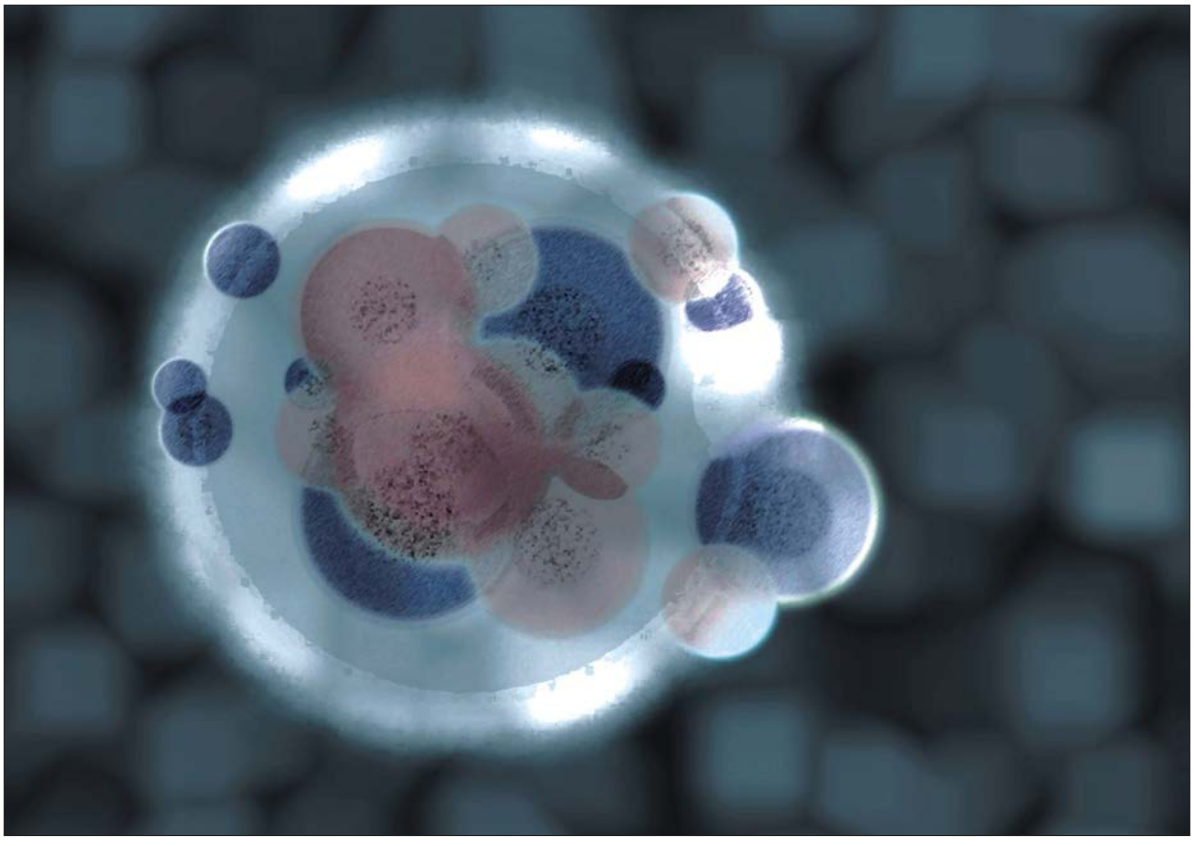

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Research Article
Utilization of Natural Antimicrobial and Antioxidant of *Moringa oleifera* Leaves Extract in Manufacture of Cream Cheese

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Abstract

Background and Objective: Cream cheese is a fresh acid-curd soft cheese which high in moisture and fat content led to has a short shelf-life. So, this study intended to prepare cream cheese by adding different ratios of *Moringa oleifera* (*M. oleifera*) extract (2.00, 3.00 and 4.00 g/100 g skimmed UF-retentate) to move up the shelf life and quality of final products. **Materials and Methods:** Antimicrobial activities of the acetone, ethanol, aqueous ethanol and water extract of *M. oleifera* dry leaves were investigated using disc diffusion method. Also, antioxidant activity and the effect of each extract on probiotic strains were studied. Moreover, the ethanol extract of *M. oleifera* using in cream cheese production. Chemical composition, antioxidant capacity, microbiological analysis, sensory properties, color measurement and rheological properties of cream cheese were determined. Data were analyzed using Duncan's multiple tests. **Results:** The proximate analysis showed that ethanol extract of *M. oleifera* had the highest total phenols and antioxidant activities and had wide range of antimicrobial properties against different pathogenic strains used in this study. Ethanol extract showed maximum zone of inhibition, which ranged between 15-22 mm at concentration 4.00 mg mL^{-1} milk. Moreover, each extract has not any effect in probiotic strains and the probiotic counts increased with increasing the concentration of *M. oleifera* extract especially with *Lactobacillus plantarum.* Total solid, fat/dry matter, acidity, soluble nitrogen/total nitrogen, diacetyl, acetaldehyde, total volatile fatty acid, total phenol content, antioxidant activity by DPPH scavenging and lactic acid bacteria count content in cream cheese increased with increasing the ratio of *M. oleifera* extract. Also, coliform, mold and yeast count in all treatments not detected fresh or during storage period. Rheological properties of cream cheese increased with increasing the ratio of *M. oleifera* extract fresh or during storage. Sensory properties of cream cheese samples enhanced and gained high scores. **Conclusion:** The ethanolic *Moringa oleifera* leaves extracts can be used as nutrition supplement and preservative agent to add healthy benefits and extend the shelf life of the cream cheese.

Key words: *Moringa oleifera* extract, antimicrobial activity, antioxidant properties, cream cheese, probiotics, lactic acid bacteria

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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

Moringa oleifera is a complete food it has an extraordinary range of medicinal benefits with high nutritional value., *M. oleifera* leaves also well-known as extremely nutritious, being a high-quality basis of protein, vitamins, minerals, amino acids and a variety of phenol compounds[1-3].

Extracts of the leaves are recognized to possess biological properties and these are frequently become vary according to the kind of solvent used to extract the vital components[4,1]. Bukar *et al.*[5] illustrated that *M. oleifera* leaves ethanol extract had the widest range of activity on the different tested bacteria. It is important to evaluate the antimicrobial properties of *M. oleifera* leaves extracted with different solvents on some selected microorganisms.

Antioxidant agents from plant are protected and contain established an amazing consideration due to their talent to protect foodstuffs and avoid rancidity caused by oxidation. Utilization of plant with antioxidant properties by animals has been descried to exceed antioxidant compounds to animal protein[6,7]. These compounds consist of phenols, flavonoids, vitamin C, vitamin E, β-carotene, flavones, zinc and selenium which have been recognized to have strong antioxidant potential[8-10]. Therefore, addition of *M. oleifera* leaves extract in different fermented dairy products can raise the growth of probiotic bacteria such as lactobacilli and bifidobacterium. Amer *et al.*[11] found that probiotic bacteria can grow well in MRS medium fortified with 8% *M. oleifera* leave extract and incubated at 37°C for 24 h.

Moringa oleifera leaves extract have positive effect on butter and butter oil composition, which inhibited the formation of free fatty acids and oxidation of butter[12,13]. Use of *M. oleifera* leaves powder in manufacture of cream cheese also improves the nutrition value, sensory quality and consumer acceptability[14]. The addition of *M. oleifera* leaves powder and *M. oleifera* oil into the ice milk lead to raise the nutritive rate also, offering extra health benefits to the consumers and possibly will protect from different category of cancer[5].

Cream cheese is a fresh acid-curd soft cheese which is structurally different from other cheese with higher smooth and spreadable reliability[15]. It is typically spreadable that also include high moisture and fat[16].

So the aim of this study was to investigate the antioxidant potential and antimicrobial properties of *M. oleifera* leaves extract with different solvents and prepare cream cheese with different ratios of *M. oleifera* leaves extract to increase the shelf life and quality of the cheese.

MATERIALS AND METHODS

Materials: Fresh skimmed UF-retentate was procured from Animal Production Research Institute, Agriculture Research Center, Dokki, Egypt and *M. oleifera* dry leaves powders were obtained from National Research Centre herb plant unit during March, 2017.

Pathogenic strains: *Bacillus cereus* B-3711, *Bacillus subtilis*, *Aspergillus flavus* 3357 and *Saccharomyces cerevisiae* Y-2223 were provided by the Northern Regional Research Laboratory Illinois, USA (NRRL). *Listeria monocytogenes* 598 was provided by the Department of Food Science, University of Massachusetts, Amherst MA, USA. *Escherichia coli* 0157: H7, *Salmonella typhimurium* and *Staphylococcus aureus* were isolated and serologically identified by dairy microbiological Lab., National Research Center. *Yersinia enterocolitica* were obtained from Hungarian National Collection of Medical Bacteria, OKI, Gyaliut 2-6, H-1966 Budapest, Hungary. *Aspergillus niger, Pseudomonas aeruginosa*, *Pencillium requeforti* J5 were obtained from Department of Microbiology, Swedish University of Agricultural Sciences, bacterial pathogenic strains were activated in trypton soya broth, incubated at 37°C for 24 h. Yeast and mold strains were activated in Malt extract broth, incubated at 25°C for 72 h.

Lactic acid strains: *Lactobacillus rhamnosus* B-445 and *Lactobacillus helveticus provided* by the Northern Regional Research Laboratory Illinois, USA, *Lactobacillus casei* and *Lactobacillus acidophilus* obtained from Chr. Hansens's Lab., Denmark. *Lactobacillus plantarum* and *Leuconostoc mesenteroides* were isolated and identified by Dairy Science Dept.,(Dairy Microbiology Lab.), National Research Center[17,18].

Methods

Preliminary study

Preparation of *M. oleifera* dry leaves extract: Mature leaves harvested from *M. oleifera* trees were dried in the shade at room temperature and milled into fine powder and stored at 25°C in the dark in tightly closed glass containers. About 50 g of *M. oleifera* dry leaves was extracted 4 times with 500 mL each of acetone, ethanol, water and ethanol:water (80:20%), respectively at room temperature with stirring for 72 h. Each extract was filtered using Whatman No. 1 filter paper and evaporated under reduced pressure to dryness below 40°C. The extract yields (w/w) were acetone 14.30%, ethanol 14.50%, water 5.50% and ethanol:water 17.50%,

respectively. The resultant extracts were analyzed directly for antioxidant and antibacterial activities[19] to choose the best extract which gave highest antimicrobial and antioxidant.

Antimicrobial activity of *M. oleifera* extracts

Preparation of different concentration from *M. oleifera* extracts: One milliliter of leaves extract with different solvent and water were dissolved individually in 5.0 mL DMSO (dimethyl sulfioxide) to get 200 mg mL^{-1} solution, after that different dilution were prepared in DMSO to get 150, 100, 75 and 50 mg mL^{-1} solution.

The antimicrobial assay of different concentration of *M. oleifera* extracts performed by agar disc diffusion method using Muller Hinton agar medium according to Singh and Sharma[20]. The Muller Hinton agar was poured into the petri dishes and allowed to solidified, the pathogenic strains 0.1 mL (approximately 10^9 cells mL^{-1}) of the tested microorganisms was spread on the surface of the agar found in petri dish using a sterile swab. The plates were rested to 2 h at 37°C to allow the agar saturated with pathogenic strains. For the agar disc diffusion method, 20 µL of each leaves concentration was impregnated on different sterile paper discs (Whatman, 106 mm) and placed on the surface of Muller Hinton agar in petri dishes gently. According to this procedure, each disc carried a loaded of different leaves extract equals to 1.00, 1.50, 2.00, 3.00 and 4.00 mg extract. The plates were incubated at 37°C for 24 h. After the incubation period the inhibition zones around the each disc was measured in millimeters.

Effect of different *M. oleifera* extracts on lactobacilli: To determine the effect of *M. oleifera* extracts on lactobacilli, different strains of lactobacilli were activated individually and grown in MRS broth medium fortified with 1.00, 1.50, 2.00, 3.00 and 4.00 mg mL^{-1} medium of different *M. oleifera* extracts at 37°C using 1% inoculums to know the best concentration for lactobacilli strains growth. The count of different lactobacilli strains were enumerated using MRS agar medium and incubated for 48 h at 37°C.

Preparation of cream cheese by using *M. oleifera* extract by ethanol: Cream cheese was made using fresh skimmed UF-retentate standardized to 15% fat using (concentrated cream 65% fat). Pasteurized at 72°C for 15 sec, cooled and adjusted to 42°C. Different concentration of *M. oleifera* extract mixed with UF-retentate in ratios of (0, 2.00, 3.00 and 4.00 g extract/100 g skimmed UF-retentate), using the electric blender (Molinex blender). Then, UF-retentate with different concentration of *M. oleifera* mixture were inoculated with *Leuconostoc mesenteroides* and *Lactobacillus plantarrum* (1:1) and packaged in plastic cups (50 mL). Cream cheese was made by the method described by Lucey[21]. Cream cheese samples were stored in refrigerator at 5±1°C for 30 days and analyzed chemically, microbiologically and rheological properties fresh and during the storage period at 5±1°C.

Cream cheese extracts preparation: Extraction procedures were done as follows: 10 g cream cheese was put into a 100 mL quick-fit conical flask. Then, 20 mL of methanol:water at 80:20 were added. After shaking on ultrasonic water bath for 30 min the residual solution was filtered in 25 mL measuring flask and completed to 25 mL by extraction solvent. The final extracts were collected separately in glass sealed containers and used for different analysis.

Total phenol content (TPC): Total phenol content of cream cheese extracts was determined calorimetrically at 625 nm using the Folin-Ciocalteu reagent according to the method described by Zheng and Wang[22] and Zahran *et al.*[23]. The solution of white soft cheese extract (0.5 mL), 20 mL of deionized water and 0.625 mL of the folin-ciocalteu reagent were added in a 25 mL volumetrical ask. After 3 min, 2.5 mL of saturated solution of Na_2CO_3 (35%) were added. The content was mixed and diluted to volume with deionized water. After 1 h, the absorbance of the sample was measured at 625 nm against a blank using a double-beam ultraviolet-visible spectrophotometer Hitachi U-3210 (Hitachi, Ltd., Tokyo, Japan). Gallic acid served as a standard for preparing the calibration curve and ranged from 2.5-20 µg/25 µL of assay solution.

Antioxidant assay: The antioxidant activity of the phenol extracts was evaluated by using the stable 2,2-Diphenyl-1-picryl-hydrazyl radical (DPPH) according to a modification method of Bandoniene *et al.*[24]. Methanolic solutions of phenol extracts (0.1 mL) and 3.9 mL methanolic solution of DPPH (0.0025 g/100 mL CH_3OH) were added in a cuvette and the absorbance at 515 nm (till stabilization) was measured against methanol using a double-beam ultraviolet-visible spectrophotometer Hitachi U-3210 (Hitachi, Ltd., Tokyo, Japan). Simultaneously, the absorbance at 515 nm of the blank sample (0.1 mL methanol+3.9 mL methanolic solution of DPPH) was measured against methanol. The radical scavenging activities of the tested samples, expressed as percentage inhibition of DPPH, were calculated according to the following formula:

$$\text{Inhibition (\%)} = \frac{100\times(A\text{-}A_0)}{A_0}$$

where, A_0 is the absorbance at 515 nm of the blank sample at time t = 0 min and A is the final absorbance of the test sample at 515 nm.

Chemical analysis: Fat, titratable acidity (T.A), total solids (T.S), fat/dry matter (F/DM), ash content and peroxide value of cream cheese samples were determined according to AOAC[25]. The pH values were measured using a digital laboratory Jenway 3510 pH meter, UK. Bibby Scientific LTD. Stone, Stafford shire, ST 15 OSA. Diacetyl, acetaldehyde, total nitrogen (T.N) and soluble nitrogen (S.N) contents were determined using the semi micro- Kjeldahl method as mentioned by Ling[26]. Total volatile fatty acids (TVFA) value was determined according to the method described by Koiskowski[27] values were expressed as mL of 0.1 M NaOH/100 g cream cheese.

Microbiological analysis: Cream cheese analyzed for *Lactobacillus plantarum* count using MRS agar according to De Man *et al.*[28]. The plates were incubated at 37°C for 48 h under anaerobic condition. *Leuconostoc mesenteroides* counts were determined on MRS agar supplemented with 30 μg mL^{-1} of vancomycin according to Mathot *et al.*,[29]. The plates were incubated at 37°C for 48 h under anaerobic conditions. Coliform bacterial counts were enumerated using violet red bile agar medium[30]. The plates were incubated at 37°C for 18 h. Yeasts and molds count were enumerated using potato dextrose agar acidified to pH 3.5 with sterile lactic acid solution (10%)[31]. The plates were aerobically incubated at 25°C for 4 days.

Texture profile analysis of cream cheese: Textural properties of experimental cream cheese were assessed using texture analyzer. Cream cheese samples were 30 mm in diameter and 20 mm in height. Samples were allowed to balance at ambient temperature for approximately 30-45 min prior to testing. The various textural parameters, hardness, springiness, cohesiveness, gumminess and chewiness were estimated according to the method of International Dairy Federation[32].

Color measurements of cream cheese: Color of cream cheese samples was measured using a Hunter colorimeter model D2s A-2 (Hunter Assoc. Lab Inc., VA, USA) following the instruction of the user manual Hunter colorimeter. This colorimeter is designed to stimulate the color matching response function of human observer. The color values were measured to the absolute values of a perfect white diffuser (white tile) as measured under the same geometric conditions. The instrument was first standardized using a white tile (top of the scale) and a black tile (bottom of the scale). A specimen of the cream cheese (flat layer) was placed at the specimen port. The values of the color namely (L, a and b) were measured using the corresponding button on the colorimeter. Where:

L: Value represents darkness from black (0) to white (100)
a: Value represents color ranging from red (+) to green(−)
b: Value represents yellow (+) to blue

Sensory evaluation: All cream cheese treatments were evaluated for sensory attributes such as flavor and appearance, body and texture, taste and spread-ability on a 9-point scale (1 = extremely weak, 3 = very weak, 5 = moderate, 7 = very strong, 9 = extremely strong). Fresh and during storage till 4 weeks at refrigerator (5±1°C) by members of Dairy department, National Research Center (NRC) according to Stone and Sidel[33].

Statistical analysis: The data were analyzed according to Statistical Analysis System Users Guide SAS Institute (SAS Institute, Inc, U.S.A). Separation among means in triplicates was carried out using Duncan's multiple tests[34]. All results data analysis in two ways accepts the chemical composition data analysis in one way. The differences were considered to be significant at $p \leq 0.05$. Data of antioxidant, total phenol content, total yield of *M. oleifera* leaves and peroxide value were expressed as mean±standard deviation of the 3 replicates.

RESULTS

Determination the antimicrobial activity of *M. oleifera* leaves extracts with different solvents and water: The antimicrobial activity of ethanol 95%, acetone, (80:20%) ethanol:water and aqueous extract of *M. oleifera* leaves with different concentration (1.00, 1.50, 2.00, 3.00 and 4.00 mg mL^{-1} DMSO) against different pathogenic strains were found in Tables 1-4. All extracts showed significantly changeable degrees of antimicrobial action on the tested microorganisms. The antimicrobial activity increased significantly by increasing the concentration of *M. oleifera* leaves extracts.

Table 1: Effect of antimicrobial of *M. oleifera* leaves extracted by ethanol 95% on pathogenic strains

	Diameter of inhibition zone (mm)				
	1.00	1.50	2.00	3.00	4.00
Pathogenic strains	mg mL^{-1}				
Bacillus cereus	9^{Ea}	12^{Da}	18^{Ca}	20^{Ba}	22^{Aa}
Bacillus subtilis	7^{Eb}	9^{Db}	13^{Cc}	15^{Bc}	19^{Ab}
Staphylococcus aureus	8^{Ea}	11^{Da}	15^{Cb}	18^{Bb}	20^{Ab}
Pseudomonas aeruginosa	5^{Ec}	7^{Dd}	10^{Cd}	13^{Bd}	16^{Ac}
Listeria monocytogenes	-	-	4^{Cf}	6^{Be}	9^{Ad}
Escherichia coli	9^{Ea}	11^{Da}	14^{Cb}	19^{Ba}	21^{Aa}
Yersinia enterocolitica	-	-	3^{Cf}	5^{Be}	7^{Ae}
Salmonella typhimurium	-	-	3^{Cf}	6^{Be}	9^{Ad}
Aspergillus niger	-	-	3^{Cf}	5^{Be}	8^{Ae}
Aspergillus flavus	-	-	-	3^{Bf}	6^{Af}
Pencillium roqueforti	-	-	-	2^{Bf}	5^{Af}
Saccharomyces cerevisiae	6^{Db}	8^{Cd}	11^{Bd}	14^{Ac}	15^{Ac}

Data expressed as mean of 3 replicates. Means in the same row showing the same capital letters are not significantly different ($p \leq 0.05$). Means in the same column showing the same small letters are not significantly different ($p \leq 0.05$)

Table 2: Effect of antimicrobial of *M. oleifera* leaves extracted by ethanol:water (80:20%) on pathogenic strains

	Diameter of inhibition zone (mm)				
	1.00	1.50	2.00	3.00	4.00
Pathogenic strains	mg mL^{-1}				
Bacillus cereus	10^{Ea}	11^{Da}	15^{C}a	16^{Ba}	18^{Aa}
Bacillus subtilis	5Ec	8^{Db}	10^{Cc}	13^{Bb}	15^{Ab}
Staphylococcus aureus	4^{Ec}	6^{Dc}	9^{Cc}	13^{Bb}	16^{Ab}
Pseudomonas aeruginosa	3^{Ed}	5^{Dc}	8^{Cd}	10^{Bc}	13^{Ac}
Listeria monocytogenes	-	-	2^{Ce}	3^{Bd}	6^{Ad}
Escherichia coli	7^{Eb}	10^{Da}	13^{Cb}	16^{Ba}	19^{Aa}
Yersinia enterocolitica	-	-	2^{Ce}	4^{Bd}	5^{Ad}
Salmonella typhimurium	-	-	-	3^{Bd}	6^{Ad}
Aspergillus niger	-	-	3^{Ce}	4^{Bd}	6^{Ad}
Aspergillus flavus	-	-	-	3^{Bd}	5^{Ad}
Pencillium roqueforti	-	-	-	-	4^{Ae}
Saccharomyces cerevisiae	-	3^{Dd}	7^{Cd}	9^{Bc}	12^{Ac}

Data expressed as mean of 3 replicates. Means in the same row showing the same capital letters are not significantly different ($p \leq 0.05$). Means in the same column showing the same small letters are not significantly different ($p \leq 0.05$)

Table 3: Effect of antimicrobial of *M. oleifera* leaves extracted by acetone on pathogenic strains

	Diameter of inhibition zone (mm)				
	1.00	1.50	2.00	3.00	4.00
Pathogenic strains	mg mL^{-1}				
Bacillus cereus	8^{Ea}	10^{Da}	15^{Ca}	17^{Ba}	19^{Aa}
Bacillus subtilis	5^{Eb}	7^{Db}	8^{Cb}	10^{Bb}	15^{Ab}
Staphylococcus aureus	2^{Ec}	4^{Dc}	7^{Cb}	10^{Bb}	14^{Ab}
Pseudomonas aeruginosa	-	2^{Dd}	5^{Cc}	7^{Bc}	12^{Ac}
Listeria monocytogenes	-	-	4^{Cc}	7^{Bc}	10^{Ad}
Escherichia coli	-	3^{Dd}	7^{Cb}	10^{Bb}	13^{Ac}
Yersinia enterocolitica	-	-	-	3^{Bd}	5^{Ae}
Salmonella typhimurium	-	-	-	2^{Bd}	5^{Ae}
Aspergillus niger	-	-	-	2^{Bd}	4^{Af}
Aspergillus flavus	-	-	-	-	3^{Af}
Pencillium roqueforti	-	-	-	-	3^{Af}
Saccharomyces cerevisiae	2^{Ec}	4^{Dc}	7^{Cb}	9^{Bb}	13^{Ac}

Data expressed as mean of 3 replicates. Means in the same row showing the same capital letters are not significantly different ($p \leq 0.05$). Means in the same column showing the same small letters are not significantly different ($p \leq 0.05$)

Ethanolic extract (Table 1) showed greatest zone of inhibition against *B. cereus* in addition to the lowly activity against *A. flavus* especially at the concentration 4.00 mg mL^{-1}. Moreover, the 1.00 mg mL^{-1} concentration showed the lowest concentration gave inhibition zone with *B. cereus*, *B. subtilis*, *S. aureus*, *E. coli*, *P. aeruginosa* and *S. cerevisiae*, not detected any inhibition zone with other tested pathogens as the same concentration.

Table 4: Effect of antimicrobial of *M. oleifera* leaves extracted by water on pathogenic strains

	Diameter of inhibition zone (mm)				
	1.00	1.50	2.00	3.00	4.00
Pathogenic strains	mg mL^{-1}				
Bacillus cereus	3^{Ea}	5^{Da}	7^{Ca}	8^{Ba}	11^{Aa}
Bacillus subtilis	-	3^{Db}	5^{Cb}	7^{Ba}	10^{Aa}
Staphylococcus aureus	-	2^{Db}	4^{Cb}	6^{Bb}	8^{Ab}
Pseudomonas aeruginosa	-	-	3^{Cc}	5^{Bb}	7^{Ac}
Listeria monocytogenes	-	-	-	3^{Bc}	5^{Ad}
Escherichia coli	-	2^{Db}	5^{Cb}	8^{Ba}	10^{Aa}
Yersinia enterocolitica	-	-	-	-	4^{Ad}
Salmonella typhimurium	-	-	-	-	3^{Ae}
Aspergillus niger	-	-	-	-	2^{Ae}
Aspergillus flavus	-	-	-	-	-
Pencillium roqueforti	-	-	-	-	-
Saccharomyces cerevisiae	-	-	2^{Cc}	5^{Bb}	7^{Ac}

Data expressed as mean of 3 replicates. Means in the same row showing the same capital letters are not significantly different ($p \leq 0.05$). Means in the same column showing the same small letters are not significantly different ($p \leq 0.05$)

Table 5: Effect of *M. oleifera* extracts with ethanol 95% on probiotic bacteria (log CFU mL^{-1})

		1.00	1.50	2.00	3.00	4.00
Probiotic strains	Control	mg mL^{-1}				
Lactobacillus acidophilus	9.00^{Ca}	9.24^{Ca}	9.26^{Cb}	9.80^{Bb}	10.03^{Bb}	10.37^{Ab}
Lactobacillus helveticus	8.10^{Cb}	8.70^{Cb}	8.96^{BCc}	9.31^{Bc}	9.50^{Bc}	10.33^{Ab}
Lactobacillus casei	8.25^{Cb}	8.46^{Cb}	8.53^{Cd}	9.03^{Cc}	9.30^{Bc}	9.90^{Ac}
Lactobacillus plantarum	9.20^{Ca}	9.38^{Ca}	9.70^{Ca}	10.16^{Ba}	10.51^{Aa}	10.70^{Aa}
Lactobacillus rhamnosus	8.00^{Cb}	8.35^{Cc}	8.50^{Cd}	8.75^{Cd}	9.20^{Bc}	9.63^{Ad}

Data expressed as mean of 3 replicates. Means in the same row showing the same capital letters are not significantly different ($p \leq 0.05$). Means in the same column showing the same small letters are not significantly different ($p \leq 0.05$)

Table 6: Effect of *M. oleifera* extract with ethanol:water (80:20) on probiotic bacteria (log CFU mL^{-1})

		1.00	1.50	2.00	3.00	4.00
Probiotic strains	Control	mg mL^{-1}				
Lactobacillus acidophilus	8.80^{Cb}	8.90^{Cb}	9.05^{BCb}	9.00^{BCb}	9.15^{Bc}	9.56^{Ab}
Lactobacillus helveticus	8.60^{Cb}	8.70^{Cc}	8.86^{BCc}	9.10^{Bb}	9.35^{Ac}	9.40^{Ab}
Lactobacillus casei	8.00^{Dc}	8.25^{Dd}	8.38^{Cd}	8.50^{Cc}	9.10^{AB}	9.30^{Ab}
Lactobacillus plantarum	9.00^{Da}	9.10^{Da}	9.35^{Da}	9.60^{Ca}	9.80^{Bab}	10.00^{Aa}
Lactobacillus rhamnosus	8.10^{Bc}	8.16^{Bd}	8.25^{Bd}	8.30^{Bc}	8.70^{Ad}	8.86^{Ac}

Data expressed as mean of 3 replicates. Means in the same row showing the same capital letters are not significantly different ($p \leq 0.05$). Means in the same column showing the same small letters are not significantly different ($p \leq 0.05$)

The same trend of results observed in Table 2 when used ethanol with water (80:20%) in extracted. The diameter of inhibition zone ranged between 18-5 mm at the 4.00 mg concentration with different tested pathogens and these diameters of inhibition significantly decreased with the lowest concentration and not detected any inhibition zone with most tested pathogens at this concentration 1.00 mg mL^{-1}.

In acetone extract (Table 3) found that *B. cereus* more sensitive to different concentration of extract which the diameter of inhibition zone against *B. cereus* ranges from 19-8 mm and the more resistant pathogen are *Y. enterocolitica and A. flavus* which not detected any inhibition zone at the concentration 1.00, 1.50 and 2.00 mg mL^{-1}.

The data also showed that the usage water in the extracted gave the lowest inhibition zone (Table 4) when compared with other solvents and the pathogens *E. coli*, *Y. enterocolitica*, *A. falvus* and *A. niger* more resistant to different concentration when used aqueous extract.

Effect of different *M. oleifera* extracts on lactobacilli: Effect of *M. oleifera* leaves extracts with different solvents and water at varied concentrations on the growth of probiotics bacteria is presented in Tables 5-8. The growth of all studied probiotic strains was affected by different extracts. All probiotics strains have significant different between different *M. olifera* leaves extracts. The obtained data showed that increasing the concentration of different extracts from control to 4 mg mL^{-1} medium lead to raise the probiotic bacterial growth. Also, it could be recognized that all studied probiotic bacteria significantly propagated well when using ethanol in the extraction (Table 5) compared with the aqueous extract (Table 8). Consequently, the lactobacilli at 4 mg mL^{-1} at 37°C especially with ethanolic extract, *Lactobacillus plantarum*

Table 7: Effect of *M. oleifera* extracts with acetone on probiotic bacteria (log CFU mL^{-1})

Probiotic strains	Control	1.00	1.50	2.00	3.00	4.00
		mg mL^{-1}				
Lactobacillus acidophilus	8.75^{Ba}	8.77^{Bb}	8.70^{Bb}	8.80^{Bb}	8.90^{Ab}	9.20^{Ab}
Lactobacillus helveticus	8.60^{Cb}	8.64^{Cb}	8.70^{Cb}	8.75^{Cb}	9.00^{Ba}	9.35^{Aa}
Lactobacillus casei	8.10^{Dc}	8.38^{Dc}	8.30^{Dc}	8.55^{Cc}	8.75^{Bb}	9.00^{Ab}
Lactobacillus plantarum	8.90^{Ca}	9.05^{Ca}	9.00^{Ca}	9.25^{Ba}	9.30^{Aa}	9.45^{Aa}
Lactobacillus rhamnosus	8.00^{Bd}	8.15^{Bd}	8.25^{Bc}	8.30^{Bc}	8.55^{Ac}	8.60^{Ac}

Data expressed as mean of 3 replicates. Means in the same row showing the same capital letters are not significantly different ($p \leq 0.05$). Means in the same column showing the same small letters are not significantly different ($p \leq 0.05$)

Table 8: Effect of *M. oleifera* extracts with water on probiotic bacteria (log CFU mL^{-1})

Probiotic strains	Control	1.00	1.50	2.00	3.00	4.00
		mg mL^{-1}				
Lactobacillus acidophilus	8.50^{Bb}	8.55^{Bb}	8.45^{Bb}	8.50^{Bb}	8.65^{Bb}	8.77^{Ab}
Lactobacillus helveticus	8.35^{Bb}	8.40^{Bb}	8.50^{Bb}	8.65^{Bb}	8.80^{Aab}	8.90^{Aa}
Lactobacillus casei	8.20^{Cb}	8.45^{Cb}	8.40^{Cb}	8.55^{Bb}	8.60^{Bb}	8.75^{Ab}
Lactobacillus plantarum	8.75^{Ba}	8.70^{Ba}	8.77^{Ba}	8.85^{Ba}	9.00^{Ba}	9.20^{Aa}
Lactobacillus rhamnosus	8.10^{Cc}	8.15^{Cc}	8.25^{Bc}	8.35^{Bc}	8.40^{Bc}	8.55^{Ac}

Data expressed as mean of 3 replicates. Means in the same row showing the same capital letters are not significantly different ($p \leq 0.05$). Means in the same column showing the same small letters are not significantly different ($p \leq 0.05$)

Table 9: Antioxidant activity (%) by DPPH, total phenol (mg g^{-1}) and total yield/100 g dry weight samples (D.W.S.)

Extraction solvent	Total yield (%) (D.W)	Total phenol content (mg g^{-1}, D.W)	Antioxidant activity % by DPPH
Ethanol	14.50±0.25	16.86±0.2946	51.12±0.56
Acetone	14.00±0.84	14.11±0.2652	47.24±0.32
Water	5.50±0.67	8.41±0.9723	34.03±0.40
Ethanol:water	17.50±0.29	14.60±0.1620	43.31±0.48

Mean±Standard deviation, D.W: Dry weight

Table 10: Chemical composition of cream cheese with different ratios of ethanol extracted

Treatments	T.S	Moisture	Fat	Fat/DM	Protein	Ash
Control	31.21^{D}	68.79^{A}	15^{A}	48.06^{A}	9.49^{C}	1.53^{D}
T1	31.32^{C}	68.68^{B}	15^{A}	47.89^{B}	9.48^{C}	1.55^{C}
T2	31.39^{B}	68.61^{B}	15^{A}	47.79^{C}	9.53^{B}	1.57^{B}
T3	31.61^{A}	68.39^{C}	15^{A}	47.45^{D}	9.71^{A}	1.61^{A}

Data expressed as mean of 3 replicates. Means in the same column showing the same capital letters are not significantly different ($p \leq 0.05$), T.S: Total solids, DM: Dry matter

exhibited a higher growth and followed by *Lactobacillus acidophilus* than others lactobacilli, which the count significantly increased around 1.5 and 1.37 log cycles for the same strains, respectively as in (Table 5). When used aqueous extract the *Lactobacillus plantarum* and *Lactobacillus acidophilus* counts increased around only 0.50 and 0.22 log cycles, respectively as in (Table 8). Also, the extraction by ethanol:water (80:20) (Table 6) found to be active in the propagate of studied probiotic strains, which the viable count improved around 1 log cycles for *Lactobacillus plantarum* and around 0.75 log cycle for *Lactobacillus acidophilus*.

Antioxidant activity by DPPH, total phenol and total yield of *M. oleifera* leaves extracted by different methods: The antioxidant activity which determined by DPPH (%) as shown in Table 9, total phenol (mg g^{-1}) and total yield/100 g dry weight samples of *M. oleifera* leaves extracted by different methods. It is clear that extracted by ethanol (95%) had highest content of antioxidant activity (51.12%) followed by acetone and (ethanol:water) (47.24, 43.03%), respectively finally water (34.03%). Also, from the same Table 9 total phenol mg g^{-1} took the same trend of antioxidant activity %. Ethanol extracted recorded highest total phenol (16.86 mg g^{-1} D.W.S), whereas (ethanol:water) and acetone intermediate (14.60, 14.11 mg g^{-1} D.W.S), respectively. On the other hand, water extract had lowest total phenol (8.41 mg g^{-1} D.W.S). Moreover, data presented from the same Table 9 shows the total yield/100 g dry weight samples by different methods. Extract with ethanol:water gained highest percent (17.50/100 g D.W.S) than other treatments followed by ethanol and acetone (14.50, 14.00/100g D.W.S), respectively. Whereas, water extract with water had lowest yield (5.5/100 g D.W.S).

Chemical composition of cream cheese with different ratios of ethanol extracted: The chemical composition of cream cheese by using different ratios of ethanol extract presented in Table 10. From the table, it was found that T3 (4.00 g/100 g cheese) extract had highest total solids

Table 11: Chemical composition of cream cheese fresh and during storage at 5±1°C for 4 weeks

Treatments	Storage (Weeks)	T.S (%)	Fat (%)	pH	T.A (%)	TVFA (mL) NaOH 0.1N/100g	SN (%)	Fat/DM	SN/TN	Diacetyl mg/100 g	Acetaldehyde mg/100 g
Control	Fresh	31.21^{De}	15^{Ac}	5.00^{Aa}	1.10^{Ae}	4.00^{De}	0.092^{Be}	48.06^{Ae}	0.97^{Ce}	10.6^{De}	9.74^{De}
T1		31.32^{Ce}	15^{Ac}	5.00^{Aa}	1.10^{Ae}	4.20^{Ce}	0.091^{Be}	47.89^{Be}	0.96^{Ce}	12.4^{Ce}	10.84^{Ce}
T2		31.39^{Be}	15^{Ab}	5.01^{Aa}	1.10^{Ae}	4.50^{Be}	0.102^{Ae}	47.79^{Cd}	1.07^{Ae}	13.6^{Be}	11.20^{Be}
T3		31.61^{Ae}	15^{Ac}	5.02^{Aa}	1.10^{Ae}	5.20^{Ae}	0.102^{Ae}	47.45^{De}	1.05^{Be}	14.0^{Ae}	12.52^{Ae}
Control	1	31.36^{Cd}	15^{Ac}	4.90^{Aab}	1.33^{Dd}	5.60^{Dd}	0.104^{Bd}	47.83^{Ad}	1.096^{Cd}	12.00^{Dd}	9.94^{Dd}
T1		31.37^{Cd}	15^{Ac}	4.90^{Aa}	1.38^{Cd}	6.00^{Cd}	0.105^{Bd}	47.82^{Ad}	1.108^{Ad}	16.60^{Cd}	13.56^{Cd}
T2		31.41^{Bd}	15^{Ab}	4.87^{Ab}	1.43^{Bd}	6.40^{Bd}	0.105^{Bd}	47.76^{Be}	1.102^{Bd}	21.12^{Bd}	18.24^{Bc}
T3		31.65^{Ad}	15^{Ac}	4.85^{Ab}	1.45^{Ad}	6.90^{Ad}	0.107^{Ad}	47.52^{Cd}	1.102^{Bd}	30.68^{Ad}	19.46^{Ac}
Control	2	32.23^{Dc}	16^{Abc}	4.86^{Ab}	1.53^{Dc}	6.20^{Dc}	0.110^{Bc}	49.64^{Bc}	1.159^{Dc}	21.40^{Dc}	11.08^{Dc}
T1		32.48^{Cc}	16^{Abc}	4.77^{Bb}	1.55^{Cc}	6.96^{Cc}	0.116^{Ac}	49.26^{Cc}	1.224^{Ac}	28.80^{Cc}	15.36^{Cc}
T2		32.53^{Bc}	16^{Ab}	4.70^{Cc}	1.57^{Bc}	7.54^{Bc}	0.116^{Ac}	49.19^{Dc}	1.217^{Bc}	31.56^{Bc}	15.42^{Bc}
T3		32.79^{Ac}	17^{Ab}	4.53^{Dc}	1.59^{Ac}	7.90^{Ac}	0.116^{Ac}	51.85^{Ac}	1.195^{Cc}	41.40^{Ac}	15.90^{Ac}
Control	3	32.40^{Db}	17^{Aab}	4.73^{Ac}	1.55^{Db}	8.00^{Db}	0.122^{Ab}	52.47^{Cb}	1.29^{Ab}	32.32^{Db}	11.34^{Db}
T1		32.60^{Cb}	17^{Ab}	4.63^{Bc}	1.57^{Cb}	9.20^{Cb}	0.122^{Ab}	52.15^{Db}	1.29^{Ab}	44.60^{Cb}	21.62^{Ca}
T2		33.06^{Bb}	18^{Aa}	4.60^{Cd}	1.59^{Bb}	9.40^{Bb}	0.122^{Ab}	54.45^{Aa}	1.29^{Ab}	46.40^{Bb}	25.34^{Ba}
T3		33.13^{Ab}	18^{Aab}	4.50^{Dc}	1.63^{Ab}	9.70^{Ab}	0.122^{Ab}	54.33^{Ba}	1.26^{Ab}	50.76^{Ab}	29.92^{Aa}
Control	4	33.07^{Da}	18^{Aa}	4.63^{Ac}	1.65^{Da}	12.4^{Da}	0.137^{Ba}	54.43^{Aa}	1.44^{Ba}	42.00^{Da}	11.88^{Da}
T1		33.17^{Ca}	18^{Aa}	4.56^{Bc}	1.72^{Ca}	15.0^{Ca}	0.139^{Aa}	54.27^{Ba}	1.47^{Aa}	50.92^{Ca}	18.12^{Cb}
T2		33.77^{Ba}	18^{Aa}	4.55^{Bd}	1.76^{Ba}	18.0^{Ba}	0.139^{Aa}	53.30^{Cb}	1.46^{Aa}	54.70^{Ba}	21.06^{Bb}
T3		35.79^{Aa}	19^{Aa}	4.46^{Cc}	1.85^{Aa}	21.0^{Aa}	0.140^{Aa}	53.09^{Db}	1.44^{Ba}	58.44^{Aa}	24.60^{Ab}

Data expressed as mean of 3 replicates. Means in the same row showing the same capital letters are not significantly different ($p \leq 0.05$). Means in the same column showing the same small letters are not significantly different ($p \leq 0.05$), T.S: Total solids, T.A: Titratable acidity, SN: Soluble nitrogen, DM: Dry matter, TN: Total nitrogen

31.61%, protein 9.71% and ash 1.61%, whereas it had lowest fat/dry matter 47.45% and moisture 68.39% than control and other treatments. Total solids, protein and ash are increased with increase the ratio of ethanol extract. Fat/dry matter took an opposite trend it decreased with increase the ratio of ethanol extract.

Chemical composition of cream cheese fresh and during storage: Table 11 illustrates total solids during storage period. Total solids increased with increase ratio of ethanol extract fresh and during storage until 4 weeks. This may be due to the composition of *M. oleifera* dry leaves extract. Fat is the same in control and treatments (T1, T2, T3) fresh and after 1 week then gradually increased until 4 weeks, also fat/DM took the same trend. pH gradually decreased during storage until 4 weeks either control and treatments. Acidity took an opposite trend of pH. Statistical analysis showed that there is a significantly different between control and treatments for TS, moisture, fat/DM, protein and ash are not significantly different for fat.

Total volatile of fatty acids (TVFA) (0.1 NaOH/100g cheese): TVFA is higher in treatments (T1, T2 and T3) than the control either fresh or during storage. Also, TVFA increased with increase the ratio of ethanol extract during storage period until 4 weeks.

Soluble nitrogen: Soluble nitrogen is higher in treatments (T1, T2 and T3) than control. Control and all treatments gradually increased during storage until 4 weeks.

Soluble nitrogen/total nitrogen: SN/TN took the same trend of soluble nitrogen. It gradually increased by increasing ratio of ethanol extract and by progress of storage period until 4 weeks.

Diacetyl: Control had lowest content of diacetyl than other treatments either fresh or during storage. Diacetyl regularly increased with the increased the ratio of ethanol extract. Control and all treatments regularly increased during cold storage period.

Acetaldehyde: Acetaldehyde content of control increased during cold storage. On the other hand acetaldehyde of treatments increased until (3 weeks) then decreased at 4 weeks. Differences between treatments and storage period for T.S, S.N, fat/DM, acetaldehyde and not significantly different for fat, T.S, T.A and acetaldehyde.

Peroxide value (PV): Results in Table 12 indicated that the control recorded highest PV (0.163 mEq. kg^{-1}) than other treatments. Whereas, (T3) recorded lowest (0.158 mEq. kg^{-1}) PV than control and other treatments. PV increased during storage until 4 weeks in either control or treatments. On the other hand at the end of storage 4 weeks control had a highest PV of 0.621 compared to other treatments.

Total phenol content (mg kg^{-1}): Table 13 illustrates that the total phenol content (mg kg^{-1}) of cream cheese during storage for 4 weeks. Control had lowest content of total phenol (43.23 mg kg^{-1}) than other treatments. Total phenol

Table 12: Peroxide value of cream cheese during storage at 5±1°C for 4 weeks

	Peroxide value (mEq. kg^{-1})			
Samples	Initial	2 weeks	3 weeks	4 weeks
Control	0.163±0.007	0.242±0.005	0.496±0.011	0.621±0.001
T1	0.161±0.002	0.177±0.003	0.205±0.007	0.217±0.007
T2	0.160±0.011	0.171±0.005	0.189±0.004	0.195±0.021
T3	0.158±0.005	0.170±0.012	0.194±0.011	0.194±0.011

Mean±Standard deviation

Table 13: Total phenol content by mg kg^{-1} of cream cheese samples during storage at 5±1°C for 4 weeks

	Total phenol content (mg kg^{-1}) sample			
Samples	Initial	2 weeks	3 weeks	4 weeks
Control	43.23±1.62	35.10±1.62	29.58±0.59	26.35±0.15
T1	108.13±0.00	85.10±0.74	75.73±0.74	70.63±0.59
T2	118.96±0.29	95.42±1.18	91.67±1.47	85.31±1.33
T3	128.65±1.03	106.88±1.18	99.48±3.68	92.40±0.74

Mean±Standard deviation

Table 14: Antioxidant activity (%) of cream cheese during cold storage at 5±1°C for 4 weeks

	Antioxidant activity (%) by DPPH			
Samples	Initial	2 weeks	3 weeks	4 weeks
Control	4.50±1.04	0.52±0.15	0.42±0.15	0.26±0.07
T1	25.81±1.85	23.87±1.33	19.69±1.33	17.75±1.11
T2	32.83±1.11	30.16±0.15	25.50±1.41	21.78±0.44
T3	43.04±0.59	36.60±0.37	32.93±0.37	28.22±0.37

Mean±Standard deviation

Table15: Viability of *Leuconostoc mesenteroides* (Log CFU g^{-1}) in cream cheese treatments during storage

	Storage period				
Treatments	Fresh	1 week	2 weeks	3 weeks	4 weeks
Control	8.31^{Aa}	8.22^{Ac}	8.16^{Bc}	8.26^{Ac}	8.13^{Bc}
T1	8.00^{Db}	8.40^{Cc}	9.00^{Bb}	9.10^{Ab}	9.16^{Ab}
T2	8.20^{Da}	8.75^{Cb}	9.13^{Bb}	9.20^{Ab}	9.30^{Ab}
T3	8.33^{Da}	9.00^{Ca}	9.28^{Ca}	10.06^{Ba}	10.31^{Aa}

Data expressed as mean of 3 replicates. Means in the same row showing the same capital letters are not significantly different ($p \leq 0.05$)

content increased by increasing the ratio of *M. oliferera* levels extract by ethanol. T3 had a highest content of total phenol 128.65 than a control and other total phenol (mg kg^{-1} sample) gradually decreased during storage until 4 weeks in control and treatments.

Antioxidant activity (%) of cream cheese during storage: Table 14 shows the antioxidant activity % of cream cheese during storage for 4 weeks. It is clear that control had lowest content of antioxidant than other treatments it recorded (4.50%). Antioxidant increased by increasing the ratio of *M. oleifera* leaves extract (2, 3, 4.00 g/100 g cheese) it reached to 25.81, 32.82 and 43.04, respectively. Whereas, antioxidant activity in control and all treatments decreased during storage until 4 weeks.

Viability of *Leuconostoc mesenteroides* in cream cheese treatments during storage: Viability of *Leuconostoc mesenteroides* in cream cheese treatments as in Table 15, indicated that the viability of *Leuconostoc mesenteroides* significantly increased gradually during cold storage period in the presence of *M. oleifera* extract especially in the treatment T3 which the viable counts of *Leuconostoc mesenteroides* increased around 2 log cycle after 4 weeks of storage compared control. But in the treatments T1 and T2 the count of the same strain increased around 1.16 and 1.10 log cycle, respectively compared with control. In these treatments, the addition of *Leuconostoc mesenteroides* as the starter culture enhanced the taste and flavor of the final cream cheese product which it able to produce acetaldehyde and diacetyl in the cream cheese during storage period.

Viability of *Lactobacillus plantarum* in cream cheese treatments during storage: In this product, addition of *Lactobacillus plantarum* as a probiotic strain to give healthy benefits to the human and improved the nutritional value of final product with the *M. oleifera* extract as in Table 16. From this table, it is clear that there is enhancement in the

Table 16: Viability of *Lactobacillus planatrum* (Log CFU g^{-1}) in cream cheese treatments during storage

	Storage period				
Treatments	Fresh	1 week	2 weeks	3 weeks	4 weeks
Control	8.17^{Cb}	8.33^{Bc}	8.30^{Bd}	8.60^{Ac}	8.45^{Ad}
T1	8.70^{Da}	9.00^{Cb}	9.34^{Bc}	9.65^{ABb}	9.73^{Ac}
T2	8.62^{Da}	9.25^{Ca}	9.55^{Cb}	9.95^{Ba}	10.13^{Ab}
T3	8.77^{Ea}	9.30^{Da}	9.88^{Ca}	10.26^{Ba}	10.40^{Aa}

Data expressed as mean of 3 replicates. Means in the same row showing the same capital letters are not significantly different ($p \leq 0.05$). Means in the same column showing the same small letters are not significantly different ($p \leq 0.05$)

Table 17: Rheological properties of fresh cream cheese and after 4 weeks of storage at 5±1°C

Samples (Fresh)	Hardness (N)	Cohesiveness A area/B	Springiness (mm)	Gumminess (N)	Chewiness (M/N)
Control	1.2	0.763	0.782	0.992	0.776
T1	1.3	0.745	0.984	1.010	0.994
T2	1.3	0.719	0.842	0.854	0.689
T3	1.4	0.657	0.788	0.819	0.673
Samples (4 weeks)					
Control	2.5	0.524	0.505	1.699	0.919
T1	3.2	0.472	0.587	1.990	1.112
T2	3.6	0.383	0.541	0.803	0.393
T3	4.1	0.238	0.490	0.595	0.349

viability of *Lactobacillus plantarum* during storage period in the treatments that fortified with different concentration of *M. oleifera* extract compared with control. Also, it clear from the result, the viability of *Lactobacillus plantarum* in the treatment T3 significantly increased around 1.63 log cycle compared with control. Also, the viable count increased around 1.00 and 1.50 log cycles in the treatments T1 and T2, respectively compared with control.

Effect of adding *M. oleifera* leaves extract on the shelf life of cream cheese treatments: From present study results, it is indicated that all treatments free from coliform during storage periods which may be attributed to the pasteurization of milk during manufacturing of cream cheese beside the potential of antimicrobial activity of *M. oleifera* leaves extract on the coliform. Also, not observed any mold and yeast count during storage periods in all treatments prepared with *M. oleifera* leaves extract, but found some molds and yeasts reached to 2.00 log CFU g^{-1} in control after 21 days of storage and reached to 2.33 log CFU g^{-1} after 4 weeks of storage.

Rheological properties of fresh cream cheese and after 4 weeks of storage: Table 17 reveals that the rheological properties of cream cheese during storage for 4 weeks. It shows that the hardness (N) values of treatments are higher than hardness of control fresh and during storage for 4 weeks. Hardness increased by increasing the present of *M. oleifera* leaves extract.

Cohesiveness A area/B: From the same table cohesiveness took the opposite trend of hardness (N). Control had higher cohesiveness than other treatments fresh and during storage for 4 weeks cohesiveness decreased by increasing the ratio of *M. oleifera* dry leaves extract.

Springiness: Control had lowest springiness (mm) is recorded (0.782 mm) fresh than other treatments springiness decreased by increasing the ratio of *M. oleifera* dry leaves extract. Also, springiness decreased during storage for 4 weeks in both control and treatments.

Gumminess (N) and chewiness: Also as presented in Table 17 gumminess took the same trend. Gumminess and chewiness were decreased by increasing *M. oleifera* dry leaves extract fresh and during storage for 4 weeks.

Sensory properties of cream cheese during storage: From Table 18 results of organoleptic properties revealed that control had gained higher score (9) for appearance means it extremely strong than other treatments fresh and during storage until 4 weeks. Scores gradually decreased during storage in control and all treatments.

Flavor cooked and granularity: Control and all treatments recorded (1) means extremely weak fresh and during storage until 4 weeks for flavor and granularity.

Table 18: Sensory properties of cream cheese during storage at 5±1°C for 4 weeks

		Treatments			
Sensory	Storage	Control	T1	T2	T3
Appearance	Fresh	9^{Aa}	8.5^{Aa}	8.3^{Aa}	8.1^{Aa}
	1 week	9^{Aa}	8.1^{Bab}	7.6^{Bb}	6.4^{BC}
	2 weeks	8.2^{ABa}	7.6^{Cb}	6.8^{Cc}	6.2^{Cd}
	3 weeks	7.8^{ABa}	7.3^{Db}	5.4^{Dc}	5.1^{Dd}
	4 weeks	7.3^{Ba}	6.5^{Eb}	5.1^{Ec}	4.6^{Ed}
Flavor cooked	Fresh	1^{Aa}	1^{Aa}	1^{Aa}	1^{Aa}
	1 week	1^{Aa}	1^{Aa}	1^{Aa}	1^{Aa}
	2 weeks	1^{Aa}	1^{Aa}	1^{Aa}	1^{Aa}
	3 weeks	1^{Aa}	1^{Aa}	1^{Aa}	1^{Aa}
	4 weeks	1^{Aa}	1^{Aa}	1^{Aa}	1^{Aa}
Taste sourness	Fresh	3.7^{Ec}	3.5^{Ed}	4.3^{Eb}	5.7^{Ea}
	1 week	4.4^{Db}	4.3^{Db}	4.1^{Dc}	5.5^{Da}
	2 weeks	5.2^{Cb}	5.0^{Cc}	4.9^{Cc}	6.8^{Ca}
	3 weeks	5.5^{Bc}	6.4^{Bb}	6.4^{Bb}	7.5^{Ba}
	4 weeks	6.2^{Ad}	6.6^{Ac}	7.0^{Ab}	7.8^{Aa}
Granularity	Fresh	1^{Aa}	1^{Aa}	1^{Aa}	1^{Aa}
	1 week	1^{Aa}	1^{Aa}	1^{Aa}	1^{Aa}
	2 weeks	1^{Aa}	1^{Aa}	1^{Aa}	1^{Aa}
	3 weeks	1^{Aa}	1^{Aa}	1^{Aa}	1^{Aa}
	4 weeks	1^{Aa}	1^{Aa}	1^{Aa}	1^{Aa}
Creamy	Fresh	8.6^{Aa}	8.6^{Aa}	8.7^{Aa}	8.6^{Aa}
	1 week	8.2^{Ba}	7.2^{Cc}	7.4^{Bb}	7.3^{Bbc}
	2 weeks	7.8^{Ca}	7.7^{Ba}	7.2^{Ca}	7.0^{Ca}
	3 weeks	7.5^{Da}	6.8^{Db}	6.5^{Dc}	6.2^{Dd}
	4 weeks	7.1^{Ea}	6.3^{Eb}	6.2^{Eb}	6.0^{Ec}
Softness	Fresh	7.3^{Aa}	7.2^{Aa}	7.0^{Aa}	7.0^{Aa}
	1 week	7.1^{Ba}	7.0^{Ba}	7.0^{Aa}	7.0^{Aa}
	2 weeks	6.8^{Ca}	6.7^{Ca}	5.9^{Bc}	6.2^{Bb}
	3 weeks	5.8^{Da}	5.3^{Db}	4.8^{Cc}	5.3^{Cb}
	4 weeks	5.3^{Ea}	5.1^{Ea}	4.3^{Db}	4.0^{Db}
Spread ability	Fresh	7.6^{Aa}	7.4^{Ab}	7.2^{Ac}	7.0^{Ad}
	1 week	6.5^{Bc}	7.2^{Ba}	7.2^{Aa}	7.0^{Ab}
	2 weeks	6.3^{Ca}	6.1^{Cb}	5.8^{Bc}	5.4^{Bd}
	3 weeks	5.7^{Da}	5.4^{Db}	5.3^{Cb}	5.0^{Cc}
	4 weeks	5.2^{Ea}	5.1^{Ea}	4.6^{Db}	4.3^{Dc}

Data expressed as mean of 3 replicates. Means in the same row showing the same capital letters are not significantly different ($p \leq 0.05$). Means in the same column showing the same small letters are not significantly different ($p \leq 0.05$)

Taste sourness: (T3) improved taste sourness it had gained (5.7) means moderate scores gradually increased during storage until 4 weeks either fresh or treatments.

Creamy, softness and spread ability: From the same Table 18 it is clear that control had gained same scores, means very strong of treatments T1, T3 and T2 had slight increase (8.7) control and all treatment scores/gradually decreased until 4 weeks.

Softness: Control had higher scores than treatment (7.3) means very strong and regularly decreased during storage until 4 weeks for control as well as treatments.

Spread ability: Softness of the samples took the same trend either fresher or during storage preserved with ethanol extracts of *M. oleifera* dry leaves had the most acceptable to consumers.

Color change of cream cheese with *M. oleifera* dry leaves extract fresh and during storage: Table 19 showed color changes of control and treatments of cream cheese, control had highest whitens storage (L) gradually increased during storage until 4 weeks (a) color ranged from red (+) to green (-). Control had higher value than treatments fresh or during storage. Value of (a) gradually increased until 4 weeks, (b) represents yellow (+) to blue (-) treatments had higher values than control. Values gradually increased during storage until 4 weeks.

Table 19: Color changes of cream cheese with *M. oleifera* leaves extract fresh and during storage for 4 weeks

Samples	Storage	L*	a*	b*
Control	Fresh	70.24	-2.74	12.11
T1		46.96	-1.28	17.38
T2		46.33	-1.99	17.07
T3		44.54	-2.08	15.28
Control	2 weeks	76.89	-0.72	10.32
T1		57.16	-1.67	23.41
T2		55.27	-1.81	21.87
T3		49.26	-2.19	20.01
Control	4 weeks	97.49	-0.12	10.06
T1		68.94	-2.24	24.37
T2		65.05	-2.25	23.47
T3		61.68	-2.28	20.65

L*: Value represents darkness from black (0) to white (100), a*: Value represents color ranging from red (+) to green(-), b*: Value represents yellow (+) to blue

DISCUSSION

The vulnerability of some strains to the extract of *M. oleifera* may be indicator to its potential as a drug and food preservative agent that can be used against these susceptible strains. The difference in strains response was possible due to the nature of the different species. Statistical analysis showed that a significant degree of inhibition between all pathogenic strains and different extract of *M. oleifera* leaves. It is noted that the ethanolic leaves extracts had strong inhibitory effect against all tested pathogens whereas, aqueous extract had slightly less inhibitory effects. Also, the fungi strains found to be stronger against *M. oleifera* extract with different solvents and water. These results confirmed by Kalpana, *et al.*[35], who found the ethanolic extract more affective against gram positive and gram negative bacteria. On the other hand Moyo *et al.*[36] found that both acetone and aqueous extracts did not show any antifungal action beside the fungal species of *C. albicans*, *P. notatum*, *A. flavus* and *A. niger* even at the concentration of 10 mg mL^{-1}.

Moreover, the obtained data showed that increasing the concentration of different extracts from control to 4 mg mL^{-1} medium led to raise the probiotic bacterial growth. This may be owing to great dietary composition of *M. oleifera* dry leaves as carbohydrates, proteins, minerals, essential amino acids and vitamins as B1, B2 and B3[11]. Additionally, increases in the level adding *M. oleifera* dry leaves led to improve the count of bacteria. These outcomes are similar to that gained by Amer *et al.,*[11] and Van Tienen *et al.,*[37], who established that the addition of *M. oleifera* leaves extract improved the probiotic strains growth. Salem *et al.,*[38] suggested that bacterial population was encouraged by adding *M. oleifera* dry leaves.

The same trend of results observed for antioxidant activity by DPPH, total phenol and total yield of *M. oleifera* leaves extracted by different methods, which the data indicated that ethanol extracted had highest total phenol. These results are in harmony with Vongsak *et al.*[39], who reported that extracted of *M. oleifera* leaves with ethanol (70%) had high DPPH scavenging activity. On the other hand water extract had lowest total phenol. These results are in agreement to Rodriguez-Perez *et al.*[40], who decided that using ethanol:water (50: 50 v/v) produced on extract of *M. oleifera* leaves with the major quantity of phenolic compounds. Also, These results are in conformity with Vongsak *et al.*[39], who reported that extracted of *M. oleifera* leaves with ethanol (70%) provide the highest yield of the extract.

Addition of *M. oleifera* extract to cream cheese did not alter completely in the chemical composition of final product but improved the properties of final product during storage. Moreover, the addition of this extract enhanced the nutrition value of the cream cheese. Also, statistical analysis showed that there is a significantly different between control and treatments for TS, moisture, fat/DM, protein and ash and are not significantly different for fat. These results are in harmony with Hassan *et al.*[41] and Salem *et al.*[38]. The same trend of results observed for TVFA and diacetyl increased with increase the ratio of ethanol extract during storage period until 4 weeks and control had the lowest content of TVFA and diacetyl. These results are in the same trend with El-Sayed *et al.*[42]. Moreover, soluble nitrogen content in all treatments increased by progress of storage period may be due to the growth and activity of starter culture as mentioned by Mahdian and Tehrani[43]. Acetaldehyde of treatments increased until 3 weeks then decreased at 4 weeks. This may be due to the demonstrated ability of numerous lactic organisms to reduce acetaldehyde to ethanol Amer *et al.*[44] and Salama[45].

On the other hand, at the end of storage 4 weeks control had a highest PV compared to other treatments. These results are agreements to Salem *et al.*[46]. Higher level of peroxide value indicate to poor keeping quality furthermore cream cheese

fortified with 4.00 g/100 g cheese had good keeping quality due to the presence of natural antioxidant[47,48]. Also, total phenol content increased by increasing the ratio of *M. oleifera* levels extract by ethanol. These due to the high level content of total phenol in the extract and these result confirmed by Salama *et al.*[3]. The high antioxidant activity of *M. oleifera* dried leaves may be owing to the presence of major amount of phyto constituents and potential therapeutic[38,48,49].

Viability of *Leuconostoc mesenteroides* in cream cheese treatments significantly increased gradually in cream cheese is resulting to the nutrition component found in *M. oleifera* extract as vitamins, essential amino acids and carbohydrates. These different nutrient compounds encourage the growth of lactic acid bacteria. Our results confirmed by Kumalaningsih *et al.*[50], who established that the presence of essential amino acids in the *M. oleifera* leaves enhanced the growth of the bacterial growth. Also, the addition of *Leuconostoc mesenteroides* as the starter culture to enhance the taste and flavor of the final cream cheese product as El-Shafei *et al.*[51], who improved the quality of yoghurt by *Leuconostoc mesenteroides* that produced more acetaldehyde and diacetyl. The same trend was observed with *Lactobacillus plantarum* which used as a probiotic strain and gives healthy benefits to the human and improved the nutritional value of final product with the *M. oleifera* extract. Also, the addition of these extract improved the viability of *Lactobacillus plantarum* in final product. These may due to that *M. oleifera* leaves contained different minerals, vitamins and phenols that can boosted the bacterial growth[2,3]. Van Tienen *et al.*,[37] established that the adding of *M. oleifera* boosted the viability of probiotic bacteria in yogurt.

From present study results, it is indicated that all treatments free from coliform during storage periods. These results recognized that *M. oleifera* leaves extract had antimicrobial properties which may be used as natural preservative material for dairy products. Salem *et al.*[38], who reported that they do not detected any yeast and mold and coliform bacteria in Labneh prepared with *M. oleifera* extract and Labneh characterized with high biological value.

The rheological properties found that hardness and cohesiveness increased by increasing the present of *M. oleifera* leaves extract. This may be due to the ratio of total solids and moisture Saad *et al.*[52]. Moreover, springiness and gumminess decreased during storage for 4 days. These may be because of the pH values and F/DM content in either control or treatments[52].

From present study data, it is indicated that the addition of *M. oliefera* leaves extract to cream cheese improved flavor and taste during storage. These results are agreements with Badoms *et al.*,[53]. Statistical analysis showed that there is a significantly different between treatments and storage for appearance, taste sourness, creamy and spread ability whereas, there is not significantly different for flavor looked and granularity.

The change in color during storage between control and other treatments may be due to increase of dietary fiber. Also, it has a good source of polyphenols, natural antioxidants and antimicrobial. These data are in line with Hassan *et al.*,[54], Saricoban and Yilmaz[55].

CONCLUSION

This study concluded that ethanol extract of *M. oleifera* leaves should be used in manufacturing of cream cheese due to its highest content of nutritious and a variety of phenol compounds. Also, the addition of *M. oleifera* leaves extract improved the probiotic strains growth in final product, enhanced flavor and taste during storage. *M. oleifera* leaves extract had excellent antimicrobial properties which may be used as natural preservative material for cream cheese. This study will help dairy producer to increase the shelf life and quality of different dairy products.

SIGNIFICANCE STATEMENT

This study discovers that the use ethanol extract of *M. oleifera* leaves in manufacture of cream cheese improved the probiotic strains growth in final product and enhanced flavor and taste during storage. *M. oleifera* leaves extract had excellent antimicrobial properties due to its highly content of phenols, which may be used as natural preservative material for cream cheese. This study will help the researcher to use *M. oleifera* leaves extract in food industry and different dairy products.

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