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Comparative Antibacterial Effects of Selected Ethnomedicinal Plants on *Escherichia coli* and *Staphylococcus aureus* Isolated from Mastitic Cow Milk in West Arsi Zone, Ethiopia

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Abstract

Objective: The study aimed to evaluate the antimicrobial activity of selected medicinal plants, *Datura stramonium* (DS), *Akokanthera schimperi* (AS), and *Balanites aegyptiaca* (BA) against *Escherichia coli*(*E. coli*) and *Staphylococcus aureus* (*S. aureus*) isolated from mastitic cow milk in the West Arsi Zone, Ethiopia. These bacterial pathogens are known contributors to bovine mastitis and often exhibit multidrug resistance (MDR). **Methods**: A laboratory-based experimental study was conducted using clinical isolates and American Type Culture Collection (ATCC) standard strains of *E. coli* and *S. aureus*. Ethanolic extracts of the three medicinal plants were prepared and tested for antibacterial efficacy using the agar disc diffusion method at a concentration of 350 mg/mL. The diameter of inhibition zones was measured to assess the antimicrobial activity of each plant extract. **Results**: All three plant extracts exhibited antimicrobial activity against the tested bacterial strains. *Akokanthera schimperi* (AS) demonstrated the highest antibacterial effect, producing the largest zones of inhibition against both *E. coli* and *S. aureus* (18.8 ± 0.03 mm for *E. coli*, 16.1 ± 0.26 mm for *S. aureus*), followed by *Datura stramonium* (DS) (14.8 ± 0.05 mm and 12.1 ± 0.17 mm respectively for *E. coli*, and *S. aureus*) and *Balanites aegyptiaca* (BA) (11.6 ± 0.3 and 7.1 ± 0.12 for *E. coli*, and *S. aureus* respectively). The results varied depending on the plant species and the bacterial strain tested. **Conclusion**: The findings indicate that traditional medicinal plants, particularly *Akokanthera schimperi* (AS), possess promising antibacterial properties against major mastitis-causing pathogens, *E. coli* and *S. aureus*. These results support the potential integration of ethnoveterinary medicine (EVM) into mastitis management strategies. Further studies, including phytochemical analysis and in vivo efficacy testing, are recommended.

Keywords: mastitis; *E. coli*; *S. aureus*; ethnoveterinary medicine (EVM); antibacterial activity; medicinal plants; Ethiopia

Introduction

Mastitis is a predominant disease in dairy cattle, primarily induced by bacterial pathogens such as *E. coli* and *S. aureus*, both of which are now extensively reported to show resistance to commonly used antibiotics, contributing to antimicrobial resistance (AMR) [1,2]. The growing AMR crisis, labeled a global health threat by the World Health Organization (WHO), has placed these two pathogens on the priority list for research and development of novel treatment options [3]. The increasing inefficacy of conventional antibiotics in treating mastitis not only exacerbates animal health problems but also threatens milk quality, food safety, and farm-level economic viability.

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In Ethiopia, where over 80% of the population relies on traditional medicine for both human and animal health care, the use of ethnomedicinal plants is a culturally entrenched and practically indispensable practice, especially in underserved rural areas [4]. Ethnoveterinary medicine (EVM) is recognized for offering cost-effective, accessible, and environmentally friendly alternatives for disease management [5–7]. Bioactive compounds found in medicinal plants such as alkaloids, flavonoids, and tannins have been shown to possess significant antimicrobial properties, suggesting their potential use against antibiotic-resistant pathogens [8].

Despite the richness of traditional knowledge, there remains a significant lack of scientific studies validating the antibacterial efficacy of medicinal plants specifically against mastitis-causing pathogens in Ethiopia. This study addresses that gap by systematically evaluating the antibacterial activity of three indigenous medicinal plants,*Datura stramonium* (DS), *Akokanthera schimperi* (AS), and *Balanites aegyptiaca* (BA), against both clinical and ATCC reference strains of *E. coli*and *S. aureus*,where the former ones are isolated from mastitic cow milk in the West Arsi Zone, Ethiopia.

The novelty of this study lies on its integration of traditional ethnoveterinary medicine (EVM) knowledge with laboratory-based scientific validation to tackle the escalating issue of antimicrobial resistance (AMR). While plant-based remedies are widely used by livestock keepers, few have undergone rigorous in vitro testing to substantiate their therapeutic claims, particularly in the context of bovine mastitis. By focusing on plants traditionally used for mastitis treatment and comparing their antibacterial effects with those of standard antibiotics, this research contributes novel, locally relevant data that could support the development of plant-based mastitis therapeutics. Ultimately, the study serves as a foundation for future pharmacological investigations and promotes a culturally grounded, sustainable response to the threat of AMR in the livestock sector.

Materials and Methods

Study Area and Design

A cross-sectional study was conducted between December 2023 and October 2024 in the West Arsi Zone, Oromia Region. A total of 200 lactating cows from various farms were diagnosed for clinical mastitis and screened using the California Mastitis Test (CMT) to detect subclinical mastitis. Out of these, 83 cows tested positive for mastitis. Milk samples from these cows were subjected to standard microbiological procedures. Bacterial identification was performed using selective agar culture media and standard biochemical tests. From these, 62 cows yielded isolates positive for either *E. coli* or *S. aureus*.

Plant Selection and Extract Preparation

The medicinal plants *Datura stramonium* (DS), *Akokanthera schimperi* (AS), and *Balanites aegyptiaca* (BA) were selected based on ethnoveterinary medicine (EVM) knowledge gathered from 40 farm owners and attendants in the study area. Structured interviews and direct observations were used to collect data regarding traditional use, plant parts utilized and perceived effectiveness for mastitis treatment. All respondents (100%) reported using these three plants to treat bovine mastitis.

Fresh plant materials, leaves of *Datura stramonium* (DS), roots of *Akokanthera schimperi* (AS), and fruits of *Balanites aegyptiaca* (BA)) were collected from natural habitats in the West Arsi Zone, Oromia Region, Ethiopia. Botanical identities were confirmed at the National Herbarium, Addis Ababa University, and voucher specimens were deposited:DS: AAU-HB-DS001, AS: AAU-HB-AS002, and BA: AAU-HB-BA003.

Plant materials were cleaned, shade-dried (22–25°C, 10–14 days), ground, and stored in airtight containers.Extraction followed the Harborne method [9], using 80% ethanol. 100 g of each powdered plant material was soaked in 500 mL ethanol for 72 h, filtered, concentrated using a rotary evaporator at 40°C, and stored at 4°C until use.

Antimicrobial Testing

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Antimicrobial activity was assessed using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar. Extracts were tested at 350 mg/mL on bacterial samples identified from the milk samples, as well as on the ATCC standard strains of *E. coli*and *S. aureus*obtained from Ethiopian Public Health Institute.Amoxicillin (20 µg/disk) served as the positive control; 5% dimethyl sulfoxide (DMSO) was used as a negative control. Inhibition zones were measured and interpreted per Clinical and Laboratory Standards Institute (CLSI) guidelines [10].

Results and Discussion

Bacterial Isolation and Ethnoveterinary Medicine (EVM) Survey

Among the 83 mastitis-positive cows, 62 were confirmed infected with either *E. coli* or *S. aureus*. All 40 farm attendants and dairy owners surveyed confirmed using medicinal plants, predominantly *Datura stramonium* (DS), *Akokanthera schimperi* (AS), and *Balanites aegyptiaca* (BA) for mastitis treatment, highlighting the reliance on ethnoveterinary medicine (EVM).

Antibacterial Activity of Plant Extracts

Disc diffusion results (Table 1) showed *Akokanthera schimperi* (AS) had the highest inhibition zones, followed by *Datura stramonium* (DS) and *Balanites aegyptiaca* (BA). Amoxicillin showed the greatest activity, confirming its standard efficacy. No inhibition was recorded for 5% DMSO.

Comparative Analysis

Table 1. Inhibition zone diameters (mm), activity ratings, and significance values.

Bacteria	Treatment	Mean ± SD (mm)	Activity	p-value
S. aureus (Clinical)	DS	12.1 ± 0.17	+	0.001**
	AS	16.1 ± 0.26	+++	0.003**
	BA	7.1 ± 0.12	+	0.045*
	Amoxicillin	20.1 ± 0.3	+++	0.0001**
	DMSO (5%)	0 ± 0	-	-
S. aureus (ATCC 29213)	DS	14.21 ± 0.1	++	0.0015**
	AS	17.3 ± 0.13	+++	0.002**
	BA	8.6 ± 0.11	+	0.04*
	Amoxicillin	22 ± 0.21	+++	0.00001**
	DMSO	0 ± 0	-	-
E. coli (Clinical)	DS	14.8 ± 0.05	++	0.001**
	AS	18.8 ± 0.03	+++	0.002**
	BA	11.6 ± 0.3	+	0.04*

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Bacteria	Treatment	Mean ± SD (mm)	Activity	p-value
	Amoxicillin	25.1 ± 0.07	++++	0.00001**
	DMSO	0 ± 0	-	-
E. coli (ATCC 25922)	DS	16.1 ± 0.04	+++	0.001**
	AS	20.5 ± 0.02	+++	0.002**
	BA	12.1 ± 0.2	+	0.03*
	Amoxicillin	26.2 ± 0.06	++++	0.00001**
	DMSO	0 ± 0	-	-

n = 62, *S. aureus*= 42, and E. *coli*= 20 * $p < 0.05$; ** $p < 0.01$.

Akokanthera schimperi (AS) demonstrated the highest activity (18.8 ± 0.03 mm for *E. coli*, 16.1 ± 0.26 mm for *S. aureus*), followed by *Datura stramonium* (DS) (14.8 ± 0.05 mm and 12.1 ± 0.17 mm respectively for the two bacteria isolates). *Balanites aegyptiaca* (BA) had weaker effects. These findings align with reports on antimicrobial properties of cardiac glycosides in *Akokanthera schimperi*(AS) and alkaloids in *Datura stramonium* (DS) [11,12]. Lower activity of *Balanites aegyptiaca*(BA)(11.6 ± 0.3 and 7.1 ± 0.12 for *E. coli*, and *S. aureus* respectively)may reflect phytochemical differences or extraction efficiency [13]. The study did not include phytochemical quantification, which is a limitation.

Conclusion

This study confirms the antibacterial potential of *Akokanthera schimperi* and *Datura stramonium* against *E. coli* and *S. aureus* isolated from mastitic cow milk. These findings validate their use in ethnoveterinary medicine (EVM) in Ethiopia. The comparatively lower efficacy of *Balanites aegyptiaca* suggests phytochemical variability. Further research should isolate active compounds and assess in vivo efficacy and safety.

Author Contributions: Abdela Edao Nura: Study design, plant collection, extracts preparation, and data collection. Behailu Assefa Wayou: Supervision, data analysis, manuscript writing and correspondence. Hussien Bonso Ahimmed: Field coordination, sample collection, lab analysis. All authors reviewed and approved the final manuscript.

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Conflict of Interest: None declared.

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