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The antibacterial activity of *Laurus nobilis* leaf extract and its potential use as a preservative for fresh lamb meat

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Lamb meats are nutritious, easily metabolisable, and offer suitable substrates for the growth and metabolism of microorganisms. The main purpose of the present study was to investigate the antagonist effect of *Laurus nobilis* leaf extract against growth of bacteria present in fresh lamb meat. The 16S rRNA gene sequence analysis was used to identify bacteria present in lamb meat samples. Eighteen bacterial species were detected in 20 samples of fresh lamb meat. The antibacterial activity of *L. nobilis* leaf extract against growth of the isolated bacteria was examined using agar well diffusion method. Gram-positive and Gram-negative showed differences in their response to *L. nobilis* leaf extract. *Staphylococcus saprophyticus* and *Proteus vulgaris* were the most bacteria affected by *L. nobilis* leaf extract. Spraying fresh lamb meat with 10% (v/v) *L. nobilis* leaf extract was found to increase the shelf-life of lamb meat kept at room and refrigeration temperatures from 1 to 3 and 6 to 13 days, respectively. *L. nobilis* essential oil seems to be a promising tool that can be used as a natural preservative for fresh lamb meat.

Key words: Fresh lamb meat, *Laurus nobilis*, 16S rRNA gene, antibacterial activity.

INTRODUCTION

Lamb meats, by their nature, are nutritious and easily metabolisable and therefore offer suitable substrates for the growth and metabolism of microorganisms (Thanigaivel and Anandhan, 2015). The microorganisms that eventually cause the spoilage of fresh meat are either present at the time of slaughter or introduced by workmen and their cutting tools, or by water and air in the dressing, cooling and cutting rooms (Newman, 2005).

Lamb meat has a short shelf-life of about one day or less at ambient temperature (15-30°C), and few days at refrigerating temperature (0-10°C) (Lucera et al., 2012). This is mainly due to the microbial growth of both pathogenic and non-pathogenic microorganisms, and/or lipid oxidation (Lucera et al., 2012). *Staphylococci*, *Corynebacterium*, *Streptococci*, *Micrococcus*, *Salmonella*, *Escherichia coli*, and yeast have been isolated from fresh

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lamb meat (Mostafa et al., 2018). In another study, it was reported that the most common bacteria found in fresh meat were bacteria of the genera *Acinetobacter* species, *Pseudomonas* species, *Brochothrix*, *Flavobacterium*, *Psychrobacter*, *Moraxella*, *Staphylococcus* species, and *Micrococcus*, lactic acid bacteria and various genera of the Enterobacteriaceae family (Pennacchia et al., 2011).

An assortment of synthetic antimicrobials has been used to decrease bacterial contamination in meat and fresh products (Rafiq et al., 2016). However, synthetic antimicrobials like butylated hydroxyanisole, and carmine have been connected with health problems such as hypersensitivity, allergies, asthma, hyperactivity, and cancer (Anand and Sati, 2013). Furthermore, synthetic antimicrobials can destroy both harmful and beneficial bacteria in the human intestine, and increase bacterial resistance to antimicrobials (Verraes et al., 2013). Natural preservation is considered as a safety parameter in foods with reduced contents of ingredients and additives that usually render to slow down microbial growth (Gharsallaoui et al., 2016).

Laurus nobilis, commonly known as Bay, is a plant belonging to the Lauraceae family (Ghadiri et al., 2014), which comprises about 2500 species (Basak and Candan, 2013). *L. nobilis* is a native of the southern parts of Europe and the Mediterranean area (Caputo et al., 2017). *L. nobilis* is an aromatic herb used broadly to add a distinctive aroma and flavor to food (Fernández et al., 2018). *Laurus* leaf has been used traditionally as herbal medicine to treat rheumatism, ear aches, indigestion, sprains, promote perspiration, and treat a variety of complaints like neuralgia and intestinal cramps (El Malti and Amarouch, 2009). Eucalyptol (1,8-cineole), sabinene, and linalool are the main components of the essential oil isolated from the leaves of *L. nobilis* (Caputo et al., 2017). All of these components are classified as monoterpenes or modified monoterpenes, which were shown to have antibacterial, antifungal, and antioxidant activities (Basak and Candan, 2013). The antimicrobial activity of the *L. nobilis* essential oils has taken great importance as an alternative for synthetic antimicrobials because they are a part of the human diet and their biodegradability suggests low poisonous residue problems. Some studies have proved the potential capacity of laurel essential oil as an antimicrobial agent and also the antioxidant property of leaves extracts (Ouibrahim et al., 2013; Ghadiri et al., 2014; Nehir El et al., 2014; Rafiq et al., 2016). *L. nobilis* was shown to have a strong antagonist activity against 20 strains of bacteria like *Salmonella*, *Staphylococcus aureus*, *Escherichia coli*, *Listeria monocytogenes* (Ouibrahim et al., 2013). Growth of *S. aureus* was also found to be inhibited by a hydroalcoholic extract of *L. nobilis* (Ghadiri et al., 2014). Furthermore, *L. nobilis* leaf extract was able to inhibit the growth of *Salmonella typhimurium* and *E. coli* O157:H7 (Rafiq et al., 2016).

The preservative effect of modified atmosphere and

vacuum packaging can be increased by *L. nobilis* essential oils in chicken meat (Esmer et al., 2011). An aqueous extract of *L. nobilis* leaf and chitosan was used as a natural edible coating to increase the shelf life of cashew (Azimzadeh and Jahadi, 2018). To our knowledge, no studies have been carried out about the antagonist effect of *L. nobilis* against bacteria present in fresh lamb meat and the possibility of using *L. nobilis* leaf extract as a natural preservative to extend the shelf life of fresh lamb meat.

The main objective of the present study was to isolate and identify bacteria from fresh lamb meat and to investigate the antibacterial activity of *L. nobilis* leaf extract against those bacteria. Furthermore, the ability of *L. nobilis* leaf extract to extend the shelf life of fresh lamb meat was evaluated.

MATERIALS AND METHODS

Fresh lamb meat samples

Twenty samples of fresh lamb meat of 200 g each were collected within 4 h of slaughtering from four different butcher shops located in Tulkarem city, Palestine. Meat samples were placed in clean, dry, and sterile glass bottles, and were transferred in a refrigerator bag to the laboratory. Microbiological analysis of meat samples was carried out within 1 h.

Isolation of bacteria

Meat samples were aseptically cut into thin smaller pieces of 10 g each and were submerged in sterile tubes that contain 90 ml of diluent saline peptone (SPO) [0.1% bacto-peptone (Difco, Detroit, MI, USA), 0.85% (w/v) NaCl (Merck, Darmstadt, Germany), 0.03% Na₂H₂PO₄ · 2H₂O (Merck) and were vortexed for 3 min. Ten-fold dilutions were prepared from each tube and spread onto three types of culture media, which included blood agar (HiMedia M1133-500G Columbia Blood Agar Base with Hemin), MacConkey agar (HiMedia M081B-100G MacConkey Agar Medium H), and chocolate agar (HiMedia M1133-500G Columbia Blood Agar Base with Hemin). Plates were incubated at 37°C for 24 h under aerobic and anaerobic conditions using candle jars (Karoki et al., 2018). The number of colony forming units (CFU) was recorded.

From a suitable dilution of each sample, a representative colony with the same characters of size, morphology, and colour was picked and recultivated on the same culture medium from which it was isolated and incubated at 37°C for 24 h under aerobic and anaerobic conditions using candle jars. Characterization of the isolates was achieved by initial morphological examination of the colonies on the plates (macroscopically) for colony appearance, size, elevation, form, edge, consistency, color, odor, opacity, hemolysis and pigmentation (Nagarajan et al., 2018), and the results were recorded. A colony from each group of colonies that has the same properties was recultivated.

Extraction of deoxyribonucleic acid (DNA)

Heat treatment method was used to extract DNA from bacteria; 2 colonies of each bacterial isolate which were grown overnight at 37°C were placed in an Eppendorf tube filled with 1 ml of ultrapure DNase/RNase-free distilled water and boiled for 10 min in a water bath. The tubes were then centrifuged for 5 min at 1000 ± g (Dashti et al., 2014). The supernatants were used as templates for

the PCR reactions.

Polymerase chain reaction (PCR)

Polymerase Chain Reaction-Ready™ (PCR-Ready™) High Specificity kit (Syntezza Company, PCR-S-192, Israel) was used. Forward primer U968-GC (5'-CGC CCG GGG CGC GCC CCG GGC GGG GCG GGG GCA CGG GGG GAA CGA GAA GAA CCT TAC-3) and reverse primer L1401 (5'-GCG TGT GTA CAA GAC CC-3') were used in the PCR reaction mixture. According to the manufacturer, for each reaction mixture, 11 µl of 0.5 µM of each primer and 3 µl of DNA were added to a PCR-Ready™ master mix tube. The reactions were performed in an automatic thermal cycler (Applied Biosystems™ Veriti™ 96-Well machine) with initial denaturation at 94°C for 3 min; 30 cycles at 94°C for 30 s, 56°C for 30 s and 68°C for 60 s; final extension at 68°C for 10 min. The PCR products were analyzed by electrophoresis in 2% (w/v) Nusieve agarose (FMC BioProducts, Rockland, ME, USA) gel in 1 × TBE [89 mM Tris-base (Sigma), 89 mM boric acid (Sigma), 2 mM EDTA (Sigma)] at 80 V for 2 h. DNA molecular marker (1Kb DNA Ladder RTU, Cat. DM010-R500, Gene DireX) was used as a marker. The amplified fragments were visualized by ethidium bromide staining and UV transillumination.

Sequence analysis of the 16S rRNA gene

The PCR products were purified using QIA quick PCR Purification Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. The PCR purified products were sent to the Molecular Genetics Laboratory in Al-Istishari Arab Hospital in Ramallah, Palestine for sequencing. The obtained sequences were aligned to the 16S rRNA gene sequences in the National Center for Biotechnology Information (NCBI) Genbank database using the BLAST algorithm (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). Accession numbers were obtained for all sequences.

Leaves of *L. nobilis*

Dry leaves of *L. nobilis* leaves were bought from the local market of Tulkarem city in Palestine. A powder was obtained by grinding leaves using a blender, where after the powder was stored in a sterile sealed bottle at room temperature (Caputo et al., 2017).

L. nobilis leaf extracts

Extracts were prepared according to Mostafa et al. (2018) with some modifications, where 100 g of fine powder of *L. nobilis* were mixed with 500 ml of 99.9% (v/v) methanol. The mixture was kept for 5 days in closed sealed vessels at room temperature, protected from light, and was shaken daily 8 to 10 times. Whereafter, the mixture was filtered by a double layer of filter papers to remove the solid extraction residues. The residues were squeezed to increase the volume of the liquid extract, centrifuged at 5000 × g for 15 min, and finally filtered again through filter paper. The liquid extract was transferred to a vacuum rotary evaporator machine (Peaken Motor Company, China), where the solvent extract was evaporated at 45°C and the extract was concentrated under reduced pressure. The essential oil was sterilized by filtration using 0.45 µm Millipore filter papers. The obtained essential oil was weighted and preserved at 4°C in a tightly sealed bottle for further use.

The antibacterial activity of *L. nobilis* leaf extract

The antibacterial activity of *L. nobilis* leaf extract was investigated

against bacteria isolated and identified from fresh lamb meat samples as described earlier. Each bacterium was cultured on Mueller-Hilton agar media and incubated at 37°C overnight. The bacterial colonies were harvested using 5 ml of sterile saline 0.85% (w/v) NaCl. The optical densities of all cultures were adjusted to match a 0.5 McFarland standard of 1×10⁸ colony-forming units (CFU/ml). Bacterial concentrations were monitored by measuring turbidity at 610 nm using GP 100 Photometer (Greiner, Germany). Each bacterium was spread using a sterile cotton swab on the Nutrient agar Petri dish. Subsequently, wells of 6 mm diameter were punched into the agar medium. Seven serial dilutions of *L. nobilis* leaf extract of 20, 15, 10, 7.5, 5, 2.5, and 1% (v/v) were prepared by diluting the extract with 40% (v/v) ethanol. Whereafter, 100 µL of each concentration of *L. nobilis* leaf extract were added to the wells. Ciprofloxacin disc (Cip) was used as a positive control, and 40% (v/v) ethanol was used as a negative control (Ghadiri et al., 2014). Agar plates were incubated at 37°C for 24 h. The antimicrobial activity of the different concentrations of *L. nobilis* leaf extract was examined by measuring the zone of growth inhibition using Vernier Calliper. The antimicrobial test was done in triplicates for each bacterium.

The antibacterial effect of *L. nobilis* leaf extract on fresh lamb meat

To investigate the antibacterial activity of *L. nobilis* leaf extract, a fresh lamb meat was obtained from a meat shop. Five hundred grams of fresh lamb meat were divided into four parts equally and placed in four sterile boxes. All four parts were divided into small pieces of about 10 g. The first two parts of meat samples were incubated at room temperature (25-30°C), where only one of them was sprayed with 10% (v/v) of *L. nobilis* extract in 40% (v/v) ethanol. The second two parts were incubated in a refrigerator (4-8°C), where only one of them was sprayed with 10% (v/v) of *L. nobilis* extract in 40% (v/v) ethanol. Bacterial counts, color, smell, pH, and any physical changes of meat samples were observed and recorded daily. pH measured by making a suspension of small pieces of meat in distilled deionized water using an Electrometrical meter (Milwaukee Electronics Kft, Hungary).

RESULTS

Isolation and identification of bacteria in fresh lamb meat by 16S rRNA gene sequence analysis

From the 20 collected fresh lamb meat samples, a total of 34 bacterial isolates were obtained and identified. Eighteen bacterial species were identified by sequence analysis of the 16S rRNA gene. The identified bacteria are shown in Table 1. It was observed that *Staphylococcus* spp., *Enterobacter* spp. and *Cedecea lapagei* were the predominant bacteria present in the collected fresh lamb meat samples. Other species of bacteria were detected in one or two fresh lamb meat samples; they include *Salmonella choleraesuis*, *Hafnia alvei*, *Acinetobacter lwoffii*, *Pseudomonas helmanticensis*, *Proteus vulgaris*, *Klebsiella pneumoniae*, and *Macrococcus epidermidis*. Bacterial counts in fresh lamb meat samples were in a range of 3 × 10³ - 1.5 × 10⁵ CFU/g, with *S. aureus* being the highest in numbers among other bacteria (Table 2).

Table 1. Bacteria isolated from fresh lamb meat samples identified by 16S rRNA gene sequence analysis.

Isolates No.	Identified bacteria	Homology (%)*	Genbank accession no.
1	<i>Staphylococcus aureus</i>	99.40	MK695866
2	<i>Staphylococcus aureus</i>	98.40	MK695940
3	<i>Staphylococcus saprophyticus</i>	99.40	MK695941
4	<i>Staphylococcus edaphicus</i>	100	MK695942
5	<i>Staphylococcus haemolyticus</i>	99.70	MK713339
6	<i>Staphylococcus aureus</i>	98.90	MK713337
7	<i>Enterobacter cancerogenus</i>	99.70	MK713337
8	<i>Staphylococcus saprophyticus</i>	100	MK713332
9	<i>Staphylococcus saprophyticus</i>	97.00	MK696049
10	<i>Pluralibacter gergoviae</i>	98.10	MK696050
11	<i>Cedecea lapagei</i>	99.70	MK696051
12	<i>Enterobacter cancerogenus</i>	99.70	MK713323
13	<i>Staphylococcus aureus</i>	99.50	MK713325
14	<i>Staphylococcus edaphicus</i>	99.80	MK713324
15	<i>Enterobacter tabaci</i>	98.00	MK713331
16	<i>Enterobacter xiangfangensis</i>	99.70	MK713330
17	<i>Proteus vulgaris</i>	100	MK713329
18	<i>Enterobacter cancerogenus</i>	99.70	MK713335
19	<i>Acinetobacter lwoffii</i>	99.80	MK689408
20	<i>Enterobacter hormaechei</i>	99.80	MK690048
21	<i>Salmonella enterica/choleraesuis</i>	98.80	MK690186
22	<i>Enterobacter hormaechei</i>	99.80	MK690181
23	<i>Cedecea lapagei</i>	99.20	MK689855
24	<i>Cedecea lapagei</i>	98.80	MK713334
25	<i>Hafnia paralvei</i>	99.80	MK684353
26	<i>Enterobacter cancerogenus</i>	99.50	MK695980
27	<i>Acinetobacter lwoffii</i>	99.50	MK713321
28	<i>Enterobacter hormaechei</i>	99.80	MK704397
29	<i>Pseudomonas helmanticensis</i>	99.80	MK695699
30	<i>Macrococcus epidermidis</i>	99.80	MK695699
31	<i>Proteus vulgaris</i>	98.70	MK685208
32	<i>Cedecea lapagei</i>	98.90	MK713322
33	<i>Pluralibacter gergoviae</i>	99.70	MK684347
34	<i>Klebsiella pneumoniae</i>	99.40	MK684237

*Percentage of identical nucleotides of the closest relative found in the Genbank database.

The antibacterial activity of *L. nobilis* leaf extract

The antibacterial activity of *L. nobilis* leaves extract were investigated against the 18 bacterial species isolated from fresh lamb meat. The inhibition zones of bacterial growth, which were measured in mm are as shown in Figure 1. It was found that *L. nobilis* leaf extract has an antibacterial activity against 9 bacterial species (Figure 1). It was observed that *Staphylococcus saprophyticus* and *P. vulgaris* were the most bacteria affected by *L. nobilis* leaf extract where their growth was decreased at all investigated concentrations, that is, 1-20% (v/v). Growth of *S. aureus* and *Staphylococcus edaphicus* was reduced at concentrations of 2.5 to 20 (v/v) of *L. nobilis*

leaf extract. *S. choleraesuis* was affected by concentrations of 5 to 20 (v/v) of *L. nobilis* leaf extract. Furthermore, concentrations of 7.5 to 20 (v/v) of *L. nobilis* leaf extract were found to have antagonist effects against growth *H. alvei*, *M. epidermidis*, and *Staphylococcus haemolyticus*. On the other hand, the growth of *P. helmanticensis* was only affected by a concentration of 20% (v/v) of *L. nobilis* leaf extract with an inhibition zone of only 8 mm. The remaining bacteria, which include, *K. pneumoniae*, *Enterobacter gergoviae*, *Cedecea lapagei*, *Enterobacter cancerogenus*, *Enterobacter tabaci*, *Enterobacter hormaechei*, *Enterobacter xiangfangensis*, *A. lwoffii*, and *Enterobacter cloacae* were not affected by any tested concentration of *L. nobilis* leaf extract.

Table 2. The number of colony forming units (CFU / g) for bacteria isolated from 20 fresh lamb meat samples.

Bacterial isolate	Number of lamb meat samples*	Average number of CFU/g
<i>Staphylococcus aureus</i>	4	1.5×10^5
<i>Staphylococcus saprophyticus</i>	3	2.5×10^4
<i>Staphylococcus edaphicus</i>	2	4×10^4
<i>Enterobacter gergoviae</i>	2	2.1×10^4
<i>Cedecea lapagei</i>	4	5×10^4
<i>Enterobacter cancerogenus</i>	4	2.5×10^4
<i>Enterobacter tabaci</i>	1	6.5×10^4
<i>Enterobacter hormaechei</i>	1	7×10^4
<i>Enterobacter cloacae</i>	2	3×10^3
<i>Salmonella choleraesuis</i>	1	1.5×10^4
<i>Hafnia alvei</i>	1	3×10^3
<i>Acinetobacter lwoffii</i>	2	3×10^4
<i>Pseudomonas helmanticensis</i>	1	3×10^3
<i>Proteus vulgaris</i>	2	5×10^3
<i>Klebsiella pneumoniae</i>	1	3×10^3
<i>Staphylococcus haemolyticus</i>	1	4×10^4
<i>Enterobacter xiangfangensis</i>	1	5×10^4
<i>Micrococcus epidermidis</i>	1	5×10^4

*Number of lamb meat samples that contain the bacterial isolates.

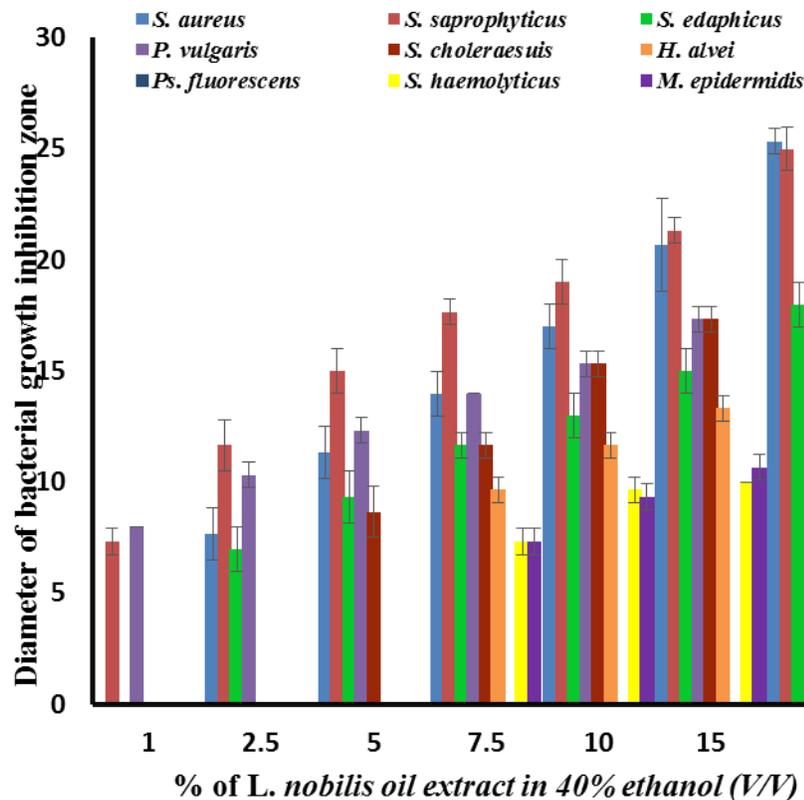


Figure 1. The antimicrobial activity of different concentrations of *Laurus nobilis* leaf extract (1, 2.5, 5, 7.5, 10, and 20%) in 40% ethanol (v/v) on growth of bacteria isolated from fresh lamb meat.

Table 3. The number of colony forming units (CFU/ g) of bacteria present in fresh lamb meat sample, treated and untreated lamb meat samples with *Laurus nobilis* extract 10 % in 40% ethanol (v/v).

Bacterial isolates ¹	Count of CFU/g in lamb meat samples				
	Fresh sample ²	Sample I ³	Sample II ⁴	Sample III ⁵	Sample IV ⁶
<i>Enterobacter hormaechei</i>	5 × 10 ³	11 × 10 ³	7 × 10 ³	6 × 10 ³	6 × 10 ³
<i>Pseudomonas helmanticensis</i>	3 × 10 ³	5 × 10 ³	4 × 10 ³	4 × 10 ³	2 × 10 ³
<i>Staphylococcus aureus</i>	8 × 10 ³	1.5 × 10 ⁴	0	1 × 10 ⁴	0
<i>Proteus vulgaris</i>	5 × 10 ³	8 × 10 ³	0	7 × 10 ³	0
<i>Cedecea lapagei</i>	1 × 10 ⁴	1.5 × 10 ⁴	1.1 × 10 ⁴	1 × 10 ⁴	1.1 × 10 ⁴
<i>Enterobacter cancerogenus</i>	2 × 10 ³	4 × 10 ³	3 × 10 ³	3 × 10 ³	2 × 10 ³
<i>Klebsiella pneumoniae</i>	3 × 10 ³	8 × 10 ³	4 × 10 ³	5 × 10 ³	5 × 10 ³

¹bacterial isolates were identified by 16S rRNA gene sequence analysis. ²CFU was determined immediately after collection of fresh meat sample. ³CFU was determined in untreated meat sample after 1 day of incubation at room temperature. ⁴CFU was determined in treated meat sample after 3 days of incubation at room temperature. ⁵CFU was determined in untreated meat sample after 6 days of incubation at 4°C. ⁶CFU was determined in treated meat sample after 13 days of incubation at 4°C.

Table 4. Physical changes observed in treated and untreated lamb meat samples with *Laurus nobilis* extract 10 % (v / v) in 40% ethanol (v/v).

Physical change	Lamb meat sample			
	Sample I ¹	Sample II ²	Sample III ³	Sample IV ⁴
Bad smell	Strong	Mild	-	-
pH	5.1	5.4	5.1	5.3
Mucoid layer	Thick	-	Thin	-
Gas bubbles	Many	-	Few	-
Dots	Green dots	Few white dots	Few white dots	Few white dots

¹Untreated meat: changes were observed after 24 h of incubation at room temperature (25-30°C). ²Treated meat: changes were observed after 3 days of incubation at room temperature (25-30°C). ³Untreated meat: changes were observed after 6 days of incubation at 4°C. ⁴Treated meat: changes were observed after 13 days of incubation at 4°C.

The effect of *L. nobilis* leaf extract on fresh lamb meat

The effects of spraying of 10% (v/v) *L. nobilis* essential oil on fresh lamb meat showed changes in microbiological and physicochemical characteristics of lamb meat. *Enterobacter hormaechei*, *P. helmanticensis*, *S. aureus*, *P. vulgaris*, *Cedecea lapagei*, *Enterobacter cancerogenus* and *Klebsiella pneumoniae* were isolated and identified from the untreated fresh lamb meat (Table 3). After spraying of lamb samples with 10% (v/v) of *L. nobilis* extract, it was found that *S. aureus* and *P. vulgaris* disappeared from the treated meat samples kept at both room temperature and refrigerating temperature (Table 3). The remaining bacteria, which include *E. hormaechei*, *P. helmanticensis*, *C. lapagei* and *E. cancerogenus*, and *K. pneumoniae* were still detected in treated meat samples kept at both temperatures but showed a decrease in their numbers compared to untreated fresh meat samples.

The observed physical changes in meat samples are shown in Table 4. It was found that the untreated lamb

meat kept at room temperature (25-30°C) showed changes after 24 h. Meat colour was red-yellowish with green dots, pH was about 5.1, strong bad smell, mucoid layer over meat and a large amount of mucus and gas bubbles were noticed on meat. On the other hand, lamb meat treated with 10% (v/v) of *L. nobilis* essential oil and kept at room temperature did not show any changes in meat appearance until 3 days of incubation. After 3 days, white dots and the mild bad smell started to be noticed in meat (Table 4). Changes in untreated lamb meat, which was kept in refrigerator, were only observed after 6 days (Table 4). Small bubbles of gas, thin mucoid layer on the meat surface and small white dots were observed. Treated meat, which was kept at the refrigerator, showed no changes during the first 13 days, whereafter, very small white dots started to appear on the meat surface.

DISCUSSION

In the present study, 34 bacteria were detected in fresh lamb meat samples (Table 1), which indicates that meat

is a rich medium for the growth of spoilage and pathogenic bacteria. *Staphylococcus* spp., *C. lapagei* and *Enterobacter* spp. were the predominant bacteria found in lamb meat. *Enterobacter* and *Pseudomonas* spp. were found to be among the predominant bacteria in lamb meat (Wang et al., 2019). According to Ahmed and Sabiel (2016), the members of the family Enterobacteriaceae are usually associated with the contamination of meat products, their incidence in meat was considered as a public health problem. It was found that *S. aureus* was the most frequent bacterium present in fresh lamb meat. *S. aureus* has the ability to colonize on raw meat and spread into meat products during the different processing stages of the meat supply chain (Velasco et al., 2019). The pathogenicity of *S. aureus* is given by bacterial structures and secondary metabolites, among which are toxins that could cause staphylococcal diseases transmitted by contaminated meat (Velasco et al., 2019).

In the present work, bacterial counts in fresh lamb meat samples were in a range of 3×10^3 - 1.5×10^5 CFU/g (Table 2). In another study, Martineli et al. (2009) found that bacterial counts in lamb meat were in a range of 1.0×10^1 to 8.0×10^4 CFU/cm for mesophiles; 1.0×10^0 to 4.4×10^4 CFU/cm for psychrophiles (Martineli et al., 2009). Contamination of raw meat with bacteria can occur during slaughtering, cutting and inadequate storage conditions.

It was reported that the extraction of *L. nobilis* showed greater antimicrobial activity against Gram-positive bacteria than Gram-negative, which were shown to have a growth sensitivity to extract of 5 tested herbs (Mostafa et al., 2018). However, in this study, the growth of some of both Gram-positive and Gram-negative bacteria was affected by *L. nobilis* leaf extract but growth of others was not. El Malti and Amarouch (2009) reported that laurel extract has a significant antimicrobial activity against growth of both Gram-positive and Gram-negative bacteria, which included *S. aureus*, *P. vulgaricus*, and *Salmonella enteridis*. In the present study, growth of *K. pneumoniae* was not affected by all concentrations of *L. nobilis* leaf extract. In another study, *K. pneumoniae* was found to have only a 7 mm zone of inhibition after treatment with *L. nobilis* leaf extract (El Malti and Amarouch, 2009). This can explain that the effect of *L. nobilis* extract was investigated against a different strain of *K. pneumoniae*.

Prevention of food spoilage and food poisoning pathogens is usually achieved by the use of chemical preservatives which have negative effects on human health. No studies have been conducted to examine the antimicrobial activity of *L. nobilis* leaf extract against bacteria present in fresh lamb meat or to evaluate the antimicrobial effects of *L. nobilis* leaf extract as a fresh lamb meat preservative. There was a very good agreement between the antimicrobial activity of *L. nobilis* leaf extract against isolated bacteria and its effect on their presence in treated lamb meat samples. *P. vulgaris* and

S. aureus were not detected in lamb meat samples treated with *L. nobilis* leaf extract (Table 3), which agrees with the investigations of the antimicrobial activity of *L. nobilis* leaf extract against those two bacteria (Figure 1). *E. hormaechei*, *C. lapagei*, *E. cancerogenus*, and *K. pneumoniae* were detected in the treated and the untreated lamb meat kept at room and refrigeration temperatures with a slight decrease in their numbers in the treated lamb meat (Table 3). The antibacterial activity of *L. nobilis* leaf extract showed that there were no effects of 1 to 20% v/v concentrations of *L. nobilis* leaf extract on the growth of those three bacteria (Figure 1). Growth of the isolated bacterium *P. fluorescens* was only affected by the highest concentration of *L. nobilis* leaf extract (20% (v/v)) with a zone of growth inhibition of 8 mm (Figure 1). The effect of spraying lamb meat with *L. nobilis* leaf extract was found to affect growth of *P. fluorescens* by decreasing its numbers from 5×10^3 to 4×10^3 CFU/g at room temperature and from 4×10^3 to 2×10^3 CFU/g at refrigerated temperature (Table 3).

It seems that treatment of lamb meat samples with *L. nobilis* leaf extract plays an important role in delaying spoilage and lipid poisoning of fresh meat. There were differences in the physical characteristics of treated and untreated lamb meat samples. Treatment of lamb meat with *L. nobilis* leaf extract kept at room and refrigeration temperatures was found to delay physical changes in meat for 3 and 13 days, respectively (Table 4). Azimzadeh and Jahadi (2018) reported that a mixture of *L. nobilis* leaf extract and chitosan has a significant effect on the growth of mold/yeast and mesophilic bacteria in cashew. Vacuum packaging combined with *L. nobilis* oil was found to reduce the growth of *E. coli* in ground chicken meat (Irkin and Esmer, 2010). In addition, the essential oil of *L. nobilis* was reported to have a significant effect in maintaining the pH and colour of minced beef meat and in reducing microbial counts (Vilela et al., 2016).

Conclusion

This study demonstrated that *L. nobilis* essential oil appears to be a promising tool that can be used as a natural preservative for fresh lamb meat. However, the present work is a preliminary study. Sequencing of the 16S rRNA gene was shown to be a good and accurate method for the identification of bacterial isolates at the species level.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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