



## Quantifying The antimicrobial Efficacy of Selected Herbal Essential Oils Against Bacteria in Simulated Beef Steak Conditions

Samar M. Ibrahim<sup>1\*</sup>, Fatin S. Hassanin<sup>2</sup>, Nahla S. Abou-Elroos<sup>3</sup> and Fahim, A. Shaltout<sup>2</sup>

<sup>1</sup> Directorate of Veterinary Medicine, Qalubiya Governorate, Egypt.

<sup>2</sup> Food Hygiene and Control Dept., Faculty of Vet. Med., Benha University, Egypt.

<sup>3</sup> Food Hygiene Dept., Animal Health Research Institute, ARC, Egypt.

### Abstract

THE CURRENT study aimed at investigating the antibacterial effect of ginger (GEO), lemon (LEO), and pomegranate (PEO) peels essential oils at concentrations of (1 and 1.5%) and their impact on experimentally inoculated beef steak with some specific foodborne pathogens referring to the bacterial counts, sensory acceptability, and shelf life during refrigeration (4±1°C). Generally, addition of the used oils revealed significant ( $P \leq 0.05$ ) inhibition of *E. coli*, *S. Typhimurium* and *S. aureus* with extending the acceptability of the tested meat samples up to 12<sup>th</sup> day of storage in relation to the concentration and type of the used oil; where the higher concentration (1.5%) showed higher inhibitory effects on the same line, where ginger had a higher antibacterial effect against *E. coli* and *S. aureus* (R%: 65.6 and 68.3, respectively), while pomegranate oil showed the highest reduction against *S. Typhimurium* (R%: 30.1%). On the other hand, lemon oil had a lower antibacterial effect than GE and PE, respectively. Reductions in the bacterial counts were time-dose dependent, where higher concentrations gave higher reductions in the bacterial counts. Regarding with the acceptability, treated beef steak samples showed acceptability up to twelve days of refrigeration. Based on the currently recorded antibacterial effect with higher acceptability time zone, the using of ginger, lemon, and pomegranate oils is strongly recommended to be used as a regular additive in meat production and preservation.

**Keywords:** Foodborne pathogens, Food preservation, Food safety.

### Introduction

Premium raw meats are valuable proteinaceous food substances that are abundant in vitamins and minerals. However, they also include unsaturated fatty acids, which are essential for a safe, palatable meat product with a high nutritional value and a long shelf life [1, 2].

Inadequate storage and packaging conditions increase the rate of protein putrefaction and lipid oxidation, which results in undesirable sensory alterations that impact the physical quality and shorten the shelf life of beef products [3, 4]. In the meat production business, natural and synthetic food additives with antibacterial and antioxidant properties are frequently employed to prevent oxidative reactions and prolong the shelf life of meat products [5, 6].

Nonetheless, customers favor the use of natural food additives in a variety of foods to improve their

quality and nutritional content, postpone oxidative lipid breakdown, and substitute safer meat products with natural preservatives [7]. Therefore, plant-based additives have received more attention due to their high concentration of bioactive ingredients that serve as thickening agents, flavorings, antioxidants, and antimicrobials [8, 9].

Throughout history, medicinal plants have been utilized for a variety of functions, such as flavoring spices and preserving food, which has raised interest in using them in place of chemical additions [10]. The efficacy of aromatic herbs and their extracts for applications involving food safety and preservation has been assessed. The active ingredients in their essential oils (EOs) and other secondary plant metabolite components were responsible for the majority of their preservation qualities. Essential oils are known to possess antibacterial and antioxidant qualities [11].

\*Corresponding authors: Samar M. Ibrahim, E-mail: samarphd2024@gmail.com Tel.: +201005764399

(Received 18 October 2024, accepted 08 December 2024)

DOI: 10.21608/EJVS.2024.329367.2437

©National Information and Documentation Center (NIDOC)

Citrus fruits are members of the *Rutaceae* family, which has over 140 genera. One of the major species in the genus *Citrus* is *Citrus limon*, or lemon [12]. Numerous useful compounds, including combinations of terpenes, sesquiterpenes, alcohols, esters, and sterols with antibacterial, antioxidant, and flavorful properties, are made from lemon essential oil [13].

Ginger is also a plant that produces essential oils. The health advantages of ginger have made it a popular spice in Indonesian society. Ginger contains 23% essential oil, gum starch, organic acid, malic acid, oxalic acid, and ginger [14]. The primary ingredients of ginger essential oil that contribute to the characteristic scent of ginger are zingiberene, gingerol, shogaol, and sap, according to Mardiansyah *et al.* [15]. Ginger EO is made up of 40 different kinds of monoterpene hydrocarbons, such as 1,8-cineole, linalool, borneol, neral, and geraniol.

Additionally, the commercial fruit crop pomegranate (*Punica granatum* L.) has been widely documented to have antibacterial properties as an herbal meat additive. It is made from various pomegranate byproducts, such as its peel, seeds, and juices [16]. The pomegranate peel possesses the highest levels of punicalagin and ellagic acid, which have been found to be the primary factors contributing to the pomegranate's antibacterial activity, according to Ambigaipalan *et al.* [17]. Furthermore, Kostka *et al.* [18] ascribed the potent antioxidant properties of pomegranate extracts to their high phenolic compound content, which includes various flavonoids, ellagic acid, pedunculagin, punicalagin, and anthocyanins (3,5-diglucoside and 3-glucoside of delphinidin, pelargonidin, and cyanidin).

Therefore, the current study was conducted to evaluate the antibacterial effect of ginger, lemon and pomegranate peel EOs against foodborne *E. coli*, *S. aureus* and *S. Typhimurium* in beef steak model during refrigeration storage.

## **Material and Methods**

### *Essential oils*

Ginger, lemon, and pomegranate peel oils were purchased from CAP PHARM Co., a company specializing in natural oil extraction. All chemicals used were food-grade. The oils were stored in amber bottles at 4°C.

### *Fresh beef meat steak*

Three kg and 300 g of the fresh beef steak used in this investigation came from El Menoufiya Governorate butcher shops. Gamma irradiation units at the Egyptian Atomic Energy Authority (EAEA), Naser City, Cairo, Egypt, sterilized the purchased beef using an Indian Gamma Cell (Ge 4000 A) with a dose of 5 kGy and a dose rate of 1.915 kGy/hr in

order to eradicate commensal microbiota according to Huq *et al.* [19].

Three equal portions of fresh beef steak, each weighing 1100 grams, were inoculated with *Salmonella Typhimurium*, *Staphylococcus aureus*, and *Escherichia coli*, respectively. Each portion was then divided into smaller subgroups of 150 grams. These subgroups were treated with different concentrations of the essential oils under investigation.

### *Bacterial strains*

*Escherichia coli*, *Staphylococcus aureus*, and *Salmonella Typhimurium* reference strains (ATCC-25922, ATCC-23235 and ATCC-19430, respectively), used in this study, were obtained from Media Unite, Food Hygiene Department, Animal Health Research Institute, Giza, Egypt.

### *Experimental grouping*

1100 g of inoculated fresh beef with *E. coli* were divided into 7 groups (150 g each) represented by:

- G1: 150 g beef meat + no treatment (Control group)
- G2: 150 g beef meat + Pomegranate oil (PEO 1.0%)
- G3: 150 g beef meat + Pomegranate oil (PEO 1.5%)
- G4: 150 g beef meat + Ginger oil (GEO 1.0%)
- G5: 150 g beef meat + Ginger oil (GEO 1.5%)
- G6: 150 g beef meat + Lemon oil (LEO 1.0%)
- G7: 150 g beef meat + Lemon oil (LEO 1.5%)

The same grouping design was followed in subgrouping of beef meat samples that were experimentally inoculated with *S. Typhimurium* and *S. aureus*.

After inoculation of beef steak groups with *E. coli* ( $10^4$  CFU/g), *S. Typhimurium* ( $10^2$  CFU/g), and *S. aureus* ( $10^3$  CFU/g), mixed thoroughly by gently squeezing the bags by hand and left for 30 minutes for complete attachment between microorganisms and beef steak samples.

The initial load of *E. coli*, *S. aureus*, and *Salmonella* was detected before the addition of essential oils, followed by addition of 1% and 1.5% of each used essential oil, followed by gentle mixing for a further 30 seconds to ensure even mixing. Each sample was packed in a polyethylene bag, labeled, and stored at 4°C.

Preparation of serial dilutions for bacteriological counting was performed according to **ISO 6887-2** [20] using 0.1% peptone water (HIMEDIA), and stomacher (Lab blender 400, Seward lab. Model No. AB 6021) for 1 minute.

Enumeration of *E. coli*, *S. aureus* and *S. Typhimurium* was conducted according to ISO 16649-2 [21], ISO 6888-1 [22] and ISO 6579 [23],

respectively. Results were recorded as CFU/g. Reduction percentage was calculated according to the equation of  $(\text{initial load} - \text{new count}) / \text{initial load} \times 100$ .

#### *Analysis of data*

Tests were performed in triplicate. Duncan's post hoc test was used to statistically analyze the data, and  $P < 0.05$  was deemed statistically significant. One-way ANOVA was performed using the SPSS software for Windows (Version 16) (SPSS Inc., Chicago, IL, USA). To compare two groups statistically, an independent T test was used.

### **Results**

Referring to the recorded results in Table 1 and Fig. 1, significant reductions in *E. coli* counts were recorded in all of the treated groups, where it was the maximum in the treated group with pomegranate oil (1.5%) with a reduction (%) of 73.4. It is worth noting that the treated group with PE 1.5% recorded the longest shelf life; where it is still apparently acceptable up to 15 days of storage, whereas the control group showed spoilage since the 6<sup>th</sup> day of storage.

Regarding to the recorded results in Table 2 and Fig. 2, significant reductions in Salmonella counts were recorded in all of the treated groups, where it was the maximum in the treated group with pomegranate oil (1.5%) with a reduction (%) of 30.1. It is worth noting that the treated group with lemon oil of both concentrations recorded the shortest shelf life, where it showed spoilage after the 9<sup>th</sup> day of storage, whereas the other treated groups were still acceptable up to 12 days of storage.

A significant reduction in *S. aureus* counts were recorded in all of the treated groups, where it was the maximum in the treated group with ginger oil (1.5%) with reduction (%) of 68.3. It is worth noted that all of the treated groups still apparently acceptable up to 12 days of storage; whereas, the control group showed signs of spoilage after the 3<sup>rd</sup> day of storage (Table 3; and Fig. 3).

### **Discussion**

Meat is a food with exceptional nutritional value since it contains a variety of vitamins, minerals, and high-quality lipoproteins [24]. However, beef has a limited shelf life and is prone to rotting quickly, even following chilling conditioning, due to its high moisture content and easily utilized nutrients [25]. Since human reaction dictates the eating quality of any food, sensory evaluation is crucial when it comes to food acceptance. This is linked to an initial loss of freshness that impacts the food's bacteriological and sensory quality [26].

According to many studies, the usage of chemicals and other produced food additives might cause malignant neoplasms as well as degenerative

illnesses. In order to cover various antibacterial qualities and extend the duration of practical usage, it became necessary to look for elective procedures. Natural plant byproducts have recently attracted a lot of attention in food conservation measures due to their ability to inhibit the growth of harmful microorganisms through their antioxidant activity [7].

Recent interest in the antioxidant qualities of polyphenols and essential oils derived from ginger, lemon, and pomegranate peels has arisen because the use of natural antioxidants is thought to be a helpful tactic in postponing or increasing the shelf-stability of food products by preventing the production of harmful oxidation products in meat products and delaying or retarding lipid oxidation [27].

Essential oils help to meet some of the growing demand for "green food" goods made from natural sources as customers choose to eat less chemical-packed food [28]. Historically, medicinal plants have been used for a wide range of purposes, including food preservation, seasoning, and spices, which has sparked interest in replacing artificial additions [10]. Essential oils may be incorporated into food products via a variety of methods, including adding, spraying, and dipping [29].

Foodborne bacteria that thrive inside food items can cause physical, chemical, and sensory changes that lead to food degradation. These microorganisms digest certain dietary ingredients and create metabolic byproducts as they grow. Eating such contaminated food can cause a variable range of illnesses, from full-body infections to moderate-to-severe food poisoning [30].

Foodborne illness is mainly caused by bacteria which represent two thirds of foodborne disease outbreaks, that occur mainly in relation to manipulation malpractices, especially *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella* spp. [31].

*Escherichia coli* is widely distributed in the environment, leading to the contamination of water and food by which the bacteria can spread. It is an organism incriminated in foodborne diseases, it is a good indicator of fecal contamination; so, its presence in meat and meat products reflects a lack of proper sanitation [32].

Salmonellae spp. are also regarded as a priority disease by food safety organizations due to the severity and frequency of illnesses they cause [33]. *Salmonella* spp. were predicted to be the leading cause of invasive foodborne disease and diarrhea worldwide, accounting for 111,000 fatalities and 93.8 million episodes of gastroenteritis each year [34]. According to estimates, foodborne *Salmonella* spp. caused the most illnesses, hospitalizations, and fatalities in the United States in 2017 [35].

However, one of the most significant foodborne illnesses is Staphylococcal food poisoning (SFP). It is brought on by consuming preformed staphylococcal enterotoxins (SEs), which are thermostable proteins produced by enterotoxigenic strains of coagulase-positive staphylococci (CPS), mostly *Staphylococcus aureus*. This is a sign of poor environmental and personal hygiene, particularly when handling [36].

Regarding the current recorded results, and addition of ginger, lemon, and pomegranate essential oils revealed significant reductions in the bacterial counts, which came in line with the recorded results of Elbalsy *et al.* [37], Eldahrawy *et al.* [38], and Zhang *et al.* [39], who recorded a significant antibacterial effect of the used herbal extracts on some food poisoning microorganisms which was reflected in the sensory quality and improved shelf life.

The rhizome of ginger, a plant of the monocotyledonous plant class with a distinctive fragrant scent, is its therapeutic component. Ginger is also a highly nutritious herb. Its primary functional categorization components are curcumin, oleoresin, oil phenols, and volatile essential oil [40]. Many studies have examined the primary bioactive components of ginger oil (GEO) in order to elucidate its antibacterial and antioxidant action against pathogenic and spoilage microorganisms in food, in light of the oil's documented antibacterial impact [41]. According to the data, the main source of ginger oil's antibacterial properties is its abundance of sesquiterpenes, including  $\alpha$ -gingerene,  $\beta$ -gingerene, and camphene. The current results were therefore consistent with those of He *et al.* [42] and Thao *et al.* [41], who found that GEO had a strong antibacterial impact against a variety of food poisoning pathogens.

One of the genus *Citrus*'s key species is *Citrus limon*, or lemon. Mixtures of  $\alpha$ -limonene, terpenes, sesquiterpenes, aldehydes, alcohols, esters, and sterols with antibacterial, antioxidant, and flavorful properties make up a variety of important products that are made from lemon essential oil [13]. In light of this, the antibacterial effect of lemon essential oil was found to be consistent with the findings of Giarratana *et al.* [43], who demonstrated that the addition of lemon oil enhanced the microbial quality and sensory scores of the treated sample in a concentration-dependent manner while testing lemon essential oil on a few specific spoilage bacteria isolated from food over a period of 15 days of refrigeration; in addition, The preservative effects of lemon essential oil (0.3 and 1.0%) on the microbial quality of salted sardines were investigated by Alfonzo *et al.* [44], who found that the treated samples significantly inhibited the growth of LAB, Enterobacteriaceae, and staphylococci. Kunová *et al.* [45] also found that the samples treated with lemon

oil significantly inhibited the growth of coliform bacteria.

Pomegranates are a well-known source of important nutrients. It includes phenolic and organic acids, flavanols, anthocyanins, hydrolyzable tannins, and condensed tannins—compounds that have been researched and linked to a host of health advantages against illnesses. Furthermore, it is distinguished by a low pH (often less than 4.0) and a comparatively high acidity (up to 20 g of citric acid per liter of juice) [46]. The wide variety of phytochemicals that pomegranate oil contains may be the reason for the observed antibacterial action of the oil against the tested foodborne pathogens. As was previously mentioned, polyphenols, primarily hydrolyzable ellagitannins and anthocyanins, are thought to be the most abundant phytochemicals in pomegranates [47].

The pH decrease brought on by organic acids, which are the main factor influencing the survival and proliferation of microorganisms, may be the source of the observed extended shelf life following the decrease in bacterial counts in the treated beef steak. Additionally, microbial cell death, which occurs when germs are deprived of cellular energy, is most likely explained by the selective inhibition of microbial ATP synthase caused by polyphenol activity [48]. Moreover, the therapeutic herbal leftovers, or byproducts, such as seeds, pomace, and peel, have been said to include bioactive constituents such as complex polysaccharides, dietary fibers, phenolic and polyphenolic chemicals, minerals, and vitamins. Fruit peels are the source of these compounds, which may be used as functional ingredients or food additives to enhance their antibacterial and antioxidant qualities or substituted for protein and fat in a range of foods that promote muscle growth. These natural additions are also said to increase the safety, quality, and shelf life of several food products, including meat and meat products [49].

Pomegranate oil clearly had a stronger antibacterial impact than ginger and lemon oil, respectively, when the antibacterial effects of the various oils were compared against certain foodborne germs. This was evident from the bacterial counts and shelf-life. The results were consistent with those of Abdel Tawab *et al.* [50], who found that pomegranate peel oil was superior to ginger oil. They attributed this to the pomegranate extract's high tannin content, which is characterized by its capacity to bind to the surface of the cell wall and penetrate its protein and polysaccharide-containing structure. By acting as oxidizing agents, polyphenols block enzymes, interact with proteins, alter the bacterial cell wall, and prevent bacteria from multiplying.

### Conclusion

In the current study, the used essential oils showed a significant promoting effect for beef

keeping quality and bacteriological profile without causing adverse effect on the sensory quality of the treated samples. Pomegranate EO showed superiority over ginger and lemon EOs as an antibacterial agent in meat model. Therefore, it is recommended to apply ginger, lemon and pomegranate plus GMP in meat production and preservation.

*Acknowledgments*

Authors want to present great appreciation for the all staff members of Food Hygiene Department,

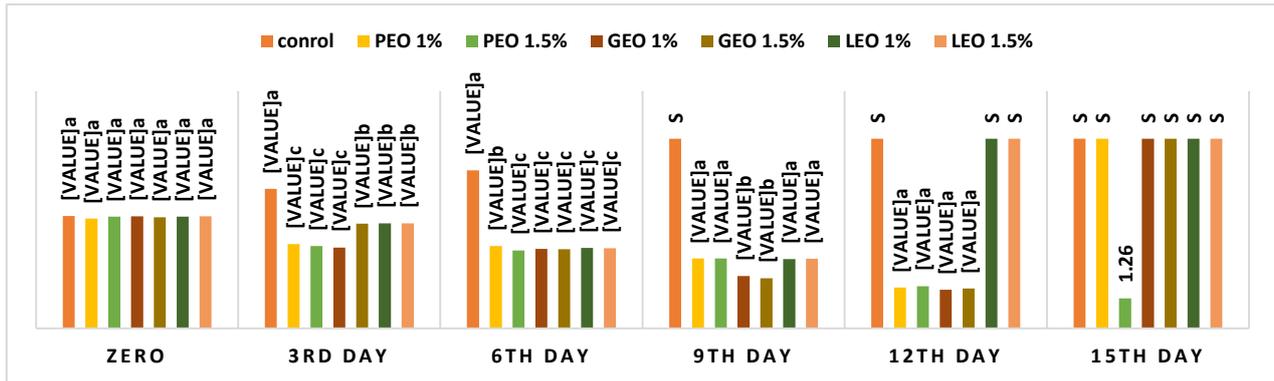
Faculty of Veterinary Medicine, Benha University, Egypt; and Animal Health Research Institute, Agriculture Research Center for their valuable help and guidance.

*Funding statement*

This study didn't receive any funding support

*Declaration of Conflict of Interest*

The authors declare that there is no conflict of interest.

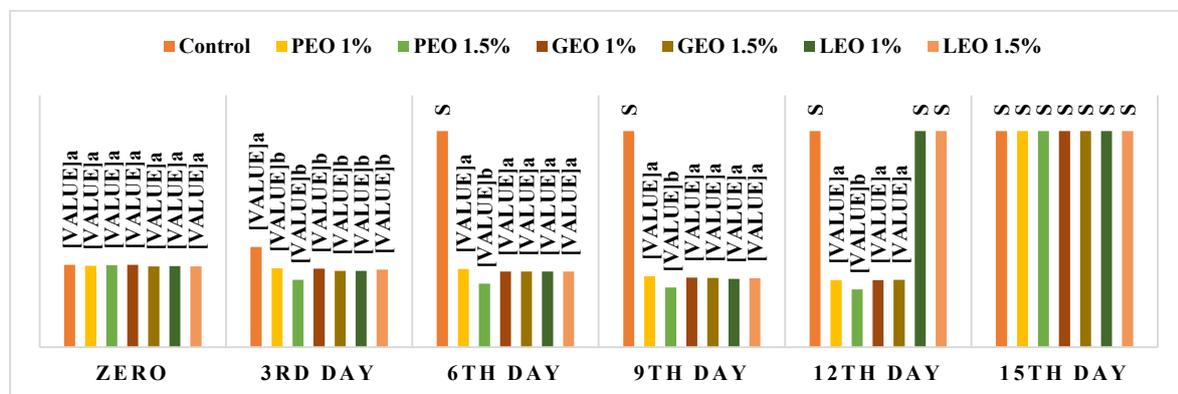


**Fig. 1. Average counts of *E. coli* (CFU/g) of the examined groups**  
 abc: different superscript letters mean significant difference ( $P \leq 0.05$ )  
 S: spoiled  
 PEO: Pomegranate essential oil, GEO: Ginger essential oil, LEO: Lemon essential oil

**TABLE 1. Percentage decrease in *E. coli* numbers compared to the control group**

G	PEO (1%)	PEO (1.5%)	GEO (1%)	GEO (1.5%)	LEO (1%)	LEO (1.5%)	P value
Zero day	2.3	0.6	0.2	1.3	0.6	0.2	0.271
3 <sup>rd</sup> day	25.1	26.8	28.1	7.0	6.5	6.5	0.042
6 <sup>th</sup> day	26.6	30.6	29.3	29.5	28.5	28.7	0.002
9 <sup>th</sup> day	37.8	37.8	53.4	55.5	38.4	38.0	0.023
12 <sup>th</sup> day	63.5	62.4	65.6	64.6	--	--	0.012
15 <sup>th</sup> day	--	73.4	--	--	--	--	

G: Group, PEO: Pomegranate essential oil, GEO: Ginger essential oil, LEO: Lemon essential oil

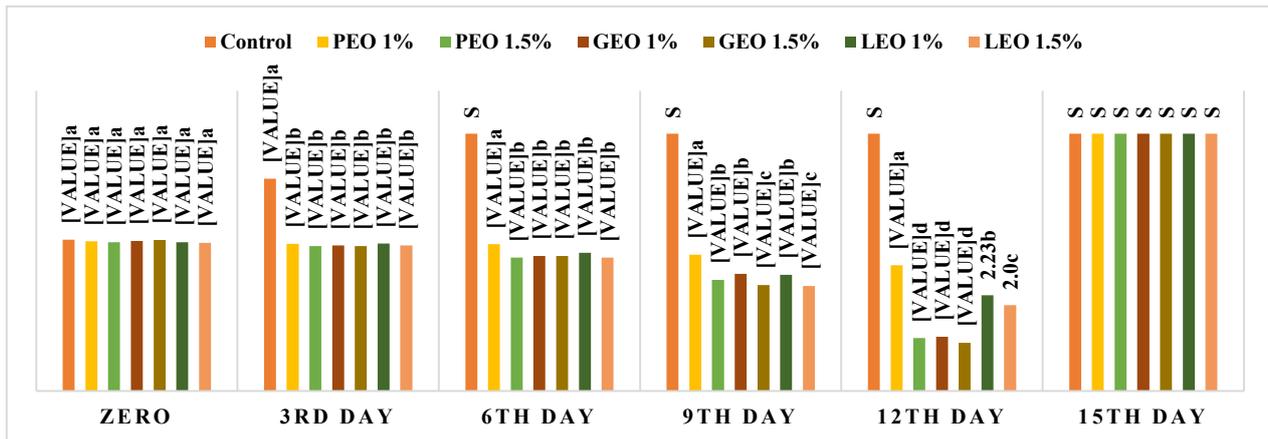


**Fig. 2. Average counts of *S. Typhimurium* (CFU/g) of the examined groups**  
 abc: different superscript letters mean significant difference ( $P \leq 0.05$ )  
 S: spoiled, PEO: Pomegranate essential oil, GEO: Ginger essential oil, LEO: Lemon essential oil

**TABLE 2. Percentage decrease in *S. Typhimurium* numbers compared to the control group**

G	PEO (1%)	PEO (1.5%)	GEO (1%)	GEO (1.5%)	LEO (1%)	LEO (1.5%)	P value
Zero day	0.4	0.4	--	2.2	1.7	2.2	0.228
3 <sup>rd</sup> day	4.4	18.3	4.8	7.4	7.4	6.1	0.042
6 <sup>th</sup> day	5.2	23.1	8.3	8.3	8.3	8.3	0.002
9 <sup>th</sup> day	14.0	27.5	15.7	16.1	17.0	16.6	0.023
12 <sup>th</sup> day	18.8	30.1	18.8	18.3	--	--	0.012
15 <sup>th</sup> day	--	--	--	--	--	--	

G: Group, PEO: Pomegranate essential oil, GEO: Ginger essential oil, LEO: Lemon essential oil

**Fig. 3. Average counts of *S. aureus* (CFU/g) of the examined groups**

abcd: different superscript letters mean significant difference ( $P \leq 0.05$ )

S: spoiled, PEO: Pomegranate essential oil, GEO: Ginger essential oil, LEO: Lemon essential oil

**TABLE 3. Percentage decrease in *S. aureus* numbers compared to the control group**

G	PEO (1%)	PEO (1.5%)	GEO (1%)	GEO (1.5%)	LEO (1%)	LEO (1.5%)	P value
Zero day	2.0	1.7	0.8	0.3	1.7	2.3	0.12
3 <sup>rd</sup> day	2.8	4.2	4.0	4.2	2.5	4.0	0.001
6 <sup>th</sup> day	3.1	11.9	10.8	10.8	8.8	11.9	0.005
9 <sup>th</sup> day	10.0	26.6	22.7	30.0	23.2	30.6	0.005
12 <sup>th</sup> day	17.0	65.2	64.3	68.3	36.8	43.3	0.001
15 <sup>th</sup> day	--	--	--	--	--	--	

G: Group, PEO: Pomegranate essential oil, GEO: Ginger essential oil, LEO: Lemon essential oil

## References

- Hassanin, F.S., Shaltout, F.A., Homouda, S. and Arakeeb, S. Natural preservatives in raw chicken meat. *Benha Vet. Med. J.*, **37**, 41-45 (2019).
- Ragab, A., Edris, A., Shaltout, F. and Salem, A. Effect of titanium dioxide nanoparticles and thyme essential oil on the quality of the chicken fillet. *Benha Vet. Med. J.*, **41**(2), 38-40 (2022).
- Edris, A., Hassan, M.A., Shaltout, F.A. and Elhosseiny, S. Chemical evaluation of cattle and camel meat. *Benha Vet. Med. J.*, **24**(2), 191-197 (2013).
- Hassanien, F.S., Shaltout, F.A., Fahmey, M.Z. and Elsukkary, H.F. Bacteriological quality guides in local and imported beef and their relation to public health. *Benha Vet. Med. J.*, **39**, 125-129 (2020).
- Saad, S.M., Shaltout, F.A., Abou Elroos, N. and El-nahas, S. Antimicrobial effect of some essential oils on some pathogenic bacteria in minced meat. *J. Food Sci. Nutr. Res.*, **2**(1), 12-20 (2019).
- Saif, M., Saad, S.M., Hassanin, F.S., Shaltout, F. and Zaghoul, M. Molecular detection of enterotoxigenic *Staphylococcus aureus* in ready-to-eat beef products. *Benha Vet. Med. J.*, **37**, 7-11 (2019).
- Aminzare, M., Hashemi, M., Ansarian, E., Bimkar, M., Azar, H., Mehrasbi, M., Daneshamooz, S., Raeisi, M., Jannat, B. and Afshari, A. Using natural antioxidants in meat and meat products as preservatives: A review. *Adv. Anim. Vet. Sci.*, **7**, 417-426 (2019).
- Shaltout, F.A., Thabet, M.G. and Koura, H.A. Impact of some essential oils on the quality aspect and shelf life of meat. *J. Nutr. Food Sci.*, **7**, 647 (2017).
- Vilas-Boas, A.A., Pintado, M. and Oliveira, A. Natural bioactive compounds from food waste:

- Toxicity and safety concerns. *Foods*, **10**, 1564-1590 (2021).
10. Martínez-Graciá, C., González-Bermúdez, C., Cabellero-Valcárcel, A., Santaella- Pascual, M. and Frontela-Saseta, C. Use of herbs and spices for food preservation: advantages and limitations. *Cur. Opinion Food Sci.*, **6**, 38-43 (2015).
  11. Laranjo, M., Fernández-León, A., Agulheiro-Santos, A., Potes, M. and Elias, M. Essential oils of aromatic and medicinal plants play a role in food safety. *J. Food Process. & Preserv.*, **2019**, 1-7 (2019).
  12. Kamal, G.M., Anwar, F., Hussain, A.I., Sarri, N. and Ashraf, M.Y. Yield and chemical composition of citrus essential oils as affected by drying pretreatment of peels. *Int. Food Res. J.*, **18**, 1275–1282 (2011).
  13. Ambrosio, C., Diaz-Arenas, G.L., Agudelo, L., Stashenko, E., Contreras-Castillo, C.J. and da Gloria, E.M. Chemical composition and antibacterial and antioxidant activity of a citrus essential oil and its fractions. *Molecules*, **26**, 2888 (2021). <https://doi.org/10.3390/molecules26102888>
  14. Sayuti, K. and Yenrina, R., 2015. Natural and synthetic antioxidants. *Andalus University Press, Padang.*, **40**, 105-115.
  15. Mardiansyah, E.A., Umniyati, S.R. and Iravati, S. Effect of ginger (*Zingiber officinale*) essential oil as a repellent against *Aedes aegypti* mosquitoes. *Berita Kedokteran Masyarakat*, **32**, 353–358 (2016).
  16. Abou-Taleb, M. Fish burger quality treated by pomegranate peels powder during cold storage. *Egy. J. Aqu. Biol. Fish.*, **26**(1), 201-215 (2022).
  17. Ambigaipalan, P., de Camargo, A. and Shahidi, F. Identification of phenolic antioxidants and bioactives of pomegranate seeds following juice extraction using HPLC-DAD-ESI-MSn. *Food Chem.*, **221**, 1883-1894 (2016).
  18. Kostka, T., Ostberg-Potthoff, J.J., Briviba, K., Matsugo, S., Winterhalter, P. and Esatbeyoglu, T. Pomegranate (*Punica granatum L.*) extract and its anthocyanin and copigment fractions-free radical scavenging activity and influence on cellular oxidative stress. *Foods*, **9**, 1617-1634 (2020).
  19. Huq, T., Vu, K., Ried, B., Bouchard, J. and Lacroix, M. Synergistic effect of gamma ( $\gamma$ )-irradiation microencapsulated and antimicrobials against *Listeria monocytogenes* on ready-to-eat (RTE) meat. *Food Microbiol.*, **46**, 507–514 (2015).
  20. ISO "International Organization for Standardization" No.6887-2. Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 2: Specific rules for the preparation of meat and meat products (2017).
  21. ISO "International Organization for Standardization" No.16649-2. Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of glucuronidase-positive *Escherichia coli* - Part 2: Colony-count technique at 44 °C using 5-bromo-4-chloro-3-indolyl-D-glucuronide (2001).
  22. ISO "International Organization for Standardization" No. 6888-1. Microbiology of food and animal feeding stuffs-Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species)-Part 1: Technique using Baird-Parker agar medium (includes amendment A1:2021) (2021).
  23. ISO "International Organization of Standardization" No.6579-1. Microbiology of the food chain-Horizontal method for detection of Salmonella -Part 1 (2017).
  24. Pal, J., Shukla, B.N., Maurya, A.K., Verma, H.O., Pandey, G. and Amitha, A. A review on role of fish in human nutrition with special emphasis to essential fatty acid. *Int. J. Fish. Aqua. Studies*, **6**, 427-430 (2018).
  25. Li, Z., Cai, M., Liu, Y., Sun, P. and Luo, S. Antibacterial activity and mechanisms of essential oil from *Citrus medica L. var. sarcodactylis*. *Molecules*, **24**, 1577-1587 (2019).
  26. Mlian, K.S., Hafiz, R.S. and Muhammad, N. Sensory evaluation of consumer's acceptability. *Hand Book of Food Sci. Technol.*, 4<sup>th</sup> Ed., pp 362-386 (2017).
  27. Lorenzo, J., Munekata, P.E., Gómez, B., Barba, F.J., Mora, L., Pérez-Santaescolástica, C. and Toldrá, F. Bioactive peptides as natural antioxidants in food products—A review. *Trends Food Sci. Technol.*, **79**, 136–147 (2018).
  28. Ashaolu, T.J. and Ashaolu, J.O. Perspectives on the trends, challenges and benefits of green, smart and organic (GSO) foods. *Int. J. Gastron. Food Sci.*, **22**, 100273 (2020).
  29. Khalafalla, F.A., Ali, F.H. and Hassan, A.R.H. Quality improvement and shelf-life extension of refrigerated Nile tilapia (*Oreochromis niloticus*) fillets using natural herbs. *Beni-Suef Univ. J. Basic Appl. Sci.*, **4**, 33-40 (2015).
  30. Amit, S., Uddin, M., Rahman, R., Islam, R.S. and Samad Khan, M. A review on mechanisms and commercial aspects of food preservation and processing. *Agric. Food Sec.*, **6**, 51-73 (2017).
  31. Bintsis, T. Foodborne pathogens. *AIMS Microbiol.*, **3**, 529-563 (2017).
  32. WHO "World Health Organization". *E. coli*. <https://www.who.int/news-room/fact-sheets/detail/e-coli>. Accessed: 19/5/2024 (2018).
  33. IFSAC. Foodborne illness source attribution estimates for 2016 for Salmonella, *Escherichia coli* O<sub>157</sub>, *Listeria monocytogenes*, and *Campylobacter* using multi-year outbreak surveillance data, United States. GA and D.C.: U.S. Department of Health and Human Services, CDC, FDA, USDA-FSIS (2018). Retrieved from <https://www.cdc.gov/foodsafety/ifsac/pdf/P19-2016-report-TriAgency-508.pdf>
  34. Havelaar, A.H., Kirk, M.D., Torgerson, P.R., Gibb, H.J., Hald, T., Lake, R.J., Praet, N., Bellinger, D.C., de Silva, N.R., Gargouri, N., Speybroeck, N., Cawthorne, A., Mathers, C., Stein, C., Angulo, F.J. and Devleeschauwer, B., World Health

- Organization Foodborne Disease Burden Epidemiology Reference Group. World Health Organization global estimates and regional comparisons of the burden of foodborne disease in 2010. *PLoS Medicine*, **12**(12), 1001923 (2015).
35. CDC. Foodborne Diseases Active Surveillance Network, FoodNet 2015 Surveillance Report (Final Data). Atlanta, Georgia: U.S. Department of Health and Human Services, CDC (2017). Retrieved from <https://www.cdc.gov/foodnet/pdfs/FoodNet-Annual-Report-2015-508c.pdf>
36. Filipello, V., Bonometti, E., Campagnani, M., Bertoletti, I., Romano, A., Zuccon, F., Campanella, C., Losio, M.N. and Finazzi, G. Investigation and follow-up of a staphylococcal food poisoning outbreak linked to the consumption of traditional hand-crafted alm cheese. *Pathogens*, **9**, 1064 (2020).
37. Elbalsy, A., Ibrahim, H., Hassan, M., and Nada, S. Effect of different concentrations of lemon oil on some food poisoning bacteria and histamine residue in fish fillet. *Benha Vet. Med. J.*, **43**, 109-113.
38. Eldahrawy, M., Salem, A. and Nabil, M. The efficiency of citrus peel powders in the improvement of meat quality during cold storage. *Benha Vet. Med. J.*, **42**, 208-213 (2022).
39. Zhang, B., Liu, Y., Peng, H., Lin, Y., and Cai, K. Effects of ginger essential oil on physicochemical and structural properties of agar-sodium alginate bilayer film and its application to beef refrigeration. *Meat Sci.*, **198**, 109051 (2023).
40. Diakos, A., Silva, M., Brito, J., Moncada, M., de mesquita, M. and Bernardo, M. The effect of ginger (*Zingiber officinale Roscoe*) aqueous extract on postprandial glycemia in nondiabetic adults: A Randomized controlled trial. *Foods*, **12**, 1037 (2023).
41. Thao, C., Tran, T., Ngan, T. and Mai, C. Extraction and volatile compounds in ginger essential oil (*Zingiber officinale Roscoe*) at laboratory scale. *Asian J. Chem.*, **35**, 3066-3070 (2023).
42. He, J., Hadidi, M., Yang, S., Khan, M., Zhang, W. and Cong, X. Natural food preservation with ginger essential oil: Biological properties and delivery systems. *Food Res. Int.*, **173**, 113221 (2023).
43. Giarratana, F., Muscolino, D., Beninati, C., Ziino, G., Giuffrida, A. and Panebianco, A. Activity of limonene on the maximum growth rate of fish spoilage organisms and related effects on shelf-life prolongation of fresh gilthead sea bream fillets. *Int. J. Food Microbiol.*, **237**, 109–113 (2016).
44. Alfonso, A., Martorana, A., Guarrasi, V., Barbera, M., Gaglio, R., Santulli, A., Settanni, L., Galati, A., Moschetti, G. and Francesca, N. Effect of the lemon essential oils on the safety and sensory quality of salted sardines (*Sardina pilchardus Walbaum* 1792). *Food Control*, **73**, 1265-1274 (2017).
45. Kunová, S., Sendra, E., Haščík, P., Vukovic, N.L., Vukic, M. and Kacáňiová, M. Influence of essential oils on the microbiological quality of fish meat during storage. *Animals*, **11**, 3145 (2021).
46. Nuncio-Jáuregui, N., Calín-Sánchez, Á., Vázquez-Araújo, L., Pérez-López, A.J., Frutos-Fernández, M.J. and Carbonell-Barrachina, Á.A. Processing pomegranates for juice and impact on bioactive components. In: Processing and Impact on Active Components in Food; Preedy, V., Ed.; Academic Press: New York, NY, USA, pp. 629–636 (2015).
47. Viuda-Martos, M., Fernández-López, J. and Pérez-Álvarez, J.A. Pomegranate and its many functional components as related to human health: A review. *Compr. Rev. Food Sci. Food Safety*, **9**, 635–654 (2010).
48. Shewail, A., Shaltout, F. and Gergis, T. Impact of some organic acids and their salts on microbial quality and shelf life of beef. *Assiut Vet. Med. J.*, **64**(159), 164-177 (2018).
49. Nirmal, N.P., Khanashyam, A.C., Mundanat, A.S., Shah, K., Babu, K.S., Thorakkattu, P., Al-Asmari, F. and Pandiselvam, R. Valorization of fruit waste for bioactive compounds and their applications in the food industry. *Foods*, **12**(3), 556 (2023).
50. Abdel Tawab, H., Niazy, M. and El Elsharkawy, D. Antibacterial effect of ginger, green tea and pomegranate versus chlorhexidine using stevia and sucrose sugar. *AL-AZHAR Dental J. For Girls*, **7**, 329-336 (2020).

## التأثير المضاد للبكتريا لبعض الزيوت العطرية العشبية في اللحم البقري

سمر محمد إبراهيم<sup>1\*</sup>، فاتن سيد حسنين<sup>2</sup>، نهلة شوقي أبو الروس<sup>3</sup> وفهيم عزيز الدين شلتوت<sup>2</sup>

<sup>1</sup> مديرية الطب البيطري بالقليوبية، جمهورية مصر العربية.

<sup>2</sup> قسم مراقبة الأغذية، كلية الطب البيطري، جامعة بنها، جمهورية مصر العربية.

<sup>3</sup> قسم صحة الأغذية، معهد بحوث الصحة الحيوانية، مركز البحوث الزراعية، جمهورية مصر العربية.

### الملخص

تهدف الدراسة الحالية إلى تقدير التأثير المضاد للبكتريا للزيوت العطرية المستخلصة من قشور الزنجبيل والليمون والرمان بتركيزات (1 و 1.5%) وتأثيرها على العمر التخزيني والقابلية الحسية لشرائح لحم البقر المخفونة تجريبياً ببكتريا الايكولاي، و السالمونيلا تيفيميوريم و الاستافيلوكوكس اوريس أثناء التخزين المبرد. بشكل عام، أظهرت إضافة الزيوت المستخدمة قدرة كبيرة على تثبيط نمو بكتريا الايكولاي و السالمونيلا تيفيميوريم و الاستافيلوكوكس اوريس مع تحسن الخصائص الحسية و إطالة العمر التخزيني لشرائح اللحم اثناء التخزين المبرد حتى اليوم الثاني عشر من التخزين نسبة إلى تركيز ونوع الزيت المستخدم؛ حيث أظهر التركيز الأعلى (1.5%) قدرة أكبر على تثبيط النمو البكتيرية حيث كان لزيوت الزنجبيل التأثير الأكبر على بكتريا الايكولاي و الاستافيلوكوكس اوريس مع اختزال في العد البكتيري بنسبة 65.6 و 68.3% على التوالي، بينما أظهر زيت قشر الليمون أعلى نسبة اختزال في العد البكتيري لبكتريا السالمونيلا (30.1%). من ناحية أخرى، أظهر زيت قشر الليمون تأثير أقل من زيوت الزنجبيل والرمان على التوالي. كان الانخفاض في أعداد البكتيريا معتمداً على الوقت والجرعة، بينما تناسب معدل انخفاض العد البكتيري تناسباً طردياً مع تركيز الزيت المستخدم محل الدراسة. بناءً على ما تم تسجيله من نتائج، يوصى بشدة باستخدام زيوت الزنجبيل والليمون والرمان كإضافات طبيعية لها القدرة على إطالة العمر التخزيني للحوم مع تحسين الخصائص البكتريولوجية لها اثناء التخزين المبرد.

**الكلمات المفتاحية:** مسببات الأمراض المنقولة بالغذاء، حفظ الأغذية، سلامة الأغذية.