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# Synergistic effects of *Psidium guajava* and procaine penicillin on MRSA, MSSA and *E. faecalis*

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**Background:** Antibiotic resistance is a significant public health challenge, exacerbated by resistant strains such as methicillin-resistant *Staphylococcus aureus* (MRSA).

**Aim:** This study investigates the synergistic antibacterial effects of *Psidium guajava* aqueous extract combined with procaine penicillin against MRSA, methicillin-sensitive *S. aureus* (MSSA) and *Enterococcus faecalis*.

**Setting:** In-vitro study conducted under controlled laboratory settings at the Mangosuthu University of Technology, Department of Biomedical Sciences, Kwa-Zulu Natal, South Africa.

**Methods:** *Psidium guajava* leaves were collected and prepared according to the German Homeopathic Pharmacopoeia, while procaine penicillin was diluted for testing. The combination treatment was evaluated using the Kirby–Bauer Antimicrobial Sensitivity Test and Minimum Inhibitory Concentration (MIC) assays.

**Results:** Results showed that the combination of *P. guajava* extract and procaine penicillin significantly enhanced the antibacterial activity against MRSA, as evidenced by larger zones of inhibition and lower MIC values compared to *P. guajava* extract and procaine penicillin on their own. However, no significant difference was observed against MSSA and *E. faecalis*.

**Conclusion:** The findings suggest that *P. guajava* extract could potentiate the efficacy of procaine penicillin against MRSA, providing a potential alternative strategy to combat antibiotic-resistant bacteria.

**Contribution:** The study revealed that *P. guajava* aqueous extract enhances the antibacterial efficacy of procaine penicillin specifically against MRSA, suggesting a potential alternative treatment strategy for antibiotic-resistant infections, validated through robust methods and highlighting the need for further research to understand the mechanisms and clinical applications.

**Keywords:** methicillin-resistant *Staphylococcus aureus*; *Psidium guajava*; procaine penicillin; antibiotic resistance; synergy.

## Introduction

Antibiotic resistance is a major worldwide health issue, aggravated by developing resistant strains such as methicillin-resistant *Staphylococcus aureus* (MRSA), which defy traditional therapies (Radebe 2017; Salam et al. 2023). The pathogenicity of these organisms is largely because of the host factors and virulence factors, including various enzymes and toxins produced by the microorganisms (Salam et al. 2023). Antibiotic-resistant diseases cause roughly 700 000 fatalities worldwide each year, highlighting the critical need for novel therapeutic solutions (Mendelson et al. 2024). This issue not only increases morbidity and death but it also raises healthcare expenditure, underscoring the crucial need of collaborative efforts to address antimicrobial resistance (Coque et al. 2023; Nsele & Thembane 2023). Traditional medicinal plants play a significant role in drug discovery and therapy in areas with limited healthcare resources, providing possible paths for reducing antibiotic resistance (Mathobela 2016; Thembane 2023). Despite early scepticism, subsequent research has shown that plant-derived compounds can enhance the efficiency of conventional antibiotics (Vaou et al. 2021). *Psidium guajava*, renowned for its rich phytochemical profile, holds promise as an antimicrobial agent (Thembane et al. 2024). Meanwhile, procaine penicillin remains a cornerstone antibiotic, despite challenges posed by resistant strains such as MRSA and *Enterococcus faecalis* (Abed et al. 2024; Khwela, Thembane & Nsele 2023; Lima et al. 2020; Nataraj & Mallappa 2021). By exploring novel combinations of these agents, this research aims to advance antimicrobial strategies against rising resistance and contribute to improved public health outcomes. Antibiotic resistance represents a major global health concern, particularly in Africa, where limited healthcare facilities

exacerbate its impact (Kariuki et al. 2022). The high healthcare costs associated with managing antibiotic-resistant infections further strain already scarce resources and infrastructure in many African countries (Murray et al. 2022). The proliferation of antibiotic-resistant bacteria such as MRSA, methicillin-sensitive *Staphylococcus aureus* (MSSA) and *E. faecalis* leads to devastating, often incurable diseases (Nandhini et al. 2022). Recent studies highlight increasing resistance levels, limiting treatment options and contributing to higher fatality rates (Allel et al. 2023). This trend necessitates urgent exploration of alternative therapeutic strategies (Rasheed & Hussein 2021).

Given the severity of antibiotic resistance and the constraints of current treatment options, investigating the efficacy of traditional medicinal plants is crucial. This research aims to evaluate the combined impact of *P. guajava* aqueous extract and procaine penicillin on MRSA, MSSA and *E. faecalis*. Exploring these alternative treatments is imperative for mitigating the growing threat of antibiotic resistance and improving public health outcomes effectively.

# Research methods and design

## Preparation of *Psidium guajava* extract

The *P. guajava* aqueous extract was prepared according to an adjusted method HAB 3a of the German Homeopathic Pharmacopoeia to ensure consistency (Benyunes 2005). Fresh *P. guajava* leaves were collected from Silverglen Nature Reserve in Chatsworth, KwaZulu-Natal, South Africa. The plants were collected early in the morning (8 am) because the cells are extra active at this time. The plant was identified by the resident horticulturalist of the nature reserve and the voucher number was deposited and stored in the nature reserve library. The plant material was minced immediately in an electrical mincer and then weighed into a glass jar. Three parts of distilled water (150 mL) were added to one part of minced plant material (50 g) resulting in 1:3 ratio. The mixture was shaken for 5 min and then left in a glass jar for 14 days with mixing performed once a day. Thereafter, it was pressed through 100% cotton and filtered through a membrane filter (Singh 2004) and stored in 100 mL glass containers at 2ºC – 8ºC until use to prevent microbial contamination.

## Preparation of procaine penicillin

Procaine penicillin dilutions were prepared by dissolving the stock concentrate in sterile distilled water in the ratio 1:3 and used for antibacterial screening as well as minimum inhibitory concentration (MIC) testing. For MIC, serial dilutions of the antibiotic solution were prepared using sterile distilled water with dilutions ranging from 1:2 up to 1:16. All MIC ranges were according to the National Committee for Clinical Laboratory Standard guidelines (Kiehlbauch et al. 2000).

## Preparation of *Psidium guajava* aqueous extract and procaine penicillin combination

To prepare the combination treatment, the prepared procaine penicillin was mixed with the *P. guajava* extract in a 1:1 volume ratio. Five millimetres of procaine penicillin was mixed with 5 mL of *P. guajava* aqueous extract. The combination was utilised for both the antibacterial screening and MIC testing. The combination dilutions were prepared by way of serial dilutions for MIC testing, using sterile distilled water. The dilutions ranged from 1:2 to 1:16. All MIC ranges were according to the National Committee for Clinical Laboratory Standard guidelines. The prepared combination treatment was used immediately after preparation.

## Antibacterial sensitivity test (screening)

The Kirby–Bauer Antimicrobial Sensitivity Test was used as a screening test to assess the antimicrobial efficacy of *P. guajava* extract and procaine penicillin on their own and in combination with each another. American Type Culture Collection (ATCC) strains of MRSA (ATCC number: 33591), MSSA (ATCC number: 25923) and *E. faecalis* (ATCC number: 29212), were grown in nutrient broth until they reached the logarithmic phase. Inoculum containing 1 × 106 colony forming units (CFU) per millilitre (mL) was introduced onto the surface of Mueller Hinton (MH) Agar plates. Inoculums were prepared by comparing the bacterial suspension with 0.5 MacFarland turbidity standard. They were distributed evenly with a sterile swab onto the MH agar. Sterile blank discs, purchased from Davies Diagnostics (Batch number: 277653), were impregnated with either the plant extract, the antibiotic solution or the combination solution. The discs were carefully placed at the centre of the labelled plate of the bacterial suspension. The plates were incubated at 37°C and examined for the zone of inhibition after 8 h, and 24 h, respectively. Sterile distilled water was used as a negative control for all the microorganisms. Vancomycin was used as a positive control for MRSA and *E. faecalis* while Penicillin G was used as a positive control for MSSA. The control plates underwent the same processing steps as the test plates. The negative control showed no zone of inhibition. A zone of inhibition in the test sample was taken as positive. The zones were measured using a ruler and reported in millimetres (mm).

## Minimum inhibitory concentration

Agar dilution technique was used to determine the MIC of procaine penicillin on its own as compared to when in combination with the *P. guajava* aqueous extract. Serial two-fold dilutions of the two treatments were prepared using sterile nutrient broth, which was then incorporated into the MH agar plates. Each dilution was inoculated with a standardised suspension of the test bacteria and incubated at 37°C for 24 h. The MIC was identified as the lowest concentration that inhibited visible bacterial growth, providing a quantitative measure of antimicrobial efficacy. Colonies were counted on plates that had bacterial growth.

## Statistical procedures

*Psidium guajava* extract and procaine penicillin were tested on their own and in combination with each other six times against each bacterium. In total, the experiment was repeated

18 times for each treatment for reproducibility. The number of replicates was determined in consultation with the statistician. A Fisher's exact test was used to compare the number of replications that responded with the organism with each treatment. Only organisms that showed significant synergistic results with the screening test were further tested for MIC. Zones of inhibition data from the screening test and MIC assays were systematically recorded and analysed. Hypothesis testing, including *t*-tests and ANOVA, was performed to determine the statistical significance of the differences observed, with a significance level set at $p < 0.05$. This approach ensured the reliability and validity of the findings regarding the enhanced antimicrobial efficacy of the combination therapy.

### Ethical considerations

This article followed all ethical standards for research without direct contact with human or animal subjects.

## Results

This study was conducted to evaluate the *in vitro* antimicrobial efficacy of *P. guajava* extract in combination with procaine penicillin. Antibacterial sensitivity screening was tested using the Kirby–Bauer method while the MICs were conducted using the agar dilution technique.

### Antibacterial screening results

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 either procaine penicillin on its own or a combination of procaine penicillin and *P. guajava* aqueous extract. Statistically significant *p*-values are indicated by an asterisk (*) on Table 1.

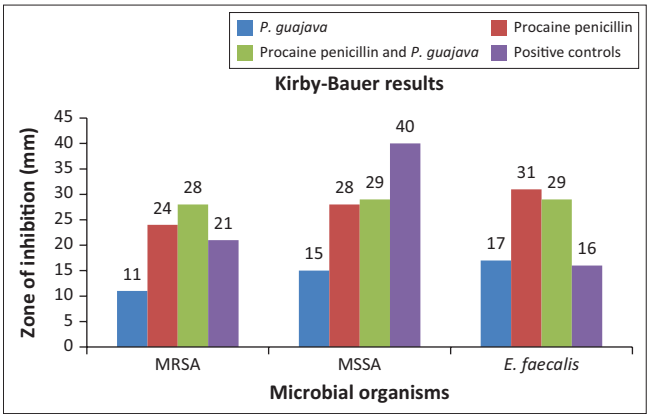
The *p*-values on Table 1 represent the statistical analysis of data obtained from the antibacterial screening results. The statistical values indicate the significant differences between values obtained during experimental work of this study. The *p*-value for MRSA (0.035) indicates a significant ($p < 0.05$) difference in diameter of zones of inhibition between the combination solution compared to the individual treatments.

### Minimum inhibitory concentration results

Table 2 presents the MIC results of *P. guajava* extract and procaine penicillin on their own and in combination with each other. The MIC was obtained by measuring visible growth of bacteria at different dilutions. Methicillin-resistant *Staphylococcus aureus* was the only organism tested for MIC as it was the only one that showed significant results during the screening test.

## Discussion

The purpose of this study was to determine whether *P. guajava* aqueous extract improves procaine penicillin's antibacterial activity against MRSA, MSSA and *E. faecalis*. The first phase




MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-sensitive *Staphylococcus aureus*; *E. faecalis*, *Enterococcus faecalis*; *P. guajava*, *Psidium guajava*.

**FIGURE 1:** Zones of inhibition for *Psidium guajava* and procaine penicillin on their own and in combination with each another on methicillin-resistant *Staphylococcus aureus*, methicillin-sensitive *Staphylococcus aureus* and *Enterococcus faecalis*.

**TABLE 1:** Analysis of anti-bacterial screening results.

| Bacteria | *P*-value |
|---|---|
| MRSA | 0.035* |
| MSSA | 0.449 |
| *E. faecalis* | 0.094 |

*, significant *p*-values.

MRSA, Methicillin-Resistant *Staphylococcus aureus*; MSSA, Methicillin-Sensitive *Staphylococcus aureus*; *E. faecalis*, *Enterococcus faecalis*.

**TABLE 2:** Minimum inhibitory concentration results for *Psidium guajava* extract and procaine penicillin on their own and in combination with each another.

| Dilution | MRSA | | |
|---|---|---|---|
| | *P. guajava* (%) | Procaine penicillin (%) | Procaine penicillin + *P. guajava* (%) |
| 1:2 | 0 | 0 | 0 |
| 1:4 | 100 | 100 | 0 |
| 1:8 | 100 | 100 | 0 |
| 1:16 | 100 | 100 | 0 |

MRSA, Methicillin-Resistant *Staphylococcus aureus*; *P. guajava*, *Psidium guajava*.

of the study assessed antibacterial efficacy by evaluating post-incubation zones of inhibition following exposure to either *P. guajava* extract and procaine penicillin on their own and in combination with each other. *Psidium guajava* extract alone showed notable activity against MSSA (15 mm) and *E. faecalis* (17 mm). When combined with procaine penicillin, it produced a larger zone of inhibition (28 mm) against MRSA, compared to *P. guajava* alone (11 mm) and procaine penicillin alone (24 mm). This indicates that *P. guajava* has potential both as a standalone antimicrobial and as an enhancer of antibiotic efficacy. However, against MSSA and *E. faecalis*, there was no significant difference in the diameter of the zones of inhibition between procaine penicillin on its own and in combination with *P. guajava* extract. This indicates that the combination may have a synergistic antibacterial impact against MRSA, with *P. guajava* aqueous extract perhaps increasing the efficacy of procaine penicillin. This is supported by a substantial *p*-value of 0.035 ($p < 0.05$) for MRSA, which suggests a significant statistical difference between procaine penicillin alone and in combination with *P. guajava* aqueous extract. In contrast, the *p*-values for MSSA and *E. faecalis* of 0.449 and 0.094, respectively, are deemed insignificant ($p > 0.05$).

Vancomycin that was used as a positive control MRSA and *E. faecalis* exhibited inhibition zones of 21 mm and 16 mm respectively, which were significantly smaller compared to the combination treatment. On the other hand, Penicillin G, the positive control for MSSA showed a greater inhibition zone of 40 mm, indicating greater sensitivity than the combination treatment.

The second phase of the study looked at the MIC of both treatments against pathogens with significant antibacterial screening results, notably MRSA. The results revealed that the combination totally inhibited bacterial growth at dilutions of 1:2, 1:4, 1:8 and 1:16, whereas procaine penicillin on its own inhibited bacterial growth at a dilution of 1:2 but was ineffective at dilutions of 1:4, 1:8 and 1:16. These data indicate that the combination of procaine penicillin and *P. guajava* aqueous extract has a potential antibacterial impact against MRSA at low concentrations.

Previous research has identified bioactive components of *P. guajava*, including phenols, flavonoids, tannins, alkaloids, saponins and triterpenes (Das & Goswami 2019; Thembane et al. 2024). Ngene et al. (2019) found that *P. guajava* leaf extracts have antibacterial activity against various gram-negative and gram-positive species, including *S. aureus*, but not *Pseudomonas aeruginosa*. Yahaya et al. (2019) also found that *P. guajava* leaf extracts suppressed the growth of *S. aureus*. Furthermore, research has indicated that mixing traditional medicine extracts with antibiotics improves antibiotic efficacy. For example, Sharaf et al. (2021) discovered synergistic effects of penicillin and potent Egyptian plant extracts against MRSA, with the combination having a substantially lower MIC than the individual treatments. Similarly, Nsele and Thembane (2023) discovered synergistic effects of mixing *Sutherlandia frutescens* plant extract and penicillin G, with the combination demonstrating much higher antibacterial activity than penicillin alone. Mitra, Hodiwala and Kar (2024) discovered that *P. guajava* leaf extract and antibiotics have synergistic effects against *Escherichia coli* (*E. coli*). The results of this study are consistent with previous research, indicating that medicinal plant extracts have the potential to improve the efficacy of conventional antibiotics.

The synergistic effects seen in the study against MRSA may be because of the combined antimicrobial actions of *P. guajava* and procaine penicillin. The bioactive compounds of *P. guajava* could improve the permeability of bacterial cell membranes, making it easier for procaine penicillin to enter and increase its inhibitory effect (Das & Goswami 2019). These interactions enhance the effectiveness of antibiotics and lower the chances of resistance by focusing on various bacterial pathways. This diverse strategy highlights the possibility of merging natural products with traditional antibiotics to create stronger therapies for antibiotic-resistant bacterial strains.

The importance of this finding is notable, particularly considering the challenges in treating MRSA infections (Mancuso et al. 2021). It is possible to anticipate a synergistic impact when dealing with MSSA instead of the MRSA strain. Moreover, the combined treatment could reduce the likelihood of bacteria developing resistance by focusing on multiple pathways. The research results emphasise the potential collaboration between *P. guajava* aqueous extract and procaine penicillin in combating MRSA. The combination therapy showed broader zones of inhibition against MRSA, suggesting greater antimicrobial effectiveness, while no notable changes were observed for MSSA or *E. faecalis*.

The results from MIC testing strongly support the potential of using combination therapy. The mixture was effective at lower doses indicating that *P. guajava* aqueous extract could reduce the necessary amount of procaine penicillin, especially beneficial for areas with limited resources. These results align with prior studies showing synergistic outcomes when antibiotics are paired with plant extracts. Enemchukwu et al. (2019) observed that *Vernonia amygdalina* and *Garcinia kola* extracts, when combined with tetracycline and metronidazole, showed synergistic effects against *Bacillus cereus* and *S. aureus*. In a similar manner, Mitra et al. (2024) emphasised the combined antimicrobial effects of *P. guajava* leaf extract with antibiotics on *E. coli*.

Herbal medicines in Africa often lack sufficient research and are poorly regulated. Traditional knowledge is typically passed down orally and lacks comprehensive documentation (Mills et al. 2005; Ngcobo et al. 2012). The findings of this study align with the ongoing efforts by respected experts in African traditional medicine in South Africa to enhance the treatment of both infectious and non-infectious diseases through the integration of traditional medicinal plants with conventional therapies (Gqaleni et al. 2011; Ngcobo et al. 2017).

## Limitations of the study

This study has various limitations that must be observed. Firstly, the findings are based on a small number of bacterial strains, which may limit their applicability to a broader spectrum of pathogens. Secondly, variations in the preparation and content of *P. guajava* extracts may affect reproducibility and consistency between studies and applications. The study also lacks precise mechanistic insights into the synergistic effects shown with *P. guajava* extract and procaine penicillin. Another disadvantage is that antimicrobial efficacy was examined *in vitro*, and the results may not be directly comparable to therapeutic efficacy *in vivo*. In addition, the possibility of bacterial resistance to the combined treatment over time was not assessed. Finally, the clinical relevance and safety of combining *P. guajava* extract with procaine penicillin have not been shown through clinical trials, which are required to validate these findings in real-world settings. Addressing these limitations in future studies is critical for verifying the potential therapeutic effects of this combination therapy.

## Conclusion

In conclusion, this study shows that medicinal plant extracts have the potential to improve the efficacy of conventional antibiotics. The combination of *P. guajava* aqueous extract with procaine penicillin showed substantial synergy against antibiotic-resistant bacteria – MRSA. This strategy shows potential for lowering treatment costs and increasing outcomes in resource-constrained places. The improved efficacy of these combinations provides a potential alternative to the limits of traditional antibiotic therapy. However, more research is needed to validate these findings in clinical settings, assure their safety and identify the chemical molecules causing the observed effects. These initiatives will help to create effective antibiotic treatments and tackle multidrug-resistant bacteria.

Future research should involve a broader and more diverse selection of bacterial strains to improve the generalisability of the findings. Detailed mechanistic studies are needed to understand the synergistic effects of *P. guajava* extract with procaine penicillin. *In vivo* investigations are required to assess the therapeutic efficacy and safety of this combination, which will be followed by human clinical trials. It is also critical to monitor the development of bacterial resistance over time. Furthermore, the efficacy of this combination therapy against a broader range of bacterial pathogens should be investigated to fully realise its therapeutic potential.

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### Competing interests

The authors declare that they have no financial or personal relationships that may have inappropriately influenced them in writing this article.

### Authors' contributions

The author's contributions to the article are as follows: N.W.N. was involved in conceptualisation, methodology, formal analysis, investigation, project administration and funding acquisition. K.P.M. and S.P.R. were responsible for formal analysis, visualisation, resources, reviewing and editing. N.T. was involved in formal analysis, original draft, visualisation, software, validation, resources, reviewing and editing.

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### Data availability

The authors confirm that the data supporting the findings of this study are available within the article.

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