Evaluation of the in Vitro Antimicrobial Efficacy of *Solanum indicum* Extract against *Staphylococcus aureus* Isolates: A Potential Alternative to Conventional Antibiotics

Nyoni-Phili Sizanobuhle L., Nyararai Yvonne O., Bhumure Ngonidzashe, Mupedziso Glossy

Department of Animal and Wildlife Sciences, Midlands State University, Gweru, Zimbabwe

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ABSTRACT

The increase of antimicrobial resistant pathogens within Zimbabwe's dairy industry has necessitated an urgent need for alternative treatments. This growing challenge directly threatens both animal health and farm productivity. Consequently, there is a critical and timely shift toward exploring plant-based solutions for mastitis management. These natural alternatives offer a promising avenue to improve dairy cow health while helping to preserve the efficacy of existing antimicrobial medicines for future use. This study evaluated the in vitro antimicrobial potential of *Solanum indicum* (Indian nightshade) leaf extract against *Staphylococcus aureus*, a primary causative agent of bovine mastitis. Milk samples from clinical routine testing were collected, and *S. aureus* was isolated and confirmed using selective media Mannitol Salt Agar, Gram staining, catalase, and coagulase tests. A methanol extract of *S. indicum* was prepared via cold maceration and tested at varying concentrations (1:0, 3:1, 1:1, 1:3 extract to solvent ratios) using the agar well diffusion method. Ampicillin and a methanol control served as positive and negative controls, respectively. The zones of inhibition obtained were 16.7mm for the highest concentration extract. The results demonstrated that the *S. indicum* extract exhibited intermediate antimicrobial activity against *S. aureus* isolates, with the highest concentration (1:0) showing a mean inhibition zone comparable to that of ampicillin. Statistical analysis revealed a significant difference ($p < 0.05$) between the extract's efficacy and the negative control. These findings suggest that *S. indicum* possesses potent bioactive compounds effective against *S. aureus*, supporting its potential as a sustainable, plant based alternative therapy for treatment of bovine mastitis infections in dairy cows.

Keywords: *Solanum indicum*, Antimicrobial resistance, Bovine mastitis, *Staphylococcus aureus*, Zone of inhibition.

INTRODUCTION

Bovine mastitis, defined as inflammation of the udder often indicated by high Somatic Cell Counts (SCC), is the most frequent and potentially fatal disease in dairy cattle, incurring substantial global economic losses through reduced milk yield, treatment costs, and premature culling (Touaitia et al, 2025; Sharun et al., 2021). The dairy industry, which is a critical component of many national economies, is severely impacted by the high prevalence of mastitis, with estimates suggesting that the disease can result in losses of up to 10% of total milk production. Furthermore, the treatment of mastitis is often costly and time-consuming, requiring significant investments in veterinary care, antibiotics, and other medications. In addition, the premature culling of affected animals can lead to additional economic losses, as well as a reduction in the overall quality and quantity of milk produced. In Zimbabwe, the challenge is compounded by the escalating prevalence of antimicrobial-resistant (AMR) pathogens, particularly *Staphylococcus aureus*, which complicates treatment and risks food safety through antibiotic residues in milk; the overuse of standard antibiotics like ampicillin has accelerated this AMR crisis, creating an urgent need for alternative antimicrobial agents (Acar & Moulin, 2012; Rushton et al., 2014; Gomes and Henriques, 2016).

Despite antibiotic treatment being a mainstay of control programs, the continuous reliance on readily available options like ampicillin underscores the necessity for new therapeutic alternatives. Medicinal plants, such as *Solanum indicum* L., which is traditionally used for infections and inflammation and contains antimicrobial

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phytoconstituents like alkaloids and flavonoids, represent a promising reservoir for novel bioactive compounds (Ajose et al, 2022; Belay, 2019; Chatterjee et al, 2024; Gamira, 2022). Additionally, the plant contains various phytoconstituents like solanine, solasornine, solamargine, solasidine, waxy material and fatty acids contributing to its therapeutic potential (Chatterjee et al, 2024) However, its specific efficacy against bovine mastitis pathogens, particularly in a standardized *in vitro* context, remains underexplored. This study therefore matters as it aims to systematically evaluate the antimicrobial activity of *S. indicum* extract against *S. aureus* isolates from dairy cows, bridging this research gap and potentially contributing to alternative treatments in the face of rising AMR.

MATERIALS AND METHODS

Study Site and Sample Collection

The study was conducted at Midlands State University, Gweru, Zimbabwe. Milk samples were aseptically collected from dairy cows at the university's Agricultural Practice section for routine lab analysis. First, the strip cup test was performed to determine the presence of clots or flakes or otherwise obviously abnormal secretions (Mohammed et al, 2019). Then, pre-dipping was performed, and one towel was used for each teat. After discarding the first three milk streams, the ends of the teats were scrubbed with 70% ethanol using cotton balls, and foremilk duplicate milk samples from the individual mammary quarters (approximately 4 mL in each vial) and composite milk samples (approximately 40 mL) were aseptically collected in sterile vials for microbiological analysis (Walker et al, 2010).

Ethical considerations

The milk samples were collected in strict compliance with animal welfare standards and institutional ethical guidelines for the use of animals in research. Procedures were designed to minimize stress and discomfort to the dairy cows. The collection was conducted for the essential purpose of routine health and quality analysis, which contributes to herd well-being and food safety.

Bacterial Isolation and Identification

Samples were serially diluted and cultured on Nutrient Agar for 24 hours at 37°C in triplicates. Single colonies from the plates were then sub-cultured on selective Mannitol Salt Agar. *Staphylococcus aureus* was confirmed through colony morphology (yellow colonies with yellow zones), *S. aureus* identifies as Gram positive cocci using Gram staining. Biochemical tests were done including catalase (positive for *S.aureus*) , coagulase tests (positive for *S.aureus*) (Oliver et al., 2004). Stock cultures were prepared in 30% glycerol and kept in -20°C till ready for use in the next set of experiments (Chauhan et al., 2020).

Preparation of Plant Extract

S. inducum fresh plants were identified by a professional botanist. Fresh *S. indicum* leaves were then picked in the local campus bushes, carefully selected, washed and oven dried at 50°C for 24 hours. 50g of leaves were pulverized separately, and subjected to cold maceration with 100ml of methanol for 72 hours in a dark room (Mendiratta et al., 2016). The filtrate was concentrated using a rotary evaporator. The crude extract was reconstituted in methanol to create four test concentrations: 1:0 (neat), 3:1, 1:1, and 1:3 (extract: solvent).

Antimicrobial Susceptibility Testing

The agar well diffusion method was employed (Akkina et al, 2020). Fresh *S. aureus* isolate was prepared to match standard 0.5 McFarland (Lalitha, 2004) and bacterial lawns were done by swabbing on the surface of MSA plates. Using a cork-borer, 6 mm wells were made on the solidified media. 20 µL of each *S. indicum* diluted extract (1:0 (neat), 3:1, 1:1, and 1:3) was placed in a labelled well. Ampicillin (1 mg/mL positive control), and methanol (negative control) were dispensed into wells on MSA. Plates were incubated at 37°C for 24 hours. The antimicrobial activity was measured as the mean diameter (mm) of the zone of inhibition (ZOI), including the well diameter (Song, 2023).

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Data Analysis

Data was analysed using one-way Analysis of Variance (ANOVA) followed by a post-hoc test (e.g., Tukey's HSD) to compare mean ZOIs across treatments. A p-value < 0.05 was considered statistically significant. Descriptive statistics (mean ± standard error of the mean) were calculated.

RESULTS

Isolation and Identification of *Staphylococcus aureus*

Staphylococcus aureus was isolated from routine testing milk samples from the University dairy facilities. The identification was based on a combination of morphological and biochemical characteristics including selected biochemical tests.

Biochemical Confirmation:

Five colonies were isolated and biochemical test results for *S. aureus* identification was done. Only three isolates confirmed positive, RTi1, RTi3, and RTi4.

	MSA	catalase	Gram stain	coagulase	Comment
RTi1	yellow colonies With yellow zones	+	purple cocci	+	presumed *S. aureus*
RTi2	red colonies With red zones	-	purple cocci	-	neg. for *S. aureus*
RTi3	yellow colonies With yellow zones	+	purple cocci	+	presumed *S. aureus*
RTi4	yellow colonies With yellow zones	+	purple cocci	+	presumed *S. aureus*
RTi5	red colonies With red zones	-	purple cocci	-	neg. for *S. aureus*

Key: (+) = Positive test result (-) = Negative test result RTi =routine test isolate

Antimicrobial Activity of *Solanum indicum* Extract

The antimicrobial efficacy of the methanolic *S. indicum* extract was evaluated using the agar well diffusion method. Activity was observed only at the highest concentration tested (1:0). Lower dilutions (3:1, 1:1, 1:3) showed no inhibitory effect on the *S.aureus* isolates. The standard antibiotic (Ampicillin) produced a significantly larger zone of inhibition, while the distilled water and methanol negative control showed no activity.

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Figure 1: *S. aureus* isolates on Mannitol Salt Agar with zones of inhibition (ZOI) of 16.7mm on the 100% (1:0) agar well. Ampicillin shows the largest ZOI of 36.5mm on the Agar well diffusion method.

Table 2: Mean Zone of Inhibition (ZOI) against *Staphylococcus aureus*

Treatment (Extract: Solvent Ratio)	Mean ZOI (mm) ± SEM
S. indicum (1:0)	16.75 ± 0.45 a
(3:1)	0.00 ± 0.00 b
(1:1)	0.00 ± 0.00 b
(1:3)	0.00 ± 0.00 b
Ampicillin (Standard)	39.78 ± 0.54 c
Distilled Water (Control)	0.00 ± 0.00 b

SEM: Standard Error of the Mean (n=3). Different superscript letters (a, b, c) indicate statistically significant differences ($p < 0.05$).

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Comparative Antimicrobial Efficacy of *S. indicum* Extract and Ampicillin (1mg/ml)

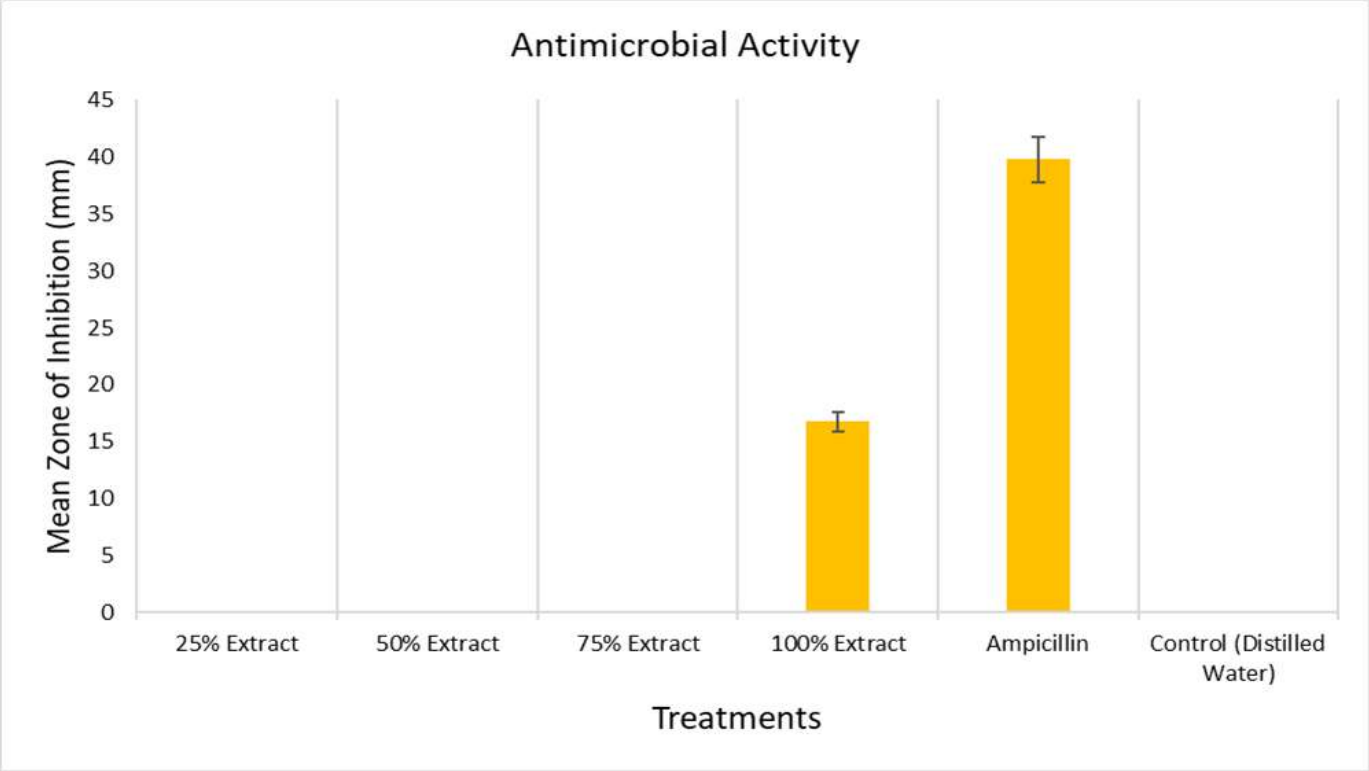

Figure 2: Ampicillin had the zone of 37mm which showed high antibiotic activity on the *S. aureus* isolates. The extract (1:0) of the *S. indicum* had a zone of inhibition of 16.7mm indicating moderate antibiotic activity.

Statistical Analysis

One-way Analysis of Variance (ANOVA) revealed a statistically significant difference in the mean zones of inhibition among the treatment groups ($p < 0.05$). Due to the absence of inhibition zones for all but the neat extract (1:0) and Ampicillin, the significant difference is attributed to the superior efficacy of Ampicillin compared to the *S. indicum* extract, which itself showed a measurable but moderate effect.

DISCUSSION

This study evaluated the in vitro antibacterial potential of *Solanum indicum* against *Staphylococcus aureus*, a primary agent of bovine mastitis. The isolates were obtained from routine testing in dairy cows without symptoms of mastitis. The findings resonate with Arunkumar et al., 2009, in which routine tests may also have presence of *S. aureus* but not in alarming counts. The key finding is that a methanolic extract of *S. indicum* exhibited a concentration-dependent antibacterial effect, with activity detectable only at the highest tested concentration (100% crude extract), producing a mean inhibition zone of 16.7 mm. These results indicate preliminary inhibitory potential. Antibacterial effects of the standard susceptibility tests are 10µg and the desired effective zone of inhibition would be > 17mm, with a zone of inhibition falling in intermediate of 14-16mm, where the plant extract activity lies (Guar et al., 2023).

The moderate activity of the crude extract aligns with several studies on Solanum species, which attribute antimicrobial effects to alkaloids such as solasodine, solamargine), flavonoids, and tannins (Sbhatu and Abraha, 2020). These compounds can disrupt bacterial cell membranes, inhibit enzyme activity, or interfere with nucleic acid synthesis (Delbrouck et al, 2023). However, the requirement for a high concentration of *S.indicum*, suggests that either the active principles are present in low quantities in the crude material, or that the extraction protocol was suboptimal requiring use of other solvents or extraction methods, failing to solubilize or preserve key heat-labile compounds (Mohan & Kakkar 2020 ; Jayanthy et al., 2016). The use of methanol, while common, may not be the ideal solvent for the full spectrum of *S. indicum's* antimicrobial phytochemicals.

Ampicillin, a β-lactam antibiotic, inhibits cell wall synthesis with high specificity and potency. The plant extract's mechanism is likely more generalized (e.g., membrane disruption), which may be less efficient and require higher concentrations (Mohan et al., 2020). This underscores a critical challenge in phytotherapy: crude extracts often cannot match the targeted potency of refined pharmaceuticals. However, this does not negate their value. In the context of antimicrobial resistance (AMR), plant compounds offer novel structures that can serve as leads for new drug development or act synergistically with existing antibiotics to overcome resistance mechanisms (Bereda, G. ,2022).

The absence of activity at lower concentrations (3:1, 1:1, 1:3) indicates low or no presence of the active phytochemical compound. This has practical implications for potential therapeutic application, as achieving such high local concentrations in vivo within the mammary gland may be challenging and could raise toxicity concerns. Future work must therefore focus on bioassay-guided fractionation to isolate and concentrate the active moiety, which would likely enhance potency and inclusion of minimum inhibitory tests (Mendiratta et al.,2016; Sbhatu & Abraha, 2020).

CONCLUSION

This study confirms that *Solanum indicum* possesses in vitro intermediate antibacterial activity against *Staphylococcus aureus*, supporting its traditional use in infection management. However, this activity is possibly concentration-dependent and moderate. It only occurs at a high concentration of the crude methanolic extract and being substantially inferior to the standard antibiotic Ampicillin. The findings indicate that while *S. indicum* contains bioactive phytonutrients with antimicrobial properties, its crude form has less inhibitory potential. Its value lies primarily as a potential source of novel compounds for future antimicrobial development and as a candidate for integration into complementary treatment strategies, especially in the face of growing antibiotic resistance in dairy livestock.

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