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The impact of Clove and thyme essential oils on *Listeria monocytogenes* isolated from meat and poultry products

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Abstract

This study detected the presence of antimicrobial resistance *L. monocytogenes* in meat and poultry products as minced meat, luncheon, and frozen chicken fillet sold in Sohag city markets. Also, study the effect of two essential oils (EOs) like clove and thyme on the isolated strains of *L. monocytogenes*. Bacteriological culture and PCR were used for *L. monocytogenes* identification in 195 meat and poultry products samples such as minced meat, luncheon, and frozen chicken fillet (65 each). Fourteen antimicrobials were tested against *L. monocytogenes* using disk diffusion method. Clove and thyme EOs were used at nine concentrations (100%, 50%, 25%, 12.5%, 6. 25%, 3. 125%, 1.56%, 0.78%, and 0.39%) to detect their antibacterial effect using well diffusion method. *L. monocytogenes* were detected in 6 (3.1%) out of 195 meat and poultry samples. Minced meat harbors the highest infection rate, followed by luncheon and frozen chicken fillet with percentage of 4.6%, 3.1%, and 1.53%, respectively. Most of *L. monocytogenes* isolates were resistant to several antimicrobial from varied groups such as streptomycin, tetracycline, ampicillin, cefotaxime, ceftriaxone, vancomycin, and amikacin. Clove and thyme EOs have inhibitory action on *L. monocytogenes* growth which significantly increased with concentration where the minimum inhibitory concentration was 3.125% for clove essential oil (EO), and 1.56% for thyme EO. The inhibitory action of Clove and thyme EOs enables them to be used in food industry as antibacterial to increase the products shelf life.

Keywords:

16S rRNA, Clove, *L. monocytogenes*, Meat products, Poultry products, Thyme.

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Introduction

L. monocytogenes is a foodborne microorganism and results in invasive listeriosis which affects mainly pregnant women, immunocompromised people, and newborns (Cherifi et al., 2020). Growth of *L. monocytogenes* at 2-4˚C reflect its presence in many processed meat products (Bahrami et al., 2020). Several methods were used to produce safe food and prolonged its shelf life such as heat treatment, irradiation, hydrostatic pressure, packaging, and preservatives (Shahbazi et al., 2018).

Increasing the level of resistance microorganisms against antimicrobials in foods especially ready to eat food raises the morbidity and mortality rates of infections and leading to serious health risks (Bloom et al., 2017). Recently, using natural additives in food industry was increased such as antimicrobials (Ribeiro et al., 2019).

Essential oils are natural plant origin materials which synthesised from varied parts of plants such as leaves, flowers, roots, seeds, barks, peels, woods, and fruits. They produced by mechanical processes, dry distillation, or steam distillation (Calo et al., 2015). EOs as secondary metabolites of plants have antifungal, antibacterial, and antibiofilm effect (Valdivieso-Ugarte et al., 2019).

Clove (*Syzygium aromaticum*) and thyme (*Thymus vulgaris*) are phytochemicals used in varied medical applications. Clove used as analgesic, antiseptic, antimicrobials, and safe food additive, while thyme used as antimicrobial, antispasmodic, and food preservative (Elafify et al., 2022). Therefore, this study aimed to detect *L. monocytogenes* incidence in meat and poultry products and their antimicrobial activity and use two EOs like clove and thyme as natural antibacterial against the isolated strains of *L. monocytogenes*.

Materials and methods

Collection and microbiological examination of samples:

Beef luncheon, minced meat and frozen chicken fillet samples (65 each) were purchased from different markets in Sohag city. Samples were prepared based on Okonko et al., 2013. The bacteriological examination of samples was done according to Arslan and Özdemir, (2020) using palcam agar from Hi media and confirmed by PCR.

Identification of *L. monocytogenes* by PCR:

The extraction of DNA from the suspected isolates was conducted according QIAamp manufacture Qiagen. The specific gene 16S rRNA of *L. monocytogenes* was detected using PCR and primers sequences F: GGA CCG GGG CTA ATA CCG AAT GAT AA and R: TTC ATG TAG GCG AGT TGC AGC CTA according to Hassanien and Shaker (2021) with some modifications in the cycling conditions as 5 min at 94°C, 35 cycles for 50s at 94°C, 50 °C for 1 min, and 60s at 72 °C, and final extension at 72 °C for 10 min. 1.5% agarose gel electrophoresis and ethidium bromide were used for examination of PCR product, and then photographed by light transilluminator (Biometra, Germany).

Antimicrobial sensitivity:

L. monocytogenes isolates were examined against 14 antimicrobials by disk diffusion method based on NCCLS (1999) using Muller Hinton agar and Oxoid antimicrobial disks.

Effect of clove and thyme essential oils on *L. monocytogenes* isolates:

The essential oils were purchased from National Research Canter, Cairo, Egypt and prepared based on wiegand et al., (2008). The stock solution was prepared by addition of dimethylsufoxide (DMSO), brain heart

infusion (BHI) and the essential oil as 2:4:2, then two-fold serial dilutions were done by BHI. The antibacterial effect was done by well diffusion technique (Nzeako et al., 2006).

Statistical analysis:

Mean and standard error of essential oils effect was analysed by SPSS 14.

Result

L. monocytogenes was examined in meat and poultry products samples by bacteriological culture and confirmed by PCR using16S rRNA gene (Table 1, Fig. 1). Among 195 samples of meat and poultry products, 6 (3.1%) have *L. monocytogenes* with higher infection rate in minced meat (4.6%), followed by beef luncheon (3.1%), and frozen chicken fillet (1.53%). *L. monocytogenes* isolates represented multidrug resistance from varied groups. All isolates showed resistance toward streptomycin and tetracycline and the highest resistance were observed against gentamycin and ciprofloxacin (83.3%), ampicillin, cefotaxime, ceftriaxone, vancomycin, and amikacin in percentage of 66.7% (Table 2, Fig. 2).

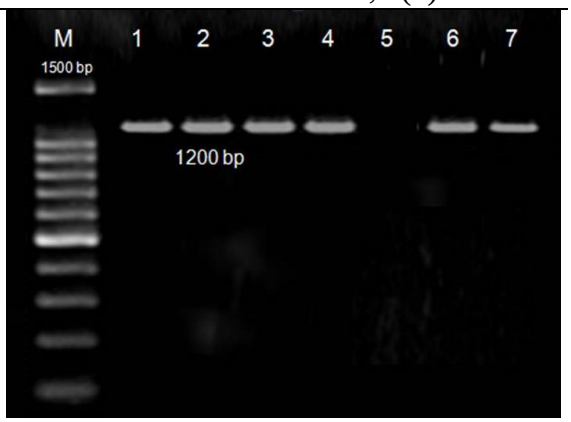

Fig. 1. 1.5% agarose gel electrophoresis of *L. monocytogenes* 16S rRNA gene by, M; marker, lanes 1-,4,6, 7; positive, 5; negative.

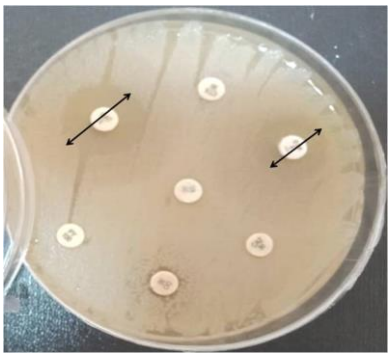

Fig. 2. Antimicrobial resistance of *L. monocytogenes* isolates.

Table 1. Incidence of *L. monocytogenes* in meat and poultry products:

Meat products	Examined samples	Microbiological culture		PCR	
		Positive samples		Positive samples	
		No	%	No	%
Minced meat	65	**4**	**6.2**	**3**	**4.6**
Beef luncheon	65	**2**	**3.1**	**2**	**3.1**
Frozen chicken fillet	65	**1**	**1.53**	**1**	**1.53**
Total	195	**7**	**3.6**	**6**	**3.1**

As shown in Table 3, clove and thyme EOs represented an inhibitory effect on multiantibiotic resistance *L. monocytogenes* isolates which increased with high concentrations. The mean inhibition zones of clove EO were 30.33 ± 2.216, 26.67 ± 2.25, 23.83 ± 2.87, 17.17 ± 2.64, 15.17 ± 1.64, and 13.83 ± 1.70 at concentrations of 100%, 50%, 25%, 12.5%, 6.25%, and 3.125 %, respectively. No zones of inhibition were reported at concentrations of 1.56%, 0.78%, and 0.39%. Thyme EO represented mean inhibition zones of 28.67 ± 1.84, 25.33 ± 2.06, 18.67 ± 1.54, 14.17 ± 2.04, 13.33 ± 2.08, 12.17 ± 1.99, and 8.83 ± 1.74 at concentration of 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, and 1.56%, respectively, while concentration of 0.78%

and 0.39% reported no inhibition zones (Table 3, Fig. 3).

Table 2. Antimicrobial sensitivity of *L. monocytogenes* isolates.

Anti-microbial	*L. monocytogenes* isolates n=6					
	S		I		R	
	N	%	N	%	N	%
AMP	1	16.7	1	16.7	4	66.7
CAR	5	83.3	0	0	1	16.7
S	0	0	0	0	6	100
CTX	1	16.7	1	16.7	4	66.7
CRO	2	33.3	0	0	4	66.7
FOX	1	16.7	3	50	2	33.3
VA	2	33.3	0	0	4	66.7
CN	0	0	1	16.7	5	83.3
AK	1	16.7	1	16.7	4	66.7
AZM	1	16.7	2	33.3	3	50
GAT	4	66.7	2	33.3	0	0
TE	0	0	0	0	6	100
CIP	1	16.7	0	0	5	83.3
OFX	2	33.3	2	33.3	2	33.3

Table 2. footnote: Ampicillin (AMP) 10µg, carbenicillin (CAR) 100µg, streptomycin (S) 10 µg , cefotaxime (CTX) 30 µg, ceftriaxone (CRO) 30µg , cefoxitin (FOX) 30µg, vancomycin (VA) 30µg, gentamycin (CN) 10 µg, amikacin (AK) 30µg , azithromycin (AZM) 15µg, gatifloxacin (GAT) 5µg, tetracycline (TE) 30µg, ciprofloxacin (CIP) 5µg , ofloxacin (OFX) 5µg .

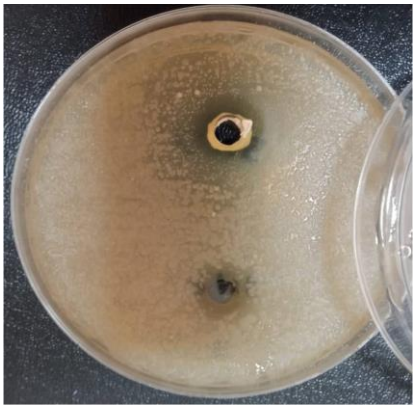

Fig. 3. Effect of Clove and thyme essential oils on *L. monocytogenes* isolates

Table 3. The impact of clove and thyme essential oil on *L. monocytogenes* isolates:

Oil concentration	Clove	Thyme	*P* value
	Mean ± SdE	Mean ± SdE	
100%	30.333±2.2160	28.666±1.837	**0.05**
50%	26.666±2.245	25.333±2.060	
25%	23.833±2.868	18.666±1.542	
12.5%	17.166±2.638	14.166±2.039	
6.25%	15.166±1.641	13.333±2.076	
3.125%	13.833±1.701	12.166±1.990	
1.56%	No zone	8.833±1.740	
0.78%	No zone	No zone	
0.39%	No zone	No zone	

Discussion

Results in Table 1 revealed that *L. monocytogenes* was detected in minced meat with percentage of 4.6%. Nearly similar results were reported by Armany et al., 2016, Mohamed et al., 2016 and Reda et al., 2016. Higher results were mentioned by Kalender, 2012 and Abdeen et al., 2021 who detected *L. monocytogenes* in a percentage of 7.2% and 14%, respectively. Contamination of minced meat by *L. monocytogenes* may be occurs during animals slaughtering, transportation, workers hands and equipment used such as mincing machine, knifes, and packaging tools. Therefore, presence of *L.*

monocytogenes reflects bad hygienic measures (Marinšek and Grebenc, 2002).

The examined luncheon and frozen chicken fillet samples harbor *L. monocytogenes* in a percentage of 3.1%, and 1.53%, respectively. Higher results were reported by Abdelmalek et al., 2009, Mohamed et al., 2016, and Ahmed et al., 2017, in contrast to Amany et al., 2016 who reported negative results for *L. monocytogenes* in luncheon samples. This variation may be related to source of meat, geographical area, sample size, and hygienic measures followed during handling, processing, and packaging. Also, curing technique, addition of spices and manufacture temperature may lower the microbial load of processed meat products (Mahmoud et al., 2019).

The majority of *L. monocytogenes* isolates exhibited resistance to several antimicrobials such as streptomycin, tetracycline, ampicillin, cefotaxime, ceftriaxone, vancomycin and amikacin which belongs to several groups (Table 2, Figure 2). This reflects that these antimicrobials were widely used in therapeutic medications or growth promoters in a haphazard manner. Presence of resistance isolates in meat products give chance of transmission of this resistance to food consumers Therefore, essential oils as natural antimicrobial agents can be used to combat the resistance of microorganisms (Hassanien and Abdel-Aziz, 2021).

Many EOs were used in food industry as a flavoring agent and increase food shelf life due to their antibacterial activities. The disadvantages of some EOs application in food are their off-flavor, and strong aroma (Pietrysiak et al., 2019). Therefore, using EOs needs information about EO properties, mechanism of action, minimum inhibitory concentration, and their interaction with food sensory and matrix properties (Hyldgaard et al., 2012).

The antimicrobial effect of EOs may be related to their penetration of cell wall of microorganism and release its components inside the cell (Burt, 2004). The EOs hydrophobicity allows them destroys the bacterial lipid layer of mitochondrion and cell membrane making them penetrable which leading to damage of cell ions and death (Lambert et al., 2001). Furthermore, the properties of EO, microorganism type, and cell wall structure affect on the activity of the EOs as antimicrobial (Nazzaro et al., 2013).

Clove and thyme EOs exhibited a significant inhibitory effect on the isolated strains of *L. monocytogenes* with minimum inhibitory concentration 3.125% for clove EO, and 1.56% for thyme Eo (Table 3, Figure 3). Similar results were reported by Elafify et al., (2022) who reported that clove and thyme Eos have antibacterial activities. Therefore, Clove and thyme EOs can be used in food industry as antibacterial but need further studies to detect the effective, safe, and acceptable concentrations.

Conclusion

Investigation of *L. monocytogenes* in meat and poultry products by specific primer and detection of their antibacterial activity is effective in control of *L. monocytogenes* infection. Clove and thyme EOs can be used for *L. monocytogenes* monitoring due to their antibacterial effect.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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