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RHIZOSPHERE MICROBIOME OF MEDICINAL PLANTS: FUNCTIONAL DIVERSITY AND PHYTOCHEMICAL ENHANCEMENT

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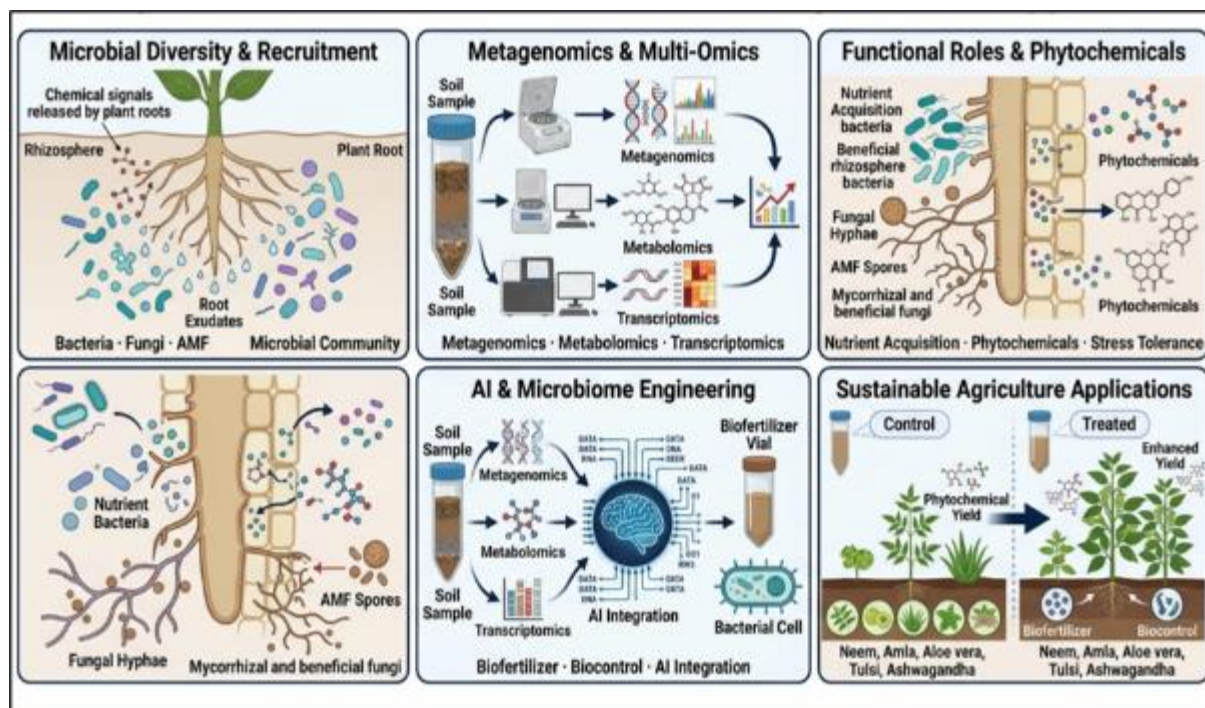
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ABSTRACT

The rhizosphere is a biologically active region where plant roots interact with diverse microorganisms that influence plant growth, soil fertility, and ecosystem functioning. In medicinal plants, these interactions are particularly important because root-associated microorganisms not only improve nutrient acquisition and stress tolerance but also affect the synthesis of pharmacologically important secondary metabolites. This review provides a comprehensive overview of the rhizosphere microbiome associated with selected medicinal plants, including *Azadirachta indica* (Neem), *Phyllanthus emblica* (Amla), *Aloe barbadensis* (Aloe vera), *Ocimum tenuiflorum* (Tulsi), and *Withania somnifera* (Ashwagandha). It discusses the ecological significance of the rhizosphere, the diversity and functional roles of beneficial microorganisms, and the influence of root exudates on microbial recruitment and community structure. Recent advances in molecular tools, particularly metagenomics and other multi-omics approaches, have considerably improved our understanding of plant microbe interactions and their contribution to phytochemical production. The review also highlights the applications of rhizosphere microorganisms in sustainable agriculture through biofertilizers, biological disease management, and enhanced stress tolerance. Despite significant progress, challenges related to environmental variability, host specificity, and the lack of standardized methodologies continue to limit field applications. Future research integrating microbiome engineering, synthetic microbial communities, and artificial intelligence is expected to improve medicinal plant cultivation, enhance phytochemical yield, and support environmentally sustainable agricultural systems.

Keywords: Rhizosphere Microbiome, Medicinal Plants, Plant–Microbe Interactions, Phytochemicals, PGPR, Metagenomics, Sustainable Agriculture, Biofertilizers.

Graphical Abstract



1 INTRODUCTION

Medicinal plants have long been recognized for their therapeutic properties and are extensively used in traditional medicine, pharmaceuticals, nutraceuticals, and cosmetic industries worldwide. Growing consumer demand for plant-based products has significantly increased their commercial and medicinal value, highlighting the need for sustainable cultivation strategies that improve both productivity and phytochemical quality. The increasing industrial utilization of medicinal plants has intensified research efforts to identify eco-friendly approaches that enhance plant growth, bioactive compound production, and soil health while minimizing dependence on synthetic agrochemicals. Among these approaches, rhizosphere microbiome-mediated cultivation has emerged as a promising strategy for improving the quality and sustainability of medicinal plant production **Figure 1**. The rhizosphere, defined as the narrow zone of soil directly influenced by plant roots, is one of the most biologically active interfaces on Earth where complex interactions occur among plant roots, soil, and microorganisms. This dynamic environment serves as a hotspot for biochemical transformations, nutrient cycling, and microbial activity, thereby playing a crucial role in maintaining soil fertility and plant health [1], [2]. The rhizosphere comprises distinct microenvironments, including the rhizoplane and endosphere, each harboring specialized microbial communities that interact continuously with plant roots. These microbial assemblages are shaped by root exudates, soil physicochemical properties, and environmental conditions, collectively influencing plant growth, nutrient availability, and ecosystem functioning [3],[4]. Recent advances in rhizosphere microbiology have highlighted the critical role of beneficial microorganisms in sustainable agriculture and environmental resilience. Rhizospheric bacteria, fungi, actinomycetes, and other microorganisms promote plant growth through diverse mechanisms such as biological nitrogen fixation, phosphate solubilization, siderophore production, phytohormone synthesis, and the secretion of extracellular enzymes [5],[6]. Plant growth-promoting rhizobacteria (PGPR) further enhance plant performance by suppressing phytopathogens through antimicrobial metabolite production and induced systemic resistance, while simultaneously improving plant tolerance to drought, salinity, heavy metals, and other abiotic stresses [7],[8]. These multifunctional microbial interactions have positioned the rhizosphere microbiome as an essential component of sustainable crop production systems. Medicinal plants represent a unique group of species capable of synthesizing a wide range of pharmacologically important secondary metabolites, including alkaloids, flavonoids, phenolics, terpenoids, glycosides, and essential oils. These compounds not only determine their therapeutic efficacy but also influence the composition and functional diversity of rhizospheric microbial communities [9],[10]. Species-specific root exudates selectively recruit beneficial microorganisms that improve nutrient acquisition, suppress pathogenic microbes, and stimulate the biosynthesis of bioactive phytochemicals [11]. Consequently, the rhizosphere microbiome performs a dual function by supporting plant growth while enhancing the quality and quantity of medicinally valuable metabolites. Furthermore, the rhizosphere itself is increasingly recognized as a reservoir of novel microorganisms capable of producing bioactive compounds with potential pharmaceutical and biotechnological applications [12]. Despite substantial progress in understanding plant–microbe interactions, significant knowledge gaps remain regarding the diversity, ecological functions, and molecular mechanisms governing rhizospheric microbial communities in medicinal plants. Microbial community composition varies considerably with plant genotype, root

exudate chemistry, soil characteristics, climatic conditions, and agricultural management practices [13]. Comparative studies have demonstrated that different medicinal plant species harbor distinct microbial assemblages; however, the ecological drivers responsible for these variations remain poorly understood [14]. Moreover, continuous cultivation may alter rhizospheric microbial diversity through changes in root exudation patterns and allelochemical accumulation, potentially affecting soil health, microbial functionality, and phytochemical productivity [15]. The advent of advanced molecular approaches, particularly high-throughput sequencing, metagenomics, metatranscriptomics, and metabolomics, has greatly expanded our understanding of rhizosphere microbial diversity and functional potential. These technologies facilitate the identification of both culturable and unculturable microorganisms while providing insights into microbial interactions, metabolic pathways, and plant-microbe communication [16]. Nevertheless, integrative studies combining microbial profiling with functional analyses remain limited, especially for medicinal plant ecosystems. Therefore, this review critically examines the rhizosphere microbiome associated with five economically and medicinally important plant species like Neem (*Azadirachta indica*), Amla (*Phyllanthus emblica*), Aloe vera (*Aloe barbadensis*), Tulsi (*Ocimum tenuiflorum*), and Ashwagandha (*Withania somnifera*). It synthesizes current knowledge on rhizospheric microbial diversity, functional interactions, molecular approaches, and their influence on phytochemical production. Additionally, the review highlights recent advances, existing knowledge gaps, and future prospects for utilizing the rhizosphere microbiome in sustainable agriculture and the cultivation of high-value medicinal plants.

2 CONCEPTUAL FRAMEWORK RHIZOSPHERE MICROBIOLOGY

2.1 Concept of Rhizosphere

The rhizosphere refers to the narrow zone of soil that is directly influenced by plant root activities and serves as a dynamic interface between plant roots and soil microorganisms. This region is characterized by intense biological and chemical interactions driven primarily by root exudates, mucilage, and sloughed-off cells, which collectively create a nutrient-rich microenvironment [11],[2]. The rhizosphere is typically divided into three zones: the endosphere (internal root tissues), the rhizoplane (root surface), and the ectorrhizosphere (soil immediately surrounding the roots). Each of these zones harbors distinct microbial communities that differ in composition and function. Root exudates play a central role in shaping rhizospheric interactions by releasing a wide array of organic compounds such as sugars, amino acids, organic acids, phenolics, and secondary metabolites. These compounds act as signaling molecules and energy sources for microorganisms, thereby influencing microbial colonization and activity [3]. The rhizosphere is therefore considered a hotspot of microbial diversity and metabolic activity, where beneficial, neutral, and pathogenic microorganisms coexist and interact with plant roots. Recent advances in metagenomic approaches have further highlighted the complexity of rhizospheric microbial communities, revealing their critical role in maintaining soil health and ecosystem stability [4].

2.2 Importance of Rhizospheric Microorganisms

Rhizospheric microorganisms are essential components of soil ecosystems, contributing significantly to plant growth, nutrient cycling, and disease suppression. These microorganisms include bacteria, fungi, actinomycetes, and archaea, many of which establish beneficial associations with plant roots. Among them, plant growth-promoting rhizobacteria (PGPR) play a pivotal role in enhancing plant productivity through various direct and indirect mechanisms [17]. Direct mechanisms include nitrogen fixation, phosphate solubilization, potassium mobilization, and the production of phytohormones such as auxins, gibberellins, and cytokinins, which promote root development and overall plant growth. Indirectly, rhizospheric microorganisms contribute to plant protection by producing antibiotics, siderophores, hydrogen cyanide, and lytic enzymes that inhibit the growth of plant pathogens [18]. Additionally, many microorganisms induce systemic resistance in plants, thereby enhancing their defense against biotic and abiotic stresses. The role of rhizospheric microorganisms extends beyond plant growth promotion to maintaining soil fertility and structure. They are actively involved in organic matter decomposition, humus formation, and biogeochemical cycling of essential nutrients such as carbon, nitrogen, and phosphorus [15]. Thus, rhizospheric microorganisms are indispensable for sustainable agriculture and environmental resilience.

2.3 Significance of Medicinal Plants in Soil Microbiology

Medicinal plants hold a unique position in soil microbiology due to their ability to produce a wide range of bioactive compounds that influence rhizospheric microbial communities. These plants synthesize secondary metabolites such as alkaloids, flavonoids, terpenoids, and essential oils, which are released into the rhizosphere through root exudation [19]. These compounds can selectively stimulate beneficial microorganisms while suppressing pathogenic populations, thereby shaping a distinct microbial community structure. The interaction between medicinal plants and rhizospheric microorganisms is often mutually beneficial. Microorganisms enhance plant growth and nutrient uptake, while plants provide carbon sources and ecological niches for microbial survival. Importantly, recent studies suggest that rhizospheric microbes can influence the biosynthesis and accumulation of medicinally important phytochemicals,

thereby enhancing the therapeutic value of these plants [20]. Furthermore, medicinal plants are often cultivated in marginal or stress-prone environments, where rhizospheric microorganisms play a crucial role in improving plant tolerance to environmental stresses such as drought, salinity, and heavy metal toxicity [21]. This highlights the importance of studying plant–microbe interactions in medicinal plant systems for both agricultural and pharmaceutical applications.

3 CONCEPTUAL FRAMEWORK OF RHIZOSPHERE MICROBIOLOGY

3.1 Rhizosphere: Structure, Components and Ecological Significance

The rhizosphere is the narrow zone of soil directly influenced by plant roots and represents one of the most biologically active interfaces in terrestrial ecosystems. Originally described by Hiltner in 1904, the rhizosphere is now recognized as a dynamic environment where continuous interactions occur among plant roots, soil particles, and diverse microbial communities. Compared with bulk soil, the rhizosphere possesses greater microbial biomass, higher enzymatic activity, and rapid nutrient turnover because of the continuous release of root-derived organic compounds [2]. Structurally, the rhizosphere is divided into three interconnected compartments: the endosphere, comprising internal root tissues colonized by endophytes; the rhizoplane, representing the root surface where microorganisms establish biofilms; and the ectorhizosphere, the thin layer of soil immediately surrounding the roots that supports intense microbial activity [22].

Plants release a wide range of compounds including carbohydrates, amino acids, organic acids, vitamins, mucilage, phenolics, and secondary metabolites into this region. These exudates alter soil physicochemical properties and create nutrient-rich microhabitats that selectively recruit beneficial microorganisms while limiting pathogen establishment [11]. Consequently, the rhizosphere functions as an ecological hotspot for nutrient cycling, organic matter decomposition, and plant defense. Rhizospheric microorganisms enhance nutrient availability through nitrogen fixation, phosphate solubilization, potassium mobilization, and micronutrient acquisition while simultaneously protecting plants against pathogens through antibiotic production and induced systemic resistance. Therefore, the rhizosphere is increasingly regarded as the functional interface linking plant physiology, soil fertility, and ecosystem sustainability, making it a fundamental component of sustainable agriculture and medicinal plant cultivation [3].

3.2 Rhizosphere Microbiome and Plant–Microbe Interactions

The rhizosphere microbiome comprises diverse communities of bacteria, fungi, archaea, actinomycetes, viruses, and protozoa that collectively regulate plant growth and soil ecosystem functions. These microorganisms establish mutualistic, commensal, and competitive interactions that influence nutrient cycling, disease suppression, and environmental adaptation. Among them, plant growth-promoting rhizobacteria (PGPR), including species of *Pseudomonas*, *Bacillus*, *Rhizobium*, and *Azospirillum*, play crucial roles by fixing atmospheric nitrogen, solubilizing phosphate, producing siderophores, synthesizing phytohormones, and lowering stress-induced ethylene through ACC deaminase activity [18]. Whereas, fungal communities also contribute substantially to rhizosphere functioning. Arbuscular mycorrhizal fungi (AMF) enhance phosphorus uptake, improve water-use efficiency, and increase plant tolerance to abiotic stresses such as drought and salinity. Likewise, beneficial fungi including *Trichoderma* spp. suppress soil-borne pathogens through competition, mycoparasitism, and production of antimicrobial metabolites. Actinomycetes participate in decomposition of complex organic matter and synthesize numerous antibiotics that contribute to biological disease control [19]. Plant–microbe interactions are largely mediated by root exudates, which serve as nutrient sources and signaling molecules that regulate microbial recruitment and colonization. Flavonoids, phenolic acids, and other secondary metabolites selectively enrich beneficial microbial taxa while suppressing pathogenic populations, thereby creating species-specific microbial assemblages. These interactions improve nutrient acquisition, stimulate secondary metabolite biosynthesis, strengthen plant immunity, and enhance resilience against environmental stresses. Recent studies emphasize that the rhizosphere microbiome functions as an extension of the plant genome, significantly influencing plant phenotype, productivity, and medicinal quality through complex biochemical communication networks [4,19].

3.3 Factors Shaping Rhizosphere Microbiome and Emerging Analytical Approaches

The composition and functional diversity of the rhizosphere microbiome are regulated by multiple interacting biotic and abiotic factors. Plant genotype is considered one of the primary determinants because different plant species and cultivars produce distinct root architectures and exudate compositions that selectively recruit specific microbial communities. Soil characteristics, including pH, texture, moisture, organic matter content, nutrient availability, and aeration, further influence microbial diversity and metabolic activity. Climatic variables such as temperature, rainfall, drought, and salinity also alter microbial community composition by favoring stress-tolerant microorganisms under adverse environmental conditions (Verma et al., 2024; Wang et al., 2023). Agricultural management practices significantly affect rhizospheric microbial ecology. Organic farming, crop rotation, conservation tillage, and application of biofertilizers generally promote microbial diversity and beneficial functional groups, whereas excessive use of

chemical fertilizers and pesticides often reduces microbial richness and disrupts ecological balance. Continuous monocropping may lead to the accumulation of soil-borne pathogens and decline in beneficial microorganisms, ultimately affecting soil health and crop productivity [15]. Recent advances in molecular biology have transformed rhizosphere research. High-throughput sequencing, metagenomics, metatranscriptomics, metabolomics, and metaproteomics now enable comprehensive characterization of both culturable and unculturable microorganisms and their functional genes. These multi-omics approaches provide valuable insights into microbial interactions, nutrient cycling, and stress adaptation. Integration of artificial intelligence, machine learning, and systems biology further facilitates prediction of microbial networks and development of synthetic microbial consortia for sustainable agriculture. Such advanced analytical tools are revolutionizing our understanding of rhizosphere ecology and offer new opportunities for microbiome engineering, precision agriculture, and enhanced cultivation of medicinal plants [4].

4 RHIZOSPHERIC MICROORGANISMS AND THEIR FUNCTIONAL DIVERSITY

Rhizospheric microorganisms constitute a highly diverse community of bacteria, fungi, archaea, actinomycetes, protozoa, and viruses inhabiting the soil immediately surrounding plant roots. These microorganisms establish intricate interactions with plants that regulate nutrient acquisition, plant growth, soil fertility, and ecosystem resilience. The rhizosphere is often regarded as the plant's "second genome" because its microbial inhabitants contribute numerous metabolic functions that complement plant physiology [4,19]. Among the microbial groups, plant growth-promoting rhizobacteria (PGPR) such as *Pseudomonas*, *Bacillus*, *Azospirillum*, *Rhizobium*, and *Burkholderia* play a pivotal role in enhancing plant productivity. These bacteria fix atmospheric nitrogen, solubilize phosphate and potassium, synthesize phytohormones including indole-3-acetic acid (IAA), produce siderophores for iron acquisition, and express ACC deaminase that alleviates ethylene-mediated stress. In addition, they suppress soil-borne pathogens through antibiotic production, lytic enzymes, biofilm formation, quorum sensing, and induction of systemic resistance (ISR) in plants [22]. Rhizospheric fungi, particularly arbuscular mycorrhizal fungi (*Glomeromycota*) and *Trichoderma* spp., enhance phosphorus uptake, improve water-use efficiency, strengthen soil aggregation, and increase tolerance to drought, salinity, and heavy metal stress. Actinomycetes, especially *Streptomyces*, contribute to organic matter decomposition and produce a wide spectrum of antimicrobial metabolites that suppress phytopathogens. Archaea participate in nitrogen transformation through ammonia oxidation, while bacteriophages regulate microbial population dynamics and maintain ecological balance. Collectively, these microorganisms drive carbon sequestration, nutrient cycling, and disease suppression while sustaining soil health [23]. Recent advances in metagenomics, metabolomics, and synthetic microbial community (SynCom) engineering have revealed that rhizospheric microorganisms function as interconnected microbial networks rather than isolated species. Understanding their functional diversity offers promising opportunities for developing next-generation biofertilizers, biocontrol agents, and climate-resilient agricultural systems, thereby reducing dependence on chemical fertilizers and pesticides while improving sustainable crop production.

5 MEDICINAL PLANTS AS DRIVERS OF RHIZOSPHERE MICROBIOME

Medicinal plants are increasingly recognized as key drivers of rhizosphere microbiome composition owing to their ability to produce a wide array of bioactive secondary metabolites and unique root exudates. Unlike conventional crops, medicinal plants synthesize compounds such as alkaloids, flavonoids, phenolics, terpenoids, saponins, and essential oils that not only contribute to their therapeutic properties but also selectively influence the diversity, abundance, and functional activities of rhizospheric microorganisms [11]. These phytochemicals act as signaling molecules and nutrient sources that recruit beneficial microorganisms while suppressing pathogenic microbes, thereby creating a plant-specific microbial niche. Root exudates released by medicinal plants shape microbial community assembly through a process known as the rhizosphere effect. Sugars, amino acids, organic acids, and secondary metabolites selectively enrich plant growth-promoting rhizobacteria (PGPR), arbuscular mycorrhizal fungi (AMF), and beneficial actinomycetes. These microorganisms, in turn, enhance nutrient acquisition through nitrogen fixation, phosphate solubilization, siderophore production, and phytohormone synthesis while improving plant resistance against pathogens and abiotic stresses [12]. Recent studies further demonstrate that medicinal plant-associated microbiomes contribute to increased biosynthesis of pharmacologically important metabolites, indicating a bidirectional relationship between plants and their microbial partners [24]. The composition of the rhizosphere microbiome varies considerably among medicinal plant species due to differences in root architecture, exudate chemistry, soil properties, climatic conditions, and cultivation practices. For example, *Azadirachta indica* (neem) recruits antimicrobial bacteria capable of suppressing phytopathogens, whereas *Withania somnifera* (ashwagandha) harbors microbial communities associated with enhanced withanolide production. Similarly, *Ocimum tenuiflorum* (tulsi) supports diverse PGPR populations owing to its phenolic-rich root exudates [11]. Advances in metagenomics, metabolomics, and synthetic microbial community engineering have further revealed that medicinal plants actively shape microbial networks that improve soil health, crop productivity, and phytochemical quality. Understanding these plant-microbiome interactions provide new opportunities for developing microbiome-based biofertilizers, sustainable medicinal plant cultivation strategies, and improved pharmaceutical raw materials.

6 COMPARATIVE RHIZOSPHERE CHARACTERISTICS OF MEDICINAL PLANTS

Medicinal plants harbor distinct rhizosphere microbiomes that are largely shaped by their botanical characteristics, root architecture, and phytochemical composition. Species such as Neem (*Azadirachta indica*), Amla (*Phyllanthus emblica*), Aloe vera (*Aloe barbadensis* Mill.), Tulsi (*Ocimum tenuiflorum*), and Ashwagandha (*Withania somnifera*) release unique root exudates containing alkaloids, flavonoids, terpenoids, phenolics, saponins, and organic acids, which selectively recruit beneficial microbial communities. These metabolites determine the abundance and functional diversity of plant growth-promoting rhizobacteria (PGPR), arbuscular mycorrhizal fungi (AMF), actinomycetes, and other beneficial microorganisms that contribute to nutrient acquisition, disease suppression, and enhanced phytochemical biosynthesis [24].

Table 1: Comparative Rhizosphere Characteristics of Selected Medicinal Plants

Medicinal plant	Dominant bioactive compounds	Dominant rhizosphere microorganisms	Major functional roles
<i>Azadirachta indica</i> (Neem)	Azadirachtin, Nimbin	<i>Bacillus</i> , <i>Pseudomonas</i> , <i>Streptomyces</i>	Disease suppression, nutrient mobilization
<i>Phyllanthus emblica</i> (Amla)	Vitamin C, tannins, gallic acid	AMF, <i>Rhizobium</i> , <i>Bacillus</i>	Nutrient uptake, antioxidant enhancement
<i>Aloe barbadensis</i>	Aloin, Aloe-emodin	Actinomycetes, <i>Bacillus</i>	Drought tolerance, soil health
<i>Ocimum tenuiflorum</i> (Tulsi)	Eugenol, Rosmarinic acid	<i>Pseudomonas</i> , <i>Trichoderma</i>	Biocontrol, phytochemical enhancement
<i>Withania somnifera</i> (Ashwagandha)	Withanolides	<i>Bradyrhizobium</i> , <i>Streptomyces</i> , PGPR	Secondary metabolite production, stress tolerance

Table 2: Functional Diversity of Major Rhizospheric Microorganisms

Microbial group	Major mechanism	Agricultural benefit	Example genera
Nitrogen-fixing bacteria	Biological nitrogen fixation	Improved nitrogen nutrition	<i>Rhizobium</i> , <i>Azospirillum</i>
Phosphate-solubilizing bacteria	Organic acid production	Enhanced phosphorus availability	<i>Bacillus</i> , <i>Pseudomonas</i>
Plant Growth-Promoting Rhizobacteria (PGPR)	IAA, siderophores, ACC deaminase	Plant growth promotion	<i>Pseudomonas</i> , <i>Bacillus</i>
Arbuscular mycorrhizal fungi	Hyphal nutrient transport	Increased P uptake and drought tolerance	<i>Glomus</i> spp.
Actinomycetes	Antibiotic and enzyme production	Disease suppression	<i>Streptomyces</i>
Endophytic microbes	Hormone production and ISR	Stress tolerance and phytochemical enhancement	<i>Enterobacter</i> , <i>Burkholderia</i>

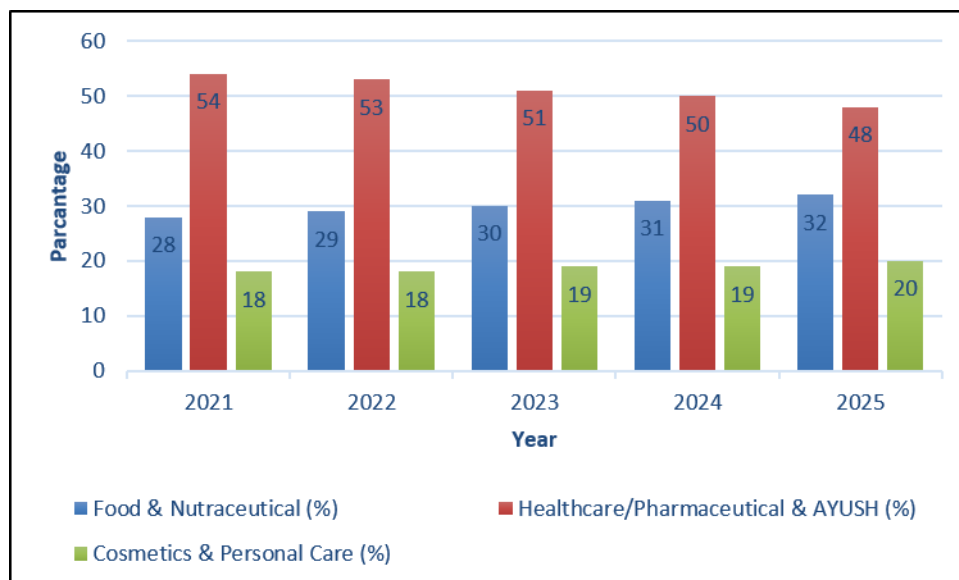


Figure 1: Estimated distribution of medicinal plant utilization in India (2021–2025)

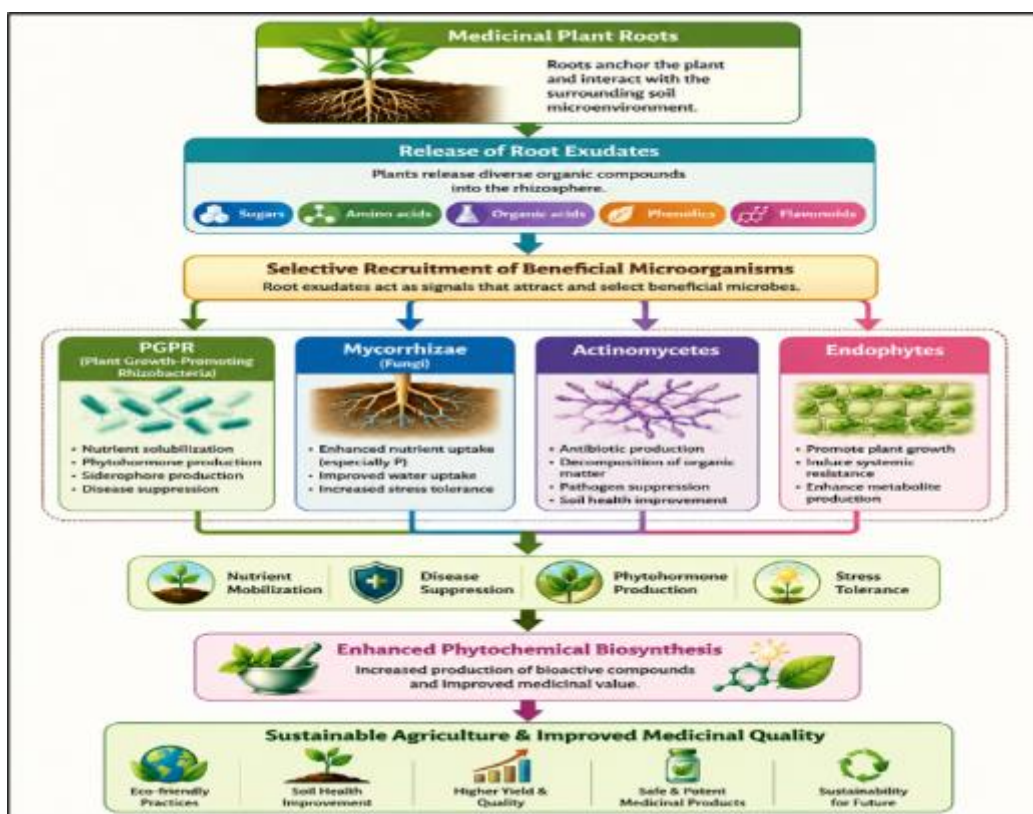


Figure 2: Functional Role of the Rhizosphere Microbiome in Medicinal Plants

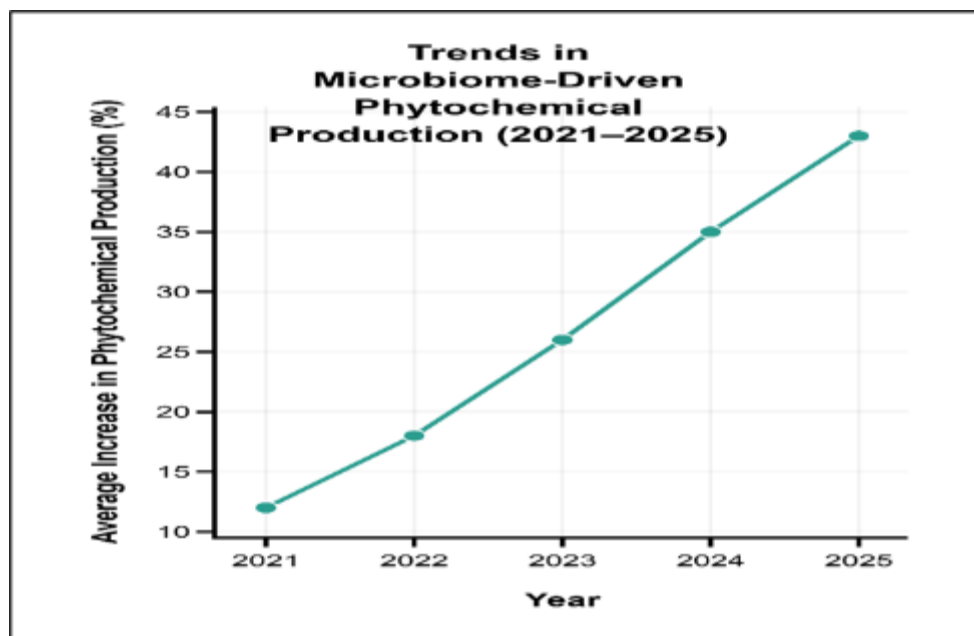


Figure 3: Influence of Rhizosphere Microbiome on Phytochemical Production (Literature-based Trend, 2021–2025)

Among these medicinal plants, Neem possesses antimicrobial limonoids such as azadirachtin and nimbin that favor populations of *Bacillus*, *Pseudomonas*, and *Streptomyces*, thereby enhancing biological control against soil borne pathogens. Amla supports diverse nitrogen-fixing bacteria and mycorrhizal fungi because of its polyphenol rich rhizosphere, improving nutrient cycling and soil fertility. Aloe vera, adapted to arid environments, generally hosts comparatively lower microbial diversity but selectively enriches drought-tolerant bacteria and actinomycetes involved in stress adaptation. Tulsi produces eugenol- and phenolic-rich exudates that promote PGPR while suppressing pathogenic fungi, whereas Ashwagandha exhibits one of the richest rhizosphere microbiomes, dominated by *Actinomycetota*, *Pseudomonadota*, *Bradyrhizobium*, and *Streptomyces*, which enhance withanolide biosynthesis, nutrient mobilization, and disease resistance. Recent shotgun metagenomic studies further demonstrate that genotype and geographical origin strongly influence the composition and functional potential of the Ashwagandha microbiome, highlighting the importance of host-specific microbial recruitment [18].

Comparatively, all five medicinal plants harbor core microbial taxa involved in nitrogen fixation, phosphate solubilization, siderophore production, phytohormone synthesis, and induced systemic resistance. However, differences in phytochemical profiles generate distinct microbial assemblages and ecological functions. Advances in metagenomics and multi-omics have confirmed that these medicinal plant-associated microbiomes are integral to improving soil health, enhancing secondary metabolite production, and supporting sustainable cultivation through microbiome-based biofertilizers and biocontrol strategies.

7 INFLUENCE OF RHIZOSPHERE MICROBIOME ON PHYTOCHEMICAL PRODUCTION

The rhizosphere microbiome plays a crucial role in regulating the biosynthesis and accumulation of phytochemicals in medicinal plants by modulating nutrient availability, hormonal signaling, and plant defense pathways. Beneficial microorganisms, particularly plant growth-promoting rhizobacteria (PGPR), arbuscular mycorrhizal fungi (AMF), and endophytic bacteria, enhance the production of secondary metabolites such as alkaloids, flavonoids, terpenoids, phenolics, glycosides, and essential oils. These microorganisms improve nutrient uptake through nitrogen fixation, phosphate solubilization, and siderophore production while stimulating phytohormone synthesis, thereby promoting plant growth and metabolic activity [24,4]. Microbial colonization also activates plant defense mechanisms, leading to induced systemic resistance (ISR) and the upregulation of genes involved in secondary metabolite biosynthesis. Recent studies have shown that rhizospheric microorganisms significantly enhance the accumulation of bioactive compounds such as withanolides in *Withania somnifera*, eugenol in *Ocimum tenuiflorum*, and azadirachtin in *Azadirachta indica*, thereby improving their medicinal quality and therapeutic potential [18]. Furthermore, microbial metabolites and signaling molecules influence metabolic pathways through complex plant-microbe interactions, resulting in improved phytochemical diversity and yield. Advances in metagenomics and metabolomics have further demonstrated that manipulating the rhizosphere microbiome offers a promising strategy for enhancing phytochemical production, supporting sustainable cultivation, and improving the pharmaceutical value of medicinal plants [11]. Recent advances in molecular biology have revolutionized the study of rhizosphere microbiology by enabling comprehensive characterization of microbial diversity, functional genes, and plant-microbe interactions. High-throughput sequencing

(HTS), particularly 16S rRNA and Internal Transcribed Spacer (ITS) sequencing, is widely employed to identify bacterial and fungal communities, including unculturable microorganisms. Shotgun metagenomics provides detailed insights into microbial genomes and metabolic pathways involved in nutrient cycling, stress tolerance, and disease suppression [4]. Complementary multi-omics approaches, including metatranscriptomics, metaproteomics, and metabolomics, reveal gene expression, protein synthesis, and metabolite production within the rhizosphere, thereby improving our understanding of microbial functionality under varying environmental conditions [24]. Quantitative PCR (qPCR), fluorescence in situ hybridization (FISH), and confocal laser scanning microscopy (CLSM) are frequently used to quantify microbial populations and visualize their spatial distribution on root surfaces. More recently, artificial intelligence (AI), machine learning, and systems biology have been integrated with multi-omics datasets to predict microbial interactions and design synthetic microbial consortia for sustainable agriculture. These advanced molecular approaches provide valuable tools for developing microbiome-based biofertilizers, enhancing medicinal plant productivity, and improving soil health under changing climatic conditions [11].

8 MOLECULAR APPROACHES IN RHIZOSPHERE MICROBIOLOGY

Recent advances in molecular biology have revolutionized the study of rhizosphere microbiology by enabling comprehensive characterization of microbial diversity, functional genes, and plant-microbe interactions. High-throughput sequencing (HTS), particularly 16S rRNA and Internal Transcribed Spacer (ITS) sequencing, is widely employed to identify bacterial and fungal communities, including unculturable microorganisms. Shotgun metagenomics provides detailed insights into microbial genomes and metabolic pathways involved in nutrient cycling, stress tolerance, and disease suppression [25]. Complementary multi-omics approaches, including metatranscriptomics, metaproteomics, and metabolomics, reveal gene expression, protein synthesis, and metabolite production within the rhizosphere, thereby improving our understanding of microbial functionality under varying environmental conditions [24]. Quantitative PCR (qPCR), fluorescence in situ hybridization (FISH), and confocal laser scanning microscopy (CLSM) are frequently used to quantify microbial populations and visualize their spatial distribution on root surfaces. More recently, artificial intelligence (AI), machine learning, and systems biology have been integrated with multi-omics datasets to predict microbial interactions and design synthetic microbial consortia for sustainable agriculture. These advanced molecular approaches provide valuable tools for developing microbiome-based biofertilizers, enhancing medicinal plant productivity, and improving soil health under changing climatic conditions [11].

9 APPLICATIONS, CHALLENGES, AND FUTURE PERSPECTIVES OF RHIZOSPHERE MICROBIOME

The rhizosphere microbiome has become an integral component of sustainable agriculture by improving crop productivity, soil fertility, and ecosystem stability. Beneficial microorganisms function as biofertilizers through biological nitrogen fixation, phosphate solubilization, and micronutrient mobilization, while also acting as biopesticides by producing antimicrobial metabolites and suppressing soil-borne pathogens. These microbial interactions enhance plant resilience against drought, salinity, heavy metals, and temperature fluctuations, thereby supporting climate-resilient and organic farming systems with reduced dependence on synthetic agrochemicals [23]. Recent research also highlights the role of rhizosphere microbial consortia in improving nutrient-use efficiency and promoting sustainable crop production under changing environmental conditions. Despite these advances, several challenges limit field-scale applications. Rhizosphere microbial communities vary with plant genotype, soil type, climatic conditions, and cultivation practices, resulting in inconsistent performance of microbial inoculants. Additional constraints include host specificity, insufficient standardization of experimental protocols, cultivation bias, and limited integration of multi-omics datasets for functional validation [26]. Future research should emphasize artificial intelligence-assisted microbiome analysis, synthetic microbial communities, precision agriculture, rhizosphere engineering, and microbiome-based bioformulations. Integrating these emerging technologies will facilitate personalized cultivation strategies for medicinal plants, improve phytochemical quality, and accelerate the development of environmentally sustainable agricultural systems [22,23].

10 CONCLUSION

The rhizosphere microbiome plays a fundamental role in regulating plant growth, nutrient cycling, soil fertility, and the biosynthesis of bioactive compounds in medicinal plants. Comparative evaluation of medicinal species such as *Azadirachta indica*, *Phyllanthus emblica*, *Aloe barbadensis*, *Ocimum tenuiflorum*, and *Withania somnifera* demonstrates that variations in root exudate composition and phytochemical profiles significantly influence rhizospheric microbial diversity and functionality. Beneficial microorganisms enhance nutrient acquisition, suppress plant pathogens, improve abiotic stress tolerance, and contribute to the accumulation of therapeutically important secondary metabolites. Recent advances in metagenomics, metabolomics, and other multi-omics approaches have substantially improved our understanding of plant-microbe interactions, providing opportunities for microbiome-based biofertilizers, biocontrol agents, and sustainable medicinal plant cultivation. Nevertheless, challenges related to environmental variability, host specificity, and methodological standardization remain. Future integration of artificial intelligence, synthetic microbial

communities, and precision microbiome engineering is expected to optimize rhizosphere management, enhance phytochemical production, and support climate-resilient, environmentally sustainable agricultural systems.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

Disclosure of conflict of interest

The authors declare no conflicts of interest regarding this manuscript.

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