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PHYTOCHEMICAL-BASED ANTIBIOFILM THERAPEUTICS FOR GUT-BRAIN HEALTH: FROM MICROBIOME MODULATION TO TRANSLATIONAL ROADMAPS.

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ABSTRACT

Intestinal biofilms play a critical role in shaping gut microbial ecology and have emerged as key drivers of dysbiosis, chronic inflammation, and impaired gut–brain communication. These structured microbial communities exhibit enhanced resistance to host defenses and antimicrobial agents, enabling persistent mucosal colonization and sustained neuroimmune activation. Growing evidence indicates that phytochemicals represent a promising class of antibiofilm agents capable of restoring microbial balance while supporting gut barrier integrity and neuroprotective signaling.

This review synthesizes current literature on biofilm-associated pathobionts and their impact on intestinal and neurological health, with a focus on the antibiofilm mechanisms of major phytochemical classes, including polyphenols, alkaloids, terpenoids, and quinones. Particular emphasis is placed on quorum-sensing inhibition, disruption of extracellular polymeric substances, modulation of microbial gene expression, and reshaping of gut microbiota composition. The role of phytochemicals in enhancing short-chain fatty acid production, strengthening epithelial tight junctions, and attenuating inflammatory pathways relevant to the gut–brain axis is critically examined.

Advances in nanotechnology-based delivery systems that improve phytochemical stability, bioavailability, and targeted intestinal release are also discussed, alongside insights from animal models used to evaluate gut–brain outcomes. Finally, translational challenges and future directions for integrating phytochemical antibiofilm strategies into functional foods and therapeutics are outlined. Collectively, this review highlights phytochemicals as multifunctional ecological modulators with significant potential for managing microbiota-driven intestinal and neurological disorders.

KEYWORDS: Phytochemicals; Antibiofilm therapeutics; Gut-brain axis; Microbiota modulation; Polyphenols; Functional foods.

INTRODUCTION

The gut-brain axis represents a dynamic bidirectional communication network that integrates the central nervous system (CNS), the enteric nervous system, the immune system, and the intestinal microbiota [1]. This complex system regulates key physiological processes such as stress response, mood regulation, appetite control, and energy metabolism. Disturbances in this axis are increasingly implicated in the pathogenesis of neurological and metabolic disorders, including depression, anxiety, Alzheimer's disease, obesity, and diabetes [2-4]. Central to this bidirectional interaction is the gut microbiota, which produces metabolites such as short-chain fatty acids (SCFAs), tryptophan derivatives, and secondary bile acids that modulate neuronal signaling, inflammation, and neurogenesis [5,6].

However, the stability and functionality of this microbial ecosystem are often compromised by biofilm formation. Biofilms are highly organized, surface-associated microbial communities encased in a self-produced extracellular polymeric substance (EPS) matrix composed of polysaccharides, proteins, lipids, and extracellular DNA [7]. Within biofilms, microorganisms exhibit phenotypic plasticity and enhanced tolerance to antibiotics, oxidative stress, and immune surveillance. These resilient microbial consortia enable chronic persistence within the gut mucosa, promoting low-grade inflammation, epithelial barrier disruption, and microbial dysbiosishallmarks of several gut and brain disorders [8,9]. Intestinal biofilms dominated by *Escherichia coli*, *Clostridium difficile*, and *Candida albicans* have been linked to inflammatory bowel disease, irritable bowel syndrome, colorectal cancer, and neuroinflammation [10].

Microbial biofilms play a dual role in health and disease. While commensal biofilms contribute to mucosal homeostasis and colonization resistance, pathogenic biofilms impede mucosal healing, increase epithelial permeability, and drive the translocation of pro-inflammatory bacterial products such as lipopolysaccharides (LPS) and peptidoglycans into systemic circulation. This systemic dissemination activates toll-like receptor (TLR)-mediated neuroimmune pathways and alters vagal signaling, ultimately perturbing brain function [11]. Thus, controlling intestinal biofilms is critical not only for maintaining microbiota balance but also for preserving the neuroimmune integrity of the gut-brain axis.

Traditional antimicrobial therapies have limited success in eradicating intestinal biofilms due to poor penetration of antibiotics through the EPS matrix, horizontal gene transfer, and the presence of dormant persister cells. Moreover, prolonged antibiotic exposure can exacerbate dysbiosis, reduce microbial diversity, and disrupt neuroactive metabolite synthesis. This has stimulated interest in phytochemicals-bioactive compounds derived from plants—as alternative or complementary antibiofilm agents. Phytochemicals such as flavonoids, alkaloids, terpenoids, quinones, and phenolic acids exhibit pleiotropic biological activities, including antioxidant, anti-inflammatory, and antimicrobial effects, alongside their ability to modulate quorum sensing (QS), inhibit EPS synthesis, and disaggregate mature biofilms [12].

Recent studies demonstrate that quercetin and curcumin inhibit biofilm formation in *Staphylococcus aureus* and *Pseudomonas aeruginosa* by downregulating QS-regulated genes (*lasI*, *rhlR*, *luxS*), while berberine interferes with bacterial cell adhesion and suppresses virulence factor production. Similarly, phenolics such as resveratrol and gallic acid repress the synthesis of polysaccharide intercellular adhesins, thereby weakening biofilm stability. In addition to their direct antibiofilm properties, phytochemicals influence microbial community composition by selectively enriching beneficial genera such as *Bifidobacterium* and *Lactobacillus* while suppressing pathobionts [13]. These shifts contribute to improved short-chain fatty acids (SCFAs) production, gut barrier integrity, and anti-inflammatory signaling-key mediators of neuroprotective gut-brain communication.

The emerging concept of phytochemical-based antibiofilm therapeutics extends beyond microbial eradication to include ecological modulation of the gut microbiome. By targeting intermicrobial communication systems such as quorum sensing and cyclic di-GMP pathways, phytochemicals reprogram microbial networks toward symbiosis rather than elimination. Such multitargeted activity minimizes resistance development and supports long-term homeostasis [14]. Furthermore, phytochemicals engage host signaling pathways, activating Nrf2-mediated antioxidant responses and downregulating NF-κB-driven inflammation, thereby reinforcing gut-brain resilience. Preclinical validation of these therapeutic principles increasingly relies on advanced *in vivo* models that recapitulate the gut microbial and immune complexity. Rodent models of intestinal dysbiosis, chemically induced colitis, and neuroinflammation have been instrumental in elucidating the systemic effects of phytochemical antibiofilm agents. Gnotobiotic and germ-free mice colonized with defined microbial consortia allow for precise tracking of phytochemical-microbiota interactions, while *Drosophila melanogaster* and zebrafish models offer high-throughput screening platforms to assess gut barrier integrity, microbial adhesion, and behavioral correlates of gut-brain modulation. These models collectively bridge the gap between molecular mechanisms and physiological outcomes, providing a translational foundation for clinical development.

Translationally, integrating these natural antibiofilm agents into functional foods, nutraceuticals, and biopharmaceutical formulations represents a promising avenue for preventive and therapeutic strategies. Advances in nanotechnology-such as PLGA-based, liposomal, and polysaccharide-based carriers—are enhancing the solubility, stability, and bioavailability of phytochemicals while allowing targeted delivery to the intestinal mucosa. Such delivery systems improve intestinal retention, facilitate controlled release at biofilm sites, and optimize therapeutic concentrations without systemic toxicity. Concurrently, metabolomic and microbiome sequencing approaches enable the personalized tailoring of phytochemical interventions based on individual microbial signatures and metabolic phenotypes [15].

In this context, phytochemical antibiofilm therapeutics embody a convergence of microbiology, neuro-gastroenterology, and natural product pharmacology. Their capacity to restore microbial equilibrium, fortify the intestinal barrier, and modulate neuroimmune signaling positions them as next-generation interventions for microbiota-linked disorders.

Role of Biofilms towards Gut and Neurological Health

Microbial biofilms are complex, structured communities of microorganisms that adhere to biotic or abiotic surfaces and are embedded in a self-produced extracellular polymeric substance (EPS) matrix. Biofilm formation represents a survival strategy adopted by bacteria and fungi under stress, enabling them to persist in hostile environments such as the gastrointestinal tract, where they encounter variations in pH, nutrient availability, immune factors, and antimicrobial agents. Unlike planktonic cells, biofilm-associated microbes exhibit altered gene expression, enhanced resistance to antibiotics, and modified metabolic activity [16]. These adaptations contribute to microbial resilience, chronic infections, and dysbiosis-associated disorders that extend beyond the gut to impact systemic and neurological health.

Stages of Biofilm Formation

Biofilm development is a dynamic, multistage process typically encompassing five stages: (i) reversible attachment, (ii) irreversible attachment, (iii) maturation I, (iv) maturation II, (v) dispersion.

In the reversible attachment stage, planktonic cells transiently adhere to surfaces such as mucosal epithelia or mucus layers via weak hydrophobic or electrostatic interactions. Flagella and pili mediate this initial contact, allowing bacteria to sense surface chemistry and environmental signals [17].During irreversible attachment, microbial cells establish firm adhesion by secreting cardinal adhesive molecules such as polysaccharide intercellular adhesin (PIA), curli fimbriae, alginate, Fap amyloid fibers, Biofilm-associated proteins (Bap), etc., and initiating EPS production. At this point, surface-sensing triggers transcriptional reprogramming that downregulates motility genes while upregulating genes related to exopolysaccharide synthesis and adhesin expression.The maturation I and II stages are characterized by microcolony formation and the establishment of a three-dimensional biofilm architecture with mushroom-like or tower-like structures separated by nutrient and oxygen channels. Within this environment, cells communicate through quorum sensing (QS) and cyclic dinucleotide signaling, leading to spatial heterogeneity and metabolic cooperation [18].The final dispersion stage involves the detachment of biofilm cells, facilitated by enzymatic degradation of the

EPS matrix and changes in cyclic-di-GMP levels, enabling recolonization at new sites(Refer to Fig.I).These developmental stages are tightly controlled by intricate genetic and biochemical regulatory networks that allow microbes to switch between sessile and planktonic lifestyles depending on environmental cues.

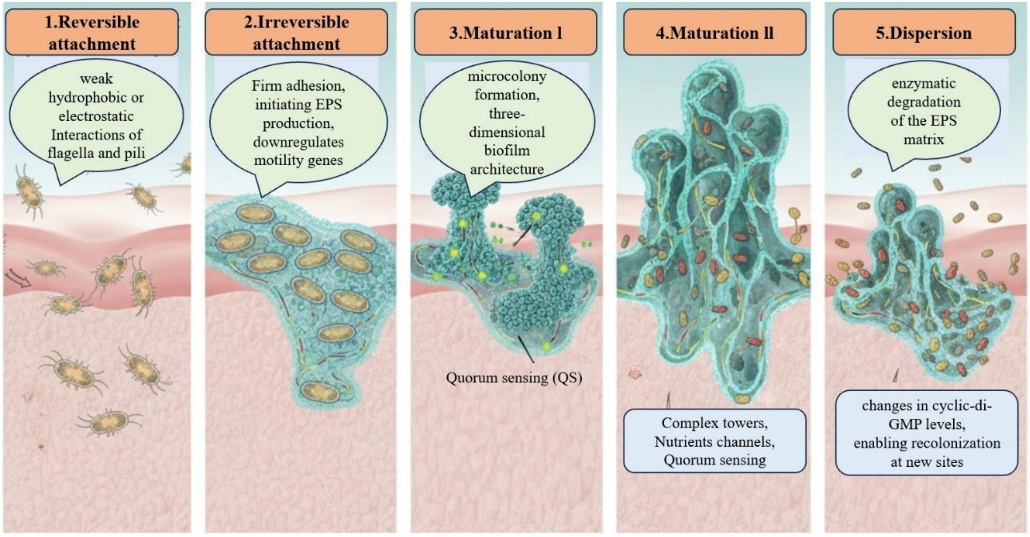

Fig. I.Stages of Biofilm Formation.

EPS Matrix and Microbial Signaling Systems

The EPS matrix accounts for up to 90% of biofilm biomass and provides mechanical stability, nutrient retention, and chemical protection. It comprises polysaccharides, proteins, lipids, and extracellular DNA (eDNA), which collectively establish a hydrated scaffold that anchors cells and impedes antimicrobial diffusion. The matrix also acts as a biochemical reservoir, harboring signaling molecules and enzymes that modulate microbial behavior.

Quorum sensing (QS) represents the primary regulatory system controlling biofilm formation. It allows microbes to sense population density through small diffusible molecules known as autoinducers. In Gram-negative bacteria, *N*-acyl-homoserine lactones (AHLs) regulate genes involved in EPS production and virulence, whereas Gram-positive bacteria primarily rely on oligopeptides that activate two-component signaling systems [35]. Cross-kingdom communication via autoinducer-2 (AI-2) further integrates bacterial and fungal signaling, contributing to polymicrobial biofilm stability [19].

In addition to QS, cyclic dinucleotides, especially cyclic di-GMP (c-di-GMP), serve as intracellular second messengers that coordinate the transition between motile and sessile states. Elevated intracellular c-di-GMP promotes the synthesis of adhesins and EPS components while repressing flagellar motility genes. Conversely, c-di-GMP degradation triggers biofilm dispersion. The spatial heterogeneity of c-di-GMP signaling enables stratified regulation of bacterial subpopulations within the biofilm, fostering resilience under stress [20].

Small non-coding RNAs (sRNAs) represent another critical regulatory layer in biofilm homeostasis. sRNAs modulate post-transcriptional gene expression, affecting EPS synthesis, nutrient metabolism, and stress responses. For instance, in *Pseudomonas aeruginosa*, sRNAs such as RsmY and RsmZ regulate the GacS/GacA pathway, influencing the balance between biofilm maturation and dispersion. Collectively, QS, c-di-GMP, and sRNA networks form an integrated signaling triad that governs biofilm architecture and adaptability.

Intestinal Biofilms: Composition and Clinical Relevance

In the gut environment, biofilms form preferentially along the mucosal surface, creating dense microbial consortia that interact intimately with host epithelial cells. Commensal biofilms composed of *Lactobacillus*, *Bacteroides*, and *Faecalibacterium* species maintain mucosal health by producing SCFAs and reinforcing tight junction proteins. However, pathogenic biofilms dominated by *Escherichia coli*, *Clostridium difficile*, *Enterococcus faecalis*, and *Candida albicans* are associated with chronic inflammation, epithelial barrier dysfunction, and disease progression [21].

In *E. coli*-dominated biofilms, the production of curli fibers and cellulose enhances adhesion to epithelial surfaces and contributes to inflammatory bowel disease (IBD) pathology. *Clostridium difficile* biofilms protect dormant spores from antibiotic exposure, explaining recurrent infections and post-antibiotic dysbiosis. Fungal biofilms, particularly those formed by *Candida albicans*, add complexity through β-glucan-rich matrices that resist antifungal penetration and modulate immune recognition. These polymicrobial biofilms often engage in synergistic interactions, where bacterial metabolites promote fungal hyphal growth, while fungal EPS provides a scaffold for bacterial colonization. Such interkingdom cooperation exacerbates mucosal damage and prolongs inflammation.

Biofilms, Chronic Inflammation, and the Gut-Brain Connection

Chronic intestinal biofilms disrupt epithelial integrity by downregulating tight junction proteins (occludin, claudin-1, ZO-1) and promoting increased intestinal permeability, often referred to as "leaky gut". This allows microbial components such as lipopolysaccharide (LPS), peptidoglycans, and flagellin to translocate into systemic circulation, activating Toll-like receptors (TLRs) and NOD-like receptors (NLRs) on immune cells. The resulting production of pro-inflammatory cytokines (TNF-α, IL-6, IL-1β) not only sustains local inflammation but also affects the blood-brain barrier (BBB) and neural function [22].

Systemic inflammation originating from intestinal biofilms has been implicated in neurodegenerative disorders, including Parkinson's disease, Alzheimer's disease, and multiple sclerosis. Microbial amyloids, such as curli fibers from *E. coli*, can trigger cross-seeding with human α-synuclein, contributing to protein aggregation in the CNS. Moreover, dysbiosis-induced alterations in SCFA and tryptophan metabolism modulate microglial activation and neurotransmitter synthesis, linking biofilm-associated gut inflammation to anxiety and depression.Recent findings demonstrate that biofilm-induced microbial metabolites influence vagal afferent signaling, altering hypothalamic-pituitary-adrenal (HPA) axis regulation and stress responses [23]. Thus, the impact of intestinal biofilms extends beyond local pathology to encompass systemic immune activation and neurobehavioral modulation.

Implications for Therapeutic Targeting

Given the multifaceted impact of biofilms on gut and brain health, disrupting their formation or promoting their dispersal represents a promising therapeutic strategy. Conventional antibiotics often fail to penetrate the EPS matrix or eradicate dormant cells, necessitating alternative approaches that target QS, c-di-GMP, or EPS biosynthesis. Phytochemicals, due to their multitargeted actions, are emerging as potent biofilm-disrupting agents that can simultaneously restore microbial diversity and modulate inflammatory pathways. By re-establishing gut barrier integrity and reducing systemic immune activation, phytochemical-based antibiofilm therapeutics offer a translational route to mitigating biofilm-driven neuroinflammatory disorders.

Phytochemicals as Antibiofilm and Microbiome Modulators

Phytochemicals constitute a diverse group of naturally occurring secondary metabolites that play a pivotal role in plant defense mechanisms and exhibit a wide range of bioactivities beneficial to human health. Beyond their antioxidant and anti-inflammatory functions, many phytochemicals act as potent antibiofilm and microbiome-modulating agents. These compounds target multiple stages of biofilm development—interfering with microbial adhesion, quorum sensing (QS), extracellular polymeric substance (EPS) synthesis, and virulence factor expressionwhile simultaneously reshaping gut microbial ecology toward symbiosis [24]. The pleiotropic mechanisms of phytochemicals are particularly advantageous for chronic intestinal conditions, where mixed-species biofilms and dysbiosis perpetuate inflammation and neuroimmune imbalance.

Phytochemicals can be broadly categorized based on their chemical scaffolds, including polyphenols, alkaloids, quinones, terpenoids, and non-flavonoid phenolics, each class exhibiting unique molecular targets and signaling effects. Their multifunctional activity not only suppresses pathogenic biofilms but also supports commensal bacteria that contribute to gut barrier integrity and neuroactive metabolite production.

Polyphenols and Flavonoids: Quercetin, Catechins, Baicalin, and Curcumin

Polyphenols, especially flavonoids, are among the most extensively studied antibiofilm phytochemicals due to their amphipathic nature and capacity to interact with microbial membranes and signaling molecules.

Quercetin, a flavonol abundant in onions, apples, and berries, disrupts biofilm formation by downregulating QS-regulated genes such as *lasI*, *rhlR*, and *pqsA* in *Pseudomonas aeruginosa*. It inhibits EPS production and reduces the synthesis of rhamnolipids and pyocyanin—key virulence factors involved in bacterial adhesion and oxidative stress. Quercetin also impairs *Escherichia coli* curli fiber formation and inhibits *Candida albicans* hyphal morphogenesis, demonstrating cross-kingdom antibiofilm activity. Furthermore, quercetin enhances intestinal barrier function by upregulating occludin and claudin-1, mitigating LPS-induced tight junction disruption. These effects collectively contribute to gut–brain homeostasis by limiting endotoxemia and systemic inflammation.

Catechins, particularly epigallocatechin gallate (EGCG) from green tea, exhibit broad-spectrum antibiofilm effects against Gram-positive and Gram-negative bacteria. EGCG interferes with the *agr* quorum-sensing system in *Staphylococcus aureus*, inhibits glucosyltransferase activity in *Streptococcus mutans*, and destabilizes pre-formed biofilms through hydrogen peroxide generation. In intestinal models, EGCG increases *Bifidobacterium* and *Lactobacillus* populations while suppressing *Clostridium perfringens*, thereby promoting microbial diversity and anti-inflammatory SCFA production [25].

Baicalin, a flavone derived from *Scutellaria baicalensis*, acts through dual antibiofilm and anti-inflammatory mechanisms. It suppresses the *luxS*-mediated AI-2 quorum-sensing pathway in *Enterococcus faecalis* and *Klebsiella pneumoniae*, thereby preventing EPS accumulation and surface adhesion. Additionally, baicalin modulates gut microbial composition by increasing butyrate-producing bacteria such as *Faecalibacterium prausnitzii* and *Roseburia spp.*, which in turn improve mucosal barrier and neuroimmune signaling .

Curcumin, a polyphenolic pigment from *Curcuma longa*, exerts strong antibiofilm activity via inhibition of quorum sensing and bacterial motility. Curcumin downregulates *lasR*, *rhlI*, and *vfr* genes in *P. aeruginosa*, reducing biofilm biomass and virulence. It also interferes with the amyloid-like curli fibers in *E. coli* biofilms and attenuates fungal adhesion by altering cell surface hydrophobicity [26].

Alkaloids: Berberine, Matrine, and Sinomenine

Alkaloids represent nitrogen-containing phytochemicals with potent antimicrobial and biofilm-modulating properties. Their structural diversity enables them to intercalate into microbial DNA, inhibit enzymatic processes, and modulate host signaling.

Berberine, isolated from *Berberis* species, has emerged as one of the most potent natural antibiofilm agents. It interferes with the FtsZ protein essential for bacterial cytokinesis and inhibits QS-controlled virulence gene expression in *P. aeruginosa* and *Vibrio harveyi*. Berberine disrupts EPS integrity by chelating divalent cations and reducing eDNA content, leading to biofilm destabilization. In gut microbiota studies, berberine selectively inhibits *Proteobacteria* while enriching *Bacteroidetes* and SCFA-producing bacteria, thereby improving metabolic and inflammatory profiles. Its anti-inflammatory effects through AMPK activation and NF-κB suppression make it a promising candidate for microbiota-mediated neuroprotection [27].

Matrine, an alkaloid from *Sophora flavescens*, exhibits antibiofilm effects primarily through QS inhibition and membrane depolarization. It suppresses *luxS* and *lasR* gene expression in *E. coli* and *Pseudomonas*, reducing biofilm biomass and exopolysaccharide synthesis. Matrine also enhances host immunity by promoting tight junction protein expression and reducing oxidative stress, thus limiting bacterial translocation.

Sinomenine, obtained from *Sinomenium acutum*, demonstrates antifungal and antibacterial biofilm inhibition by reducing intracellular c-di-GMP levels and impairing adhesion-associated genes. Sinomenine's immunomodulatory activity, suppressing pro-inflammatory cytokines and enhancing mucosal IgA production, contributes to a balanced gut microbiome and reduced neuroinflammation.

Quinones: Aloe-Emodin and Cryptotanshinone

Quinones are aromatic compounds with redox-active structures capable of generating reactive oxygen species (ROS) that disrupt microbial homeostasis.

Aloe-emodin, an anthraquinone from *Aloe vera*, impedes *Staphylococcus aureus* and *Candida albicans* biofilm formation by interfering with surface hydrophobicity and downregulating adhesion-related genes (*icaA*, *ALS3*). It also inhibits AI-2 quorum sensing and disrupts mature biofilm architecture through ROS-mediated matrix degradation. Aloe-emodin enhances the abundance of *Lactobacillus* and *Bifidobacterium* species in gut models while reducing *Enterococcus faecalis*, aligning with its role in microbial balance [28].

Cryptotanshinone, a diterpenoid quinone from *Salvia miltiorrhiza*, inhibits *Pseudomonas* and *Staphylococcus* biofilms via suppression of the *lasI/R* and *agrA* systems, respectively. It downregulates polysaccharide biosynthesis enzymes and reduces eDNA release, thereby weakening EPS integrity. Cryptotanshinone also acts on host pathways by inhibiting MAPK and STAT3 signaling, providing dual anti-inflammatory and neuroprotective benefits in microbiota-associated pathologies.

Terpenoids: Andrographolide, Thymol, and Carvacrol

Terpenoids are lipophilic compounds that target microbial membranes and signaling cascades, often acting synergistically with antibiotics [29].

Andrographolide, the major diterpenoid from *Andrographis paniculata*, exerts antibiofilm activity by suppressing *lasI*, *rhlR*, and *pqsR* genes, thereby inhibiting QS in *P. aeruginosa*. It reduces alginate and pyocyanin synthesis, disrupts established biofilms, and sensitizes bacteria to antibiotics. In gut models, andrographolide increases the abundance of butyrate-producing bacteria and enhances tight junction integrity, mitigating colonic inflammation.

Thymol and carvacrol, monoterpenes derived from thyme and oregano oils, destabilize microbial membranes and downregulate EPS production. Thymol inhibits *luxS*-mediated QS signaling in *Streptococcus mutans* and *E. coli*, while carvacrol disrupts c-di-GMP accumulation, triggering biofilm dispersion. Both compounds reduce eDNA release and interfere with bacterial adhesion proteins such as fimbriae and flagellin [30]. Thymol also enhances gut microbial richness and reduces LPS-induced systemic inflammation, suggesting benefits in microbiota-brain axis modulation.

Non-Flavonoid Phenolics: Resveratrol, Mangiferin, and Gallic Acid

Non-flavonoid phenolics are structurally distinct yet mechanistically versatile phytochemicals that contribute to biofilm attenuation and microbiome restoration.

Resveratrol, a stilbene found in grapes and berries, inhibits QS-controlled biofilm formation in *P. aeruginosa*, *Vibrio harveyi*, and *Listeria monocytogenes* by repressing *lasI* and *luxS* expression. It reduces EPS synthesis, suppresses flagellar motility, and interferes with eDNA release. In animal studies, resveratrol supplementation modulated gut microbiota by enhancing *Akkermansia muciniphila* and reducing *Desulfovibrio* species, contributing to reduced endotoxemia and improved cognitive performance.

Mangiferin, a xanthone glycoside from *Mangifera indica*, disrupts biofilm formation by interfering with glucosyltransferases and inhibiting bacterial adhesion. It attenuates oxidative stress and enhances mucin secretion in intestinal epithelial cells, fortifying barrier function. Mangiferin also promotes the growth of beneficial microbes and suppresses pro-inflammatory species, thereby contributing to gut-brain axis equilibrium [31].

Gallic acid, a simple phenolic acid found in tea, berries, and gallnuts, inhibits EPS formation in *Pseudomonas* and *Staphylococcus* species and promotes dispersal of pre-formed biofilms. It scavenges free radicals within biofilms, thereby impairing oxidative stress-dependent signaling essential for biofilm maturation. In vivo, gallic acid modulates the Firmicutes/Bacteroidetes ratio, enhances SCFA levels, and attenuates neuroinflammation through microglial regulation. Refer to Fig.II

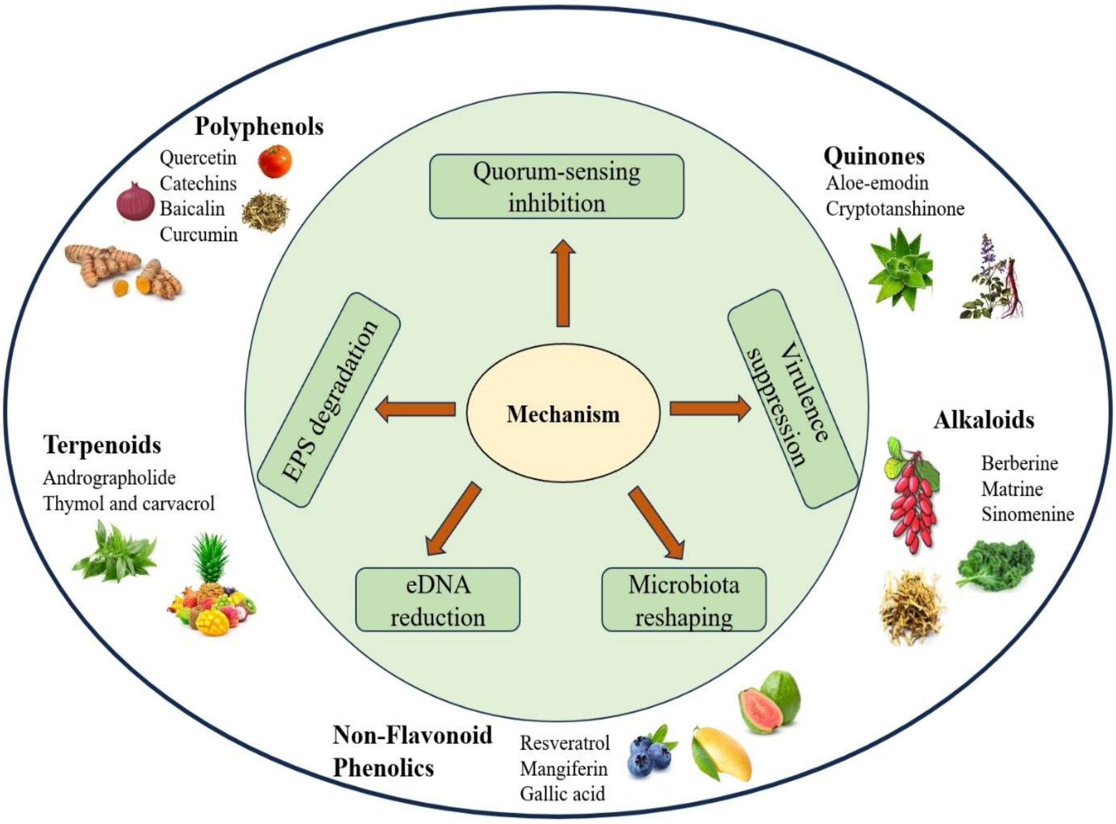

Fig.IIMechanism of various Phytochemicals and their effect on the cellular level.

Mechanistic Integration and Microbiota Reshaping

Across phytochemical classes, several shared mechanistic pathways underpin their antibiofilm efficacy:

1. Quorum-sensing inhibition: Flavonoids (quercetin, curcumin) and alkaloids (berberine) suppress autoinducer synthesis or receptor binding, reducing virulence and EPS production.
2. EPS degradation: Phenolics (gallic acid, aloe-emodin) disrupt polysaccharide networks and matrix-bound proteins, facilitating biofilm dispersion.
3. Virulence suppression: Terpenoids and quinones downregulate genes encoding toxins, adhesion proteins, and siderophores.
4. eDNA reduction: Alkaloids and resveratrol reduce extracellular DNA release, weakening matrix cohesion.
5. Microbiota reshaping: Many phytochemicals selectively promote beneficial taxa (*Lactobacillus*, *Bifidobacterium*, *Akkermansia*) and inhibit pathogens (*Enterobacteriaceae*, *Clostridium difficile*), fostering microbial eubiosis [32].

Through these overlapping mechanisms, phytochemicals act as ecological modulators rather than conventional antimicrobials, aligning with a systems biology approach to gut-brain axis therapeutics. Their multitargeted actions, combined with host anti-inflammatory and antioxidant effects, make them ideal candidates for integration into functional foods and nutraceutical formulations designed to prevent or mitigate microbiota-linked neurological disorders.

The Gut Microbiome-Biofilm Interface

The gut microbiome represents a complex ecological network composed of trillions of microorganisms that maintain a dynamic equilibrium with the host. Within this ecosystem, biofilms serve as a structural and metabolic scaffold that profoundly shapes microbial interactions, community stability, and host signaling. In a healthy gut, commensal biofilms formed by *Lactobacillus*, *Bacteroides*, *Akkermansia*, and *Faecalibacterium* species create a protective mucosal layer that enhances nutrient metabolism and modulates immune homeostasis. Conversely, under dysbiotic conditions, pathogenic biofilms dominated by *Escherichia coli*, *Clostridium difficile*, and *Candida albicans* disrupt epithelial barrier function, promote local inflammation, and impair neuroimmune communication along the gut-brain axis. The transition from beneficial to pathological biofilms represents a pivotal inflection point in gastrointestinal and neurological disease pathophysiology.

Gut Microbial Ecology Under Biofilm Dominance

Biofilm formation in the gut constitutes a strategic microbial adaptation that confers persistence and ecological competitiveness. Within biofilms, microbial cells are embedded in an extracellular polymeric matrix that shields them from host defenses and environmental fluctuations [33]. The metabolic cooperation within these structures enables resource sharing and enhances stress tolerance through quorum sensing and metabolite cross-feeding. Facultative anaerobes such as *E. coli* consume oxygen at the mucosal interface, generating localized anaerobic niches that favor the proliferation of obligate anaerobes like *Clostridium* and *Bacteroides*. This hierarchical spatial organization contributes to the formation of stable but dysbiotic biofilm consortia under inflammatory stress.

Pathogenic biofilms exert multiple deleterious effects on intestinal homeostasis. For instance, adherent-invasive *E. coli* (AIEC) isolated from Crohn’s disease patients exhibits strong adhesion to the ileal mucosa and invades epithelial cells via type 1 pili and outer membrane adhesins. *Clostridium difficile* forms persistent biofilms that encapsulate spores within an EPS matrix, protecting them from antibiotics and facilitating recurrence. Fungal species such as *Candida albicans* form synergistic biofilms with *Staphylococcus* and *E. coli*, reinforcing mucosal adhesion and promoting chronic inflammation. These polymicrobial communities release toxins, proteases, and lipopolysaccharides (LPS) that impair tight junction proteins (claudin-1, occludin, ZO-1), leading to increased epithelial permeability or “leaky gut” [34]. The resulting antigenic translocation activates mucosal immune responses and systemic cytokine release, mechanistic links between gut biofilms and neuroinflammatory diseases.

Microbial Metabolites and Neuroimmune Signaling: SCFAs, Tryptophan Derivatives, and Neuroactive Molecules

Metabolites derived from gut microbes act as principal mediators of gut-brain communication. Among these, short-chain fatty acids (SCFAs) - acetate, propionate, and butyrateproduced by the fermentation of dietary fibers, play a critical neuroprotective role. Butyrate supports colonic epithelial energy metabolism, reinforces tight junction integrity, and exerts anti-inflammatory effects through histone deacetylase (HDAC) inhibition and G-protein-coupled receptor activation (GPR41 and GPR43). These receptors are also expressed in microglia and vagal afferents, suggesting a direct pathway linking microbial metabolism to brain signaling [35].

Biofilm-mediated dysbiosis often results in SCFA depletion and a concurrent increase in branched-chain and aromatic amino acid metabolites that promote inflammation. This metabolic shift contributes to altered blood–brain barrier permeability and neuroimmune activation.

Tryptophan metabolism represents another vital interface between microbiota and neural function. Under healthy conditions, commensal bacteria convert tryptophan into indole derivatives (indole-3-propionic acid, indole-3-aldehyde) that activate aryl hydrocarbon receptors (AhR) and maintain mucosal immune tolerance. Dysbiosis and biofilm dominance divert tryptophan metabolism toward the kynurenine pathway, increasing neurotoxic metabolites such as quinolinic acid and 3-hydroxykynurenine [36]. Elevated kynurenine levels correlate with depression, cognitive impairment, and microglial hyperactivation.

Additionally, certain gut microbes synthesize neurotransmitters or their precursors directly-*Lactobacillus* and *Bifidobacterium* produce γ-aminobutyric acid (GABA), *Escherichia coli* synthesizes serotonin (5-HT), and *Bacillus* species generate dopamine. These microbial-derived neuromodulators interact with enteric neurons and vagal pathways, thereby influencing mood, cognition, and stress responses. The biofilm-associated disruption of these pathways contributes to neurobehavioral disorders linked to gut dysbiosis.

Role of Phytochemicals in Restoring Microbial Diversity and the Firmicutes/Bacteroidetes Ratio

Phytochemicals have emerged as ecological modulators capable of reversing biofilm-induced dysbiosis and restoring microbial equilibrium. Polyphenols, flavonoids, and alkaloids selectively inhibit biofilm-forming pathogens while enriching beneficial commensals that maintain gut barrier and neuroimmune integrity.

Dietary polyphenols such as quercetin, catechins, and resveratrol increase the relative abundance of *Bacteroidetes* and *Akkermansia muciniphila*, while reducing the *Firmicutes/Bacteroidetes* ratio - an index often elevated in metabolic and neurodegenerative disorders. These compositional shifts correlate with enhanced SCFA production and reduced intestinal permeability. Curcumin supplementation has been shown to promote butyrate-producing bacteria (*Roseburia*, *Eubacterium rectale*) and suppress pro-inflammatory taxa like *Enterobacteriaceae*.

Berberine modulates microbial ecology by reducing pathogenic *Proteobacteria* and increasing beneficial *Bacteroides* and *Lactobacillus*, which collectively enhance gut-brain signaling through GABAergic and serotonergic modulation. Similarly, baicalin and mangiferin improve microbial richness and restore tight junction integrity via IL-10-mediated anti-inflammatory pathways [37]. These phytochemicals exert multitargeted effects, direct antibiofilm action, antioxidant defense activation (via Nrf2), and metabolic reprogramming toward eubiosis - collectively fostering an environment conducive to gut-brain axis homeostasis.

Microbiota-Derived Modulation of Neurotransmitter Synthesis and Immune Tone

The gut microbiota modulates central neurotransmission both directly, through metabolite synthesis, and indirectly, through immune and endocrine signaling [38]. Dysbiosis resulting from biofilm dominance alters these interactions, often tilting the balance toward neuroinflammation. Conversely, phytochemical-mediated microbiota restoration can normalize neurotransmitter biosynthesis and immune tone.

For example, catechin-rich diets elevate *Lactobacillus rhamnosus* populations, increasing GABA synthesis and attenuating anxiety-like behavior in animal models [39]. Resveratrol upregulates tryptophan hydroxylase-1 (TPH1), enhancing serotonin biosynthesis in enterochromaffin cells via microbial modulation [40]. Butyrate-producing bacteria stimulated by quercetin and berberine inhibit microglial activation and reduce pro-inflammatory cytokines (IL-6, TNF-α), contributing to neuroprotection [41].

In parallel, phytochemicals modulate immune tone through suppression of NF-κB and activation of regulatory T cells. Mangiferin and curcumin enhance IL-10 and TGF-β signaling, while polyphenols strengthen mucosal IgA production, supporting immune tolerance and preventing bacterial translocation. These effects, integrated through the microbiota immune-neural triad, underpin the neuroprotective and mood-stabilizing outcomes observed with phytochemical-rich diets.

Translational Implications

Understanding the biofilm-microbiome interface provides a mechanistic foundation for designing next-generation therapeutics and functional foods aimed at gut-brain modulation. The capacity of phytochemicals to simultaneously dismantle biofilms, reshape microbial networks, and restore metabolic communication highlights their translational potential. Integrating metabolomic and microbiome profiling with phytochemical-based interventions can enable precision strategies for neurodegenerative and metabolic disorders where microbial dysbiosis plays a pathogenic role.

Phytochemical-Based Nanotherapeutics and Delivery Systems

The clinical translation of phytochemicals as antibiofilm and gut-brain modulatory agents is often hindered by their intrinsic limitations in solubility, chemical stability, and bioavailability. Despite extensive evidence of potent in vitro activity, many phytochemicals, including curcumin, quercetin, berberine, and thymol, exhibit poor pharmacokinetic performance due to low aqueous solubility, rapid metabolism, and limited gastrointestinal absorption [42]. Furthermore, the physicochemical environment of the gut, characterized by fluctuating pH and enzymatic activity, leads to significant pre-systemic degradation. These challenges restrict therapeutic concentrations at biofilm or mucosal sites and hinder consistent modulation of microbiota composition.

Nanotechnology-based delivery systems have emerged as a transformative approach to overcome these barriers by enhancing phytochemical solubility, mucosal penetration, and controlled intestinal release. Polymeric nanoparticles, liposomes, nanoemulsions, micelles, and solid lipid nanoparticles (SLNs) have been extensively employed to improve the stability and targeted delivery of natural antibiofilm compounds [43]. These carriers facilitate sustained release, protect phytochemicals from gastrointestinal degradation, and allow site-specific accumulation in the colon, crucial for maximizing antibiofilm efficacy at mucosal biofilm niches.

Overcoming Solubility and Bioavailability Barriers

The absorption of hydrophobic phytochemicals across the intestinal epithelium is limited by poor dissolution and extensive first-pass metabolism [44]. For instance, curcumin has an oral bioavailability of less than 1% due to rapid glucuronidation and sulfation [45]. Similarly, berberine exhibits low plasma concentrations because of P-glycoprotein efflux and poor permeability across enterocytes [46]. Nanosizing these compounds enhances their surface area and improves interaction with mucosal membranes, leading to better absorption and bioactivity [47]. Encapsulation within biodegradable polymers such as PLGA (poly(lactic-co-glycolic acid)) or PEGylated matrices not only enhances solubility but also prolongs circulation time and prevents oxidative degradation [48].

Nano-Curcumin Systems

Curcumin has been extensively studied as a model compound for phytochemical nanodelivery. Nano-curcumin formulations, including polymeric nanoparticles, nanogels, and solid lipid nanoparticles, have demonstrated superior biofilm inhibition and anti-inflammatory properties compared to free curcumin [49]. Curcumin-loaded PLGA nanoparticles (150-200 nm) exhibited enhanced uptake by intestinal epithelial cells and stronger inhibition of *Pseudomonas aeruginosa* and *Staphylococcus aureus* biofilms through downregulation of *lasI* and *rhlR*quorum-sensing genes [50].

In gut-related applications, nano-curcumin improved mucosal adhesion, upregulated tight junction proteins, and increased the abundance of *Lactobacillus* and *Akkermansia* species, restoring microbiota balance in colitis and depression models [51]. Liposomal curcumin formulations demonstrated improved systemic exposure, with 5 to 10-fold higher plasma concentrations and extended half-life, facilitating consistent gut-brain axis modulation [52]. Moreover, curcumin-loaded chitosan nanoparticles achieved colon-targeted release due to pH-responsive polymer degradation, maintaining high local concentrations within the large intestine [53].

Berberine-Liposome and Polymeric Nanocarriers

Berberine’s therapeutic potential in microbiota modulation and antibiofilm therapy has been extensively validated; however, its poor intestinal absorption and first-pass metabolism limit efficacy [54]. Liposomal and nanoemulsion formulations of berberine have emerged as promising strategies to circumvent these limitations. Liposomal berberine exhibited improved encapsulation efficiency, membrane fusion capability, and enhanced cellular uptake in intestinal and neuronal models.

In animal studies, berberine-liposomes restored microbial diversity and increased SCFA production more effectively than free berberine, suggesting superior biofilm penetration and microbiome modulation [55]. Incorporation into PLGA and PEG-PCL copolymer nanoparticles further enhanced bioavailability and reduced hepatic metabolism, improving pharmacokinetic exposure by up to 15-fold [56]. Notably, berberine nanoformulations have demonstrated neuroprotective benefits by decreasing microglial activation, attenuating oxidative stress, and normalizing kynureninetryptophan metabolismkey pathways in gut-brain signaling.

Recent work has also introduced berberinemetalorganic framework (MOF) composites and solid lipid nanoparticles for dual antibiofilm and anti-inflammatory activity, where the nanoscale architecture facilitated biofilm penetration and slow drug release over 48 to 72 hours.

PLGA-Thymol and Other Terpenoid Nanoformulations

Terpenoids such as thymol, carvacrol, and andrographolide exhibit strong antibiofilm and antioxidant properties but are limited by high volatility and poor stability in aqueous media [57]. Encapsulation within PLGA nanoparticles or lipidpolymer hybrids stabilizes these compounds, reduces volatility, and ensures controlled intestinal release.

PLGA-thymol nanoparticles have demonstrated superior antibiofilm efficacy against *E. coli* and *Listeria monocytogenes*, showing a 3 to 4-fold reduction in biofilm biomass compared to free thymol [58]. The controlled release profile (over 48 hours) ensures prolonged antibacterial action and reduces cytotoxicity to intestinal epithelial cells. Furthermore, PLGA-thymol systems showed enhanced mucoadhesion, facilitating localized action at the mucosal surface and preventing pathogen recolonization [59].

Nano-carvacrol formulations improved colonic targeting and suppressed inflammatory cytokines (IL-6, TNF-α) in dextran sulfate sodium (DSS)-induced colitis models. Andrographolide-loaded nanocarriers improved blood-brain barrier penetration and protected neuronal integrity via Nrf2 activation, linking gut anti-inflammatory activity with neuroprotection.

Controlled Release and Colon-Targeted Delivery

Colon-targeted systems are particularly advantageous for managing gut biofilms, as they allow high local drug concentrations with minimal systemic exposure. pH-sensitive polymers such as Eudragit® S100, chitosan, and alginate have been used to design nanoformulations that release their payload in response to the colonic pH range (6.8-7.4) [60]. This ensures that phytochemicals like curcumin, berberine, and thymol are protected from degradation in the stomach and small intestine, and are released precisely where biofilm accumulation is most prevalent [61].

Mucoadhesive nanocarriers, particularly those incorporating chitosan or hyaluronic acid, extend residence time on the intestinal surface and improve interaction with mucosal biofilms. Additionally, hybrid systems combining polymeric nanoparticles with liposomes or micelles allow dual release kinetics, an initial burst to disrupt surface biofilms, followed by sustained release to prevent recolonization [62].

Safety and Pharmacokinetic Advantages

Nanocarrier-based phytochemical systems offer improved safety profiles by enabling dose reduction without compromising efficacy. Biodegradable polymers such as PLGA, PEG, and PCL are FDA-approved for oral and parenteral use and are metabolized into non-toxic by-products [63]. Nanoformulations reduce gastrointestinal irritation and cytotoxicity often associated with high doses of free phytochemicals [64].

Pharmacokinetic evaluations reveal significant improvements: nano-curcumin and nano-berberine exhibit enhanced oral bioavailability (6-20× higher AUC), extended plasma half-lives, and improved tissue distribution in the colon and brain. Importantly, these formulations minimize systemic accumulation, reducing hepatic stress and off-target effects. Toxicological studies report no significant alterations in hematological or biochemical parameters following chronic oral administration of phytochemical-loaded nanoparticles [65].

In summary, phytochemical-based nanotherapeutics address critical limitations of conventional bioactive delivery by offering controlled release, targeted intestinal deposition, and systemic safety. Their capacity to maintain effective concentrations at biofilm sites while modulating microbiota composition and neuroimmune pathways positions them as next-generation platforms for gut–brain health therapeutics. Future directions include integrating nanotechnology with omics-based microbiome mapping and responsive smart delivery systems to achieve personalized, microbiota-guided phytochemical therapy.

Experimental Animal Models for Evaluating Phytochemical-Based Antibiofilm and Gut-Brain Therapeutics

Animal models serve as essential platforms for elucidating the mechanistic and functional outcomes of phytochemical-based antibiofilm and microbiota-modulating interventions. They bridge in vitro findings with clinical applications by simulating microbial ecology, intestinal physiology, and neurobehavioral responses. Rodents, zebrafish, *Drosophila melanogaster*, and *Caenorhabditis elegans* represent the most widely utilized systems for exploring the gut-microbiomebrain interface and for assessing the efficacy and safety of phytochemical formulations [66].

Rodent Models

Rodents remain the gold standard in gut-brain research due to their well-characterized microbiota, behavioral repertoire, and immunological similarity to humans [67]. Germ-free, antibiotic-treated, and fecal microbiota transplantation (FMT) mouse models have been instrumental in establishing causality between dysbiosis and neurological dysfunction [68]. Phytochemicals such as quercetin, berberine, and curcumin have demonstrated restoration of microbial diversity, increased SCFA production, and attenuation of neuroinflammatory markers (IL-6, TNF-α) in rodent models of colitis, obesity, and depression [69]. Nano-curcumin and berberine-liposomes further enhanced bioavailability and improved cognitive performance in chronic stress and Alzheimer’s models.

Zebrafish Models

Zebrafish (*Danio rerio*) have emerged as versatile vertebrate models to study hostmicrobiome interactions and neurobehavioral outcomes under controlled environmental conditions [70]. Their transparent larvae allow real-time imaging of microbial colonization and biofilm formation within the gut. Zebrafish exposed to high-fat diets or LPS show disrupted gut barrier integrity and altered microbiota composition similar to mammalian dysbiosis [71]. Phytochemicals like resveratrol, thymol, and curcumin modulate microbiota composition and prevent anxiety-like behaviors via anti-inflammatory and antioxidant pathways. Their rapid development and high-throughput screening potential make zebrafish ideal for evaluating gut-targeted nanophytochemical formulations.

Drosophila melanogaster and *Caenorhabditis elegans*

Invertebrate models such as *Drosophila melanogaster* and *C. elegans* provide genetic tractability and cost-effective systems for studying microbial symbiosis, oxidative stress, and neuroprotection [72]. The *Drosophila* gut microbiome, though simpler, displays conserved signaling through NF-κB and Toll pathways similar to mammals [73]. Polyphenols and alkaloids improve gut barrier function, extend lifespan, and suppress microbial virulence in *Drosophila* models of infection and neurodegeneration.

C. elegans, a nematode with a transparent body and a defined neuronal network, facilitates high-throughput screening of phytochemicals for antibiofilm and anti-aging properties. Berberine and quercetin disrupt bacterial biofilms and reduce pathogen colonization in *C. elegans*, concurrently activating DAF-16/FOXO and SKN-1/Nrf2 pathways, key regulators of oxidative defense and longevity [74]. These models provide mechanistic insights into conserved molecular pathways and hostmicrobe-metabolite interactions relevant to gut-brain communication.

Translational Relevance

Together, these models provide complementary perspectives-rodents for systemic and behavioral integration, zebrafish for imaging and gut physiology, and invertebrates for genetic and molecular dissection [75]. Multi-model validation is increasingly recognized as a cornerstone for preclinical translation of phytochemical-based antibiofilm therapeutics. Incorporating microbiota profiling, metabolomics, and neurobehavioral assays within these models enables robust evaluation of gut-brain axis modulation and supports rational design of functional foods and nanophytomedicines for human application [76]. Refer to Table 1.

Table I. Summary of animal models used to evaluate phytochemical-based antibiofilm and gut-brain therapeutics.

Model	**Key Advantages**	**Phytochemicals Studied**	**Endpoints Assessed**	**Representative References**
Rodents (mice/rats)	Mammalian microbiota, behavioral readouts, and immunological similarity	Quercetin, curcumin, berberine, resveratrol	Microbiota diversity, SCFAs, cytokines, behavior	[69-71]
Zebrafish (*Danio rerio*)	Transparent larvae, imaging gut biofilm dynamics, high-throughput assays	Curcumin, thymol, resveratrol	Gut permeability, oxidative stress, and anxiety-like behavior	[72-73]
Drosophila melanogaster	Conserved immune and stress signaling, rapid screening	Polyphenols, catechins, berberine	Gut barrier function, oxidative stress, and lifespan	[74-75]
Caenorhabditis elegans	Simple gut-brain circuitry, genetic tractability	Quercetin, berberine, curcumin	Biofilm inhibition, neuroprotection, longevity	[76]

Translational Roadmaps: From Functional Foods to Therapeutics

The growing understanding of the gut-microbiomebrain axis and the antibiofilm potential of phytochemicals has catalyzed efforts to transition these bioactives from laboratory discoveries to clinically relevant interventions. Translational success requires integrating phytochemical antibiofilm strategies into functional foods, synbiotics, and nutraceutical formulations while ensuring regulatory compliance, personalized efficacy, and safety [77]. The ultimate goal is to develop sustainable and evidence-based products that bridge microbial modulation with measurable improvements in neurological and metabolic health.

Integration into Functional Foods, Synbiotics, and Nutraceuticals

Functional foods and nutraceuticals represent practical vehicles for delivering phytochemicals with gut-targeted effects. The combination of prebiotics, probiotics, and phytochemicals-collectively termed synbiotics-offers synergistic benefits by enhancing microbial colonization, disrupting pathogenic biofilms, and promoting beneficial metabolite production [78]. For instance, formulations combining polyphenols (such as quercetin or catechins) with *Lactobacillus* and *Bifidobacterium* strains enhance SCFA synthesis, restore epithelial barrier function, and attenuate inflammation in both animal and clinical studies [79].

Nanoencapsulation technologies are increasingly applied to functional foods to stabilize phytochemicals during processing and gastrointestinal passage. Lipid- and protein-based nanocarriers protect sensitive molecules like curcumin, resveratrol, and thymol, ensuring controlled intestinal release. Advances in spray-drying, microfluidization, and biopolymer complexation allow scalable production of phytochemical-loaded matrices suitable for beverages, yogurts, and nutrition bars. Industrial translation also requires optimization of sensory acceptability and bioactive retention during storage.

Regulatory Frameworks and Safety Assessments

The global regulatory landscape for phytochemical-based functional foods and therapeutics remains heterogeneous. In the European Union, the European Food Safety Authority (EFSA) classifies health claims for bioactives under the Nutrition and Health Claims Regulation (EC No. 1924/2006), requiring substantiated evidence of efficacy and safety [80]. In the United States, phytochemicals in functional foods are regulated as dietary supplements under the Dietary Supplement Health and Education Act (DSHEA), which mandates safety but not pre-market approval [81].

Emerging phytochemical nanocarriers and antibiofilm formulations present unique regulatory challenges because of their hybrid food-drug nature. The U.S. FDA’s nanomaterial guidance (2022) emphasizes the need for physicochemical characterization, stability testing, and toxicological evaluation. Preclinical safety assessment should include genotoxicity, subchronic toxicity, and reproductive safety studies in accordance with OECD guidelines [82]. Additionally, evaluation of microbiome alterations and metabolomic shifts is increasingly considered an integral part of safety assessment to ensure no adverse ecological perturbations[83-85].

Standardization remains another key barrier to translation. Variability in phytochemical composition due to botanical source, extraction method, and formulation can significantly influence bioactivity. Establishing reference standards, validated analytical methods (LC-MS/MS, NMR), and harmonized labeling protocols will be critical for reproducibility and consumer trust [86].

Personalized Nutrition and Omics-Driven Microbiome Profiling

Precision nutrition approaches are redefining the way phytochemical interventions are designed and evaluated. Integrating metagenomics, metabolomics, and transcriptomics enables personalized matching of phytochemical profiles with individual microbial compositions [87]. For instance, interindividual variability in polyphenol metabolism (via gut-derived urolithins or phenyl-γ-valerolactones) significantly determines therapeutic efficacy [88].

Microbiome-based diagnostics and clinical biomarkers such as SCFA ratios, tryptophan–kynurenine balance, or inflammatory cytokine signatures can serve as endpoints to evaluate phytochemical efficacy [89]. Artificial intelligence and machine learning algorithms are increasingly applied to predict microbial responses and optimize formulation composition for target populations.

The future trajectory of personalized nutrition lies in digital-twin models that integrate host genomics, dietary intake, and microbiome data to simulate outcomes of phytochemical interventions in silico before clinical validation [90]. Such models will accelerate the transition from empirical formulation to data-driven precision therapeutics.

Industrial and Clinical Translation Strategies

Industrial translation of phytochemical-based antibiofilm therapeutics requires an integrated pipeline encompassing formulation optimization, stability validation, and clinical demonstration. Scalable manufacturing using green technologies-such as supercritical CO_2 extraction and biodegradable polymer synthesis-ensures sustainability and compliance with environmental regulations [91].

On the clinical front, randomized controlled trials (RCTs) remain the gold standard for establishing health claims. Recent RCTs using polyphenol-rich diets and curcumin nanoformulations have demonstrated improved mood, cognitive performance, and inflammatory biomarkers, validating the gut-brain modulation hypothesis [92]. Collaborative consortia combining academia, biotech startups, and food industries are accelerating this translation through multi-center, multi-omics clinical trials.

The integration of phytochemical antibiofilm science into functional food and therapeutic sectors will hinge on regulatory flexibility, validated biomarkers, and personalized strategies. Future translational success will depend on aligning industrial innovation with clinical rigor and ecological safety, ensuring that phytochemical-based interventions evolve from promising natural remedies to precision microbiome therapeutics for sustainable gut-brain health.

Challenges and Future Perspectives

Despite remarkable advances in elucidating the gut-microbiome, brain axis, and the antibiofilm potential of phytochemicals, several translational challenges continue to impede their full clinical realization. Chief among these are reproducibility, dosage standardization, and host variability, which collectively influence efficacy and consistency across studies [93]. Variability in phytochemical composition due to differences in plant genotype, extraction technique, and formulation leads to inconsistent biological outcomes. Moreover, interindividual variation in microbiota composition and metabolic phenotype significantly affects phytochemical biotransformation and responsiveness [94]. These factors complicate dose-response relationships, making it difficult to establish standardized clinical dosage regimens for functional foods and nutraceuticals.

The bioavailability paradox also persistsdespite potent *in vitro* activity; many phytochemicals demonstrate suboptimal systemic exposure *in vivo*. Nanotechnology-based delivery systems have improved intestinal stability and targeted release, yet long-term safety data and scalable production remain limited [95]. Furthermore, most preclinical studies rely on rodent models that incompletely recapitulate human microbial diversity and neuroimmune complexity. Translational progress thus demands advanced experimental platforms that integrate host physiology, microbial dynamics, and biofilm ecology.

Emerging technologies such as gut-on-chip microfluidic systems, zebrafish imaging models, and humanized gnotobiotic mice offer physiologically relevant settings to model host–microbe–phytochemical interactions [96]. These models enable real-time visualization of microbial biofilm formation, metabolite flux, and neuronal signaling under controlled conditions. The integration of these tools with metabolomics and single-cell sequencing will deepen understanding of phytochemical mechanisms and host-specific responses.

Artificial intelligence (AI), bioinformatics, and systems pharmacology represent transformative frontiers for predictive modeling and phytochemical discovery. Machine learning algorithms trained on microbiome and metabolomic datasets can identify bioactive signatures predictive of gut–brain modulation, while network pharmacology approaches map multi-target interactions across microbial and host pathways [97]. These strategies will facilitate the rational design of phytochemical combinations with synergistic antibiofilm, anti-inflammatory, and neuroprotective potential.Future directions should emphasize multi-omics integration, longitudinal clinical trials, and data-driven personalization to ensure reproducibility and regulatory acceptance. As technological and analytical frontiers converge, phytochemical-based antibiofilm therapeutics are poised to evolve from empirical interventions into precision-engineered solutions for maintaining gut–brain homeostasis and preventing complex chronic diseases.

CONCLUSION

Phytochemical-based antibiofilm therapeutics represent a synergistic convergence of natural product pharmacology, microbiome science, and neurogastroenterology, offering a holistic strategy to restore gut-brain homeostasis [98]. Through mechanisms encompassing quorum-sensing inhibition, extracellular polymeric substance disruption, and microbial community remodeling, phytochemicals such as polyphenols, alkaloids, and terpenoids effectively counteract dysbiosis-driven inflammation and neuroimmune imbalance [99]. Their multitargeted actions extend beyond microbial suppression to encompass modulation of host signaling pathways, enhancement of barrier integrity, and normalization of metabolic communication via short-chain fatty acids and tryptophan derivatives.

Advances in animal models-including rodents, zebrafish, *Drosophila*, and *C. elegans*—have been instrumental in elucidating phytochemical effects on microbial ecology, neurobehavioral outcomes, and immune cross-talk [100]. Complementarily, emerging nanotechnological platforms and gut-on-chip systems enable precise modeling and targeted delivery of phytochemical formulations, bridging preclinical mechanisms with translational outcomes.

Looking ahead, the integration of these natural antibiofilm agents into functional foods, synbiotic formulations, and nutraceuticals represents a transformative step toward preventive and personalized gut-brain therapeutics. Omics-driven approaches and AI-enabled microbiome analytics will refine formulation design, ensuring reproducibility, dose optimization, and population-specific efficacy.

Collectively, phytochemical antibiofilm therapeutics embody the evolution of natural medicine into next-generation precision interventions, combining ecological modulation with molecular innovation. Their successful translation into dietary and therapeutic paradigms will redefine microbiota-centered strategies for sustainable neurological and metabolic health.

Ethical approval

NA

Competing interests

The authors have no competing interests to declare that are relevant to the content of this article.

Author Contributions

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