

Anatomical Specialization of *Myristica fragrans* Houtt. Extracts: Targeted Antimicrobial Efficacy Against Veterinary Cutaneous Pathogens

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Abstract

The escalating global crisis of antimicrobial resistance (AMR) in veterinary practice necessitates the urgent development of sustainable, plant-derived topical agents for managing cutaneous infections. This study systematically evaluated the antimicrobial potency of 75% ethanol extracts from three distinct anatomical fractions of nutmeg (*Myristica fragrans* Houtt.); the pericarp, seed coat (mace), and kernel; against major veterinary pathogens, including *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *S. epidermidis*, and *Streptococcus pyogenes*. Using the agar well diffusion method, the extracts demonstrated significant anatomical specialization in their inhibitory profiles. The pericarp extract exhibited the broadest antimicrobial spectrum at 500 mg/mL, with inhibition zones reaching up to 29.40 ± 1.31 mm; however, its activity was attenuated at lower concentrations (≤ 125 mg/mL). In contrast, the kernel extract demonstrated remarkable inhibition efficacy, maintaining potent inhibitory zones (14.70–17.30 mm) against *S. aureus*, *S. epidermidis*, and *S. pyogenes* even at the lowest concentration of 125 mg/mL, suggesting the presence of highly active, dilution-stable compounds. Thin-layer chromatography profiling revealed that each fraction possesses distinct phytochemical fingerprints, correlating with their varying biological activities. These findings substantiate the necessity of targeted extraction strategies over whole-fruit processing to optimize therapeutic yield. Consequently, nutmeg-derived kernel and pericarp fractions represent promising natural candidates for integration into veterinary dermatological formulations to mitigate infection and address the AMR challenge.

Keywords: *Myristica fragrans*, Veterinary Dermatology, Antimicrobial Resistance (AMR), Anatomical Specialization, Targeted Extraction, Cutaneous Pathogens

Introduction

The global escalation of antimicrobial resistance (AMR) represents one of the most formidable challenges in modern medicine, with projections suggesting it could cause up to 1.91 million deaths

annually by 2050. Within the veterinary sector, cutaneous infections caused by pathogens such as *Staphylococcus aureus* (including MRSA) and *Pseudomonas aeruginosa* are major drivers of compromised animal welfare and economic loss.

Conventional therapies are increasingly failing due to resistance mechanisms like biofilm formation and efflux pump overexpression, necessitating an urgent search for sustainable alternatives¹. In response to this crisis, plant-derived natural products have garnered substantial attention as alternative or adjunctive antimicrobial agents. Numerous botanical extracts—including those from *Pongamia pinnata*, *Coriandrum sativum*, and *Aloe vera*—have demonstrated antibacterial efficacy against multidrug-resistant skin pathogens through mechanisms involving plasma membrane disruption, inhibition of nucleic acid synthesis, and interference with biofilm formation². Additionally, compounds such as terpenoids and phenylpropanoids have been shown to restore antibiotic susceptibility by inhibiting bacterial efflux pumps³. However, the clinical translation of these extracts remains severely limited by several disadvantages: (i) high minimum inhibitory concentrations (MICs) relative to conventional antibiotics, often requiring milligram-per-milliliter doses; (ii) predominantly Gram-positive-specific activity due to inability to penetrate the outer membrane of Gram-negative pathogens; (iii) insufficient antibiofilm potency at sub-MIC concentrations; (iv) cytotoxicity to mammalian cells at antimicrobial doses; (v) poor aqueous solubility and chemical instability; and (vi) a paucity of in vivo efficacy and toxicity validation⁴. These limitations underscore the urgent need to identify plant species with superior potency, broader spectrum coverage, and clinically relevant mechanisms of action.

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The integumentary system, particularly the skin, serves as the primary interface between the organism and its external environment, providing a multifaceted barrier against microbial invasion, chemical insults, and mechanical trauma. In veterinary species, the skin's defensive architecture comprises the stratum corneum—a mechanical shield of terminally differentiated keratinocytes

embedded in a lipid matrix—the acid mantle maintaining a slightly acidic pH, sebum with antimicrobial lipids, and sweat containing lysozyme and dermcidin. Disruption of these protective layers through wounds, surgical incisions, burns, or immunosuppression renders animals susceptible to cutaneous and systemic infections. Opportunistic pathogens exploit such breaches with remarkable efficiency. *P. aeruginosa*, a Gram-negative bacillus, readily colonizes compromised skin, penetrates cutaneous breaches, and disseminates via lymphatic or hematogenous routes to cause systemic infection in veterinary patients. *S. aureus*, a Gram-positive coccus, is frequently implicated in suppurative skin and soft tissue infections in animals, producing toxins and enzymes that facilitate tissue destruction and abscess formation. *Staphylococcus epidermidis*, typically a commensal organism, can acquire pathogenic potential under immunocompromised conditions or in nosocomial veterinary settings, contributing to biofilm-associated infections. *Streptococcus pyogenes*, another Gram-positive coccus, initiates infection through minor abrasions, progressing from superficial pustules to bullous lesions and desquamation, with severe cases culminating in necrotizing fasciitis and systemic dissemination mediated by microbial toxins. Collectively, these pathogens exemplify the diverse strategies by which microorganisms exploit compromised skin barriers in veterinary species, underscoring the need for novel therapeutic interventions that reinforce host defenses and mitigate infection risk^{6–11}.

Myristica fragrans Houtt. (nutmeg; family *Myristicaceae*) emerges as a candidate of profound

pharmacological interest due to its complex and unique phytochemical profile. Unlike many botanical extracts that exhibit the limitations described above, nutmeg contains specific constituents—including myristicin, macelignan, trimyristin, and the monoterpene alcohols α -terpineol and terpinen-4-ol—that have demonstrated multi-target efficacy against both Gram-positive and resilient Gram-negative isolates implicated in skin and soft tissue infections, including methicillin-resistant *S. aureus* (MRSA), *P. aeruginosa*, *S. pyogenes*, and *S. epidermidis*. Notably, α -terpineol and its derivatives demonstrate synergistic interactions with streptomycin, reducing MIC values via membrane disruption and collapse of the proton motive force. Furthermore, *M. fragrans* extracts and essential oils have shown significant antibiofilm activity against MRSA and inhibit bacterial efflux pumps, thereby reversing resistance phenotypes. The ethyl acetate extract of *M. fragrans* flesh has exhibited particularly potent activity, with MIC values as low as 0.625 mg/mL against *Streptococcus* species. Beyond its antimicrobial properties, *M. fragrans* holds traditional ethnomedicinal precedent for treating skin and gastrointestinal ailments, and its phytochemical profile suggests favorable drug-like properties pending ADMET validation. A defining feature of nutmeg is its distinct anatomical specialization: the pericarp accumulates higher proportions of oxygenated monoterpenes, whereas the kernel and seed coat (mace) are enriched in phenylpropanoids such as myristicin and elemicin. This tissue-specific metabolite distribution suggests that targeted extraction could optimize therapeutic yield, enhance phytochemical stability, and overcome the batch-to-

batch variability and rapid attenuation issues commonly observed in whole-fruit preparations. Consequently, this study systematically evaluates these anatomical fractions as precision-based interventions for veterinary dermatology^{12–16}.

The present study evaluated the antimicrobial efficacy of 75% ethanol extracts from three anatomical fractions of *Myristica fragrans* Houtt.—the pericarp, seed coat, and kernel—against four clinically relevant veterinary cutaneous pathogens: *P. aeruginosa*, *S. aureus*, *S. epidermidis*, and *S. pyogenes*. Thin-layer chromatography was employed to establish distinct phytochemical fingerprints across fractions, thereby substantiating the anatomical specialization of secondary metabolite accumulation. Collectively, these findings establish a scientific basis for targeted extraction strategies and support the development of nutmeg-derived topical agents as sustainable alternatives to conventional antibiotics in veterinary dermatology.

Methods

1. Chemicals and Reagents

All chemicals and reagents used in this study were of analytical grade. Ethanol (75% and 99.9%), hexane, diethyl ether, isopropyl alcohol (AR grade), dimethyl sulfoxide (DMSO) and deionized water were purchased from Sigma-Aldrich (Singapore). Reference antibiotics—oxacillin, ceftazidime, clindamycin, and ceftriaxone—were utilized as positive controls for antimicrobial susceptibility testing.

2. Microbial Strains

Four clinically relevant bacterial strains were investigated: *P. aeruginosa*, *S. aureus*, *S. epidermidis*, and *Streptococcus pyogenes*. All strains were maintained on appropriate culture media and

incubated under standardized conditions prior to experimental use.

3. Plant Material and Extraction Procedure

The dried nutmeg (*M. fragrans* Houtt.) fruits were purchased from a licensed Thai herbal drugstore (herbal pharmacy) that routinely supplies authenticated medicinal plants for traditional and research purposes. Fresh nutmeg fruits were carefully collected and manually separated into three anatomical fractions: the pericarp (fruit husk), seed coat (mace), and kernel (nutmeg seed). Each part was macerated into 75% ethanol with a solid-to-solvent ratio of 1:5 (w/v), an optimized method for extracting a wide range of bioactive compounds. The extraction process was conducted at ambient temperature with intermittent agitation to maximize compound release while minimizing degradation of thermolabile constituents. Following extraction, the crude solutions were filtered through Whatman filter paper No. 1 to remove insoluble residues and ensure clarity of the extracts. The filtrates were then concentrated under reduced pressure using a rotary evaporator (Labconco, Labconco Corporation, USA) maintained at 40°C, which facilitated solvent removal while preserving volatile and sensitive phytochemicals. Finally, the concentrated extracts were stored at 4°C in airtight containers until further analysis, ensuring stability and preventing microbial contamination. This optimized protocol provided reproducible crude extracts suitable for subsequent phytochemical profiling and antimicrobial evaluation.

4. Antimicrobial Susceptibility Testing: Agar Well Diffusion Method

The antimicrobial activity of nutmeg-derived extracts was assessed using a modified agar well

diffusion assay to evaluate their inhibitory potential against clinically relevant pathogens. Tested microorganisms were first propagated in nutrient broth and incubated at 37°C with shaking at 150 rpm until reaching the logarithmic growth phase, ensuring optimal metabolic activity for susceptibility testing. Bacterial suspensions were standardized to the 0.5 McFarland turbidity standard, corresponding to approximately 1.5×10^8 CFU/mL, using 0.85% normal saline to achieve reproducibility across experiments. Mueller-Hinton agar plates were evenly inoculated with sterile cotton swabs to establish uniform bacterial lawns. Wells of 6 mm diameter were aseptically created using a sterile cork borer, and 100 μ L aliquots of crude extracts were dispensed at concentrations of 500, 250 and 125 mg/mL, dissolved in 1% DMSO. Negative control wells received only 1% DMSO, while standard antibiotics, oxacillin, clindamycin, ceftriaxone, ceftazidime, served as positive controls to benchmark activity. Plates were incubated at 37°C for 18–24 hours, after which inhibition zones were measured in millimeters using digital calipers to ensure precision. All experiments were performed in triplicate, providing statistical reliability and minimizing experimental variability. This standardized protocol enabled comparative evaluation of nutmeg fractions and established a robust foundation for subsequent antimicrobial profiling.

5. Thin-Layer Chromatography (TLC) Analysis

Preliminary phytochemical profiling of nutmeg extracts was carried out using analytical TLC to establish chemical fingerprints for different anatomical fractions. Small aliquots of each extract (2 μ L) were carefully spotted onto silica gel TLC plates measuring 20 $\times$ 20 cm, aluminum-backed, with a 0.2 mm layer thickness to ensure reproducibility and resolution. The plates were

developed in a saturated chamber using hexane:diethyl ether (5:5, v/v) for pericarp and those with 9:1, v/v for seed coat and kernel as the mobile phase, a solvent system selected for its ability to separate non-polar and moderately polar constituents. Following chromatographic development, the plates were visualized under ultraviolet (UV) light at 254 nm using a UVITEC documentation system, allowing detection of fluorescent and UV-absorbing compounds. Distinct bands corresponding to different phytochemical classes were observed, and retention factor (R_f) values were calculated for each resolved component to provide quantitative measures of compound migration. These R_f values served as preliminary indicators of metabolite distribution across nutmeg fractions, supporting subsequent purification and characterization strategies. All tests were performed in triplicate.

6. Statistical Analysis

All experiments were conducted in triplicate, and results are expressed as mean $\pm$ standard deviation (SD). Statistical significance was determined using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test, with $p < 0.05$ considered statistically significant.

Results

1. Nutmeg-Derived Essential Oils Physical Characteristics and Extraction Yield

Macroscopic properties—including color, viscosity, and physical state (solid, semi-solid, or liquid)—serve as preliminary indicators of tissue-specific phytochemical composition. Maceration extraction of three anatomical fractions of nutmeg (*Myristica fragrans* Houtt.)—the pericarp, seed coat

(mace), and kernel—using 75% ethanol yielded crude extracts with distinct macroscopic profiles, reflecting differential accumulation of bioactive constituents. On a fresh-weight basis, the seed coat yielded the highest (7.22%), followed by the kernel (4.81%) and pericarp (3.82%). The pericarp extract was observed as a viscous, dark-brown semi-solid. In contrast, the seed coat extract exhibited a translucent reddish-brown oily consistency with a pronounced aromatic profile. The seed coat extract was observed as a translucent reddish-brown oil with a pronounced aromatic odor. Notably, the kernel extract presented as a dense, nearly black viscous liquid, which serves as a physical indicator of its substantial deposition of lipophilic pigments and highly concentrated resinous compounds. The kernel extract was observed as a dense, nearly black viscous liquid. (Figure 1).

The macroscopic differences among the extracts underscore the anatomical specialization of nutmeg tissues and provide preliminary evidence for chemical heterogeneity that underpins their distinct biological properties (Table 1). The macroscopic properties differed markedly among the three anatomical fractions. The percentage yields varied by fraction, ranging from 3.82% to 7.22% on a fresh-weight basis and from 5.59% to 27.35% on a dry-weight basis (Table 1). Collectively, the distinct physical characteristics of the pericarp, seed coat, and kernel extracts establish a foundation for correlating morphological traits with chemical composition, thereby guiding subsequent chromatographic profiling and antimicrobial evaluation. The physical characteristics and extraction yields were recorded as baseline data for each fraction.

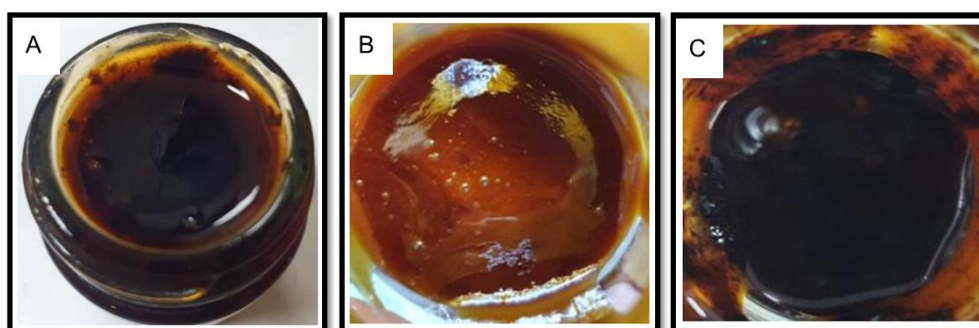


Figure 1 Physical characteristics of the crude extract from nutmeg (*Myristica fragrans*) obtained by maceration extraction. (A) pericarp extract; (B) seed coat extract; and (C) kernel extract

Table 1 Percentage yield (% w/w) of crude ethanol extracts from different anatomical fractions of *M. fragrans* Houtt.

Crude extract	% Yield	
	% Yield (fresh weight basis)	% Yield (dry weight basis)
Pericarp	3.82	27.35
Seed coat	7.22	22.79
Kernel	4.81	5.59

2. Antimicrobial Efficacy Against Cutaneous Pathogens

The antimicrobial activity of crude ethanol extracts from three anatomical fractions of nutmeg—the pericarp, seed coat (mace), and kernel—was systematically evaluated against four clinically significant cutaneous pathogens using the agar well diffusion assay at concentrations of 500 and 250 mg/mL, with 1% DMSO as negative control and oxacillin, clindamycin, ceftriaxone, and ceftazidime as positive controls. As shown in Figure 2, the pericarp extract exhibited the broadest antimicrobial spectrum at 500 mg/mL, producing inhibition zones ranging from 18.90 ± 2.05 mm against *P. aeruginosa* to 29.40 ± 1.31 mm against *S. pyogenes*, with activity persisting at 250 mg/mL (13.70 – 18.40 mm) but becoming undetectable at ≤ 125 mg/mL, indicating concentration-dependent potency. In contrast, the kernel extract demonstrated antimicrobial activity, maintaining potent inhibitory zones (14.70 – 17.30 mm) against *S. aureus*, *S. epidermidis*, and *S. pyogenes* even at the lowest concentration of 125 mg/mL. Furthermore, the pericarp extract showed specialized efficacy against the Gram-negative pathogen *P. aeruginosa* (18.90 ± 2.05 mm at 500 mg/mL), a finding

of particular significance given the intrinsic resistance of Gram-negative bacteria to many plant-derived agents.

The seed coat (mace) extract of nutmeg demonstrated moderate antimicrobial potency when evaluated against clinically relevant skin pathogens using the agar well diffusion assay. At the highest concentration tested (500 mg/mL), the extract produced inhibition zones of 17.80 ± 1.25 mm against *S. aureus* and 19.80 ± 0.68 mm against *S. pyogenes*, indicating measurable activity but less pronounced than that observed with pericarp extracts (Figure 3). The antimicrobial effect was clearly concentration-dependent, as reduced activity was observed at lower concentrations. At 125 mg/mL, inhibition zones remained detectable against *S. aureus* (12.50 ± 0.68 mm), *S. pyogenes* (11.40 ± 0.34 mm), and *P. aeruginosa* (11.70 ± 0.75 mm), though the magnitude of inhibition was diminished compared to higher concentrations. Interestingly, *S. epidermidis* exhibited resistance at this lower concentration. At 125 mg/mL, no detectable inhibition zone was observed for the seed coat extract against *S. epidermidis*. These findings highlight the variable antimicrobial spectrum of nutmeg anatomical fractions and emphasize the importance of concentration in determining efficacy. While the seed coat extract does not exhibit the broad-spectrum activity of the pericarp. At 500 mg/mL, the seed coat extract produced inhibition zones of 17.80 ± 1.25 mm against *S. aureus* and 19.80 ± 0.68 mm against *S. pyogenes*, with reduced activity at lower concentrations. Overall, the results support further investigation into fraction-specific bioactive compounds and their potential application in veterinary dermatology and wound management.

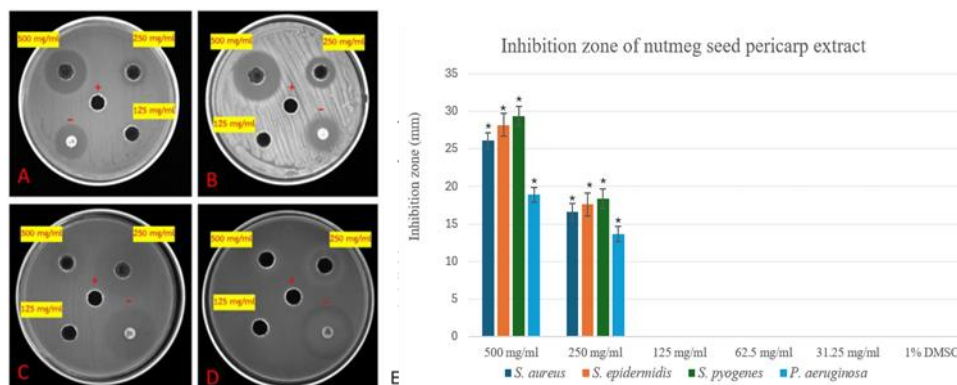


Figure 2 Antibacterial activity of the nutmeg pericarp extract in inhibiting the growth of bacteria

- (A) *S. aureus*, (B) *S. epidermidis*, (C) *S. pyogenes*, (D) *P. aeruginosa* and (E) bar graph showing inhibition zone of pericarp extract, whereas (+) = DMSO, (-) A = Oxacillin (1 µg), (-) B = Clindamycin (2 µg), (-) C = Ceftriaxone (30 µg) and (-) D = Ceftazidime (30 µg).
- (E) The ethanol extract of nutmeg pericarp exhibited potent antimicrobial activity against all tested cutaneous pathogens in a concentration-dependent manner. At the highest concentration of 500 mg/mL, the extract showed the maximum zone of inhibition against *S. pyogenes* (29.40±1.31 mm), followed by *S. epidermidis* and *S. aureus*. The inhibitory effects at 500 and 250 mg/mL for all pathogens were statistically significant compared to the negative control (1% DMSO) ($P < 0.05$). Notably, the extract also inhibited the Gram-negative pathogen *P. aeruginosa* at high concentrations, although no inhibitory activity was detected at concentrations ≤ 125 mg/mL across all species.

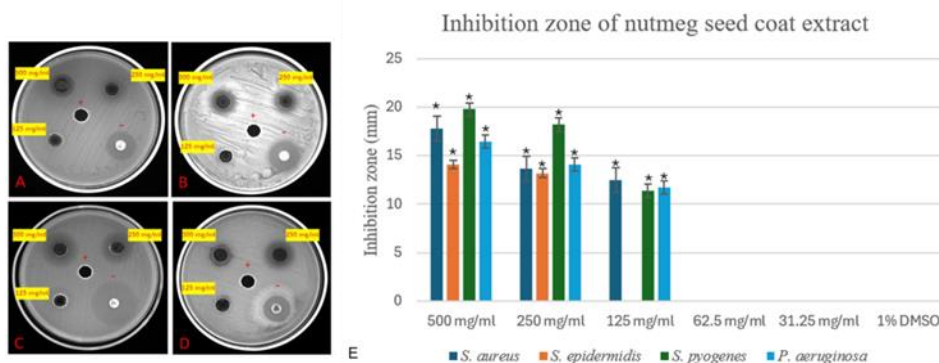


Figure 3 Antibacterial activity of the nutmeg seed coat extract in inhibiting the growth of bacteria

- (A) *S. aureus*, (B) *S. epidermidis*, (C) *S. pyogenes*, (D) *P. aeruginosa* and (E) bar graph showing inhibition zone of seed coat extract, whereas (+) = DMSO, (-) A = Oxacillin (1 µg), (-) B = Clindamycin (2 µg), (-) C = Ceftriaxone (30 µg) and (-) D = Ceftazidime (30 µg).
- (E) Antimicrobial activity of nutmeg seed coat (mace) ethanol extract against four cutaneous pathogens at various concentrations. Data are presented as mean ± SD *Statistically significant difference compared to the negative control (1% DMSO) at $p < 0.05$. No inhibitory activity was observed at concentrations ≤ 62.5 mg/mL or in the 1% DMSO. negative control (0 mm).

The kernel extract of nutmeg demonstrated the most sustained antimicrobial activity across the dilution series, as illustrated in Figure 4. At the highest concentration tested (500 mg/mL), inhibition zones were observed at 19.90 ± 0.35 mm for *S. pyogenes*, 18.20 ± 0.34 mm for *S. aureus*, 17.80 ± 1.20 mm for *S. epidermidis*, and 14.40 ± 0.34 mm for *P. aeruginosa*. Remarkably, antimicrobial activity persisted even at lower concentrations. At 125 mg/mL, the extract continued to inhibit *S. aureus* (16.60 ± 0.35 mm), *S. epidermidis* (14.70 ± 0.40 mm), and *S. pyogenes* (17.30 ± 0.70 mm), with *S.*

pyogenes showing exceptional tolerance to dilution. At a concentration of 125 mg/mL, the kernel extract continued to exhibit measurable inhibition zones, indicating retained inhibitory activity against *S. aureus*, *S. epidermidis*, and *S. pyogenes*. The kernel extract exhibited measurable antimicrobial activity across all tested concentrations (125–500 mg/mL). These findings emphasize the importance of anatomical specialization in nutmeg tissues and support targeted extraction strategies to optimize bioactive yield.

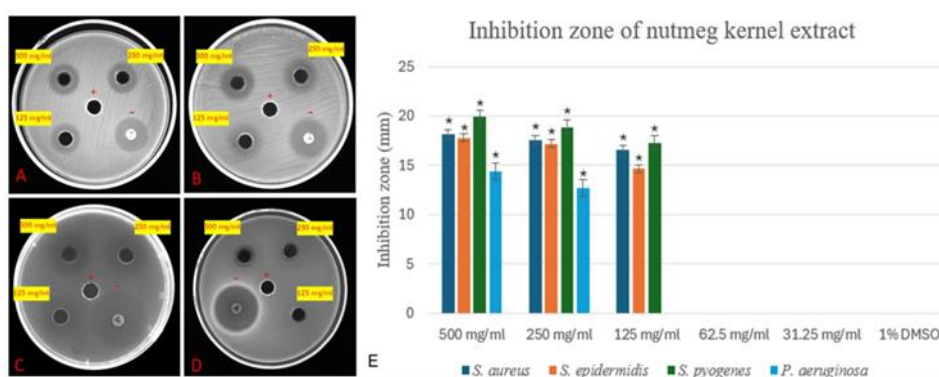


Figure 4 Antibacterial activity of the nutmeg kernel extract in inhibiting the growth of bacteria (A) *S. aureus*, (B) *S. epidermidis*, (C) *S. pyogenes*, (D) *P. aeruginosa* and (E) bar graph showing inhibition zone of kernel extract, whereas (+) = DMSO, (-) A = Oxacillin (1 μ g), (-) B = Clindamycin (2 μ g), (-) C = Ceftriaxone (30 μ g) and (-) D = Ceftazidime (30 μ g). (E) Antimicrobial activity of nutmeg (*Myristica fragrans*) kernel ethanol extract against four cutaneous pathogens. Data are presented as mean $\pm$ SD.*Statistically significant difference compared to 1% DMSO at $p < 0.05$. No inhibitory activity was observed at concentrations ≤ 62.5 mg/mL or in the 1% DMSO negative control (0 mm).

3. Phytochemical Profiling and Fractionation

Thin-layer chromatography (TLC) profiling was undertaken to generate preliminary chemical fingerprints of nutmeg (*M. fragrans*) extracts derived from the pericarp, seed coat, and kernel, thereby providing insight into the tissue-specific distribution of secondary metabolites. As

shown in Figure 5, the chromatographic patterns differed markedly among the anatomical fractions, underscoring the chemical heterogeneity of the fruit. The pericarp extract, analyzed using hexane:diethyl ether (5:5, v/v) as the mobile phase, exhibited multiple well-resolved bands. In contrast, the seed coat and kernel extracts were developed under

more non-polar conditions (hexane:diethyl ether, 9:1, v/v), and both displayed distinct retention factor (Rf) profiles. Notably, the kernel fraction demonstrated unique migration of polar constituents under the chromatographic conditions employed. These differential TLC signatures provide strong evidence for anatomical specialization in metabolite accumulation, each fraction exhibited a distinct retention factor (Rf) profile. The Rf values were

calculated for each resolved band to document metabolite distribution across fractions. Collectively, the TLC analysis not only confirmed the chemical diversity of nutmeg anatomical fractions but also established a foundation for targeted extraction and fractionation approaches, thereby enhancing the potential for developing plant-based therapeutic agents in veterinary dermatology and wound management.

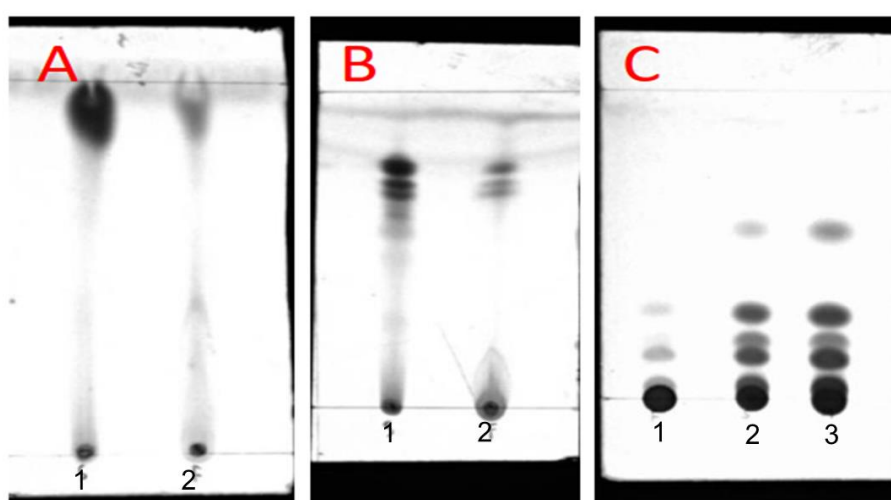


Figure 5 Chromatograms of ethanol crude extracts from various parts of nutmeg; (A) Ethanol:ethyl acetate (5:5) for seed coat (lane 1) and kernel (lane 2), (B) Hexane:acetone (5:5) for seed coat (lane 1) and kernel (lane 2) and (C) Hexane:diethyl ether (9:1) for pericarp (lane 1), seed coat (lane 2), and kernel (lane 3).

Discussion

The ethanol maceration extracts derived from the pericarp, seed coat (mace), and kernel of *M. fragrans* Houtt. exhibited marked differences in yield, physical characteristics, and antimicrobial potency against four clinically relevant cutaneous bacterial pathogens. These observations underscore the tissue-specific distribution of bioactive secondary metabolites within the nutmeg fruit and support the rationale for targeted utilization of individual anatomical fractions rather than whole-fruit

processing. On a fresh-weight basis, the seed coat (mace) provided the highest extraction yield (7.22% w/w), followed by the kernel (4.81%) and pericarp (3.82%). When corrected for dry weight, however, the pericarp demonstrated superior efficiency (27.35%), indicating that its lower absolute oil content is largely attributable to higher moisture levels rather than diminished biosynthetic capacity. This pattern is consistent with previous studies showing that pericarp tissue is enriched in monoterpene alcohols and oxygenated derivatives,

whereas the seed coat and kernel accumulate higher proportions of non-polar phenylpropenes and monoterpene hydrocarbons^{17–21}.

The observed physical differences among nutmeg extracts—viscous dark-brown semi-solid for the pericarp, translucent reddish-brown oil for the seed coat, and dense, nearly black viscous liquid for the kernel—reflect tissue-specific differential accumulation of pigments, oleoresins, and lipophilic constituents. Such macroscopic variation corresponds to distinct phenylpropanoid and terpenoid profiles: pericarp extracts typically exhibit lower levels of myristicin and safrole but elevated concentrations of oxygenated monoterpenes such as terpinen-4-ol and α -terpineol, whereas the kernel accumulates markedly higher proportions of lipophilic phenylpropanoids (particularly myristicin and safrole) along with oleoresins. The dense, nearly black viscous character of the kernel extract arises from the formation of a semi-stable, high-molecular-weight matrix that physically entraps these dominant bioactive constituents, thereby decreasing their volatility and protecting them from rapid oxidative degradation or evaporative loss. This results in a slower, diffusion-controlled release profile, with the near-black coloration attributable to concentrated pigments and minor polymerized phenolic derivatives. In practical bioactivity contexts, including antimicrobial, antioxidant, and insect-repellent applications, the kernel's viscous medium functions as a natural reservoir that sustains effective concentrations over extended periods, conferring superior prolonged residual efficacy compared with the more fluid, terpenoid-rich pericarp and seed-coat fractions, which undergo

comparatively rapid attenuation. Consequently, these physical attributes serve as a macroscopic proxy for tissue-specific metabolic specialization that translates directly into practically advantageous, long-lasting performance^{22–24}.

Antimicrobial screening via the agar well diffusion method revealed that all three extracts possessed concentration-dependent inhibitory activity against *P. aeruginosa*, *S. aureus*, *S. epidermidis*, and *S. pyogenes*. At 500 mg/mL, the pericarp extract produced the broadest spectrum of activity, with inhibition zones ranging from 18.90 ± 2.05 mm (*P. aeruginosa*) to 29.40 ± 1.31 mm (*S. pyogenes*). Activity persisted at 250 mg/mL but was abolished at ≤ 125 mg/mL. In contrast, the seed coat extract exhibited moderate potency, achieving maximum zones of 17.80 ± 1.25 mm (*S. aureus*) and 19.80 ± 0.68 mm (*S. pyogenes*) at 500 mg/mL, with residual activity detectable at 125 mg/mL against three of the four pathogens. The kernel extract was notable for its sustained efficacy at lower concentrations: inhibition zones of 14.70–17.30 mm remained evident at 125 mg/mL against *S. aureus*, *S. epidermidis*, and *S. pyogenes*. In contrast to the more concentration-dependent profiles of the other fractions, the kernel extract demonstrated a remarkable sustained antimicrobial efficacy, maintaining potent inhibitory zones against *S. aureus*, *S. epidermidis*, and *S. pyogenes* even at the lowest tested concentration of 125 mg/mL. This dilution-stable property suggests an enrichment in highly potent bioactive molecules, most likely phenylpropanoids such as myristicin and elemicin, which were identified as key constituents of the kernel's dense, pigment-rich matrix^{25,26}. Existing research indicates that phenylpropanoid-

rich fractions can exert concentration-independent membrane-disrupting effects, allowing them to sustain significant antimicrobial potency even when substantially diluted^{26,27}. This hypothesis is further substantiated by the unique polar constituent migration observed in the kernel's TLC fingerprints, confirming that its specialized phytochemical composition provides a robust and persistent therapeutic profile suitable for clinical topical applications²⁵. These findings align with numerous reports documenting the antibacterial efficacy of *M. fragrans* extracts and essential oils against both Gram-positive and Gram-negative skin pathogens, although direct comparisons are complicated by variations in extraction solvent, concentration, and assay methodology^{24,28}.

Several earlier studies employing disk-diffusion or broth-dilution methods have reported inhibition zones of 10–25 mm for nutmeg seed or mace extracts against *S. aureus* and *P. aeruginosa* at concentrations comparable to those tested here. The superior performance of the pericarp extract against the Gram-negative pathogen *Pseudomonas aeruginosa* is particularly noteworthy, given that Gram-negative organisms are generally less susceptible to plant-derived antimicrobial agents due to the protective barrier of their complex outer membrane. This specialized efficacy is likely attributable to the high relative abundance of monoterpene alcohols, specifically terpinen-4-ol, which are concentrated within the pericarp tissue. These compounds are known to effectively compromise outer-membrane integrity by integrating into the lipid bilayer, thereby increasing its fluidity and permeability. This disruption facilitates the

irreversible leakage of essential intracellular ions and metabolites, and may further allow for synergistic interactions with other lipophilic constituents within the extract to achieve complete microbial inhibition. The enhanced activity may therefore be linked to the higher relative abundance of monoterpene alcohols such as terpinen-4-ol in pericarp tissue, compounds known to compromise outer-membrane integrity and synergize with other lipophilic constituents. In contrast, the kernel extract demonstrated notable sustained efficacy at lower concentrations (125 mg/mL), with inhibition zones of 14.70–17.30 mm persisting against *S. aureus*, *S. epidermidis*, and *S. pyogenes*. This observation suggests enrichment in highly potent, less volatile antimicrobials—possibly myristicin or related phenylpropanoid derivatives—whose activity is maintained upon dilution due to the viscous matrix acting as a natural^{25–27}. The enhanced activity may be attributable to the higher relative abundance of monoterpene alcohols such as terpinen-4-ol in pericarp tissue, compounds known to compromise outer-membrane integrity and synergize with other lipophilic constituents. The kernel's ability to retain activity at 125 mg/mL suggests enrichment in highly potent, less volatile antimicrobials—possibly myristicin or elemicin derivatives—whose efficacy persists upon dilution. This observation is consistent with reports indicating that phenylpropanoid-rich fractions can exert concentration-independent membrane-disrupting effects, thereby sustaining antimicrobial potency even at reduced levels^{28–30}.

Thin-layer chromatography (TLC) fingerprints provided preliminary chemical evidence for the anatomical specialization observed in

bioactivity. Pericarp extracts resolved multiple bands under moderately polar conditions (hexane:diethyl ether 5:5 v/v), indicating a diverse array of polar and semi-polar metabolites. Seed coat and kernel extracts, developed under non-polar conditions (9:1 v/v), displayed distinct R_f profiles, with the kernel showing unique polar constituent migration. TLC fingerprints provided preliminary chemical evidence for the anatomical specialization observed in bioactivity. Pericarp extracts resolved multiple bands under moderately polar conditions (hexane:diethyl ether 5:5 v/v), indicating a diverse array of polar and semi-polar metabolites. Seed coat and kernel extracts, developed under non-polar conditions (9:1 v/v), displayed distinct R_f profiles, with the kernel showing unique polar constituent migration. These differential chromatographic signatures corroborate GC-MS and HPLC data from independent laboratories showing that pericarp oil is dominated by monoterpenes ($\approx 60\%$) and oxygenated monoterpenes ($\approx 29\%$), whereas seed and mace oils are richer in sabinene, myristicin, and safrole. Such tissue-specific chemotypes explain the observed variation in antimicrobial spectra and potency and highlight the importance of fraction-specific extraction for optimizing therapeutic applications. Thin-layer chromatography (TLC) fingerprints corroborated these biological findings, displaying distinct phytochemical fingerprints for each fraction. The kernel's unique polar constituent migration on TLC supports its observed sustained efficacy upon dilution. The multifaceted antimicrobial action of nutmeg; ranging from membrane disruption by monoterpenes (α -pinene, β -pinene) to the inhibition of quorum sensing and efflux pumps by

phenylpropenes (myristicin); minimizes the likelihood of resistance development, positioning these extracts as ideal candidates for veterinary topical therapy^{31,32}.

The antimicrobial mechanisms of nutmeg constituents are multifaceted. Monoterpenes (α -pinene, β -pinene, sabinene) and their oxygenated derivatives increase bacterial membrane fluidity and permeability, leading to leakage of intracellular ions and metabolites. Phenylpropenes such as myristicin and elemicin further inhibit bacterial efflux pumps and disrupt quorum-sensing systems, effects that are particularly pronounced against methicillin-resistant *S. aureus* and biofilm-forming pathogens. The broader activity of pericarp extracts against *P. aeruginosa* may reflect synergistic interactions between terpinen-4-ol and trace phenolic aldehydes, compounds previously isolated from pericarp and shown to inhibit both lipid peroxidation and cyclooxygenase enzymes. These multi-target actions reduce the likelihood of resistance development compared with single-site antibiotics and position nutmeg extracts as attractive candidates for adjunctive or alternative therapies in an era of rising antimicrobial resistance³³.

Despite these promising results, several limitations must be acknowledged and factors considered for clinical translation. The study employed crude ethanol extracts rather than purified essential oils or isolated compounds, meaning synergistic or antagonistic interactions among constituents cannot be excluded; furthermore, although 1% DMSO served as a negative control with no observable inhibition, solvent effects at higher concentrations remain a potential

confounder. Antimicrobial data were generated solely by the agar well diffusion method, which—while standardized—does not provide minimum inhibitory concentration (MIC) or minimum bactericidal concentration (MBC) values, and the investigation was conducted strictly under in-vitro conditions, leaving the influence of the complex skin microenvironment (including sebum composition and host immune status) to be elucidated. To establish a comprehensive pharmacological profile, future research must prioritize: (1) quantitative potency through broth microdilution assays to determine MIC and MBC values; (2) in-vivo validation via wound-healing and safety studies in animal models to evaluate efficacy and dermal toxicity under physiological conditions; and (3) compound identification using bioassay-guided fractionation and GC-MS/HPLC-MS to isolate the specific bioactive molecules responsible for the observed anatomical specialization. Additionally, although the four test strains represent major cutaneous pathogens, clinical isolates from diverse geographic origins and resistance phenotypes should be evaluated to confirm generalizability; synergistic interactions with conventional antibiotics (e.g., oxacillin, ceftazidime) warrant investigation, as sub-MIC nutmeg fractions may restore susceptibility in multidrug-resistant isolates; and stability testing under accelerated storage conditions is required to ensure shelf-life suitability for pharmaceutical or cosmeceutical formulations.

The findings of this study translate directly into novel therapeutic avenues for veterinary dermatology and wound management. Given the rising challenge of Antimicrobial Resistance (AMR)

in livestock and pets, there is an urgent need for natural adjuncts that reduce reliance on systemic drugs. These extracts demonstrate significant potential as bioactive components in topical formulations, targeting primary veterinary pathogens including *S. aureus* and *S. pseudintermedius*. The documented ability of nutmeg constituents to enhance re-epithelialization in vivo, coupled with their efficacy against high-burden human wound isolates, underscores their translational value as a safe, effective intervention for complex animal skin infections.

Conclusions

This investigation substantiates the profound pharmacological significance of anatomical specialization within *M. fragrans* Houtt. The study demonstrates that the pericarp, seed coat, and kernel possess distinct phytochemical fingerprints that correlate directly with their specialized antimicrobial profiles. Specifically, the pericarp exhibits superior broad-spectrum activity, notably against the resilient Gram-negative *P. aeruginosa*, whereas the kernel maintains exceptional, dilution-stable efficacy even at the lowest tested concentration of 125 mg/mL. From an industrial and pharmaceutical perspective, these findings provide a compelling rationale for adopting fraction-specific extraction strategies over conventional whole-fruit processing. By isolating specific anatomical parts, the veterinary pharmaceutical industry can achieve targeted concentrations of localized bioactive compounds, thereby optimizing therapeutic yield and ensuring superior potency in topical formulations. This targeted approach not only enhances cost-

effectiveness by maximizing the utility of each fraction but also provides a sustainable, low-resistance-risk solution to the escalating antimicrobial resistance crisis in veterinary dermatology. Ultimately, the utilization of specialized nutmeg fractions represents a significant advancement toward precision-based natural interventions, offering a robust alternative to systemic antibiotics for the management of cutaneous infections in livestock and companion animals.

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