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Bioactivity of Clove (*Syzygium aromaticum*) against Some Taxa of Enterobacteriaceae Isolated from Fresh Ground Beef

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INVESTIGATION of antibacterial activity of some spices against some enteric bacterial species isolated from fresh ground beef was the aim of this study as well as the identification of the active metabolites of the selected active spice. A number of morphologically varied 90 enteric bacterial isolates were recovered on MacConkey agar from 20 fresh ground beef (cows) samples collected from butcher shops located at Suez City, Egypt. The collected 90 bacterial isolates were characterized into six groups based on their phenotypic characteristics according to Bergey's manual and were identified genetically using 16S rRNA gene sequencing to *Serratia nematodiphila, Proteus penneri, Enterobacter cloacea, Shigella sonnei, Providencia rettgeri* and *Escherichia fergusonii* and deposited in GenBank with accession numbers KY712436, KY712438, KY712439, KY712440, KY712441 and KY712442, respectively. The aqueous extracts of seven spices: Cloves, thyme, cinnamon, ginger, anise, red pepper and curry were tested for their antibacterial activity against the isolated bacteria and the obtained results indicated that clove extract was the most active antibacterial among the other tested spices. The GC-MS analysis of the clove extract metabolites revealed that the active compound was the eugenol.

Keywords: Antibacterial, Enterobacteriaceae, Clove, Eugenol, Beef.

Introduction

The consumption of meat contaminated by foodborne pathogens leads to foodborne diseases and represent a huge public health problem nowadays in the developing countries (Kiessling, et al., 2002; Adwan et al., 2015 and Rasmey et al., 2018). It has been reported that about 50% of the foodborne illness cases every year in the developing countries are due to contaminated meat and poultry (Ahmed & Sarangi, 2013). Meat is a suitable nutritional medium for microorganisms due to its high content of moisture, nitrogenous compounds, minerals and growth factors (Thanigaivel & Anandhan, 2015). There are various infectious agents responsible for these diseases, but the most predominant bacterial species were related to the family Enterobacteriaceae (Addis & Sisay, 2015). A major concern in food hygiene is the contamination of meat with *Salmonella*, *Shigella*, *Escherichia*, *Proteus* and *Klebsiella* species that originate from the raw meat or through processing and storage (Paterson, 2006 and Gwida et al., 2014). In addition to the diseases caused by these bacteria, they are responsible for meat spoilage and lead to large economic losses. Their metabolic activity of the meat leads to the change of odor, color, taste, texture defects and nutritional value and have therefore a negative impact on the poultry meat production (Höll et al., 2016). Therefore, the effective preservatives must be used to inhibit or reduce the contamination of meat products by bacteria during storage.

There are various chemical preservatives such as nitrates, nitrites and benzoates salts were widely added to the food products to inhibit the growth and survival of food pathogens during storage (Sharma, 2015 and Zhao et al., 2017). However, the uses of chemical preservatives and antibiotics in food over the last years leds into the development of resistant bacterial strains. Such practice is unacceptable manner due to their side effects on human health (Karlowsky et al., 2003 and Chikwendu et al., 2008). Also, it has been reported that the preservation of food by high salt content may increase the hypertension

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and risk of cardiovascular disease (Desmond, 2006). This increases the demand for high quality and minimally processed food products with prolonged shelf-life, free from or with small quantities of added chemical antimicrobial agents (Sharma, 2015). So, using of natural compounds such as herbs extracts and spices has been received more attention as an alternative to the chemical additives (Tajkarimi et al., 2010).

Herbal spices have been used as food additives as natural flavouring agents since ancient times. Over the last years, various studies reported the antimicrobial activity of spices against the foodborne pathogens. Several spices, such as clove, cinnamon, garlic, ginger, allspice, caraway, cumin, oregano, rosemary, sage and thyme, have been reported to have significant antimicrobial activities (Burt, 2004; Kwon et al., 2008 and Gutierrez et al., 2009). The flavor and antibacterial properties of the spices are related to the presence of alkaloids, phenolics, flavonoids, anthocyanins, glycosides, steroids, essential oils, coumarins and tannins (Gottardi et al., 2016).

The main goal of the present study was to evaluate the occurrence and frequency of Enterobacteriaceae in the fresh ground beef as well as to determine the antibacterial activity of some spices against the isolated foodborne pathogens. Also, the elucidation of the active compounds of the most potent spice was addressed.

Materials and Methods

Collection of samples

Twenty fresh ground beef (cows) samples were randomly purchased from different butchershops located at Suez City, Egypt. The samples were collected in sterilized polyethylene bags and transferred under aseptic conditions to the laboratory to be analyzed during one hour of collection.

Isolation and enumeration of bacteria

Nutrient agar (NA) and MacConkey agar (MAC) media were used to isolate and count of the contaminated bacteria in the collected ground beef samples. Twenty five grams of each sample were homogenized in 225ml of the sterile buffered peptone water and incubated at 37°C for 1h. Ten ml of this suspension were immediately drawn into 90ml sterile buffered peptone water and serially diluted to10^{-5}. By using pour plate method, 1ml of each dilution was transferred aseptically into each plate and incubated at 37°C for 48h. The developed colonies were counted and those of morphological variations were picked up and sub-cultured onto fresh agar medium. The bacterial cultures were purified and transferred onto tryptic soya agar (TSA) slants and preserved at 4°C.

Characterization and identification of the isolated bacteria

Phenotypic characterization

The colour, shape, surface, margin, elevation and opacity of the bacterial colony were recorded on MAC agar medium. The shape of microscopic cells was examined after Gram staining. Also, the isolated bacteria were subjected to physiological and biochemical tests such as oxidase, catalase production, urease, indole, starch hydrolysis, gelatin liquefaction, methyl red (MR), Voges-Proskauer (VP), glucose and lactose fermentation according to Bergey's Manual of Determinative Bacteriology**.**

Genotypic characterization

DNA extraction: The pellets of vegetative cells were collected by centrifugation at 5000rpm for 20min and vortexed vigorously for 1min after adding of 300µl lysis buffer and 2µl RNAase A. Then 8µl of proteinase K was added to the mixture and mixed, incubated at 60°C for 10min, cooled down for 5min. Three hundred µl binding buffer were added and centrifuged for 5min at 10,000g. After that the lysate was pipetted directly into the spin column and the flow was discarded after centrifugation for 1min at 10,000g. Then 500µl washing buffer was added into spin column, centrifuged for 30sec at 10,000g and the flow through was discarded and this step was repeated again. To remove residual washing buffer, they were centrifuged again at 10,000g for 1min. Finally, the columns were filled with 40-50µl elution buffer, incubated at room temperature for 1min, centrifuged at 10,000g for 2min and DNA was stored at -20°C.

PCR amplification: The PCR amplification was performed by using Qiagen Proof-start Tag Polymerase kit (Qiagen, Hilden, Germany). The primers (16SF: 5′-GAGTTTGATCCTGGCTTAG-3′ and 16SR: 5′-GGTTACCTTGTTACGACTT-3′) were used. Two µl of template DNA (20ng/µl), 12.5µl PCR master mix, 20pmol (2µl) each of forward and

reverse primers and 8.5µl of water DNAase free water were added together to complete the reaction volume to 25µl. Then the complete reaction mixture was incubated at automated thermo-cycler TC-3000 (Biotechnology Research Center, Suez Canal University, Ismailia). The mixture was denaturated at 94°C for 5min, then 37 cycles of denaturation were done at 94°C for 30sec, then the mixture was annealed at 51°C for 30sec and then an extension was done at 72°C for 30sec. At last, a final extension was conducted at 72°C for 5min. The products of PCR were analyzed by electrophoresis on 1.5% (w/v) agarose in 1X TAE buffer and gels. The photos were captured by gel documentation system and analyzed by Gel Docu advanced ver.2 software. The PCR products of 1500bp were purified from the gel with QIA quick gel extraction kit (Qiagen, Hilden, Germany).

DNA sequencing: The purified PCR products were cycle sequenced with didesoxy mediated chain-termination (Sanger et al., 1977). The sequences were analyzed and assembled to assess the degree of DNA similarity using BLAST search program at the NCBI website: http:// www.ncbi.nlm.nih.gov/BLAST/ and CLUSTALW program (http://clustalw.ddbj.nig.ac.jp/top-ehtml). Phylogenetic trees of the 16S rRNA gene sequences of the unknown bacteria with the related 16S rRNA gene sequences from different standard bacteria strains obtained from GenBank were displayed by the TREE VIEW program.

Nucleotide sequence accession numbers: The nucleotide sequences of the isolates number 2006, 2051, 2059, 2103, 2115 and 2434 were deposited in the GenBank database with accession numbers KY712436, KY712438, KY712439, KY712440, KY712441 and KY712442, respectively.

Spices extract preparation

Seven dry spices: Clove (*Syzygium aromaticum*), thyme (*Thymus vulgaris*), cinnamon (*Cinnamomum verum*), ginger (*Zingiber* officinale), anise (*Pimpinella anisum*), red pepper (*Capsicum annuam*) and curry (*Murraya koenigii*) were obtained from local markets in Suez City, Egypt. Fifty grams of each spice were mixed with 200ml distilled water and shaking at 140rpm for 24h. The extracts were filtered through a fine mesh cloth, centrifuged at 4000rpm for 20min, and evaporated under vacuum and stored at 4°C.

Determination of the antibacterial activity of the spices extract

The antibacterial activity of the aqueous extracts of the spices was performed by the well diffusion method. A 100µl of each extract were injected intoan agar well of plates previously seeded with the tested bacterial isolate. The plates were left for 1h in a refrigerator, then incubated at 37°C for 24h and were examined and the diameter of inhibition zones were recorded (Sethi et al., 2013). Also, the chloroform, ethanol, acetone and ethyl acetate extracts of clove were prepared by the same method and tested for their antibacterial activity against the isolated bacteria.

The minimum inhibitory concentration (MIC) of aqueous, chloroform, ethanol, acetone and ethyl acetate extracts of clove against the isolated enteric species was determined by testing different concentrations (1–750mg/ml) using the well diffusion method.

Gas chromatography-mass spectrometry (GC-MS) analysis of the ethyl acetate extract of clove

The GC-Ms analysis of the clove ethyl acetate extract was carried out using gas chromatography–mass spectrometry instrument stands at the Central Laboratory, National Research Center, Egypt with the following specifications. Instrument, a TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp., USA), coupled with a THERMO mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The GC-MS system was equipped with a TG-5MS column (30m x 0.32mm i.d., 0.25µm film thickness). Analyses were carried out using helium as the carrier gas at a flow rate of 1.0ml/min. The following temperature program: 50°C for 1min, rising at 5°C/min to 280°C and held for 5min. The injector and detector were held at 220 and 200°C, respectively. Diluted samples (1:10 Diethyl ether, v/v) of 1μL of the mixtures were always injected. Mass spectra were obtained by electron ionization (EI) at 70eV, using a spectral range of m/z 50-500. The separated metabolites were identified by comparing with NIST mass – spectral library data.

Results and Disscusion

Isolation and enumeration of Enterobacteriaceae

Ninety bacterial isolates belonging to the family Enterobacteriaceae were recovered on MAC agar medium from 20 ground beef samples. All the isolated bacteria were Gram negative, rod

shaped cells and non spore former. Various studies have shown the importance of the constituents of MAC medium as a selective medium for Enterobacteriaceae due to the presence of crystal violet and bile salts which inhibit the growth of Gram positive bacteria and also the presence of lactose sugar differentiate between the lactose fermentable and non-fermentable Gram negative bacteria (Joosten, et al., 2008; Becker et al., 2009 and Doulgeraki et al., 2011). According to the morphological characters of the colonies such as color, shape, margin, elevation and texture, the recovered bacterial isolates were divided into six groups (I, II, III, IV, V and VI) as presentedintable 1. The colonies of the bacterial isolatesof groups II, III and VI were colorless on MAC agar which indicates that they are non-lactose fermenter. On the other hand the members of bacterial groups I, IV and V were lactose fermenter and appeared as pink or red colonies. Also, the biochemical characteristics of all groups such as fermentation of glucose, indole, methyl red, Voges-Proskauer, citrate (IMViC) tests, H_2S production, urease, catalase production, oxidase production and other tests were studied and the obtained results revealed that all isolated bacteria belong to the family Enterobacteriaceae (Table 1). Based on the morphological and biochemical characteristics, the bacterial groups I, II, III, IV, V and VI were identified at the genus level as *Enterobacter*, *Providencia*, *Proteus*, *Serratia*, *Esherichia* and *Shigella* spp., respectively. For identification of the isolated bacteria at the species level, one isolate from each group was selected and their DNA was extracted and PCR amplified (Fig. 1). The resulted PCR products were subjected to 16S rRNA gene sequencing and were compared to 16S rRNA gene sequences of GenBank database using BLAST search analysis. The isolates no. 2059 (I), 2115 (II), 2051 (III), 2006 (IV), 2434 (V), and 2103(VI) were identified as *Enterobacter cloacea*, *Providencia rettgeri*, *Proteus penneri, Serratia nematodiphila, Escherichia fergusonii*, and *Shigella sonnei*, respectively. The phylogenetic tree of their positions among the related bacterial species was inferred from 16S rRNA sequence data by the neighbor-joining method (Fig. 2). The diversity of microbial population has been reported by many studies dealing with meat (Ercolini et al., 2006; Joosten et al., 2008; Becker et al., 2009; Doulgeraki et al., 2011 and Rasmey et al., 2018).

TABLE 1. Morphological and biochemical characterization of the isolated bacterial groups.

Characteristics	I	II	III	IV	V	VI
Colony morphology	**Large, circular, shiny, mucoid, entire, raised, red center, colorless periphery**	**Circular, smooth, entire, raised, colorless**	**Small, circular, smooth, entire, flat, colorless, translucent**	**Irregular, mucoid, entire, umbonate, red color**	**Dry, circular, smooth, entire, flat, pink color**	**Small, irrgular, mucoid, jagged edges, flat, colorless, translucent**
Cells shape	Rod	Rod	Rod	Rod	Rod	Rod
Gram reaction	-	-	-	-	-	-
Lactose fermentation	+	-	-	+	+	-
Glucose fermentation	+	+	-	+	+	-
Urease	-	-	+	-	-	-
Catalase	+	+	+	+	+	+
Indole	-	-	+	-	+	-
VP	+	+	-	+	-	-
MR	-	-	+	-	+	-
Citrate	+	+	+	+	-	-
Gelatin liquefaction	+	-	+	+	-	-
H_2S production	-	-	+	-	-	-
Motility	+	+	+	+	+	-
Oxidase	-	-	-	-	-	-
Starch hydrolysis	-	-	-	-	-	-

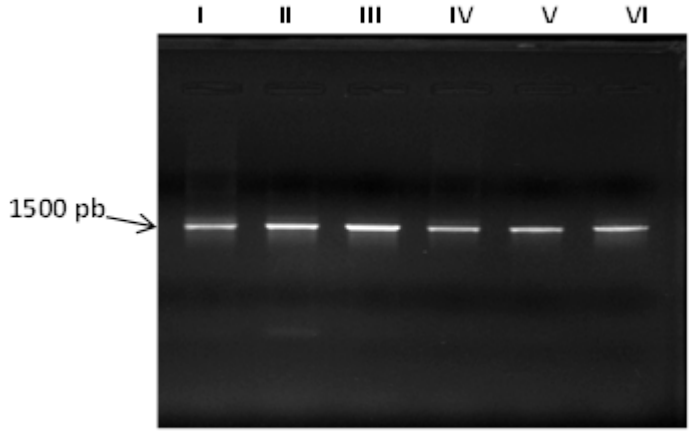


Fig. 1. PCR gel electrophoresis showing 16S rRNA gene bands (at 1500pb) of the isolated bacterial groups.

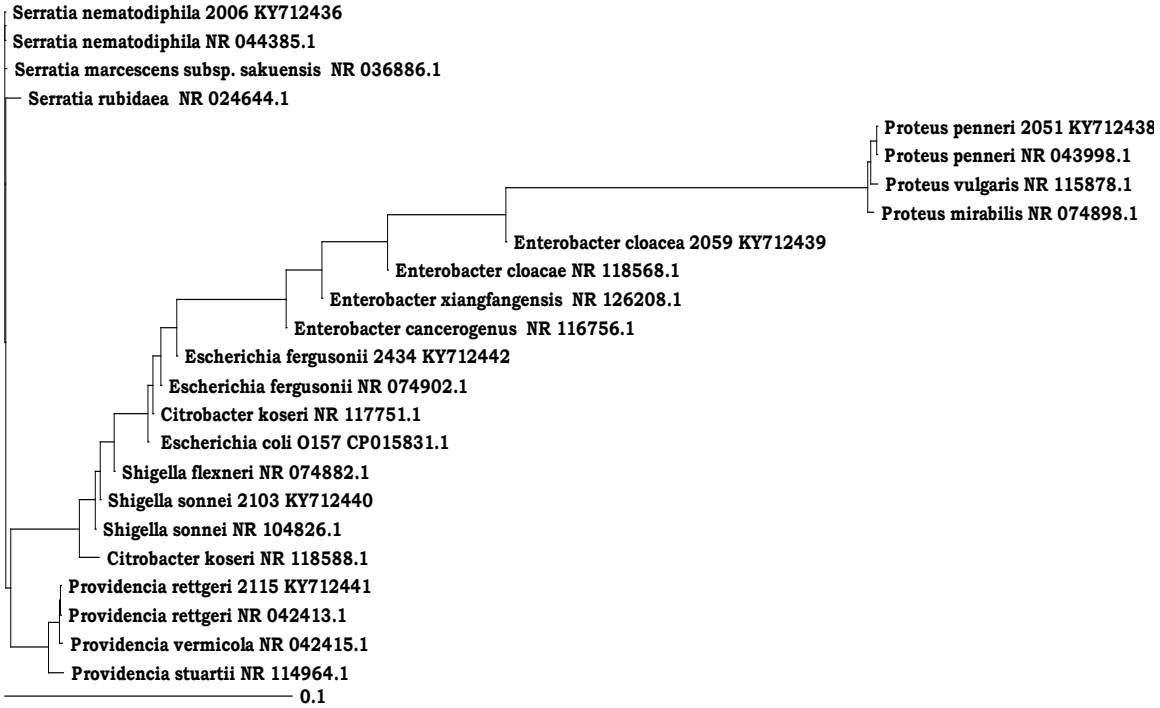


Fig. 2. The phylogenetic tree of the tested bacterial isolates based on 16S rRNA gene sequences showing their position among the related strains in GenBank database.

The total bacterial count ranged from 24±1.7X10^5 to 291±2.15X10^5 of the collected fresh ground beef samples. However, the count recoreded on the MAC medium revealed that the most dominant species was *Enterobacter cloacea* and *Providencia rettgeri* with frequency of 85% and 55%, respectively (Table 2). While, the frequency of *Proteus penneri*, *Serratia nematodiphila*, *Escherichia fergusonii* and *Shigella sonnei* were 35, 20, 20 and 10%, respectively. The heavy load of the Enterobacteriaceae members in the collected ground beef might be due to its available nutrients such as amino acids, minerals, vitamins, water and fats (Ferraz et al., 2010). Ground beef contamination by spoilage bacteria may be resulted from food processing equipment and meat handlers (Schroeder et al., 2004). The combination of meat tissues from different animals is also one of the reasons for ground beef contamination (LeJeune & Christie, 2004).

Antibacterial activity of spices against the isolated enteric bacteria

Seven spices were tested include clove

(*Syzygium aromaticum*), thyme (*Thymus vulgaris*), cinnamon (*Cinnamomum verum*), ginger (*Zingiber officinale*), anise (*Pimpinella anisum*), red pepper (*Capsicum annuam*) and curry (*Murraya koenigii*) for their antibacterial effect on the recovered bacteria from ground beef. The results in Table 3 show that the clove aqueous extract was the most potent antibacterial with an inhibition zone diameter of 24.5± 0.6, 22± 0.28, 14.5±0.29, 22.5±0.29, 15.5±0.29 and 22.5±0.29 mm against *Enterobacter cloacea*, *Providencia rettgeri*, *Serratia nematodiphilia*, *Escherichia fergusonii*, *Proteus penneri* and *Shigella sonnei*, respectively. Several studies indicated that spices had a deleterious effect on foodborne bacteria (Gutierrez et al., 2009; Škrinjar & Nemet, 2009; Tajkarimi et al., 2010 and Ahene et al., 2011). Zhang et al. (2009) found that the clove extract has a strong antibacterial activity against the four common meat spoilage and pathogenic bacteria; *Listeria monocytogenes, Escherichia coli, Pseudomonas fluorescens* and *Lactobacillus sake*. Cloves are aromatic herbs and have pleasant yet spicy. They are the flowering bud of the tree *Eugenia aromatic*, which dried and become brown and used for medicinal purposes (Beuchat, 2000). Clove is effective against different bacterial species such as *Escherichia coli*, *Salmonella enteric* and *Staphylococcus aureus* (Beuchat, 2000 and Cressy et al., 2003). It has also antifungal, anticarcinogenic, antiallergic, antimutagenic, antioxidant and insecticidal properties (Ogata et al., 2000; Friedman et al., 2002; Tworkoski, 2002 and Park & Shin, 2005).

TABLE 2. Colony forming units (CFU) and frequency (% out of 20 samples) of the isolated bacteria from the collected meat samples.

Meat samples	Nutrient agar	CFUx10^5					
		MacConkey agar					
		Enterobacter cloacea	***Providencia rettgeri***	***Proteus penneri***	***Serratia nematodiphila***	***Escherichia fergusonii***	***Shigella sonnei***
1	49±1.15	19±0.57	10±1.2	-	-	-	-
2	321±2.3	8±1.3	13±0.68	8±0.68	-	-	-
3	291±2.15	14±1.7	-	26±1.15	-	3±0.68	23±1.7
4	117±2.15	5±0.57	-	4±0.68	5±0.7	6±1.15	-
5	24±1.7	-	-	-	-	-	23±1.2
6	74±2.5	16±2.5	-	-	-	58±1.3	-
7	166±1.15	18±2.1	24±1.5	-	40±1.2	-	-
8	144±2.15	-	-	-	51±1.3	-	-
9	87±1.15	24±1.15	19±1.15	-	-	-	-
10	61±0.58	31±0.57	21±1.15	-	-	-	-
11	218±1.15	90±1.7	-	16±1.7	-	-	-
12	94±0.57	23±1.3	66±2.5	41±1.7	2±0.68	-	-
13	234±1.33	104±1.15	-	-	-	-	-
14	128± 1.15	-	63±1.3	64±1.15	-	-	-
15	163±1.7	19±0.3	61±1.15	-	-	-	-
16	82±1.7	67±1.15	-	-	-	-	-
17	46±0.86	18±1.7	-	14±1.7	-	13±1.2	-
18	60±1.15	17±1.15	16±0.58	-	-	-	-
19	78±1.15	17±1.3	20±1.3	-	-	-	-
20	213±1.15	46±1.5	4±0.3	-	-	-	-
Total (x10^5)	2610	248	83	17	67	151	81
Frequency (% out of 20)		85	55	35	20	20	10

TABLE 3. Antibacterial activity of the aqueous extracts of the tested spices against the isolated bacteria.

Spices	Diameter inhibition zone of (mm)					
	Enterobacter cloacea	***Providencia rettgeri***	***Serratzia nematodiphilia***	***Escherichia fergusonii***	***Proteus penneri***	***Shigella sonnei***
Clove	24.5±0.6	22±0.28	14.5±0.29	22.5±0.29	15.5±0.29	22.5±0.29
Thyme	20.5±0.86	13.5±0.12	-	15±0.86	-	-
Red pepper	17±0.29	-	-	18.5±0.29	-	-
Cinnamon	-	-	21±0.57	-	17±0.87	19.5±0.29
Anise	-	-	13.5±0.28	-	14.5±0.57	20±0.28
Curry	-	20.5±0.29	15.5±1.15	-	16.5±0.57	-
Ginger	-	-	16±0.28	-	15.5±0.28	-

Both of cinnamon and anise extract had a harmful effect on the growth of *Serratia nematodiphilia*, *Proteus penneri* and *Shigella sonnei*. Also, the thyme extract exhibit inhibitory effect on *Enterobacter cloacea*, *Providencia rettgeri* and *Escherichia fergusonii*. The red pepper extract was effective in reducing the growth of *Enterobacter cloacea* and *Escherichia fergusonii* with an inhibition zone diameter of 17±0.29 and 18.5±0.29mm, respectively. Ginger had a moderate antibacterial effect on growth of *Serratia nematodiphila* and *Proteus penneri* with an inhibition zone diameter of 16±0.28 and15.5± 0.28mm, respectively. It is well documented that showed that ginger had no any antibacterial effect on the growth of the tested bacteria (Keskin & Toroglu, 2011 and Akrayi, 2014). In a study by Škrinjar & Nemet (2009), thyme has a potent antibacterial effect on *Enterbacter cloaceae* and *Listeria* spp. Asimi et al. (2013) showed that cinnamon was the most vigorous antibacterial against the tested bacterial strains.

Antibacterial activity of different solvent extracts of clove against the isolated enteric bacteria

Clove was extracted by the different solvents such as ethanol, ethyl-acetate, acetone and chloroform and the results were recorded in Table 4. All the extracts of the different solvents have antibacterial activity against all the tested bacteria with different inhibition zone diameters. Witkowska et al.(2013) reported that the choice of the solvent used to extract the spices has an influence on the antimicrobial activity. The ethyl-acetate extract was the most effective with the lowest MIC against most of the tested species as shown in Table 5. The lowest MIC of ethyl-acetate extract was 2.5mg/ml against *Escherichia fergusonii* while the lowest MIC of acetone extract was 5mg/ml against *Enterobacter cloacea* and the lowest MIC of chloroform extract was 250mg/ml against *Proteus penneri*. On the other hand, the lowest MIC of the clove aqueous extract was 150mg/ml with *Enterobacter cloacea*. The antibacterial activity of spices could be attributed to the presence of antioxidant compounds (Sebranek et al., 2005).

GC-MS analysis of ethyl-acetate extract of clove

The obtained GC-MS analysis chromatogram (Fig. 3) of the ethyl-acetate extract of clove indicatedthat the major constituent components were eugenol ($C_{10}H_{12}O_2$), Phenol,2-methoxy-4-(2-propenyl)-acetate ($C_{12}H_{14}O_3$), trans-Caryophylene ($C_{15}H_{24}$), 4-Allyl-2-methoxyphenoxy(trimethylsilane)($C_{13}H_{20}O_2Si$), Humulene ($C_{15}H_{24}$) and 3-Methyl-2,5,6-trimethoxy-1-indanone ($C_{13}H_{16}O_4$) with 23.18, 28.56, 4.55, 2.74, 1.21 and 0.01 %, respectively (Table 6). According to Juliani et al. (2002), the content of the clove bud essential oil was eugenol 74.4%, β-caryophyllene 7.5% and eugenyl acetate 15.8%. Also, Fichi et al. (2007) reported that the clove extract contained eugenol 59.3%, β–caryophyllene 24.9% and eugenyl acetate 4.2%. Razafimamonjison et al. (2014) found that the major componentsof clove extract were eugenol, β-caryophyllene and humulene. These findings indicate that the antibacterial activity of clove spice might be due to the presence of the phenolic compounds especially the eugenol, β-caryophyllene and humulene compounds.

TABLE 4. Antibacterial activity of different solvents extracts of clove against the isolated enteric bacteria.

Bacterial species	Diameter inhibition zone of (mm)			
	Ethanol	Chloroform	Ethyl-acetate	Acetone
Providencia rettgeri	21± 0.57	9±0.57	16.5± 0.88	24± 0.57
Enterobacter cloacea	32.5± 1.0	38.5± 3.5	40.5± 0.57	33± 0.88
Serratia nematodiphilia	21.5± 0.57	17± 0.17	21± 0.57	21± 0.67
Escherichia fergusonii	29± 0.57	36.5± 0.33	30.5± 0.33	25± 0.57
Proteus penneri	21± 0.28	19.5± 0.16	20± 0.33	20.5± 0.33
Shigella sonnei	19.5± 0.33	11±0.57	13.5± 0.44	21± 0.17

TABLE 5. MIC of clove using different solvent for the six groups.

Bacterial isolates	MIC (mg/ml) of clove extracts				
	Water	Ethanol	Acetone	Chloroform	Ethyl acetate
Providencia rettgeri	500	500	500	500	150
Proteus penneri	500	5	150	250	50
Serratia nematodiphila	500	500	500	500	500
Shigella sonnei	500	500	500	500	50
Escherichia fergusonii	500	250	500	500	2.5
Enterobacter cloacea	150	500	5	500	150

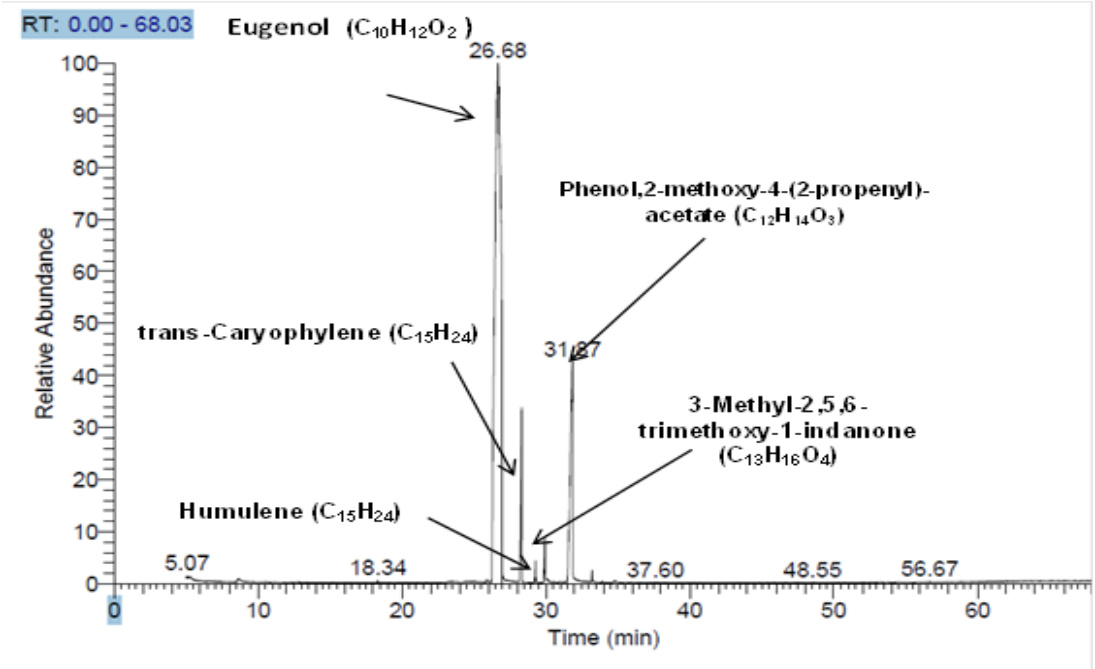
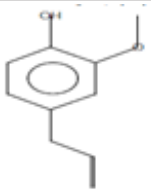
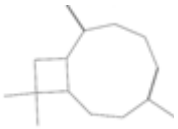
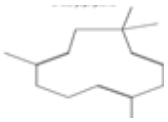
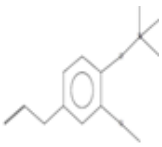
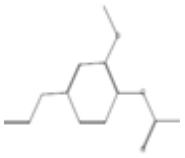
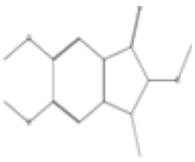


Fig. 3. GC-MS analysis of the ethyl acetate extract of clove.

TABLE 6. GC-MS analysis of the ethyl acetate extract of clove.

Peak	RT	Area%	MW	MF	Compound	Structure
1	26.68	23.18	164	$C_{10}H_{12}O_2$	Eugenol	
2	28.32	4.55	204	$C_{15}H_{24}$	trans-Caryophylene	
3	29.28	1.21	204	$C_{15}H_{24}$	Humulene	
4	29.92	2.74	236	$C_{13}H_{20}O_2Si$	(4-Allyl-2-methoxyphenoxy) trimethylsilane	
5	30.15	0.01	236	$C_{13}H_{16}O_4$	3-Methyl-2,5,6-trimethoxy-1-indanone	
6	31.85	28.56	206	$C_{12}H_{14}O_3$	Phenol,2-methoxy-4-(2-propenyl)-,acetate (CAS)	

RT: Retention time, MW: Molecular weight, MF: Molecule formula.

Conclusion

Ground beef is heavily contaminated by different members of Enterobacteriaceae such as *Serratia nematodiphila, Proteus penneri, Shigella sonnei, Providencia rettgeri, Escherichia fergusonii* and *Enterobacter cloacea*. These bacterial species represent a harmful effect on food safety and the health of the consumer. Many natural spices are cheap and effective antibacterial against the food-borne pathogens and can be used widely instead of chemical additives in food processing. It is worth mentioning that the most active antibacterial spice is the clove due to the presence of the phenolic compounds B-caryophylene, humulene and eugenol in its chemical composition.

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النشاط الحيوي للقرنفل (سيزيجيم اروماتيكم) ضد بعض الأصناف من عائلة البكتيريا المعوية المعزولة من اللحم المفروم الطازج

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يعد تقييم النشاط المضاد البكتيرى لبعض التوابل ضد بعض الأنواع البكتيرية المعوية المعزولة من لحم البقر المفروم الطازج وكذلك تعريف المركبات النشطة فى التوابل النشطة المنتخبة هو الهدف من هذه الدراسة. تم عزل عدد 90 عزلة بكتيرية معوية متنوعة مورفولوجيا على بيئة أجار ماكونكي من عدد 20 عينة من اللحم البقري الطازج تم تجميعها من محلات الجزارة في مدينة السويس، مصر. تم توصيف العزلات البكتيرية المعزولة والبالغ عددها 90 إلى 6 مجموعات بناءً على خصائصها المظهرية وفقًا لدليل بيرجي لتصنيف البكتيريا، ثم تم تعريفها جينيًا باستخدام تسلسل الجين 16س حمض الريبونيكوليك الريبوسومى إلى سيراتيا نيماتوديفيليا وبروتيوي بيننيري و انتيروباكتير كلواكيا وشيجيلا سوننياى و بروفيدنيكيا ريتيجيرى و ايشيريشيا فيرقيسونى وتم تسجيلها في بنك الجينات تحت ارقام ، KY712440 ، KY712439 ، KY712438 ، KY712436 KY712441 و KY712442 ، على التوالي. تم اختبار مقدرة التضاد البكتيرى للمستخلصات المائية لسبعة توابل وهى القرنفل، الزعتر، القرفة، الزنجبيل، اليانسون، الفلفل الأحمر والكاري ضد البكتيريا المعزولة، وقد أشارت النتائج التي تم الحصول عليها إلى أن مستخلص القرنفل هو الأكثر فعالية كمضاد بكتيرى بين مستخلصات التوابل المختبرة الأخرى. وقد تبين من التحليل الكروموتوجرافى GC-MS لمستخلص القرنفل أن المركب الفعال بهذا المستخلص هو الأوجينول.