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Shelf-life improvement of raw milk using ethanolic extracts of selected medicinal plants (*Moringa stenopetale, Artemesia anua and Mentha Spicata)*

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ABSTRACT

The current study was designed to examine the Shelf life extension ability and antioxidant activity of the ethanolic extracts processed from Moringa stenopetale, Artemesia anua and Mentha Spicata. The raw milk and preservative plants was collected from Chencha woreda, Arba Minch. Purposive sampling techniques were used for collection of milk and plant samples. The crude extracts were isolated from three plant species using Soxhlet extraction method and characterized for the presence of bio-active compounds qualitatively and quantitatively. He accelerated shelf-life testing method was used to estimate the shelf-life of milk samples. Deterioration was tracked using measurements of the peroxide values and sensory characteristics. The DPPH free radical scavenging assay was used to determine the antioxidant capacity of various extracts, and the absorbance was measured at 517 nm. Mentha Spicata had the highest TPC value of 2.842 ± 0.109 mg GAE/g, while Moringa stenopetale had the lowest at 0.285± 0.0098 mg GAE/g. Similarly, Moringa stenopetale had the greatest TTC value of 69.86 ± 1.25 mg QE/g, while Mentha Spicata had the lowest at 46.2 ± 0.7 mg QE/g. The extracts of Moringa stenopetale, Artemesia anua, and Mentha Spicata showed good antioxidant activities, with IC_{50} values of 39.490 ± 0.029, 61.189± 0.058, and 68.062± 0.014 g/mL, respectively. The shelf-life of the milk sample improved with Moringa stenopetale was 6.99 days at 27 °C. Generally, Moringa stenopetale had the longest shelf life of all the plant extracts tested for milk samples.

1. Introduction

Food safety is a difficult and ongoing issue that has gained significant social attention as a result of numerous outbreaks of foodborne illnesses. The majority of foodborne illnesses are brought on by the presence of harmful bacteria in food, which can be dangerous to people's health. The complex biochemical composition, high water activity, and nutrient content of milk create an ideal habitat for the development of pathogenic and spoilage microbes. Consuming raw or pasteurized milk that has been improperly stored or that has gone bad could be harmful to your health because it could be contaminated with pathogenic bacteria. These germs can cause a variety of symptoms, including diarrhea and abdominal pain, and in severe situations, [1].

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Abbreviations

PVperoxide Value
TPCTotal Phenolic Content
DPPH2,2-diphenyl-1-picrylhydrazyl
AMU PG Arba Minch University Post Graduate
IC_{50}Inhibitory concentration at 50 %
NSSNormal Saline solution
CFUColony Forming Unit
IDFInternational Dairy Federation

Bacteria in raw milk can come from a variety of places, including the air, milking equipment, feed, soil, cow feces, and grass [2]. Furthermore, 90% of milk and milk products are infected with diseases that affect humans. One of the reasons for public health rules prohibiting the use of informal milk markets and the consumption of raw or unpasteurized milk is the risk of infection from milk-borne zoonotic illnesses such as tuberculosis and brucellosis, [3]. Spoilage of milk manifests itself as a change in flavor, undesired coagulation of milk proteins, and an increase in free fatty and amino acid content. Fresh raw milk contains less than 5000 cfu/ml immediately after sanitary milking [4,5]. The deterioration of milk and other dairy products has been linked to a number of bacteria. Pseudomonas species and Bacillus species are the most common isolated organisms found in raw or heat-treated (pasteurized) milk at the time of spoilage [6]. It is known that many bacteria, including Bacillus cereus, Salmonella spp., *Staphylococcus aureus*, and *E. coli*, contaminate milk and dairy products. These bacteria produce a variety of enterotoxins that can make people sick[7].

In the process of milk preservation, several techniques were known to be used. Preservative plants are extensively employed in rural settings, particularly in some pastoralist groups, to preserve milk. Antimicrobial activity has been reported in a variety of plants, herbs, and spice extracts against bacteria, yeast, and molds. Smallholders must either look for items with a longer shelf life or change their current processing processes to acquire products with a longer shelf life due to problems with storage stability with dairy products. Making ensuring that the manufacturing and production processes are clean is the first thing that manufacturers or product handlers can do to guarantee a high-quality product. The end goods will be of superior quality and have a longer shelf life since there will be fewer rotting organisms in dairy products. Using extraordinary caution while handling, storing, and selling products is another technique to ensure good quality [8]

Charcoal from *Olea europea*, *Lagenaria siceraria*, and *Olea africana* has been documented as being used to flavor and protect milk storage gourds from spoiling microbes [9]. Previously several researches was conducted and reported to extend the shelf life of milk like Plantaricin FB-2 as indicated in [5,10], using ultraviolet treatment [11], combined thermal and Pulse Electric field. However, in rural regions, these preservatives are scarce, and cooling systems are impractical due to a lack of infrastructure [12,13]. Traditional procedures were used in rural areas without refrigerators to confirm the quality and safety of their milk. Smallholders preserve drinking milk with a variety of spices [14]. Raw milk quickly spoils in hotter areas unless it is cooled or, ideally, preserved with a preservative. Milk has a built-in inhibitory mechanism that, if chilled to 4 °C within the first two to 3 h, protects practically all of its original quality. During this time, the bacteria count does not increase significantly. Milk's purity is maintained for processing and consumption by quickly cooling it [15].

Preservative plants have been used for milk preservation and milk container fumigation in Ethiopia for a long time. But, there is no documented research or evidence available on the preservation of fresh milk. This study was aimed to examine the Shelf life extension ability and antioxidant activity of the ethanolic extracts processed from *Moringa stenopetale*, *Artemesia anua* and *Mentha Spicata*. In this study, Ethanolic extract was extracted from *Moringa stenopetale*, *Artemesia anua* and *Mentha Spicata* and was added to raw milk to improve the shelf life. The Phytochemical, Chemical Constituents of extracts and antioxidant activity of the ethanolic extracts was analyzed. To ascertain the impact of plant extracts on the shelf life, the bacterial load assessment of raw milk following the addition of those plant extracts during storage was evaluated along with sensory evaluation. This study proved that the ethanolic extracts of Moringa stenopetale, Artemesia anua, and Mentha Spicata are efficient preservatives. These extracts, particularly Moringa stenopetale, can be used as a reference for future predictions of microbial growth in raw food.

2. Materials and methods

2.1. Description of the study area

Chencha is one of the woreda in the Southern Nations, Nationalities, and Peoples' Region of Ethiopia. It is one of Gamo Zone woreda and bordered on the south by Arba Minch Zuria, on the west by Dita, on the north by Kucha and Boreda, and on the east by Mirab Abaya. Chencha woreda is situated between 1300 m and 3250 m above sea level and Chencha town is 37 km north of Arbaminch town. Geographical location of Chencha is between 37° 29′ 57″ East to 37° 39′ 36″ West and between 6°8′55″ North and 6° 25′30″ South.

2.2. Apparatus and instruments

The apparatus used for this experiment was UV–vis spectrophotometer (specord/50 plus, Analytical jina, Germany), Electronic

balance (ABS 22O–4N, Germany), Distiller (Lasany LPH-4, India), Autoclave (HIRAYAMA, Japan), Soxhlet Extractor (EXBG-500-001, UK) and Rotary Evaporator (RE-201D, China).

2.3. Collection and processing of plant samples

The leaves of *Mentha Spicata, Moringa stenopetale and Arthemisia Anua* were collected from the study areas. The plant materials were collected after gaining consent from the AMU PG coordination office, and the letter was approved by the agriculture office of Arba-minch Zuria Woreda (Chencha). Sceientific name of medicinal plants were identified by botanist in the Addis Ababa university, Ethiopia national herbarium. The collected plant parts were separately washed using tap water followed by sterilized distilled water and cut into smaller sizes of about 1–2 cm long. The washed plant parts were then shade-dried at room temperature for 15 days, grinded using an electric grinder into a fine powder, and finally kept in a refrigerator (4 °C) for further use.

2.4. Extraction of plant components

In a grinder, the dried leaves were powdered individually. In a thimble 60 g of powdered samples were added and placed inside the Soxhlet device. They were then extracted with ethanol in a series of steps using the hot Soxhlet extraction technique at temperature of 80 °C. For extracting the crude extracts of the materials, the device was operated for 6 h till the greenish tinted solvent appeared in the siphon. After complete extraction, the solvent was evaporated at 78 °C in a rotary vacuum evaporator at a pressure of 200 mbar. After that, the extracts were dried in a water bath. After solvent evaporated, the remaining crude extracts were diluted with 10 mL sterile distilled water and kept in an airtight bottle in the refrigerator until use [16].

2.5. Collection of milk ***samples***

Lactating cows were specifically chosen from the study Kebele. Zebu cattle breeds (Z) were milked by hand in the morning and evening. Four streams of milk were removed to decrease the threat of contamination from germs in the teat. The udder and teats were brushed to remove any dirt, trash or bedding particles. The teats were predipped in water with effective dip and left for 10 min before removing it. Each teat was dried with cotton towel and the dip was removed. The teat end was scrubbed and orificed with cotton gauze. The teat end was cleaned until it is entirely clean and white to avoid recontamination of teat ends. Before taking the sample, the collection vial was opened and disallowed to skin or dirt particles to enter the container. The collection vial was kept at a 45-degree angle to prevent debris (hair, manure, dirt) from falling into the vial. The collection vial was filled only half way to decrease the risk of contamination. Using disposable syringes, 20 mL milk samples were transferred from the stock to sterile screw cap test tubes. The samples were transported to the Chemistry laboratory in an ice box for analysis.

2.6. Milk sample preparation

Following physical separation protocols [17], 0.02 g of crude extracts of the plants were mixed in 10 mL of milk samples using a pressure homogenizer in 100 mL of beaker and then collected into clean 300 mL reagent bottles for analysis of total bacterial load, peroxide value, and organoleptic test.

2.7. Qualitative determination of the chemical constituents of extracts

The existence of naturally occurring biologically active substances such as alkaloids, tannins, phenols, saponins, and flavonoids was screened in extracts from *Mentha Spicata*, *Arthemesia anua*, and *Moringa stenopetale* leaves. Three plant extracts were subjected to qualitative phytochemical analysis using the protocols of (Zaidi et al., 2015, Adetuyi et al., 2001).

2.7.1. Test for saponins

In a test tube with 0.5 g of crude extract, 5 mL of deionized water was added. For 2 min, the mixture was Shaken Vigorously. The presence of saponins was detected by persistent foaming when shaken.

2.7.2. Test for alkaloids

A mixture of crude extract (3 mL) and 1% HCl (2 mL) was heated on a steam bath for 20 min. After cooling, the mixture was filtered. Wagner's reagents were added in very little amounts. The turbidity of the resultant precipitate revealed the presence of alkaloids.

2.7.3. Test for phenols

One grams of aqueous extract was mixed in 5 mL of ethanolic mixture, and two drops of 5% $FeCl_3$ solution were added. A greenish precipitate showed the presence of phenols.

2.7.4. Test for tannins

The presence of tannins was indicated by the appearance of the dull white precipitate that was produced when 10% of 1 mL freshly prepared KOH was added to 1 mL of aqueous extract.

2.7.5. Test for flavonoids

2 mL of a 2% NaOH solution were added to 2 mL of extract. The presence of flavonoids was indicated by a deep yellowish hue that became colorless when a few drops of weak acid were added.

2.8. Quantification of the constituents of extracts

2.8.1. Standard gallic acid preparation

According to Singleton et al., 's 1999 procedure, the Folin-Ciocalteu technique is commonly used to estimate the total phenolic contents (TPC). 10 mg of standard gallic acid was dissolved in 10 mL of methanol to form 1 mg/mL of solution. From the standard solution, a series of working gallic acid solutions in methanol (25, 50, 75, and 100 mg/L) were prepared. A final volume of 10 mL was prepared by adding 5 mL of 10% Folin-Ciocalteu reagent (FCR) and 4 mL of 7% Na_2CO_3 to each concentration. The resulting blue colored solution was then well mixed and incubated for 30 min At 40 °C in a water bath and then, the absorbance of the solution was measured at 760 nm. The FCR reagent oxidizes phenols in plant extracts and changes into the dark blue color, which is then measured by UV–visible spectrophotometer. All the experiments were carried out in triplicates measurements, and the average absorbance values were obtained at different concentrations of gallic acid which were used to plot the calibration curve.

2.8.2. Total phenolic contents (TPC)

The extracts were prepared in the range of concentrations (25, 50, 75, and 100 mg/L). Following the instructions for standard gallic acid, each concentration of the extracts' absorbance was recorded. For each analysis, the samples were made in triplicate, and the average absorbance value was used to plot the calibration curve and ascertain the concentration of phenolics in the extracts. The extracts' total phenolic content was calculated as mg gallic acid equivalents (GAE) per gram of dry sample weight (mg/g) [18]. The TPC in all the samples were calculated using equation (1):

$$C = c\,\frac{V}{m} \tag{1}$$

Where C = total phenolic content mg GAE/g dry extract, c = Concentration of gallic acid obtained from calibration curve in mg/mL, V = volume of extract in mL, and m = mass of extract in gram.

2.8.3. Tannin contents

The Folin-Ciocalteu method was used to evaluate the tannin contents of the extracts. To a 25 mL volumetric flask containing 7.5 mL of distilled water, 0.5 mL of Folin-Ciocalteu phenol reagent, 1 mL of 35% sodium carbonate solution, and 10 mL of distilled water, 0.1 mL of the sample extracts was added. After thoroughly shaking, the mixture was left at room temperature for 30 min. Tannic acid reference solutions (20, 40, 60, 80, and 100 mg/L) were used to calibrate UV/Visible spectrophotometer and the absorbances of test and standard solutions were measured 760 nm [19].

2.8.4. Flavonoid content

100 mL of 80% aqueous ethanol were regularly used to extract 10 g of the plant sample while it was at room temperature. 125 mm whatman filter paper No. 42 was used to filter the entire solution. The filtrate was then put into a crucible, evaporated over a water bath to become dry, and weighed to a constant weight and %flavonoid was calculated using equation (2) [20].

$$\%\ \text{Flavonoid} = \frac{Wc + g - Wc}{Wg}\ \text{x}100 \tag{2}$$

Where Wc + g = weight of crucible with extract; WC = weight of crucible only; Wg = weight of plant sample.

2.8.5. Alkaloid content

The extracts were weighed (5 g), added to a 250 mL beaker containing 200 mL of 10% acetic acid in ethanol, and let to stand for 4 h. This was filtered, and the extract was then concentrated to 1/4 of its original volume by placing it in a water bath. The extract was then treated with concentrated ammonium hydroxide, which was applied drop by drop until the precipitation was finished. After allowing the entire solution to settle, the precipitated material was collected, cleaned with diluted ammonium hydroxide, and then filtered. Then, the dried residue was measured for the alkaloid.

2.8.6. Saponin content

After being pulverized, the dried leaves of several plants were placed in a conical flask with 20 g of each and 100 cm^3 of 20% aqueous ethanol. The samples were heated at roughly 55 °C over a hot water bath for 4 h with continuous stirring. The mixture was filtered and the residue re-extracted with another 200 mL 20% ethanol. The combined extracts were reduced to 40 mL over water bath at about 90 °C. The concentrate was added into a 250 mL separatory funnel, 20 mL of diethyl ether was added, and the mixture was vigorously shaken. While the ether layer was discarded, the aqueous layer was recovered. A second round of purification was conducted by adding 60 mL of n-butanol. Two separate washes with 10 mL of 5% aqueous sodium chloride were performed on the combined n-butanol extracts. In a water bath, the residual solution was warmed. The samples were dried in an oven after evaporation to a consistent weight, and the saponin content was determined as a percentage.

2.9. Antioxidant activities

2.9.1. Radical scavenging potential of DPPH

The antioxidant qualities of the extracts were evaluated invitro using the DPPH free radical scavenging test [21]. By dissolving 0.39 mg of DPPH in 100 mL of methanol in a volumetric flask, 0.1 M of the DPPH solution was prepared and stored at 4 °C for the next analysis. Solutions of 1 mg/mL of the extracts were prepared as stock solutions and a series of working solutions (25, 50, 75, and 100 μg/mL) were prepared from it.

2.9.2. Evaluation of antioxidant potential

DPPH solution of 1 mL was added to the previously prepared sample solution and placed at room temperature for 30 min in the dark. 1 mL methanol and 1 mL DPPH solutions were combined to make a control. Finally, a spectrophotometer set to 517 nm was used to determine the absorbance of the prepared solution. The ascorbic acid was used as a reference material. A graph of concentration vs. percentage inhibition was used to determine 50% inhibitory concentrations of extracts (IC50 values). The percentage inhibition was used to quantify the radical scavenging potential of the DPPH. The IC50 values of the extracts and standards were calculated graphically and percentage of inhibition was calculated using equation (3) - [22].

$$I\ (\%) = \frac{A_C - A_0}{A_C} \times 100 \tag{3}$$

Where A_C represents the absorbance of the control solution (1 mL methanol + 1 mL DPPH), A_0 represents the absorbance of the sample solution, and I (percent) represents the percentage of inhibition. The data were given as mean standard deviation (n = 3).

2.10. Bacterial load assessment

For total bacterial counts, 1 mL of sample was transferred in a sterile test tube filled with 9 mL of normal saline solution (NSS). After being serially diluted up to $1{:}10^{-6}$, the samples were thoroughly mixed before being pour plated with 15–20 mL standard plate count agar solution. The plated sample may solidify before being incubated for 48 h at 37 °C. After a 48-h incubation period at 37 °C, the colonies were counted continuously for 7 days. The colonies on selected plates were counted with a colony counter, including those as small as a pinhead. Plates holding 30 to 300 colonies were discovered and reported. Plates with over 300 colonies were labeled TMTC (too many to count), whereas plates with fewer than 30 colonies were labeled TFTC (too few to count) (too few to count). The plate counts were expressed in colony forming units of suspension per milliliter (CFU/ml), and each sample's average CFU/ml was reported. Samples were rated as very good, good, or fair if the total bacterial count was less than 2×10^5 CFU/ml, between 2×10^5 and 1×10^6 CFU/ml, or between 1×10^6 and 5 × 106 CFU/ml, respectively. Samples with a bacterial count greater than 5 × 106 CFU/ml were rated as poor quality [23].

2.11. Peroxide value determination

The peroxide value was determined using the ferric thiocyanate method explained by the IDF (International Dairy Federation) [24]. 5 mL of high purity ethanol was used to prepare sample blank, 100 μL of high purity iso-hexane, 100 μL of 30% ammonium thiocyanate solution, and 100 μL Fe^{2+} solutions, blank samples were prepared. A stopwatch was used to ensure that the reaction time was exactly 3 min after addition of iron. At 500 nm, absorbance was measured in comparison to pure ethanol. Every working day, fresh Fe^{2+} solution was produced. The identical method used to create blank samples was used to create milk samples, except that 100 mL of sample was dissolved in iso-hexane instead of 100 mL of iso-hexane. Minimum 0.02 g of milk was weighed directly into a small test tube, and 1 mL of iso-hexane was added as a solvent to create the sample solution.

A standard curve was made based on 0.1 mg/mL Fe^{3+} standard work solution PV was calculated using equation (4):

$$\mathrm{PV}\ \left(\mathrm{mEq\ peroxide\ kg^{-1}}\right) = \frac{\left(\mathrm{A_{sample}} - \mathrm{A_{blank}}\right) \times \mathrm{L} \times \mathrm{V}}{(55.845 \times \mathrm{S} \times 0.1) \times 0.5} \tag{4}$$

Where L is the slope of the standard curve constructed as m Fe^{3+} = f (A), V is the volume of iso-hexane used to dissolve milk (mL), S is the amount of milk sample (g), 55.845 is the molar weight of iron (g/mol), 0.1 is the volume of the sample dissolved in iso-hexane added to the ethanol (mL), and 0.5 is the correction factor.

2.12. Shelf life prediction

The experimental data was used to forecast shelf life using an approach similar to that described by researchers [25]. Milk fat must contain 0.2 meq oxygen/kg fat, according to the International Dairy Federation. A two-step ordinary linear least squares fit was performed to fit the Arrhenius kinetic model. The rate constant k and the initial concentration PVo were calculated using regressions of the quality function (peroxide value) vs. time at each temperature.

$$PV - PV_0 = kt. \text{Zero order} \tag{5}$$

$$\ln PV = \ln PV_0 - kt \text{ first order} \quad (6)$$

Zero order and first order models were used to simulate the change in peroxide concentration in milk over time. To estimate k and PVo, the model equations (5) and (6) with the least root mean square error was chosen. The Pre-exponential factor A and Ea/R were then calculated using equation (7) as regressions of ln k vs. 1/T.

$$lnk = \ln A - \frac{E_a}{RT} \quad (7)$$

2.13. Sensory evaluation

2.13.1. Consent of participants

Our participation on sensory evaluation is completely voluntary. We had the opportunity to ask questions about this research and we have received satisfactory answers. We understand the general purposes, risks and methods of this research. We consent to participate in the research project.

A composite scoring test was used for sensory analysis, according to. Flavor, odor, and color were among the sensory attributes. Flavor accounted for a substantial share of the total score (out of 60 points), followed by odor (out of 30 points), and color (out of 30 points) on the scale (out of 10 point). The composite score for each panelist was obtained by summing their individual scores. Participants had to be at least 18 years old, drink milk at least once a week, and be eager to participate in sensory testing. The panelists were chosen depending on the research participants' availability.

2.14. Data analysis

Every study was done in triplicate, and the outcomes were given as Mean ± Standard Deviation. Graphs and regression coefficients were displayed using Origin 7, Graphad prism 7, and Excel 2010 as well as a one-way ANOVA with a significance threshold of p 0.05 and a Tukey post hoc test using IBM SPSS 20 software.

3. Result and discussions

3.1. Qualitative determination of the chemical constituency

Phytochemicals are plant-derived chemical substances that have a physiological influence on humans. Alkaloids, flavonoids, phenolic acids, tannins, and saponins are some of the most important bioactive phytochemicals. In the present study, preliminary phytochemical screening for *Mentha Spicata*, *Artemesia anua* and *Moringa stenopetale* leave revealed that the presence and absence of alkaloids, flavonoids, saponins and tannin (Table 1).

Ethanol extract of the plants showed that the presence of phytochemically active compounds such as saponins, alkaloids, phenols, tannins, flavonoids. In the extracts of *Artemesia anua* Alkaloids, Saponins, Tannin and Flavonoids were present; whereas Phenols was absent. In related research In accordance with the current study, Ajah and Eteng (2010), Kumar and Upadhya (2013), Owuna et al. (2013), and Enas et al. (2015) reviewed qualitative estimations of *Artemesia annua* with phytochemical studies and reported several compounds of confirmed biological activity from *Artemesia annua*, including tannins, alkaloids, flavonoids, and saponins. The existence of tannins, alkaloids, phenols, flavonoids, and saponins was demonstrated in *Mentha Spicata*. Naseem et al., 2011 and Zaidi et al., 2015 reported similar findings. Poudel, D. K. et al., 2022 reported that an extract of the *Moringa stenopetala* plant included saponins, alkaloids, phenols.

3.2. Quantitative determination of the chemical constituents

Plant secondary metabolites, or phyto-constituents, are a valuable and unique source of nutrients for dietary supplements and medications. Numerous studies have documented their varied roles [26]. Alkaloids, saponins, and tannins, according to Ref. [27] are important because they can be used as antimicrobials to treat a variety of diseases. The quantitative study of secondary metabolites in three crude plant extracts is shown in Table 2.

Alkaloids: An extensive range of physiological effects, including antibacterial, antimitotic, anti-inflammatory, analgesic, local

Table 1
Determination of the chemical constituents of plant extracts; Qualitative.

Chemical Constitutes	Plant Extract		
	Mentha Spicata	Moringa Stenopetale	Artemesia Anua
Alkaloids	+	+	+
Tannins	+	+	+
Flavonoids	+	+	+
Saponins	+	+	+
Phenols	+	+	–

anesthetic, hypnotic, psychotropic, and antitumor activity, are produced by the class of naturally occurring organic compounds known as alkaloids. Alkaloids were detected in 54%, 86%, and 64% of *Mentha spicata*, *Artemesia anua*, and *Moringa stenopetale* crude extracts, respectively. With a $p < 0.05$ significance difference, the results imply that *Artemesia anua* has a greater alkaloids value than *Mentha spicata* and *Moringa stenopetale*. **Saponins** have a lipid soluble aglycone and a water soluble sugar chain, making them amphiphilic. Saponins amphiphilic nature allows them to have a wide range of biological effects [28]. Saponins, on the other hand, have been recommended as a treatment for diabetes, obesity, and osteoporosis [29]. Saponin content was measured in *Mentha spicata*, *Artemesia anua*, and *Moringa stenopetale* and the value was found to be 18.2%, 37.8% and 30.6% respectively. **Flavonoids** are a group of plant metabolites with antioxidant and cell signaling properties that are thought to provide health benefits. These chemicals can be found in a variety of fruits and vegetables [30]. The flavonoids content of *Mentha spicata*, *Artemesia anua*, and *Moringa stenopetale* was (20.3 ± 1.0%, 58± 1.0%, and 61± 1.0%, respectively). With a significance of $p < 0.05$, the results demonstrated that *Moringa stenopetale* had a higher flavonoid value than *Artemesia anua* and *Mentha spicata*.

Total phenolic content activity is a method for assessing the amount of phenolic content in samples. Plant phenolic compounds have redox properties that allow them to serve as antioxidants [31]. Total phenolic content of *Mentha spicata*, *Artemesia anua*, and *Artemesia anua* form ethanolic extracts were expressed as mg GAE/gm in the table below. Values shown are in terms of mean ± standard deviation as shown in Table 2. The total phenolic content of *Mentha spicata*, *Artemesia anua*, and *Artemesia anua was* determined using Folin-Ciocalteu method. The total phenolic content of *Mentha spicata*, *Artemesia anua*, and *Artemesia anua* was 2.842 ± 0.2 mg GAE/gm, 1.619± 0.0 mg GAE/gm, and 0.285 mg GAE/gm, respectively, with a significance of $p < 0.05$. *Mentha Spicata* had the highest value, followed by *Moringa stenopetale* and *Artemesia anua*. The content of phenolic compound in the present study was compared with P [18]. who reported ethanolic extract has higher phenolic content compared to hot water extract. Generally, recovery of phenolic compound is believed to depend on type of solvent used, its polarity index and the solubility of phenolic compounds in the extraction solvent [18].

Tannins are water-soluble polyphenols present in a variety of plant diets. They have been associated with decreased feed intake, growth rate, feed efficiency, net metabolizable energy, and protein digestibility in experimental animals [32]. The Folin-Ciocalteu method was used to determine the total tannin content of *Mentha Spicata*, *Artemesia anua*, and *Moringa stenopetale* as shown in Table 2. Total tannin concentration of *Mentha Spicata*, *Artemesia anua*, and *Moringa stenopetale* was 46.2 ± 0.7 mg TAE/gm, 51.66± 1.5 mg TAE/gm, and 69.86± 1.3 mg TAE/gm, respectively. *Moringa stenopetale* had the highest significance of p 0.05, followed by *Mentha Spicata* and *Artemesia anua*.

3.3. Antioxidant properties

The DPPH free radical scavenging experiment was used to determine the antioxidant activity of the leaves, and their reducing power was determined using the concentrations that generated 50% inhibition (IC_{50}) values, as shown in Fig. 1. The mean percentage of DPPH free-radical scavenging activity at various extract concentrations is shown in Table 3 and Fig. 2.

In addition to phenolic compounds, additional compounds such as ascorbic acid, tocopherols, and others also contribute antioxidants. Several extracts showed an increase in concentration-dependent radical scavenging activity. Different extracts had varying levels of antioxidant activity, and ascorbic acid was utilized as a control. The IC_{50} of ascorbic acid was 14.86 ± 0.005 μg/mL. A higher IC_{50} value indicates lower radical scavenging activity or antioxidant capabilities [33]. The *Mentha spicata* extract showed the highest antioxidant capacity when compared to the *Artemesia anua* and *Moringa stenopetale* extracts. *Mentha spicata* has a high polyphenolic functional content, which contributes to its high oxygen-derived free radical scavenging action. Because of its antioxidant properties, which make it easier to use as food additives in a variety of edible materials, it reduces the rancidity and spoiling of fats and oils [5]. With a significance of $p < 0.05$, the IC_{50} values of *Artemesia anua* and *Moringa stenopetale* leaf extracts were 61.189 ± 0.058 μg/mL and 39.490 ± 0.029 μg/mL, respectively. *Mentha spicata* extract had the greatest IC_{50} value of 68.062 ± 0.014 μg/mL, indicating that it has the highest antioxidant activity. Improvements in polyphenols are primarily caused by the breakdown of the grain cell wall and subsequent enzyme activities that result in the liberation of bound phenolic compounds, which increase antioxidant activity, according to Ref. [34].

3.4. Total plate count

The composition and structural configuration of plant volatile oils, their functional groups, and potential synergistic interactions and reactions between components would all be relevant factors in relation to the antimicrobial activity of essential oils and plant

Table 2
Quantitative determination of the chemical constituency.

Phytochemicals	Types of Plants		
	Artemesia anua	*Moringa stenopetale*	Mentha *Spicata*
% of Alkaloid	86.00 ± 1.0	64.00 ± 1.0	54.00 ± 1.0
% of saponins	37.80 ± 1.0	30.60 ± 1.0	18.20 ± 1.0
% of Flavonoid	58.00 ± 1.0	61.00 ± 1.0	20.30 ± 1.0
Total Phenolic Content as mg GAE/gm)	0.28 ± 0.0	1.62 ± 0.0	2.84 ± 0.2
Total tannin content as mg GAE/gm)	51.66 ± 1.5	69.86 ± 1.3	46.20 ± 0.7

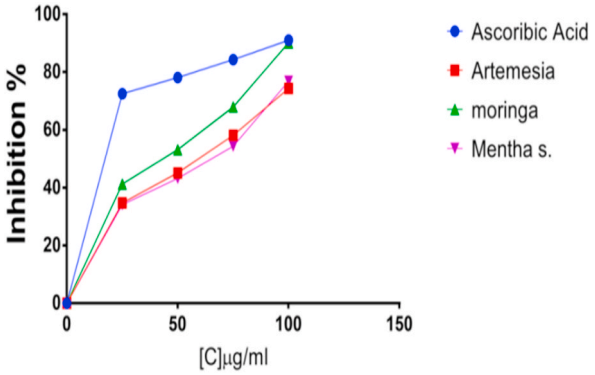

Fig. 1. Plot of radical scavenging percentage between ascorbic acid and plant samples.

Table 3
Percentage of inhibition of ascorbic acid and extract samples with their IC_{50} values.

Series of standard Concentration (µg/mL)	Percent inhibition			
	Ascorbic acid	*Artemesia anua*	*Moringa stenopetale*	*Mentha Spicata*
100	91.04 ± 0.01	74.31 ± 0.02	90.16 ± 0.04	76.85 ± 0.01
75	84.32 ± 0.05	58.12 ± 0.02	67.91 ± 0.04	54.33 ± 0.01
50	78.12 ± 0.15	45.135 ± 0.06	53.16 ± 0.03	43.21 ± 0.01
25	72.51 ± 0.01	34.72 ± 0.01	41.27 ± 0.03	34.11 ± 0.01
0	0	0	0	0
IC_{50}	14.86 ± 0.005	61.189 ± 0.058	39.490 ± 0.029	68.062 ± 0.014

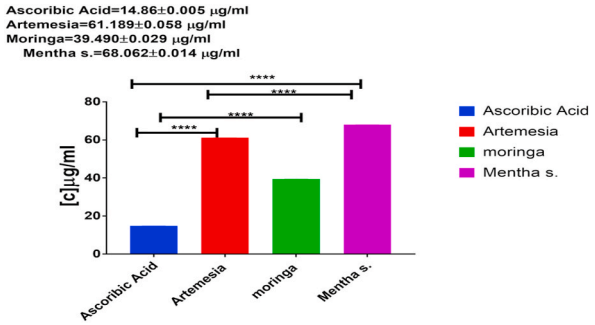

Fig. 2. IC_{50} (in µg/mL) value of Ascorbic Acid and plant leaf extracts.

extracts [35]. To assess changes in the number of organisms over time, tests can be undertaken. The Total plate counts results for milk with plant extract and milk without extract are summarized in Table 4.

equation (8) formula to calculate total bacterial count is as follows;

$$\text{CFU} \Big/ \text{ml} = \frac{(\text{No colonies*dilution factor})}{\text{volume of culture plated}} \tag{8}$$

The overall bacterial count was classified as very good if it did not exceed 2×10^5 CFU/ml, good if it was between 2×10^5 and 1×10^6 CFU/ml and fair if it was between 1×10^6 and 5×10^6 CFU/ml. A bacterial count of more than 5×10^6 CFU/ml was found in low-quality samples [36]. The results of the present study demonstrate that the mean TPC of the zero day and first day milk samples were both rated as excellent. Milk from each home holder with *Moringa stenopetale* extract performed significantly better than control milk and milk with other extracts. This means that *Moringa stenopetale* leaf extract is more effective against bacteria than *Artemesia anua* and *Mentha Spicata*. This is due to the fact that the IC_{50} value of *Moringa stenopetale* was 39.490 ± 0.029 g/mL, suggesting the strongest antioxidant characteristics, as well as a greater amount of total phenolic and flavonoid components, demonstrating their association with antioxidant activity according to previous reported literature [37]. Similar research was reported on Aegle marmelos leaf extracts that exhibit varied degrees of antibacterial activity against a variety of microorganisms [38].

The presence of tannins, flavonoids, and saponins in the ethanol extract could explain its potent antibacterial activity. Antibacterial capabilities abound in these medicinally useful components. Tannins prevent the development of cell walls by creating irreversible

Table 4
Summary of bacterial counts presented as CFU/ml from milk sample.

Milk Sample	TIME (Day)								
	0			1			3		
	Dilution Plated	No- of Colonies	Average (CFU/ml)	Dilution Plated	No- of Colonies	Average (CFU/ml)	Dilution Plated	No- of Colonies	Average (CFU/ml)
Z (control	10^{-4}	221	22.1×10^4	10^{-4}	231	23.1×10^4	10^{-4}	241	24.1×10^6
Za	10^{-5}	118	11.8×10^6	10^{-5}	128	12.8×10^6	10^{-5}	138	13.8×10^7
Zm	10^{-5}	10 (TFTC)	**0**	10^{-5}	11 (TFTC)	**0**	10^{-5}	12 (TFTC)	**0**
Zn	10^{-5}	33	3.3×10^6	10^{-5}	43	43×10^6	10^{-5}	53	53×10^6

Milk Sample	Time (day)					
	5			7		
	Dilution plated	No- of Colonies	Average (CFU/ml)	Dilution plated	No- of Colonies	Average (CFU/ml)
Z (control)	10^{-4}	251	25.1×10^6	10^{-4}	TMTC	$>5 \times 10^6$
Za	10^{-5}	148	14.8×10^6	10^{-5}	158	15.8×10^7
Zm	10^{-5}	135	$\mathbf{13.5 \times 10^6}$	10^{-5}	145	$\mathbf{14.5 \times 10^7}$
Zn	10^{-5}	63	63×10^6	10^{-5}	73	73×10^6

Zm = milk treated with Moringa **Za** = Milk treated with Artemesia **Zn** = milk treated with Mentha.

compounds with prolene-rich proteins [39]. Saponins have the capacity to cause cells to release proteins and enzymes [40]. Plants produce flavonoids in response to microbial infection, and they've been found to be potent antibacterial agents against a number of pathogens in vitro. They can form complexes with extracellular and soluble proteins, as well as bacterial cell walls [41].

3.5. Shelf life stability

The peroxide value and organoleptic parameters of each milk product were followed over time with the three distinct plant extracts added to the milk for the shelf stability experiment. The ASLT method was used, and temperature was the primary acceleration factor, as it had been in all previous ASLT approaches.

Peroxide value (PV) for Zebu cattle (Z) breed milk samples developed at a higher rate in control milk than in other treated milk samples with *Mentha spicata, Artemesia anua*, and *Moringa stenopetale* extracts at various temperatures. Throughout the storage period, the Peroxide Value (PV) of both the treated and control milk samples increased. The control sample (Z) had the highest PV of 0.079 (m_{eq} oxygen/kg fat) respectively at 27 °C at the end of the investigational period (7th day), compared to the samples treated with *Mentha spicata, Artemesia anua*, and *Moringa stenopetale* extracts, which had values 0.032, 0.027, and 0.022 (m_{eq} oxygen/kg fat) for treatments Zn, Za, and Zm, respectively. Furthermore, milk treated with *Moringa stenopetale* had the lowest Peroxide value than milk treated with *Mentha spicata* (68.062 ± 0.014 g/mL) or *Artemesia anua*, (61.189 ± 0.058 g/mL) which could be due to the presence of a lower IC_{50} value for *Moringa stenopetale* (39.490 ± 0.029 g/mL), which showed the highest antioxidant properties (Fig. 3). Additionally, there was a larger concentration of total phenolic and flavonoid compounds (Table 2), which prevented oxidation. These findings are consistent with those of [42], who found that *Moringa oleifera* oil had a lower peroxide value than palm oil, showing that the oil is resistant to oxidative damage. When the figures were compared, it was discovered that they were significantly different ($P < 0.05$).

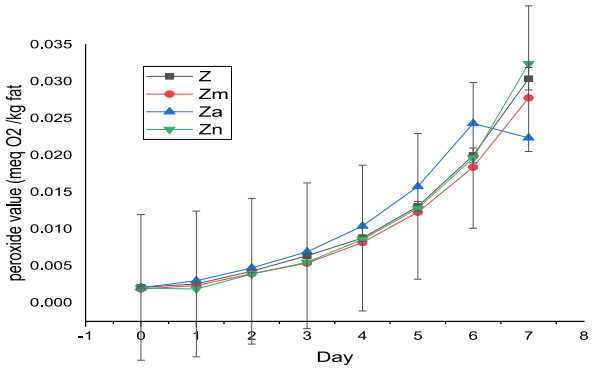

Fig. 3. Peroxide value (PV) for Zebu cattle (Z) milk samples for 1 week at 27 °c.

3.6. Sensory evaluation

Sensory analysis rates a food's flavor, color, and aroma. It is helpful for assessing the shelf life of foods since it may be used to monitor and document changes that become apparent over time. The food should be assessed given the storage and consumption conditions [43].

The results of organoleptic attributes demonstrated that adding *Mentha Spicata*, *Artemesia anua*, and *Moringa stenopetale* to the overall score had a substantial effect on flavor, odor, and color, as shown in Table 5. The milk sample that had been treated with *Moringa stenopetale* received the highest score when it was fresh or after several storage periods. Furthermore, the flavor, odor, and appearance of all milk samples treated with *Mentha Spicata*, *Artemesia anua*, and *Moringa stenopetale* were all satisfactory. A similar finding was reported by Badoms et al., 2014. According to the study, consumers preferred cheese samples preserved with ethanol extracts of Moringa leaves. Furthermore, the outcome of the sensory analysis matches the result of the peroxide value. Milk containing *Artemesia anua*, and *Mentha Spicata* extract received the lowest score. When it came to organoleptic features, *Moringa stenopetale* milk came out on top. Because of the presence of numerous antioxidant components such as ascorbic acid, flavonoids, and phenolic acids, Moringa oleifera leaves operate as a good source of natural antioxidants [44].

3.7. Shelf-life prediction

Peroxide concentration change with time for Zebu cattle breeds (Z) milk results was applied to zero order, first order and second order models. According to the fit, root mean square error was minimum for first order model at 27 °C. Thus, the model-representing rate of change of peroxide value will be calculated using equation (9):

$$lnPV = lnPV_0 - kt \tag{9}$$

Where k is the peroxide generation rate constant (meq × kg $^{-1}$*day^{-1}), t is the time (days), PV_O is the predicted beginning peroxide value (meq/kg), and PV is the peroxide value in meq/kg at time t.

Regressions of ln *k* vs. 1/T were conducted to obtain the Pre-exponential factor A and E_a/R using equation (10).

$$lnk = \ln A - \frac{E_a}{RT} \tag{10}$$

Where A represents the pre-exponential factor (meq × kg $^{-1}$*day^{-1}); Ea represents the activation energy (kJ/mol); and T represents the oven temperature (K). R is the gas constant (8.314 J/mol × K), and k is the predicted peroxide production rate constant. A straight line with a slope of -Ea/R and intercept ln kA was drawn by plotting lnk against the reciprocal absolute temperature (1/T). Assuming the product is stored at room temperature (27 °C) with a peroxide value of 0.2 meq/kg as the end-of-shelf-life indicator quality index. When the Peroxide value Ln (PV) reached the quality limit point the shelf life was calculated using the equation ($t = \frac{1}{k}(ln\,pvo - lnpv)$ in day was given in Table below.

From Table 6 it is shown that the Shelf life of Z Milk was found to be 1.532 day and after addition of *Artemesia anua*, *Mentha Spicata* and *Moringa stenopetale* it was determined to be 1.668 day, 3.28 day and 6.99 day respectively. From the Result it was found that addition of *Moringa stenopetale* become better shelf life when compared to *Artemesia anua* and *Mentha Spicata* which is because of the lowest IC_{50} value of *Moringa stenopetale* (39.490 ± 0.029 μg/mL). The highest antioxidant properties; higher content of total phenolic and flavonoids compounds, indicate their relationship with antioxidants as indicated in the previously reported literature [45]. This finding is consistent with [46]; who investigated the shelf life of milk using soybean oil and propolis extracts obtained through various extraction procedures. A similar finding was also reported by Yajuan Li et al., 2022 who investigated shelf life extension of raw and Pasteurized Milk with Plantaricin FB-2, Ramli A.N.M et al., 2020 who investigated Antibacterial and antioxidative activity of the essential oil and seed extracts of *Artocarpus heterophyllus* for effective shelf-life enhancement of stored meat.

4. Conclusion

Designing appropriate techniques for milk products to improve the shelf life of fresh milk is prudent. Medicinal plants that contain bioactive components are known to have antibacterial properties and are useful for preservation purposes. The current study's findings regarding the ethanolic extracts of *Artemesia anua*, *Mentha spicata*, and *Moringa stenopetala* suggested ability for obtaining bioactive substances that may act as pathogenic bacterial inhibitors and improve the shelf life of raw milk. Total phenolic and flavonoid content analysis in this study proved that sample extract from *Artemesia anua*, *Mentha Spicata* and *Moringa stenopetale* contains antioxidant compound from phenolic group and flavonoid group. The effectiveness of sample extract is determined by increasing in percentage of DPPH scavenging activity. The DPPH and reducing power assay can be used to provide additional information regarding changes occur in milk sample. According to the result obtained the higher levels of total phenolic, tannins, and flavonoids and Lower IC_{50} value of *Moringa stenopetale* extract (39.490 ± 0.029 g/mL) makes the extracts of *moringa stenopetale* good antioxidant and antibacterial activity. The sensory evaluation of the preserved milk samples was observed that all preservations were in good acceptance, but the milk samples preserved with *Moringa stenopetale* having a high acceptance value on a 3-point scale (color, odor, and flavor). Generally, *Moringa stenopetale* extract treatment extends the shelf life raw milk better than *Artemesia anua* and *Mentha Spicata* extracts.

Table 5
Sensory analysis results for accelerated milk shelf stability using three plant extracts.

Score of sample from oven one at 25°c					Score of samples from oven two at 30°c			
Milk sample (storage time)	Color	Odor	Flavor	Total	Color	Odor	Flavor	Total
Za (day 1)	5 ± 0.01	18 ± 0.01	49 ± 0.01	72 ± 0.01	5 ± 0.01	18 ± 0.01	10 ± 0.01	33 ± 0.01
Za (day 3)	5 ± 0.01	18 ± 0.01	10 ± 0.01	33 ± 0.01	5 ± 0.01	10 ± 0.01	10 ± 0.01	25 ± 0.01
Zm (day 1)	5 ± 0.01	10 ± 0.01	59 ± 0.01	**74 ± 0.01**	5 ± 0.01	18 ± 0.01	10 ± 0.01	**33 ± 0.01**
Zm (day3)	5 ± 0.01	18 ± 0.01	10 ± 0.01	**33 ± 0.01**	5 ± 0.01	10 ± 0.01	10 ± 0.01	**25 ± 0.01**
Zn (day 1)	5 ± 0.01	10 ± 0.01	49 ± 0.01	64 ± 0.01	5 ± 0.01	10 ± 0.01	10 ± 0.01	25 ± 0.01
Zn (day3)	5 ± 0.01	10 ± 0.01	10 ± 0.01	25 ± 0.01	5 ± 0.01	10 ± 0.01	8 ± 0.01	23 ± 0.01

Table 6
Shelf life of Zebu cattle breeds (Z) milk results at 27 °C.

S.No	Control milk		Milk treated with *Artemesia anua*	Milk treated with *Mentha Spicata*	Milk treated with *Moringa stenopetale*
1	Milk Sample	Z	Za	Zn	Zm
	Shelf life (day)	1.532 day	1.668 day	3.28 day	6.99 day

Ethical approval

Human/animal testing is unnecessary in this study. Human subject is not involved in this study. Patients are also not involved in this study. After reviewing the research protocol, Arba Minch University Institutional Research Ethics Review Committee (IRERC) evaluated and accepted the research work to be conducted.

Author contribution statement

Amsalu Matewos, Tolera Seda Badessa, Alemu Mekonnen: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Temesgen Dingamo: Analyzed and interpreted the data; Wrote the paper.

Data availability statement

Data included in article/supp. Material/referenced in article.

Conflict of interest

The Authors of this article have no any conflict of interest.

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