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Antimicrobial effect of some essential oils on *Staphylococcus aureus* in minced meat

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A B S T R A C T

consumers increasingly demand of using natural preservatives as alternative to those chemical additives that has been questioned in last years. So, the effect of some essential oils as thyme, clove and garlic as antimicrobial agent against *Staphylococcus aureus* and their role in enhance shelf life of minced meat were studied. The sensory analysis indicated significant advantages in using thyme, clove and garlic oils in refrigerated minced beef. All used essential oils had considerable effectiveness in decreasing *S. aureus* count. In addition, the results indicated that the bacterial counts decreased as the concentration of the oil increased, accordingly, the concentration 1.5% of each oil gave the best effectiveness and the thyme oil showed the highest action followed by clove and garlic oils.

Keywords: Antimicrobial efficiency, essential oils, *Staphylococcus aureus,* minced meat.

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1. INTRODUCTION

Meat and meat products are of great concern in the human diet. Meat is supplying the human body with easily digestible proteins and supplies all nutrients that contribute significantly to the dietary balance of meal (Azab, 2010). Once the animal has been slaughtered, the meat is fabricated into wholesale or retail cuts. Trim and other cuts of meat are then further processed and ground. This increases the surface area of the meat which allows the increased adherence and growth of the bacteria (Skandamis and Nychas, 2001 and Donsí et al., 2011). Foodborne diseases continue to be a common serious threat to public health all over the world, both industrialized and developing countries suffer large numbers of illnesses (WHO, 2002; Gayoso et al., 2005). Microbial activity is a primary mode of deterioration of many foods and often responsible for the loss of quality and safety. Concerning pathogenic and spoilage microorganisms in foods, they increase due to the increase in outbreaks of food-borne diseases (Singh et al., 2000; Rahman and Kang, 2009). Some food poisoning bacteria as *Staphylococcus aureus* exhibit a serious effect on the consumer health (Saleh and Salah El-Dien, 2005). *Staph. aureus* is versatile pathogen of human and animals and causes a wide variety of diseases ranging in severity from slight skin infection to more sever diseases such as pneumonia and septicaemia of particular relevance to the food processing industry is the ability of some *staph.aureus* strains to produce heat stable enterotoxins that cause staphylococcal food poisoning which ranks as one of the most prevalent causes of gastroenteritis worldwide (Dinges et al ., 2000 and Hejazi , 2013). Refrigeration storage is usually the most common preservative method of fresh meat and meat products. In order to extend refrigerated storage time, antimicrobial and antioxidant additives especially those of synthetic origin, are added to beef products.

However, consumers increasingly demand use of natural products as alternative preservatives in foods, as the safety of synthetic additives has been questioned in last years (Singh et al*.,* 2003; Grande et al., 2007 and Abdel-Hamied et al*.,* 2009). With the rise in bacterial resistance to antibiotics, there is considerable interest in the development of other classes of antimicrobials for the control of infection. Recently, there has been a considerable interest in extracts and essential oils (EOs) from common culinary herbs, spices and aromatic plants which characterized by a notable antimicrobial activity. Such substances can be used to delay or inhibit the growth of pathogenic and/or toxin producing microorganisms in foods (Marino et al*.,* 2001 and Kuorwel et al., 2011).

Thyme essential oil is stated to possess bactericidal properties. It is commonly used in foods mainly for its flavor and aroma. It is active against *Staph. aureus, E. coli* and spoilage flora in meat products. The significant rate of antibacterial activities of thyme oil is mostly attributable to the phenolic compounds and to the hydrocarbons which can be bactericidal or bacteriostatic depending on their effective concentration (Rassooli et al*.,* 2006; Kotan et al., 2010**;** Helmy, 2012 and Küçükbay et al., 2014)**.** Clove essential oil has a wide spectrum of actions not only antibacterial, antiviral, antifungal and antiprotozoal, but also have beneficial effects on the cardiovascular and immune system. It has the ability to inhibit the growth of *Staph. aureus* in meat products. The antibacterial activity of clove is attributed to eugenol with a small addition of cariophyllene and humulene. (Daniel et al*.,* 2009. Garlic oil provides antimicrobial benefits, where garlic oil is rich in organosulfur compounds and their precursors inhibiting the growth of a lot of pathogens as APC, *E. coli* and *Staph. aureus.* and subsequently extending the shelf life of the product, so the garlic extracts are potentially useful in preserving meat products. The application of these garlic derived compounds in meat or other food systems could enhance color, lipid and microbial safety (Uhart et al., 2006 and Jolly and Menon, 2015).

Therefore, the present work was carried out to evaluate the efficiency of thyme, clove and garlic essential oils as antimicrobial agents against *Staphylococcus aureus* in minced meat. Also, enhancement of shelf life of minced meat using some essential oils as thyme, clove and garlic.

2. MATERIAL AND METHODS.

2.1. Essential oils

The ready-made herbal oils of thyme (*Thymus vulgaris*), clove (*Syzygium aromaticum*) and garlic (*Allium Sativum*) used in this study were purchased. All the used chemicals were of analytical reagent grade. These oils were stored in amber-colored bottles at 4°C until use.

2.2. Minced beef.

A total of 3 kg of the fresh minced beef used in this study was purchased from different butcher shops in El Menofiya Governorate. Aiming to eliminate natural bacterial populations, the purchased meat was sterilized in Gumma irradiation units, The Egyptian Atomic Energy Authority (EAEA), Naser City, Cairo, Egypt by Gumma irradiation (dose 5 kgy and dose rate 1.915 kgy/hr) using Indian Gamma Cell (Ge 4000 A), (Huq et al*.,* 2015).

2.3. Bacterial strain and Culture Media

Gram positive *Staphylococcus aureus* reference strain, used in this study, was obtained from Media Unite, Food Hygiene Department, Animal Health Research Institute, Dokki, Giza, Egypt and Baird-Parker Agar was supplied by Merck, Darmstadt, Germany for enumeration and identification of *Staph. aureus.*

2.4. Sensory evaluation

It was carried out according to Pearson and Tauber (1984).

2.5. Preparation of bacterial strains

Four to five isolated colonies of each of the tested strain were picked by sterile inoculating loop and inoculated in tubes of sterile peptone water 0.1% (Merck, Germany) (5 ml in each) and were then incubated at 37°C/24 hrs. From this culture, dilutions up to 10^{10} were plated on Baired Parker agar (Merck, Germany) to determine the cell concentration (Barbosa et al., 2009). The cell count was adjusted to10^6 cfu/ml for *Staph. aureus* (the infective dose of enterotoxin may be achieved when the population of *Staph. aureus* reaches a level of > 10^5 CFU/g) (Stewart et al., 2003). With tube dilution methods, the number of cfu/ml was considered as initial inoculum load to inoculate into fresh minced beef samples. The tested strains were inoculated on decontaminated meat by pouring and swabbing over the minced beef surfaces (Dorsa, 1997). Subsequently, the inoculated minced beef samples were kept for 20 minutes to allow attachment and absorption of the inoculated bacteria (Dubal et al., 2004).

2.6. Antimicrobial Activity Test

The meat samples were immediately prepared and inoculated with *Staph. aureus* (10^6cfu/g) (Kantachote and Charernjiratrakul 2008) then mixed thoroughly by gently squeezing the bags by hand. The meat was divided into equal groups (150 g each). Essential oils of thyme, clove and garlic (%v/g) were added to the minced beef groups to achieve final concentrations of 0.5, 1 and 1.5%. PBS was used as control. The essential oils were mixed with the minced beef samples for a further 30 seconds to ensure even mixing. All the samples with oils and the controls were packed in polyethylene bags, labeled and stored at 4ºC. Sensory (color, odor, texture and overall acceptability) and *Staph. aureus* counts analyses were conducted after 3 hours and every day (24 hrs) during storage, using the serial dilutions and spread plate technique (Jay, 1992). Tests were performed in triplicate.

2.7. Statistical analysis

The data was statistically treated by one-way ANOVA using SPSS program for windows (Version 16) (SPSS Inc. Chicago, IL and USA) and Duncan's post hoc test with $p < 0.05$ considered to be statistically significant.

3. RESULTS.

3.1. Sensory evaluation of untreated (control) and treated minced beef samples inoculated with Staph. aureus during cold storage at 4ºC.

The results showed that the sensory attributes of different treated minced beef samples during cold storage were improved by using different concentrations (.5%, 1% and 1.5 %) of thyme, clove and garlic oils, compared to the control samples under the same storage conditions at 3hrs,1st, 2nd, 3rd, 4th and 5th day of the storage period. The sensory properties of different treated minced beef samples were enhanced by increasing the concentrations of thyme and clove oils as the samples containing 1.5% thyme and clove oils, demonstrated the highest enhancement of sensory attributes, while the samples treated with 0.5% of the same oils demonstrated lower enhancement. The .5% garlic oil demonstrated sensory attributes enhancement more than 1.5% garlic oil. It was observed that the samples treated with thyme and clove oils revealed the highest improvement of sensory attributes, while the samples treated with garlic oil demonstrated the lowest one as shown in table (1).

3.2. The antibacterial effects of different concentrations of essential oils on S. aureus count in artificially inoculated minced beef samples.

Staphylococcus aureus counts in minced beef samples treated with different concentrations of thyme, clove and garlic oils were showed in tables (2, 4 and 6). The initial count of *Staph. aureus* in minced beef samples after inoculation was 10.86±9.24 log CFU/g. in the control samples, the counts were 8.75±6.91, 8.93±8.29, 9.63±9.06 and 10.50±9.24log CFU/g after 3hrs, 1day, 2days and 3days, respectively but not be detected after 4 days.

Table (2) showed that at the concentration of 0.5% thyme, the *Staph. aureus* counts were 8.23±7.19, 7.52±6.56 and 6.82±7.43 log CFU/g after 3hrs, 1day and 2days respectively, but not be detected after 3 days. At the concentration of 1%, the counts were 7.01±5.72, 6.02±4.72, 5.52±4.64, and 5.01±3.80 log CFU/g after 3hrs, 1day, 2days and 3days respectively but not detected after 4 days. At the concentration of 1.5%, the counts were 5.98±4.95, 4.01±2.86, 3.25±1.60, 3.00±2.09 and 2.52±1.64 log CFU/g after 3hrs, 1day, 2days, 3days and 4days respectively, but not be detected after 5 days.

In case of using clove oil (table 4) at a concentration of 0.5, 1 and 1.5%, the counts were 8.71±7.80, 7.78±6.48 and 6.83±6.27 log CFU/g after 3hrs, while after 1day the counts were 8.07±6.82, 6.76±5.57 and 6.05±5.03 log CFU/g respectively, but after 2 days the counts were 7.20±6.14, 6.04±4.00 and 4.95±4.17 log CFU/g respectively. After 3 days the counts were 5.99±4.93, 5.02±3.72 and 3.98±3.02 log CFU/g respectively. The count was 3.88±3.16 log CFU/g at the concentration of 1.5% after 4 days, but the *Staph. aureus* not detected after 4 days at the concentrations of 0.5% and 1% and after 5 days at the concentration of 1.5%.

Table (6) showed that at the concentration of .5% garlic, the counts were 10.10±8.89, 8.87±7.00, 8.83±7.90 and 7.72±6.93 log CFU/g after 3hrs, 1day, 2days and 3days respectively, but not be detected after 4 days. At the concentration of 1%, the counts were 9.20±8.21, 8.54±7.00, 7.75±6.30 and 6.60±5.75 log CFU/g after 3hrs, 1day, 2days and 3days respectively, but not be detected after 4 days. At the concentration of 1.5%, the counts were 8.00±6.96, 7.77±7.43, 7.00±6.29, 6.20±4.93 and 5.59±4.89 log CFU/g after 3hrs, 1day, 2days, 3days and 4 days respectively, but not be detected after 5 days.

All results showed significant growth inhibition of *Staph. aureus* in the minced beef samples treated with different concentrations of thyme, clove and garlic oils during cold storage as shown in tables (3, 5 and 7).

Table (1): Sensory evaluation of untreated (control) and treated minced beef samples inoculated with different concentrations of some E.Os and *Staph. aureus* during cold storage at 4ºC.

Groups		3 hrs	1st day	2nd day	3rd day	4th day	5th day
Control		6	5	4	3	2	1
Thyme oil	0.5%	7	6	5	4	3	2
	1%	8	7	6	5	4	3
	1.5%	8	8	7	6	5	4
Clove oil	0.5%	7	5	4	3	2	1
	1%	7	6	5	4	3	2
	1.5%	8	7	6	5	4	3
Garlic oil	0.5%	8	6	5	4	3	2
	1%	7	6	5	4	3	2
	1.5%	6	5	4	3	2	1

Score System for Sensory Evaluation (Pearson and Tauber, 1984): 1: Very very poor, 2: Very poor, 3: Poor, 4: Fair, 5: Medium, 6: Good, 7: Very good, 8: Very very good, 9: Excellent, Table (2): The antimicrobial effect of different concentrations of thyme essential oil on counts of Staph. aureus (log CFU/g) in artificially inoculated minced beef samples.

Groups		3 hrs	1st day	2nd day	3rd day	4th day	5th day
Control		8.75±6.91	8.93±8.29	9.63±9.06	10.50±9.24	ND	ND
Thyme oil	0.5%	8.23±7.19	7.52±6.56	6.82±7.43	ND	ND	ND
	1%	7.01±5.72	6.02±4.72	5.52±4.64	5.01±3.80	ND	ND
	1.5%	5.98±4.95	4.01±2.86	3.25±1.60	3.00±2.09	2.52±1.64	ND

Initial load of Staph. aureus at zero hour = 10.86±9.24 log CFU/g. ND: Not Detected. The values represent Mean ± SD of three experiments.

Table (3): Analysis of variance (ANOVA) of Staph. aureus counts in artificially inoculated minced meat samples with thyme oil.

Source of variance	D.F	S.S	M.S	F. value
Total	75	235451.47		
Between Doses (D)	3	23642.75	7880.92	4.36++
Between Time (T)	6	97716.15	16286.03	9.01++
(D) × (T) interaction	18	27330.16	1518.34	0.84NS
Error	48	86762.41	1807.55	

D.F = Degrees of freedom ++ = High significant differences (P<0.01)
S.S = Sum squares NS = Non significant differences
M.S = Mean squares

Table (4): The antimicrobial effects of different concentrations of clove essential oil on counts of Staph. aureus (log CFU/g) in artificially inoculated minced beef samples.

Groups		3 hrs	1st day	2nd day	3rd day	4th day	5th day
Control		8.75±6.91	8.93±8.29	9.63±9.06	10.50±9.24	ND	ND
Clove oil oil	0.5%	8.71±7.80	8.07±6.82	7.20±6.14	5.99±4.93	ND	ND
	1%	7.78±6.48	6.76±5.57	6.04±4.00	5.02±3.72	ND	ND
	1.5%	6.83±6.27	6.05±5.03	4.95±4.17	3.98±3.02	3.88±3.16	ND

Initial load of *Staph. aureus* at zero hour = 10.86±9.24 log CFU/g. ND: Not Detected
The values represent Mean ± SD of three experiments.

Table (5): Analysis of variance (ANOVA) of Staph. aureus counts in artificially inoculated minced meat samples with clove oil.

Source of variance	D.F	S.S	M.S	F.value
Total	75	458020.54		
Between Doses (D)	3	199220.37	6406.79	2.99+
Between Time (T)	6	130878.56	21813.09	10.18++
(D) × (T) interaction	18	25070.06	1392.82	0.65NS
Error	48	102851.49	2142.74	

D.F = Degrees of freedom ++ = High significant differences (P<0.01)
S.S = Sum squares + = Significant differences (P<0.05)
M.S = Mean squares NS = Non significant differences

Table (6): The antimicrobial effects of different concentrations of garlic essential oil on counts of Staph. aureus (log CFU/g) in artificially inoculated minced beef samples.

Groups		3 hrs	1st day	2nd day	3rd day	4th day	5th day
Control		8.75±6.91	8.93±8.29	9.63±9.06	10.50±9.24	ND	ND
Garlic oil oil	0.5%	10.10±8.89	8.87±7.00	8.83±7.90	7.72±6.93	ND	ND
	1%	9.20±8.21	8.54±7.00	7.75±6.30	6.60±5.75	ND	ND
	1.5%	8.00±6.96	7.77±7.43	7.00±6.29	6.20±4.93	5.59±4.89	ND

Initial load of *Staph. aureus* at zero hour = 10.86±9.24 log CFU/g ND: Not Detected
The values represent Mean ± SD of three experiments.

Table (7): Analysis of variance (ANOVA) of Staph. aureus counts in artificially inoculated minced meat samples with Garlic oil.

Source of variance	D.F	S.S	M.S	F.value
Total	75	114993.89		
Between Doses (D)	3	9843.72	3281.24	2.61^{+}
Between Time (T)	6	44805.78	7467.63	5.94^{++}
(D) × (T) interaction	18	18103.39	1005.75	0.80^{NS}
Error	48	60344.62	1257.18	

4. DISCUSSION

The meat preservatives restrict microbial activity that cause deterioration and spoilage of meat and meat products (Yadav and Singh, 2004), but The major problem with their application is their carcinogenic nature. So, natural compounds derived from herbs or plants are recommended to be used either completely or partially substituting chemical preservatives (Gammariello et al., 2008 and Hyldgaard et al*.,* 2012).

The present study tried to evaluate the efficacy of different concentrations (.5%, 1% and 1.5%) of thyme, clove and garlic essential oils against *Staphylococcus aureus* in minced beef.

The results obtained in this study concluded that, the sensory properties of different treated minced beef samples during cold storage (4ºC) were improved by using different concentrations (.5%, 1% and 1.5%) of thyme, clove and garlic oils, compared to the control samples after 3 hrs, 1st, 2nd, 3rd, 4th and 5th day of the storage period. The sensory properties of the samples were enhanced by increasing the concentrations of oils during the storage period. As, samples containing 1.5% thyme, clove and garlic oils, respectively demonstrated the highest enhancement of sensory attributes, while the samples treated with 0.5% of the same oils demonstrated the lowest enhancement. The samples treated with thyme and clove oils revealed the highest improvement of sensory attributes, while the samples treated with garlic oil demonstrated the lowest one (table 1). These results are in synchronization with those reported by El-Desouky et al*.,* (2006); Sallem et al., (2010) and Amine, (2013).

Concerning to the antimicrobial effect of different concentrations of tested essential oils on *Staph. aureus* count in artificially inoculated minced beef samples, the results showed that the control samples had the highest counts of *Staph. aureus* at any time of cold storage compared to other treatments. Thyme essential oil showed maximum antibacterial activity followed by clove oil then garlic oil. The inhibition of *Staph. aureus* is related to the concentration of the studied essential oils, since they declined and even inhibited completely, when increasing the concentration of the studied essential oils (tables 2, 4 and 6). These results were in agreement with these of Babu et al*.,* (2011), Amine, (2013) and Jolly and Menon, (2015).

Table (3), Table (5) and Table (7) illustrated that the statistical analysis of the data by one-way ANOVA indicated that there is high significant differences (*P*<0.01) between the antimicrobial effect of different concentrations of thyme, clove and garlic essential oils on Staph. *aureus* counts as the concentration of 1.5% cause significant inhibition of Staph. *aureus*, also there is significant differences (P<0.05) between time of storage. But there are no significant differences between doses of oil and the time of storage.

The better effectiveness of tested essential oils against Gram-positive *S. aureus* may be due to volatile action of essential oils and due to absence of lipo-polysaccharide layer in Gram positive bacteria that might function as an effective barrier against any incoming biomolecule. There might be another possibility that essential oils may successfully inhibit microbial respiration and increase the plasma membrane permeability, which resulted in death of bacterial cells after massive ion leakage. It may also happen due to hydrophilic nature of bacterial cell wall (Burt, 2004; Stojanović-Radić et al., 2010 and Hyldgaard et al., 2012).

In conclusion, using of natural essential oils as antimicrobial agents in meat industry enhance safety and shelf life meat by controlling of food poisoning bacteria. So, it is recommended to replace chemical preservatives by natural ones as thyme, clove and garlic oils.

5. REFERENCES

Abdel-Hamied, A.A., Nassar, A. G. ,El-Badry, W. 2009. Investigations on Antioxidant and Antibacterial Activities of Some Natural Extracts. World J. Dai. and Food Sci. 4: 01-07.

Amine, Reham, A. 2013. Screening of antibacterial activity of Cinnamon, Clove and Rosemary essential oils against common food borne pathogens in minced beef. Benha Veterinary Medical Journal, Vol. 25, NO. 2:151-164.

Azab, R.M. 2010. Enteropathogenic E.coli in some meat products with special reference to O157. M. V. Sc. Thesis, (Meat Hygiene) Fac. Vet. Med., Zagazig Univ.

Babu, A., Sundari, A.,Indumathi, J. , Srujan, R.V. , Sravanthi, M. 2011. Study on the Antimicrobial activity and Minimum Inhibitory Concentration of Essential Oils of Spices. Veterinary World, 4 (7): 311-316.

Barbosa, L.N., Rall, V.L., Fernades, A., Ushimaru, P. , Da Silva I. and Fernandes, A. 2009. Essential oils against foodborne pathogens and spoilage bacteria in minced meat. Foodborne Pathogens and Disease 6:725 – 728.

Burt, S. 2004. Essential oils: their antibacterial properties and potential applications in foods-a review. International Journal of Food Microbiology 94, 223– 253.

Daniel, A.N., Sartoretto, S.M., Schmidt, G. , Caparroz-Assef, S.M. ,Bersani-Amado, C. A. , Cuman, R. K.N. 2009. Anti- inflammatory and antinociceptive activities A of eugenol essential oil in experimental animal models. Revista Brasileira de Farmacognosia, 19: 212- 217.

Dinges, M.M., Orwin, P.M. ,Schlivert, p.M. 2000. Enterotoxins of *Staphylococcus aureus*. Clin. Microbiol. Rev., 13:16-34.

Donsí, F., Annunziata, M., Sessa, M.,Ferrari, G. 2011. Nanoencapsulation of essential oils to enhance their antimicrobial activity in foods. Food Science and Technology, 44, 1908–1914.

Dorsa, W.J.1997. New and established carcass decontamination procedures commonly used in the beefprocessing industry. J. Food Protec., 60: 1146-1151.

Dubal, Z.B., Paturkar, A.M., Waskar, V.S., Zende, R.J., Latha, C. 2004. Effect of food grade organic acids on inoculated Staph. aureus, L. monocytogenes, E. coli and Sal.

typhimurium in sheep/goat meat stored at refrigeration temperature. J. Meat Sci., 66: 817-821.

El-Desouky, A. L., Bahlol, H. E. M., Sharoba, A. M. A. 2006.Effect of some essential oils and preservatives on the growth of E. coli O157: H7 and quality of refrigerated minced meat. Annuals of Agric. Sci. Moshtohor, 44 (4): 1675- 1695.

Gammariello, D. et al. 2008. Effects of natural compounds on microbial safety and sensory quality of Fior di Latte cheese, a typical Italian cheese. J. Dairy Sci., 91 (11): 4138-4146.

Gayoso, C. W. 2005. Sensitivity of fungi isolated from onychomycosis to Eugenia cariophyllata essential oil and eugenol. Fitoterapia, 76, ;247-249.

Grande, M.J., Lopez, R.L., Abriouel, H., Valdivia, E., Ben Omar,N. ,Maqueda, M. ,Martinez- Canamero, M. , Galvez, A. 2007. Treatment of vegetable sauces with enterocin AS-48 alone or in combination with phenolic compounds to inhibit proliferation of Staphylococcus aureus. Journal of Food Protection 70 (2), 405–411.

Hejazi, M. A. 2013. Microbial changes in cattle carcasses stored at chilling condition. M. V. Sc. (Meat hygiene)Fac. Vet. Med., Alexandria Univ.

Helmy, Shahinaze, A. 2012. Extending the shelf life of minced meat by some essential oils under refrigeration and freezing storage. International Journal of Academic Research, 4 (6) :14-21.

Huq, T., Vu, K., Ried, B., Bouchard, J., Lacroix, M. 2015. Synergistic effect of gamma (γ)-irradiation and microencapsulated antimicrobials against Listeria monocytogenes on ready-to-eat (RTE) meat. Food MicrobiologyVolume 46, 507–514.

Hyldgaard, M., Mygind, T., Meyer, R. L. 2012. Essential oils in food preservation: mode of action, synergies, and interactions with food matrix components. Front Microbiogy, 3, 12. doi: 10.3389/ fmicb. 2012.00012.

Jay, J.M. (1992): Modern Food Microbiology, 4th Ed. NewYork: Chapman and Hall.

Jolly, D., Menon, V. 2015.Antibacterial effect of garlic and ginger extracts on Escherichia coli and Listeria monocytogenes. International Journal of Applied and Pure Science and Agriculture (IJAPSA) 01, 2. -: 2394-5532, : 2394-823x.

Kantachote, D., Charernjiratrakul, W. 2008. Selection of lactic acid bacteria from fermented plant beverages to use as inoculants for improving the quality of the finished product. Pakistan J. Biol. Sci., 1-8.

Kotan, R., Çakır, A., Dadasoglu, A., Aydin, T., Cakmakci, R., Özer, H. ,Kordali, S. , Mete E. , Dikbas, N. 2010.Antibacterial activities of essential oils and extracts of Turkish Achillea, Satureja and Thymus species against plant pathogenic bacteria, J. Sci. Food. Agr. 90, 145-160.

Küçükbay, F., Kuyumcu, S., Çelen, S., Azaz, A., Arabac, T. 2014.Chemical Composition of the Essential Oils of Three Thymus Taxa from Turkey with Antimicrobial and Antioxidant Activities. Rec. Nat. Prod. 8:2; 110-120.

Kuorwel, K., Cran, M., Sonneveld, K., Miltz, J., Bigger, S. 2011. Essential Oils and Their Principal Constituents as Antimicrobial Agents for Synthetic Packaging Films. Journal of Food Science, 76 (9).

Marino, M., Bersani, C., Comi, G. 2001. Impedance measurements to study the antimicrobial activity of essential oils from Lamiaceae and Compositae. International Journal of food Microbiology 67, 187–195.

Pearson, M. A., Tauber, W. F. 1984. Processed meat. 2nd Ed., pp: 93. AVI Pub. Com., Inc. West port, Connection.

Rahman, A., Kang, S. 2009. In vitro control of food-borne and food spoilage bacteria by essential oil and ethanol extracts of Lonicera japonica Thunb. Food Chemistry 116, 670–675.

Rassooli, I., Rezaei, M. B., Allameh, A. 2006. Ultrastructural studies on antimicrobial efficacy of thyme essential oils Listeria monocytogenes, Int. J. Infect. Dis. 10, 236-241.

Saleh, M. A., Salah El-Dien, W.M. 2005. Microbiological studies on some meat products at sharkia governorate markets. Zag. Vet. J., 33(3) 141-151.

Salem-Amany, M., Amine-Reham, A., A. Gehan, S. 2010. Studies on Antimicrobial and Antioxidant Efficiency of Some Essential Oils in Minced Beef. Journal of American Science, 6 (12).

Singh, R., Chandra, R., Bose, M., Luthra, P. M. 2000. Antibacterial activity of Curcuma longa rhizome extract on pathogenic bacteria. Current Science, 83(6), 25.

Singh, A., Singh, R.K., Bhunia, A.K.,Singh, N. 2003. Efficacy of plant essential oils as antimicrobial agents against Listeria monocytogenes in hotdogs. LWT Food Science and Technology 36, 787–794.

Skandamis, P.N., Nychas, G.J.E. 2001 Effect of oregano essential oil on microbiological and physico-chemical attributes of minced meat stored in air and modified atmospheres. Journal of Applied Microbiology 91, 1011 –1022.

Stewart, C.M.; Cole, M.B. and Schaffner, D.W. (2003): Managing the risk of staphylococcal food poisoning from cream- filled baked goods to meet a food safety objective. J. Food Prot., 66: 1310-25.

Stojanović-Radić, Z., Nešić, M., Čomić, L., Niko Radulović, N. 2010. Antimicrobial activity and cytotoxicity of commercial rosemary essential oil (Rosmarinus officinalis L.). Biologica Nyssana 1 (1-2): 83-88.

Uhart, M., Maks, N., Ravishankar, S. 2006. effect of spices on growth and survival of Salmonella Typhimurium DT 104 in ground beef stored at 4 and 8ºc. J. Food Safety, 26:115-125.

WHO, 2002. World Health Report 2002: World Health Report: Reducing Risks to Health Noncommunicable Diseases, World Health Organization.

Yadav, A. S. and Singh, R. P. 2004. Natural preservatives in poultry meat products. Natural Product Radiance, 3(4).