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In-vitro Antibacterial Activity of Common Spices against Isolated Lactic Acid Bacteria and Their Sensory Quality Role in Fermented Camel Milk

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ABSTRACT

The aim of this study was to evaluate the antibacterial activities of common spices on the growth of lactic acid bacteria (LAB) isolated from Ethiopian traditionally fermented camel milk and the consumer acceptability of adding isolated LAB to fermented camel milk. The experiment was conducted at Haramaya University between April and December 2021 with fifteen isolated LABs obtained from the Holeta Agricultural Biotechnology Research Center and eight spices commonly used in Ethiopia. The antibacterial activity of each extracted spice was assessed using the disc diffusion method. Spice extracts were evaluated on different test-isolated LABs at 12.5%, 25%, 50%, 75%, and 100% to check if they had antibacterial activity. The sensory evaluation of the fermented camel milk samples was done by seventeen experienced panelists. The results revealed that different extracted spices at varied concentrations influenced the growth of isolated LABs. Ground elder and fenugreek at 100%, 75%, and 50% concentration for *P. acidilactici* 226NB had the greatest inhibitory effect recorded. Ground elder, black pepper, garlic, turmeric, and rue inhibited the growth of some LABs at a concentration of 25%. Black cumin seeds, fenugreek, and ground elder at 12.5% concentration inhibited the growth of *E. faecium* 210NB, *P. acidilactici* 226NB, and *P. pentosaceus* 301A, respectively. The sensory evaluation showed isolated LABs received ratings of 'moderately liked' and 'slightly liked' colour, aroma, texture, taste and overall acceptability from panelists. All methanol extracted spices were positive, while glycosides were negative for *Allium Sativum*, according to phytochemical results.

Keywords: Camel milk, Inhibition zone, Isolates, Lactic acid bacteria, Spices.

INTRODUCTION

Camel milk is one of the most important dairy products for human nutrition in lowland areas of Ethiopia (Afar, Somali and Borena), with possible medicinal effects and as a natural supply of probiotics (Al-Otaibi et al., 2013). Lactic acid bacteria (LAB), which are potential sources of biological materials and can be employed in dairy technology, are the most valuable dominating microflora in camel milk (Khedid et al., 2009).Camel milk provides a very nutritive medium for the growth of a wide range of bacteria with key technical properties, health-promoting properties, and the ability to produce a wide range of antibacterials that could be employed as food preservatives (Quigley et al., 2013). Spices are added to dairy products in Ethiopia to improve product quality (i.e., flavor, taste, and shelf life) (Aysheshim et al., 2015).

Spices are a plant substance of indigenous or exotic origin that have been used as food condiments even in ancient societies recognized to enhance flavor, colour, aroma, and preservation of foods (Shan et al., 2005; Oli, 2011; Al-Wabel and Fat'hi, 2012), and for medicinal value in recent decades (Nabavi et al., 2015; Zheng et al., 2016; Maharjan et al., 2019). Various spices are utilized as additives to food processing around the world and in Ethiopia because they have antibacterial properties against pathogenic and spoilage fungi and bacteria. Rue (*Ruta chlepenis*), garlic (*Allium sativum*), mustard (*Brassica carinata*), rosemary (*Salvia rosmarinus*), basil (*Ocimum basilicum*), cumin (*Cuminum cyminum*), ajwain seed (*Trachyspermum ammi*), fenugreek (*Trigonella foenum-graecum*), coriander (*Coriandrum*

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sativum), korerima (*Aframomum korerima*), black seed (*Nigela sativa*), ginger (*Zingiber officinale*), Ethiopian cardamom (*Aframomum corrorima*), turmeric (*Curcuma domestica*) (Gebreselassie et al., 2012; Seifu, 2013; Oli, 2011; Gottardi et al., 2016; Irshad et al., 2017).

Spices have antibacterial and antibiotic effects to a variety of bacterial pathogens (Nanasombat et al., 2002; Oli, 2011; Panpatil et al., 2013) due to the presence of naturally derived components (Nanasombat et al., 2002; Oli, 2011; Panpatil et al., 2013; Maharjan et al., 2019). Although the antibacterial properties of spices on various food pathogens have been studied, the effects of spices added to fermented camel milk on the growth of lactic acid bacteria have not. As a result, research to understand which spices have the most impact on LAB survival rate/growth is critical. Customer approval of fermented camel milk is influenced by taste, colour, scent, texture, appearance, and general acceptability. In these situations, LAB plays an essential role in increasing organoleptic quality. As a result, the effect of adding LABs to fermented camel milk on organoleptic properties has yet to be investigated in Ethiopia. Therefore, the purpose of this study was to evaluate the in-vitro antibacterial activity of common spices of Ethiopian against the growth of isolated lactic acid bacteria and the consumer's acceptance of the addition of isolated lactic acid bacteria to fermented camel milk.

MATERIALS AND METHODS

Spices collection:

Between April 2021 and December 2021, the study was carried out in the dairy and meat technology laboratory at Haramaya University. Purposively, eight common Ethiopian spices (i.e., *Capsicum annum, Nigella sativa, Trigonella foenum-Graceum, Cuminum cyminum, Piper nigrum, Allium sativum, Curcuma longa and Ruta chalepensis*) were purchased from Bate and Haramaya town and transported to Haramaya University (Table 1).

Lactic acid bacteria used for test:

Tested LABs used in the study were obtained from Holeta Agricultural Biotechnology Research Center (HABRC). Purposively, fifteen isolated LABs(i.e. *L. delbrueckii* subsp. *bulgaricus* 61A, *L. brevis* 9S,*L. acidophilus* 42A, *L.casei* subsp. *casei*143NB, *L. salivarius* 216M, *L. helveticus* 107J, *L. plantarum* 99J, *P. acidilactici* 226NB, *S. cremoris* 273L, *L.lactis* subsp*lactis* 84J, *L. lactis* subsp. *cremoris*187NB, *E. faecalis* 85J, *E. faecium* 210NB, *P. damnosus* 21M, *P. pentosaceus* 301A) that was isolated from fermented camel milk (camel milk samples were collected from Somali Regional State, Afar Regional State, and Negelle Borena District of Oromia Regional State) were used to test antibacterial activities of spices.

Spices extract preparation for antibacterial activity:

The spices were cleaned, descaled as needed to eliminate any dirt or debris, washed in sterile distilled water, dried, and allowed to air dry until they were fully dry. Spices were crushed (ground) with a mill under aseptic conditions in order to obtain spice extracts. The extracts were sieved through a fine mesh cloth (0.45-micron sterile filter) and then sterilized with a membrane filter. Each spice powder (50g) was extracted individually in 250ml methanol and distilled autoclaved water for 24 hours. The filtered solvent was evaporated under reduced pressure using a rotary evaporator at 55°C to remove the solvent after 24 hours of shaking, and the dried extract was stored in the refrigerator (at 4°C) until analysis.

Determination of the antibacterial activity of extract of spices on isolated LAB:

The examination was performed as per the procedure outlined by Kapilan (2015) with slight modifications. The purpose of the study was to determine whether commonly used Ethiopian spices could inhibit or have an antibacterial impact on LAB isolates. The antibacterial activity of each extracted spice was evaluated using the disc diffusion method. To determine the diameter of each spice's inhibitory zone for isolated LABs, equipment, and Mueller Hinton agar (MHA) media were sterilized in an autoclave at 121°C for 30 minutes. The extract was considered as having a concentration of 100%. Spice extracts were tested at 12.5%, 25%, 50%, 75%, and 100% on various test isolated LABs to see if they had antibacterial

Table 1: Spices that were employed in the LAB evaluation

Common name of spices	Amharic name	Scientific name	Parts used for extracted
Paprika Pepper	Berbere	*Capsicum Annum*	Fruit
Black Cumin Seeds	Tikurazmud	*Nigella Sativa*	Seed
Fenugreek	Abish	*Trigonella Foenum-Graceum*	Seed
Ground Elder	Nechazmud	*Cuminum cyminum*	Seed
Black Pepper	Qundo Berbere	*Piper Nigrum*	Seed
Garlic	NechShinkurt	*Allium Sativum*	Bulb
Turmeric	Ird	*Curcuma Longa*	Leaves
Rue	Tena'adam	*RutaChalepensis*	Leaves

activity. The number of cells in 1ml of inoculation suspension evaluated by the 0.5McFarland Nephelometer turbidity standard was adjusted to 10^7-10^8cfu/ml. The inoculated LABs were cultivated for 24 hours at 30/37°C on sterile MRS/M17 agar. Methanol extracts of spices were applied to sterilized 6mm diameter paper discs in a volume of 20 µl of 12.5%, 25%, 50%, 75%, and 100% aseptically, and then after solidification placed on 10^7-10^8cfu/ml seeded/inoculated with LABs MHA surfaces. As a negative and positive control, a 20µl aliquot of dimethyl sulfoxide (DMSO) and Gentamycin were placed on sterilized paper discs, respectively, and left the plate for 15 minutes to allow excess pre-diffusion. The antibacterial assay plates were then incubated at 30/37°C for 24-48 hours, depending on the growth requirements of each isolate. The zones of inhibition in millimeters for each sample were measured and recorded to determine the antibacterial activity of spices (including the 6 mm disc).

Consumer acceptability test:

Consumer acceptability test was conducted by Resurrecin (1998) procedure with some modification. Fresh camel milk was gathered from the Babile Oromiya Regional State for the experiment, and fifteen isolated LABs were used. Prior to the experiment, optimization was carried out to decide the proper ratio of LAB to pasteurized camel milk. On this basis, 3% LAB and 97% pasteurized camel milk were chosen. Camel milk was collected and pasteurized for 30 minutes at 90°C before being cool down to 35-42 °C at room temperature. Then, in 97ml of pasteurized camel milk, 3ml of overnight growing LAB was added (0.5McFarland turbidity standard corrected). After 24h at room temperature, the labeled packed fermented camel milk (inoculated pasteurized camel milk) was randomly delivered to panelists for sensory evaluation. Each panelist was given a pen to record the results of her/ his impressions, as well as a bottle of water to sip in between samplings to keep her/his mouth clean. Taste, colour, aroma, texture, appearance, and overall acceptability of fermented camel milk samples were assessed on a seven-point hedonic scale (1 = strongly disliked; 2 = moderately disliked; 3 = slightly disliked; 4 = indifferent/ neither like nor dislike; 5 = slightly liked; 6 = moderately liked, and 7 = strongly liked).

Phytochemical extract:

Alkaloids test:

Extracted from 2g of each spice sample using 5% tetraoxosulphate (VI) acid ($H2SO_4$) (20 cm^3) in 50% ethanol by boiling for 2 minutes and filtering through Whatman filter paper number 42 (125 mm). In a separating funnel, 5 cm^3 of 28% ammonia solution (NH_3) was used to make the filtrate alkaline. Further solution extraction was carried out using an equal amount of chloroform (5.0 cm^3), which was extracted with two 5 cm^3 parts of 1.0 M dilute tetraoxosulphate (VI) acid. The following test was subsequently performed using the final acid extract. The presence of alkaloid was determined by mixing 0.5 cm^3 of Dragendorff's reagent (Bismuth potassium iodide solution) with 2 cm^3 of acid extract (Hikinoet al., 1984).

Flavonoids test:

Each sample (0.30 g) was weighed into a beaker and extracted for 2 hours with 30 cm^3 distilled water before being filtered using Whatman filter paper number 42 (125 mm). Five cm^3 of 1.0 M dilute ammonia solution was added to 10 cm^3 of each spices extract's aqueous filtrate, followed by 5 cm^3 of concentrated tetraoxosulphate (VI) acid. The presence of flavonoids is shown by the appearance of a yellow colouration that faded after standing (Sofowara, 1993).

Tannins test:

Each spice sample (0.30 g) was weighed into a test tube and cooked for 10 min in a water bath with 30 cm^3 of water to conduct the test. After boiling, Whatman filter paper number 42 (125 mm) was used for filtration. Three drops of 0.1% ferric chloride were applied to 5 cm^3 of the filtrate. A positive test was indicated by a brownish green or blue-black colouration (Ejikemeet al., 2014).

Saponin test:

Distilled water (30 cm^3) was added to wood powder samples (0.30 g) and boiled for 10 minutes in water bath and filtered using Whatman filter paper number 42 (125 mm). A mixture of distilled water (5 cm^3) and filtrate (10 cm^3) was agitated vigorously for a stable persistent froth. The formation of emulsion on addition of three drops of olive oil showed positive result (Ejikemeet al., 2014).

Steroid test:

A beaker containing 0.3g of each extracted spice was filled with 20cm^3 of methanol, and the mixture was let to stand for two hours. Following the addition of 2cm^3 of concentrated tetraoxosulphate acid, 5 cm^3 of the methanol recovered from each sample was treated with acetic anhydride (2cm^3). When the color shifted from violet to blue or green, it meant that steroids were present (Ejikeme et al., 2014).

Terpenoids test:

Each wood powder sample (0.30 g) was weighed into a beaker and extracted for 2 hrs with 30 cm^3 of water. To make a layer, 5 cm^3 of each extract was

mixed with 2 cm^3 chloroform and 3 cm^3 concentrated tetraoxosulphate (VI) acid. The presence of a reddish-brown colouration near the contact indicated that terpenoids were present (Ejikeme et al., 2014).

Glycosides test:

Fifty mg of extract was hydrolyzed for 2 hrs in a water bath with strong hydrochloric acid, filtered, and the hydrolysate was subjected to the following assays. Three ml chloroform was added to 2 ml filtered hydrolysate and agitated. The chloroform layer was removed, and 10% ammonia solution was added to it. The presence of glycosides was indicated by a pink colour (Evans, 1997).

Phenolic test:

The extract (50 mg) was diluted in 5 ml distilled water before being mixed with 2 ml of a 1% gelatin solution containing 10% NaCl. The presence of phenolic compounds was indicated by a white precipitate (Evans, 1997).

Statistical analysis:

All experiments were performed in triplicate and the data were recorded on Excel spread sheet. Data were presented as mean ± standard deviation (SD) calculated from the results of triplicate determinations by using descriptive statistics (inhibition zones were calculated as means ± SD) while one-way ANOVA was used to examine the sensory evaluation at a 95% level of significance. At $p \leq 0.05$, probability values were considered statistically significant.

$Yij = \mu + \beta i + eij$
Where:
Yij = individual observation for each test
μ = the overall mean
β = the $i^{th}$for different isolated LABs effect
eij = the error.

RESULTS

Antibacterial activity of spice extracts on isolated LAB:

The different concentration extracts of commonly used spices against isolates of LAB in diameter (milligram) of the growth inhibition zone indicated in (Table 2 & 3; Fig 1). In methanol extracted concentration of 100% black cumin seeds (Tikurazmud), fenugreek (Abish), ground elder (Nechazmud), turmeric (Ird), and rue (Tena'adam), the growth of LABs was shown to be inhibited. The highest diameter inhibition zone was 17±2mm in 100% concentration of ground elder (Nechazmud) for isolate *P. acidilactici* 226NB.All isolated LABs demonstrated an inhibitory zone in methanol extracted 75% concentrations of black cumin seeds (Tikurazmud), fenugreek (Abish), and ground elder (Nechazmud). The highest inhibition zone recorded was 14±2mm in 75% concentration of fenugreek (Abish) for isolated *P. acidilactici* 226NB.

A 50% concentration of black cumin seeds (Tikurazmud) inhibited the growth of all isolated LAB except *L. lactis*s ubsp *lactis* 84J; and except for *L. helveticus* 107J, and *E. faecium* 210NB, all isolated LABs displayed inhibitory zones in 50% concentration extracted fenugreek (Abish). Inhibition zones were detected for all isolated LAB in a 50% concentration of ground elder (Nechazmud). *P. pentosaceus* 301A was found in the clear zone in50% concentration of black pepper (Qundo Berbere). Extracted paprika pepper (Berbere) in a 25% concentration had an inhibition zone for *P. acidilactici* 226NB. In general, the growth of LABs was showed more inhibited by extracted black cumin seeds (Tikurazmud), fenugreek (Abish), ground elder (Nechazmud), andrue (Tena'adam) compared to other extracted spices in concentration 25%. *E. faecium* 210NB (13), *P. acidilactici* 226NB, and *P. pentosaceus* 301A demonstrated a clear zone in 12.5% methanol extracted black cumin seeds (Tikurazmud), fenugreek (Abish), and ground elder (Nechazmud). Negative controls (DMSO) did not show a zone of inhibition against any of the isolated LABs, although gentamicin (positive control) (20µl) showed an inhibition (8±3-38±1 mm).

Sensory evaluation:

The mean value of sensory evaluations on a seven-point hedonic scale is summarized in (Table 4). In general, the results revealed that sensory evaluation of pasteurized fermented camel milk using isolated LAB had significant differences ($p<0.05$). The average colour score for pasteurized fermented camel milk prepared with *L. acidophilus* 42A was highest. On the other hand, for the mean value for colour based on seven hedonic scale points, most of the isolated LAB for fermented pasteurized camel milk ranged from 5.5-6.2 (moderately liked) and there was no significant difference. The colour of fermented pasteurized camel milk *S. cremoris* 273L, *L. lactis* subsp. *cremoris* 187NB, and *E. faecium* 210NB was scored in the range of 4.8-5.4 (somewhat liked) by seventeen panelists, with no significant difference.

Regarding the mean score of panelists for aroma of pasteurized camel milk fermented by most of isolated LABs was between 5.5-5.9 (moderately liked) with no significant difference ($p > 0.05$). However, the mean of 17 panelists for aroma of pasteurized camel milk fermented by *P. acidilactici* 226NB, *E. faecium* 210NB and *P. damnosus* 21M was 4.6-5.2 (slightly liked). Also, the overall acceptability for texture of pasteurized camel milk fermented by most of isolated LABs

Table 2: Antibacterial activity for 50%, 75%, and 100% concentration of methanol extracted spices against fifteen isolated LABs

ES	Diameter of inhibition zone (in mm)														
LABs	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
100%															
T1	-	-	-	-	-	-	-	10±2	3±2	4±1	4±2	5±2	4±2	-	6±1
T2	4±3	7±2	7±1	6±3	9±1	5±3	5±1	3±1	3±1	11±2	2±1	11±1	13±3	13±1	13±2
T3	5±1	3±1	2±.3	3±1	4±1	3±1	4±1	15±1	5±2	11±1	6±1	5±4	4±1	3±2	12±1
T4	3±1	5 ±4	9±1	6±2	4±1	6±3	7±2	17±2	6±1	7±2	5±2	7±3	9±2	10±3	15±2
T5	-	-	-	-	-	-	-	-	-	-	-	-	3±1	4±2	7±2
T6	-	-	-	-	-	-	-	-	-	-	-	-	6±1	1±1	5±1
T7	2±1	3±1	3±1	1±1	1±2	1±2	1.±1	1±1	2±2	3±1	2±1	1±1	3±1	4±1	3±0
T8	4±1	5±2	2±1	10±2	9±1	10±2	5±4	8±3	4±1	2±1	8±3	2±1	6±1	7±3	5±2
T9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T10	32±1	17±6	36	38±1	35±3	26±4	28±2	30±2	27±3	14±2	19±3	18±2	24±2	17±5	9±2
75%															
T1	-	-	-	-	-	-	-	6±1	2±1	5±1	4±1	5±1	1±1	-	3±2
T2	2±0	4±3	3±1	2±1	4±2	1±1	2±1	2±1	3±0	8±1	2±1	8±1	11±1	10±3	11±1
T3	4±1	3±2	1±1	2±2	3±1	3±1	3±1	14±2	3±1	5±2	3±1	2±0	3±2	2±1	8±2
T4	2±1	4±2	7±2	5±1	3±0	6±2	6±2	12±3	4±2	6±1	5±2	5±1	7±3	7±2	11±2
T5	-	-	-	-	-	-	-	-	-	-	-	-	1±0	2±1	5±1
T6	-	-	-	-	-	-	-	-	-	-	-	-	3±1	-	3±0
T7	1±1	0.3± 1	-	-	1±1	1±1	-	-	-	1±1	3±1	-	2±1	2±2	2±2
T8	3±1	4±2	1±1	7±1	6±2	6±2	2±1	2±1	2±1	-	-	-	4±1	3±1	2±1
T9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T10	24±3	15±8	34±3	20±3	36±4	33±2	27±3	13±1	30±2	18±2	21±12	13±10	20±3	9±3	12±2
50%															
T1	-	-	-	-	-	-	-	4±1	-	2±1	2±1	3±1	-	-	1±1
T2	1±0	2±1	2±0	1±1	3±1	1±1	1±1	1±1	1±0	-	1±0	4±1	7±1	8±1	5±1
T3	2±1	5±1	1±0	1±0	2±1	-	1±1	9±2	1±1	4±1	1±1	1±1	-	1±1	4±1
T4	1±1	3±0	3±1	4±2	2±0	3±1	4±2	7±1	2±1	2±1	3±0	2±1	3±0	5±2	8±1
T5	-	-		-	-	-	-		-	-	-	-	-	-	3±0
T6	-	-		-	-	-	-	-	-	-	-	-	1±0	-	2±1
T7	-	-	-	4±2	-	-	-	-	-	-	2±1	-	-	2±1	1±1
T8	1±0	3±1	1±0	1±0	4±2	3±0	1±0	1±1	-	-	-	-	2±1	2±1	1±1
T9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T10	21±5	27±6	21±1	10±2	36±3	33±4	34±2	19±3	30±6	10±1	21±3	25±3	11±1	23±4	24±5

were under the score of 5.5-6.3 (moderately like); and pasteurized fermented camel milk by *S. cremoris* 273L, *L. lactis* subsp. *cremoris* 187NB, and *E. faecium* 210NB were 4.5-5.2 (slightly liked). Except for *L. acidophilus* 42A, *E. faecalis* 85J, and *E. faecium* 210NB isolates, all other samples were classified as moderately liked (5.0-5.3). In general, pasteurized fermented camel milk by *P. pentosaceus* 301A, followed by that fermented by *L. delbrueckii* subsp. *bulgaricus* 61A, *L. acidophilus* 42A, *L. casei* subsp. *casei* 143NB, *L. plantarum* 99J, and *E. faecalis* 85J, with *E. faecium* 210NB being the least overall acceptable.

Phytochemical analysis of common spices:

The phytochemical analysis results of extracted spices are presented in (Table 5). Phenols, tannins, saponins, alkaloids, flavonoids, steroids, and terpenoids were found in methanol extracted spices, according to phytochemical investigations. Except for Nech Shinkurt (garlic), methanol extracted spices revealed the presence of glycosides (reducing sugar).

DISCUSSION

The variation in antibacterial activities of spice extracts on LABs in present result may be attributed to the different environmental growth conditions of spices, climate, soil composition, age, and vegetation cycle stage on the quality, quantity, and composition of extracted product (Ozcan & Erkmen, 2001; Del Nobile et al., 2009). In addition, different studies have found that the type of solvent has an important role in the process of extracting and the difference between their effects may be due the quantity of the phenolic compounds (Akrayi, 2014). The current result of inhibition zone also varies due plant extracts contain large amounts of polyphenolic compounds, which are well known as antioxidants but may also

Table 3: Antibacterial activity for 12.5% and 25% concentration of methanol extracted spices against fifteen isolated LABs

ES	Diameter of inhibition zone (in mm)														
LABs	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
25%															
T1															
T2	-	-	-	-	-	-	-	1±1	-	-	-	-	-	-	-
T3	-	1±1	1±1	-	2±1	-	-	-	-	-	-	1±1	3±1	2±1	2±1
T4	1±0	2±0	-	-	1±1	-	-	3±2	-	1±1	-	-	-	-	2±1
T5	-	2±0	1±1	2±1	1±1	1±1	2±1	2±1	-	-	2±0	-	1±0	2±1	3±2
T6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1±1
T7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1±0
T8	-	-	-	1±0	-	-	-	-	-	-	-	-	-	1±1	-
T9	-	1±1	-	-	2±0	1±0	-	-	-	-		-	1±1	1±1	-
T10	-	-	-	-	-	-	-		-	-	-	-	-	-	-
12.5%															
T1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T2	-	-	-	-	-	-	-	-	-	-	-	-	1±1	-	-
T3	-	-	-	-	-	-	-	1±0	-	-	-	-	-	-	-
T4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1±1
T5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T10	20±4	29±9	21±2	8±3	34±2	30±5	34±4	14±3	11±4	13±8	15±8	18±8	11±3	23±2	22±4

T_1= Paprika Pepper (Berbere), T2= Black Cumin Seeds (Tikurazmud), T_3=Fenugreek (Abish), T_4= Ground Elder (Nechazmud), T_5= Black Pepper (Qundo Berbere), T6= Garlic (NechShinkurt), T_7 = Turmeric (Ird), T_8= Rue (Tena'adam), T_9 =DMSO, T_{10}=Gentamicin,1=*L. delbrueckii* subsp. *bulgaricus* 61A, 2=*L. brevis* 9S, 3=*L. acidophilus* 42A, 4= *L.casei* subsp. *casei*143NB, 5= *L. salivarius* 216M, 6= *L. helveticus* 107J, 7= *L. plantarum* 99J, 8= *P.acidilactici* 226NB, 9= *S. cremoris* 273L, 10= *L. lactis*subsp*lactis* 84J, 11= *L. lactis* subsp. *cremoris*187NB, 12= *E. faecalis* 85J, 13= *E. faecium* 210NB, 14= *P. damnosus* 21M, 15= *P. pentosaceus* 301A, ES= extracted spices, LABs= lactic acid bacteria.

Table 4: Mean score of sensory evaluation of pasteurized camel milk fermented by isolates LAB based on seven-point hedonic scales for 24hrs

Isolates	Colour	Aroma	Texture	Taste	Overall acceptability
*L. delbrueckii*subsp. *bulgaricus* 61A	5.9^{a}	5.8^{ba}	5.8^{ba}	5.9^{a}	6.0^{a}
L. brevis 9S	5.5^{ba}	5.8^{ba}	5.8^{ba}	5.6^{ba}	5.6^{b}
L. acidophilus 42A	6.2^{a}	5.6^{bac}	6.0^{ba}	5.3^{ab}	5.9^{ba}
L.casei subsp. *casei* 143NB	6.0^{a}	5.7^{bac}	6.0^{ba}	6.0^{a}	5.9^{ba}
*L. salivarius*216M	6.0^{a}	5.9^{a}	5.9^{ba}	5.6^{ba}	5.8^{ba}
L. helveticus 107J	5.9^{a}	5.6^{bac}	5.8^{ba}	5.5^{ba}	5.8^{ba}
L. plantarum 99J	6.0^{a}	5.8^{bac}	5.8^{ba}	5.6^{ba}	5.9^{ba}
P.acidilactici 226NB	5.5^{ba}	5.2^{bdc}	5.7^{ba}	5.6^{ba}	5.7^{b}
S. cremoris 273L	5.4^{ba}	5.6^{bac}	5.2^{bcd}	5.5^{ba}	5.6^{b}
*L.lactis*subsp*lactis* 84J	5.8^{a}	5.7^{bac}	5.5^{bc}	5.6^{ba}	5.8^{ba}
L. lactis subsp. *cremoris* 187NB	4.8^{b}	5.5^{bac}	4.6^{cd}	5.5^{ba}	5.6^{b}
E. faecalis 85J	5.6^{ba}	5.8^{bac}	5.6^{ba}	5.3^{ba}	5.9^{ba}
E. faecium 210NB	4.9^{b}	4.6^{d}	4.5^{d}	5.0^{b}	4.9^{c}
P.damnosus 21M	5.5^{ba}	5.1^{dc}	5.5^{ba}	6.0^{a}	5.8^{ba}
P.pentosaceus 301A	5.8^{a}	5.8^{bac}	6.3^{a}	5.7^{ba}	6.3^{a}

Row with different superscripts is significantly different at P<0.05

be described as natural antibacterial agents (Efenberger-Szmechtyk et al., 2020). According to research on spice extracts and antibiotics, the inhibitory effects against bacteria depend on the plant material's solvent, the type and concentration of spice extracts, and the type of microorganisms that were tested (Burt, 2004). The spices used in the study had a polyphenol that inhibited the growth of microorganisms, especially bacteria; and the sensitivity of microorganisms to polyphenols

Table 5: Phytochemical analysis of selected spices extracted

Qualitative Phytochemicals	Extracted spices							
	T1	T2	T3	T4	T5	T6	T7	T8
Phenols	+	+	+	+	+	+	+	+
Tannins	+	+	+	+	+	+		+
Saponins	+	+	+	+	+	+		+
Alkaloids	+	+	+	+	+	+		+
Flavonoids	+	+	+	+	+	+		+
Steroids	+	+	+	+	+	+		+
Terpenoids	+	+	+	+	+	+		+
Glycosides (reducing sugar)	+	+	+	+	+	-		+

T_1= Berbere, T_2= Tikurazmud, T_3= Abish, T_4= Nechazmud, T_5= Qundo berbere, T_6= NechShinkurt, T_7= Ird and T_8= Tena'adam.

Fig. 1: inhibition zone of spices against LAB

depends on the species and strain, as well as on the molecular structure of the phenolic compounds (Efenberger-Szmechtyk et al., 2020). Also, various spices and herbal essential oils can influence the growth and activity of LAB (Kozłowska et al., 2015). In the current result, where low concentration of extracted showed an inhibition zone, it might be that some spice extracts well known to contain phenolic components show the strongest antimicrobial activity (Farag et al., 1989); and spices containing antibacterial compounds are among the most used natural antimicrobial agents in foods (Indu et al., 2006).

In comparison to Saeed et al. (2013), the inhibition zone recorded in this study was lower, but this author recorded a higher inhibition zone for extracts of black pepper, aniseed, coriander, and herb that exhibit antibacterial action against all tested organisms (16mm to 22mm).According to Sah et al. (2020), a 100% concentration of garlic extracts affect the growth of bacteria and antibacterial activity appeared to be dependent on the type of bacteria, and Gram-positive bacteria were more sensitive to spice extracted. Even though some isolated LAB had inhibition zone for 100%, 75% and 50% of extracted paprika pepper (Berbere) (T_1),the present study showed the opposite of the results of Agaoglu et al. (2007), who reported that crushed red pepper and anise had no antibacterial effect against the strains tested. In addition, Chaudhry, and Tariq (2006) found that the aqueous decoction of black pepper showed higher antibacterial activity (75%) compared to aqueous decoction of herb (53.4%) and aniseed (18.1%) while aqueous decoction of coriander didn't show any antibacterial effects.

The present result in line with report by Altuntas & Korukluoglu (2019) that all strains except *L. acidophilus*742 were inhibited at varying levels by 10µl ofgarlic extract. Similar results were obtained with Booyens & Thantsha (2013), who reported that *L. acidophilus* did not show a region of inhibition against 30µl of garlic extract. However, Tas (2008) reported that the inhibitory zone diameter of *L. acidophilus* LAB 108 was 27 mm when 100µl of garlic extract was used. The investigation by Akabanda et al. (2014) on consumer sensory evaluation revealed that for Nunu fermented milk with various starter cultures had diverse degrees of acceptance in terms of sensory qualities such as flavor, odor, colour, texture, and overall acceptability. In addition, Nunu made with a single *L. helveticus* starter culture or a combination of *L. fermentum* and *L. helveticus* or *L. fermentum* and *L. plantarum* starter cultures had much higher overall acceptance (Akabanda et al., 2014). The current finding agreed with Farahani et al. (2017) report that stated that the highest overall score for sensory evaluation was showed by *L. helveticus*, *L.*

paracasei subsp. *paracasei*, *L. delbrueckii* subsp. *lactis* and *E.faecium*. Ayad et al. (2001) summarized those different strains had influenced the sensory qualities. However, some scholars in the previous research considered diameters of inhibition less than five or six as having favorable antibacterial properties or having no effect (Kasimala et al., 2014; Rahman et al., 2021).

Spices have a unique aroma and flavor due to the presence of different compounds known as phytochemicals or secondary metabolites (Avato et al., 2000; Panpatil et al., 2013). According to Shahidi and Ambigaipalan (2015), black pepper contains a wide range of phytochemicals. The result obtained was in line with Mehmood et al. (2009) who reported that the mean colour score for yoghurt *L. bulgaricus*, *L. lactis*, *S. thermophilus*, and *L. casei* when used gave a good quality product and satisfactory colour. Many classes of phytochemicals including isoflavonoids, anthocyanins, and flavonoids, are associated with spices (Shan et al., 2007). Spices have a unique aroma and flavor that derives from compounds called phytochemicals or secondary metabolites (Avato et al., 2000).

In conclusion, the findings demonstrated that different methanol extracted spices had distinct antibacterial activity and inhibitory zones at different concentrations. Ground elder (Nechazmud) for *P. acidilactici* 226NB had the highest inhibition zone (17±2mm) for 100% concentration. *P. acidilactici* 226NB had the largest inhibition zone for fenugreek (Abish) at 75% and 50% concentration. LAB growth was more inhibited by extracted black cumin seeds (Tikurazmud), fenugreek (Abish), ground elder (Nechazmud), and rue (Tena'adam) compared to other extracted spices in25% concentration. In 12.5% concentration of black cumin seeds (Tikurazmud), fenugreek (Abish), and ground elder (Nechazmud) on *E. faecium 210NB, P. acidilactici 226NB, and P. pentosaceus* 301A showed inhibited growth, respectively. In general, black cumin seeds (Tikurazmud), fenugreek (Abish), and ground elder (Nechazmud) had more antibacterial activity compared to the remaining spices. *P. pentosaceus* 301A isolated LAB adding to pasteurized camel milk preferred moderately like scored for colour, aroma, taste, texture and overall acceptability by selected panelists. Phytochemical investigations identified phenols, tannins, saponins, alkaloids, flavonoids, steroids, and terpenoids in methanol extracted spices.

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COMPETING INTERESTS

The authors have declared that they have no competing interest.

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