Extension the storage period of minced beef by using some antibacterial medicinal plant extracts

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Abstract

Objectives: This study was carried out using different solvent extracts of leaves of Pelargonium (*Pelargonium zonale*) and basil (*Ocimium basilicum*) to improve shelf-life and safety of minced beef.

Methods: The different solvents *viz.* water, 70% aq. acetone and *n*-hexane extracts of the *Pelargonium zonale* (*P. zonale*) and *Ocimium basilicum* (*O. basilicum*) leaves were assessed against various pathogenic bacterial strains including Gram-negative bacteria of *Escherichia coli* O157:H7, *Klebsiella pneumonia*, *Proteus vulgaris* and *Serratia marcescens*, and Gram-positive bacteria of *Bacillus cereus*, *Micrococcus luteus*, *Micrococcus roseus* and *Staphylococcus aureus*. Agar wells diffusion method was used for the antibacterial assessments. The study also included addition of the most active water extract of *P. zonale* and *n*-hexane extract of *O. basilicum* to the minced beef. The microbiological changes including the changes in the total bacteria, Psyhcrophilic bacteria, *Staphylococcus aureus* and Coliform group counts were considered for evaluation of the preservative effects of the added plants extracts on extending shelf life of the stored minced beef at 4 ±1°C for 15 days.

Results: In general, antibacterial effects of the *P. zonale* extracts were more prominent than those of the *O. basilicum* extracts. Wide inhibition zones were observed with water extracts of *P. zonale* against the tested bacteria, except *St. aureus* and *M. roseus*. Addition of the aforementioned effective extracts reduced the total bacteria, psychrophilic bacteria, *Staphylococcus aureus* and Coliform group counts compared with that of the control sample during the storage period. Significant decreases in these counts are observed in minced beef containing 0.3% of *P. zonale* water extract. A 0.3% *n*-hexane extract of *O. basilicum* also noticeable reduced these counts compared with the high counts observed in minced beef without any additives (control samples).

Conclusion: It could be concluded that both of *P. zonale* water extract and *O. basilicum n*-hexane extract have powerful effect as antibacterial agents and could be employed as safe natural preservatives to improve the shelf-life of minced beef.

Keywords: *Pelargonium zonale*, *Ocimium basilicum*, medicinal plant extracts, antibacterial activity, minced beef.

1. Introduction

Minced meat is highly appreciated food because of its convenience for preparation of a lot of delicious dishes. However, its shelf- life is limited because of the large exposed surface area which facilitates its spoilage. The rate of deteriorative changes depends on meat composition, hygienic practices during cutting, grinding, preparation and storage conditions. The most important factor in meat spoilage is the microbial contamination which can affects minced meat safety and physical character **(Brooks *et al.*, 2008)**. Since ancient times, spices and herbs have been used for their perfume and flavor as seasoning additives and as preservatives due to their strong antimicrobial and antioxidant properties **(Tassou *et al*, 2004, Shan *et al*, 2007 Coma, 2008; and Véronique, 2008)**. The use of natural preservatives to increase the shelf-life of meat products is a promising technology since many plant derived substances show strong antimicrobial activity **(Botsoglou *et al.*, 2002**). The demand for safe foods, coupled with the consumers preference of foods free from synthetic additives has increased the interest for natural preservatives in recent years **(Roller, 2004)**. Biopreservatives, a wide range of natural products from both plants and microorganisms are useful in extending the shelf life of foods, reducing or eliminating pathogenic bacteria, and increasing overall quality of food products **(Draughon, 2004)**. Nowadays, most studies have concerned with evaluation of the antimicrobial activity of naturally occurring botanicals by *in vitro* experiments. In recent years the essential oils and extracts of many plant species have become popular, and the attempts to characterize their bioactive principles have been gained great interest in many food-processing applications **(Özkan *et al.*, 2010; Djenane *et al.*, 2011)**. The genus Pelargonium (*F: Geraniaceae*) contains 280 species, most of them are native to South Africa and few are in Tropical Africa **(Mabbereley, 1997).** Phytochemical studies of plant species of *Pelargonium* attracted the attention of many authors. They are rich in essential oils **(Lalli *et al.*, 2006)**, phenolic constituents including flavonoids **(Kokkalou and Souleles, 1988)**, tannins **(Kolodziej *et al*. 1995)**, phenolic acids **(Contour and Louguet. 1985)**. Many *Pelargonium* species were also successfully employed in modern phytotherapy for their antibacterial, antitubercular, antifungal, antihelmintic, insecticidal, antiplasmodial, antioxidant, anticancer activities. **(Lis-Balchin *et al*. 1998; Saraswathi, *et al.*, 2011**). Although the antibacterial effects of essential oils of *P. zonale*, in the best of our knowledge none was mentioned about the antimicrobial activities of solvent extracts of *P. zonale* leaves, a widely growing plant in Egypt, and one of the two plants included in this investigation. The genus *Ocimum* belonging to family Lamiaceae is widely distributed in tropical and warm temperate regions of the world **(Keita *et al.*, 2000)**. Both of the *Ocimum* oil and other solvent extracts of the plant were shown to exhibit antibacterial activities against gram positive and gram negative bacteria by various researchers **(Bozin *et al.*, 2006)**.

The present study is comparative study of the antibacterial effects of various solvent extracts, directly taken from the plant samples, of leaves of *P. zonale* and *O. basilicum*. Additionally, the effects of addition of the most active antibacterial extracts on microbiological changes of stored minced beef during refrigerator storage for 15 days were also reported.

2. Materials and methods

2.1. Material

The leaves of *P. zonale* and *O. basilicum* were collected in May 2014 from Farm of Faculty of Agriculture, Al-Azhar University, Assuit, Egypt.

Fresh beef (bottom round) was purchased from local market at Assiut city, Egypt and transported under refrigeration to the laboratory within 30 min. Then, meat was cut and minced with a grinder through a 4 mm plate diameter (AC110V, China) just before analysis and treatments.

The tested microorganisms used in this study were obtained from Botany Department, Faculty of Scince, Al-Azhar University, Assuit, Egypt.

2.2. Experimental methods

2.2.1. Preparation of extracts

The procedures of **Pokorny *et al.*, (1997**) for extraction were allowed in present work as follows: The dried leaves (by oven 50 ºC for 24 hours) of *P. zonale* and *O. basilicum* leaves were individually ground by an electrical grinder to pass through 60 mesh sieve. About 10 g of each dry powder was defatted by 30 min sonication in 100 mL of *n*-hexane followed by overnight maceration of the sonicated material at room temperature. The marc after defatting was allowed to dry then re-extracted by repeated maceration in 100 mL of H_2O : $(CH_3)_2CO$ (3:7, v/v) at room temperature till exhaustion. The H_2O extract was obtained from another 10 g of dry powder rep the same defatting procedures. The obtained various extracts were then filtrated and the filtrates were dried under reduced pressure till dryness at 40 ºC to yield *n*-hexane, 70% aq. acetone and water extract respectively. The obtained dried extracts were placed in sealed bottles and stored at 4 ºC until its use.

2.2.2. Determination of antibacterial activity

Antibacterial activity was determined by the well agar diffusion method according to the (**NCCLS, 1993**). Stock cultures of all bacterial species were grown in nutrient broth at 35º C for 18-24 h to obtain final concentrations of 10^8 CFU/mL. Petri plates containing 20 mL of nutrient agar medium were inoculated with 0.2 mL of stock cultures of the bacterial strains which were individually spread on the surface of the solid wet agar plates. Wells (6 mm diameter) were cut in the agar using sterile cork borer. Serial concentrations

(5→10→15→20→25 mg/mL) of all test extracts were prepared in dimethyl sulfoxide (DMSO). Volumes of 40 µL of each extract of different concentrations were carefully added into the wells using sterilized micro-pipettes. DMSO without the extracts was used as a negative control and Cephradine (CE); 30 µg/disk and Cefotaxime (CTX); 30 µg/disk were used as standard antibacterial agent. Petri plates of nutrient agar were inoculated with bacterial species in triplicates. The different concentrations of each extracts as well as DMSO were added separately to the holes at plates in triplicates. The plates were then incubated at 37 ºC. After 24 h of incubation, plates were evaluated for the presence or absence of visible growth on the agar plate. The absence of colonies on all tested plates was considered as an inhibitory effect and the inhibition zones diameter (mm) were recorded.

2.2.3. Microbial count

A 10 g of each minced beef sample were mixed with 90 mL of sterile saline solution (9 g NaCl/ 1L distilled water) in a blender, under sterile conditions to give 1/10 dilution. Serial dilutions were prepared to be used for counting several types of bacteria. The total bacteria, psychrophilic bacteria, *Staphylococcus aureus* and Coliform group counts were determined according to procedures by **A.P.H.A, 1976** and **Difco, 1984.**

3. Results and discussion

3.1. Antibacterial activity of plant extracts

In present work, the different extracts of *P. zonale* and *O. basilicum* were evaluated for their antibacterial activities against several Gram positive and Gram negative bacteria as a predisposing step for selecting the promising antibacterial extract, and examining its behavior as candidate in extension of storage period of minced beef.

3.1. 1. Antibacterial activity of *P. zonale* extracts

The results of the anti-bacterial activities of the *n*-hexane, 70% aqueous acetone and water extracts of *P. zonale* at different concentrations (5, 10, 15, 20 and 25 mg/ml) are summarized in Table 1. Obviously, the Gram-positive bacteria are more sensitive for *P. zonale* extracts than Gram-negative bacteria. Also, wide inhibition zones were observed with water extract of *P. zonale* against the tested bacteria, except *St. aureus* and *M. roseus* at concentration 5, 10 and 15 mg/ml while these last strains showed good susceptibility to the 70% aq. acetone extract (Table 1). The water extract exhibited a fairly high antibacterial effect against spectrum of microorganisms in comparison with the used standard antibiotics. In recent studies it was reported that extracts of pelargonium species is rich in highly oxygenated coumarins, flavonoids, flavonoid *C*-glycoside, phenolic acids, and simple phenols with gallic acid as the principal plant metabolite **(Williams *et al*., 2000)**. Hydrolyzable tannins and high molecular weight proanthocyanidins are also reported in several plant species of *Pelargonium* (**Kolodziej and Kayser, 1998, Kolodziej 2007**). For these chemical constituents, which are also the common group of compounds in *P. zonale*, pharmacological studies have demonstrated antibacterial, antioxidant and *in vitro* immunomodulatory activities **(Kayser and Kolodziej, 1997, Adewusi and Afolayan, 2009)**.

3.1.2. Antibacterial activity of *O. basilicum* extracts

The antibacterial activities of *O. basilicum* extracts (water, 70% aqueous acetone and *n*-hexane) at 5, 10, 15, 20 and 25 mg/ml concentrations were examined against the same eight bacterial strains. Data in Table (2) revealed that despite the lack of inhibition zone at all concentrations of *O. basilicum* water extracts against most tested microorganisms, the *n*-hexane extract recorded inhibitory effects against *Klebsiella pneumonia, Bacillus cereus and Staphylococcus aureus*. The predominant antimicrobial activity of the *n*-hexane extract, which mainly composed of the essential oils and the non-polar metabolites, are in accord with the previously reported antimicrobial activity of the essential oils of *O. basilicum* **(Amir *et al*., 2011; Dinanath, *et al*., 2011)**

In general, antibacterial effects of the *P. zonale* extracts are more prominent than those of the *O. basilicum* extracts. The water extract of former plant and *n*-hexane extract of the later showed a remarkable antibacterial activity so they were selected to evaluate its activity in extension of storage period of minced beef.

Table 1. Inhibition zones (mm ± s.e.) of *P. zonale* extracts against the selected microorganisms

Bacterial strains	Water extract (mg/mL)					70% aq. acetone (mg/ml)					*n*-hexane extract (mg/ml)					CE µg/disk	CTX µg/disk
	5	10	15	20	25	5	10	15	20	25	5	10	15	20	25	30	30
Gram-negative	Inhibition zone (mm ± s.e.)																
Proteus vulgaris	10 ± 0.88	12 ± 0.00	14 ± 0.00	15 ± 0.58	17 ± 1.53	9± 0.33	9± 0.33	10± 0.66	10± 0.33	12± 0.33	6±0 .00	7±0 .00	9± 0.33	10± 0.67	10± 0.00	6± 0.00	26 ± 0.67
Escherichia coli O157 :H7	12± 0.33	13± 0.67	14± 0.33	14± 0.33	15± 0.67	8± 0.00	9± 0.00	10± 1.53	11± 0.58	11± 0.33	7±0 .00	7±0 .33	7±0. 67	7±0. 33	7±0. 33	6± 0.00	19 ± 0.67
Klebsiella pneumoniae	10± 0.00	11± 0.58	14± 0.33	12± 0.68	13± 0.66	9±0. 58	11± 0.33	13± 0.33	15± 0.00	15± 0.00	7±0 .00	9±0 .33	10± 0.58	10± 0.33	10± 0.66	6± 0.00	13 ± 0.58
Serratia marcescens	7±0. 00	9±0. 00	10± 0.66	10± 0.33	11± 0.58	9±0. 58	10± 0.33	10± 0.33	10± 0.00	13± 0.33	7±0 .00	7±0 .33	10± 0.66	11± 0.33	11± 0.58	6± 0.00	47 ± 1.53
Gram-positive																	
Micrococcus roseus	6±0. 00	6±0. 67	7±0. 00	9±0. 33	10± 0.00	7±0. 00	10± 0.67	12± 0.67	12± 0.00	13± 0.58	7±0 .33	7±0 .58	7±0. 00	7±0. 00	7±0. 00	16± 0.33	19 ± 0.67
Micrococcus luteus	14± 0.58	15± 0.33	20± 1.53	21± 0.33	21± 0.00	6±0. 00	7±0. 00	8±0. 00	9±0. 00	10± 0.67	7±0 .00	7±0 .67	7±0. 00	7±0. 33	7±0. 33	11± 0.67	14 ± 0.88
Bacillus cereus	12± 0.66	12± 0.00	12± 0.33	14± 0.67	15± 0.67	13± 0.33	13± 0.67	14± 0.67	15± 0.58	16± 0.58	6±0 .00	7±0 .67	9±0. 58	10± 0.00	11± 0.58	6± 0.00	14 ± 0.58
Staphylococcus aureus	6±0. 33	6±0. 00	6±0. 67	8±0. 00	12± 0.33	8±0. 33	10± 1.53	10± 0.66	10± 0.66	12± 0.67	8±0 .00	8±0 .00	8±0. 00	8±0. 33	10± 0.66	6± 0.00	15 ± 0.33

Table 2. Inhibition zones (mm ± s.e.) of *O. basilicum n*-extracts against the selected microorganisms

Bacterial strains	Water extract (mg/ml)					70% aq. acetone extract (mg/ml)					*n*-Hexane extract (mg/ml)					CE µg/disk	CTX µg/disk
	5	10	15	20	25	5	10	15	20	25	5	10	15	20	25	30	30
Gram-negative	Inhibition zone (mm ± s.e.)																
Proteus vulgaris	6 ± 0.0	6 ± 0.33	6 ± 0.33	6 ± 0.0	6 ± 0.67	7 ± 0.33	7 ± 0.67	8 ± 0.00	8 ± 0.00	8 ± 0.00	7 ± 0.58	7 ± 0.0	7 ± 0.67	7 ± 0.33	7 ± 0.33	6 ± 0.0	26 ± 0.67
Escherichia coli O157 :H7	6 ± 0.00	6 ± 0.33	6 ± 0.00	6 ± 0.67	6 ± 0.67	6 ± 0.00	6 ± 0.33	6 ± 0.33	6 ± 0.33	6 ± 0.67	6 ± 0.67	7 ± 0.0	7 ± 0.0	7 ± 0.0	7 ± 0.0	6 ± 0.0	19 ± 0.67
Klebsiella pneumoniae	6 ± 0.00	6 ± 0.33	6 ± 0.67	6 ± 0.67	6 ± 0.67	7 ± 0.33	7 ± 0.33	7 ± 0.33	7 ± 0.67	7 ± 0.67	8 ± 0.0	8 ± 0.33	8 ± 0.00	8 ± 0.33	8 ± 0.33	6 ± 0.00	13 ± 0.58
Serratia marcescens	6 ± 0.00	7 ± 0.67	7 ± 0.58	7 ± 0.00	7 ± 0.00	6 ± 0.00	9 ± 0.58	10 ± 0.00	10 ± 0.88	10 ± 0.66	7 ± 0.33	7 ± 0.33	7 ± 0.67	7 ± 0.58	7 ± 0.67	6 ± 0.00	47 ± 1.53
Gram-positive																	
Micrococcus roseus	6 ± 0.00	6 ± 0.00	6 ± 0.00	6 ± 0.00	6 ± 0.00	7 ± 0.00	7 ± 0.00	7 ± 0.67	8 ± 0.33	8 ± 0.33	7 ± 0.0	8 ± 0.66	9 ± 0.33	9 ± 0.58	9 ± 0.33	16 ± 0.33	19 ± 0.67
Micrococcus luteus	6 ± 0.00	6 ± 0.33	6 ± 0.00	6 ± 0.67	7 ± 0.00	7 ± 0.67	7 ± 0.58	8 ± 0.66	8 ± 0.00	8 ± 0.00	6 ± 0.0	6 ± 0.00	6 ± 0.00	6 ± 0.00	6 ± 0.00	11 ± 0.67	14 ± 0.88
Bacillus cereus	6 ± 0.00	6 ± 0.00	6 ± 0.00	6 ± 0.00	6 ± 0.00	6 ± 0.67	7 ± 0.33	7 ± 0.67	7 ± 0.00	8 ± 0.33	7 ± 0.6	9 ± 0.33	10 ± 0.88	10 ± 0.00	10 ± 0.66	6 ± 0.00	14 ± 0.58
Staphylococcus aureus	6 ± 0.67	6 ± 0.00	6 ± 0.00	6 ± 0.33	6 ± 0.33	8 ± 0.33	8 ± 0.66	8 ± 0.00	8 ± 0.00	8 ± 0.33	8 ± 0.66	9 ± 0.58	9 ± 0.00	10 ± 0.33	12± 0.33	6 ± 0.00	15 ± 0.33

3.2. Microbiological evaluation of minced beef

The bacteriological load of meat products depends on the microbial load of the raw meat used for mincing, sanitary conditions and temperature of storage.

3.2.1. Changes in total bacterial count of minced beef treated with *P*. zonale and *O*. basilicum extracts during storage at 4 ± 1 °C for 15 days

Data listed in table 3 point to the changes in total bacterial counts in minced beef samples formulated with *P*. *zonale* water extract, and *O*. *basilicum n*-hexane extract at levels 0.1, 0.2 and 0.3% compared to untreated control samples during storage at 4±1 °C up to 15 days. The total bacterial counts were gradually increased during cold storage for all samples with different ratios depending on the concentration of the plants extracts. Also, the total bacterial counts for treated samples were lower than the control samples at any time of cold storage. The *P*. *zonale* water extract (0.3%) have the lowest total bacterial count followed by *O*. *basilicum n*-hexane extract (0.3%) (Table 3). The decrease in total bacterial count may be attributed to the antimicrobial effects of the compounds in these extracts.

Table 3. Changes in total bacterial count (log CFU/g ± SD) of minced beef treated with *P*. zonale and *O*. basilicum extracts during storage at 4 ± 1 °C up to 15 days

Treatments	Storage periods (days)					
	0	3	6	9	12	15
						log CFU/g ± SD
Control	6.82 ± 0.15	6.96 ± 0.17	7.43 ± 0.19	8.24 ± 0.17	8.92 ± 0.22	10.11 ± 0.26
P. zonale water extract						
0.1%	6.42 ± 0.14	6.55 ± 0.17	7.15 ± 0.18	7.91 ± 0.17	8.21 ± 0.21	9.13 ± 0.24
0.2%	6.19 ± 0.09	6.34 ± 0.11	6.41 ± 0.11	6.80 ± 0.09	6.92 ± 0.12	6.98 ± 0.12
0.3%	5.64 ± 0.09	5.31 ± 0.09	5.37 ± 0.09	5.56 ± 0.08	5.64 ± 0.09	5.71 ± 0.09
O. *basilicum n*-hexane extract						
0.1%	6.62 ± 0.08	6.87 ± 0.12	7.41 ± 0.12	8.33 ± 0.11	8.87 ± 0.15	9.78 ± 0.17
0.2%	6.43 ± 0.14	6.56 ± 0.17	6.87 ± 0.19	6.95 ± 0.15	7.21 ± 0.19	7.77 ± 0.20
0.3%	6.21 ± 0.13	6.38 ± 0.16	6.45 ± 0..17	6.79 ± 0.15	6.86 ± 0.18	6.87 ± 0.18

3.2.2. Changes in psychrophilic bacterial count of minced beef treated with *P*. zonale and *O*. *basilicum* extracts during storage at 4 ± 1°C for 15 days

The values of Psychrotrophic bacterial counts of different untreated and treated minced beef samples during cold storage at 4±1 °C up to 15 days were shown in table 4. The untreated control samples showed the highest Psychrotrophic bacterial counts as compared to the others containing *P*. *zonale* water extract, and *O*. *basilicum n*-hexane extract at different concentrations (Table 4). Psychrophilic bacterial count in all stored samples increased with the storage time. However, the samples treated with plants extract showed marked reduction in the psychrophilic bacterial count with different ratios depending on the

concentration of the extracts. The treated samples at concentration 0.3% showed the lowest counts at zero time and any time of cold storage (Table 4).

Table 4. Changes in Psychrophilic bacterial count (log CFU/g ± SD) of minced beef treated with *P. zonale* and *O. basilicum* extracts during storage at 4 ±1 °C up to 15 days

Treatments	Storage periods (days)					
	0	3	6	9	12	15
						log CFU/g ± SD
Control	7.13 ± 0.15	7.68 ± 0.20	7.84 ± 0.20	9.35 ± 0.20	10.16 ± 0.26	10.64 ± 0.28
P. zonale water extract						
0.1%	6.29 ± 0.13	6.42 ± 0.17	6.45 ± 0.17	6.67 ± 0.15	6.88 ± 0.18	7.35 ± 0.19
0.2%	5.82 ± 0.08	5.87 ± 0.10	5.9 ± 0.09	6.36 ± 0.09	6.56 ± 0.11	6.63 ± 0.12
0.3%	5.11 ± 0.06	5.16 ± 0.10	5.23 ± 0.10	5.43 ± 0.07	5.62 ± 0.09	6.09 ± 0.11
O. basilicum *n*-hexane extract						
0.1%	7.04 ± 0.09	7.41 ± 0.13	7.52 ± 0.13	7.88 ± 0.11	8.44 ± 0.15	8.43 ± 0.15
0.2%	6.27 ± 0.13	6.46 ± 0.17	6.47 ± 0.17	6.60 ± 0.14	7.22 ± 0.19	7.93 ± 0.21
0.3%	6.15 ± 0.13	6.18 ± 0.16	6.25 ± 0..17	6.34 ± 0.14	6.46 ± 0.17	6.52 ± 0.18

3.2.3. Changes in *Staphylococcus aureus* count of minced beef treated with *P. zonale* and *O. basilicum* extracts during storage at 4 ± 1°C for 15 days

Data presented in table 5 showed *Staphylococcus aureus* count of different treated and untreated minced beef samples during cold storage at 4 ± 1°C up to 15 days. All samples treated with extracts at different levels showed lower *Staphylococcus aureus* count than control sample. The treated samples with *P. zonale and O. basilicum* extracts at concentration 0.3% respectively showed the lowest counts in this parameter at zero time and during cold storage (Table 5).

Table 5. Changes in *Staphylococcus aureus* count (log CFU/g ± SD) of minced beef treated with *P. zonale* and *O. basilicum* extracts during storage at 4 ± 1 °C up to 15 days

Treatments	Storage periods (days)					
	0	3	6	9	12	15
						log CFU/g ± SD
Control	4.64 ± 0.09	4.76 ± 0.12	4.89 ± 0.13	9.35 ± 0.20	5.43 ± 0.14	5.75 ± 0.15
P. zonale water extract						
0.1%	4.15 ± 0.09	4.50 ± 0.11	4.76 ± 0.13	6.67 ± 0.15	5.08 ± 0.13	5.16 ± 0.14
0.2%	4.36 ± 0.06	4.42 ± 0.08	4.59 ± 0.08	6.36 ± 0.09	4.8 ± 0.08	4.86 ± 0.08
0.3%	4.26 ± 0.06	4.22 ± 0.08	4.08 ± 0.08	5.43 ± 0.07	3.16 ± 0.06	2.2 ± 0.04
O. basilicum *n*-hexane extract						
0.1%	4.61 ± 0.07	4.66 ± 0.08	4.72 ± 0.08	7.88 ± 0.11	4.99 ± 0.08	5.04 ± 0.09
0.2%	4.31 ± 0.09	4.29 ± 0.11	4.42 ± 0.12	6.60 ± 0.14	4.41 ± 0.12	4.09 ± 0.11
0.3%	4.14 ± 0.09	4.16 ± 0.11	4.26 ± 0.11	6.34 ± 0.14	4.12 ± 0.11	4.02 ± 0.11

3.2.4. Changes in coliform group counts of minced beef treated with *P*. zonale and *O*. *basilicum* extracts during storage at 4 ±1°C for 15 days

Table (6) represent the coliform group counts in minced beef formulated with *P*. zonale water extract and *O*. *basilicum* *n*-hexane extract under levels 0.1, 0.2 and 0.3 % compared to control sample during storage at 4 ± 1°C up to 15 days. Results showed that the coliform group counts in control sample had higher difference population of these bacteria at zero time and during storage periods. Whereas, the sample contained *P*. zonale and *O*. *basilicum* extracts had the lowest number of coliform group especially sample contained 0.3% of *P*. zonale water extract. From the results it could be noticed that the gradually decrease in coliform group counts which might be due to attributed to the antimicrobial effect of these plant extracts.

Table 6. Changes in Coliform group counts (log CFU/g) of minced beef treated with *P*. zonale and *O*. *basilicum* extracts during storage at 4 ± 1 °C up to 15 days

Treatments	Storage periods (days)					
	0	3	6	9	12	15
						log CFU/g ± SD
Control	4.26 ± 0.09	4.65 ± 0.12	4.89 ± 0.13	5.64 ± 0.12	6.43± 0.16	7.34 ± 0.14
P. zonale water extract						
0.1%	3.85 ± 0.09	3.94 ± 0.11	4.42 ± 0.11	4.24 ± 0.09	4.02 ± 0.11	3.66 ± 0.10
0.2%	3.36 ± 0.06	3.48 ± 0.06	3.59 ± 0.06	3.36 ± 0.03	3.04 ± 0.04	2.46 ± 0.04
0.3%	3.22 ± 0.05	3.32 ± 0.05	3.12 ± 0.05	3 ± 0.04	2.74 ± 0.04	2.2 ± 0.03
O. *basilicum* *n*-hexane extract						
0.1%	4.21 ± 0.05	4.25 ± 0.07	4.37 ± 0.07	3.34 ± 0.06	4.48 ± 0.05	4.51 ± 0.08
0.2%	3.66 ± 0.07	3.58 ± 0.10	3.49 ± 0.10	3 ± 0.06	2.63 ± 0.07	3.43 ± 0.08
0.3%	3.59 ± 0.07	3.74 ± 0.10	3.41 ± 0.09	3.12 ± 0.06	3.02 ± 0.08	2.98 ± 0.08

4. Conclusion

P. *zonale* and *O*. *basilicum* are widely spread perennial herbs which are easy to obtain in large quantities for industrial applications. In the light of the results obtained, it obvious that *P*. *zonale* water extract and *O*. *basilicum* *n*-hexane extract are promising as natural antimicrobial agents that could be an alternative to synthetic food additives in extending shelf life of minced beef. Despite the negative discoloration, the nice odour imparted from the plant extracts is highly appreciated.

5. References

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اطالة مدة التخزين للحم البقر المفروم باستخدام بعض مستخلصات النباتات الطبية المضادة للبكتريا

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الملخص العربى

اهداف البحث: أجراء هذه الدراسة باستخدام مستخلصات المذيبات المختلفة من أوراق (البلارجونيوم *Pelargonium zonale* والريحان *Ocimium basilicum*) لتحسين العمر الافتراضي وسلامة لحوم البقر المفروم. الطرق المستخدمة: المذيبات المختلفة من الماء، الأسيتون70٪، الهكسان، لعمل مستخلصات من أوراق البلارجونيوم والريحان، ثم تقييم هذه المستخلصات ضد سلالات مختلفة من البكتيريا الممرضة بما فيها البكتريا سالبة لجرام من (*Escherichia coli O157:H7, Klebsiella pneumonia, Proteus vulgaris* and *Serratia marcescens*) والبكتريا موجبة لجرام من (*Bacillus cereus, Micrococcus luteus, Micrococcus roseus* and *Staphylococcus aureus*)، باستخدام طريقة "agar wells diffusion" المستخدمة لقياس تضاد البكتريا. وشملت الدراسة أيضاً إضافة المستخلص المائي الأكثر نشاطا من البلارجونيوم ومستخلص الهكسان من الريحان بالإضافة إلى ذلك تم دراسة تأثير اضافة هذة المستخلصات إلى لحم البقر المفروم ، التغيرات الميكروبيولوجية بما فى ذلك التغير فى العد الكلى للبكتيريا و البكتيريا المحبة للبرودة و البكتريا العنقودية الذهبية و بكتريا القولون تم اعتبارها لتقييم التأثير الحافظ لإضافة مستخلصات هذه النباتات حول إطالة مدة التخزين للحوم البقر المفروم المخزنة في 4 ± 1 درجة مئوية لمدة 15 أيام.

النتائج: بشكل عام، كانت التأثيرات المضادة للبكتريا لمستخلصات البلارجونيوم أكثر وضوحا من مستخلصات الريحان، وقد لوحظت مناطق تثبيط واسعة مع مستخلصات البلارجونيوم المائية ضد الأنواع البكتيرية المختبرة، فيما عدا البكتريا العنقودية الذهبية و .M. roseus ، جميع المستخلصات المضافة ادت إلى خفض العد الكلى للبكتيريا، وكذلك البكتيريا المحبة للبرودة ، والبكتريا العنقودية الذهبية ومجموعة القولون، وذلك مقارنة بالكنترول خلال فترة التخزين . كان هناك انخفاض ملحوظ فى عدد الميكروبات لعينات لحم البقر المفروم المحتوية على 0.3٪ اضافة لمستخلصات البلارجونيوم المائية والريحان الهكسانية مقارنة بالكنترول.

الخلاصة: يمكن أن نخلص إلى أن المستخلص المائى للبلارجونيوم وكذلك مستخلص الهكسان للريحان يمكن أن تستخدم كمواد حافظة طبيعية وآمنة وبشكل آمن لإطالة مدة التخزين للحوم البقر المفرومة.