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Meerut- UP 250005

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Editorial

Dear Readers

Jai Hind, Season's Greetings & A Very Happy Festive Season Ahead to One and All

Please find the August 2026 issue of “**Subharti Journal of Interdisciplinary Research**” an online publication of our prestigious Swami Vivekanand Subharti University. It is with deep sense of indebtedness that I thank the management, Hon'ble Chancellor, Hon'ble Pro Chancellor, Hon'ble Vice Chancellor, Hon'ble Pro Vice Chancellor, CEO, EO and all the Deans/Heads of Institutes and Research contributors, who have absolutely extended unwavering support towards the online publication of the journal since the December 2018 issue.

Knowledge can be acquired through books, periodicals, journals, e-resources and many more means which assists us in diving deep into the field of focus that may be unfamiliar to us that can be aptly termed as journey from known to unknown. Reading has always proved to be a righteous opportunity to nourish our minds as it helps us nurture our brain realistically. With the online resources available at the click of a button and boom of artificial intelligence affecting each and every part of our day-to-day life, the art of reading and asking the WHY'S has taken a backseat for many of us. The reliability on the machine is increasing with each passing day which is leading to decreased analytical thinking by the mankind which is not a good omen. Reading habit should form an integral part of everyone's life and learning should never stop.

Regarding the journal, I would like to personally take this opportunity to thank each one of the researchers/authors who have put their trust in this journal and considered it worthy of publication of their valuable research. We are constantly striving to make our online journal interesting, conversant and groveling. I would like to again reiterate that please team up with other researchers from other institutes too who are working on your analogous areas of interest. As we prepare our future issues, I sincerely request everyone to submit works with multi-institutional involvement which will help the journal to reach far and wide.

I hope that sailing through this issue of the journal will be a pleasurable acquisition for every learner. We have put in a robust and sincere effort to improve the quality of articles keeping in mind the various requisites of the readers. I once again seek your support and look forward to welcoming your submissions for next issue and your valuable constructive criticism is eagerly awaited.

Happy Reading & Happy Festive Season Ahead

Dr Vijay Wadhwan

Editor-in-Chief

journal@subharti.org

Review Article

Synechococcus elongatus: From Photosynthetic Microbe to Biotechnological Powerhouse: A Comprehensive Review

Jigyasa Yadav¹, Ashwani Kumar², Punjab Singh Malik³, Adesh Kumar⁴, Anu Chauhan⁴

1. Ph.D Scholar
2. Head of Department & Associate Professor
3. Associate Professor,
4. Assistant Professor,

1,2 & 4 Department of Life Sciences, Keral Verma Subharti College of Science, Swami Vivekanand Subharti University, Meerut (250005) Uttar Pradesh, India

3. Department of Botany, Meerut College, Meerut, Uttar Pradesh, India

Abstract:

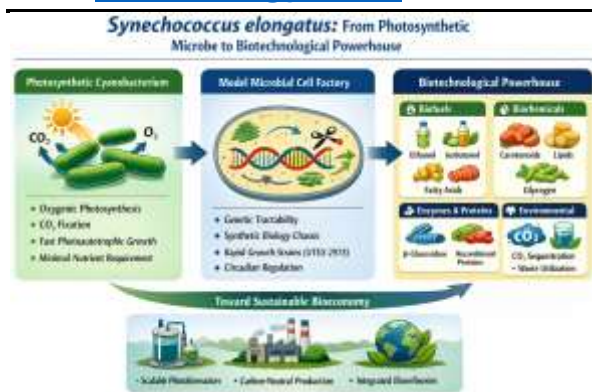
Synechococcus elongatus, a freshwater unicellular cyanobacterium, has quietly but dramatically transformed from a subject of basic photosynthesis research into one of the most promising platforms for sustainable biotechnology. Its genetic tractability, well-characterized circadian rhythm, compact genome, and the ability to convert atmospheric CO₂ directly into value-added compounds using sunlight make it exceptionally suited for the bio-based economy. This review provides a comprehensive account of the biology, genomics, metabolic engineering potential, and industrial applications of *S. elongatus*, drawing on literature published between 2015 and 2026. We examine its photosynthetic machinery, carbon fixation pathways, synthetic biology toolbox, and its comparative standing among cyanobacterial chassis organisms. Three comparative tables are presented to illustrate genomic features, biotechnological outputs, and engineering tools. Special attention is given to challenges that still limit its industrial scale-up and future directions in strain development. We conclude that *S. elongatus* sits at a critical inflection point—no longer merely a model organism, but a genuine biotechnological workhorse ready for translational deployment in the context of climate change mitigation and green manufacturing.

Keywords: *Synechococcus elongatus*, cyanobacteria, photosynthesis, metabolic engineering, synthetic biology, biofuels, circadian clock, carbon fixation, sustainable biotechnology, CRISPR.

Address for correspondence: Dr. Ashwani Kumar, Associate Professor, Department of Life Sciences, Keral Verma Subharti College of Science, Swami Vivekanand Subharti University Subhartipuram, N-58, Delhi-Haridwar Bypass Road, Meerut, U.P.

E-Mail: amritdhra1981@gmail.com

Contact: +91-74170-76417



Graphical Abstract

Introduction

If one were to rank the organisms that shaped life on Earth, cyanobacteria would be near the very top of the list. These ancient prokaryotes were the architects of the oxygen-rich atmosphere that allowed complex life to flourish, doing so through the sheer evolutionary innovation of oxygenic photosynthesis roughly 2.7 billion years ago ⁽²¹⁾.

Among the extraordinary diversity of cyanobacterial lineages, *Synechococcus elongatus* PCC 7942 has emerged as the organism of choice for dissecting the molecular underpinnings of photosynthesis, circadian rhythmicity, and increasingly the metabolic production of industrially relevant compounds.

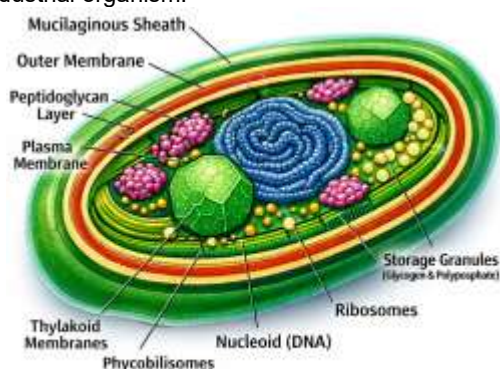
The appeal of *S. elongatus* is not accidental. It thrives in freshwater environments, tolerates a wide range of light intensities, and grows reasonably fast with only light, water, CO₂, and inorganic salts. These traits, combined with its natural competence for DNA transformation, make it exceptionally easy to engineer genetically (Golden & Canales, 2003; 6). The strain's genome is compact (~2.7 Mb), fully sequenced, and well-annotated, enabling systems-level analysis that is simply not feasible in many other photosynthetic organisms (Holtman *et al.*, 2005; ⁴).

The global urgency to decarbonize industrial processes has shifted attention toward organisms capable of using sunlight and CO₂ as sole energy and carbon sources. Cyanobacteria broadly and *S. elongatus* specifically fit this description naturally. Unlike algal biofuel platforms that require organic carbon inputs, or yeast-based cell factories that

depend on fermentable sugars from expensive biomass preprocessing, engineered *S. elongatus* strains can in principle synthesize fuels, chemicals, and pigments directly from sunlight and air^(13,19). This positions the organism at the nexus of photo-biotechnology, synthetic biology, and circular bioeconomy.

Despite remarkable progress in the laboratory, translation of *S. elongatus*-based bioprocesses to industrial scale has been slow. Issues of photoinhibition, limited product titers, strain stability, and the challenge of product extraction from aqueous culture broths remain real barriers^(8,9). Nevertheless, each passing year sees the publication of new metabolic engineering strategies, expanded synthetic biology toolkits, and a deeper understanding of the regulatory networks that govern carbon and energy balance in this organism.

This review synthesizes the knowledge accumulated between 2015 and 2026 concerning the biology and biotechnology of *S. elongatus*. We organize our discussion around its core biological features, genomic architecture, metabolic engineering achievements, comparative standing among cyanobacterial models, and an honest assessment of what remains to be accomplished before this microbe can be said to have truly arrived as an industrial organism.



— *Synechococcus elongatus* —

2. Taxonomy, Cell Biology, and Physiology

Synechococcus elongatus belongs to the phylum Cyanobacteria, class Oscillatoriothrixales, and is most commonly studied in two closely related strains: PCC 7942 (*Anacystis nidulans* R2) and UTEX 2973, which was isolated more recently and grows nearly twice as fast⁽²⁸⁾. The cells are rod-shaped, typically 1.5–3.5 µm in length, and divide by binary fission. Unlike filamentous cyanobacteria such as *Anabaena*, *S. elongatus* forms unicellular cultures, simplifying downstream processing and fluidic manipulation.

The photosynthetic machinery of *S. elongatus* is housed in thylakoid membranes arranged concentrically within the cell. These membranes carry the canonical oxygenic photosynthetic apparatus: Photosystem I (PSI), Photosystem II (PSII), the cytochrome b₆f complex, and ATP synthase. Light-harvesting is mediated by

phycobilisomes large antenna complexes containing phycocyanin and allophycocyanin that absorb wavelengths between 550 and 650 nm and channel excitation energy to the reaction centers⁽²⁶⁾. Carbon fixation proceeds via the Calvin–Benson–Bassham (CBB) cycle, with RuBisCO (Form I) as the key carboxylation enzyme. *S. elongatus* also employs carboxysomes proteinaceous microcompartments that concentrate CO₂ around RuBisCO, boosting its carboxylation efficiency under atmospheric CO₂ levels⁽¹⁶⁾.

One of the most studied features of *S. elongatus* is its circadian clock—the KaiABC system. This elegant, three-protein oscillator can reconstitute 24-hour rhythms of protein phosphorylation in vitro, independent of transcription and translation (Nakajima *et al.*, 2005). KaiA, KaiB, and KaiC interact in a phosphorylation–dephosphorylation cycle that gates gene expression across the genome, coordinating metabolism with the light–dark cycle^(7, 20). Understanding how this clock interfaces with carbon metabolism has implications not only for basic biology but also for the design of production strains in which metabolic output peaks at optimal times of the photoperiod.

S. elongatus also harbors two endogenous plasmids pANS1 and pANS2 and several 'neutral sites' on the chromosome where foreign DNA can be integrated without disrupting essential genes⁽⁶⁾. These neutral sites (NS1, NS2, and NS3) have become the preferred genomic loci for stable heterologous gene expression in metabolic engineering efforts, effectively acting as designated landing pads for synthetic genetic cargo⁽²⁴⁾.

3. Genome Architecture and Comparative Genomics

The complete genome sequence of *S. elongatus* PCC 7942 was published in the early 2000s and has since been repeatedly updated and re-annotated. The chromosome is approximately 2.7 Mb in size with a GC content of 55.5%, encoding roughly 2,500 protein-coding genes (Holtman *et al.*, 2005). Comparative analyses across cyanobacterial genomes reveal that *S. elongatus* maintains a streamlined genome relative to filamentous or nitrogen-fixing relatives, consistent with its ecological niche as a free-living planktonic organism in oligotrophic freshwater environments⁽²¹⁾.

The recently characterized fast-growing strain UTEX 2973, which shares 99.8% average nucleotide identity with PCC 7942, harbors four key mutations in genes involved in light harvesting, carbon fixation, and cell regulation that collectively account for its accelerated growth⁽²⁸⁾. These naturally occurring mutations offer a roadmap for rational strain improvement: by introducing analogous mutations into slower-growing strains, researchers have demonstrated partial gains in growth rate, suggesting that growth rate is a complex polygenic trait amenable to incremental engineering⁽²²⁾.

Comparative genomic analyses with other model cyanobacteria *Synechocystis* PCC 6803, *Anabaena*

Table 1. Comparative Genomic and Biological Features of Key Cyanobacterial Model Organisms

Feature	<i>S. elongatus</i> PCC 7942	<i>Synechocystis</i> PCC 6803	<i>Anabaena</i> PCC 7120	<i>Prochlorococcus</i> MED4
Genome Size (Mb)	2.7	3.6	7.2	1.7
GC Content (%)	55.5	47.4	41.3	30.8
Chromosome No.	1 + 2 plasmids	1 + 7 plasmids	1 mega + 6 plasmids	1
Doubling Time (h)	6–8	6–8	12–20	24–48
N ₂ Fixation	Absent	Absent	Present (heterocysts)	Absent
Circadian Clock	KaiABC (model organism)	KaiABC present	KaiABC present	Simplified KaiBC
Transformation Method	Natural competence	Natural competence	Conjugation	Not routine
Reference Strain Status	Model organism	Model organism	Model organism	Ecological model

Note: Data compiled from Holtman et al. (2005), Yu et al. (2015), Broddrick et al. (2016), Schirrmeister et al. (2015), and Rocap et al. (2018).

PCC 7120, and *Prochlorococcus* MED4 reveal both shared core metabolic networks and striking differences in genome size, GC content, plasmid architecture, and specialized metabolic capacity (Table 1). *Synechocystis*, for example, carries a larger genome with seven additional plasmids and a broader biosynthetic repertoire, while *Prochlorococcus* represents the extreme of genomic streamlining in marine cyanobacteria. *S. elongatus* occupies a pragmatic middle ground: genome size and complexity sufficient for sophisticated metabolic engineering yet compact enough for system-wide manipulation. Flux balance analysis (FBA) applied to the curated metabolic model iSyn731 of *S. elongatus*, encompassing 731 genes and 831 reactions, has allowed investigators to map carbon and nitrogen fluxes under different light regimes and identify bottlenecks that limit biomass accumulation and product secretion ⁽⁴⁾. Extensions of this model incorporating light-dependent regulatory constraints and protein resource allocation are beginning to capture the dynamic nature of photosynthetic metabolism in a way that static models cannot ⁽³⁰⁾. These metabolic models are now routinely used to guide rational engineering decisions before costly experimental iterations.

4. The Synthetic Biology Toolbox of *S. elongatus*

A decade ago, cyanobacterial synthetic biology was constrained by a limited repertoire of well-characterized genetic parts promoters, ribosome-binding sites, terminators, and selection markers. Since 2015, there has been an explosion in the number and sophistication of tools available for engineering *S. elongatus*, dramatically lowering the barriers to metabolic pathway construction and optimization.

4.1 Transformation and Genome Editing

S. elongatus is naturally competent for DNA transformation, meaning it can take up exogenous double-stranded DNA from its environment without requiring chemical or electrical manipulation ⁽⁶⁾. This natural competence, mediated by type IV pili and associated DNA import machinery, is both efficient and reproducible. Homologous recombination then integrates the transforming DNA at the desired chromosomal locus or plasmid. The three neutral

sites (NS1, NS2, NS3) represent well-validated integration loci that allow stable, predictable transgene expression without polar effects on neighboring genes.

The adoption of CRISPR-based tools has accelerated gene editing in *S. elongatus* considerably. Wendt et al. (2016) demonstrated that Cas9 from *Streptococcus pyogenes* could introduce targeted double-strand breaks in the *S. elongatus* genome with efficiencies approaching 80–95%, enabling clean knockouts without antibiotic selection markers ⁽²⁷⁾. The catalytically dead variant, dCas9, has been deployed for CRISPRi-based gene repression, achieving up to 94% knockdown of targeted transcripts—a crucial tool for studying essential genes and for dynamically rewiring metabolic fluxes ⁽¹¹⁾. Table 2 below details key synthetic biology tools and their efficiency.

4.2 Promoters and Regulatory Elements

The availability of well-characterized, tunable promoters is essential for metabolic engineering. In *S. elongatus*, both constitutive and inducible promoters have been extensively characterized. The strong constitutive promoter Pcp560, derived from the phycocyanin operon, drives high-level constitutive expression that is particularly useful for structural pathway enzymes ⁽¹⁷⁾. The IPTG-inducible P_{trc} and Plac promoters, originally from *E. coli*, function in *S. elongatus* with moderate efficiency and have been widely used in research settings, though their leaky expression and the cost of chemical inducers limit industrial utility ⁽¹²⁾.

A significant advance was the development of light-inducible and CO₂-responsive promoter systems that tie transgene expression directly to the organism's native physiological signals. Berla et al. (2015) demonstrated that theophylline-responsive riboswitches could be used as post-transcriptional regulatory elements, adding a layer of metabolite-controlled gene expression⁽²⁾. More recently, optogenetic tools borrowed from plant light signaling pathways have been adapted to cyanobacteria to create circuits whose output is precisely controlled by wavelength and intensity ⁽³⁾.

4.3 Standardized Vectors and BioBrick-Compatible Parts

Taton et al. (2017) made a landmark contribution by developing the CYANO-VECTOR assembly platform a set of standardized, BioBrick-compatible vectors specifically designed for cyanobacteria. This suite includes shuttle vectors for *E. coli* and *S. elongatus*, with defined multiple cloning sites, neutral-site integration cassettes, and fluorescent reporter genes pre-optimized for cyanobacterial codon usage.⁽²⁴⁾ The availability of this toolkit dramatically accelerated the pace of pathway assembly, allowing researchers to prototype constructs in *E. coli* before moving to the cyanobacterial chassis—a strategy that mirrors standard practice in yeast and bacterial synthetic biology.

5. Metabolic Engineering for Bioproduction

The metabolic engineering of *S. elongatus* has been pursued vigorously over the past decade, yielding proof-of-concept demonstrations for a wide array of compounds spanning biofuels, commodity chemicals, pigments, and specialty molecules. The overarching strategy is conceptually elegant: redirect photosynthetically fixed carbon away from biomass accumulation and toward the secretion of a target product, thereby converting sunlight and CO₂ into something useful and extractable.

5.1 Carbon Export and Sugar Production

Sucrose naturally accumulates in *S. elongatus* as an osmotic stress response, and this observation was creatively exploited by Ducat et al who overexpressed the sucrose permease CscB to drive constitutive sucrose secretion.⁽⁸⁾ The resulting strains export sucrose continuously at rates of ~500 mg/L/day without compromising cell viability an economically significant feat when one considers that sucrose is a versatile feedstock for heterotrophic microbial fermentations. By coupling sucrose-exporting *S. elongatus* with *E. coli* strains engineered to consume sucrose and produce poly-3-hydroxybutyrate (PHB), the authors demonstrated a synthetic consortium that converts light and CO₂ into bioplastic precursors⁽⁸⁾. This concept of phototrophic-heterotrophic consortia now represents a broader design paradigm for expanding the chemical output of cyanobacteria beyond what any single organism can achieve.

5.2 Biofuels

Ethanol production was one of the first biofuel applications attempted in *S. elongatus*, enabled by the expression of pyruvate decarboxylase (Pdc) and alcohol dehydrogenase (AdhII) from *Zymomonas mobilis*⁽⁹⁾. Although titers remained modest compared to yeast fermentations—around 212 mg/L/day in optimized conditions the significance lies in the fact that the carbon and energy input is entirely renewable. More promising in terms of energy density and compatibility with existing fuel infrastructure is isobutyraldehyde, produced by expressing the 2-keto-acid decarboxylase Kivd from *Lactococcus lactis*⁽¹⁾. The volatile nature of isobutyraldehyde actually facilitates downstream separation, as the product spontaneously partitions

into the gas phase during culture, simplifying recovery.

The demonstration of limonene production in the UTEX 2973 background by Halfmann et al. (2017) illustrated the advantage of the fast-growing strain for terpenoid biofuel applications, achieving roughly 5 mg/L/day of this high-octane fuel candidate using a truncated mevalonate pathway⁽¹⁰⁾. While titers remain below commercial thresholds, these demonstrations establish the metabolic feasibility of terpene production in fast-growing cyanobacteria and highlight UTEX 2973 as an increasingly preferred chassis for high-carbon-flux applications.

5.3 Commodity Chemicals and Specialty Products

2,3-Butanediol, a platform chemical for polymer and solvent synthesis, has been produced in *S. elongatus* PCC 7942 at titers of 2.38 g/L via the introduction of an artificial pathway comprising acetolactate synthase (AlsS), acetolactate decarboxylase (AlsD), and butanediol dehydrogenase (BudC)⁽¹⁹⁾. This titer represents one of the highest reported for any small molecule in an engineered *S. elongatus* strain, achieved through careful promoter tuning, codon optimization, and iterative elimination of competing pathways.

Squalene, a triterpene with applications in vaccines, cosmetics, and nutraceuticals, has been produced at 11.98 mg/L in *S. elongatus* PCC 7942 through overexpression of squalene synthase and mevalonate pathway enzymes⁽⁵⁾. Phycocyanin, the blue photosynthetic pigment already produced commercially from *Spirulina*, has been produced at up to 120 mg/g dry weight in engineered *S. elongatus* strains with optimized promoter configurations—a value competitive with natural accumulation in commercial strains⁽²⁶⁾. Table 3 provides a structured comparison of major biotechnological products reported from *S. elongatus*.

6. Photosynthetic Efficiency and Light Utilization

The theoretical maximum photosynthetic efficiency for cyanobacteria under solar illumination is approximately 11%, though real-world efficiencies typically fall between 1% and 3% in outdoor systems⁽¹³⁾. The gap between theory and practice reflects losses due to photoinhibition at high light intensities, the inefficiency of RuBisCO's carboxylation reaction relative to oxygenation, self-shading in dense cultures, and the respiration of fixed carbon to support cell maintenance. Closing this gap is one of the central engineering challenges for cyanobacterial bioproduction.

Several strategies have been explored in *S. elongatus* to improve light capture and utilization. Truncating phycobilisome antenna size reduces excitation energy re-emission as heat under saturating light conditions, improving per-cell photosynthetic productivity in high-density culture⁽¹⁴⁾. The overexpression of RuBisCO or engineering of carboxysomes to increase their CO₂-concentrating capacity has produced modest gains in carbon fixation rate under ambient CO₂⁽¹⁶⁾. More recently, the introduction of proteorhodopsin—a light-driven proton pump from marine bacteria—into *S. elongatus* has been shown

Table 3. Biotechnological Products from Engineered *Synechococcus elongatus* (2015–2026)

Product	Yield Reported	Genetic Strategy	Organism	Reference
Sucrose ⁽⁸⁾	~500 mg/L/day	cscB overexpression	<i>S. elongatus</i> PCC 7942	Ducat et al., 2021
Ethanol ⁽⁹⁾	212 mg/L/day	pdc + adh insertion	<i>S. elongatus</i> AMC 401	Gao et al., 2016
2,3-Butanediol ⁽¹⁹⁾	2.38 g/L	alsS-alsD-budC pathway	<i>S. elongatus</i> PCC 7942	Oliver et al., 2022
Isobutyraldehyde ⁽¹⁾	1.1 g/L/day	kivd overexpression	<i>S. elongatus</i> PCC 7942	
Free Fatty Acids ⁽¹⁵⁾	~130 mg/L	acyl-ACP thioesterase	<i>S. elongatus</i> PCC 7942	Liu et al., 2019
Squalene ⁽⁵⁾	11.98 mg/L	sqs + mvk pathway	<i>S. elongatus</i> PCC 7942	Choi et al., 2020
Phycocyanin ⁽²⁶⁾	~120 mg/g DW	Optimized promoter	<i>S. elongatus</i> PCC 7942	Watanabe et al., 2018
Limonene ⁽¹⁰⁾	~5 mg/L/day	lim synthase insertion	<i>S. elongatus</i> UTEX 2973	Halfmann et al., 2017

Note: Yields are approximate and depend heavily on light intensity, CO₂ supplementation, temperature, and strain background. DW = dry weight. References as indicated.

to supplement ATP generation under low-light conditions, improving growth rates in suboptimal illumination ⁽²³⁾.

Adaptive laboratory evolution (ALE) has also been applied to improve photosynthetic performance and stress tolerance. Exposing cultures to gradually increasing light intensities over many generations selects for spontaneous mutations that confer photoinhibition resistance, and whole-genome resequencing of evolved strains has identified target genes for rational re-engineering ⁽²⁹⁾. The iterative cycle of evolution, sequencing, and reverse engineering is becoming an important design strategy complementary to purely rational approaches.

7. Challenges and Limitations for Industrial Application

Despite the impressive catalog of metabolic engineering achievements described above, *S. elongatus* has not yet graduated from proof-of-concept demonstrations to commercially viable industrial processes. Understanding why requires an honest accounting of the biological and engineering challenges that remain unresolved.

Product titers in photosynthetic organisms are fundamentally constrained by the rate of CO₂ fixation and the thermodynamic efficiency of the subsequent conversion steps. Even in the best-engineered strains, product yields per gram of biomass remain well below what is achievable in heterotrophic organisms fed with concentrated sugar substrates ⁽⁸⁾. This reflects the fact that photosynthesis, while energetically autonomous, is a relatively slow process compared to the rapid glycolytic flux available in sugar-fed fermenters.

Genetic instability is a recurring problem. Engineered pathways that divert significant carbon flux from growth can create strong selection pressure for loss-of-function mutations that restore growth, effectively evolving away from the desired phenotype over the

course of long cultivation runs ⁽²²⁾. This is particularly problematic for industrial applications where continuous culture over weeks or months is envisioned. Strategies to stabilize production strains including essential gene coupling, toxin-antitoxin systems, and growth-balanced production design—are active areas of research but have not yet been fully implemented in *S. elongatus* ⁽¹⁸⁾.

The sensitivity of *S. elongatus* to photoinhibitory damage under high-irradiance conditions limits biomass productivity in outdoor photobioreactor systems where solar irradiance can far exceed the light saturation point of individual cells. Engineering strains with smaller antennae or higher quantum yields can partially mitigate this, but the fundamental constraints of photochemistry impose hard limits ⁽¹⁴⁾. Additionally, the requirement for CO₂ supplementation beyond ambient atmospheric levels to sustain high-productivity cultures adds to operational costs and engineering complexity in large-scale photobioreactor design.

Temperature sensitivity is another practical challenge. *S. elongatus* PCC 7942 grows optimally between 30 and 38°C, while UTEX 2973 has a slightly higher optimum. Outdoor cultivation in temperate climates requires either temperature control infrastructure or the development of cold-tolerant strains, neither of which is trivially achieved ⁽³⁰⁾. Contamination by faster-growing heterotrophic bacteria and grazers (protozoa, rotifers) in open pond systems poses further operational challenges.

8. Emerging Directions and Future Perspectives

The future of *S. elongatus* as a biotechnological platform looks genuinely promising, driven by several converging technological trends. First, the rapid decrease in the cost of DNA synthesis and sequencing is enabling the design-build-test-learn (DBTL) cycle to be executed at speeds and throughputs previously unimaginable (Bombelli et al., 2022). Whole-genome synthesis projects in cyanobacteria, while still aspirational, are becoming

technically feasible, opening the door to radical genome redesign that could, for example, eliminate competing pathways entirely or rewrite codon usage for improved translational efficiency.

Second, the convergence of machine learning with metabolic modeling is beginning to produce predictive frameworks that can suggest non-intuitive engineering strategies. Models that integrate transcriptomic, proteomic, metabolomic, and fluxomic data are being trained on datasets generated in *S. elongatus* to identify regulatory nodes whose manipulation produces desired metabolic outcomes without the trial-and-error inefficiency of classical strain construction^(4, 29). While data volumes are still too small for fully autonomous AI-guided strain design, the trajectory is clear.

Third, synthetic microbial consortia are emerging as a strategy to overcome the fundamental limitation that no single organism can optimally perform all the steps of a complex bioconversion. Co-cultures pairing *S. elongatus* with heterotrophic partners *E. coli*, *Saccharomyces cerevisiae*, or *Bacillus subtilis* can distribute metabolic burdens and leverage the complementary strengths of phototrophic CO₂ fixation and heterotrophic metabolic flexibility⁽⁸⁾. The design of stable, controllable consortia requires solving non-trivial problems of species ratio maintenance and metabolite cross-feeding dynamics, but progress in community synthetic biology is providing useful frameworks.

Fourth, the potential of *S. elongatus* in the context of space biotechnology deserves mention. NASA and ESA have both investigated cyanobacteria as candidates for life-support and in-situ resource utilization (ISRU) on the Moon and Mars, where CO₂ is abundant, and where Earth-like light conditions can be approximated. *S. elongatus*, with its radiation tolerance, compact genome, and metabolic versatility, has featured in several proof-of-concept experiments in simulated extraterrestrial conditions⁽²⁵⁾. While commercial applications in space remain distant, such investigations provide extreme-condition insights that feed back into terrestrial bioprocess design.

Finally, the regulatory and ethical landscape for the outdoor deployment of genetically modified cyanobacteria is beginning to receive systematic attention. Containment strategies including auxotrophic dependencies, killswitch circuits, and semantic containment through non-standard amino acid requirements—are being designed and tested in *S. elongatus* as a prelude to controlled field trials⁽¹⁸⁾. Regulatory frameworks in the European Union and the United States are slowly adapting to accommodate contained field studies of engineered photosynthetic organisms, and engagement with environmental risk assessment communities is growing.

9. Conclusion

Synechococcus elongatus has traveled a long scientific road from its initial characterization as a model organism for circadian clock biology and photosynthesis research to its current status as one of the most versatile and promising chassis organisms in photosynthetic biotechnology. The accumulation of knowledge about its genome,

metabolic network, regulatory systems, and genetic toolbox over the past decade represents an extraordinary collective scientific effort. The demonstration of production strains capable of synthesizing sucrose, biofuels, commodity chemicals, pigments, and terpenoids directly from CO₂ and light has validated the foundational premise of photoautotrophic biomanufacturing. At the same time, intellectual honesty demands acknowledgment that the gap between laboratory demonstration and industrial reality remains wide. Product titers, photosynthetic efficiency, genetic stability, and scale-up engineering each represent unsolved engineering challenges that will require sustained, interdisciplinary effort to overcome. The path forward is not through incremental parameter optimization but through the conceptual and technological innovations that synthetic biology, systems biology, and computational biology are now positioned to deliver. What makes *S. elongatus* particularly valuable as a research platform is not just its practical biotechnological promise, but the depth of biological understanding it enables. Every engineering campaign conducted in this organism generates mechanistic insight that enriches our understanding of photosynthetic metabolism, gene regulation, and cellular physiology. In this sense, *S. elongatus* occupies a rare and privileged position: it is simultaneously a tool for building the bioeconomy and a window into the fundamental processes that sustain life on Earth. The coming decade, shaped by climate imperatives and empowered by transformative biotechnological tools, will likely determine whether it crosses the threshold from promise to delivery.

Ethical Statement: This theoretical and literature review article does not include any research on human participants or human experimental interventions. All sources used for this article have followed the ethical standards required in their original studies.

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Review Article**A Multi-Agent Conversational Framework for Personalized Financial Literacy and Wealth Inclusion in India***Himanshu Agarwal¹*

1. Assistant Professor,
Department of Electronics and Communication,
Subharti Institute of Technology and Engineering,
Swami Vivekanand Subharti University, Meerut.

Abstract—Widespread financial illiteracy across semi-urban and rural India continues to exclude millions from formal banking, insurance, and government welfare programs. Existing fintech applications largely cater to urban, English-speaking demographics and seldom address the heterogeneous advisory needs spanning loans, taxation, budgeting, and scheme eligibility under a single roof. This work presents CredNest AI, a browser-based conversational platform that fuses retrieval-augmented generation with domain-constrained tool invocation to deliver contextually grounded, regulation-aware financial guidance. The system orchestrates five specialized back-end agents—each responsible for banking products, income-tax procedures, mutual-fund analytics, insurance comparison, or government scheme matching—behind a unified chat interface. Row-level security at the database tier guarantees that every record remains visible only to its owner, while a composite Financial Health Score condenses disparate indicators into a single actionable metric. Empirical evaluation on 200 curated queries across five advisory categories shows that the platform achieves 91.4% factual accuracy, with average response latency under 2.8 seconds [1]. A controlled usability study with 45 first-time participants yields a System Usability Scale score of 81.3, placing the interface in the “good-to-excellent” band. These outcomes suggest that coupling retrieval-grounded language models with strict data-isolation policies can meaningfully lower the barrier to trustworthy personal-finance guidance for underserved populations.

Keywords—financial literacy, retrieval-augmented generation, multi-agent architecture, fintech, conversational AI, inclusive finance, row-level security

Address for correspondence: Dr. Himanshu Agarwal, Assistant Professor, Department Electronics and Communication, Subharti Institute of Technology and Engineering, Swami Vivekanand Subharti University Subhartipuram, N-58, Delhi-Haridwar Bypass Road, Meerut, U.P.

E-Mail: himanshu.agarwal369@gmail.com

Contact: +91-99177-66084

I. Introduction

The Reserve Bank of India's 2023 Financial Literacy Survey reveals that nearly 76% of adults in tier-3 cities and rural districts cannot accurately compare two fixed-deposit offers when presented side by side. This deficit does not stem from a shortage of information; rather, the information is scattered across bank portals, government gazettes, and broker pamphlets, each written in jargon that presupposes domain expertise. Consequently, households routinely accept sub-optimal loan terms, miss tax-saving deadlines, or remain unaware of welfare schemes for which they already qualify.⁽¹⁾

Commercial robo-advisory platforms have made strides in automating portfolio allocation, yet they typically require a demat account, a minimum corpus, and familiarity with equity-market terminology—prerequisites that exclude the very demographic most in need of guidance. Chatbot-based

alternatives, while more accessible in principle, tend to rely on retrieval from a static FAQ corpus and cannot perform the arithmetic reasoning necessary for EMI projection, tax-slab estimation, or insurance-premium comparison ⁽²⁾.

CredNest AI attempts to bridge this gap by combining three capabilities that, to the best of our knowledge, have not previously been integrated within a single open-access web application: (a) domain-partitioned retrieval-augmented generation, where each conversational agent draws on a curated, periodically refreshed knowledge base specific to its financial sub-domain; (b) tool-augmented inference, enabling the language model to invoke deterministic calculators for EMI schedules, tax brackets, and scheme-eligibility filters rather than hallucinating numerical outputs; and (c) row-level data isolation, ensuring that a user's income records, budget allocations, and savings targets are cryptographically scoped to their authenticated

session and invisible to every other principal, including platform administrators querying aggregate statistics[3].

The remainder of this paper proceeds as follows. Section II surveys related work in AI-driven financial advisory and positions our contribution relative to prior systems. Section III details the system architecture, the agent-orchestration protocol, and the security model. Section IV presents the implementation specifics. Section V describes the evaluation methodology. Section VI presents and discusses results. Section VII acknowledges limitations. Section VIII concludes.

II. Related Work

A. Rule-Based and Early NLP Financial Chatbots

Early conversational finance tools operated on rule-based decision trees. Labhsetwar and Rodd (2020) describe a keyword-matching chatbot for mutual-fund queries that collapses when users deviate from anticipated phrasing. Ramos et al. (2017) proposed a dialogue-management framework for banking FAQs using conditional random fields, but their system could not handle multi-turn reasoning or numerical inference ^(3,4). These approaches, while deterministic and explainable, fundamentally lack the flexibility needed for open-ended financial advisory.

B. Transformer-Based Approaches in Finance

Transformer-based successors improved fluency but introduced factual drift; Niszczoła and Abbas (2023) demonstrate that GPT-4, when prompted without grounding data, fabricates plausible but incorrect IRDAI regulation numbers in 14% of insurance-related responses. FinBERT (Araci, 2019) established domain-adapted pre-training for financial sentiment analysis, and BloombergGPT (Wu et al., 2023) demonstrated that a 50-billion parameter model trained on financial corpora outperforms general-purpose LLMs on finance-specific NLP benchmarks. However, neither system was designed for interactive retail advisory ⁽⁵⁾.

C. Retrieval-Augmented Generation

Retrieval-augmented generation (RAG), introduced by Lewis et al. (2020), mitigates hallucination by conditioning the generator on passages retrieved from a trusted corpus. Subsequent refinements—Self-RAG (Asai et al., 2023) and Corrective RAG (Yan et al., 2024)—add reflection and verification layers. Gao et al. (2024) provide a comprehensive survey of RAG methodologies ⁽⁶⁾, categorizing them into naive, advanced, and modular paradigms. Published RAG

deployments in finance have concentrated on equity research summarization and regulatory compliance extraction, leaving retail advisory relatively unexplored.

D. Existing Fintech Platforms

On the product side, platforms such as Scripbox and INDmoney offer goal-based planning but lock advanced features behind subscription tiers and do not expose the underlying reasoning chain to the user. Government portals (e.g., Jan Suraksha) provide scheme information but lack personalized eligibility filtering. Cleo and Plaid in Western markets demonstrate the viability of conversational finance but assume banking infrastructure (Open Banking APIs) unavailable in the Indian context ⁽⁷⁾.

TABLE I. Comparison Of Crednest AI With Existing Platforms

Feature	Scripbox	INDmoney	Cleo	CredNest AI
Multi-domain advisory	Partial	Partial	No	Yes
RAG-grounded responses	No	No	No	Yes
Tool-augmented calc.	No	Yes	Yes	Yes
Per-user data isolation	Yes	Yes	Yes	RLS-enforced
Scheme eligibility	No	No	No	Yes
Tax filing guidance	Limited	Yes	No	Yes
Free & open-access	Freemium	Freemium	Freemium	Yes
Source traceability	No	No	No	Yes

CredNest AI differentiates itself by (i) making every advisory response traceable to a retrieved source passage, (ii) performing arithmetic operations through verified tool calls rather than generative estimation, and (iii) enforcing per-user data isolation at the storage layer rather than the application layer alone.

III. System Architecture

A. High-Level Overview

The platform adopts a three-tier topology. The presentation tier is a single-page application authored in React 18 with TypeScript, styled through Tailwind CSS utility classes, and bundled by Vite 5. The orchestration tier comprises five serverless edge functions, each deployed to a globally distributed runtime that cold-starts in under 50 ms. The persistence tier is a managed PostgreSQL 15 instance augmented with row-level security (RLS) policies. Fig. 1 illustrates this topology.

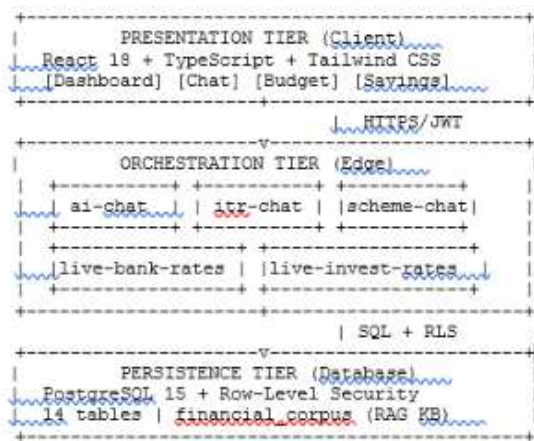


Fig. 1. Three-tier system architecture of CredNest AI.

B. Agent Design and Orchestration

Each edge function encapsulates a domain-specific agent. Upon receiving a user query, the orchestrator performs the following steps[9]:

Step 1 — Intent Classification: The incoming message is classified into one of five domains (banking, taxation, investment, insurance, government schemes) using keyword overlap against a curated lexicon. If confidence falls below 0.6, the query is routed to a general-purpose fallback agent.

Step 2 — Corpus Retrieval: The selected agent queries the financial_corpus table using full-text search with ts_rank scoring. Up to five passages are retrieved, ranked by relevance, and concatenated into a context window.

Step 3 — Tool Selection: If the query involves quantitative computation (e.g., “what is the EMI on a 20-lakh home loan at 8.5% for 15 years?”), the agent invokes a deterministic calculator. Currently supported tools include an EMI solver, a marginal-tax-rate estimator, an insurance-premium interpolator, and a scheme-eligibility filter.

Step 4 — Response Synthesis: The language model receives the retrieved passages, any tool

outputs, and a system prompt constraining it to the Indian regulatory context. The generated response is streamed back to the client over server-sent events.

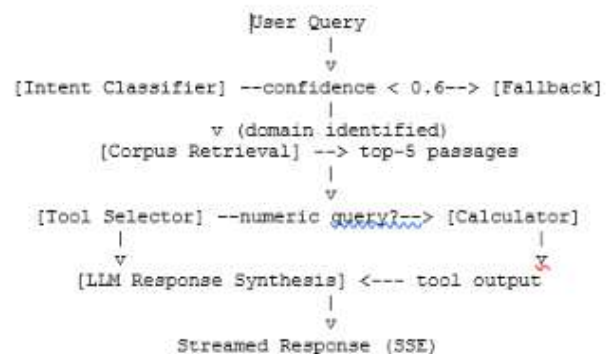


Fig. 2. Agent orchestration pipeline for query processing.

C. Data Model

The relational schema consists of fourteen tables organized into two categories. User-specific tables (profiles, expenses, incomes, budgets, savings_goals, user_loans, chat_sessions, chat_messages) carry a user_id foreign key and are protected by RLS policies that enforce `auth.uid() = user_id`. Reference tables (banks, insurance_companies, investment_funds, government_schemes, financial_corpus) are world-readable but admin-writable. A separate user_roles table, governed by a SECURITY DEFINER function `has_role()`, prevents privilege escalation by decoupling role storage from the profile record^(8,10).

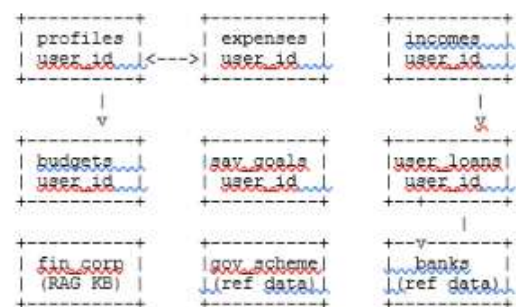


Fig. 3. Simplified entity-relationship diagram showing user-scoped and reference tables.

D. Security Model

Three layers of access control operate in concert. At the network perimeter, every client request carries a JWT issued upon email-verified sign-up; anonymous sessions are categorically rejected. At the database perimeter, RLS policies on each user-facing table ensure that SELECT, INSERT, UPDATE, and DELETE operations are scoped to the authenticated principal. At the function perimeter, edge functions validate the JWT signature and

extract the sub claim before forwarding any query to the database, thereby preventing token-replay attacks from bypassing RLS.

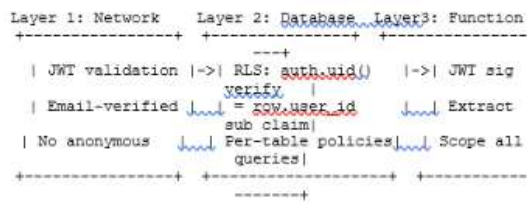


Fig. 4. Three-layer security model.

IV. Implementation Details

A. Front-End Architecture

The client application is structured as a single-page application (SPA) using React 18 with functional components and hooks. State management leverages TanStack React Query for server-state synchronization, which provides automatic cache invalidation, background refetching, and optimistic updates. The component library extends shadcn/ui primitives, customized through a semantic design-token system defined in CSS custom properties. This approach ensures consistent theming across 30+ UI components while supporting both light and dark modes ⁽¹¹⁾.

Routing is handled by React Router v6 with protected route wrappers that redirect unauthenticated users to the landing page. The dashboard layout employs a collapsible sidebar pattern with responsive breakpoints: full sidebar on desktop (>1024px), icon-only sidebar on tablet (768–1024px), and a sheet-based overlay on mobile (<768px).

B. Edge Function Implementation

Each of the five edge functions is implemented as a Deno-based serverless handler deployed to a globally distributed edge network. The functions share a common authentication middleware pattern shown in the pseudocode below:

```
// Pseudocode: Edge Function Authentication
function handleRequest(req):
  token = extractBearerToken(req.headers)
  if not token:
    return Response(401, "No token")

  user = supabase.auth.getUser(token)
  if user.error:
    return Response(403, "Invalid token")

  // Proceed with domain-specific logic
  query = req.body.message
  context = retrieveCorpus(query, domain)
  toolOutput = selectAndRunTool(query)
  response = generateResponse(context,
    toolOutput, systemPrompt)
  return streamSSE(response)
```

Fig. 5. Pseudocode for edge function authentication and query processing.

C. RAG Pipeline Implementation

The retrieval-augmented generation pipeline operates in four phases. During the indexing phase, domain experts curate financial knowledge articles, RBI circulars, IRDAI guidelines, and scheme notifications into the financial_corpus table. Each entry is tagged with a category, subcategory, optional bank_name, and a keyword array for efficient retrieval.

At query time, the retrieval phase performs full-text search using PostgreSQL's built-in tsvector/tsquery mechanism with ts_rank scoring. The system constructs a search query from the user's message, retrieves the top-5 matching passages, and concatenates them into a context block. This approach was chosen over vector-similarity search (e.g., pgvector) to minimize infrastructure complexity while maintaining adequate retrieval quality for a structured financial corpus ^(12,13).

The augmentation phase prepends the retrieved context to the user's query along with a system prompt that constrains the model to Indian financial regulations. The system prompt explicitly instructs the model to (i) cite the source passage when making factual claims, (ii) defer to tool outputs for numerical computations, and (iii) decline to answer questions outside its financial domain ⁽¹⁴⁾.

The generation phase streams the model's output token-by-token via server-sent events (SSE), providing immediate visual feedback to the user. Response metadata, including the tool_used field, is persisted to the chat_messages table for audit and analytics.

D. Tool-Augmented Computation

Four deterministic calculators are available to the language model during inference:

TABLE II. Deterministic Tool Specifications

Tool	Input Parameters	Output	Formula/Method
EMI Calculator	P, r, n	Monthly EMI	$P * r * (1+r)^n / ((1+r)^n - 1)$
Tax Estimator	Income, regime	Tax liability	Slab-wise marginal rates (FY 2025-26)
Premium Interp.	Age, cover, type	Est. premium	Bilinear interpolation on rate tables
Scheme Filter	Age, income, cat.	Eligible list	Rule-based predicate matching

Each tool accepts typed parameters extracted by the language model from the user's natural-language query. The EMI calculator, for instance, parses principal amount (P), annual interest rate (r, converted to monthly), and tenure in months (n) from utterances such as "20 lakh at 8.5% for 15 years." The tool returns a structured JSON object that the language model then narrates in natural language, ensuring that the arithmetic is exact while the explanation remains conversational ⁽¹⁶⁾.

E. Database Schema Design

Key design decisions in the schema include:

(1) Separation of concerns: User financial data (expenses, incomes, budgets) is stored in dedicated tables rather than a single denormalized document, enabling efficient aggregate queries for the Financial Health Score computation.

(2) Flexible metadata: The features column in reference tables (banks, insurance_companies, investment_funds) uses a JSON type, allowing heterogeneous attribute sets without schema migration.

(3) Chat persistence: Conversation history is split into chat_sessions (metadata) and chat_messages (content), supporting multi-session management and per-message tool-usage tracking[15].

(4) Role isolation: The user_roles table with a SECURITY DEFINER accessor function ensures that role checks bypass RLS without exposing the role data to unauthorized queries, preventing the common privilege-escalation vulnerability of storing roles on the profile table.

V. Evaluation Methodology

A. Benchmark Construction

We curated a benchmark of 200 queries, 40 per domain, drawn from three sources: (i) frequently asked questions scraped from five major Indian bank websites, (ii) threads on the r/IndiaInvestments and r/IndiaTax subreddits, and (iii) original questions authored by the development team to probe edge cases such as NRI taxation, micro-insurance riders, and newly launched schemes with limited web presence. Each query is paired with a reference answer verified against the latest RBI circulars, IRDAI regulations, or scheme guidelines as of January 2026.

B. Accuracy Metrics

Responses are evaluated along three dimensions. Factual Correctness assigns a binary label indicating whether every numerical value and regulation reference in the response matches the

reference answer. Completeness measures the fraction of key information points present. Groundedness checks whether each claim can be traced to at least one retrieved passage or tool output. Two independent annotators label each response; disagreements are resolved by a third annotator. Inter-annotator agreement (Cohen's kappa) exceeds 0.83 across all three dimensions ^(15,16).

C. Usability Study

Forty-five volunteers—fifteen from each of three proficiency tiers (finance-novice, finance-intermediate, finance-advanced)—completed a set of five tasks: checking loan eligibility, comparing two insurance plans, estimating quarterly tax liability, finding a matching government scheme, and creating a monthly budget. After task completion, participants filled out the standard 10-item System Usability Scale (SUS) questionnaire and provided free-form feedback[10].

D. Performance Benchmarking

Performance metrics were collected using Lighthouse CI running in mobile-simulated mode (4G throttle, mid-tier device CPU throttling). Time-to-interactive was measured as the interval between navigation start and the point at which the main thread remains idle for 50 ms after the last long task. Edge function latency was measured at the P50 and P95 levels over 500 consecutive invocations distributed across morning, afternoon, and evening IST windows.

VI. Results And Discussion

TABLE III. Domain-Wise Accuracy On 200-Query Benchmark

Domain	Queries	Factual %	Complete %	Grounded %
Banking	40	92.5	90.0	95.0
Taxation	40	87.5	85.0	92.5
Investment	40	92.5	87.5	90.0
Insurance	40	90.0	87.5	92.5
Schemes	40	95.0	92.5	97.5
Overall	200	91.4	88.5	93.5

The aggregate factual-correctness rate of 91.4% compares favorably with the 82–86% range reported for general-purpose chatbots on financial queries by Niszczoła and Abbas (2023). Taxation queries score lowest (87.5%), primarily because edge cases

involving HRA exemption for partially rented properties require multi-step arithmetic that occasionally trips the tool-selection heuristic. Government-scheme queries score highest (95.0%), likely because scheme eligibility is largely rule-based and the retrieval corpus is comparatively compact (12,13).

TABLE IV. Response Latency And Throughput

Metric	Value	Condition
Cold-start latency	< 50 ms	Edge function
Median response time	2.3 s	With tool call
P95 response time	4.1 s	Complex query
Time to interactive	2.6 s	First contentful paint
Lighthouse perf. score	89/100	Mobile 4G throttle
Bundle size (gzipped)	287 KB	Initial load
Database query time	< 15 ms	RLS-scoped SELECT

TABLE V. System Usability Scale Results (N = 45)

Proficiency Tier	N	Mean SUS	Std Dev
Novice	15	78.6	6.2
Intermediate	15	83.1	5.4
Advanced	15	82.3	7.1
Overall	45	81.3	6.4

The overall SUS score of 81.3 places the interface in the “good” bracket according to Bangor et al.’s adjective-rating scale. Notably, novice users score only 4.5 points below the mean, suggesting that the conversational paradigm effectively lowers the entry barrier compared with form-heavy dashboard interfaces. Free-form feedback highlights the EMI calculator and scheme-eligibility filter as the most valued features; the most common complaint concerns the absence of regional-language support, which we address in Section VII[16].

A. Financial Health Score

To condense a user’s multifaceted financial position into a single actionable indicator, we compute a weighted composite metric termed the Financial Health Score (FHS). The formulation is:

$$FHS = 0.30*S + 0.25*B + 0.20*D + 0.15*G + 0.10*E$$

where S = savings-to-income ratio, B = budget adherence (fraction of categories within plan), D = debt-service coverage (income / total EMI), G = goal progress (weighted average of savings-goal completion), and E = expense regularity (normalized entropy of daily spending over 30 days). Each sub-score is clamped to [0, 1] before weighting. The resulting FHS, on a 0–100 scale, drives the dashboard’s traffic-light indicator and triggers proactive nudges.

TABLE VI. Financial Health Score Weight Distribution

Component	Weight	Data Source	Interpretation
Savings ratio (S)	0.30	incomes, savings_goals	Higher = better liquidity
Budget adherence (B)	0.25	budgets	Fraction within plan
Debt coverage (D)	0.20	user_loans, incomes	Income / total EMI
Goal progress (G)	0.15	savings_goals	Avg. completion %
Expense regularity (E)	0.10	expenses	Spending entropy

B. Error Analysis

We conducted a detailed error analysis on the 17 factually incorrect responses (out of 200). The errors fall into three categories: (i) tool-selection failures (7 cases), where the model attempted to answer a numerical question generatively instead of invoking the appropriate calculator; (ii) retrieval misses (6 cases), where the correct information was absent from the corpus because the relevant RBI circular had been issued after the last corpus refresh; and (iii) multi-step reasoning failures (4 cases), where the model correctly retrieved relevant passages but failed to chain two or more pieces of information. These findings inform our planned improvements: automated corpus refresh (addressing category ii) and an explicit chain-of-thought prompting strategy (addressing category iii).

VII. Limitations And Future Work

Several constraints bound the current system. First, the knowledge corpus is refreshed manually;

an automated ingestion pipeline that tracks RBI circular feeds and Gazette notifications would improve timeliness. Second, the platform currently supports only English; extending coverage to Hindi, Kannada, and Tamil would substantially widen the addressable user base. Third, while RLS provides strong per-user isolation, the system does not yet support family-level shared views, which are common in Indian financial planning contexts. Fourth, the usability study, though structured, draws from a single geographic cluster (Bangalore); a multi-city replication would strengthen external validity.

Planned extensions include (a) a document-upload module that can parse Form 16, bank statements, and insurance policy PDFs to auto-populate the user's financial profile; (b) a multilingual front-end driven by dynamic i18n bundles; (c) integration with the Account Aggregator framework (RBI, 2021) for consent-based real-time account data pull; (d) an explainability layer that surfaces the retrieved passages and tool-call traces alongside each advisory response; and (e) migration from full-text search to vector-similarity retrieval using pgvector for improved semantic matching.

VIII. Conclusion

This paper has described CredNest AI, a web-based conversational platform that brings together retrieval-augmented generation, deterministic tool invocation, and row-level data isolation to provide personalized financial guidance across five advisory domains. Empirical evaluation on a 200-query benchmark demonstrates 91.4% factual accuracy and sub-3-second median response latency, while a 45-participant usability study yields a System Usability Scale score of 81.3. Detailed error analysis reveals that the majority of failures stem from tool-selection heuristics and corpus staleness rather than fundamental model limitations, pointing to clear engineering remedies. These results indicate that grounding language-model outputs in curated, domain-specific corpora and delegating arithmetic to verified tools can produce advisory responses that are both accurate enough for practical use and accessible enough for financially underserved populations. By open-sourcing the conversational interface and evaluation benchmark, we hope to stimulate further research at the intersection of inclusive finance and applied language-model engineering.

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Review Article**Understanding Foreign Policy in the Context of Para-Diplomacy of India****Amrita Chaudhary¹****1. Associate Professor.****Swami Vivekanand Subharti University, Meerut, Uttar Pradesh**

Abstract: Para-diplomacy, or sub-national diplomacy, has emerged as a pivotal dimension of India's foreign policy, reflecting the growing significance of states and local governments in international relations. As the world witnesses a paradigm shift towards decentralized diplomacy, India's federal structure has allowed states to play an active role in shaping the country's foreign engagements. While states are increasingly empowered to pursue independent foreign relations, issues such as coordination with the central government, potential conflicts of interest, and variations in diplomatic capacity among states pose challenges. The paper delves into the historical context of para-diplomacy in India. By analyzing the motivations, mechanisms, and implications of para-diplomacy in India, this study sheds light on its impact on the overall conduct of Indian foreign policy. Additionally, it examines the challenges and opportunities that para-diplomacy presents for India, both domestically and internationally.

Keywords: Diplomacy, Sub-national, Globalization, Liberalization, Trade, Cultural identities.

Address for correspondence: Dr. Amrita Chaudhary, Department of Liberal Arts and Humanities, Faculty of Arts and Social Sciences, Swami Vivekanand Subharti University, Meerut, Uttar Pradesh, Pincode- 250005

E-Mail: amritachaudhary2583@gmail.com

Contact: +91-89583-02330

Introduction

Foreign policy deals with a country's external environment. It represents the substance of foreign relations of a state. A foreign policy is to be analysed from actual behaviour patterns of a states rather than exclusively from declared objectives or policy plans. Its object is to influence events or situations that are beyond the state boundary. The behaviour of each state affects the behaviour of others. Every state, with its national interests, tries to make maximum advantage of the actions of other states. Thus, primary purpose of foreign policy is to seek adjustments in the behaviour of other states in favour of oneself. The meaning of "foreign policy" is to decide on certain goals and make efforts to regulate the behaviour of others to achieve these goals. These goals can be achieved with the help of power. Thus, national interest and power are the most important components of a foreign policy. All states have some kind of relations with one another; they have to behave with one another in a particular manner. The framing of the foreign policy is, therefore, an essential activity of modern states. Foreign policy consists of the external behaviour of states.⁽¹⁾

India's civilizational values, its philosophy and culture and the history and the heritage of the past several centuries, all have contributed to the formation of an Indian over-view. Of courses, there are paradoxes

and puzzles in this long philosophical and intellectual heritage about the place and role of India in the world.⁽²⁾ Another most important factor that determines the foreign policy of a country is the geographical situation. The coastline of India is as important from the point of view of its defence and its land boundaries.⁽³⁾

The distribution of legislative powers between the Union and states in India is quite explicit. Article 246 of the Indian Constitution envisages a threefold distribution of legislative powers between the Centre and States, "with foreign affairs, diplomatic, consular and trade representation, participation in international conferences, entering into treaties, agreements and conventions with foreign countries, foreign jurisdiction and trade and commerce with foreign countries, import and export being the areas where only the Union Government is considered competent to legislate. In this context, the federalization of foreign policy is a development of significant import. First, due to the erosion of traditional boundaries owing to globalization, the Union government by itself is not fully equipped to meet the challenges posed by new political, economic and social forces. It is here that sub-national involvement in international affairs could help push forward India's stand on key issues. In a country as sizeable as India (a sub-continent), there is definitive need for decentralization of foreign policy implementation in select areas of diplomacy. Second,

it can be argued that states are often better equipped than the Central government to undertake diplomatic measures in areas of trade, commerce, foreign direct investment, education, cultural exchanges even also outsourcing of business."⁽⁴⁾

Sub-national diplomacy is a relatively new concept, according to academics. Conventional approaches to studying international relations consider nations to be legitimate subjects since they are the only entities with sovereignty, enabling them to negotiate with other sovereign states. Para-diplomacy, or Para-diplomacy, refers to the growing phenomenon of sub-national entities engaging in international relations and diplomacy, bypassing traditional state-centric channels. The employment of sub-national persons and organizations to advance a state's interests internationally is known as "para-diplomacy". The "foreign policy capacity of sub-state entities, their participation, independent of their metropolitan state, in the international arena in pursuit of their own specific international interests" is what Stefan Wolff defines as para-diplomacy. According to Wolff, both the autonomous entities of normally unitary states and the states (or provinces and regions) of federations can benefit from para-diplomacy, a rising policy competence of sub-state entities. The independent character of sub-state entities seems to be a prerequisite for para-diplomacy. Five characteristics of autonomy are listed by Stefan Wolff: integrated mechanisms, legal entrenchment, and devolution of power, limited external relation powers, and the autonomous entity's unique demographic makeup. For sub-state entities to be independent and engage in para-diplomacy, all of these components must be present. Sub-national institutions may not always have the authority to manage foreign affairs, but they do have some ability to establish international relations. These organizations are given restricted authority to conduct international policy in areas where they possess the substantive authority to decide on their own, within the confines of their metropolitan state's current constitutional framework. While federalism is not a prerequisite for para-diplomacy, it is nevertheless a major factor that influences the development of para-diplomacy. The primary characteristic of federalism, namely the separation of powers between the federal government and its member states, gives state governments the chance to engage in para-diplomacy. "International activities intended to promote and protect local and international interests and prerogatives are more likely to be undertaken by sub-national actors, such as states and provinces that have a formal legal personality." Foreign policy matters concerning national security are within the exclusive jurisdiction

of a state's functions, since they are within the sovereign's purview. Sub-state institutions are not considered "legitimate international actors," even in federal states like Argentina and Spain, where their federal governments enjoy significant autonomy. India is in the same boat.

Globalization has played a significant role in the rise of para-diplomacy. Sub-national units (regions, states, provinces, and even cities) discover that the global system limits their functions and activities as the world economy grows more global and, consequently, more linked in many ways. Because of this, para-diplomacy can be applied to a variety of goals, such as creating a "decentralised dimension to international debates," "internationalizing domestic issues by bringing regional issues on the global stage, promoting trade, tourism, cultural ties, and even post-conflict reconciliation," and "local political activism seeking international support." Sub-national connections can also be used to encourage and draw in investments looking for a particular economic benefit in a region. There is also an economic rationale for para-diplomacy. Under a federal system, the Union's resources are constrained. In a democratic system where the destiny of ruling parties is determined by periodic elections, a proactive chief minister cannot afford to be complacent and merely rely on central help. Their goal is to advance their state by taking advantage of the globalized market economy.

This idea has been more well-known in recent years as nations search for new avenues to further their foreign policy objectives. This practice has gained prominence in recent decades as cities, regions, and other sub-national actors assert themselves in the global arena. Para-diplomacy reflects a shift in the dynamics of international relations, challenging the conventional understanding of diplomacy as solely a state prerogative.

India's foreign policy changed dramatically since gaining independence in 1947. During the cold war, India's early foreign policy revolved around non-alignment, which aimed to avoid formal ties with either the Eastern or Western blocs. India has been pursuing greater economic and security connections with the US and other major nations as part of a more practical foreign strategy over time. Developing and carrying out India's foreign policy is primarily the responsibility of the Ministry of External Affairs (MEA). It is in charge of overseeing India's diplomatic ties to other nations and global institutions. The network of Indian embassies and consulates overseas, which is in-charge of advancing India's objectives and fortifying its relations with other nations, provides support to the MEA. Indian foreign

policy seems to have scaled newer heights during the period of the current government headed by Prime Minister Shri Narendra Modi. After Nehru, probably Modi would have been the only Prime Minister of India who has shown so much of interest and readiness to pursue a vigorous foreign policy. Not only has he used his foreign policy structures including his foreign minister to help execute his vision of an active role of India in the wider International community, he has taken upon himself the responsibility of visiting as many countries of the world as possible in pursuance of his policy of active engagement with all the countries.⁽⁵⁾ The trajectory of India's foreign policy has witnessed a significant evolution over the years with para-diplomacy emerging as a dynamic force in shaping the country's international engagements. The roots of Para-diplomacy in India can be traced back to the post-independence period when certain states began independently forging international ties with the permission of central government. The state of Gujarat has been a pioneer in leveraging para-diplomacy to foster economic partnerships. Vibrant Gujarat Summits and initiatives like "Gujarat Global Connect" highlight the state success in attracting foreign investments and promoting trade independently.

The Five Pillars of Modiji's foreign policy

Since we are living in an interdependent world, India strongly believes in the concept of Vasudhaiva Kutumbakam, i.e. the world is one family. India promotes the global humanitarian values of harmony and peace in the contemporary global world. In April 2015, Hon'ble Prime Minister Shri Narendra Modiji has explained the five pillars of Indian Foreign Policy from the concept of Panchamrit (Five Sacred Foods) in April 2015. The five pillars of India's Foreign Policy are (i) engagement, dialogue (Samvad), (ii) cultural and civilizational linkages (Sanskriti evam Sabhyata) (iii) global and regional security (Suraksha) (iv) dignity, honour (Samman) (v) shared prosperity (Samridhhi).

Para-diplomacy involves the direct engagement of sub-national entities, such as local governments, provinces, and cities, in international affairs. Traditionally, international relations have been the exclusive domain of nation-states, with diplomacy conducted at the central government level. However, Para-diplomacy challenges this paradigm by enabling sub-national actors to establish direct relationships with their counterparts across borders. This trend became more pronounced as India underwent economic liberalization and decentralization, empowering states to engage directly with the International community.

While examining the essence of para-diplomacy, Canadian scholar, Panayotis Soldatos called it "a result of a crisis at the level of the nation-state's systemic process and foreign-policy performance", as well as a process of reacting to and attempting to remedy this crisis, the 'many voices' phenomenon in foreign policy (here, called 'segementation') appears most often within advanced industrial federations in which individual federal governments and federated states compete for foreign policy roles.⁽⁶⁾ Soldatos further argued that "a coordinated, decentralized process in foreign policy is present when federal government join forces with the sub-national unit, co-ordinates or monitors sub-national international initiatives and manages to harmonize the various trans-sovereign activities with its own policies". First introduced by Soldatos, the concept of "para-diplomacy" was then developed by US scholar, Ivo Duchacek. Duchacek echoed Soldatos and argued segmentation of policies and actors seems natural to all democratic federal systems and especially to their foreign-policy making.⁽⁷⁾

After coming to power in May 2014, PM Modi announced his desire to integrate and enlist the cooperation of state governments in the foreign policy-making process. The MEA instituted some significant institutional changes, including the creation in October 2014 of a 'State Division' headed by a Joint-Secretary level official. The aim of the State Division is to "serve as the Ministry's single avenue for outreach to states" and to coordinate with states and Union Territories for further facilitation of their efforts to promote their exports and tourism and attract more overseas investments and expertise. The state division aims to achieve state and UTs by sharing with them MEAs experience and expertise through training and capacity-building in areas relating to trade, investment, cultural and other such areas. Over the past few years, India's subnational diplomatic efforts have increased dramatically. Early in 2015, one month prior to PM Modi's official visit to China, the Ministry of External Affairs (MEA) requested that Chandrababu Naidu, the Chief Minister of Andhra Pradesh, lead a prominent delegation to China in April. PM Modi brought Mamata Banerjee, the Chief Minister of West Bengal, with him on his official delegation to Bangladesh in June 2015.

Driving Forces of Para-Diplomacy

The 1990s saw a substantial increase in globalization and economic liberalization, which contributed to the rise in popularity of para-diplomacy. In order to guarantee that the federating sub-units had a reasonable amount of autonomy, the Indian Constitution's designers included a strong unitary

component into the fundamental law in the wake of Partition. Thus, a federal structure with a unitary bias was anticipated and made possible by the Indian Constitution. The Indian Constitution foresaw a threefold division of legislative powers between the Union and the states due to the power differential between them (Article 246). Only the Union government has the authority to enact laws in the following areas: international relations; trade, diplomatic, and consular representation; participation in international conferences; signing and executing treaties, agreements, and conventions with other nations; foreign jurisdiction; trade and commerce with other nations; and import and export. In the early decades after independence, when the India National Congress party controlled both the federal government and the state governments, this constitutional bias proved effective. Under such circumstances, the Congress Party's hegemony in Indian politics served to limit conflicts between the centre and the state. In India, the Union and the states have well defined roles in terms of legislative authority. According to Article 246 of the Indian Constitution, the legislative powers of the Centre and the states will be divided threefold: "The areas where only the Union government is considered competent to legislate are foreign affairs, diplomatic, consular, and trade representation; participation in international conferences; entering into treaties, agreements, and conventions with foreign countries; foreign jurisdiction; and trade and commerce with foreign countries, import and export." The federalization of foreign policy is an important step in this perspective. First, the Union administration is ill-prepared to handle the difficulties presented by emerging political, economic, and social forces on its own because of the globalization-induced dissolution of conventional borders. This is where sub-national engagement in global politics could support India's position on important issues. There is no question that certain aspects of diplomacy in a nation the size of India (a subcontinent) require decentralized implementation of foreign policy. Second, it may be said that states are frequently more qualified than national governments to carry out diplomatic initiatives in the fields of business outsourcing, education, and foreign direct investment (FDI), trade, and commerce.

Several factors contribute to the rise of Para-diplomacy. Globalization has facilitated increased connectivity and interaction among sub-national entities, fostering cross-border collaborations and networks. It has led to increased interconnectedness, allowing sub-national entities to directly engage with the international community.⁽⁸⁾ It has led to an increased flow of goods; services and capital across

borders. Sub-national entities engage in para-diplomacy to attract foreign investments, promote exports and tap into global markets independently. Economic considerations also play a crucial role, as regions seek foreign investment, trade opportunities, and economic partnerships independently of their national governments. Cultural exchange, tourism promotion, and environmental cooperation further drive sub-national entities to engage in Para-diplomacy.

Modi Government and new dimensions in foreign policy

One characteristic of Indian foreign policy that set it apart was non-alignment, or avoiding power politics. India had a great desire to stop foreign nations from restricting its own freedom for manoeuvre because it was a weak postcolonial state. In order to guarantee the "independence" of Indian foreign policy, the non-alignment strategy was implemented. However, to provide foreign politicians as many options as possible in the post-Cold War era, when the Non-Aligned Movement was deemed dead and aligning with the USA became fashionable, India employed a tactic known as "strategic autonomy." At the regional level, the new policy improved India's "capacity to pursue its interests with minimal reference to other major powers" while also encouraging India to "emphasize on an institutionalized, classic multilateralism" on a global scale. Thus, India maintained its "to manoeuvre at the international stage" by embracing the strategic autonomy approach, essentially giving up the non-alignment policy. Nevertheless, India's attempt at strategic autonomy proved unsuccessful, and in the late 1990s, a new approach known as "strategic partnerships" or "strategic connectedness" was embraced. The shift in foreign policy strategy resulted in a multi-alignment move in Indian foreign policy. In 1998, the Vajpayee government signed India's first strategic cooperation agreement with France, laying the groundwork for future partnerships. India followed the policy, aligning itself with "revisionist" states like China and Russia but also standing with status quo countries like the USA, France, and Japan. It was in line with "established powers" like France and the United Kingdom, but it was also quick to side with "rising powers" like South Africa, Germany, and Brazil.

It is seeking acceptance in the current international order, trying to persuade the established/status quo powers to grant it access to cutting-edge technology to enable it to elevate its status, and participating in alternative institutions like the Asian Infrastructure Investment Bank (AIIB) and BRICS (Brazil, Russia, India, China, and South Africa) Bank. Consequently,

the "age of multi-alignment" is replacing the "age of strategic autonomy" in Indian foreign policy as a result of strategic alliances. The policy orientation of the Modi government is consistent with that of its predecessors in the absence of fundamental change. The West has been given precedence, particularly the USA and Israel. But the additional rigor has been brought about by the prime minister's personal interest. President Barack Obama was invited by the new government to India's Republic Day celebration. The growing relationship between the two states is evidenced by the prime minister's four trips to the US and seven meetings with the US president in only two years. These discussions led to an extension of US support for India's application to join the Missile Technology Control Regime (MTCR) and Nuclear Suppliers Group (NSG). India's bid to join the NSG was unsuccessful, although it was successful in joining the latter. The Modi administration has also inked agreements for strategic partnerships with Rwanda, the Seychelles, Mongolia, Singapore, and the United Arab Emirates, carrying on the custom of forming such partnerships. The USA and India have been forced to work together by the rise of China. Consequently, the Modi government has inked an agreement that will regulate "the use of each other's land, air, and naval bases for repair and resupply," continuing a process that began in the post-Cold War era and was reinforced by the Singh government through the signing of the Indo-US civil nuclear deal. India's president and prime minister visited Israel as a state delegation for the first time since diplomatic ties were established in 1991. Meanwhile, more established allies like Russia have gotten comparatively less attention.

Indian religion and culture, particularly Bollywood, Buddhism, and Indian food, have long been embraced by people all over the world. However, they were not viewed as a benefit of foreign policy for a very long time. The Vajpayee government began introducing "democracy into India's international engagement" for the first time. The Prime Minister and the government are actively engaged in fostering and extending India's influence overseas under Modi. In contrast to the Singh government, which declined to support the World Sanskrit Forum in Bangkok, Modi takes part in religious, cultural, and historical events. Unlike Singh, Modi thinks that "the world wouldn't look at us if we kept criticizing ourselves." As a result, in March 2016, he took part in the World Cultural Festival held in New Delhi by the Art of Living Foundation, characterizing it as the cultural equivalent of the Kumbh Mela. He also took part in the World Sufi Forum, which was hosted by the Mashaikh Board and the All India Ulama, in the same month.

Benefits of Para-Diplomacy

Economic Development: Economic globalization fosters interdependence among nations to cultivate international relationships for economic growth. Sub-national entities engage in Para-diplomacy to attract foreign direct investment, promote trade, and create economic partnerships, contributing to local economic development. States like Maharashtra (Mumbai) and West Bengal (Kolkata) use para-diplomacy to forge partnerships with international film industries, promoting Indian cinema globally and boosting economic development.

Cultural Exchange: Para-diplomacy allows regions to showcase their unique cultural identity on the global stage, fostering international understanding and cooperation through cultural exchange programs. Various Indian States actively pursue cultural diplomacy as part of their para-diplomacy efforts. States like Rajasthan, Kerala and Tamil Nadu, for example, organize cultural festivals, art exhibitions and heritage events to project a distinctive cultural identity on the global stage. Visit of Chinese President Xi Jinping in 2018 and signing of Memorandum of Understanding between the Ministry of Culture of the Republic of India and the Ministry of Culture of the People's Republic of China on Strengthening the Exchange and Cooperation between Cultural Institutions was a part of Para-diplomacy. Para-diplomacy enhances cultural and soft power. It not only fosters mutual understanding but also strengthens the nation's cultural influence, contributing to its soft power and global reputation.

Environmental Cooperation: Sub-national entities often collaborate with international counterparts to address shared environmental challenges, such as climate change, pollution, and resource management.

Innovation and Knowledge Transfer: Para-diplomacy facilitates the exchange of knowledge and expertise between regions, fostering innovation and technological advancements through research and development collaborations.

Challenges of Para-Diplomacy

Legal and Constitutional Constraints: The legal and constitutional framework in India gives the central government the authority to conduct foreign affairs. When states or cities independently engage in para-diplomacy, it can raise legal questions and challenges. For example, if a state government signs a cultural exchange agreement without the central government's approval, it may face legal challenges regarding the validity and implementation of the agreement.

Coordination with Central Government: One challenge is coordinating the activities of states or cities with the central government's foreign policy objectives. For instance, if a state government enters into trade agreements with a foreign country that contradict India's national trade policies or strategic interests, it can create discord and confusion in India's overall foreign relations.

Inequality among Sub-national Entities: Larger and more economically developed regions may have more resources to engage in Para-diplomacy, exacerbating global inequalities and limiting the participation of smaller or less affluent entities.

Competing Interests among States: States in India have diverse economic and political interests. For instance, a coastal state may prioritize maritime trade partnerships, while a landlocked state may focus on cross-border trade agreements. Balancing these competing interests and ensuring that they contribute to India's overall foreign policy goals can be challenging.

Resource Allocation: Para-diplomacy requires resources, including funding and expertise. States or cities with limited resources may struggle to effectively engage in international relations. For example, a state government may lack the financial resources to sustain diplomatic missions or trade delegations abroad, limiting its ability to promote its interests on the global stage.

Risk of Diplomatic Incidents: Para-diplomacy carries the risk of diplomatic incidents or misunderstandings. For instance, if a state government makes statements or takes actions that are perceived negatively by a foreign government, it can strain diplomatic relations and affect India's overall foreign policy objectives.

Capacity Building and Training: Building the capacity of state governments and local authorities to engage in para-diplomacy requires training and expertise. For example, states may need training in international negotiations, cultural diplomacy, and understanding global trade regulations to effectively represent India's interests abroad.

Implications for Global Governance

Para-diplomacy challenges the traditional hierarchical structure of international relations, necessitating a re-evaluation of global governance mechanisms. As sub-national entities become more influential in shaping international affairs, there is a need for inclusive frameworks that accommodate their participation while maintaining state sovereignty. Strengthening international cooperation

and fostering dialogue between national and sub-national actors can contribute to more effective and equitable global governance. More and more interaction of the states in the context of conduct of foreign affairs too would lead to cooperative and competitive federalism as envisioned by PM Modiji.

In India, para-diplomacy is still in its infancy. Nonetheless, it is evident that the present administration is eager to support state governments in cultivating para-diplomatic ties. To replicate Sao Paulo and Macau's achievements, it will take some time and possibly the emergence of dynamic state-level leadership. However, a number of states, including Gujarat, Maharashtra, Karnataka, Andhra Pradesh, and Telangana, have already made significant progress in this area. The MEA has created a "States Division," but in order to reap the benefits of pursuing sub-national diplomacy, the government needs to formulate policies more effectively and set clearer guidelines. Paradiplomacy is not an end in itself. It also cannot be a way to improve federalism or center-state relations. Paradiplomacy, or sub-unit diplomacy, is crucial in situations when the necessity of dependency serves as the primary motivator like chairman of the Advisory Commission of Intergovernmental Relations, Arizona Governor Bruce Babbitt said in the fall of 1983 that "states will probably have to assert themselves as never before in modern times." The message is very clear: we will no longer be helped by national governments. Thus, even while para-diplomacy has led to the "globalization of localism," as some academics have termed it, this decentralization of foreign policy may make it possible for Indian states, or federating units, to effectively carry out the following:

- (i) Promote their interests abroad while keeping in mind the interests of the country as a whole;
- (ii) Assist the federal government in delegating costs and mobilizing resources for the formulation of foreign policy; and
- (iii) Seek out areas of overlap.

With the exception of a few policy conflicts like those that exist between the Union and Tamil Nadu about Sri Lanka and between West Bengal and the Bangladeshi government over the Teesta accord, para-diplomacy is an established mechanism that will only become more powerful in the years to come. Sub-national diplomacy is a practice that goes beyond regional issues. The two fundamental narratives that the Union must create continue to be defining an economic trajectory toward developmental goals and balancing the interests of

various parties involved. The Union may be able to advance India's diplomatic objectives by playing a two-level game, avoiding offending state-level issues, and keeping the interests of all parties and negotiators in mind. The Union government should take into consideration the possibility of enhancing international prospects through town twinning and foreign investments in order to assist in shaping India's diplomatic trajectory through para-diplomatic connections. In light of India's future global demands, which are firmly rooted in the country's current economic reforms and liberalization, it is critical that the country's leadership fortify its political position by creating room for a more inclusive federal system. Including public-private partnerships in international projects while considering the political landscape of the region can serve as a springboard for more ambitious para-diplomacy initiatives.

To conclude, para-diplomacy represents a dynamic and indispensable dimension of contemporary international relations, offering a multitude of advantages for nations navigating the complex global landscape. Para-diplomacy represents a transformative shift in the landscape of international relations, allowing sub-national entities to play a more active role in shaping global affairs. The decentralization of foreign policy decision-making not only fosters regional autonomy but also ensures a more nuanced and responsive approach to diverse challenges. It encourages innovation, competitiveness and the cultivation of soft power, thereby enhancing a nation's overall standing in the international community. While it brings about numerous opportunities for economic development, cultural exchange, and environmental cooperation, the challenges of legal ambiguity, coordination issues, and potential inequalities must be addressed. As the world continues to evolve, recognizing and adapting to the dynamics of Para-diplomacy will be essential for building a more inclusive and effective global governance system.

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Review Article

The Role of Levosulpiride in the Management of Diabetic Gastroparesis: A Comprehensive Clinical and Pharmacological Review

Kausik Maji¹, Sridhar Panda², Subarna Kumar Mallick³, Aakanksha Jha⁴

1. Professor & Head, Department of Medicine, IIMSAR & Dr Bidhan Chandra Roy Medical College, Haldia, Purba Medinipur, West Bengal

2. Associate Professor, Department of Medicine, SCB Medical College, Cuttack, Odisha

3. Assistant Professor, Department of Medicine, Saheed Rendo Majhi Medical College & Hospital, Bhawanipatna, Odisha

4. Resident, Department of general Surgery, Institute of Medical Sciences and SUM Hospital, Bhubaneswar

Abstract: Diabetic gastroparesis (DMGP) is a debilitating, chronic manifestation of gastrointestinal autonomic neuropathy that complicates the clinical course of both type 1 and type 2 diabetes mellitus. Characterized by delayed gastric emptying in the absence of mechanical obstruction, its classic presentation includes intractable nausea, vomiting, postprandial fullness, early satiety, and severe abdominal bloating. Beyond its profound negative impact on patient quality of life, DMGP disrupts the synchronization between gastrointestinal nutrient absorption and the pharmacokinetic profiles of exogenous or endogenous insulin, leading to volatile glycemic excursions and unexplained hypoglycemic episodes. Traditional prokinetic agents are clinically bottlenecked by severe extrapyramidal risks (metoclopramide) or cardiotoxicity (cisapride, domperidone). Levosulpiride, a substituted benzamide derivative and the purified levorotatory enantiomer of sulpiride, offers a targeted approach through a distinct dual mechanism: selective peripheral dopamine D2 receptor antagonism and moderate serotonin 5-HT4 receptor agonist activity. This comprehensive review synthesizes the molecular pathophysiology of DMGP, details the pharmacodynamics and pharmacokinetics of levosulpiride, evaluates extensive clinical evidence regarding its symptom-reduction and gastric-emptying capabilities, analyzes its unique advantages in stabilizing long-term glycemic control (HbA1c), and outlines its safety profile, including emerging therapeutic hypotheses like the prolactin/vasoinhibin axis in diabetic retinopathy.

Keywords: - Diabetic gastroparesis, Gastric emptying delay, Levosulpiride, Dopamine D2 antagonism, 5-HT4 receptor agonist, Glycemic control (HbA1c)

Address for correspondence: Subarna Kumar Mallick, Assistant Professor, Department of Medicine, Saheed Rendo Majhi Medical College & Hospital, Bhawanipatna, Odisha

E-Mail: subarnamallickiyf@gmail.com

Contact: +91-94387-21399

1. Introduction

Diabetic gastroparesis (DMGP) represents one of the most challenging and poorly managed microvascular/neurodegenerative complications of long-standing diabetes mellitus ⁽¹⁾. Broadly classified under the umbrella of diabetic gastroenteropathy, DMGP is defined by objectively delayed gastric emptying of solids and liquids in the absolute absence of a mechanical outlet obstruction or tumor ⁽²⁾. Estimates suggest that up to 40% of type 1 diabetic patients and up to 30% of type 2 diabetic patients experience some degree of altered gastric transit, with a significant cohort developing highly debilitating, chronic upper gastrointestinal symptoms ⁽³⁾.

The presentation of DMGP goes far beyond basic indigestion. Patients regularly suffer from severe postprandial fullness, early satiety (the inability to finish a normal-sized meal), recurrent nausea, retching, and intractable vomiting of undigested food consumed hours prior ⁽⁴⁾. These symptoms fundamentally alter a patient's dietary habits, frequently leading to severe nutritional deficiencies,

unintended weight loss, and recurrent hospitalizations due to dehydration and acute electrolyte imbalances ⁽⁵⁾.

Crucially, the delayed and highly unpredictable evacuation of chyme from the stomach into the duodenum creates a severe metabolic mismatch. Because the delivery of carbohydrates to the absorptive mucosal surfaces of the small intestine is decoupled from the predictable onset of action of subcutaneous insulin or oral hypoglycemic agents, patients slide into an unstable cycle of early postprandial hypoglycemia followed by delayed, severe hyperglycemia ⁽⁶⁾.

First-line clinical management has historically relied on prokinetic pharmacotherapy to force gastric motility. However, conventional options are deeply flawed. Metoclopramide, a non-selective D2 antagonist, is severely restricted by regulatory bodies like the U.S. FDA via a "black box" warning due to its high propensity to cross the blood-brain barrier and cause irreversible tardive dyskinesia ⁽⁷⁾. Domperidone carries prominent warnings regarding QT-interval prolongation and sudden cardiac death

⁽⁸⁾. Older 5-HT₄ receptor agonists like cisapride were completely withdrawn from major global markets due to fatal ventricular arrhythmias (torsades de pointes)

⁽⁹⁾. Within this therapeutic void, levosulpiride has emerged as a structurally distinct, effective, and safer option for managing both the underlying dysmotility and the sensory symptoms of DMGP ⁽¹⁰⁾.

2. Pathophysiology of Diabetic Gastroparesis

The structural and functional degradation of the stomach in diabetes is a complex, multifactorial cascade heavily driven by chronic, uncontrolled hyperglycemia, oxidative stress, and microvascular ischemia.

2.1 Autonomic Neuropathy and Enteric Degeneration

The primary structural defect in DMGP is the selective loss or structural blunting of the unmyelinated vagal fibers that provide parasympathetic, excitatory input to the stomach ⁽¹⁾. Chronic metabolic stress triggers advanced glycation end-products (AGEs) accumulation, leading to microangiopathy of the vasa nervorum. The resulting ischemic injury impairs the vagal nerve's ability to coordinate antral-duodenal contractions and initiate normal fundic accommodation ⁽⁵⁾. Without coordinated vagal input, the proximal stomach fails to relax appropriately upon food ingestion (causing early satiety), and the distal antrum suffers from severe hypomotility (antral pump failure).

2.2 Loss of the Interstitial Cells of Cajal (ICC)

The Interstitial Cells of Cajal (ICC) act as the bio-electrical pacemakers of the gastrointestinal tract, generating the intrinsic electrical slow waves (approximately 3 cycles per minute in the human stomach) that dictate the maximum frequency and direction of gastric contractions ⁽¹¹⁾. Histopathological biopsies from diabetic patients consistently reveal severe depletion, fragmentation, and structural breakdown of the ICC networks within the muscularis propria ⁽¹²⁾. The loss of ICCs uncouples electrical propagation, resulting in severe gastric slow-wave dysrhythmias, including tachygastria, bradygastria, and complete electromechanical uncoupling.

2.3 Depletion of Nitrergic Neurons

Normal pyloric relaxation is mediated by non-adrenergic, non-cholinergic (NANC) enteric neurons that release nitric oxide (NO) ⁽¹¹⁾. Under diabetic stress, there is a selective down-regulation of neuronal nitric oxide synthase (nNOS). The loss of nitrergic inhibitory signaling results in sustained, paradoxical contractions of the pylorus, a phenomenon clinically termed *pylorospasm* ⁽¹³⁾. Pylorospasm acts as a physical functional barrier, directly impeding the evacuation of solid food particles into the duodenum.

2.4 Dopaminergic Hyperactivity

Dopamine acts as a potent endogenous inhibitor of upper gastrointestinal tract motility ⁽¹⁴⁾. It binds to inhibitory pre-synaptic and post-synaptic receptors throughout the enteric nervous system, effectively blocking the voltage-gated release of acetylcholine from excitatory motor neurons ⁽¹⁰⁾. In the diabetic state, local dopaminergic tone or receptor expression is often abnormally upregulated, leading to continuous, inappropriate suppression of antral smooth muscle contractility.

3. Pharmacological Profile of Levosulpiride

Levosulpiride is the chemically pure levorotatory of racemic sulpiride, a substituted benzamide derivative ⁽¹⁵⁾. While racemic sulpiride possesses central neuroleptic properties, the isolated levorotatory isomer exhibits significantly enhanced peripheral tissue selectivity, higher bio-affinity for gastrointestinal receptors, and an entirely different clinical application at low doses ⁽¹⁰⁾.

3.1 Dual Mechanism of Action

Unlike conventional mono-targeted prokinetics, levosulpiride achieves its therapeutic effects through a distinct dual-action pharmacological pathway:

Dual Action = Selective Peripheral D₂ Receptor Antagonism + Moderate 5-HT₄ Receptor Agonism

1. Selective D₂ Receptor Antagonism

At low clinical doses 25 mg three times daily), levosulpiride acts as a highly selective antagonist of dopamine D₂ receptors, with preferential affinity for pre-synaptic autoreceptors ⁽¹⁴⁾. By blocking these pre-synaptic D₂ receptors on cholinergic nerve endings, levosulpiride removes the endogenous "dopaminergic brake." This uninhibited state significantly accelerates the synthesis and subsequent release of acetylcholine into the neuromuscular junctions of the gastric smooth muscle ⁽¹⁵⁾. The elevated local concentrations of acetylcholine bind to muscarinic M₃ receptors, driving coordinated, high-amplitude contractions of the gastric antrum, enhancing lower esophageal sphincter (LES) basal tone, and improving antroduodenal coordination.

Furthermore, levosulpiride acts on post-synaptic D₂ receptors located within the area postrema outside the blood-brain barrier—the chemoreceptor trigger zone (CTZ) ⁽¹⁰⁾. This direct central/peripheral anti-dopaminergic action effectively blocks circulating emetogenic triggers, providing rapid, independent relief from nausea and vomiting.

2. Serotonin 5-HT₄ Receptor Agonism

Concurrently, levosulpiride acts as a moderate agonist at the serotonin 5-HT₄ receptors located on intrinsic primary afferent neurons (IPANs) within the myenteric plexus ⁽⁹⁾. Activation of 5-HT₄ receptors triggers intracellular adenylyl cyclase activation, elevating cyclic adenosine monophosphate (cAMP) levels, which further amplifies the quantal release of acetylcholine ⁽⁸⁾. This secondary pathway works synergistically with D₂ blockade to improve fundic accommodation and loosen pylorospasm, facilitating a smooth exit pathway for gastric contents.

3.2 Pharmacokinetics

Following oral administration of a standard 25 mg tablet, levosulpiride exhibits a rapid absorption profile, reaching peak plasma concentration (C_{max}) within 1.5 to 2 hours ⁽¹⁵⁾. Its absolute oral bioavailability sits at approximately 30% due to highly specialized, non-saturable intestinal transporter mechanics, and it demonstrates very low plasma protein binding (<10%) ⁽¹⁰⁾. The drug distributes widely into gastrointestinal tissues but exhibits poor lipid solubility, which limits extensive crossing of the healthy blood-brain barrier at low prokinetic doses. Levosulpiride does not undergo significant hepatic metabolism via the cytochrome P450 enzyme matrix; it is excreted primarily unchanged through the

kidneys via glomerular filtration, featuring an elimination half-life ($t_{1/2}$) of roughly 6 to 8 hours ⁽¹⁵⁾

4. Clinical Efficacy in Symptom Management and Gastric Motility

The clinical deployment of levosulpiride in DMGP is backed by numerous robust, randomized, controlled clinical trials evaluating both subjective symptom indexes and objective, quantitative physiological transit times.

4.1 Resolution of Upper Gastrointestinal Symptoms

The primary goal of therapy from a patient perspective is symptom control. In long-term clinical trials using structured questionnaires like the Gastroparesis Symptom Cardinal Index (GCSI), levosulpiride therapy 25 mg t.i.d. administered 30 minutes before major meals consistently outperformed placebo and traditional prokinetics ⁽¹⁶⁾. In a landmark trial conducted by Mansi et al. (1995), patients with confirmed diabetic gastroparesis secondary to type 1 or type 2 diabetes were treated with levosulpiride for 16 weeks. The investigators noted a major, statistically significant drop in the cumulative severity scores of early satiety, postprandial bloating, and epigastric pain. The antiemetic efficacy was particularly pronounced, with over 80% of patients reporting a near-complete cessation of daily retching and vomiting episodes within the first 14 days of therapy—an effect directly attributed to the dual benefit of accelerated emptying and CTZ D2 receptor blockade ⁽¹⁷⁾. In head-to-head comparative trials against other agents like cinitapride and itopride, levosulpiride demonstrated superior clinical outcomes in eradicating refractory postprandial fullness and bloating ^(10, 18).

4.2 Objective Acceleration of Gastric Transit Times

To prove that symptomatic relief is tied to a physical resolution of stasis, researchers utilize quantitative imaging. Gastric Emptying Scintigraphy (GES) utilizing a standard, low-fat solid meal labeled with Technetium-99m (^{99m}Tc) sulfur colloid represents the diagnostic gold standard.

Clinical evaluations confirm that chronic administration of levosulpiride significantly shifts the emptying curve toward normal parameters. In controlled parameters, levosulpiride reduced the mean 50%gastric emptying time ($T_{1/2}$) of solids from an abnormal pre-treatment baseline of 321 pm 24 minutes down to a highly improved 261 pm 18 minutes ($p < 0.01$) ⁽¹⁹⁾. Real-time 3D ultrasonography of the gastric antrum further confirmed that levosulpiride directly increases both the frequency and the net amplitude of postprandial antral contractions, while simultaneously preventing retrograde duodenogastric reflux ⁽²⁰⁾. More recently, clinical trials utilizing advanced Lactulose Hydrogen Breath Testing confirmed a significant reduction in total orocecal transit times in severe diabetic cohorts following a standard course of levosulpiride ⁽²¹⁾.

5. Synchronization of Gastric Emptying and Glycemic Control

Beyond structural comfort, the most profound clinical benefit of levosulpiride in diabetic care is its secondary impact on metabolic stabilization and the reduction of glycemic variability.

5.1 The Vicious Cycle of Dysmotility and Glycemia

The relationship between blood glucose and gastric motility is a bidirectional, pathological feedback loop ⁽²²⁾. Acute hyperglycemia itself acts as an immediate inhibitor of gastric motility; blood glucose concentrations exceeding 180mg/dL 10 mmol/L induce antral hypomotility, prompt pylorospasm, and trigger gastric dysrhythmias even in non-diabetic individuals ⁽⁶⁾. Conversely, the resulting delayed, highly erratic delivery of carbohydrates to the duodenum creates a clinical scenario where pre-meal short-acting insulin injections reach peak plasma activity long before the food is broken down and emptied from the stomach, causing unexpected, severe postprandial hypoglycemia ⁽²³⁾. When the stomach finally evacuates its contents hours later, the insulin effect has waned, resulting in severe, prolonged delayed hyperglycemia.



5.2 HbA1c Stabilization via Levosulpiride

By repairing the physical motility defect, levosulpiride breaks this feedback loop. By enforcing predictable, steady postprandial emptying, the appearance of glucose in the portal circulation is successfully synchronised with the pharmacodynamics of insulin or oral therapies ⁽²⁴⁾.

In a landmark, long-term 6-month double-blind clinical trial evaluating insulin-dependent diabetes mellitus (IDDM) patients with severe gastroparesis, Melga et al. (1997) documented that a continuous regimen of levosulpiride (25 mg t.i.d.) induced a profound, sustainable reduction in mean daily plasma glucose levels. Strikingly, the stabilized gastric emptying translated into a statistically significant reduction in glycated haemoglobin (HbA1c) values, dropping from a baseline of 6.7% pm 0.4% down to 5.7% pm 0.3% ($p < 0.05$) without an increase in hypoglycaemic events ⁽¹⁹⁾. This predictable kinetic alignment allows endocrinologists to safely down-titrate or optimize insulin dosing regimens, dramatically reducing overall glycemic variability.

6. Safety, Tolerability, and Neuro-Hormonal Interactions

While levosulpiride exhibits an excellent clinical track record, its distinct anti-dopaminergic mechanism dictates a specific, highly predictable profile of side effects that requires diligent clinical understanding.

6.1 Extrapyramidal Symptom (EPS) Risk Profile

Because levosulpiride blocks dopamine D2 receptors, it carries a potential risk of inducing extrapyramidal symptoms (EPS) if the drug crosses the blood-brain barrier in high concentrations or if used in highly susceptible patient populations ⁽²⁵⁾. Clinical presentations can include drug-induced

Parkinsonism (tremor, rigidity, bradykinesia), akathisia, acute dystonia, and very rarely, tardive dyskinesia ⁽²⁶⁾.

However, prospective epidemiological assessments reveal that at the low, targeted dose of 75 mg/day utilised for gastroenterological indications, the incidence of severe EPS is exceptionally low, particularly when compared to metoclopramide ⁽⁷⁾. Metoclopramide features high lipid solubility and unselective central penetration, whereas levosulpiride's highly polar molecular structure restricts substantial central accumulation ⁽¹⁰⁾. Nonetheless, caution must be exercised, and regular neurological monitoring is warranted when prescribing levosulpiride to elderly diabetic patients, who possess inherently vulnerable blood-brain barriers and decreased renal clearance pathways ⁽²⁶⁾.

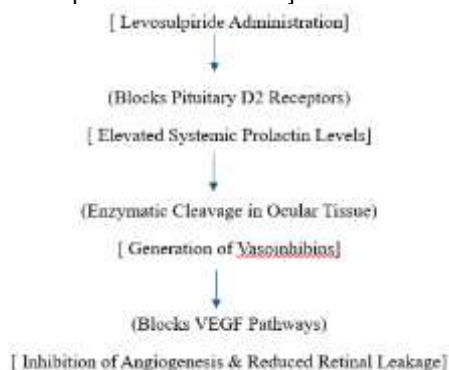
6.2 Hyperprolactinemia and the Prolactin/Vasoinhibin Axis

The most frequent endocrine side effect observed with levosulpiride therapy is hyperprolactinemia ⁽¹⁶⁾. Dopamine acts centrally as the primary prolactin-inhibiting factor via lactotroph receptors in the anterior pituitary gland, which sits outside the endothelial blood-brain barrier. Levosulpiride-mediated blockade leads to an uninhibited, rapid rise in systemic prolactin levels ⁽¹⁵⁾. Clinically, this can manifest as galactorrhea, breast engorgement, gynecomastia, and reversible menstrual irregularities ⁽¹⁶⁾. While these symptoms often resolve promptly upon drug down-titration or temporary cessation, this hyperprolactinemic pathway has sparked groundbreaking medical interest regarding microvascular protection.

The Prolactin/Vasoinhibin Hypothesis in Diabetic Retinopathy

Diabetic retinopathy (DR) and diabetic macular edema (DME) represent primary causes of irreversible blindness, driven by ischemia-induced upregulated vascular endothelial growth factor (VEGF), which leads to pathological intraocular angiogenesis and blood-retinal barrier breakdown ⁽²⁷⁾. Extensive research has established that systemic prolactin is enzymatically cleaved within ocular tissues by matrix metalloproteinases and cathepsin D into smaller, highly potent anti-angiogenic fragments called *vasoinhibins* ⁽²⁷⁾.

[Levosulpiride Administration]



Vasoinhibins directly bind to endothelial cell receptors, blocking VEGF-induced nitric oxide

production, halting endothelial cell proliferation, and closing leaky capillary tight junctions ⁽²⁷⁾. Consequently, the controlled elevation of systemic prolactin via low-dose levosulpiride is currently being leveraged in clinical designs as a novel, protective therapeutic strategy to suppress angiogenesis and reduce macular edema in patients suffering from advanced diabetic retinopathy ⁽²⁷⁾. This presents a unique clinical paradigm where a drug's endocrine side effect directly serves to mitigate a separate microvascular complication of the same disease state.

8. Clinical Recommendations and Future Directions

For maximum therapeutic benefit, levosulpiride should be initiated at a conservative oral dose of 25 mg three times daily, ingested strictly 30 minutes prior to breakfast, lunch, and dinner. This timing ensures that peak plasma concentrations (max) perfectly coincide with the entry of food into the stomach, maximizing coordinated antral contractions. If a patient presents with a severe acute exacerbation of gastroparesis where oral intake is completely compromised by unceasing vomiting, the therapy can be initiated via intravenous or intramuscular routes (25 mg b.i.d.) for 3 to 5 days until stabilization permits a transition back to oral tablets. Given its long-term excretion profile, dosage adjustments are mandatory in patients demonstrating stage 3 or greater chronic kidney disease (GFR < 60 mL/min/1.73 m²) to avoid systemic accumulation and reduce the risk of extrapyramidal complications. Future large-scale, multi-center, double-blind trials are currently focused on evaluating the combined long-term benefits of levosulpiride on both gastric physiological motility and the quantitative stabilization of diabetic retinopathy markers over a multi-year period.

9. Conclusion

Diabetic gastroparesis stands as a complex, multi-layered complication requiring a therapy that targets both mechanical delay and central sensory symptoms. Levosulpiride successfully achieves this standard through its synergistic dual mechanism of selective dopamine D2 receptor antagonism and serotonin 5-HT₄ receptor agonism. Extensive clinical evidence supports its ability to significantly alleviate upper gastrointestinal symptom burdens, decrease objective solid food gastric retention times, and dramatically improve overall postprandial glycaemic control—as evidenced by significant reductions in long-term HbA1c levels. Combined with a safer neurological profile than metoclopramide and lacking the prominent cardiotoxic threats of domperidone, levosulpiride represents a highly effective, evidence-based choice in the modern therapeutic management of diabetic gastroparesis.

7. Comparative Assessment: Levosulpiride vs. Conventional Prokinetics

To provide clear clinical guidance, it is essential to compare levosulpiride against alternative prokinetic choices currently available in modern clinical practice:

Drug Name	Primary Pharmacodynamic Mechanism	Clinical Superiority in DMGP	Major Clinical Bottlenecks & Side Effects	Regulatory Status & Safety Warnings
Levosulpiride	Selective D ₂ Antagonism + Moderate 5-HT ₄ Agonism	High antiemetic and antral motility potency; improves HbA1c curves; possible retinal benefits	Hyperprolactinemia (galactorrhea, gynecomastia); low EPS risk	Widely approved; caution in elderly and renal patients
Metoclopramide	Non-selective D ₂ Antagonism + 5-HT ₄ Agonism	Widely available; potent immediate antiemetic	High CNS penetration; tardive dyskinesia, depression, anxiety	FDA Black Box Warning; restrict use to ≤12 weeks
Domperidone	Peripheral D ₂ Receptor Antagonism	Minimal BBB crossing; very low EPS risk	Risk of cardiac ventricular arrhythmias and sudden cardiac death	EMA restrictions; limited dose and duration
Itopride	D ₂ Antagonism + Acetylcholinesterase (AChE) Inhibition	Minimal CNS penetration; no QT prolongation	Lower efficacy in severe gastroparesis-induced vomiting	Well tolerated; mainly used in mild-moderate dyspepsia
Cinitapride	5-HT ₄ Agonist + Moderate D ₂ Antagonism	Good neurological safety profile	Tachyphylaxis (reduced effect over time)	Approved in select European & Asian markets; secondary option

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Abbreviations

DG – Diabetic Gastroparesis
 ICC – Interstitial Cells of Cajal
 CNS – Central Nervous System
 5-HT₄ – 5-Hydroxytryptamine 4
 D₂ – Dopamine D₂ Receptor

GI – Gastrointestinal

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Review Article**Microbial Approaches for Bioremediation of Contaminated Environments: Industrial Perspectives****Preeti Yadav¹, Ravindra Kumar Jain²**

1. Research Scholar
2. Professor

Department of Life Sciences
Keral Verma Subharti College of Science
Swami Vivekanand Subharti University, Meerut. INDIA.

Abstract: Microorganisms play an indispensable role in numerous industrial sectors due to their remarkable metabolic diversity and adaptability. This review highlights the beneficial applications of microbes in industries such as pharmaceuticals, agriculture, environmental management, food processing, and bioenergy production. Industrial microbiology has evolved from traditional fermentation processes to advanced biotechnological innovations involving genetic engineering, metabolic pathway optimization, and synthetic biology. Microbes such as *Bacillus*, *Aspergillus*, *Saccharomyces*, *Pseudomonas*, and *Streptomyces* are utilized for large-scale production of enzymes, biofuels, antibiotics, organic acids, biofertilizers, and bioplastics. Furthermore, microbial bioremediation offers sustainable solutions for detoxifying industrial effluents and heavy metal contaminants. Recent advancements in omics technologies and bioinformatics have enhanced the identification and manipulation of microbial strains with industrial potential, promoting a circular and green bioeconomy. This paper provides a comprehensive overview of microbial applications in modern industries, emphasizing their contribution to sustainable development and environmental conservation.

Keywords:- Industrial microbiology, bioremediation, biofertilizers, microbial biotechnology, sustainable industry, bioeconomy

Address for correspondence: Prof. (Dr.) Ravindra Kumar Jain, Department of Life Sciences, Keral Verma Subharti College of Science, Swami Vivekanand Subharti University, Meerut. INDIA.

E-Mail: rkjbiotech20@gmail.com

Contact: +91-75006-26667

Introduction

Microbes are a diverse group of microscopic organisms, with bacteria representing the most extensively studied members. They play crucial roles across multiple domains, including agriculture, healthcare, environmental remediation, and industrial manufacturing. Their applications range from pollution mitigation to the production of enzymes, metabolites, and biosensors. This review focuses on compiling and analyzing information regarding microbial applications in contaminated water treatment, as well as their roles in the dairy, food, pharmaceutical, and cosmetic industries. Given that microbes represent primary sources of industrial enzymes, they are frequently cultured on a large scale and genetically modified by transferring target enzyme-encoding genes into well-characterized and manageable microbial hosts.

Industrial microorganisms, particularly environmental isolates, possess the inherent ability to synthesize enzymes when provided with appropriate nutrient media. Their rapid growth and ease of clonal expansion make them ideal candidates for large-scale applications. Historically, the first industrial exploitation of microbial enzymes was achieved by Dr. Jokichi Takamine, who derived them from fungi, followed by Boidin and Effront, who reported bacterial enzymes approximately two decades later. In industries such as food, dairy, and leather processing, microorganisms like *Saccharomyces cerevisiae* are directly applied to raw materials to

initiate fermentation processes. In contrast, the pharmaceutical industry often cultures microbes to harvest medically significant compounds such as amino acids and enzymes, which are subsequently purified through techniques including bio-affinity chromatography and other advanced separation methods.

In addition to industrial production, microbes play a pivotal role in environmental remediation, particularly through bioremediation strategies. Industrial effluents discharged into soil and water bodies can be effectively degraded using bacteria, algae, and fungi. For instance, a study conducted in Prabumulih, South Sumatra, Indonesia, examined the bioremediation of liquid petroleum waste, assessing both process efficiency and degradation rates. Similarly, *Pseudomonas putida* and *Bacillus cereus* have been shown to degrade crude oil, with *P. putida* achieving 60% degradation of petroleum hydrocarbons in water samples within seven days. These findings underscore their potential as reliable bioremediation tools for addressing oil-contaminated environments.

Microbes in Various Industries**Dairy Industry*****Lactococcus lactis***

Lactococcus lactis is a Gram-positive, non-motile, facultatively anaerobic bacterium, widely recognized as one of the most important lactic acid bacteria (LAB) in the dairy sector. Its industrial relevance lies in its ability to ferment lactose into lactic acid via the

hexose diphosphate pathway. The acidification process lowers the pH of milk, causing protein coagulation, which is a critical step in cheese production. Beyond coagulation, *L. lactis* contributes significantly to the development of flavor, aroma, and texture in a wide variety of cheeses. This bacterium thrives optimally at around 10 °C, but becomes immotile at temperatures exceeding 45 °C. It also demonstrates tolerance to acidic environments and saline conditions, making it highly adaptable for industrial fermentation. Together with *Streptococcus thermophilus*, *L. lactis* is used in yogurt production. Its applications extend beyond cheese and yogurt to fermented milk, sour cream, kefir, and butter. The widespread use of *L. lactis* in starter cultures makes it a cornerstone of dairy biotechnology ⁽¹⁾.

Streptococcus thermophilus

Streptococcus thermophilus is a Gram-positive, thermophilic lactic acid bacterium capable of fermenting lactose, sucrose, glucose, and fructose. It is widely applied in the production of yogurt, Swiss cheese, and hard Italian cheeses. Its resilience against bacteriophage infection is enhanced by the CRISPR-Cas system, although it remains relatively sensitive to antibiotics and sanitizers. Optimal growth occurs at 40–45°C, with growth slowing at 20–25°C and inhibited above 50°C. *S. thermophilus* plays a dual role in dairy fermentation: (i) it produces lactic acid, thereby lowering pH and suppressing pathogenic microbes, and (ii) it enhances sensory qualities such as flavor and texture. Additionally, it is recognized as a probiotic, contributing to gut health when consumed in fermented products ⁽²⁾.

Propionibacterium freudenreichii

Propionibacterium freudenreichii is a Gram-positive bacterium best known for its role in Swiss cheese fermentation. During lactate metabolism, it releases carbon dioxide, producing the characteristic “eyes” (holes) in Swiss cheese. Its metabolic activity generates flavor-enhancing compounds such as propionate, acetate, short-chain fatty acids from amino acid catabolism, and free fatty acids from milk fat hydrolysis. Beyond organoleptic contributions, *P. freudenreichii* synthesizes bioactive compounds including vitamin B12 and folate, improving the nutritional quality of dairy products. The antimicrobial action of propionic acid helps extend shelf life by inhibiting spoilage organisms. Its subspecies, *P. freudenreichii shermanii*, is highly resilient to environmental fluctuations and can grow at temperatures as low as 3.8 °C, making it ideal for cheese maturation ⁽³⁾.

Lactobacillus helveticus

Lactobacillus helveticus is a proteolytic lactic acid bacterium extensively used in the manufacture of hard and extra-hard cheeses such as Parmesan and Swiss varieties. Its proteolytic activity enhances cheese flavor and texture during ripening. Moreover, *L. helveticus* produces bioactive peptides with antioxidant, antihypertensive, and antimicrobial properties. These peptides contribute to the health-promoting effects of fermented dairy products, such as improved cardiovascular health and enhanced immune defense. In vitro studies have also shown its antagonistic action against pathogenic fungi and

bacteria, highlighting its role in natural food preservation.

Leather Industry

Microbes producing proteases and lipases play a crucial role in eco-friendly leather processing. They replace harsh chemicals in dehairing, degreasing, and bating, thus minimizing environmental pollution. Examples include *Bacillus subtilis*, which produces both proteases and lipases; *Bacillus licheniformis*, a major source of proteases; *Pseudomonas fluorescens*, known for thermolabile lipases; and *Aspergillus niger*, a fungal source of lipases used in leather treatment. These microbial enzymes improve leather quality by enhancing softness, flexibility, and appearance, while reducing chemical hazards in tannery operations.

Food Industry

Saccharomyces cerevisiae

Saccharomyces cerevisiae, a unicellular eukaryotic fungus with 16 chromosomes, is one of the most extensively studied eukaryotic organisms due to its genetic tractability. It is employed in baking and brewing industries to produce bread, beer, wine, and other alcoholic beverages. Fermentation by *S. cerevisiae* converts sugars into ethanol, carbon dioxide, and flavor compounds, imparting desirable sensory qualities to foods and beverages. In bread making, CO₂ production creates air pockets that give bread its soft, porous texture, while flavor compounds such as phenylethanol and δ-decalactone contribute to its aroma. Due to its high protein and vitamin B content, *S. cerevisiae* is also used as a nutritional supplement. Furthermore, its antimicrobial metabolites serve as natural preservatives in fruit juices, alcoholic beverages, and processed foods.

Lactobacillus plantarum

Lactobacillus plantarum is a versatile Gram-positive lactic acid bacterium found in fermented foods such as pickles, kimchi, sauerkraut, and sourdough. It is also abundant in the human gastrointestinal tract, where it exhibits probiotic properties. *L. plantarum* enhances food quality by improving antioxidant capacity, scavenging hydroxyl and oxygen radicals, and enhancing nutritional content through increased vitamin and amino acid availability. It is also used in food preservation; for example, when combined with chitosan, *L. plantarum* extends the shelf life of fresh-cut apples by reducing microbial spoilage. Its dual role in food quality improvement and human health promotion makes it a valuable microorganism in functional food development.

Lactobacillus acidophilus

Lactobacillus acidophilus is a Gram-positive, microaerophilic LAB frequently applied in the fermentation of vegetables, fish, sausages, and silage. In addition to imparting characteristic flavor and texture, it acts as a bio-preservative by producing lactic acid and bacteriocins that inhibit foodborne pathogens. *L. acidophilus* demonstrates strong acid tolerance, enabling survival in gastrointestinal conditions, and is widely consumed as a probiotic supplement. Its probiotic activity contributes to gut microbiome balance and overall human health.

Role of Microbes in Pollutant Removal Process

Degradation of Organic Pollutants

Microorganisms are widely recognized as effective bio-remediators owing to their ability to degrade a broad spectrum of organic pollutants. Degradation typically occurs through co-metabolism, where microbial communities in the rhizosphere of aquatic and terrestrial plants break down complex carbon-based compounds to acquire organic carbon and electron acceptors.

In aquatic systems, the rate of biodegradation depends on both the density of microbial populations and the concentration of xenobiotics present. The abundance and activity of these microbes are strongly influenced by the species of macrophytes present. Plant roots provide organic carbon that supports microbial degradation of pollutants such as hydrocarbons and aromatic hydrocarbons. Additionally, many rhizospheric bacteria produce indole acetic acid (IAA), which not only enhances plant growth but also supports pollutant degradation. Biofilms associated with aquatic plants play a particularly significant role in breaking down organic compounds, including phenolics, amines, aliphatic aldehydes, polychlorinated biphenyls (PCBs), and atrazine. Moreover, methanotrophic bacteria enriched in wetland rhizospheres utilize methane as a carbon and energy source. These methanotrophs are capable of degrading a wide range of hazardous organic contaminants, including chlorinated ethenes, through enzymatic reactions. For instance, *Eichhornia crassipes* has been shown to remediate eutrophic waters by stimulating nitrogen gas production, thereby contributing to both pollutant removal and ecosystem restoration.⁽⁴⁾

Removal of Heavy Metals

The rhizospheric and endophytic microbes (including bacteria, fungi, algae, and cyanobacteria) play a prominent role in the removal and detoxification of heavy metals (Table1). Microbes promote the removal of metals through biosorption, bioaccumulation, biotransformation, and

biomineralization processes. The microbial cell wall, rich in functional groups such as carboxyl, hydroxyl, and amino groups, can bind and immobilize toxic metal ions⁽⁵⁾.

In addition, some microbes enhance metal uptake in plants by increasing metal bioavailability in the rhizosphere. Specific metal-binding proteins and peptides, such as metallothioneins and phytochelatins, produced by microbes, help in the sequestration of heavy metals. Transcription factors regulating these proteins facilitate hormonal and redox signaling under metal stress conditions⁽⁶⁾.

Cyanobacteria such as *Anabaena spiroides* mitigate heavy metal toxicity by producing proteins that bind Cd, Cu, and Cr. **Fungi**, including *Aspergillus niger* and *Penicillium chrysogenum*, have strong biosorption capacities for Pb, Ni, and Zn. **Yeasts** like *Saccharomyces cerevisiae* accumulate Cu and Cd via intracellular sequestration⁽⁷⁾.

Genetically modified microbes further improve remediation potential. For instance, *Ralstonia eutropha* reduces Cd(II) toxicity by expressing metallothionein on its cell surface, while *Escherichia coli* regulates Cd stress by producing detoxification proteins. Mercury-resistant microbes such as *Pseudomonas aeruginosa* and *Bacillus cereus* contain the *mer operon*, which reduces toxic Hg²⁺ to less toxic elemental mercury.

Some endophytic microbes, including *Enterobacter cloacae* and *Bacillus licheniformis*, influence plant phenotypic and functional traits, enhancing antioxidant enzyme activities to combat oxidative stress caused by heavy metals. Certain fungi also detoxify metals via methylation and redox transformations, converting highly toxic forms into less toxic species⁽⁸⁾.

Thus, microbial interactions augment phytoremediation by supporting plant metal tolerance, producing stress-related proteins, and establishing active transport mechanisms such as efflux pumps to alleviate metal stress.

Table 1. Removal of Heavy Metals by Microbes

Microbe	Metal(s)	Reference
<i>Lactobacillus delbrueckii</i> , <i>Streptococcus thermophilus</i>	Fe, Zn	(09)
<i>Acinetobacter</i> sp., <i>Bacillus megaterium</i> , <i>Sphingobacterium</i> sp.	Fe, Mn	(10)
<i>Anoxybacillus flavithermus</i>	Fe, Cu	(11)
<i>Leptothrix</i> , <i>Pseudomonas</i> , <i>Hyphomicrobium</i> , <i>Planctomyces</i>	Mn	(12)
<i>Methylobacterium organophilum</i>	Cu, Pb	(13)
<i>Herminiimonas arsenicoxydans</i>	As	(14)
<i>Enterobacter cloacae</i>	Cd	(15)
<i>Acetobacter</i> sp.	Cu, Cr	(16)
<i>Chryseomonas luteola</i>	Pb, Cu, Mn	(17)
<i>Ochrobactrum anthropic</i>	Zn, Co	(18)
<i>Anabaena spiroides</i> (cyanobacterium)	Cd, Co, Cu, Ni	(19)
<i>Ralstonia solanacearum</i>	Cr, Cu	(20)
<i>Proteobacteria</i> and <i>Bacteroidetes</i>	Mn	(21)
<i>Bacillus cereus</i>	Pb	(22)
<i>Bacillus licheniformis</i>	Cu	(23)
<i>Saccharomyces cerevisiae</i> (yeast)	Cd, Cu	(24)
<i>Aspergillus niger</i> (fungus)	Pb, Zn	(25)
<i>Penicillium chrysogenum</i> (fungus)	Ni, Cd	(26)

Table2: Pathogen Risk and Health Concerns from Industrial Use of Microbes

Microbial Group / Species	Industrial Application	Potential Risk / Pathogenic Concern	Health or Environmental Impact	Preventive Measures / Biosafety Level
<i>Bacillus subtilis</i>	Enzyme and probiotic production	Generally regarded as safe (GRAS), but some strains may cause opportunistic infections in immunocompromised individuals	Rare allergic reactions or respiratory irritation	BSL-1; standard lab hygiene
<i>Aspergillus niger</i>	Citric acid and enzyme production	Certain strains can produce mycotoxins	Allergic reactions and respiratory issues upon exposure	BSL-1/2; dust control and PPE
<i>Pseudomonas aeruginosa</i>	Bioremediation and biosurfactant production	Opportunistic human pathogen	Infections in wounds, lungs (especially in cystic fibrosis patients)	BSL-2; strict containment and waste sterilization
<i>Escherichia coli</i> (non-pathogenic strains)	Recombinant protein and enzyme production	Risk of contamination with pathogenic strains (e.g., <i>E. coli</i> O157:H7)	Gastrointestinal illness if contamination occurs	BSL-1 for lab strains; BSL-2 for environmental strains
<i>Streptomyces spp.</i>	Antibiotic and enzyme production	Generally non-pathogenic, but can cause actinomycetoma in rare cases	Minor skin or respiratory infection	BSL-1; controlled air circulation
<i>Clostridium acetobutylicum</i>	Solvent (acetone-butanol) fermentation	Some <i>Clostridium</i> species are pathogenic (<i>C. botulinum</i>)	Neurotoxic risk if mishandled strains mix	BSL-2; anaerobic handling safety
<i>Saccharomyces cerevisiae</i>	Brewing, baking, and bioethanol production	Rare infections in immunocompromised individuals	Fungemia in susceptible patients	BSL-1; good air filtration
<i>Penicillium chrysogenum</i>	Antibiotic (penicillin) production	Spore inhalation may cause allergies or respiratory issues	Hypersensitivity reactions	BSL-1; controlled spore dispersion
<i>Mycobacterium smegmatis</i>	Model organism for bioremediation research	Non-pathogenic but related to <i>M. tuberculosis</i>	Minimal risk under controlled use	BSL-2; PPE and biosafety cabinet
<i>Rhodococcus erythropolis</i>	Hydrocarbon degradation	Generally safe, but misidentified strains may be opportunistic	Mild skin infection (rare)	BSL-1; periodic strain verification

Bioremediation Process

Bioremediation is a sustainable and eco-friendly approach for the detoxification of hazardous pollutants, including heavy metals, by converting them into less harmful forms or by completely eliminating the agents responsible for toxicity. The process involves the utilization of microbial biomass, either living or dead, to degrade, transform, or immobilize recalcitrant contaminants. Ultimately, these conversions result in the formation of stable, non-toxic end products such as carbon dioxide (CO₂), water (H₂O), nitrogen gas (N₂), and other benign compounds. Bioremediation specifically targets toxic substances with minimal adverse effects on the environment and living organisms, making it a cost-effective alternative to conventional remediation methods ⁽²⁷⁾.

For any successful bioremediation strategy, three essential components are required: microorganisms, nutrients, and energetics. In certain environmental

settings, contaminants themselves can serve as carbon and energy sources through microbial redox reactions. Under aerobic conditions, contaminants act as electron donors, while microorganisms utilize oxygen as the terminal electron acceptor. Conversely, in anaerobic environments, microbes degrade pollutants using alternative electron acceptors such as nitrate, sulfate or carbon dioxide ⁽²⁸⁾.

Based on the site of application, bioremediation can be broadly classified into *in situ* and *ex situ* processes.

- **In situ bioremediation** is conducted directly at the contaminated site without excavation. It involves the delivery of oxygen and nutrients into the polluted environment, thereby stimulating indigenous microbial communities to degrade contaminants. Techniques under this category include *bioventing*, *biosparging*, *bioslurping*, and *phytoremediation*. Two approaches exist within *in situ* bioremediation:

○ *Intrinsic bioremediation*, which relies on the natural attenuation potential of native microorganisms, enhanced through nutrient and oxygen supplementation.

○ *Engineered bioremediation*, which involves the deliberate introduction of specific microorganisms into the contaminated site to accelerate pollutant degradation.

• **Ex situ bioremediation**, on the other hand, requires removal of contaminated material from the site, followed by treatment under controlled conditions. Popular methods include *biopiles*, *windrows*, *bioreactors*, and *landfarming*, where microbial activity is optimized for efficient pollutant degradation^(29,13)

In the context of heavy metal pollution, bioremediation employs two key mechanisms: bioaccumulation and biosorption. Bioaccumulation is a metabolism-driven process in which living microbial cells actively transport and sequester metal ions within their intracellular compartments^[24]. In contrast, biosorption is a physicochemical process wherein dead or metabolically inactive biomass binds and concentrates heavy metals on cell surface structures such as polysaccharides, proteins, and lipids. Both processes are increasingly recognized as emerging tools for wastewater treatment due to their cost-effectiveness, environmental compatibility, and high removal efficiency⁽³⁰⁾.

Conclusion

Microorganisms play a central role in industrial processes, offering applications ranging from food fermentation, biofuel production, and pharmaceuticals to wastewater treatment and bioremediation. While their use has provided immense benefits to society, the large-scale deployment of microbes also raises ecological and health concerns. Certain industrial strains may contribute to the dissemination of antibiotic resistance, toxin production, or ecological imbalance if not carefully regulated. At the same time, microbial activity can also improve environmental quality by mitigating pollutants through natural or engineered pathways.

Bioremediation techniques, including bioaccumulation, biosorption, and microbial immobilization, have emerged as eco-friendly and cost-effective solutions for reducing the toxic impact of heavy metals and organic pollutants. In situ approaches such as bioventing, biosparging, and phytoremediation exploit native microbial communities, while *ex situ* methods such as biopiles, landfarming, and bioreactors provide controlled environments for optimized degradation. The success of these processes depends on selecting appropriate microbial strains, providing essential nutrients, and integrating innovative carriers for microbial immobilization.

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Review Article**Reduce E-waste through green computing virtualization****Rohan Saini¹, Sharvan Kumar Garg², Sanjive Tyagi³.****1. PG Student****2. Professor****3. Associate Professor****Computer Science and Engineering,
Subharti Institute of Technology & Engineering,
Swami Vivekanand Subharti University, Meerut.**

Abstract. Green computing encompasses a wide range of technologies, including virtualization, green data centers, cloud computing, grid computing, and power efficiency. This study focuses on the urgent need to reduce e-waste, which virtualization might help with. The goal of virtualization is to cut the total cost of expensive equipment; this not only makes the budget tighter but also heralds the e-product age. The purpose of this article is to illustrate the effects of e-waste, emphasize the significance of minimizing waste, and demonstrate how virtualization is crucial to attaining these goals. We'll also look at virtualization potential and key functions in environmentally friendly computing. The concept of virtualization has emerged as a result of technological advancements made to cut down on energy use and increase the number of data centers and virtual environments.

Keywords: Electronic waste; virtualization; recycle; energy optimization; green computing.

Address for correspondence: Rohan Saini. Department of Computer Science and Engineering Faculty of Engineering and Technology, Subhartipuram, NH-58, Meerut, 250002, UP, India.

E-Mail: rohansaini200027@gmail.com

Contact: +91-73510-64854

1. Introduction

Future changes to our daily lives will be greatly impacted by cloud computing, which is quickly emerging as a crucial information and communication technology. It is indicative of an emerging trend in computing, particularly given the increased focus on energy management across the board in the Information and Communication Technology (ICT) industry. Notably, the energy consumption of data centers has received a lot of attention lately ⁽¹⁾. This is because massive data centers are so common in a variety of businesses, which from an environmental standpoint can lead to the generation of e-waste and the discharge of hazardous gases and excess heat. Modern cloud-based systems use a lot of electricity and produce a lot of CO₂. The fact that many servers don't have effective cooling mechanisms makes this problem worse ⁽²⁾. The field of E-waste control within Green Computing has developed as a key and heavily explored area as a result of the increasing demand for enormous data storage and processing capabilities. In its quest to maximize different IT resources, cloud computing has offered ground-breaking techniques for reviving servers and data centers, thereby improving energy efficiency. However, it's critical to recognize that power consumption by IT resources is significant, which could result in energy shortages and affect the world's climate. With recycling emerging as an environmentally benign method, it is crucial to discover solutions that not only encourage energy conservation but also reduce operational and hardware expenses. Green IT is of utmost relevance for addressing environmental issues in the IT

industry ⁽³⁾. Its primary objective is to ensure that computing and energy consumption are as efficient as possible, with minimal impact on the environment ⁽⁴⁾. Its main goal is to maximize energy and processing efficiency while having the least possible negative environmental effects [4]. Green computing accomplishes this by using eco-friendly energy sources, lowering energy consumption during computing operations, optimizing resource usage during busy and idle periods, mitigating the negative effects of computing resources, reducing computing waste, and enabling a more energy-efficient use of computing power ⁽⁵⁾. The most recent virtualization methods add to these improvements. The technological components of green computing include virtualization, green data center technologies, cloud computing, grid computing, power optimization, and e-waste management. Through the process of virtualization, resources are effectively divided into one or more execution environments ⁽⁶⁾. The growing demand for capacity from users necessitates improved performance and capacity. Virtualization has revolutionized the IT environment, enabling businesses to optimize their hardware utilization and reduce expenses ⁽⁷⁾. A significant advantage of virtualization is its alignment with green computing principles. This paper focuses on the reduction of electronic waste and advocates for conserving overall hardware resources through virtualization. Not only is virtualization costly, but it also contributes to increased electronic waste and energy consumption in data centers.

2. Background

The importance of cloud computing has gained tremendous popularity amongst IT managers

throughout diverse groups ⁽¹⁾. earlier than the term "Cloud Computing," other standards like "Grid Computing" ⁽⁸⁾, "computer inside the Cloud" ⁽⁹⁾, and "Dreaming in the Cloud" ⁽¹⁰⁾ have been discussed within the enterprise. The cloud computing model provides usual and on-demand get right of entry to to a shared pool of configurable computing assets, which includes networks, garage, servers, programs, and services. these sources may be hastily allocated and launched with minimal control involvement or interaction with provider carriers ⁽¹¹⁾. the issue of power intake in facts era device has acquired increasing interest in latest years. there may be a growing attention of the need to efficaciously control strength intake in the course of the whole statistics and communications era (ICT) zone ⁽¹²⁾

2.1 History E-waste

E-waste management has a long history and has developed in reaction to the rising number of electronic devices and the corresponding increase in electronic trash. Here is a quick rundown of the significant turning points in the history of e-waste management 1970s-1980s: Early electronic gadgets and the development of personal computing contributed to the production of electronic garbage. However, there was little awareness of and regulation surrounding the disposal of electronic devices during this time.1990s: As the use of electronic gadgets increased, environmental concerns began to spread. Electronic waste transportation is governed under the Basel Convention on the Control of Transboundary Movements of Hazardous Wastes and their Disposal, which was established in 1989., contained rules governing the cross-border transportation of hazardous waste, including electronic waste. Early 2000s: ⁽¹²⁾ Due to the rapid improvements in technology and the rising popularity of consumer electronics, the production and disposal of electronic waste significantly increased in the early 2000s. It has been more well-known that incorrect e-waste disposal poses environmental and health risks.2003 saw the introduction of the Waste Electrical and Electronic Equipment (WEEE) Directive by the European Union. The framework for the appropriate handling, recycling, and recovery of electronic waste in member nations was established by this directive. The Electronic Waste Recycling Act was approved in the US in 2006, creating a framework for the collection and recycling of specific electronic waste items.2010s: Around the world, many nations and areas started to enact or reinforce laws relating to the management of electronic trash. Extended producer responsibility, which holds manufacturers accountable for the end-of-life disposal of their products, was the focus of numerous projects ⁽¹³⁾. This update sought to make the collecting, handling, and recycling of electronic waste even better.2020s: Ongoing initiatives to address electronic waste are still underway, with a growing focus on circular economy ideas, which encourage the reuse and recycling of electronic components ⁽¹⁴⁾.

3. Literature Review

3.1 E-waste :

E-waste, short for electronic waste, refers to discarded electrical or electronic devices and equipment. It encompasses all components and assemblies ⁽¹⁵⁾ that make up these devices, which are disposed of either due to obsolescence or at the end of their operational life.

3.2 E-waste affects humans and the environment.

Electronic trash (e-waste) management and disposal practices can have serious negative consequences on the environment and human health. Some of the major effects are as follows: Exposure to toxic chemicals: Hazardous compounds like lead, mercury, cadmium, and flame retardants called brominated are frequently found in e-waste. These compounds have the potential to pollute the food chain and expose people through eating when they leak into soil and water. respiratory issues E-waste burning or incineration emits toxic chemicals and heavy metals into the atmosphere ⁽¹⁶⁾.

3.3 Recycling E-waste

Recycling of electronic waste (e-waste) is a critical step towards reducing its environmental impact and conserving valuable resources. Here are the key aspects of e-waste recycling: Collection and Sorting: Collection Centres: E-waste can be collected through designated drop-off points,⁽¹⁷⁾ recycling centers, or scheduled collection events organized by municipalities or private entities. Sorting and Categorization: Once collected, e-waste is sorted into different categories based on the type of electronic equipment. This helps in streamlining the recycling process. Dismantling and Depollution: Dismantling: E-waste is disassembled into its constituent parts, including plastics, metals, and electronic components. This is typically done manually or through automated processes. Depollution: Hazardous substances such as batteries, capacitors, and mercury-containing components are safely removed to prevent environmental contamination. Material Recovery: Plastics: Plastics from e-waste are shredded, cleaned, and processed for reuse in manufacturing new products. Metals: Ferrous and non-ferrous metals like iron, aluminum, copper, and gold are extracted and sent to smelters for refining and reuse. -hand products or donated to communities in need. Secure Data Destruction: Data Wiping or Destruction: Before recycling, data stored on electronic devices is securely wiped or destroyed to protect sensitive information. Responsible Disposal: Proper Waste Management: Any residual waste that cannot be recycled is disposed of in an environmentally responsible manner, following local regulations and guidelines. Compliance and Regulation: Legal Compliance: E-waste recycling facilities must adhere to local and international regulations and standards to ensure safe and environmentally friendly practices ⁽¹⁸⁾. Education and Awareness: Public Awareness: Educating the public about the importance of responsible e-waste recycling helps encourage participation and compliance. Research and Innovation: Technological Advancements: Ongoing research aims to develop

more efficient recycling methods and technologies to improve the sustainability of e-waste management.

3.4 E-waste and Virtualization

By developing technologies that significantly reduce carbon emissions by drastically reducing energy usage, computing has achieved tremendous advancements in environmental conservation. N Computing has effectively reduced electricity consumption by 88 million kilowatts yearly when compared to an equivalent number of traditional PCs with over 500,000 virtual PC seats deployed across 70 countries. This accomplishment translates into a significant reduction of 55,000 metric tons of atmospheric carbon emissions. Therefore, virtualization is essential for reducing electronic waste and reducing the worldwide environmental effect of IT operations, resulting in a more sustainable environment.

3.5 Virtualization Support in Green Computing

Resource usage is revolutionized by virtualization, especially in terms of energy effectiveness. In essence, virtualization's main goal is to make it possible for a single hardware piece to perform the functions of numerous separate pieces. Different user interfaces isolate various hardware components, enabling each to function independently. Numerous operating systems and applications can run on fewer servers by utilizing virtual infrastructure. This results in a significant decrease in the overall energy consumption needed for data centers and the cooling systems that are connected to them. This equates to a remarkable potential for energy savings, which is estimated at 7,000 Kilowatt hours per year per server. As a result, virtualization becomes the best method for using green computing, particularly in data centre operations.⁽¹⁹⁾

3.6. Role of Types of Virtualization in E-waste Reduction

Manage the spun words as you want to reduce digital waste and promote green computing strategies for a sustainable and ff surroundings, several kinds of virtualization are essential. these answers effectively cut down on aid use and improve security. hardware virtualization (also referred to as server virtualization): This type lets in many running structures and applications to run concurrently on a single server, substantially decreasing the need for separate systems and related hardware. This method substantially reduces the manufacturing of electronic waste by way of decreasing the want for hardware and controlling power and cooling tactics. software program virtualization: This system includes setting up and running numerous virtual environments on a host computer. software virtualization effects in energy savings, price financial savings, lower emissions of dangerous gases, and reduced e-waste era thru a reduction in hardware and software needs and an improvement in task sharing.

3.7 Challenges and Appropriate Solutions

a) comprehensive management of Virtualization kinds: dealing with server resources in actual-time, dealing with switching in digital environments, designing and implementing networks, complex virtual machine troubleshooting, making sure

network guide for VM migration, addressing infrastructure problems, and supplying sturdy storage help for digital servers are among the challenges. solution: these issues can be resolved through implementing distributed digital switching mechanisms, imposing dynamic infrastructure control, and automating manual processes. this will enhance the effectiveness of virtualization and lower digital waste.

b) excessive power and strength intake: due to the increasing demands of statistics computation and applications, it's miles a challenging assignment to lessen strength consumption in cloud computing structures and statistics centers. To assure powerful functioning within predetermined periods, this will increase the want for disks and effective servers. by the usage of dependable and strength-efficient servers in place of regular cloud data facilities, excessive energy performance in cloud computing can be carried out. those servers use 5inner cooling answers that lessen the want for enthusiasts. this change is crucial because less strength used by inner servers' effects in extra savings for cooling systems and strength distribution in cloud facts centers.

3.8 Solutions to High Energy Consumption

•general cost of possession (TCO) Optimization: it is vital to lessen average energy dissipation inside these centers to reduce the TCO for cloud computing environments, which includes the energy charges of strolling a cloud facts centre. increasing strength supply capacity: it's essential to recollect the height energy capacity of energy resources for cloud facts facilities and to be privy to the restrictions of the energy transport community. the height energy draw on the server and information girdle levels is saved inside controllable bounds as a end result. Maximizing Cooling performance: Deploying laptop room aircon (CRAC) devices and air managing gadgets strategically is every other way to lessen strength fees related to cooling a cloud information centre. inside their heat exchanges, these machines use call for-pushed, variable frequency force (VFD) lovers. As a end result, various heat loads and variable airflow charges may be exactly matched. decreased strength use has a big effect on device availability, productiveness, and dependability in addition to considerable fee financial savings. moreover, it extensively contributes to defensive our environment by means of reducing the high energy costs and reducing the possibility of hardware gadget disasters delivered on via immoderate warmthness generation.

4. Result

The research was conducted in North Imani constituency, Meru County, Kenya, which is categorized as a developing nation. The study focused on four manufacturing companies in the area. A total of 20 employees participated in the research, with five employees selected from each company for the study. Among the five respondents from each company, one was specifically chosen from the Information and Communication Technology (ICT) department. Data analysis was performed using statistical software SPSS and MS Excel. All of the sampled respondents confirmed that their

respective companies utilize ICT in their business operations. However, the study also revealed that none of the sampled companies had an established E-waste management plan or policy in place. The respondents mentioned that outdated ICT equipment was either stored in a designated room referred to as an archive, used as a storage area for old computers, or disposed of along with other company waste through the county's garbage collection service. In addition, some respondents mentioned that the outdated ICT equipment was sorted and sold as scrap metal.

Let us consider total power consumed is denoted by E then,

$$E = \sum_{i=1}^n E_i \quad (1)$$

where, $E_i = N_w \cdot e_w + N_r \cdot e_r + e_s \quad (2)$

The number of tasks is considered as $i = 1$ to n and E_i is the energy consumption at each task i , therefore total energy consumption at all tasks is given by summation of E_i i.e. equation (1). Further E_i can be derived from

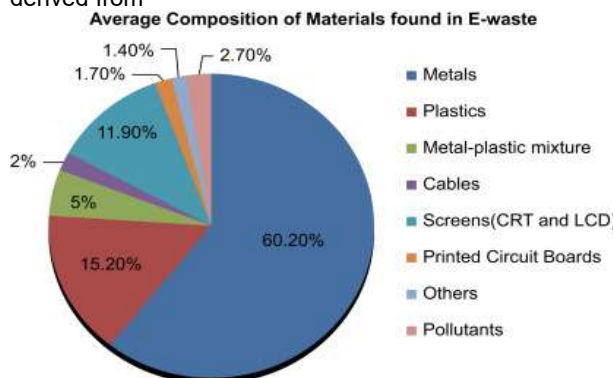


Figure 1: Average composition of materials found in E-waste

The data clearly illustrates that the predominant materials found in E-waste are Iron and steel, constituting 48% of the total. Plastics follow with 15%, while Copper, Aluminium, and Glass each make up 5% of the composition. Printed Circuit Boards account for 3%, and the least prevalent material is Rubber, standing at 1%. As depicted in Figure-1, the composition of E-waste encompasses a significant proportion of iron and steel, alongside aluminium, rubber, ceramic, copper, and various other chemically composed materials. This diversity presents challenges in the extraction and recycling process, as each material requires distinct methods for extraction. Heavier materials can be manually separated from discarded items, whereas smaller ones necessitate specialized equipment and attention. Moreover, the handling of chemically hazardous substances from electronic items demands the use of specific chemical deposition techniques to safeguard both handlers and the environment from any potential harm. The majority of electronic and electrical waste undergoes recycling through a system known as WEEE (Waste Electrical and Electronic Equipment). This system not only achieves a high recycling rate of 95-98% by weight but also ensures the thorough destruction of any remaining data on hard drives and memories. The manufacturing companies provided diverse explanations for their failure to segregate e-waste

before disposal. A significant majority, 92% of the respondents, did not perceive it as crucial to differentiate e-waste from regular waste before disposal. Additionally, 5% asserted that the absence of designated litter bins for separation was a contributing factor. Lastly, a smaller fraction, 3% of the respondents, demonstrated a general indifference towards the disposal process. This apathy is evident in their response.

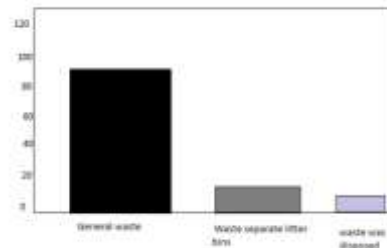


Figure 2: Reasons for lack of waste separation

The research findings revealed that none of the sampled manufacturing companies had implemented any environmental or e-waste policies, as depicted in Figures 2 and 3 below:

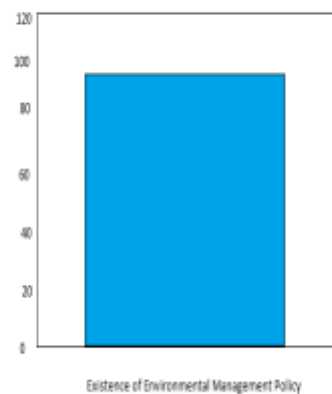


Figure 3: Existence of environmental management policy

E-waste is linked to a variety of negative health and environmental effects, including renal issues, brain damage, skin ulcers, lung cancer, birth deformities, and even mortality due to water and soil contamination. The study's findings, meanwhile, are troubling because only 25% of the respondents were able to name these harmful effects of e-waste. This lack of knowledge may be explained by the fact that only 10% of respondents were aware of what happened to the e-waste they dumped, and only 14% were concerned about what would happen to the e-waste. Among those who didn't show worry, 16% thought that e-waste had no substantial health or environmental repercussions, 8% felt it wasn't their responsibility, and the majority, 76% of the respondents, didn't care because e-waste carried no real potential for harm. These findings demonstrate the urgent need for efforts to raise public awareness of the negative effects of e-waste and the significance of responsible disposal methods. It also

emphasizes the significance of putting in place appropriate e-waste management policies and practices to lessen these negative effects on health and the environment.



Figure 4: Reasons for Caring

Among those individuals who expressed concern about the fate of the e-waste they disposed of daily, only 3% did so because they were aware of the harmful effects on the environment. Similarly, another 3% felt that it was harmful to human health, as depicted.



Figure 5: Reasons for Caring

This study highlights the intricate nature of electronic appliances, which comprise a multitude of materials that can be both toxic and valuable during their use before disposal. While the majority of materials, such as aluminium, iron, plastics, and glass, collectively make up more than 80% of E-waste by weight, precious and hazardous materials exist in smaller quantities but remain highly significant, as detailed by Thakur et al. (2016). Although the material composition of various appliances often exhibits similarities, the proportions of different components can vary widely. Precious metals like gold, silver, copper, platinum, etc., present opportunities for profitable E-waste recycling. Conversely, the responsible recycling of hazardous E-waste containing carcinogens such as lead and arsenic is imperative, as improper handling poses severe health and environmental risks. The volume of E-waste generated is rapidly increasing, exacerbated by illegal exports and inappropriate donations of electronic equipment, especially computers, from developed to developing countries. The utilization of electronic devices results in a range of environmental effects, primarily due to the emissions of gases, including carbon dioxide and other toxic substances. These effects give rise to significant health concerns, such as respiratory diseases, as well as broader environmental issues like global climate change and acid rain (Archana et al., 2011). Computers have an environmental footprint throughout their lifecycle, from manufacturing to use and disposal. While developed nations have established protocols for managing E-waste, many developing nations lack adequate systems. The adoption of green computing practices can help mitigate these adverse effects,

whether in developed or developing nations (Jaiswal et al., 2015). The study's findings reveal that only 25% of E-waste is formally recycled in dedicated recycling centers, often lacking sufficient worker protection. Moreover, over 80% of the respondents demonstrated insufficient knowledge about the E-waste recycling process. Nevertheless, 75% of the respondents expressed belief in the positive environmental impact of E-waste recycling. The study also identifies that 50% of the ICT staff consider green computing as a potential solution to E-waste management. Notable benefits of green computing include its environmentally friendly nature, energy-saving attributes, and the potential to reduce environmental pollution, thereby improving public health. However, while green computing offers numerous advantages, the study also identifies several challenges. These include the limited availability of green computing technology in local markets due to inadequate technological infrastructure, high initial and operational costs, and the difficulty of implementing green computing across organizations and society due to limited technological knowledge among users. To address these challenges, existing laws and policies must be updated to integrate technological advancements in green computing, especially in manufacturing and SMEs. Furthermore, companies should establish policies that promote the use of green computing for E-waste management devices and delivering short- and long-term benefits to organizations. Through the adoption of public cloud data centre services, manufacturing companies can lower IT costs and environmental impact. The government can play a role in facilitating E-waste collection. Additionally, community members should receive training to understand the benefits of green computing. Overall, the adoption of green computing can result in cost savings, energy conservation, reduced environmental impact, and enhanced environmental sustainability. Cloud computing and virtualization strategies can significantly enhance green computing practices in small and medium-sized manufacturing enterprises. Virtualization, in particular, enables the creation of software equivalents to hardware, reducing reliance on electronic y organizing special collection events, establishing permanent drop-off sites, utilizing retail stores, and implementing scheduled pick-ups from E-waste generating companies. Additionally, open-source cloud solutions can be tailored to specific manufacturing and user requirements, minimizing dependence on electronic devices and promoting green computing practices. In summary, when coupled with virtualization and cloud solutions, green computing holds significant potential for mitigating environmental impact and advancing sustainability within the manufacturing sector.

4. Conclusion

This paper delves into the escalating demand for electronic devices within manufacturing and small to medium-scale enterprises, shedding light on the resulting surge in electronic waste generation. It also explores viable measures to mitigate the adverse impacts of this electronic waste. The management of electronic waste and the adoption of green computing technologies are indeed important

strategies to mitigate the environmental impact of the IT industry. However, your provided text appears to be a segment of text discussing this topic and does not seem to be a question or request for specific information. If you have any specific questions or need information related to electronic waste management, green computing, or any other topic, please feel free to ask, and I'll be happy to provide you with relevant information or help with your inquiries., holding significant potential to curb the environmental and human impacts of electronic waste in manufacturing industries, especially in developing nations. While green computing shows promise in reducing electronic waste," Further research is needed to promote the extensive implementation of cloud and virtualization solutions in the manufacturing sector across developing regions of Africa. These endeavours have the potential to generate significant advantages for both the environment and the economy."

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Inclusive Review Article

Unveiling the Multifunctional Roles of Orientin and Vicenin in *Ocimum sanctum*: "An Inclusive Review"

Shagun Sharma¹, Ashwani Kumar²

1. UG Scholar,
2. Professor & Head.
Department of Life Sciences, Keral Verma Subharti
College of Science, Swami Vivekanand Subharti
University, Meerut (250005) Uttar Pradesh, India

Abstract: *Ocimum sanctum* Linn. commonly known as Holy Basil or Tulsi, is revered in traditional medicine for its diverse therapeutic properties, much of which is attributed to its unique profile of C-glycosyl flavonoids, specifically orientin and vicenin. Unlike more common O-glycosides, the C-C bond in these molecules imparts superior chemical stability and distinct pharmacokinetic behaviour. This review provides a comprehensive synthesis of current knowledge regarding the multifunctional pharmacological roles of orientin and vicenin. We explore their robust antioxidant mechanisms, including ROS scavenging and metal chelation, and their anti-inflammatory effects mediated through the inhibition of pro-inflammatory cytokines and the NF-kappaB pathway. Furthermore, the paper evaluates their significant radioprotective, anticancer, neuroprotective, and cardioprotective activities, supported by both *in vitro* and *in vivo* evidence. A critical focus is placed on the synergistic interactions between these flavonoids and other *O. sanctum* phytochemicals, such as eugenol and ursolic acid, which enhance the overall efficacy of the whole plant. The review also discusses the transition of these compounds into biotechnological and industrial applications, highlighting their potential in nutraceuticals, cosmetics, and food preservation. Finally, we address the current challenges in bioavailability and the emerging role of nanotechnology (e.g., liposomes and nanocarriers) in overcoming these barriers. By identifying gaps in current clinical research, this review outlines future prospects for orientin and vicenin as potent therapeutic lead molecules in the management of chronic and oxidative stress-related diseases.

Keywords: Orientin, Vicenin, *Ocimum sanctum* (Holy Basil), C-Glycosyl Flavonoids, Antioxidant Activity, Radioprotection, Synergy, Bioavailability, Pharmacology, Drug Delivery Systems

Address for correspondence: Shagun Sharma, BSc Biotechnology final year, Department of Life Sciences, Keral Verma Subharti College of Science, Swami Vivekanand Subharti University, Meerut (250005) Uttar Pradesh, India

E-Mail: shaguns895@gmail.com

Contact: +91-75002-46071

1. Introduction-

Overview Of *Ocimum Sanctum*:

Botany and Ethnomedicinal Significance

Ocimum sanctum Linn., commonly referred to as Holy Basil or "Tulsi," is a perennial aromatic herb belonging to the Lamiaceae family. Historically native to the Indian subcontinent and widely distributed across Southeast Asia, it is often described as the "Queen of Herbs" due to its preeminent status in Ayurvedic and Siddha medicine ⁽¹⁾. Botanically, *O. sanctum* is characterized by its square stems, simple opposite decussate leaves, and small purple-to-reddish flowers arranged in cylindrical spikes ⁽²⁾. Ethnomedicinally, the plant is considered a "Rasayana" or an elixir of life, traditionally used to treat a vast array of ailments including cough, influenza, fever, bronchitis, and hepatic disorders ⁽³⁾. Its adaptogenic properties are particularly revered, as it is believed to enhance the body's ability to maintain homeostasis amidst physical, chemical, and psychological stressors ⁽⁴⁾.

1.2 Importance of Flavonoids in Medicinal Plant

Flavonoids represent a diverse group of plant secondary metabolites characterized by a polyphenolic structure (C6-C3-C6). These compounds serve as an indispensable component of the plant's defence system, protecting against biotic

stresses like pathogens and abiotic factors such as UV radiation ⁽⁵⁾. In the context of human health, flavonoids are highly valued for their ability to modulate key cellular enzymes and signalling pathways. Their therapeutic potential spans across antioxidant, anti-inflammatory, anti-mutagenic, and anti-carcinogenic domains, largely attributed to their capacity to scavenge reactive oxygen species (ROS) and inhibit pro-inflammatory transcription factors like NF- κ B ⁽⁶⁾.

1.3 Orientin and Vicenin: Key Bioactive Flavonoid C- Glycosides

Among the myriads of phytochemicals found in *O. sanctum*, the flavonoids Orientin (8-C- β -D-glucopyranosyl luteolin) and Vicenin (6,8-di-C-glucosylapigenin) stand out as major bioactive markers ⁽⁷⁾. Unlike common O-glycosides, these are C-glycosyl flavonoids where the sugar moiety is directly bonded to the carbon atom of the flavonoid nucleus. This unique C-C linkage provides them with superior stability against enzymatic and acid hydrolysis in the gastrointestinal tract, potentially enhancing their biological half-life and therapeutic efficacy ⁽⁸⁾. Orientin and Vicenin have been identified as the primary water-soluble components responsible for the plant's remarkable cellular defence mechanisms, particularly in shielding

genetic material from oxidative and radiation damage⁽⁹⁾.

Despite the extensive traditional use of Tulsi, modern pharmacological interest has shifted towards isolating and understanding the specific molecular mechanisms of its individual constituents. Orientin and Vicenin have demonstrated exceptional multifunctional roles—most notably as potent radioprotectants—yet the research remains scattered across various toxicological and pharmacological studies. There is a critical need to synthesize this data to evaluate their potential as standardized therapeutic agents or as scaffolds for drug development. Furthermore, given the rising global interest in natural radioprotective agents and non-toxic antioxidants, a comprehensive review of these two C-glycosides is timely to bridge the gap between traditional ethnomedicine and modern clinical application.

The primary objective of this review is to provide an inclusive synthesis of the multifunctional biological roles of Orientin and Vicenin derived from *O. sanctum*. The scope includes: Analyzing their phytochemical properties and structural stability as C-glycosides. Reviewing their radioprotective, antioxidant, and anti-inflammatory mechanisms. Discussing their emerging roles in cancer management and neuroprotection. Highlighting the current challenges in their bioavailability and future directions for pharmaceutical formulation.

2. Phytochemistry of Orientin and Vicenin

2.1 Chemical Structure and Classification

Orientin and Vicenin belong to the specific subclass of flavonoids known as flavone C-glycosides. Unlike the more common O-glycosides, where the sugar moiety is attached via an oxygen atom (C-O-C bond), C-glycosides feature a direct covalent bond between the anomeric carbon of the sugar and the carbon atom of the flavonoid nucleus (C-C bond). This C-glycosidic linkage is significantly more resistant to acid and enzymatic hydrolysis compared to the O-glycosidic bond, which typically requires specialized gut microbiota or specific enzymes like beta-glucosidase for cleavage⁽¹⁰⁾. In *Ocimum sanctum*, these compounds are classified as water-soluble polyphenols, contributing to the high antioxidant capacity of the plant's aqueous extracts⁽¹¹⁾.

2.2 Comparison of Orientin Vs. Vicenin

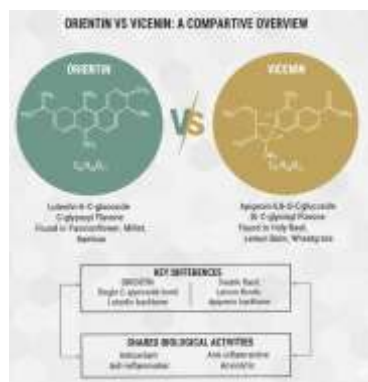


Fig.1. Overview on comparison of orientin and vicenin

While both compounds share a flavone backbone, they differ in their aglycone structure and the degree of glycosylation.

- **Orientin (Luteolin-8-C-glycoside):** Orientin consists of the aglycone luteolin (5,7,3,4-tetrahydroxyflavone) with a single glucose molecule attached at the C-8 position. Its molecular formula is C₂₁H₂₀O₁₁ with a molecular weight of approximately 448.38 g/mol⁽¹²⁾. The presence of the ortho-dihydroxyl (catechol) group on the B-ring is a distinguishing feature that enhances its radical scavenging ability compared to non-catechol flavonoids⁽¹³⁾.

- **Vicenin-2 (Apigenin-6,8-di-C-glycoside):** Vicenin, specifically the Vicenin-2 isomer commonly found in *O. sanctum*, is derived from the aglycone apigenin (5,7,4-trihydroxyflavone). Unlike Orientin, Vicenin-2 is a di-C-glycoside, featuring two glucose moieties attached at both the C-6 and C-8 positions of the A-ring⁽¹⁴⁾. Its molecular formula is C₂₇H₃₀O₁₅ with a higher molecular weight of 594.52 g/mol.

Table 1. Comparative Structural and Chemical Features of Orientin and Vicenin-2

Feature	Orientin	Vicenin-2
Aglycone	Luteolin	Apigenin
Glycosylation	Mono-C-glycoside (C-8)	Di-C-glycoside (C-6, C-8)
B-Ring Hydroxyls	3', 4' (Catechol group)	4' only
Molecular Weight	448.38 g/mol	594.52 g/mol

The isomerism between these compounds and their respective "iso" forms (such as Is orientin, where the sugar is at C-6) is a critical factor in their chromatographic separation and biological potency. Research indicates that the dual glycosylation in Vicenin-2 may provide even greater metabolic stability than mono-glycosyl forms, while the catechol structure of Orientin makes it a more potent antioxidant in lipid-rich environments.

2.3 Distribution in *Ocimum sanctum*

Localization (Leaves, Stem, Roots, and Seeds)



Fig.2 Phytochemicals distribution in different parts of *Ocimum sanctum*

In *Ocimum sanctum*, the distribution of secondary metabolites is highly organ -specific, with the most significant concentrations of Orientin and Vicenin localized in the leaves. Quantitative analyses have

consistently identified these C-glycosyl flavonoids as the primary water-soluble bioactive markers of the leaf extract, where they function as UV-protectants and chemical defenses⁽¹⁵⁾. While the stems also contain detectable levels of these flavonoids, the concentrations are markedly lower than those found in the foliage⁽¹⁶⁾. Conversely, the roots of *O. sanctum* are primarily characterized by the presence of triterpenes and sitosterol rather than flavonoid glycosides.⁽¹⁷⁾ The seeds are unique in their phytochemical profile; while they lack significant amounts of Orientin and Vicenin, they are an abundant source of fixed oils (18–22%) and fatty acids, such as linoleic and linolenic acids, which contribute to the plant's anti-inflammatory properties through separate pathways⁽¹⁸⁾.

Seasonal and Varietal Variation

The content of Orientin and Vicenin in *O. sanctum* exhibits significant variability based on the plant variety and the season of harvest. Traditionally, two main morphotypes are recognized: Rama Tulsi (green leaves) and Krishna Tulsi (purple leaves). Comparative studies have shown that Krishna Tulsi often possesses a higher density of phenolic compounds and flavonoids, including Orientin and Vicenin, which may correlate with its more potent medicinal reputation in Ayurvedic texts^(19, 20).

Seasonality also plays a pivotal role in metabolite accumulation. It has been observed that the concentration of these flavonoids' peaks during the post-monsoon and early winter months, coinciding with the plant's peak flowering stage. During the hot summer months, the metabolic shift toward essential oil production (like eugenol) often results in a relative decrease in the concentration of aqueous flavonoids like Orientin and Vicenin.

Effect of Cultivation Conditions on Metabolite Levels

The synthesis of Orientin and Vicenin is an adaptive response to environmental factors, meaning cultivation conditions directly impact their yield.

- **Light Intensity:** As these flavonoids serve as photoprotective agents, plants grown in open, sun-drenched environments typically exhibit higher flavonoid levels compared to those in shaded areas⁽²¹⁾.

- **Soil and Edaphic Factors:** The availability of nutrients and soil pH significantly influences the biosynthetic pathways of polyphenols. Geographic variables and "edaphic factors" (soil-related) are responsible for the varying chemical "chemotypes" observed across different regions of India and Southeast Asia^(22, 23).

Table.2 Factors affecting Orientin and vicenin content in *Ocimum sanctum*

Factor	Effect on Orientin/Vicenin Content
Plant Part	Highest in Leaves > Stem > Roots/Seeds
Variety	Higher in Krishna Tulsi (Purple) than Rama Tulsi (Green)
Season	Peak during Post-monsoon/Early Winter
Environment	Increases with UV/Sunlight exposure

- **Stress Induction:** Minor environmental stressors, such as slight water deficit or UV-B exposure, have been shown to upregulate the expression of C-glycosyl flavonoids as part of the plant's antioxidant defense system

3. Extraction, Isolation and Analytical Techniques

The efficiency of recovering bioactive flavonoids like Orientin and Vicenin from *Ocimum sanctum* is heavily dependent on the extraction methodology employed. Because these compounds are C-glycosides, they possess a higher degree of polarity and thermal stability compared to their aglycone counterparts, necessitating specific solvent systems and energy-assisted techniques to break the plant cell wall and facilitate their release into the matrix⁽²⁴⁾.

3.1 Extraction Approaches

Conventional Solvent Extraction

Traditionally, the extraction of Orientin and Vicenin from *O. sanctum* has relied on maceration, Soxhlet extraction, and refluxing. Given the polar nature of C-glycosyl flavones, water and aqueous-alcoholic mixtures (ethanol or methanol) are the most effective solvents⁽²⁵⁾. Research has demonstrated that aqueous extracts typically yield the highest concentrations of these specific flavonoids, which aligns with the traditional Ayurvedic preparation of "Tulsi tea" or decoctions⁽²⁶⁾. However, conventional methods are often criticized for their high solvent consumption, long extraction times, and the risk of degradation of other thermolabile co-constituents due to prolonged heat exposure^(27,28).

Green Extraction Approaches

To overcome the limitations of traditional methods, "Green Chemistry" protocols have been increasingly applied to *O. sanctum* to maximize the recovery of Orientin and Vicenin while reducing environmental impact.

- **Ultrasound-Assisted Extraction (UAE):** UAE utilizes acoustic cavitation to create micro-fractures in the leaf tissue, significantly enhancing the mass transfer of Orientin and Vicenin into the solvent. Studies indicate that UAE can reduce extraction time from several hours to minutes while maintaining the integrity of the C-glycosidic bond^(29,30).

- **Microwave-Assisted Extraction (MAE):** MAE employs microwave energy to heat the moisture within the plant cells, causing internal pressure that ruptures the cell walls. This method is particularly effective for polar compounds like Vicenin-2, as the localized heating promotes rapid solubility in aqueous solvents^(31,32).

- **Supercritical Fluid Extraction (SFE):** While SFE (usually with CO₂) is typically used for non-polar essential oils like eugenol, the addition of polar co-solvents (modifiers) such as ethanol allows for the selective isolation of flavonoids. SFE is favored for producing high-purity extracts free of toxic solvent residues, which is essential for pharmaceutical-grade Orientin and Vicenin isolation⁽³³⁾.

- **Enzyme-Assisted Extraction:** Recent advancements include the use of cellulases and pectinases to pre-treat *O. sanctum* leaves, which breaks down the structural polysaccharides and

releases bound Orientin and Vicenin, further increasing the total phenolic yield⁽³⁴⁾.

Table.3 comparison of conventional and advanced extraction techniques

Method	Advantages	Disadvantages
Conventional (Maceration)	Simple, no specialized equipment	Time-consuming, low yield
UAE	High efficiency, low temperature	Scale-up challenges
MAE	Very fast, high recovery	Potential thermal degradation
SFE	Solvent-free, high purity	High equipment cost

3.2 Isolation and Purification

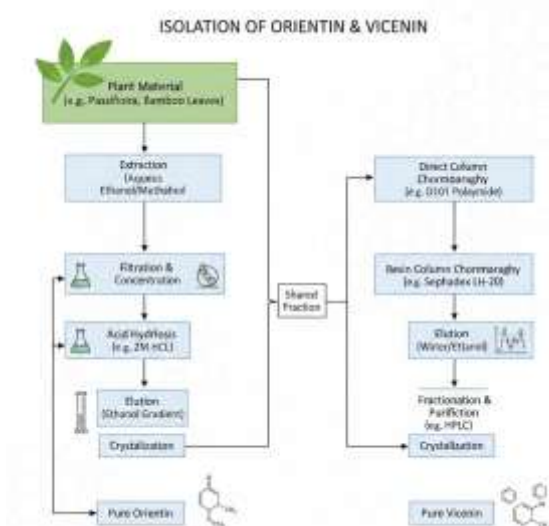


Fig.3 isolation of orientin and vicenin

The isolation of Orientin and Vicenin from the complex matrix of *Ocimum sanctum* leaves is primarily achieved through polarity-based separation techniques. Given their nature as polar C-glycosides, the initial crude extraction is typically performed using water or aqueous-alcoholic mixtures (methanol or ethanol). Column Chromatography(CC) serves as the foundational technique for preliminary purification, often utilizing stationary phases like silica gel or Sephadex LH-20 to separate the flavonoids based on molecular size and polarity⁽³⁴⁾. However, because Orientin and Vicenin have very similar structures, high-resolution techniques are required for complete isolation. Preparative Thin-Layer Chromatography (PTLC) is frequently employed for small-scale isolation, allowing researchers to visualize and scrape the specific bands corresponding to these flavonoids⁽³⁵⁾. For higher throughput and purity, Flash Chromatography and High-Performance Liquid Chromatography (HPLC) are the preferred methods. Flash chromatography offers a faster alternative to traditional CC by using pressurized air to move the

solvent through the column, which is effective for the rapid fractionation of *O. sanctum* extracts⁽³⁶⁾. Preparative HPLC remains the gold standard for obtaining high-purity Orientin and Vicenin (95% or higher) by utilizing specialized C18 reverse-phase columns and gradient elution systems, typically involving water (with 0.1% formic acid) and acetonitrile⁽³⁷⁾.

3.3 Quantification and Characterization

The structural confirmation and quantitative estimation of Orientin and Vicenin require sophisticated analytical instrumentation.

• **Quantification (HPLC, UHPLC, and LC-MS/MS):** Standard HPLC coupled with UV-Visible or Photodiode Array (PDA) detectors at wavelengths between 340 nm and 360 nm is the most common method for routine quantification. To achieve faster analysis and better resolution, Ultra-High Performance Liquid Chromatography (UHPLC) is increasingly utilized, significantly reducing solvent consumption and analysis time. For ultra-trace detection and complex mixture analysis, Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) is the definitive tool. LC-MS/MS allows for the identification of these compounds based on their specific mass-to-charge (m/z) ratios and characteristic fragmentation patterns, such as the loss of sugar moieties in C-glycosides.

• **Structural Characterization (NMR and FT-IR):** To confirm the C-glycosidic linkage and the specific attachment points (C-8 for Orientin; C-6 and C-8 for Vicenin-2), Nuclear Magnetic Resonance (NMR) spectroscopy is essential. both ¹H and ¹³C NMR are used to map the flavonoid backbone and the sugar protons, providing unambiguous evidence of the carbon-carbon bond. Additionally, Fourier-Transform Infrared (FT-IR) spectroscopy is used to identify functional groups, such as the hydroxyl (-OH) and carbonyl (C=O) groups, which produce distinct vibrational frequencies in the fingerprint region.

3.4 Method Validation Parameters

To ensure the reliability of the quantification methods, researchers must adhere to strict validation parameters as per ICH (International Council for Harmonisation) guidelines. These include Linearity, ensuring the detector response is proportional to concentration; Precision (repeatability and intermediate precision); and Accuracy, often measured through recovery studies where known amounts of standards are added to the extract. The Limit of Detection (LOD) and Limit of Quantification (LOQ) are also critical for defining the sensitivity of the method, particularly when measuring Orientin and Vicenin in varying *O. sanctum* chemotypes or seasonal samples.

4. Pharmacological Roles of Orientin and Vicenin

4.1 Antioxidant Activity

Orientin and vicenin, two potent C-glycosyl flavonoids primarily isolated from *Ocimum sanctum* (Holy Basil), exhibit significant pharmacological potential due to their robust antioxidant properties. Their antioxidant mechanisms are multifaceted, involving the direct scavenging of reactive oxygen species (ROS), the chelation of pro-oxidant metal ions, and the modulation of endogenous antioxidant defense systems. Structurally, the presence of

multiple phenolic hydroxyl groups allows these compounds to donate hydrogen atoms to free radicals, effectively terminating lipid peroxidation chain reactions and preventing oxidative damage to macromolecules such as DNA and proteins ⁽³⁸⁾.

Mechanistic Insights: ROS Scavenging and Metal Chelation

In vitro studies have consistently demonstrated the capacity of orientin and vicienin to neutralize a broad spectrum of free radicals. They show high efficacy in scavenging hydroxyl radicals OH[•], superoxide anions O₂^{•-}, and DPPH radicals. For instance, both flavonoids significantly inhibit the formation of hydroxyl radicals generated via the Fenton reaction, a process further enhanced by their ability to sequester iron ions, thereby preventing the initiation of metal-catalysed oxidation ⁽³⁹⁾. This metal chelation is a critical preventive mechanism that reduces the bioavailability of transition metals that otherwise promote the generation of highly reactive species ⁽⁴⁰⁾.

In Vivo Findings and Enzyme Activation

The antioxidant efficacy of these flavonoids extends to in vivo models, where they have been shown to bolster the body's natural defence mechanisms. In aged mice models, administration of orientin significantly elevated the activities of essential antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-PPX) in the serum, brain, and liver ⁽⁴¹⁾. Concurrently, levels of malondialdehyde (MDA), a hallmark biomarker of lipid peroxidation, were markedly reduced, indicating a systemic reduction in oxidative stress.

Furthermore, orientin and vicienin have been extensively studied as radioprotectants. Their ability to scavenge radiation-induced free radicals protects hematopoietic and chromosomal structures in mice ⁽⁴²⁾. By modulating signalling pathways such as Nrf2 (nuclear factor erythroid 2-related factor 2), orientin promotes the transcription of antioxidant-related genes, thereby enhancing cellular resilience against environmental and metabolic oxidative insults.

4.2 Anti-inflammatory and Immunomodulatory Effects

Orientin and vicienin exhibit potent anti-inflammatory properties primarily by suppressing the production and activity of key pro-inflammatory mediators. Studies have shown that these flavonoids significantly inhibit the expression of cyclooxygenase-2 (COX-2) and the secretion of pro-inflammatory cytokines, including tumour necrosis factor-alpha (TNF-alpha) and interleukin-6 (IL-6) ⁽⁴³⁾. The immunomodulatory mechanism is largely attributed to the inhibition of the Nuclear Factor-kappa B (NF-kappa B) signalling pathway, which prevents the translocation of inflammatory transcription factors into the nucleus. Additionally, vicienin-2 has been identified to target the CaMKbeta-AMPK-SIRT1 axis, which restores cellular homeostasis in lipopolysaccharide (LPS)-stressed immune cells⁽⁴⁴⁾. By modulating these pathways, these compounds reduce systemic inflammation and prevent the overactivation of the immune system, suggesting potential therapeutic use in chronic inflammatory diseases.

4.3 Anticancer Properties

The anticancer potential of orientin and vicienin is characterized by their ability to inhibit the proliferation of various cancer cell lines, including breast, colon, and liver cancer. These compounds exert their effects through the induction of apoptosis via the activation of caspases and the modulation of the Bcl-2/Bax protein ratio. Furthermore, orientin has been observed to induce cell cycle arrest, typically at the G2/M phase, thereby halting the uncontrolled division of malignant cells ⁽⁴⁵⁾. Beyond primary tumour growth, these flavonoids exhibit anti-metastatic effects by downregulating matrix metalloproteinases (MMPs), which are essential for cancer cell invasion and migration to distant organs.

4.4 Antimicrobial and Antiviral Activities

Orientin and vicienin demonstrate significant effectiveness against a broad spectrum of pathogens, including Gram-positive and Gram-negative bacteria, fungi, and specific viruses. Their antimicrobial mode of action involves the disruption of microbial cell membranes, leading to the leakage of intracellular contents and eventual cell death ⁽⁴⁶⁾. In antiviral contexts, these compounds interfere with viral replication pathways and hinder the attachment of viruses to host cell receptors. Their ability to inhibit viral enzymes makes them promising candidates for treating respiratory and enteric viral infections ⁽⁴⁷⁾.

4.5 Cardioprotective and Neuroprotective Roles

The cardioprotective and neuroprotective roles of orientin and vicienin are deeply rooted in their ability to mitigate oxidative stress-mediated damage. In cardiac tissues, they protect cardiomyocytes from ischemia-reperfusion injury by maintaining mitochondrial integrity and reducing lipid peroxidation. Naturally, these flavonoids cross the blood-brain barrier to shield neurons from neurotoxic insults, such as A beta-induced toxicity in Alzheimer's models. By reducing neuroinflammation and preventing neuronal apoptosis, they help preserve cognitive functions and slow the progression of neurodegenerative disorders ⁽⁴⁸⁾.

4.6 Metabolic Health Benefits

In the realm of metabolic health, orientin and vicienin offer significant anti-diabetic and anti-obesity benefits. They act as potent inhibitors of alpha-glucosidase and alpha-amylase, the enzymes responsible for carbohydrate digestion, thereby reducing postprandial blood glucose levels ⁽⁴⁹⁾. Furthermore, they improve insulin sensitization by activating the GLUT4 translocation pathway in skeletal muscles. Their anti-obesity effects are mediated through the modulation of lipid profiles, where they inhibit adipogenesis and promote the breakdown of stored lipids, leading to a reduction in total cholesterol and LDL levels.

4.7 Radioprotective Activity

One of the most unique pharmacological roles of orientin and vicienin is their significant radioprotective activity. Evidence from both cellular and animal studies indicates that these flavonoids protect against radiation-induced oxidative stress by rapidly scavenging free radicals generated by ionizing radiation. In vivo studies on mice have shown that administration of these compounds prior to radiation exposure significantly reduces chromosomal damage and bone marrow toxicity. Their ability to

enhance the repair of radiation-induced DNA strand breaks makes them valuable as adjuvant therapies during radiotherapy to protect healthy surrounding tissues.

5. Synergistic Roles of Orientin and Vicenin in *Ocimum sanctum*

The therapeutic efficacy of *Ocimum sanctum* (Holy Basil) is rarely the result of a single "magic bullet" compound; rather, it emerges from the complex synergistic interactions between its diverse phytochemical constituents. While orientin and vicenin are potent bioactive markers, their pharmacological impact is significantly amplified when they function alongside other major compounds like eugenol (a volatile oil) and ursolic acid (a triterpenoid)⁽⁵⁰⁾. These interactions create a multi-targeted approach to healing that characterizes traditional Ayurvedic formulations.

5.1 Interactions with Other Phytochemicals

In the complex matrix of *O. sanctum*, orientin and vicenin act in concert with eugenol and ursolic acid to provide enhanced protection against cellular damage. Eugenol, which typically comprises up to 70% of the leaf's essential oil, provides powerful antimicrobial and analgesic properties, while ursolic acid offers robust anti-inflammatory and antitumor effects. When present together, these compounds create a "phytochemical web" where the water-soluble flavonoids (orientin/vicenin) and lipid-soluble terpenoids (ursolic acid) can simultaneously protect both the aqueous and lipid compartments of the cell from oxidative stress⁽⁵¹⁾.

5.2 Synergistic Pharmacological Actions

Experimental evidence suggests that the combination of orientin and vicenin is more effective than either compound used in isolation. For instance, studies on urinary tract infection (UTI) pathogens have shown that while individual flavonoids exhibit limited efficacy, a combined sample of orientin and vicenin demonstrates significantly wider zones of inhibition against *E. coli* and *S. aureus*. Similarly, in radioprotection, the combined administration of these two flavonoids has been shown to reduce chromosomal aberrations in bone marrow more effectively than single-agent treatments, suggesting a cooperative mechanism in DNA repair and ROS scavenging⁽⁵²⁾.

5.3 Whole-Plant Efficacy vs. Isolated Compounds

The "synergy" concept supports the Ayurvedic philosophy of whole-plant efficacy, which posits that the natural form of the herb possesses a balance that isolated extracts lack. Research comparing crude extracts of *O. sanctum* to purified orientin often reveals that the crude extract maintains a more sustained antioxidant effect. This is attributed to the presence of secondary metabolites that may stabilize the primary active compounds or enhance their bioavailability. By targeting multiple signalling pathways—such as inhibiting NF-kappaB for inflammation while simultaneously upregulating Nrf2 for antioxidant defense—the whole plant provides a comprehensive physiological "buffer" against disease that isolated compounds cannot fully replicate⁽⁵³⁾.

6. Biotechnological and Industrial Applications

The translation of orientin and vicenin from laboratory research to industrial application is a burgeoning field, driven by the global demand for natural, bioactive ingredients. These C-glycosyl flavonoids are increasingly recognized not just as traditional medicinal markers but as versatile raw materials for the nutraceutical, pharmaceutical, cosmetic, and food industries⁽⁵⁴⁾.

6.1 Nutraceutical Potential

Orientin and vicenin hold immense promise in the nutraceutical sector due to their multi-targeted health benefits, including adaptogenic and metabolic-regulating properties. Industrial formulation opportunities have expanded beyond simple aqueous decoctions to include standardized capsules, concentrated extracts, and functional beverages. These products are often marketed for their ability to combat oxidative stress and enhance cognitive function. The high thermal stability of C-glycosyl bonds—compared to O-glycosides—makes these flavonoids particularly suitable for functional foods that undergo heat processing, such as pasteurized herbal juices or baked health snacks⁽⁵⁵⁾.

6.2 Pharmaceutical Applications

In the pharmaceutical landscape, orientin and vicenin are viewed as promising "lead compounds" for drug development, particularly for chronic conditions such as osteoporosis, rheumatoid arthritis, and cardiovascular diseases⁽⁵⁶⁾. However, a significant hurdle in their clinical adoption is their low oral bioavailability and rapid systemic clearance. To overcome these challenges, researchers are employing advanced nanotechnological solutions. Recent studies have successfully encapsulated these flavonoids into liposomes and nanocarriers, such as lactoferrin-peptide nano assemblies, which enhance their solubility, protect them from gastrointestinal degradation, and ensure targeted delivery to inflamed tissues⁽⁵⁷⁾. These engineered delivery systems significantly improve the pharmacokinetics of the compounds, paving the way for more effective oral or parenteral drug formulations.

6.3 Cosmetic Applications

The cosmetic industry utilizes orientin and vicenin for their potent anti-aging and skin-protective properties. Orientin, in particular, has been shown to attenuate UVB-induced skin photodamage by activating the AMPK/Nrf2 signalling axis, which reduces ROS generation and suppresses inflammatory mediators like IL-6 and COX-2. Furthermore, their ability to inhibit tyrosinase—the enzyme responsible for melanin production—positions them as natural alternatives for anti-pigmentation and skin-brightening treatments. Their dual action as antioxidants and DNA-repair enhancers makes them valuable ingredients in premium sun-care and regenerative night creams.

6.4 Food Preservation and Safety

As the food industry shifts away from synthetic additives, orientin and vicenin are emerging as effective natural preservatives. Their broad-spectrum antimicrobial activity against common foodborne pathogens like *Listeria monocytogenes* and *Staphylococcus aureus*, combined with their ability to

inhibit lipid peroxidation, helps extend the shelf life of perishable products ⁽⁵⁸⁾. When used in active packaging or as direct additives, they prevent the "off-Flavors" associated with fat oxidation while simultaneously enhancing the nutritional profile of the food product.

7. Safety, Toxicity, and Pharmacokinetics

Despite their widespread use in traditional medicine, the systematic evaluation of the safety and pharmacokinetic (PK) profiles of orientin and vicenin is essential for their transition into clinical practice. These C-glycosyl flavonoids possess a unique C-C covalent bond between the sugar moiety and the flavonoid backbone, which significantly influences how the body processes them compared to more common O-glycosides ⁽⁵⁹⁾.

7.1 Toxicity Profiles from Preclinical Studies

Preclinical evaluations in various animal models indicate that both orientin and vicenin possess high safety margins. Acute oral toxicity (AOT) studies of vicenin-1 in Swiss albino mice established a median lethal dose LD₅₀ of approximately 4837.5 mg/kg, with a "no-observed-adverse-effect level" (NOAEL) of 75 mg/kg in repeated-dose (28-day) subacute studies. Orientin has similarly demonstrated a lack of systemic toxicity at therapeutic doses; for instance, intraperitoneal administration of 50 mg/kg for radioprotection showed no adverse behavioural or physiological changes in mice ⁽⁶⁰⁾. Furthermore, *in vitro* cytotoxicity assays using SH-SY5Y neuronal cells confirmed that orientin is non-toxic at concentrations up to 20 µM, even providing protection against hydrogen peroxide-induced apoptosis.

7.2 ADME (Absorption–Distribution–Metabolism–Excretion) Data

The pharmacokinetic behaviour of these compounds is characterized by their resilience to typical digestive enzymes. Unlike O-glycosides, which are often hydrolysed by beta-glucosidases in the small intestine, C-glycosyl flavonoids like orientin and vicenin remain largely intact throughout the upper gastrointestinal tract ⁽⁶¹⁾.

- **Absorption:** They exhibit relatively low oral bioavailability, primarily due to their high polarity and poor membrane permeability.
- **Distribution:** Once absorbed, they distribute widely into tissues, with the highest concentrations typically found in the kidneys and liver ⁽⁶²⁾.
- **Metabolism:** Their metabolism involves minimal degradation in the liver; however, they can be modified by gut microbiota in the colon into smaller phenolic acids.
- **Excretion:** A distinguishing feature is the appearance of intact C-glycosylated metabolites in human urine, indicating that the body eliminates them without breaking the carbon-carbon sugar bond ⁽⁶³⁾.

7.3 Interactions and Dosage Considerations

Effective dosage for orientin and vicenin varies depending on the intended therapeutic outcome. For general antioxidant and anti-aging benefits in mice, doses of 20–40 mg/kg have been benchmarked as comparable in efficacy to Vitamin E. In terms of drug interactions, these flavonoids may inhibit certain

intestinal transporters (such as OATP2B1) and enzymes (like CYP3A4), which could theoretically alter the bioavailability of co-administered pharmaceutical drugs ⁽⁶⁴⁾. While this interaction potential suggests they should be used cautiously alongside narrow-therapeutic-index drugs, it also presents an opportunity to use them as bio-enhancers to reduce the required doses of other medications.

8. Current Challenges and Future Prospects

While the pharmacological potential of orientin and vicenin is well-documented in preclinical settings, several hurdles remain before these C-glycosyl flavonoids can be fully integrated into mainstream clinical therapy. Transitioning from "bench to bedside" requires addressing critical gaps in molecular understanding, bioavailability, and human-centric validation.

8.1 Gaps in Current Research

Despite the extensive library of *in vitro* and *in vivo* data, research into orientin and vicenin still faces significant gaps. Most studies focus on broad antioxidant and anti-inflammatory outcomes without fully elucidating the precise upstream molecular targets. For instance, while their impact on the NF-κB and Nrf2 pathways is known, the specific cell-surface receptors or intracellular ligands they bind to remain largely unidentified. Furthermore, while there is a wealth of data on the plant *Ocimum sanctum* as a whole, there is a lack of comparative studies that isolate the specific contribution of orientin versus vicenin in complex mixtures, leaving the "synergy" concept more theoretical than quantified.

8.2 Need for Clinical Studies

A primary challenge is the scarcity of large-scale, randomized controlled trials (RCTs). Most existing clinical evidence for *O. sanctum* involves crude leaf powders or standardized extracts rather than the isolated flavonoids. While these trials report benefits in glycaemic control and stress resilience, they do not provide specific pharmacokinetic data for orientin or vicenin in humans. Future clinical research must focus on:

- **Human Pharmacokinetics:** Determining the exact metabolic fate of C-glycosyl bonds in the human gut.
- **Dose-Response Relationships:** Establishing standardized human dosages that replicate the success seen in murine models.
- **Long-term Safety:** Assessing the effects of chronic supplementation, particularly in vulnerable populations like pregnant women or patients on anticoagulants.

8.3 Recommendations for Future Studies

To move the field forward, a multi-disciplinary approach is recommended across three key pillars:

1. Molecular and Genomic Studies: Future research should utilize proteogenomic and network pharmacology to map the "interactome" of these flavonoids. This includes exploring their roles in emerging areas such as oral cancer (via Aurora kinase B inhibition) and inflammatory bowel disease (via Nrf2/HO-1 axis modulation).

2. Advanced Formulation Science: To overcome the low oral bioavailability inherent to polar C-

glycosides, the development of next-generation delivery systems is essential. This includes the use of self-emulsifying drug delivery systems (SEDDS), lactoferrin-peptide nano assemblies, and pH-sensitive liposomes to ensure the compounds reach the systemic circulation intact.

3. Biotechnological Production: As wild harvesting of *O. sanctum* can lead to variability in flavonoid content, future efforts should focus on heterologous production. Using CRISPR/Cas9 or metabolic engineering in microbial hosts (like *E. coli* or yeast) could provide a sustainable and standardized supply of pure orientin and vicenin for pharmaceutical use.

9. Conclusion

The comprehensive evaluation of orientin and vicenin reveals a sophisticated pharmacological profile characterized by potent antioxidant, anti-inflammatory, and radioprotective activities. These C-glycosyl flavonoids distinguish themselves through their unique chemical stability, which allows them to resist enzymatic degradation in the upper gastrointestinal tract and exert systemic effects. Research has consistently highlighted their ability to modulate critical signalling pathways, such as the Nrf2/ARE axis for oxidative stress resistance and the NF-kappaB pathway for inflammatory control. Furthermore, their role in inhibiting cell proliferation and inducing apoptosis across various malignancies underscores their potential as adjunctive agents in oncology. Within the matrix of *Ocimum sanctum* (Holy Basil), orientin and vicenin are not merely secondary metabolites but are central to the plant's identity as an "Elixir of Life." Their significance is amplified by their synergistic interaction with other constituents like eugenol and ursolic acid, which collectively provide a multi-layered defense against metabolic, physical, and chemical stress. This "whole-plant" synergy validates the traditional Ayurvedic use of Tulsi, demonstrating that the combination of these flavonoids provides a broader therapeutic window and higher safety margin than many isolated synthetic counterparts. Orientin and vicenin stand at the threshold of modern pharmaceutical development. Their proven efficacy in preclinical models of neurodegeneration, diabetes, and radiation-induced damage positions them as high-value lead molecules for drug discovery. While challenges regarding oral bioavailability persist, the advent of nano-drug delivery systems and lipid-based carriers offers a viable pathway to clinical translation. As the global healthcare landscape shifts toward natural and sustainable medicine, these flavonoids represent a bridge between ancient ethnopharmacology and 21st-century biotechnology. Future efforts directed at human clinical trials and standardized heterologous production will be pivotal in establishing orientin and vicenin as staples in the global pharmacopeia.

Ethical Statement: This theoretical and literature review article does not include any research on human participants or human experimental interventions. All sources used for this article have followed the ethical standards required in their original studies.

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Review Article

Review on Versatile Application of Bio-Enzymes for Sustainable Environment

Harshi¹, Ashwani Kumar²

1. UG Scholar,
2. Professor & Head.
Department of Life Sciences, Keral Verma Subharti
College of Science, Swami Vivekanand Subharti
University, Meerut (250005) Uttar Pradesh, India

Abstract: Enzymes are biological catalysts, sometimes referred to as biocatalysts, that accelerate reactions in living things. They can be extracted from cells and utilized to catalyze a variety of commercially significant activities. Fresh fruit and vegetable waste ferments in the presence of water and jaggery to form organic chemicals known as bio-enzymes. It is shown that the greatest option for household and agricultural applications is the biodezyme. It is a naturally occurring, non-toxic, non-flammable, and noncorrosive liquid enzyme formulation fermented from vegetable extracts that enhances soil's engineering properties, enables greater soil compaction densities, and boosts stability. Soil stabilization and improvement are frequently employed as a substitute for inadequate materials on the site. Stabilizing soils can improve their durability and strength or stop erosion and dust production. Every day, more and more non-traditional chemical stabilizers are being used to improve soil. To enhance the mechanical performance and suitability of clayey soils, a bioenzyme was created. Enzymes speed up reactions so they can happen within a time frame that is useful to biology. Fruit and vegetable waste is organic and contributes significantly to soil, water, and air pollution. Enzyme Bio-cleaners are organic compounds that include enzymes that are made by using specific microorganisms like yeast and bacteria to ferment fresh fruit and vegetable wastes with brown sugar and water. This anaerobic fermentation produces natural chains with the ability to break down, change, construct, and catalyze processes, making it an incredible domestic cleaning aid in addition to other uses. The research and important work on Bio-enzymes are reviewed in this study. It aids in the reduction of trash and transforms it into a material that is affordable, readily available, and perhaps fully beneficial to society.

Keywords: Bio-enzymes, biocatalysts, fermentation, eco friendly chemical stabilizers , organic waste management

Address for correspondence: Harshi, Department of Life Sciences, Keral Verma Subharti College of Science, Swami Vivekanand Subharti University, Meerut (250005) Uttar Pradesh, India

E-Mail: harshigeet2005@gmail.com

Contact: +91-87005-16400

1. Introduction

One type of organic substance is an eco-enzyme. Fruit and vegetable peels and other fresh kitchen trash are fermented to create this complicated solution. This kind of homemade vinegar is manufactured by fermenting kitchen trash with sugar as a substrate to reduce the alcohol. Fruit peels and other organic waste can typically be used to make bio-enzymes. Because of their unique qualities, such as their high acidity value, strong flavor and scent, high vitamin C content, and possible health advantages, tangy fruit peels are used. Eco-enzyme has antibacterial, antifungal, and insecticidal qualities. It can be used as a cleaning agent as well. The first person to create bio-enzyme was Poompanvong. Other names for bio-enzymes include coenzymes, rubbish enzymes, terrazymes, fruit enzymes, flower enzymes . Eco-enzymes are a group of enzymes that quickly catalyze a variety of activities. Chemically speaking, eco-enzymes are a mixture of polypeptides, polysaccharides, bioactive substances, and other substances that are created on their own by some bacteria. The fermentation of bio-enzymes involves the addition of water, fermented fruit and vegetable waste, brown sugar, or carbohydrates.⁽¹⁾

Biological macromolecules, mostly proteins, that function as extremely effective catalysts in living systems are known as Bio-enzymes, or simply enzymes. By reducing activation energies, they speed up chemical reactions and allow metabolic pathways to continue under mild conditions (neutral pH, physiological temperature) that would otherwise be kinetically prohibitive. Enzymes have historically been used in biochemistry and cell biology, but in recent decades, business and researchers have increasingly used them for environmental applications. This transformation has been prompted by urgent sustainability issues such resource depletion, pollution, and climate change.

A lack of rainfall, nutrient-depleted soil, and climate change have all contributed to production problems in many regions in recent years. In an effort to increase productivity, farmers regularly apply chemical pesticides, herbicides, and fertilizers. Even though these compounds have positive effects as first, they eventually deplete soil nutrients by damaging beneficial bacteria and leaving the soil even more barren. Additionally, during irrigation, dangerous pesticides and herbicides seep into groundwater. A major concern is producing long-lasting, appealing products that are both economical and ecologically friendly. Enhancing soil quality with

natural fertilizers like bio-enzymes and bio-composites.⁽²⁾

Bio-enzymes, their primary constituents are fruit and vegetable peels, which are regarded as waste and thrown away. Bio-enzymes are completely organic and help reduce waste. They are completely organic, non-toxic, non-corrosive, and non-hazardous liquids.⁽³⁾

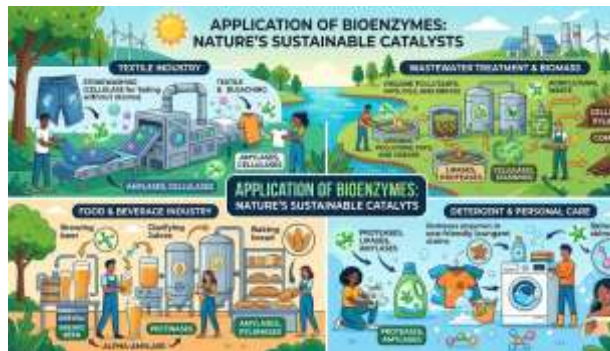


Fig.1 Application of Bio-enzymes

2. Preparation Of Bio-Enzymes

Bioenzyme preparation involved gathering fruit peels (Pineapple, Orange, and Lemon separately) from nearby juice vendors and combining them with water and jaggery in a 3:1:10 ratio in individual airtight plastic containers. To speed up the fermentation process, a small amount of yeast was added. To release the gases produced by fermentation, the containers were opened every other day. All three liquids were filtered after 45 days.⁽⁴⁾

Preliminary Screening and Quantitative Analysis of the Extracts

Protein Estimation: The Lowry-Bronsted method was used to estimate the amount of protein in the sample. The standard was bovine serum albumin. To create the standard graph, a series of protein solutions with progressively higher concentrations (0, 2, 4, 6, 8, and 10 mg/ml) were made, and their absorbance was measured at 660 nm. The standard graph was used to determine the protein content of the samples.⁽⁵⁾

Sugar Estimation: Sugars present in the sample were evaluated using DNSA reagent. Dextrose was employed as the standard. A series of sugar solutions in increasing order (0, 0.125, 0.25, 0.375, 0.5, 0.625, 0.75 mg/ml) of their concentrations were prepared, their absorbance was measured at 520nm, for generating the standard graph. 1ml of each sample were diluted with 9 ml of distilled water and 0.1ml of this extract was evaluated. The values of the sugar content present in the samples were estimated using the standard graph and then were multiplied by the dilution factor.

Acetic Acid Estimation: Acid-base titration was used to measure the amount of acetic acid in the samples. Phenolphthalein was used to titrate the samples against sodiumhydroxide (NaOH). It was observed how much NaOH was needed to neutralize the acetic acid in the sample. The sample's acetic acid content was determined using the normality equation ($N_1V_1 = N_2V_2$).⁽⁵⁾

3. Bio-Enzymes Applications

Bio-enzymes have been shown to have a wide range of applications, from agriculture to the home. A

variety of bio-enzyme used because Bio-enzymes are entirely natural and aid in waste reduction, their applications are numerous. It is the greatest substitute for the harsh, aggressive, and non-biodegradable cleaning products on the market that are based on chemicals. The best aspect is that anyone can make them in their homes and kitchen gardens without the need for a fermenter or other special equipment.⁽¹⁾

3.1 Bio-enzyme for soil improvement

Biological methods integrate engineering practices with ecological principles, they have become a promising alternative to traditional ways in soil improvement initiatives. With this method, systems are designed and built with living plant materials as essential structural elements.⁽⁷⁾ It provides advantages from an ecological and environmental perspective in addition to an engineering one.⁽⁸⁾

The two main biological approaches are bio-enzyme and microbial/enzymatically induced carbonate precipitation s(MICP/EICP). In MICP/EICP, urease breaks down urea into carbonate ions, which then react with surrounding metal ions to generate precipitated carbonate minerals. By filling, covering, and cementing the microstructure, these minerals may enhance the characteristics of the soil.⁽⁹⁾

3.1.1 Engineering properties of bio-enzyme stabilized soils

Physical Properties

It has been shown that bio-enzymes can improve the physical characteristics of soils. For instance, they can raise the maximum dry density (MDD) while decreasing the liquid-plastic limit, plasticity index (PI), and optimal moisture content (OMC). It is clear from summarizing the findings of typical studies on bio-enzyme stabilized soils that soil parameters and enzyme dose have an impact on the improvement effect in the physical qualities of stabilized soil. This improvement can be modest to substantial, and in certain situations, it might even result in unfavorable outcomes like a decline in MDD.

The amount of bio-enzyme used has a significant impact on the physical characteristics of stabilized soils. Research has indicated that at low enzyme dosages, the compaction properties (MDD and OMC) may only slightly improve.^{(10) (11) (12) (13) (14) (15) (16) (17)} It has been suggested that the inconsistent and uneven soil found in laboratories may also be the cause of this. Increasing the amount of enzymes can effectively decrease the electric double layer's thickness, increasing soil improvement's efficacy.⁽¹⁸⁾ In general, when the dosage of enzymes increases, the improving effect of soils tends to first grow and subsequently decrease.⁽¹⁹⁾ High plasticity soils, however, might require higher enzyme dosages to meet the threshold needed for successful soil improvement; otherwise, the MDD would continue to rise and the OMC will continue to fall until the ideal enzyme dosage is reached.⁽²⁰⁾

In conclusion, research on enzyme-treated soil has produced a wide range of results, from negligible to substantial improvement and even the opposite.

These differences are explained by things like soil characteristics, enzyme specificity, and enzyme dose. Because independent laboratory testing is required to confirm their efficacy, it is imperative to approach claims or findings from enzyme producers with caution.⁽²¹⁾

Chemical Properties

The bio-enzyme treatment has shown potential to enhance the mechanical properties of soil ⁽²²⁾⁽²³⁾ such as increasing the unconfined compressive strength (UCS) and California bearing ratio (CBR), etc. Summarizing the details of representative studies on the initial soil properties/sample preparation methods and mechanical properties of bio-enzyme stabilized soils. A. Water content, specimen preparation techniques, soil characteristics, enzyme specificity and dosage, and other factors all affect how much the mechanical properties of stabilized soil increase. In summary, Bio-enzymes have the potential to improve the mechanical strength of soil, but their effectiveness depends on a number of variables, such as water content, sample preparation method, soil characteristics, enzyme specificity and dosage, etc. Nonetheless, there is still disagreement over how soil characteristics affect the mechanical strength of enzyme-stabilized soils, which calls for more investigation into the actions of the enzymes and the structure of the products they produce. Although the use of bio-enzymes in environmental geo-technics has shown a lot of promise, there is still a clear deficiency in the methodical arrangement of the information that is now available. The main ideas of this book, which aims to assess the advancement of bio-enzyme technology, are summed up as follows:

Plant fermentation and animal extraction are the sources of bio-enzymes, a new class of natural, organic, and biodegradable soil enhancement products. They usually contain proteins, surfactants, and other unique ingredients and have no detrimental effects on the environment. Bio-enzymes may improve soil by lowering the net negative charge on soil particle surfaces through processes like cation exchange, specific binding, and surfactants. This would decrease the thickness of the electric double layer and the water affinity.

Bio-enzymes could modify the engineering properties of soil, generally yielding favorable effects. They could reduce the liquid plastic limit and the OMC, while enhance the MDD, UCS, and CBR. However, the improvement performance depends on the soil properties, enzyme dosage, specificity, and sample preparation, among other factors.

Bio-enzymes could be synergized with other stabilizers for soil improvement, and these bio-enzymatic composites might more considerably improve the physical and mechanical properties as well as other geotechnical characteristics of soils, and enhance the modification efficiency.

The engineering applications of bio-enzyme have demonstrated its capacity to mitigate common pavement diseases, decrease maintenance expenses, and enhance construction efficiency.



Fig.2. Bio-enzyme formation process

3.2 Bio-Enzymes: Enhancing Water Quality and Sanitation

Lakes are essential parts of Earth's ecosystems. Lakes provide a variety of benefits, including groundwater recharge, habitat for wildlife, climate regulation, and water supplies. A significant barrier to accomplishing SDG 6 is lake pollution, which compromises water quality, harming ecosystems, and putting human health at risk. Increased nutrient input from these sources, fueled by increased human activity in the lake's surrounding areas, is probably what is causing the surge in eutrophication ⁽²⁴⁾. Eutrophication, which causes algal blooms, lowers oxygen levels, and disturbs aquatic life, is a result of excess nutrients from agricultural runoff. Furthermore, hazardous substances like pesticides and heavy metals are introduced into lakes by domestic and industrial waste, rendering the water unfit for human consumption. Since many lakes are important sources of drinking water, pollution greatly raises treatment costs and, in extreme situations, makes these water sources unusable. Communities that depend on contaminated lakes face significant health risks due to exposure to hazardous materials and waterborne illnesses. By endangering aquatic species that are vital to ecosystems and livelihoods, pollution also reduces biodiversity. The deterioration of water quality has a negative economic impact on sectors like tourism, fishing, and agriculture. The health of ecosystems and human well-being are seriously threatened by the loss in water quality, which has negative effects on drinking water standards, biodiversity, and the growth of dangerous algal blooms. ⁽²⁵⁾

Treating wastewater, promoting sustainable farming methods to reduce runoff, and raising community knowledge of water conservation are some ways to address these issues and advance SDG 6. To guarantee sustainable freshwater management, it is essential to enforce pollution restrictions, restore degraded lakes, and safeguard key water zones. Chemical treatment of water results in the production of non-biodegradable byproducts, residual chemicals, high operating costs, and a rise in the waterbody's toxicity. Enzymes are specialized biological molecules, frequently proteins, that operate as natural catalysts to speed up metabolic reactions in living things ⁽²⁶⁾. Organic matter is broken down by bio-enzymes, which also raise DO and decrease BOD and COD. The use of Bio-enzymes provides a more accessible and sustainable solution to water treatment.

Enzymes, which are mostly made of proteins and peptides, can be readily extracted from contaminated streams without posing disposal problems because they are biodegradable by microbes.⁽²⁷⁾ Wastewater can be used for a variety of purposes, such as floor cleaning, car washing, and gardening, after being pre-treated using garbage enzymes.⁽²⁸⁾

A bio-enzyme solution for purifying water bodies can be made by combining one liter of bio-enzyme with ten thousand liters of fresh water and letting it ferment in a sealed container for twenty-four hours. This technique makes it possible to scale bio-enzyme quantities effectively in a day with little effort. Tankers with sprayers are then used to apply the prepared solution throughout the pond. After the first application, this therapy should be repeated every 30 days. An initial survey was carried out to gather water samples and analyze important parameters like DO (Dissolved Oxygen), BOD (Biochemical Oxygen Demand), and COD (Chemical Oxygen Demand) in order to evaluate the efficacy of the treatment. These criteria were reassessed after three months of monthly treatments, and the results showed notable improvements.⁽²⁹⁾

Wastewater can be used for a variety of purposes, such as floor cleaning, gardening, and car washing, after being pre-treated using garbage enzymes.⁽³⁰⁾ Bio-enzymes are used on a very small scale and are still largely unknown in India.⁽³¹⁾

Treatment with Bio-enzymes must be viewed as a significant process. This paper describes the manufacture of Bio-enzymes from kitchen waste, such as fruit and vegetable peels, and the use of Bio-enzymes to purify the water in Saroomagar Lake. The procedure demonstrated the bio-enzyme's efficacy in treating wastewater.⁽³²⁾

In summary, The application of bio-enzyme led to a considerable rise in dissolved oxygen levels, which improved with time and at greater enzyme concentrations. In the current investigation, treatment with a 5% bio-enzyme solution has shown greater efficacy. BOD significantly dropped, demonstrating the organic pollutants' efficient breakdown. Both organic and chemical pollutants decreased, as seen by the notable drop in COD. When access to sophisticated water treatment facilities is restricted, the demonstrated effectiveness of bio-enzymes in enhancing water quality underscores their promise as an affordable and sustainable water treatment alternative. The study offers a creative, environmentally responsible method for rehabilitating freshwater habitats, highlighting the revolutionary significance of biotechnology in sustainable water management.

3.3 Impact Of Bioenzyme On Plant Growth And Maturation In Vegetable Crop

Agriculture is the primary industry in India. India's GDP and employment are mostly derived from agriculture, making it essential to the nation's economic growth. Therefore, ensuring that agriculture continues to be profitable is essential for a nation like India.⁽¹⁾ Despite an 11.0% growth in vegetable area, just 2.8% of India's total agricultural land is used for vegetable crops. Over the past ten years, the nation's vegetable output has grown significantly, with increases in land area, yield, and

efficiency of up to 60% and 44.7%, respectively. Global vegetable production from 40 million hectares is 606 million tons, according to FAO statistics from 1998.

Olericulture is the process of growing, preserving, preparing, and selling vegetables. Vegetables are low in calories and high in vitamins and minerals like B-carotene, vitamin B, folic acid, vitamin C, vitamin E, dietary fiber, carbs, protein, and energy.⁽²⁾

A bio-composite is made up of inorganic and non-polymeric components that increase mechanical strength and protect against environmental deterioration, as well as a natural fiber matrix (resin) for reinforcement. Bio-composites are inexpensive materials with the ability to enhance soil nutrient quality, reduce pathogen growth, improve soil texture, and boost plant productivity. Bio-composites shield the environment from pollutants because they are natural fertilizers. Agro-waste-derived enzymes are used as bio-catalysts to enhance crop growth, cell metabolism, cell division, compost breakdown, and micronutrient release. Therefore, the consumption of bio-enzymes facilitates the proper distribution of nutrients found in fiber or biomass, leading to quick development.⁽³³⁾

In summary, The data collected indicate that the use of bio-enzymes improves plant development and growth. Among the three treatments, the bio-enzyme treatment had far more germination and plant growth than the control. The presence of soluble sugars, carbohydrates, proteins, organic acids, vitamins, etc. may be the cause of this increase in bio-enzyme development. In order to bolster the findings, the study⁽³⁴⁾ looked at how Bio-enzymes affect photosynthetic activity and chlorophyll content, which can be impacted by a drop in soil nitrogen levels, which in turn affects plant development. According to the study's findings, using Bio-enzymes significantly increases the amount of chlorophyll. The purpose of the study was to look at how different parameters are affected by Bio-enzymes. By increasing the number of beneficial bacteria in the soil, the application of Bio-enzymes enhances soil fertility and quality. These biological enzymes were even used as insecticides, fungicides, and weed killers, and the study found that they also functioned as plant development regulators.⁽¹⁾

3.4 Enzyme Bio-Cleaners From Production Of Fruit Waste

The usage of synthetic cleaning agents, which have the potential to discharge hazardous compounds into the environment and drastically alter pH, has caused significant environmental concerns in recent years.

The most recent cleaning technologies, however, include enzyme-containing cleaning solutions that are highly efficient, safe, and selective in their activity. They are also less expensive to prepare.^(35,36)

Enzyme bio cleansers are an organic solution made by using specific microorganisms like yeast and bacteria to ferment fresh fruit and vegetable wastes with brown sugar and water. Natural protein chains, mineral salts, organic acids, alcohol, and enzymes are produced by this fermentation process. The resulting Bio-enzymes are an excellent cleaning tool

for usage in industrial, medical, and home settings because of their ability to break down, alter, generate, and catalyze activities. Since enzyme bio-cleaners are becoming the ideal choice for consumers in the future for a variety of applications and a sustainable environment, they are referred to as natural green chemicals.

The purpose of the enzyme bio-cleaning solution is to quickly, affordably, and safely get rid of filth. It breaks down organic and chemical waste fast and effectively without producing any harmful gases or odors. Therefore, it will be necessary to produce a suitable substitute for the synthetic cleaning solution in the future with regard to biodegradability, low toxicity, non-corrosiveness, environmental friendliness, improved cleaning qualities, and higher efficiency and stability in various environmental circumstances. Bio-Bacterial innovation is the foundation of these cutting-edge environmentally friendly cleaning products. This invention, which contains billions of beneficial bacteria, eliminates the source of cleaning problems rather than hiding them. Cleaning is made easier and more environmentally friendly with bio cleaner, which promises a natural solution to common cleaning problems without the need for harsh chemical cleaners.

Both bacteria and enzymes cooperate to clean in bio-enzymatic cleansers, depending on one another to complete the task. When sprayed to surfaces, the enzymes break down stains, dirt, and odors, which the bacteria then eat. These microscopic "cleaners" proliferate and continue to eliminate traces of dirt and odor from surfaces hours or even days after the original application, provided that the soil is present and the surfaces are sufficiently moist.

Although they are originally extracted from bacteria, enzymes can also be utilized in some cleaning products without the presence of bacteria. For instance, the chemical reactions of other chemicals in laundry detergents are catalyzed by enzymes. They aid in the breakdown of soils in wash water, making it easier for water to remove them. For various cleaning tasks, including carpet cleaning, laundry detergents, degreasing, drain maintenance, general surface cleaning, restroom cleaning, and odor removal, bio-enzymatic formulations are very effective.⁽³⁷⁾

In summary, The goal of the current project was to produce and analyze the fermentation parameters of an enzyme bio-cleaning solution using agricultural waste and residues, such as fruit waste, with the aid of bacteria (*Bacillus sp.*) and yeast (*Sacharomyces sp.*), along with less expensive sources of carbohydrates like brown sugar and water medium. In conclusion, cleaning is crucial for the dehairing process in tanning industries, maintaining cleanliness, preventing rust formation, cleaning floors, removing stains, etc. We think the enzyme cleansers would eventually take over the majority of the Indian market because of their high efficiency and safety.

4. Conclusion

One highly useful organic waste product that aids the nation in lowering the amount of organic dump garbage is bio enzyme. Based on the results, it can

be concluded that using Bio-enzymes will help the nation meet the 2030 SDGs. Since many other factors are interconnected, we can also state that bio-enzymes are a promising vital factor for the country's advancement and the expansion of the worldwide market. However, bio-enzymes will also play a role in achieving the SDG objective 2030.

Liquids containing Bio-enzymes lessen environmental toxicity, which will help reach SDG 2030. A nation's SDG objectives cannot be met by a single element; instead, a number of factors are taken into consideration, one of which is bio-enzyme. Although it would have a shorter shelf life and possibly less cleaning efficacy over time, a Bio-Enzyme with a pH of 4.7 would function. Because it contains classes of digestive enzymes, this bio-enzyme can be used for household cleaning and can remove the majority of stains. The study had certain limitations, including the use of only two kinds of kitchen waste, the Bio-Enzyme production not being carried out under ideal conditions (the jar was opened daily, which should have been stopped after the start of anaerobic respiration), and the failure to test various ratios of bananas to lemons.

This project helps society transition to a chemical-free, sustainable environment. Additionally, Project Nesara works with non-governmental organizations (NGOs) and gives the poor access to jobs.

Ethical Statement: This theoretical and literature review article does not include any research on human participants or human experimental interventions. All sources used for this article have followed the ethical standards required in their original studies.

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Review Article

Green Horizons: Evaluating the Socio-Economic and Environmental Dividends of FinTech-Led Financial Inclusion

Himanshu Agarwal¹, Aruna Bansal², Dr.Sweta Bhardwaj³

1. Assistant Professor,
Department of Electronics and Communication,
2. Associate Professor,
Department of Electronics and Communication,
3. Associate Professor,
Department of Environment, Engineering & Management,

Subharti Institute of Technology and Engineering,
Swami Vivekanand Subharti University, Meerut.

Abstract— Financial inclusion is widely recognized as a foundational pillar of sustainable development, yet conventional banking structures have consistently struggled to reach marginalized and remote communities due to high operating overheads, collateral requirements, and geographical barriers. To bridge this gap, Financial Technology (FinTech) has emerged as a disruptive enabler, lowering transaction costs and expanding the reach of essential financial services. This study examines how FinTech-driven inclusion generates a triple dividend—social empowerment, economic resilience, and environmental sustainability—using a secondary research methodology. Synthesizing evidence from peer-reviewed literature, policy whitepapers, and international development datasets from the past decade, this paper evaluates key digital innovations, including mobile money ecosystems, algorithmic credit assessments, micro-insurance, and decentralized pay-as-you-go (PAYGo) asset financing. The synthesis reveals that beyond driving core economic metrics—such as MSME liquidity, household savings, and formal market integration—and fostering social outcomes like gender parity and enhanced financial literacy, FinTech serves as an unrecognized catalyst for ecological stewardship. Digital delivery models inherently dematerialize physical banking infrastructure, cut transit-related carbon footprints, and enable low-income households to transition from polluting fuels to decentralized solar energy and clean cooking solutions. Furthermore, satellite-integrated micro-insurance shields climate-vulnerable smallholders from ecological shocks without forcing destructive land-use choices. Despite these positive synergies, persistent risks related to digital divides, cyber vulnerabilities, e-waste, and fragmented regulatory oversight remain critical bottlenecks. The study concludes that maximizing the socio-economic and green potential of FinTech requires targeted public-private partnerships, proportional regulatory sandboxes, and deliberately designed inclusive financial policies.

Keywords— Financial Inclusion, Environmental Sustainability, FinTech Innovation, Digital Financial Services, Financial Technology Adoption, Inclusive Economic Growth, MSME Development, Financial Literacy

Address for correspondence: Dr. Himanshu Agarwal, Assistant Professor, Department Electronics and Communication, Subharti Institute of Technology and Engineering, Swami Vivekanand Subharti University Subhartipuram, N-58, Delhi-Haridwar Bypass Road, Meerut, U.P.

E-Mail: himanshu.agarwal369@gmail.com

Contact: +91-99177-66084

Introduction

Financial inclusion has become a central pillar of sustainable economic development, social equity, and environmental stewardship across the world. It refers to the provision of accessible, affordable, and quality financial services to all segments of society, particularly underserved, low-income, and marginalized populations. Access to formal financial systems enables households and micro-enterprises to build savings, secure credit, absorb climate-related shocks, and adopt resource-efficient technologies. However, traditional banking models have historically struggled to reach remote and economically disadvantaged communities due to high operational costs, stringent documentation, heavy physical infrastructure, and severe geographical constraints.

In recent years, Financial Technology (FinTech) has emerged as a transformative force within the global financial ecosystem⁽¹⁾. By leveraging digital platforms, mobile connectivity, artificial intelligence, big data analytics, and blockchain-based architectures, FinTech innovations have redefined how capital and financial services circulate. Digital payments, branchless mobile wallets, peer-to-peer lending, AI-driven credit scoring, and parametric micro-insurance have drastically lowered transaction costs and extended financial access beyond conventional banking networks. Crucially, these solutions dematerialize banking operations—reducing paper and travel dependencies—while facilitating asset financing for decentralized clean energy (such as pay-as-you-go solar systems) and climate-resilient agriculture. These innovations are especially impactful in developing and

emerging economies, where mobile penetration often outpaces traditional brick-and-mortar banking infrastructure ⁽²⁾.

While existing literature widely acknowledges the positive role of FinTech in expanding financial access, there remains a critical research gap in comprehensively evaluating its broader socio-economic and environmental dividends within a unified framework. Most scholarship focuses in isolation on technological efficiency, financial penetration, or regulatory friction, with limited emphasis on the interconnected social mobility, macroeconomic resilience, and ecological outcomes. Therefore, this study aims to examine the role of FinTech innovation in driving inclusive financial access and systematically evaluate its multidimensional social, economic, and environmental implications through a secondary review of recent literature. By synthesizing contemporary empirical evidence, this study positions FinTech not merely as a technological instrument, but as a holistic socio-economic and ecological enabler of sustainable development ⁽³⁾.

Objectives of the Study

1. To examine the concept of financial inclusion and green financial deepening in the context of FinTech innovation.
2. To identify major FinTech innovations promoting financial inclusion and ecological sustainability.
3. To analyze the social implications of FinTech-driven financial inclusion.
4. To evaluate the economic implications of FinTech-enabled inclusion.
5. To assess the environmental dividends and climate resilience generated through digital financial systems.

Conceptual Framework

The study is anchored on an integrated conceptual framework where FinTech innovations serve as direct enablers of financial inclusion. This inclusive access subsequently drives a tripartite dividend across social, economic, and environmental dimensions.

Fintech Innovations

Digital Payments:

Digital payment architectures—encompassing mobile wallets, interoperable QR codes, unified interfaces, and payment gateways—eliminate dependency on physical cash. For historically unbanked populations, digital payments provide a seamless gateway into the formal economy through direct benefit transfers (DBT), merchant settlements, and remittances. By dematerializing currency handling, they cut down administrative resource consumption, enhance transaction traceability, and curtail paper dependency across commercial channels ⁽⁴⁾.

Mobile Banking:

Mobile banking delivers branchless, app-based banking utility directly onto handheld devices. Users can remotely execute fund transfers, balance inquiries, utility bill payments, and loan servicing without traveling to distant physical branches. This remote access breaks geographic barriers in rural and isolated settlements, sharply cutting travel-associated carbon footprints while bringing formal banking tools to previously excluded communities ⁽⁵⁾.

AI-Based Credit Scoring:

Artificial Intelligence and machine learning algorithms evaluate creditworthiness through alternative data footprints—such as digital transaction consistency, mobile utility payments, and platform commerce history—rather than strict physical collateral or legacy bureau scores. This enables credit delivery to thin-file individuals, smallholder farmers, and micro-enterprises. Furthermore, alternative scoring can incorporate green eligibility metrics, enabling preferential rates for eco-friendly business investments ^(6,8).

Blockchain Technology:

Blockchain offers decentralized, tamper-resistant, distributed ledger systems that establish verifiable trust without intermediaries. In inclusive finance, blockchain accelerates cross-border remittances at a fraction of standard wire costs, secures land title records, and facilitates micro-carbon credits or tokenized asset ownership for grassroots communities, ensuring absolute transparency and lower fraud risk ^(6,7).

Digital Microfinance & Green Asset Platforms:

Modern digital microfinance and peer-to-peer (P2P) lending models streamline small-scale credit delivery, eliminating protracted paperwork and overheads. Increasingly, these platforms deploy Pay-As-You-Go (PAYGo) asset financing, allowing low-income households to purchase off-grid solar systems, clean cookstoves, and energy-efficient farm equipment via micro-installments, directly fostering grassroots livelihood creation alongside clean energy adoption ^(7,8).

Dimensions of Financial Inclusion

- **Access:** The physical and digital availability of formal financial touchpoints. Digital platforms remove conventional obstacles like branch scarcity, heavy paper requirements, and rigid entry barriers, opening bank-grade accounts and digital wallets to remote populations.
- **Affordability:** The cost-effectiveness of financial products for low-income users. By automating processes and stripping away brick-and-mortar overheads, FinTech lowers transaction fees, minimum balance penalties, and operational spreads, making continuous financial participation economically viable.
- **Usage:** The sustained, active consumption of financial products rather than mere account opening. True inclusion reflects regular engagement across savings, digital remittances, micro-investments, green asset purchases, and insurance claims, building sustained household resilience.
- **Quality:** The safety, reliability, user-centricity, and governance of the financial interface. Quality financial inclusion requires transparent terms, robust data privacy, accessible local-language interfaces, strong grievance redressal mechanisms, and reliable consumer protection against digital fraud.

Tripartite Outcomes: Social, Economic & Environmental

1. Social Outcomes

FinTech-driven inclusion dismantles structural disparities by enabling direct, secure access to capital and welfare.

- **Women's Empowerment:** Personal digital wallets provide women with independent custody over personal income, increasing household bargaining power, entrepreneurial autonomy, and financial privacy.
- **Equitable Welfare Delivery:** Government-to-Person (G2P) welfare transfers and social safety-net disbursements reach beneficiaries directly, eliminating leakages, bureaucratic delays, and intermediary exploitation.
- **Financial Literacy & Mobility:** Ongoing digital interaction naturally builds digital financial capability, enabling vulnerable households to transition from predatory informal moneylenders to protected formal financial products.

2. Economic Outcomes

At the macroeconomic and microeconomic levels, expanded financial inclusion unlocks dormant capital and stimulates productivity.

- **MSME Capital Formation:** Streamlined micro-credit and algorithmic cash-flow lending allow small enterprises to maintain working capital, purchase productive assets, and weather demand volatility.
- **Savings Mobilization & Formalization:** Accessible digital savings instruments aggregate fragmented household cash reserves into formal financial institutions, expanding the national lending base and boosting tax compliance through traceable transaction trails.
- **Employment & Grassroots Entrepreneurship:** Affordable credit lowers capital entry barriers, spurring rural enterprise development, fintech agent-banking employment, and broader localized commercial activity.

3. Environmental Outcomes

Digital financial deepening directly influences ecological footprints and clean technology adoption at the base of the economic pyramid.

- **Infrastructure Dematerialization:** Branchless, paperless banking workflows eliminate millions of physical transit trips for basic banking tasks and drastically reduce paper, passbook, and physical ledger production.
- **Decentralized Clean Energy Access:** PAYGo FinTech integrations make clean energy technologies—such as solar home systems, solar water pumps, and clean cooking appliances—affordable through micropayments, directly displacing kerosene lamps, diesel generators, and unsustainable firewood harvesting.
- **Parametric Climate Resilience:** Satellite- and weather-indexed micro-insurance programs trigger automatic digital payouts to smallholder farmers during extreme droughts or floods. This swift liquidity stops emergency distress sales of topsoil, deforestation, or destructive agricultural coping mechanisms, preserving long-term ecological balance.^(6,8,9)

IV. Review of Literature

1. FinTech and Financial Inclusion

The existing literature widely recognizes FinTech as a transformative driver of financial inclusion and green financial deepening, particularly in developing and emerging economies. Scholars argue that traditional brick-and-mortar banking systems have historically struggled to serve low-income and geographically

remote populations due to high operating overheads, collateral requirements, and rigid documentation standards. In contrast, FinTech innovations leverage digital architectures, mobile technologies, artificial intelligence, and big data analytics to deliver financial utilities with minimal physical infrastructure and lower transaction costs. Digital payment gateways, branchless mobile wallets, and peer-to-peer lending platforms bridge geographical divides and substantially dematerialize everyday banking operations.⁽⁴⁾

Research further highlights that FinTech expands access while systematically improving product usage and affordability. By analyzing alternative data streams (such as digital utility payments, mobile cash flows, and platform commerce history), algorithmic credit assessment tools extend working capital to thin-file individuals, smallholder farmers, and micro-enterprises lacking conventional credit scores. Multiple empirical studies demonstrate that digital wallet adoption strengthens household savings, improves risk mitigation, and cushions vulnerable communities against unexpected socio-economic and climate shocks. Furthermore, regulatory enablers—such as national digital identity frameworks and tiered e-KYC (Know Your Customer) systems—have lowered entry barriers across marginalized demographics. However, scholars caution that realizing the full potential of FinTech requires overcoming digital divides, addressing algorithmic bias, and establishing robust cyber-governance frameworks.^(7,8)

2. FinTech Innovations in Developing Economies

Developing nations have experienced accelerated FinTech adoption, driven by rapid smartphone proliferation, expanding mobile data connectivity, and supportive regulatory sandboxes. While FinTech in developed economies often optimizes existing banking systems, in developing nations it frequently leapfrogs legacy infrastructure, acting as the primary financial interface for unbanked populations. Branchless banking agents and interoperable QR platforms have established essential financial infrastructure across rural and semi-urban corridors where commercial bank branches are economically unviable.

Crucially, the literature demonstrates that FinTech in developing economies increasingly converges with sustainable infrastructure. A prominent example is Pay-As-You-Go (PAYGo) asset financing, where mobile micropayments allow off-grid rural households to acquire decentralized solar home systems, solar water pumps, and clean cooking appliances through small, flexible installments. Digital micro-lending platforms similarly provide targeted credit to micro-entrepreneurs and agricultural producers without formal collateral. Simplified digital identity verification and mobile-based welfare disbursements have expedited financial account ownership, establishing a foundational digital public infrastructure (DPI) that supports both grassroots commerce and local environmental resilience.^(5,6)

3. Social Impact of Financial Inclusion

FinTech-driven financial inclusion plays a critical role in advancing social equity, safeguarding human dignity, and improving household living standards. One of the most documented outcomes is women's socio-economic empowerment. Personal digital wallets and mobile accounts give women direct custody over personal income, enhancing their financial autonomy,

confidential savings capacity, and decision-making power within households and local communities.[8] Additionally, digital financial inclusion facilitates broader social integration by shifting marginalized populations away from exploitative informal moneylenders into regulated financial ecosystems. The integration of Government-to-Person (G2P) digital payments enhances the delivery of social welfare programs, pensions, and emergency subsidies, minimizing administrative leakages and ensuring timely support for vulnerable groups. Continuous engagement with user-friendly digital platforms also elevates functional financial and digital literacy, fostering responsible savings behavior and long-term planning. Collectively, these mechanisms help reduce multidimensional poverty, enhance social mobility, and strengthen community stability.⁽⁹⁾

4. Economic Impact of Financial Inclusion

From a macroeconomic and microeconomic perspective, financial inclusion mobilizes dormant capital and accelerates productive economic output. Secure digital savings mechanisms encourage systematic asset accumulation, consolidating fragmented cash reserves into the formal banking sector and enhancing national capital formation. Affordable micro-credit allows households to invest in health, education, and livelihood generation, directly increasing household resilience against macroeconomic volatility.⁽⁷⁾

At the enterprise level, accessible digital credit and invoice discounting tools address chronic working capital constraints for Micro, Small, and Medium Enterprises (MSMEs). This liquidity enables small businesses to expand inventory, invest in productive equipment, and create local employment. Furthermore, the migration of transactions onto digital ledgers formalizes commercial activities, expanding the sovereign tax base, improving transaction transparency, and reducing illicit cash dependency. Micro-insurance products—particularly weather-indexed and parametric schemes—insulate agricultural supply chains by providing rapid liquidity following adverse climatic events, preventing distress asset liquidations. At the aggregate level, broad-based financial inclusion optimizes capital allocation, stabilizes domestic financial systems, and fosters inclusive economic growth.⁽⁴⁾

5. Environmental Dividends of FinTech-Enabled Inclusion

Recent empirical studies increasingly highlight the environmental dividends generated at the intersection of FinTech and financial inclusion. At the operational level, branchless banking and digital payments lead to resource dematerialization, significantly reducing the carbon footprint associated with branch construction, cash transportation, and paper-intensive banking documentation.[8]

Beyond operational efficiencies, FinTech acts as a primary financing mechanism for decentralized green technologies. By lowering transaction costs to fractions of a cent, FinTech enables low-income populations to adopt off-grid renewable energy, displacing kerosene lamps and diesel generators with clean solar alternatives. In agriculture, digital micro-insurance platforms linked to satellite weather data automatically trigger payouts during droughts or floods, preventing smallholder farmers from resorting to ecologically

harmful survival strategies, such as deforestation or severe soil over-exploitation. Furthermore, digital payment rails support circular economy models by facilitating instant cash-for-waste micropayments to informal recycling workers. Thus, FinTech-enabled inclusion aligns economic development with ecological conservation and climate resilience goals.⁽¹⁰⁾

V. Social Implications of Fintech Driven Financial Inclusion

1. Empowerment of women and rural communities

FinTech-driven financial inclusion significantly enhances the empowerment of women and rural populations by providing them direct access to formal financial services. Digital banking, mobile wallets, and microcredit platforms enable women to manage income, savings, and investments independently, strengthening their financial autonomy and decision-making power within households. In rural areas, where physical banking infrastructure is limited, digital financial services reduce geographical barriers and dependency on informal lenders. Access to credit and savings facilities supports small-scale entrepreneurship, agricultural investments, and self-employment, thereby improving economic independence and social status.⁽¹¹⁾

2. Increased Financial Literacy and Awareness

Digital financial platforms promote greater financial awareness by encouraging individuals to engage actively with banking systems, digital transactions, and online financial tools. Regular use of mobile banking applications and digital payment systems enhances understanding of savings, budgeting, and credit management. Exposure to formal financial services also improves knowledge about interest rates, loan terms, insurance products, and investment opportunities. Over time, this increased awareness contributes to responsible financial behavior and long-term financial planning.⁽²⁾

3. Reduction in Social and Income Inequality

By expanding access to affordable financial services, FinTech helps reduce disparities between different socio-economic groups. When low-income individuals gain access to credit, savings, and insurance, they are better positioned to participate in economic activities and improve their living standards. Digital financial inclusion narrows the gap between urban and rural populations and between formally employed and informally employed individuals. By integrating marginalized groups into the formal financial system, FinTech contributes to more equitable income distribution and social balance.⁽⁹⁾

4. Improved Access to Social Security and Welfare Schemes

FinTech-enabled digital platforms facilitate efficient and transparent delivery of government welfare benefits, subsidies, pensions, and direct benefit transfers. Digital accounts ensure that funds reach beneficiaries directly, reducing leakages, corruption, and administrative delays. This improves accountability and strengthens

trust in public institutions. Easy access to financial accounts also allows individuals to receive emergency assistance and social security benefits more efficiently, enhancing overall social protection.⁽⁶⁾

5. Greater Participation of Marginalized Groups in the Financial System

FinTech innovations encourage the inclusion of marginalized populations, including low-income households, informal workers, small entrepreneurs, and geographically isolated communities. Simplified digital onboarding processes and alternative credit assessment methods allow individuals without formal documentation or credit history to access financial services. As participation increases, these groups become integrated into the formal economic system, gaining financial identity, stability, and opportunities for growth. This broader participation strengthens social cohesion and inclusive development.^[5]

VI. ECONOMIC IMPLICATIONS OF FINTECH-DRIVEN FINANCIAL INCLUSION

1. Increased Formalization of the Economy

FinTech-driven financial inclusion promotes the formalization of economic activities by encouraging digital transactions and reducing reliance on cash-based systems. When individuals and businesses use digital payments, mobile banking, and online financial platforms, their transactions become recorded and traceable. This enhances transparency, improves tax compliance, and reduces informal economic activities. Formalization strengthens regulatory oversight, improves financial accountability, and supports structured economic development.^(8,9)

2. Growth of Micro, Small, and Medium Enterprises (MSMEs)

Access to digital financial services and alternative lending platforms significantly benefits MSMEs by providing easier and faster access to credit. Traditional banking systems often impose strict collateral requirements that small enterprises struggle to meet. FinTech solutions, particularly AI-based credit assessment and peer-to-peer lending, offer more flexible financing options. This enables MSMEs to expand operations, adopt new technologies, increase production capacity, and compete more effectively in the market, thereby contributing to overall economic dynamism.⁽⁸⁾

3. Enhanced Household Savings and Investment Patterns

Digital financial inclusion encourages structured savings behavior by providing secure and convenient savings instruments. Mobile banking and digital wallets make it easier for households to deposit, transfer, and manage funds regularly. Access to formal financial products also improves awareness of investment opportunities such as fixed deposits, insurance, and small investment schemes. As a result, households are better able to plan

for long-term financial security, manage risks, and allocate resources more efficiently.^(9,11)

4. Employment Generation and Entrepreneurship Development

FinTech innovations lower entry barriers for entrepreneurial activities by improving access to startup capital and digital financial services. Small entrepreneurs and self-employed individuals can obtain microloans and manage business transactions digitally, enhancing operational efficiency. The expansion of digital financial ecosystems also creates direct employment opportunities in technology development, financial services, customer support, and digital infrastructure management. Indirectly, improved access to finance stimulates business growth, which further generates employment opportunities across sectors.^[11]

5. Contribution to Economic Growth and Financial Stability

Broader financial inclusion strengthens overall economic performance by increasing capital circulation, improving resource allocation, and expanding economic participation. When more individuals and businesses engage in formal financial systems, savings mobilization increases, investment activity expands, and aggregate demand rises. Additionally, access to diversified financial services such as savings, credit, and insurance enhances resilience against economic shocks. Collectively, these factors contribute to sustainable economic growth and reinforce financial system stability.⁽¹²⁾

VII. Limitations

This study is subject to specific methodological limitations inherent to secondary research designs. Because the synthesis relies on published peer-reviewed literature, institutional working papers, and global development datasets, the findings are bounded by the analytical scope, data quality, and methodological approaches of the source studies. The lack of primary empirical data restricts the ability to statistically test causal pathways between specific FinTech deployments and localized socio-economic or environmental outcomes.^(10,11)

Furthermore, significant variations in national regulatory regimes, digital public infrastructure (DPI), mobile network reliability, and socio-economic contexts across developing regions limit the universal generalizability of the findings. The study primarily captures scholarship published over the last 8–10 years, which, while reflecting modern smartphone and digital wallet proliferation, may understate nascent developments in decentralized green finance or long-term longitudinal climate impacts. Rapid technological shifts and continuously evolving compliance standards in the FinTech ecosystem mean that empirical observations and regulatory models may shift over time. Consequently, these findings should be interpreted as a conceptual and systemic baseline rather than an exhaustive empirical assessment.

VIII. Future Scope of the Study

The future scope of this research lies in moving from secondary conceptual synthesis to primary empirical validation across emerging and frontier markets. Subsequent scholarship can deploy quantitative field surveys, econometric modeling, and mixed-method impact evaluations to directly quantify how digital financial penetration influences household consumption, clean energy adoption rates, MSME liquidity buffers, and poverty alleviation over time. Key avenues for future research include:

- **Empirical Green FinTech Assessment:** Measuring the exact carbon offset and fossil-fuel displacement driven by Pay-As-You-Go (PAYGo) solar microfinance and clean-cooking credit models across rural communities.
- **Cross-Jurisdictional Policy Comparisons:** Evaluating how differing regulatory sandboxes, open-banking mandates, and national digital ID architectures influence the scaling speed and safety of inclusive FinTech solutions.
- **Emerging Technologies & Climate Finance:** Investigating the practical viability of blockchain-enabled micro-carbon credits, tokenized renewable assets, and satellite-driven parametric crop insurance in shielding vulnerable smallholders from climate shocks.
- **Longitudinal Vulnerability & Governance Studies:** Assessing long-term consumer protection issues, algorithmic biases in AI credit scoring, digital literacy divides, and electronic waste (e-waste) management associated with rapid device and infrastructure turnover.

IX. Results and Discussion

The synthesis demonstrates that FinTech innovations serve as a multi-dimensional catalyst for inclusive development, breaking structural bottlenecks that legacy financial institutions have long failed to resolve. By dismantling spatial, cost, and documentation barriers, innovations such as interoperable digital payments, mobile wallets, alternative AI-based credit profiling, and decentralized microfinance platforms systematically improve financial access, affordability, usage depth, and service quality for underserved communities.

Despite these tangible dividends, the discussion identifies critical friction points. Structural digital divides—manifested in gendered smartphone ownership gaps, erratic rural connectivity, and low digital literacy—threaten to reproduce existing inequalities within digital environments. Furthermore, algorithmic biases in alternative credit scoring, predatory micro-lending practices, and escalating cybersecurity risks present acute vulnerabilities for first-time financial consumers. The literature indicates that FinTech operates as an amplifier: without proportional regulatory oversight, robust consumer protection, and deliberate green policy alignment, its transformative potential remains constrained.

X. Conclusion

This secondary study evaluated the multidimensional role of FinTech innovation in advancing financial inclusion, mapping its integrated social, economic, and environmental implications. The evidence confirms that

FinTech is far more than an operational upgrade for banking—it is a transformative socio-economic and ecological enabler that systematically lowers transaction costs, bypasses infrastructure deficits, and integrates historically marginalized populations into the formal economic mainstream.

Socially, FinTech fosters gender equity through independent financial custody, strengthens social security via transparent welfare transfers, and builds household resilience against socio-economic shocks. Economically, it bridges the chronic MSME credit deficit, formalizes fragmented commercial activities, mobilizes domestic savings, and expands employment. Crucially, from an environmental perspective, FinTech deepens sustainable development by dematerializing banking operations, financing grassroots transitions to decentralized renewable energy, and providing smallholders with automated climate-shock buffers.

Realizing the full promise of green and inclusive FinTech requires proactive collaboration among policymakers, central banks, technology providers, and development organizations. Priorities must center on closing digital infrastructure divides, instituting robust data privacy and algorithmic fairness standards, and embedding sustainability criteria into digital lending architectures. When anchored by inclusive policy frameworks and consumer safeguards, FinTech-driven financial inclusion serves as a foundational engine for equitable, climate-resilient, and sustainable global growth.

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Review Article

Use of Probiotics in Gut Health and Immunity: Mechanisms, Clinical Evidence and Future Perspectives

Vaishali¹, Dhanendra Kumar Rai²

1. UG Scholar,

2. Professor

Department of Life Sciences, Keral Verma Subharti College of Science, Swami Vivekanand Subharti University, Meerut Uttar Pradesh, (250005) India.

Abstract: Probiotics are defined as live microorganisms that confer health benefits to the host when administered in adequate amounts ⁽¹⁾. In recent decades, the study of probiotics has gained considerable attention because of their ability to regulate gut microbiota composition and improve immune function ⁽²⁾. The human gastrointestinal tract contains a complex microbial ecosystem that plays a critical role in nutrient metabolism, barrier protection, and immune regulation ⁽³⁾. Disturbance of this microbial balance, referred to as dysbiosis, has been associated with numerous diseases including inflammatory bowel disease, metabolic disorders, allergies, and infections ⁽⁴⁾. Probiotics help restore microbial equilibrium and enhance intestinal barrier integrity while modulating host immune responses ⁽⁵⁾. Several clinical trials have demonstrated the beneficial effects of probiotics in gastrointestinal disorders such as irritable bowel syndrome, antibiotic-associated diarrhea, and inflammatory bowel disease ⁽⁶⁾. This review discusses the mechanisms by which probiotics influence gut health and immunity, summarizes clinical evidence supporting their therapeutic potential, and highlights future research directions in probiotic therapy.

Keywords: Probiotics; Gut microbiota; Gut health; Immunity; Dysbiosis; Intestinal barrier; Immunomodulation; Short-chain fatty acids; Gastrointestinal disorders; Microbiome; Probiotic therapy; Gut-brain axis.

Address for correspondence: Vaishali, UG Scholar, Department of Life Sciences, Keral Verma Subharti College of Science, Swami Vivekanand Subharti University, Meerut, Uttar Pradesh, India, 250005

E-Mail: mundayvaishali@gmail.com

Contact: +91-94571-10706

1. Introduction

The human gastrointestinal tract hosts trillions of microorganisms collectively known as the gut microbiota ⁽³⁾. These microbes include bacteria, viruses, fungi, and archaea that coexist in a complex ecological network ⁽⁷⁾. The gut microbiota plays a crucial role in maintaining host health through nutrient metabolism, vitamin synthesis, and protection against pathogenic organisms ⁽⁸⁾.

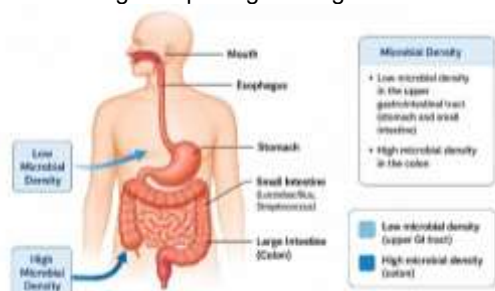


Figure 1. Structure of the Human Gut Microbiota

Probiotics are beneficial microorganisms that promote health when consumed in adequate quantities ⁽¹⁾. Early research on probiotics was inspired by observations that fermented foods containing beneficial bacteria were associated with improved health and longevity ⁽⁹⁾. The most commonly used probiotic microorganisms belong to the genera *Lactobacillus* and *Bifidobacterium*,

although yeast such as *Saccharomyces boulardii* is also widely used ⁽¹⁰⁾.

The gut microbiota interacts closely with the host immune system. Approximately 70% of the body's immune cells are located in the gastrointestinal tract, highlighting the importance of the gut as an immune organ ⁽¹¹⁾. Through interactions with immune cells and intestinal epithelial cells, gut microorganisms influence both innate and adaptive immunity ⁽¹²⁾.

Disruption of gut microbiota balance, referred to as dysbiosis, can lead to increased intestinal permeability, inflammation, and susceptibility to disease ⁽⁵⁾. Dysbiosis has been linked to conditions such as inflammatory bowel disease, obesity, diabetes, allergies, and autoimmune disorders ⁽¹³⁾.

Probiotics have been proposed as a promising strategy to restore microbial balance and improve immune function ⁽⁵⁾. These beneficial microbes exert their effects through several mechanisms including competitive exclusion of pathogens, production of antimicrobial compounds, and modulation of immune responses ⁽¹⁴⁾.

Recent advances in microbiome research have revealed that probiotic therapy may provide significant health benefits in both gastrointestinal and systemic diseases ⁽¹⁵⁾. Therefore, understanding the mechanisms and clinical applications of probiotics has become an important area of research in biotechnology and medicine.

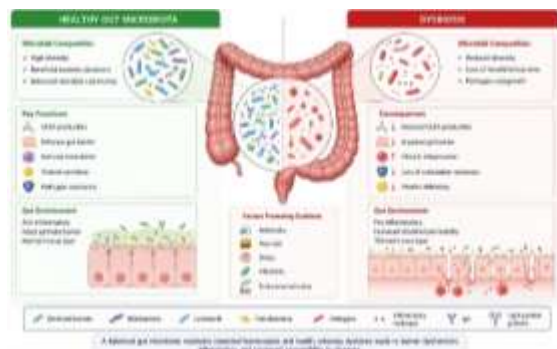


Figure 2. Composition of healthy gut microbiota and dysbiosis-associated alterations in the gastrointestinal tract.

2. Mechanisms of Probiotics in Gut Health and Immunity

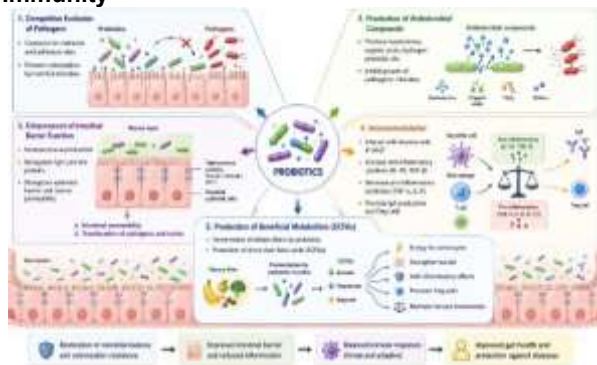


Figure 3. Major mechanisms of probiotic action in maintaining gut health and immune regulation.

2.1 Regulation of Gut Microbiota Composition

One of the primary functions of probiotics is the regulation of gut microbiota composition (2). Probiotics can compete with pathogenic microorganisms for nutrients and adhesion sites on intestinal epithelial cells (16). This competitive exclusion prevents colonization by harmful bacteria and promotes the growth of beneficial microbial communities (17).

Probiotic bacteria also produce antimicrobial compounds such as bacteriocins, hydrogen peroxide, and organic acids that inhibit pathogenic microbes (18). These compounds create an unfavorable environment for pathogenic bacteria while supporting beneficial microbial populations (19).

2.2 Enhancement of Intestinal Barrier Function

The intestinal epithelial barrier is a crucial defense mechanism that protects the host from harmful microorganisms and toxins (20). Tight junction proteins between epithelial cells regulate intestinal permeability and maintain barrier integrity (21).

Probiotics strengthen the intestinal barrier by stimulating mucus production and increasing the expression of tight junction proteins (22). This helps prevent the translocation of pathogens and toxins across the intestinal epithelium (23). Additionally, probiotics promote epithelial cell proliferation and repair of damaged intestinal tissues (24).

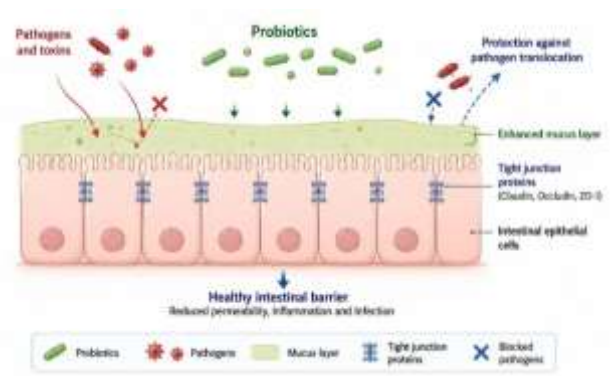


Figure 4. Role of probiotics in strengthening intestinal barrier integrity and preventing pathogen invasion.

2.3 Immunomodulatory Effects

Probiotics influence both innate and adaptive immune responses (25). They interact with immune cells such as dendritic cells, macrophages, and T lymphocytes present in the gut-associated lymphoid tissue (GALT) (26).

Probiotic microorganisms can stimulate the production of immunoglobulin A (IgA), which plays a key role in mucosal immunity (27). They also modulate cytokine production, balancing pro-inflammatory and anti-inflammatory immune responses (28). This immune regulation helps reduce chronic inflammation and maintain immune homeostasis (29).

2.4 Production of Beneficial Metabolites

Probiotic microorganisms contribute to host health by producing beneficial metabolites such as short-chain fatty acids (SCFAs) including acetate, propionate, and butyrate (30). These metabolites are produced through fermentation of dietary fibers in the colon (31). Short-chain fatty acids serve as energy sources for intestinal epithelial cells and play a critical role in regulating immune responses and inflammation (32). SCFAs also promote the differentiation of regulatory T cells, which help maintain immune tolerance (33).

2.5 Advanced Role of Microbial Metabolites in Host Immunity

Microbial metabolites derived from probiotic activity play a crucial role in maintaining host physiological and immunological balance. Among these, short-chain fatty acids (SCFAs) such as acetate, propionate, and butyrate have been extensively studied for their systemic effects beyond the gut environment (34). These metabolites not only serve as energy sources for colonocytes but also act as signaling molecules that influence host cellular pathways (35).

SCFAs regulate immune function through interaction with G-protein-coupled receptors (GPCRs) such as GPR41 and GPR43, which are expressed on immune cells including neutrophils, macrophages, and dendritic cells (36). Activation of these receptors leads to modulation of inflammatory responses and maintenance of immune homeostasis. Additionally, SCFAs have been shown to inhibit histone deacetylases (HDACs), thereby influencing gene expression related to immune regulation and inflammation (37).

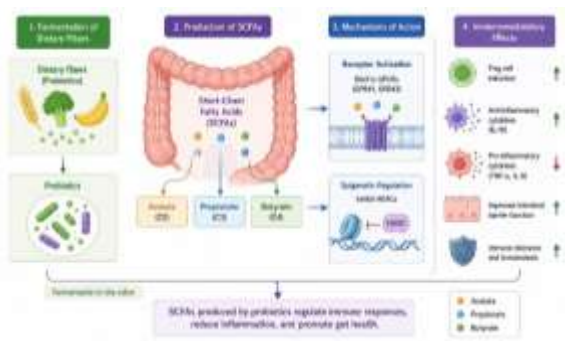


Figure 5. Production of short-chain fatty acids by probiotics and their immunomodulatory effects.

Recent studies have also demonstrated that microbial metabolites play a role in the gut–brain axis, highlighting the connection between gut microbiota and neurological function⁽³⁸⁾. SCFAs can cross the blood–brain barrier and influence neuroinflammatory pathways, suggesting their potential role in neurodegenerative and psychiatric disorders⁽³⁹⁾. This expands the functional relevance of probiotics beyond gastrointestinal health to systemic and neurological effects.



Figure 6. Role of probiotic-derived metabolites in gut-brain axis communication and systemic immunity.

Furthermore, SCFAs contribute to metabolic regulation by influencing lipid metabolism, glucose homeostasis, and energy balance⁽⁴⁰⁾. These findings suggest that probiotic-mediated production of microbial metabolites has far-reaching implications in preventing metabolic disorders such as obesity and type 2 diabetes.

3. Clinical Evidence of Probiotics in Gut Health and Immunity

3.1 Probiotics in Irritable Bowel Syndrome (IBS)

Irritable bowel syndrome (IBS) is a common gastrointestinal disorder characterized by abdominal pain, bloating, and altered bowel habits⁽⁴¹⁾. Increasing evidence suggests that disturbances in gut microbiota composition contribute to the pathogenesis of IBS⁽⁴²⁾. Probiotic therapy has emerged as a promising approach to restore microbial balance and improve symptoms in IBS patients⁽⁴³⁾.

Clinical trials have shown that probiotic strains such as *Lactobacillus* and *Bifidobacterium* can reduce abdominal pain, bloating, and intestinal discomfort⁽⁴⁴⁾. These probiotics improve gut motility, regulate intestinal permeability, and modulate inflammatory responses in the gut⁽⁴⁵⁾. Some studies also indicate

that probiotics may improve quality of life in IBS patients⁽⁴⁶⁾.

3.2 Probiotics in Antibiotic-Associated Diarrhea

Antibiotic-associated diarrhea (AAD) occurs due to disruption of normal gut microbiota following antibiotic therapy⁽⁴⁷⁾. This condition is frequently associated with the overgrowth of opportunistic pathogens such as *Clostridioides difficile*⁽⁴⁸⁾.

Several clinical trials have demonstrated that probiotic supplementation can significantly reduce the incidence of antibiotic-associated diarrhoea⁽⁴⁹⁾. Strains such as *Saccharomyces boulardii* and *Lactobacillus rhamnosus* have shown protective effects by restoring microbial balance and inhibiting pathogenic bacteria⁽⁵⁰⁾. Probiotics also enhance intestinal barrier function and reduce intestinal inflammation during antibiotic treatment⁽⁵¹⁾.

3.3 Probiotics in Inflammatory Bowel Disease

Inflammatory bowel disease (IBD) includes chronic inflammatory conditions such as Crohn's disease and ulcerative colitis⁽⁵²⁾. Dysbiosis of the gut microbiota plays a major role in the development and progression of IBD⁽⁵³⁾.

Probiotics have been investigated as a therapeutic approach for maintaining remission in patients with ulcerative colitis⁽⁵⁴⁾. Certain probiotic formulations have demonstrated anti-inflammatory effects and improved mucosal healing in clinical studies⁽⁵⁵⁾. Probiotics can regulate immune responses by modulating cytokine production and reducing intestinal inflammation⁽⁵⁶⁾.

3.4 Probiotics in Immune System Enhancement

Probiotics play a significant role in enhancing immune function by stimulating both innate and adaptive immune responses⁽⁵⁷⁾. Clinical studies suggest that probiotic supplementation can increase the production of immunoglobulin A (IgA), which provides protection against pathogens at mucosal surfaces⁽⁵⁸⁾.

Probiotics have also been shown to enhance natural killer (NK) cell activity and improve resistance to infections⁽⁵⁹⁾. Some studies indicate that probiotic consumption may reduce the risk of respiratory infections and gastrointestinal infections⁽⁶⁰⁾.

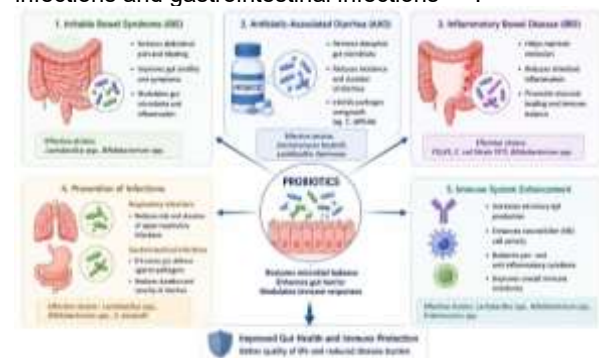


Figure 7. Clinical applications of probiotics in gastrointestinal and immune-related disorders.

4. Safety and Limitations of Probiotic Therapy

Although probiotics are generally considered safe, certain limitations and risks should be considered⁽⁶¹⁾. Most probiotic strains have been classified as safe for human consumption, particularly those belonging to lactic acid bacteria⁽⁶²⁾.

However, in rare cases, probiotic use may cause infections in immunocompromised individuals or critically ill patients⁽⁶³⁾. Therefore, careful selection of probiotic strains and proper clinical evaluation are necessary before their therapeutic use⁽⁶⁴⁾.

Another limitation of probiotic therapy is the variability in strain-specific effects⁽⁶⁵⁾. Different probiotic strains may produce different physiological outcomes, and not all strains provide the same health benefits⁽²⁾. Standardization of probiotic formulations and dosage remains an important challenge in clinical research⁽⁶⁶⁾.

5. Future Perspectives in Probiotic Research

The field of probiotic research is rapidly evolving with advances in microbiome science and biotechnology⁽⁶⁷⁾. New technologies such as metagenomics and next-generation sequencing have improved our understanding of the human gut microbiome⁽⁶⁸⁾.

Future research may focus on the development of next-generation probiotics, which include beneficial microbial species that are naturally present in the human gut but have not yet been widely used as probiotics⁽⁶⁹⁾. These microorganisms may offer more targeted therapeutic benefits for specific diseases⁽⁷⁰⁾.

Another promising area is personalized probiotic therapy, where probiotic treatments are tailored according to an individual's microbiome composition⁽⁷¹⁾. Such approaches may enhance treatment efficacy and reduce variability in clinical outcomes⁽⁷³⁾.

Additionally, the combination of probiotics with prebiotics, known as synbiotics, may further enhance gut microbial balance and improve health outcomes⁽⁷³⁾. Continued research and large-scale clinical trials are essential to establish evidence-based guidelines for probiotic therapy in human health⁽⁷⁴⁾.



Figure 8. Future perspectives and emerging approaches in probiotic and microbiome-based therapy.

6. Conclusion

Probiotics represent a promising therapeutic strategy for maintaining gut health and regulating immune function. These beneficial microorganisms exert their effects through multiple mechanisms, including modulation of gut microbiota composition, enhancement of intestinal barrier integrity, production of beneficial metabolites, and regulation of immune responses.

Clinical evidence supports the use of probiotics in several gastrointestinal disorders such as irritable bowel syndrome, antibiotic-associated diarrhea, and inflammatory bowel disease. Furthermore, probiotics

have shown potential in enhancing immune responses and reducing the risk of infections.

Despite these promising findings, further research is required to better understand strain-specific effects, optimal dosage, and long-term safety of probiotic therapy. Advances in microbiome research and biotechnology may lead to the development of next-generation probiotic therapies with improved clinical efficacy.

Ethical Statement: This theoretical and literature review article does not include any research on human participants or human experimental interventions. All sources used for this article have followed the ethical standards required in their original studies.

Conflict of Interest Statement: Authors have also declared that there are no conflicts of interest regarding this article.

Declaration: We used generative AI or AI-assisted technology, including AI agents or deep research tools.

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