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Therapeutic Potential of Allium sativum Against Subclinical Mastitis in Dairy Buffaloes: Antimicrobial Mechanisms, Effects on Udder Health and Future Perspectives

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ABSTRACT

Subclinical mastitis (SCM) is a major production-limiting disease in dairy buffaloes, characterized by intramammary infection, increased somatic cell count (SCC) and deterioration of milk quality without visible clinical abnormalities. Increasing antimicrobial resistance among mastitis-associated pathogens has encouraged the investigation of plant-derived antimicrobial agents. *Allium sativum* (garlic) contains bioactive organosulfur compounds, particularly allicin, ajoene and diallyl sulfides, which demonstrate antibacterial, antibiofilm and antioxidant properties. Experimental investigations have demonstrated the inhibitory activity of garlic extracts against multidrug-resistant *Staphylococcus aureus* and other mastitis-associated bacteria isolated from buffalo milk. Preliminary evidence from dairy cattle also suggests potential applications of garlic-containing preparations in mastitis management. This review examines the phytochemical composition, antimicrobial mechanisms, effects on mammary health, available experimental evidence and limitations of garlic-based interventions. Although *A. sativum* represents a promising phytotherapeutic candidate, standardized formulations and controlled clinical trials in buffaloes are required to establish its therapeutic efficacy and safety.

Keywords: *Allium sativum*; Buffalo; Subclinical mastitis; Allicin; Antimicrobial resistance; *Staphylococcus aureus*; Phytotherapy

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Introduction

The dairy and livestock sector is an important component of Pakistan's agricultural economy, with buffaloes (*Bubalus bubalis*) contributing substantially to national milk production. Mastitis remains one of the most economically significant diseases affecting dairy animals because of its adverse effects on milk yield, composition, animal health and farm profitability. Subclinical mastitis (SCM) is particularly challenging because infected animals generally exhibit no visible udder inflammation or obvious abnormalities in milk appearance. Nevertheless, intramammary infection produces inflammatory changes, increases SCC and compromises milk quality [1].

Major bacterial organisms associated with buffalo mastitis include *Staphylococcus* spp., *Streptococcus* spp. and *Escherichia coli*. Persistent intramammary infections caused by *S. aureus* are particularly important because of their ability to establish chronic infections and exhibit antimicrobial resistance [1,2].

Conventional mastitis management frequently involves antimicrobial therapy. However, inappropriate antibiotic administration and repeated exposure to antimicrobial agents can contribute to the selection of resistant bacterial populations. Antimicrobial residues in milk also represent a food safety concern when recommended withdrawal periods are not observed. These challenges have increased interest in ethnoveterinary medicine and plant-derived antimicrobial compounds.

Allium sativum (garlic), belonging to the family Amaryllidaceae, has traditionally been used for its medicinal properties. Its bioactive organosulfur compounds exhibit antibacterial activity against several pathogenic microorganisms, including resistant bacterial strains [3]. Moreover, investigations involving buffalo mastitis isolates have demonstrated the inhibitory potential of garlic extracts against multidrug-resistant bacterial pathogens [4]. These findings provide a scientific basis for evaluating garlic as a potential complementary approach to SCM management.

2. Phytochemical Composition of *Allium sativum*

Garlic contains several biologically active organosulfur compounds that contribute to its antimicrobial and antioxidant properties. The major constituents include allicin, ajoene, diallyl sulfide, diallyl disulfide and diallyl trisulfide.

Allicin is one of the principal bioactive compounds responsible for the antibacterial activity of freshly disrupted garlic. It is generated when the enzyme alliinase converts alliin into allicin following mechanical disruption of garlic tissue. Allicin is chemically reactive and relatively unstable, undergoing transformation into other organosulfur compounds depending on temperature, pH, storage conditions and extraction procedures [3].

Consequently, the biological activity of garlic preparations depends on their extraction method, chemical composition and concentration of active constituents. Standardization is therefore essential for developing reproducible veterinary formulations.

3. Antimicrobial Mechanisms of Garlic Against Mastitis-Associated Pathogens

3.1. Direct antibacterial activity

Garlic-derived organosulfur compounds demonstrate bacteriostatic and bactericidal activities against several Gram-positive and Gram-negative bacterial species. Their antimicrobial effects have been reported against *S. aureus*, *E. coli* and other pathogenic microorganisms [3].

Allicin is particularly important because it can interact with bacterial proteins and interfere with essential metabolic processes. Its broad biochemical activity makes it a promising candidate for investigating alternative antimicrobial strategies.

3.2. Enzymatic inhibition and thiol modification

One of the principal antibacterial mechanisms of allicin involves its interaction with free sulfhydryl groups (-SH) of cysteine residues in bacterial proteins.

Allicin can modify thiol-containing enzymes through S-thioallylation, interfering with metabolic pathways, cellular functions and bacterial survival. This mechanism affects multiple protein targets rather than a single specific bacterial enzyme [3].

3.3. Antibiofilm activity

Biofilm formation is an important virulence-associated characteristic of mastitis pathogens, particularly *S. aureus*. Biofilms consist of bacterial communities embedded within an extracellular polymeric matrix, which can reduce antimicrobial penetration and protect microorganisms from host immune responses.

Experimental investigations have demonstrated antibiofilm properties of garlic-derived organosulfur compounds. These compounds may interfere with bacterial adhesion, biofilm development and associated cellular communication mechanisms [3].

However, their ability to eradicate established intramammary biofilms in naturally infected buffaloes remains insufficiently investigated.

3.4. Potential activity against antimicrobial-resistant bacteria

Garlic extracts have demonstrated inhibitory activity against multidrug-resistant bacterial isolates obtained from buffalo mastitic milk [4].

Their potential is particularly relevant in the context of antimicrobial resistance. Nevertheless, it would be scientifically incorrect to conclude that garlic universally overcomes beta-lactamase production, altered antimicrobial targets or other established resistance mechanisms. Its activity must be evaluated against individual pathogens and their resistance profiles.

4. Effects on Udder Health and Milk Composition

Subclinical mastitis produces pathological and biochemical changes within the mammary gland. Inflammatory cell migration, epithelial damage and alterations in mammary permeability contribute to changes in milk composition.

4.1. Somatic cell count and mammary health

SCC is an important indicator of mammary gland health. During intramammary infection, leukocytes migrate into milk, resulting in increased SCC.

A study involving dairy buffaloes demonstrated a progressive increase in SCC with increasing severity of SCM. Mean SCC increased from 2.06×10^5 cells/mL in the negative group to 121.01×10^5 cells/mL in the most severely affected group [5].

Garlic-based preparations may potentially support udder health through their antimicrobial activity. However, a reduction in SCC following garlic administration must be demonstrated through controlled clinical investigations.

4.2. Milk lactose, fat and protein

Intramammary infections can interfere with normal mammary epithelial function and milk synthesis. Damage to the mammary epithelial barrier may alter the movement of milk constituents and reduce lactose concentration.

Sharif et al. reported a progressive reduction in milk lactose with increasing severity of subclinical mastitis in buffaloes. Mean lactose concentration decreased from 5.10% in the negative group to 2.66% in the most severely affected group [5].

Changes in milk fat and protein may also occur during mastitis because of inflammatory damage and alterations in mammary metabolism. However, the ability of garlic treatment to restore these components in buffalo milk has not been adequately established.

4.3. Milk pH and electrical conductivity

During mammary inflammation, disruption of epithelial tight junctions can alter the movement of sodium, chloride and bicarbonate between blood and milk. These changes may increase milk electrical conductivity and pH.

Electrical conductivity, SCC and milk yield are therefore useful indicators when evaluating mammary health [1,6].

Successful control of intramammary infection may contribute to the restoration of normal mammary epithelial function. Nevertheless, improvements in milk pH and electrical conductivity following garlic administration require direct experimental confirmation.

5. Antioxidant and Immunomodulatory Potential

Oxidative stress is an important component of the inflammatory response associated with mastitis. Activated leukocytes produce reactive oxygen species (ROS), which contribute to bacterial killing but may also cause oxidative damage to mammary epithelial cells.

Garlic-derived organosulfur compounds have demonstrated antioxidant and redox-modulating activities in experimental investigations [3].

Potential biological effects include modulation of oxidative stress, protection against excessive cellular oxidation and support of antioxidant defence mechanisms.

Future buffalo mastitis studies should evaluate total antioxidant capacity (TAC), total oxidant status (TOS), malondialdehyde (MDA), superoxide dismutase (SOD) and catalase (CAT) to investigate the possible antioxidant effects of garlic-based preparations.

These effects should be regarded as potential mechanisms rather than established clinical outcomes in buffaloes.

6. Experimental Evidence and Therapeutic Potential

6.1. In-vitro evidence in buffalo mastitis pathogens

Naseer et al. evaluated garlic (*A. sativum*), ginger (*Zingiber officinale*) and red chilli (*Capsicum annuum*) extracts individually and in combination against multidrug-resistant *S. aureus* and *Streptococcus pyogenes* isolated from buffalo mastitic milk.

The investigation employed agar well diffusion and minimum inhibitory concentration (MIC) assays. Garlic demonstrated inhibitory activity against both bacterial species. Its 75% and 100% concentrations significantly inhibited *S. aureus*, while the 100% concentration also demonstrated significant activity against *S. pyogenes*. The combination of garlic and red chilli produced greater inhibitory activity than the other tested botanical combinations [4].

These findings provide direct laboratory evidence supporting the antimicrobial potential of garlic against multidrug-resistant buffalo mastitis isolates. However, the results demonstrate in-vitro antibacterial activity rather than clinical cure in infected animals.

6.2. Evidence from other mastitis investigations

Absar et al. investigated aqueous and methanolic extracts of *A. sativum* and *Chenopodium album* against mastitis-associated bacteria. Aqueous garlic extract produced a 20-mm inhibition zone against *S. aureus*. The combination of garlic and *C. album* extracts demonstrated inhibitory activity against selected organisms, including *E. coli* and *Streptococcus uberis* [7].

Although these findings support the antibacterial potential of garlic, the study was published as a conference proceeding and should be interpreted as preliminary experimental evidence.

6.3. Evidence from dairy cattle

Antanaitis et al. investigated subclinical mastitis management in dairy cattle using two treatment protocols: a garlic-extract-containing SCC bolus combined with a non-steroidal anti-inflammatory drug (NSAID), and intramammary antibiotic treatment combined with an NSAID.

The investigation involved 32 cows with SCM, divided into two treatment groups, and evaluated milk yield, electrical conductivity, rumination time and SCC over a 14-day period [6].

The findings provide preliminary clinical information regarding garlic-containing preparations in dairy cattle. However, the intervention involved a multicomponent formulation and concomitant anti-inflammatory treatment. Therefore, the independent contribution of garlic cannot be established conclusively, and these findings cannot be directly extrapolated to buffaloes.

6.4. Overall interpretation of available evidence

The available literature indicates promising antimicrobial activity of garlic against mastitis-associated pathogens. However, three levels of evidence should be distinguished:

1. **In-vitro evidence:** Demonstrates bacterial growth inhibition under laboratory conditions.
2. **Preclinical evidence:** Evaluates biological activity, pharmacological properties and safety in experimental models.
3. **Clinical evidence:** Establishes bacteriological cure, SCC reduction, improvement in milk production and safety in naturally infected animals.

At present, direct buffalo-specific evidence primarily supports in-vitro antibacterial activity. Further controlled clinical investigations are required to establish the therapeutic efficacy of garlic in buffalo SCM.

7. Limitations and Future Research Perspectives

Despite its promising biological properties, several limitations must be addressed before garlic-based therapeutics can be recommended for routine veterinary practice.

Standardization of extracts: Different garlic preparations contain variable concentrations of allicin and other organosulfur compounds. Future investigations should establish standardized extraction methods and quantify active constituents.

Dosage determination: Effective concentrations should be established through MIC, minimum bactericidal concentration (MBC) and dose-response studies.

Clinical validation: Randomized controlled trials in naturally infected buffaloes are necessary to evaluate bacteriological cure, SCC, milk yield, milk composition, recurrence and adverse reactions.

Safety and milk quality: Oral and intramammary formulations require separate safety assessments. Studies should investigate local tissue tolerance, systemic toxicity, possible effects on milk flavour and processing properties, and the presence of garlic-derived compounds in milk.

Combination therapy: Potential interactions between garlic-derived compounds and conventional antimicrobials should be investigated through checkerboard assays, time-kill studies and subsequent clinical trials. Synergistic activity should not be assumed without experimental confirmation.

8. Conclusion

Allium sativum represents a promising plant-derived source of bioactive antimicrobial compounds with potential applications in the management of subclinical mastitis in dairy buffaloes. Its organosulfur constituents, particularly allicin, demonstrate antibacterial, antibiofilm and redox-modulating activities against several pathogenic microorganisms.

Experimental studies involving multidrug-resistant bacteria isolated from buffalo mastitic milk provide encouraging evidence of its antibacterial potential. Preliminary research in dairy cattle also supports further investigation of garlic-containing formulations. However, laboratory inhibition cannot be considered equivalent to bacteriological cure or clinical recovery. Further research should focus on standardized garlic formulations, effective dosage determination, pharmacological characterization, safety assessment and well-designed clinical trials in dairy

buffaloes. Integrating scientifically validated phytotherapeutic agents with appropriate mastitis diagnosis, hygienic milking practices and responsible antimicrobial stewardship may contribute to sustainable udder-health management and the reduction of unnecessary antimicrobial exposure in dairy production systems.

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