Investigating Plant-Derived Antimicrobials as a Low-Cost Prevention for Pneumonia

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Abstract

Pneumonia remains a leading cause of preventable mortality worldwide, particularly in low-income regions where antibiotic access is limited and antimicrobial resistance is rising. Plant-derived antimicrobials represent a potentially low-cost and sustainable alternative. This study aimed to evaluate the antimicrobial activity of four plant extracts—*Moringa oleifera, Abelmoschus esculentus (okra), Acacia senegal, and Ocimum basilicum (basil)*—against *Escherichia coli* K-12 using the Kirby-Bauer disk diffusion method. Extracts were prepared using both water and ethanol solvents and tested on Mueller-Hinton agar plates. However, due to prolonged spectrophotometer malfunction during the experimental period and procedural timing limitations involving extract preparation and agar freshness, consistent and interpretable inhibition data could not be obtained. Observations suggest that extract potency may decline without rapid plating after preparation, and bacterial lawn consistency depends heavily on recently prepared Mueller-Hinton agar. Although results were inconclusive, this investigation identified critical methodological considerations for future plant-based antimicrobial research and refined experimental protocol for improved reliability.

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Introduction

Pneumonia is a leading cause of morbidity and mortality, particularly in low-income regions where access to antibiotics is limited. According to the World Health Organization, pneumonia accounts for approximately 14% of all deaths among children under the age of five. In Chad, where life expectancy remains among the lowest globally, pneumonia contributes significantly to preventable mortality. Conventional antibiotics, though effective, are frequently unaffordable, inaccessible, or increasingly ineffective due to the rise of antimicrobial resistance (AMR). These challenges highlight the urgent need for alternative, low-cost, and sustainable approaches to disease prevention and treatment. Plant-derived antimicrobials represent a promising alternative. Many medicinal plants contain bioactive compounds—such as alkaloids, phenolics, flavonoids, and terpenoids—that disrupt bacterial cell membranes, inhibit biofilm formation, and interfere with metabolic pathways. Because these plants are locally accessible and affordable in many regions, they may serve as realistic preventative tools.

This study sought to evaluate whether selected plant extracts demonstrate measurable antimicrobial activity against the Biosafety Level 1 bacterial strain using agar disk diffusion methodology, intending to model their potential as low-cost preventative agents for pneumonia.

Prior Literature Review

The growing threat of antimicrobial resistance has intensified the search for alternative solutions, with plant-derived antimicrobials emerging as a promising alternative. The article *Plant-Derived Antimicrobials and Their Crucial Role in Combating Antimicrobial Resistance* (Angelini, 2024) emphasizes the importance of plant secondary metabolites—such as alkaloids, flavonoids, terpenoids, and phenolics—in reducing bacterial viability. This review explains that these compounds can disrupt microbial cell membranes, inhibit quorum sensing, and prevent biofilm formation, thereby offering mechanisms distinct from conventional antibiotics. The author highlights that such natural products are both sustainable and accessible, positioning them as viable candidates for use in low-resource regions.

Direct experimental evidence supports these conclusions. The study, *In vitro studies of the antimicrobial properties and qualitative phytochemical analysis of selected Chadian medicinal plants* (Abdel-Razakh, 2024), tested extracts from species including *Acacia nilotica*

and *Calotropis procera*. Results demonstrated measurable inhibition against bacterial strains, while phytochemical screening confirmed the presence of tannins, saponins, and flavonoids—compounds strongly associated with antimicrobial activity. This validation of traditional Chadian medicine highlights the practical potential of locally available plants as low-cost interventions against infectious diseases. Evidence also extends specifically to pneumonia-related pathogens. The article *Investigation into Finding a Novel Antibiotic against S. pneumoniae Using Four Plant Extracts* (Hossain, 2020) reported that several plant-derived preparations produced zones of inhibition against *Streptococcus pneumoniae*. These findings align with the broader review *Recent Advances of the Medicinal Plant Compound for Combating Pneumonia Disease: A Review* (Patel and Sharma, 2021), which documents how phytochemicals may reduce pneumonia burden through both antimicrobial and immunomodulatory mechanisms. Together, these sources demonstrate the applicability of plant-derived treatments to respiratory infections. Finally, broader reviews illustrate the clinical potential of plant antimicrobials beyond pneumonia. For instance, *Back to Nature: Medicinal Plants as Promising Sources for Antibacterial Drugs in the Post-Antibiotic Era* (Abdallah et al., 2023) emphasizes that phytochemicals possess dual antimicrobial and anti-inflammatory functions, making them attractive both for direct therapeutic application and as adjuncts in wound healing or infection prevention.

Collectively, this body of literature provides strong evidence that plant-derived antimicrobials are scientifically validated, cost-effective, and contextually appropriate for use in resource-limited settings. The convergence of global reviews, ethnobotanical studies, and pneumonia-focused investigations establishes a solid foundation for exploring plant-based antimicrobials as preventative measures against pneumonia.

Global Impact

The adoption of plant-derived antimicrobials has important implications for global health, especially in regions where pneumonia remains a leading cause of mortality and access to antibiotics is limited. By providing low-cost, locally sourced alternatives, these treatments could reduce reliance on imported pharmaceuticals and strengthen community healthcare systems. In addition to clinical benefits, the use of medicinal plants supports cultural traditions and lowers economic barriers, making interventions more sustainable and contextually relevant. Plant-based antimicrobials also offer novel mechanisms of action that may slow the rise of

antibiotic resistance. This aligns with international health priorities, including the United Nations Sustainable Development Goal 3 (Good Health and Well-Being), which seeks to reduce preventable deaths and expand access to essential medicines.

Overall, plant-derived antimicrobials represent a practical and equitable strategy for improving pneumonia outcomes in resource-limited settings while contributing to global efforts against antimicrobial resistance.

Risk Analysis

Several limitations must be acknowledged in the context of this investigation. First, plant extracts are inherently variable; the concentration of active phytochemicals can fluctuate depending on growing conditions, harvesting time, and extraction methods. Such variability may influence the reproducibility of results and complicate the standardization of any promising treatments. Second, although the present study will not involve human subjects, safety considerations remain important. Some medicinal plants contain compounds that can be toxic or cause adverse effects, which would need to be addressed before clinical application. Third, the antimicrobial effects observed in this study may be less pronounced than those of conventional antibiotics. Zones of inhibition may be small or inconsistent, raising questions about the clinical strength of the extracts and the feasibility of using them as stand-alone therapies.

Another limitation concerns the potential for bacteria to develop resistance to plant-based compounds if they are used widely, though this phenomenon has not yet been studied extensively. Most critically, the study itself is restricted by biosafety regulations. Because only Biosafety Level 1 organisms can be tested, the chosen surrogate *Escherichia coli K-12,* does not fully replicate the pathogenicity of pneumonia-causing bacteria like *Streptococcus pneumoniae* or *Haemophilus influenzae*. As a result, while the findings will provide valuable preliminary evidence, they cannot directly confirm clinical effectiveness against pneumonia. Taken together, these risks emphasize the need to interpret results cautiously and to pursue further research in higher-level laboratory and clinical settings before plant-derived antimicrobials can be considered viable alternatives to conventional antibiotics.

Materials and Methods

Materials

Dried plant materials included *Moringa oleifera* leaves, *Abelmoschus esculentus* (okra) powder, *Acacia senegal* powder, and *Ocimum basilicum* (basil) powder. Extraction solvents consisted of 70% ethanol and distilled water. The bacterial strain used was *Escherichia coli* K-12 (BSL-1). Mueller-Hinton agar plates were used for antimicrobial susceptibility testing. Additional materials included sterile 6 mm filter paper disks, ampicillin (10 µg) antibiotic disks for positive controls, ethanol-only disks for negative controls, sterile cotton swabs, sterile inoculating loops, sterile forceps, 50 mL centrifuge tubes, filter paper, and parafilm. All measurements were recorded in a laboratory notebook and a digital spreadsheet.

Instruments

Equipment included an autoclave for sterilization, a 37 °C incubator for bacterial growth, an analytical balance for measuring plant mass, a mortar and pestle for grinding plant material, micropipettes with sterile tips, sterile forceps for disk placement, digital calipers for zone measurement, and a UNICO UV-2150 spectrophotometer for optical density measurements of bacterial cultures. During the experimental period, the spectrophotometer was nonfunctional for approximately one month, resulting in delayed inoculation standardization and interrupted data collection.

Methods

Plant Extraction

Each plant sample was weighed and ground using a mortar and pestle to increase surface area for extraction. Approximately equal masses of each plant were soaked in 70% ethanol for 24 hours to extract ethanol-soluble phytochemicals. A parallel extraction using distilled water was also performed to compare solvent-dependent differences in compound solubility.

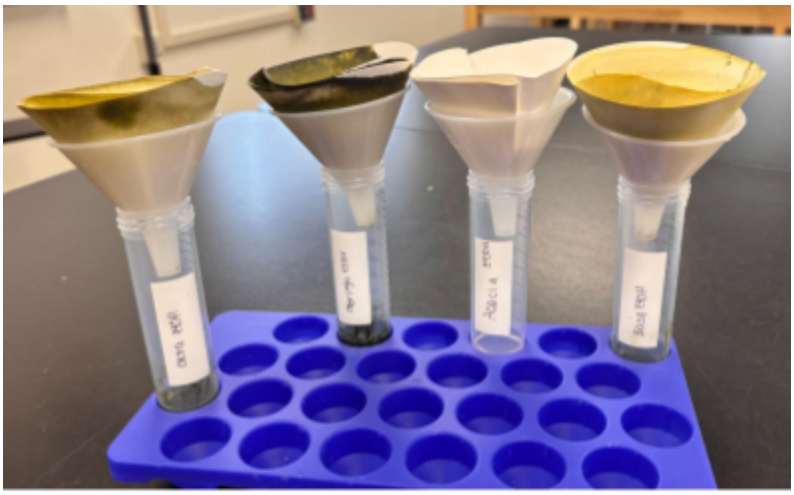

Figure 1. Ethanol-based plant extract filtration.
Filtered ethanol extracts of okra, moringa, acacia, and basil collected in labeled centrifuge tubes following 24-hour extraction.

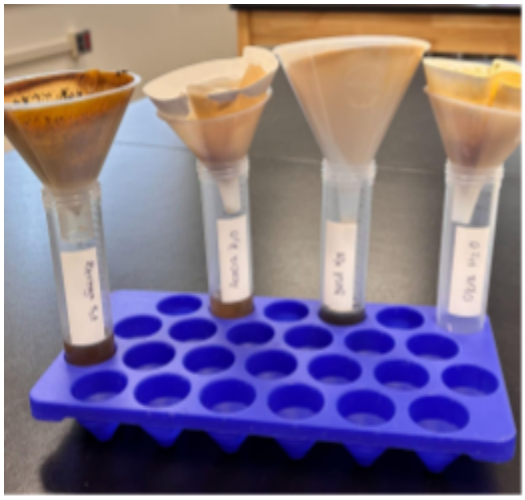

Figure 2. Water-based plant extract filtration.
Filtered distilled water extracts of moringa, acacia, basil, and okra demonstrating solvent-dependent differences in coloration and particulate retention.

Following extraction, both ethanol and water mixtures were filtered through sterile filter paper using funnels and collected into labeled sterile centrifuge tubes.

Disk Preparation

Sterile 6 mm filter paper disks were soaked in filtered plant extract for approximately 10 minutes to allow absorption of phytochemicals. Disks were then allowed to dry briefly under sterile conditions prior to placement on agar plates.

Bacterial Lawn Preparation

Escherichia coli K-12 cultures were grown overnight in LB broth at 37 °C. Optical density was intended to be standardized to approximately 0.5 McFarland turbidity using a spectrophotometer; however, due to prolonged equipment malfunction, consistent turbidity measurements were not possible. Bacterial cultures were instead inoculated based on visual estimation, which may have contributed to variability in lawn density. Mueller–Hinton agar plates were inoculated using sterile swabs to create uniform bacterial lawns.

Disk Diffusion Assay

Extract-infused disks, along with positive (ampicillin) and negative (ethanol-only) controls, were placed on inoculated plates using sterile forceps. Plates were sealed and incubated at 37 °C for 24 hours. Zones of inhibition were intended to be measured in millimeters using digital calipers.

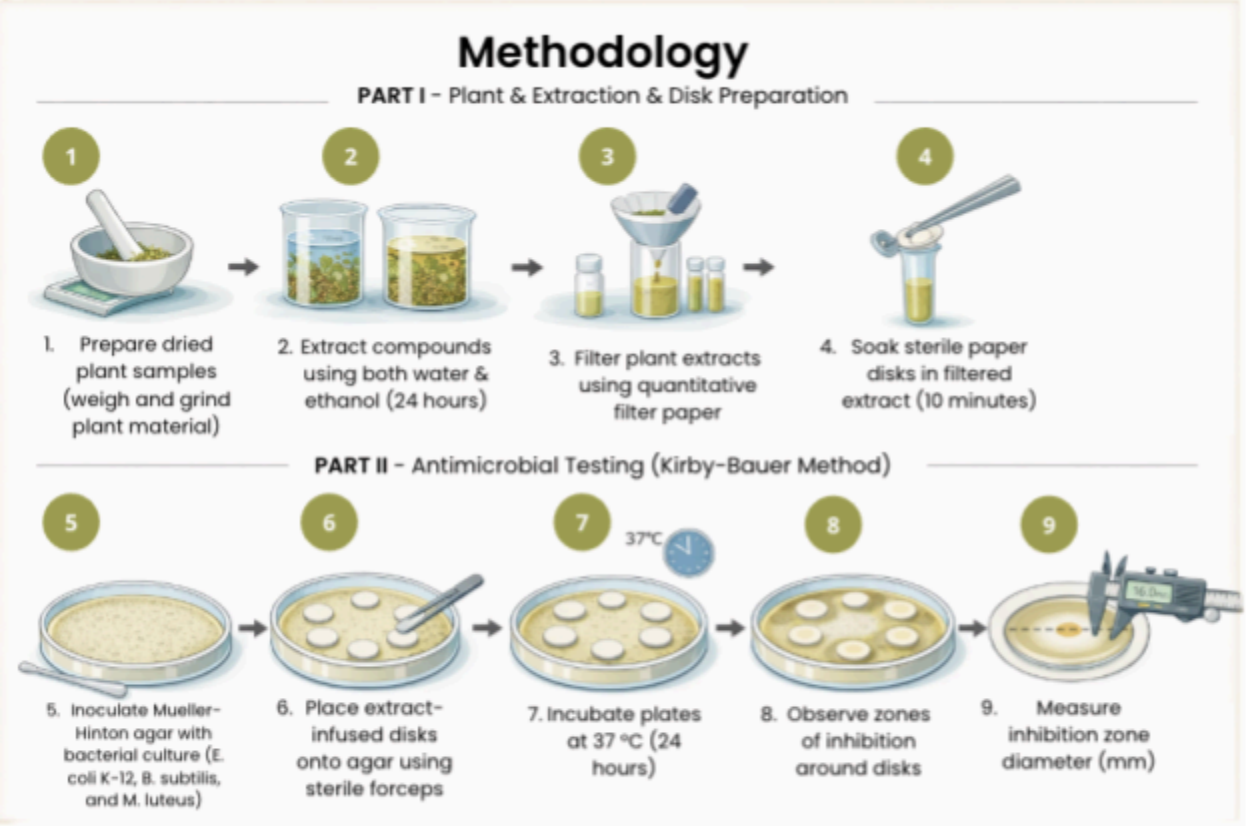

Figure 3. Experimental workflow for plant-derived antimicrobial testing.
Diagram summarizing extraction, disk preparation, inoculation, incubation, and zone measurement steps.

Results

Following 24 hours of incubation at 37 °C, agar plates were examined for measurable zones of inhibition surrounding extract-infused disks. Across trials, no consistently defined zones exceeding the diameter of the filter disks were observed. In several plates, slight discoloration or faint clearing was noted immediately adjacent to certain extract-infused disks; however, these areas were inconsistent between trials and did not produce reproducible, measurable inhibition zones in millimeters. As a result, quantitative zone diameter data could not be validly recorded. In addition, variability in bacterial lawn density was observed between

plates. Some plates exhibited uneven growth patterns, including patchy or clustered colony formation rather than a uniform lawn. This variability likely resulted from the inability to standardize bacterial turbidity using spectrophotometric measurement prior to inoculation.

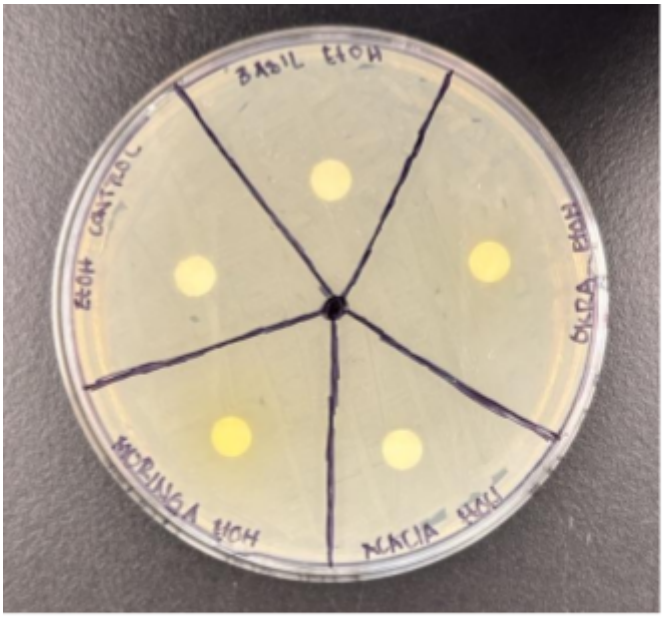

Figure 4. Representative Mueller–Hinton agar plate following disk diffusion assay.
Agar plate inoculated with *Escherichia coli* K-12 showing extract-infused disks after 24-hour incubation. No clearly measurable zones of inhibition are present beyond disk diameter.

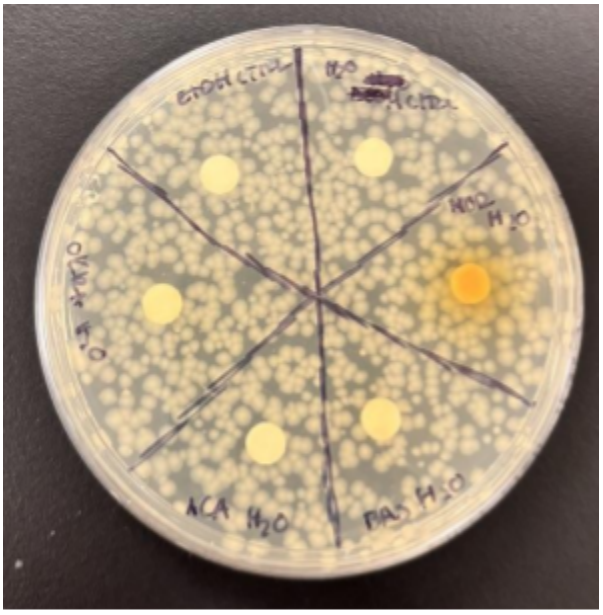

Figure 5. Agar plate demonstrating uneven bacterial lawn formation.
Plate exhibiting non-uniform bacterial growth, limiting reliable measurement of inhibition zones.

Further observation revealed that plates prepared more than 48 hours prior to inoculation demonstrated greater inconsistency in lawn formation compared to freshly prepared plates. Surface moisture variation was visible in some samples, potentially influencing bacterial spread and disk diffusion dynamics. Because inhibition measurements were not reproducible across trials, statistical analysis was not performed.

Discussion

The results of this investigation were inconclusive, as no consistently measurable zones of inhibition were observed across trials. However, the absence of clear inhibition does not necessarily indicate that the tested plant extracts lack antimicrobial properties. Rather, several methodological variables likely influenced the reliability of the disk diffusion assay.

One major limitation was the inability to standardize bacterial turbidity using spectrophotometric measurement. The Kirby–Bauer disk diffusion method requires inoculation at approximately 0.5 McFarland turbidity to ensure consistent lawn density. Due to prolonged spectrophotometer malfunction, bacterial cultures were inoculated based on visual estimation. Even minor deviations in turbidity can significantly alter lawn thickness, which directly affects diffusion dynamics and the visibility of inhibition zones. Uneven lawn formation observed on multiple plates suggests that inoculum variability contributed to inconsistent results. Timing between extraction and plating also emerged as a critical factor. Phytochemical stability may decline following filtration, particularly when extracts are exposed to light, oxygen, or prolonged storage. Delays between extraction, disk soaking, and plating may have reduced active compound concentration at the time of testing. Rapid turnaround between extraction and assay application would likely improve reliability in future trials. Additionally, the age of Mueller–Hinton agar plates appeared to influence bacterial growth patterns. Plates prepared more than 48 hours before inoculation exhibited uneven surface moisture distribution, leading to patchy lawn formation. Because diffusion-based assays rely on uniform agar composition and moisture content, plate freshness is essential for valid measurement. It is also important to consider solvent extraction efficiency. Ethanol and water extract different classes of phytochemicals, and the concentration of active compounds was not quantified in this study. Without determining extract concentration or performing serial dilutions, it is not possible to conclude whether the absence of inhibition was due to insufficient compound potency or procedural limitations. Although quantitative antimicrobial activity was not demonstrated, this investigation successfully identified critical procedural sensitivities in plant-based susceptibility testing. The findings emphasize that reproducible disk diffusion results depend on strict turbidity control, fresh agar media, rapid extract handling, and standardized concentration protocols.

Future studies should incorporate functional spectrophotometric standardization, immediate plating following extraction, agar plates prepared within 24 hours of use, and concentration gradient testing to determine minimum inhibitory thresholds. Testing against additional Biosafety Level 1 organisms may also help determine whether susceptibility varies between bacterial species. Overall, while antimicrobial activity was not conclusively demonstrated, the methodological refinements identified in this study provide a stronger framework for future investigation of plant-derived antimicrobials as potential low-cost preventative agents.

Conclusion

This investigation sought to evaluate whether selected plant extracts demonstrate measurable antimicrobial activity using the Kirby–Bauer disk diffusion method as a preliminary model for low-cost pneumonia prevention strategies. While reproducible zones of inhibition were not observed, the study identified critical procedural factors that strongly influence antimicrobial susceptibility testing outcomes. The inability to standardize bacterial turbidity, variability in agar freshness, and delays between extraction and plating likely contributed to the inconclusive results. These findings highlight the sensitivity of diffusion-based assays to strict methodological control. Although antimicrobial efficacy was not definitively demonstrated in this experiment, the investigation refined experimental design parameters necessary for future testing. Plant-derived antimicrobials remain a promising area of research, particularly in resource-limited regions where pneumonia burden and antimicrobial resistance are high. With improved standardization and concentration analysis, further investigation may clarify the potential role of locally sourced plant extracts in low-cost infection prevention strategies.

Overall, this study contributed methodological insight into plant-based antimicrobial testing and established a foundation for more controlled and reproducible future research.

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