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Clove (*Syzygium aromaticum*) extract as a natural bio-preservative in milk: antimicrobial and organoleptic evaluation

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The food industry increasingly struggles to achieve product biological safety while avoiding artificial chemical preservatives that raise significant health concerns among consumers. The purpose of this study was to determine the optimal concentration threshold of ethanolic clove (*Syzygium aromaticum*) extract that maximizes bacterial control in refrigerated bovine milk while successfully preserving its baseline organoleptic attributes. To achieve this, serial dilutions of ethanolic and aqueous extracts were evaluated via the agar well diffusion method against *Staphylococcus aureus* and *Escherichia coli*, and inoculated milk samples stored at 4±1°C were monitored for Total Aerobic Count alongside descriptive sensory analysis using a nine-point hedonic scale. The experimental results demonstrated that the untreated control group exhibited the highest microbial load, reaching 6.29 and 7.17 log CFU/mL on Day 1 and Day 2 of storage, respectively. Conversely, the incorporation of clove extracts resulted in a significant, concentration-dependent reduction in bacterial profiles ($p<0.05$). Milk formulated with the maximum 100 % extract concentration demonstrated the highest antimicrobial efficacy, maintaining the lowest Total Aerobic Count values at 3.65 and 4.36 log CFU/mL. The agar well diffusion assay further verified that the ethanolic extract possessed a significantly more potent antibacterial activity compared to the aqueous variant, yielding superior zones of inhibition of 22 mm against *Staphylococcus aureus* and 21 mm against *Escherichia coli*, whereas the aqueous variant produced moderate zones of 20 mm. Notably, at the lowest 25 % concentration, neither extract displayed any detectable inhibitory activity. In the sensory evaluation, the inclusion of the ethanolic extract at 25 %, 50 %, and 75 % concentrations caused no statistically significant modifications ($p>0.05$) in milk aroma, color, or consistency. However, the 100% concentration level induced a critical organoleptic trade-off, significantly compromising product flavor (decreasing from 8.6±0.54 to 7.4±0.19) and overall acceptability (decreasing from 8.6±0.54 to 7.4±0.24). In conclusion, the 75 % concentration of ethanolic clove extract represents the mathematically established optimum for dairy bio-preservation, achieving sufficient bacterial control – reducing the Total Aerobic Count to 4.42 log CFU/mL on Day 1– without triggering consumer rejection.

Keywords: bovine milk, bio-preservation, clove extract, Total Aerobic Count, antibacterial efficacy, organoleptic attributes.

Екстракт гвоздики (Syzygium aromaticum) як натуральний біоконсервант у молоці: антимікробна та органолептична оцінка

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Харчова промисловість дедалі більше змагається за забезпечення біологічної безпеки продуктів, уникаючи використання штучних хімічних консервантів, які викликають серйозне занепокоєння щодо здоров'я споживачів. Метою цієї роботи було визначити поріг оптимальної концентрації спиртового екстракту гвоздики (*Syzygium aromaticum*), який забезпечує максимальний контроль чисельності бактерій в охолодженому коров'ячому молоці та успішне збереження його базових органолептичних показників. Для досягнення цього послідовні розведення спиртового та водного екстрактів оцінювали методом дифузії в агарі проти *Staphylococcus aureus* та *Escherichia coli*, а зразки інокульованого молока, що зберігалися за температури 4±1°C, контролювали за показником загальної кількості аеробних бактерій разом із проведенням описового сенсорного аналізу за дев'ятибальною гедоністичною шкалою. Експериментальні результати засвідчили, що необроблена контрольна група демонструвала найвище мікробне навантаження, досягаючи 6,29 та 7,17 log КУО/мл на першу та другу добу зберігання відповідно. Навпаки, внесення екстрактів гвоздики призвело до значного, залежного від концентрації, зниження кількості бактерій ($p<0,05$). Молоко з додаванням екстракту з максимальною концентрацією 100 % виявило найвищу антимікробну ефективність, забезпечуючи найнижчі показники загальної кількості аеробних бактерій – 3,65 та 4,36 log КУО/мл. Аналіз методом дифузії в агарі додатково підтвердив, що спиртовий екстракт має значно потужнішу антибактеріальну активність порівняно з водним варіантом, забезпечуючи більші зони затримки росту – 22 мм проти *Staphylococcus aureus* та 21 мм проти *Escherichia coli*, тоді як водний варіант сформував помірні зони діаметром 20 мм. Примітно, що за найнижчої концентрації 25 % жоден з екстрактів не виявив помітної пригнічувальної активності. Під час сенсорного оцінювання внесення спиртового екстракту в концентраціях 25 %, 50 % та 75 % не викликало статистично значущих змін ($p>0,05$) аромату, кольору чи консистенції молока. Проте рівень концентрації 100 % призвів до критичного органолептичного компромісу, достовірно погіршивши смак продукту (зниження з 8,6±0.54 до 7,4±0.19 балів) та його загальну прийнятність (зниження з 8,6±0.54 до 7,4±0.24 балів). На завершення, 75 % концентрація спиртового екстракту гвоздики є математично обгрунтованим оптимумом для біоконсервації молочної продукції, оскільки забезпечує достатній мікробіологічний контроль – знижуючи загальну кількість бактерій до 4,42 log КУО/мл на першу добу зберігання – без ризику відторгнення продукту споживачами.

Ключові слова: коров'яче молоко, біоконсервація, екстракт гвоздики, загальна кількість аеробних бактерій, антибактеріальна ефективність, органолептичні показники.

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Introduction

Food is currently recognized not only for its nutritional value but also for its capacity to enhance overall well-being. According to [1], individuals actively seek to include healthy foods in their diets to promote longevity. The dairy sector illustrates this evolution through the development of novel processing techniques aimed at enhancing the dietary value of its derivatives. Milk derivatives are highly abundant in vital elements, containing essential minerals, fatty acids, proteins, lactose, and vitamins [2].

The worldwide sector for dairy-based drinks, although still niche compared to yogurt and plain milk, has experienced steady growth. Nutritional dairy drinks, including those containing probiotics and prebiotics, have become increasingly popular. These beverages generally fall into two categories: those formulated from fruit juices and dairy products (especially whey), and those fortified with bioactive compounds such as sterols, vitamins, peptides, prebiotics, polyphenols, probiotics, fish oil, and minerals [3]. Many flavored milk-based drinks contain ingredients such as almonds, chocolate, coffee, sugar, or various natural and artificial flavors and colors to enhance consumer appeal, particularly among children and teenagers. All these drinks undergo thermal processing, such as pasteurization or sterilization [4]. However, synthetic pigments and artificial flavors raise significant health concerns and potential hazards. Conversely, natural herbal remedies mitigate these risks while offering further nutritional advancements. Aromatic plants, for instance, garlic, cardamom, ginger, cinnamon, and clove, are readily available and can serve as effective natural flavor enhancers, improving taste profiles and lowering production expenditures compared to artificial flavorings.

Clove (*Syzygium aromaticum*), a member of the Myrtaceae family, is a widely esteemed spice, ranking second in global commerce [6]. It is a prominent medicinal plant owing to its ability to treat various diseases. Clove contains phytochemicals that are crucial for plant development and exhibits potent antibacterial, antifungal, antiviral, antioxidant, and anti-inflammatory effects [5]. Furthermore, it significantly reduces the risk of gastric ulcers by decreasing inflammation in the stomach lining, making it an effective natural remedy for gastrointestinal distress [9]. Similarly, cinnamon has been used for centuries to treat inflammation and improve digestion due to its powerful antibacterial, anti-inflammatory, and antioxidant properties [7]. One of the main reasons for cinnamon's medicinal benefits is its bioactive compounds, especially its essential oils, which contain high concentrations of eugenol, exhibiting high efficacy against a wide range of viruses, fungi, and bacteria [8].

The aim of the study

The purpose of this study was to determine the optimal concentration threshold of ethanolic clove (*Syzygium aromaticum*) extract to maximize bacterial control in refrigerated milk while preserving its baseline organoleptic attributes.

Materials and methods

Plant material and origin

Plant samples of clove (*Syzygium aromaticum*) were purchased from a local market in Diyala Province, Iraq, in 2026. The specimens were initially cleaned to remove foreign matter and subsequently pulverized into a fine powder utilizing an electric grinder (***Fig. 1***).

Fig. 1. Clove (*Syzygium aromaticum*) buds before and after the milling process

Preparation of clove (Syzygium aromaticum) extracts

The extraction procedure followed a modified method described by [10], utilizing two distinct solvents: distilled water and a 10 % ethanol solution. Initially, fresh clove buds were thoroughly washed, drained, and dried in a vacuum oven at 40°C for 12 hours. Once completely dehydrated, the buds were milled into a fine powder. This powder was continuously stirred with the respective solvent at room temperature for two hours. The resulting mixture was filtered through Whatman No. 1 filter paper. The filtrate was subsequently concentrated using a rotary evaporator at 40°C, and the residual solution was distilled to yield the primary aqueous extract.

In a parallel preparation aimed at evaluating an alternative extraction thermal profile, 10 grams of the ground clove powder were suspended in 100 mL of distilled water and stirred continuously using a magnetic stirrer for 30 minutes at 50°C. The suspension was subsequently centrifuged at room temperature for 10 minutes at a standardized speed. The supernatant was separated, and the remaining solid residue was removed by filtration through Whatman No. 1 paper to obtain the alternative hot aqueous extract. Both extract variants were

transferred to sterile amber glass containers and stored under refrigeration at 4±1°C until further antimicrobial analysis.

To achieve a standardized stock solution (designated as 100 % concentration), the obtained clove extract was reconstituted in the respective solvent to yield a final stock concentration of 100 mg/mL. To ensure sufficiency for all experimental stages, this ratio was proportionally scaled up utilizing 100 g of the crude plant material per 1000 mL of solvent during the initial extraction phase for both the aqueous and ethanolic groups. Working concentrations were subsequently prepared via serial dilution of this standardized stock solution: 75 % (75 mL stock + 25 mL solvent), 50 % (50 mL stock + 50 mL solvent), and 25 % (25 mL stock + 75 mL solvent). All prepared working dilutions were transferred to sterile amber glass jars and stored under refrigerated conditions at 4±1°C prior to antibacterial sensitivity testing on Mueller-Hinton agar against *Staphylococcus aureus* and *Escherichia coli*.

Antimicrobial activity of clove extracts

The antibacterial properties of both ethanolic and aqueous clove extracts were evaluated using the agar well diffusion method, following the approach described by [11]. Two bacterial strains – the Gram-positive *Staphylococcus aureus* and the Gram-negative *Escherichia coli* – were selected to determine microbial sensitivity. To prepare the bacterial suspension, four to five colonies were isolated from each pure culture and transferred into test tubes containing 4 mL of sterile normal saline. The turbidity of each suspension was adjusted to match the 0.5 McFarland standard, corresponding to approximately 1×10^8 colony-forming units per milliliter (CFU/mL). A sterile cotton swab was immersed in the suspension and evenly spread across the surface of Mueller-Hinton agar plates. The plates were allowed to dry for 10 minutes, and wells were subsequently introduced. Following the application of the extracts, the plates were incubated at 37°C for 24 hours. The diameters of the zones of inhibition surrounding each well were measured and recorded in millimeters (mm), as outlined by [12].

Bacterial enumeration protocol for milk samples

Bacterial analysis of the milk samples was performed in strict accordance with the ISO 4833-1:2013 standard guidelines using the pour plate technique. Milk samples were collected aseptically into sterile containers and immediately transferred to storage at 4±1°C. Microbial enumeration was subsequently carried out on Day 1 and Day 2 of the storage period.

To prepare the serial dilutions, each milk sample was thoroughly agitated under a laminar flow hood to ensure a homogenous distribution of microorganisms. Sequentially, a 1 mL aliquot of the milk sample was aseptically transferred into a sterile test tube containing 9 mL of sterile 0.1 % peptone water to establish a 10^{-1} dilution, which was then vortexed for 10 seconds. This procedure was repeated by transferring 1 mL from the 10^{-1} dilution into a subsequent 9 mL of sterile diluent to obtain a 10^{-2} dilution. Serial tenfold dilutions were continued up to 10^{-6}, depending on the anticipated microbial load.

For the plating procedure, 1 mL of each prepared dilution was pipetted into sterile Petri dishes in triplicate. Approximately 15 mL of liquefied Plate Count Agar (PCA), cooled to 44±1°C, was dispensed into each dish. The plates were gently rotated to facilitate uniform mixing of the inoculum with the medium and allowed to solidify on a level surface. Upon solidification, the plates were inverted and incubated at 37°C for 48 hours.

Following the incubation period, Petri dishes containing between 30 and 300 colonies were selected for enumeration using an electronic colony counter. If no visible colonies were observed at the lowest dilution (10^{-1}), the result was documented as < 10 CFU/mL, representing the limit of detection. The total number of colony-forming units per milliliter (CFU/mL) of milk was calculated according to the following formula:

$$\text{CFU/mL} = \frac{C \times D}{V}$$

where:
- C is the number of colonies counted on the selected plate;
- D is the reciprocal of the dilution factor;
- V is the volume of the inoculum plated (1 mL).

The obtained data were subsequently transformed into logarithmic form ($\log_{10}$) CFU/mL) and documented for each sample on Day 1 and Day 2 of cold preservation.

To ensure the reliability of the method, rigorous quality control protocols were implemented. A negative control consisting of the sterile diluent plated on Plate Count Agar (PCA) was included in each analytical batch to monitor potential contamination. Concurrently, *Escherichia coli* ATCC 25922 was utilized as a positive control strain with a known countable range to validate medium performance and verify the consistency of incubation conditions.

Preparation of clove-flavored milk and experimental groups

To ensure bacteriological safety, 20 fresh bovine milk samples were subjected to thermal treatment. Initially, sugar was dispersed into the milk samples at a ratio of 9 % (w/v) at 50°C under continuous agitation to ensure complete dissolution, following the method described by [13]. The mixtures were subsequently heated to 90°C and held for 5 minutes. Following heat treatment, the milk was rapidly cooled to 45°C to prevent thermal decomposition of its nutritional constituents.

The heat-treated milk was then inoculated with the ethanolic extract of clove (*Syzygium aromaticum*). Five distinct experimental treatment groups were established based on the final concentration of the extract in the milk matrix:

- *Control group*: Untreated milk (0 mg/mL, no extract added);
- *25 % Extract group*: 1 mL of a 25 mg/mL dilution (prepared from 25 mL stock + 75 mL alcohol) added to 100 mL of milk, yielding a final concentration of 0.25 mg/mL;
- *50 % Extract group*: 1 mL of a 50 mg/mL dilution (prepared from 50 mL stock + 50 mL alcohol)

added to 100 mL of milk, yielding a final concentration of 0.50 mg/mL;

- *75 % Extract group*: 1 mL of a 75 mg/mL dilution (prepared from 75 mL stock + 25 mL alcohol) added to 100 mL of milk, yielding a final concentration of 0.75 mg/mL;
- *100 % Extract group*: 1 mL of the pure stock extract (100 mg/mL) added to 100 mL of milk, yielding a final concentration of 1.00 mg/mL.

Each treated milk sample was thoroughly homogenized under aseptic conditions and transferred into sterile, transparent container vials. All samples were stored under refrigeration at 4±1°C for 2 days to monitor and evaluate the preserving effect of the clove extract on the shelf life of the milk product.

Organoleptic Evaluation

Sensory evaluation of the milk samples was performed by a trained panel consisting of five expert examiners from the veterinary medical staff of the Public Health Division. While larger consumer panels are typically used for market preference studies, a small, highly trained panel of five to ten assessors is scientifically validated for descriptive organoleptic analysis of dairy and milk-based products, ensuring low intra-panel variability. The panelists underwent rigorous sensory training and were thoroughly acclimated to the specific evaluation criteria.

Prior to sensory analysis, each milk sample was coded with a unique barcode to ensure a blind study design. Following the evaluation protocols outlined by [14], the specimens were assessed for flavor, odor, color, texture, and overall acceptability. A nine-point hedonic scale was utilized for scoring, ranging from 1 (dislike extremely) to 9 (like extremely). To eliminate carryover effects and ensure data accuracy, panelists thoroughly rinsed their mouths with purified water between samples, and sessions were conducted at staggered time intervals. Each panel member recorded evaluations independently in isolated booths to ensure objectivity and uniformity in the outcomes, in accordance with [13].

Statistical analysis

The experimental data were subjected to statistical analysis utilizing the SAS software package (Statistical Analysis System, version 9.1). Differences among treatment means were evaluated via two-way analysis of variance (ANOVA), followed by the Least Significant Difference (LSD) post-hoc test for multiple comparisons. All data are presented as mean ± standard error (SE). Statistical significance was defined a priori at a p-value < 0.05 [15].

Results and discussion

Microbiological analysis of milk during storage

The Total Aerobic Count (TAC) serves as a critical indicator of product shelf life, given that elevated levels of these microorganisms inevitably accelerate product spoilage. To determine the preservation capacity of the plant material, the variations in the TAC of milk samples treated with distinct concentrations of ethanolic clove extract during refrigerated storage were systematically evaluated, as summarized in ***Table 1***.

Table 1

Effect of different concentrations of ethanolic clove extract on the Total Aerobic Count (log CFU/mL) of milk samples

Concentration	Day 1	Day 2
Control	6.29[a]	7.17[a]
25%	6.08[a]	7.02[a]
50%	5.65[b]	6.19[b]
75%	4.42[c]	4.81[c]
100%	3.65[d]	4.36[d]
LSD	0.19	

Note: different lowercase superscript letters within the same column indicate statistically significant differences ($p<0.05$).

At the initial evaluation points, the untreated control samples exhibited the highest microbial loads, reaching 6.29 and 7.17 log CFU/mL on Day 1 and Day 2, respectively. Conversely, data analysis presented in ***Table 1*** confirms that the incorporation of clove extracts resulted in a significant, concentration-dependent reduction in bacterial counts ($p\leq0.05$). Milk samples flavored with 100 % clove extract demonstrated the highest antimicrobial efficacy, maintaining the lowest microbial profiles at 3.65 and 4.36 log CFU/mL.

To visually track the dynamics of this inhibitory effect over the cold storage period, a comparative graphical projection of the concentrations against time intervals was established, as illustrated in ***Fig. 2***.

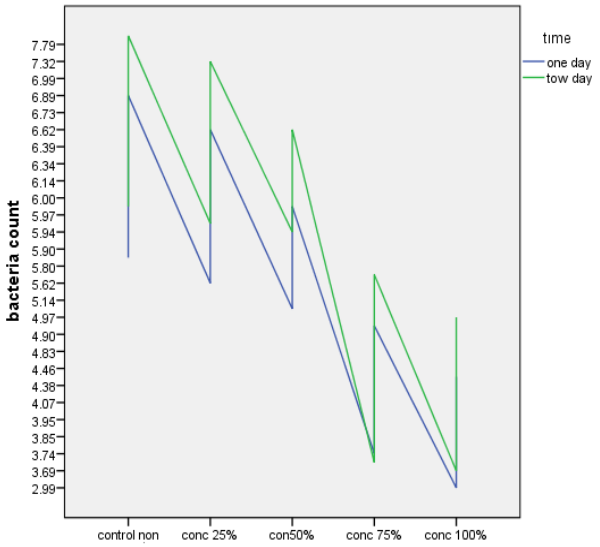

Fig. 2. Effect of different concentrations (25 %, 50 %, 75 %, and 100 %) of ethanolic clove (*Syzygium aromaticum*) extract over different storage periods (Day 1 and Day 2) on the Total Aerobic Count (log CFU/mL) of milk

The linear trends in ***Fig. 2*** illustrate that while the 25 % extract concentration showed non-significant divergence from the control group, concentrations of 50 % and above achieved a sharp, statistically validated drop in the log values. The post-hoc Least Significant

Difference (LSD) test established distinct homogenous groups, showing that doubling the extract strength progressively intensifies the preservation effect.

To provide primary microbiological proof of this suppression, the physical colony growth on the respective culture media was documented, as presented in ***Fig. 3***.

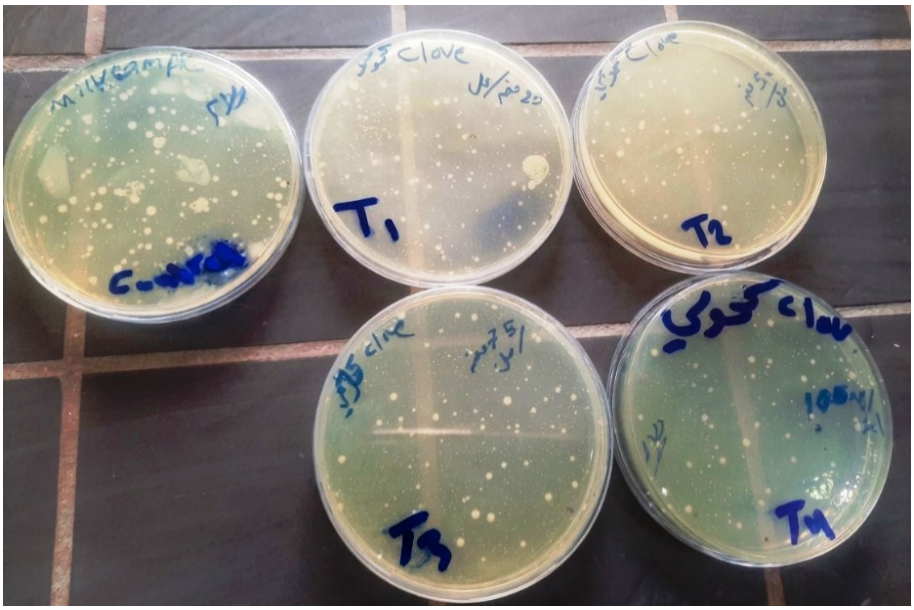

Fig. 3. Total Aerobic Count (TAC) plates of milk samples after treatment with different concentrations of ethanolic clove (*Syzygium aromaticum*) extract (Control: untreated; T1: 25 %; T2: 50 %; T3: 75 %; T4: 100 % concentration)

The visual comparison of the Petri dishes in ***Fig. 3*** confirms a profound reduction in colony-forming units, shifting from a dense, confluent bacterial lawn in the control plate to isolated, sparse colonies in the T3 and T4 treatment groups. This direct visualization perfectly corroborates the numerical parameters verified in Table 1, demonstrating that the ethanolic extract structurally inhibits the logarithmic replication phase of aerobic flora in the milk matrix.

Antibacterial efficacy of clove (Syzygium aromaticum) extracts

To establish the baseline sensitivity of specific bacterial profiles before milk application, the antimicrobial efficiency of two distinct variants of clove extracts (aqueous and ethanolic) was evaluated against prominent Gram-positive and Gram-negative indicators, with the resulting zone configurations shown in ***Fig. 4***.

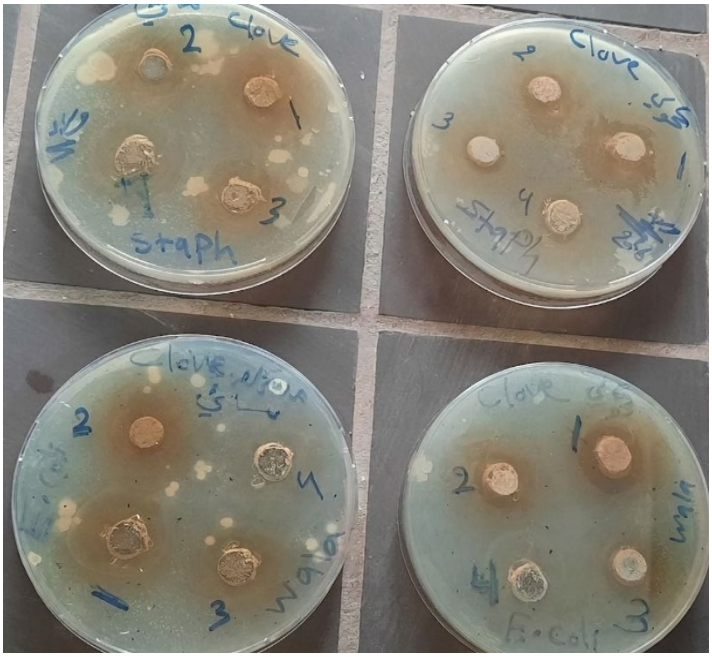

Fig. 4. Antibacterial activity of clove (*Syzygium aromaticum*) extracts evaluated via the agar well diffusion method against *Staphylococcus aureus* (left plates) and *Escherichia coli* (right plates) using ethanolic (top) and aqueous (bottom) extracts at various concentrations (1: 100 %, 2: 75 %, 3: 50 %, 4: 25 %)

The experimental results from the well diffusion assay illustrated in ***Fig. 4*** indicated that the ethanolic extract exhibited a substantially stronger antibacterial effect against both bacterial species compared to the aqueous extract ($p \leq 0.05$). Notably, at the lowest evaluated concentration (25 %), neither the aqueous nor the ethanolic extract displayed any detectable inhibitory activity (0 mm) against either of the tested microbial species. To perform a rigorous comparative analysis of the extraction yield efficacy specifically against the Gram-positive target, the measured inhibition diameters for *Staphylococcus aureus* were plotted in ***Fig. 5***.

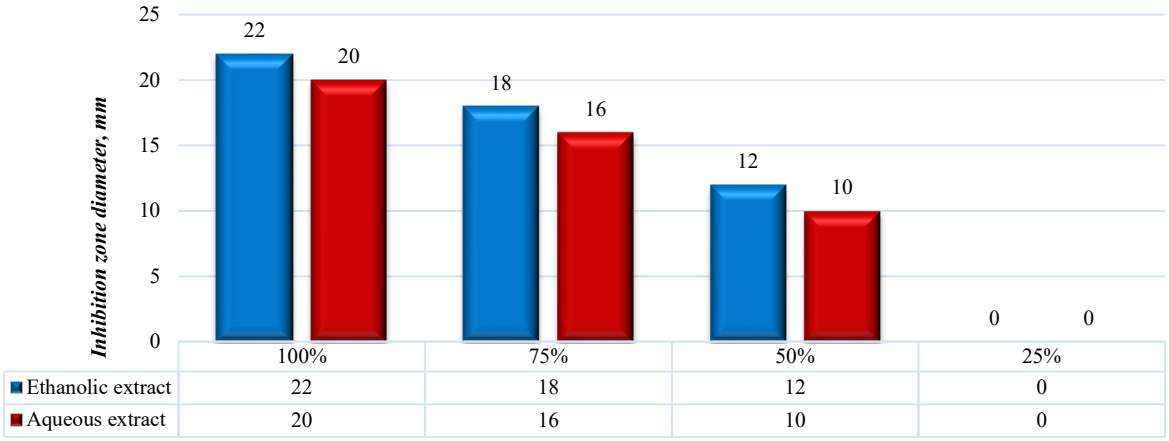

Fig. 5. Comparative antibacterial effect of ethanolic (alcoholic) and aqueous clove (*Syzygium aromaticum*) extracts at various concentrations against *Staphylococcus aureus*

As detailed in the comparative bar chart in ***Fig. 5***, the ethanolic extract demonstrated a highly significant antibacterial influence against *S. aureus*, producing superior inhibition zones of 22 mm at the 100 % concentration level, whereas the aqueous extract displayed a moderate reduction diameter of 20 mm. This variance underscores the higher solubility of hydrophobic phenolic compounds in organic solvents. To verify whether this susceptibility trend remains consistent when encountering the structural obstacles of Gram-negative cell walls, a parallel assessment was performed for *Escherichia coli*, as charted in ***Fig. 6***.

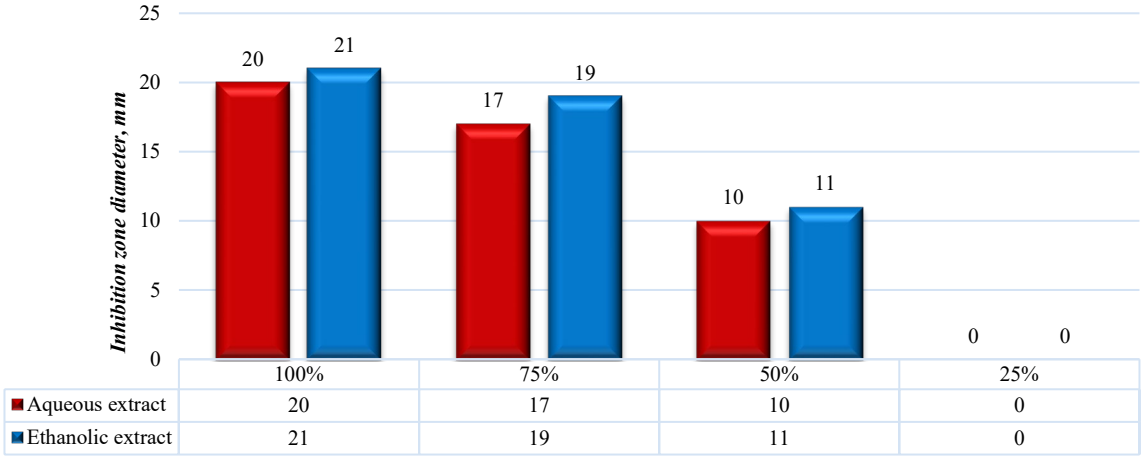

Fig. 6. Comparative antibacterial effect of ethanolic (alcoholic) and aqueous clove (*Syzygium aromaticum*) extracts at various concentrations against *Escherichia coli*

The data represented in ***Fig. 6*** show a similar trend for *E. coli*, with the ethanolic extract generating a peak zone of 21 mm compared to 20 mm for the aqueous variant at maximum strength.

The findings of this research align with prior investigations conducted by [16], which demonstrated that the ethanolic extract of clove exhibits clear inhibition zones against both Gram-negative and Gram-positive bacteria, indicating robust antimicrobial activity. This suggests that the inhibitory activity depends primarily on the chemical nature and constituents of the extract itself rather than on structural differences in the bacterial cell walls. Consequently, several dairy matrices have been the focus of clove incorporation in previous literature, including ice cream [17], probiotic yogurt powder [18], fermented milk beverages [19], and soft cheese [20].

The high phenolic content of clove induces the coagulation of bacterial cellular components, exerting a potent germicidal effect against numerous pathogenic microorganisms by disrupting active transport mechanisms, the proton motive force, and

electron flow [5]. This mechanism of action emphasizes the potency of ethanolic clove extract in suppressing microbial proliferation and supports its potential application as a natural antimicrobial agent in food preservation systems. Furthermore, [21] confirmed these findings, demonstrating that ethanolic extracts possess higher efficacy compared to aqueous variants. This enhanced efficiency is further supported by an earlier study conducted by [22], which indicated that the increased solubility of plant-derived bioactive compounds in polar organic solvents is a key determinant of their antimicrobial capacity.

A previous study by [23] also demonstrated that both ethanolic and aqueous clove extracts exhibit substantial antibacterial effects, specifically against *Klebsiella pneumoniae* and *E. coli*. The choice of extraction solvent significantly influences the overall yield of bioactive molecules; ethanol is documented to be more efficient than water due to the superior solubility of hydrophobic phenolic substances in organic media. Previous literature has similarly established that clove extracts prepared with ethanol have the potential to enhance both the nutritional value and health-promoting properties of milk and derivative dairy products [24]. Additionally, related investigations conducted by [25] demonstrated the efficacy of ethanolic extracts of *Syzygium aromaticum* at a concentration of 10 mg/mL against several pathogenic bacteria, including *S. aureus*, *E. coli*, *Pseudomonas aeruginosa*, and *Bacillus cereus*, yielding inhibition zones of 15.8 mm, 11.9 mm, 13.4 mm, and 14.6 mm, respectively.

Furthermore, [26] highlighted that clove has garnered considerable attention for its pivotal role in mitigating antimicrobial resistance. As drug-resistant bacterial infections become increasingly prevalent, the capacity of clove constituents to enhance the efficacy of traditional antibiotics positions it as an essential complementary therapeutic agent. Research indicates that clove can help restore antibiotic activity against resistant pathogens, such as *Proteus* spp., which are frequently associated with urinary tract and wound infections. By increasing bacterial sensitivity to conventional antibiotics, clove aids in combating multidrug-resistant strains, thereby offering a viable contribution to addressing the escalating global challenge of antimicrobial resistance within healthcare systems.

Organoleptic assessment of flavored milk samples

While achieving microbial stability is a primary objective in bio-preservation, the incorporation of potent botanical extracts must not degrade the baseline consumer characteristics of the beverage. To determine the practical viability of the formulation, the organoleptic attributes of milk flavored with various concentrations of ethanolic clove extract – specifically evaluating aroma, flavor, color, texture (consistency), and overall acceptability – were systematically monitored by the expert panel and are summarized in ***Table 2***.

Table 2

Organoleptic characteristics of flavored milk samples supplemented with different concentrations of ethanolic clove extract, *mean±SE.*

Sensory attributes	Aroma	Flavor	Color	Consistency	Overall acceptability
Control	8.4±0.54^{a}	8.6±0.54^{a}	8.6±0.54^{a}	8.2±0.44^{a}	8.6±0.54^{a}
25% Extract	8.4±0.24^{a}	8.6±0.31^{a}	8.6±0.19^{a}	8.4±0.24^{a}	8.6±0.19^{a}
50% Extract	8.0±0.44^{a}	8.4±0.24^{a}	8.4±0.19^{a}	8.4±0.24^{a}	8.4±0.19^{a}
75% Extract	7.8±0.19^{a}	8.4±0.24^{a}	8.2±0.19^{a}	8.4±1.41^{a}	8.0±0.31^{a}
100% Extract	7.6±0.24^{a}	7.4±0.19^{b}	7.8±0.19^{a}	8.6±0.18^{a}	7.4±0.24^{b}
LSD	1.18	1.87	1.17	0.85	0.73
Significance	NS	$p<0.05$	NS	NS	$p<0.05$

Note: NS: non-significant ($p>0.05$). Different lowercase superscript letters within the same column indicate statistically significant differences ($p<0.05$).

The experimental results compiled in ***Table 2*** revealed no statistically significant differences ($p>0.05$) in the monitored sensory properties compared to the untreated control group, with the sole exceptions of flavor and overall acceptability scores at the maximum (100 %) concentration level. This study aligns with previous literature, such as the report by [20], which documented excellent sensory retention in beef cuts and soft cheeses processed with clove and alternative herbal extracts. Consistent with those reports, the current data suggest that ethanolic clove extract can be successfully utilized to enhance the organoleptic attributes of various food matrices, including dairy-based beverages. Throughout the storage period, the inclusion of the extract at any evaluated concentration did not notably alter the visual color values of the milk. This observation is in close agreement with [13], who reported that milk-based beverages fortified with plant extracts exhibit minimal or negligible color modifications. Additionally, as noted by [27], clove has a well-established history of use as a natural preservative in various food matrices, serving as a viable alternative to synthetic chemical additives.

The compiled experimental outcomes indicated that while the 100 % clove extract concentration exhibited the most robust antibacterial effect, it simultaneously exerted a negative impact on the sensory attributes of the milk matrix, particularly reducing overall acceptability scores due to an over-concentrated flavor profile. In contrast, the 75% concentration demonstrated effective antibacterial efficacy while successfully maintaining acceptable sensory quality. Therefore, selection of the optimal extract concentration must be based on a balanced evaluation of both microbiological stability and sensory quality, rather than prioritizing antibacterial potency alone. From both industrial manufacturing and consumer perspectives, natural preservatives must effectively inhibit bacterial proliferation while preserving the desired organoleptic characteristics of the product. For this reason, the 75 % concentration of ethanolic clove extract

was determined to be optimal and most suitable for commercial application, achieving sufficient bacterial control without compromising consumer acceptance.

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Based on the established optimal parameters, several prospective recommendations for future research can be outlined:

- To comprehensively understand the long-term preserving capacity of ethanolic clove extract, future investigations should evaluate its potential to extend the refrigerated storage life of dairy products over a prolonged period of one to two weeks.
- Recent literature supports the development of nanotechnology-based delivery systems for clove extracts; such nanoparticles could significantly improve the absorption and stability of eugenol, maximize targeted antibacterial efficacy, and effectively bypass microbial drug resistance pathways.
- Additional rigorous screening is needed to evaluate potential synergistic interactions between clove extracts and standard pharmaceutical antibiotics, which could significantly augment conventional antimicrobial effectiveness in healthcare and food preservation systems.

Scientific novelty statement

While the antimicrobial properties of clove extract are widely recognized, this study provides the first systematic evidence establishing a precise optimal concentration threshold (75 %) that maximizes the reduction of bacterial loads in refrigerated milk – achieving a decrease from 6.29 to approximately 4.42 log CFU/mL – while simultaneously preserving all key organoleptic attributes (flavor, aroma, color, and consistency) without statistically significant differences from the untreated control. Furthermore, this investigation uniquely demonstrates that concentrations exceeding this threshold (100 %), despite offering the maximum antibacterial potency (inhibition zones of 22 mm against *Staphylococcus aureus* and 21 mm against *Escherichia coli*), create a critical organoleptic trade-off by significantly compromising flavor profiles and overall consumer acceptability. These findings deliver a practical, evidence-based solution for the dairy industry aimed at replacing synthetic chemical additives with natural plant-derived alternatives without triggering consumer rejection.

Conclusions

The agar well diffusion analysis demonstrated that the ethanolic clove (*Syzygium aromaticum*) extract possesses a significantly more potent antibacterial activity compared to the aqueous variant, yielding maximum zones of inhibition of 22 mm against *Staphylococcus aureus* and 21 mm against *Escherichia coli* at 100 % concentration, whereas both extracts displayed no inhibitory activity (0 mm) at 25 % concentration. In the sensory evaluation, the incorporation of the ethanolic extract at concentrations of 25 %, 50 %, and 75 % caused no statistically significant modifications ($p>0.05$) in milk aroma, color, or consistency compared to the untreated control group. However, the maximum 100 % concentration level induced a critical organoleptic trade-off, significantly compromising the product flavor (decreasing from 8.6±0.54 to 7.4±0.19) and overall acceptability (decreasing from 8.6±0.54 to 7.4±0.24). Consequently, the 75 % concentration was established as the optimal formulation for bio-preservation, as it successfully balanced high microbiological efficacy – achieving a substantial reduction in the Total Aerobic Count from 6.29 to 4.42 log CFU/mL on Day 1 and from 7.17 to 4.81 log CFU/mL on Day 2 of refrigerated storage – with the complete retention of acceptable product sensory quality.

DECLARATIONS

Ethical Statement

Not applicable. This study was conducted entirely *in vitro* using bacterial strains and bovine milk samples, and did not involve any live animal subjects or human tissues.

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Conflict of Interest

The authors declare no conflict of interest.

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Declaration of AI and AI-assisted technologies

The authors declare that no generative AI or AI-assisted technologies were used during the preparation, writing, translation, or language polishing of this manuscript. The final content was fully authored and reviewed by the authors, who take sole responsibility for the work.

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