Research Article

Evaluation of Herbal Remedies for Bovine Subclinical Mastitis: Impact on Milk Quality and Antimicrobial Activity

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Abstract | Bovine mastitis poses a significant challenge to the dairy industry, resulting in substantial economic losses. Subclinical mastitis, characterized by inconspicuous inflammation, is particularly prevalent and detrimentally impacts milk quality and yield. This study investigates the occurrence of subclinical mastitis within buffalo herds in Hyderabad's cattle colony, concentrating on discernible indicators of mastitis milk and the therapeutic potential of ethnoveterinary plant extracts. Among 120 milk samples tested using the California Mastitis Test (CMT), 66.66% were positive for subclinical mastitis. The antimicrobial susceptibility of Berberis lyceum, Capsicum annuum, and Asafoetida extracts was tested against Staphylococcus aureus and Streptococcus agalactiae using the disc diffusion method. The highest prevalence of subclinical mastitis was observed in 5-year-old buffaloes (83.33%) and those in their 4th parity (75.00%). Examination of milk properties revealed increased acidity, electrical conductivity, and specific gravity in mastitis-affected milk. Antimicrobial testing showed that Berberis lyceum exhibited inhibition zones of 1.5±0.57 mm and 1.6±1.10 mm, Capsicum annuum had 1.6±1.10 mm and 1.03±0.10 mm, and Asafoetida displayed 1.1±0.10 mm and 1.5±0.57 mm against S. aureus and S. agalactiae, respectively. These results indicate limited antibacterial efficacy of the herbal extracts. The study highlights the high prevalence of subclinical mastitis and the need for alternative therapeutic strategies. While herbal extracts exhibited weak antibacterial activity, further research is required to explore higher concentrations, synergistic effects, and potential formulations to improve therapeutic efficacy.

Keywords | Mastitis, Ethnoveterinary plant, Herbal therapy, Antimicrobial susceptibility, Dairy production

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INTRODUCTION

Bovine mastitis is a major economic burden on the dairy sector, primarily caused by bacterial infections (Adkins and Middleton, 2018). The disease encompasses subclinical and clinical forms, differing in symptom severity and visibility Subclinical mastitis, often asymptomatic and difficult to detect, is diagnosed by measuring somatic cell count (SCC) in milk, an indicator of udder inflammation (Hossain *et al*., 2017).Clinical mastitis, more conspicuous and severe, manifests as milk alterations, such as clots or flakes, along with udder infection and inflammation indicators like fever, redness, pain, and swelling (Adkins and Middleton, 2018). Both forms detrimentally affect milk quality and production, elevate costs for treatment and replacement, and compromise animal welfare (Halasa *et al*., 2007). Major bacterial pathogens of bovine mastitis include *Streptococcus* spp., *Staphylococcus aureus*, and *Corynebacterium bovis*, with *S. aureus* being particularly problematic due to its persistence and ability to cause chronic infections (Wente *et al*., 2020). Although antibiotics remain the primary treatment, their effectiveness is limited by bacterial resistance, udder tissue changes, and poor management practices. Additionally, antibiotic residues in milk pose health risks to consumers (Hossain *et al*., 2017; Halasa *et al*., 2007).

Furthermore, excessive antibiotic use poses a dual threat by fostering drug-resistant bacteria and introducing residues into milk and meat products, thereby endangering public health and the environment (Rug *et al*., 2010). Given these concerns, alternative treatments for bovine mastitis are imperative, necessitating solutions that are both safe and economically feasible. A promising avenue lies in natural products, particularly those endowed with antimicrobial properties like ethnoveterinary plant extracts. Recognizing the urgency of antimicrobial resistance, the World Health Organization (WHO) champions the incorporation of traditional medicines to combat this global health hazard (Hassan and Yousif, 2014). In this context, WHO encourages member countries to promote indigenous medicinal practices (DeJong *et al*., 2018), where traditional medicine involves utilizing diverse natural resources, such as plants and seaweeds, to address microbial-induced diseases. While traditional medicine's use waned with the advent of antibiotics, the bioactive compounds within natural products offer efficacious remedies with reduced risk of adverse effects for humans and animals (Saranraj *et al*., 2016; Kalidasan and Kanna, 2017). Various plants, including Black Cumin, Curcuma zeodharia, Curcuma manga, and Curcuma aeruginosa, have been historically employed to aid the healing of mammary gland parenchymal tissues (Nurdin and Arief, 2009; Nurdin *et al*., 2011). These herbs have long been employed to counter inflammation in humans, offering advantages of affordability, accessibility, and safety (Nurdin and Arief, 2009; Nurdin *et al*., 2011). Herbal preparations, rooted in centuries of traditional practice, have found favour among pastoralists and farmers for treating livestock ailments, presenting a potential source of novel pharmaceuticals (Lans *et al*., 2020). With this backdrop, the present study seeks to assess specific milk indicators' potential for early bovine subclinical mastitis detection, alongside exploring the therapeutic efficacy of herbal preparations for subclinical mastitis. Given the rising threat of antimicrobial resistance, the WHO encourages the integration of traditional herbal medicine in both human and veterinary healthcare, particularly in managing bacterial infections (DeJong *et al*., 2018; Hassan and Yousif, 2014) the present study aims to evaluate milk quality indicators for early detection of subclinical mastitis and assess the therapeutic potential of herbal extracts as alternative treatments.

MATERIALS AND METHODS

Sample collection

Thirty buffaloes were randomly selected from the cattle colony in Hyderabad for the assessment of subclinical mastitis (SCM). The sample size was determined based on previous prevalence studies and statistical considerations. A total of 120 milk samples were collected from all four quarters of the buffaloes. The California Mastitis Test (CMT) was used for the detection of SCM following the standardized protocol recommended by the National Mastitis Council (NMC, 2004). Each mammary quarter contributed 5 to 10 mL of milk, which was mixed with an equal volume of CMT reagent in a CMT paddle and gently agitated. Results were interpreted based on gel formation, with samples graded on a 0 to 3 scale. A score of ≥1+ was considered positive for subclinical mastitis. For comparison, milk samples from 10 clinically healthy buffaloes were also collected as controls. Positive CMT samples were aseptically transferred to sterile test tubes, stored in an icebox, and transported to the Central Veterinary Diagnostic Laboratory (CVDL), Tandojam for further analysis.

Physical characteristics of mastitis milk

To evaluate milk quality, pH, specific gravity, and acidity were measured.

pH Measurement: Milk pH was determined using a Microprocessor pH 211 meter with a glass electrode. The probe was immersed in the milk sample, and the constant stabilized reading was recorded. Specific Gravity: Measured using a pycnometer by weighing both distilled water and milk samples at 20°C, following the formula: Specific gravity = Weight of milk sample at 20°C/ Weight of distilled water at 20°C. Acidity Test: Conducted following AOAC (2000) guidelines. 9 mL of milk was mixed with phenolphthalein indicator, and 0.1 N NaOH solution was added until a light pink color appeared. Acidity % = (Volume of 0.1 N NaOH used × 0.009)/ (Volume of sample taken) × 100.

Media preparation

Staphylococcus aureus and *Streptococcus agalactiae* were cultured using Nutrient Agar, Blood Agar, and Mueller-Hinton Agar. Media were prepared following manufacturer guidelines and sterilized by autoclaving at 121°C for 15 minutes at 15 lbs. pressure.

Bacterial culturing and staining

Primary cultures were established by streaking milk samples onto Nutrient Agar and Blood Agar. Plates were incubated at 37°C for 24 hours. Colonies were purified by subculturing, followed by Gram staining and biochemical tests:

- Catalase test: Differentiated *Staphylococcus aureus* (catalase-positive) from *Streptococcus agalactiae* (catalase-negative).
- Oxidase test: Identified bacterial respiratory enzyme activity.
- Coagulase test: Confirmed *S. aureus* using plasma clot formation.

For quality control, reference strains (*Staphylococcus aureus ATCC 25923* and *Streptococcus agalactiae ATCC 13813*) were used as positive controls**,** while sterile broth served as a negative control.

Preparation of herbal extracts

Aqueous extracts of Berberis lyceum, Capsicum annuum, and Asafoetida were prepared as follows: Fifty grams (50 g) of dried plant material was powdered and mixed with 400 mL of distilled water in a 500 mL conical flask. The mixture was shaken at 30-minute intervals over 12 hours and then filtered using Whatman No. 1 filter paper. The extracts were refrigerated at 4°C until further use. To ensure standardization, the final concentration of the herbal extracts was 50 mg/mL.

Antimicrobial susceptibility testing

The disc diffusion method was used to evaluate the antibacterial activity of the herbal extracts against S. aureus and S. agalactiae, following Clinical and Laboratory Standards Institute (CLSI, 2021) guidelines. Mueller-Hinton Agar plates were prepared and dried at 37°C for 30 minutes before use. Bacterial suspensions were adjusted to 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL). Sterile 6 mm filter paper discs were impregnated with 50 µL of each herbal extract (50 mg/mL) and placed onto inoculated agar plates. Plates were incubated at 37°C for 24 hours, and inhibition zones were measured in millimeters (mm). Results were classified as sensitive, intermediate, or resistant, based on CLSI (2021) interpretation guidelines. Positive controls: Amoxicillin (10 µg) and Tetracycline (30 µg). Negative control: Sterile distilled water.

RESULTS

Overall prevalence of bovine subclinical mastitis in dairy farms of cattle colony Hyderabad

The investigation into the prevalence of subclinical mastitis in buffaloes within the cattle colony of Hyderabad led to the examination of 120 milk samples through the California Mastitis Test (CMT). Among all the milk samples, 80 (66.66%) were identified as positive for subclinical mastitis. An analysis of the prevalence of bovine subclinical mastitis based on age is provided in Table 1. The data reveals that the occurrence of bovine subclinical mastitis was prominent among buffaloes aged 4 to 8 years. Notably, the highest prevalence rate of 83.3% was observed within the 5-year age group, while the lowest was 33.33% among buffaloes aged 8 years. However, no significant ($p > 0.05$) variance was detected among the different age groups.

Table 1: Age wise prevalence of bovine subclinical mastitis in the dairy farms of cattle colony Hyderabad.

Age of buffaloes	Number of milk samples examined	Number of positive milk samples	Prevalence %	P value
4 years	20	15	75.00	0.3191
5 years	30	25	83.33	
6 years	25	20	80.00	
7 years	20	10	50.00	
8 years	15	5	33.33	
> 8 years	10	5	50.00	

Parity-wise prevalence of bovine subclinical mastitis in dairy farms of cattle colony Hyderabad

The parity-wise prevalence of subclinical mastitis among buffaloes within the cattle colony of Hyderabad. The data underscores that the highest prevalence rate of 75.00% was recorded among buffaloes in the 3rd and 4th parity, while the lowest rate of 50.00% was seen in the 1st and 5th parity. No significant difference was observed in terms of parity among the animals.

Quarter-wise prevalence of bovine subclinical mastitis in dairy farms of cattle colony Hyderabad

The data concerning quarter-wise prevalence of subclinical mastitis among buffaloes in the cattle colony of Hyderabad is presented. The prevalence rate was found to be 93.30% in the hind right quarters and the lowest at 43.33% in the front left quarters. The frequency of infection was notably higher (35.00%) in the hind right quarters Table 2. Non-significant differences were observed among all quarters of buffaloes.

Physical indicators/characteristics of mastitis milk

The results of milk pH, specific gravity, acidity, and electrical conductivity in mastitis milk are displayed in Table 3. The mean pH of mastitis milk was recorded as 6.38.

Additionally, the specific gravity, acidity, and conductivity values were 1.029, 0.13, and 6.19, respectively.

Table 2: Quarter wise prevalence of bovine subclinical mastitis in the dairy farms of cattle colony Hyderabad.

Quarters	Number of milk samples examined	Number of positive milk samples	Prevalence %	Frequency %	P value
Front left	30	13	43.33	16.25	0.0526
Front right	30	15	50.00	18.75	
Hind left	30	24	80.00	30.00	
Hind right	30	28	93.30	35.00	

Table 3: Mean values of milk pH, specific gravity, acidity, and electrical conductivity of mastitis milk samples.

Physical indicators	Value recorded (Mean±SD)	Normal values (Range)
Milk pH	6.38±0.15	6.70-6.81
Specific gravity	1.029±0.05	1.029-1.032
Acidity	0.13±0.02	0.10-0.26%
Electrical conductivity	6.19±97.21	4.0-5.0 mS

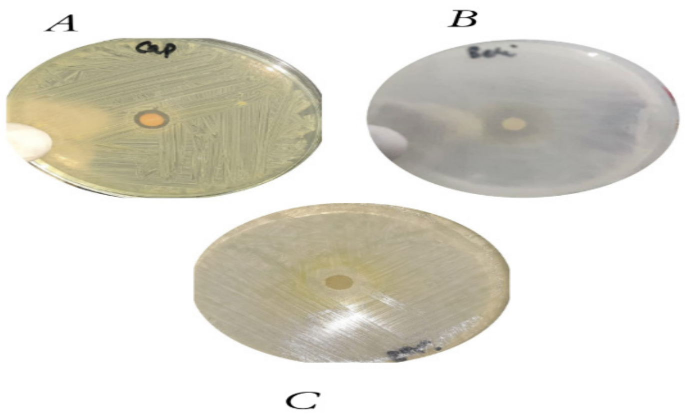

Figure 1: Inhibitory zones of herbal extracts against bacterial strains isolated from bovine milk samples. The figure shows three circular objects that represent different herbal extracts used for antimicrobial sensitivity testing. Object A is Capsicum annuum (yellowish with a rough texture and an orange dot in the center), object B is Berberis lyceum (white with a smooth texture and a black dot in the center), and object C is Asafoetida (yellowish with a rough texture and a Tan dot in the center and a blue mark on the bottom right side). The diameter of each object is 6 mm. The figure illustrates the inhibitory zones of these extracts against two bacterial strains, Staphylococcus aureus (top row) and Streptococcus agalactiae (bottom row), isolated from bovine milk samples. The results indicate that all plant extracts exhibited weak sensitivity against both bacterial strains, with Berberis lyceum showing the largest inhibition zones (1.5±0.57 mm for S. aureus and 1.6±1.10 mm for S. agalactiae) and Asafoetida showing the smallest inhibition zones (1.1±0.10 mm for S. aureus and 1.5±0.57 mm for *S. agalactiae*).

Antimicrobial sensitivity of herbal extracts

The antimicrobial sensitivity of Berberis lyceum, Capsicum annuum, and Asafoetida against *Staphylococcus aureus* and *Streptococcus agalactiae* isolated from bovine milk samples. The results indicated that all plant extracts exhibited weak sensitivity against both *Staphylococcus aureus* and *Streptococcus agalactiae*. Specifically, Berberis lyceum inhibited zones of 1.5±0.57 mm and 1.6±1.10 mm against *Staphylococcus aureus* and *Streptococcus agalactiae*, respectively. Capsicum annuum showed inhibitory zones of 1.6±1.10 mm and 1.03±0.10 mm against *Staphylococcus aureus* and *Streptococcus agalactiae,* respectively. Asafoetida demonstrated inhibition zones of 1.1±0.10 mm and 1.5±0.57 mm against *Staphylococcus aureus* and *Streptococcus agalactiae*, respectively as shown in Figure 1.

DISCUSSION

Mastitis poses a substantial challenge in the dairy sector, attributed to diverse factors such as inadequate feeding practices, suboptimal husbandry, and improper animal housing and handling (Procópio *et al*., 2019; Waminal *et al*., 2021). It manifests in clinical and subclinical forms, with the latter leading to elevated milk somatic cell counts, diminished milk yield, and alterations in milk composition that impact overall quality (Reis *et al*., 2013; Piepers *et al*., 2007; Blowey and Edmondson, 2010). This study focuses on the prevalence of subclinical mastitis among buffaloes in the cattle colony of Hyderabad. Our findings reveal that out of 120 milk samples collected from all quarters of forty randomly selected buffaloes, 80 (66.66%) tested positive for subclinical mastitis. Notably, an elevated prevalence rate of 83.33% was observed in 5-year-old buffaloes, while those in their 4th parity displayed a significant infection rate of 75.00%. Furthermore, a notably higher prevalence rate (93.33%) was recorded in the right hind quarters of the buffaloes. These results resonate with previous research that has highlighted heightened rates of subclinical mastitis in both cows and buffaloes (Misrak *et al*., 2017; Mukhamadieva *et al*., 2022; Mekonnen *et al*., 2017; Khan and Muhammad, 2005). Beyond assessing subclinical mastitis prevalence, we examined the physical indicators of mastitis-affected milk. Our findings demonstrated mean milk pH, specific gravity, acidity, and electrical conductivity of CMT-positive milk as 6.36±0.15, 1.029±0.50, 0.13±0.02, and 6.19±97.91, respectively. Additionally, we evaluated the antimicrobial sensitivity of Berberis lyceum, Capsicum annuum, and Asafoetida against Staphylococcus aureus and Streptococcus agalactiae, employing the disc diffusion method. The results revealed that all plant extracts exhibited modest sensitivity against both bacteria. Berberis lyceum showcased inhibition zones of 1.4mm and 1.6mm, Capsicum annuum presented inhibition zones of 0.7mm and 1.03mm, and Asafoetida displayed inhibition zones of 1.1mm and 1.5mm against Staphylococcus aureus and Streptococcus agalactiae, respectively. Notably, these herbal extracts have been recognized for their clinical efficacy in addressing localized bacterial infections, ulcers, and burns (Ali *et al*., 2015). Berberis lyceum holds a traditional role in treating a wide range of human ailments in Pakistan, India, and Bangladesh (Ali *et al*., 2015). Capsicum annuum finds application in managing pain related to conditions like osteoarthritis and rheumatoid arthritis (Aronson *et al*., 2016). Asafoetida's traditional usage encompasses treating various conditions including whooping cough, asthma, ulcers, epilepsy, stomach-aches, flatulence, bronchitis, intestinal parasites, and influenza, underscoring its diverse therapeutic potential (Amalraj and Gopi, 2016).

CONCLUSIONS

Our investigation sheds light on the substantial prevalence of subclinical mastitis among buffaloes in Hyderabad's cattle colony, with particular emphasis on 5-years old buffaloes in their 4^{th} parity. Notably, mastitis-affected milk displayed elevated specific gravity, acidity, and electrical conductivity in comparison to normal milk. Additionally, our evaluation of herbal extracts indicated limited sensitivity of Beriberis lyceum, Capsicum annuum, and Asafoetida against Staphylococcus aureus and Streptococcus agalactiae. These outcomes underscore the imperative for continued exploration of alternative approaches to address subclinical mastitis. By addressing this concern, we can enhance the health and well-being of dairy animals and subsequently bolster milk production.

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NOVELTY STATEMENT

This study is the first to assess subclinical mastitis prevalence in buffaloes of Hyderabad's cattle colony while evaluating the antimicrobial potential of Berberis lyceum, Capsicum annuum, and Asafoetida. It reveals a high mastitis burden and weak antibacterial efficacy of these herbal extracts. The findings highlight the need for improved plant-based formulations or alternative therapeutic strategies.

AUTHOR'S CONTRIBUTION

AK and AHM, MS: Equal contribution to this research work.

AK: Analysed the data and wrote the paper.

MI, MU, MK, and AM: Completed the sampling and performed the test.

AK, KS, HI and SH: Performed the biochemical tests.

AHM and AK: Designed the study and funded for the research work.

Conflict of interest

The authors have declared no conflict of interest.

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