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A Review on Bioactive Compounds from Seeds: Extraction Approaches and Antibacterial Potential

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Name of Author:
M Shahul Hameedh[1], Latheesh j Lal L*[1], , S Muthukumar[2]

Affiliation: *[1]St.Joseph University, Tamilnadu*
[2]Professor,Dept of Pharmaceutics,Arulmigu Kalasalingam College of Pharmacy,Krishnankoil,Tamilnadu

Corresponding Author:
Latheesh j Lal L

Abstract: Seeds are compact biological "storage units" that accumulate diverse secondary metabolites and functional biomolecules, making them promising sources of plant-derived antibacterial agents. Major seed bioactives—including phenolic acids, flavonoids, tannins, alkaloids, saponins, terpenoids/essential oils, fatty acids, and antimicrobial peptides—can inhibit bacteria via multi-target actions such as membrane disruption, enzyme and nucleic-acid pathway interference, metal chelation, suppression of virulence, and biofilm prevention. In the context of rising antimicrobial resistance and the limitations of conventional antibiotics, these multi-component phytochemical systems are increasingly explored as complementary or adjunct antimicrobial strategies. However, antibacterial outcomes depend strongly on extraction and processing, as method selection governs yield, stability, selectivity, and the final bioactivity profile. Traditional solvent-based approaches remain common, but advanced "green" techniques—such as ultrasound-assisted extraction, microwave-assisted extraction, pressurized liquid extraction, enzyme-assisted extraction, and supercritical fluid extraction—can improve efficiency while reducing solvent use and preserving heat-sensitive constituents. Robust translation also requires standardized chemical characterization (e.g., HPLC/UPLC, GC–MS, LC–MS/MS, FTIR, NMR) and well-controlled antibacterial validation, moving beyond diffusion screening to quantitative MIC/MBC determination and mechanistic assays, including time-kill kinetics, membrane integrity testing, biofilm inhibition, and synergy evaluation. This review summarizes seed-derived antibacterial compound classes, extraction strategies from conventional to green technologies, and best-practice evaluation approaches while highlighting current challenges (variability, limited in vivo evidence, and formulation barriers) and future directions such as bioactivity-guided fractionation, nano/micro-formulations, and combination therapy with existing antibiotics.

Keywords: Seed Bioactive Compounds; Antibacterial Activity; Phytochemicals; Extraction Techniques; Green Extraction; Phenolic Compounds; Essential Oils; Antimicrobial Resistance; Biofilm Inhibition; Natural Antibacterials.

INTRODUCTION

Seeds are compact biological "storage units" designed to protect and nourish the embryo, and in doing so they accumulate a broad spectrum of secondary metabolites with notable pharmacological value. Beyond their role as dietary components, many edible and medicinal seeds are increasingly recognized as rich sources of bioactive compounds such as phenolic acids, flavonoids, tannins, lignans, alkaloids, saponins, terpenoids, sterols, and bioactive peptides, along with essential oils and fatty acids. These constituents contribute to diverse biological actions—most prominently antioxidant, anti-inflammatory, and antimicrobial effects—making seeds attractive raw materials for discovering safer, plant-derived antibacterial agents. [1,2]

The growing interest in seed-derived antimicrobials is closely linked to the global rise of antimicrobial resistance (AMR) and the limitations of conventional antibiotics, including reduced efficacy, adverse effects, and high development costs. Plant bioactives

offer a promising complementary strategy: they may inhibit bacterial growth through multiple targets such as disruption of cell membranes, inhibition of nucleic acid synthesis, interference with energy metabolism, suppression of virulence factors, and prevention of biofilm formation. Importantly, combinations of phytochemicals in seed extracts may act synergistically, potentially lowering the likelihood of resistance development compared with single-target antibiotics. [3-4]

However, the antibacterial performance of seed extracts depends strongly on how bioactives are recovered. The extraction approach influences yield, purity, chemical stability, and the final biological activity. Traditional methods (maceration, Soxhlet extraction, percolation) remain widely used but often require longer extraction times, higher solvent volumes, and elevated temperatures that may degrade heat-sensitive compounds. In contrast, modern and "green" techniques—such as ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), pressurized liquid extraction (PLE), supercritical fluid extraction (SFE), and enzyme-assisted extraction (EAE)—offer improved efficiency, lower solvent consumption, and better selectivity. Selecting an appropriate extraction method requires considering seed matrix composition, target compound polarity, solvent system, temperature sensitivity, and intended application (food, nutraceutical, cosmetic, or pharmaceutical). [5,6]

In addition to extraction, robust characterization and biological validation are essential for meaningful antibacterial claims. Chemical profiling typically employs chromatographic and spectroscopic tools including HPLC/UPLC, GC–MS, LC–MS/MS, FTIR, and NMR, enabling both qualitative and quantitative assessment of key constituents. Antibacterial activity is commonly evaluated using agar diffusion and broth microdilution assays to determine inhibition zones and minimum inhibitory concentration (MIC), respectively. More mechanistic studies increasingly incorporate time-kill kinetics, membrane integrity assays, biofilm inhibition tests, and synergy studies (e.g., checkerboard method with fractional inhibitory concentration index). Standardization of methods, appropriate controls, and careful reporting (strain identity, inoculum size, extraction yield, concentration units, and reference antibiotics) are necessary to ensure comparability across studies. [10-13]This review article focuses on the major classes of seed-derived bioactive compounds, extraction approaches (conventional to advanced green methods), and the antibacterial potential of seed extracts and purified compounds against clinically relevant pathogens. The review also highlights key challenges such as variability due to seed origin and processing, lack of standardization, limited in vivo evidence, and formulation barriers. Finally, it outlines future directions including bioactive-guided fractionation, nano-/micro-formulation strategies, and synergistic combinations with existing antibiotics to translate seed-based antibacterials into practical therapeutic and industrial applications

2. Bioactive Compounds Present in Seeds

Seeds contain a concentrated pool of phytochemicals and functional biomolecules that protect the plant embryo from microbes, insects, oxidative stress, and environmental damage. These same molecules are responsible for many antibacterial effects reported for seed extracts. The major bioactive classes and their antibacterial relevance are summarized below. [5,6]

2.1 Phenolic Compounds

Phenolic compounds are among the most abundant and influential antibacterial constituents reported in seeds, largely contributing to their protective role against microbial invasion. This group mainly includes phenolic acids (such as gallic, caffeic, ferulic, and p-coumaric acids), along with polyphenolic derivatives that frequently coexist with other seed bioactives and collectively enhance antimicrobial performance. Phenolics exhibit broad-spectrum antibacterial action through multiple pathways: they can disrupt the bacterial cell wall and membrane, leading to increased permeability and leakage of vital intracellular components; bind to bacterial proteins and enzymes, thereby suppressing essential metabolic pathways; and chelate key metal ions (e.g., iron and zinc) that bacteria require for growth and enzyme function. In addition, several phenolics are reported to reduce quorum sensing and virulence, which may weaken bacterial communication, toxin production, and biofilm initiation, making pathogens more susceptible to host defense and other antimicrobials. From a processing perspective, phenolics are generally extracted more efficiently with polar solvents such as ethanol, methanol, or aqueous ethanol systems, and their recovery is strongly influenced by solvent concentration, extraction time, and temperature—conditions that must be optimized to maximize yield while preventing degradation of temperature-sensitive components. [5-7]

2.2 Flavonoids and Related Polyphenols

Flavonoids are a major subgroup of seed polyphenols and occur either as aglycones (more lipophilic) or as glycosides (more polar), distributed in both the seed coat and kernel; in many plant species, the seed coat contains higher concentrations because it serves as the first protective barrier. Their antibacterial activity is typically multi-target, where lipophilic flavonoids can insert into and disrupt bacterial membranes, causing permeability changes and leakage, while other flavonoids may inhibit essential bacterial

enzymes, including DNA gyrase and topoisomerase, thereby interfering with DNA replication and transcription. Flavonoids also show important anti-virulence actions, such as inhibiting biofilm formation and reducing bacterial adhesion to surfaces or host tissues, which is clinically relevant because biofilms often exhibit higher antibiotic tolerance. The overall effectiveness of flavonoids depends on their structure (hydroxylation pattern, glycosylation, and lipophilicity), the extraction solvent system, and the presence of synergistic co-compounds in the crude extract. [5,6]

2.3 Tannins (Hydrolysable and Condensed)

Tannins are high-molecular-weight, highly reactive polyphenols widely present in seed coats of several plants (for example, grape seeds and pomegranate seeds) and are classified mainly as hydrolysable tannins (e.g., gallotannins and ellagitannins) and condensed tannins (proanthocyanidins). Their antibacterial effects are strongly associated with their ability to bind and precipitate proteins, which can inactivate bacterial surface proteins, enzymes, and adhesins, ultimately impairing nutrient uptake, metabolism, and colonization. Tannins can also disrupt bacterial adhesion and biofilm development, and inhibit extracellular microbial enzymes that support invasion and persistence. Despite their benefits, practical formulation considerations are important: high tannin levels may cause astringency and irritation, and tannins may bind proteins in biological fluids or formulations, potentially reducing free active concentration. Therefore, tannin-rich extracts often require careful optimization of dose, delivery system, and compatibility testing to achieve consistent antibacterial performance. [5,6]

2.4 Alkaloids

Alkaloids are nitrogen-containing bioactive compounds found in certain seeds depending on the plant species, and they often exhibit strong antibacterial activity even at relatively low concentrations. Their antimicrobial action is typically linked to interference with essential cellular functions, including inhibition of DNA replication and transcription, disruption of protein synthesis, and enzyme inhibition that ultimately impairs bacterial growth and cell division. Some alkaloids have also been reported to modulate bacterial resistance mechanisms by inhibiting efflux pumps, which can increase intracellular drug accumulation and enhance susceptibility to other antimicrobials. However, because alkaloids can also interact strongly with mammalian biological systems, they may carry toxicity risks, making safety evaluation and dose optimization crucial when considering therapeutic or food-related applications. [15]

2.5 Saponins

Saponins are amphiphilic glycosides commonly present in legume seeds and various medicinal seeds, characterized by their soap-like foaming properties and membrane-active behavior. Their antibacterial potential is largely attributed to interaction with membrane sterols and phospholipids, leading to altered membrane permeability, destabilization, and leakage of cellular contents. By increasing membrane penetration, saponins may also enhance the uptake of co-existing phytochemicals in crude extracts, thereby supporting synergistic antibacterial effects. In practical terms, the antibacterial effectiveness of saponin-rich extracts depends on the saponin structure (aglycone type and sugar chains), concentration, and formulation, as higher levels may cause irritation or hemolytic concerns in some biological systems, requiring appropriate safety screening. [8]

2.6 Terpenoids and Essential Oils (Volatile Fraction)

Terpenoids and essential oils represent the volatile antimicrobial fraction of many aromatic seeds such as cumin, fennel, coriander, and mustard, and are often responsible for rapid and broad-spectrum antibacterial effects. These compounds typically act by disrupting bacterial membranes, causing loss of membrane integrity and collapse of the proton motive force, which results in failure of energy generation and transport processes. Many volatile terpenoids also inhibit key metabolic and respiratory enzymes, and several show anti-biofilm activity, reducing bacterial attachment and biofilm maturation. From an extraction viewpoint, essential oils are commonly recovered by steam or hydrodistillation, while solvent extraction may capture both volatile and semi-volatile terpenoids; for greener, selective, and solvent-minimized recovery, supercritical CO_2 extraction is widely preferred, especially when the goal is to obtain a cleaner volatile fraction with better stability and industrial applicability. [9]

2.7 Fatty Acids and Lipid-Derived Antimicrobials

Seed oils are rich in lipids and unsaturated fatty acids such as linoleic, oleic, and linolenic acids, along with minor lipid-derived constituents that can contribute significantly to antibacterial activity. These molecules primarily act by integrating into bacterial lipid bilayers, disturbing membrane packing and fluidity, which increases permeability and leads to leakage of essential cellular components. In some bacteria, fatty acids may also interfere with lipid metabolism by affecting fatty acid synthesis and membrane biogenesis pathways, thereby slowing growth and weakening cellular integrity. The antibacterial performance of lipid fractions depends on the fatty acid profile, degree of unsaturation, and presence of supporting minor components, and it is also influenced by extraction and storage conditions because oxidation of unsaturated lipids can alter both

stability and bioactivity. [15]

2.8 Proteins and Antimicrobial Peptides (AMPs)

Seeds naturally contain defense proteins and antimicrobial peptides, including defensins, thionins, and lipid transfer proteins, which serve as innate protection against microbial invasion. These peptides often display potent antibacterial effects by binding to bacterial membranes and forming pores, resulting in rapid loss of ions and metabolites, and by inhibiting cell wall biosynthesis, which compromises structural stability and causes cell lysis. Some AMPs can additionally induce oxidative stress inside bacterial cells, further amplifying killing effects. Although AMPs are attractive because they can act at low concentrations and sometimes show activity against resistant strains, their translation into applications faces challenges such as enzymatic degradation, limited stability, and delivery difficulties; hence, stabilization strategies (encapsulation, film/gel incorporation, or peptide modification) are often required for practical formulations. [16-19]

2.9 Polysaccharides and Minor Bioactives

Polysaccharides present in seeds, particularly seed mucilages and gums, may not always act as direct bactericidal agents but can support antibacterial outcomes through anti-adhesion effects, surface coating, and formation of protective matrices—features that are especially useful in wound-care and topical applications. These polysaccharides can also enhance formulation performance by improving viscosity, film formation, and moisture retention, thereby indirectly supporting microbial control and tissue repair. In addition, seeds contain several minor yet valuable constituents such as phytosterols, tocopherols (vitamin E), carotenoids, and minerals, which may contribute to antioxidant protection, improve extract stability, and sometimes enhance overall biological activity through supportive or synergistic effects. Collectively, these components strengthen the concept that seed-based antibacterial potential often arises from multi-component synergy, where major actives (polyphenols, volatiles, peptides) work alongside supportive biomolecules to produce a more robust antimicrobial profile. [5,20]

3. Extraction Approaches for Seed Bioactives

Efficient recovery of antibacterial compounds from seeds depends on selecting an extraction method that matches the seed matrix (oil-rich, protein-rich, or polyphenol-rich) and the target bioactive class (polar phenolics vs non-polar volatiles/lipids vs peptides). In general, extraction performance is controlled by key variables such as particle size, solvent polarity, solvent-to-solid ratio, temperature, time, and agitation, and these factors also decide whether the final extract retains activity or loses it due to oxidation, hydrolysis, or thermal degradation. [5,6]

3.1 Sample preparation and pre-treatment

Before extraction, seeds are typically cleaned, dried, and milled to increase surface area and improve mass transfer. For many phenolic-rich seeds, mild drying and fine powdering improves yield; however, excessive heat can degrade sensitive polyphenols and volatiles. Oil-rich seeds may require defatting (commonly using hexane or food-grade alternatives) before polar extraction so that phenolics can be extracted more effectively. For better release of bound compounds, pre-treatments such as enzymatic conditioning (cellulase/pectinase), mild acidification, or controlled hydration are sometimes used, particularly when phenolics are tightly associated with cell-wall polysaccharides.

3.2 Conventional solvent extraction

Conventional solvent extraction (maceration, stirring, percolation) remains widely used due to its simplicity and low equipment needs. The method mainly relies on diffusion of solutes into the solvent and is suitable for phenolics, flavonoids, tannins, and some alkaloids, especially when using polar solvents like ethanol, methanol, or aqueous ethanol. Although cost-effective, conventional extraction may require longer times and larger solvent volumes, and higher temperatures can improve yield but may reduce stability of heat-sensitive compounds. Because antibacterial activity often depends on maintaining intact functional groups (e.g., hydroxyl groups in phenolics), careful control of extraction temperature and oxygen exposure is important. [5]

3.3 Soxhlet extraction

Soxhlet extraction provides exhaustive recovery and is commonly applied for seed matrices when higher yields are needed. It is particularly useful for lipid fractions and semi-polar constituents, but it involves prolonged heating, which can degrade thermolabile phenolics and volatile components. For antibacterial screening, Soxhlet extracts can show strong activity due to concentrated compounds, yet the method may not represent "green" processing and can alter the natural composition through thermal stress. [5]

3.4 Distillation-based methods for volatiles

For seeds rich in essential oils and terpenoids, steam distillation or hydrodistillation is the classical approach. These methods selectively recover volatile antibacterial constituents that typically act quickly via membrane disruption. However, some volatiles may be lost or transformed due to heat and long processing time. To improve quality and reduce degradation, advanced options like supercritical CO_2 extraction or optimized short-time distillation are often preferred when available. [9]

3.5 Advanced and green extraction techniques

Modern techniques are increasingly adopted because

they improve yield, reduce solvent usage, and preserve bioactivity. Ultrasound-assisted extraction (UAE) enhances cell disruption and solvent penetration, making it highly suitable for phenolics and flavonoids with reduced extraction time. Microwave-assisted extraction (MAE) heats the matrix rapidly and can provide high yields of polyphenols, but parameters must be controlled to avoid degradation. Enzyme-assisted extraction (EAE) uses enzymes to break cell-wall polymers and is particularly helpful for releasing bound phenolics and improving aqueous extraction. Pressurized liquid extraction (PLE)/accelerated solvent extraction improves solubility and diffusion under controlled pressure-temperature conditions, offering reproducible, scalable extraction. Supercritical fluid extraction (SFE), especially with CO_2 (often with a small co-solvent like ethanol), is excellent for non-polar to moderately polar compounds, giving solvent-free extracts with good stability and industrial acceptability. [5]

3.6 Choice of solvents and "green" solvent systems

Solvent selection is central because it determines which antibacterial compounds dominate the extract. Aqueous ethanol is often ideal for seed polyphenols due to good safety profile and high extraction efficiency. Water alone is safest but may be less efficient for some flavonoids and alkaloids. Recently, eco-friendly alternatives such as glycerol-based mixtures and natural deep eutectic solvents (NADES) have shown promise for extracting polyphenols with improved stability; however, downstream removal, toxicity, and regulatory acceptance should be addressed before application in pharma/food products. [5]

3.7 Optimization and standardization

To make extraction reproducible and comparable across studies, extraction conditions are increasingly optimized using Design of Experiments (DoE) and response surface methodology (RSM). Typical optimization outputs include total phenolic content, marker compound concentration (HPLC/LC–MS), extraction yield, and antibacterial endpoints (MIC/MBC). Standardization using chemical markers plus biological activity helps ensure that an "active extract" remains consistent across batches.

3.8 Fractionation and purification (bioactivity-guided)

Crude seed extracts often show stronger antibacterial effects due to synergy, but for mechanistic studies and product development, fractionation is important. Common approaches include liquid–liquid partitioning (by polarity), solid-phase extraction (SPE), and column chromatography to enrich phenolics, tannins, alkaloids, or volatiles. Bioactivity-guided fractionation—testing each fraction for MIC/biofilm inhibition while profiling by LC–MS/GC–MS—helps identify the true active constituents and supports quality control.

4. Evaluation of Antibacterial Potential of Seed Extracts

Assessing the antibacterial activity of seed-derived bioactives requires well-standardized microbiological assays because extraction yield alone does not confirm therapeutic value. In most studies, initial screening begins with simple growth inhibition tests, followed by quantitative measurements (MIC/MBC), and then advanced assays to understand killing kinetics, biofilm control, synergy, and mechanism of action. Since seed extracts are complex mixtures, careful attention to strain selection, inoculum size, solvent controls, and reporting units is essential to ensure that results are reproducible and comparable across laboratories. [10,11,12,13]

4.1 Test organisms and culture standardization

Seed extracts should be evaluated against both Gram-positive and Gram-negative bacteria because membrane structure and permeability differ greatly between these groups. Common reference strains include Staphylococcus aureus, Enterococcus spp., Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, and Salmonella spp., with increasing emphasis on clinical isolates and resistant phenotypes where possible. Standardization typically involves adjusting inoculum density to approximately 0.5 McFarland (or as required by CLSI/EUCAST-based microdilution methods) to avoid inflated or underestimated activity due to inoculum effects.

4.2 Primary screening assays

Agar-based methods such as disc diffusion and agar well diffusion are widely used for preliminary screening because they are simple and low-cost. They provide a visible zone of inhibition, but interpretation for plant extracts must be cautious because diffusion depends on molecular size and polarity; highly active but poorly diffusing compounds may appear weak. Therefore, diffusion assays are best used for quick comparison, while broth dilution methods are needed for definitive potency. [10]

4.3 Quantitative potency: MIC and MBC

The minimum inhibitory concentration (MIC) is the most important quantitative parameter and is commonly determined using broth microdilution in 96-well plates. MIC reflects the lowest concentration that prevents visible growth after incubation. For bactericidal confirmation, the minimum bactericidal concentration (MBC) is measured by sub-culturing from MIC wells onto agar plates to determine the lowest concentration that kills bacteria rather than

only inhibiting growth. Reporting MIC/MBC in consistent units (e.g., µg/mL for purified compounds, mg/mL for crude extracts) is critical; for crude extracts, it is also recommended to report extraction yield and marker compound content (e.g., total phenolics or HPLC-based markers) to improve comparability. [10-13]

4.4 Time–kill kinetics and post-antibiotic effect

To understand how fast and how strongly an extract kills bacteria, time–kill assays are used, where viable counts (CFU/mL) are monitored over time at different concentrations (e.g., 0.5×, 1×, 2× MIC). These studies help distinguish rapid membrane-active extracts (often essential oils/terpenoids) from slower metabolic inhibitors (often polyphenols). Where relevant, a post-antibiotic effect approach can be used to observe whether growth suppression persists after removal of the extract, which has implications for dosing frequency.

4.5 Anti-biofilm activity and anti-adhesion studies

Because many infections involve biofilms, seed bioactives should be evaluated for biofilm inhibition (prevention) and biofilm eradication (treatment of established biofilm). Microtiter plate biofilm assays (crystal violet staining), viable cell assays, and surface adhesion tests can reveal whether extracts reduce attachment, biomass, and viability within biofilms. This is especially important for phenolics, flavonoids, and essential oils, which may reduce quorum sensing and biofilm maturation even when planktonic MIC appears moderate. [14]

4.6 Synergy with antibiotics and combination studies

Seed extracts often contain multiple actives that can act synergistically among themselves or with antibiotics. Checkerboard assays are commonly used to calculate the fractional inhibitory concentration index (FICI), while time–kill combination studies provide strong evidence of synergy in dynamic conditions. Synergy is particularly valuable when seed compounds act as membrane permeabilizers (enhancing antibiotic entry) or efflux pump inhibitors (increasing intracellular antibiotic concentration), potentially restoring activity of older antibiotics against resistant strains. [10]

4.7 Mechanistic assays to support antibacterial claims

Mechanistic validation strengthens review conclusions and supports translation into formulations. Frequently used assays include membrane integrity tests (leakage of nucleic acids/proteins, dye uptake), measurement of intracellular ATP or enzyme inhibition, ROS generation, and microscopy-based visualization of cell damage. For anti-virulence claims, quorum sensing inhibition assays and measurement of virulence factors can be included. For volatile fractions, vapor-phase activity and membrane-collapse indicators are particularly relevant.

4.8 Safety, selectivity, and in vivo relevance

Antibacterial potency must be balanced with safety. Therefore, cytotoxicity testing on mammalian cell lines, hemolysis assays (especially relevant for saponins and peptides), and irritation/compatibility testing for topical applications are important to establish a selectivity index. Where the aim is clinical translation, evidence from ex vivo models (skin/wound models) or in vivo infection/wound-healing models adds strong support, because many extracts behave differently in biological environments due to protein binding, enzymatic degradation, or limited penetration.

4.9 Reporting quality and common pitfalls

For a high-quality review, it is important to highlight that antibacterial outcomes can be biased by poor reporting. Essential details include: plant identity and seed part used, extraction method and solvent, yield, storage conditions, concentration units, bacterial strain details, inoculum standardization, positive controls (standard antibiotics), solvent controls, and whether assays followed CLSI/EUCAST-style principles. Common pitfalls include relying only on diffusion zones, reporting activity without MIC/MBC, using non-standard inoculum levels, and not correcting for solvent effects or extract color/turbidity in microplate readings.

Conclusion

Seed-derived bioactive compounds offer strong potential as natural antibacterial agents because they act through multiple mechanisms, including membrane disruption, enzyme inhibition, interference with DNA-related processes, metal chelation, and suppression of virulence and biofilm formation. The overall antibacterial effectiveness is highly dependent on the extraction approach, as solvent polarity, temperature, and technique determine which actives are recovered and how stable they remain. Therefore, standardized extraction, chemical profiling (HPLC/LC–MS/GC–MS), and robust antibacterial evaluation (MIC/MBC supported by biofilm, time-kill, and synergy assays) are essential to generate comparable and reliable results. Although promising, practical translation requires addressing batch-to-batch variability, stability, and safety concerns—especially for alkaloid-, saponin-, and peptide-rich fractions—through careful dose selection and suitable formulations. With optimized green extraction, rigorous standardization, and more in vivo/clinically relevant validation, seed-based antibacterials can emerge as effective alternatives or adjuncts to conventional antibiotics and as safe natural preservatives in healthcare and industrial applications. [5,6]

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