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REVIEW

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Recent advances and applications in the antibacterial activity of *Piper betle* leaf extract

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Abstract: *Piper betle* leaves contain diverse phytochemicals including phenolics, alkaloids, and flavonoids that demonstrate significant inhibitory effects against both Gram-positive and Gram-negative bacteria, particularly *Staphylococcus aureus* and *Escherichia coli*. The review analyzes extraction methodologies, mechanistic aspects of antimicrobial action, and efficacy against specific pathogens. Applications across multiple domains are explored, including clinical treatments for ocular and dental infections, advanced wound healing systems, food preservation through biodegradable packaging, and functional materials development. Despite promising results, challenges remain in standardization, active compound identification, and delivery system optimization. By synthesizing research from the past five years, this review provides a foundation for future development of *P. betle*-based antimicrobial applications that may help address the global challenge of antimicrobial resistance.

Keywords: Piper betle, antibacterial activity, biofilm inhibition, natural preservatives, antimicrobial resistance

Introduction

Antimicrobial resistance represents one of the most pressing global health challenges of the 21st century. The World Health Organization (WHO) reported that drug-resistant bacterial infections caused 1.27 million deaths worldwide in 2019, highlighting the urgency of this crisis [1]. This indicates alarming levels of antimicrobial resistance in common pathogens such as *Escherichia coli* and *Klebsiella pneumoniae*, making infections increasingly challenging to treat with conventional antibiotics.

This trend has intensified the search for alternative antimicrobial agents, particularly those derived from natural sources with established historical use. Among these potential resources, *Piper betle* L. (betel leaf) has emerged as a promising candidate due to its extensive traditional applications and growing scientific validation of its antibacterial properties [2]. This climbing vine, belonging to the Piperaceae family, is characterized by heart-shaped leaves with a deep green color and distinctive aroma. It is widely cultivated throughout Southeast Asia, South Asia, and parts of East Africa, where it has been integral to cultural practices for centuries, including its use as a traditional antiseptic mouthwash.

The biological activities of *P. betle* stem from its rich phytochemical profile, which includes numerous secondary metabolites such as steroids, alkaloids, saponins, flavonoids, and phenolics. These compounds collectively contribute to the plant's antioxidant, anti-inflammatory, and antibacterial properties. The antibacterial efficacy extends to Gram-positive and Gram-negative bacteria, with studies demonstrating significant inhibitory effects against pathogens, including *E. coli*, *Shigella dysenteriae*, and *Staphylococcus aureus*. The antimicrobial spectrum varies with the extraction solvent employed; for instance, chloroform extracts have shown particular efficacy against Gram-negative bacteria such as Klebsiella, Citrobacter, and Proteus species [3].

The mechanisms underlying the antibacterial activity of *P. betle* are multifaceted, involving disruption of bacterial cell membranes, interference with metabolic pathways, inhibition of virulence factor production, and modulation of biofilm formation. This mechanistic diversity contributes to the broad-spectrum activity observed across different bacterial species, potentially reducing the likelihood of resistance development. Phytochemical analysis has identified several compounds that contribute to these antimicrobial properties, including hydroxychavicol, eugenol, chavibetol, and various flavonoids [4,5]

With increasing attention toward herbal-based treatments, *P. betle* leaf extract offers promising alternatives for pharmaceutical development. The extract can be incorporated into various formulations,

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including gels, creams for topical applications, or oral preparations, potentially addressing infections while circumventing some challenges associated with conventional antibiotics. The contemporary relevance of *P. betle* extends beyond direct pharmaceutical applications to diverse fields including food preservation, materials science, and agriculture. In the food industry, *P. betle* has been explored as a natural preservative in biodegradable packaging systems and direct food treatments [6,7]. Materials science applications include incorporating textile treatments, nanoparticle synthesis, and advanced delivery systems, creating functional materials with inherent antimicrobial protection [8,9].

Despite significant potential demonstrated across these applications, several challenges require continued research attention, including standardization of extraction procedures, identification of active compound profiles, optimization of delivery systems, and comprehensive safety evaluations. Addressing these challenges requires interdisciplinary approaches integrating traditional knowledge with modern analytical techniques, pharmaceutical science, materials engineering, and clinical evaluation.

This review aims to comprehensively examine the current state of knowledge regarding the antibacterial properties of *P. betle*, with a particular focus on its activity against clinically significant pathogens, including *Staphylococcus aureus* and *Escherichia coli*. By synthesizing evidence from diverse research conducted over the past five years, this review provides insights into extraction methodologies, mechanistic aspects of antimicrobial action, efficacy against specific bacterial pathogens, and applications across healthcare, food preservation, and materials development. This systematic analysis illuminates established applications and emerging opportunities for leveraging *P. betle*'s antibacterial properties in addressing contemporary challenges related to infection management and antimicrobial resistance while providing a strong foundation for future therapeutic developments.

Methods

This study employed a systematic literature review to analyze the antibacterial potential of *Piper betle* leaves. Data collection utilized PubMed (https://www.ncbi.nlm.nih.gov/) and Springer (https://link.springer.com) databases, which were selected to ensure comprehensive coverage of relevant, credible, and current literature on the topic.

Literature searches were conducted using the keywords "*Piper betle* leaf extract" AND "Antibacterial" AND "Extraction." The selection process adhered to PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines to ensure methodological rigor and transparency [10].

Inclusion criteria comprised: original research articles, English-language publications, publication years 2020-2024, focus on antibacterial effects of *Piper betle* leaf extract. Exclusion criteria comprised: review articles, publications older than 5 years, studies with topics unrelated to the antibacterial properties of *Piper betle*.

Following the systematic screening and eligibility assessment, nine research articles were identified for comprehensive analysis. These publications specifically investigated the therapeutic potential and antibacterial activities of *Piper betle* leaf extract through pharmacological approaches. The complete PRISMA selection workflow is illustrated in Figure 1.

Extraction methods and antimicrobial mechanisms

The antibacterial efficacy of *Piper betle* leaf is significantly influenced by extraction methodologies. Various solvents have been employed to extract bioactive compounds, with notable differences in antimicrobial potency. Comparative analysis of water, ethanol, and hexane solvents revealed that ethanolic extracts demonstrate superior antibacterial activity against soft rot disease-causing bacteria such as *Erwinia caratovora* subsp. caratovora (ECC), with a minimum inhibitory concentration (MIC) of 1.562 mg/mL, substantially lower than hexane extracts (6.25 mg/mL) and aqueous extracts (>12.50 mg/mL) [10]. Time-kill assays further confirmed the bactericidal mode of action, with complete elimination of ECC occurring within 6-8 hours post-treatment, depending on concentration levels.

The effectiveness of extraction processes can be enhanced through post-extraction modifications. Dechlorophyllization techniques represent an important refinement step that addresses the challenge of chlorophyll contamination, which can affect color, stability, and potentially mask bioactive compounds. A comparative evaluation of dechlorophyllization methods revealed that sedimentation processes significantly reduced chlorophyll content while simultaneously enhancing antimicrobial properties.

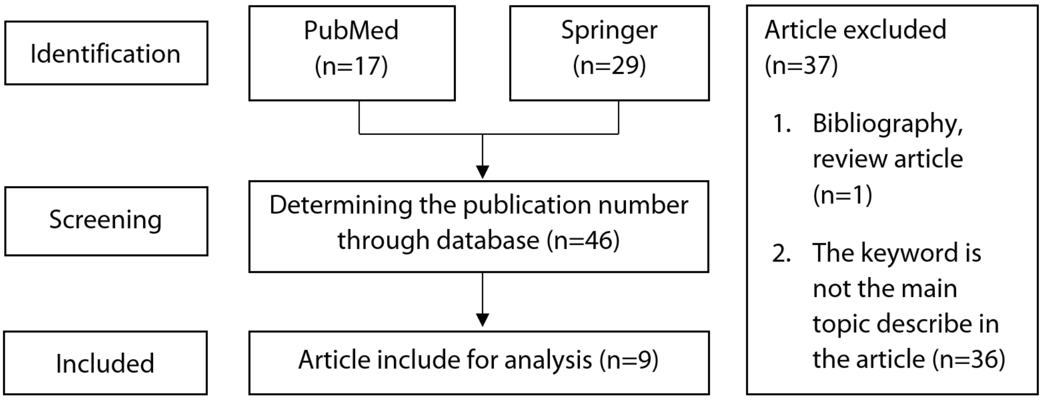

Figure 1. PRISMA diagram related to the analysis of antibacterial activity of *Piper betle*

Table 1. Studies on antibacterial activity of *Piper betle* leaf extracts

Extract type	Target bacteria	Key findings	Applications	Ref
Aqueous extract incorporated into pectin/chitosan films	*S. aureus*, *P. aeruginosa*, *B. cereus*, *K. pneumoniae*	Antibacterial activity increased with higher extract concentration; well-organized homogeneous film structure	Food packaging for purple eggplants	[6]
Chloroform extract	*Bacillus gaemokensis* MW067143	70.11% biofilm inhibition; 57.98% biofilm demolition; synergistic with oxosteroid compounds	Dental caries treatment	[5]
Ethanolic extract	*Staphylococcus aureus* ATCC 29213	MIC: 2500 µg/mL; MBC: 5000 µg/mL; biofilm inhibition at 1250 µg/mL	Potential topical antibacterial and antiseptic	[11]
Ethanolic extract (0.5-3% concentrations)	*S. aureus* from conjunctivitis patients	Dose-dependent inhibition zones; significant antibacterial potential	Treatment of bacterial conjunctivitis	[12]
Multiple extracts and bead formulation	*S. aureus*, *E. coli*	100% antibacterial efficiency; detected eugenol, quercetin, apigenin, kaempferol, ascorbic acid, hydroxychavicol	Wastewater treatment	[4]
Water, ethanol, and hexane extracts	*Erwinia caratovora* subsp. *caratovora*	Ethanol extract most effective (MIC: 1.562 mg/mL); bactericidal action within 6-8 hours	Control of soft rot disease in crops	[13]
Dechlorophyllized ethanolic extracts	Various food spoilage bacteria	Sedimentation process enhanced antimicrobial activity while reducing chlorophyll content	Shelf-life extension of Nile tilapia fillets	[7]
Silver nanoparticles from leaf extract	*E. coli*, *S. aureus*, *C. albicans*, *A. niger*	Synergistic antimicrobial effect; no negative impact on leather properties	Hygienic footwear production	[8]
Ethanolic extract in chitosan hydrogels	*E. coli*, *S. aureus*	70.67-99.94% bacterial inhibition; controlled release following Korsmeyer-Peppas model	Wound treatment via local subcutaneous injection	[9]

When compared to organic solvent-based approaches, the sedimentation method yielded extracts with lower minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values against multiple bacterial species [7]. This finding highlights the potential of green processing techniques to produce safer extracts with improved bioactivity profiles and reduced pigmentation.

Phytochemical analysis has been instrumental in identifying the bioactive constituents responsible for the antibacterial properties of *P. betle* leaf. GC-MS analysis has detected approximately 20 distinct phytocompounds, including tannins, steroids, phenolic compounds, and alkaloids, confirmed through TLC and FTIR techniques. Among these, certain oxosteroids such as Spirost-8-en-11-one,3-hydroxy(3β,5β,14β,20β,22β,25R) have shown promising antibacterial potential, particularly against *Bacillus gaemokensis* isolated from dental caries [5]. High-performance liquid chromatography (HPLC) has further identified six primary compounds: eugenol, quercetin, apigenin, kaempferol, ascorbic acid, and hydroxychavicol, which contribute to the observed antimicrobial effects [4].

The mechanisms underlying the antibacterial activity of *P. betle* extracts operate through multiple pathways involving cellular structure disruption and metabolic interference. Initial studies using scanning electron microscopy have documented significant morphological alterations in bacterial cells following treatment, with cellular disintegration being a prominent feature. Preliminary molecular investigations suggest that bioactive compounds in the extracts interact with various bacterial targets, potentially interfering with essential cellular processes including cell wall synthesis, division, and metabolic activities [5]. These mechanistic insights provide a foundation for understanding the broad-spectrum activity observed across different bacterial species.

The antibacterial efficacy of *P. betle* extracts demonstrates a clear concentration-dependent relationship, with higher concentrations generally showing enhanced inhibitory action. This dose-response pattern has been consistently observed across multiple studies and provides valuable insights for determining optimal concentrations for various applications [9,10].

Recent advances in extraction technology have focused on optimizing both the process and the application formats. Novel approaches include the encapsulation of ethanolic betel leaf extracts into thermo-sensitive chitosan hydrogels, which undergo sol-gel transition at physiological temperatures (37.0–40.0°C) with gelation times of 9.5–13.0 minutes. These systems exhibit controlled diffusion of bioactive compounds following the Korsmeyer-Peppas model, with 50.91–60.29% release of the initial loading content. The released solutions maintain potent antibacterial activity against *E. coli* and *S. aureus*, with inhibition rates of 70.67–99.94% [9]. Such delivery systems offer promising platforms for controlled release in therapeutic applications, particularly for the management of infected wounds.

Antibacterial activity against specific pathogens

Gram-positive bacteria

The antimicrobial efficacy of *P. betle* against Gram-positive bacteria has been extensively investigated, with *Staphylococcus aureus* being one of the most thoroughly studied targets. Ethanolic leaf extracts have shown considerable inhibitory activity against various *S. aureus* strains, including methicillin-sensitive *S. aureus* ATCC 29213, with clearly defined minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values [11]. Different extraction methods and concentrations have demonstrated varying degrees of effectiveness, generally showing dose-dependent relationships between extract concentration and inhibition zone diameters [12].

The antibacterial properties of *P. betle* have also been observed against other Gram-positive species. Silver nanoparticles biosynthesized from *P. betle* leaf extracts exhibited strong antimicrobial effects against *Bacillus cereus* when incorporated into leather materials. This innovative application demonstrates the potential for *P. betle* derivatives in creating antimicrobial surfaces and materials [8].

The antibacterial spectrum extends to other Gram-positive bacteria beyond *S. aureus*. Notably, chloroform extracts of *P. betle* have demonstrated significant activity against Bacillus species, including *B. gaemokensis* isolates. Fractionation studies have identified specific compounds with enhanced antimicrobial potency compared to crude extracts, suggesting the presence of particularly active constituents that could be isolated for more targeted applications [5].

Gram-negative Bacteria

The antibacterial spectrum of *P. betle* extends to Gram-negative bacteria, which are often more resistant to antimicrobial agents due to their additional outer membrane. *Escherichia coli*, a common Gram-negative pathogen, has shown susceptibility to *P. betle* extracts across multiple studies. When applied to leather materials, silver nanoparticles derived from *P. betle* leaf extracts demonstrated significant inhibitory effects against *E. coli*, comparable to their activity against Gram-positive species [8].

In agricultural applications, *P. betle* has shown promise against plant pathogens that cause significant economic losses. Ethanolic extracts exhibited superior antibacterial activity against *Erwinia caratovora* subsp. caratovora (ECC), a causative agent of soft rot disease affecting various crops. Time-kill assays revealed complete eradication of ECC within 6 hours at 4-fold MIC (6.248 mg/mL) and within 8 hours at 2-fold MIC (3.124 mg/mL), indicating a bactericidal mode of action [13]. This finding highlights the potential for developing *P. betle*-based biopesticides as alternatives to synthetic chemical treatments.

Additional studies have confirmed the effectiveness of *P. betle* against other Gram-negative species, including *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. Passion fruit peel pectin/chitosan composite films incorporated with *P. betle* leaf extract demonstrated increasing antibacterial activity against these pathogens proportional to the concentration of the extract [6]. Such findings support the application of *P. betle* in biodegradable food packaging with antimicrobial properties.

Antibiofilm activity

Beyond planktonic bacteria, *P. betle* extracts have shown remarkable efficacy against bacterial biofilms, which represent a significant challenge in both clinical and industrial settings due to their enhanced resistance to antimicrobial agents. At subinhibitory concentrations (1250 μg/mL), ethanolic *P. betle* extracts demonstrated biofilm inhibition activity against *S. aureus* ATCC 29213, although their biofilm eradication capability was less pronounced compared to conventional antibiotics such as oxacillin [11].

The antibiofilm activity of *P. betle* extracts represents a particularly valuable property for addressing biofilm-associated challenges in various contexts. Studies have demonstrated significant inhibition of biofilm formation and substantial destruction of established biofilms across multiple bacterial species. Microscopic examination has confirmed that *P. betle* extracts not only prevent biofilm formation but also disrupt the structural integrity of existing biofilms, potentially by interfering with intercellular communication and extracellular matrix components [5,11]. This ability to combat bacterial biofilms distinguishes *P. betle* from many conventional antimicrobial agents that show limited efficacy against these complex bacterial communities.

Recent advances in delivery systems have further enhanced the anti-biofilm potential of *P. betle* extracts. Encapsulation in thermo-sensitive chitosan hydrogels has demonstrated sustained release of bioactive compounds with maintained antibacterial efficacy against both *E. coli* and *S. aureus*, with inhibition rates ranging from 70.67% to 99.94% [6]. Such controlled-release systems offer promising platforms for the management of biofilm-associated infections, particularly in wound treatment applications.

The broad-spectrum antibacterial and anti-biofilm properties of *P. betle* extracts against various pathogens highlight its significant potential for development into natural antimicrobial formulations. The demonstrated efficacy against both Gram-positive and Gram-negative bacteria, including drug-resistant and biofilm-forming strains, positions *P. betle* as a valuable resource in the ongoing search for alternatives to conventional antimicrobial agents.

Medical and clinical applications

Treatment of infections

Ocular infections, particularly bacterial conjunctivitis, present significant challenges in ophthalmology due to increasing antibiotic resistance profiles. *P. betle* leaf extracts have demonstrated promising potential for ophthalmological applications, particularly against common conjunctival pathogens. The established antibacterial efficacy against ocular isolates of *Staphylococcus aureus* suggests possibilities for developing topical ophthalmic preparations, offering alternatives for patients experiencing treatment failure with conventional antibiotics or those seeking natural therapeutic options [12].

The oral cavity represents another clinical domain where *P. betle* extracts show therapeutic promise. Comprehensive research on dental applications has demonstrated efficacy against cariogenic bacteria, with particular potential for addressing biofilm-associated dental infections. The ability of *P. betle* extracts to both prevent biofilm formation and disrupt established biofilms makes them particularly valuable for dental applications, where biofilm-mediated diseases predominate. Advanced molecular investigations have elucidated potential mechanisms of action, identifying specific bacterial targets and pathways affected by bioactive compounds in the extracts. These findings provide a scientific basis for the traditional use of *P. betle* in oral health practices and suggest directions for developing modern dental formulations [5].

The clinical relevance of *P. betle* extracts extends to addressing biofilm-associated infections, which present particular challenges in healthcare settings due to their enhanced resistance profiles. Ethanolic extracts have demonstrated significant activity against methicillin-sensitive *Staphylococcus aureus* ATCC 29213, with minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of 2500 μg/mL and 5000 μg/mL, respectively. At subinhibitory concentrations (1250 μg/mL), these extracts effectively inhibited biofilm formation, although their biofilm eradication capability was less pronounced compared to conventional antibiotics such as oxacillin. These findings suggest potential applications in preventing biofilm formation on medical devices and implants, where infection remains a significant clinical challenge [11].

Wound healing applications

The management of infected wounds represents a critical area where novel antimicrobial approaches are urgently needed. Recent advances in delivery systems have significantly enhanced the therapeutic potential of *P. betle* extracts for wound treatment. Innovative research has successfully encapsulated ethanolic betel leaf extracts into thermo-sensitive chitosan hydrogels, creating sophisticated delivery systems specifically designed for infected wound management. These advanced formulations maintain the antimicrobial efficacy of *P. betle* while providing controlled release profiles suitable for wound healing timelines. The clinical advantage of such systems lies in their ability to deliver consistent antimicrobial action directly at the infection site while conforming to irregular wound geometries [9].

The potential for subcutaneous injection of these thermo-sensitive hydrogels represents a significant advancement for wound management, particularly for irregularly shaped or difficult-to-access wounds. Upon injection, the solution transitions to a gel state at body temperature, conforming to the wound shape and providing sustained release of antimicrobial compounds directly at the infection site. This targeted delivery approach potentially reduces systemic side effects while maximizing local therapeutic efficacy [9].

Beyond direct antimicrobial effects, *P. betle* extracts may contribute to wound healing through additional mechanisms. The presence of bioactive compounds with antioxidant and anti-inflammatory properties potentially creates a microenvironment conducive to tissue regeneration while controlling infection. This multifunctional therapeutic profile offers advantages over conventional antimicrobial treatments that may impede healing processes through cytotoxic effects on regenerating tissues [5,9].

The integration of *P. betle* extracts into advanced biomaterials continues to expand potential clinical applications. Silver nanoparticles biosynthesized from *P. betle* leaf extracts have been successfully embedded into leather matrices for potential applications in medical footwear. These modified materials maintain their physical properties while gaining significant antimicrobial efficacy against common skin pathogens, including *Staphylococcus aureus*. This approach holds particular promise for preventing foot infections in vulnerable populations, such as individuals with diabetes who face elevated risks of foot ulcers and subsequent infections [8].

Biomaterial development and novel applications

Nanoparticle synthesis

Green synthesis methodologies have emerged as environmentally favorable alternatives to conventional chemical synthesis approaches for nanoparticle production. *P. betle* leaf extract has demonstrated remarkable efficacy as a reducing and stabilizing agent in the biosynthesis of silver nanoparticles (AgPBL). This eco-friendly approach leverages the phytochemical constituents naturally present in the leaf extract to facilitate the reduction of silver ions to elemental silver and subsequently stabilize the resulting nanoparticles. The process occurs under mild conditions without requiring hazardous chemicals, representing a significant advantage over traditional synthesis methods that often utilize toxic reducing agents and generate harmful byproducts [8].

The resulting silver nanoparticles exhibit enhanced antimicrobial properties compared to either silver or *P. betle* extract alone, suggesting synergistic interactions that amplify their biological activity. This synergism likely results from multiple mechanisms of action, including disruption of bacterial cell membranes by silver ions and simultaneous interference with cellular processes by bioactive compounds from the extract. Characterization of these bio-synthesized nanoparticles has confirmed their stability and appropriate physicochemical properties for integration into various material systems, while maintaining their antimicrobial efficacy over extended periods [8].

The application potential of these bio-synthesized nanoparticles extends beyond direct antimicrobial applications to include catalysis, sensing technologies, and biomedical imaging. Their green synthesis methodology aligns with growing emphasis on sustainable production processes in nanotechnology, potentially reducing environmental impacts associated with nanoparticle manufacturing while maintaining functional performance [8].

Functional materials with antimicrobial properties

The incorporation of *P. betle*-derived antimicrobial agents into various materials has created functional products with inherent protection against microbial colonization. An innovative application involves the modification of natural materials like leather with bio-synthesized silver nanoparticles from *P. betle* leaf extract (AgPBL). These modified materials demonstrate significant antimicrobial properties against both bacteria and fungi while maintaining their essential physical characteristics and performance properties.

The integration of *P. betle*-based antimicrobial systems into traditional materials exemplifies how natural antimicrobial compounds can enhance conventional products. Standardized antimicrobial testing has confirmed efficacy against a broad spectrum of microorganisms, including both Gram-positive and Gram-negative bacteria, as well as fungi and yeasts. This versatility makes *P. betle*-derived antimicrobial systems suitable for applications ranging from consumer products to specialized medical materials [8].

A particularly valuable characteristic of these functional materials is the preservation of their original performance properties alongside the added antimicrobial benefits. This balanced approach ensures that antimicrobial enhancement does not compromise the material's primary functions, making the technology commercially viable for sectors where both antimicrobial protection and material performance are essential requirements [8].

Controlled release systems

The development of these delivery systems represents a truly interdisciplinary approach, combining principles from pharmaceutical technology, polymer science, and antimicrobial research. The systems are designed to address specific challenges in antimicrobial delivery, including maintaining stability of bioactive compounds, achieving appropriate release kinetics, and ensuring compatibility with the intended application environment. The engineering of these delivery platforms demonstrates how traditional herbal extracts can be transformed into modern therapeutic systems through advanced material science approaches [9].

These controlled-release systems significantly expand the application potential of *P. betle* extracts beyond their traditional uses. By enabling precise control over the temporal and spatial release of antimicrobial compounds, these systems can optimize efficacy while minimizing waste and potential side effects. This approach represents an important bridge between traditional herbal medicine and modern pharmaceutical technology, potentially increasing the acceptance and adoption of plant-based antimicrobials in contemporary healthcare applications [9].

Conclusion

This review highlights the significant potential of *P. betle* leaf extracts as natural antibacterial agents across diverse applications. The evidence from recent studies demonstrates robust efficacy against both Gram-positive and Gram-negative bacteria, including *Staphylococcus aureus* and *Escherichia coli*. The antimicrobial mechanisms involve multiple pathways, contributing to broad-spectrum activity and potential advantages in addressing antimicrobial resistance. Extraction methodology significantly influences efficacy, with ethanolic extracts generally showing superior performance, though varying solvents may optimize activity against specific microorganisms. The applications span healthcare (dental, ocular, and wound treatments), food preservation (packaging films and direct food treatments), agricultural protection (crop disease management), and functional materials development (antimicrobial textiles and controlled-release systems). Despite promising results, further research is needed to standardize extraction procedures, identify optimal delivery systems, and establish comprehensive safety profiles. As antimicrobial resistance continues to challenge global health systems, *P. betle* leaf extracts represent a valuable resource for developing natural alternatives that align with the growing consumer preference for plant-based solutions while potentially addressing some limitations of conventional antimicrobials.

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Author contribution

LW: investigation, visualization, original draft writing; GBO: editing, draft writing KTW: visualization, draft writing, editing; KRDY: supervision, conceptualization, methodology.

Declaration of interest

The authors declare there are not competing interests.

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