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Antimicrobial efficacy of selected plant extracts against common dairy spoilage microorganisms

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Abstract

Background: Dairy products are highly susceptible to microbial spoilage due to their rich nutritional values, leading to reduced shelf life and economic losses. Increasing consumer demand for natural and chemical free preservatives has driven research toward plant-based bio-preservatives with antimicrobial properties.

Objective: The present study aimed to evaluate the efficacy of selected plant extracts as natural bio-preservatives against common dairy spoilage microorganisms.

Method: Plant extracts such as neem (*Azadirachta indica*), tulsi (*Ocimum sanctum*), and garlic (*Allium sativum*) were prepared using both aqueous and ethanolic extraction methods. The antimicrobial activity was assessed against selected dairy spoilage microorganisms, including *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas spp.* using agar well diffusion and minimum inhibitory concentration (MIC) methods. The effectiveness of extracts was further evaluated in a model dairy system under refrigerated storage conditions.

Results: All plant extracts exhibited inhibitory effects against the tested microorganisms, with garlic extract showing the highest antimicrobial activity, followed by neem and tulsi. Ethanolic extracts demonstrated significantly higher inhibition zones compared to aqueous extracts ($p < 0.05$). Application of plant extracts in the dairy model system resulted in a noticeable reduction in microbial load and extended shelf life.

Conclusion: The study confirms that plant extracts possess significant antimicrobial potential and can be effectively utilized as natural biopreservatives to control dairy spoilage microorganisms, thereby enhancing product safety and shelf life.

Keywords: Bio-preservatives, plant extracts, antimicrobial activity, dairy spoilage microorganisms, natural preservatives, shelf life

Introduction

Dairy products serve as an excellent growth medium for a wide range of microorganisms due to their high moisture content, near-neutral pH, and abundant nutrients such as lactose, proteins, and fats. Common spoilage microorganisms include psychrotrophic bacteria like *Pseudomonas spp.*, coliforms such as *Escherichia coli*, and Gram-positive pathogens like *Staphylococcus aureus*. These microbes secrete proteolytic and lipolytic enzymes, leading to off-flavors, gas production, curdling, and biofilm formation, ultimately reducing shelf life to 5–7 days under refrigeration (Ray & Bhunia, 2014) [11].

Traditionally, chemical preservatives (e.g., sodium benzoate, potassium sorbate) have been used to extend dairy shelf life. However, growing consumer awareness regarding the potential health risks and a preference for "clean-label" products have accelerated the search for natural alternatives. The clean-label trend has been extensively reviewed, with plant-based antimicrobials emerging as the most promising substitutes for synthetic preservatives in dairy applications (Choi *et al*., 2024) [3]. Plant extracts, rich in secondary metabolites such as alkaloids, tannins, flavonoids, and organosulfur compounds, offer promising antimicrobial properties (Tajkarimi *et al*., 2010) [13]. Much research confirms that plant metabolites effectively inhibit dairy spoilage microbes and limitations of chemical preservatives.

Among these, neem (*Azadirachta indica*) is known for its broad-spectrum antibacterial activity due to nimbidin and azadirachtin; tulsi (*Ocimum sanctum*) contains eugenol and ursolic acid; and garlic (*Allium sativum*) produces allicin, a potent thiosulfinate. However, comparative studies on their efficacy specifically against dairy spoilage microbiota under refrigerated conditions remain limited.

Therefore, this study was conducted to: (i) evaluate the *in vitro* antimicrobial activity of aqueous and ethanolic extracts of neem, tulsi, and garlic against three key dairy spoilage microorganisms; and (ii) validate their bio-preservative potential in a pasteurized milk model system during refrigerated storage. Recent reviews have highlighted the growing interest in natural bioactive compounds from plants as substitutes for synthetic additives in dairy processing (Popescu, 2021) [10]

Materials and Methods

1. Collection and Authentication of Plant Materials

Fresh leaves of neem (*Azadirachta indica*) and tulsi (*Ocimum sanctum*) and fresh garlic cloves (*Allium sativum*) were collected from agricultural field, Nanded.

2. Preparation of Plant Extracts

Plant materials were washed with distilled water, shade-dried at 25–30°C for 7 days, and ground into a fine powder (80-mesh size).

Aqueous extract were prepared by mixing 50 g of powder with 250 mL of sterile distilled water, boiled at 60°C for 30 minutes, then filtered through Whatman No. 1 filter paper, and concentrated using a rotary evaporator at 50°C.

Ethanolic extract were prepared by mixing 50 g of powder in 250 mL of 70% ethanol for 48 hours at room temperature with intermittent shaking, followed by filtration and concentration.

All extracts were stored in sterile amber vials at 4°C until use. Stock solutions (100 mg/mL) were prepared in 10% DMSO.

3. Test Microorganisms

Standard strains of *Escherichia coli* (MTCC 443), *Staphylococcus aureus* (MTCC 96), and *Pseudomonas aeruginosa* (MTCC 2453) were obtained from the Microbial Type Culture Collection (MTCC), Chandigarh, India. Cultures were revived in nutrient broth and incubated at 37°C for 24 hours. The inoculum density was adjusted to 0.5 McFarland standards (corresponding to a cell density of 1.5×10^8 CFU/mL).

4. Agar Well Diffusion Assay

Mueller-Hinton agar plates were spread uniformly with each bacterial suspension. Wells of 6 mm diameter were punched and filled with 50 µL of each extract (100 mg/mL). Standard tetracycline disc from Himedia, Mumbai (30 µg/disc) as a Positive control and negative control was 10% DMSO was used. Plates were incubated at 37°C for 24 hours. The diameter of the inhibition zone in millimeters (mm) was measured in triplicate.

5. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

MIC of extract was determined by the broth micro dilution method. Two-fold serial dilutions of each extract (0.78 to 100 mg/mL) were prepared in Mueller-Hinton broth. Each well received 20 µL of bacterial suspension (~10^8 CFU/mL). Plates were incubated at 37°C for 24 hours. MIC was the lowest concentration with no visible growth. MBC was determined by subculturing wells with no growth onto nutrient agar; MBC was the lowest concentration killing ≥99.9% of initial inoculum.

6. Model Dairy System (Milk Preservation Assay)

Pasteurized whole milk (3.5% fat) was procured from a local dairy and confirmed to be free from initial contamination (plate count < 10^2 CFU/mL). Milk samples (100 mL each) were divided into:

Control: No extract
Treatment groups: 0.5% and 1.0% (w/v) of each extract (based on preliminary MIC results)
Samples were inoculated with a cocktail of *E. coli*, *S. aureus*, and *Pseudomonas spp*. (each at ~10^4 CFU/mL final concentration). All samples were stored at 4°C for 14 days. On days 0, 3, 7, 10, and 14, aliquots were serially diluted and plated on plate count agar. Total viable counts (log CFU/mL) were recorded. Shelf life was defined as the time to reach 10^7 CFU/mL (spoilage threshold). Sensory evaluation (odor, color, coagulation) was performed by five trained panelists on day 7 using a 5-point hedonic scale.

7. Statistical Analysis

All experiments were performed in triplicate. Data are expressed as mean ± standard deviation (SD). One-way ANOVA followed by Tukey's post hoc test was used to compare means. A p-value < 0.05 was considered statistically significant. Statistical analysis was performed using SPSS version 25.0.

Results

1. Antimicrobial Activity by Agar Well Diffusion

All three plant extracts exhibited concentration-dependent antimicrobial activity against all tested pathogens (Table 1). Ethanolic extracts produced significantly larger inhibition zones than aqueous extracts ($p < 0.05$). Garlic ethanolic extract showed the highest activity against *S. aureus* (22.4 ± 1.2 mm), followed by *Pseudomonas* spp. (19.8 ± 0.9 mm) and *E. coli* (18.5 ± 1.0 mm). Neem and tulsi ethanolic extracts showed moderate activity, with zones ranging from 11.5 to 16.3 mm.

Table 1: Inhibition zone diameters (mm) of plant extracts against dairy spoilage microorganisms

Extract Type	Microorganism	Garlic (mm)	Neem (mm)	Tulsi (mm)
Aqueous	*E. coli*	12.3 ± 0.8	9.1 ± 0.6	8.4 ± 0.5
Aqueous	*S. aureus*	14.1 ± 0.9	10.2 ± 0.7	9.3 ± 0.6
Aqueous	*Pseudomonas*	13.2 ± 0.7	9.8 ± 0.5	8.9 ± 0.4
Ethanolic	*E. coli*	18.5 ± 1.0	14.2 ± 0.8	13.1 ± 0.7
Ethanolic	*S. aureus*	22.4 ± 1.2	16.3 ± 0.9	15.2 ± 0.8
Ethanolic	*Pseudomonas*	19.8 ± 0.9	15.1 ± 0.7	13.8 ± 0.6

Positive control (tetracycline): 25–30 mm; Negative control: no zone. Values = mean ± SD (n=3).

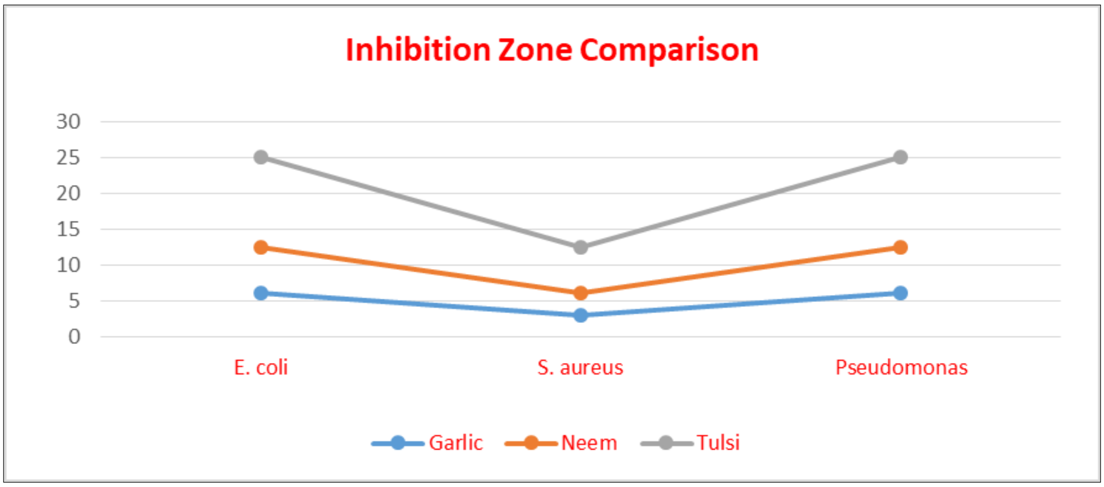

Fig 2: Comparative antimicrobial activity of ethanolic plant extracts against dairy spoilage microorganisms.

2. Minimum Inhibitory Concentration (MIC) and MBC

Garlic ethanolic extract exhibited the lowest MIC values (Table 2). Notably, *S. aureus* was the most susceptible (MIC = 3.12 mg/mL for garlic ethanolic), while *Pseudomonas* showed relatively higher resistance (MIC = 6.25 mg/mL). Ethanolic extracts consistently showed 2–4 times lower MICs than aqueous extracts.

Table 2: MIC and MBC values (mg/mL) of pla**nt extracts**

Extract	Parameter	*E. coli*	*S. aureus*	*Pseudomonas* spp.
Garlic (E)	MIC	6.25	3.12	6.25
Garlic (E)	MBC	12.5	6.25	12.5
Garlic (A)	MIC	12.5	6.25	12.5
Neem (E)	MIC	12.5	6.25	12.5
Tulsi (E)	MIC	25.0	12.5	25.0

(E) = Ethanolic, (A) = Aqueous MIC=Minimum Inhibitory Concentration, MBC= Minimum Bactericidal Concentration

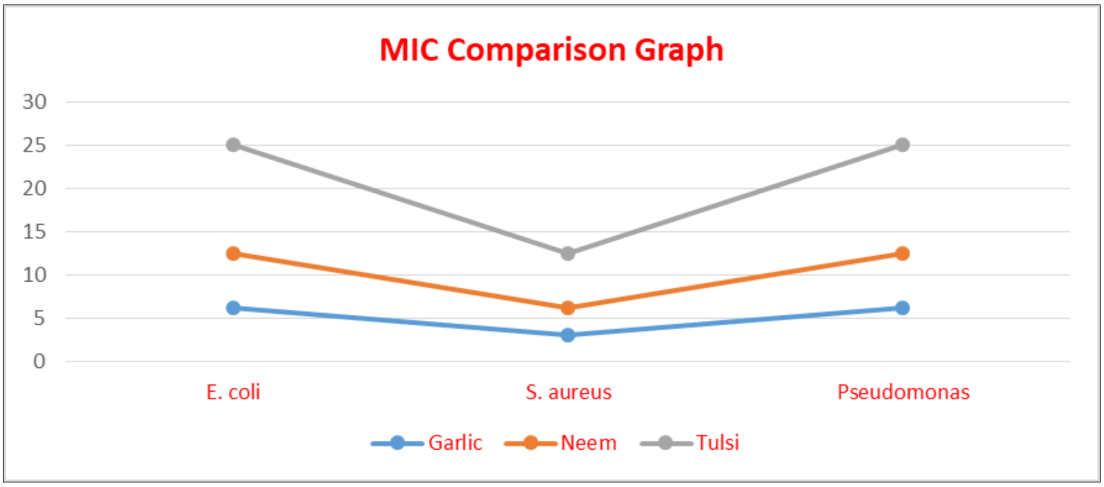

Fig 3: Minimum inhibitory concentration (MIC) of plant extracts against selected microorganisms.

3. Model Dairy System (Milk Preservation)

In the milk model, all extracts significantly reduced microbial growth compared to the control ($p < 0.05$). Figure 1 summarizes the total viable counts over 14 days.

Control milk reached the spoilage threshold (10^7 CFU/mL) by day 5.

0.5% garlic extract extended shelf life to day 9.

1.0% garlic extract extended shelf life to day 11 (6 days longer than control) and reduced microbial load by 3.2 log CFU/mL on day 7.

Neem and tulsi (1.0%) extended shelf life to days 8 and 7, respectively.

Sensory evaluation on day 7 revealed that milk with 1% garlic extract had a detectable but acceptable garlicky odor (score 3.4/5), while neem and tulsi extracts did not negatively affect sensory properties (scores 4.2 and 4.5, respectively). No coagulation or off-color was observed in any treated group until day 10.

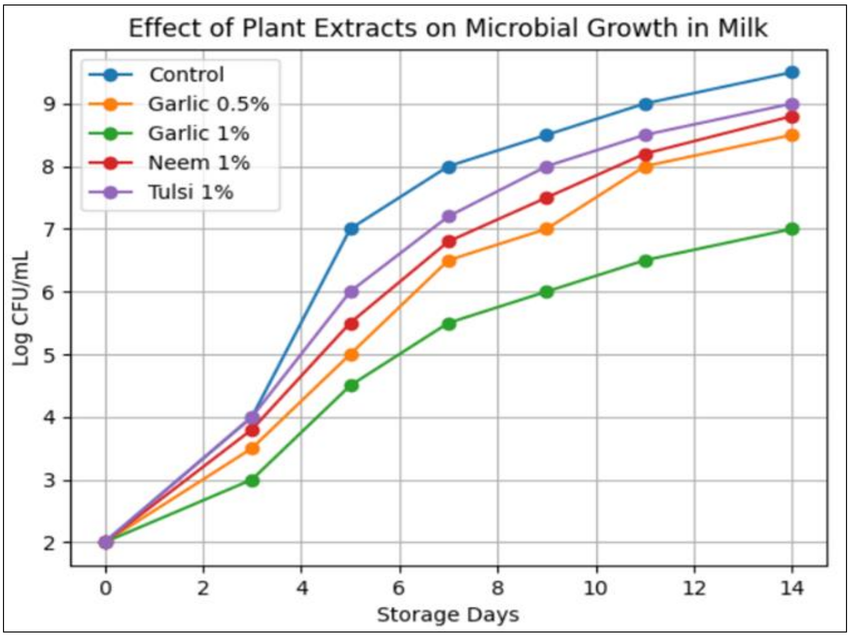

Fig 1: Effect of plant extracts (garlic, neem, tulsi) on microbial growth (log CFU/mL) in pasteurized milk during refrigerated storage (4°C).

The graphical representation (Figure 1) illustrates the effect of plant extracts on microbial growth in milk during 14 days of refrigerated storage. The control sample showed a rapid increase in microbial load, reaching the spoilage threshold (10^7 CFU/mL) by day 5. In contrast, milk samples treated with plant extracts exhibited significantly slower microbial growth. Among the treatments, 1% garlic extract demonstrated the highest inhibitory effect, maintaining microbial counts below spoilage levels up to day 11. Neem and tulsi extracts also showed moderate preservation effects, indicating their potential as natural bio-preservatives.

Discussion

The present study demonstrates that plant extracts, particularly garlic in ethanolic form, possess strong antimicrobial activity against key dairy spoilage bacteria, including Gram-positive (*S. aureus*) and Gram-negative (*E. coli*, *Pseudomonas*) organisms. This aligns with previous reports where allicin from garlic disrupted bacterial cell wall synthesis and inhibited sulfhydryl-dependent enzymes (Ankri & Mirelman, 1999) [1]. The superior activity of ethanolic extracts over aqueous extracts is consistent with the higher solubility of hydrophobic bioactive compounds (e.g., allicin, eugenol, nimbidin) in ethanol. "This observation aligns with a recent review on plant extracts in the dairy industry, which emphasized that ethanol-based extraction maximizes recovery of membrane-active antimicrobials (Mahmoud *et al*., 2023) [9]." These compounds are known to penetrate the lipid bilayer of bacterial membranes, causing increased permeability and cell lysis (Burt, 2004) [2]. "Our MIC values for garlic ethanolic extract (3.12–6.25 mg/mL) are consistent with those reported by Ibrahim and Salisu (2023) [4], who observed MICs of 4.0–8.0 mg/mL against similar dairy-related pathogens, confirming the reproducibility of garlic's potent antibacterial effect." allowing easier access of plant polyphenols to the cytoplasmic membrane.

Comparable results have been reported by Khan *et al*. (2020) [6, 7], who found that 1.5% neem and tulsi aqueous extracts extended raw milk shelf life by 17–18 hours at room temperature, demonstrating the versatility of these extracts across different storage conditions.", especially for short-shelf-life products like fresh milk. However, the mild garlic odor may limit its use in flavored dairy products (e.g., yogurt, cheese), where it could be desirable, or in combination with masking agents. Neem, while effective, imparted a slight bitterness at 1% concentration, suggesting that lower concentrations (0.5%) or combinations with tulsi might offer a balance between efficacy and sensory acceptability. A limitation of this study is the use of a single dairy matrix (pasteurized milk). Future research should evaluate these extracts in other dairy products (e.g., paneer, cheese, curd) and assess their synergy with existing preservation methods (e.g., refrigeration, modified atmosphere packaging). Furthermore, toxicological evaluation and stability studies of the bioactive compounds during storage are warranted."Our findings are consistent with Khan *et al*. (2020) [6, 7], who reported that 1.5% neem and tulsi aqueous extracts extended raw milk shelf life up to 17–18 hours at room temperature. Similarly, Tamil Selvam Krishnan (2016) [14] demonstrated that *Ocimum sanctum* extract at 300 mg/mL significantly reduced total viable counts in raw milk after 24 hours of storage

Conclusion

This study confirms that ethanolic extracts of garlic, neem, and tulsi possess significant antimicrobial potential against *E. coli, S. aureus,* and *Pseudomonas spp*. Garlic extract, at 1% concentration, was the most effective, reducing microbial load and extending the shelf life of refrigerated milk by 6 days. These findings support the use of plant extracts as natural bio-preservatives to control dairy spoilage microorganisms, thereby enhancing product safety and shelf life while meeting consumer demand for clean-label ingredients.

Future recommendations

"The use of oregano essential oil at 2.0 mg/L has recently been shown to extend pasteurized milk shelf life while maintaining quality parameters (Liu *et al*., 2026) [8]. Future studies should explore such essential oils in combination with the extracts evaluated in the present work."

To optimize extract concentrations to balance antimicrobial efficacy with sensory neutrality.

Investigate synergistic combinations of extracts (e.g., garlic + tulsi) for broad-spectrum activity.

Conduct challenge studies in commercial dairy products under industrial storage conditions.

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