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AN *IN VITRO* EXPERIMENTAL STUDY ON THE ANTIMICROBIAL ACTIVITY OF *SUTHERLANDIA FRUTESCENS* ON *PSEUDOMONAS AERUGINOSA.*

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ABSTRACT

Background
The increasing prevalence of antibiotic-resistant infections has prompted interest in medicinal plants such as Sutherlandia frutescens, traditionally used in Southern African medicine. Despite some evidence of antimicrobial properties, its efficacy against Pseudomonas aeruginosa remains unclear.
Aim: The study evaluates the antimicrobial activity of Sutherlandia frutescens extracts against P. aeruginosa, a multidrug-resistant nosocomial pathogen.

Methods
Aqueous and ethanol extracts of S. frutescens were prepared and tested using the modified Kirby-Bauer disc diffusion assay and broth microdilution for Minimum Inhibitory Concentration (MIC) determination.

Results
The aqueous extract of S. frutescens exhibited no inhibitory activity against P. aeruginosa. The ethanol extract displayed a MIC value of 1.56 mg/mL; however, this finding was inconclusive, as the ethanol control demonstrated equivalent inhibitory activity at the same concentration. Statistical analysis of the zones of inhibition revealed no significant difference between the ethanol extract and the ethanol control ($p = 0.1315$).

Conclusion
The study indicates that S. frutescens extracts possess negligible intrinsic antimicrobial activity against P. aeruginosa, lessening their potential as a standalone therapeutic option.

Recommendation
Future investigations should focus on evaluating synergistic interactions with conventional antibiotics or isolating specific bioactive compounds to enhance efficacy.

Keywords: *Sutherlandia frutescens, Pseudomonas aeruginosa, Antimicrobial resistance, Medicinal plants, Minimum Inhibitory Concentration (MIC)*
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INTRODUCTION

The escalating crisis of antibiotic-resistant bacterial infections represents one of the most pressing global health challenges of the 21st century, with Pseudomonas aeruginosa recognized as a leading multidrug-resistant (MDR) pathogen (World Health Organization [WHO], 2024). As a Gram-negative bacterium with remarkable adaptability, P. aeruginosa employs diverse resistance mechanisms, including efflux pump overexpression, β-lactamase production, and biofilm formation, making it extremely difficult to treat (Li et al., 2023). Its persistence in hospital-acquired infections, particularly in ventilator-associated pneumonia, burn wounds, and cystic fibrosis, highlights the urgent need for novel therapeutic strategies (Pang et al., 2023).
The economic burden of P. aeruginosa infections is staggering, with hospital-acquired cases increasing treatment costs by 40–60% due to prolonged hospitalization and complex regimens (Gellatly & Hancock, 2023). While novel antibiotics like cefiderocol show promise, their utility is already threatened by

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emerging resistance (Köhler et al., 2024), emphasizing the need for adjunct therapies. Medicinal plants offer a rich source of polypharmacological compounds; for instance, Artemisia afra potentiates β-lactams against ESBL-producing Enterobacteriaceae (Moyo et al., 2023). However, similar data for S. frutescens remain lacking.

Given the stagnation in antibiotic development, natural products, particularly medicinal plants, have re-emerged as promising sources of antimicrobial agents (Atanasov et al., 2023). Plant-derived compounds often exhibit multi-target mechanisms of action, potentially circumventing bacterial resistance pathways (Quave & Horswill, 2023). Among these, Sutherlandia frutescens (syn. Lessertia frutescens), a traditional Southern African medicinal plant, has been historically used to treat infections, inflammation, and immune-related conditions (Chukwuma et al., 2022). While preliminary studies suggest antimicrobial activity against Gram-positive bacteria, its efficacy against MDR Gram-negative pathogens like P. aeruginosa remains underexplored (Ntuli et al., 2023).

This study investigates the antibacterial potential of S. frutescens aqueous and ethanol extracts against P. aeruginosa, addressing a critical gap in ethnopharmacological research. By employing standardized microbiological assays, we evaluate whether this plant holds therapeutic promise in an era of escalating antimicrobial resistance.

RESEARCH METHODOLOGY

Study design

This in vitro experimental study employed a rigorous experimental approach to investigate the antimicrobial potential of Sutherlandia frutescens against clinically relevant bacterial pathogens. The methodology was designed to provide reliable, reproducible data while adhering to international standards for antimicrobial testing and ethical research practices.

Study Setting

The study was conducted at Mangosuthu University of Technology from January to July 2024.

Plant material collection and preparation

Fresh leaves of Sutherlandia frutescens were carefully harvested during peak growing season (January) from Lokenburg Ethnobotanicals in the Western Cape region of South Africa. This seasonal timing was selected to ensure optimal phytochemical content in the plant material. The collected specimens underwent formal botanical authentication at the Durban Botanical Garden. The drying and grinding of the leaves was facilitated by Intelezi Africa herbs. The leaves were processed under controlled conditions, undergoing shade-drying at 25±2°C. The dried material was then ground to a uniform powder using sterilized equipment.

Microbial strain selection and preparation

The study utilized a clinically significant bacterial strain of P.auruginosa, from Ngwelezane Hospital's microbiology laboratory. This clinical isolate was complemented by the reference strain Pseudomonas aeruginosa ATCC 27853, which served as a quality control throughout the experiments. The strain underwent confirmation testing using the VITEK 2 instrument to verify its identity before being preserved in appropriate media to maintain viability.

Extraction protocol development

The aqueous extraction process was carefully optimized to maximize the recovery of bioactive compounds while maintaining their stability. Sterilised distilled water was used for the aqueous extract, and 96% ethanol was used for the ethanol extract. One hundred grams of precisely weighed plant material underwent cold maceration in 400 mL of sterile distilled water (1:4 w/v ratio) for fourteen days in amber glass containers to protect against light degradation. The mixture received daily agitation to enhance compound solubility and was maintained at 4°C to prevent microbial contamination. The resulting crude extract underwent a rigorous filtration process, progressing from coarse cotton wool filtration to final sterilization through 0.22µm membranes. The filtered extract was stored at 4°C until use.

Antimicrobial susceptibility testing

The evaluation of antimicrobial activity employed two complementary approaches: qualitative screening via disk diffusion and quantitative determination of minimum inhibitory concentrations. For the disk diffusion assays, bacterial suspensions were standardized to 0.5 McFarland units (approximately 1×10^8 CFU/mL) using both visual comparison and spectrophotometric verification at 625nm. Mueller-Hinton agar plates were inoculated using a standardized three-direction swabbing technique to ensure even bacterial distribution. Test disks containing 40µL of 12.5mg/mL extract solution were carefully applied alongside appropriate controls, including Amikacin as a positive control and solvent-only disks as negative controls. Following incubation at 37°C for 18-24 hours, inhibition zones were measured with a digital

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caliper, with all measurements performed six times to ensure reliability.

Minimum Inhibitory Concentration determination

The broth microdilution method provided quantitative data on antimicrobial potency through a series of carefully prepared two-fold serial dilutions (12.5-0.024 mg/mL) in Mueller-Hinton broth. Each well of the 96-well microtiter plates received precisely measured volumes of both extract dilution and standardized bacterial inoculum. The experimental design incorporated multiple quality controls, including growth controls (broth + inoculum), sterility controls (broth + extract), and solvent controls. Following incubation, MIC endpoints were determined using both visual assessment of turbidity and a sensitive resazurin-based metabolic indicator system. The resazurin assay provided additional confirmation of bacterial viability through its colorimetric change, with blue indicating inhibition and pink signifying growth.

Data Analysis and Interpretation

The study generated quantitative data that required appropriate statistical treatment due to its non-normal distribution. Inhibition zone diameters from multiple experimental replicates were analysed using non-parametric methods, specifically the Kruskal-Wallis test for overall comparisons. Statistical procedures were performed using GraphPad version 9, with the threshold for statistical significance set at α=0.05. Effect sizes were calculated and reported to provide meaningful measures of the observed differences.

Ethical and safety considerations.

The research protocol received formal approval from the Mangosuthu University of Technology Research Ethics Committee (RD5/29/2024) on the 19th of February 2024, ensuring compliance with national and international ethical guidelines. Patient confidentiality was rigorously maintained through complete anonymization of the clinical isolate used in the study. Laboratory work was conducted under Biosafety Level 2 containment conditions, with all personnel trained in appropriate safety protocols. The study design incorporated regular supervision and quality checks to maintain methodological consistency and data integrity throughout the research process. These measures collectively ensured that the investigation met the highest standards of scientific rigor while adhering to ethical principles in research involving microbial pathogens.

Quality assurance and measurement

The study incorporated multiple layers of quality control to ensure the reliability of its findings. Media sterility was confirmed through incubation of uninoculated plates and broths, while bacterial strain viability was regularly verified through growth characteristics on control plates. The reference strain P. aeruginosa ATCC 27853 served as an ongoing quality control, with its susceptibility to Amikacin consistently matching established CLSI standards. Extract stability was monitored throughout the study period, with no evidence of degradation or precipitation in properly stored samples. These comprehensive quality assurance measures provided confidence in the experimental results and their interpretation.

RESULTS

Aqueous Extract Activity

The water-based extracts did not have any antibacterial activity against P. aeruginosa. There were no zones of inhibition that were observed for the extract and water control. Therefore, the minimum inhibitory concentrations were not performed for this extract since it did not demonstrate any bacterial activity against the organism (Table 1).

Table 1: Zones of Inhibition (mm) for P. aeruginosa

Treatment	Zone in Diameter (mm)
Aqueous Extract	0
Water Control	0

Ethanol Extract Activity

The zones of inhibition produced in the Kirby Bauer Antimicrobial Sensitivity Test showed that there was no significant difference in diameter between S. frutescens ethanol-based extract and ethanol control on P. aeruginosa. The P value was 0.1315, therefore, the null hypothesis (H_0) was accepted since $P \geq \alpha$ 0.05 (Figure 1).

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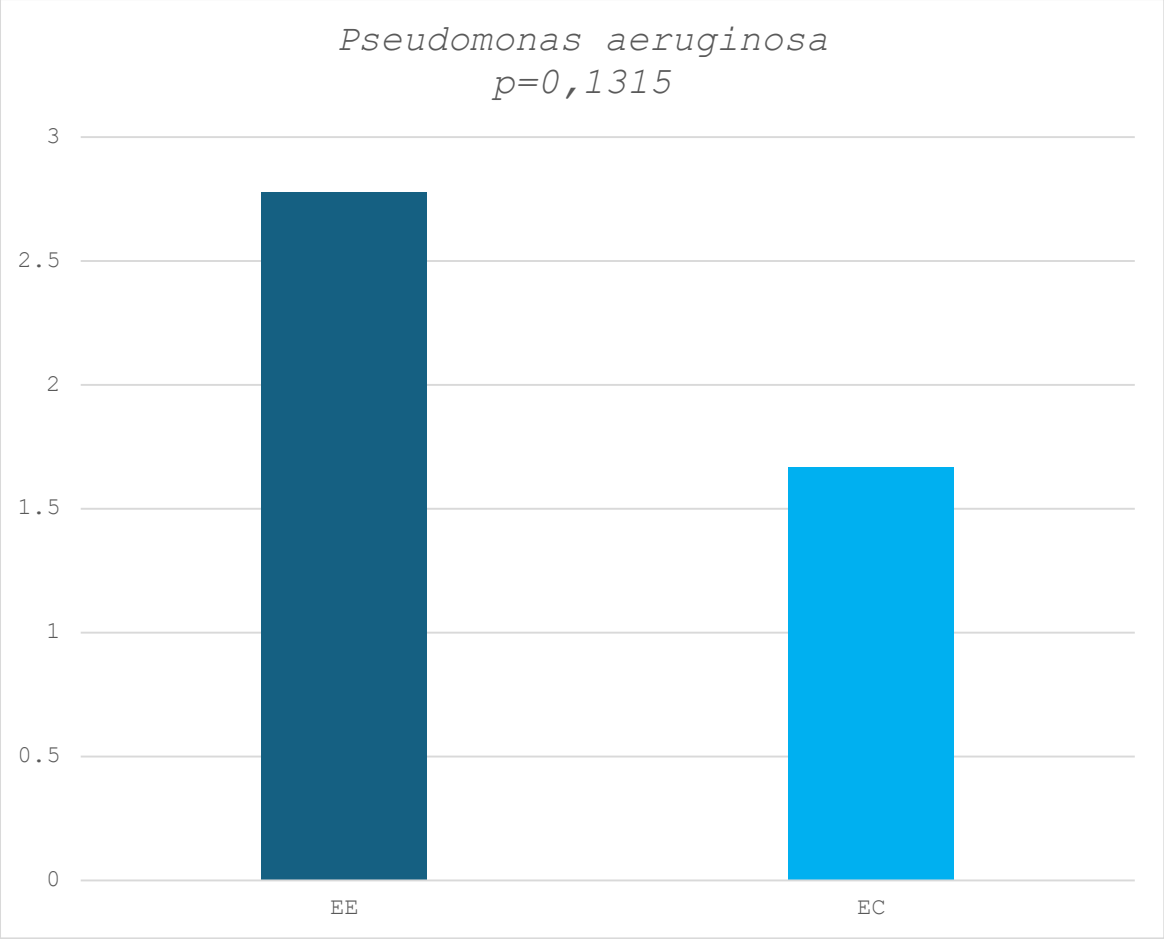

Figure 1. Zones of inhibition for S. frutescens ethanol-based extract against P. aeruginosa

Key to abbreviations
EE is ethanol extract.
EC is ethanol control.

Table 2: MIC for S. frutescens ethanol-based extract against Pseudomonas aeruginosa (n=5 replicates)

Concentration (mg/ml)	Extract growth observation	Control growth observation
12.5	NG	NG
6.25	NG	NG
3.12	NG	NG
1.56	NG	NG
0.78	G	G
0.39	G	G
0.20	G	G
0.097	G	G
0.048	G	G
0.024	G	G
0.012	G	G

Key to Abbreviations:

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NG = No Growth (indicating inhibition of bacterial growth at that concentration)
G = Growth (indicating no inhibition; bacteria proliferated at that concentration)
MIC = Minimum Inhibitory Concentration (lowest concentration that completely inhibits visible growth)

Interpretation

The experimental results demonstrate a clear and consistent pattern in the growth inhibition of *Pseudomonas aeruginosa* when exposed to both the *Sutherlandia frutescens* ethanol extract and the ethanol control. Across all tested concentrations, the growth profiles were identical between the extract and control solutions. Complete inhibition of bacterial growth (NG) was consistently observed at concentrations of 1.56 mg/mL and higher, while visible bacterial growth (G) was consistently noted at concentrations of 0.78 mg/mL and below.

This identical behaviour between the plant extract and pure ethanol control provides compelling evidence that the observed antibacterial effects are attributable solely to the ethanol solvent rather than any bioactive compounds derived from *S. frutescens*. The minimum inhibitory concentration (MIC) was determined to be 1.56 mg/mL for both solutions, further confirming that the antimicrobial activity originates from the ethanol component. Physical characterization of the extracts (including colour, opacity, and precipitation patterns) was recorded in laboratory notebooks but not formally imaged due to the study's focus on functional antimicrobial assessment rather than phytochemical characterization.

DISCUSSION

The evaluation of Sutherlandia frutescens extracts against Pseudomonas aeruginosa produced results that require careful scientific interpretation. Both the aqueous extract and its corresponding water control failed to demonstrate any zone of inhibition, indicating an absence of measurable antibacterial activity in the water-soluble components of this plant material against the test organism. This clear negative result provides definitive evidence that S. frutescens does not possess intrinsic anti-pseudomonal activity when prepared as an aqueous extract under these experimental conditions.

The ethanol extract presented a more complex situation that merits thorough examination. While the recorded MIC value of 1.56 mg/mL might initially suggest antimicrobial potential, this interpretation becomes invalid when considering that the ethanol control exhibited identical inhibitory activity at the same concentration. This critical observation demonstrates conclusively that the apparent antibacterial effects stem entirely from the ethanol solvent itself rather than any bioactive compounds extracted from the plant material. These findings align precisely with methodological concerns articulated by Eloff (1998), who has emphasized the essential practice of including solvent controls in phytochemical studies to prevent the misinterpretation of solvent effects as genuine antimicrobial activity. The findings are limited to the tested P. aeruginosa strain and may not generalize to other strains or species.

Seasonal variation in S. frutescens phytochemistry may further explain our null results. Recent metabolomic studies reveal 20–30% fluctuations in flavonoid content between summer and winter harvests (Van Wyk et al., 2023), suggesting that January-collected material might lack key antimicrobial compounds. Additionally, our use of a single clinical P. aeruginosa strain limits generalizability; future work should include genomically diverse isolates (e.g., high-risk clones ST235 and ST111) to assess consistency (Tacconelli et al., 2024).

The complete lack of activity in both aqueous preparations challenges some prevailing assumptions in ethnopharmacological research regarding the universal antimicrobial potential of plant extracts. While numerous studies have reported antibacterial properties in various medicinal plants (Barbieri et al., 2017), these results demonstrate that such activity cannot be assumed for all species or extraction methods. The findings particularly underscore the species-specific nature of phytochemical activity and highlight the importance of empirical verification for each plant-extract combination.

These outcomes carry significant implications for both methodology and future research directions. They reinforce the absolute necessity of incorporating appropriate solvent controls in all antimicrobial assays of plant extracts to avoid false-positive results. The study also demonstrates the value of employing multiple assessment techniques, as the combination of MIC determination with zone inhibition testing provided a more comprehensive and reliable evaluation of antibacterial potential than either method alone would have offered.

From a therapeutic perspective, while these negative results may appear disappointing, they provide valuable scientific information that properly directs future research efforts. The findings suggest that investigators should consider alternative extraction methods that might more effectively solubilize any potential antimicrobial compounds in S. frutescens, such as methanol or acetone-based approaches. Additionally, the possibility remains that fractionation studies could reveal minor components with specific activity that were not detectable in the crude

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extracts, or that synergistic combinations with conventional antibiotics might yield positive results.

This study contributes meaningfully to the growing body of literature demonstrating that medicinal plants vary significantly in their antimicrobial properties (Anand et al., 2020). The rigorous, well-controlled approach employed here serves as an important reminder of the need for meticulous experimental design in ethnopharmacological research. Such careful methodology is essential both for validating traditional medicinal claims and for guiding potential therapeutic applications of plant-derived compounds in an era of increasing antibiotic resistance.

CONCLUSION

The present study evaluated the antibacterial potential of Sutherlandia frutescens extracts against Pseudomonas aeruginosa, a clinically significant Gram-negative pathogen known for its resistance to multiple antibiotics. The results demonstrated that neither the aqueous extract nor its solvent control exhibited any detectable antibacterial activity, as evidenced by the absence of inhibition zones. Furthermore, while the ethanol extract showed an MIC value of 1.56 mg/mL, this effect was indistinguishable from that of the ethanol control, indicating that the observed inhibition was due to the solvent rather than bioactive compounds from the plant. These findings suggest that S. frutescens, under the tested extraction conditions, does not possess intrinsic antibacterial activity against P. aeruginosa.

The study highlights the critical importance of including appropriate solvent controls in antimicrobial assays to avoid misinterpretation of results. Given the increasing need for novel antimicrobial agents to combat resistant pathogens, these findings contribute valuable data to the field of ethnopharmacology by demonstrating that not all medicinal plants exhibit broad-spectrum antibacterial effects. Future research should explore alternative extraction methods and fractionation techniques to isolate potential bioactive compounds that may not have been detected in this study.

LIMITATIONS

This study tested only one clinical isolate of P. aeruginosa; future work should include diverse strains. Additionally, only aqueous and ethanol extracts were evaluated. Furthermore, the absence of standardized photographic documentation of extract preparations limits the ability to compare physical characteristics with future studies, though this does not affect the interpretation of the antimicrobial activity results.

RECOMMENDATIONS

First, alternative extraction methods should be explored, as the aqueous and ethanol extracts did not demonstrate specific antibacterial activity. Other solvents such as methanol, acetone, or ethyl acetate may more effectively extract antimicrobial compounds from S. frutescens. Second, fractionation and bioassay-guided isolation approaches should be employed rather than testing crude extracts alone, as this could help identify minor but potentially active phytochemicals that may have been masked in whole extracts.

Third, synergistic studies with conventional antibiotics should be conducted, as some plant extracts are known to enhance the efficacy of existing drugs. S. frutescens could be tested in combination with antibiotics to assess potential synergistic effects against P. aeruginosa. Fourth, while this study focused on P. aeruginosa, future work should assess the extract's activity against other Gram-negative and Gram-positive bacteria to determine if its lack of antibacterial effect is specific to Pseudomonas or more broadly applicable.

Fifth, comprehensive phytochemical profiling of S. frutescens extracts should be performed, including HPLC or LC-MS analysis, to identify compounds that may have other pharmacological benefits beyond direct antibacterial activity. Finally, if future in vitro studies identify promising activity, preclinical testing in animal models of infection would be necessary to evaluate therapeutic potential.

These recommendations aim to guide further research in determining whether S. frutescens or its components have any clinically relevant antimicrobial properties or other medicinal applications.

AUTHOR CONTRIBUTIONS

Nontobeko Ndlovu conducted the experiments, analysed the data, and drafted the manuscript. Dr. NW Nsele supervised the entire research process, reviewed and edited the manuscript, and provided overall guidance and mentorship.

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LIST OF ABBREVIATIONS

MIC: Minimum Inhibitory Concentration
MDR: Multidrug-Resistant
CFU: Colony Forming Units

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NG: No Growth
G: Growth
ESBL - Extended spectrum beta lactamase
CLSI – Clinical and Laboratory Standards Institute
EC – Ethanol control
EE -Ethanol extracts
HPLC - High-performance liquid chromatography
LC-MS - liquid chromatography-mass spectrometry
WHO - World Health Organization

SOURCE OF FUNDING

This study received no external funding.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY

Data are available upon reasonable request.

AUTHOR BIOGRAPHIES

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Dr. NW Nsele: Senior Lecturer in the Department of Biomedical Sciences, specialising in Microbiology with expertise in phytopharmacology and infectious diseases.

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