

In-vitro Antimicrobial Activity of *Syzygium cumini* L. and *Moringa oleifera* Against Waterborne Bacteria for Safe Water Use

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ABSTRACT

Background: Waterborne pathogens pose a substantial global threat to public health, particularly in regions with limited access to safe drinking water. Plant-based antimicrobials offer a promising alternative to conventional chemical disinfectants, which may pose safety concerns.

Objective: This scientific study investigates the in-vitro antibacterial efficacy of hydroalcoholic extracts from *Syzygium cumini* L. and *Moringa oleifera* plant leaves against key waterborne pathogens: *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella* spp., and *Vibrio cholerae*.

Methods: Antibacterial activity was assessed using the agar well diffusion method to measure zones of inhibition, as well as minimum inhibitory concentration (MIC) assays.

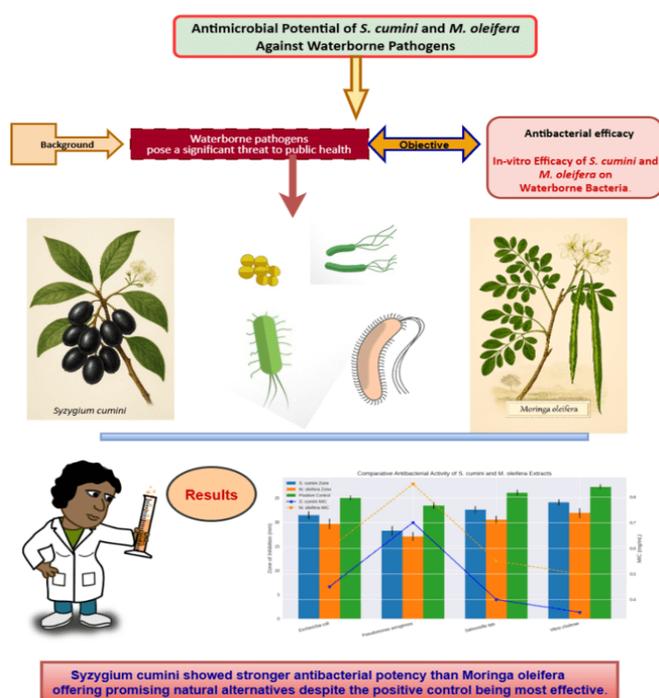
Results: Both plant extracts of *S. cumini* and *M. oleifera* demonstrated notable antibacterial activity. *S. cumini* exhibited superior inhibition zones (24.1 ± 0.6 mm against *V. cholerae*) and lower MIC values (0.35 mg/mL), indicating stronger potency compared to *M. oleifera*. The positive control (Ampicillin) presented the uppermost inhibition across all strains, but the plant extracts offered promising natural alternatives.

Conclusion: Hydroalcoholic extracts of *S. cumini* and *M. oleifera* possess significant antibacterial potential against waterborne pathogens. Their application could enhance natural water safety, especially in resource-limited conditions. Further studies are warranted to explore formulation and field-level efficacy.

Keywords: *Syzygium cumini*, *Moringa oleifera*, Water-borne bacteria, Antibacterial activity, In-vitro antimicrobial assay.

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Graphical Abstract

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INTRODUCTION

Urgency of Safe drinking water

Access to microbiologically safe drinking water has remained a persistent global health challenge, especially in developing regions. Researchers are actively exploring safe alternatives, particularly in regions vulnerable to bacterial contamination, such as South Asia and parts of Africa (Emran, Barma, Khan, & Roy, 2024).

According to the World Health Organization (WHO), contaminated drinking water contributes to over half a million diarrheal deaths annually. Young children under the age of five are affected very badly due to the scarcity of drinking water in

developing countries. The problem is getting worse because of climate change, a growing population, and poor sanitation systems. The problem of scarcity of safe drinking water puts pressure on clean water sources and permits harmful germs to spread in the water we drink (Machona, Morara, Morara Ogendi, & Ahana, 2025). Water-borne pathogens and Bacteria such as *E. coli*, *P. aeruginosa*, *S. spp.*, and *V. cholerae* are key contributors to gastrointestinal and intestinal tract disease outbreaks. These pathogens are a major health problem both for human health and the sanitation in the home.

In current environmental conditions, finding safe and alternative ways to clean drinking water is vital. Use of chemical disinfectants can be costly or harmful to the environment. Scientists are presently looking at natural solutions. A few traditional Indian plants like *S. cumini* (Jamun) and *M. oleifera* (Sahjan) have been used for centuries in home remedies. *S. cumini* and *M. oleifera* are known to fight microorganisms and may have the potential to kill harmful bacteria found in dirty water. Using these plants is very simple, eco-friendly, and economical to make water safer, particularly in rural areas where an advanced water purifier is not available.

Ethnomedicinal Significance of *S. cumini* and *M. oleifera*

There are various limitations of using chemical disinfectants; therefore, plant-derived antimicrobials have recently gained attention in the scientific field as a sustainable, eco-friendly alternative. To overcome microbiologically unsafe drinking water, we use *S. cumini* (Jamun) and *M. oleifera* (Drumstick tree) as a potential risk-free alternative to this health problem Fig.1.

S. cumini Plant Profile and Antimicrobial Property

Antimicrobial property

S. cumini, commonly known as Jamun or Indian blackberry. This traditional Indian plant is a fast-growing evergreen tree native to Southeast Asia. This plant comes under the Myrtaceae family and is famous for its purplish-black fruits with rich anthocyanins and tannins, phytoconstituents (Jagetiya, 2024). Traditionally, various parts of these plants, such as seeds, bark, leaves, and pulp used for antidiabetic, antioxidant, and antimicrobial properties. *S. cumini* is cultivated widely in tropical regions for both nutritional and medicinal applications, particularly in handling intestinal disorders, diabetes, and microbial infections (Adithya, Nayeem, Sagar, & Kumar, 2025).

Copper nanoparticles (CuNPs) were synthesized using *S. cumini* leaf extract and applied to cotton fabric for antimicrobial and UV protection. The coated fabric showed strong resistance against bacteria and fungi, while maintaining its physical strength. Additionally, the CuNPs enhanced the fabric's color absorption, making it both protective and visually improved (Boruah, Phukan, Kalita, Gangwar, & Jose, 2025).

This comparative study assessed the antibacterial activity of *S. cumini* seed extract and *Carica papaya* leaf extract against frequent and prevalent germs. Using agar diffusion approaches, both extracts showed antimicrobial effects, but *S. cumini* demonstrated stronger inhibition zones. The results support its potential as a natural antibacterial agent, highlighting its relevance in advancing plant-based options to traditional antibiotics (Bhatia & Upadhyay, 2025).

This study assessed the antibacterial effectiveness of *S. cumini* fruit extract in herbal mouthwash formulations against *Streptococcus mutans*, a fundamental contributor to dental caries. Among the four tested formulas, Formula 4 showed the strongest antibacterial activity and was clinically tested. Results revealed a notable reduction in bacterial colonies and good formulation stability, suggesting *S. cumini*'s potential for oral medical care applications (Chismirina *et al.*, 2025).

This research highlights the use of *S. cumini* seed-derived lignocellulosic biomass as a sustainable filler in PVC composites. The resulting films showed improved biodegradability, antimicrobial activity against *Staphylococcus aureus*, and enhanced hydrophobicity, making them suitable for eco-friendly medical applications. Structural and morphological analyses confirmed strong filler-polymer interactions and stable composite integrity (Arun, Muthukrishnan, Thiagamani, Khan, & Alzahrani, 2025).

This experimental study assessed the ethanol extract of *S. cumini* (jamblang) stem bark for its secondary metabolites and antimicrobial activity. GC-MS identified essential compounds like 7-Tetradecenal (Z), Octadecanoic acid, and n-Hexadecanoic acid. The extract showed dose-dependent inhibition against *C. acnes*, *S. aureus*, and *C. albicans*, with balanced antimicrobial activity compared to controls, indicating its potential as a natural antimicrobial agent (Meutia, Ismail, & Fitri, 2025).

This study used *in silico* approaches to evaluate 83 *S. cumini* phytochemicals against antimicrobial-resistant *Staphylococcus aureus*. Docking and molecular dynamics simulations identified Beta-Glucogallin (BEG) and Dihydro Dehydro Coniferyl alcohol (DIH) as promising inhibitors of AgrC, forming stable hydrogen bonds with fundamental amino acids. These compounds showed robust binding affinities, supporting their potential as natural anti-AMR agents pending experimental validation (Bhavyashree, Vaishnavi, Shravani, & Sabat, 2025).

This study used molecular docking and dynamics simulations to evaluate *S. cumini* phytochemicals against AMR *Staphylococcus aureus*, targeting penicillin-binding protein 2a (PBP2a). Beta-Glucogallin (BEG) and Dihydro Dehydro Coniferyl alcohol (DIH) showed stable binding, robust hydrogen bonding, and beneficial



Fig 1: *S. cumini* plant

drug-likeness profiles. Compared to FDA-approved drugs, these compounds demonstrated promising antibacterial and anti-inflammatory potential, warranting further experimental validation (Pillanjinayya, Nagaraja, Subbaraya, & Sabat, 2025).

Pandey *et al.*, 2025 evaluated the bioactive potential of *S. cumini* (Jamun) seed kernel extracts, highlighting the methanolic extract as the richest in flavonoids, polyphenols, and carbohydrates. It showed strong antioxidant activity, effective xanthine oxidase inhibition, and potent antibacterial effects, especially against *S. epidermidis* with MIC and MBC values as low as 0.32 and 0.52 mg/mL, respectively. These findings suggest promising therapeutic applications of *S. cumini* seed extract in combating microbial infections and oxidative stress (Pandey *et al.*, 2025).

Kotakadi, *et al.*, 2024 examined the use of *S. cumini* seed extracts, especially the methanolic extract rich in quercetin, for the green synthesis of magnesium oxide nanoparticles (MgONPs). The biologically synthesized MgONPs demonstrated strong antioxidant activity ($IC_{50} = 22.46 \mu\text{g/mL}$) and effective antimicrobial action against several bacterial strains, notably *S. typhimurium*. Comprehensive characterization confirmed the role of bioactive compounds in shaping the structure and function of these nanomaterials (Kotakadi, *et al.*, 2024).

Cebrian *et al.*, 2024 developed a film-forming solution (FFS) infused with *S. cumini* extract to explore its potential in postoperative care. The extract showed strong antioxidant and antimicrobial activity, while the FFS demonstrated good skin adhesion, stable pH, and thermal stability. These results suggest that *S. cumini*-based FFS could serve as an effective and eco-friendly therapeutic aid for post-surgical wound management (Cebrian *et al.*, 2024). This study reports the green synthesis of EDAS-(CuO-Ag) nanocomposite utilizing *Syzygium cumini* seed extract as both a decreasing and stabilizing agent. Characterization via UV-Vis, FT-IR, XRD, HRTEM, BET, and XPS validated its structure. The nanocomposite exhibited improved photocatalytic deterioration of methylene blue and superior antibacterial activity, with effectiveness ranking: CuO < CuO-Ag < EDAS-(CuO-Ag) (Kathija, Kavitha, Eswaran, & Badhusha, 2024).

This study utilized *S. cumini* leaf extract to green-synthesize gold nanoparticles (ScAu-NPs) as a potential treatment for multidrug-resistant urinary tract pathogens. The ScAu-NPs were spherical, rich in phenolic and aromatic compounds, and showed strong antibacterial activity against resistant strains like *E. coli*, *K. pneumoniae*, and *S. aureus*. These findings support the use of ScAu-NPs in future drug development to combat antibiotic-resistant infections (Diksha, Gupta, Gupta, Banerjee, & Kalita, 2023).

This study investigated the antioxidant, antimicrobial, and phytochemical properties of *Syzygium cumini* leaf extracts using n-hexane, ethyl acetate, and methanol as solvents. The n-hexane extract showed the strongest antioxidant activity (IC_{50} : 23.79%) and contained flavonoids, terpenoids, and steroids, while all three extracts demonstrated antimicrobial effects against *E. coli*, *S. aureus*, and *C. albicans*. These findings highlight the therapeutic potential of *S. cumini* leaves as a natural source of bioactive compounds (Junairiah, Fatimah, Nurhariyati, & Zuraidassanaaz, 2023).

This study examined the antimicrobial potential of methanol, ethanol, and aqueous extracts from the bark of *Syzygium cumini*,

revealing the presence of diverse phytochemicals like flavonoids, alkaloids, and terpenoids. Methanolic and ethanolic extracts showed strong inhibitory effects against both gram-negative and gram-positive bacteria, while aqueous extracts were less effective. Notably, the methanolic seed extract exhibited superior antimicrobial activity, supporting the eco-friendly use of *S. cumini* in managing bacterial infections (Junairiah *et al.*, 2023).

This study analyzed the essential oil from *S. cumini* fruit using GC and GC-MS, identifying 46 components, with major ones including 1,2,3-Propanetriol and octadecanoic acid. Bioactivity prediction revealed strong antibacterial potential, suggesting its usefulness against meningitis-causing bacteria. Further experimental validation is needed to confirm the therapeutic efficacy of these compounds (Junairiah *et al.*, 2023).

This study analyzed the necessary and fundamental oil of *S. cumini* leaves, identifying α -pinene (53.21%) as the important and significant compound via GC-MS. The oil showed balanced antibacterial activity against *E. coli* ATCC 25922 (MIC: 512 $\mu\text{g/mL}$) and improved antibiotic effectiveness against multidrug-resistant *E. coli* 06 and *S. aureus* 10 when combined with gentamicin, erythromycin, and norfloxacin (Fernandes *et al.*, 2022).

This study highlighted the AgNPs exhibited outstanding *in-vitro* antioxidant, antibacterial, and anti-inflammatory activities, suggesting their potential as eco-friendly therapeutic agents for infections, inflammation, and oxidative stress-related diseases.

This study assessed the antimicrobial activity of lyophilized hydroalcoholic extracts and silver nanoparticles (AgNPs) derived from *S. cumini* seed and flower. GC-MS identified 7 and 17 phytochemicals in HEScSeed and HEScFlower, respectively. AgNPs showed variable morphology and improved antimicrobial effectiveness against oral and medical germs at reduced MICs (31.2–2,000 $\mu\text{g/mL}$) compared to crude extracts (648.4–5,187.5 $\mu\text{g/mL}$), indicating potent bacteriostatic and fungistatic potential (de Carvalho Bernardo *et al.*, 2021).

This study assessed the antimicrobial, antibiofilm, and cytotoxic effects of lyophilized ethanolic extract (HEScL) and silver nanoparticles (AgNPs-HEScL) from *S. cumini* leaves. Characterization validated nanoparticle formation and stability. AgNPs-HEScL showed stronger antimicrobial and antibiofilm activity at reduced MICs (31.2–250 $\mu\text{g/mL}$) compared to HEScL (1,296.8–10,375 $\mu\text{g/mL}$), with minimal cytotoxicity in NOK-SI cells, supporting their potential for dental and medical applications (Bernardo *et al.*, 2022).

This review takes a close look at *S. cumini* and how it fits into dentistry. Different parts of the plant show real antibacterial and antifungal effects against germs that cause oral problems, which back up why people have used it for dental issues for so long. The results point to a clear therapeutic potential for both preventing and managing oral diseases, and honestly, it's a good reminder that medicinal plants still have a place in modern dental care (Junior *et al.*, 2021).

***M. oleifera* Plant Profile and Antimicrobial Property**

M. oleifera, commonly known as the drumstick tree, is a fast-growing, drought-tolerant plant widely cultivated in tropical

climates *M. oleifera* is widely cultivated across hot and humid climates. It belongs to the family Moringaceae, valued as a super vegetable. All parts of the plant, such as leaves, seeds, pods, and roots, are rich in vitamins, minerals, and flavonoid compounds. *M. oleifera* displays antioxidant, antimicrobial, anti-inflammatory, and hepatoprotective activities Fig. 2 (Abdull Razis, Ibrahim, & Kntayya, 2014).

Antimicrobial Property

This study developed a novel green-synthesized nanocomposite (Mo-Ag-Kao) by immobilizing *M. oleifera*-derived silver nanoparticles onto kaolinite clay. The composite retained kaolinite's structure, showed high thermal stability (up to 700 °C), and exhibited potent antibacterial activity against both *Staphylococcus aureus* and *Escherichia coli*. Using kaolinite as a carrier improved nanoparticle stability and enhanced antimicrobial efficacy, offering a promising route for eco-friendly antibacterial (Abdull Razis, Ibrahim, & Kntayya, 2014).

This study examined the antibacterial potential of *M. oleifera* leaf and seed extracts against drug-resistant strains of *S. aureus*, *E. coli*, and *P. aeruginosa*. Among eight solvent-based extracts, aqueous seed extract showed the strongest activity, with inhibition zones up to 32.5 mm and the minimum MIC values (50–200 mg/mL). Findings suggest *M. oleifera* as a hopeful and encouraging origin of natural antibiotic-resistance inhibitors (Apenteng-Takyiako *et al.*, 2025).

This study highlights the dual role of *M. oleifera* leaf aqueous extract (MLAE) as an antimicrobial agent and a biostimulant under salt stress. MLAE inhibited different bacterial and fungal germs and substantially enhanced tomato seedlings under salinity. It improved antioxidant activity, reduced oxidative harm, and increased protein and proline levels, affirming its eco-friendly potential for integrated crop management and plant protection (Abdelhameed, Galilah, & Metwally, 2025).

This study developed a nanoemulsion of *M. oleifera* seed oil (MoSO) employing ultrasonic emulsification and assessed its antimicrobial and preservative effects. Optimized MoSO nanoemulsions (17.84 nm, PDI 0.226) inhibited *Staphylococcus aureus* (MIC: 125 µL/mL) by disrupting membranes and metabolism. Transcriptomic analysis revealed gene regulation linked to ribosomal and metabolic pathways. Treated chicken meat showed reduced spoilage indicators, confirming MoSO's potential as a natural food preservative (Ma *et al.*, 2025).

This study identified three innovative bacterial endophytes, *Serratia marcescens* (KR-27), *Klebsiella aerogenes* (KL-4), and *Lelliottia amnigena* (KS-7), from *M. oleifera* that generate exopolysaccharides (EPS), phytohormones (IAA, GA3, SA), antioxidants, and solubilize phosphate. These strains showed antagonistic activity against phytopathogens, highlighting their potential as eco-friendly biofertilizers and biopesticides for sustainable agriculture (Gul *et al.*, 2025).

This study reports the green synthesis of silver oxide nanoparticles (Ag₂O NPs) utilizing *M. oleifera* leaf extract. Characterization validated spherical and rod-shaped NPs (2–126 nm) with functional groups assisting stability. The Ag₂O NPs showed robust antimicrobial activity, particularly against *E. coli* (14 mm inhibition zone), and demonstrated photocatalytic decline of Congo red (84%) and ciprofloxacin (46%), highlighting their biomedical and environmental potential (Tabassum,

Hossain, Sachchu, Uddin, & Ahmed, 2025).

This study optimized a synergistic mixture of *Moringa oleifera*, cinnamon, and black seed, necessary and fundamental oils, showing potent antibacterial activity against resistant *Staphylococcus aureus*, outperforming tetracycline, with minimal cytotoxicity and robust bioactive profiles validated by GC/MS and statistical modelling (Abu-Hussien *et al.*, 2025).

This study developed biocomposite films utilizing orange peel vital and crucial oil, chitosan, and *M. oleifera* gum. CME-5, containing 2.5 ml oil, showed ideal and perfect performance: homogeneous dispersion, new chemical linkages, amorphous structure, improved UV absorption, thermal stability, low water solubility, and superior tensile strength; highlighting its potential for eco-friendly packaging applications (Thomas, Jesu Rethinam, Srithar, Lydia, & Unnikrishnan, 2025).

This study highlights the anticancer potential of biogenically synthesized silver nanoparticles (MOAgNPs) applying *M. oleifera* bioactives against AGS gastric cancer cells. Characterization verified stable, spherical nanoparticles (30–35 nm, –24.9 mV zeta potential). MOAgNPs induced ROS generation, apoptosis, and downregulated fundamental metabolic and apoptotic proteins, achieving an IC₅₀ of 55.213 µg/mL. These results suggest MOAgNPs as promising agents for directed gastric cancer therapy (Antony & Chanthini, 2025).

This study introduces a green synthesis procedure for zinc oxide nanoparticles (ZnO-NPs) utilizing *M. oleifera* leaf extract, avoiding toxic chemicals and high energy input. Characterization validated crystalline ZnO-NPs with robust antibacterial and antioxidant activity. The approach provides a sustainable, scalable choice for producing multifunctional nanoparticles with biomedical and environmental applications (Sarwar, Nazli, Munir, Aslam, & Khalofah, 2025).



Fig 2: *Moringa oleifera* plant

This review highlights *M. peregrina* as a multifunctional "miracle tree" with potent biological activities: antimicrobial, antiviral, anticancer, antioxidant, and immunomodulatory. Beyond medicinal functions, it serves as a natural cleaning agent, fertilizer, gum origin, and biopesticide. The study highlights its broad applications and promotes further inquiry into its bioactive compounds for therapeutic and agricultural innovations (Majali, Althunibat, & Qaralleh, 2025).

This study highlights the potent anticancer, anti-inflammatory, antioxidant, and antibacterial attributes of *Moringa oleifera* seed extract (MSE). Rich in bioactives like cycloisolongifolene and chamazulene, MSE showed particular cytotoxicity against cancer cells (IC_{50} : 4.85–9.15 $\mu\text{g/mL}$) while sparing normal cells. It induced apoptosis via p53/p21 upregulation and Bcl-2 downregulation, arrested cell cycle, reduced nitric oxide, improved SOD activity, and inhibited germs and biofilms dose-dependently (El-Fakharany, Elsharkawy, El-Maradny, & El-Gendi, 2024).

Hamed *et al.*, 2024 developed edible chitosan (CS) films enriched with purified flavonoids (PF) from *M. oleifera* leaves, showing improved physical, mechanical, and bio-functional properties. Incorporation of 4% PF significantly enhanced water vapor barrier, antioxidant activity, and antimicrobial effects, particularly in preserving packed beef burgers. These findings highlight the potential of CS-MOPF films as sustainable packaging materials to extend food shelf life (Hamed *et al.*, 2024).

This study evaluated the phytochemical, antioxidant, and antibacterial properties of *M. oleifera* leaf nanosuspension (MON) for potential use in peri-implantitis therapy. The MON extract contained key bioactive compounds and showed antibacterial activity against pathogens like *Aa*, *Pg*, *Pi*, and *Fn*, with effective MIC and MBC values at 25% and 12.5% concentrations. Although its antioxidant capacity was lower than vitamin C, the nanosuspension demonstrated promising therapeutic potential for oral infections (Nugraha *et al.*, 2023).

Royani *et al.*, 2023 evaluated the antibacterial activity of *M. oleifera* leaf extracts against *Pseudomonas aeruginosa*, a biofilm-forming bacterium responsible for metal deterioration. The 100% methanol extract showed the highest yield, phenolic (16.26%) and flavonoid (23.32%) content, and demonstrated antibacterial activity with a MIC of 6144 $\mu\text{g/mL}$, while lower methanol concentrations showed no activity. These findings highlight the importance of solvent concentration in extracting bioactive compounds and suggest potential applications of *M. oleifera* in controlling biofilm-related corrosion (Royani *et al.*, 2023).

This study evaluated how oven treatment at 80°C and 100°C affects the bioactive compounds in *M. oleifera* leaf mucilage. Heating at 80°C preserved more phenols, flavonoids, tannins, and sugars, while higher temperatures led to greater nutrient loss and reduced antibacterial activity against probiotic *Lactobacillus* strains. The findings suggest that lower temperature processing retains therapeutic properties and supports probiotic compatibility (Meziani, Aissani, Khemis, Oomah, & Zaidi, 2023).

This study explored the green synthesis of calcium oxide nanoparticles (CaO NPs) using aqueous *M. oleifera* leaf extract as a natural reducing and stabilizing agent. The biosynthesized

CaO NPs were spherical (average size: 32.08 nm), structurally pure, and showed enhanced crystallinity upon heat annealing. Notably, they exhibited promising antimicrobial activity against gram-positive bacteria, highlighting their potential for eco-friendly applications in medicine and materials science (Jadhav *et al.*, 2022).

This systematic review analyzed twelve in-vitro studies on the antibacterial potential of *M. oleifera*, focusing on extraction methods and testing formats. Whole seeds and methanolic leaf extracts showed better antibacterial activity, particularly against *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, than dehusked seeds and aqueous extracts. However, inconsistent methodologies and limited inhibition results highlight the need for standardized liquid media testing and future research on combining *M. oleifera* with antibiotics for enhanced therapeutic outcomes (van den Berg & Kuipers, 2022).

This study evaluated the phytochemical composition and antibacterial activity of aqueous and ethanol leaf extracts of *M. oleifera*. Ethanol extract showed a higher yield (62.87%), richer flavonoid content, and stronger antibacterial efficacy (MIC/MBC: 6.25 mg/mL) compared to aqueous extract. These findings affirm *M. oleifera*'s potential as a natural antimicrobial agent against clinical bacterial pathogens (Enerijiofi, Akapo, & Erhabor, 2021).

Study Objective and Methodology

This scientific study evaluates the *in-vitro* antibacterial activity of hydroalcoholic extracts of *S. cumini* and *M. oleifera* against key

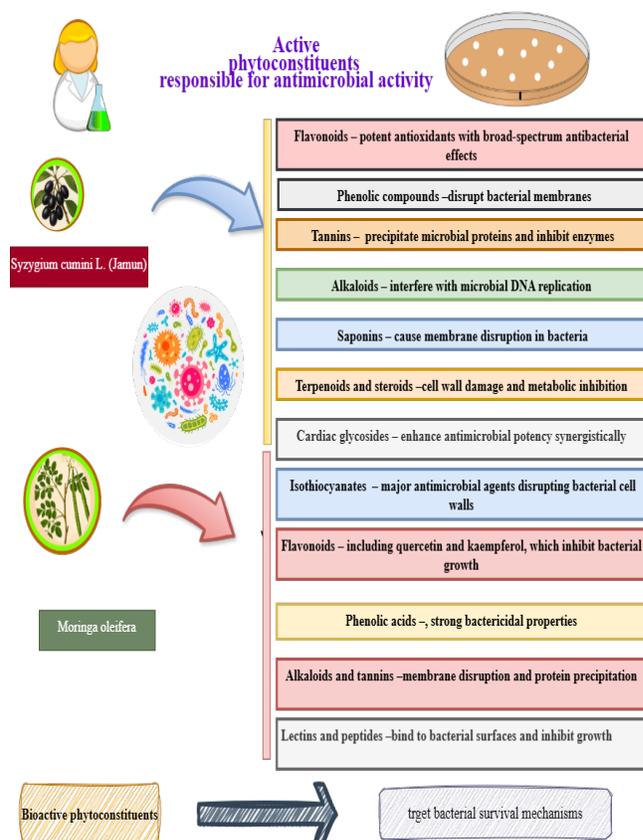


Fig. 3: Active phytoconstituents in *S. cumini* and *M. oleifera* for antimicrobial activity

waterborne pathogens *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella* spp., and *Vibrio cholerae*. Special emphasis is placed on correlating antimicrobial outcomes with the presence of bioactive phytoconstituents such as flavonoids, alkaloids, tannins, and isothiocyanates known to disrupt bacterial survival mechanisms, as illustrated in Fig. 3. Using zone of inhibition and minimum inhibitory concentration (MIC) assays, we investigate and compare the effectiveness and explores the effectiveness of plants in natural water sanitisation. The findings of this antimicrobial study may contribute to the development of phototherapeutic strategies for water disinfection. By carefully examining the data, it is found that *S. cumini* and *M. oleifera* possess significant antibacterial potential against waterborne pathogens.

MATERIALS AND METHODS

Infographic summary of the material method (Fig. 4)

Fresh leaves of *S. cumini* and *M. oleifera* were extracted using 70% ethanol and tested against waterborne bacteria via agar well diffusion and MIC assays. The method targeted key phytoconstituents, flavonoids, alkaloids, and isothiocyanates known for disrupting bacterial membranes and inhibiting growth, as outlined in the experimental workflow and phytochemical diagram.

Plant Material Collection and Preparation

Fresh leaves of *S. cumini* L. and *M. oleifera* Lam. were collected from healthy, pesticide-free plants in the Tiloi region of Uttar Pradesh, India, in Dt 07 September 2025. Further authenticated by Botanist Mr. Adarsh Pandey (Email: Adarshpandey009@gmail.com) from the Department of Botany, Babu Mahipati Singh Mahavidyalay. The leaves were washed thoroughly with distilled water, shade-dried at room temperature, and ground into a fine powder using a mechanical grinder.

Extraction Procedure

Hydroalcoholic extracts of *S. cumini* and *M. oleifera* were prepared using a standardized maceration technique to ensure optimal recovery of bioactive compounds. For each plant, 50 g of finely powdered material was soaked in 500 mL of 70% ethanol solution. The maceration process was carried out over a period of 72 hours at room temperature with intermittent shaking to facilitate solvent penetration and enhance extraction efficiency. After completion of the extraction period, the mixture was first filtered through a clean muslin cloth to remove coarse plant debris. This was followed by fine filtration using Whatman No. 1 filter paper to obtain a clear filtrate. The filtrate was then concentrated under reduced pressure using a rotary evaporator to remove excess solvent without degrading thermolabile compounds. The concentrated extracts were transferred into sterile, airtight containers and stored at 4°C until further use.

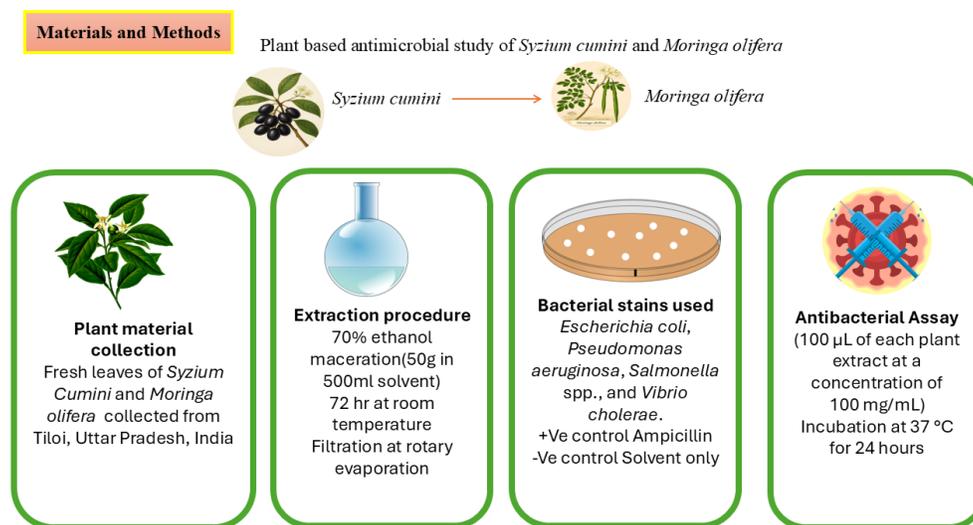


Fig. 4: Infographic diagram of material and methods

Table 1: Antibacterial activity of *S. cumini* and *M. oleifera* extracts against selected waterborne bacteria

Bacterial Strain	<i>S. cumini</i> Zone of Inhibition (mm)	<i>M. oleifera</i> Zone of Inhibition (mm)	Positive Control (mm)	<i>S. cumini</i> MIC (mg/mL)	<i>M. oleifera</i> MIC (mg/mL)
<i>E. coli</i>	21.4 ± 0.8	19.6 ± 1.1	25.0 ± 0.5	0.45	0.60
<i>P. aeruginosa</i>	18.2 ± 1.0	17.0 ± 0.9	23.4 ± 0.7	0.70	0.85
<i>S. spp.</i>	22.6 ± 0.7	20.5 ± 0.8	26.1 ± 0.6	0.40	0.55
<i>V. cholerae</i>	24.1 ± 0.6	21.9 ± 1.0	27.3 ± 0.5	0.35	0.50

in experimental procedures. This method was chosen for its simplicity, cost-effectiveness, and ability to preserve a wide range of phytochemicals, including flavonoids, phenolics, and alkaloids. The hydroalcoholic solvent system (70% ethanol) was selected based on its proven efficacy in extracting both polar and moderately nonpolar compounds. The resulting extracts were later subjected to phytochemical screening, antioxidant assays, and antimicrobial evaluations to determine their therapeutic potential and suitability for biomedical applications (Sasidharan, Chen, Saravanan, Sundram, & Latha, 2011).

Bacterial Strains

- *Escherichia coli*
- *Pseudomonas aeruginosa*
- *Salmonella spp.*
- *Vibrio cholerae*
- Mueller-Hinton agar
- Sterile Petri dishes
- Incubator (37°C)
- Positive control: (Ampicillin)
- Negative control: Solvent only

Antibacterial Assay

The antibacterial activity of hydroalcoholic extracts of *S. cumini* and *M. oleifera* was assessed using the agar well diffusion method. Mueller-Hinton agar plates were uniformly inoculated with standardized bacterial suspensions adjusted to a 0.5 McFarland turbidity standard to ensure consistency in microbial load (Biemer, 1973). Wells of 6 mm diameter were aseptically punched into the agar surface and filled with 100 μ L of each plant extract at a concentration of 100 mg/mL. For comparative analysis, Ampicillin discs were used as the positive control, while the solvent alone served as the negative control to rule out any inherent antimicrobial effect of the vehicle. The inoculated plates were incubated at 37 °C for 24 hours under aerobic conditions. After incubation, the antibacterial efficacy was determined by measuring the diameter of the zone of inhibition around each well in millimetres (mm). The results provided a clear indication of the antimicrobial potential of the extracts against the tested bacterial strains (Shahab & Pribadhi, 2025).

Observation & Measurement

MIC values were determined using the broth dilution method. Serial dilutions of each extract (ranging from 0.1 to 1.0 mg/mL) were prepared in nutrient broth. Each tube was inoculated with 100 μ L of bacterial suspension and incubated at 37°C for 24 hours. The MIC was recorded as having the lowest concentration that showed no visible bacterial growth. Each experiment was conducted three times to ensure reproducibility. The outcomes are presented as mean values accompanied by their respective standard deviations. Statistical evaluation was carried out using GraphPad Prism, applying one-way ANOVA and Tukey's multiple comparison tests, with significance set at $p < 0.05$.

RESULTS AND DISCUSSION

Statistical significance was assessed through a one-way analysis of variance, complemented by Tukey's multiple comparison

procedure, with differences deemed significant at a probability threshold below 0.05.

Zones of inhibition (mean \pm SD, mm) produced by agar well diffusion assay against *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella spp.*, and *Vibrio cholerae*. Extract concentrations were standardized at 100 mg/mL, with ampicillin (10 μ g/mL) as a positive control and solvent as a negative control. Results highlight the superior potency of *S. cumini* extract, particularly against *V. cholerae*, compared to *M. oleifera*. Statistical significance was determined using one-way ANOVA ($p < 0.05$).

MIC values (mg/mL) determined via broth dilution assay for *E. coli*, *P. aeruginosa*, *Salmonella spp.*, and *V. cholerae*. Data are presented as mean \pm SD from triplicate experiments. *S. cumini* exhibited consistently lower MIC values (0.35–0.50 mg/mL) compared to *M. oleifera* (0.55–0.80 mg/mL), confirming stronger antibacterial potency. Ampicillin served as the reference antibiotic control. These findings reinforce the potential of *S. cumini* as a natural water-sanitizing agent.

DISCUSSION

The present study evaluated the antibacterial potential of *S. cumini* and *M. oleifera* hydroalcoholic extracts against selected waterborne pathogenic bacteria, including *Escherichia coli*, *P. aeruginosa*, *Salmonella spp.*, and *V. cholerae* (Wiegand, Hilpert, & Hancock, 2008). The results revealed that both plant extracts exhibited significant antibacterial activity, with *S. cumini* showing comparatively higher efficacy across all tested strains (Table 1; Fig. 5).

The zone of inhibition ranged from 18.2 ± 1.0 mm to 24.1 ± 0.6 mm for *S. cumini* and 17.0 ± 0.9 mm to 21.9 ± 1.0 mm for *M. oleifera*. Among the tested organisms, *V. cholerae* was the most susceptible, followed by *Salmonella spp.*, *E. coli*, and *P. aeruginosa*. The maximum inhibition zone was recorded for *S. cumini* against *V. cholerae* (24.1 ± 0.6 mm), which was statistically significant ($p < 0.05$) compared to *M. oleifera*. The positive control

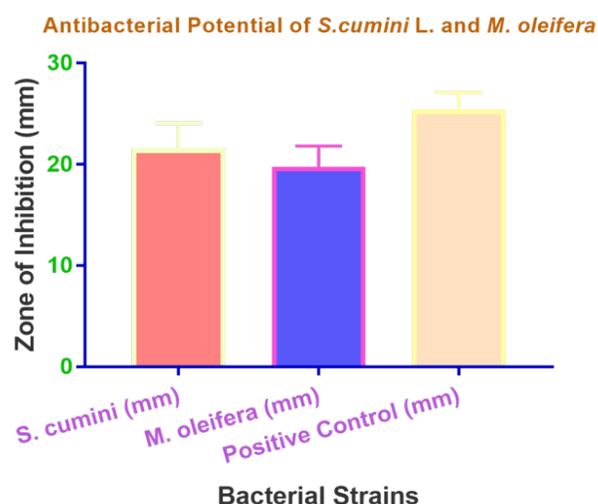


Fig 5: Comparative antibacterial activity of hydroalcoholic extracts of *Syzygium cumini* and *Moringa oleifera*

showed higher inhibition values (23.4–27.3 mm), yet the extracts demonstrated a comparable antibacterial response, indicating their potential as natural antimicrobial agents.

The Minimum Inhibitory Concentration (MIC) results further supported these findings (Fig. 6). *S. cumini* exhibited lower MIC values (0.35–0.70 mg/mL) compared to *M. oleifera* (0.50–0.85 mg/mL), indicating stronger antibacterial potency. The lowest MIC (0.35 mg/mL) was again observed against *V. cholerae*, suggesting that the active phytoconstituents in *S. cumini* may effectively disrupt bacterial cell integrity or interfere with metabolic processes. These results align with previous reports highlighting the antimicrobial activity of *S. cumini* phenolics, flavonoids, and tannins, which are known to cause protein precipitation and membrane disruption in Gram-negative bacteria.

The moderate activity of *M. oleifera* observed in this study may be attributed to the presence of bioactive compounds such as isothiocyanates, alkaloids, and saponins, which act synergistically to inhibit bacterial growth. However, the slightly

reduced efficacy compared to *S. cumini* suggests differences in phytochemical concentration or polarity of extractable compounds in the hydroalcoholic medium.

The pictorial presentation (Fig. 7) clearly demonstrated the formation of distinct inhibition zones around wells treated with both extracts, confirming the visual antimicrobial effect. The statistical analysis using one-way ANOVA followed by Tukey's post hoc test ($p < 0.05$) validated the significant differences between the treatment groups.

Overall, the results highlight that both *S. cumini* and *M. oleifera* possess considerable antibacterial properties against common waterborne pathogens. The stronger performance of *S. cumini* suggests its potential use as a natural antimicrobial agent for developing eco-friendly formulations aimed at water disinfection or preventing bacterial contamination. Further studies involving phytochemical profiling, mechanism elucidation, and formulation standardization are warranted to establish these plants as viable alternatives to synthetic antimicrobials (Ushimaru, Silva, Di Stasi, Barbosa, & Fernandes Junior, 2007).

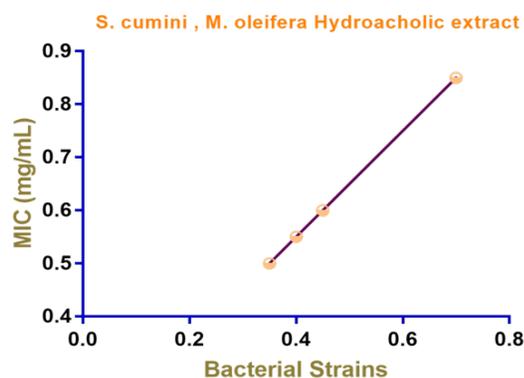


Fig 6: Minimum inhibitory concentration (MIC) values of *Syzygium cumini* and *Moringa oleifera* extracts against waterborne pathogens

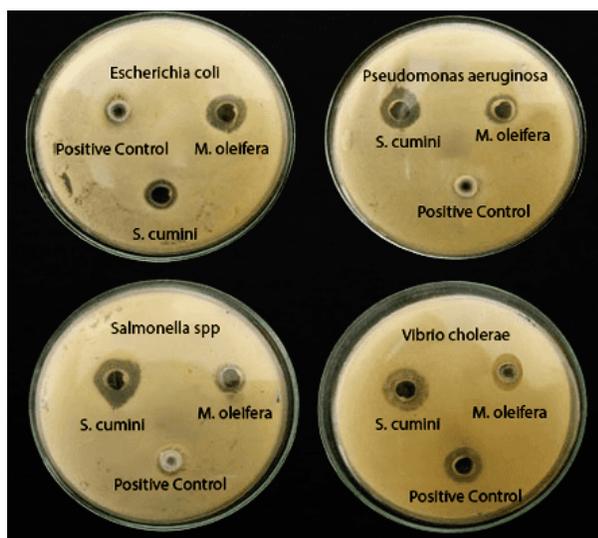


Fig. 7: Pictorial presentation of the zone of inhibition produced by hydroalcoholic solvent extracts of *S. cumini* and *M. oleifera*

SUMMARY AND CONCLUSION

This in-vitro investigation evaluated the antibacterial efficacy of hydroalcoholic extracts of *S. cumini* L. and *M. oleifera* against four prominent waterborne bacterial pathogens: *E. coli*, *P. aeruginosa*, *S. spp.*, and *V. cholerae*. The results demonstrated that both plant extracts exhibited notable antibacterial activity, with *S. cumini* consistently showing larger zones of inhibition and lower minimum inhibitory concentrations (MICs) compared to *M. oleifera*. Among the tested strains, *V. cholerae* was the most susceptible to both extracts, while *P. aeruginosa* showed relatively higher resistance. These findings reinforce the potential of *S. cumini* and *M. oleifera* as natural antibacterial agents for water purification. Their efficacy against common waterborne pathogens suggests a viable role in community-level water treatment, especially in rural and resource-limited settings where access to chemical disinfectants is constrained.

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CONFLICT OF INTEREST

We declare that there is no conflict of interest regarding the publication of this paper.

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