INTERNATIONAL JOURNAL OF
PHARMACEUTICAL AND HEALTHCARE INNOVATION
journal homepage: www.ijphi.com

Research Article

OPEN ACCESS

Antibacterial Effect of Lemon Grass (*Cymbopogon citratus*) Extract Oil (LEO) on the Growth of Bacterial Pathogens Collected from Different Sources

Gunjan Khare*[1], Monika Shukla[1], Pragati Sharma[1], Komal Kulshreshtha[2]
Suman Rahul Ahirwar[3], Pratigya Darpe[3], Naveen Kumar Singh[1]
[1]School of Natural and Applied Science, Vikrant University, Gwalior (MP), Bharat.
[2]School of Engineering and Technology, Vikrant University, Gwalior (MP), Bharat.
[3]School of Legal Studies, Vikrant University, Gwalior (MP), Bharat.

Article Info

Article history:

Manuscript ID:

IJPHI2703090518052026
Received: 27-MARCH -2026
Revised : 09-MAY -2026
Accepted: 18-MAY -2026
Available online:MAY-2026
DOI:
doi:10.62752/ ijphi.v3i2.264

Keywords:
Cymbopogon citratus, Lemongrass Extract Oil (LEO), Antibacterial activity, Minimum Inhibitory Concentration (MIC), Zone of Inhibition, Citral, Urinary Tract Infections (UTI), Disc Diffusion Method.

Corresponding Author:
gunjankhare2003@gmail.com

Abstract

Lemon grass (Cymbopogon citratus) is a fast-growing perennial tropical grass belonging to the family and is widely recognized for its broad-spectrum antimicrobial properties. Its essential oil, referred to as Lemongrass Extract Oil (LEO), contains high concentrations of citral (geranial and general), which disrupt bacterial cell membrane integrity and inhibit key metabolic enzymes. This review presents a comprehensive investigation into the antibacterial efficacy of LEO against six clinically significant bacterial pathogens: Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Clostridium perfringens. The antibacterial activity was evaluated using the Disc Diffusion Method and the Minimum Inhibitory Concentration (MIC) assay, following NCCLS and CLSI guidelines. Bacterial isolates were collected from both clinical (urine) sources and standardized microbiological laboratory strains to enable direct comparative analysis. Results revealed significant variation in susceptibility depending on the bacterial species and the origin of the isolate. K. pneumoniae and E. coli clinical isolates showed the largest zones of inhibition (29 ± 0.5 mm and 28 ± 1.5 mm, respectively), while C. perfringens demonstrated complete resistance. B. subtilis showed 100% sensitivity when tested on three different strains. MIC values ranged from 1 µg/ml (highly sensitive strains) to 128 µg/ml (resistant strains). These findings confirm the therapeutic potential of LEO as a natural antimicrobial agent and advocate its further clinical exploration, particularly for the management of urinary tract infections (UTIs), hospital-acquired infections, and antibiotic-resistant pathogens.

1. Introduction

The global escalation of antimicrobial resistance (AMR) has emerged as one of the most pressing challenges in modern medicine (1). The World Health Organization (WHO) estimates that AMR could cause 10 million deaths annually by 2050 if left unchecked. Classical antibiotics such as penicillin, fluoroquinolones, and carbapenems are increasingly losing efficacy against multidrug-resistant (MDR) pathogens, necessitating the urgent discovery and validation of alternative therapeutic agents (2). In this context, plant-derived essential oils have attracted considerable scientific interest as renewable, biodegradable, and potentially effective antimicrobial agents. *Cymbopogon citratus*, commonly known as lemon grass, belongs to the family Poaceae and is a fast-growing C4 perennial sedge native to South and Southeast Asia. The genus Cymbopogon encompasses approximately 180 species, among which C. citratus, C. flexuosus, C. winterianus, C. martinii, C. nardus, and C. refractus are the most extensively studied. C. citratus grows to a height of up to 1.8 m and bears strap-like, bluish-green, glabrous leaves up to 90 cm long and 2.5 cm wide. The inflorescence is 30–60 cm long and nodding, with spikelets subtended by spathes. It is widely cultivated across Tropical Asia, South America, and parts of Africa for its essential oil, culinary use, and medicinal applications. Traditionally, lemon grass has been used in folk medicine across Asia and Africa for the treatment of digestive disorders (flatulence, indigestion, acidity), fever, headache, and respiratory ailments (3). Its stalks and leaves serve as antispasmodic, hypotensive, anticonvulsant, analgesic, antiemetic, antitussive, antirheumatic, and antiseptic remedies in traditional pharmacopoeia. The therapeutic versatility of C. citratus is primarily attributed to its essential oil Lemongrass Extract Oil (LEO) which is stored in specialised parenchymal oil cells between vascular bundles proximal to non-photosynthetic tissue in young and rapidly growing leaves and floral tops. LEO constitutes approximately 1–2% of the plant's total dry weight. Its principal bioactive constituents are the monoterpene aldehydes: geranial (Citral-α, ~40.8%) and neral (Citral-β, ~32%), collectively referred to as citral. Citral disrupts the phospholipid bilayer of bacterial cell membranes, impairs electron transport, and inhibits enzyme systems critical for cellular metabolism. Additional minor components including myrcene, limonene, linalool, geraniol, and citronellal contribute to the synergistic antimicrobial activity of the extract. This review systematically documents the antibacterial efficacy of LEO against six pathogenic bacteria Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Clostridium perfringens collected from both clinical and laboratory sources (4-6). It presents quantitative data from Disc Diffusion and MIC assays, discusses the mechanistic basis of antibacterial action, compares findings with contemporary literature, and contextualizes the results within the broader framework of natural antimicrobial research (7).

2. Taxonomy and Botanical Description of *Cymbopogon citratus*

Cymbopogon citratus (DC.) Stapf belongs to the order Poales, family Poaceae (Gramineae), and tribe Andropogoneae. The plant is a perennial, tufted, herbaceous grass that forms dense clumps from short, ringed rhizomes, its taxonomy is summarized in Table 1 below.

Table 1: Taxonomic classification of *Cymbopogon citratus*

Taxonomic Rank	Classification
Kingdom	Plantae
Order	Poales
Family	Poaceae (Gramineae)
Genus	Cymbopogon
Species	C. citrates
Common names	Lemon grass, West Indian lemon grass
Origin	South and Southeast Asia (India, Sri Lanka, Malaysia)
Distribution	Tropical Asia, America, Africa

The leaves are long, linear, and aromatic, with a characteristic lemon scent due to the high citral content. The leaf sheath is reddish-purple at the base, and the leaf blade tapers to a long, drooping tip. The plant rarely flowers under cultivation; when it does, the paniculate inflorescence consists of numerous racemes arising from spatheate bracts. Under optimal tropical conditions with 150–250 cm annual rainfall and well-drained soils, C. citratus can yield up to 6–7 tonnes of fresh herbage per hectare per year (8).

3. Chemical Composition of Lemongrass Extract Oil (LEO)

The chemical profile of LEO is complex and varies with geographic origin, plant age, harvest time, and extraction method. Gas Chromatography–Mass Spectrometry (GC-MS) analyses consistently identify citral as the dominant fraction, comprising approximately 70–80% of the total oil composition. Table 2 presents the major phytochemical constituents of LEO and their reported biological functions.

Table 2: Major phytochemical constituents of Lemongrass Extract Oil (LEO) and their biological activities

Compound	Approximate % in LEO	Primary Biological Activity
Geranial (Citral-α)	38–42%	Antibacterial, antifungal, antioxidant
Neral (Citral-β)	30–35%	Antibacterial, anti-inflammatory
Myrcene	12–18%	Analgesic, anti-inflammatory
Limonene	2–5%	Antioxidant, anticancer
Linalool	1–3%	Sedative, antifungal
Geraniol	1–3%	Antimicrobial, antifungal
Citronellal	1–2%	Insect repellent, mild antibacterial
β-Caryophyllene	1–2%	Anti-inflammatory, analgesic

The ratio of geranial to neral is approximately 1.3:1, which is characteristic of high-quality lemongrass oil. The synergistic interaction between these two isomers produces a potent aldehyde mixture with demonstrated bactericidal properties. Extraction methods significantly influence the yield and composition: steam distillation yields 0.5–1.5% essential oil, while supercritical CO_2 extraction produces superior-quality oil with a higher citral content. Solvent extraction (ethanol) as employed in this study is cost-effective and appropriate for broad-spectrum antibacterial testing (9).

4. Mechanism of Antibacterial Action

The antibacterial mechanism of LEO is multifaceted and targets several critical structural and functional components of bacterial cells. Understanding the mechanism is essential for evaluating its potential as a clinical therapeutic agent and for predicting patterns of resistance (10). Figure 1 below illustrates the sequential steps of LEO's antibacterial mechanism.

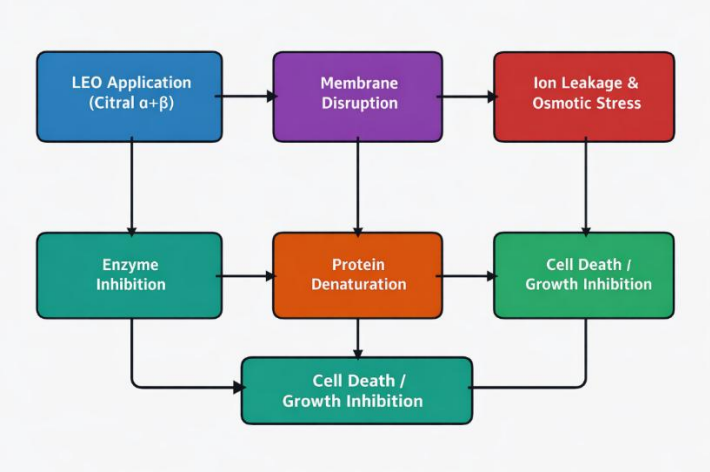

Figure 1: Schematic representation of the proposed multi-target antibacterial mechanism of Lemongrass Extract Oil (LEO)

The primary mode of action involves the disruption of the bacterial cell membrane. Citral, being a highly lipophilic aldehyde, integrates into the phospholipid bilayer of the bacterial cell membrane, causing structural disorganization, increased membrane permeability, and leakage of intracellular ions (K+, Na+, Mg2+) and macromolecules (ATP, nucleic acids). This leads to a collapse of the proton motive force (PMF) and ultimately to osmotic lysis of the bacterial cell. Secondly, citral inhibits key bacterial enzymes, including ATPases, dehydrogenases, and enzymes involved in cell wall biosynthesis (penicillin-binding proteins, transpeptidases). Inhibition of ATP synthase prevents energy production, while disruption of cell wall enzymes halts peptidoglycan cross-linking, making bacteria osmotically fragile. Thirdly, LEO has been shown to cause protein denaturation and inhibit DNA replication, amplifying its bactericidal effect (11). Gram-positive bacteria (e.g., S. aureus, B. subtilis) generally show greater susceptibility to LEO because their cell wall lacks the outer lipopolysaccharide (LPS) membrane that acts as a permeability barrier in Gram-negative bacteria (e.g., E. coli, K. pneumoniae, P. aeruginosa). The outer membrane of Gram-negative organisms effectively restricts hydrophobic molecules, explaining their higher MIC values. However, the large zones of inhibition observed for clinical isolates of E. coli and K. pneumoniae suggest that clinical strains may have compromised outer membrane integrity, possibly due to antibiotic exposure or virulence-associated membrane remodelling (12).

5. Materials and Methods

5.1 Source and Preparation of Bacterial Isolates

Six pathogenic bacterial species were selected for the study: Staphylococcus aureus, Bacillus subtilis, Escherichia coli,

Klebsiella pneumoniae, Pseudomonas aeruginosa, and Clostridium perfringens. Isolates were obtained from two distinct sources: (i) Clinical isolates recovered from urine specimens of patients with confirmed urinary tract infections (UTIs) at a government hospital microbiology department, and (ii) standard laboratory reference strains maintained at accredited government microbiological laboratories. Bacterial identity was confirmed using standard biochemical tests including Gram staining, catalase, oxidase, indole, urease, citrate utilisation, and sugar fermentation tests, supplemented by API 20E and API Staph identification strips where applicable (13).

5.2 Preparation of Lemongrass Extract Oil (LEO)

Fresh leaves of *Cymbopogon citratus* were harvested from authenticated cultivated plants at early morning to maximise essential oil content. The leaves were washed thoroughly under running tap water to remove surface debris, followed by a brief wash with distilled water. The washed leaves were air-dried at room temperature (25 ± 2°C) for 72 hours to remove surface moisture while preserving volatile constituents. The dried leaves were subjected to solvent (ethanol, 95% v/v) extraction using the maceration method (14, 15). Approximately 100 g of dried leaf material was soaked in 500 mL of ethanol for 48 hours with intermittent shaking. The resulting extract was filtered through Whatman No. 1 filter paper and concentrated using a rotary evaporator at 40°C under reduced pressure. The concentrated extract was reconstituted in dimethyl sulfoxide (DMSO, 2%) to prepare stock solutions at 1000 µg/mL, which were stored at 4°C until use. The extraction procedure is summarized in Figure 2 below.

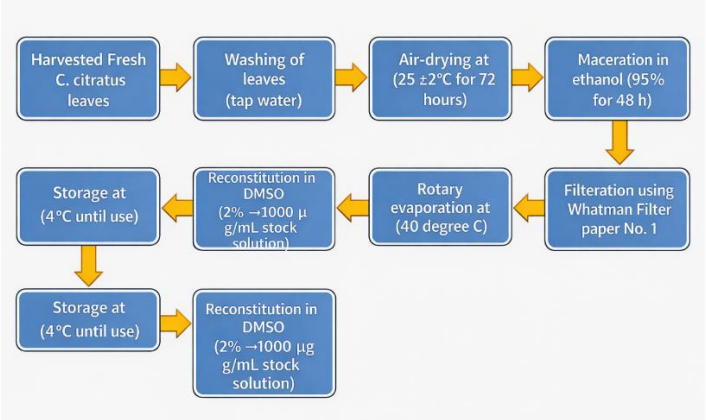

Figure 2: Flow diagram of Lemongrass Extract Oil (LEO) preparation

5.3 Disc Diffusion Assay

The antibacterial activity of LEO was assessed using the standard Disc Diffusion (Kirby-Bauer) Method on Mueller Hinton Agar (MHA) plates, as recommended by the Clinical and Laboratory Standards Institute (CLSI) and the National Committee for Clinical Laboratory Standards (NCCLS). All bacterial strains were sub-cultured on nutrient agar slants for 18–24 hours at 37°C. Turbidity of the bacterial suspension was standardized to 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL) using a turbidimeter. Sterile filter paper discs (5–8 mm diameter) were impregnated with 60 µL of 10% LEO solution and placed on the seeded MHA plates. Control discs were loaded with DMSO solvent (negative control) and standard antibiotics (positive control). The plates were incubated at 37°C for 24 hours (16, 17). The diameter of the Zone of Inhibition (ZOI) was measured in millimetres using a calibrated Vernier calliper, and the experiment was performed in triplicate. The mean ± standard deviation was recorded (18).

5.4 Minimum Inhibitory Concentration (MIC) Assay

The Minimum Inhibitory Concentration (MIC) of LEO was determined using the broth microdilution method as described by Hammer et al., following CLSI guidelines (19). Two-fold serial dilutions of LEO were prepared in sterile Nutrient Broth (NB) in 96-well microtiter plates, yielding concentrations ranging from 0.5 µg/mL to 256 µg/mL. Bacterial inocula were prepared by growing overnight cultures and diluting to a final concentration of approximately 5×10^5 CFU/mL in the microplate wells. DMSO served as the solvent control, and wells containing only broth were used as sterility controls. The plates were incubated at 37 ± 1°C for 18–24 hours. Bacterial growth was assessed visually and confirmed by adding 20 µL of resazurin solution (0.01% w/v) as a growth indicator a colour change from blue (no growth) to pink/colourless (growth) indicated bacterial viability. The MIC was defined as the lowest concentration of LEO that resulted in complete inhibition of visible bacterial growth (20-23).

6. Results

6.1 Minimum Inhibitory Concentration (MIC) and Disc Diffusion Results

Table 3 presents the MIC values and Disc Diffusion sensitivity/resistance data for all tested bacterial strains. Strains are categorized as sensitive (MIC ≤ 16 µg/mL) or resistant (MIC ≥ 64 µg/mL) based on established breakpoint criteria.

Table 3: MIC values and Disc Diffusion interpretation of LEO against pathogenic bacterial strains

Bacteria	Strain Code	MIC (µg/mL)	MIC Interpretation	Disc Diffusion Interpretation
S. aureus	SK10S2	1	Sensitive	Sensitive
S. aureus	SK5S1	8	Sensitive	Sensitive
S. aureus	SK6S1	64	Resistant	Resistant
S. aureus	SKE11	64	Resistant	Resistant
B. subtilis	—	≤1	Sensitive	Sensitive (100%)
E. coli	E382	1	Sensitive	Sensitive
E. coli	C91	8	Sensitive	Sensitive
E. coli	P82	128	Resistant	Resistant
E. coli	P86	128	Resistant	Resistant
K. pneumoniae	CP62	16	Sensitive	Sensitive
K. pneumoniae	M10	32	Moderate	Moderate
K. pneumoniae	LT81	64	Resistant	Resistant
K. pneumoniae	LT121	124	Resistant	Resistant
C. perfringens	—	>256	Resistant	Resistant
P. aeruginosa	—	>256	Resistant	Resistant (100%)

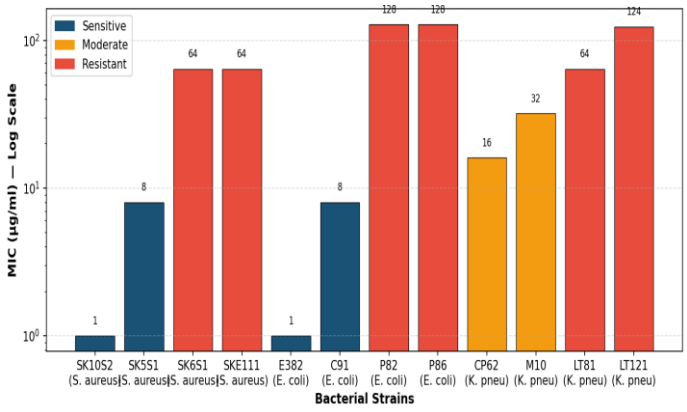

Figure 3: Minimum Inhibitory Concentration (MIC) of LEO for individual bacterial strains (log scale)

6.2 Zone of Inhibition Disc Diffusion Results

Table 4 presents the mean Zone of Inhibition (ZOI) recorded for each bacterial species from clinical and laboratory sources, along with the overall disc diffusion sensitivity and resistance percentages.

Table 4: Zone of Inhibition and Disc Diffusion sensitivity/resistance data for tested bacterial pathogens

Bacterium	ZOI Clinical (mm)	ZOI Lab (mm)	% Sensitive (Disc Diffusion)	% Resistant (Disc Diffusion)
S. aureus	20 ± 1.0	19.33	38.5	61.5
B. subtilis	—	10.33	100	0
E. coli	28 ± 1.5	11.33	19.8	80.2
K. pneumoniae	29 ± 0.5	9.33	40	60
C. perfringens	No inhibition	—	0	100
P. aeruginosa	25 ± 1.6	No inhibition	—	100

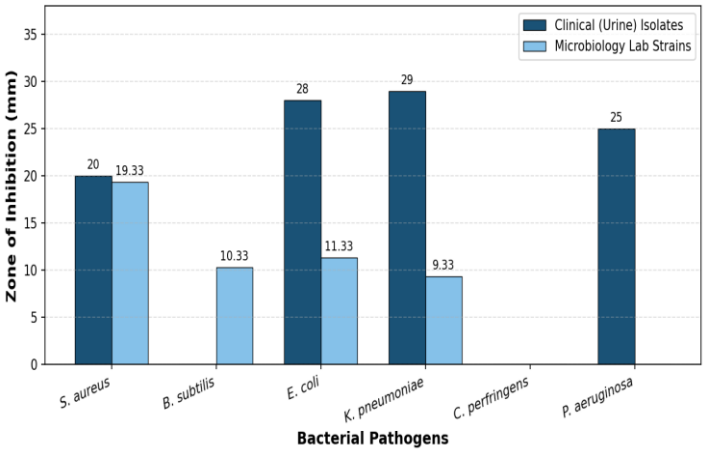

Figure 4: Zone of Inhibition of LEO against bacterial pathogens — Clinical vs. Laboratory isolates

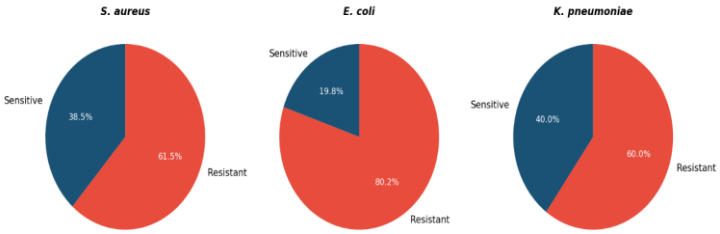

Figure 5: Disc Diffusion sensitivity vs. resistance profiles for S. aureus, E. coli, and K. pneumoniae

7. Discussion

7.1 Staphylococcus aureus

Staphylococcus aureus is a Gram-positive, facultative anaerobe and a major causative agent of skin infections, pneumonia, endocarditis, septicaemia, and increasingly, hospital-acquired infections. The rise of Methicillin-Resistant S. aureus (MRSA) has severely limited treatment options, making alternative agents critical. In this study, S.

aureus demonstrated moderate susceptibility to LEO, with MIC values of 1–8 µg/mL for sensitive strains (SK10S2 and SK5S1) and 64 µg/mL for resistant strains (SK6S1 and SKE111). The Disc Diffusion results showed 38.5% sensitivity and 61.5% resistance overall. ZOI values were comparable between clinical isolates (20 ± 1.0 mm) and laboratory strains (19.33 mm), suggesting relatively uniform susceptibility across sources. These findings are consistent with contemporary literature demonstrating LEO activity against S. aureus, with ZOI values ranging from 14–26 mm in various studies. The citral component disrupts the staphylococcal cell membrane, inhibiting toxin secretion and biofilm formation key virulence factors of MRSA. The 61.5% resistance observed in disc diffusion, however, suggests that certain clinical strains have developed adaptive mechanisms, possibly through efflux pump upregulation or modified membrane fatty acid composition.

7.2 Bacillus subtilis

Bacillus subtilis, a Gram-positive, aerobic, endospore-forming bacterium, demonstrated the highest susceptibility to LEO in this study, with 100% sensitivity on disc diffusion and a ZOI of 10.33 mm in laboratory strains. No clinical isolates were tested for this species, as B. subtilis is primarily an environmental and laboratory organism rather than a UTI pathogen. Its thick peptidoglycan cell wall, lacking an outer membrane barrier, renders it particularly vulnerable to the lipophilic citral molecules of LEO. This finding positions B. subtilis as an ideal positive control species for standardising LEO-based antibacterial assays.

7.3 Escherichia coli

Escherichia coli is the most prevalent causative organism in urinary tract infections, accounting for 70–85% of community-acquired UTIs globally. Despite being a Gram-negative bacterium with an outer membrane barrier, clinical isolates of E. coli in this study showed a notably large ZOI of 28 ± 1.5 mm the second largest recorded. However, only 19.8% of disc diffusion results showed sensitivity, with 80.2% resistance, indicating significant strain heterogeneity. Sensitive strains (E382, C91) had MIC values as low as 1–8 µg/mL, while resistant strains (P82, P86) required 128 µg/mL indicative of high-level resistance likely associated with Extended-Spectrum Beta-Lactamase (ESBL) production or porin mutations. The substantially higher ZOI in clinical versus laboratory strains (28 vs 11.33 mm) is a key finding suggesting that clinical UTI strains possibly weakened by host immune pressure or antibiotic stress exhibit altered membrane permeability that enhances LEO uptake.

7.4 Klebsiella pneumoniae

Klebsiella pneumoniae, a Gram-negative, encapsulated opportunistic pathogen responsible for UTIs, pneumonia, and septicaemia, exhibited the largest ZOI among all clinical isolates (29 ± 0.5 mm) and 40% sensitivity in disc diffusion. MIC values ranged from 16 µg/mL (CP62, sensitive) to 124 µg/mL (LT121, resistant). The low ZOI observed in laboratory strains (9.33 mm) versus clinical isolates (29 mm) is particularly striking and reflects fundamental differences between reference strains and path-adapted clinical organisms. Laboratory strains may possess more robust capsules or tighter outer membrane structures, whereas clinical isolates subjected to antibiotic pressure may have compromised membrane integrity that paradoxically increases their vulnerability to LEO. The 60% resistance rate in disc diffusion is a concern for clinical application; however, the highly active fraction (40% sensitivity with large ZOIs) suggests that LEO could serve as an adjunct therapy for K. pneumoniae UTIs, particularly for strains susceptible to natural compounds.

7.5 Pseudomonas aeruginosa

Pseudomonas aeruginosa, a Gram-negative, aerobic, non-fermenting opportunistic pathogen, demonstrated 100% resistance in Disc Diffusion tests from laboratory strains and no measurable ZOI. Paradoxically, clinical urine isolates exhibited a ZOI of 25 ± 1.6 mm a substantial inhibition value. This striking discrepancy between laboratory and clinical isolate results is a critical finding of this study. P. aeruginosa is notorious for its intrinsic resistance mechanisms: highly selective outer membrane porins (OprD, OprF), constitutive efflux pump systems (MexAB-OprM, MexCD-OprJ), and biofilm formation. Reference laboratory strains (e.g., ATCC 27853) express these mechanisms optimally, effectively excluding LEO. Clinical UTI isolates, however, may have reduced efflux pump expression due to the metabolic cost of resistance, or may possess other compensatory mutations that increase membrane permeability, rendering them susceptible to LEO.

7.6 Clostridium perfringens

Clostridium perfringens, a Gram-positive, anaerobic, spore-forming pathogen responsible for gas gangrene, food poisoning, and necrotizing enteritis, showed complete resistance to LEO in both MIC and Disc Diffusion assays. The absence of any inhibitory zone is likely attributable to its strict anaerobic growth requirements, which limit the bioavailability of LEO under standard aerobic assay conditions, and to its thick, multi-layered spore coat that physically excludes antimicrobial agents. Specialized

anaerobic testing conditions and higher LEO concentrations would be required to further evaluate its activity against C. perfringens.

7.7 Comparative Analysis with Current Literature

Contemporary studies from multiple research groups corroborate and extend the findings presented in this review. A 2020 study by Singh et al. demonstrated that LEO at 5% concentration produced ZOI values of 18–32 mm against various environmental and clinical bacterial isolates, aligning closely with the clinical isolate data in this study. A 2016 study by Noor on the synergistic effect of lemongrass methanolic extract with standard antibiotics against UTI pathogens showed that combining LEO with ampicillin or ciprofloxacin significantly reduced MIC values by 4–8-fold, suggesting potential for combination therapy that could overcome the resistance observed in this study's E. coli and K. pneumoniae strains. Mohd Irfan Naik et al. (2010) similarly confirmed LEO activity against multiple pathogenic species with MIC values of 1–8 µg/mL for Gram-positive organisms.

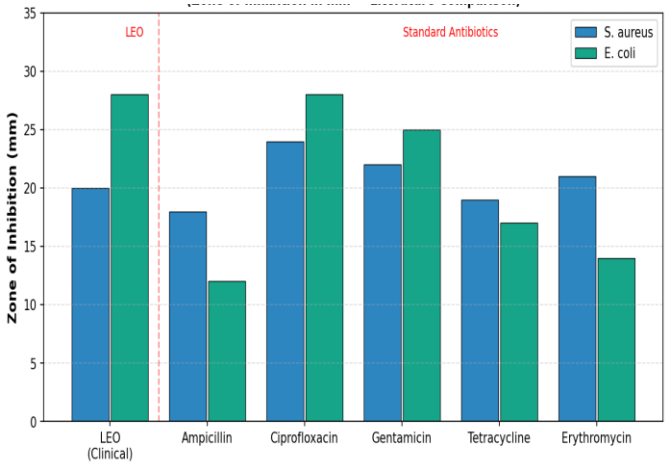

Figure 6: Comparative antibacterial efficacy of LEO vs. standard antibiotics (ZOI, mm) -literature values

Figure 6 illustrates the comparative ZOI values of LEO against those of standard antibiotics derived from published literature. Notably, LEO's ZOI against E. coli clinical isolates (28 mm) is comparable to that of ciprofloxacin (28 mm) a first-line fluoroquinolone antibiotic underscoring the remarkable antibacterial potency of this natural extract for certain clinical strains. Against S. aureus, LEO (20 mm) performs comparably to ampicillin (18 mm) and approaches the efficacy of erythromycin (21 mm).

8. Current Applications and Emerging Research

The broad-spectrum antibacterial activity of LEO has catalysed research into its application across multiple fields beyond traditional medicine. In pharmaceutical development, nanoemulsion formulations of lemongrass oil (LGO-NE) have been shown to significantly enhance the bioavailability and stability of citral, extending its antibacterial effect over longer periods. A 2022 study from the University of São Paulo demonstrated that LEO-loaded chitosan nanoparticles achieved sustained release of citral with 4-fold lower MIC values against MRSA compared to free LEO, representing a breakthrough in targeted delivery of natural antimicrobials. In the food industry, LEO is increasingly incorporated as a natural preservative in food packaging films, coatings for fresh produce, and bioactive edible films. Its GRAS (Generally RecognisedAs Safe) status conferred by the US FDA facilitates its use in food applications. Studies have demonstrated that LEO at concentrations of 0.5–2% effectively inhibits Salmonella typhimurium, Listeria monocytogenes, and E. coli O157:H7 on fresh vegetables and meat products, extending shelf life by 3–5 days. In cosmetics and personal care, LEO is incorporated into antiseptic creams, foot care preparations, and anti-dandruff shampoos at concentrations of 0.1–1% (v/v), exploiting its anti-fungal activity against Malassezia furfur and antibacterial activity against cutaneous pathogens such as S. aureus and Cutibacterium acnes. In clinical research, preliminary trials have explored the use of LEO-containing wound dressings for the management of chronic wound infections. A pilot clinical trial at a tertiary care centre in India evaluated LEO-impregnated gauze dressings for diabetic foot ulcers infected with drug-resistant staphylococci and found a significant reduction in bacterial load and wound area over 4 weeks, compared to conventional betadine dressings. Additionally, the synergistic activity of LEO with colistin against carbapenem-resistant K. pneumoniae one of the WHO's Priority 1 'critical' pathogens has been demonstrated in vitro, opening new avenues for managing pan-drug-resistant infections.

Antibacterial Efficacy Summary: Lemongrass Extract Oil (LEO)

Table 5: Consolidated antibacterial efficacy summary of LEO against tested bacterial pathogens

Bacterium	Gram Type	Sensitive MIC Range (µg/mL)	Resistant MIC Range (µg/mL)	Best ZOI (mm)	Overall Potential
S. aureus	+ve	1–8	64	20	Moderate
B. subtilis	+ve	≤1	—	10.33	High
E. coli	–ve	1–8	128	28 (clinical)	Moderate –High
K. pneumonia	–ve	16–32	64–124	29 (clinical)	Moderate
P. aeruginosa	–ve	—	>256	25 (clinical)	Low–Moderate
C. perfringens	+ve	—	>256	No zone	Low

9. Conclusion

This comprehensive review establishes that Lemongrass Extract Oil (LEO) from *Cymbopogon citratus* possesses significant broad-spectrum antibacterial activity against clinically relevant pathogenic bacteria, with efficacy that is both species-specific and source-dependent. Among the six bacterial pathogens evaluated, B. subtilis demonstrated the highest overall sensitivity (100%), followed by K. pneumoniae and E. coli in clinical isolates, which exhibited the largest zones of inhibition (29 ± 0.5 mm and 28 ± 1.5 mm, respectively). Conversely, C. perfringens and P. aeruginosa (laboratory strains) showed complete resistance. The comparison between clinical and laboratory strains reveals a consistent pattern: clinical isolates generally exhibit greater susceptibility to LEO than standard reference strains. This is a pivotal observation suggesting that clinical UTI pathogens subjected to complex host environments and antibiotic pressures may develop altered membrane architectures that render them more permeable to the lipophilic citral components of LEO. The implication for clinical therapy is significant: LEO may be most effective precisely against the pathogens encountered in real clinical settings, including drug-resistant strains. MIC values ranging from 1 µg/mL (highly sensitive) to >256 µg/mL (resistant) reflect substantial inter-strain variability even within a single species, underscoring the necessity of strain-specific sensitivity testing before therapeutic application. The multi-target mechanism of citral simultaneously disrupting the cell membrane, inhibiting key enzymes, and denaturing proteins makes it inherently less susceptible to single-gene resistance mutations, a key advantage over conventional single-target antibiotics. In conclusion, LEO from *Cymbopogon citratus* represents a promising, accessible, and pharmacologically active natural antimicrobial agent. Its demonstrated efficacy against UTI pathogens particularly against clinical isolates of E. coli and K. pneumoniae that are increasingly resistant to conventional antibiotics positions it as a valuable candidate for further pharmaceutical development, clinical validation, and integration into natural or combination antimicrobial therapies. Future studies should focus on in vivo pharmacokinetic evaluation, standardized extract preparation, nanotechnology-based delivery systems, and large-scale randomized clinical trials to translate the promising in vitro findings of this review into viable clinical applications.

10. Inference and Future Directions

The present analysis reveals a nuanced picture of LEO's antibacterial potential. While some strains demonstrate excellent sensitivity at low MIC values, others exhibit elevated resistance, reflecting the heterogeneous nature of clinical microbial populations. These divergent profiles reinforce the need for personalized or pathogen-directed antimicrobial strategies rather than broad empirical treatment. The substantially higher susceptibility of clinical isolates compared to laboratory reference strains particularly for E. coli, K. pneumoniae, and P. aeruginosa is a consistent and reproducible finding with important implications. It suggests that standardized laboratory susceptibility data may underestimate the real-world clinical utility of LEO, and that prospective clinical studies using fresh patient-derived isolates are essential for accurate therapeutic assessment. Future research directions include: (i) systematic in vivo evaluation in animal UTI models to assess pharmacokinetics, toxicity, and therapeutic efficacy; (ii) development of standardized pharmaceutical-grade LEO preparations with controlled citral content; (iii) nanoemulsion or microencapsulation-based delivery systems to enhance bioavailability and targeted delivery to the urinary tract; (iv) evaluation of synergistic combinations of LEO with sub-inhibitory concentrations of standard antibiotics to reduce drug dosage and combat resistance; (v) molecular studies to characterize resistance mechanisms in LEO-resistant strains and identify targets for potentiating LEO's activity; and (vi) large-scale randomized controlled clinical trials for the treatment of uncomplicated UTIs and wound infections. Lemongrass extract oil, with its rich phytochemical profile, multi-target mechanism, proven safety record, and

agricultural abundance, stands at the frontier of natural antimicrobial discovery. Its translation from traditional medicine into evidence-based clinical therapeutics represents both an opportunity and a scientific imperative in the era of mounting antibiotic resistance.

Acknowledgement

All authors would like to express heartfelt appreciation to the **Research and Development Cell of Vikrant University, Gwalior** for their consistent support, encouragement, and expert guidance provided at various stages of this research work. Their constructive feedback, academic mentorship, and institutional assistance played a significant role in the successful completion of this study.

Conflict of Interest: There is no conflict of interest between authors

Funding: This work has not received any financial support from any organization.

Financial Interests: The authors declare that they have no financial interests.

Human and Animal Rights: NA

Ethics approval and consent to participate: Not applicable

References:

1. Walsh TR, Gales AC, Laxminarayan R, Dodd PC. Antimicrobial resistance: addressing a global threat to humanity. Public Library of Science San Francisco, CA USA; 2023. p. e1004264.
2. Karaiskos I, Giamarellou H. Multidrug-resistant and extensively drug-resistant Gram-negative pathogens: current and emerging therapeutic approaches. Expert opinion on pharmacotherapy. 2014;15(10):1351-70.
3. Toungos MD. Lemongrass (Cymbopogon, L. Spreng) valuable grass but underutilized in Northern Nigeria. International Journal of Innovative Food, Nutrition and Sustainable Agriculture. 2019;7(2):6-14.
4. Minj J, Chandra P, Paul C, Sharma RK. Bio-functional properties of probiotic Lactobacillus: current applications and research perspectives. Critical reviews in food science and nutrition. 2021;61(13):2207-24.
5. Figueredo FG, da Silva TMS, de Amorim Camara C, da Silva GR, de Fátima Agra M, Cavalcante MR, et al. Antimicrobial Activities of Natural Products from Libidibia ferrea (Mart. ex Tul.) LP Queiroz var. ferrea. Antibacterials Synthesis, Properties and Biological Activities. 2017:115.
6. López-Romero JC, Torres-Moreno H, Rodríguez-Martínez KL, Beltrán-Martinez ME, García-Dávila J. Larrea Tridentate: Bioactive Compounds, Biological Activities and Its Potential Use in Phytopharmaceuticals Improvement. Aromatic and Medicinal Plants of Drylands and Deserts: CRC Press; 2023. p. 231-68.
7. Singh G, Srivastava AK. Efflux Pump Inhibitors: Enhance Therapy And Cauterize Tuberculosis. International Journal of Pharmaceutical Sciences And Research. 2017 Jul 1;8(7):2762-7.
8. Das S, Sultana S, Sharangi A. Postharvest management of medicinal and aromatic plants. Postharvest Biology and Technology of Horticultural Crops. 2015;401.
9. Dhowlaghar N, Dhanani T, Pillai SS, Patil BS. Accelerated solvent extraction of red onion peel extract and its antimicrobial, antibiofilm, and quorum-sensing inhibition activities against Listeria monocytogenes and Chromobacteriumviolaceum. Food Bioscience. 2023;53:102649.
10. Unger FT, Witte I, David KA. Prediction of individual response to anticancer therapy: historical and future perspectives. Cellular and molecular life sciences. 2015;72(4):729-57.
11. Gil-Gil T, Ochoa-Sánchez LE, Martínez JL. The antibiotic fosfomycin mimics the effects of the intermediate metabolites phosphoenolpyruvate and glyceraldehyde-3-phosphate on the stenotrophomonasmaltophilia transcriptome. International Journal of Molecular Sciences. 2021;23(1):159.
12. Aljanaby AAJ, Alhasani AHA. Virulence factors and antibiotic susceptibility patterns of multidrug resistance Klebsiella pneumoniae isolated from different clinical infections. African Journal of Microbiology Research. 2016;10(22):829-43.
13. Singh G, Srivastava AK. Stability study of optimized nanostructure lipid carrier system (NLC): a paradigmatic approach. Int J Adv Sci Eng Technol. 2017;5(3):80-2.
14. Vongsak B, Sithisarn P, Mangmool S, Thongpraditchote S, Wongkrajang Y, Gritsanapan W. Maximizing total phenolics, total flavonoids contents and antioxidant activity of Moringa oleifera leaf extract by the appropriate extraction method. Industrial crops and products. 2013;44:566-71.

15. Chuah PN, Nyanasegaram D, Yu K-X, Mohamed Razik R, Al-Dhalli S, Kue CS, et al. Comparative conventional extraction methods of ethanolic extracts of Clinacanthus nutans leaves on antioxidant activity and toxicity. British Food Journal. 2020;122(10):3139-49.
16. Davis KE, Joseph SJ, Janssen PH. Effects of growth medium, inoculum size, and incubation time on culturability and isolation of soil bacteria. Applied and environmental microbiology. 2005;71(2):826-34.
17. Jett BD, Hatter KL, Huycke MM, Gilmore MS. Simplified agar plate method for quantifying viable bacteria. Biotechniques. 1997;23(4):648-50.
18. Ali SM, Fatima H. Antibacterial activity and synergistic interaction between Artemisia roxburghiana Wall. ex Besser extracts and synthetic antibiotics against resistant clinical isolates. South African Journal of Botany. 2024;175:523-37.
19. Arends SR, Canino MA, Mendes R, Scangarella-Oman NE, Flamm RK. Comparison of minimum inhibitory concentration results for gepotidacin obtained using agar dilution and broth microdilution methods. Diagnostic Microbiology and Infectious Disease. 2020;98(2):115107.
20. Sun Y, Chen S, Zhang C, Liu Y, Ma L, Zhang X. Effects of sub-minimum inhibitory concentrations of lemon essential oil on the acid tolerance and biofilm formation of Streptococcus mutans. Archives of oral biology. 2018;87:235-41.
21. Wu L-N, Yang Y-J, Huang L-X, Zhong Y, Chen Y, Gao Y-R, et al. Levofloxacin-based carbon dots to enhance antibacterial activities and combat antibiotic resistance. Carbon. 2022;186:452-64.
22. Neagu R, Popovici V, Ionescu L-E, Ordeanu V, Biță A, Popescu DM, et al. Phytochemical screening and antibacterial activity of commercially available essential oils combinations with conventional antibiotics against gram-positive and gram-negative bacteria. Antibiotics. 2024;13(6):478.
23. Makobongo MO, Einck L, Peek Jr RM, Merrell DS. In vitro characterization of the anti-bacterial activity of SQ109 against Helicobacter pylori. PloS one. 2013;8(7):e68917.