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EVALUATION OF THE ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY OF CORIANDER ESSENTIAL OIL AND SEED EXTRACT AND APPLICATIONS IN PRESERVING FRESH REFRIGERATED CHICKEN MEAT.

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ABSTRACT

Food borne pathogens and oxidation processes represent a serious spoilage factors affecting consumers health and economy. The objective of the present study was to evaluate and compare the antimicrobial and antioxidant activity of coriander essential oil (CEO) and seeds extract (SE) in vitro and studying the effect of applying CEO at the acceptable levels (1 and 2 ml/100gm) on the antioxidant activity and E.coli count of fresh skinless chicken breast meat during refrigeration. Results revealed that CEO is superior than SE in all tested parameters. GC mas analysis distinguished several organic compounds of CEO known for its antibacterial activity such as α-Pinene (8.01%), 3- Carene (0.94%), β-Pinene (7.46%), D-Limonene (4.9%), camphor (5.53), and its antioxidant activity such as γ-Terpinene (3.05%), Carveol (8.69%). The total phenolic compound and total antioxidant activity of CEO (9.84 g GAE /L and 140g AAE /L respectively) were higher than those of SE (7.3 g GAE /kg and 49.6 g AAE/kg). Also the antibacterial activity of CEO was higher than that of SE in all tested bacteria. The MIC of the CEO were ordered *Staphylococcus aureus* and *Enterococcus faecalis* (0.0312% (v/v) <*Bacillus cereus* (0.25) < *Pseudomonas aeruginosa* (0.75) <*Escherichia coli* and *Salmonella typhimurium* (1%). The DPPH scavenging activity of poultry meat treated with CEO at concentration

1and 2ml/100g has no significant (*P>0.05*) differences compared to nitrite treated group during 9 and 11 days respectively, indicating that the CEO (2ml/100g) has the same antioxidant effect as well as the synthetic preservative nitrite. The CEO treated chicken meat at both concentrations (1and 2ml/100g) showed significantly (*P<0.05*) lower *E.coli* count compared to positive control indicating more preserving efficacy than nitrite. Overall, the results of this study indicate that coriander essential oil with its antioxidant and antimicrobial activity can be applied in fresh chicken breast meat to improve its safety during storage besides its nutritional value which guarantees healthy food.

Key words: antibacterial, antioxidant, chemical component, chicken meat, coriander, *E.coli* count, essential oil, seed extract, , sensory evaluation.

INTRODUCTION

Pathogenic and food spoilage bacteria have been considered as the primary causes of food-borne diseases and food loss, resulting in negative effect in economic resources of any country **(Mith *et al.*, 2014)**

Poultry meat production and consumption is steadily increasing worldwide **(Recanati *et al.*, 2015),** hence the microbial safety of poultry meat products is an important issue in the context of increasing consumption and production.

The bacteria from animal microbiota, fecal residue from the gut of birds, the slaughterhouse environment, and the equipment used contaminate carcasses, their subsequent cuts, and processed meat products. Some of these bacterial contaminants can grow or survive during food processing and storage. Their growth and metabolic activity during shelf life leading to colour, odour, taste, or texture defects which means spoilage and losses of food products. (**Rouger *et al.*, 2017).** Of importance is the coliforms especially *Escherichia coli* and *Salmonella* have been described as the leading causes of food-borne illnesses worldwide **(Adeyanju and Ishola, 2014).**

Oxidation in lipid and protein fractions of meat has been demonstrated as the main, non-microbial cause of quality deterioration leading to economic problems in the meat industry. It compromises the nutritional quality, limits shelf life, increases toxicity and

decreases the market value of meat and meat products. Lipids and proteins in meat are easily susceptible to oxidative damages due to the rapid depletion of endogenous antioxidants after slaughter. However, the rate and extent of oxidation can be retarded, reduced or prevented through the application of natural antioxidant **(Falowo *et al*., 2014).**

Physical, chemical or biological processes have been widely applied in food industries to guarantee the food safety and to extend the shelf life of food products This imply a wide range of food grade chemicals has been added during food manufacture to extend shelf-life by stabilizing chemical change or by preventing or inhibiting microbial growth, yet food safety issues remains **(Mith *et al*., 2014).**

Recently, because of greater consumer awareness and concern regarding synthetic chemical additives, consumers increasingly seek for natural and healthier products. In addition, consumers are used to the presence of herbs and spices commonly added to provide flavor and aroma in poultry meat. Therefore, essential oils or plant extracts can be considered as a good choice of natural alternative preservatives **(Aziz and Karboune, 2016).**

Essential oils containing bioactive compounds were reported in many plants to have antioxidant activity and antimicrobial activity against food-borne pathogens and food spoilage bacteria. In addition, supplementing meat with medicinal plants rich-antioxidants can act as functional or nutraceutical food to promote consumers' health and wellness compared to the use of synthetic antioxidants **(Falowo *et al*., 2014).** This added health and nutritional benefit could be a distinctive advantage of natural antioxidants applied to meat processing.

Coriander (*Coriandrum sativum* L.) is a well-known herb widely used in folk medicine, in the pharmacy and as a spice. The C. sativum essential oil and extracts possess promising antibacterial, antifungal and anti-oxidative activities as various chemical components in different parts of the plant play a great role in maintaining the shelf-life of foods by preventing their spoilage **(Mandal and Mandal, 2015).** In addition, the potent antioxidant property of the C. *sativum* provides a key mechanism behind its protective effects against neurodegenerative diseases, cancer, and metabolic syndrome. These nutritional and therapeutic values along with its integration in daily life render the coriander a distinguished functional food of interest **Prachayasittikul *et al*., (2018)**.

Several studies have reported the bioactivity of coriander essential oil or seed extract separately and also a few studies were concerned with their application in meat products to provide safety food. The aim of this study was to investigate and compare chemical composition, antioxidant and antibacterial activity against food born bacteria of coriander essential oil (CEO) and seed extracts (SE) *in vitro* to determine which one is more efficient. Hence, studying the effect of applying CEO at the sensory acceptable levels on the antioxidant activity and *E.coli* count of fresh chicken meat during refrigeration.

MATERIALS AND METHODS

1. Plant Samples:

Coriander seeds and essential oil (CEO) were grade purchased from local market in Giza, Egypt.

2. Chemicals:

All the chemicals used were of analytical grade which were purchased from Sigma Chemical Co.

3. Preparation of ethanolic extract (SE):

Coriander seeds were cleaned from solid materials and finely grounded by electric grounder and stored in plastic bags at 4ºC. 100 grams of coriander seeds powder were macerated in 300 ml of ethanol (99%) overnight at room temperature, followed by 3 hour on magnetic stirring and macerated again overnight then filtered. The residue was mixed with 200ml ethanol and filtered. The filtrate was collected to the previous extract and dried at room temperature (35 ºC) in dark and for 2hr at 40ºC. The ethanolic extract was collected and stored at refrigerator.

4. Gas chromatography-mass (GC-mass-mass) identification for CEO and SE:

The analysis was carried out using a GC (Agilent Technologies 7890A) interfaced with a mass- selective detector (MSD, Agilent 7000) equipped with an polar Agilent HP-5ms (5%-phenyl methyl poly siloxane) capillary column (30m × 0.25 mm I.D. and 0.25 µm film thickness). The carrier gas was helium with the linear velocity of 1ml/min.

5. Determination of total phenolic Contents (TPC):

Contents of total phenolics of coriander CEO and SE were estimated spectrophotometrically using the Folin–Ciocalteu assay **(Singleton and Rossi, 1965).** 0.3 mL [1mg/mL] of sample was combined with Folin–Ciocalteu reagent [1.5 ml; diluted 10 times] and sodium carbonate [1.2 ml; 7.5% w/v]. Samples were incubated for 30 min at room temperature, and then the absorbance was measured at 765 nm. A standard curve was plotted using different concentrations of Gallic acid. The absorbance obtained was converted to gallic acid equivalent (GAE) using gallic acid standard curve.

6. Determination of total flavonoid content (TFC):

The total flavonoid content was determined using the Dowd method as adapted by **Arvouet-Grand *et al*. (1994).** 5 mL of 2% aluminium trichloride ($AlCl_3$) in ethanol was mixed with the same volume of the sample solution (1 mg/mL). Absorption readings at 415 nm using spectrophotometer were taken after 10 minutes against a blank sample consisting of a 5 mL extract solution with 5 mL ethanol without $AlCl_3$. The total flavonoid content was determined using a standard curve with quercetin (0-50mg/L) as the standard. Total flavonoid content is expressed as quercetin equivalent (QE).

7. Determination of total antioxidant activity (TAA):

The total antioxidant activity of coriander CEO and SE was determined using the phosphomolybdenum method according to the procedure described by **(Prieto *et al.*, 199**9). Each sample solution [0.1 ml, 0.5 mg/ml] was combined with 0.3 ml of reagent solution [0.6 mol/L sulphuric acid, 28 mmol/L sodium phosphate and 4 mmol/L ammonium molybdate]. The reaction mixture was incubated at 95°C for 90 min. After the mixture had cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank using a spectrophotometer UVD-3500. The antioxidant activity was expressed as ascorbic acid equivalent (AAE).

8. Sensory evaluation:

The sensory evaluation included the odour, softness, juiciness, taste, color as well as general acceptance of cooked chicken meat. Four treatments and control were tested. All treated with chopped onion and chicken spices. The control was without coriander essential oil and the four other treatments were with CEO at ratio 1, 2,4or 5 ml/

100gm. All treatments were cooked in electric roaster for 12 min in the form of 15 g chicken meat pieces. Evaluation was performed by 20 persons. Samples served in random sequence with white bread and water were provided after each sample to cleanse the palate. The characteristics of the evaluated samples were compared with those parameter listed on a previously compiled table and grades were awarded accordingly.

9. Susceptibility test and minimum inhibition concentration (MIC):

The Susceptibility Tests were performed according to NCCLS recommendations **(National Committee for clinical laboratory Standards, 1993)**. Screening tests regarding the inhibition zone were carried out by the well diffusion method **(Hindler *et al*., 1994).** The inoculum suspension was prepared from colonies grown overnight on an agar plate, and inoculated into Mueller-Hinton broth. A sterile swab was immersed in the suspension and used to inoculate Mueller-Hinton agar plates. The CEO and ES were dissolved in dimethyl sulfoxide (DMSO) with ratio 1:1 to test the susceptibility to each microorganism. For MIC of ECO, serial dilutions (with DMSO) below the sensory accepted ratio (2%) were tested. Well diameter was 6mm and 100µl was tested. The inhibition zone was measured around wells after 24h at 37^{o}C. Control using DMSO was adequately done. Ampicillin 20mg/ml or Propionic acid 10% were used as positive control.

10. Application of CEO on breast chicken meat:

Fresh raw skinless chicken breast meat was purchased from a local market in Egypt and was transferred to the laboratory within 1 h of slaughtering. Chicken breast meat was cut into small pieces about 5gm each. The samples were divided into 4 groups and with three replicates per group. The samples were subjected to the following treatments:

Group C: negative control without any preservative.

Group N: positive control treated with 120 mg/kg $NaNO_2$ (the permissible limit according to Codex General Standard for Food Additives **(Codex GSFA, Rev. 2018).**

Group TH: treated with CEO (2 ml/100gm).

Group TL: treated with CEO (1 ml/100gm).

After treatment, samples were left for 1 min to drip and were then separately packaged in sterile bags and stored at refrigerator. Samples were analyzed on storage day 0, 2,4,7,9 and 11 for antioxidant activity and other samples on day 0, 7 and10 for E.coli count.

a. DPPH free radical scavenging activity of fresh chicken meat:

Each fresh chicken meat (5 gm) in distilled water (15 ml) was homogenized for 2min. 9ml of chloroform was added and the mixture was shaken vigorously 2 min to separate the lipids. DPPH free radical scavenging activity was estimated in the aqueous supernatant. According to **Jang *et al*., (2008),** 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity was estimated by spectrophotometric method. The solution of DPPH in methanol (200 μM) was freshly prepared. 1ml of tested sample was added to 1ml of methanolic solution of DPPH. A control sample contained distilled water instead of sample was prepared. After 30 min at room temperature in dark, the absorbance of the samples was measured at 517 nm using UV/Vis spectrophotometer. The percentage of DPPH radical scavenging was calculated as: [1-(absorbance value of sample/absorbance value of control)] x100. The measurements were triplicated and their scavenging effects were calculated based on the percentage of DPPH scavenged.

b. Total E.coli colonies counts:

To determine the *E. coli* in samples, the assay was developed using pour plate method. 1 ml from serial dilutions containing viable microorganisms was plated onto E. coli Chromo Select Agar media. The plates were incubated for 48 h at 37°C, the colonies were then counted and the average of two replicates from the same dilution was calculated directly by colony forming unit (CFU/g) **(Frampton *et al*., 1988).**

11. Statistical analysis

The data were expressed as means ± standard error (SE). The data were subjected to analysis of variance (ANOVA) using the CoStat software to determine the significance at level ($P<0.05$). The differences were classified by Duncan multiple comparison test **(Gmez and Gomez, 1984)**

RESULTS AND DISCUSSION

1. Composition of the essential oil:

The composition of the essential oil and seed extract determined by gas chromatography (GC/MS) are presented on **Table (1)** as relative percentage of the total chromatogram area. Twenty eight compounds were identified in the essential oil. The most abundant chemical compounds aaccording to the percent area sum were linalool oxide (18.36%) followed by terpinolene (9.07 %). **Michalczyk *et al*, (2012)** reported linalool as the abundant component in essential oil of coriander. The % area sum of α-pinene (8.1%) and D-limonene (4.99%) was similar to that reported by **Tsagkli *et al*., (2012)** (5.5-9.3% and 4.7-6.3%, respectively) in Romania while there are variation in the % area sum of other component.

On the other hand, 27 compounds were identified in the seed extract. The most abundant chemical compounds found according to the % area sum were 13-Octadecenal (32.87%) and 4',6-Dimethoxy-isoflavone-7-O-β-D-glucopyranoside (15.79%) and Dihydro-3-oxo-β-ionol (7.13%).

The variation of the chemical compositions of essential oils and plant extract may be affected by environmental factors such as seasonal and climate variations, geographical conditions, and growth stages of the plant in addition to the genotype of the same plant species **(Jaradat *et al*, 2017).**

CEO contain several compounds such as α-Pinene (8.01%), 3-Carene (0.94%), β-Pinene (7.46%), D-Limonene (4.99%), camphor (5.53), which reviewed as antimicrobials and γ-Terpinene (3.05%), Carveol (8.69%) as antioxidant that extending the shelf life of products (**Aziz and Karboune, 2016).** In addition, α- terpineol (1.04%) , γ-terpinene (3.05%) found in CEO in the present result have been reported by **Oyedemi *et al*., (2009)** to have bactericidal effect against both gram positive and gram negative bacteria including *E.coli* by disrupting their outer membrane**.** On the other hand, Quercetin7,3'4'- trimethoxy (3.07%) and 3,4,5-Trimethoxy cinnamic acid (1.49%) found in SE are derivatives of Quercetin and cinnamic acid which reported as antioxidant compound (**Aziz and Karboune, 2016).**

Table (1): Phytochemical compounds of coriander CEO and SE

Phytochemical compounds	%Area sum		Phytochemical compounds	%Area sum	
	CEO	SE		CEO	SE
α-Pinene	8.01	Nd	geranyl-1-terpinene	0.41	Nd
3- Carene	0.94	Nd	Farnesol	0.21	0.61
Trans-β-Ocimene	1.41	Nd	Cis-13-Eicosenoic acid	Nd	0.73
β-Pinene	7.46	Nd	Ethyl linoleate	0.7	1.07
γ-Terpinene	3.05	Nd	Linoleic acid	0.24	0.5
Carveol	8.69	Nd	4'-Benzyloxy-5,7-dimethoxyflavone	Nd	4.78
Terpinolene	9.07	Nd	4',6-Dimethoxyisoflavone-7-O-β-D-glucopyranoside	0.23	15.79
D-Limonene	4.99	Nd	Elaidic acid	0.18	5.05
(+)-2-Carene	4.98	Nd	(E) -2- Hexadecenal	Nd	0.51
3-thujanol	2.2	Nd	Dihydro-3-oxo-β-ionol	Nd	7.13
Verbenol	7.93	Nd	13-Octadecenal,(z)-	Nd	32.87
Linalool oxide	18.36	Nd	Arachidonic acid	Nd	0.63
Isopulegol	2.8	Nd	Quinine	Nd	0.39
camphor	5.53	Nd	Cis-Trismethoxyresveratrol	Nd	0.86
Geranyl vinyl ether	1.19	Nd	3,5,7-Trihydroxy-3',4',5'-trimethoxyflavone	Nd	3.75
terpineol	1.04	Nd	3,4,5-Trimethoxy cinnamic acid	Nd	1.49
8-Hydroxylinalool	0.79	Nd	Vitexin	Nd	0.43
(R)-lavandulyl acetate	2.04	Nd	Heptacosane	Nd	1.9
Decanoic acid	1.26	Nd	6,2',3'-Trimethoxyflavanone	Nd	0.87
Nerol	2.91	Nd	Phytanic acid	Nd	2.52
Cis-Vaccenic acid	1.16	0.91	2-Decanol	Nd	2.78
Quercetin7,3'4'-trimethoxy	Nd	3.07	Cholesta-5,7-dien-3-ol, (3β)-	Nd	3.09
Thunbergol	Nd	2.07	2-Butenedioic acid, 2-methyl-, (E)-	Nd	2.01
Phytol	0.65	1.68	D-Amygdalin	Nd	2.49

Nd: Not detected

2. Total phenols, Total flavonoid and Total antioxidant capacity:

In the present work, the antioxidant properties of CEO and SE were evaluated through determining their total antioxidant capacity, total phenolic content and total flavonoids (**Table 2**). Total antioxidant capacity is a better way of depiction of combined effect of phenols, flavonoids and other reducing compounds in the plant extracts and is expressed in terms of ascorbic acid equivalents (AAE) **(Kumar *et al.*, 2014).**

Table (2) Total phenols, Total flavonoid and Total antioxidant capacity of Essential Oil (CEO) and Seed Extract (SE) of Coriander

	TPC	TFC	TAA
Coriander Essential oil CEO	9.84 ± 0.002 (gm GAE /L)	0.0	140±0.005 (gm AAE /L)
Coriander seed extract SE	7.3 ±0.001 (gmGAE /kg)	4.180± 0.004 (gm QE/kg)	49.6±0.001 (gm AAE/kg)

From **Table 2**, it is clear that TAC is higher than that of SE. This may be attributed to the high total phenolic content of CEO than SE, where TPC could be used as an important indicator of the antioxidant capacities which may be used as a preliminary screen for plant extract when intended to be used as natural sources of antioxidants in functional foods **(Viuda-Martos *et al.*, 2009)**. Phenolic compounds play an important role in stabilizing lipid peroxidation **(Subhadradevi *et al.*, 2010).** The TPC of CEO in the present result (9.84g GAE /L) is much more than that reported by **Alves-Silva *et al* (2013) and Wangesteen *et al.* (2004)** (52.3 mg GAE/L, 0.14 g GAE/100 g, respectively). On the other hand **Farah *et al.*, (2015)** reported almost the same phenolic content of SE (7.2gGAE/kg) as the present result (7.3 GAE/kg). The phenolic and terpenoid compounds present in the chemical composition of essential oils are closely associated with their antioxidant function, mainly due to their high redox potential exerted by various possible mechanisms: free radical-scavenging activity, hydrogen donors, transition metal chelating activity, and/or singlet oxygen quenching capacity **(Alves-Silva *et al.*, 2013).** In agreement with the present results, CEO has been reported to possess high iron chelating activity in Ferrous ion-chelating ability assay (94%) **(Alves-Silva *et al.*, 2013).** Flavonoid are non-volatile compounds content

represent a class of the phenolic compounds based on the aromatic ring structures and have antioxidant activity **(Li *et al*., 2018).** Flavonoids presented in the present results only in the seed extract and not in the CEO. This high phenolic content along with antioxidant activity of both CEO and SE provides maintenance of the physiological functions of all body systems and contribute in prevention of several diseases **(Wilson *et al*., 2017)**.

3. Antibacterial activity of CEO and ES

The results of antibacterial activity of coriander essential oil (CEO) and seed extract (SE) are presented in **Table 3**. CEO and ES were tested against three Gram positive (*Staphylococcus aureus, Streptococcus faecalis and Bacillus cereus*) and three Gram negative bacteria (*Escherichia coli, Pseudomonas aeruginosa and salmonella typhimurium*).

Table (3) Antimicrobial activity coriander essential oil and seed extracts extract, expressed as inhibition zone diameter.

Bacterial species	Gram reaction	Inhibition zone diameter mm (inhibition %)			
		SE	CEO	Ampicillin 20mg/ml	Propionic acid 10%
Escherichia coli	G^-	9 (36)	25(100)	25	--
Pseudomonas aeruginosa	G^-	9 (34)	42(161)	26	--
Staphylococcus aureus	G^+	10(47)	20 (95)	21	--
Streptococcus faecalis	G^+	0.0	31(114)	27	--
Salmonella typhimurium	G-	0.0	25(75)	--	33
Bacillus cereus	G^+	0.0	26(108)	--	24

Values in parentheses are the inhibition percentages compared to standard antibacterial agent.

All strains studied were inhibited by CEO, with different degrees of Inhibition while the SE showed activity against 3 organisms only. The highest activity of CEO was against *Pseudomonas aeruginosa* (161%) and the lowest activity was against *Salmonella typhimurium* (75%) compared to control antibiotic. Similar inhibitory effect of SE against *Staph aureus* (47%) was reported by **(Farah *et al*., 2015).**

The results revealed that coriander essential oil (CEO) exhibits better antibacterial effect than seed extract (SE) against both Gram negative and Gram positive bacteria. Similarly, Coriander essential oil

has been reported to inhibit a broad spectrum of micro-organisms **(Mandal and Mandal (2015).** The chemical composition of the CEO in the present work supporting these results as the CEO contains several compounds which reported to have antibacterial activity as previously mentioned.

4. Sensory evaluation

From the present results, coriander essential oil revealed superior adequacy over seed extract in the tested parameters. Additionally, extracted essential oils and oleoresins are preferred over crude spices in the meat industry due to their better stability during storage, microbial safety, high concentration of flavor components, reduced storage space, ease of handling, no seasonal variation, and standardization **(Jayasena and JO, 2013).** Hence, in the present work CEO was applied on chicken meat to study its effect on the antioxidant status of chicken breast meat.

The results of sensory evaluation of the cooked chicken samples treated with different concentration of CEO are presented in **Table (4).** A score of 100% reflected the highest quality, while samples of the poorest (unacceptable) quality were awarded 40%. The acceptability threshold was established at 60% **(Michalczyk *et al*., 2012).** The sensory analysis indicates that the lowest tested concentrations (1, 2ml/100gm) of CEO was the most accepted compared to the other treatments, however it was significantly ($p<0.05$) different from control. In the same way the overall acceptability of products with oregano oil added at rat of (0.9%) was significantly lower than the control sample up to the 6^{th} day of storage, but significantly higher thereafter **(Govaris *et al*., 2010).** In addition, essential oil, like any form of seasoning, might be considered unpleasant by certain consumers, thus lowering their evaluation of a product. However, for those consumers who do not particularly enjoy the flavour of meat itself, the addition of essential oils at an appropriate dose could make meat products more attractive **(Michalczyk *et al.*, 2012).** Besides, frequently encountered problem concerning the application of additives in food is that the amount of essential oil necessary for effective food preservation exceeds the sensory acceptable level **(Reyes-Jurado *et al*., 2014).**

Table (4) Changes in sensory evaluation of cooked chicken meat with different concentration of CEO.

Treatment	Odour	Softness	Juiciness	Colour	Taste	Acceptance
control	89 a	82 a	80 a	72 a	88 a	86 a
1 ml/100gm	79 b	75 b	70 b	76 a	80 b	70 b
2ml/100gm	76 b	74 b	65 c	76 a	75 c	66c
4ml/100gm	65 c	76 b	68 bc	71 a	46 d	55 d
5ml/100gm	67 c	71 b	57 d	72 a	36 e	45 e

Values in a column with different letters are significantly different ($p<0.05$).

5.Minimum inhibitory concentration (MIC) of Coriander essential oil

The Minimum inhibitory concentration of CEO were tested at serial concentrations below the highest sensory accepted ratio (2%) and presented in **Table (5).**

Table 5. Minimum inhibitory concentration (MIC) of coriander essential oil (CEO).

Tested micro organism	Concentration of CEO % (v/v)
Gram positive bacteria	
Staphylococcus aureus	0.0312
Bacillus cereus	0.250
Enterococcus faecalis	0.0312
Gram negative bacteria	
Pseudomonas aeruginosa	0.75
Escherichia coli	1
Salmonella typhimurium	1

The bacterial strains that showed the higher sensitivity to the inhibitory action of CEO were ordered *Staphylococcus aureus* and *Enterococcus faecalis* < *Bacillus cereus* which are gram positive bacteria followed by the gram negative bacteria *Pseudomonas aeruginosa* < *Escherichia coli* and *Salmonella typhimurium* which have been reported to be more resistant to other essential oils than Gram-positive bacteria **(Silveira *et al.*, 2014).** Similarly, **Silva *et al.***

(2011) reported different susceptibility profiles between Gram-positive and Gram-negative bacteria to CEO and regarding this to some factors taken together such as difference in the bacterial envelope of each bacteria and chemical composition of coriander oil. Also, **Mith *et al*., (2014)** considered CEO to kills both Gram-positive and Gram-negative bacteria by disrupting membrane function, and so all its functions are compromised not only as a barrier but also as a matrix for enzymes and as an energy transducer. Due to this inhibitory effect of CEO against these food-borne and food- spoilage bacteria, CEO can be used as natural food preservative to reduce these bacteria and control their contaminations in foods.

6. Application of CEO on refrigerated chicken breast meat

a. DPPH scavenging activity of chicken breast meat

The stable free radical DPPH has been used in order to evaluate the antioxidant effect of CEO on chicken breast meat when applied at the two sensory accepted levels (1,2 ml/100g) and compared to synthetic preservative sodium nitrite that have strong antioxidant and antimicrobial activity **(Sindelar and Milkowski, 2011)**. The antioxidant activity has been determined based on the scavenging of the stable 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical. It is a discoloration assay, which is evaluated by the addition of the antioxidant to a DPPH solution and the ability to scavenge the stable free radical of DPPH was measured in the absorbance at 517 nm **(Blois, 1958)**.

The effect of storage period on the DPPH scavenging activity of chicken breast meat of all groups is depicted in **Figure1**. The results indicated that increasing the storage period resulted in a proportional decrease in the DPPH scavenging activity of chicken breast meat in all groups. Similar results were also reported by **Hassanin *et al*. (2015).** This occurs due to uneven generation of free radicals reactive oxygen species (ROS) and reactive nitrogen species (RNS) during meat storage which triggers oxidative and/or nitrosative stress leading to damage of macromolecules including the lipid and protein fractions in meat and affect meat quality **(Falowo *et al*., 2014)**.

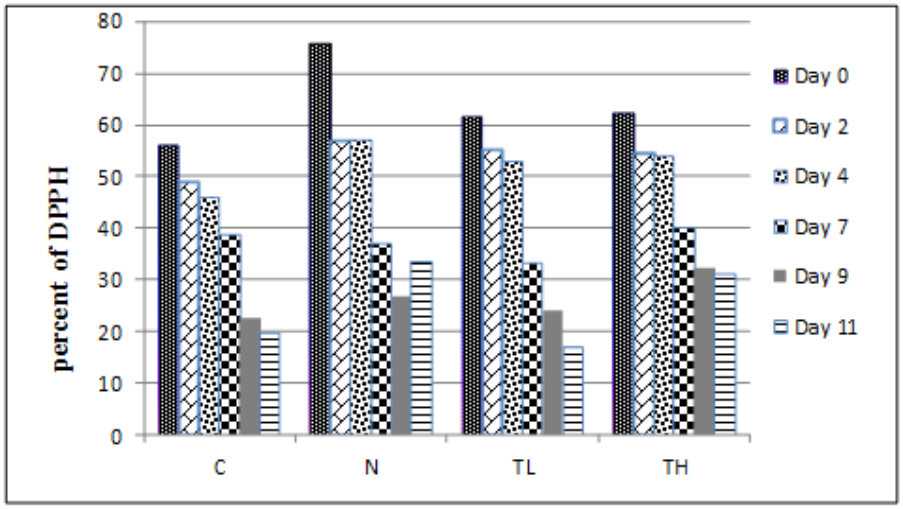

Figure 1. Effect of storage time on DPPH scavenging activity of chicken breast meat, C: negative control, N: positive control (nitrite), TL:1% CEO, TH:2% CEO.

The results of free radical-scavenging effect of chicken breast meat supplemented with CEO **(Table 6)** showed that the negative control group has the lowest value of DPPH scavenging activity in all days. After 2 and 4 days of storage there were no significant differences (*P>0.05*) between the DPPH scavenging activities of breast chicken meat of TL, TH groups (treated with CEO) and the positive control treated with nitrite, while the negative control group had significantly (*P<0.05*) the lowest scavenging activity. After 9 days of storage, group TH showed significantly the highest value of DPPH scavenging activities compared to other groups. In addition, no significant difference (*P>0.05*) was found between group TL and positive control from day 2 until day 9. In the same way, there is no significant difference between group TH and positive control group from day 2 to the end of storage period. Indicating that, the coriander essential oil has the same antioxidant effect as well as the preservative nitrite for 9 days of storage at ratio 1% and for 11days of storage at ratio 2%. This may be explained on the light of **(Falowo *et al.*, 2014)** who regarded the phenolic compounds as effective sources of antioxidants that inhibit oxidation in muscle foods. This coincident with the high phenolic content and the organic compounds of CEO

reported in the present results which have antioxidant activity as early mentioned.

Table 6. DPPH scavenging activity of chicken breast meat at different storage time.

Time	C Negative control	N Positive control	TL (1% CEO)	TH (2% CEO)
Day 0	55.8486 ±3.80 c	75.5726 ±4.84 a	61.3614 ±3.14 b	62.2271 ±0.16 b
Day 2	48.7549 ±1.41 b	56.7497 ±2.62 a	55.1273 ±0.61 a	54.4446 ±2.58 a
Day 4	45.9127 ±15.5b	57.0461 ±11.58 a	52.7165 ±3.91 a	53.7867 ±0.14 a
Day7	38.6406 ±2.57 a	36.9404 ±1.09 ab	33.3278 ±0.02 b	39.9616 ±6.31 a
Day9	22.5820 ±1.45 b	26.7199 ±8.59 b	24.2054 ±2.47 b	32.3553 ±4.74 a
Day11	19.8307 ±4.56 b	33.4686 ±4.65 a	16.9891 ±3.52 b	31.0150 ±0.09 a

lues in a row (within the same day) with different letters are significantly different ($p<0.05$).

b. *E. coli* count of chicken breast meat

E. coli, a natural inhabitant of the intestinal tracts of humans and warm-blooded animals, is used as an indicator bacterium because it acquires antimicrobial resistance faster than other conventional bacteria. Its presence therefore reliably reflects faecal contamination, indicating a possible contamination by enteric pathogens **(Adeyanju and Ishola, 2014).** The results of *E. coli* count of chicken breast meat treated with CEO are presented in **Table (7)**. At the first day there was no significant difference between TH and TL groups (treated with CEO), however they are significantly (*P<0.05*) lower than the negative and positive control groups.

Table 7. E. coli count of chicken breast meat at different storage time (10CFU/g)

Time	C Negative control	N Positive control	TH (2% CEO)	TL (1% CEO)
Day0	16.66±1.5 a	15.33±3.5 a	4 ±1 b	5.66±1.5 b
Day 7	39±8.8a	32.66±0.5 b	11.66±0.5 c	14.66±0.6 c
Day 10	57±8 a	45.66±1.1 b	28±3 d	33.33±2.5 c

Values in a row (within the same day) with different letters are significantly different ($p<0.05$).

This indicates that the CEO has instant killing effect on the *E.coli* at the two tested ratio. After 7 and 10 days of storage the negative control group showed significantly the highest *E.coli* count compared to other groups. After 7 days, there was no significant differences (*P>0.05*) between TH and TL group (treated with CEO), however they were significantly (*P<0.05*) lower than positive control group. After 10 days the treated groups still have lower *E. coli* count compared to the positive and negative groups; however TH group has significantly lower count compared to all groups. These results are clarified in **Figure (2**) which indicate that the coriander essential oil inhibits the growth of E.coli with equal or bitter efficacy than the synthetic preservative (sodium nitrite). These results are in agreement with the antimicrobial activity of the organic components that have been reported in the present results. This antimicrobial activity could be provoked by the major compounds of the EO or due to a synergistic effect between the major compounds and the minor ones **(Jayasena and JO, 2013).**

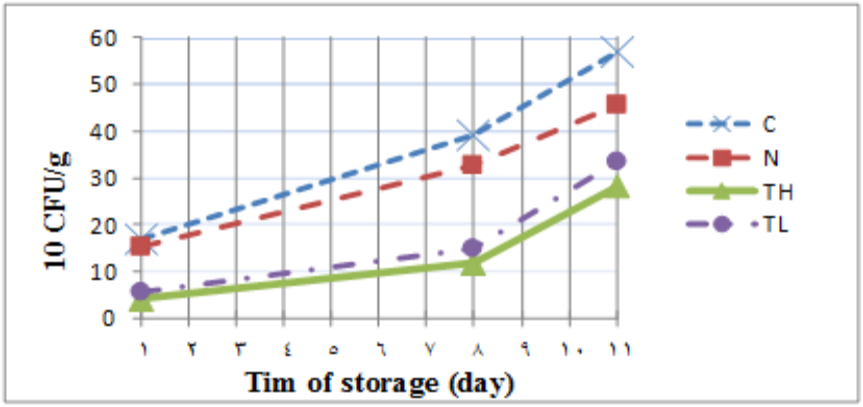

Figure 2. Effect of added CEO to chicken breast meat on the E.coli count (C: negative control, N: positive control (nitrite), TH: 2% CEO, TL:1% CEO).

Antimicrobial mechanisms may be an attack on the cell membrane's phospholipid bilayer, the disruption of the enzymatic systems, the compromising of the genetic material of the bacteria, the coagulation of the cytoplasm, the damage of lipids and proteins, or inhibiting the activity of protective enzymes and sequentially inhibiting one or more biochemical pathways **(Alves-Silva *et al.*, 2013). Silva *et al.*, (2011)** regarded, through the flow cytometric

analysis, the potent antibacterial activity of CEO against Gram-positive and Gram-negative bacteria is due to membrane permeability and consequent bacterial cell death.

CONCLUSION

Coriander essential oil has been proven to possess higher antioxidant and antibacterial activities than seed extract. Coriander essential oil could be considered as a good source of natural compounds with significant antioxidant and antimicrobial activities, which allow it to be used in food preservation systems. The use of coriander essential oil as a natural antioxidant for chicken breast meat showed promising results at the sensory accepted levels. It was effective for retarding the oxidative damage and the growth of E coli of chicken breast meat during refrigeration. Thus coriander essential oil with both antioxidant and antimicrobial activities can be considered as a natural preservative. And it could be applied as ingredients in chicken meat processing not only as a food flavouring agent, but also as a replacement of the synthetic additives to preserve chicken meat quality and preventing economic loss.

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تقييم النشاط الميكروبي والمضاد للأكسدة للزيت الاساسي ومستخلص بذور الكزبرة وتطبيقات علي حفظ صدور الدجاج المبردة

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تمثل مسببات الأمراض التي تنتقل عن طريق الأغذية وعمليات الأكسدة عوامل تلف خطيرة تؤثر على صحة المستهلكين والاقتصاد. الهدف من هذه الدراسة هو تقييم ومقارنة النشاط المضاد للميكروبات ومضادات الأكسدة للزيت الاساسي ومستخلص بذور الكزبرة في المختبر، ودراسة تأثير إستخدام المستويات المقبولة حسياً من الزيت الأساسي (1 و 2 مل / 100 جم) على نشاط مضادات الأكسدة وعدد بكتريا *E.coli* في لحم صدور الدجاج خالي الجلد الطازج أثناء التبريد.

أظهرت النتائج أن كفاءة الزيت الاساسي أعلى من مستخلص البذور في جميع التحاليل التي تم إختبارها. تميز تحليل GC mas للزيت الأساسي بالعديد من المركبات العضوية المعروفة بنشاطها المضاد للبكتيريا مثل α-Pinene (8,01٪)، 3- Carene (0,94٪)، β-Pinene (7,46٪)، D-Limonene (4,9٪) و camphor (5,53٪) ونشاطها المضاد للأكسدة مثل Carveol (8,69٪) وγ-Terpinene (3,05٪).

كان إجمالي المركبات الفينولية و النشاط الكلي المضاد للأكسدة في الزيت الاساسي (9,84 جم مكافئ حمض الجاليك/لتر و 140 جم مكافئ حمض الأسكوربيك/ لتر على التوالي) وكان هذا أعلى من مثيله في مستخلص البذور 7,3 مكافئ حمض الجاليك/كجم و 49,6 جم مكافئ حمض الاسكوربيك/ كجم.

كان النشاط المضاد للبكتيريا في الزيت الأساسي أعلى من نشاط مستخلص البذور في جميع البكتيريا المختبرة. وكانت نتيجة اختبار اقل تركيز مثبط للبكتريا (MIC) للزيت الاساسي ضد بعض انواع البكتريا بالترتيب التالي: *Staphylococcus aureus* و *Enterococcus faecalis*(0,0312٪) أقل من *Bacillus cereus* (0,25٪) أقل من *Pseudomonas aeruginosa* (0,75٪) أقل من *Escherichia coli* و *Salmonella typhimurium* (1٪).

كانت نتيجة مقياس نشاط الارتباط بالشوارد الحرة DPPH للحم الدجاج المعامل بالزيت الاساسي عند التركيزات المقبولة حسياً (1 و 2 مل / 100 جم) هو عدم وجود فروق معنوية ($P> 0.05$) بالمقارنة مع المجموعة المعالجة بالنتريت خلال 9 و 11 يومًا (على التوالي) ، مما يشير إلى أن الزيت الاساسي للكزبرة بتركيز 2 مل / 100 جم له نفس التأثير المضادات للأكسدة مثله مثل المادة الحافظة الصناعية (النتريت).

اوضح العد البكتيري لبكتيريا *E.coli* في لحم الدجاج المعامل بتركيزات (1 و 2 مل / 100 جم) وجود انخفاض معنوي ($P<0.05$) في عدد البكتيريا مقارنة مع مجموعة الدجاج القياسية الموجبة مما يدل على ان فاعلية الزيت الاساسي تتفوق علي فعالية المادة الحافظة (النتريت).

عموما ، تشير نتائج هذه الدراسة إلى أن زيت الكزبرة الأساسي بما له من خواص مضادة للأكسدة ومضادة للبكتريا فإنه يمكن إضافته إلى لحم الدجاج الطازج لتحسين سلامته أثناء التخزين إلى جانب قيمته الغذائية التي تضمن الغذاء الصحي.

الكلمات المفتاحية : الكزبرة، الزيت الاساسي، مستخلص البذور، مضاد للأكسدة، مضاد للبكتريا، التقييم الحسي، ، المكونات الكيمائية، لحم الدوجن، عد بكتريا الايكولاي.