

## Research Article

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# Antimicrobial Effect of Some Essential Oils on Some Pathogenic Bacteria in Minced Meat

Saad M Saad<sup>1</sup>, Fahim A Shaltout<sup>1\*</sup>, Nahla A Abou Elroos<sup>2</sup>, Saber B El-nahas<sup>3</sup>

<sup>1</sup>Food Control Department, Veterinary Medicine, Benha University, Egypt

<sup>2</sup>Animal Health Research Institute, Shebin El koom branch, Egypt

<sup>3</sup>Food Inspector at Veterinary Quarantine, Cairo Airport, Egypt

**\*Corresponding Author:** Fahim A Shaltout, Food Control Department, Veterinary Medicine, Benha University, Egypt, E-mail: [fahimshaltout@hotmail.com](mailto:fahimshaltout@hotmail.com)

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## Abstract

Plant essential oils (EOs) serve as a “safe” alternative to chemical or synthetic antimicrobials and antioxidants to struggle with the food borne pathogens or spoilage organisms, inhibiting lipid oxidation so, the effect of some essential oils as thyme (*thymus vulgaris*), cinnamon (*cinnamomum zeylanicum*) and garlic (*Allium sativum*) at concentrations (1%) as natural preservatives as well as their ability to increase the shelf life of minced meat, and also their effects on bacterial agents as *Staphylococcus aureus* and *E. coli* and sensory properties of minced meat after 3 hrs, 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> day during cold storage at 4°C. The obtained results showed that treated samples revealed decreasing values of *S. aureus* and *E. coli* counts and improving sensory properties than untreated samples (control) ones. Also, thyme oil at concentration (1%) was more effective than cinnamon and garlic oils.

**Keywords:** Essential oils; Thyme oil; Cinnamon oil; Garlic; Minced meat

## 1. Introduction

Meat and meat products are the most palatable of highly nutritive value foods for human being as they are important sources for protein, fat, essential amino acids, minerals, vitamins and other nutrients [1]. On the other hand, they are considered as an ideal culture medium for growth of many organisms because of the high moisture, the high percentage of nitrogenous compounds, plentiful supply of minerals, some fermentable carbohydrates (glycogen) and of a favorable pH for most microorganisms [2]. Poor hygienic practices in food processing plants may result in the contamination of food products with pathogens causing a serious risk for human health. Moreover, the complete elimination of pathogens from food processing environments is a difficult task, in part because bacteria can attach to food contact surfaces and form biofilms where they survive even after cleaning and disinfection [3]. Natural

products, such as essential oils (EOs) which are produced by the secondary metabolism of herbs and/or spices and their constituents have uses in human consumption as functional food (nutraceuticals, biopolymers) and food additives (flavourings, antioxidant and antimicrobial) [4]. Food industry is facing great challenges to produce safe and at the same time food without synthetic chemical preservatives. So essential oils make their way into the scientific focus, due to their antibacterial, antifungal and antiviral activity, as well as antioxidant properties, they are used to prevent foodborne diseases, to extend shelf-life and to improve some meat characteristics [5]. These generally recognized as safe (GRAS) natural substances which inhibit lipid oxidation in foods and thus offer the promise of providing natural food additives to food products [4]. Thyme is commonly used in foods mainly for its flavor and aroma. Also, thymol, which is found in thyme, has been commercially available as part of mouthwash for more than hundred years. Cinnamon can be used as spice because of its sweet flavoring and spicy characteristics. It also plays an important role in pharmacological effects such as: anti-inflammatory, antimicrobial, antioxidant and cytotoxic properties [6]. Garlic is one of the most commonly used ingredients as a flavor enhancement. Garlic has a wide spectrum of actions, not only antibacterial, antifungal and antiprotozoal, but also it has beneficial effects on the cardiovascular and immune systems. Therefore, the present work was carried out to evaluate the efficiency of thyme, Cinnamon and garlic essential oils as antimicrobial agents in control of *Staphylococcus aureus* and *E. coli* in minced meat and enhancement of shelf life of these meat using some essential oils as thyme, Cinnamon and garlic.

## 2. Materials and Methods

### 2.1 Essential oils

The ready-made herbal oils of (1%) thyme (*Thymus vulgaris*), (1%) cinnamon (*cinnamomum zeylanicum*) and (1%) garlic (*Allium Sativum*) used in this study were purchased from Elgamhoria Co., Sharkia, Egypt. 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 2400 gm 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, Minced meat samples put under the UV light in the cabinet for 20 minutes in order to be completely sterilized from any microorganisms attached to its surface.

### 2.3 Bacterial strain and culture media

*Escherichia coli* count determined by plating appropriate dilutions on EMB agar, *Staphylococcus aureus* count was determined on Baird Parker agar, both obtained from Media Unite, Food Hygiene Department, Animal Health Research Institute, Dokki, Giza, Egypt.

### 2.4 Preparation of sample

The samples were immediately prepared and divided into two equal groups (1200 gm each) One group was inoculated with *E-coli* (O<sub>26</sub>:H<sub>111</sub>) ( $2 \times 10^6$  cfu/g) and the other group was inoculated with *Staphylococcus aureus* ( $5 \times$

$10^6$  cfu/g), then mixed thoroughly by gently squeezing the bags by hand. Each main group was subdivided into 4 equal subgroups (100 g each), the number of cfu/ml was considered as initial inoculum load to inoculate into fresh minced beef samples. The inoculated minced beef samples were kept for 20 minutes to allow attachment and absorption of the inoculated bacteria [7]. Essential oils of thyme, cinnamon and garlic (%v/g) were added to the minced beef groups to achieve final concentration (1%). 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 4°C. Sensory (color, odor, texture and overall acceptability) and *Staph. aureus* and *E-coli* counts analyses were conducted after 3 hours and 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> day during storage, using the serial dilutions and spread plate technique [8].

## 2.5 Statistical analysis

ANOVA was carried out on data of the sensory, chemical and microbiological evaluations.

## 3. Results

Results in Table 1 showed that in case of using thyme oil (1%) the scores of color evaluation were 8, 6, 5, 4, 3 and 2 after 3 hrs, 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> day of the storage period respectively, while in case of using cinnamon oil (1%) the scores were 7, 6, 5, 4, 3 and 2 after 3 hrs, 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> day of the storage period respectively, but in case of using garlic oil (1%) the scores were 7, 6, 5, 4, 3, 2 after 3 hrs, 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> day of the storage period respectively.

Groups	Oil conc.	3 hrs	1 <sup>st</sup> day	2 <sup>nd</sup> day	3 <sup>rd</sup> day	4 <sup>th</sup> day	5 <sup>th</sup> day
Control	-	6	4	4	3	2	1
Thyme	1%	8	6	5	4	3	2
Cinnamon	1%	7	6	5	4	3	2
Garlic	1%	7	6	5	4	3	2

Score System for Sensory Evaluation [9]: 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 1:** Effect of (1%) thyme, cinnamon and garlic essential oils on color of inoculated minced beef during cold storage at 4°C.

The data recorded in Table 2 demonstrated that in case of using (1%) concentration of thyme oil the scores of odor evaluation were 8, 7, 6, 5, 4 and 2 after 3 hrs, 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> day of the storage period respectively, while in case of using (1%) concentrations of cinnamon oil the scores were 7, 6, 5, 4, 3 and 2 after 3 hrs, 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> day of the storage period respectively, but in case of using (1%) concentration of garlic oil the scores were 6, 5, 5, 3, 3 and 2 after 3 hrs, 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> day of the storage period respectively.

Groups	oil conc.	3 hrs	1 <sup>st</sup> day	2 <sup>nd</sup> day	3 <sup>rd</sup> day	4 <sup>th</sup> day	5 <sup>th</sup> day
Control	-	7	6	5	4	2	2
Thyme	1%	8	7	6	5	4	2
Cinnamon	1%	7	6	5	4	3	2
Garlic	1%	6	5	5	3	3	2

Score System for Sensory Evaluation [9]: 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:** Effect of (1%) thyme, cinnamon and garlic essential oils on odor of inoculated minced beef during cold storage at 4°C.

The data in Table 3, in case of using thyme oil at the concentrations (1%) the scores of texture were 8, 6, 5, 4, 3 and 2 after 3 hrs, 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> day of the storage period respectively, while in case of using (1%) concentration of cinnamon oil, the scores were 7, 6, 5, 4, 3 and 2 after 3 hrs, 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> day of the storage period respectively, but in case of using 1% concentration of garlic oil the scores were 7, 6, 5, 4, 3 and 2 after 3 hrs, 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> day of the storage period respectively.

Groups	oil conc.	3 hrs	1 <sup>st</sup> day	2 <sup>nd</sup> day	3 <sup>rd</sup> day	4 <sup>th</sup> day	5 <sup>th</sup> day
Control	-	6	5	4	3	2	1
Thyme	1%	8	6	5	4	3	2
Cinnamon	1%	7	6	5	4	3	2
Garlic	1%	7	6	5	4	3	2

Score System for Sensory Evaluation [9]: 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 3:** Effect of (1%) thyme, cinnamon and garlic essential oils on texture of inoculated minced beef during cold storage at 4°C.

Concerning to the overall acceptability, the results obtained in Table 4 revealed that in case of using (1%) concentrations of thyme oil the scores were 8, 7, 6, 5, 4 and 3 after 3 hrs, 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> day of the storage period respectively, while in case of using 1% concentration of cinnamon oil the scores were 7, 6, 5, 4, 3 and 2 after 3 hrs, 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> day of the storage period respectively, but in case of using 1% concentration of garlic oil the scores were 7, 6, 5, 4, 3 and 2 after 3hrs, 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> day of the storage period respectively. The results recorded in Table 5 In case of using the concentration of (1%) of thyme oil, *S. aureus* counts were 7.01, 6.02, 5.52, 5.01 and 3.52 log<sub>10</sub> cfu/g after 3 hrs, 1 day, 2 days, 3 days and 4 days of inoculation respectively, but not detected after 5 days of inoculation, comparing to *S. aureus* counts in the control samples which were 8.75, 8.93, 9.63, 10.50 and 10.71 log<sub>10</sub> cfu/g after 3 hrs, 1 day, 2 days 3 days and 4 days of inoculation respectively, but not detected after 5 days of inoculation.

Groups	oil conc.	3 hrs	1 <sup>st</sup> day	2 <sup>nd</sup> day	3 <sup>rd</sup> day	4 <sup>th</sup> day	5 <sup>th</sup> day
Control	-	6	5	4	3	2	1
Thyme	1%	8	7	6	5	4	3
Cinnamon	1%	7	6	5	4	3	2
Garlic	1%	7	6	5	4	3	2

Score System for Sensory Evaluation [9]: 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 4:** Effect of (1%) thyme, cinnamon and garlic essential oils on overall acceptability of inoculated minced beef during cold storage at 4°C.

Groups	oil conc.	3 hrs	1 <sup>st</sup> day	2 <sup>nd</sup> day	3 <sup>rd</sup> day	4 <sup>th</sup> day	5 <sup>th</sup> day
Control	-	8.75 ± 6.91	8.93 ± 8.29	9.63 ± 9.06	10.50 ± 9.24	10.71 ± 10.51	ND
Thyme	1%	7.01 ± 5.72	6.02 ± 4.72	5.52 ± 4.64	5.01 ± 3.80	3.52 ± 2.96	ND
Cinnamon	1%	7.01 ± 5.72	6.02 ± 4.72	5.52 ± 4.64	5.01 ± 3.80	3.52 ± 2.96	ND
Garlic	1%	9.20 ± 8.21	8.54 ± 7.00	7.75 ± 6.30	6.60 ± 5.75	6.06 ± 4.65	ND

Initial load of *S. aureus*=10.86 ± 9.24 log CFU/g; The values represent Mean ± SD of three experiments.

**Table 5:** Antimicrobial effect of (1%) thyme, cinnamon and garlic essential oils in control of *Staphylococcus aureus* (log cfu/g)artificially inoculated in minced beef during cold storage at 4°C.

Groups	Oil conc.	3 hrs	1 <sup>st</sup> day	2 <sup>nd</sup> day	3 <sup>rd</sup> day	4 <sup>th</sup> day	5 <sup>th</sup> day
Thyme	1%	35.451	44.567	49.171	53.867	67.587	-
Cinnamon	1%	28.361	37.753	44.383	53.775	57.735	-
Garlic	1%	15.285	21.363	28.637	39.227	44.199	-

**Table 6:** Reduction % of *S.aureus* count artificially inoculated into minced beef samples treated with (1%) thyme, cinnamon and garlic oils.

In case of using cinnamon oil at a concentration of (1%), *S. aureus* counts were 7.01, 6.02, 5.52, 5.01 and 3.52 log<sub>10</sub> cfu/g after 3 hrs, 1 day, 2 days, 3 days and 4 days of inoculation respectively, but not detected after 5 days of inoculation, comparing to *S. aureus* counts in the control samples which were 8.75, 8.93, 9.63, 10.50 and 10.71 log<sub>10</sub> cfu/g after 3 hrs, 1 day, 2 days, 3 days and 4 days of inoculation respectively, but not detected after 5 days of inoculation. At the concentration of 1% garlic oil, *S. aureus* counts were 9.20, 8.54, 7.75, 6.60 and 6.06 log<sub>10</sub> cfu/g after 3 hrs, 1 day, 2 days, 3 days and 4 days of inoculation respectively, but not detected after 5 days of inoculation, comparing to *S. aureus* counts in the control samples which were 8.75, 8.93, 9.63, 10.50 and 10.71 log<sub>10</sub> cfu/g after 3 hrs, 1 day, 2 days, 3 days and 4 days of inoculation respectively, but not detected after 5 days of inoculation. Table 7.

Groups	oil conc.	3 hrs	1 <sup>st</sup> day	2 <sup>nd</sup> day	3 <sup>rd</sup> day	4 <sup>th</sup> day	5 <sup>th</sup> day
Control	-	6.06 ± 7.04	5.42 ± 7.50	7.24 ± 7.54	5.86 ± 8.00	6.98 ± 9.13	ND
thyme	1%	4.13 ± 5.04	2.42 ± 4.54	2.90 ± 4.20	3.27 ± 3.25	2.95 ± 3.10	ND
cinnamon	1%	4.78 ± 6.08	4.05 ± 5.10	0.78 ± 4.53	2.75 ± 4.10	2.42 ± 4.05	ND
garlic	1%	4.75 ± 6.11	6.27 ± 5.91	4.00 ± 5.51	3.56 ± 4.66	3.00 ± 4.49	ND

Initial load of *E. coli* = 7.90 ± 5.70 log cfu/g; The values represent Mean ± SD of three experiments.

**Table 7:** Antimicrobial effect of (1%) thyme, cinnamon and garlic essential oils in control of *Escherichia coli* (log cfu/g) artificially inoculated in minced beef during cold storage at 4°C.

Groups	Oil conc.	3 hrs	1 <sup>st</sup> day	2 <sup>nd</sup> day	3 <sup>rd</sup> day	4 <sup>th</sup> day	5 <sup>th</sup> day
Thyme	1%	36.203	42.531	46.835	58.861	60.759	-
Cinnamon	1%	23.038	35.443	42.659	48.101	48.734	-
Garlic	1%	22.658	25.189	30.253	41.013	43.165	-

**Table 8:** Reduction % of *E. coli* count artificially inoculated into minced beef samples treated with (1%) thyme, cinnamon and garlic oils.

At the concentration of (1%) thyme oil, *E. coli* counts were 4.13, 2.42, 2.90, 3.27 and 2.95 log<sub>10</sub> cfu/g after 3 hrs, 1 day, 2 days, 3 days and 4 days of inoculation respectively, but not detected after 5 days of inoculation, comparing to *E. coli* counts in the control samples which were 6.06, 5.42, 7.24, 5.86 and 6.98 log<sub>10</sub> cfu/g after 3 hrs, 1 day, 2 days, 3 days and 4 days of inoculation respectively, but not detected after 5 days of inoculation. In case of using cinnamon oil at a concentration of (1%), *E. coli* counts were 4.78, 4.05, 0.78, 2.75 and 2.42 log<sub>10</sub> cfu/g after 3 hrs, 1 day, 2 days, 3 days and 4 days of inoculation respectively, but not detected after 5 days of inoculation. At the concentration of (1%) garlic oil, *E. coli* counts were 4.75, 6.27, 4.00, 3.56 and 3.00 log<sub>10</sub> cfu/g after 3 hrs, 1 day, 2 days, 3 days and 4 days of inoculation respectively, but not detected after 5 days of inoculation, comparing to *E. coli* counts in the control samples which were 6.06, 5.42, 7.24, 5.86 and 6.98 log<sub>10</sub> cfu/g after 3 hrs, 1 day, 2 days, 3 days and 4 days of inoculation respectively, but not detected after 5 days of inoculation.

#### 4. Discussion

The meat preservatives restrict microbial activity that cause deterioration and spoilage of meat and meat products [10], 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 [11, 12]. Essential oils will result in immediate reduction of bacterial population [13] and might be more effective against food borne pathogens and spoilage bacteria when applied directly on foods ready to be used, containing a high protein level at acidic pH, as well as, lower levels of fat or carbohydrates [14]. The sensory properties of different treated minced beef samples during cold storage (4°C) were improved by using concentration (1%) of thyme,

cinnamon and garlic oils, compared to the control samples after 3 hrs, 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> day of the storage period.

Concerning to the antimicrobial effect of (1%) concentration of tested essential oils on *Staph. aureus* and *E. coli* count in artificially inoculated minced beef samples, the results showed that the control samples had the highest counts of *Staph. aureus* and *E. coli* at any time of cold storage compared to other treatments. The antimicrobial properties of essential oils against various pathogenic microorganisms are contributed to the presence of a large number of alkaloids, phenols, terpenes derivatives compounds and other antimicrobial compounds. These compounds possess hydrophobic characteristics, which enable them to partition the lipids of bacterial cell membrane and mitochondria and interact with different targets of microbial cell (e.g., cell wall and cytoplasmic membrane), causing loss of cellular constituents, collapse of membrane structure, loss of membrane integrity, dissipation of proton motive force, sequential inhibition of respiration and ion transport processes, impairment of a variety of protective enzymes, involved in the production of energy or synthesis of structural components in microbial cells, possibly through reaction with sulfhydryl compounds or through more non specific interactions with the protein, alteration in the morphology, structure and function, modification in the transport of nutrients, membrane disruption, extensive leakages from bacterial cells or exit of critical molecules and ions leading to cell death [15]. The antimicrobial activities of thyme (*thymus vulgaris*) is likely due to their high content of thymol which is among the most efficient herbal antibacterial agent known the working of thymol against *S. aureus* was hypothesized to that thymol binds to membrane proteins hydrophobically and by means of hydrogen bonding, thereby changing the permeability characteristics of the membrane. Thymol was found to be more inhibitive at pH 5.5 than 6.5. At low pH the thymol molecule would be undissociated and therefore more hydrophobic, and so may bind better to the hydrophobic areas of proteins and dissolve better in the lipid phase [16, 17].

Garlic oil (*Allium sativum*) provides antimicrobial benefits, where garlic oil is rich in organosulfur compounds and their precursors (allicin, diallyl sulfide and diallyltrisulfides) inhibiting the growth of a lot of pathogens as *E. coli* and *S. aureus*, 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 [18, 19]. 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 [11, 20, 21]. Essential oils possess a significant antibacterial activity against *S. aureus* and *E. coli* in the following order: thyme>cinnamon>garlic.

## 5. Conclusion

The obtained results in this study concluded that thyme, cinnamon and garlic oils can be used as natural meat preservatives with antimicrobial activities against food borne pathogens and therefore may be useful 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, cinnamon and garlic oils.

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