



In vitro experimental antimicrobial and phytochemical evaluation of unsukumbili (*Hypericum aethiopicum*) against MRSA in wound sepsis.

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Abstract

Background

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a leading cause of nosocomial infections, contributing to severe wound sepsis and high treatment failure rates due to multidrug resistance, resulting in a strain that the healthcare system faces as an uncontrollable, fatal pandemic with a high mortality rate. The increasing prevalence of MRSA necessitates alternative therapeutic strategies, including plant-derived antimicrobials.

Aim: This study evaluated the antimicrobial efficacy of aqueous and ethanolic extracts of *Hypericum aethiopicum* (unsukumbili) against Methicillin-resistant *Staphylococcus aureus* (MRSA), assessing its potential for wound infection management.

Methodology

An in vitro experimental design was employed, following standardized antimicrobial testing protocols. *H. aethiopicum* leaves were extracted using ethanol and water, and antimicrobial activity was assessed via disk diffusion, Minimum Inhibitory Concentration (MIC), and Minimum Bactericidal Concentration (MBC) assays.

Results

Ethanolic extracts exhibited superior antibacterial activity (mean inhibition zone: 26mm) compared to aqueous extracts (4 mm). No significant difference was observed between fresh and dried leaf extracts. The MIC for MRSA was achieved at a 1:8 dilution, demonstrating potent bactericidal effects.

Conclusion

H. aethiopicum ethanol extract shows promising activity against MRSA, suggesting its potential as an alternative treatment for Methicillin-resistant *Staphylococcus aureus* (MRSA) infected wounds.

Recommendation

Further studies are needed to isolate bioactive compounds and assess in vivo efficacy, identifying levels of the plant toxicity against human cells.

Keywords: Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC), *Hypericum aethiopicum*, Methicillin-resistant *Staphylococcus aureus* (MRSA), Wound infections, Antimicrobial resistance, Phytochemical analysis.

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Introduction

The presence of a critical challenge in clinical settings caused by wounds that are infected by Methicillin-resistant *Staphylococcus aureus* (MRSA), particularly in

immunocompromised and post-surgical patients (Turner et al., 2023). The rise of antibiotic-resistant strains has limited treatment options, the increasing morbidity and healthcare costs (WHO, 2022). Traditional medicinal plants, such as *Hypericum aethiopicum*, offer a potential



alternative due to their bioactive antimicrobial properties. *Hypericum aethiopicum*, known to have high levels of phytochemical profile, has been identified in previous studies as having wound therapeutic properties (Moodley et al., 2021).

Given the ever-increasing healthcare costs and resource limitations in African nations (Murray et al., 2022), the validation of ethnomedicinal therapies such as *H. aethiopicum* is both appropriate and essential. This experiment examines the antibacterial components of *H. aethiopicum* ethanol extracts against MRSA, targeting to contribute to alternative wound management approaches amid intensifying antimicrobial resistance (Lima et al., 2020; Allel et al., 2023).

This study investigates the efficacy of *H. aethiopicum* extracts against MRSA, addressing the urgent need for novel antimicrobial agents in wound care.

Materials and methods

Study design

An in vitro experimental study was conducted in Durban, KwaZulu-Natal, at Mangosuthu University of Technology (MUT) between 2021 and 2022, combining antimicrobial susceptibility testing with phytochemical profiling. This university, where the study was conducted, is known to stand out as a key institution for students aspiring to pursue careers in the Biomedical sciences, with a focus on academic excellence, practical training, and research innovation. MUT prepares its graduates to become leaders in healthcare and Biomedical research, making a tangible impact on society's health and well-being.

Plant collection and extraction

Fresh *H. aethiopicum* leaves were collected from Silverglen Nature Reserve, South Africa (KwaZulu-Natal). Ethanol and aqueous extracts were prepared following the German Homoeopathic Pharmacopoeia (Method 3a) (Benyunes, 2005). In extraction, the plant material was minced and 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 strain and culture

MRSA (ATCC 29213) was commercially 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 distribution on the base of the agar.

Antimicrobial assay

Disk Diffusion: 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 vancomycin 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/MBC: MIC was determined via broth dilution, followed by incubation at 37°C for 16 hours. Tubes showing no turbidity indicated bacterial inhibition, and MBC was determined by sub-culturing from the nutrient broth that displayed bacterial inhibition onto fresh nutrient agar, incubated at 37°C for 24 hours, and checking for bacterial regrowth.

Phytochemical analysis

Ethanolic extracts were subjected to IR spectroscopy and GC-MS to identify active phytochemical constituents.

Statistical analysis

Fisher's Exact Test and Kruskal-Wallis Test were applied in the analysis of data ($p < 0.05$ considered significant).

Ethical considerations

Ethical clearance was obtained from the Mangosuthu University Ethics Committee (RD5/29/2024)

Results

Antibacterial activity

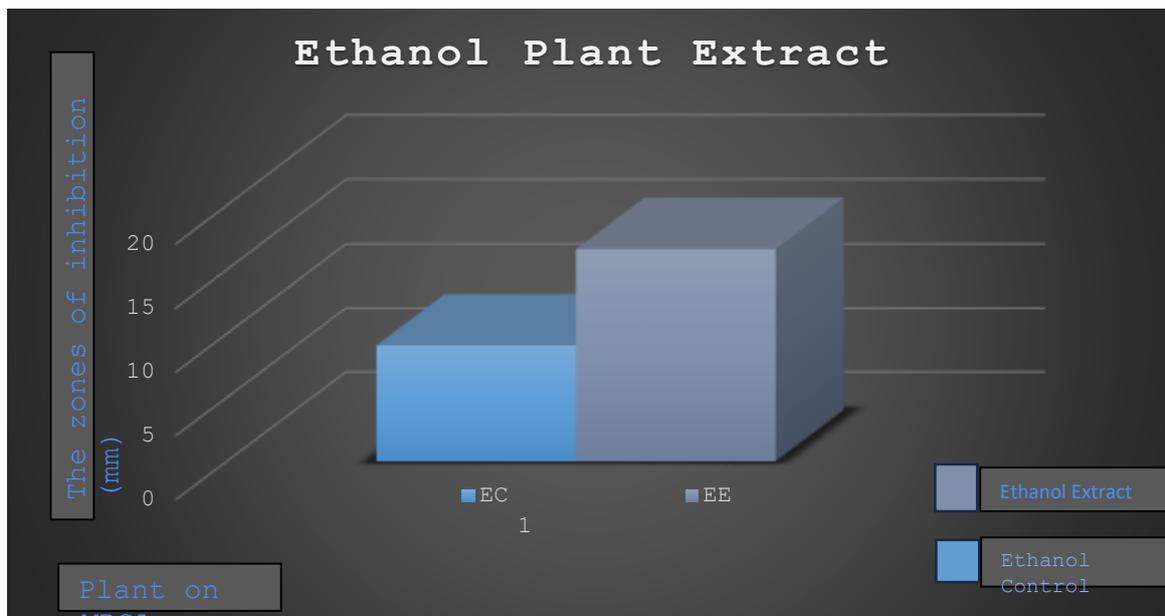


Figure 1: Zones of inhibition for *H. aethiopicum* ethanol extract on MRSA.

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.

Ethanol extracts showed significant inhibition (28 mm, $p = 0.035$) against MRSA.

Table 1: Antibacterial screening results

	P-value
MRSA	0.035

MIC and MBC

The ethanol extract of *H. aethiopicum* completely inhibited bacterial growth at 1:4 dilution and higher concentrations. These results underscore the extract's potency at low dosages.

Discussion

The promising *in vitro* results of *H. aethiopicum* ethanol extract against Methicillin-Resistant *Staphylococcus aureus* provide a critical foundation for its potential therapeutic application. However, the generalizability of these findings, that is, the extent to which they can be reliably applied to real-world clinical settings and broader bacterial populations, is contingent upon further rigorous investigation.

Currently, the study's generalizability is constrained by its *in vitro* nature. While the established zones of inhibition

and significantly low MIC values offer robust preliminary evidence of bactericidal activity, these results are derived from a controlled laboratory environment. Direct extrapolation to complex human biological systems, with factors like pharmacokinetics, tissue penetration, immune response, and potential toxicity, is not yet warranted. The extract's efficacy must be validated *in vivo* using animal models and subsequently in human clinical trials to confirm its therapeutic potential for managing actual Methicillin-Resistant *Staphylococcus aureus* infections.

Furthermore, the generalizability across diverse bacterial populations requires confirmation. The findings are based on specific Methicillin-Resistant *Staphylococcus aureus* strains tested in the laboratory. To be considered a broad-spectrum or reliably effective option, the extract's activity must be demonstrated against a wider array of Methicillin-Resistant *Staphylococcus aureus* genotypes and phenotypes, as well as other clinically relevant Gram-positive pathogens commonly found in wound sepsis. The



mention of its potential to reduce resistance development is a compelling hypothesis, but its generalizability depends on long-term exposure studies across diverse bacterial strains to assess the actual rate and mechanisms of resistance emergence.

Finally, the applicability of these findings is particularly relevant for specific clinical contexts. As noted, the extract holds significant promise for settings with high rates of antimicrobial resistance and limited healthcare infrastructure, where accessible, plant-based remedies are urgently needed. For this potential to be fully realized, subsequent research must focus on standardizing the extraction process, ensuring batch-to-batch consistency, and developing stable formulations suitable for topical wound application in resource-limited environments.

In conclusion, while the *in vitro* findings are a vital and encouraging first step, their generalizability to clinical practice is currently limited. The study successfully provides the necessary scientific underpinning for further pharmacological authentication, but broader endorsement through studies involving diverse bacterial strains, *in vivo* models, and eventual clinical trials is essential to translate these preliminary results into a widely applicable and accessible therapeutic intervention.

Conclusion

H. aethiopicum ethanol extract demonstrates potent anti-MRSA activity, supporting further pharmacological development. This could stipulate a cost-effective therapeutic substitute, predominantly in underserved communities. However, wide-ranging phytochemical reporting and clinical trials are essential to approve safety and therapeutic applicability.

Limitations

This study was restricted to a solitary bacterial strain, limiting the generalizability of the discoveries. Furthermore, the precise bioactive compounds were not isolated or identified. All experiments were conducted *in vitro*; hence, *in vivo* efficacy and potential toxicity remain unverified.

Recommendations

Forthcoming studies should discover:

- In vivo toxicity and efficacy studies
- Bioactive compound isolation
- Broader-spectrum testing

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List of abbreviations

MRSA: Multidrug-Resistant *Staphylococcus aureus*

MIC: Minimum Inhibitory Concentration

MBC: Minimum Bactericidal Concentration

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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Conflict of interest and author declarations

All authors have reviewed and approved the final manuscript.

The work is original, unpublished, and not under consideration elsewhere.

No conflicts of interest, financial or otherwise, are declared.

Author contributions

Mally S.R. MaDanger Mncube 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. Dr S Ghuman was appointed the main supervisor for the study.

Data availability

Data are available upon reasonable request.

Author biographies

Mally S.R MaDanger Mncube: 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 caused by micro organisms.

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