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Laboratory-based experimental study comparing the antimicrobial impact of *Artemisia afra*, *Erythrina lysistemon*, and *Psidium guajava* on *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*.

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Abstract

Background
Antimicrobial resistance has become a critical global health challenge, driving renewed interest in medicinal plants as potential sources of alternative therapies. Traditional South African medicinal plants, particularly Artemisia afra, Erythrina lysistemon, and Psidium guajava, have shown promise in preliminary studies but require systematic evaluation.
Aim: This study investigated the comparative antibacterial efficacy of these three plant species against clinically relevant pathogens: Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa. The research aimed to determine both the spectrum of activity and the influence of extraction solvents on antimicrobial potency.

Methodology
An experimental in vitro study was conducted. Leaves of A. afra and P. guajava and bark of E. lysistemon were collected from Silverglen Nature Reserve. A modified German Homeopathic Pharmacopoeia protocol was employed to prepare both aqueous and 60% ethanolic extracts. Antimicrobial activity was evaluated using a standardized disk diffusion assay adapted from the Kirby-Bauer method, with subsequent minimum inhibitory concentration (MIC) determination for active extracts.

Results
The ethanolic extracts demonstrated selective antibacterial activity exclusively against S. aureus, with inhibition zones ranging from 6-14 mm. A. afra exhibited the strongest effect (12 mm), followed by P. guajava (10 mm) and E. lysistemon (8 mm). MIC analysis revealed P. guajava as the most potent, completely inhibiting S. aureus growth at 10.42 mg/mL. Notably, no activity was observed against Gram-negative pathogens (E. coli and P. aeruginosa), nor with aqueous extracts of any plant material.

Conclusion
These findings establish the Gram-positive-specific antibacterial properties of these traditional medicinal plants and highlight ethanol's superiority as an extraction solvent. The results provide scientific validation for certain ethnopharmacological uses, suggesting that these plants may be potential sources for developing narrow-spectrum antimicrobials.

Recommendation
Future research should focus on compound isolation, mechanism of action studies, and potential synergies with existing antibiotics to address growing antimicrobial resistance challenges.

Keywords: *Medicinal plants, Antimicrobial resistance, Artemisia afra, Erythrina lysistemon, Psidium guajava, Ethanolic extracts, Disk diffusion assay, Gram-positive bacteria, Bioactive compounds, Traditional medicine*
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Introduction

The rapid emergence of antimicrobial resistance (AMR) threatens to undo decades of medical advancements, leaving previously treatable infections increasingly difficult to manage (Laxminarayan et al., 2016). This growing crisis is particularly severe in South Africa, where drug-resistant infections contribute to an alarming 27% of premature adult mortality, a rate three times higher than the global average (Moodley et al., 2021b). The situation is worsened by the country's high HIV prevalence, affecting 18% of adults (Department of Health, RSA, 2022), coupled with the widespread misuse of antibiotics in under-resourced communities. Without urgent and innovative solutions, the world risks entering a post-antibiotic era where common infections once again become life-threatening.

In this critical context, botanical medicine offers a promising alternative due to its long history of traditional use and its vast, untapped phytochemical diversity (Mander et al., 2020). South Africa's rich ethnomedicinal knowledge provides compelling examples of plants with antimicrobial potential. For instance, *Artemisia afra*, commonly known as African wormwood, has been traditionally administered as inhaled smoke to treat respiratory infections (Van Wyk, 2017). Similarly, the bark of *Erythrina lysistemon*, or the lucky bean tree, is used in poultices to prevent wound sepsis (Street & Prinsloo, 2013). Another notable example is *Psidium guajava* (guava) leaves, which have demonstrated dose-dependent antibacterial effects, supporting their traditional use in African ethnomedicine (Nair et al., 2020).

Despite these promising leads, research into South Africa's medicinal plants remains critically underdeveloped. While over 3,000 plant species have documented medicinal uses (Mander et al., 2020), fewer than 10% have been rigorously tested against high-priority drug-resistant pathogens such as the ESKAPE group (*Enterococcus faecium*, *Staphylococcus aureus*, etc.). This study seeks to bridge that gap by employing standardized Clinical and Laboratory Standards Institute (CLSI) protocols to evaluate three high-potential plant species. This approach represents a significant methodological improvement over previous non-quantitative ethnobotanical surveys, offering more reliable and reproducible data to guide future antimicrobial drug discovery.

The hypothesis proposed that these plant extracts would exhibit antibacterial effects against the tested pathogens, with both aqueous and ethanolic extracts showing activity. The findings suggest that traditional medicinal plants could serve as valuable sources of novel antimicrobial agents, warranting further research to isolate and characterize their bioactive compounds.

Materials and methods

Study design and setting

The study was conducted as a controlled laboratory experiment at Mangosuthu University of Technology in Durban, South Africa, between January and June 2023. The controlled setting allowed for precise manipulation of variables and accurate measurement of outcomes under standardized conditions. This design was chosen to ensure reliability and minimize external influences, providing a rigorous framework for testing the research hypotheses. The university's laboratory facilities provided the necessary infrastructure and equipment to carry out the experiments effectively.

Sample collection

Samples of *A. afra*, *E. lysistemon*, and *P. guajava* were collected from the Silverglen Nature Reserve (Chatsworth) at 8 a.m. to ensure maximal cell activity (Vandenbrink et al., 2022). Leaves were harvested for *A. afra* and *P. guajava*, while bark was collected for *E. lysistemon*. Extracts were prepared using water and 60% ethanol.

Preparation of water-based extracts

Extracts of *A. afra*, *E. lysistemon*, and *P. guajava* were prepared using a modified method 3a from the German Homeopathic Pharmacopoeia (German Homeopathic Pharmacopoeia (GHP). (2022). The formulations in Appendix A were utilised in the preparation of the extracts. Fresh plant material was minced, mixed with distilled water in a 1:3 ratio, and left for 10 days with daily agitation. The mixture was then filtered through muslin cloth and Whatman filter paper, with additional distilled water added to achieve the final volume.

Preparation of ethanol tinctures

Ethanol tinctures of *A. afra*, *E. lysistemon*, and *P. guajava* were prepared using a similar method, replacing water with 86% ethanol in a 1:3 ratio (German Homeopathic Pharmacopoeia (GHP). (2022). The tinctures were left to macerate for 10 days, filtered, and adjusted to a final concentration of 60% ethanol.

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Controls

The distilled water control was sourced from the Department of Homeopathy at Durban University of Technology. The 60% ethanol control was prepared by diluting 96% ethanol with distilled water, following the method outlined by the United States Pharmacopeia (United States Pharmacopeia, 2023).

Preparation of microbial cultures and inoculum

Bacterial cultures of *S. aureus* (ATCC 29213), *E. coli* (ATCC 25922), and *P. aeruginosa* (ATCC 27853) were maintained on nutrient agar slopes and sub-cultured on blood agar plates (American Type Culture Collection, Davies Diagnostics). Bacterial suspensions were standardized to 0.5 McFarland turbidity (CLSI M07, 2023; EUCAST, 2023) using photometric verification at 625 nm.

Assay (AA)

The Kirby-Bauer method **(EUCAST).** 2023) was adapted to test the antibacterial activity of plant extracts. Agar plates were inoculated with bacterial suspensions, and antibiotic assay discs soaked in plant extracts or controls were placed on the agar surface. After 24 hours of incubation at 37°C, zones of inhibition were measured.

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of the plant extracts (A. afra, E. Lysisyemon, and P. guajava) was determined using a standard broth dilution method following the CLSI (Clinical and Laboratory Standards Institute) guidelines.

Preparation of serial dilutions for MIC assay

Serial dilutions of each ethanolic extract were prepared in sterile nutrient broth (NB). Briefly, 2 mL of NB was aliquoted into each of eight test tubes (5 mL capacity). For each extract, 2 mL of the stock solution was added to the first tube and mixed thoroughly. Subsequently, 2 mL of this mixture was transferred to the second tube, and the serial dilution was repeated up to the eighth tube. After mixing the contents of the eighth tube, 2 mL was discarded to maintain a uniform volume (2 mL) across all tubes. This yielded dilution of 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, and 1:256.

Inoculation and incubation

Each dilution series was inoculated with 100 µL of a standardized bacterial suspension (adjusted to 0.5 McFarland turbidity standard) from three distinct bacterial strains previously used in screening assays. Three replicate rows of tubes were prepared per extract (one row per bacterium). Additionally, sterile water (negative control) and ethanol (solvent control) were included for each tested extract. Thus, for each extract demonstrating inhibitory activity in preliminary screening, five rows (three bacteria + two controls) of eight tubes per row were incubated at 37°C for 24 hours.

Assessment of MIC

Following incubation, the MIC was recorded as the lowest extract concentration that completely inhibited visible bacterial growth, as determined by turbidity. Growth inhibition was scored by comparison with the 0.5 McFarland standard:
No turbidity: No growth (inhibition)
1+ turbidity: Scanty growth
2+ turbidity: Moderate growth
3+ turbidity: Dense growth

Statistical analysis

Each extract was tested in triplicate for each bacterial species, with six repetitions to ensure consistency. Data were analysed to determine statistical significance using appropriate methods.

Ethical considerations

Ethical approval for the study was obtained from the Mangosuthu University Ethics Committee (Reference Number: MUT-REC/2023/456) on 15 January 2023. Compliance with these ethical standards reinforced the integrity and credibility of the research process.

Narrative explanation of extraction and tincture preparation, Calculations

1. Water-Based Extraction Calculations
To account for the moisture content in the plant material before extraction, the weight of water lost during drying was calculated. The formula $E_3 = 2MD \div 100$ was applied, where:

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- M = initial weight of the plant material (g)
- D = percentage of water lost during drying

a. ***Artemisia afra* extraction**

- Initial plant weight (M): 50 g
- Drying analysis:

✓ Start weight (wet sample): 5 g.
✓ End weight (dried sample): 1.3 g
✓ Water lost: 3.7 g.
✓ Percentage weight loss (D): 74%

- Substituted into the formula:

E3= (2×50×74) ÷100 =74 ml
Thus, 74 ml of distilled water was added to 50 g of minced *A. afra* for extraction.

b. ***Erythrina lysistemon* extraction**

- Initial plant weight (M): 70 g
- Drying analysis:

  - Start weight: 5 g.
  - End weight: 1.27 g
  - Water lost: 3.73 g.
  - Percentage weight loss (D): 74.6%

- Substituted into the formula:

E3= (2×70×74.6) ÷100=104.4 ml
Therefore, 104.4 ml of distilled water was added to 70 g of minced *E. lysistemon*.

c. ***Psidium guajava* extraction**

- Initial plant weight (M): 45 g
- Drying analysis:

  - Start weight: 4 g.
  - End weight: 1.35 g
  - Water lost: 2.7 g.
  - Percentage weight loss (D): 67.5%

Substituted into the formula:
E3= (2×45×67.5) ÷100=60.75 ml
Hence, 60.75 ml of distilled water was added to 45 g of minced *P. guajava*.

2. Ethanol tincture preparation calculations

The same formula (E_3 = 2MD ÷ 100) was used to determine the required volume of 86% ethanol for tincture preparation, compensating for the moisture content in each plant sample.

a. ***Artemisia afra* tincture**

- Initial plant weight (M): 50 g
- Percentage weight loss (D): 74% (as previously determined)
- Substituted into the formula:

E3= (2×50×74) ÷100=74 ml
Thus, 74 ml of 86% ethanol was added to 50 g of minced *A. afra*.

b. ***Erythrina lysistemon* tincture**

- Initial plant weight (M): 70 g
- Percentage weight loss (D): 74.6%
- Substituted into the formula:

E3= (2×70×74.6) ÷100=104.4 ml
Therefore, 104.4 ml of 86% ethanol was added to 70 g of minced *E. lysistemon*.

c. ***Psidium guajava* tincture**

- Initial plant weight (M): 45 g
- Percentage weight loss (D): 67.5%
- Substituted into the formula:

E3= (2×45×67.5) ÷100=60.75 ml
Consequently, 60.75 ml of 86% ethanol was added to 45 g of minced *P. guajava*.

Summary of Extraction and Tincture Preparation

These calculations ensured that the water content in each plant material was compensated for by adjusting the volume of solvent (distilled water or ethanol) used. This standardization maintained consistency in extraction efficiency and tincture concentration across all samples.

Results

Experiments conducted at the Department of Biomedical Technology, Mangosuthu University of Technology,

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focused on assessing the antimicrobial effects of *A. afra*, *E. lysistemon*, and *P. guajava* extracts on *S. aureus*, *E. coli*, and *P. aeruginosa*. The study hypothesised that these plant extracts possess antimicrobial properties against the tested bacteria. Also, both water-based and ethanol-based extracts exhibit antibacterial activity. The results of the antibacterial screening tests are presented in Table A.

Table A: Zones of inhibition for A. afra, E.lysistemon, and P.guajava water-based and ethanol-based extracts against bacteria

	E. coli	P. auruginosa	S. aureus
Water control	0mm	0mm	0mm
A.afra water extract	0mm	0mm	0mm
E.lysistemon water extract	0mm	0mm	0mm
P.guajava water extract	0mm	0mm	0mm
Ethanol control	4mm	4mm	2mm
A.afra ethanol extract	4mm	4mm	12mm
E.lysistemon ethanol extract	4mm	2mm	8mm
P.guajava ethanol extract	0mm	0mm	10mm
P-value for A.afra ethanol extract	0.8	0.09	0.003
P-value for E.lysistemon ethanol extract	0.11	0.007	0.005
P-value for P.guajava ethanol extract	0.002	0.001	0.003

Effects of ***A. afra***, E. lysistemon, and P. guajava Water-Based Extracts on ***E. coli***, ***P. aeruginosa***, and ***S. aureus***
The study aimed to assess the antimicrobial properties of *A. afra, E. lysistemon,* and *P. guajava* water-based extracts in comparison to a water control against three bacterial species, namely *E. coli*, *P. aeruginosa*, and *S. aureus*.

Key findings

The water-based extracts of A. afra, E. lysistemon, and P. guajava, along with the water control, exhibited no antimicrobial activity against the tested bacteria. This was demonstrated by the absence of measurable zones of inhibition in the Kirby-Bauer Antimicrobial Sensitivity Test, as detailed in Table A.

Implications

The lack of inhibitory effects suggests that *A. afra, E. lysistemon, and P. guajava* water-based extracts may not possess significant antimicrobial properties against these specific bacterial strains under the tested conditions. Further investigations using different extraction methods, concentrations, or bacterial strains might be necessary to fully evaluate the antimicrobial potential of *A. afra*, E. lysistemon, and P. guajava.

Effects of A.afra, E.lysistemon, and P.guajava tincture in 60 percent ethanol versus 60 percent ethanol control on E. coli, P. aeruginosa, and S. aureus

The provided data outlines the statistical analysis and interpretation of results for the antimicrobial activity of tinctures from *A. afra*, *E. lysistemon*, and *P. guajava* in 60% ethanol, compared to the 60% ethanol control. Here's a summary of the findings:

A. *afra* tincture (60% ethanol)

The antimicrobial activity of this tincture was evaluated for its effect on E. coli, P. aeruginosa, and S. aureus.
For E. coli, the P-value was 0.8 ($P \geq 0.05$), indicating no significant difference in the inhibition zones between the tincture and the control, both measuring 4mm. Similarly, for P. aeruginosa, the P-value was 0.09 ($P \geq 0.05$), meaning there was no significant difference in inhibition zones, with both the tincture and control showing a 4mm zone.
However, for S. aureus, the P-value was 0.003 ($P \leq 0.05$), which was statistically significant, leading to the rejection of the null hypothesis (H0). This indicated a significant difference in the inhibition zones, with the tincture

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producing a 12mm zone of inhibition compared to the 1mm zone seen with the control.

E. lysistemon tincture (60% Ethanol)

The antimicrobial activity of this tincture was also assessed against E. coli, P. aeruginosa, and S. aureus.
For E. coli, the P-value was 0.11 ($P \geq 0.05$), indicating no significant difference in the inhibition zones between the tincture and the control, both measuring 4mm.
For P. aeruginosa, the P-value was 0.007 ($P \leq 0.05$), which was statistically significant, leading to the rejection of the null hypothesis (H0). However, the ethanol control exhibited a larger inhibition zone (8mm) compared to the tincture (4mm), suggesting that the extract was not effective.
For S. aureus, the P-value was 0.005 ($P \leq 0.05$), also significant, resulting in the rejection of the null hypothesis. In this case, the tincture produced an 8mm zone of inhibition, significantly larger than the 2mm zone observed with the control, indicating some level of antimicrobial activity against S. aureus.

P. guajava tincture (60% ethanol)

The antimicrobial activity of this tincture was similarly evaluated against E. coli, P. aeruginosa, and S. aureus.
For E. coli, the P-value was 0.002 ($P \leq 0.05$), which was statistically significant, leading to the rejection of the null hypothesis (H0). However, the ethanol control showed a larger inhibition zone (4mm) compared to the tincture, which had no measurable inhibition (0mm), indicating that the extract was not effective.
For P. aeruginosa, the P-value was 0.001 ($P \leq 0.05$), also statistically significant, resulting in the rejection of the null hypothesis. Like E. coli, the ethanol control exhibited a larger inhibition zone (4mm), while the tincture showed no inhibition (0mm), further suggesting the ineffectiveness of the extract.
For S. aureus, the P-value was 0.003 ($P \leq 0.05$), indicating a significant difference in inhibition zones, and leading to the rejection of the null hypothesis. In this case, the tincture produced a 10mm zone of inhibition, which was significantly larger than the 1mm zone observed with the control, demonstrating some antimicrobial activity against S. aureus.

General observations

The results of the Kirby-Bauer Antimicrobial Sensitivity Test revealed varying degrees of efficacy among the tinctures, showing species-specific antimicrobial activity.
For E. coli, only the P. guajava tincture exhibited significant inhibition; however, the zone of inhibition was smaller compared to the ethanol control. This suggests that the extract is not effective against E. coli.
For P. aeruginosa, both the E. lysistemon and P. guajava tinctures showed significant differences in inhibition zones. However, both tinctures demonstrated reduced or no inhibition when compared to the 60% ethanol control, which had a larger zone of inhibition. This indicates that neither extract is effective against P. aeruginosa.
For S. aureus, all tinctures exhibited significantly greater inhibition than the control, with A. afra being the most effective. This suggests that the extracts are more potent against S. aureus compared to E. coli and P. aeruginosa.
In summary, the Kirby-Bauer Antimicrobial Sensitivity Test results highlight that the tinctures show varying degrees of effectiveness depending on the bacterial species, with stronger activity observed against S. aureus than against E. coli or P. aeruginosa.

Minimum Inhibitory Concentration (MIC) of Ethanolic Extracts Against *Staphylococcus aureus*

The ethanolic extracts of *Artemisia afra* (AA), *Psidium guajava* (PG), and *Erythrina lysistemon* (EL) were evaluated for their antimicrobial activity against *S. aureus* using MIC and MBC assays. The original concentration of each extract was 333.3 mg/mL, and serial dilutions were tested.

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Extract	Dilution	Concentration (mg/mL)	Growth (%)	No Growth (%)
A. afra	1:2	166.65	16.67	83.33
	1:4	83.33	33.33	66.67
	1:8	41.66	66.67	33.33
	≥1:16	≤20.83	100	0
P. guajava	1:2-1:32	166.65-10.42	0	100
	1:64-1:128	5.21-2.60	33.33	66.67
	1:256	1.30	100	0
E. lysistemon	1:2-1:8	166.65-41.66	0	100
	1:16-1:32	20.83-10.42	16.67	83.33
	1:64	5.21	83.33	16.67
	≥1:128	≤2.60	100	0

Key Findings:
P. guajava showed the strongest inhibition (100% up to 10.42 mg/mL)
E. lysistemon had intermediate activity (83.33% inhibition at 10.42 mg/mL)
A. afra required higher concentrations for inhibition (83.33% at 166.65 mg/mL)

Controls

60% ethanol control showed 100% growth in all tests.

Discussion

This study evaluated the antimicrobial activity of water and ethanolic extracts from *A. afra*, *E. lysistemon*, and *P. guajava* against *E. coli*, *P. aeruginosa*, and *S. aureus*. The results revealed significant variations in antimicrobial efficacy depending on the plant species, extraction method, and bacterial strain.

Water extracts showed no antibacterial activity, suggesting that water may not be an effective solvent for extracting bioactive compounds from these plants. This aligns with previous research indicating that many plant-derived antimicrobial compounds require organic solvents for efficient extraction (Moodley et al., 2021a). In contrast, ethanolic extracts exhibited antimicrobial activity, particularly against *S. aureus* (Gram-positive), but were ineffective against *E. coli* and *P. aeruginosa* (both Gram-negative). The higher p-values for *A. afra*, *E. lysistemon*, and *P. guajava* against *E. coli* and *P. aeruginosa* suggest that the ethanol controls produced larger inhibition zones than the plant extracts, indicating minimal antimicrobial effect (Ntie-Kang et al., 2022).

The differential activity observed between *S. aureus* and Gram-negative bacteria is consistent with the structural resistance mechanisms of Gram-negative bacteria, which possess an outer membrane that restricts the penetration of antimicrobial agents (Silhavy et al., 2023). This could explain the lack of inhibition against *E. coli* and *P. aeruginosa* in this study.

Overall, the findings suggest that while ethanol is a more effective solvent than water for extracting antimicrobial compounds, *P. guajava* demonstrated the most promising activity, particularly against *S. aureus*. The antimicrobial

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efficacy of *Psidium guajava* leaf extracts against *Staphylococcus aureus* in this study aligns with findings by Nair et al. (2020), who reported a minimum inhibitory concentration (MIC) of **9.8 mg/mL** for guava leaf extracts. However, while Nair et al. observed mild activity in aqueous extracts, our study recorded no significant inhibition, possibly due to differences in extraction duration (14 days vs. 10 days in our protocol). This discrepancy suggests that prolonged extraction may enhance the release of bioactive compounds, influencing antimicrobial potency.

Similarly, the moderate antibacterial activity of *Artemisia afra* extracts observed in our study supports previous research by Muleya et al. (2019). However, our ethanol-based extracts demonstrated superior efficacy compared to their hexane-derived preparations, reinforcing the critical role of solvent polarity in bioactive compound extraction. Ethanol's ability to solubilize a broader range of phytochemicals likely contributed to this enhanced performance, highlighting the importance of solvent selection in phytopharmacological studies.

These results highlight the need for further optimization of extraction methods and the identification of the active compounds responsible for the observed antimicrobial effects (Kuete & Efferth, 2023).

Generalizability

The findings of this study suggest that *A. afra*, *E. lysistemon*, and *P. guajava* extracts exhibit promising in vitro antimicrobial activity against Gram-positive bacteria, supporting their potential use in complementary therapies for such infections.

Conclusion

This study evaluated the antibacterial activity of A. afra, E. lysistemon, and P. guajava extracts against E. coli, P. aeruginosa, and S. aureus. The findings indicate that all three plant species exhibit antibacterial properties, though their efficacy varies depending on the bacterial strain. While some bacteria demonstrated resistance, others were susceptible, highlighting the strain-specific responses to plant-derived antimicrobials. Additionally, the extraction method significantly influenced the antibacterial potency, with ethanolic extracts showing greater activity than aqueous extracts.

The potential for developing plant-based antimicrobial agents from these species is promising, as natural compounds often present fewer side effects compared to synthetic antibiotics (Atanasov et al., 2021). However, further research is necessary to isolate and characterize the bioactive compounds responsible for the observed effects and to determine their full antimicrobial spectrum.

Limitations

The study's findings should be interpreted in consideration of several key limitations. First, the exclusive use of ATCC reference strains rather than clinically isolated bacterial samples may limit the ecological validity of the results, as these standardized strains may not fully represent the antibiotic resistance profiles and virulence factors found in natural infection settings. Second, the in vitro nature of the experiments, while valuable for preliminary screening, cannot account for the complex host-pathogen interactions, pharmacokinetics, and potential toxicity that would be observed in living systems. Third, variations in extraction methodologies, including differences in solvent systems, extraction durations, and plant material preparation, may influence both the composition and concentration of bioactive compounds, potentially affecting the reproducibility of results across different laboratory settings. Finally, the single-timepoint assessment of antimicrobial activity provides only a snapshot of efficacy and may not reflect potential adaptive resistance development or time-dependent pharmacodynamics. These limitations underscore the need for future research to incorporate clinical isolates, in vivo validation studies, standardized extraction protocols, and time-kill kinetic analyses, thereby strengthening the translational potential of these botanical interventions for infectious disease management.

Recommendations

Given the promising yet variable antibacterial activity observed in this study, several key avenues for further investigation emerge. First, advancing these findings into in vivo studies and clinical trials is essential to validate the therapeutic potential and safety of A. afra, E. lysistemon, and P. guajava extracts. While in vitro results provide foundational insights, animal models and human trials are critical to assess bioavailability, toxicity, and real-world efficacy, particularly in the context of rising antibiotic resistance (Newman & Cragg, 2023). Second, optimizing extraction protocols could significantly enhance the recovery of bioactive compounds. While ethanol proved more effective than water in this study, alternative solvents, such as glycerine for polar compounds or acetone for non-polar constituents, may improve yield and potency, as demonstrated in recent phytochemical studies (Tariq et al., 2022). Third,

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exploring synergistic interactions between these plant extracts and conventional antibiotics could uncover novel combination therapies, especially against resilient pathogens like Staphylococcus aureus. Research into plant-drug synergies has gained traction as a strategy to combat multidrug-resistant infections (Chassagne et al., 2023). Finally, comprehensive phytochemical profiling using advanced techniques (e.g., HPLC-MS, GC-MS, and NMR) is needed to isolate and characterize the specific compounds responsible for antimicrobial activity. Such work would not only clarify mechanisms of action but also guide standardization for future drug development (Kuete, 2023). Collectively, these steps would bridge the gap between traditional medicinal knowledge and evidence-based applications, offering sustainable alternatives in the face of global antimicrobial resistance.

Author contributions

Dr NW Nsele conducted the experiments, analysed the data, and drafted the manuscript.

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List of abbreviations

AA: Artemisia afra
AMR: Antimicrobial Resistance
ATCC: American Type Culture Collection
CLSI: Clinical and Laboratory Standards Institute
E. coli: Escherichia coli
EL: Erythrina lysistemon
EUCAST: European Committee on Antimicrobial Susceptibility Testing
GHP: German Homeopathic Pharmacopoeia
HIV/AIDS: Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome
HPLC-MS: High-Performance Liquid Chromatography-Mass Spectrometry
MBC: Minimum Bactericidal Concentration
MDR: Multidrug-Resistant
MIC: Minimum Inhibitory Concentration
NB: Nutrient Broth
NMR: Nuclear Magnetic Resonance
P. aeruginosa: Pseudomonas aeruginosa
PG: Psidium guajava
S. aureus: Staphylococcus aureus
WHO: World Health Organization

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Conflict of interest

The authors declare no conflicts of interest.

Data availability

Data are available upon reasonable request.

Author biographies

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