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Research Article
Functional Evaluation of Moringa Seed Extracts as Plant-Based Coagulants for Camel Milk

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Abstract

Background and Objective: Cheese production from camel milk is limited by poor coagulation, leading to weak curd structures. Plant-based coagulants, such as *Moringa oleifera* seeds, have shown potential, but little is known about *Moringa peregrina*, a related species native to the Middle East and Northeast Africa. This study evaluated crude seed extracts of *M. peregrina* as a novel plant-based coagulant for camel milk and compared their performance with *M. oleifera*. **Materials and Methods:** Extracts were biochemically characterized, and their antimicrobial and antioxidant activities were assessed. Milk-clotting performance was tested under varying pH and temperature conditions. Trisodium citrate was evaluated as a milk-standardizing agent. Statistical analysis was performed using SPSS v19 with ANOVA and mean comparisons at $p<0.05$. **Results:** Both extracts exhibited strong antimicrobial activity against Gram-positive bacteria, notably *Staphylococcus aureus* and *Bacillus cereus*. Antioxidant activity was higher in *M. oleifera* (63.32%) than *M. peregrina* (49.93%). Temperature had a greater impact on clotting than pH, with firm curd formation at 60°C and weaker coagulation at 45°C, especially for *M. peregrina*. Trisodium citrate (≥30 mmol/L) inhibited curd formation. High milk-clotting to proteolytic activity ratios indicated strong enzyme specificity. **Conclusion:** *M. peregrina* seed extract is a promising plant-based coagulant for camel milk, offering a viable alternative to animal rennet. Future studies should focus on enzyme purification, molecular characterization, and detailed evaluation of cheese curd quality.

Key words: Camel milk, *Moringa oleifera*, *Moringa peregrina*, antioxidant activity, antimicrobial effect, total phenols, sustainability

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Camel milk has gained recognition as a vital nutritional resource in arid and semi-arid regions, offering unique health benefits and economic opportunities for pastoral communities[1]. Rich in essential nutrients and bioactive compounds, it serves as an important dietary staple for millions and holds promise for functional food development[2]. However, the unique physicochemical properties of camel milk especially its distinct casein composition present challenges for traditional dairy processing, particularly in cheese making where effective coagulation is crucial[3]. Conventional cheese production relies on rennet (chymosin) from calf stomach linings to coagulate cow's milk. However, camel milk does not respond well to this method, resulting in weak curd formation and low cheese yields due to lower κ-casein content and structural differences in its casein micelles[3,4]. This highlights the need for alternative coagulation strategies tailored to camel milk's unique characteristics[2,3].

Otherwise, the use of recombinant enzymes remains limited in certain countries due to religious and dietary restrictions. Moreover, the declining availability and increasing cost of calf rennet, coupled with the growing global demand for cheese, have driven the search for alternative milk-clotting enzymes. These alternatives are also influenced by religious and ethical considerations, including vegetarianism[5,6]. Given the challenges associated with producing high-quality cheese from camel milk, recent research has focused on plant-derived coagulants as potential substitutes for traditional rennet[7-11].

Several plant-based enzymes are currently employed in cheesemaking, with studies comparing their effects with those of animal rennet on rheological and sensory properties. However, due to their high proteolytic activity (which often leads to bitterness) plant coagulants are not yet fully suitable for widespread cheese production[6]. Plant proteases are classified by their hydrolytic mechanisms into aspartic, serine, and cysteine proteases[12]. Coagulants derived from plants such as *Zingiber officinale*[13], *Ficus carica*[14], and *Withania coagulans*[8] have been applied in camel milk cheese production, yielding acceptable results. In the same context, Moringa seed extract, rich in nutrients and bioactive compounds beneficial for human health, has shown promise as a plant-based alternative to animal rennet[15,16].

Moringa extracts are known for their rich mineral content, including potassium, phosphorus, sodium, zinc, magnesium, and calcium. It also contains a significant amount of oil, with a high saturated fatty acid content, including palmitic acid, behenic acid, stearic acid, and arachidic acid. Furthermore, the extract contains bioactive compounds such as isothiocyanates, flavonoids, and polyphenols, which contribute to its health benefits. Overall, Moringa seed extract is considered a powerful superfood with various nutrients and bioactive compounds that can support human health[17-19]. *Moringa oleifera* seed extract has been found to contain milk clotting enzymes that can be used in cheese production. Research has shown that the seed extract of *M. oleifera* generates suitable milk clotting activity for cheesemaking. Additionally, the milk clotting enzyme (MCE) of *M. oleifera* from prepared seed waste has been used in goat soft cheese production, indicating its potential as a coagulant. Furthermore, studies have explored the caseinolytic and milk-clotting activities from *M. oleifera* flowers, indicating the versatility of this plant in producing milk-clotting enzymes. The partial purification of milk-clotting enzyme from the seeds of *M. oleifera* has also been investigated, highlighting the ongoing interest in utilizing this natural source for milk clotting. The extraction process for these enzymes has been studied, with different types of extracting solutions being evaluated to find the most reliable, quick, and efficient enzyme extracting solution[15,16,20].

Because limited information is available on *M. peregrina*, a related species native to the Middle East and Northeast Africa, this study aims to evaluate the effectiveness of crude extracts from Moringa seeds as plant-based coagulants for camel milk. It investigates the coagulation efficiency and functional performance of *M. peregrina* seeds in comparison to *M. oleifera* seeds. The goal is to identify a viable, sustainable, and locally accessible alternative for processing camel milk. The findings of this research could contribute to the development of new camel milk products and support the use of plant-based coagulants in dairy technology.

MATERIALS AND METHODS

Materials: The current research was conducted between May and August 2025. Raw camel milk (Total solids: 12.60±0.14%, Protein: 2.71±0.25%, Fat: 4.22±0.39%, Ash: 0.83±0.01% and Lactose: 4.48±0.24%) was freshly collected during the morning milking of Wadah species (aged from 5 to 8 years) from local breeders in Al-Asyah area, Qassim Region, Saudi Arabia. Immediately after milking, collected milk was kept under cooling conditions (4±1°C) and directly transferred to the lab (Food science and human nutrition department at girls' section, College of Agriculture and Food, Qassim University, Saudi Arabia) for further preparations. *Moringa peregrina* and *Moringa oleifera* seeds were obtained from Qataf Radwa Organic Farm in AlUla city, Saudi Arabia. Tri-sodium citrate (TSC: Merck, Darmstadt, Germany) was purchased from Bayouni Trading Co. Ltd., Riyadh St., Cross 21 Bayouniya Alkhobar, Khobar, Riyadh, Saudi Arabia.

All chemicals used in the study were of analytical grade. Chemicals for each experimental method are mentioned in detail under its method of analysis, described subsequently.

Moringa seed's crude extract preparation: The crude extract of *M. oleifera* and *M. peregrine* seeds was prepared following the method described by Terefe *et al.*[3] and Sánchez-Muñoz *et al.*[20] which is illustrated in Fig. 1. Briefly, mature seeds from each *Moringa* spp. were brought directly after harvesting, and the outer shells were manually removed to extract the kernel. The kernels were ground using an electric grinder to produce a coarse powder. The powder was then dried in an air oven at 40°C for one hour to reduce moisture content while preserving enzymatic activity.

After that, fifty grams of the dried powder were mixed with 500 mL of saline solution consisting of 5% Sodium Chloride (NaCl) dissolved in a 0.1 mol/L sodium acetate buffer (pH 5.0). The mixture was stirred for 30 minutes using a magnetic stirrer, then treated in an ultrasonic bath (42 kHz) for 15 minutes. Afterward, it was filtered through double-layer cheesecloth to remove solid residues, and then centrifuged at 10,000 rpm for 20 minutes at 4°C) using a Hermle Labortechnik GmbH, Wehingen, Germany centrifuge with SER. # 220.72, Type 09/144 rotor centrifuge. The resulting crude extract was used for the rest of the study.

Study design: Crude extracts were prepared from the seeds of both *M. oleifera* and *M. peregrina*. These extracts were then evaluated for their antioxidant properties, specifically total phenolic content (TPC) and DPPH radical scavenging activity. Additionally, the extracts were tested for their antimicrobial effects against both gram-positive (*Staphylococcus aureus* and *Bacillus cereus*) and gram-negative (*Escherichia coli*, *Salmonella typhimurium*, and *Klebsiella pneumoniae*) pathogenic bacterial strains.

Furthermore, the milk clotting activity (MCA) of both *Moringa* spp. crude extracts was assessed under varying conditions, including different pH levels (5.5 and 6.2), temperatures (45°C and 60°C). To assess the MCA, camel milk was treated as illustrated in Fig. 2. Additionally, a separate examination was conducted to determine the effect of varying concentrations (10, 20, 30, and 40 mmol/L) of TSC on camel milk coagulation under consistent conditions of pH (~6) and temperature (45±3°C).

Methods of analysis

DPPH radical scavenging activity: A DPPH radical scavenging activity of the samples was determined according to the modification mentioned by Mudgil *et al.*[21]. The decolorization of DPPH free radicals after scavenging was monitored by measuring the absorbance at 517 nm after 30 min of

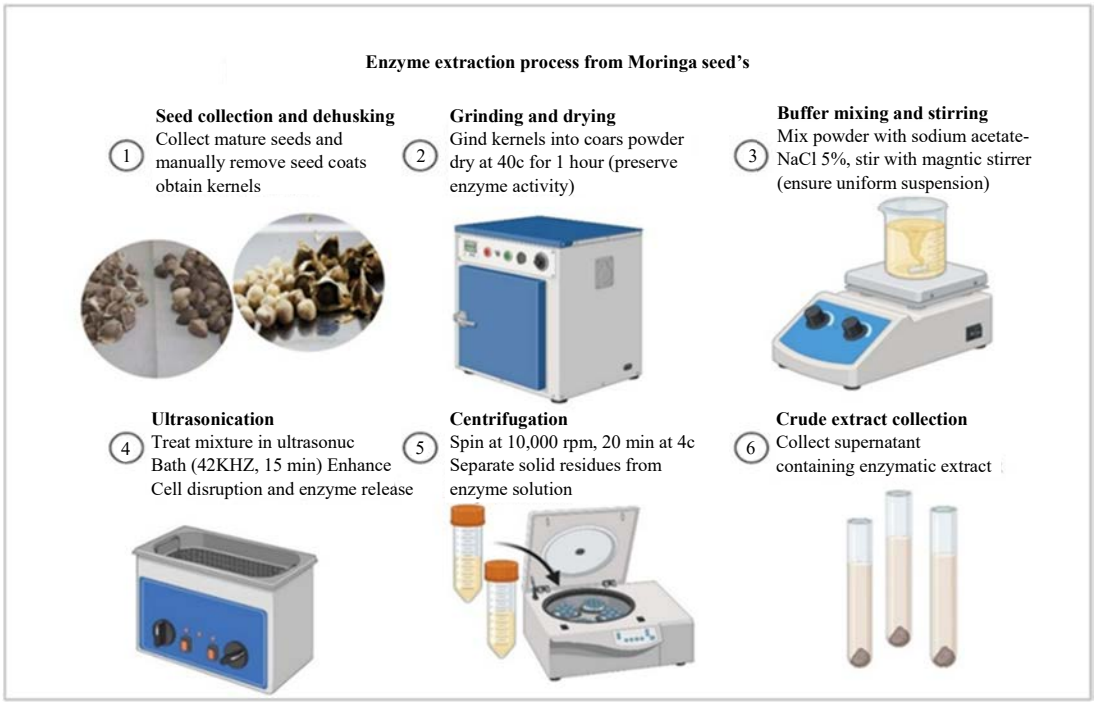

Fig. 1: Illustration of the crude extract preparation from Moringa seeds

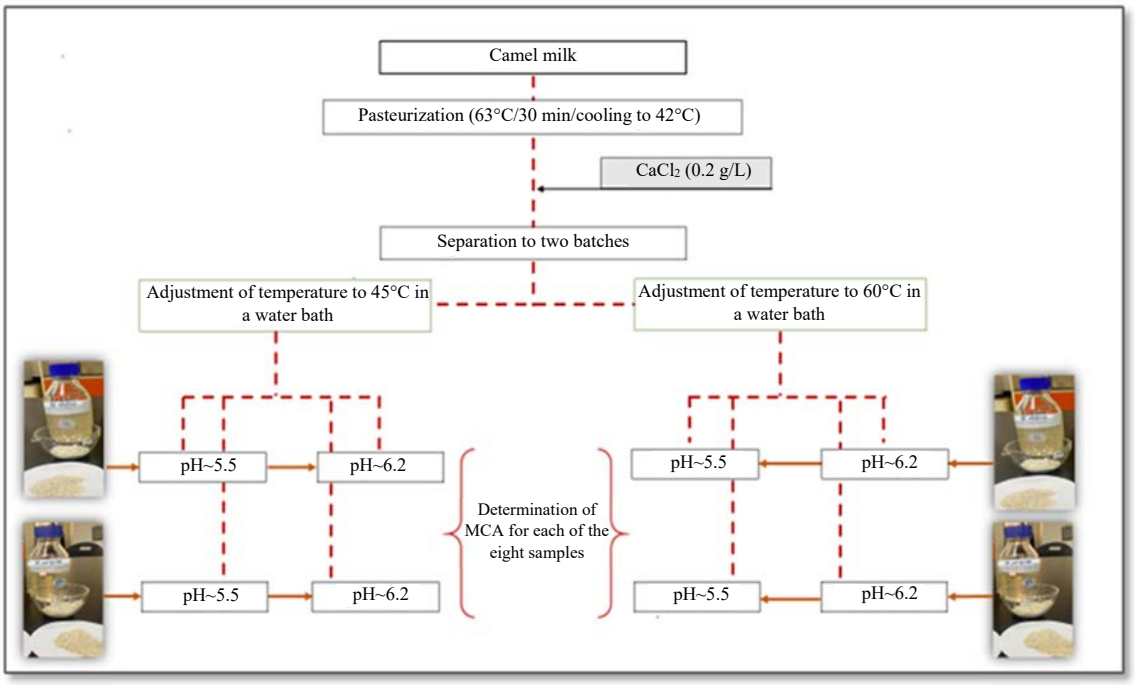

Fig. 2: Preparation of camel milk for MCA assessment

incubation at 37°C using a 96-well microplate reader (Multiskan Sky, Thermo Fisher Scientific, Cambridge, MA, USA). A 200 µL of each soluble nitrogen extract was mixed with 800 µL of DPPH reagent (0.1 mmol/L in 95% methanol) in a 96-well microplate reader and left in the dark for 30 min before the measurement. The radical scavenging activity percentage (DPPH radical scavenging activity of extracts %) was calculated as follows:

$$\text{DPPH radical scavenging activity (\%)} = \frac{(A_0 - A_s)}{A0 \times 100}$$

where, A_0 is the absorbance at 517 nm of blank (the blank was distilled water) and As is the absorbance at 517 nm of extract. Also, a standard curve equation sing Trolox was used to utilized in calculating the µm Trolox equivalent in one ml sample.

Total phenolic content (TPC): The total phenolic content (TPC) of the prepared extract from each sample was determined using the Folin-Ciocalteu reagent, following the method described by Bettaieb *et al.*[22]. In brief, 400 µL of the methanolic extract was mixed with 1000 µL of the Folin-Ciocalteu reagent in Eppendorf tubes and allowed to react for 5 minutes. Next, 300 µL of an alkali solution (7.5% sodium carbonate, Na_2CO_3) was added. The mixture was then incubated in the dark for 60 minutes at 23°C, after which it was centrifuged at 10,000 × g for 10 minutes at 4°C. Following centrifugation, 120 µL of the supernatant from each tube was transferred to a new plate, and the absorbance was measured at 765 nm using a microplate reader (BioTek, Winooski, VT, USA). TPC values were calculated by plotting the results on a standard curve of gallic acid (GA) and expressed as gallic acid equivalents (GAE), reported for the Moringa crude extract as mg GAE/mL and for the cheese sample as mg GAE/g.

Anti-microbial effect of Moringa seed's crud extracts: The antimicrobial activity of *M.* oleifera and *M. peregrina* seed extracts was evaluated using the agar well diffusion technique[23,24], with a well diameter of 6.0 mm (100 µL of extract was tested). This assay was conducted at the Regional Centre for Mycology and Biotechnology (RCMB) at Al-Azhar University, Cairo, Egypt, on 11 August, 2025. The screening tests focused on the inhibition zones produced against pathogenic microorganisms, specifically *Staphylococcus aureus* (RCMB 010010) and *Bacillus cereus* (RCMB 027) as Gram-positive bacteria, as well as *Escherichia coli* (RCMB 010052, ATCC 25955), *Salmonella typhimurium* (RCMB 006, ATCC 14028), and *Klebsiella pneumoniae* as Gram-negative bacteria. The standard antibiotic gentamicin (4 mg/mL) served as the positive control sample for clear differentiation.

Milk clotting activity (MCA) of Moringa seed's crud extracts: The milk clotting activity was measured following the method described by Omrani *et al.*[25]. One millilitre (mL) of crude extract from *Moringa* spp. seeds was added to 10 mL of camel

milk after the addition of Calcium Chloride ($CaCl_2$) at a concentration of 0.2 g/L. The pH and temperature were adjusted as needed. The milk clotting time was recorded in seconds once discrete particles became clearly visible[26]. The milk clotting activity (MCA) was then calculated using the following equation:

$$MCA\ (u / mL) = (\frac{2400}{t}) \times (\frac{S}{E})$$

where, t is clotting time (sec), S is volume of milk (mL) and E is volume of crude extract (mL)

Statistical analysis: The statistical analysis was carried out using the SPSS program (ver. 19) with multi-function utility regarding the experimental design and multiple comparisons using ANOVA with comparison of differences between the means of the treatments at the significance level of $p<0.05$ carried[27].

RESULTS AND DISCUSSION

DPPH radical scavenging activity and TPC of the seed's crude extract from *Moringa* spp.: The DPPH radical scavenging activity (measured in µg TE/mL) and TPC (expressed as mg GAE/mL) of crude extracts from *M. oleifera* and *M. peregrina* seeds are shown in Fig. 3. The crude extract of *M. oleifera* seed demonstrated a greater ability to scavenge free radicals compared to those of *M. peregrina*, with values of approximately 63.32% (2.17 µmoL TE/mL) and 49.93% (1.56 µmoL TE/mL), respectively. A similar pattern was observed in the total phenolic content of the seed extracts. This indicates a positive correlation between DPPH radical scavenging activity and TPC in the extracts, which aligns with the findings of a study conducted by Ghada *et al.*[28]. They investigated the biochemical composition of *M. oleifera* seeds and other parts of the plant, as well as their antioxidant potential. Their results demonstrated a significant relationship between total phenolic content and antioxidant activity, suggesting that phenolics play a crucial role in the radical scavenging capacity. In contrast to our findings, Bawadekji *et al.*[29] noted similar phytoconstituents in both *M. peregrina* and *M. oleifera*, with their antioxidative properties being nearly equivalent. A study by Abdalla *et al.*[30] showed that the ground seeds of Moringa, specifically *M. oleifera* and *M. peregrina*, contain polyphenols such as gallic acid, chlorogenic acid, methyl gallate, and caffeic acid, which provide several health benefits. While the study of Jahan *et al.*[31] on different three extracts (methanol, acetone and water) of *M. oleifera* seed revealed that the water extract showing significant antioxidant activity due to its high free radical scavenging capacity and phenolic and flavonoid content. The antioxidant effects of crude extracts from *Moringa* spp. seeds are generally correlated with total phenolic compounds. Specifically, gallic acid, quercetin, and ferulic acid have been identified as major contributors[32].

Antimicrobial effect of Moringa seed's crude extracts from *Moringa* spp.: The antimicrobial effects of crude seed extracts from *Moringa* spp. against selected bacterial pathogens are presented in Table 1 and Fig. 4. Both *M. oleifera* and *M. peregrina* seed extracts exhibited larger inhibition zones against the gram-positive strains *Staphylococcus aureus* and *Bacillus cereus*. Specifically, the inhibition zones measured

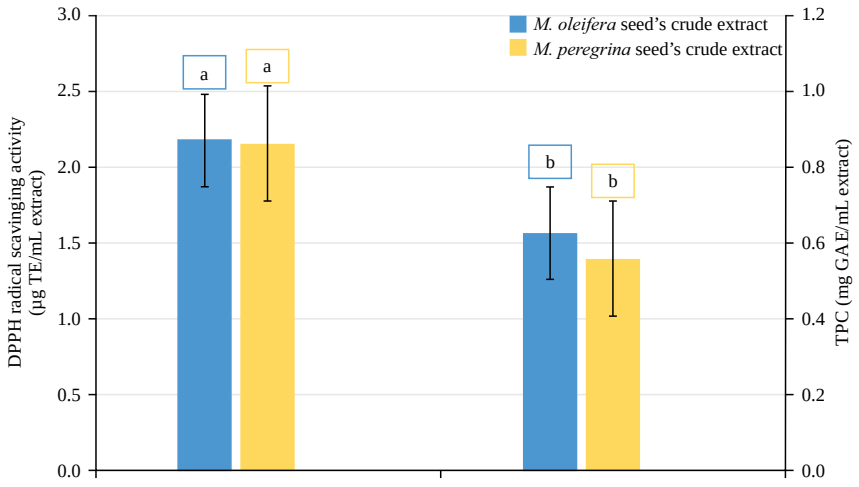

Fig. 3: DPPH and TPC of crude extracts from *Moringa* spp. seeds

Mean±SE with different small letters for each parameter are significantly different at $p<0.05$

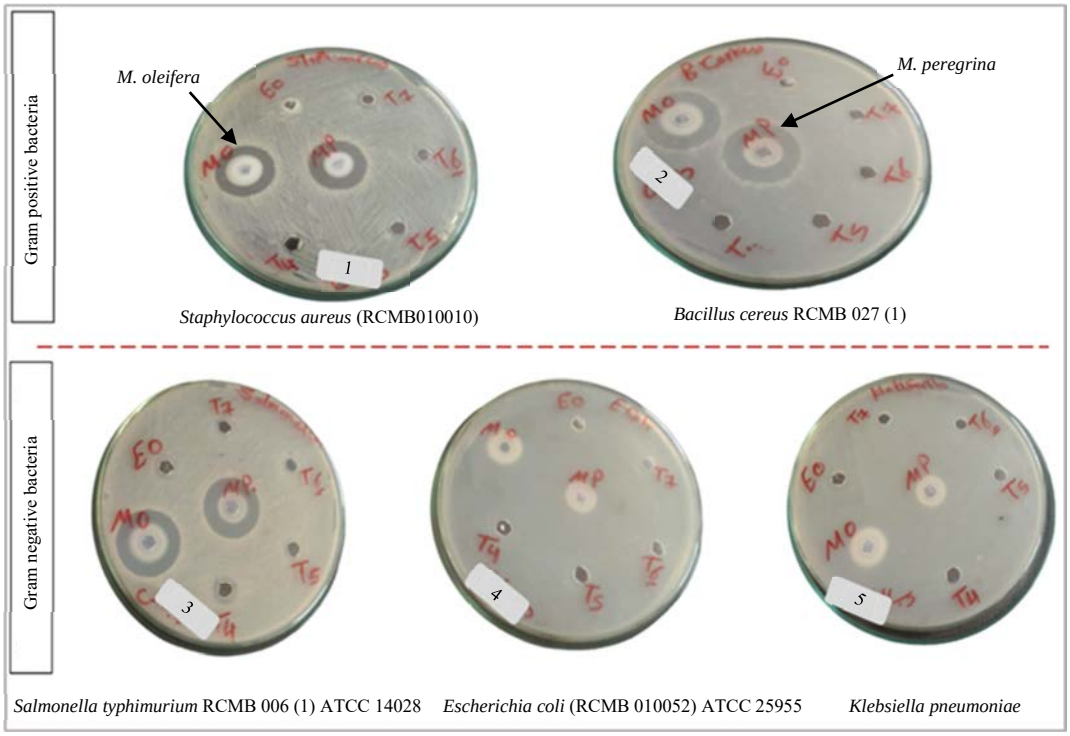

Fig. 4: Effect of crude extracts from *Moringa* spp. seeds on the inhibition zone of gram-positive and gram-negative bacterial strains

Table 1: Effect of crude extracts from Moringa spp. seeds on gram-positive and gram-negative bacterial strains

	Inhibition zone (mm)		
	Crude extract's source		
Tested microorganism	*M. oleifera*	*M. peregrina*	Gentamicin (4 mg/mL)
Gram positive bacteria			
Staphylococcus aureus (RCMB010010)	27.00±1.00	26.00±1.00	24.00±1.00
Bacillus cereus RCMB 027 (1)	34.00±1.00	33.00±1.00	24.00±0.06
Gram negative bacteria			
Escherichia coli (RCMB 010052) ATCC 25955	NA	NA	29.00±0.01
Salmonella typhimurium RCMB 006 (1) ATCC 14028	31.00±0.58	31.33±0.58	17.00±0.03
Klebsiella pneumonia	NA	NA	21.00±0.04

*NA: No activity

27.00±1.00 mm and 26.00±1.00 mm for *M. oleifera* and *M. peregrina*, respectively, against *S. aureus*, and 34.00±1.00 mm and 33.00±1.00 mm for *M. oleifera* and *M. peregrina*, respectively, against *B. cereus*. These results indicate that both extracts performed better than Gentamicin, which served as the positive control. Also, there were no significant differences in the inhibition zone diameters between the two extracts. Additionally, both extracts were effective against *Salmonella typhimurium*, with inhibition zones measuring 31.00±0.58 mm for *M. oleifera* and 31.33±0.58 mm for *M. peregrina*. However, no inhibition was observed against *Escherichia coli* or *Klebsiella pneumoniae* with either of the crude extracts. In parallel to our result, previous research has shown that the aqueous extract of *M. oleifera* exhibits an antimicrobial effect against gram-positive pathogens such as *Staphylococcus aureus*, but does not have the same effect on gram-negative pathogens[33-35]. In a study conducted by Majali *et al.*[36], the ethanolic extract from *M. peregrina* seeds demonstrated antibacterial activity against both gram-positive and gram-negative bacteria. This suggests that the seeds of *M. peregrina* may affect protein structure or transcription levels, rather than specifically disrupting the cell wall structure. Additionally, it was found that the methanol extract of *M. oleifera* showed no antimicrobial effect against *Escherichia coli*, while it did exhibit activity against *Staphylococcus aureus*[37].

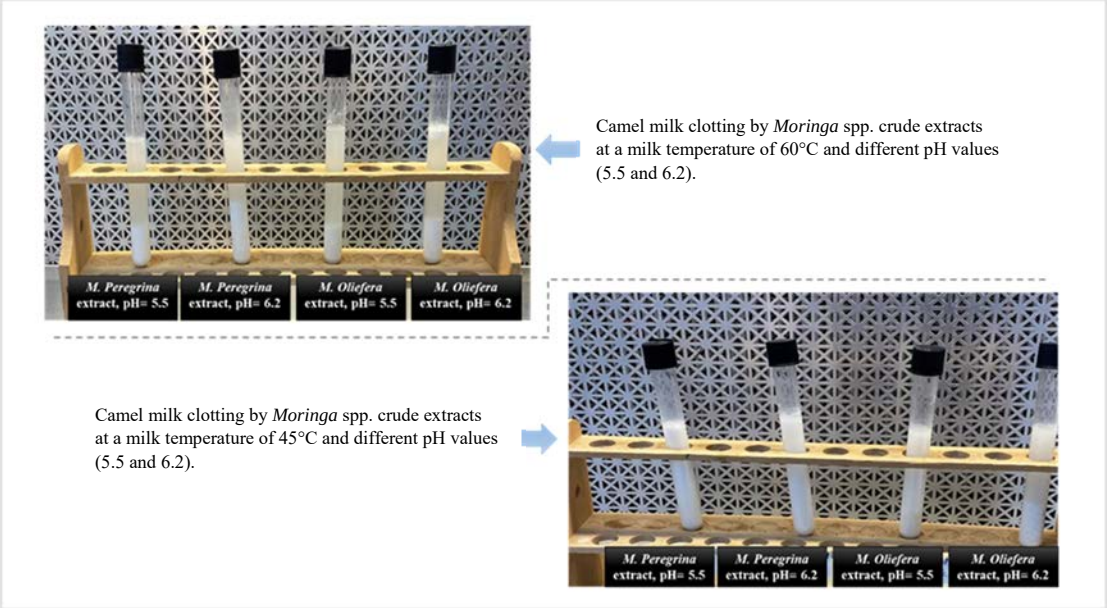

Fig. 5: Photos of the impact of crude extract from *Moringa* spp. seeds on the coagulation of camel milk

This difference could be attributed to phytochemicals such as phenols[38] and lipophilic compounds that bind to the cytoplasmic membrane of bacterial cells, leading to their destruction. In general, these effects are often more pronounced against gram-positive bacteria than against gram-negative ones[37].

Camel-MCA of crude extracts from *Moringa* spp. seeds at varying temperatures degrees and pH levels: Because not enough data regarding the clotting activity of *M. peregrina* on camel milk was found, the impact of crude extracts from both *M. oleifera* and *M. peregrina* seeds on camel-MCA was assessed. Various pH levels (6.2 and 5.5) and temperatures (45°C and 60°C) were studied, and the data are illustrated in Fig. 5-6. MCA is inversely related to coagulation time. Thus, higher MCA values indicate increased clotting efficiency and shorter coagulation times[3,39].

Observations from the photos in Fig. 5 indicate that all test tubes at temperatures at 60°C and at both pH levels displayed a clear and firm coagulum (precipitate) along with a clear supernatant. Additionally, the MCA of both seed's crude extracts of *Moringa* spp. showed no significant differences, even at varying pH levels (Fig. 6a). However, when the temperature was reduced to 45°C, a different trend emerged for both pH values, as illustrated in Fig. 6b. The results revealed that *M. oleifera* had higher clotting activity on camel milk compared to *M. peregrina* across all tested conditions, particularly at 45°C. The camel-MCA of the crude extract from *M. oleifera* seeds was significantly greater, measuring 7.2 U/mL at pH 6.2 and 3.4 U/mL at pH 5.5. In contrast, camel-MCA of *M. peregrina* was lower, at 3.2 U/mL at pH 6.2 and 2.9 U/mL at pH 5.5. Also, no significant differences were detected in camel-MCA for the same Moringa seeds extract at different pH values under the same temperature. Although the study[39] was made on bovine milk, their findings could support our results. They discovered that the MCA of *M. oleifera* seed extract was significantly higher at a pH of 5.5 and a temperature of 42°C. It was concluded that *M. oleifera* extract is a viable alternative for milk clotting in the production of white cheesemaking in bovine milk[20] and camel milk[10] as well.

Based on the results obtained, temperature appeared to have a more significant impact than pH on the MCA of camel milk when using Moringa seed extract within the studied range. This finding aligns with previous research indicating that plant-derived milk-clotting enzymes are highly sensitive to temperature changes, while moderate alterations in pH typically produce less noticeable effects[3,39]. Therefore, temperature seems to play a crucial role in the effectiveness of clotting camel milk when utilizing Moringa seed's extract as a coagulant. This finding could have significant implications for improving processing conditions in camel-milk cheese production.

Effect of TSC on the clotting of camel milk by crude extracts of *Moringa* spp. seeds: The effect of different concentrations of TSC (10, 20, 30, and 40 mmol/L) on milk coagulation (Table 2) was evaluated after adjusting camel milk at

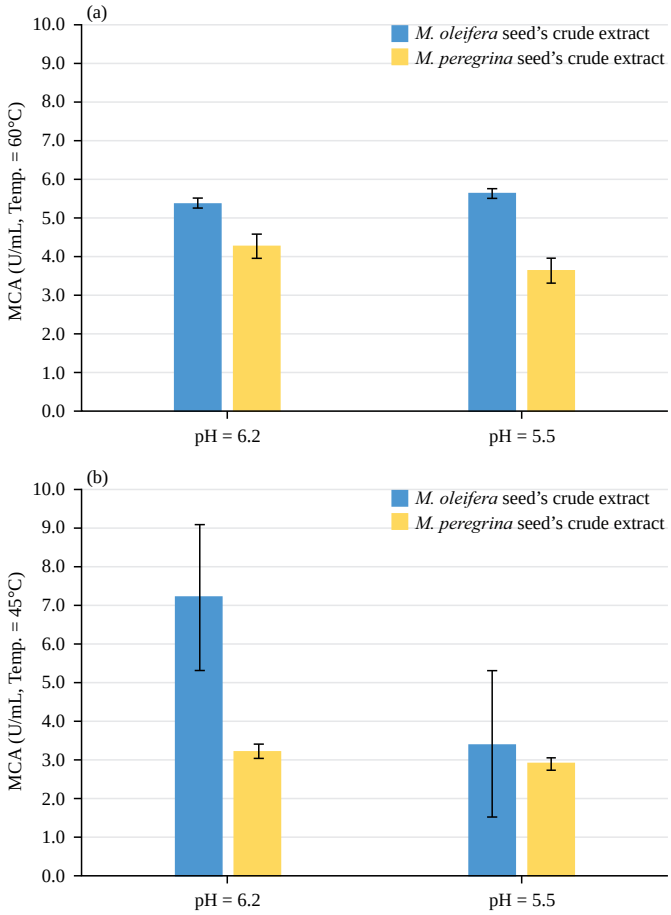

Fig. 6(a-b): Camel-MCA of the seed's crude extract from *M. oleifera* and *M. peregrina* at different pH levels and temperature degrees. (a) MCA at 60°C when at different pH levels (6.2 and 5.5) and (b) MCA at 45°C when at different pH levels (6.2 and 5.5)

MCA (mean±SE) with different small letters at the same pH are significantly different at $p<0.05$, means with different capital letters for the same extract at different pH are significantly different at $p<0.05$

Table 2: Coagulation characteristics of camel milk with different TSC concentrations

TSC (mmol/L)	Crude extract's source	Time of first clotting observation (min.)	Observation
10	*M. oleifera* seed	160	No clear curd was observed, but just precipitation
	M. peregrina seed	168	at the bottom of the container
20	*M. oleifera* seed	Non	After about 3 hours, no coagulum was observed
	M. peregrina seed	170	No clear curd was observed, but just precipitation at the bottom of the container
30	*M. oleifera* seed	165	No clear curd was observed, but just precipitation
	M. peregrina seed	197	at the bottom of the container
40	*M. oleifera* seed	177	No clear curd was observed, but just precipitation
	M. peregrina seed	172	at the bottom of the container

pH = 6.2 and temperature 45°C. At lower concentrations (10 and 20 mmol/L), delayed and weak coagulation was observed, while no curd formation occurred in treatments with added 30 or 40 mmol/L, confirming the strong calcium-chelating behavior of TSC. Citrate binds to Ca^{2+} ions and dissolves colloidal calcium phosphate from casein micelles, which reduces the amount of micellar calcium needed for aggregation after the cleavage of κ-casein. In bovine milk, adding citrate can eliminate over 33% of colloidal calcium phosphate, releasing approximately 20% of casein from the micelles. This process completely inhibits gelation until $CaCl_2$ is re-added[40]. At concentrations of 20 mmol/L or higher,

citrate prevents rennet coagulation at a pH of around 6.6 by chelating Ca^{2+} ions and dissolving colloidal calcium phosphate[41]. The addition of citrate also increases rennet coagulation time and results in the formation of the weakest gels, regardless of micelle size[42].

CONCLUSION

This study demonstrates the potential of *Moringa oleifera* and *Moringa peregrina* seed extracts as sustainable coagulants in dairy applications. These extracts possess antimicrobial and antioxidant properties while effectively clotting camel milk, particularly at optimal temperatures of 60°C. Both extracts showed significant inhibition of Gram-positive bacteria, and the study highlights the importance of preserving enzyme-casein interactions in clean-label cheese formulations, especially with trisodium citrate. Additionally, a cost-effective method for partially purifying a milk-clotting enzyme from *Moringa peregrina* seeds was applicable. Given Moringa's resilience in arid environments and the growing interest in camel milk as a climate-friendly dairy option, these findings advocate for using indigenous plant resources to enhance sustainable dairy processing and regional food systems.

SIGNIFICANCE STATEMENT

This study highlights Moringa peregrina seed extract as a novel, plant-based coagulant for camel milk, addressing limitations of weak curd formation. Its strong milk-clotting activity, antimicrobial potential, and enzyme specificity offer a sustainable alternative to animal rennet, supporting cheese production in regions where camel milk is abundant. These findings can guide further research on enzyme purification, molecular characterization, and improving cheese quality.

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