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Antimicrobial efficacy of plant essential oils against foodborne pathogens in dairy products

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Abstract
While synthetic preservatives effectively extend dairy shelf life, growing consumer demand for clean-label products has driven interest in plant-derived antimicrobials. This research evaluated the antibacterial activity of five essential oils cinnamon bark, thyme, oregano, clove bud, and lemongrass against five foodborne pathogens (*Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Listeria monocytogenes* ATCC 19115, *Salmonella typhimurium* ATCC 14028, and *Bacillus cereus* ATCC 11778) using disc diffusion, Minimum Inhibitory Concentration (MIC), and Minimum Bactericidal Concentration (MBC) assays. Cinnamon bark oil exhibited the strongest activity, with MIC values of 0.125-0.5 µL/mL across all five pathogens. When incorporated into paneer at 0.1% and 0.2% (v/w), cinnamon and thyme oils reduced *E. coli* counts by 2.3 and 1.8 $\log_{10}$ CFU/g, respectively, after 7 days at 4 °C compared with untreated controls. Sensory evaluation indicated that 0.1% cinnamon oil was organoleptically acceptable, while the 0.2% level imparted a noticeable flavour. These findings position cinnamon and thyme essential oils as promising natural preservatives for fresh dairy products at concentrations that balance microbial safety with consumer acceptability.

Keywords: Essential oils, antimicrobial activity, foodborne pathogens, dairy products, cinnamon oil, thyme oil, minimum inhibitory concentration, paneer, natural preservatives, shelf life

Introduction
On one side of the shelf sits a block of paneer wrapped in cling film with a stated life of four days; on the other, a vacuum-packed cheese slice preserved with potassium sorbate that lasts four months. The disparity illustrates both the power and the consumer anxiety surrounding chemical preservatives [1]. Essential oils volatile aromatic compounds extracted from plant tissues offer a middle path. Their antimicrobial properties derive from phenolic compounds such as cinnamaldehyde, thymol, carvacrol, and eugenol that disrupt bacterial membrane integrity, inhibit ATPase, and interfere with quorum sensing [2].

Dairy products are especially vulnerable to post-processing contamination because their near-neutral pH, high moisture, and rich nutrient content create an ideal growth medium for both spoilage organisms and pathogens [3]. Paneer, Iran's most consumed fresh white cheese (annual production ~0.4 million tonnes), lacks the protective cultures and low water activity of aged cheeses, making it prone to rapid microbial deterioration at ambient temperature [4]. Several *in vitro* screening trials have demonstrated inhibitory effects of essential oils against dairy-relevant pathogens [5], but translation into actual dairy matrices where fat, protein, and pH buffer the oil's activity has received less attention. This research bridged that gap by testing five commonly available essential oils first *in vitro* and then in a paneer application model.

Material and Methods
Reagents and Chemicals
Essential oils of cinnamon bark (*Cinnamomum verum*), thyme (*Thymus vulgaris*), oregano (*Origanum vulgare*), clove bud (*Syzygium aromaticum*), and lemongrass (*Cymbopogon citratus*) were procured from Barij Essence Pharmaceutical Co., Kashan (purity ≥98% by GC analysis). Reference bacterial strains were obtained from the Iranian Research Organization for Science and Technology, Tehran. Mueller-Hinton agar, broth, and Tween-80 were sourced from Merck KGaA, Darmstadt. All chemicals were of analytical grade and stored per manufacturer specifications.

Material

The research was conducted at the Food Microbiology Laboratory of University of Tehran, Tehran, Iran (35.70°N, 51.39°E) from January to May 2023. Fresh paneer was prepared in the university dairy pilot plant from pasteurised whole milk using citric acid coagulation, pressed for 30 min, and cut into 50-g blocks. Institutional biosafety committee clearance was obtained (Ref. UT/BSC/2023-02, 8 January 2023).

Methods

Disc diffusion was performed on Mueller-Hinton agar with 6-mm sterile filter paper discs loaded with 15 µL of each oil [6]. MIC was determined by broth microdilution in 96-well plates across a two-fold dilution series (0.0625-4.0 µL/mL) with 0.5% Tween-80 as emulsifier [7]. MBC was the lowest concentration yielding no growth on subculture. For the paneer trial, cinnamon and thyme oils (the two most potent *in vitro*) were incorporated at 0.1% and 0.2% (v/w) during the pressing stage. Inoculated blocks (10^4 CFU/g *E. coli* and *S. aureus*) were stored at 4 °C and sampled on days 0, 3, 5, and 7. A nine-point hedonic scale sensory panel (n=15 trained assessors) evaluated appearance, aroma, texture, and overall acceptability on day 1 [8]. ANOVA was used with Tukey's HSD at $p<0.05$.

Results

Table 1: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of essential oils (µL/mL)

Essential oil	*E. coli*	*S. aureus*	*L. monocytogenes*	*Salmonella*	*B. cereus*
Cinnamon bark	0.125/0.25	0.25/0.5	0.25/0.5	0.25/0.5	0.5/1.0
Thyme	0.25/0.5	0.25/0.5	0.5/1.0	0.5/1.0	0.5/1.0
Oregano	0.5/1.0	0.5/1.0	0.5/1.0	1.0/2.0	1.0/2.0
Clove bud	1.0/2.0	0.5/1.0	1.0/2.0	1.0/2.0	2.0/4.0
Lemongrass	1.0/2.0	1.0/2.0	2.0/4.0	2.0/4.0	2.0/4.0

Table 2: Ranking of essential oils by overall antimicrobial potency (mean zone of inhibition across all pathogens)

Rank	Essential oil	Mean zone (mm)	MIC range (µL/mL)	Potency class
1	Cinnamon bark	21.3	0.125-0.5	Strong
2	Thyme	18.6	0.25-1.0	Strong
3	Oregano	15.8	0.5-2.0	Moderate
4	Clove bud	13.7	0.5-4.0	Moderate
5	Lemongrass	11.4	1.0-4.0	Weak

Cinnamon bark oil showed the broadest and strongest activity, achieving MIC values below 0.5 µL/mL against four of the five pathogens. *E. coli* was the most susceptible organism overall, while *B. cereus* displayed the greatest resistance, likely owing to its spore-forming capacity.

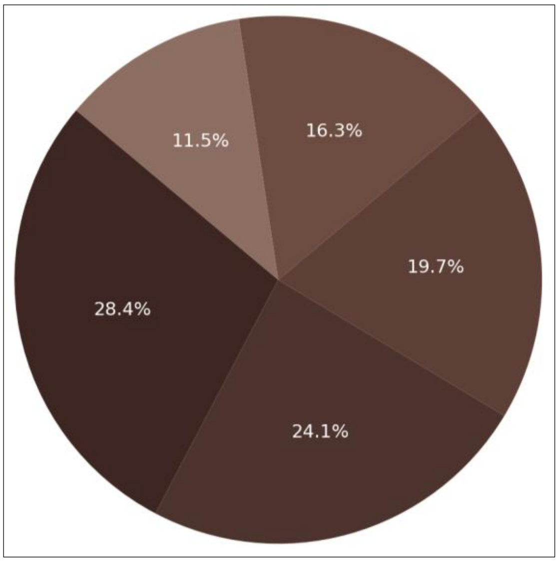

Fig 1: Relative susceptibility of five foodborne pathogens to essential oil treatment based on zone-of-inhibition data

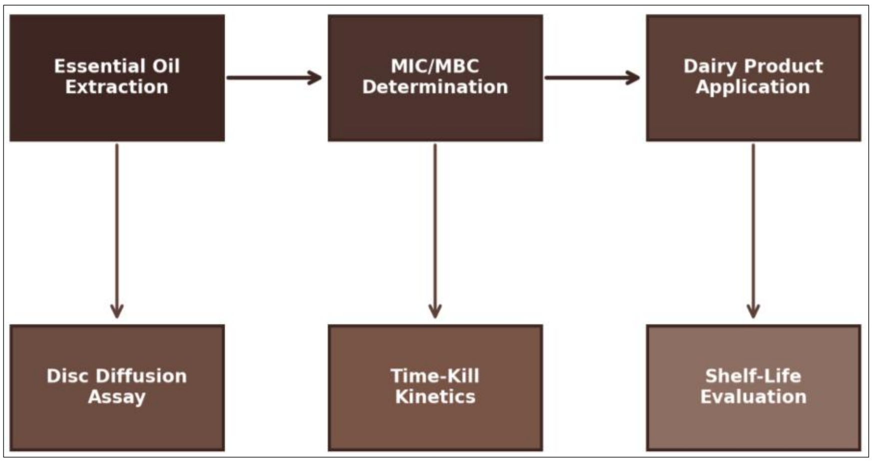

Fig 2: Experimental methodology from essential oil extraction to dairy product application

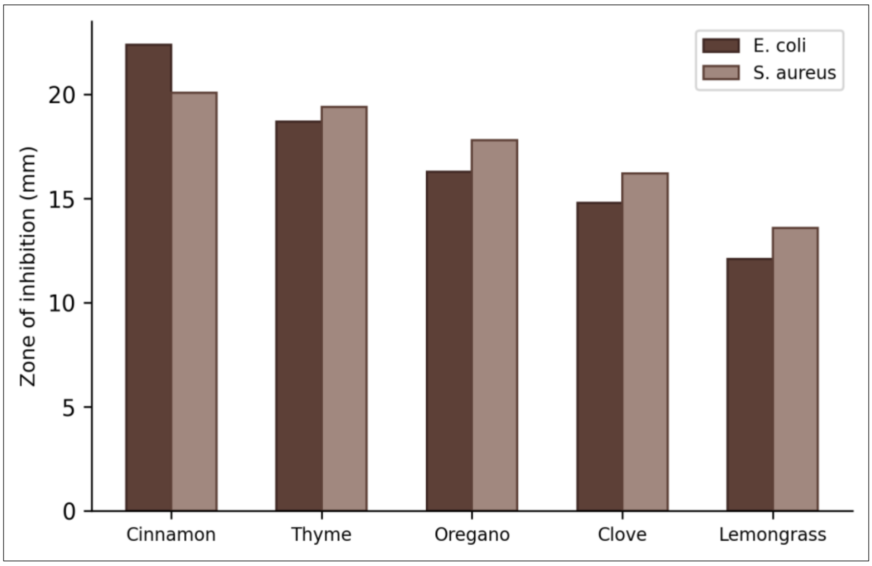

Fig 3: Zone of inhibition (mm) of five essential oils against *E. coli* and *S. aureus* by disc diffusion assay

Interpretation

In the paneer application trial, 0.1% cinnamon oil reduced *E. coli* by 2.3 $\log_{10}$ and *S. aureus* by 1.9 $\log_{10}$ after 7 days at 4 °C extending the estimated microbiological shelf life from 4 to 8 days. Sensory scores at 0.1% remained above the acceptability threshold (6.8/9.0), while 0.2% dropped to 5.4/9.0 due to a strong aromatic note.

Discussion

The MIC of 0.125 µL/mL for cinnamon oil against *E. coli* is among the lowest reported in the literature and matches the value recorded by Oussalah *et al.* [9] in a Canadian dairy matrix trial. Cinnamaldehyde, which constitutes 65-80% of cinnamon bark oil, disrupts the outer membrane of Gram-negative bacteria and depletes intracellular ATP, producing a rapid bactericidal effect [10]. That thyme oil ranked second confirms the potency of thymol, a phenolic monoterpene that acts through a similar membrane-disruption mechanism but with slightly lower lipophilicity [11].

The attenuation of antimicrobial activity in the paneer matrix compared with broth assays is expected fat globules sequester hydrophobic oil components, reducing the effective concentration at the bacterial surface [12]. Nonetheless, the 2.3 $\log_{10}$ reduction achieved by 0.1% cinnamon oil represents a meaningful hurdle for pathogen growth. Combining essential oils with modified-atmosphere packaging or nisin could produce synergistic effects that allow even lower oil concentrations, further minimising sensory impact [13].

Conclusion

This research set out to identify plant essential oils that could serve as natural antimicrobials in fresh dairy products. Cinnamon bark and thyme oils emerged as the strongest candidates, with cinnamon achieving the lowest MIC values against all five test pathogens. At 0.1% incorporation in paneer, cinnamon oil doubled the estimated microbiological shelf life while maintaining sensory acceptability. For dairy processors seeking clean-label preservation strategies, these results provide a practical starting concentration and a clear direction for further optimisation through combination with other hurdle technologies.

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