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Full Length Research Paper

The Inhibition Effect of Thyme and Coriander Essential Oils on of *Campylobacter Jejuni* and Some of Their Virulence Genes on Chicken Burger and Chicken Shawerma Products

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Microbial food-borne illness are widespread around the world. *Campylobacter jejuni* considering one of the most common contaminant of chicken meat products. This study aimed to; evaluate the uses of (coriander and thyme) extracts (EOs) in different methods when added to the popular chicken meat products; chicken burgers and chicken shawerma and storage at 4±1 °C for 14 days against *C. jejuni* pathogenicity such as; (*Iam*) and (*virB11)*virulence genes.100 grams of dried leaves and seeds of coriander and thyme water extracted. *C. jejuni,* (*Iam*) and (*virB11)* were collected, enrichment to determine antimicrobial activity by sensitivity test of thyme and coriander EOs compared with ciprofloxacin. 7 kilograms of chicken (burger and shawerma) mixed with 1%,2%,3% concentrations of coriander or/and thyme and control samples. Results revealed that negative effect of EO against *C. jejuni,* with 2cm inhibition zone around the disc immersed by 3% thyme EO and 4 cm inhibition zone around mixture of coriander and thyme Eos disc. The higher level of *C. jejuni* were observed on control (untreated) different chicken samples then *C. jejuni* and(*Iam* & *VirB11*) genes level decreased gradually from 1% conc. Until reach the highest inhibition effect on 3% thyme EO treated. The mixture between both types of EOs 3% concentration. The effect was nearly similar on both types of chicken products (chicken burger & chicken shawerma). In conclusion, this survey revealed that thyme and coriander EOs mixture can be used to stop the growth of *campylobacter jejuni* and some *C. jejuni* (*Iam* & *VirB11*)virulence genes as Gram –ve bacteria especially in high concentration in vitro conditions and may be suitable for preventing the foodborne disease especially in highly perishable food. Further research needed for study and enhancement the application of other herbs essential oils.

Keywords: Natural preservatives, VirB11, Chicken meat, food poisoning, Thyme, Sensitivity Test.

INTRODUCTION

Nowadays, there is highly interesting of consciousness between almost consumers to consume food with high nutritional value, with natural preservatives and free from chemical preservatives and any microbial hazards. Chicken meat industry is the biggest supplier of acceptable animal protein with high meat yield, low shrinkage in cooking and great source of amino acids, vitamins and minerals for human utilization as chicken meat (Schonfeldt and Gibson, 2008; Hassanin *et. al.*, 2014;Thanissery, *et. al.,* 2014; Chelebi *et. al.,* 2015 and Oulkeir, *et. al.,* 2017).

The preservatives effect of different herbs and spice essential oils' (EOs) appear as; antioxidant and antimicrobial properties which inhibit food pathogen and spoilage microorganisms as well as enhancing the flavor and odor of the food. In addition to be safer, more healthy and less subject to hazards than food containing artificial food additives specially in meat which is highly susceptible to microbial growth such as different meat types, which consider favorable media for spoilage and food borne diseases in human, resulting in serious health problems(El-Dosoky, *et. al.,* 2018).

C. jejuni is the major pathogen causing foodborne diseases worldwide (Scallan, *et. al.,* 2011).The incident of C*. jejuni* is one of the most common causes of bacterial enteritis in human. Poultry products play an important role in transmission of Campylobacter bacteria to humans (Berrang, *et. al.,* 2001). *Campylobacter* is most often detected in fresh broiler meat and in the EU the prevalence of these bacteria in broiler carcasses identified at the retail level varied from 3.1% to 58.8%, depending on the Member State (Datta, *et. al.,* 2003). Several studies showed that certain bacterial factors are essential for the pathogenesis of *C. jejuni* including the motility and adherence to intestinal mucosa, capability of the bacteria to invade enterocytes as well as toxin production (Nuijten, *et. al.,* 2000 and Dasti, *et. al.,* 2003).Recently, some genes have been recognized as responsible for the expression of pathogenicity such as; invasion associated markers (*Iam*), *virB11* and genes which are genetic markers for *C. jejuni* (APHA, 2002).The *iam* gene has isolated from two important hosts, chickens and humans (Korsak, *et. al.,* 2004).

Spices are aromatic plant utilized in different food dishes all over the world for improving taste and preserving by antimicrobial properties. Spices may have more effectiveness than antibiotics which may developed resistance against it. In some cases addition of different spices have the synergistic effect which be higher antimicrobial effectiveness but also mixing of other extracts may have antagonistic or equal effects. Coriander (*Coriandrum sativum L.*), is a soft growing plant about 50 cm tall and has potent volatile leaf oil and a stronger aroma which have a different taste from the seeds. Its flavor diminished by gain or loss temperature and there were it usually consumed raw. Raw coriander leaves are 92% water, 4% carbohydrates, 2% protein, and less than 1% fat in addition to its high content in vitamin;(A, C,K) with dietary fiber, and many minerals; (calcium, selenium, iron, magnesium and manganese).Thyme is an aromatic green herb characterized by its aromatic flavor. Thyme retains its flavor on drying better than many other herbs. Oil of thyme, the essential oil of common thyme (Thymus vulgaris), contains 20–54% thymol (Ibrahim, and El-Shehawy, 2013). Thyme essential oil also contains a range of additional compounds, such as p-cymene, myrcene, borneol, and linalool(Friedman *et al.,* 2002).Thymol, an antiseptic, is an active ingredient in various commercially produced mouthwashes such as Listerine(Grieve, 2011).Before the advent of modern antibiotics, oil of thyme was used to medicate bandages(Pierce, 1999 and Ramsewak*, et. al.,* 2003).

The objective of the present study was to; evaluate the uses of (coriander and thyme) extracts (EOs) in different methods; (1%, 2% and 3%) when they added to some popular chicken meat products; chicken burgers and chicken shawerma and storage at 4±1 °C for 14 days against *C. jejuni* pathogenicity such as invasion associated markers (*Iam*) genes and *virB11* which are genetic markers for *C. jejuni*.

MATERIAL AND METHODS

1. Essential Oil Extraction (AOAC, 2005)

The extraction of coriander and thyme essential oils by; 100 grams of dried leaves and seeds of coriander and thyme dipped in 400ml cold distilled water in hydro-distilled for 3 hours using a Clevenger type apparatus. The collected oils dried by passing over anhydrous sodium sulfate on a filter paper (Whatman No.1) in a glass funnel. The oils were stored in a sealed glass bottle at 4˚c until subsequent tests. The extracted oils from thyme and coriander added to meat products (chicken burger and chicken shawerma) at 5 different methods; ($^{(i)}$ control sampling, $^{(ii)}$1%. $^{(iii)}$2% concentrations and $^{(iv)}$3% concentration(v/w).

2. *Campylobacter jejuni* isolates(Banes-Marshall, *et. al.,* 2001)

C. jejuni (RM1221, 11601MD) were collected from chicken skin and intestine of turkey respectively. The organisms were enrichment under microaerobic conditions in Muller Hinton broth at 42°C/24 h to obtain 10^7 cfu/mL. then strains were cultured under microaerobic condition on Muller Hinton agar plates. Disc Diffusion Method used to determine antimicrobial activity according to the zone of

Table (1): Primers sequences, target genes, amplicon sizes and annealing temperature of PCR reactions.

Target gene	Primers sequences (5` to 3`)	Amplified segment (bp)	Annealing	Reference
flaA	AATAAAAATGCTGATAAAACAGGTG	855	53˚C	**Datta *et al*., 2003**
	TACCGAACCAATGTCTGCTCTGATT			
VirB11	TCTTGTGAGTTGCCTTACCCCTTTT	494	53˚C	
	CCTGCGTGTCCTGTGTTATTTACCC			
iam	GCGCAAATATTATCACCC	518	46˚C	**Wieczorek *et al*., 2012**
	TTCACGACTACTACTATGCGG			

inhibition measurement. Sterile filter paper discs (6.0 mm diameter) were soaked with 12 µL of individual undiluted thyme oil &/or coriander oil) and placed on the surface of inoculated agar plates. All plates were left at room temperature for 30 min to allow diffusion of oil before inverting the plates for incubation under microaerobic conditions at 42°C/48 h. Control plates lacked exposure to any EO. Two plates were free from any EO, 2 discs contained 12 µL of thyme EO, another 2 discs contained 12 µL of coriander EO, 2 discs contained mixing of (6 µL coriander EO and 6 µL thyme EO respectively) and 2 discs of ciprofloxacin (one of fluoroquinolone antibiotics family which considering the antibiotics of choice against *C. jejuni*.) Minimum inhibitory concentration (MIC) or minimum bactericidal concentration (MBC) of coriander, thyme and their mix was determined using a macro-broth dilution technique. Serial 2-fold dilutions of the EO were made in tubes using BHI broth for Muller Hinton broth for Campylobacter, in a total volume of 1mL, 2 mL & 3 mL of dimethyl sulfoxide was added to increase the solubility of the EO. Overnight broth cultures (0.1 mL, 0.2 mL& 0.3 mL of 10^7 –10^8 cfu/ mL) of Campylobacter were then added to the tubes which incubated at 42°C/48 h. under microaerobic conditions then determined the inhibition. Results are reported as mean values from 2 replicate trials with each dilution used.

3. Meat Samples Preparation

7 kilograms of chicken meat products as following; (3.50 Kg chicken burger and 3.50 Kg chicken shawerma). 3.50 kilograms of chicken burger divided as following; (i)750 gm mixed with 1% concentrations of coriander or/and thyme (250 gm for each), (ii)750 gm mixed with 2% concentrations of coriander or/and thyme (250 gm for each) and (iii)750 gm mixed with 3% concentrations of coriander or/and thyme (250 gm for each).(iv)1000 gm control chicken burger and chicken shawerma (500 gm for each).

4. Microbiological Quality (APHA, 1992)

Samples homogenized in a Seward stomacher (400R/UK) and serial dilutions (10^{-1}) then samples inoculated in Preston enrichment broth were incubated at 37ºC/ 24 hours, then 0.1 ml of the broth was streaked onto modified Campylobacter selective agar base Cefoperazone Charcoal Desoxycolate Agar (Oxoid) containing antibiotic supplement (Oxoid) at 42ºC for 48 hours under microaerophilic conditions (5% O_2, 10% CO_2, and 85% N_2) using Campylobacter gas generating kits (Oxoid) (Quinn *et al.,* 1994). Examined microscopically by Gram's stain to observe the morphological arrangement and staining reaction and pure cultures of the isolates were biochemically identified using catalase test, oxidase test, urea hydrolysis test, hydrogen sulphide (H_2S) production, citrate utilization test and rapid hippurate hydrolysis test according to Quinn *et al.*(1994). 10 pure positive isolates for C. *jejuni* further enterotoxins and pathogenicity gene identification by PCR according to primer in table (no. 1).

5. **Statistical Analysis (GraphPad Instant, 2009):** The statistical program, GraphPad Instant version 3 for window, was used for determination of means, the analysis of variance between the different data and treatment in this study were determined using standard error and analysis of variance (*P<0.05*).

RESULTS

1. Effect of Different Coriander and/or Thyme EOs Concentration against Campylobacter *jejuni* in comparing with ciprofloxacin in Sensitivity Test: Photos (1, 2 & 3) which declared the different inhibition zones on sensitivity test against *C. jejuni* using different concentrations of coriander EO and/or thyme EOs as following: the 1st photo describe the completely negative effect of all concentration of coriander EO against *C. jejuni* while the 2nd photo viewed clear inhibition zone about 2cm in diameter around the disc immersed by 3% thyme EO while nearly absent of inhibition zone around the other

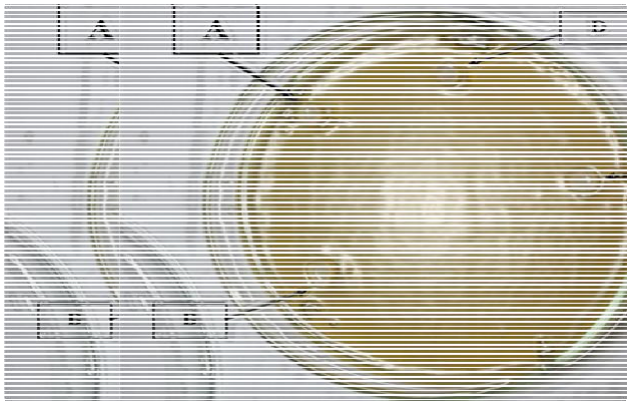

Photo (1): Effect of Different Coriander Concentration against Campylobacter *jejuni*

A) No inhibition zonne around the disc containing 3% coriander EO
B) No inhibition zonne around the disc containing 2% coriander EO
C) No inhibition zonne around the disc containing 1% coriander EO
D) No inhibition zonne around the control disc.

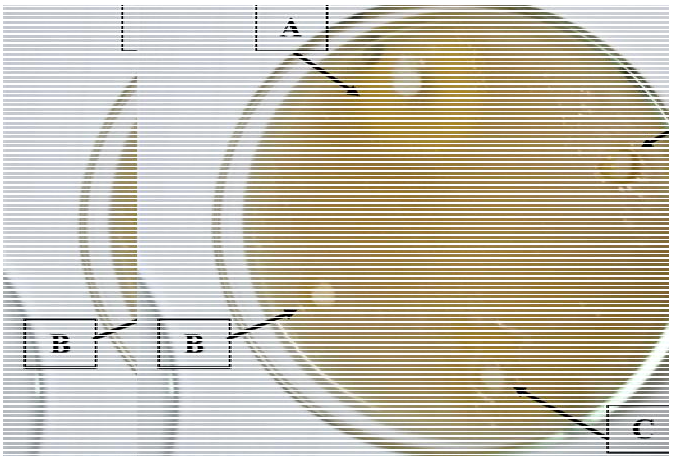

Photo (2): Effect of Different Thyme Concentration against Campylobacter *jejuni*

A) About 2 cm inhibition zone around the disc containing 3% Thyme EO
B) No inhibition zonne around the disc containing 2% Thyme EO
C) No inhibition zonne around the disc containing 1% Thyme EO
D) No inhibition zonne around the control disc.

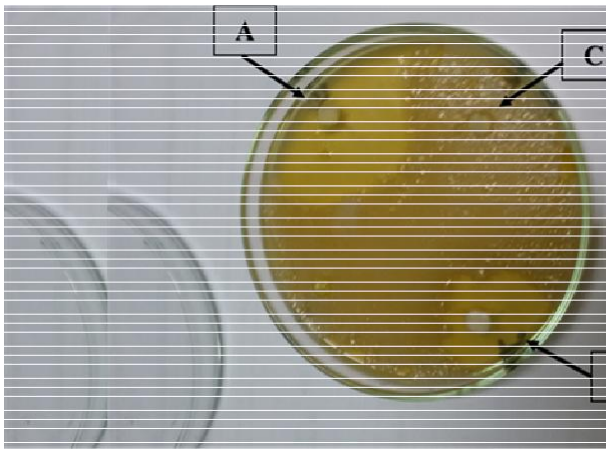

Photo (3): Comparison between the Effect of Coriander& Thyme Mixture and Ciprofllloxacin against Campylobacter *jejuni*

A) About 4 cm inhibition zone around the disc containing Coriander EO & Thyme EOO Mixture
B) About 2 cm inhibition zone around the Ciprofloxacin disc
C) No inhibition zonne around the control disc.

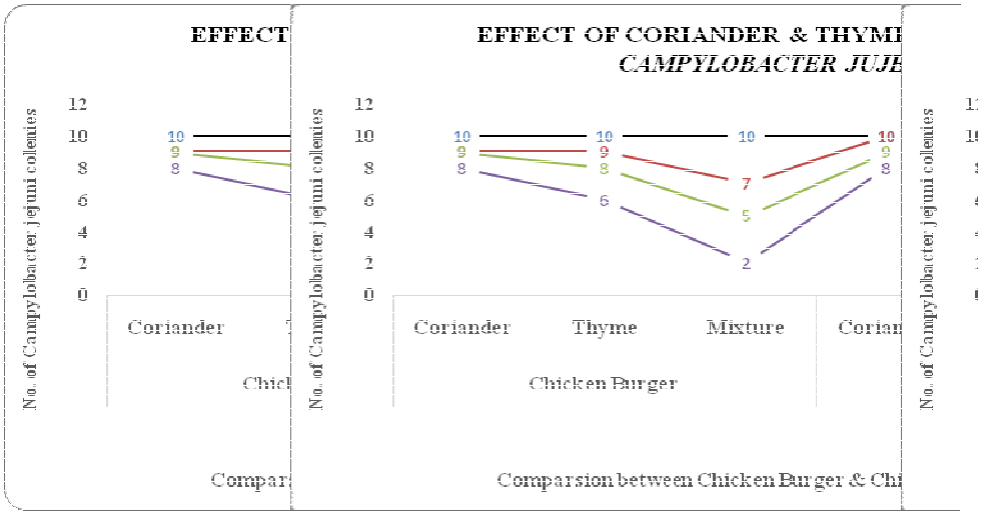

Figure (1): Effect of Different Concentrations of Coriaander EO & Thyme EO and Their Mixture against *Campylobaccter jejuni*

concentrations of thyme EO. The 3rd photorepresented the very wide clear zone (about 4 cm) in diametter around the disc immersed with the mixture of coriander and thyme EO followed by narrower inhibition zone (aboout 3 cm) in diameter surrounded ciprofloxacin disc (one of fluoroquinolone antibiotics family which coonsidering the antibiotics of choice against *C. jejuni*.) while there weren't any inhibition zone around the control (plan) disc.

2. Effect of Different Concentrations of Coriander EO & Thyme EO and Their Mixture against *Caampylobacter jejuni*: Figure (1) illustrated the different reduuction effect of different concentrations of coriander EO &/or thyme EO as following; The higher level of *C. jejuni* were observed on control (untreated) different chicken samples then *C. jejuni* level decreased gradually from 1% conc. Until reach the highest inhibition effect on 3% thyme EO treated. The mixture between both types of EOs specially when used by 3% concentration. The effect was nearly similar on both types of chicken products (chhicken burger & chicken shawerma) as following; the numbers of colonies were about 10% colonies in all control samples while addition of 1%, 2%, 3% of coriander EO reeduced the no. of colonies to; 9%, 9%, 8% respectively in chicken burger and 10%, 9%, 8% colonies respectively inn chicken shawerma. The addition of 1%, 2%, 3% of thym e EO were more effective on reduction of the no. of colonies to; 9%, 8%, 6% respectively in chicken burger and 8%, 7%, 6% colonies respectively in chicken shawwerma. The best results

Table (2): Effect of Coriander EO & Thyme EO and Their Mixture by Different Concentrations against *Campylobacter jejuni* virulence genes

Treatment	*Iam*			*VirB11*		
	Coriander	Thyme	Mixture	Coriander	Thyme	Mixture
Control	3%	3%	3%	3%	3%	3%
1%	3%	3%	1%	3%	3%	2%
2%	3%	3%	1%	3%	2%	1%
3%	3%	2%	0%	3%	2%	0%

observed by mixing between both types of EOs by 1%, 2%, 3% concentration on the samples as following; 7%, 5%, 2% respectively in chicken burger and 8%, 4%, 2% respectively in chicken shawerma.

3. **Effect of Different Concentrations of Coriander EO & Thyme EO and Their Mixture against *Campylobacter jejuni* virulence genes:** Table (2) declared the different inhibition effect of different concentrations of coriander EO &/or thyme EO on 2 major food posing causes of *C. jejuni* (Iam & VirB11) virulence genes. From the table (2) it's completely clear that coriander EO hasn't any effect on *C. jejuni* (*Iam* &/or *VirB11*) virulence genes while addition of thyme EO inhibit *C. jejuni* (*Iam & VirB11*) virulence genes as following; from 3% in control, 1% & 2% concentrations samples to 2% on (*Iam*) only when use by 3% thyme EO concentration and from 3% in control, 1% concentration samples to 2% of (*Vir B11*) virulence genes after addition of thyme EO by 2% & 3% concentrations. While the best results recorded by using of mixture between both EOs which were as following; (*Iam*) virulence genes reduced from 3% in control samples to 1% in case of 1% & 2% concentrations and complete inhibition observed on samples treated by 3% of both EOs mixtures. (*VirB11*) virulence genes reduced from 3% in control samples to 2%, 1%, zero% in case of 1%, 2% & 3% concentrations respectively.

DISCUSSION

Recently herbal essential oils become more applicable for improvement of different food products taste and as natural preservatives in many food manufacturing due to their antibacterial, antifungal, anti-carcinogenic and antioxidant characters, (Bondi, *et. al.,* 2017). The antimicrobial effect mainly different between a variety of herbs, almost herbs didn't studied enough to detect their effect against different microorganisms as antimicrobial substance. Many herbs essential oils has a synergistic effect when used in combinations (Swamy *et al.,* 2016), such as coriander and thyme.

Effect of different coriander and/or thyme EOs concentration against *Campylobacter jejuni* in comparing with Amoxicillin in sensitivity test declared the different inhibition zones on sensitivity test against *C. jejuni* using different concentrations of coriander EO and/or thyme EOs declared the completely negative effect of all concentration of coriander EO against *C. jejuni* while nearly absent of inhibition zone around the other concentrations of thyme EO. The mixture of coriander and thyme EO followed by narrower inhibition zone (about 3 cm) in diameter surrounded amoxicillin disc (the antibiotic of choice against *C. jejuni*). Nearly similar results obtained by (Ibrahim and El-shehawy, 2013)who showed that the inhibition percentage values ranged from 35.9 to 39.6 and 25.2 to 37.6% for thyme, at 0, 10, 20, 30 and 40% thyme respectively against gram negative bacteria when added to minced beef meat with pathogenic bacteria and stored at 4±1°C for 6 days.

Slightly lower results reported by Khatoon, *et. al.,* (2014) in Pakistan who measured the inhibition zone of Ciprofloxacin (fluoroquinolone) about 1.61 cm against *Campylobater jejuni*. HyoBi *et al.,* (2011) reported nearly similar results of Ciprofloxacin (fluoroquinolone) against Gram –ve bacteria in Korea. Al-Allaf *et al.,* (2009)observed similar effect of Ciprofloxacin (fluoroquinolone) against Gram –ve bacteria in Iraq. In the other hand (Karami-Obsoo, 2010) studied the antimicrobial effect of thyme EO on gram –ve bacteria and reported positive inhibition effect of thyme against gram negative bacteria. Friedman *et al.,* (2002) measured the minimum inhibitory concentration (MIC) of thyme extract against some Gram-negative bacteria which range about 3.5% - 4.5%. Higher results reported by Dolaz *et al.* (2002) and Nasar-Abbas & Kadir Halkman (2004) conducted the antimicrobial effect of thyme as highly effective against gram negative bacteria with lower inhibitory effect on Gram-positive. Inhibitory effects were exaggerated with increase in herbs extract concentrations. Luangtongkum, *et. al.,* (2009) found about MIC against *Campylobacter jejuni* by Ciprofloxacin about 0.025µg/ml.

C. jejuni infect digestive tract of many domestic poultry spices and be more severe in chicks which called "enter-invasive transient diarrhea" resulting as watery diarrhea, focal hepatic necrosis, jejunum distention, focal hemorrhage or the absence of clinical signs. *C. jejuni* shaded after 2-3 weeks from bird infection through feces (Wagenaar *et. al.,* 2013 and Jørgensen *et al.,* 2002).

Effect of Different Concentrations of Coriander EO & Thyme EO and Their Mixture against *Campylobacter jejuni* the different reduction effect of different concentrations of coriander EO &/or thyme EO as following; The higher level of *C. jejuni* were observed on control (untreated) different chicken samples then *C. jejuni* level decreased gradually from 1% conc. Until reach the highest inhibition effect on 3% thyme EO treated. The mixture between both types of EOs specially when used by 3% concentration. The effect was nearly similar on both types of chicken products (chicken burger & chicken shawerma) as following; the numbers of colonies were about 10 colonies in all control samples while addition of 1%, 2%, 3% of coriander EO reduced the no. of colonies to; 9, 9, 8 respectively in chicken burger and 10, 9, 8 colonies respectively in chicken shawerma. The addition of 1%, 2%, 3% of thyme EO were more effective on reduction of the no. of colonies to; 9, 8, 6 respectively in chicken burger and 8, 7, 6 colonies respectively in chicken shawerma. The best results observed by mixing between both types of EOs by 1%, 2%, 3% concentration on the samples as following; 7, 5, 2 respectively in chicken burger and 8, 4, 2 respectively in chicken shawerma. Nearly similar results obtained by (Ercan and Ekrem, 2011 and Ibrahim & El-shehawy, 2013)who showed that the inhibition percentage values ranged from 35.9 to 39.6 and 25.2 to 37.6% for thyme, at 0, 10, 20, 30 and 40% thyme respectively against gram negative bacteria when added to minced beef meat with pathogenic bacteria and stored at 4±1°C for 6 days. Dorman and Deans, (2000) and Lambert *et al.,* (2001) studied a lot of herbs EOs antimicrobial effect and concluded that thyme consider one of the widest spectrum antibacterial herb. These inhibition effect refers to the presence of the hydroxyl group at a different location on the phenolic ring which able to disintegrate the outer cell membrane of the Gram negative bacteria which allow releasing lip polysaccharides (LPS) and increasing the permeability of the cytoplasmic membrane to ATP. Ibrahim and El-shehawy, (2013)detected that the thyme antibacterial effect refer to its component of total phenolic and total flavonoids compounds. On the other hand; Delaquis *et al.,* (2002), Duman *et al.*, (2010) and Silva, *et. al.,* (2011) reported weak antibacterial effect of coriander (*Coriandrum sativum*) essential oil against Gram-positive and Gram-negative bacteria due to its content of linalool EO which affect the bacterial cell membrane permeabilization and damage bacterial cell wall. Rattanachaikunsopon & Phumkhachorn, (2010) indicated that coriander EO has bactericidal activity against *Campylobacter jejuni*. Trombetta *et al.,* (2005) stated that coriander affect in Gram-negative bacteria, the outer membrane has an asymmetrical lipid distribution, with the phospholipids in the inner layer and the lip polysaccharides, which confer a strong negative charge to the outer membrane, in the outer layer. Friedman *et al.,* (2002) observed that during storage period, population of the pathogen in the negative control was lowest.

CONCLUSION

A lot of herbal plant uses not only as a food flavoring agent, but also as a food preservative to prevent bacterial spoilage of foods. Thyme and (thyme / coriander mixture) essential oils can be uses as natural preservatives in highly perishable food especially a protein-rich food type due to their antimicrobial effectiveness. Thyme and coriander EOs mixture can be used to stop the growth of *campylobacter jejuni* and some *C. jejuni* (*Iam* & *VirB11*) virulence genes as Gram –ve bacteria especially in high concentration in vitro conditions and may be suitable for preventing the foodborne disease especially in highly perishable food. Further research needed for study and enhancement the application of other herbs essential oils.

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