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Antibacterial Effect of *Origanum majorana* L. (Marjoram) and *Rosmarinus officinalis* L. (Rosemary) Essential Oils on Food Borne Pathogens Isolated from Raw Minced Meat in Egypt

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Abstract: Bacterial activity is a primary mode of deterioration of many foods and is often responsible for the loss of quality and safety of these foods. In this study, a total of 100 raw refrigerated minced meat samples were collected from retail stores in Egypt and examined for the presence of food borne microorganisms. Antimicrobial susceptibility of the isolates was determined using the disc diffusion assay. Alarming level of resistance was observed to some tested antibiotics reflecting multi-drug resistant strains. The dominant type of resistance was detected to amoxiclav (amoxicillin / clavulanic acid). The effect of Origanum majorana L. (marjoram) and *Rosmarinus officinalis* L. (rosemary) essential oils in comparison to erythromycin on the growth of *Salmonella*, *E. coli*, *Shigella, Citrobacter*, *Proteus* and *Pseudomonas* species isolates was investigated. All the isolates were susceptible to marjoram essential oil. *Salmonella* was the most sensitive strain tested to the antibacterial action of marjoram essential oil. Finally, marjoram essential oil can play an important role as antimicrobial agent in refrigerated minced meat and potentially it might be used as a natural preservative ingredient for longer periods without the need to use hazardous preservatives in food industry.

Key words: Minced Meat · Antibacterial Resistance · *Origanum majorana* L. (Marjoram) · *Rosmarinus officinalis* L. (Rosemary) · Bacteria

INTRODUCTION

Food borne infections and illnesses have become a major international health problem as it is the cause of major illness and deaths worldwide [1]. Outbreaks of food borne diseases and gastrointestinal illness such as dysentery and diarrhea have been associated with the consumption of meat that are contaminated by *Salmonella*, *Escherichia coli*, *Shigella sonnei* and *Listeria monocytogenes* through unhygienic practices [2]. Meat is the main source of food borne diseases in humans [3]. The principal pathogens which can be transmitted through meat include: *Bacillus cereus, Campylobacter* species*, Clostridium perfringens, Clostridium botulinum, Escherichia coli, Listeria monocytogenes, Salmonella* species*, Staphylococcus aureus, Yersinia enterocolitis, Aeromonas, Brucella, Clostridium difficile, Enterobacter* and *Shigella* [4].

Food contamination with antibiotic resistant bacteria could be a major threat to public health as the antibiotic resistance determinants can be transferred to other pathogenic bacteria, causing compromise in the treatment of severe infections [5]. The prevalence of antimicrobial resistance among food-borne pathogens has increased during recent decades [6]. Natural antimicrobial compounds have several beneficial effects as they reduce the need for antibiotics, control microbial contamination in food, improve shelf-life extension technologies to eliminate undesirable pathogens and/or delay microbial spoilage, decrease the development of antibiotic resistance by pathogenic microorganisms or strengthening immune cells in humans [7].

Spices and herbal essential oils (Eos) are used by the food industry as natural agents for extending the shelf life of foods. A variety of plant- and spice-based antimicrobials is used for reducing or eliminating

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pathogenic bacteria and increasing the overall quality of food products. Edible medicinal and herbal plants such as oregano, rosemary have been successfully used alone or in combination with other preservation methods [7]. Food processing technologies such as chemical preservatives cannot eliminate food pathogens or delay microbial spoilage totally [8]. Cold distribution of perishable food can help, but it cannot guarantee the overall safety and quality of the product. Moreover, changes in dietary habits and food processing practices and increasing demand for ready-to-eat products [7].

Among several essential oils that may be useful as antimicrobial agents, Origanum majorana L. (marjoram) essential oil belonging to the family Lamiaceae possesses antimicrobial properties against food borne bacteria and mycotoxigenic fungi and therefore, it may have the greatest potential for use in industrial applications [9, 10]. They are characterized by a wide range of volatile secondary metabolites. It is commercially used as a spice. It is traditionally used to treat asthma, indigestion, headache, rheumatism, dizziness, gastrointestinal disorder and migraine [11].

Rosmarinus officinalis L. essential oil, common name rosemary, belongs to the family Lamiaceae and it is a very ancient plant of several applications. Some of its traditional uses include antispasmodic, analgesic, antirheumatic, diuretic and antiepileptic. On the other hand, *Rosmarinus officinalis* has several applications especially in the food processing and preserving industry because of its natural antioxidant and antimicrobial effect [11].

The objectives of this study were to determine the antimicrobial resistance activities of the food borne pathogens isolated from raw refrigerated minced meat obtained from retail stores in Egypt and to evaluate the antimicrobial activities of two essential oils (marjoram and rosemary) against the isolated food borne pathogens. Also, this study aimed to investigate the effect of marjoram (*Origanum majorana* L.) essential oil on the quality of meat during cold storage.

MATERIALS AND METHODS

Sample Collection: Raw refrigerated minced meat samples (n=100) were randomly collected from local supermarkets in Cairo and Giza governorates, Egypt and transported on ice to the laboratory.

Isolation and Identification of Bacterial Pathogens: Twenty five gram of each meat sample was homogenized in 225 ml of peptone water (PW) and incubated at 37°C overnight, then pre-enrichment in maximum recovery broth and Rappaport vassiliadis broth (Oxoid) then streaked on *Salmonella Shigella* agar (SS Agar) (Oxoid), Mannitol salt agar (Oxoid), MacConkey's agar (Oxoid) and defibrinated sheep blood agar media (Oxoid, Ltd., Hampshire, UK). All plates were incubated at 37°C for 24 hours. Identification was occurred by culture characteristics and bacterial films stained with Gram's technique (BD, Maryland, USA). Identification of the recovered isolates was done according to Forbes *et al.* [12]. API system (20 E and NE) (Biomerieux SA, Marcy l'Etoile, France) was used to identify members of family *Enterobacteriaceae* using manufacturer's instructions that inserted within each diagnostic kit. For the isolation of *Campylobacter*, 1 g of each sample was added to 9 ml of Preston *Campylobacter* selective enrichment broth (Oxoid) supplement with *Campylobacter* selective supplement SR 117 (Oxoid), *Campylobacter* growth supplement SR 84 (Oxoid) and 5% defibrenated horse blood [13]. The inoculated broth was incubated under microaerophilic conditions at 42°C for 24 hours. Then the enrichment culture was streaked onto *Campylobacter* blood-free selective agar base (Oxoid) supplemented with *Campylobacter* selective supplement SR 155 (Oxoid) and incubated under microaerophilic conditions at 42°C for (3-5) days. Suspected *Campylobacter* isolates were confirmed using standard biochemical procedure including catalase, oxidase and hippurate hydrolysis tests [14].

Antimicrobial Susceptibility Testing (Ast) of the Recovered Isolates: The isolates were tested for their antibiotic resistance patterns using the disc diffusion method. Steps of the test and interpretation were relying on the instructions of the NCCLS [15]. The used antimicrobial discs (Oxoid) were imipenem (IPM10 µg), gentamicin (CN10 µg), amoxiclav (amoxicillin / clavulanic acid) (AMC30 µg), tobramycin (TOB10 µg), ciprofloxacin (CIP5 µg) and oxytetracycline (OT30 µg). In addition to vancomycin (VA30 µg) was used for *Staphylococci* isolates. Multidrug Resistant (MDR) strains were also determined. MDR strain is defined as that the pathogen which is resistant to ≥ 3 groups of antibacterial agents [16].

Antimicrobial Activity of *Origanum majorana* L. (Marjoram) and *Rosmarinus officinalis* (Rosemary) Essential Oils: The antimicrobial activity of marjoram and rosemary essential oils was studied using agar well diffusion method in comparison with erythromycin [17].

Extraction of essential oils: The aerial parts of wild marjoram (*Origanum majorana* L.) and rosemary (*Rosmarinus officinalis*) were collected from Egypt at full flowering stage. The collected plants were dried in shade and ground in a grinder. The dried plant samples (500 g) were subjected to hydrodistillation (plant material in boiling water) using a Clevenger-type apparatus for 4h [18]. Hydrodistillation of plants yielded 2.3% (v/w) of essential oil. The 2 essential oils preparations were stored at 4°C in dark glass bottles until used.

Method: The prepared essential oils were tested for their antimicrobial activities against 62 isolates. Aliquots (100µl) of each bacterial cell culture suspension matching with 0.5 McFarland was spread over the surface of pre-dried Mueller-Hinton agar plates with a sterile glass spreader. Eighty µl of each type of oil was added into wells of agar plates directly. Erythromycin discs were used to assess the susceptibility of tested isolates to compare with the result of essential oils efficiency. All Petri dishes were sealed with sterile laboratory parafilm to avoid eventual evaporation of the essential oils. The plates were left for 30 min at room temperature to allow the diffusion of oil. The plates were re-incubated at 37°C for 24 h. After incubation, plates were observed for antimicrobial activities by measuring the diameters of the zones of inhibition in millimeter for each of the essential oil. For an accurate analysis, tests were run in triplicate for each isolate to avoid any error. Standard reference strains of *S. Typhimurium* ATCC 14028 were used as a control.

Microbiological Evaluation of Treated and Untreated Minced Meat with *Origanum majorana* L. (Marjoram) Essential Oil During Cold Storage with and Without *Salmonella* Inoculation [19]: Raw minced meat was divided into 4 groups as following:

Group 1: contained 15 g of raw minced meat.

Group 2: contained 15 g of raw minced meat treated by 1.5 ml marjoram essential oil.

Group 3: contained 15 g of raw minced meat inoculated by 1.5 ml of *Salmonella* in concentration of $1.5x10^8$ CFU/ml.

Group 4: contained 15 g of raw minced meat treated by 1.5 ml marjoram essential oil and inoculated by 1.5 ml of *Salmonella* in concentration of $1.5x10^8$ CFU/ml.

Each group was mixed well in polyethylene bags using stomacher, then labeled and stored at -4°C. The groups were analyzed at each 10 days interval during the storage period. To evaluate the antimicrobial potency of marjoram essential oil in the food system as a preservative, heterotrophic aerobic bacteria counting on plate count agar and *Salmonella* counting on SS agar were carried out on the four tested groups.

Statistical Analysis: ANOVA was carried out on data of the sensory evaluation applying the function of two factors with replicates. "Excel" Software of Microsoft office 2000. L.S.D. analysis was adapted according to statistical procedures for agriculture research [20]. Data were expressed as mean ± SE.

RESULTS

Prevalence of Food Borne Pathogenic Microorganisms: A total of 100 samples of raw refrigerated minced meat were collected from retail stores in Egypt and examined for the presence of food borne microorganisms; *Campylobacter* was not isolated from any of the analyzed samples. Meanwhile, the prevalence of *Salmonella* species was 19(19%), *E. coli* was 13(13%), *Shigella* species was 12(12%), *Staphylococcus* species was 8(8%), *Enterobacter* species was 8(8%), *Pseudomonas* species was 2(2%), *Citrobacter* species was 1(1%) and *Proteus* species was 1(1%) as shown in Figure (1).

Antimicrobial Susceptibility Testing: Seven different antibiotics were used against 62 identified bacterial species isolated from raw minced meat samples. The results presented in Table (1) showed that the ciprofloxacin was the most effective antibiotics against 62 bacterial isolates followed by imipenem, amoxiclav, gentamicin, tobramycin and oxytetracycline. Resistance to one or more antimicrobial agents was found. The dominant type of resistance was detected to amoxiclav (amoxicillin / clavulanic acid) followed by oxytetracycline, tobramycin, gentamicin and imipenem. Results clearly showed that Gram-negative isolates were more resistant than the Gram-positives; it was observed that there's marked resistance of *Shigella* and *Pseudomonas* that were 100% for both of them followed by *Salmonella*, *E. coli* and some strains of *staphylococci* to amoxiclav (amoxicillin / clavulanic acid) that were 84.20, 76.92 and 25.00% respectively.

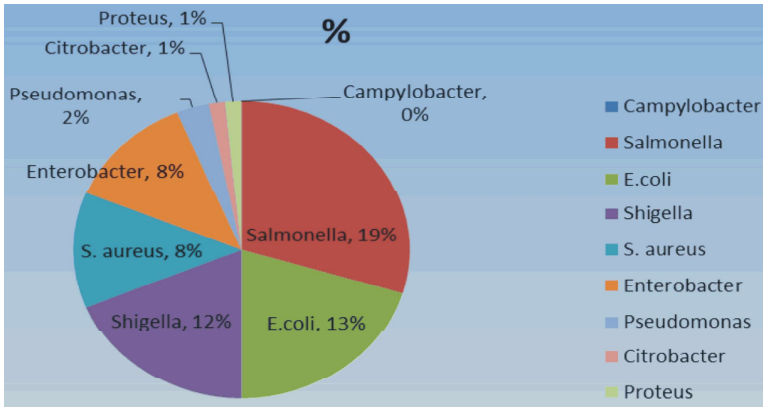

Fig 1: Occurrence of food borne pathogenic bacteria isolated from minced meat in Egypt.

Table 1: Antimicrobial susceptibility testing of meat isolates

Tested isolates (No)	Sensitivity type (%)	IPM	VA	CIP	TOP	AMC	CN	OT
Salmonella (19)	S %	42.11	-	100.00	21.05	0.00	42.10	-
	I %	57.89	-	-	0.00	15.79	36.84	-
	R%	0.00	-	-	78.95	84.20	21.05	100.00
	ND%	-	100.00	-	-	-	-	-
E. coli (13)	S%	76.92	-	100.00	30.76	-	23.08	-
	I%	23.08	-	-	46.15	61.54	76.92	23.08
	R%	0	-	-	23.08	76.92	-	76.92
	ND%	-	100.00	-	-	-	-	-
Shigella (12)	S%	66.67	-	100.00	-	-	-	-
	I%	-	-	-	33.33	-	66.67	66.67
	R%	33.33	-	-	66.67	100.00	33.33	33.33
	ND%	-	100.00	-	-	-	-	-
Pseudomonas (2)	S%	100.00	-	100.00	-	-	-	-
	I%	-	-	-	100.00	-	100.00	100.00
	R%	-	-	-	-	100.00	-	-
	ND%	-	100.00	-	-	-	-	-
Enterobacter (8)	S%	-	-	100.00	50.00	100.00	-	50.00
	I%	100.00	100.00	-	50.00	-	50.00	50.00
	R%	-	-	-	-	-	50.00	-
	ND%	-	-	-	-	-	-	-
S.aureus (8)	S%	25.00	37.50	100.00	37.50	62.50	87.50	50.00
	I%	50.00	62.50	-	50.00	12.50	-	-
	R%	25.00	-	-	12.50	25.00	12.50	50.00
	ND%	-	-	-	-	-	-	-

IPM (10) = Imipenem, VA (30 mcg) = Vancomycin, CIP (5mcg) = Ciprofloxacin, TOB (10mcg) = Tobramycin, AMC (30 mcg) = Amoxiclav (Amoxicillin / Clavulanic acid), CN (10 mcg) = Gentamicin, OT (30) = Oxytetracycline, ND= Not detectable.
*S=sensitive, I=intermediate, R=resistance

Table 2: Antibacterial activities of *Origanum majorana* L. (marjoram) and *Rosmarinus officinalis* L. (rosemary) essential oils in comparison to erythromycin using disc diffusion method:

	Inhibition zone diameter (mm)		
Microorganisms (number)	Marjoram	Rosemary	Erythromycin
Shigella (4)	16.75	Resistance	Resistance
Salmonella (6)	19.20	Resistance	Resistance
Pseudomonas (2)	14.00	Resistance	Resistance
Proteus (1)	15.00	Resistance	Resistance
E. coli (3)	19.00	Resistance	Resistance
Citrobacter (1)	16.00	Resistance	Resistance
Reference strain *S.Typhimurium*ATCC 14028	13.00	Resistance	Resistance

Table 3: Inhibitory effect of *Origanum majorana* L. (marjoram) essential oil as a preservative on total bacterial count and *Salmonella* either normally present or inserted in minced meat during storage period at -4 °C for 1 month period with an interval 10 days between each examination:

	Bacterial count CFUs/gm							
Groups	Group 1		Group 2		Group 3		Group 4	
Storage time	SS	PCA	SS	PCA	SS	PCA	SS	PCA
Zero day	3×10^3	TNTC	No Growth	3.5×10^3	3×10^7	TNTC	No Growth	5.6×10^3
Day 10	10×10^2	TNTC	No Growth	3.5×10^3	5×10^6	TNTC	No Growth	4.4×10^3
Day 20	No Growth	8.5×10^5	No Growth	1×10^3	3.5×10^5	7.5×10^6	No Growth	1.4×10^3
Day 30	No Growth	5.6×10^5	No Growth	No Growth	8×10^4	1.1×10^6	No Growth	No Growth

TNTC= Too Numerous to Count.

Group 1: 15 gm of raw minced meat only.

Group 2:15 gm of raw minced meat treated by 1.5 ml 100% Marjoram essential oil.

Group 3: 15 gm of raw minced meat inoculated by 1.5 ml of *Salmonella* in concentration of $1.5x10^8$ CFU/ml.

Group 4: 15 gm of raw minced meat treated by 1.5 ml 100% Marjoram essential oil and inoculated by 1.5 ml of *Salmonella* in concentration of $1.5x10^8$ CFU/ml.

Antibacterial Activities of *Origanum majorana* L. (Marjoram) and *Rosmarinus officinalis* L. (Rosemary) Essential Oils in Comparison to Erythromycin Using Disc Diffusion Method: The effect of marjoram and rosemary essential oils on the growth of *Salmonella*, *E. coli*, *Shigella, Citrobacter*, *Proteus* and *Pseudomonas* species isolates was illustrated in Table (2). It is clear that rosemary and erythromycin did not show any inhibitory effect against the isolates. However, all tested isolates either Gram negative or Gram positive were susceptible to the action of 100% marjoram essential oil with a range of inhibition zone diameter values from 13.00 to 19.20mm. The results revealed that *Salmonella* was the most sensitive strain tested to the antibacterial action of marjoram essential oil with the strongest inhibition zone (19.20mm) followed by *E. coli* (19.00 mm), *Shigella* (16.75mm), *Citrobacter* (16.00mm), *Proteus* (15.00mm) and *Pseudomonas* species(14.00mm).

Effect of *Origanum majorana* L. (Marjoram) Essential Oil as a Preservative on the Microbiological Quality of Cold Stored Minced Meat: Table (3) shows the effect of marjoram essential oil as a preservative on the microbiological aspects of minced meat (*Salmonella* and total bacterial count) during 4 intervals examination (day zero, day 10, day 20 and day 30) at -4°C for 1 month. The data indicated that marjoram showed 100% bactericidal effect on the end of the storage period.

DISCUSSION

In the present study, *Campylobacter* was not isolated from any of the analyzed samples. Meanwhile, the prevalence of *Salmonella* species was 19(19%), *E. coli* was 13(13%), *Shigella* species was 12(12%), *Staphylococcus* species was 8(8%), *Enterobacter* species was 8(8%), *Pseudomonas* species was 2(2%), *Citrobacter* species was 1(1%) and *Proteus* species was 1(1%) as shown in Figure (1). It was reported that 69% of cases of bacterial food borne diseases are caused by Gram negative bacteria [21]. *Enterobacter* species (28.6%), *Klebsiella* species (5.7%), *Escherichia coli* (8.6%), *Bacillus* species (14.2%) and *Pseudomonas* species (42.9%) was recorded from meat samples [22]. A total of thirty-one bacterial isolates covering six genera from raw meat was isolated and characterized as *Bacillus* (9.68%), *Enterobacter* (9.68%), *Escherichia* (25.81%), *Klebsiella* (22.58%), *Pseudomonas* (9.68%) and *Staphylococcus* species (22.58%) [23], these microorganisms causes toxic infection after multiplication in meat foods [24]. *Escherichia* species are of the most concern in undercooked meat products, such as beef meat [25]. The presence of *E. coli* in raw meat might have originated from animal tissues or contaminated tools used in slaughtering and related treatment or cutting process. *Escherichia coli* is commonly used as surrogate indicator, its presence in food generally indicate direct and indirect fecal contamination [21]. Meat may be contaminated with *Salmonellae* throughout the slaughtering, dressing and boning process, starting with the carcass during knife incision for hide removal [25]. *S. aureus* has a huge impact on animals' health and welfare and causes major economic losses in livestock's production [26]. The prevalence of *S. aureus* in meat products indicates the presence of cross contamination, which is usually related to human skin and clothing and this level of food contamination by this pathogen might lead to food intoxications [27]. If meat product is temperature abused, this pathogen is

able to produce heat-resistant enterotoxins [25]. The data concluded that minced meat is considered important source of bacterial pathogens causing severe gastroenteritis in human.

Antibiotic resistance levels are elevated among food borne pathogens. Although, it is difficult to prove a direct role of drug resistance in bacteria contaminating food with increased clinical cases of resistant infections, the presence of such resistant bacteria in food items could play a role in the spread of antimicrobial resistance amongst food borne pathogens [28].

Seven different antibiotics were used in the present study against 62 identified bacterial species isolated from raw minced meat samples. The results presented in Table (1) showed that the ciprofloxacin was the most effective antibiotics against 62 bacterial isolates, followed by imipenem, amoxiclav, gentamicin, tobramycin and oxytetracycline. Resistance to one or more antimicrobial agents was found. The dominant type of resistance was detected to amoxiclav (amoxicillin / clavulanic acid) followed by oxytetracycline, tobramycin, gentamicin and imipenem. Results clearly showed that there's marked resistance of *Shigella* and *Pseudomonas* that were 100% for both of them followed by *Salmonella*, *E. coli* and some strains of *staphylococci* to amoxiclav (amoxicillin / clavulanic acid) that were 84.20, 76.92 and 25.00% respectively.

In the present study Gram-negative isolates were more resistant than the Gram-positives; this is expected because of intrinsic nature of gram-negative cell wall also they harbor series of antibiotic resistance genes which can be transferred horizontally to other bacterial species [29]. Most of investigated *Salmonella* isolates were sensitive to colistine sulphate, ciprofloxacin, amoxicillin, sulfa-trimethoprim, ampicillin, danofloxacin and gentamicin; on the other hand, most of the isolates were multiple drug resistance [30].

In veterinary medicine, antimicrobial agents are used in therapy, prophylaxis and as growth promoters and this may be responsible for generation of resistant bacteria. During the two past decades, the emergence of antibiotic-resistant bacteria has become a serious problem worldwide and wide usage of antibiotics in the diet of domestic animals has made drug resistant bacteria which could be transferred to human beings. The problem of resistant strains to multiple drugs (MDR) is increasing and most studies in different countries have shown high resistance of strains to several antibiotics [31].

Bacterial gain of antimicrobial resistance is an alarming situation where treatment is getting limited to currently sensitive antibiotics. The prophylactic use of many antimicrobials in animal feed can also lead to acquired antibiotic resistance [32]. Plant extracts can be used in the treatment of infectious disease caused by resistant microbes [33]. Some herbs are used as meat effects which have been reported to have bactericidal or bacteriostatic additives [34]. The inhibitory effects of herbs are mostly because of their content of volatile oils; the volatile oils can act as pro-oxidant affecting inner cell membranes and organelles such as mitochondria in eukaryotic organisms. Mitochondria like structure and membranes structure in prokaryotic organisms are also affected by volatile oil depending on type and concentration [35].

This investigation was directed to study the effect of *Origanum majorana* L. (marjoram) and *Rosmarinus officinalis* L. (rosemary) essential oils in comparison to erythromycin on the growth of *Salmonella*, *E. coli*, *Shigella, Citrobacter*, *Proteus* and *Pseudomonas* isolates using agar well diffusion method. The results illustrated that rosemary and erythromycin did not show any inhibitory effect against the isolates. It was reported that the weak antimicrobial activities of rosemary essential oils against pathogenic and nonpathogenic bacteria were likely due to the lack of verbenone [36]. The antibacterial activity potential of rosemary essential oil against bacteria was related to the rate of verbenone, camphor and linalool rather than to the increased content of 1, 8-cineole. In fact, major or trace compounds contained in an essential oil might increase or decrease its antimicrobial activity because it should be taken into consideration the possible synergistic and antagonistic effect of compounds in the oil [37]. The low activity of the essential oil against the tested isolates suggests either that the crude extracts held very low concentration of active antibacterial compounds or that the crude extract contained compounds that inhibited the antibacterial activity of the effective compounds.

On the other hand, all tested isolates were susceptible to the action of 100% *Origanum majorana* L. (marjoram) essential oil with a range of inhibition zone diameter values from 13.00 to 19.20 mm. The results revealed that *Salmonella* was the most sensitive strain tested to the antibacterial action of marjoram essential oil with the strongest inhibition zone (19.20mm) followed by *E. coli* (19.00 mm), *Shigella* (16.75mm), *Citrobacter* (16.00mm), *Proteus* (15.00mm) and *Pseudomonas* species

(14.00mm). It was reported that marjoram essential oil possesses antimicrobial properties against food borne pathogens and spoilage microorganisms when tested *in vitro* and therefore, it may have the greatest potential for use in industrial applications [9, 38]. The main component of marjoram essential oils was carvacrol which represented at 81.5 % in marjoram [39]. It was explained that globally, the antimicrobial mode of action of the marjoram essential oil is considered to arise mainly from their hydrophobic potential to introduce into the bacterial cell membrane [36]. Moreover, marjoram essential oil components can penetrate into the interior of the cell and interact with intracellular sites critical for bacterial activities [40]. More precisely, they are able to inhibit glucosyl transferase enzyme activity, which is responsible in bacteria adhesion to its sites [41].

The inhibitory effect of *Origanum majorana* L. (marjoram) essential oil on total bacterial count and *salmonella* count in minced meat during storage period at - 4°C for 1 month was studied. The data indicated that *Origanum majorana* L. (marjoram) essential oil showed 100% bactericidal effect at the end of the storage period. Therefore, these results indicated that both the effect of lower temperature and addition of marjoram essential oil significantly allows for preservation of minced meat samples for longer periods without the need to use hazardous preservatives. It was reported that the fresh or dried marjoram leaves and their essential oil are widely used in the food industry as a food ingredient [42]. In order to reduce health hazards, food poisoning outbreaks, lipid oxidation and subsequent economic losses, the use of natural marjoram essential oils as green antioxidant and antimicrobial agents seems to be an attracting manner both in industrial applications and scientific research [38]. It was concluded that *Origanum majorana* is considered a broad spectrum antibacterial agent against Gram-positive and Gram-negative pathogenic micro-organisms; *Origanum majorana* is an alternative for synthetic preservatives routinely used in the food industry [7].

CONCLUSIONS

The results indicated that minced meat are considered important sources of food borne pathogens as well demonstrated the role of this meat as a reservoir of antibiotic resistant bacteria that can be transferred to humans. The growing concern about safety of foods has recently directed into the development of natural antimicrobials as a preservatives to control food borne pathogens. Antimicrobial activities of marjoram (*Origanum majorana* L.) essential oils could be used as natural preservative to control microbial contamination; improve quality of minced meat; and replace synthetic preservatives. *In-vivo* extension studies will be required to find appropriate doses of essential oils showing both antimicrobial activity and very low detrimental effect on eukaryotic cells.

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