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In Vitro experimental assessment of *Hypericum aethiopicum* (Unsukumbili) ethanolic extract against *Pseudomonas aeruginosa* in wound sepsis: Antimicrobial susceptibility and phytochemical profiling study.

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Abstract

Background
Nosocomial infections, particularly those caused by *Pseudomonas aeruginosa*, remain a significant public health concern, particularly in resource-limited settings. This Gram-negative pathogen is frequently implicated in wound sepsis and is noted for its resistance to multiple antibiotics.
Aim: This study aimed to investigate the antimicrobial potential of aqueous and ethanolic extracts of *Hypericum aethiopicum* (locally known as unsukumbili) against *P. aeruginosa*, with a focus on its application in wound therapy.

Methodology
This study was conducted at Mangosuthu University of Technology in the Department of Biomedical Sciences. The study was carried out *in vitro* employing a vigorous experimental a. This in vitro experimental study employed a rigorous experimental method to investigate the antimicrobial potential of *Hypericum aethiopicum* against clinically relevant bacterial pathogens. Methodology was designed to provide reliable, reproducible data while adhering to international standards for antimicrobial testing and ethical research practices.
Plant sample collection and preparation: Leaf samples of *H. aethiopicum* were harvested early in the morning (8:00 am), where plant cells are said to be active, and processed following the German Homoeopathic Pharmacopoeia standards. Antimicrobial activity was evaluated using the Kirby-Bauer disk diffusion method, and the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined.

Results
Ethanolic extracts exhibited significantly greater antibacterial activity than aqueous extracts (ethanolic inhibition zone was 24mm while aqueous inhibition zone was 8mm). No substantial difference was observed between fresh and dried leaf samples within each solvent type. MIC assays revealed complete inhibition of *P. aeruginosa* growth at 1:8 dilution and higher.

Conclusion
The findings suggest that *H. aethiopicum* ethanol extract demonstrates promising antimicrobial activity against *P. aeruginosa*, indicating potential for development as an alternative treatment for infected wounds.

Recommendation
Further studies are required to evaluate its toxicity, bioactive compounds, and in vivo therapeutic efficacy.

Keywords: *Hypericum aethiopicum, Pseudomonas aeruginosa, Wound healing, Traditional medicine, Antimicrobial resistance, Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC)*
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Introduction

Wound infections, particularly those affecting diabetic and burn patients, represent a growing global health challenge, with *Pseudomonas aeruginosa* frequently implicated in such cases (Salam *et al*., 2023). The pathogen's resistance to multiple antibiotics contributes to increased mortality and has necessitated limb amputations in severe cases (Mendelson *et al*., 2024). The burden of managing non-healing wounds significantly strains healthcare systems, particularly in low- and middle-income countries (Kariuki *et al*., 2022).

In response, traditional medicinal plants offer an accessible and potentially effective alternative for infection control and wound healing, especially in settings where modern medical treatments are inaccessible or unaffordable (Vaou *et al*., 2021; Kumar *et al*., 2018). *Hypericum aethiopicum*, a plant with a rich phytochemical profile, has been identified in previous studies as having wound healing properties (Thembane *et al*., 2024).

Given the escalating healthcare costs and resource limitations in African nations (Murray *et al*., 2022), the validation of ethnomedicinal therapies such as *H. aethiopicum* is both timely and necessary. This study examines the antibacterial properties of *H. aethiopicum* ethanol extracts against *P. aeruginosa*, aiming to contribute to alternative wound management strategies amid rising antimicrobial resistance (Lima *et al*., 2020; Allel *et al*., 2023).

Materials and methods

Study design

This research employed an *in vitro* experimental laboratory study design combining antimicrobial susceptibility testing with phytochemical analysis. This study was conducted at Mangosuthu University of Technology (MUT) in Durban, KwaZulu-Natal, South Africa, between January 2021 and December 2021.

Plant collection and extraction

Fresh *H. aethiopicum* leaves were collected from Silverglen Nature Reserve, KwaZulu-Natal, South Africa. Extraction followed Method 3a of the German Homoeopathic Pharmacopoeia (Benyunes, 2005). For ethanol extraction, minced plant material was mixed with 100% ethanol in a 1:3 ratio, agitated daily, and stored at 4°C for seven days. Filtration was carried out using muslin cloth and Whatman filter paper, and filtrates were preserved in sterile bottles.

Bacterial culture and inoculum preparation

The *P. aeruginosa* ATCC 27853 strain was obtained from Davies Diagnostics. Bacterial suspensions were prepared in nutrient broth and adjusted to 0.5 McFarland turbidity standards. Mueller-Hinton agar plates were inoculated using sterile swabs to ensure even bacterial lawn distribution.

Antimicrobial assay

The antimicrobial efficacy was assessed using the Kirby-Bauer disk diffusion method (Cappuccino & Sherman, 1992). Sterile antibiotic assay discs were impregnated with plant extract and placed on inoculated plates. Positive controls included amikacin discs, and 100% ethanol served as a negative control. Plates were incubated at 37°C for 24 hours, and zones of inhibition were measured in millimetres.

MIC and MBC determination

The MIC was determined using serial dilution in nutrient broth, followed by incubation at 37°C for 16 hours. Tubes showing no turbidity indicated bacterial inhibition. MBC was established by sub-culturing from these tubes onto fresh nutrient agar and checking for bacterial regrowth.

Phytochemical analysis

Both aqueous and ethanolic extracts were subjected to IR spectroscopy and GC-MS to identify active phytochemical constituents.

Statistical analysis

Data were analysed using Fisher's Exact Test and Kruskal-Wallis Test. MIC and MBC values were validated across multiple replicates (n=50). A p-value < 0.05 was considered statistically significant.

Results

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Antibacterial activity

The disk diffusion assay demonstrated that ethanol extracts of *H. aethiopicum* produced significant zones of inhibition against *P. aeruginosa* (mean diameter: 24 mm; p = 0.035). In contrast, aqueous extracts exhibited negligible antibacterial effects.

Antibacterial screening results

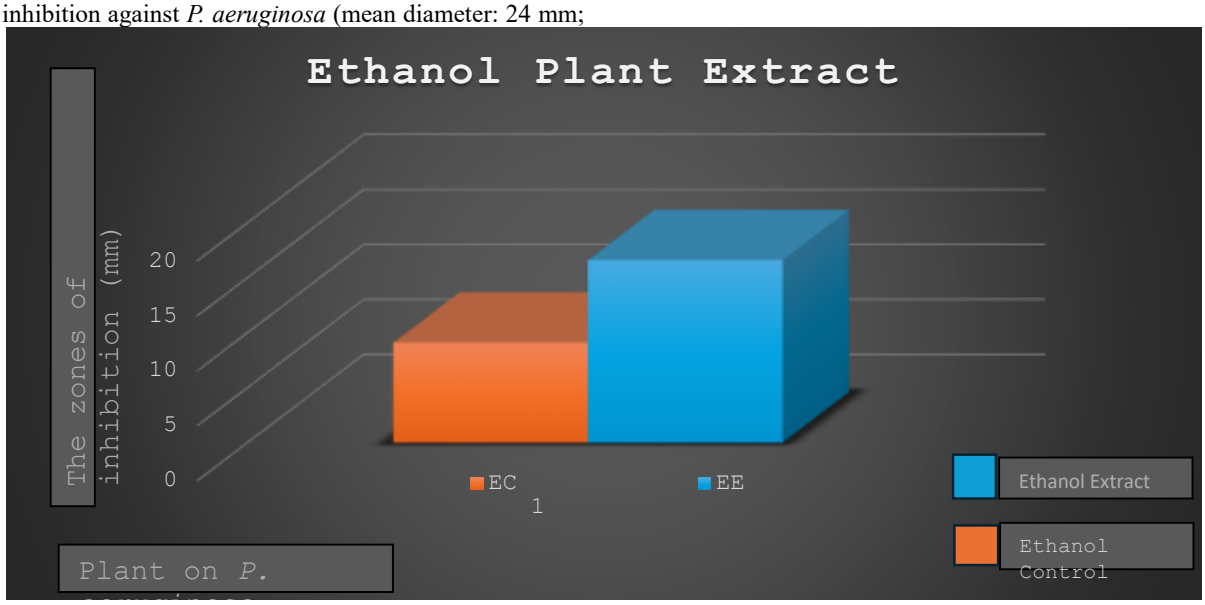

Figure 1: Zones of inhibition for *H. aethiopicum* ethanol extract on *P. aeruginosa*

The zones of inhibition for the antibacterial screening test are displayed in Figure 1. The inhibitory effects on the bacteria were measured by the size of the zone of inhibition post-treatment with *H. aethiopicum* ethanol extract. Statistical significance of the p-value is indicated in Table 1.

Table 1: Analysis of Anti-bacterial screening results

	P-value
P. aeruginosa	0.035

MIC and MBC

The ethanol extract of *H. aethiopicum* completely inhibited bacterial growth at 1:8 dilution and higher concentrations. These results underscore the extract's potency at low dosages.

Discussion

The current study supports the therapeutic potential of *H. aethiopicum* ethanol extract in managing infections caused by *P. aeruginosa*. The observed zones of inhibition and low MIC values indicate the extract's bactericidal effect at minimal concentrations, aligning with earlier findings on plant-derived antimicrobial compounds (Das & Goswami, 2019; Nsele & Thembane, 2023).

The phytochemicals in *H. aethiopicum* likely disrupt bacterial membranes and inhibit cellular replication pathways, reducing the chance of resistance development (Golkar *et al*., 2016). The significant antibacterial activity demonstrated in vitro provides a foundation for further pharmacological validation, particularly in settings with high rates of antimicrobial resistance and limited healthcare infrastructure (Mills *et al*., 2005; Ghuman *et al*., 2019).

While this study provides robust *in vitro* evidence for *H. aethiopicum*'s antimicrobial properties, its generalizability depends on further validation across diverse bacterial strains and clinical contexts. The findings nonetheless offer a critical foundation for developing accessible, plant-based therapies against wound sepsis in antimicrobial-resistant settings.

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Conclusion

The findings underscore the potential of *H. aethiopicum* ethanol extract as a natural antimicrobial agent against *P. aeruginosa*. This could provide a cost-effective therapeutic alternative, particularly in underserved regions. However, comprehensive phytochemical profiling and clinical trials are necessary to confirm safety and therapeutic applicability.

Limitations

The study was limited to a single bacterial strain, restricting the generalizability of the findings. Additionally, the precise active compounds were not isolated or identified. All experiments were conducted *in vitro*; hence, *in vivo* efficacy and potential toxicity remain unverified.

Recommendations

Future investigations should explore:

- *In vivo* efficacy and safety profiles
- Broader spectrum testing against multiple bacterial pathogens
- Isolation and characterisation of active compounds
- Monitoring for resistance development over prolonged use

Author contributions

Mally S.R. MaMkhize conducted the experiments, analysed the data, and drafted the manuscript. Dr. NW Nsele and Dr. S Ghuman supervised the entire research process, reviewed, and edited the manuscript, and provided overall guidance and mentorship.

Acknowledgements

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List of abbreviations

MIC: Minimum Inhibitory Concentration
MBC: Minimum Bactericidal Concentration
MDR: Multidrug-Resistant
NG: No Growth
G: Growth
EC: Ethanol control
EE: Ethanol extracts
HPLC: High-performance liquid chromatography
WHO: World Health Organization
GC-MS: Gas chromatography
IR spectroscopy: Infrared

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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

Mally S.R MaMkhize: Medical Technologist specialised in Clinical Pathology registered with HPCSA, recently acquired Masters degree in Health Sciences at Durban University of Technology and currently pursuing the PhD degree at Tshwane University of Technology, employed at Mangosuthu University of Technology as a Laboratory Medical Technologist in Biomedical Sciences, specialising in Microbiology with expertise in the indigenous research therapies and infectious diseases.

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

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