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Review article

Punica granatum as a source of novel antibacterial agents for combating bovine mastitis and multidrug resistance

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Abstract

Antimicrobial resistance has emerged as a major challenge for both human and veterinary health, highlighting the need for novel therapeutic approaches. Bovine mastitis remains one of the most economically significant infectious diseases affecting dairy production worldwide. In this narrative review, we examine current evidence regarding the antibacterial potential of *Punica granatum* L. (pomegranate) and its major bioactive constituents, particularly punicalagin, against clinically relevant multidrug-resistant bacterial pathogens. Recent studies indicate that pomegranate-derived extracts possess antibacterial activity against several mastitis-associated organisms, including *Staphylococcus aureus* and *Escherichia coli*. Experimental evidence suggests that punicalagin may contribute to membrane destabilization, increased membrane permeability, interference with biofilm formation, and modulation of bacterial stress-response pathways, although the relative importance of these mechanisms appears to vary among bacterial species and experimental systems. Several studies have also reported synergistic interactions between pomegranate-derived compounds and conventional antibiotics against selected multidrug-resistant pathogens. Despite these promising findings, the current evidence remains dominated by *in vitro* investigations, while *in vivo* efficacy, pharmacokinetic characterization, toxicological assessment, and clinical validation remain limited.

Keywords: Anti-virulence, Efflux pump inhibitors, Ellagitannins, Membrane disruption, Natural products, Punicalagin

Introduction

The medical community faces a public health crisis of growing proportions: the rapid emergence and global dissemination of bacterial strains resistant to existing antibiotics. The failure to introduce new classes of antibacterial drugs into clinical use in recent decades represents a critical vulnerability, threatening a return to the pre-antibiotic era [1, 2]. This challenge is intensified by the inherent resilience of pathogens, including the ESKAPE group (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species), which encompasses both Gram-positive and Gram-negative bacteria, many of which possess barriers such as the outer membrane or efflux mechanisms that hinder the action of antibacterial compounds [3]. The urgent need, therefore, is not only for new molecules but for compounds with innovative mechanisms of action, capable of overcoming these bacterial defenses or restoring the effectiveness of our existing arsenal [1].

The severity of the antimicrobial resistance (AMR) crisis is underscored by the economic and health burden of common but increasingly intractable infections. Mastitis, an inflammatory condition of the mammary gland, serves as a compelling and economically devastating model of the challenges posed by multidrug-resistant (MDR) bacterial infections. In the dairy industry, bovine mastitis is recognized as one of the most costly diseases, resulting in reduced milk production, compromised milk quality, and increased veterinary expenses [4]. It is important to note that the responsible pathogens, such as *S. aureus* and *E. coli*, are often zoonotic, posing a direct risk to public health through the food chain and contributing to the global pool of resistance genes [5].

The management of bovine mastitis goes beyond direct infection control, encompassing hygienic and prophylactic measures, including the appropriate use of antiseptics and disinfectants and the application of antimicrobials [6]. However, the use of antibiotics in dairy production has generated increasing concern due to the potential public health risks, including the presence of antimicrobial residues in milk and the selection of resistant pathogens. In addition to impacts on human health, antibiotic residues can reach the environment, contaminate soil and water resources, and adversely affect local microbiota and groundwater quality. This scenario poses a significant threat to global health and underscores the need to adopt the One Health approach, which integrates animal, human, and environmental health as a fundamental strategy for mitigating the risks associated with antimicrobial use [1, 7]. The search for alternatives to traditional antibiotics, such as natural compounds, therefore becomes imperative for the sustainability of public and environmental health.

A renewed emphasis on natural compounds and their derivatives is necessary. Most of our most effective antibiotics have historically been derived from natural resources. However, this abundant resource remains underutilized, especially given contemporary drug discovery methods. This narrative review focuses on the strong evidence supporting the use of pomegranate as a powerful, natural source of antibacterial agents. An urgent paradigm shift in treatment and prevention approaches is necessary due to the magnitude of the mastitis problem, which is predicted to cost the global dairy industry billions of dollars annually, as well as the growing public health concern surrounding antibiotic residues. Finding new compounds that are both efficient and environmentally sustainable is becoming essential, not optional [1]. Here, we aim to synthesize the current understanding of *Punica* (*P.*) *granatum*'s efficacy against mastitis-causing MDR bacteria, delve into the mechanistic insights into its action, and discuss its potential role in novel delivery systems and combination therapies, positioning it as a candidate for new drug discovery and development. Our analysis highlights how the effects of its key compounds offer a multi-target approach that is more resilient to the development of resistance than single-target synthetic drugs.

Punica granatum as a reservoir of antibacterial phytochemicals

Pomegranate, *Punica granatum* L. (family *Lythraceae*), has been valued in traditional medicine, with its many parts, fruit, juice, flowers, and peel, used to treat a variety of ailments, including infections, inflammation, and digestive problems. Modern phytochemistry has corroborated traditional knowledge by identifying this fruit as a high source of beneficial chemicals, primarily polyphenols. While the juice is well known for its antioxidant effects, the highest concentrations of antibacterial compounds are found in the often discarded peel (Table 1) and, to a lesser extent, in the flowers and leaves [8, 9].

The main class of compounds associated with the observed antibacterial activity is that of ellagitannins, with Punicalagin being the most abundant and physiologically active [3]. However, the contribution of other compounds present in the extract should not be underestimated. Punicalagin is a large hydrolyzable tannin found mainly in two isomers: Punicalagin A and Punicalagin B. It is easily degraded into smaller, highly active molecules, particularly ellagic acid and gallagic acid, which contribute significantly to overall bioactivity [10]. In addition to ellagitannins, pomegranate extracts contain a complex mixture of other compounds that likely contribute to the observed synergistic effects, including flavonoids (quercetin, kaempferol, and luteolin), anthocyanins (responsible for the fruit's red color, comprising delphinidin, cyanidin, and pelargonidin glycosides), and phenolic acids (gallic acid, caffeic acid, and ferulic acid). The complexity of this phytochemical profile suggests the hypothesis that the total extract, or a standardized fraction, may offer a broader spectrum of activity and, theoretically, a lower risk of resistance development compared to a single isolated compound, a concept known as phytocomplex synergy. While promising, this hypothesis requires more robust *in vivo* validation. Recent studies have also highlighted the antibacterial potential of the leaf extract, demonstrating efficacy against MDR *E. coli* and further expanding the utility of the entire plant [11].

Table 1: Overview of *P. granatum* plant parts, their main bioactive constituents, and corresponding observed biological effects.

Plant part	Main bioactive compounds	Observed biological effect	References
Peel	Punicalagin (A & B), ellagic acid, gallic acid	Rapid bactericidal effect, membrane disruption, efflux pump inhibition (*in vitro*)	[3, 8, 12, 15]
Flower	Punicalagin, polysaccharides	Anti-inflammatory effect, mastitis improvement (*in vivo*)	[9, 13]
Leaf	Flavonoids, phenolic acids	Activity against Gram-negative MDR pathogens (*in vitro*)	[11]

Antibacterial efficacy against mastitis pathogens

The *in vitro* efficacy of *P. granatum* extracts against the major etiological agents of mastitis has been consistently demonstrated in the literature. Studies indicate that hydroalcoholic and aqueous extracts from peel and flowers possess significant inhibitory and bactericidal activity against these pathogens under laboratory conditions [3, 14].

In vitro validation and potency

A critical aspect of evaluating a potential antibacterial agent is determining its minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The literature reports MIC values for pomegranate peel extracts ranging widely, from 0.5 to 10 mg/mL, against common mastitis-causing bacteria [14]. It is fundamental to note that this 20-fold variability reflects substantial differences in extraction methods, solvents used, plant cultivars, and bacterial strains tested. This lack of standardization poses a significant challenge to direct comparisons between studies and to the evaluation of the clinical significance of these values. For a more robust interpretation, it is essential that future studies include reference comparator antibiotics and use

standardized interpretive criteria. Despite these caveats, the observed *in vitro* potency, particularly against *S. aureus* and *E. coli* strains isolated from clinical mastitis cases, suggests potential relevance warranting further investigation. Table 2 illustrates the broad-spectrum efficacy of the extracts, with potency demonstrated in different plant parts and against Gram-positive and Gram-negative bacteria [9, 11, 14, 15].

Table 2: Summary of minimum inhibitory concentrations (MIC) of *P. granatum* extracts against main mastitis pathogens.

Pathogen	Pomegranate part	Extract type	MIC/MBC range (mg/mL)	References
S. aureus	Peel	Hydroalcoholic	0.5 - 5.0	[14, 15]
E. coli	Peel	Hydroalcoholic	1.0 - 10.0	[14, 15]
Staphylococcus spp.	Flower	Methanolic	0.625 - 2.5	[9]
E. coli (MDR)	Leaf	Methanolic	1.25	[11]

S. aureus = *Staphylococcus aureus*, *E. coli* = *Escherichia coli*, MDR = multidrug-resistant.

A particularly noteworthy finding is the rapid bactericidal activity observed in some studies, in which complete growth inhibition was achieved within substantially shorter contact durations, sometimes in just 30 seconds [15]. This rapid action is a highly desirable characteristic in an antibacterial agent, as it minimizes the time available for bacteria to develop a resistance response and is critical for topical applications, such as teat dips in dairy farming. Furthermore, the relevance of *P. granatum* extends beyond mastitis, as its extracts demonstrate effective antimicrobial activity against other members of the ESKAPE group, broadening its potential applications in systemic MDR infections [16].

Antivirulence and biofilm inhibition

One of the most significant virulence factors in chronic infections, including mastitis, is biofilm formation, a complex structured community of bacteria encased in a self-produced polymeric matrix. Biofilms provide a physical barrier against antibiotics and the host immune system, making infections highly persistent. *In vitro* studies demonstrate the antibiofilm capacity of pomegranate extract against biofilms produced by relevant bacterial pathogens, such as *S. aureus*, methicillin-resistant *S. aureus* (MRSA), *E. coli*, and *P. aeruginosa* [17, 18].

Recent integrated omics studies suggest that Punicalagin can significantly inhibit the growth and biofilm formation of *S. aureus in vitro* [19]. This inhibition has been associated with the compound's ability to interfere with the bacterial quorum sensing (QS) system, the cell-to-cell communication network that regulates the expression of virulence genes and biofilm initiation [2]. By disrupting QS, Punicalagin could, theoretically, disarm bacteria, making them more susceptible to elimination by the host immune system or co-administered antibiotics. While this proposed dual action, direct bactericidal effect, and antivirulence activity suggest that Punicalagin may be a sophisticated therapeutic agent, it is crucial to emphasize that these observations are based on *in vitro* models and require rigorous *in vivo* validation before general therapeutic conclusions can be drawn [2, 9].

Mechanistic insights

To be considered a true candidate for new drug development, a compound must demonstrate a distinct and effective mechanism of action. Punicalagin and other polyphenols from *P. granatum* have been investigated for their multifaceted effects, which appear to target bacterial structural defenses, particularly the outer membrane of Gram-negatives and efflux pump systems that drive MDR [2]. However, it is crucial to rigorously contextualize mechanistic findings, explicitly linking each assertion to the bacterial species and experimental systems from which they derive (Table 3). One of the best-characterized mechanisms proposed for Punicalagin is its ability to destabilize the integrity of the bacterial cell membrane [2, 15]. This mechanism has been suggested to be particularly relevant against Gram-negative bacteria, where the outer membrane serves as a critical barrier. Based primarily on studies performed in *Vibrio parahaemolyticus*, Punicalagin is proposed to interact with the lipopolysaccharide layer, leading to its destabilization [3].

It is important to note that this interaction is a hypothesis based on *in vitro* evidence and requires further investigation to elucidate the precise molecular details and *in vivo* relevance. *In vitro* assays using fluorescent probes, such as 1-N-phenylnaphthylamine (NPN) uptake, indicate that Punicalagin can increase outer membrane permeability in certain bacterial strains [3]. This alteration in permeability has been associated with subsequent damage to the underlying cytoplasmic membrane. Studies have shown that exposure to Punicalagin can cause membrane depolarization and the consequent leakage of vital intracellular contents, including potassium ions (K^+), proteins, and nucleic acids [2, 3]. The loss of these essential components is incompatible with bacterial survival, which may explain the rapid bactericidal effect observed *in vitro*. By compromising this outer barrier, Punicalagin not only exerts its own bactericidal effect but can also make bacteria more susceptible to other agents.

Table 3: Proposed antibacterial mechanisms associated with *P. granatum*-derived compounds.

Proposed mechanism	Representative biological effects	Bacterial models	Experimental evidence	References
Membrane destabilization	Increased membrane permeability, membrane depolarization, leakage of intracellular constituents	*V. parahaemolyticus* and selected Gram-negative bacteria	NPN uptake assays, membrane potential measurements, potassium leakage assays	[2, 3, 15]
Modulation of resistance-associated pathways	Altered expression of stress-response and resistance-related genes	*S. aureus*	Transcriptomic and integrated omics analyses	[12, 19]
Biofilm inhibition	Reduced biofilm biomass and biofilm formation	*S. aureus*, MRSA, *E. coli*, *P. aeruginosa*	Biofilm quantification assays	[2, 19]
Anti-inflammatory activity	Reduced inflammatory cytokine production	Experimental mastitis models	Cytokine quantification and histopathology	[13]

V. parahaemolyticus = *Vibrio* parahaemolyticus, *S. aureus* = *Staphylococcus aureus*, MRSA = methicillin-resistant *Staphylococcus aureus*, *E. coli* = *Escherichia coli*, *P. aeruginosa* = *Pseudomonas aeruginosa*, NPN = 1-N-phenylnaphthylamine.

This approach of destabilizing outer membranes is often highlighted as a necessary strategy to make standard antibiotics more effective against Gram-negative pathogens [15].

One of the main mechanisms of MDR, especially in ESKAPE Gram-negative pathogens, is the overexpression of efflux pumps, transmembrane protein complexes that actively expel antibiotics and other toxic compounds from the bacterial cell, thereby reducing intracellular drug concentration below the therapeutic level. Integrated transcriptomic analyses in *S. aureus* suggest that Punicalagin may modulate regulatory networks associated with efflux pump activity [12, 19]. This study demonstrated that Punicalagin can interfere with key efflux pump systems in *S. aureus*, including the two-component regulatory systems BraRS and GraRS, which regulate the expression of efflux pump components such as VraG and VraF. By modulating these regulatory pathways, Punicalagin could, in *S. aureus*, disarm the efflux machinery, potentially preventing the expulsion of co-administered antibiotics. While these findings are promising for *S. aureus*, generalization to other MDR pathogens, including ESKAPE Gram-negatives, requires specific experimental validation for each species. The dual action of membrane disruption (increased passive entry) and efflux pump inhibition (reduced active efflux) represents a sophisticated approach that addresses multiple aspects of MDR and spots Punicalagin as a promising anti-MDR agent [15].

A strategy for combination therapy

The concept of combination therapy, which involves repurposing old drugs or using co-adjuvant compounds, is a key area of research. Punicalagin has been shown, using *in vitro* studies, to significantly restore the sensitivity of MDR strains, including MRSA and various *Enterobacteriaceae*, to conventional antibiotics [4]. Punicalagin's potential lies in its role as a sensitizing agent [2, 12, 20]. This synergistic effect may result from the combined influence of membrane perturbation, altered permeability, and modulation of bacterial stress-response pathways, although the precise molecular basis remains incompletely understood. By compromising the bacterial cell wall or membrane and inhibiting efflux pumps, Punicalagin can effectively circumvent key bacterial resistance mechanisms, allowing the co-administered antibiotic to reach its intracellular target at a lethal concentration [20].

The Fractional Inhibitory Concentration Index (FICI) value (Table 4) provides a quantitative assessment of the interaction between two antimicrobial agents; according to established interpretive criteria, a value ≤ 0.5 indicates synergistic activity. This quantitative evidence provides initial support for the use of Punicalagin as an adjuvant therapy, due to its combined antibacterial and antivirulence properties [14, 15].

Table 4: Reported interactions between punicalagin and selected conventional antibiotics in experimental studies**.**

Pathogen	Antibiotic	FICI	Conclusion	References
S. aureus (MRSA)	Oxacillin	0.5	Synergistic	[4]
K. pneumoniae (MDR)	Ciprofloxacin	0.5	Additive/Synergistic	[12]
E. coli (MDR)	Ampicillin	0.5	Additive/Synergistic	[12]
S. aureus (clinical isolate)	Gentamicin	0.25	Synergistic	[14]

S. aureus = *Staphylococcus aureus*, MRSA = methicillin-resistant *Staphylococcus aureus*, *K. pneumoniae* = *Klebsiella pneumoniae*, MDR = multidrug-resistant, *E. coli* = *Escherichia coli*, FICI = Fractional Inhibitory Concentration Index.

However, it is fundamental that these *in vitro* results be validated by *in vivo* studies to confirm clinical relevance. This approach offers a promising path for the treatment of mastitis, in which a combination of Punicalagin and an antibiotic could reduce the required dose of the conventional drug, mitigate the risk of antibiotic residues in milk and meat, and decrease the selective pressure for the development of resistance. The use of a natural product derivative as an adjuvant is an attractive strategy that aligns with the growing demand for sustainable and innovative therapeutic solutions. Furthermore, the inherent anti-inflammatory properties of pomegranate polyphenols, although not explicitly discussed in the context of their antibacterial action, represent a valuable secondary benefit in the treatment of mastitis, a disease fundamentally characterized by inflammation. This dual action capability, combating infection and simultaneously mitigating tissue damage, highlights the multi-target therapeutic potential of *P. granatum* extracts, a characteristic rarely found in conventional antibiotics [15]. However, the *in vivo* validation of these properties and the demonstration of their clinical relevance are key steps that remain largely unexplored.

Future perspectives and novel formulations

The journey from a natural product extract to a clinically approved drug is long and complex. Current evidence supports continued investigation of *P. granatum*-derived compounds through well-designed preclinical studies to address existing gaps in pharmacological and translational knowledge. The immediate focus must shift from *in vitro* validation to preclinical *in vivo* models and the development of advanced delivery systems. A critical area for future investigation is the standardization of extracts. Given the variability in phytochemical composition across cultivars, geographic origins, and extraction methods, a robust, standardized extract with a guaranteed Punicalagin content is essential for reproducible clinical trials and eventual commercialization. This standardization, coupled with rigorous toxicological assessment, will be the final hurdle in translating the promise of the pomegranate from the laboratory bench to the farm and the clinic.

In vivo validation and efficacy

While *in vitro* data are promising, the true test of any new antibacterial agent lies in its efficacy and safety *in vivo*. Recent studies have begun to bridge this gap. For instance, research has demonstrated that a polysaccharide fraction derived from pomegranate flowers can significantly improve mastitis by *in vivo* models, primarily by modulating the inflammatory response and reducing bacterial load [13]. This suggests that different components of the pomegranate plant can be leveraged in a therapeutic approach to address both the infection and the associated inflammation. Furthermore, the development of novel delivery systems is principal for practical application. For mastitis prophylaxis in dairy cattle, a key innovation is the use of film-forming systems based on pomegranate peel extract [5]. These systems are designed to be applied topically to the teats, forming a protective, sustained-release barrier that prevents bacterial entry and provides a localized, high concentration of the active compounds. This approach offers a potential prophylactic strategy that is non-antibiotic and reduces the risk of residues in the milk supply.

Nanotechnology and targeted delivery

Nanoformulations, such as liposomes, nanoparticles, and nanofibers, can encapsulate active compounds, protecting them from degradation and facilitating their penetration into deep-seated infection sites, including mammary gland tissue and bacterial biofilms. Several studies have explored the preparation of nanofibers containing pomegranate peel extract for application as antibacterial wound dressings, demonstrating the feasibility of incorporating the extract into advanced biomaterials [21]. Similarly, the development of nano-herbal formulations aims to leverage enhanced drug-delivery selectivity and improved antibacterial activity observed when the extract is delivered at the nanoscale. This approach not only maximizes the therapeutic effect but also minimizes potential systemic toxicity, paving the way for a new generation of highly effective, targeted anti-mastitis treatments [22, 23].

Toxicological, pharmacokinetic, and regulatory challenges

Despite the promising antibacterial properties reported for *P. granatum*-derived compounds, several important barriers must be addressed before these molecules can be considered viable therapeutic candidates for veterinary or human use. One of the most significant challenges concerns pharmacokinetics and bioavailability [24]. Punicalagin, the principal ellagitannin found in pomegranate peel, possesses a high molecular weight and limited membrane permeability, characteristics that may restrict systemic absorption following oral administration [25]. Furthermore, punicalagin undergoes extensive hydrolysis in the gastrointestinal tract, yielding ellagic acid and, subsequently, urolithins via microbial metabolism [26]. Consequently, the bioactive molecules reaching target tissues may differ substantially from the parent compound evaluated in many *in vitro* studies. This metabolic complexity complicates the interpretation of pharmacological data and raises important questions regarding the relationship between *in vitro* antibacterial activity and *in vivo* efficacy.

A second challenge is the lack of pharmacokinetic studies in dairy cattle. While antibacterial activity has been demonstrated under laboratory conditions, little information is available on the concentrations of punicalagin or related metabolites achievable in mammary gland tissues following topical, oral, or intramammary administration. As a result, it remains unclear whether the inhibitory concentrations reported *in vitro* are physiologically attainable under practical farming conditions. Although several studies have reported minimum inhibitory concentrations for punicalagin and pomegranate-derived extracts, these values alone do not establish therapeutic feasibility. In the absence of pharmacokinetic/pharmacodynamic (PK/PD) studies, it remains unknown whether these compounds can attain and maintain concentrations above the reported MIC values at the site of infection following topical, intramammary, or systemic administration. Furthermore, factors such as tissue penetration, protein binding, formulation stability, and residence time within the mammary gland may substantially influence antibacterial efficacy. Consequently, direct extrapolation of *in vitro* susceptibility data to clinical effectiveness should be avoided until appropriate PK/PD investigations have been performed. Future investigations should therefore focus on determining absorption, distribution, metabolism, excretion, and tissue persistence profiles in relevant veterinary species [27].

Toxicological evaluation represents another critical knowledge gap. Although pomegranate-derived products are generally regarded as having favorable safety profiles and have been widely consumed as foods and traditional remedies, safety data obtained from dietary exposure cannot be directly extrapolated to therapeutic applications. Antibacterial activity often requires concentrations substantially higher than those encountered through normal dietary consumption. In addition, tannin-rich preparations may exhibit dose-dependent biological effects, including alterations in nutrient digestibility, protein interactions, and gastrointestinal physiology. Comprehensive toxicological assessments, including acute toxicity, repeated-dose toxicity, local tissue tolerance, reproductive safety, and residue studies, remain necessary before clinical implementation can be considered [28].

The potential presence of residues in milk also warrants careful consideration. One of the principal motivations for developing alternative mastitis therapies is to reduce antibiotic residues in dairy products. However, before pomegranate-derived formulations can be adopted commercially, regulatory authorities will require evidence regarding residue persistence, withdrawal periods, consumer safety, and potential impacts on milk quality. At present, these data remain largely unavailable. Another important limitation concerns extract standardization. The phytochemical composition of *P. granatum* extracts can vary substantially depending on cultivar, geographical origin, environmental conditions, harvest timing, storage conditions, and extraction methodology. Such variability may directly influence antibacterial potency and reproducibility. Therefore, future translational efforts should prioritize the development of standardized formulations with well-characterized phytochemical profiles, including quantitative determination of major bioactive constituents such as punicalagin, ellagic acid, and related polyphenols [29].

From a regulatory perspective, plant-derived therapeutics face unique challenges because they often contain complex mixtures of bioactive compounds rather than single active pharmaceutical ingredients. Regulatory agencies generally require robust evidence of efficacy, safety, manufacturing consistency, and quality control [30]. Consequently, substantial investment in formulation development, pharmacological characterization, toxicological assessment, and controlled clinical trials will be necessary before pomegranate-derived antibacterial products can achieve regulatory approval.

Conclusions

The growing prevalence of antimicrobial resistance among both human and veterinary pathogens has intensified the search for alternative and complementary antimicrobial strategies. Within this context, *Punica granatum* has emerged as a promising source of bioactive compounds with potential relevance for combating mastitis-associated pathogens and selected MDR bacteria. The available literature demonstrates that pomegranate-derived extracts, particularly those enriched in punicalagin and related polyphenols, exhibit antibacterial, anti-biofilm, and antibiotic-sensitizing activities under experimental conditions. Mechanistic studies suggest that these effects may involve membrane destabilization, interference with bacterial stress-response systems, inhibition of biofilm formation, and modulation of resistance-associated pathways. However, the relative contributions of these mechanisms appear to vary across bacterial species and experimental systems, and many mechanistic hypotheses remain incompletely understood.

Despite encouraging laboratory findings, important limitations currently restrict translational interpretation. Most available evidence originates from *in vitro* investigations, whereas robust *in vivo* efficacy data remains scarce. Furthermore, major knowledge gaps persist regarding pharmacokinetics, toxicology, formulation optimization, tissue distribution, residue profiles, and regulatory requirements, particularly in dairy cattle. Variability among plant materials and extraction procedures also poses challenges to standardization and reproducibility. Future research should prioritize standardized characterization of extracts, mechanistic

validation in clinically relevant pathogens, pharmacokinetic and toxicological studies, and well-designed animal trials to establish efficacy under practical conditions.

From a One Health perspective, exploring plant-derived antibacterial compounds may contribute to broader efforts to reduce reliance on antimicrobials and mitigate the spread of antimicrobial resistance across human, animal, and environmental interfaces. However, the successful translation of pomegranate-derived compounds into clinical or veterinary applications will depend upon rigorous experimental validation and comprehensive assessment of safety, efficacy, and regulatory feasibility.

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