



SCREENING OF ANTIBACTERIAL ACTIVITY OF CINNAMON, CLOVE AND ROSEMARY ESSENTIAL OILS AGAINST COMMON FOOD BORNE PATHOGENS IN MINCED BEEF

Reham, A. Amin

Food Control Department, Fac. Vet. Med., Benha University

ABSTRACT

Cinnamon, clove and rosemary essential oils were evaluated for their effects on the growth and survival of *Staphylococcus aureus* and *Escherichia coli* artificially inoculated into minced beef. Fresh minced beef samples were inoculated with (~ 106 CFU/ml) (6 log CFU/g) of *S. aureus* and (~ 104 CFU/ml) (4 log CFU/g) of *E. coli* and left for 30 minutes at room temperature (25°C) to allow attachment and absorption of bacteria. Initial counts of *S. aureus* and *E. coli* in minced beef samples immediately after inoculation were 10.86 and 7.91 log CFU/g, respectively. Essential oils of cinnamon (*Cinnamomum zeylanicum*), clove (*Syzygium aromaticum*) and rosemary (*Rosmarinus officinalis*) (%v/g) were added to the minced beef samples to achieve final concentrations of 0.5, 1 and 1.5%. Sensory (color, odor and texture) and bacteriological (*S. aureus* and *E. coli* counts) analyses were conducted after 3, 6 hrs and every day (24 hrs) during cold storage at 4°C. Initial counts in minced beef samples decreased following treatment with 1.5% of cinnamon, clove and rosemary oils for 3 hours by 5.56 log CFU/g (51.19 %), 4.01 log CFU/g (36.92 %) and 2.84 log CFU/g (26.15 %), respectively, of *S. aureus* and 3.58 log CFU/g (45.26 %), 2.88 log CFU/g (36.41 %) and 1.23 log CFU/g (15.55 %), respectively, for *E. coli*. Growth of *S. aureus* in minced beef samples was completely inhibited after treatment with cinnamon oil 1% after 3 days, cinnamon oil 1.5% after 2 days, clove oil 1% after 4 days and clove oil 1.5% after 3 days. As compared with several other mild preservation procedures, treatment with cinnamon, clove and rosemary essential oils is inexpensive and uncomplicated method. Results of the present study are envisaged to be useful for commercial applications of these essential oils as potential food biopreservatives and anti- *E. coli* and *S. aureus* agents in minced meat and other foods, depending upon the desired flavor of such products.

KEY WORDS: Essential oils, Cinnamon, Clove, Rosemary, *S. aureus*, *E. coli*, Minced beef, Gram positive bacteria, Gram negative bacteria

(BVMJ-25(2): 151-164, 2013)

1- INTRODUCTION

In spite of modern improvements in food production techniques, food safety is still an important aspect of public health. It has been estimated that as many as 30 % of people in industrialized countries suffer from a food borne disease each year [1]. Such outbreaks are a challenging issue for the food industry and underpin the need for a paradigm shift in the methods used to prevent or minimize such occurrences. There is a current worldwide demand to explore new

alternatives to control food borne diseases, giving priority to methods that reduce disease incidence and avoid negative and side effects on human health. Western society is experiencing a trend of “green” consumerism, desiring fewer synthetic additives and chemical residues in foods together with their increased safety and quality [2].

The use of phytochemicals as natural antimicrobial agents is gaining popularity as an emerging technology that could be

used by the industry to extend the storage life of food and overcome these food safety issues and hence offering a potential alternative to synthetic preservatives [3].

Essential oils are natural, concentrated, hydrophobic, volatile and aromatic, oily liquids obtained from plant materials, showing different colors ranging from pale yellow to emerald green and from blue to dark brownish red. Down the ages essential oils from an estimated 3000 plant species are known, of which about 300 are commercially important - destined chiefly as food flavoring agents and impart pungent stimuli [4].

Essential oils of plants are of growing interest both in the industry and scientific research because of their antibacterial, antifungal, antiviral, insecticidal, anti-inflammatory and antioxidant properties [5].

Cinnamon can be used as spice, especially in the Indian diet, because of its sweet flavoring and spicy characteristics, easily absorbed and do not have any adverse effects. It also plays an important role in pharmacological effects such as: carminative, stomachic, astringent, stimulant, anti-inflammatory, antimicrobial, antioxidant, antispasmodic, antiulcer completely inhibiting both sensitive and resistant strain of *Helicobacter pylori*, cytotoxic properties and and treating the common cold [6].

Clove oil is dental analgesic, carminative, stimulant and antiseptic. Clove oil can be used in food as a flavoring agent, in folk medicine as an antiepileptic remedy, as a fragrance in personal care products, in aromatherapy, transdermal drug delivery systems, pharmacy, perfumery and cosmetics [7].

Rosemary (*Rosmarinus officinalis* L.) is plant belonging to the family Labiateae. It is widely used as a carminative, flavoring agent and culinary herb, especially in Mediterranean dishes, and is also used as a fragrant additive in soaps and other cosmetics [8].

For the establishment of antimicrobial properties of essential oils, conventional in vitro studies as the agar diffusion and broth dilution methods are usually applied. The main disadvantages of the results of in vitro studies are the volatility, water insolubility and complexity of essential oils, their volatile components are likely to evaporate with the dispersing solvent during the incubation time, while their poorly soluble components do not diffuse well in the agar broth [9], difficult to compare to each other because of the different test methods, different methods of extraction, and variation in chemical phytoconstituents in plants due to different agroclimatic, but they still remain the most common techniques.

To the best of our knowledge, there is no profound knowledge on antibacterial activities of the essential oils in food (in vivo). Therefore, the main goal of the present work was carried out to:

1. Evaluate the efficacy of cinnamon, clove and rosemary essential oils as antimicrobial agents in vivo and to determine their minimal inhibitory and bactericidal activities against two pathogenic bacterial strains i.e. *S. aureus* and *E. coli*.
2. Optimize the concentrations of these essential oils in minced beef due to their limited application in high concentrations because of their strong aroma causing unfavorable organoleptic properties.

2. MATERIAL AND METHODS:

2.1. Essential oils:

The ready-made herbal oils of cinnamon (*Cinnamomum zeylanicum*), clove (*Syzygium aromaticum*) and rosemary (*Rosmarinus officinalis*) were purchased from Nevertary Co., Cairo, Egypt. All the used chemicals were of analytical reagent-grade. These oils were stored in amber-coloured bottles at 4°C until use.

2.2. Preparation of bacterial strains:

Antibacterial activity of cinnamon, clove and rosemary essential oils

The essential oils were individually tested against *S. aureus* and *E. coli*. These strains were obtained from Laboratory of Bacteriology, Department of Microbiology, Faculty of Veterinary Medicine, Benha University, Egypt. Four to five isolated colonies of each of the tested strains 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 [10]. From this culture, dilutions up to 10¹⁰ were plated on Baird Parker agar and Eosin Methylene Blue (EMB) agar (Merck, Germany) to determine the cell concentration. The cell count was adjusted to 10⁶ cfu/ml for *S. aureus* [11] (the effective dose of enterotoxin may be achieved when the population of *S. aureus* reaches a level of > 10⁵ CFU/g) [12] and adjusted to 10⁴ cfu/ml for *E. coli* [13] with tube dilution methods. The number of cfu/ml was considered as initial inoculum load to inoculate into fresh minced meat samples. The tested strains were inoculated on decontaminated meat by pouring and swabbing over the meat surfaces [14]. Subsequently, the inoculated meat samples were kept for 20 minutes to allow attachment and absorption of the inoculated bacteria [15].

2.3. Antimicrobial Activity Test:

A grand total of 3 kg of fresh minced beef was collected from the butcher shop in Tanta, Gharbia governorate, Egypt. The samples were taken and transferred directly to the laboratory using an ice box under complete aseptic conditions without undue delay. The samples were immediately prepared and divided into two main equal groups (1.5 Kg each). One group was inoculated with *S. aureus* (10⁶ cfu/g) [11], and the other group was inoculated with *E. coli* (10⁴ cfu/g) [13], then mixed thoroughly by gently squeezing the bags by hand. Each main group was subdivided into ten equal subgroups (150 g each). Essential oils of cinnamon (*Cinnamomum zeylanicum*),

clove (*Syzygium aromaticum*) and rosemary (*Rosmarinus officinalis*) (%v/g) were added to the minced beef subgroups 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 4°C. Sensory (color, odor and texture) and bacteriological (*S. aureus* and *E. coli* counts) analyses were conducted after 3, 6 hours and every day (24 hrs) during storage, using the serial dilutions and spread plate technique [16]. Tests were performed in triplicate.

2.4. Sensory examination:

It was carried out according to Pearson and Tauber [17].

2.5. Bacteriological analysis:

Bacterial counts were applied using standard methods [18]. *Staphylococcus aureus* count was determined on Baird Parker agar (Merck, Germany). Plates were incubated at 37°C/48 hours and black shiny colonies with narrow white margins surrounded by a clear halo zone extending into the opaque medium were enumerated. *Escherichia coli* count was determined by plating appropriate dilutions on EMB agar (Merck, Germany). Plates were incubated at 37°C/24 hours and greenish metallic colonies with dark purple center were enumerated.

2.6. 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:

Table (1): Sensory evaluation of untreated (control) and treated minced beef samples inoculated with *S. aureus* during cold storage at 4°C

Groups		3 hrs	6 hrs	1st day	2nd day	3rd day	4th day	5th day	6th day
Control		6	5	4	2	1	1	1	1
Cinnamon	0.5%	7	6	5	4	3	2	1	1
	1%	8	7	6	5	4	3	2	1
	1.5%	9	8	7	6	5	4	3	2
Clove	0.5%	6	5	4	3	6	1	1	1
	1%	7	6	5	4	3	2	1	1
	1.5%	8	7	6	5	4	3	2	1
Rosemary	0.5%	5	4	3	2	1	1	1	1
	1%	6	5	4	3	2	1	1	1
	1.5%	7	6	5	4	3	2	1	1

Table (2): Sensory evaluation of untreated (control) and treated minced beef samples inoculated with *E. coli* during cold storage at 4°C

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

Score System for Sensory Evaluation (Pearson and Tauber, 1984):

- | | |
|-------------------|-------------------|
| 9: Excellent | 4: Fair |
| 8: Very very good | 3: Poor |
| 7: Very good | 2: Very poor |
| 6: Good | 1: Very very poor |
| 5: Medium | |

Antibacterial activity of cinnamon, clove and rosemary essential oils

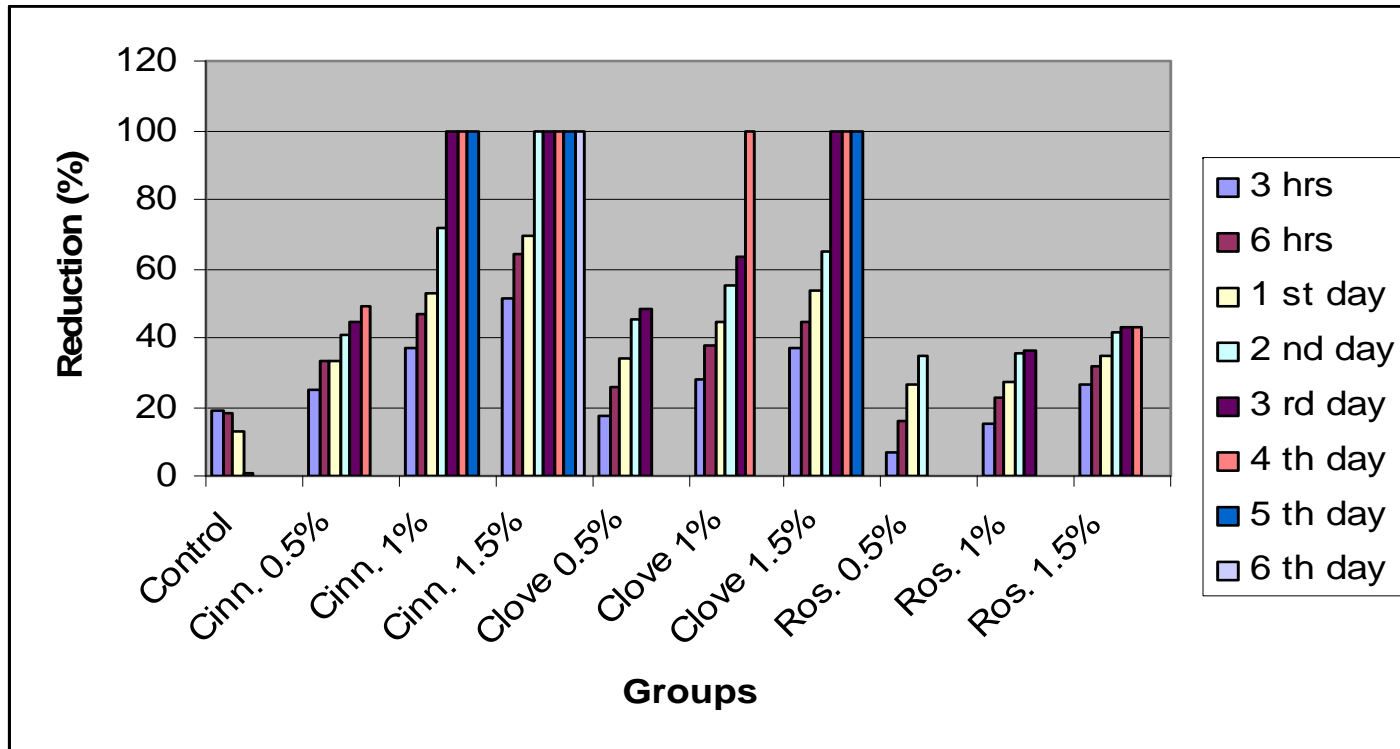
Table (3): The effects of different concentrations of essential oils on counts of *S. aureus* (log CFU/g) in artificially inoculated minced beef samples

Groups		3 hrs	6 hrs	1st day	2nd day	3rd day	4th day	5th day	6th day
Control		8.79±0.17bc	8.91±0.06a	9.44±0.83a	10.80±0.59a	-	-	-	-
Cinnamon oil	0.5%	8.19±0.55bc	7.23±0.47d	6.73±0.68b	6.41±0.88b	6.01±1.73a	5.53±0.86	-	-
	1%	6.85±0.72d	5.74±0.41f	5.11±0.62d	3.04±.81d	ND	ND	ND	-
	1.5%	5.30±0.80e	3.92±0.82g	3.30±0.87e	ND	ND	ND	ND	ND
Clove oil	0.5%	8.99±0.59bc	8.09±0.31bc	7.16±0.56bc	5.98±0.59b	5.61±1.11ab	-	-	-
	1%	7.85±0.97cd	6.73±0.19de	6.02±0.42cd	4.87±0.68c	3.95±0.67b	ND	-	-
	1.5%	6.85±0.52d	6.03±0.47ef	5.03±0.51d	3.79±0.73d	ND	ND	ND	-
Rosemary oil	0.5%	10.15±0.58a	9.16±0.36a	7.95±0.73b	7.11±0.25b	-	-	-	-
	1%	9.23±0.47ab	8.39±0.67ab	7.89±0.80b	7.00±0.18b	6.91±0.31a	-	-	-
	1.5%	8.02±0.58c	7.41±0.24cd	7.05±0.14bc	6.32±.41b	6.22±0.47a	6.20±0.43	-	-

Initial load of *S. aureus* at zero hr = 10.86±5.18 log CFU/g ND: Not Detected

The values represent Mean ± SD of three experiments. Means within a column followed by different letters are significantly different ($P < 0.05$).

Fig. (1): Reduction % of *S. aureus* count artificially inoculated into minced beef samples treated with different concentrations of essential oils



Antibacterial activity of cinnamon, clove and rosemary essential oils

Table (4): The effects of different concentrations of essential oils on counts of *E. coli* (log CFU/g) in artificially inoculated minced beef samples

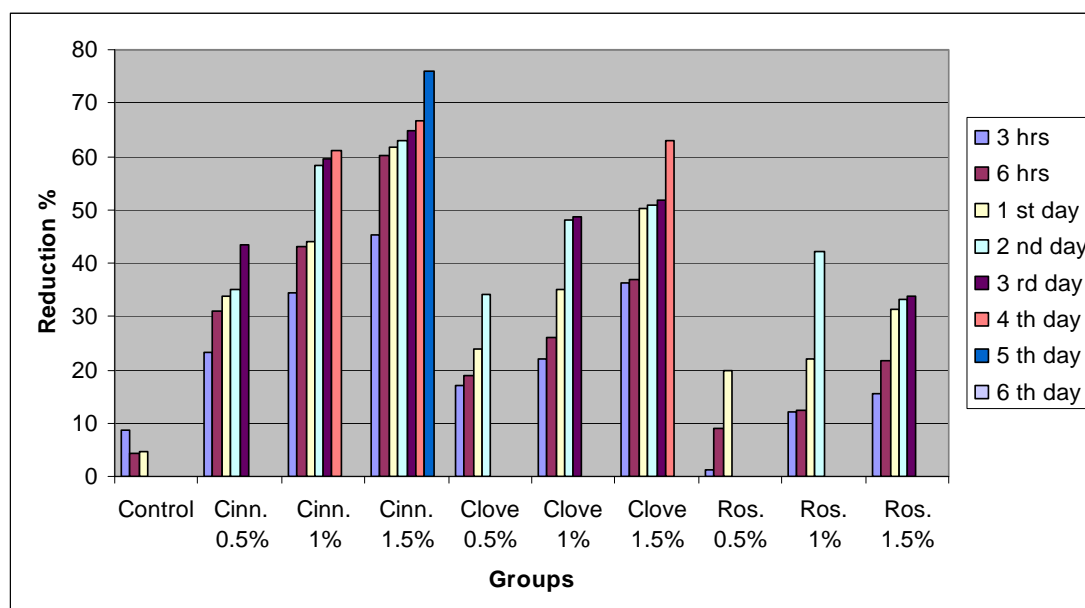
Groups		3 hrs	6 hrs	1st day	2nd day	3rd day	4th day	5th day	6th day
Control		7.22±0.57a	7.56±0.52a	7.55±0.52a	-	-	-	-	-
Cinnamon	0.5%	6.08±0.93e f	5.45±0.13d e	5.23±0.62ef g	5.14±0.65a bc	4.47±0.67a b	-	-	-
oil	1%	5.18±0.15f g	4.49±0.62f g	4.42±0.63f g	3.30±0.56b c	3.20±0.56a b	3.09±0.60 a	-	-
	1.5%	4.33±0.24g	3.16±0.28g	3.02±0.25g	2.93±0.33c	2.79±0.44b	2.63±0.58 a	1.90±0.17	-
Clove	0.5%	6.56±0.95c d	6.41±0.25b c	6.01±0.16c d	5.20±0.60a b	-	-	-	-
Oil	1%	6.16±0.12e f	5.84±0.03c d	5.13±0.54d ef	4.10±0.56a b	4.07±0.58a	-	-	-
	1.5%	5.03±0.56e f	5.00±0.57e f	3.94±0.59ef	3.88±0.61a bc	3.81±0.63a b	2.92±0.88 a	-	-
Rosemary	0.5%	7.82±0.01a b	7.21±0.50a	6.34±0.18b c	-	-	-	-	-
Oil	1%	6.96±0.69b c	6.94±0.39b	6.18±0.60b	4.57±0.50a	-	-	-	-
	1.5%	6.68±0.45d e	6.20±0.12c	5.44±0.58c de	5.28±0.60a b	5.23±0.60a	-	-	-

Initial load of *E. coli* at zero hr = 7.91±0.006 log CFU/g ND: Not Detected

The values represent Mean ± SD of three experiments.

Means within a column followed by different letters are significantly different (P < 0.05).

Fig. (2): Reduction % of *E. coli* count artificially inoculated into minced beef samples treated with different concentrations of essential oils



It is obvious from results obtained in table (1 & 2) that the sensory attributes of different treated minced beef samples during cold storage (4°C) were improved by using different concentrations of cinnamon, clove and rosemary oils, compared to the control samples during the storage period. Generally, the samples treated with cinnamon and clove oils revealed the highest improvement of sensory attributes, while the samples treated with rosemary oil demonstrated the lowest one. In addition, the samples experimentally inoculated with *S. aureus* showed better improvement of sensory attributes than samples experimentally inoculated with *E. coli*.

Table (3, 4) showed *S. aureus* and *E. coli* counts in minced beef samples treated with different concentrations of cinnamon, clove and rosemary oils. The studied essential oils showed various degrees of inhibition against the two bacterial strains (Fig. 1 & 2). The present data exhibited the potential of plant essential oils as natural food preservatives against *S. aureus* and *E. coli* in minced beef. The initial counts of *S. aureus* and *E. coli* in minced beef samples after inoculation were 10.86 and 7.91 log CFU/g. All results showed significant

growth inhibition of both organisms in the minced beef samples ($p \leq 0.05$).

At the concentration of 0.5%, cinnamon, clove and rosemary oils reduced the growth of *S. aureus* to 5.53 log CFU/g after 4 days, 5.61 log CFU/g after 3 days and 7.11 log CFU/g after 2 days, respectively. In comparison, cinnamon oil reduced the growth of *E. coli* to 4.47 log CFU/g after 3 days, while clove oil reduced its growth to 5.20 log CFU/g after 2 days and rosemary oil reduced its growth to 6.34 log CFU/g after 1st day. At the concentration of 1%, cinnamon, clove and rosemary oils reduced the growth of *S. aureus* to 3.04 log CFU/g after 2 days, 3.95 log CFU/g after 3 days and 6.91 log CFU/g after 3 days, respectively. In comparison, cinnamon oil reduced the growth of *E. coli* to 3.09 log CFU/g after 4 days, while clove oil reduced its growth to 4.07 log CFU/g after 3 days and rosemary oil reduced its growth to 4.57 log CFU/g after 2 days. At the concentration of 1.5%, cinnamon, clove and rosemary oils reduced the growth of *S. aureus* to 3.30 log CFU/g after 1st day, 3.79 log CFU/g after 2 days and 6.20 log CFU/g after 4 days, respectively (Fig. 1). In comparison, cinnamon oil reduced the growth of *E. coli*

to 1.90 log CFU/g after 5 days, while clove oil reduced its growth to 2.92 log CFU/g after 4 days and rosemary oil reduced its growth to 5.23 log CFU/g after 3 days. Moreover, the growth of *S. aureus* in minced beef samples was completely inhibited after treatment with cinnamon oil 1% after 3 days, cinnamon oil 1.5% after 2 days, clove oil 1% after 4 days and clove oil 1.5% after 3 days (Fig. 2). Additionally, the present data indicated that cinnamon oil showed maximum activity followed by clove oil followed by rosemary oil. The inhibition of *S. aureus* and *E. coli* 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.

4. DISCUSSION:

Essential oils have been used for many thousands of years, in food preservation, pharmaceuticals, alternative medicine and natural therapies [19]. It is necessary to investigate those plants scientifically to improve the quality of healthcare. Essential oils are potential sources of novel antimicrobial compounds especially against bacterial pathogens. The data presented here showed the potential of selected plant essential oils as potent inhibitors of *S. aureus* and *E. coli* in minced beef, but their effectiveness varied.

Spoilage characteristics develop in food as microorganisms digest the sugars, complex carbohydrates, proteins, and fats of food producing undesirable effects in the food if the spoilage microorganisms grow to significant levels. Typically, the threshold level for observation of food spoilage by odor, taste, or sight is not reached until the spoilage microflora exceeds about 10^7 organisms/g of food. A wide variety of microorganisms including *S. aureus* produce lipolytic enzymes that hydrolyze lipids, producing readily oxidizable substrates that have a rancid odor. Many spoilage bacteria as *E. coli* produce proteolytic enzymes that hydrolyze

proteins in foods leading to offensive odor. Most groups of microorganisms can spoil food by growing on the surface. Refrigerated cured meats and cooked products can become slimy or sticky to the touch because of the growth of microorganisms. This particular spoilage defect is caused simply by the accumulation of very high numbers of microbial cells and not by any specific metabolic activity of the microbes. Similarly, color changes in food can occur because of the surface growth of microorganisms [20].

Bactericidal or bacteriostatic activity of essential oils, in vitro and in food assays, against bacterial pathogens has been reported [21, 22 and 23].

A greater concentration of essential oils is generally needed to achieve the same effect in food in comparison to in vitro assays. It is believed to be due to the intrinsic (pH, salt, antioxidants and other additives) and extrinsic properties (temperature, vacuum and modified atmosphere packaging, characteristic of microorganisms, intra and inter species communication within a microbial population that is present in a food matrix) of the food which can influence the antimicrobial efficacy of the plant antimicrobials [24]. The more complex growth environment in food provides the microbial cells with greater protection from antimicrobial agents. It has been reported that the fat in food could form a protective coat around bacteria, thereby protecting them from antimicrobial agents [25]. The lipid fraction of the food has been suggested to absorb the antimicrobial agent, thus decreasing the concentration in the aqueous phase and hence its bactericidal action. Furthermore, the reduced water content in food compared to laboratory media could hamper the transfer of antimicrobial agents to the active site in the microbial cell [26].

The results obtained in this study concluded that the examined essential oils are more active against Gram-positive (*S.*

aureus) than Gram-negative bacteria (*E. coli*). Results are in synchronization with those reported by Burt [4], Stojanović-Radić et al. [27] and Shareef [28]. While in other studies, Gram-positive bacteria were more resistant to the essential oils than Gram-negative bacteria [29, 22 and 30]. On the other hand, essential oils were found to be equally effective against both Gram-positive and Gram-negative organisms [31, 22 and 32].

The better effectiveness of essential oils against Gram-positive bacteria than Gram-negative bacteria 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 [33]. There might be another possibility that essential oils may successfully inhibit microbial respiration and increase the plasma membrane permeability, which results in to death of bacterial cells after massive ion leakage [34]. It may also happen due to hydrophilic nature of bacterial cell wall. On the other hand, Gram-negative bacteria have intrinsic resistance against toxic components, since they have a permeability barrier in the outer cell envelope against toxic agents. Hydrophobic macromolecules, such as essential oil constituents, are unable to penetrate the barrier. On the other hand, essential oils usually express low aqueous solubility, which prevents them from reaching a toxic level in cellular membranes [35].

The antimicrobial activity of essential oils have been attributed to the presence of some active constituents in the essential oils, mainly the phenolic compounds with a hydroxyl group (-OH) [36]. 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 [4], loss of membrane integrity, dissipation of proton motive force, sequential inhibition of respiration and ion transport processes [37], 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 [38]. Essential oils can act against resistance mechanisms in bacteria and tumor cells via plasmid curing and inhibition of efflux processes and may act indirectly by stimulating the defense mechanisms and the immune response. Certain components of essential oils can act as uncouplers, which interfere with proton translocation over a membrane vesicle and subsequently interrupt ADP phosphorylation. They can also interfere with membrane-integrated or associated enzyme-proteins, stopping their production or activity. Essential oils are also able to inhibit the synthesis of DNA, RNA, proteins and polysaccharides in bacterial cells [39].

Cinnamon oil was reported to consist of many components such as cinnamaldehyde, eugenol, and linalool. Cinnamaldehyde was the predominant active compound found in cinnamon oil. Cinnamaldehyde leads to inhibition of N-3-oxohexanoyl-L-homoserine lactone (3-oxo-C6-HSL) and AI-2 and has the potential to affect bacterial QS regulated processes [40]. It has also been shown that, eugenol limits the growth of microorganisms by inhibiting the production of certain enzymes needed for growth [38].

The antibacterial activity of clove is attributed to eugenol (2-methoxy-4-allyl phenol) with a small addition of

caryophyllene and humulene. The antimicrobial action of clove oil is related to its ability to inactivate microbial adhesion, enzymes and cell envelope proteins [41].

α -pinene is reported as the major component of rosemary essential oil, followed by 1,8 - cineole, camphene, β -myrcene, camphor and borneole. It was determined that rosemary essential oil exhibits antimicrobial activity by passing through the cell wall and cytoplasm membranes and disrupting their structure as a typical lipophilic substance [27]. Since damaged cell membrane increases its permeability, small molecular weight molecules such as phosphates and potassium ions first leach out from the cell, followed by larger molecules, such as DNA and RNA. 1, 8 - cineole exhibits a strong toxic effect on eukaryotic cells. Another potent compound is β -caryophyllene, which may affect accumulation of some substances by increasing permeability of the plasma membrane. In that way, it affects and increases cytotoxic effect of compounds with which it interacts [42].

5. REFERENCES

1. World Health Organization "WHO" (2002): Food safety and foodborne illness. Fact sheet 237. Geneva: Switzerland.
2. Bautista-Banos, S.A.; Hernandez-Lauzardo, M. G.; Velazquez-del Valle, M. et al. (2006): Chitosan as a potential natural compound to control pre and postharvest diseases of horticultural commodities. *Crop. Prot.*, 25: 108-18.
3. Gould, G.W. (1996): Industry perspectives on the use of natural antimicrobials and inhibitors for food applications. *J. Food Protec.*, (Suppl): 82-86.
4. Burt, S. (2004): Essential oils: their antibacterial properties and potential applications in foods, a review. *Int. J. Food Microbiol.*, 94: 223-53.
5. Kordali, S.; Kotan, R.; Mavi, A.; Cakir, A.; Ala, A. and Yildirim, A. (2005): Determination of the chemical composition and antioxidant activity of the essential oil of *Artemisia dracunculus* and of the antifungal and antibacterial activities of Turkish *Artemisia absinthium*, *A. dracunculus*, *Artemisia santonicum*, and *Artemisia spicigera* essential oils. *J. Agric. Food Chem.*, 53: 9452-9458.
6. Mishra, N. and Behak, K.K. (2010): Antimicrobial activity of some spices against selected microbes. *Int. J. Pharmacy and Pharmaceutical Sci.*, 2(3): 187-196.
7. Daniel, A.N.; Sartoretto, S.M.; Schmidt, G.; Caparroz-Assef, S.M.; Bersani-Amado, C. A. and 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.
8. Rastogi, R. P. and Mehrotra, B. N. (2002): *Glossary of Indian Medicinal Plants*. National Institute of science communication, New Delhi, India.
 9. Kalemba, D. and Kunicka, A. (2003): *Curr. Med. Chem.*, 10: 213-829.
 10. Saeed, S. and Tariq, P. (2005): Antibacterial activities of *Mentha piperita*, *Pisum sativum* and *Momordica charantia*. *Pak. J. Bot.*, 37(4): 997-1001.
 11. Kantachote, D. and 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.
 12. 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.
 13. Barbosa, L.N.; Mores Rall, V.L.; Fernandes, A.H.; Ushimaru, P.I.; Probst, I.S. and Fernandes, A. (2009): Essential oils against foodborne pathogens and spoilage bacteria in minced meat. *Foodborne Pathog. Dis.*, 6(6): 725–728.
 14. Dorsa, W.J. (1997): New and established carcass decontamination procedures commonly used in the beef-processing industry. *J. Food Protec.*, 60: 1146-1151.
 15. Dubal, Z.B.; Paturkar, A.M.; Waskar, V.S.; Zende, R.J. and Latha, C. et al. (2004): Effect of food grade organic acids on inoculated *S. aureus*, *L. monocytogenes*, *E. coli* and *S. typhimurium* in sheep/goat meat stored at refrigeration temperature. *J. Meat Sci.*, 66: 817-821.
 16. Jay, J.M. (1992): *Modern Food Microbiology*, 4th Ed. New York: Chapman and Hall.
 17. Pearson, M.A. and Tauber, W.F. (1984): *Processed meat*. 2nd ed., AVI Pub. Com., Inc. West port, Connection. pp: 93.
 18. Food and Drug Administration "FDA" (2001): U.S. Food and Drug Administration: *Bacteriological Analytical Manual Online*.
 19. Lis-Balchin, M., Deans, S. G. (1997): Bioactivity of selected plant essential oils against *Listeria monocytogenes*. *J. Appl. Bacteriol.*, 82:759-762.
 20. Sperber, W.H. (2009): Introduction to the microbiological spoilage of foods and beverages. In: Sperber, W. H. and Doyle, M. P. (Eds). *Compendium of the Microbiological Spoilage of Foods and Beverages*, Food Microbiology and Food Safety. DOI 10.1007/978-1-4419-0826-1_1, C_Springer Science+Business Media, LLC.
 21. Kim, J.W.; Kim, Y.S. and Kyung, K. H. (2004): Inhibitory activity of essential oils garlic an onion against bacteria and yeasts. *J. Food Prot.*, 67: 499–504.
 22. Gupta, C.; Garg, A. P.; Uniyal, R. C. and Kumari, A. (2008): Antimicrobial activity of some herbal oils against common food-borne pathogens. *African J. Microbiol. Res.*, 2: 258-261.
 23. Matan, N.; Rimkeeree, H.; Mawson, A. J.; Chompreeda, P.; Haruthaithanasan, V. and Parker, M. (2006): Antimicrobial activity of cinnamon and clove oils under modified atmosphere conditions. *Int. J. Food Microbiol.*, 107 (2): 180-185.
 24. 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.
 25. Smith-Palmer, A.; Stewart, J. and Fyfe, L. (2001): The potential application of plant essential oils as natural food preservatives in soft cheese. *J. Food Microbiol.*, 18: 463-70.
 26. Hayouni, E.; Imed, C.; Manaf, A. et al. (2008): Tunisian *Salvia officinalis* L. and *Schinus molle* L. essential oils:

- their chemical compositions and their preservative effects against *Salmonella* inoculated in minced beef meat. *Int. J. Food Microbiol.*, 125: 242-51.
27. Stojanović-Radić, Z.; Nešić, M. Čomić, L. and Niko Radulović, N. (2010): Antimicrobial activity and cytotoxicity of commercial rosemary essential oil (*Rosmarinus officinalis* L.). *Biologica Nyssana* 1 (1-2): 83-88.
 28. Shareef, A. A. (2011): Evaluation of antibacterial activity of essential oils of *Cinnamomum* sp. and *Boswellia* sp. *J. Basrah Researches (Sciences)*, 37 (5.A):60-71.
 29. Friedman, M.; Henika, P.R. and Mandrell, R.E (2002): *J. Food Protec.*, 65:1545.
 30. Babu, A. J.; Sundari, A.R.; Indumathi, J. ; Surjan, R.V.N. and Sravanthi, M. (2011): *Veterinary World*, 4:311.
 31. Prabuseenivasan, S.; Jayakumar M. and Ignacimuthu, S. (2006): In vitro antibacterial activity of some plant essential oils. *BMC Complementary and Alternative Medicine* 2006, 6:39-46.
 32. Upadhyay, R.K.; Dwivedi, P. and Ahmad, S. (2010): Screening of antibacterial activity of six plant essential oils against pathogenic bacterial strains. *Asian J. Medical Sciences*, 2(3): 152-158.
 33. Delaquis, P.J.; Stanich, K.; Girard, B. and Mazza, G. (2002): Antimicrobial activity of individual and mixed fractions of dill, cilantro, coriander and eucalyptus essential oils. *Int. J. Food Microbiol.*, 74: 101-109.
 34. Walsh, S.E.; Maillard, J.Y.; Russel, A.D.; Catrenich, C.E.; Charbonneau, D.L. and Bartolo, R. J. (2003): Activity and mechanism of action of selected biocidal agents on Gram - positive and negative bacteria. *J. Appl. Microbiol.*, 94: 240-247.
 35. Mann, C.M.; Cox, S.D. and Markham, J. L. (2000): *Lett. Appl. Microbiol.*, 30:294-297.
 36. Ferdeş, M. and Ungureanu, C. (2012): Antimicrobial activity of essential oils against four food-borne fungal strains. *U.P.B. Sci. Bull., Series B*, 74 (2): 87-98.
 37. Santiesteban-Lopez, A.; Palou, E. and López-Malo, A. (2007): Susceptibility of food-borne bacteria to binary combinations of antimicrobials at selected a(w) and pH. *J. Appl. Microbiol.*, 102: 486–497.
 38. Parasa, L. S.; Tumati, S. R.; Prasad, C. and Kumar, L.C.A. (2012): In vitro antibacterial activity of culinary spices aniseed, star anise and cinnamon against bacterial pathogens of fish. *Int. J. Pharmacy and Pharmaceutical Sciences*, 4(3): 667-670.
 39. Schelz, Z.; Hohmann, J. and Joseph Molnar, J. (2010): Recent advances in research of antimicrobial effects of essential oils and plant derived compounds on bacteria. *Ethnomedicine: A Source of Complementary Therapeutics*, 179-201.
 40. Niu, C.; Afre, S. and Gilbert, E. S. (2006): Subinhibitory concentrations of cinnamaldehyde interfere with quorum sensing. *Lett. Appl. Microbiol.*, 43(5): 489-494.
 41. Marín, S.; Velluti, A.; Ramos, A. J. and Sanchis, V. (2004): Effect of essential oils on zearalenone and deoxynivalenol production by *Fusarium graminearum* in non-sterilized maize grain. *J. Food Microbiol.*, 21: 313-318.
 42. Legaule, J. and Pichette, A. (2007): Potentiating effect of β -caryophyllene on anticancer activity of α - humulene, isocaryophyllene and paclitaxel. *J. Pharmacy and Pharmacology*, 59 (12): 1643-1647.



كلية معتمدة 2013

دراسة النشاط المضاد للبكتيريا لزيوت القرفة والقرنفل والروزمارى ضد مسببات الأمراض الشائعة المنقولة عن طريق الأغذية فى لحم البقر المفروم

ريهام عبد العزيز أمين

قسم مراقبة الأغذية، كلية الطب البيطرى، جامعة بنها

الملخص العربي

قامت فكرة البحث على دراسة مدى تأثير بعض الزيوت العطرية المسموح باستخدامها فى الأغذية ومنها زيت القرفة، زيت القرنفل، وزيت الروزمارى على نمو وتكاثر بكتيريا المكور العنقودى الذهبى وبكتيريا الايشيريشيا كولاي فى اللحم المفروم الطازج. وذلك بحقن عينات اللحم البقرى المفروم الطازجة ببكتيريا المكور العنقودى الذهبى (لوغاريتم 6 /جم) وبكتيريا الايشيريشيا كولاي (لوغاريتم 4 /جم) وتم ترك العينات لمدة 30 دقيقة فى درجة حرارة الغرفة (25 درجة مئوية). وقد كان العدد الأولى لبكتيريا المكور العنقودى الذهبى وبكتيريا الايشيريشيا كولاي فى عينات اللحم المفروم مباشرة بعد الحقن هو 10,91 و 7.91 لوغاريتم / جم من عينات اللحم المفروم، على التوالي. وقد انخفض العدد البكتيرى لهذه البكتيريا فى العينات المضاف إليها زيت القرفة، زيت القرنفل، وزيت الروزمارى بعد 3، 6 ساعة، ويومياً لمدة 6 أيام متتالية. وقد أكد التحليل الحسى مزايا كبيرة فى استخدام زيت القرفة وزيت القرنفل فى لحم البقر المفروم المبرد حيث انخفض العدد البكتيرى للمكور العنقودى الذهبى فى عينات اللحم المفروم المعالجة 1.5% من القرفة، زيت القرنفل، وزيت الروزمارى بعد 3 ساعات بمعدل 5.56 لوغاريتم / جم بنسبة مئوية قدرها (51.19%) و 4.01 لوغاريتم / جم بنسبة (36.92%) و 2.84 لوغاريتم / جم بنسبة (26.15%) على التوالي. بينما انخفض العدد البكتيرى للايشيريشيا كولاي فى عينات اللحم المفروم المعالجة 1.5% من القرفة، زيت القرنفل، وزيت الروزمارى بعد 3 ساعات بمعدل 3.58 لوغاريتم / جم بنسبة مئوية قدرها (45.26%) و 2.88 لوغاريتم / جم بنسبة (36.41%) و 1.23 لوغاريتم / جم بنسبة (15.55%) على التوالي. وقد توقف نمو بكتيريا المكور العنقودى الذهبى فى عينات اللحم المفروم تماماً باستخدام زيت القرفة بنسبة 1% و 1.5% لمدة 3 أيام ويومين على التوالي، وزيت القرنفل 1% و 1.5% لمدة 4 أيام و 3 أيام، على التوالي. وبالإضافة إلى ذلك، أكدت الدراسة وجود فروق معنوية عالية بين الزيوت المختلفة وأن ميكروب المكور العنقودى الذهبى كان أكثر حساسية للزيوت العطرية المستخدمة مقارنة بميكروب الايشيريشيا كولاي. بالإضافة إلى أن أعداد البكتيرية كانت تنخفض بزيادة تركيز تلك الزيوت، حيث أن تركيز 1,5% قد أعطي أفضل فعالية، وأكدت الدراسة أن زيت القرفة أعطي أفضل فعالية يليه زيت القرنفل وأخيراً زيت الروزمارى مما ساعد على زيادة فترة الصلاحية للحوم البقر المفروم المبردة والمعالجة بهذه الزيوت، مقارنة بالمجموعة الغير معالجة. ونوصى الدراسة بتفعيل استخدام هذه الزيوت العطرية فى التطبيقات التجارية فى صناعة الأغذية للفضاء على هذه الملوثات لما لها من أثر ضار على الصحة العامة حيث أنها أحد الملوثات التى تسبب التسمم الغذائى، ولحسن الحظ ان هذه الزيوت العطرية غير مكلفة وغير معقدة.

(مجلة بنها للعلوم الطبية البيطرية: عدد 25(2):151-164, ديسمبر 2013)