

Bioactive Profiling of *Moringa oleifera* (Shigru): A Critical Review on the Antimicrobial Synergy of Stem Bark and Seed Extracts

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Abstract:

Moringa oleifera Lam. (Shigru) is a widely used medicinal plant in Ayurvedic and folk-health traditions and is increasingly investigated for antimicrobial activity. This critical review examines the bioactive profile of stem bark and seed extracts with special emphasis on possible synergistic antimicrobial interactions. Stem bark is comparatively rich in tannins, phenolics, alkaloids and sterol-like constituents, whereas seeds contain glucosinolate-derived isothiocyanates, lectins, flavonoids and fixed oils. These molecules may act through complementary pathways such as microbial membrane damage, protein precipitation, oxidative stress, enzyme inhibition, interference with energy metabolism and suppression of DNA/protein synthesis. Available in-vitro evidence suggests that *Moringa* extracts inhibit Gram-positive bacteria, Gram-negative bacteria and selected fungi; recent reports also indicate extract-antibiotic synergy, especially with beta-lactam antibiotics against resistant organisms. From an Ayurvedic perspective, the *Katu-Tikta Rasa*, *Ushna Virya*, *Tikshna Guna* and *Krimighna* karma of *Shigru* provide a rational traditional framework for use in infection-associated conditions. However, direct stem bark-seed synergy remains underexplored and requires standardized extraction, phytochemical fingerprinting, checkerboard/FIC studies, toxicity testing and translational formulation research. This review concludes that stem bark and seed extracts are promising complementary antimicrobial resources, but robust experimental and clinical validation is essential before therapeutic claims are made. ^[1-8]

Keywords: *Moringa oleifera*; *Shigru*; antimicrobial synergy; stem bark; seed extract; Ayurveda; phytochemistry; antibiotic resistance.

Introduction

Antimicrobial resistance has become a serious challenge to public health and clinical therapeutics. The gradual decline in antibiotic effectiveness has encouraged renewed interest in medicinal plants, especially those containing multiple classes of bioactive secondary metabolites. A plant extract is not a single molecule; it is a dynamic mixture where phenolics, flavonoids, alkaloids, tannins, glucosinolates and oils may act together on different microbial targets. This multi-component character is particularly attractive when microbial resistance involves more than one cellular pathway. ^[1,4,5,21]

Moringa oleifera Lam., popularly known as drumstick tree and classically known as *Shigru*, is native to South Asia and extensively cultivated in India and other tropical regions. In Ayurveda, *Shigru* is valued for *Katu* and *Tikta Rasa*, *Laghu-Tikshna Guna*, *Ushna Virya* and *Katu Vipaka*. These attributes correspond broadly with *Kapha-Vatahara*, *Krimighna*, *Shothahara* and *Lekhana* actions, making the plant relevant in infection, swelling, inflammatory disorders and obstructive pathology. [2-5,19,20,22,23]

Modern investigations support the traditional importance of *M. oleifera* by reporting antioxidant, anti-inflammatory, hepatoprotective, antidiabetic, wound-healing and antimicrobial effects. Although leaves are frequently studied due to nutritional value, seeds and stem bark are equally important. Seeds are chemically remarkable for isothiocyanates such as moringin, while stem bark is a useful source of tannins, phenolics and alkaloids. The present review focuses on antimicrobial potential of these two plant parts and critically examines the plausibility of their synergy. [2-8,11,12]

Aim and Objectives

- To compile the Ayurvedic and modern scientific profile of *M. oleifera* (*Shigru*) with emphasis on stem bark and seed extracts.
- To summarize major phytochemical classes present in stem bark and seeds and correlate them with antimicrobial mechanisms.
- To critically review available evidence on antibacterial, antifungal and extract-antibiotic synergistic activity.
- To identify research gaps and propose a practical standardization pathway for antimicrobial formulation development.
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Review Methodology

A narrative critical review approach was followed. Literature was identified from scientific databases and publisher platforms including PubMed/PMC, Frontiers, Springer, MDPI, Oxford Academic, PLOS, De Gruyter and Ayurveda-oriented journals. Search terms included *Moringa oleifera* antimicrobial, *Moringa* seed isothiocyanate, *Moringa* stem bark phytochemical, *Shigru* Ayurveda, *Moringa* antibiotic synergy and moringin antibacterial mechanism. Articles were prioritized when they provided phytochemical data, antimicrobial assays, MIC/MBC values, mechanism-based findings, synergy assays or pharmacognostical relevance. Classical Ayurvedic references were included to support traditional drug attributes and therapeutic indications. [4,5,22-24]

Botanical and Ayurvedic Profile

M. oleifera belongs to family Moringaceae and is a fast-growing, drought-tolerant tree. The stem bark is externally greyish and internally pale to brownish, while seeds are commonly winged and enclosed in elongated pods. In Ayurveda, *Shigru* is categorized as a sharp, hot and penetrating drug useful where *Kapha* and *Vata* involvement, swelling, channel obstruction and *Krimi*-like pathology are present. The Ayurvedic interpretation does not replace microbiology, but provides a clinically meaningful traditional lens for understanding why this plant has long been selected in infection-associated and inflammatory conditions. [4,5,12,19,20,22,23]

Table 1: Botanical and Ayurvedic profile of *Moringa oleifera* (Shigru)

Parameter	Description	Relevance to Review
Botanical name	<i>Moringa oleifera</i> Lam.	Authentic identity for reproducible research
Family	Moringaceae	Botanical classification and pharmacognosy
Sanskrit name	<i>Shigru</i>	Classical name used in Ayurvedic literature
<i>Rasa</i>	<i>Katu, Tikta</i>	Traditional <i>Kapha</i> -reducing and antimicrobial-like relevance
<i>Guna</i>	<i>Laghu, Tikshna</i>	Penetrating, channel-clearing action
<i>Virya</i>	Ushna	Relevant in <i>Kapha-Vata</i> dominant conditions
<i>Vipaka</i>	<i>Katu</i>	Supports <i>Lekhana</i> and metabolic stimulation
Classical actions	<i>Krimighna, Shothahara, Lekhana</i>	Traditional correlate of antimicrobial, anti-inflammatory and cleansing actions
Parts reviewed	Stem bark and seeds	Underexplored combination for synergy

Phytochemical Rationale

The antimicrobial value of *M. oleifera* is not due to one compound alone. Stem bark and seeds provide overlapping but distinct chemical profiles. Stem bark commonly contains tannins, phenolics, alkaloids, saponins and sterol-like compounds. Such constituents can denature microbial proteins, disturb membrane integrity and interfere with enzyme systems. Seeds are important because they contain glucosinolate-derived isothiocyanates, particularly moringin, along with lectin-like proteins, flavonoids and fatty components. This difference supports the logical hypothesis that bark and seed extracts may complement each other. [1-8,10-12]

Isothiocyanates are electrophilic molecules capable of interacting with microbial proteins and redox systems. Moringin has been shown to affect cell wall and membrane biosynthesis, oxidative stress response, energy metabolism and DNA-related processes in *Listeria monocytogenes*. Lectins may bind microbial surfaces and disturb cell viability, while flavonoids such as quercetin and kaempferol can contribute antioxidant, membrane-active and enzyme-modulating effects. Bark tannins may add protein-precipitating and anti-adhesive activity. [6-8,10]

Table 2: Comparative phytochemical profile of stem bark and seed extracts

Phytochemical class	Stem bark	Seed	Probable antimicrobial role	Comments
Tannins	Prominent	Variable	Protein precipitation, enzyme inhibition, membrane effects	Important for bark-based activity
Phenolic acids	Reported	Reported	Oxidative stress modulation and membrane destabilization	May contribute to broad-spectrum action
Alkaloids	Reported	Reported	Interference with nucleic acid and enzyme pathways	Often solvent-dependent
Flavonoids	Moderate	Prominent	Membrane interaction, enzyme inhibition, quorum modulation	Quercetin/kaempferol reported in seed studies
Isothiocyanates	Low/limited	Characteristic	Protein reactivity, membrane/cell-wall damage, multi-target stress	Key marker for seeds; moringin is important
Lectins/proteins	Limited	Reported	Binding to microbial cell surfaces and growth inhibition	Relevant for seed antimicrobial action
Fixed oils/fatty acids	Low	Present	Membrane interaction and formulation value	May assist topical/nanoformulation development
Sterols	Reported	Reported	Membrane interaction;	Requires quantitative confirmation

			supportive bioactivity	
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Antimicrobial Evidence: Stem Bark

Stem bark has received less attention than leaves, yet it is pharmacologically meaningful. Available studies describe antimicrobial activity of stem bark extracts against Gram-positive bacteria, Gram-negative bacteria and yeast strains. Solvent selection appears critical; acetone and alcoholic extracts often show stronger activity than simple aqueous extracts because they dissolve moderately polar phenolics, tannins and alkaloidal constituents more effectively. Phytochemical and pharmacognostical studies on *Shigru* stem bark also support proper authentication, microscopy and chromatographic fingerprinting before antimicrobial testing. ^[9,11,12]

The bark may be valuable in a combination strategy because tannins and phenolics act differently from seed isothiocyanates. Tannins can reduce microbial adhesion, precipitate proteins and disrupt extracellular enzymes, while phenolics may disturb membrane permeability and metabolic activity. If seed components weaken the microbial envelope, bark phenolics and tannins may enter more easily and produce a stronger combined effect. This is the biological basis for testing stem bark-seed combinations using checkerboard, time-kill and biofilm assays. ^[7,11,12,15-17]

Antimicrobial Evidence: Seeds

Seeds are among the most promising antimicrobial parts of *M. oleifera*. The isolation of 4-(alpha-L-rhamnosyloxy)benzyl isothiocyanate and related compounds from seeds provides a strong phytochemical explanation for their activity. These isothiocyanates have shown activity against selected Gram-positive bacteria and dermatophytes. A mechanistic study of moringin against *Listeria monocytogenes* demonstrated multi-target antibacterial effects involving cell wall and membrane damage, oxidative stress, energy metabolism and DNA replication pathways. ^[6-8,10]

Seed extracts have been evaluated against common bacterial pathogens such as *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. Phytochemical screening frequently detects alkaloids, tannins, saponins, phenols and flavonoids. Computational studies involving quercetin and kaempferol suggest possible enzyme-target interactions, although docking evidence should be interpreted as supportive rather than conclusive. ^[8-10,15]

Figure 1: Proposed multi-target antimicrobial mechanisms

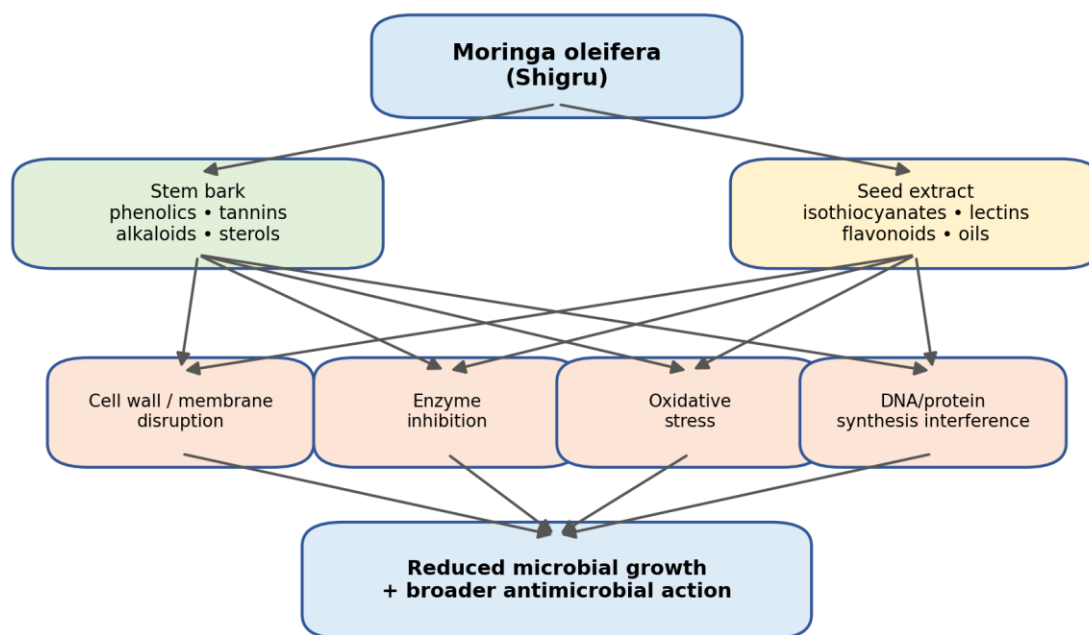


Figure 1: Proposed multi-target antimicrobial mechanisms of Shigru stem bark and seed extracts.

Antimicrobial Spectrum and Synergistic Interactions

The studies reviewed indicate activity against Gram-positive and Gram-negative bacteria, although sensitivity varies by organism, solvent, concentration, assay method and plant-part quality. Gram-positive bacteria may be more susceptible to certain isothiocyanates because of differences in cell envelope architecture, while Gram-negative bacteria are often more difficult due to the outer membrane barrier and efflux systems. Several *Moringa* extracts still show activity against Gram-negative strains, suggesting that mixed phytochemicals may overcome some permeability barriers. [1,6-10,13,14,18,21]

Synergy means that the combined effect of two agents is greater than the expected additive effect of each agent alone. In antimicrobial research, synergy is commonly evaluated through checkerboard assays and expressed as the fractional inhibitory concentration index. A FICI below 0.5 is generally interpreted as synergy. In the context of *M. oleifera*, three synergy possibilities are important: stem bark plus seed extract, extract plus conventional antibiotics and *Moringa* combined with other medicinal plants or essential oils. Recent studies indicate that *Moringa* seed extract can potentiate selected beta-lactam antibiotics against multidrug-resistant *Escherichia coli* and essential oil mixtures containing *Moringa* can show synergistic effects against *Staphylococcus aureus*. These findings justify systematic bark-seed testing. [15-17]

Table 3: Reported antimicrobial relevance of *Moringa oleifera* extract types

Plant part/extract	Target organisms reported	Assay types	Observed relevance	Limitations
Stem bark acetone/alcoholic extracts	MRSA, Klebsiella, Pseudomonas, yeasts	MIC, zone of inhibition, kill kinetics	Broad-spectrum potential; solvent-sensitive activity	Limited replication across regions
Seed methanolic/ethanolic extracts	E. coli, S. aureus, K. pneumoniae, P. aeruginosa	Agar diffusion, MIC/MBC, phytochemical tests	Strong antibacterial promise; unique isothiocyanates	Variable extraction/dose reporting
Isolated seed isothiocyanates	Gram-positive bacteria, dermatophytes	Disk diffusion, MIC, MBC	Mechanistically plausible bactericidal role	Limited pure compound studies
Moringin	<i>Listeria monocytogenes</i>	MIC, transcriptomics, mechanistic assays	Multi-target antibacterial action	Needs broader pathogen testing
Extract + antibiotics	MDR E. coli, MRSA-related models	Synergy/adjuvant testing	Potential antibiotic-potentiating effect	Clinical evidence absent

Figure 2: Stem bark-seed synergy model

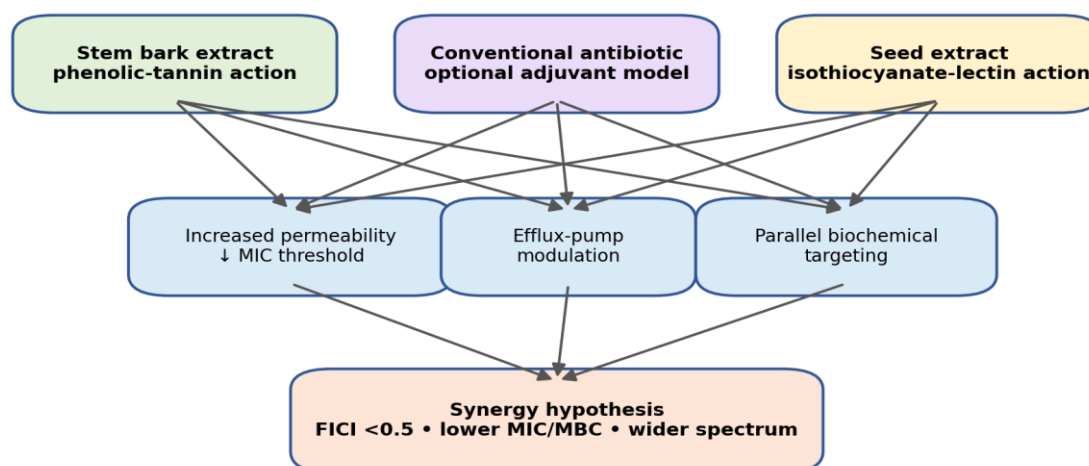


Figure 2: Synergy model for Shigru stem bark-seed combinations and extract-antibiotic adjuvant strategies.

Probable Mechanisms Behind Bark–Seed Synergy

- Membrane permeability enhancement: seed isothiocyanates and lipophilic constituents may disturb bacterial envelopes, allowing bark phenolics and tannins to reach intracellular or periplasmic targets more effectively. [6,7,11,12]
- Protein and enzyme targeting: bark tannins can precipitate proteins, while isothiocyanates can react with thiol-containing proteins; these parallel effects may suppress microbial metabolism at multiple levels. [6,7,11]
- Oxidative and metabolic stress: moringin-associated oxidative stress and energy pathway disruption may weaken microbial adaptation and increase vulnerability to phenolic compounds. [7]
- Efflux-pump and biofilm modulation: flavonoids, phenolics and tannins may influence efflux-mediated tolerance, microbial adhesion, extracellular polymeric substances and quorum-associated behaviour. [14,16,17]

Ayurvedic Interpretation and Integrative Relevance

Ayurveda does not describe microbes in the exact language of modern bacteriology, yet the concepts of *Krimi*, *Dushta Vrana*, *Shotha* and *Ama*-related pathology provide an interpretive bridge. *Shigru* possesses *Katu-Tikta Rasa* and *Ushna Virya*; therefore, it is traditionally suitable for *Kapha* accumulation, sluggish metabolism, swelling and obstruction. These descriptors are consistent with experimentally observed antimicrobial and anti-inflammatory potential, although they should not be used as a substitute for controlled evidence. [22,23]

The idea of synergy is also familiar to Ayurveda through polyherbal formulations and multi-part plant usage. A single plant may exhibit different actions through root, bark, leaf, flower, fruit and seed. Combining stem bark and seed extracts of *Shigru* could reflect this integrative logic: bark provides astringent phenolic-tannin action, while seed provides pungent, penetrating isothiocyanate-rich activity. Such a combination is scientifically testable and philosophically compatible with Ayurvedic pharmaceuticals. [22,23]

Extraction, Standardization and Evidence Grading

The antimicrobial outcome of any *Shigru* extract depends strongly on extraction design. Aqueous extraction may reflect traditional decoction logic, but hydroalcoholic, ethanolic, methanolic and acetone extracts often recover higher quantities of moderately polar phenolics, tannins, flavonoids and alkaloids. Seeds require additional consideration because fixed oil, proteins and glucosinolate-derived compounds may be partitioned differently depending on solvent polarity, temperature and extraction time. Therefore, a future stem bark-seed synergy study should compare standardized extracts prepared under reproducible conditions and report yield, appearance, pH, total phenolic content, total flavonoid content and marker-compound fingerprints. [4,5,8,11,12]

A practical design would begin with authenticated stem bark and seeds collected from the same geographical area and season. After drying and pulverization, both materials may be extracted separately using water, ethanol-water and methanol-water systems. HPTLC can provide preliminary fingerprinting, while HPLC/LC-MS may quantify moringin or related isothiocyanates in seed extract and phenolic/tannin markers in bark extract. This approach fits Ayurveda-oriented quality-control expectations that value both classical identity and modern analytical validation. [5,7,8,12,24]

Safety, Toxicity and Formulation Considerations

Although *M. oleifera* is widely consumed, concentrated extracts cannot be assumed safe at all doses. Toxicity depends on plant part, solvent, dose, duration, route of administration and phytochemical concentration. Antimicrobial development should include cytotoxicity assays, hemolysis testing, Ames testing where appropriate, acute and subacute toxicity models, dermal irritation tests for topical products and quality-control checks for heavy metals, pesticides and microbial contamination. ^[4,5,11,14]

Formulation strategy should follow the intended use. For topical infections and wound-supportive applications, gels, ointments, hydrogels and nanoemulsions may be considered. For oral use, caution is needed because antimicrobial claims require clinical validation, gut microbiome safety and dose standardization. Nanotechnology-based approaches may improve stability and bioavailability but introduce additional safety and regulatory questions. ^[4,5,14]

Table 4: Proposed standardization parameters for future bark-seed antimicrobial research

Stage	Recommended parameters	Purpose	Preferred tools
Raw drug authentication	Macroscopy, microscopy, voucher specimen, geo-tagging	Avoid misidentification/adulteration	Pharmacognosy, herbarium validation
Extraction	Solvent ratio, time, temperature, yield, moisture	Ensure reproducibility	Maceration/Soxhlet/ultrasound
Fingerprinting	Marker compounds, total phenolics/flavonoids/tannins	Chemical comparability	HPTLC, HPLC, LC-MS, GC-MS
Antimicrobial testing	MIC, MBC/MFC, time-kill, biofilm assay	Quantify antimicrobial action	CLSI-informed microdilution
Synergy testing	Checkerboard, FICI, time-kill combinations	Prove synergy/additivity/antagonism	MIC-based combination design
Safety	Cytotoxicity, hemolysis, acute/subacute toxicity	Establish safety margin	MTT, cell culture, animal models
Formulation	Stability, pH, viscosity, release profile	Develop usable product	Gel/nanoemulsion/hydrogel analysis

Figure 3: Workflow for standardization and future translation



Figure 3: Recommended workflow for future standardization and translation of Shigru antimicrobial formulations.

Critical Appraisal

The strongest evidence currently supports the antimicrobial potential of *M. oleifera* as a species and the distinctive activity of seed isothiocyanates. Stem bark possesses a rational phytochemical basis and preliminary antimicrobial support, but the evidence base is smaller and more heterogeneous. The most important gap is the lack of direct, well-controlled studies evaluating stem bark and seed extracts together. Without checkerboard assays, time-kill curves and standardized phytochemical fingerprints, statements about bark-seed synergy must remain cautiously framed as a hypothesis rather than a proven fact. [6-8,11,15-17]

Another limitation is variability in methodology. Agar diffusion is useful for screening but strongly affected by compound diffusion and extract viscosity. MIC and MBC data are more informative, especially when combined with positive controls, solvent controls and replicate clinical isolates. Future studies should include resistant strains, biofilm-forming organisms and clinically relevant endpoints. Clinical translation requires formulation, pharmacokinetic and safety evidence. [4,5,9-11]

Future Scope

- Direct checkerboard evaluation of authenticated *Shigru* stem bark and seed extracts in fixed and variable ratios.
- Identification of chemical markers such as moringin for seed extract and tannin/phenolic markers for bark extract.
- Testing against MDR *E. coli*, MRSA, *Pseudomonas aeruginosa*, *Candida* species and wound-associated biofilms.
- Comparison of aqueous, hydroalcoholic, methanolic, ethanolic and acetone extracts.
- Development of topical formulations after cytotoxicity and dermal safety screening.
- Integration of Ayurvedic formulation principles with modern quality-by-design validation.

Conclusion

Moringa oleifera (Shigru) represents a valuable bridge between Ayurvedic knowledge and contemporary antimicrobial research. Stem bark and seed extracts contain complementary phytochemical groups that may act on microbial membranes, proteins, enzymes, oxidative balance and genetic machinery. Seed isothiocyanates, particularly moringin, provide a strong mechanistic basis for antibacterial action, while bark tannins and phenolics may broaden and reinforce antimicrobial effects. The combined use of stem bark and seed extracts is scientifically promising, but direct synergy remains to be proven through standardized experimental designs. With careful authentication, phytochemical fingerprinting, synergy testing, safety evaluation and formulation development, *Shigru* may contribute meaningfully to integrative antimicrobial strategies in the era of drug resistance. [1-8,11,15-17,22,23]

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