



Antibacterial Activity of Green Tea Leaf Extract Against Dairy-Associated Bacteria and Its Application in Pasteurized Milk

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Abstract

Bacterial contamination in pasteurized milk remains a potential cause of milk-borne diseases. Pasteurization may not eliminate all bacteria, thereby posing health risks. This study evaluated the potential of green tea leaf extract as an antibacterial agent to enhance pasteurization treatment by reducing contaminant bacteria and assessing its effect on milk flavor after treatment. Green tea leaves were extracted using the decoction technique. Antibacterial activity was evaluated using the agar well diffusion method, and bacterial reduction after pasteurization was measured using the total plate count (TPC) test. Panelist acceptance was assessed organoleptically based on color, aroma, and taste. The results showed that green tea leaf extract at a concentration of 5% had a very strong inhibitory effect (≥ 20 mm) against *Staphylococcus aureus* ATCC 25923 and *Staphylococcus aureus* SM 3.2.3, reducing bacterial counts by 5.2 log CFU ml⁻¹. Organoleptic testing of pasteurized milk treated with 1 to 5% extract showed consistently favorable scores for color, aroma, and taste. These findings indicate that green tea leaf extract has considerable potential to reduce *S. aureus* contamination and enhance the effectiveness of pasteurization for commercial application.

Keywords: *Camellia sinensis*; food safety; milk quality; natural antimicrobial; sensory evaluation

INTRODUCTION

The high number of reported cases of milk-borne diseases in various countries has prompted particular concern regarding the need to increase vigilance toward dairy products on the market. Consumption of milk contaminated with bacteria can lead to milk-borne diseases such as gastroenteritis, food poisoning, salmonellosis, campylobacteriosis, enteritis, brucellosis, and Shiga toxin-producing *Escherichia coli* (STEC) infections (Hirose et al., 2023; Kapoor et al., 2023; Hazaa and Khalil, 2025; Weinstein et al., 2025).

The level of bacterial contamination in dairy products varies significantly across different regions, and numerous cases of contamination

have continued to be reported in recent years. Commonly found bacteria include *Staphylococcus* spp., *Escherichia coli*, *Salmonella typhi*, *Pseudomonas* spp., *Listeria monocytogenes*, *Enterobacter* spp., *Bacillus cereus*, and many others (Bastam et al., 2021; Elbassiony, 2021; Wang et al., 2021; Hoffmann et al., 2022; Desye et al., 2023; Mengstu et al., 2023; Chang et al., 2024). Factors such as the environment, post-pasteurization handling, production equipment, and unsanitary distribution and storage practices are sources of contamination by these bacteria (Martin et al., 2018; Fusco et al., 2020; Calahorrano-Moreno et al., 2022; Du et al., 2022; Desye et al., 2023; Medjahdi et al., 2025).

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One of the simplest methods of processing raw milk is pasteurization. Pasteurization involves heating milk at a specific temperature for a specific duration to kill bacteria, making the milk safer to consume (Sharifi et al., 2021). Pasteurization can reduce the number of Gram-negative bacteria, such as *E. coli*, *Salmonella* spp., *Enterobacter* spp., and *Pantoea* spp., by 5 to 6 log CFU ml⁻¹ (Pearce et al., 2012). For Gram-positive bacteria such as *Staphylococcus* spp. and *Micrococcus* spp., the reduction in bacterial count ranges from 4 to 6 log CFU ml⁻¹ (Nascimento et al., 2023). Although pasteurization reduces the bacterial count in milk, it does not guarantee that dairy products are free from contamination. Thermotolerant bacteria and certain spore-forming bacteria pose a significant problem in the dairy industry, increasing the risk of contamination. Furthermore, the risk of post-pasteurization contamination is also a major challenge.

Quality standards for pasteurized milk in Indonesia are regulated by the Indonesian National Standard (SNI) 8984:2021 and the Indonesian Food and Drug Authority Regulation (PerBPOM) No. 13/2019, which concern microbial contamination in food. According to SNI 8984:2021, the maximum limit for total plate count (TPC) in pasteurized milk is 10⁴ CFU ml⁻¹ (BSN, 2021). Meanwhile, according to the BPOM Regulation, the threshold for TPC in pasteurized milk is 10⁴ to 10⁵ CFU ml⁻¹ (BPOM, 2019). Suboptimal implementation of milk quality standards can also increase the risk of contamination.

In light of these issues, a synergistic approach is required to reduce the risk of contamination in pasteurized milk while enhancing its flavor by utilizing natural plant extracts as antibacterial agents. Several plant extracts possess antibacterial properties against various pathogenic bacteria. Green tea leaves are a natural ingredient with potential as an antibacterial agent. Indonesia is one of the world's largest producers of green tea, making it readily available. Green tea is a key agricultural commodity in Indonesia due to its high economic value (Wahyuni et al., 2025). Green tea contains bioactive compounds, namely polyphenols, which consist of flavonoids and catechins as their main components (Rafique et al., 2023). Based on the previous research, the bioactive catechin compounds in green tea leaf extract exhibit strong antibacterial activity against both Gram-positive and Gram-negative

bacteria. These bacteria showed significant inhibition zones in the agar diffusion method (Taherpour et al., 2016; Khan et al., 2021; Alghamdi, 2023).

Most studies on the antibacterial activity of green tea leaf extract have been conducted only on agar media. Research on the effect of adding green tea leaf extract to directly enhance the effectiveness of milk pasteurization in reducing the number of contaminating bacteria has not been extensively studied. The uniqueness of this study lies in the detection of the antibacterial efficacy of green tea leaf extract in reducing the number of contaminating bacteria in milk while maintaining acceptable sensory quality. It is hoped that this study can serve as a foundation for developing innovative dairy products enriched with green tea leaf extract to improve the safety of pasteurized milk and prevent milk-borne diseases.

MATERIALS AND METHOD

Materials

The materials used in this study included dried green tea leaves from the Tambi Tea Plantation in Wonosobo; bacterial isolates identified as dairy pathogens, namely *Staphylococcus aureus* SKMF 1.2.1, *S. aureus* SM 3.2.3, *S. aureus* SKMC 2.2.4, *Micrococcus caseolyticus* SKMF 1.1.2, *Salmonella* spp., *Enterobacter sakazakii* S8H3, and *Pantoea* spp. S21B; standard cultures of *S. aureus* ATCC 25923, *S. epidermidis* ATCC 14990, and *E. coli* ATCC 25922; and fresh cow's milk from the Central Laboratory for Tropical Livestock Research, Faculty of Animal Science, Universitas Gadjah Mada.

Extraction of green tea leaves

The dried green tea leaves were initially ground using a grinder and subsequently sieved through a 200-mesh sieve. The extraction process employed the decoction method, conducted at a temperature of 100 °C for a duration of 10 minutes, with varying concentrations of 1%, 2%, 3%, 4%, and 5% (w/v) dissolved to a final volume of 100 ml. The extracts were centrifuged at 5,000 rpm for 15 minutes and filtered using a sterile 0.22 µm membrane filter (Khan et al., 2019).

Bacterial rejuvenation

Bacterial isolates were grown in Brain Heart Infusion Broth (BHIB) medium and incubated at 37 °C for 18 to 24 hours. The BHIB bacterial culture was then inoculated onto a Brain Heart

Infusion Agar (BHIA) slant and incubated at 37 °C for 18 to 24 hours (FDA, 2001).

Gram staining

Bacterial cultures from BHIA medium were collected, smeared onto microscope slides in sterile distilled water, and then fixed. The slides were stained with crystal violet for 1 minute, followed by Lugol's solution for 1 minute, decolorized with acetone-alcohol, and counterstained with safranin for 1 minute. The slides were then dried and examined under a microscope (Bishop and McCue, 2021).

Preparation of the test bacterial suspension

The bacterial culture on BHIA was subcultured onto a BHIA slant and incubated at 37 °C for 18 to 24 hours. One to two loopfuls of the fresh culture were then suspended in 5 ml of sterile 0.85% NaCl solution until a turbidity equivalent to the 0.5 McFarland standard was achieved (CLSI, 2024).

Preparation of the positive control solution

The antibiotic ciprofloxacin was used as the positive control. A 200 mg 100 ml⁻¹ ciprofloxacin injection solution was diluted to 0.1 mg ml⁻¹ (100 µg ml⁻¹) to achieve a final amount of 5 µg per well. This concentration was used as a study-specific positive control and was not considered directly equivalent to a standardized disk-diffusion assay (CLSI, 2024).

Antibacterial activity test (agar well diffusion)

The antibacterial activity test was performed using the well diffusion method on Mueller–Hinton agar (MHA). A bacterial suspension was spread evenly over the surface of the medium, and 9-mm-diameter wells were then made. A positive control of ciprofloxacin, a negative control of sterile distilled water, and samples of green tea leaf extract (1%, 2%, 3%, 4%, and 5%) were added to the wells in volumes of 50 µl, followed by incubation at 37 °C for 24 hours (Balouiri et al., 2016; Manandhar et al., 2019; Chavez-Esquivel et al., 2021). The diameter of the

inhibition zone was measured and categorized according to the Davis and Stout classification presented in Table 1.

Total plate count test

Total bacterial counts were determined before and after pasteurization and following the addition of green tea leaf extract. Fresh milk was evaluated before pasteurization, whereas the pasteurized control was evaluated immediately after the process. Green tea leaf extract was subsequently added to pasteurized milk at concentrations of 1%, 2%, 3%, 4%, and 5%. Pasteurization was performed at 65 °C for 30 minutes. Samples were serially diluted in 0.1% peptone water, and 100 µl of the appropriate dilution was inoculated onto BHIA using the spread plate method. Plates were incubated at 37 °C for 24 to 48 hours, after which colonies were counted and expressed as CFU ml⁻¹. The reduction attributable to pasteurization was calculated relative to the raw-milk bacterial count, whereas the additional difference associated with extract supplementation was calculated relative to the pasteurized control (Taye et al., 2021; FDA, 2025).

Sensory testing

Sensory testing was conducted on two types of formulations: one containing green tea leaf powder (0.10%, 0.25%, 0.50%, 1.00%, and 2.00%) and the other containing green tea leaf water extract (1%, 2%, 3%, 4%, and 5% w/v). Evaluations used a 5-point hedonic scale as follows: 1 = strongly dislike, 2 = dislike, 3 = neutral, 4 = like, and 5 = strongly like. The testing was conducted with 30 panelists, who assessed color, aroma, and taste (Lim et al., 2022).

Data analysis

Data analysis of the sensory test results was performed using SPSS. The Kruskal–Wallis test, followed by post hoc tests, was applied at a significance level of $p < 0.05$ to determine differences in panelists' ratings for the color, aroma, and taste parameters across concentrations. The sensory scores were expressed as mean values ± standard deviations (SD) (Triandini et al., 2024).

RESULTS AND DISCUSSION

Antibacterial activity of green tea leaf extract

Test results for antibacterial activity at concentrations of 1 to 5% yielded inhibition zones

Table 1. Classification of inhibition zone diameters

Inhibition zone diameter (mm)	Category
> 20	Very strong
10–19	Strong
5–10	Moderate
< 5	Weak

Source: Pratiwi et al. (2022)

of 4.26 to 21.37 mm against *S. aureus*, 2.77 to 5.79 mm against *S. epidermidis*, and 12.92 to 18.87 mm against *M. caseolyticus*. The highest inhibitory effect was observed only at a 5% extract concentration, with an inhibition zone of 21.16 mm for *S. aureus*. Testing showed that the addition of the 5% extract reduced contaminating bacteria by 5.2 log CFU ml⁻¹. In contrast, the control treatment (pasteurization without the addition of the extract) reduced them by only 2.4 log CFU ml⁻¹. These test results indicate that adding green tea leaf extract can increase the effectiveness of the milk pasteurization process by 216%. This result far exceeds that of conventional pasteurization.

The findings of Jallou et al. (2022) on pasteurized milk tested with extract concentrations of 0.1 to 1 mg ml⁻¹ for *Thymus vulgaris* L., rosemary (*Rosmarinus officinalis* L.), and *Ammoides* (*Ammoides pusilla* L.), combined with temperature treatments at 55, 65, and 75 °C, showed a reduction of the count by 0.11 to 0.85 log CFU ml⁻¹. According to a study by Bikheet et al. (2025) using clove water extract, the inhibition zones formed against Gram-positive bacteria measured 16 to 18 mm. Compared to those results, the inhibition zones formed in this study were superior, particularly at a concentration of 5%.

However, the inhibition zones for *S. epidermidis* ATCC 14990 in Table 2 ranged from weak to moderate. As shown in Figure 1, these results differ from those of other Gram-positive test bacteria, possibly due to the genetic properties of *S. epidermidis*, which is highly adept at forming biofilms that inhibit absorption and reduce the effectiveness of antibacterial compounds. The biofilm in *S. epidermidis* functions to block antibacterial agents; even as the concentration increases, the extract remains unable to penetrate the *S. epidermidis* biofilm, resulting in inhibition zones that tend to be weak to moderate (Ahmad et al., 2022; Oliveira et al., 2023).

The results of the antibacterial activity test against Gram-negative bacteria, presented in Table 3 and Figure 2, show inhibition zones ranging from weak to moderate. Test results at concentrations of 1 to 5% showed that *E. coli* produced inhibition zones of 0 to 7.42 mm, *Salmonella* spp. produced zones of 2.60 to 5.94 mm, *E. sakazakii* produced zones of 3.21 to 10.27 mm, and *Pantoea* spp. produced zones of 2.42 to 7.01 mm. The inhibition zones formed against

Gram-negative test bacteria were relatively small. Other studies using aqueous extracts from various plants have shown that the inhibition zones formed against Gram-negative dairy pathogens ranged from 9.68 to 12.60 mm (Mushemesh et al., 2023).

Green tea catechins, when interacting with bacterial cells, attack the lipid membrane, destabilizing its structure and increasing permeability. This mechanism results in the leakage of K⁺ ions, ATP, proteins, and intracellular components, leading to cell death (Amirdivani and Baba, 2015). The results for Gram-positive test bacteria showed antibacterial activity ranging from strong to very strong, whereas those for Gram-negative test bacteria ranged from weak to moderate. In Gram-negative bacteria, the outer lipopolysaccharide layer acts as a barrier to catechins as they travel toward their primary target—the lipid membrane—resulting in lower antibacterial activity than in Gram-positive bacteria (Sessa, 2022; Zhang, 2022; Xu et al., 2026). Overall, the antibacterial activity of green tea leaf extract against the test bacteria showed the optimal inhibition zone at a concentration of 5%. The results indicate that antibacterial activity increases with rising concentrations.

The potential of green tea leaf extract against bacteria causing milk-borne diseases

The reduction in bacterial count before and after pasteurization is shown in Figure 3. The control treatment demonstrated that the pasteurization method could not eliminate the entire bacterial population, but merely reduced the bacterial count in the milk. The bacterial count in the control treatment before pasteurization was 7.7 log CFU ml⁻¹, which decreased to 5.3 log CFU ml⁻¹ after pasteurization. The 1% extract treatment reduced the bacterial count to 3.8 log CFU ml⁻¹, whereas the 2% extract treatment reduced it to 3.5 log CFU ml⁻¹. At a 4% concentration, the count decreased to 2.7 log CFU ml⁻¹, whereas at a 5% concentration, the greatest reduction was observed, with the bacterial count falling to 2.5 log CFU ml⁻¹. The bacterial counts obtained for the extract treatments at concentrations ranging from 1 to 5% were in accordance with the quality standards set out in SNI 8984:2021 and PerBPOM No. 13/2019, namely 10⁴ to 10⁵ CFU ml⁻¹.

Figure 4 shows a reduction in the bacterial count in milk following pasteurization, with the reduction increasing progressively as the

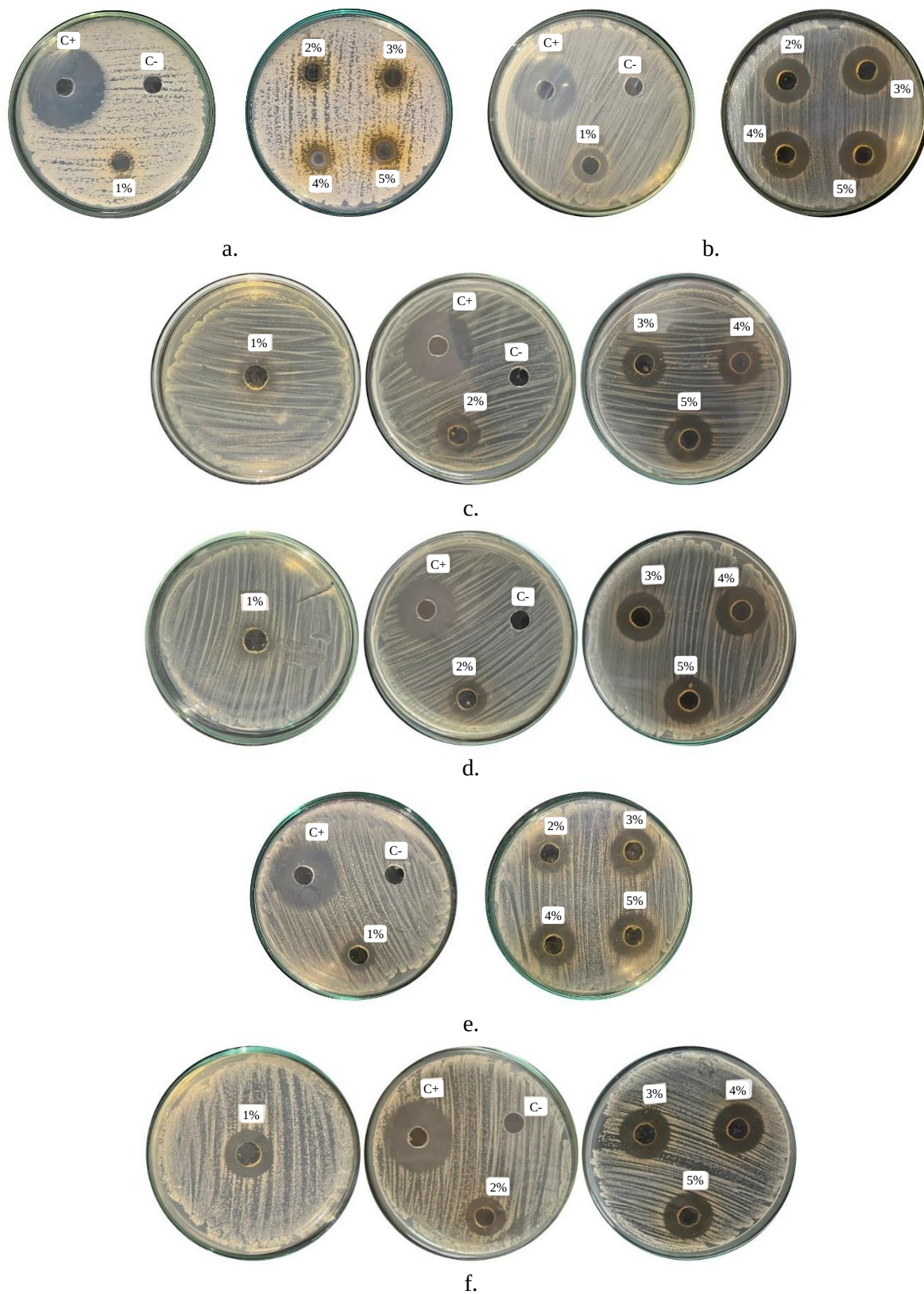


Figure 1. Inhibition zone results for: a) *S. epidermidis* ATCC 14990, b) *S. aureus* ATCC 25923, c) *S. aureus* SM 3.2.3, d) *S. aureus* SKMF 1.2.1, e) *S. aureus* SKMC 2.2.4, and f) *M. caseolyticus* SKMF 1.1.2

Note: C = treatment control, 1–5% = extract concentration

Table 2. Inhibition zone diameters for Gram-positive test bacteria

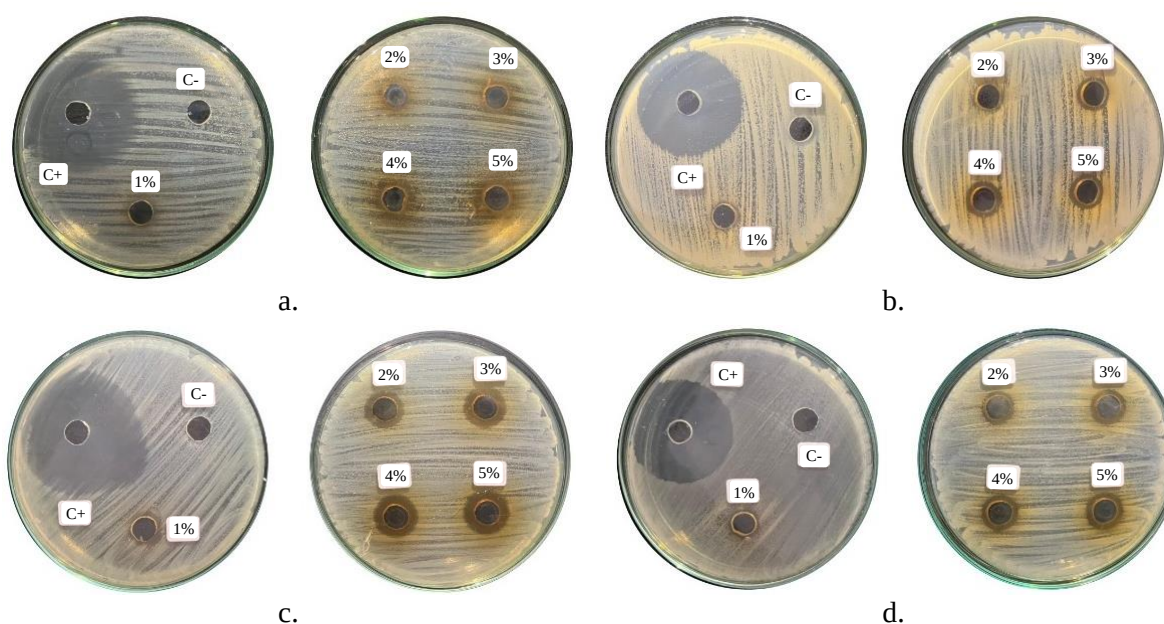
Test bacteria	Treatment (%)	Inhibition zone diameter (mm)	Classification
<i>Staphylococcus epidermidis</i> ATCC 14990	Control +	42.68	Very strong
	Control -	0.00	-
	1	2.77	Weak
	2	3.38	Weak
	3	4.10	Weak
	4	4.77	Weak
	5	5.79	Moderate
<i>Staphylococcus aureus</i> ATCC 25923	Control +	30.03	Very strong
	Control -	0.00	-
	1	12.19	Strong
	2	18.17	Strong
	3	20.20	Very strong
	4	21.37	Very strong
	5	21.16	Very strong
<i>Staphylococcus aureus</i> SM 3.2.3	Control +	33.17	Very strong
	Control -	0.00	-
	1	5.85	Moderate
	2	16.57	Strong
	3	16.70	Strong
	4	16.43	Strong
	5	20.11	Very strong
<i>Staphylococcus aureus</i> SKMF 1.2.1	Control +	25.93	Very strong
	Control -	0.00	-
	1	4.26	Weak
	2	13.77	Strong
	3	19.03	Strong
	4	19.02	Strong
	5	19.56	Strong
<i>Staphylococcus aureus</i> SKMC 2.2.4	Control +	31.94	Very strong
	Control -	0.00	-
	1	8.83	Moderate
	2	12.23	Strong
	3	16.18	Strong
	4	17.39	Strong
	5	17.58	Strong
<i>Macroccoccus caseolyticus</i> SKMF 1.1.2	Control +	32.03	Very strong
	Control -	0.00	-
	1	12.92	Strong
	2	13.42	Strong
	3	19.17	Strong
	4	18.64	Strong
	5	18.87	Strong

concentration of green tea leaf extract rises. A concentration of 5% resulted in the greatest reduction in bacterial count, amounting to 5.2 log CFU ml⁻¹, whereas other studies on pasteurized milk without the addition of extract showed a reduction of only around 2 to 4 log CFU ml⁻¹ (Sobeih et al., 2020; Mladenović et al., 2021;

Coleman et al., 2023; Aladhadh and Abdallah, 2025). Based on the study by Liu et al. (2022), which used a 1:20 (w/v) volume ratio equivalent to a 5% concentration, the polyphenol content was 27.10±0.05%, and the catechin content was 21.30±0.05%, with these compounds serving as the primary antibacterial agents.

Table 3. Inhibition zone diameters for Gram-negative test bacteria

Test bacteria	Treatment (%)	Inhibition zone diameter (mm)	Classification
<i>Escherichia coli</i> ATCC 25922	Control +	49.59	Very strong
	Control -	0.00	-
	1	0.00	-
	2	0.00	-
	3	0.00	-
	4	6.83	Moderate
	5	7.42	Moderate
<i>Salmonella</i> spp.	Control +	44.22	Very strong
	Control -	0.00	-
	1	2.60	Weak
	2	3.97	Weak
	3	4.64	Weak
	4	5.88	Moderate
	5	5.94	Moderate
<i>Enterobacter sakazakii</i> S8H3	Control +	51.38	Very strong
	Control -	0.00	-
	1	3.21	Weak
	2	7.55	Moderate
	3	9.18	Moderate
	4	9.77	Moderate
	5	10.27	Moderate
<i>Pantoea</i> spp. S21B	Control +	43.78	Very strong
	Control -	0.00	-
	1	2.42	Weak
	2	4.70	Weak
	3	5.97	Moderate
	4	6.59	Moderate
	5	7.01	Moderate

Figure 2. Inhibition zone results for: a) *E. coli* ATCC 25922, b) *Salmonella* spp., c) *E. sakazakii* S8H3, and d) *Pantoea* spp. S21B

Note: C = treatment control, 1–5%= extract concentration

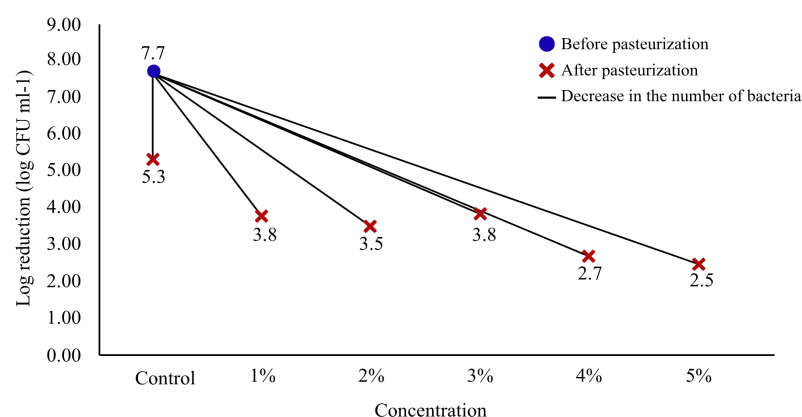


Figure 3. Bacterial count (log CFU ml⁻¹) in milk before and after pasteurization

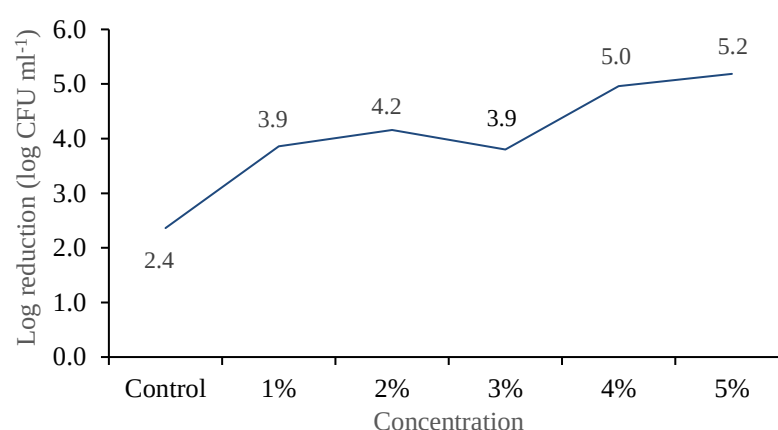


Figure 4. Log reduction in bacterial count in milk following pasteurization

The 5.2 log CFU ml⁻¹ reduction achieved at the 5% concentration is classified as very high compared with other studies using antimicrobials such as epinecidin-1, LAB metabolites, and plant extracts. The use of the antimicrobials epinecidin-1 and LAB metabolites reduced bacterial counts by only approximately 1.4 log CFU ml⁻¹ and 4.6 log CFU ml⁻¹, respectively (Salimnejhad et al., 2023; Wang et al., 2026). Other studies using plant extracts of *Melissa officinalis*, *Salvia officinalis*, and *Mentha spicata* reduced bacterial counts by only 0.6 to 1.4 log CFU ml⁻¹ (Silva et al., 2023).

The addition of green tea leaf extract to milk results in a positive interaction between milk casein and the polyphenols in the extract. Milk plays an important role in protecting and stabilizing green tea catechins against oxidation and degradation caused by environmental factors by forming a protective matrix around the catechins (Wijegunawardhana et al., 2024). Milk supplemented with green tea leaf extract also has a positive effect on fat stability. According to research by Ruengdech et al. (2025), adding green

tea leaf extract to milk reduces the formation of hydroperoxides (primary oxidation products) and malondialdehyde (secondary oxidation products), thereby minimizing the risk of rancidity and extending the milk's shelf life. It is hoped that this research will serve as a basis for optimizing the pasteurization process by adding green tea leaf extract to reduce contamination levels, thereby producing pasteurized milk products that are safe for consumption.

Sensory analysis of milk with green tea leaf extract

Sensory analysis was performed on 2 formulation types. For Formulation I, green tea leaf powder samples were used at concentrations of 0.10%, 0.25%, 0.50%, 1.00%, and 2.00%. These concentrations were determined based on previous research showing that green tea leaf water extract at 10 to 40 mg ml⁻¹ is effective as an antibacterial agent (Wali et al., 2020). According to research by Hariadi et al. (2023), green tea leaf extract at concentrations of 0.4 to 0.6% already affects the color, taste, and aroma

of the beverages. Other research indicates that concentrations of 0.05 to 0.25% added green tea leaf extract remain within normal limits in oat-based milk drinks (Qin et al., 2023).

Based on Table 4, the *p*-values obtained for the color and aroma parameters were ≥ 0.05 , with mean values of 4.03 ± 0.787 and 3.98 ± 0.755 , respectively. The panelists' assessments of these parameters did not differ significantly, with relatively little variation in the data. The addition of green tea leaf powder to the milk samples had the same effect on color and aroma across all concentrations. Meanwhile, for the taste parameter, the *p*-value obtained was < 0.05 with a mean of 3.56 ± 1.192 ; consequently, the ratings across concentrations differed significantly and showed high variation. The results of the taste parameter test were followed by a post hoc test to identify specifically which concentrations differed significantly.

Based on Table 5, the follow-up tests for the taste parameter showed that Group A (control, 0.1%, 0.25%, and 0.5%) was the most preferred by the panelists, with no significant differences among the samples within this group. Meanwhile, the 1% concentration (Group B) began to be disliked by the panelists, as it showed a significant difference from Group A. The 2% concentration was the least preferred, as it differed significantly from both Group A and Group B. In Formulation

I, the green tea leaf powder added to the milk did not dissolve during pasteurization, which significantly affected the taste, making it more bitter as the concentration increased.

Based on the antibacterial activity inhibition zones of the green tea leaf extract, the sensory test for Formulation II used an aqueous green tea leaf extract at concentrations of 1%, 2%, 3%, 4%, and 5%. The results of the sensory test, presented in Table 6, showed that *p*-values for the parameters of color, aroma, and flavor were all ≥ 0.05 , indicating no significant differences. The overall mean panelist ratings for color (4.18 ± 0.766), aroma (4.17 ± 0.751), and taste (4.23 ± 0.768) were consistent, with low variation. As the concentration increased, the color, aroma, and taste of the milk did not affect the panelists' level of preference.

The use of green tea leaf water extract added to the milk did not produce an excessively bitter taste sensation compared to Formulation I, and was therefore preferred by the panelists. This is because the green tea leaf water extract contains only a portion of the water-soluble compounds, meaning that the resulting bitterness is not as dominant as when green tea leaf powder is used (Orita et al., 2023). Particles of green tea leaf powder mixed with milk continue to release bitter compounds, resulting in a flavor that the panelists find less enjoyable (Hwang et al., 2021).

Table 4. Statistical analysis results of the sensory evaluation of Formulation I

Parameters	<i>p</i> -value	Mean $\pm$ SD
Color	0.166	4.03 ± 0.787
Aroma	0.325	3.98 ± 0.755
Taste	0.000	3.56 ± 1.192

Table 5. Results of the post hoc analysis of the taste parameters for Formulation I

Treatment (%)	Mean rank
Control	128.42 ^a
0.10	112.50 ^a
0.25	100.08 ^a
0.50	102.25 ^a
1.00	64.38 ^b
2.00	35.37 ^c

Note: Values in the same column followed by different superscript letters are significantly different ($p < 0.05$)

Table 6. Statistical analysis results of the sensory evaluation of Formulation II

Parameters	<i>p</i> -value	Mean $\pm$ SD
Color	0.744	4.18 ± 0.766
Aroma	0.656	4.17 ± 0.751
Taste	0.612	4.23 ± 0.768

Study limitations

Several limitations should be considered when interpreting these findings. First, the TPC assay provides an overall measure of viable cultivable bacteria and does not identify individual bacterial species responsible for the observed reduction. Second, the green tea extract was added after pasteurization; therefore, the reduction observed in the treated milk reflects the combined effects of pasteurization and subsequent extract exposure rather than the effect of the extract alone. Third, the study did not quantify the concentration of catechins or other active compounds in the extract. Finally, the sensory evaluation requires further clarification regarding panelist recruitment, ethical approval, randomization, and the statistical treatment of repeated observations. Future studies should use defined bacterial strains in milk, characterize the active compounds, evaluate extract stability, and determine microbiological and sensory outcomes under standardized storage conditions.

CONCLUSIONS

The antibacterial activity of green tea leaf extract demonstrated the strongest inhibitory effect against the Gram-positive bacterium *S. aureus*, with an inhibition zone ≥ 20 mm. In contrast, against Gram-negative bacteria from the *Enterobacteriaceae* family, it exhibited weak to moderate inhibition (2.60 to 10.27 mm). The highest antimicrobial activity at a 5% concentration was capable of reducing the bacterial count by 5.2 log CFU ml⁻¹, which is 2.8 log CFU ml⁻¹ higher than the control (2.4 log CFU ml⁻¹). Milk with aqueous green tea leaf extract at concentrations of 1 to 5% was most preferred, receiving consistent ratings for color, aroma, and taste. Further research is needed, including repeated testing using higher concentrations of green tea leaf extract, evaluating the stability of the bioactive compounds, and assessing the safety of the dairy product. In addition, other extracts should be added to enhance antimicrobial activity against Gram-negative bacteria.

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DECLARATION OF GENERATIVE AI USE

Artificial intelligence (AI) tools, namely Scopus AI and Copilot, were used to assist and facilitate the search for reference articles relevant to this research topic.

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