless.

Preparation of methylene blue solution

Weighed 10 g of methylene blue powder and dissolved it in 100 mL of distilled water to prepare a 10% solution. It is mixed well until the dye is completely dissolved.

In anaerobic culture media (in eppendorf tubes), methylene blue is added as a solution.

Loss of blue colour confirms the absence of oxygen, indicating that suitable anaerobic conditions have been achieved. If the indicator remains blue, it signifies oxygen leakage or incomplete anaerobiosis.

4. Preparation of Bacterial Growth Curves (Aerobic Conditions)

I. Control (Without Plant Extract)

All preparations were carried out under aseptic conditions inside a laminar airflow hood.

1. Preparation of culture tubes:
   15 mL Falcon tubes were prepared, each containing 15 mL of LB (Luria–Bertani) medium. Two sets of tubes were used, one for Inoculum A and one for Inoculum B.
2. Inoculation:
   150 µL (0.15 mL) of bacterial inoculum was added to each tube using a micropipette. One tube was inoculated with inoculum A, and the other with inoculum B.
3. Initial absorbance (0 min):
   Immediately after inoculation, the 0-minute absorbance reading was recorded using a spectrophotometer.
   The spectrophotometer was first set to dark zero and blanked using LB medium without inoculum.
4. Wavelength:
   Optical density was measured at 660 nm.
5. Incubation:
   The inoculated Falcon tubes were placed in an incubator shaker maintained at 37°C under aerobic conditions.
6. Hourly readings:
   Absorbance readings were taken every half an hour for a total duration of 4 hours.
   For each reading, a small aliquot of the culture was aseptically transferred into a cuvette inside the laminar airflow hood, and the absorbance was measured. The aliquot used was discarded after measurement, and the Falcon tube was returned to the incubator shaker.
7. Data recording:
   All absorbance values were recorded in a tabular format, and bacterial growth curves were plotted using absorbance versus time.

II. Experimental Setup (With Plant Extracts)

Preparation of extract-treated media:

Each experimental tube contained the following:

- 13.5 mL LB medium
- 1.5 mL plant extract (Turmeric / Jeera / Ajwain / Peppermint)
- 150 µL (0.15 mL) bacterial inoculum (A or B)

The following experimental tubes were prepared:

1. Turmeric + Inoculum A
2. Turmeric + Inoculum B
3. Ajwain + Inoculum A
4. Ajwain + Inoculum B
5. Jeera + Inoculum A
6. Jeera + Inoculum B
7. Peppermint + Inoculum A
8. Peppermint + Inoculum B

Growth Curve Analysis:

For each extract-treated tube, the same procedure as described for the control was followed:

- Initial absorbance recorded immediately after inoculation
- Incubation at 37°C in an incubator shaker
- Absorbance was measured at 660 nm.
- Readings taken every 30 mins for 4 hours
- Data recorded and growth curves plotted to compare bacterial growth in the presence and absence of plant extracts

5.**Preparation of Bacterial Growth Curves (Anaerobic Conditions)**

All preparations were carried out under aseptic conditions inside a laminar airflow hood. Cultures were maintained in closed Eppendorf tubes at room temperature (28–30°C) to simulate anaerobic conditions, supporting probiotic Lactobacilli growth (optimum 30–40°C, pH 5.5–6.2). Optical density (OD) was measured at 660 nm.

I. Control (Without Plant Extract)
1. Preparation of culture tubes:
Eppendorf tubes were prepared, each containing 2 ml of LB (Luria–Bertani) medium autoclaved beforehand. Two sets were used: one for Inoculum A and one for Inoculum B (prepared from agar plate colonies).
2. Inoculation:
0.02 ml Bacterial inoculum (A or B) was added to each tube with sterile distilled water (no extract). Blanks contained only LB medium and water.
3. Initial absorbance (0 min):
Immediately after inoculation, the 0-minute OD reading was recorded using a spectrophotometer (set to dark zero and blanked with LB medium).
4. Incubation:
Closed Eppendorf tubes were kept undisturbed at room temperature (28–30°C) under anaerobic conditions.
5. Short-term readings: OD measured every 30 minutes for 5 hours (e.g., 10:40 A.M. to 3:40 P.M.) until saturation.
6. Long-term readings: OD monitored daily for 4 days until saturation.
For each reading, a small aliquot was aseptically transferred to a cuvette, measured, and discarded.
7. Data recording:
All OD values were tabularized, and growth curves plotted (OD vs. time) for Controls A and B.

5. RESULT AND DISCUSSION

1.Isolation of probiotic

- 2 colonies were identified—Colony A appeared to be opaque and yellowish. Colony B appeared to be translucent and whitish.

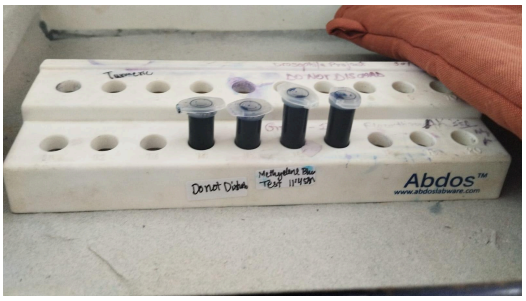


Fig 5: Colony A and Colony B observed on agar plates

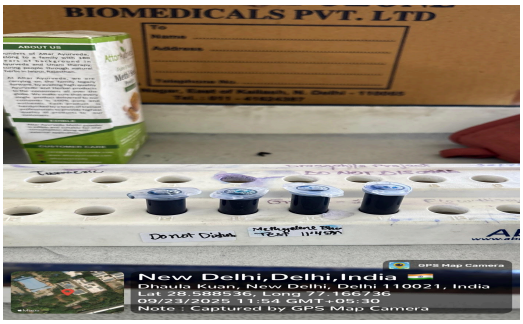
Fig 6 a-f: Results of the Methylene Blue Test show that media filled with culture provide adequate anaerobicity till 72 hours.



11:50 a.m. (a)



3:00 p.m. (b)



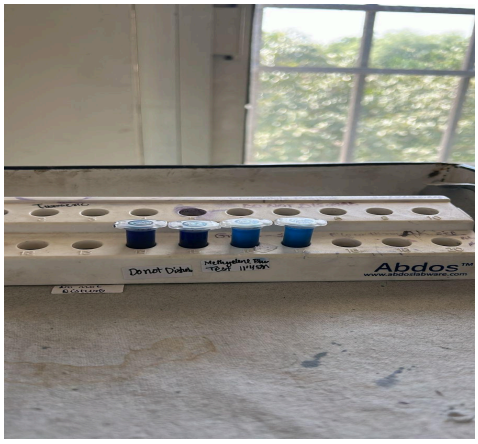
24 hrs later (c)



48 hrs later (d)



72 hrs later (e)



96 hrs later (f)

Section 1 - Growth curves of controls for culture A and B

TABLE 2: AEROBIC CONTROL

TIME/MIN	AEROBIC (CONTROL)	
	CULTURE A	CULTURE B
0 minutes	0.00824298	0.014667
30 minutes	0.01233333	0.035822
60 minutes	0.03048915	0.028269
90 minutes	0.0169898	0.018154
120 minutes	0.04246645	0.059968
150 minutes	0.09247505	0.088777
180 minutes	0.15899868	0.156747
210 minutes	0.15949823	0.162657
240 minutes	0.1464815	0.221517

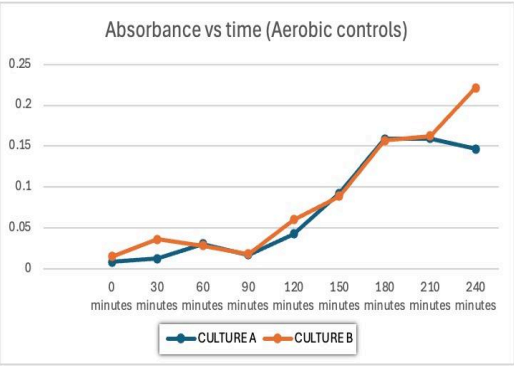


Figure 7: CONTROL - CULTURE A & B - Aerobic

TABLE 3: ANAEROBIC CONTROL

TIME/MIN	ANAEROBIC (CONTROL)	
	CULTURE A	CULTURE B
0 minutes	0.025	0.018
30 minutes	0.018	0.02
60 minutes	0.028	0.003
90 minutes	0.016	0.033
120 minutes	0.003	0.049
150 minutes	0.033	0.03
180 minutes	0.008	0.022
210 minutes	0.006	0.054
240 minutes	0.008	0.073
270 minutes	0.018	0.068
300 minutes	0.012	0.072

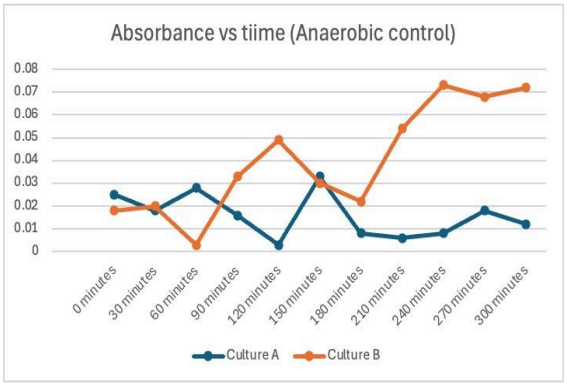


Figure 8 : CONTROL - CULTURE A & B - Anaerobic

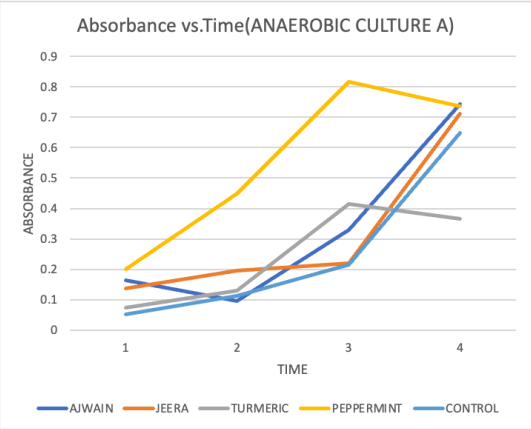
In the lag phase, we observed the growth of culture B to be faster than that of culture A. The log phase showed consistent growth of both the cultures, and the cultures tracked closely. Culture A reached the stationary phase earlier than culture B. Culture B had a higher carrying capacity compared to culture A. Under anaerobic conditions, culture B showed better and sustained growth, whereas culture A showed minimal and unstable growth.

Discussion: Aerobic conditions support rapid and high-density growth for both cultures, whereas anaerobic conditions restrict growth. Culture B has greater aerobic efficiency than Culture A and in the case of anaerobic conditions also, culture B shows better tolerance than Culture A.

TABLE 4: ANAEROBIC WATER EXTRACTS FOR CULTURE A

TIME/MIN	CULTURE A(ANAEROBIC)				
	AJWAIN	JEERA	TURMERIC	PEPPERMINT	CONTROL
0 hour	0.165	0.137	0.075	0.201	0.053
3 hours	0.097	0.195	0.131	0.448	0.113
6 hours	0.33	0.22	0.415	0.816	0.214
50 hours	0.743	0.712	0.365	0.737	0.649

Figure 9: ABSORBANCE VS. TIME (ANAEROBIC WATER EXTRACTS FOR CULTURE A)

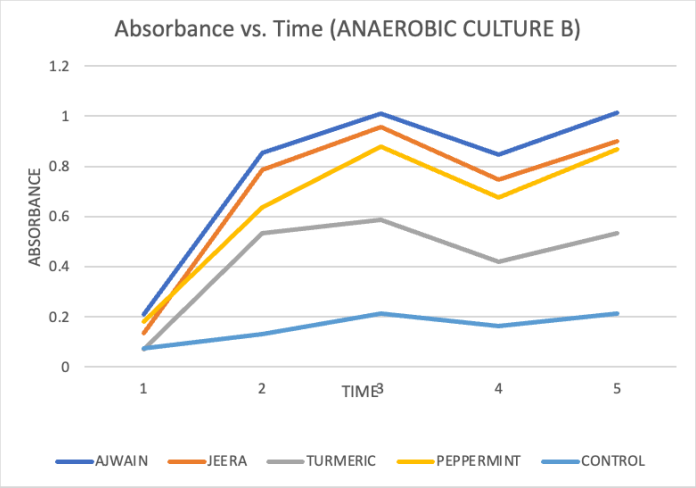


Peppermint, ajwain, and jeera enhanced growth of Culture A relative to the anaerobic control, while turmeric exhibited inhibitory effects.

TABLE 5: ANAEROBIC WATER EXTRACTS FOR CULTURE B

TIME/MIN	CULTURE B(ANAEROBIC)				
	AJWAIN	JEERA	TURMERIC	PEPPERMINT	CONTROL
0 hour	0.209	0.136	0.07	0.18	0.075
15 hours	0.852	0.786	0.534	0.637	0.132
18 hours	1.009	0.956	0.588	0.877	0.214
21 hours	0.848	0.746	0.418	0.675	0.163
24 hours	1.014	0.901	0.534	0.867	0.213

Figure 10: ABSORBANCE VS. TIME (ANAEROBIC WATER EXTRACTS FOR CULTURE B)



All herb extracts show increased absorbance in comparison to anaerobic Culture B control. Ajwain and Jeera showed the most enhancement followed by Peppermint, and lastly turmeric, but none exhibit strong inhibitory activity.

Discussion: Culture B showed a relatively better response compared to Culture A with all the extracts. Ajwain and Jeera were the most effective enhancers, followed by peppermint ,and then turmeric with inhibitory or minimal effects.

SECTION 2 - Growth curves of water extracts (10%) with Culture A and B

TABLE 6: AEROBIC WATER EXTRACTS FOR CULTURE A MEAN +/- Std. Error

Time	T Mean +/-	*J Mean +/-*	A Mean +/-	P Mean +/-
0min	-0.019	0.094	0.154	0.005
30mins	0.124	0.191	0.221	0.09049822702
60mins	0.077	0.118	0.206	0.068
90mins	0.105	0.250	0.231	0.1294984849
120mins	0.077	0.302	0.157	0.1174991409
150mins	0.042	0.312	0.246	0.093
180mins	0.133	0.368	0.577	0.216
210mins	0.030	0.159	0.284	0.066

TABLE 7: AEROBIC WATER EXTRACTS FOR CULTURE B MEAN +/- Std.Error

Time	T MEAN +/-	A MEAN +/-	J MEAN +/-	P MEAN +/-
0min	0.1302855948	0.2473042061	0.132120387	0.021
30mins	0.2242004704	0.3151824477	0.2806737378	0.048
60mins	0.2627490573	0.4254833387	0.2356552538	0.089
90mins	0.2209607109	0.4158713532	0.1399655386	0.067
120mins	0.2286933919	0.3160982243	0.2051595587	0.125
150mins	0.2099783269	0.3698432188	0.102106088	0.042
180mins	0.333	0.5207264303	0.166	0.134
210mins	0.086	0.345367326	0.273	0.661
240mins	0.914	1.014	0.583	0

Figure 11: ABSORBANCE VS. TIME (AEROBIC WATER EXTRACTS FOR CULTURE A)

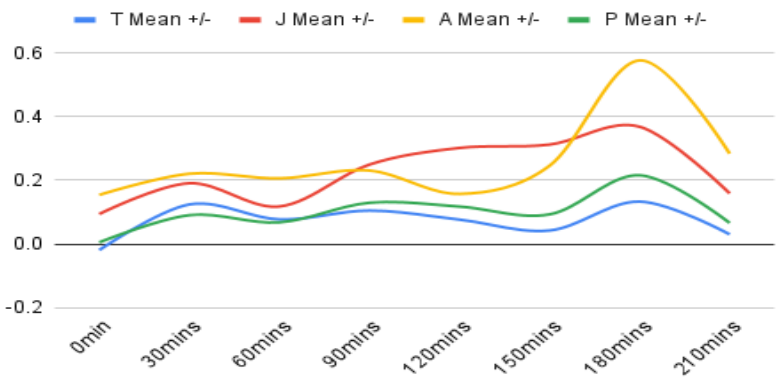


Figure 12: ABSORBANCE VS. TIME (AEROBIC WATER EXTRACTS FOR CULTURE B)

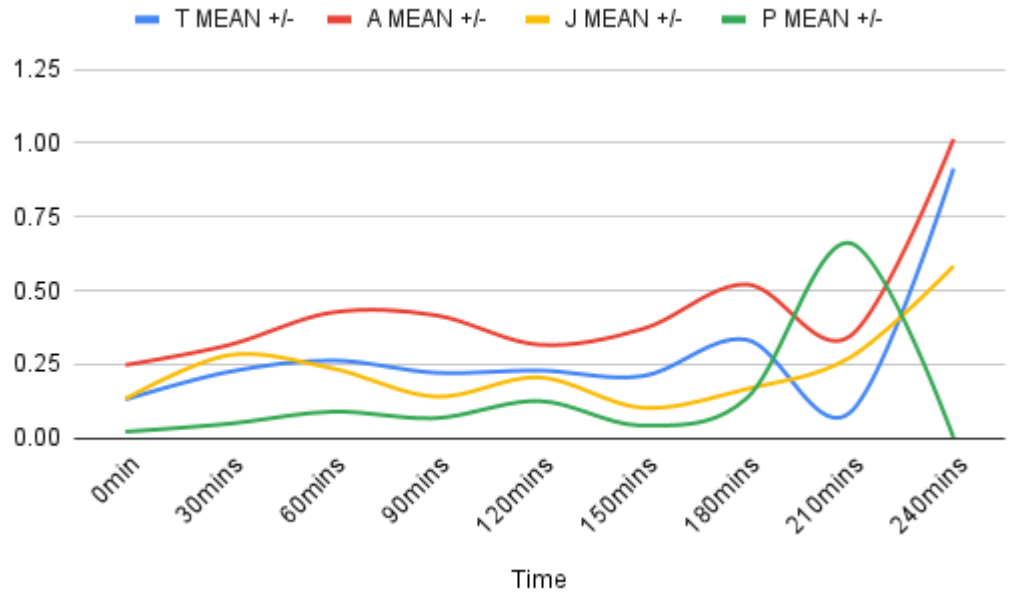
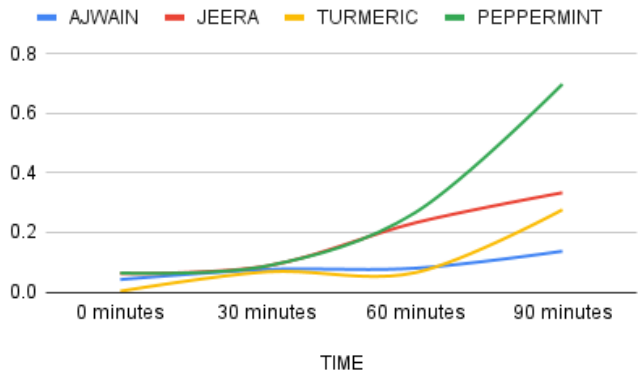


TABLE 8: CONTROL FOR EXTRACTS WITHOUT INOCULUM

TIME	AJWAIN	JEERA	TURMERIC	PEPPERMINT
0 minutes	0.042	0.062	0.003	0.064
30 minutes	0.076	0.088	0.068	0.088
60 minutes	0.08	0.232	0.065	0.266
90 minutes	0.137	0.333	0.276	0.697

Figure 13: ABSORBANCE VS. TIME (CONTROL FOR EXTRACTS WITHOUT INOCULUM)



Culture A (Probiotic Culture A + Extracts)

Out of all the treatments in Culture A, the herb, Ajwain (A) showed the greatest growth response, attaining the highest mean value. Moderate and long-lasting growth enhancement was produced by cumin/carom extract (J). The stimulatory effect of peppermint extract (P) was comparatively lower and delayed. There was little growth stimulation and erratic trends with turmeric extract (T). Growth kinetics showed a brief lag phase, a clear exponential phase, and a decline that might indicate metabolite buildup or nutrient depletion. Order of effectiveness (Culture A): A > J > P > T

Culture B (Probiotic Culture B + Extracts)

When compared to Culture A, Culture B showed noticeably greater growth responses. With a dramatic exponential rise, Ajwain (A) generated the greatest overall growth. Extract from Turmeric (T) showed strong late-phase stimulation, suggesting increased metabolic utilisation. Cumin/Carom (J) showed steady but modest growth. Peppermint (P) showed a rise at first, then a fall, indicating concentration-dependent inhibitory effects. Order of effectiveness (Culture B): A > T > J > P

Control – Extracts Without Inoculum

All herbal extracts exhibited slight but time-dependent increases in measured values from 0 to 90 minutes when probiotic inoculum was not present. The greatest increase was seen in peppermint extract, which went from 0.064 at 0 minutes to 0.697 at 90 minutes. Jeera extract increased gradually and moderately, reaching 0.333 after 90 minutes. Only after 90 minutes did turmeric extract start to show a discernible increase (0.276). With a slight increase from 0.042 to 0.137 over 90 minutes, ajwain extract showed the least overall change. No extract demonstrated a classical biological growth pattern (lag–log–stationary phases). Overall trend (Control): Peppermint > Jeera > Turmeric > Ajwain

Discussion: The increased reaction in Culture B with herbs points to strain-specific metabolic adaptability, perhaps as a result of improved essential oil and polyphenol utilisation. Turmeric's potent effect in Culture B may be due to curcuminoid metabolism, which differs among probiotic strains. The moderate but consistent effect of cumin/carom supports its function in promoting microbial metabolism and digestive enzymes. The different responses to peppermint indicate that it serves two purposes: it stimulates growth at low concentrations and functions as an antimicrobial agent at higher concentrations.

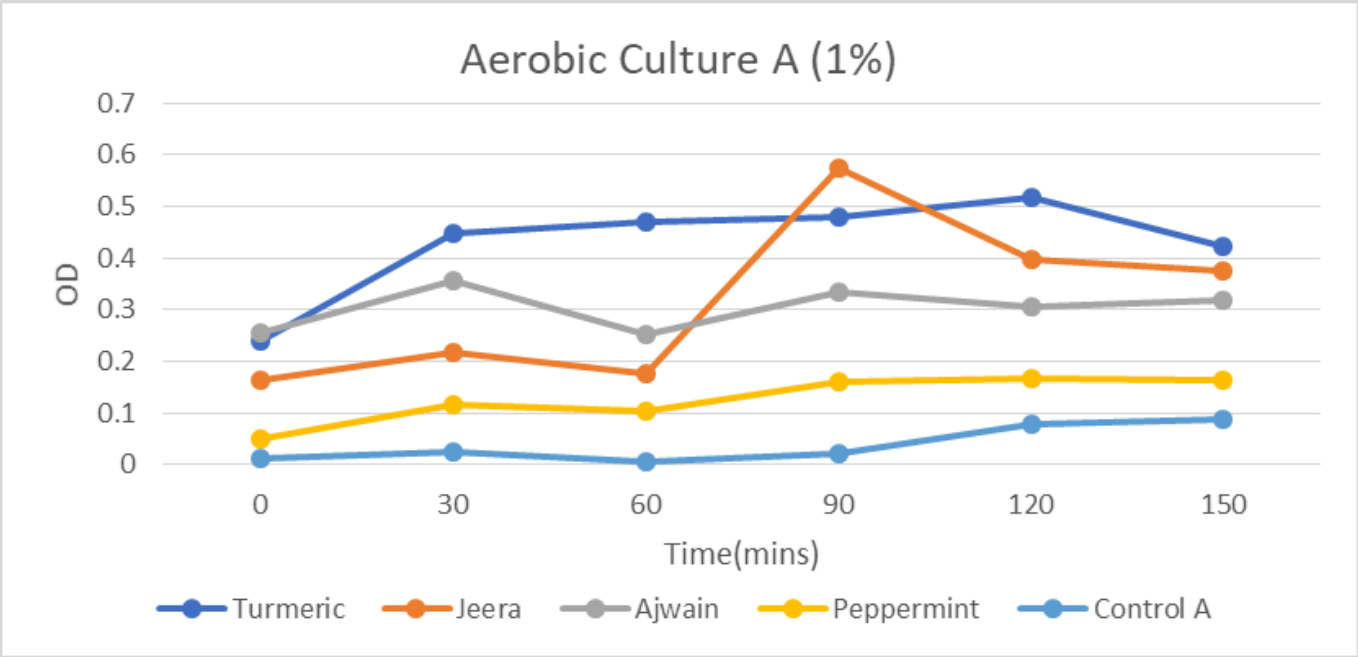
All extracts under control conditions showed a gradual increase, which suggests non-biological contributions rather than microbial activity. The lack of an inoculum implies that these changes are caused by the extracts' inherent optical properties, the phytochemicals' gradual solubilisation, and the medium's mild chemical interactions. Peppermint's high essential oil and phenolic content, which can boost absorbance without microbial involvement, may be the reason for the noticeable increase in peppermint values. Turmeric and jeera exhibit delayed increases, indicating slow extraction or diffusion of bioactive compounds rather than metabolic processes. Ajwain's minimal variation makes it less susceptible to measurement interference, implying relatively stable optical behaviour. Importantly, none of the extracts demonstrated sustained or exponential biological amplification, confirming that herbal extracts do not replicate the kinetics of probiotic growth.

Section 3 - Growth Curves of steam distilled extracts (1%) with Culture A and B

TABLE 9: AEROBIC 1% AQUEOUS EXTRACT OF CULTURE A

Time (mins)	Turmeric	Jeera	Ajwain	Peppermint	Control A
0	0.24	0.164	0.254	0.049	0.013
30	0.4465	0.2175	0.3545	0.1145	0.025
60	0.47	0.175	0.251	0.102	0.005
90	0.478	0.575	0.333	0.1615	0.022
120	0.516	0.398	0.3045	0.167	0.077
150	0.4215	0.376	0.3195	0.164	0.088

Figure 14: ABSORBANCE VS. TIME (AEROBIC 1% STEAM DISTILLED EXTRACTS FOR CULTURE A)



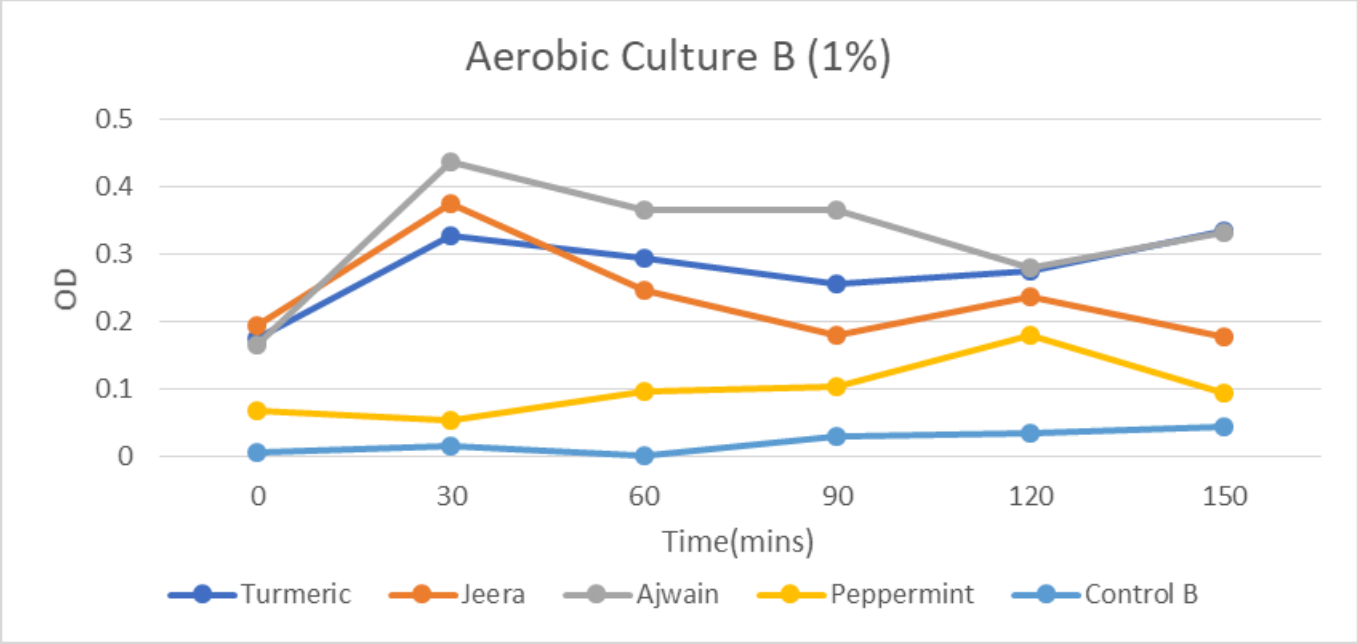
Looking at the data, every herbal extract absorbed more light than the Aerobic Culture A reference. What stood out was turmeric - it had the greatest rise in absorption levels. After that came jeera, then ajwain, each stepping down slightly in effect. In contrast, peppermint barely changed the reading at all. When it came to blocking activity, none of them performed strongly.

Discussion: Few surprises marked the response of Culture A - turmeric drove growth strongest. Following behind, jeera then ajwain still lifted development well. Even so, peppermint brought weaker gains, edged close to neutral. Hardly any suppression appeared at all through that trial.

TABLE 10: AEROBIC 1% STEAM DISTILLED EXTRACT OF CULTURE B

Time (mins)	Turmeric	Jeera	Ajwain	Peppermint	Control B
0	0.174	0.195	0.166	0.068	0.006
30	0.328	0.374	0.436	0.054	0.016
60	0.293	0.246	0.364	0.096	0.002
90	0.257	0.179	0.365	0.104	0.031
120	0.275	0.238	0.28	0.181	0.036
150	0.333	0.178	0.331	0.094	0.045

Figure 15: ABSORBANCE VS. TIME (AEROBIC 1% STEAM DISTILLED EXTRACTS FOR CULTURE B)



Compared to the aerobic culture B control, every herbal extract demonstrated higher absorbance. Most noticeable gains came from ajwain and jeera. After these, turmeric brought moderate improvement. Peppermint produced the smallest change. Inhibition remained weak across all samples.

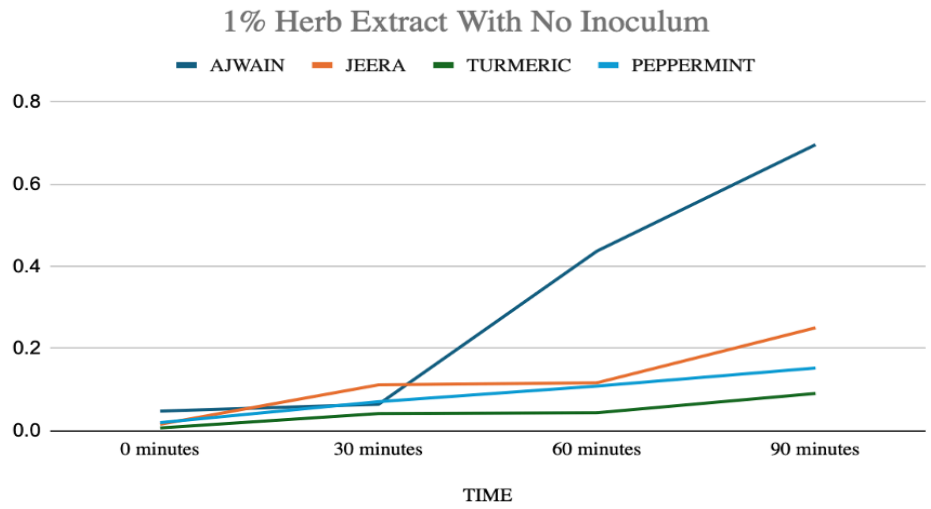
Discussion: Culture B responded more intensely than Culture A. Notably, ajwain along with jeera boosted activity the most. Turmeric came next in effectiveness. In contrast, peppermint had little to no suppressive impact.

TABLE 11: AEROBIC CONTROL FOR 1% STEAM DISTILLED EXTRACT OF CULTURE A AND CULTURE B

Time (mins)	Control A	Control B
0	0.013	0.006
30	0.025	0.016
60	0.005	0.002
90	0.022	0.031

120	0.077	0.036
150	0.088	0.045

Figure 16: ABSORBANCE VS. TIME AEROBIC CONTROL 1% STEAM DISTILLED EXTRACTS (Without Inoculum)



Over time, each herbal extract displayed rising absorbance when no inoculum was added. What stood out was how ajwain led this trend, with jeera coming next, then peppermint, with turmeric trailing behind. Without any microbes in the mix, shifts in light absorption likely stem from the natural visual traits of the plant materials themselves. Eventually, it became clear—no biological activity drove these results.

Discussion: Absorbance rose slowly in the aerobic control with 1% water-based herb extract, even without added microbes. This shift likely stems from how the extract interacts with light—its natural optical traits play a role. Residual compounds or hidden internal microorganisms within the plant material could also contribute. Without any outside microbial strain introduced, real growth-driven changes seem improbable. Instead, dormant endophytes or substances they left behind might influence readings. Still, proving these organisms are alive and functioning demands deeper analysis. Isolating them in lab cultures or using genetic tools would clarify their involvement. Such steps remain necessary before drawing firm conclusions about their role.

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