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THE ANTIMICROBIAL EFFECT OF SOME AROMATIC PLANT EXTRACTS ON *Campylobacter* SPP. IN CHICKEN MEAT

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ABSTRACT

The presence of *Campylobacter* spp was assessed in raw chicken at retail level. It was found in whole chicken in leg parts and was identified as *Campylobacter jejuni*. The mint essential oil (EO) extraction gave the highest antimicrobial activity with inhibition zone (more than 20 mm) against *C. jejuni*, while onion extract gave active inhibition zone ranged between 16 to 19 mm. The lowest Minimal Inhibitory Concentration (MIC) value against *C. jejuni* was (0.01%) and (0.14%) for mint EO and onion extract, respectively. Mint EO was analyzed using gas chromatography-mass spectrometry technique. The main components obtained were Carvone (56.87%), Cineole (6.89%), D-limonene (6.48%), 1-6-Dihydrocarveol, Carvyl acetate-E (5%) and Carveol substances (4.25%). Mint EO and onion extract were tested in chicken leg meat stored for 7 days at 4ºC after their inoculation with *C. jejuni* at a level of 10^6 cfu/g meat. *C. jejuni* count of untreated chicken leg meat was 2.14 (log cfu/g) after 7 days of storage at 4°C and *C. jejuni* count of mint EO treated chicken leg meat was not detected after 7 days of storage at the same temperature. *C. jejuni* count of onion extract treated chicken leg was 1.3 log cfu/g. The total viable aerobic bacterial count in the untreated sample was (5.88 log cfu/g) which exceeded the maximum acceptable level (5.3 log cfu/g) after 3 days of storage at 4 °C. On contrast, the total viable aerobic and anaerobic bacterial counts of chicken leg treated with 2% of mint EO and Onion extract were below the maximum acceptable levels up to 7 days. Thiobarbituric acid (TBA) value of untreated sample (control) sharply increased during the refrigerated storage at 4ºC from 0.16 to 3.60 (mg malonaldehyde/Kg) at the end of storage (day 7). While, TBA value of the samples treated with 2% of mint EO or onion extract gradually increased to a lower extent, and did not exceed the maximum acceptable level (2.4 mg malonaldehyde/Kg). The results revealed that, the total volatile basic nitrogen (TVBN) of untreated sample (control) sharply increased during refrigerated storage (36.10 mg/100 g). While, the TVBN of treated chicken leg increased slowly and did not exceed maximum acceptable levels (20mg/100g). The results indicated that, the addition of 2% mint EO or 2% onion extract reduced *Campylobacter jejuni* counts and also improved the microbiological and chemical quality, and extended the shelf-life of raw chicken meat.

Key words*: *Campylobacter jejuni, chicken meat, mint EO, onion extract, shelf-life extension.

1. INTRODUCTION

Gram-negative bacterium *Campylobacter* is responsible for an estimated 400 million human cases of entero-colitis worldwide, making it the leading cause of bacterial foodborne disease and a major causative agent of traveler's disease (Allos, 2001). Disease caused by *Campylobacter* usually is manifested as diarrhea, fever, malaise and severe abdominal pain (Nachamkin, *et al.*, 1992). However, it may lead to Guillain-Barre syndrome, which is a serious neurological disease with symptoms that include flaccid paralysis (Smith, 2002).

In a study involved infected children in the rural Egyptian area‘‘Abou-Homos”, the overall incidence of diarrhea caused by *Campylobacter* was 0.6 episodes per person a year. This is the highest incidence rate ever estimated in a developing country (Rao *et al.*, 2001) and is 10 times higher than that previously reported in

another Egyptian area (Zaki *et al*., 1986). Rao *et al*. (2001) suggested that, poor domestic hygienic practices, manifested by the presence of animals and uncovered garbage in the cooking areas, were associated with a risk of *Campylobacter* diarrhea in the Abou-Homos area. However, this was not supported by experimental work tracing *Campylobacter* in the surrounding environment, foods and animals. Consumption and handling of chicken have been identified as important risk factors for campylobacteriosis. In relation to chicken, foodborne exposure to *Campylobacter* takes place through the consumption of undercooked, internally contaminated chicken meat or via cross-contamination to ready-to-eat foods or hands during preparation of raw chicken (Wingstrand *et al*., 2006).

Much attention lately has been focused on extracts from herbs and spices which have been used traditionally to improve the sensory characteristics and to reduce the risk of pathogenic microorganisms and extend the shelf-life of foods (Botsoglou *et al*., 2003). The purpose of the present study was to isolate and identify *Campylobacter* spp from chicken meat at retail and to evaluate antimicrobial activity of some selected plant extracts (mint and onion) against *Campylobacter* isolates, and to study the effect of plant extraction treatment on the shelf-life of chicken leg meat during refrigerating storage.

2. MATERIALS AND METHODS

2.1. Material

Fifty raw chickens (whole chicken, chicken breast with skin and chicken leg pieces) were purchased from May to September 2010, from local supermarket chains and local butchers' shops (randomly selected from Giza region, Egypt). Raw chicken purchased from supermarkets were pre-packaged, whilst raw chicken purchased from local butchers' shops were packaged at the point of sale. All samples were placed in separate sterile plastic bags to prevent spilling and cross contamination and were immediately transported to the laboratory in a cooler with ice packs and all raw chickens were sampled on the day of purchase. Mint and Onion were purchased from the local market and prepared for extraction.

2.2.Isolation and identification of bacterial isolates

2.2.1. Isolation

Samples were chilled stored and examined within 24 h., 25 g of each sample was homogenized in 225 ml of Preston broth (nutrient broth no. 2 CM0067B, *Campylobacter* selective supplement SR0117E and lyzed horse blood SR0048, Oxoid) used a Stomacher (Stomacher 400, Lab. Blender, London, UK) . Incubation of Preston broth for 17 to 19 h at 42 °C took place under microaerophilic conditions by using (Campygen, CN0025 or CN0035, Oxoid) and then made a serial dilutions in Peptone Water (CM0009B, Oxoid). Then, 0.1 ml of each serial dilution was streaked onto an mCCDA plate (modified Charcoal Deoxycholate Agar, *Campylobacter* blood-free selective medium, CM0739 and CCDA selective supplement, SR0155, Oxoid) and was incubated for 24 to 72 h at 42 °C in a microaerophilic atmosphere conditions (Ghafir *et al*., 2007).

2.2.2. Identification method

One presumptive *Campylobacter* colony from each selective agar plate was subcultured and tested by standard microbiological and biochemical procedures**,** differentiated at species level by Gram stain, oxidase and catalase activities, hippurate hydrolysis, hydrogen sulfide production and susceptibility to nalidixic acid by using a commercially available species differentiation kit (API CAMPY, bioMérieux, Marcy-l'Etoile, France).

2.3. Preparation of plant extract

Two hundred and seventy grams of fresh white onion were peeled, chopped and macerated with (methanol 70%) during 72 h at room temperature. Extract was filtered and sterilized using a 0.45 µm pore size cellulose acetate membrane filter (Cole-Parmer-47 mm), then alcohol was evaporated under vacuum and concentrated extract stored at 4 ºC and used within few days.

Essential Oil (EO) of mint was obtained by hydro distillation of shade-dried *M. arvensis* leaves (200 g) with 1.4 l of distilled water (1:7 w/v). The distillate (1 l) was extracted twice with dichloromethane (5:1 v/v). The combined organic phases were dried with anhydrous sodium sulfate and filtered. Dichloromethane was evaporated in a rotary evaporator at 30 ºC, under reduced pressure. The EO was weighed, stored at 4 ºC in sealed ampoules and used within a few days (Freire *et al*., 2012).

2.4. Antimicrobial assay

The prepared plant extracts were screened *in vitro* for their antibacterial activity against *Campylobacter jejuni* by agar diffusion method (Baratta *et al*., 1998 and NCCLS, 1999). 0.5 ml suspension of *C. jejuni* was uniformly spread using sterile cotton swab on a sterile Petri dish

Blood agar media (No.2 CM0271B Oxoid) supplemented with lyzed horse blood (SR0048, Oxoid). Fifty µl of plant extracts dissolved in dimethyl sulfoxide (DMSO) were added to each well (7 mm diameter holes cut in the agar gel, 20 mm apart from one another). A hole filled with DMSO was also used as a control. The plates were left for 1 h at room temperature as a period of pre-incubation diffusion to minimize the effects to variation in time between the applications of the different solutions. The plates were incubated for 24 h at 37ºC, under microaerophilic conditions. After incubation, confluent bacterial growth was observed. Inhibition of the bacterial growth was measured in mm. Reference commercial discs were used Ciprofloxacin 5µl (CT0425B, Oxoid). Tests were performed in duplicate.

2.5. Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration (MIC) of the test compounds was determined by agar diffusion method (Baratta *et al*., 1998 and NCCLS, 1999). Solutions of the plant extracts were made using DMSO as the solvent. From this stock solution, concentrations from 2% till 0.07% of the tested plant extract solutions were poured inside the holes. A hole filled with DMSO was also used as a control. Plates were incubated at 37°C for 24 h. After incubation period, plates were observed for the growth of microorganisms. The lowest concentration of the plant extracts inhibited the growth of the given bacteria was considered as a minimum inhibitory concentration (MIC) of the test compounds against that bacteria.

2.6. Identification of the compounds in the crude EO

The volatile compounds were isolated, identified, and quantified on a Thermo Scientific ISQ Single Quadrupole GC/MS (THERMO Comp., USA), coupled with a THERMO mass spectrometer detector (GC-MS ISQ). The GC-MS system was equipped with a Trace GOLD TG-Wax MS GC Columns (30 m X 0.25 mm i.d., 0.25 µm film thicknesses).

Analyses were carried out using Helium as carrier gas at a flow rate of 0.5 ml/min at a split ratio of 1:10 and the following temperature program: 40ºC for 1 min, rising at 4.0ºC /min to 150ºC and held for 6 min, rising at 4ºC /min to 200ºC and held for 1min. The injector and detector were held at 200 and 200ºC, respectively. Diluted samples (1:10 Hexane, v/v) of 0.2 µl of the extracts were always injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 45-450. Most of the compounds were identified using analytical methods: mass spectra (authentic chemicals and Wiley spectral library collection).

2.7. Decontamination treatment with mint essential oil and onion extraction

Inoculated chicken legs were treated with 2% EO of mint or onion extractions for 5 min, separately. Meat samples were dipped in the stomacher bag containing 250 ml of 2% extraction solution, with maximum 2 cuts consequently submerged into one batch of the solution, and afterwards let drain for five seconds. Immediately after the treatment, chicken legs were packed in plastic food trays and stored at 4 °C, for 7days.

2.8. Microbial analysis

The legs used for the artificial inoculation with *C. jejuni* were placed in plastic bags at 4 ºC and inoculated with *C. jejuni* by dipping into the working culture (10^6 CFU/ml) for 2 min. A maximum of one leg was consecutively submerged into one batch of 200 ml of the working culture. Previously tested enumeration of *C. jejuni* in the working culture, before and after inoculation, showed that there were no significant changes in the number of *C. jejuni* cells in the working culture. Cells were allowed to adhere to the surface of carcasses for 1 h at 4°C before decontamination treatment. Twenty five grams of the sample were homogenized in 225 ml of Preston broth (nutrient broth no. 2 CM0067B, *Campylobacter* selective supplement SR0117E and lyzed horse blood SR0048, Oxoid) using a Stomacher (Stomacher 400, Lab. Blender, London, UK). Incubation of Preston broth was took place for 17 to 19 h at 42°C under microaerophilic conditions by using (Campygen, CN0025 or CN0035, Oxoid) and then a serial dilutions in Peptone Water (CM0009B, Oxoid) were prepared. Then, 0.1 ml of each serial dilution was streaked onto an mCCDA plate and incubated at 42 °C for 24 to 72 h in a microaerophilic atmosphere condition. Confirmation of presumptive *C. jejuni* colonies was made by microscopic observation (Rajkovic *et al*. 2010). Total viable counts (TVC) aerobic and anaerobic were determined using Plate Count Agar (PCA, Merck, Darmstadt, Germany) and AnaeroGen (AN0025A, Oxoid) for anaerobic atmosphere conditions after incubation for 48–72 h at 30 °C.

2.9. Chemical analysis

2.9.1. Thiobarbituric acid (TBA) measurement

The 2-thiobarbituric acid tests (TBA) were used to determine the extent of lipid oxidation in the samples. The method for analysis was described by Du and Ahn (2002).

2.9.2. Total volatile basic nitrogen (TVBN)

Total volatile basic nitrogen was isolated from sample by steam distillation as the method described by Erdawati (2010).

2.10. Statistical analysis

The effects of the treatment on the shelf life of chicken leg were tested statistically by analyzed microbial and chemical data using the general linear model procedure (SAS, 1998) and Duncan's range test (Duncan, 1955) for determination of the significance.

3. RESULTS

The presence of *Campylobacter* spp was assessed from raw chicken at retail level and it was found in whole chicken in leg parts. The presumptive *Campylobacter* colony from selective mCCDA plate appeared as shown in Fig. (1). Isolate was Gram negative rods, S-shaped and motile. The results of the biochemical test are shown in Table (1). According to the API compy tests the isolate was oxidase positive, catalase (CAT, positive) and was identified as *Campylobacter jejuni*.

The mint EO extraction gave the highest antimicrobial activity with an inhibition zone more than 20 mm against *C. jejuni*, while, onion gave active inhibition zones 16-19 mm. To confirm the last results the MIC values of the most active plant extracts for Mint (*Mentha arvensis*) and onion (*Allium cepa*) were determined. MIC data of both mint and onion are shown in Fig. (2). MIC of Mint against *Campylobacter jejuni* 2, 0.4, 0.2 0.16, 0.14 and 0.01%, expressed by inhibition zone diameter 26 , 26, 16, 16, 12 and 8 mm respectively. While, the MIC values for onion 2, 0.4, 0.2, 0.16 and 0.14%, expressed by an inhibition zone diameter 16, 16, 12, 12 and 8 mm respectively. The results obtained in the qualitative and quantitative analyses of mint are shown in Table (2). Results showed that, thirty constituents have been identified in mint oil. Carvone substances represent the highly percenage reached up to 56.87%. Also, both of D-limonene and Cineole gave 6.48% and 6.89%. While, 1-6-Dihydrocarveol, Carvyl acetate-E and Carveol substances gave 5%, 4.25% and 3.6%, respectively. On the other hand, five substances showed moderate levels, i.e. Caryophyllene (2.15%), Sabinene (1.95%), Dihydrocarryl acetate (1.73%), α-Bourbonene (1.47%) and α-pinene (0.79%). The rest of constituents (19 compounds) gave a concentration less than 0.66%. Total *C. jejuni* counts of chicken leg treated with 2% of mint EO and 2% of onion extract at 4°C storage temperature are shown in Fig. (3) and Table (3). The counts of *C. jejuni* for untreated chicken leg were 2133, 966, 73.3, 14.3 and 0.14 ($x10^3$ cfu/g) at 0, 1, 3, 5 and 7 days of refrigerated storage at 4°C, respectively. While, such counts of treated chicken leg with 2% mint EO were 0.11, 0.08, 0.03, ND and ND ($x10^3$ cfu/g) at 0, 1, 3, 5 and 7 days of refrigerated storage at 4°C, respectively. The counts of treated chicken leg with 2% onion were 21.67, 1.73, 0.10, 0.05 and 0.02 ($x10^3$ cfu/g) at 0, 1, 3, 5 and 7 days of refrigerated storage at 4°C, respectively. The activity of microorganisms is the most important factor limiting the shelf life of meat. Total viable aerobic and anaerobic bacterial counts ($x10^3$ cfu/g) of chicken leg treated with 2% of mint EO or 2% of onion extract at 4°C storage temperature are shown in Tables (4 and 5), respectively. The total viable aerobic bacterial counts of untreated chicken leg were 19.05, 138.038, 758.577, 18620.871 and 37153.523 ($x10^3$ cfu/g) at 0, 1, 3, 5 and 7 days of refrigerated storage at 4°C, respectively. The total viable aerobic bacterial counts of chicken legs treated with 2% mint EO were 0.069, 0.079, 0.089, 0.128 and 0.245 ($x10^3$ cfu/g) at 0, 1, 3, 5, and 7 days of refrigerated storage at 4°C, respectively. While, the total viable aerobic bacterial counts of treated chicken legs with 2% onion extract were 0.045, 0.047, 0.058, 0.061 and 0.079 ($x10^3$ cfu/g) at 0, 1, 3, 5 and 7 days of refrigerated storage at 4°C, respectively. The total viable anaerobic bacterial counts of untreated chicken legs were 0.069, 0.079, 0.128, 0.616 and 0.794 ($x10^3$ cfu/g) at 0, 1, 3, 5 and 7 days of refrigerated storage at 4°C, respectively. The total viable anaerobic bacterial counts of chicken legs treated with 2% mint EO were 0.044, 0.047, 0.049, 0.069 and 0.1 ($x10^3$ cfu/g) at 0, 1, 3, 5, and 7 days of refrigerated storage at 4°C, respectively. The total viable anaerobic bacterial counts of chicken legs treated with 2% onion extract were 0.023, 0.025, 0.027, 0.044 and 0.058($x10^3$ cfu/g) at 0, 1, 3, 5 and 7 days of refrigerated storage at 4°C, respectively. Table (6) illustrates the changes occurred in TBA values (mg malonaldehyde/Kg) of refrigerated chicken leg treated with 2% mint EO or onion extract stored at 4°C for 7 days. The TBA values of untreated sample were 0.16, 0.78, 1.50, 2.90 and 3.60 at 0, 1, 3, 5 and 7 days at 4°C, respectively and TBA values of chicken legs treated with 2% mint EO were 0.09, 0.10, 0.14, 0.20 and 0.22 at 0, 1, 3, 5 and 7 days at 4°C, respectively. While, The TBA values of chicken legs treated 2% onion extract were 0.08, 0.10,

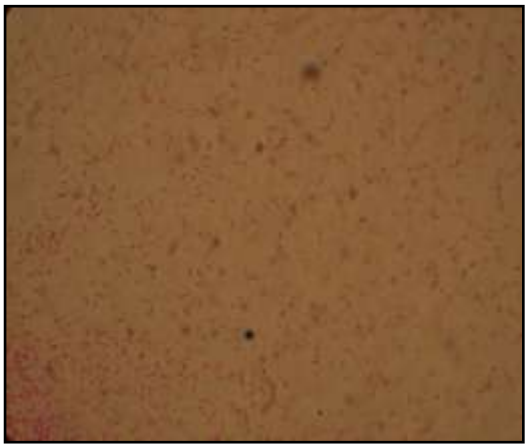

Fig. (1): Morphological properties of *Campylobacter jejuni* **grown on mCCDA and examined by light microscope (X100) after 24 hours.**

Table (1): Biochemical identification test for Campylobacter spp according to commercially available species differentiation kit (API CAMPY, bioMérieux, Marcy-l'Etoile, France).

URE	NIT	EST	HIP	GGT	TTC	PyrA	ArgA	AspA	PAL	H2S	GLU	SUT	NAL	CFZ	ACE	PEO	MLT	CIT	ERO	CAT
-	+	+	+	-	-	-	-	-	+	-		+	-	+	+	-	-	-	-	+

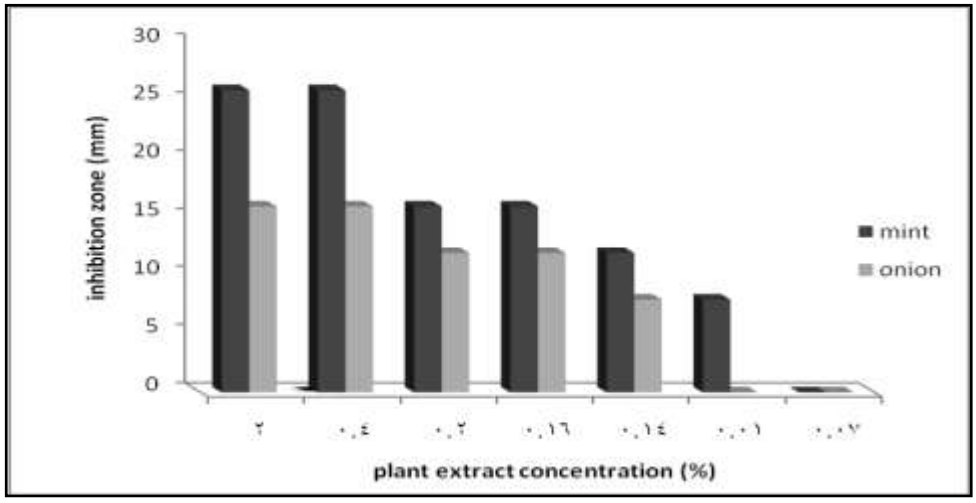

Fig. (2): Minimum inhibitory concentration (MIC) percentage of mint and onion extracts on *Campylobacter jejuni*.

Table (2): Main constituents of the essential oil for *Mentha arvensis* **as identified by GC/MS analysis.**

RT	Area%	Compound Name
5.43	0.79	α-pinene
5.81	1.95	Sabinene
6.85	0.53	B-Myrcene
7.62	6.48	D-Limonene
8.01	6.89	Cineole
9.70	0.27	O-Cymene
13.74	0.39	3-Octanol
15.50	0.27	1-Octen-3-ol
16.04	0.75	Terpineol-cis-á
16.28	0.40	Dihydroedulan II
16.95	1.47	α-Bourbonene
19.36	2.15	Caryophyllene
20.21	0.46	Terpinen-4-ol
20.56	0.84	p-Menth-8-en-2one-trans
21.58	0.91	á-Copaene
22.26	1.73	Dihydrocarryl acetate
22.77	0.34	Germacrene D
23.01	0.34	endo-Borneol
23.14	0.24	á-Terpineol
23.81	0.29	Neodihydrocarveol
24.59	56.87	Carvone
24.73	5.00	1-6-Dihydrocarveol
25.18	4.25	Carvyl acetate-E
26.32	0.55	Calamenene
27.05	0.79	cis-Carveol
27.87	3.65	Carveol
31.16	0.31	Caryophyllene oxide
33.78	0.41	Cubenol
36.85	0.29	Spathalenol
40.62	0.41	á-Cadinol

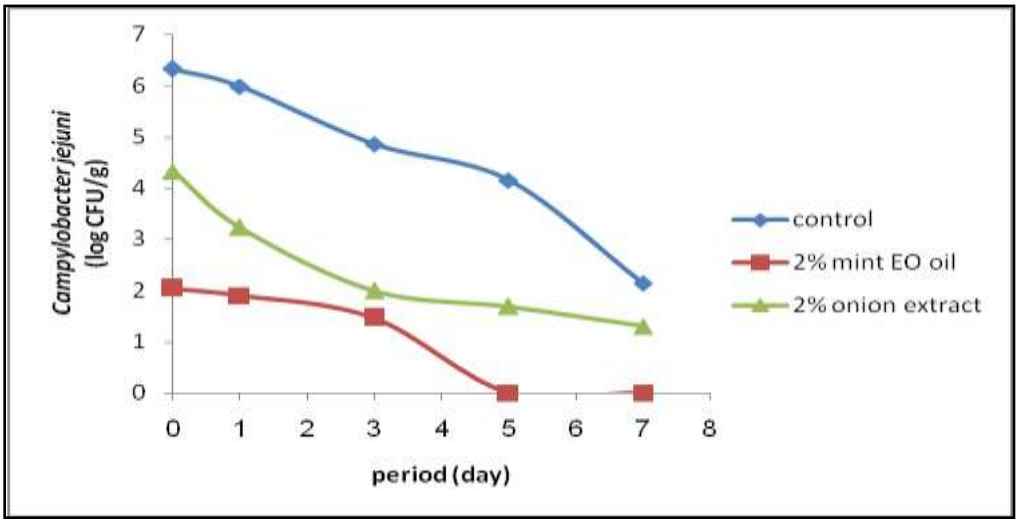

Fig. (3): *Campylobacter jejun***i counts enumerated in chicken legs treated with mint EO or onion extract and stored at 4◦C.**

Table (3): *Campylobacter jejuni* **counts (mean±SD) enumerated in chicken legs (x103 cfu/g) treated with mint EO or onion extracts and stored at 4◦C.**

Treatment	Storage period (days)				
	0	1	3	5	7
Control	2133^{a}±0.02	966^{a}±0.01	73.3^{a}±0.01	14.3^{a}±0.00	0.14^{a}±0.02
Mint EO 2%	0.11^{b}±0.04	0.08^{b}±0.2	0.03^{b}±0.02	ND^{b}	ND^{b}
Onion extract 2%	21.67^{c}±0.02	1.73^{c}±0.02	0.10^{c}±0.00	0.05^{c}±0.01	0.02^{c}± 0.00

Means in the same column for the same storage day with a different letter are significantly different (*P*<0.05).
ND= Not Detected.

Table (4): Total aerobic bacterial counts (mean±SD) enumerated in chicken legs (x103 cfu/g) treated with mint EO or onion extracs and stored at 4◦C.

Treatment	Storage period (days)				
	0	1	3	5	7
Control	19.054a±0.08	138.038a±0.04	758.577a±0.08	18620.871a±0.06	37153.523a±0.05
Mint EO 2%	0.069b±0.04	0.079b±0.05	0.089b±0.05	0.128b±0.06	0.245b±0.04
Onion extract 2%	0.045c±0.03	0.047c±0.00	0.058c±0.01	0.061c±0.02	0.079c± 0.05

Means in the same column for the same storage day with a different letter are significantly different (P<0.05).

Table (5): Total anaerobic bacterial counts (mean±SD) enumerated in chicken legs (x103 cfu/g) treated with mint EO and onion extracts and stored at 4◦C.

Treatment	Storage period (days)				
	0	1	3	5	7
Control	0.069a±0.04	0.079a±0.02	0.128a±0.00	0.616a±0.02	0.794a±0.00
Mint EO 2%	0.044b±0.01	0.047b±0.02	0.049b±0.00	0.069b±0.02	0.1b±0.00
Onion extract 2%	0.023c±0.02	0.025c±0.00	0.027c±0.04	0.044c±0.01	0.058c±0.00

Means in the same column for the same storage day with a different letter are significantly different at (P<0.05).

Table (6): TBA values (mg malonaldehyde/Kg) of chicken legs treated with mint EO or onion extracts during refrigerated stored at 4◦C for 7 days.

Treatment	Storage period (days)				
	0	1	3	5	7
Control	0.16a±0.01	0.78a±0.01	1.50a±0.00	2.90a±0.00	3.60a±0.1
Mint EO 2%	0.09b±0.00	0.10b±0.00	0.14b±0.02	0.20b±0.01	0.22b±0.01
Onion extract 2%	0.08c±0.00	0.10c±0.02	0.11c±0.00	0.15c±0.01	0.18c±0.01

Means in the same column for the same storage day with a different letter are significantly different (P<0.05).

Table (7): Total volatile bases nitrogen (mg/100 g) of chicken legs treated with mint EO or onion extract during refrigerated stored at 4◦ C for 7 days.

Treatment	Storage period (days)				
	0	1	3	5	7
Control	0.90a±0.01	1.70a±0.01	14.7a±0.2	28.3a±0.2	36.1a±0.01
Mint EO 2%	0.70b±0.00	0.90b±0.00	1.70b±0.1	2.40b±0.01	3.90b±0.01
Onion extract 2%	0.80c±0.00	0.80c±0.00	0.90c±0.00	1.20c±0.1	2.0c±0.01

Means in the same column for the same storage day with a different letter are significantly different (P<0.05).

0.11, 0.15 and 0.18 at 0, 1, 3, 5 and 7 days at 4°C respectively.

The changes in total volatile bases nitrogen (TVB-N) (mg N/100 g) during refrigerated storage of chicken legs treated with 2% mint EO or onion extract at 4°C are shown in Table (7). Results show that, (TVB-N) (mg N/100 g) values of untreated samples were 0.90, 1.70, 14.7, 28.3 and 36.10 at 0, 1, 3, 5 and 7 days at 4 °C, respectively and (TVB-N) (mg N/100 g) values of chicken legs treated with 2% mint EO were 0.7, 0.9, 1.70, 2.40 and 3.90 at 0, 1, 3, 5 and 7 days at 4°C, respectively. While, (TVB-N) (mg N/100 g) values of chicken legs treated with 2% onion extract were 0.80, 0.80, 0.90, 1.20 and 2.0 at 0, 1, 3, 5 and 7 days at 4 °C**,** respectively.

4. DISCUSSION

The obtained results of *Campylobacter* spp. in raw chicken and whole chicken especially in leg piece parts are in agreement with that reported by Corry and Atabay (2001). They found that, the major sources of human infection by *Campylobacter* spp are the handling and consumption of poultry and poultry products. Also, Suzuki and Yamamoto (2009) reported that, *Campylobacter jejuni* was the dominant species isolated from retail poultry. In spite of the limited data available on the effect of some medicinal plants on *C. jejuni.* Tyagi and Malik (2011), reported that, the antimicrobial efficacy of Mentha essential oils has been found to vary from moderate to significant often correlating with the composition of the oil. Also, Abdul Hannan *et al*. (2010) reported that onion has significant bioactive compound effect against *Campylobacter jejuni* and concluded that, onion alcohol extraction has significant antimicrobial effect against *Vibrio cholera*. Also, the results for MIC of two plants forward *C. jejuni* were in agreement with those reported by Babu *et al.* (2011) and Djenane *et al.* (2012) who recorded that, each of Cinnamon oil , Cloves oil and *Inula graveolens* oil had a MIC values mostly identical with the obtained results against *C. jejuni*. High percentage of Carvone revealed activity of such constituents against pathogenic bacteria. These results are in agreement with Aggarwal *et al.* (2002) and Vivek *et al.* (2009). They found that Carvone was the major component in *Mentha arvensis* and concluded that, the Carvone and Limonene had antimicrobial activity. Also, Gallucci *et al.* (2010) reported that Carvone was active against *Staphylococcus aureus* methicillin sensitive. The survival of *Campylobacter* spp. on chicken meat also may be an important factor for at-home contamination due to improper food handling. The results showed reduction of more than 2.0 (log cfu/g) of *C. jejuni* when treated with mint EO or onion extract at zero time of storage. Our results are in agreement with the data reported by Nauta *et al*. (2009) and Loretz *et al*. (2010) who reported that the reduction of *Campylobacter* by 2.0 logs on chicken meat might represent a reduction in *Campylobacter* risk *via* chicken meat consumption, as estimated by several risk assessments. It is clearly noticed that, at 3 days of storage at 4 °C, the total viable aerobic bacterial counts in the untreated sample (5.88 log cfu/g) exceeded the maximum acceptable levels (5.3 log cfu/g) according to E.O.S.(1988). By contrast, the total viable aerobic and anaerobic bacterial counts of chicken legs treated with 2%, of mint EO or onion extract were below the maximum acceptable levels even after 7 days. TBA value of untreated sample (control) sharply increased during the refrigerated storage at 4ºC from 0.16 to 3.60 (mg malonaldehyde/Kg) at the end of storage (day 7). While, samples treated with 2% of mint EO or onion extracts gradually increased to a lower extent, and did not exceed the maximum acceptable levels (2.4 mg malonaldehyde/Kg) according to E.O.S. (1988). The results revealed that, the TVBN of the untreated sample (control) was sharply increased during refrigerated storage (36.10 mg/100 g). While, TVBN of the treated chicken legs increased slowly and did not exceed the maximum acceptable levels (20 mg/100g) according to E.O.S. (1988).

It could be concluded that, the addition of 2% mint EO or 2% onion extracts resulted in reduction of *Campylobacter jejuni* counts and also improved the microbiological and chemical quality, and extended the shelf-life of raw chicken meat.

5. REFERENCES

Abdul Hannan A., Humayun T., Hussain M.B., Yasir M. and Sikandar S. (2010). *In vitro* antibacterial activity of onion (*Alluim cepa*) against clinical isolates of *Vibiro cholera.* J. Ayub. Med. Coll. Abbottabad, 22 (2): 160-164.

Aggarwal K.K., Khanuja S.P.S., Ahmad A. Santha Kumar T.R., Gupta V.K. and Kumar, S. (2002). Antimicrobial activity profiles of the two enantiomers of limonene and carvone isolated from the oils of *Mentha spicata* and *Anethum sowa*. Flavour and Fragrance Journal, 17(1):59-63.

Allos B.M. (2001). *Campylobacter jejuni* infections: update on emerging issues and trends. Clin. Infect. Dis., 32(8):1201–1206.

Babu A.J., Sundari1 A.R., Indumathi1 J., Srujan R.V.N. and Sravanthi, M. (2011). Study on the antimicrobial activity and Minimum Inhibitory Concentration of essential oils of spices. Veterinary World, 4(7): 311-316.

Baratta M.T., Dorman H. J.D., Deans S.G., Figueiredo A.C., Barroso J.G. and Ruberto G. (1998). Antimicrobial and antioxidant properties of some commercial essential oils. Flavour and Fragrance Journal, 13:235-244.

Botsoglou N.A., Grigoropoulou S.M., Botsoglou E., Govaris A. and Papageorgiou G. (2003). The effects of dietary oregano essential oil and a-tocopheryl acetate on lipid oxidation in raw and cooked turkey during refrigerated storage. Meat Sci., 65:1193-1200.

Corry J.E. and Atabay H.I., (2001). Poultry as a source of *Campylobacter* and related organisms. Symposium Series Society for Applied Microbiology, 96S–114S.

Djenane D., Yangüela J., Gómez D. and Roncales P. (2012). Perspectives on the use of essential oils as antimicrobials against *Campylobacter jejuni* CECT7572 in retail chicken meats packaged in microaerobic atmosphere. Journal of Food Safety, 32:37-47.

Du M. and Ahn D.U. (2002). Effect of antioxidants on the quality of irradiated sausages prepared with turkey meat. Poultry Science, 81: 1251-1256.

Duncan D.B. (1955). Multiple ranges and multiple F test. Biometerics, 11:1-42.

E.O.S. (1988). The Egyptian Organization for Standardization and Quality Control. Chilled Chicken. (1651).

Erdawati B. (2010). Development of chitosan-nanoparticles film based materials for controlled quality of minced beef during refrigerated storage. The Third Nanoscience and Nanotechnology Symposium. Abdullah, M. and Khairurrijal (Eds), Jakarta. pp.172-178.

Freire M.M., Jham G.N., Dhingra O.D., Jardim, C.M., Barcelos R.C. and Valente V.M. (2012). Composition, antifungal activity and main fungitoxic components of the essential oil of *Mentha piperita* L. Journal of Food Safety, 32:29-36.

Gallucci N., Oliva M., Carezzano E., Zygadlo J. and Demo M. (2010). Terpenes antimicrobial activity against slim producing and non–producing staphylococci. Molecular Medicinal Chemistry, 21:132-136.

Ghafir Y., China B., Dierick K., De Zutter L. and Daube G. (2007). A seven-year survey of *Campylobacter* contamination in meat at different production stages in Belgium. International Journal of Food Microbiology, 116:111–120.

Loretz M., Stephan R. and Zweifel C. (2010). Antimicrobial activity of decontamination treatments for poultry carcasses: a literature survey. Food Control, 21:791-804.

Nachamkin I., Blaser M. and Tompkins L. (1992). *Campylobacter jejuni*: Current Status and Future Trends. Washington, DC, USA: ASM Press.

Nauta M. J., Van derwal F. J., Putirulan F. F., Post J., Van de Kassteele J. and Bolder N. M. (2009). Evaluation of the testing and scheduling strategy for control of *Campylobacter* in broiler meat in The Netherlands. International Journal of Food Microbiology, 34:216-222.

NCCLS-National Committee for Clinical Laboratory Standards. (1999). Methods for Determining Bactericidal activity of antimicrobial agents. Approved Guideline M26-A. National Committee for Clinical Laboratory Standards, Wayne, Pa. 320 p.

Rajkovic A., Tomic N., Smigic N., Uyttendaele M., Peter Ragaert P. and Devlieghere F. (2010). Survival of *Campylobacter jejuni* on raw chicken legs packed in high-oxygen or high-carbon dioxide atmosphere after the decontamination with lactic acid and sodium lactate buffer. International Journal of Food Microbiology, 140:201-206.

Rao M., Naficy A., Savarino S., Abu-Elyazeed R., Wierzba T. and Peruski L., (2001). Pathogenicity and convalescent excretion of *Campylobacter* in rural Egyptian children. American Journal of Epidemiology, 154(2):166-173.

SAS (1998). SAS User's Guide, SAS Institute Inc., Cary, NC., USA.

Smith J. (2002). *Campylobacter jejuni* infection during pregnancy: Long-term consequences of associated bacteremia, Guillain-Barre syndrome, and reactive arthritis. Journal of Food Protection, 65(4):696–708.

Suzuki H. and Yamamoto S. (2009). A review-*Campylobacter* contamination in retail poultry meats and by-products in Japan. A literature survey. Food Control. 20:531–537.

Tyagi A.K. and Malik A. (2011). Antimicrobial potential and chemical composition of *Mentha piperita* oil in liquid and vapor phase against food spoiling microorganisms. Food Control, 22:1707-1714.

Vivek S., Nisha S., Singh H.S., Devendra S.K., Vijaylata P., Bikram S. and Raghbir G.C. (2009). Comparative account on GC-MS analysis of *Mentha arvensis* L. corn mint. from three different locations of north India. INT. J.DRUG DEV. and RES., 1(1):1-9.

Wingstrand A., Neimann J., Engberg J., Nielsen E.M., Gerner-Smidt P., Wegener H.C. and Molbak K. (2006). Fresh chicken as main risk factor for campylobacteriosis, Denmark. Emerging Infectious Diseases 12:280-285.

Zaki A., DuPont H., Alamy M., Arafat R., Amin K. and Awad, M. (1986). The detection of enteropathogens in acute diarrhea in a family cohort population in rural Egypt. American Journal of Tropical Medicine and Hygiene, 35(5):1013-1022.

تأثير مضادات الميكروبات المستخلصة من بعض النباتات العطرية
على ميكروب الكمبيلوباكتر في لحوم الدجاج

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ملخص

بدراسة تواجد ميكروب *Campylobacter* spp. بالدجاج الكامل أو المجزأ خاصة فى الأجزاء الخلفية والمعروض محليا بالاسواق وجد انه يتبع سلالة *Campylobacter jejuni* .

كما وجد أن مستخلص زيت النعناع أعطي أعلى نشاط كمضاد لنمو هذه السلالة حيث أعطى منطقة خالية من النمو قطرها يزيد عن 20 ملليتر ، و أعطى مستخلص البصل منطقة قطرها 16 الى 19 ملليتر، وقد ثبت أيضا أن أدنى التركيزات المثبطة لهذا الميكروب من النعناع والبصل هو 0.01% ، 0.05 % على التوالي.

ولهذا تم تحليل مستخلص زيت النعناع باستخدام طريقة Gas chromatography-mass spectrometry وكانت أهم المركبات التى تم التعرف عليها هى: كارفون (56.87٪)، سينول (6.89٪)، D-الليمونين (6.48٪)، 1-6-Dihydrocarveol، Carvyl خلات-E (5٪) وCarveol المواد (4.25٪).

ولهذا تم اختبار تاثير كل من مستخلص زيت النعناع ومستخلص البصل على أجزاء الدجاج الخلفية والمخزنة لمدة سبعة ايام على 4 درجة مئوية بعد تلقيحها بميكروب *C. jejuni* تركيزه 10^6 خلية ميكروبية/جرام لحم. وتم تقدير عدد البكتريا موضع الدراسة مع مقارنتها بالعينات غير المعاملة بالمستخلص حيث ان الاخيرة وجد بها 2,14 لوغارتم خلية ميكروبية / جرام لحم بينما لم يتم وجود أى اعداد لهذا الميكروب فى العينات المقابلة بمستخلص زيت النعناع . كما أعطى الدجاج المعامل بمستخلص البصل والمخزن على 4 درجة مئوية أعداد تصل الى 1.3 لوغارتم خلية ميكروبية / جرام لحم بينما أعطت البكتريا الكلية الهوائية أعداد تصل الى 5.88 لوغارتم خلية ميكروبية لكل جرام لحم فى الدجاج غير المعامل بعد ثلاثة ايام من التخزين على 4 درجة مئوية. وتعتبر هذه الأعداد أعلى من الحد المسموح به بالتواجد فى الدجاج والمقدر ب 5.3 لوغارتم خلية ميكروبية لكل جرام لحم

على الجانب الأخر كانت أعداد البكتريا الكلية الهوائية واللاهوائية فى أجزاء الدجاج والمعاملة بكل من مستخلص زيت النعناع ومستخلص البصل أقل من الحد المسموح به فى الدجاج حتى اليوم السابع من التخزين. كما اثبتت قيم قياسات Thiobarbituric acid فى العينات غير المعاملة زيادة حادة خلال التخزين على 4 درجة مئوية بحيث زادت من 0.16 الى 3.6 ملليجرام لكل كيلو جرام دجاج فى نهاية خلال فترة التخزين. بينما أعطت العينات المعاملة ب 2% من كل من مستخلص زيت النعناع أو مستخلص البصل زيادة تدريجية اقل من المسموح به وهو 2.4 ملليجرام لكل كيلو جرام لحم.

كما أشارت النتائج المتحصل عليها أيضا أن volatile basic nitrogen الكلى فى العينات غير المعاملة إزداد بصورة حادة خلال فترة التخزين حيث وصل الى 36.1 ملليجرام لكل 100 جرام دجاج. بينما أعطى TVBN فى عينات الدجاج المعامل بالمستخلصات زيادة طفيفة حيث لم تزد عن الحد المسموح به وهو 20 ملليجرام لكل كيلو جرام لحم.

وعليه فأن النتائج المتحصل عليها من المعاملة ب 2% من مستخلص زيت النعناع أو مستخلص البصل تخفض أعداد ميكروب *Campylobacter jejuni* وأيضا تحسن الخواص الكيميائية للدجاج مما زاد من فترة حفظ الدجاج.