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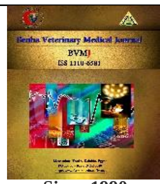

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Original Paper

Bactericidal effect of some herbal extracts against meat-borne *Staphylococcus aureus*

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ABSTRACT

Natural extracts of Green tea, Rosemary, and Ginger were evaluated for their effects on the growth and survival of *Staphylococcus aureus* artificially inoculated into minced meat. Fresh minced meat samples were inoculated with (~ 7 $\log_{10}$ CFU/g) of *S. aureus* and left for 30 minutes at room temperature to allow attachment and absorption of bacteria; where the initial count of *S. aureus* in minced meat samples immediately after inoculation was 7.66 $\log_{10}$ CFU/g. Natural aqueous extracts of green tea leaves, rosemary, and ginger roots were added to the minced meat samples to achieve final concentrations of 0.7, 1, and 2% (% v/g). Sensory profile and *S. aureus* counts were conducted on zero, 3rd, 5th, 7^{th,} and 9th day during cold storage at 4°C. Initial counts of *S. aureus* in minced meat samples decreased following treatment with 2% of green tea, rosemary, and ginger extracts on 3rd day by 6.27, 25.46, and 30.81%, respectively. Ginger extract (2%) showed the best antimicrobial effect with the extension of shelf-life of refrigerated minced meat to nine days compared to all groups especially the control untreated group, followed by rosemary 2% (till 7th day). As compared with several other mild preservation procedures, treatment with green tea, rosemary, and ginger extracts is an inexpensive and uncomplicated method. Results of the present study are envisaged to be useful for commercial applications of these plant extracts as potential food bio- preservatives and anti-*S. aureus* agents in minced meat and other foods, depending upon the desired flavor of such products.

1. INTRODUCTION

Consumers and the food industry around the world are most concerned about food safety. Fresh minced meat is a very perishable product that can lead to foodborne illnesses and intoxications due to the growth and multiplication of pathogenic and spoilage bacteria. It is thought that inadequate or nonexistent hand, equipment, and surface washing and disinfection in areas connected to food are one of the main causes of many outbreaks. Public concern with foodborne bacterial profiles has been sparked by both direct and secondary food poisoning. Meat rotting caused by microbial development is manifested as slime production, degradation, biochemical and enzymatic deteriorations, off-odor, and visual changes, all of which indicate large economic losses (Awny et al., 2010; Dave and Ghaly, 2011).
The second most frequent infection linked to food poisoning outbreaks that arise from eating contaminated food with staphylococcal enterotoxins is *Staphylococcus aureus*. Therefore, its existence in the food manufacturing chain may frequently be a sign of inadequate cleanliness. Because enterotoxins are very thermostable and cannot be eliminated by typical cooking or pasteurization, they can result in food poisoning (Nagarajappa et al., 2012).
Consumer expectations for preservatives in food additives have increased due to the ongoing strengthening of safety regulations. They demand preservative benefits that are evident as well as assurances of safety and non-toxicity (Xu et al., 2016). Nevertheless, numerous studies have demonstrated that artificial preservatives are frequently highly harmful and may even cause deformities and the emergence of cancer (Kumar et al., 2013).
There is a "green" consumerism trend in Western society, where people want food to be safer, of higher quality, and contain fewer artificial additives and chemical residues. To improve the quality and prolong the shelf life of meat products, there is currently a growing interest in adding plant extract or antioxidants during the production phase (Akter et al., 2022).
The plant known as Rosemary, which is a member of the Labiateae family, is used extensively as a flavoring, carminative, and culinary herb, particularly in Mediterranean dishes (Rastogi and Mehrotra, 2002). It is also added as a fragrant ingredient to soaps and other cosmetics, and because of its phenolic compounds, carnosic acid, and carnosol, it has a wide range of antimicrobial activity against different microorganisms (Del Campo et al., 2000).
Ginger is a type of perennial herb that is found in nature and is a member of the Zingiberaceae family. Due to its distinct spicy flavor and scent, ginger is used as a spice in a variety of dishes and drinks. Furthermore, according to Kieliszek et al. (2020), it is a great source of numerous bioactive chemicals, including bioactive phenols (gingerols, shogaols, and zingerones). Its distinct scent and biological activity encourage it for a wide range of use possibilities in the pharmaceutical and food industries. Specifically, ginger

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extract is widely regarded as a safe natural substance with potential uses in the management of respiratory and gastrointestinal disorders (Mahboubi, 2019). The antioxidant, antifungal, and antibacterial properties of ginger extract have been the subject of much research, with an expanding number of uses in food preservation (Ju et al., 2018).

Catechin and epicatechin, which are found in green tea, have been the subject of in-depth research and have been demonstrated to be efficacious against a variety of food-borne diseases (Taguri et al., 2004). Numerous research has been conducted on the health advantages of green tea's polyphenols. Green tea has been shown to have antibacterial, antimycotic, antiviral, and antitoxic properties against bacterial hemolysins in addition to its antioxidant properties (Okubo et al., 1989).

This study was designed to determine the functional potential of Rosemary, Ginger, and Green tea plant extracts by evaluating their inhibitory activity against *S. aureus* in fresh minced meat during refrigeration at 4±1 °C.

2. MATERIAL AND METHODS

The research was performed after approval of the ethical committee of Faculty of Veterinary Medicine, Benha University (BUFVTM 05-10-23).

2.1. Plant extracts

The ready-made herbal aqueous extracts of Rosemary, Ginger, and Green tea were obtained from Makin Co., Cairo, Egypt. All the used chemicals were of analytical reagent grade. The extracts were stored at 4°C in amber-colored bottles till used.

2.2. Preparation of bacterial strains according to Saeed and Tariq (2005) and Kantachote and Charernjiratrakul (2008)

The Animal Health Research Institute', Department of Microbiology, Laboratory of Bacteriology, Dokki, Giza, Egypt, provided a locally isolated strain of *Staphylococcus aureus*. The bacterial strain was added to 0.1% sterile peptone water tube and was incubated for 24 hours at 37 °C. To find the cell concentration, dilutions up to 10^{-10} of this culture were plated on Baird Parker agar (OXOID). Using tube dilution techniques, the cell count for *S. aureus* was adjusted to 7 $\log_{10}$ CFU/ml. For samples of minced meat, the first inoculum load to be added was measured in CFU/g.

2.3. Challenge trial

A total of 2,500 g of minced meat were bought from local market located in Toukh city, Qalubiya governorate, Egypt. Before being used, the minced meat samples were kept in the refrigerator at 4ºC for two hours. After some time, *S. aureus* was aseptically added to the minced beef samples at a concentration of roughly 7 $\log_{10}$ CFU/g. The samples were then kept at room temperature for 15 minutes. To allow bacterial cell attachment, *Staphylococcus aureus* was inoculated on minced meat samples by pouring and mixing over the meat surfaces (Dorsa, 1997). The samples were then kept at room temperature for 20 minutes (Dubal et al., 2004). The initial load of the inoculated samples was determined by counting the number of *Staphylococcus aureus* in each of the ten equal groups of inoculated minced beef samples (250 g each). Then, the inoculated minced meat samples were treated with aqueous plant extracts of Rosemary, Ginger, and Green tea to obtain final concentrations of 0.7%, 1%, and 2% (%v/g), respectively. The control was double-distilled sterile water. To ensure equal mixing, the plant extracts were mixed with the minced beef samples for another thirty seconds. Finally, the inoculated minced beef samples were placed in separate, heat-sealed polyethylene bags that were sterile, labeled, and refrigerated at 4 °C.

Sensory analysis and *S. aureus* counts were conducted for 9 days of refrigerated storage to estimate the shelf-life and antibacterial effects of the used essential oils. Tests were performed in triplicate.

2.4. Sensory analysis

The sensory quality, represented by color, odor, and texture, of each sample was determined and recorded according to a numerical scale with 10 representing "highest quality." Ten employees on the panel were knowledgeable about the qualities of meat (Kanatt et al., 2010).

2.5. Staphylococcus aureus count

It was performed by plating 0.1 ml of previously prepared tenth-fold serial dilutions according to ISO 6887-1: (2017) on *Baird Parker agar* using standard methods (FDA, 2001). *Staphylococcus aureus* was enumerated, converted to log, and expressed as ($\log_{10}$ CFU/g) of the sample.

2.6. Statistical analysis

Using the SPSS software V 19.0, the recorded results were analyzed using one-way ANOVA. Utilizing Duncan's post hoc test, the significance of the variations in mean values was ascertained. The means ± SD of the results were displayed. The reduction % was calculated as follow:

Initial count − current count ÷ Initial count × 100

3. RESULTS

In comparison to the control samples, there was an overall improvement in the sensory qualities (color, odor, and texture) of the treated meat groups during cold storage (4ºC) using varying amounts of tea, rosemary, and ginger aqueous extracts (Table 1). In general, samples treated with extracts from ginger and rosemary showed the greatest improvement in sensory qualities, while those treated with extract from the tea plant showed the lowest improvement. The control group was acceptable through the second storage day. However, samples treated with 2% rosemary were okay through the seventh day, and samples treated with 2% ginger were acceptable through the ninth day. Up until the third, fifth, and seventh days, respectively, sensory qualities were slightly enhanced by 1% of green tea, 1% of rosemary, and 1% of ginger.

Table (2) displayed the *S. aureus* counts in minced beef samples that were subjected to varying doses of extracts of ginger, rosemary, and green tea. Different levels of inhibition against *S. aureus* were seen in the plant extracts under study.

Table 1 Effect of different plant aqueous extracts concentrations on over all acceptability of examined minced meat samples experimentally inoculated with *S. aureus* during cold storage at 4±1 °C.

Groups	Zero day	3rd day	5th day	7th day	9th day
Control	7	1	1	1	1
Green tea 0.7%	7	4	1	1	1
Green tea 1%	7	5	1	1	1
Green tea 2%	7	6	4	1	1
Rosemary 0.7%	7	5	4	1	1
Rosemary 1%	7	6	4	1	1
Rosemary 2%	7	6	5	4	1
Ginger 0.7%	7	5	4	3	1
Ginger 1%	7	6	5	4	1
Ginger 2%	7	7	6	5	4

beef samples had 7.66 $\log_{10}$ CFU/g of *S. aureus*. The minced beef samples exhibited considerable growth inhibition of *S. aureus*, as indicated by all results ($p < 0.05$). 9: Excellent, 8: Very very good, 7: Very good, 6: Good, 5: Medium, 4: Fair, 3: Poor, 2: Very poor, 1: Very very poor,

The results of this study demonstrated the plant extracts' potential as natural food preservatives against *S. aureus* in minced beef. The minced beef samples exhibited considerable growth inhibition of *S. aureus*, as indicated by all results ($p < 0.05$).

Table 2 The effects of different concentrations of plant extracts on *S. aureus* (log_{10} CFU/g) inoculated in the examined minced meat samples

Groups	Zero day	3rd day	5th day	7th day	9th day
Control	7.66±0.51^{a}	---	---	---	---
Tea 0.7%	7.43 ±0.09^{b}	7.32±0.04^{b}	---	---	---
Tea 1.0%	7.30 ± 0.12^{b}	7.28±0.5^{b}	---	---	---
Tea 2.0%	7.22 ±0.03^{b}	7.18±0.14^{c}	7.16±0.14^{c}	---	---
Rose 0.7%	7.20±0.05^{b}	7.12±0.04^{b}	7.10±0.04^{b}	---	---
Rose 1.0%	7.18±0.07^{b}	6.32±0.08^{c}	6.00±0.09^{b}	---	---
Rose 2.0%	7.16±0.21^{b}	5.71±0.64^{d}	5.24±0.22^{a}	5.10±0.22^{a}	---
Ginger 0.7%	7.15±0.13^{b}	5.50±0.31^{b}	5.47±0.28^{b}	5.40±0.25^{b}	---
Ginger 1.0%		5.45±0.05^{c}	5.40±0.05^{c}	5.38±0.05^{c}	---
Ginger 2.0%	7.10±0.10^{b}	5.30±0.13^{d}	4.81±0.21^{b}	4.75±0.18^{a}	4.26±0.14

Initial load of *S. aureus* on the 1st day = 7.66±0.42 log_{10} CFU/g. The values represent the Mean ± SD of three experiments. Means within a column followed by different letters are significantly different ($P < 0.05$).

Moreover, Fig. (1) showed that the growth of *S. aureus* in minced beef samples was completely inhibited after treatment with plant extracts of green tea 2% after 5 days, rosemary 1% after 5 days, ginger 1% after 7 days, and ginger 2% after 9 days.

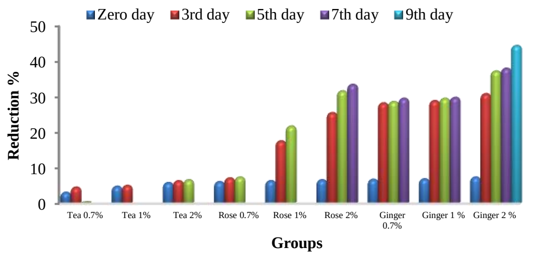

Fig. 1 Reduction % of *Staphylococcus aureus* (CFU/g) in the examined minced meat samples treated with different concentrations of plant extracts

Also, Table (3) illustrated that the reduction percentages were concentration dependent where it significantly increased with an increase in the concentration of the used substances. Additionally, the present data indicated that ginger plant extract showed maximum activity followed by rosemary and green tea plant extracts, respectively. The inhibition of *S. aureus* is related to the concentration of the studied plant extracts since they declined and even inhibited completely when increasing the concentration of the studied plant extracts.

Table 3 Reduction % of *Staphylococcus aureus* (log_{10} CFU/g) in the examined minced meat samples treated with different concentrations of plant extracts

Groups	Zero day	3rd day	5th day	7th day	9th day
Green Tea 0.7%	3.00	4.44	--	--	--
Green Tea 1%	4.70	4.96	--	--	--
Green Tea 2%	5.74	6.27	6.53	--	--
Rosemary 0.7%	6.01	7.05	7.31	--	--
Rosemary 1%	6.27	17.49	21.67	--	--
Rosemary 2%	6.53	25.46	31.59	33.42	--
Ginger 0.7%	6.66	28.2	28.59	29.5	--
Ginger 1%	6.79	28.85	29.5	29.77	--
Ginger 2%	7.31	30.81	37.21	37.90	44.39

4. DISCUSSION

Human foodborne illnesses are primarily caused by *Staphylococcus aureus* (Kadariya et al., 2014). Several studies have been conducted recently to test the effectiveness of natural plant extracts or their oils in enhancing sensory qualities and inhibiting bacterial development in place of artificial preservatives. Table (1) illustrated how the treated minced meat samples' sensory qualities during cold storage at 4°C were much better than those of the control group. Additionally, the concentration of the plant extracts—particularly ginger, which was at 2%, followed by rosemary, which was at 2%, and green tea, which was at 2% increase the proportion of *S. aureus* counts. These results were nearly similar to those reported by Amin (2013) and Hassanin et al. (2016) who revealed that ginger extract induced high sensory scores for meat samples; and Salim et al. (2016) who mentioned that samples containing the highest concentration of green tea extract showed much enhancement of sensory profile.

Table (2) illustrated that the initial *S. aureus* count of control minced meat samples during cold storage at 4±1 °C (7.66±0.42) log_{10} CFU/g was significantly reduced with all concentrations of the examined plant extracts. Then, by increasing the concentration of rosemary by 2%; Tables (2 and 3) showed a reduction in *S. aureus* count. The results nearly agreed with those of Stojanović-Radić et al. (2010) and Amin (2013).

The best result for *S. aureus* reduction was achieved with ginger 2% till 9th day of inoculation. The results came in agreement with those of Natta et al. (2008) and Amany (2018) who discussed the highest efficacy of ginger 2% for *S. aureus* inhibition and reduction in minced meat samples.

The predominant constituent in rosemary extract is reportedly α-pinene, which is followed by 1,8-cineole, camphene, β-myrcene, camphor, and borneole. As a typical lipophilic drug, rosemary extract was found to demonstrate antibacterial activity by permeating the cytoplasm and cell wall membranes and rupturing their structure (Stojanović-Radić et al., 2010). Small molecular weight molecules like phosphates and potassium ions leak out of the cell first, followed by bigger molecules like DNA and RNA, since damaged cell membranes increase their permeability. 1, 8 - On eukaryotic cells, cineole has a potent poisonous effect. ß-caryophyllene is another powerful molecule that can affect the accumulation of some chemicals by making the plasma membrane more permeable. Thus, it influences and amplifies the cytotoxic impact of substances it comes into contact with (Legaule and Pichette, 2007).

Sesquiterpenoids and monoterpenoids, two types of volatile chemicals present in ginger extract, have phenolic properties, according to Mesomo et al. (2013). Zingiberene, α-farnesene, 6-gingerol, and α-curcumin are some of the active ingredients that have been associated with ginger extract's antibacterial activity. According to Zhang et al. (2017), these ingredients attack cell membranes and cell walls to change the permeability and release of intracellular components.

According to Zhang et al. (2023), ginger extract acts directly on the cell membrane, destroying its structure and increasing its permeability. This causes the bacteria to lose their fundamental structural functions and leak macromolecules like bacterial proteins and nucleic acids, which in turn causes a decline in the bacteria's metabolic activity and ultimately results in bacterial cell death. Furthermore, the separated mitochondria and the lipophilic portion of the membrane may interact with the hydrophobic chemicals in ginger extract, impairing their integrity and functionality (enzyme activity, energy metabolism, protein, and nucleic acid) (Beristain-Bauza et al., 2019). Consequently, the microbial cells may be affected by ginger extract in several ways, ultimately resulting in their death.

The compounds associated with the antibacterial activities of green tea extract are catechins and caffeine, which are its main constituents. The caffeine in the tea inhibits regular cell division, which halts the growth of microorganisms and the germination of spores (Aneja and Gianfagna, 2001). Green tea extract has been demonstrated to prevent some major foodborne pathogens, including Vibrio spp., Shigella spp., Staphylococcus spp., and Salmonella spp. (Gadang et al., 2008).

Our results affirm that ginger aqueous extract can significantly inhibit the growth of *S. aureus*, which can serve as a theoretical basis for effective antimicrobial activity in our study.

5. CONCLUSIONS

In conclusion, the obtained results revealed that using extracts of green tea, rosemary, and ginger enhanced the sensory profile of the minced meat by their antibacterial effect on *S. aureus*. Furthermore, it was noticed that ginger extract is more effective than rosemary and green tea against *S. aureus*.

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