

In vitro experimental study evaluating the antibacterial activity of aqueous extract of *Sutherlandia frutescens* against *Shigella flexneri*.

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ABSTRACT

Background

Shigella flexneri is a highly infectious bacterium responsible for gastrointestinal infections and has developed alarming levels of antibiotic resistance over the years. It particularly poses a threat in developing countries. Traditional medicinal plants offer potential alternatives to conventional antimicrobial therapies.

Aim

To investigate the antibacterial activity of the aqueous extract of *Sutherlandia frutescens* against *Shigella flexneri*.

Methods

This *in vitro* experimental study was conducted at the Mangosuthu University of Technology, Department of Biomedical Sciences, KwaZulu-Natal, South Africa, between June and December 2024. The aqueous extract of *Sutherlandia frutescens* was prepared using standard methods. The extract was assessed using the Kirby-Bauer Antimicrobial Sensitivity, Minimum Inhibitory Concentration (MIC), and Minimum Bactericidal Concentration (MBC) assays.

Results

Sutherlandia frutescens aqueous extract demonstrated statistically significant antimicrobial activity against *Shigella flexneri* with p-values less than 0.05. The aqueous extract showed moderate activity with high MIC (1.56 mg/ml) and equivalent MBC (1.56 mg/ml) values, suggesting balanced bacteriostatic and bactericidal activity.

Conclusion

The findings of the study show that *S. frutescens* exhibits promising antimicrobial activity against *Shigella flexneri*, supporting its potential as a natural antimicrobial agent for treating gastrointestinal infections.

Recommendations

Future studies should investigate the identity of the specific bioactive compounds responsible for the antibacterial activity of *S. frutescens*. Furthermore, studies should aim to evaluate the efficacy and safety of the *S. frutescens* aqueous extract using *in vivo* models.

Keywords: Aqueous extract, *Sutherlandia frutescens*, antimicrobial activity, *Shigella flexneri*, Minimum Inhibitory Concentration.

Submitted: 2025-03-14 **Accepted:** 2025-03-25 **Published:** 2025-03-31

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INTRODUCTION

Globally, healthcare systems are facing an escalating threat from antimicrobial resistance (AMR), which reduces the effectiveness of essential antimicrobial agents. This growing concern is largely attributed to excessive usage of these drugs and inadequate infection prevention and control practices (Hutchings, 2019). In response to the escalating threat of antimicrobial resistance, researchers have begun exploring alternative approaches to combat AMR, including the potential use of plant extracts. These

plants could have the potential to enhance the efficacy of antimicrobials against resistant microorganisms (Ashraf *et al.*, 2023).

One area of concern is the emergence of extensively drug-resistant (XDR) *Shigella* species, which are resistant to all frontline antibiotics. International studies have reported a worrying increase in antibiotic resistance among *Shigella* isolates, highlighting the need for enhanced monitoring and management (Ranjbar & Farahani, 2019). First detected in 2015, XDR *Shigella* strains have since become a

significant public health concern. Reports have indicated an alarming increase in their prevalence. According to the Centers for Disease Control and Prevention (CDC), XDR *Shigella* strains accounted for 5% of all tested samples in 2022 in the United States, a one percent increase from 2019 (CDC, 2023). This troubling trend calls for immediate action to address the growing threat of multidrug-resistant *Shigella* species (Cock & Vuuren, 2020).

Multidrug-resistant *Shigella* species have become a pressing public health issue, owing to their increased difficulty in treatment. Antimicrobial resistance has spread rapidly across the globe and is not limited by national borders or geographical distances. The World Health Organization prioritizes *Shigella* for new antibiotic development due to its alarming multidrug resistance (WHO, 2024). Multidrug-resistant *Shigella* strains demand immediate attention as treatments lose effectiveness (Ranjbar & Farahani, 2019). Globally, *Shigella* poses a threat to public health, impacting people across all age groups. The most vulnerable population is children in low and middle-income countries, who suffer significantly from the disease.

Traditional medicine has emerged as a crucial supplement to primary healthcare. It presents as a credible substitute for a considerable percentage of the world's population. Studies indicate that many people worldwide employ traditional medicine to address and manage a range of health issues (Ndhlovu *et al.*, 2021). In South Africa, traditional medicine is widely used, with approximately 27 million people utilizing it. This is largely due to the country's rich biodiversity, with an estimated 3000 to 4000 plant species possessing therapeutic value (Nsele & Moodley, 2022). The reliance is particularly evident in rural areas, where limited access to conventional healthcare facilities and transportation makes traditional medicine an essential alternative (Ndhlovu *et al.*, 2021).

One plant of interest in this context is *Sutherlandia frutescens* (*S. frutescens*), a hardy medicinal plant native to South Africa. Known for its adaptability to diverse environments, including drought-prone areas, *S. frutescens* is used in traditional medicine to treat a wide range of ailments, from colds and fevers to stomachaches and chickenpox (Korth, 2021). The plant is widely used in traditional medicine by various ethnic groups in South Africa. Its adaptogenic properties also support stress resilience. Scientific analysis reveals that the plant's bioactive compounds, including tannins, alkaloids, triterpenoids, and flavonoids, demonstrate potent antimicrobial activity against various disease-causing microorganisms (Sishuba, 2022).

Shigellosis, a severe form of bacterial gastroenteritis caused by *Shigella* species, remains a major global health challenge. While the illness typically resolves within a few days, complications can arise, particularly in cases of *Shigella flexneri* (*S. flexneri*) infections. About 3 percent of

those infected with *S. flexneri* can develop Reiter's syndrome. This is a painful autoimmune reaction that affects the joints, eyes, and urinary tract (Uddin *et al.*, 2023). It can persist for months or years after the initial *Shigella* infection has cleared. *Shigella* species are categorized into four main groups: *S. dysenteriae*, *S. flexneri*, *S. boydii*, and *S. sonnei*. The epidemiology of *Shigella* infections indicates that *S. flexneri* is predominantly found in low-income countries, while *S. sonnei* is the dominant species in industrialized countries (Garcia-Williams *et al.*, 2023). According to recent data, *Shigella* species are responsible for approximately 270 million cases of diarrhea annually. The global burden of *Shigella* infections is substantial, causing approximately 212,000 deaths annually. The majority (90%) of these deaths occurring

In developing countries, 64,000 of these deaths affect young children under five years old (Micoli *et al.*, 2022).

Recent estimates indicate that Shigella infections cause approximately 600,000 to 700,000 deaths globally each year, with the majority occurring in low- and middle-income countries (Garcia-Williams *et al.*, 2023). The global burden of shigellosis is particularly severe in Africa. A study by Nyarkoh and colleagues (2024) concluded that *S. flexneri* is the most prevalent species causing shigellosis in Africa, followed by *S. sonnei*. Among the *Shigella* species, *S. flexneri* is the most deadly and is responsible for the majority of shigellosis-related deaths. Limited access to clean water, sanitation, and affordable antibiotics contributes to the high prevalence of *Shigella* infections in low-income regions where antibiotic resistance further complicates treatment (Micoli *et al.*, 2022).

For over a century, researchers have sought an effective vaccine against this debilitating disease. Despite numerous clinical trials assessing various vaccine candidates, none have been approved for use against *Shigella* (MacLennan *et al.*, 2022). The challenge in vaccine development is creating a multivalent vaccine effective against multiple bacterial serotypes. Also producing a robust immune response in children, who are significantly affected by the infection (MacLennan *et al.*, 2022). As a result, the need for effective, affordable treatment options remains critical. Given these complexities, the development of a vaccine for *Shigella* has become a global health priority. Investigating alternative therapies such as plant-based antimicrobials may offer valuable insights into the fight against the antimicrobial resistance of *Shigella* species.

RESEARCH METHODS AND DESIGN

Study design and setting

The current study was an *in vitro* experimental study conducted in a research laboratory at the Mangosuthu University of Technology, Department of Biomedical Sciences. The experiments were carried out between June and December of the year 2024.

Sample collection

The *S. frutescens* plant was collected from a sustainable farm, Lokenburg Ethnobotanicals, in the Western Cape of South Africa. The leaves of the plant were obtained in dried form. The drying and grinding of the plant leaves was facilitated by a reputable supplier, Intelezi African Herb, ensuring the material used for analysis was of high quality. This helped to ensure the accuracy and reliability of the results.

Extraction process

S. frutescens was prepared according to an adjusted method 3a of the German Homeopathic Pharmacopeia. A 1:4 ratio of the dried plant to distilled water was obtained by mixing 100 g of the dried plant powder with 400 ml of distilled water in a 1000 ml jar. The mixture was then shaken by hand for 5 minutes at room temperature until the powder fully dissolved. Afterward, it was left to macerate for 14 days at room temperature, with daily stirring for about 2 minutes. After maceration, the solution was strained using a gauze and then filtered through filter paper. The resulting liquid, known as the plant extract, was stored at 4°C for further use.

Distilled water

The distilled water was obtained from the Department of Biomedical Technology at Mangosuthu University of Technology.

Preparation of Media

Mueller Hinton agar from Davies Diagnostics was prepared as per the manufacturer's instructions for both the solid and nutrient broth agar. Thirty-eight grams of the respective Mueller Hinton powder was weighed using an analytical balance and placed into 1000 ml glass bottles. One liter of distilled water was then added to the mark of the glass bottle using a measuring cylinder. The contents of the bottle were mixed until the powder dissolved completely. The bottles were sterilized by autoclaving for 15 minutes at 121°C. The agar was then poured into agar plates to solidify for solid agar. For nutrient broth, the media was dispensed into bijou bottles before autoclaving for 15 minutes at 121°C. This process ensured that the nutrient broth was sterile and free of any microorganisms or other contaminants that could affect the results of the experiment.

Microbial Cultures

The *S. flexneri* microbial cultures were obtained from King Edward Hospital (ATCC 12021) and were maintained on chocolate agar at 4°C.

Bacterial Sensitivity Testing

To determine the sensitivity of *S. flexneri* to *S. frutescens* extracts, the method used was analogous to the modified Kirby Bauer Antimicrobial Sensitivity test procedure (Nsele, 2019). An inoculum of 1×10^6 colony forming units per millimeter was prepared and then placed onto a Mueller Hinton agar plate. The bacterial suspension was compared to that of a one McFarland turbidity standard to ensure that it was of the correct concentration. A sterile swab was then used to evenly inoculate and distribute the bacteria across the Mueller Hinton agar plate. Sterile antibiotic assay discs, which were previously soaked in the extracts and controls, were placed carefully in the center of the labeled plate. The plates were then incubated at 37°C for 18-24hr and examined for zones of inhibition. For the controls, Amikacin, supplied by Davies Diagnostics, was used as a positive control, and distilled water was used as a negative control.

Determination of Minimum inhibitory concentration (MIC)

Microtiter plates with 12 wells were used to determine MIC. A hundred microliters of the prepared broth was added to all 12 wells of the microtiter plates, and then 10 μ l of the extract (aqueous extract) was added to the first well. This was mixed well, and then 10 μ l was aspirated from the first well and transferred to the second. This process was repeated for all wells up until the last well in which the 10 μ l aspirated was discarded. Then, 20 μ l of the McFarland standard (organism) was added to all 12 wells of the microtiter plates, ensuring that mixing was done in each plate. The plate was then covered and incubated overnight at 37 °C for 18-24 hours.

Determination of Minimum Bactericidal Concentration (MBC)

The MBC was determined from the microtiter wells of MIC that had no growth during the MIC testing. These were inoculated on the Mueller Hinton plate to determine the MBC. The Mueller Hinton plate was divided into sections, inoculating loop was used to streak the plate from the aqueous extract that had no growth. The plate was incubated overnight at 37°C for 18-24hrs. The plate was inspected the next morning to determine the MBC for the aqueous extract.

Data analysis

The plant aqueous extract was tested five times per day for five days against the strain of *S. flexneri*. The average zone of inhibition produced (measured in millimeters), MIC, and MBC were recorded. The Kruskal-Wallis test was used to compare the zones of inhibition around the antibiotic discs as well as the minimum inhibitory concentration. To determine if samples come from the same distribution, one nonparametric alternative to a one-way ANOVA is the Kruskal-Wallis test (Ostertagova *et al.*, 2014). This test is a

two-sample Wilcoxon-Mann Whitney test. This test will compare if there is a statistically significant difference in the size of zones of inhibition between the antimicrobial agents and aid in determining which antimicrobial agent is most effective in inhibiting the growth of bacteria.

Ethical Consideration

This research study followed all ethical standards for research without direct contact with human or animal subjects. Ethical approval was granted by the Mangosuthu University of Technology research ethics committee on 28 February 2024 (REF: RD5/04/2024).

RESULTS

Effects of *S. frutescens* aqueous-based versus aqueous control on *S. flexneri*

The statistical analysis revealed a significant difference in the diameter of the zone of inhibition between the aqueous extract of *S. frutescens* (an average of 8 mm) and the water control against *S. flexneri*, with a P-value of <0.0001 . Since the P-value is below the significance level (α), the alternative hypothesis (H1) is accepted. This indicates that *S. frutescens* aqueous extract exhibited a significant antibacterial effect on *S. flexneri*. The zones of inhibition shown in Figure 1 of the Kirby Bauer Antimicrobial Sensitivity test support this finding.

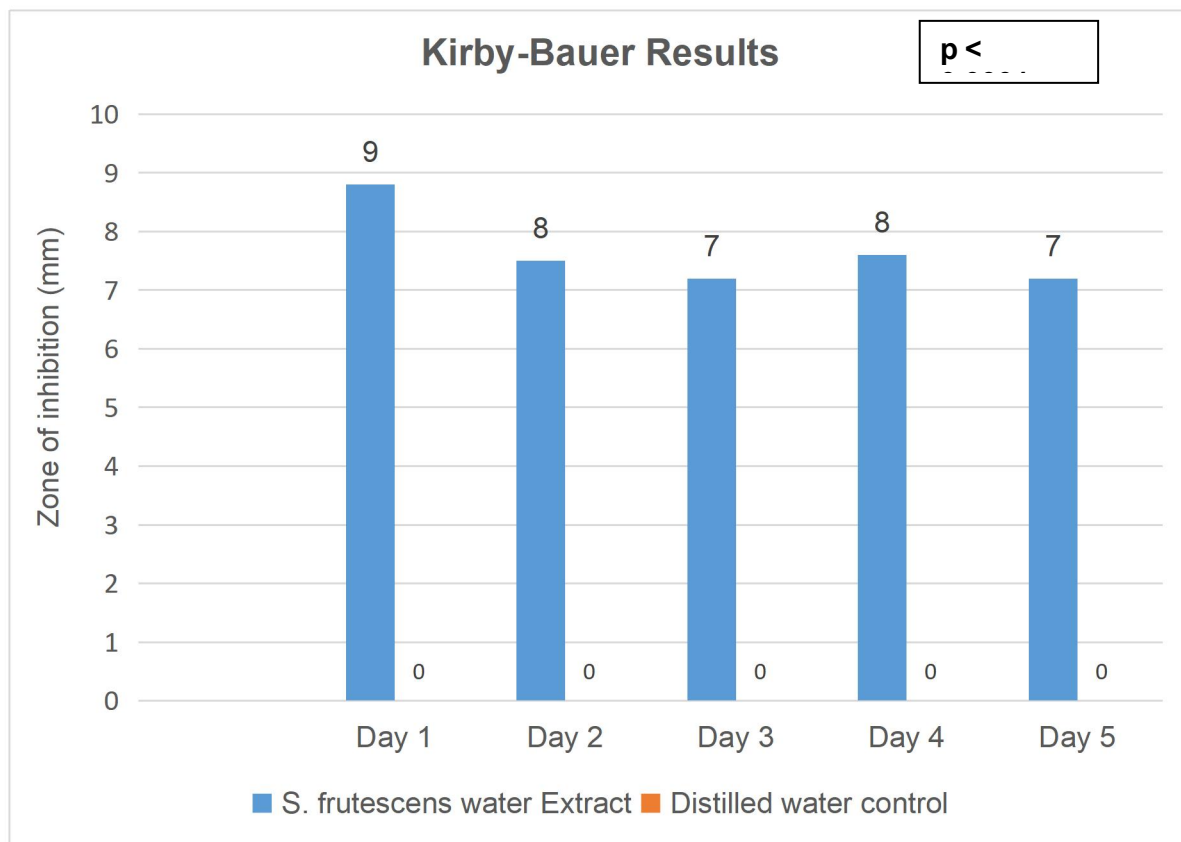


Figure 1: Zones of inhibition for *S. frutescens* aqueous extract against *S. flexneri*

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) for *S. frutescens* aqueous-based extract against bacteria (*S. flexneri*)

The MIC of *S. frutescens* aqueous extract against *S. flexneri* was determined to range from 12.50 mg/ml to 0.78 mg/ml, with no growth (NG) observed up to 0.78 mg/ml (Table 1). Growth (G) was observed starting at a concentration of 0.78 mg/ml. The MBC for the aqueous extract of *S. frutescens* was found to be 1.56 mg/ml.

Dilution (mg/ml)	<i>S. frutescens</i> aqueous extract
12.50	NG
6.25	NG
3.13	NG
1.56	NG
0.78	G
0.39	G
0.20	G
0.01	G
0.05	G

Table 1: MIC and MBC results for *S. frutescens* aqueous extract against *S. flexneri*.
NG: No Growth; G: Growth

DISCUSSION

The results of this study provide compelling evidence of the antibacterial activity of *S. frutescens* aqueous extract against *S. flexneri*. The antimicrobial efficacy of *S. frutescens* was evaluated using a water-based extraction method, which demonstrated a significant reduction in bacterial growth. Statistical analysis revealed a highly significant difference ($p < 0.0001$) between the aqueous extract and the control group, indicating that the antibacterial effect is highly unlikely to be due to chance. This result strongly supports the potential therapeutic application of *S. frutescens* as a treatment for infections caused by *S. flexneri*.

The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values obtained in this study further substantiate the antimicrobial potential of *S. frutescens* extract. The MIC, defined as the lowest concentration of antimicrobial agent required to inhibit visible microbial growth, was determined to be 1.56 mg/ml. Correspondingly, the MBC, the lowest concentration that kills 99% of the bacteria, was also observed at 1.56 mg/ml. These findings suggest that *S. frutescens* aqueous extract not only inhibits the growth of *S. flexneri* but also effectively kills the bacteria at relatively low concentrations. This aligns with the traditional use of *S. frutescens* as a medicinal plant for treating bacterial infections, including those caused by *Shigella* species.

The antibacterial activity observed in this study is consistent with previous research on the bioactive properties of *S. frutescens*. Specifically, compounds such as flavonoids, phenolic acids, and other secondary metabolites are likely contributors to the observed antimicrobial effects (Zonyane-Egbichi, 2022). These compounds are known for their antibacterial and

antioxidant properties. Their presence in *S. frutescens* is likely responsible for the inhibition and bactericidal effects in this study.

It is noteworthy that the use of water as a solvent for extracting active compounds from *S. frutescens* is not only scientifically relevant but also culturally significant. Aqueous-based extraction methods are commonly employed by traditional healers in Southern Africa, who have long relied on plant-based remedies for the treatment of various ailments (Ndhlovu *et al.*, 2021). This practice is beneficial not only because water is a non-toxic, readily available solvent but also because it may enhance the solubility and bioavailability of the plant's bioactive compounds. Previous studies have indicated that aqueous extracts can improve the stability and efficacy of active compounds, thereby maximizing their therapeutic potential (Cheng *et al.*, 2021). Moreover, the practical advantage of aqueous extracts makes them an appealing option for broader therapeutic applications, especially in resource-limited settings. In areas where access to pharmaceutical antibiotics is limited, the use of traditional water-based plant extracts like *S. frutescens* may offer an affordable and accessible alternative for managing bacterial infections like shigellosis caused by *S. flexneri*.

The findings of this study provide valuable insights regarding the antibacterial activity of *S. frutescens* aqueous extract against *S. flexneri*. However, the study was carried out under controlled laboratory conditions, which may not replicate the actuality of complex *in vivo* infections. While the extract demonstrated significant antimicrobial effects, factors such as bacterial strain variability, host immune response, and potential interactions with other compounds in the body could influence its effectiveness.

CONCLUSION

The antimicrobial evaluation of *S. frutescens* extracts demonstrated significant activity against *S. flexneri*, validating its traditional use in treating infections. The aqueous extract's balanced bacteriostatic and bactericidal properties highlight the potential of this plant as a natural antimicrobial agent. Future research should focus on optimizing extraction methods, investigating combination therapies, and evaluating *in vivo* efficacy to fully exhibit *S. frutescens*'s therapeutic potential. Additionally, elucidating the mechanisms of action and identifying the bioactive compounds responsible for observed antimicrobial activity will be crucial in advancing this plant's potential as a natural antimicrobial agent. This study contributes to expanding the body of evidence supporting the use of traditional medicinal plants in modern healthcare.

LIMITATIONS OF THE STUDY

While the findings of this study have demonstrated that the aqueous extract of *S. frutescens* has antibacterial activity, there are a few limitations to the study. Firstly, the study was conducted in a controlled laboratory setting using microbial cultures. *In vitro* studies may not accurately reflect the complex interactions within a living organism (Katerere & Eloff, 2005). Secondly, the study focused on a single bacterial strain, *S. flexneri*. Different bacterial strains may respond differently to *S. frutescens* extracts; thus, there's a narrow scope of results with limited generalizability to other pathogens (Vaou *et al.*, 2021). Thirdly, the study did not investigate the underlying mechanisms of antibacterial activity, limiting understanding of the extract's mode of action as well as the stability and shelf life of the extracts (Horablaga *et al.*, 2023).

RECOMMENDATIONS

Future research should explore the specific bioactive compounds responsible for the antibacterial activity, assess the plant's synergistic effects with conventional antibiotics, and optimize extraction methods to enhance potency. Additionally, further studies should aim to address the limitations of the study by investigating the antibacterial activity of *S. frutescens* aqueous extract against a range of bacterial strains, using *in vivo* models to evaluate its efficacy and safety and elucidating the mechanisms of action of the extract. These findings could support the potential development of *S. frutescens*-based therapeutic formulations for managing bacterial infections, particularly in regions with high antibiotic resistance.

LIST OF ABBREVIATIONS

AMR – Antimicrobial Resistance
 CDC – Centers for Disease Control and Prevention
 MBC – Minimum Bactericidal Concentration
 MIC – Minimum Inhibitory Concentration

XDR – Extensively Drug-Resistant

ACKNOWLEDGEMENT

This article is extracted from the full thesis that was written by the main author with the title "Antibacterial activity of *Sutherlandia frutescens* against *Shigella flexneri*".

Author's contribution

All authors of this article were involved in the whole process of writing this article, from the introduction to the references.

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Funding information

This research had no funding to support it.

Data availability

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

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Competing Interests

The authors have declared that no competing interests exist.

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Publisher Details:

Student's Journal of Health Research (SJHR)

(ISSN 2709-9997) Online

(ISSN 3006-1059) Print

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