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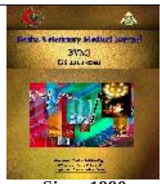

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Antibacterial effect of pepper and cumin extracts on some pathogens contaminating chicken meat

Saad, M. Saad1., Hemmat, M. Ibrahim1., Mohamed, A. Hassan1., Suhair, N. Shehab Eldin2.

[1] *Food control Dept., Fac. Vet. Med., Benha University*

[2] *Directorate of Veterinary Medicine, Qalubiya governorate*

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ABSTRACT

Prevention of food poisoning bacteria is usually achieved by using of some chemical preservatives which have public hazards coming through the improper use of it including presence of chemical residues in food, and acquisition of microbial resistance. Based on such safety concerns, the public need to replace it with more potentially effective, healthier natural alternative preservatives are increased. In the present study, two plant extracts, represented by black pepper and cumin extracts were examined to inhibit the growth of foodborne *Staphylococcus aureus*, *Escherichia coli* and *Salmonella Typhi* strains that were artificially inoculated into minced chicken meat samples. Results revealed that the used pepper and cumin extracts significantly reduced the count of tested strains by 10^2 CFU/g with reduction percent exceeded 99.0%, which proved that the used herbal extracts were potentially effective with variable efficiency against the tested bacterial strains; so, it can be used as natural alternative preservatives to control food poisoning diseases and preserve food stuff avoiding health hazards of chemically antimicrobial agent applications.

1. INTRODUCTION

Thousands of human deaths are reported yearly due to foodborne illness in developing countries Sapkota et al. (2012). Most of food related illness is associated with bacteria and/or its toxins contaminating foods especially *E. coli* and *S. Typhi* as Gram negative bacteria (Pandey and Singh, 2011). Moreover, *Staphylococcus aureus*, as Gram positive bacteria, was reported as the 3rd foodborne pathogen associated with food poisoning which essentially referred to its wide variety of enterotoxins production (Normanno et al., 2007).

Control of foodborne pathogens is commercially performed by addition of chemical preservatives (Shan et al., 2007). As they have antibacterial activity, many researches were conducted to replace them by natural preservatives to avoid their hazards due to their repeated applications such as accumulation of chemical residues, microbial resistance, and side effects on human health (Nasar-Abbas and Kadir, 2004; Bialonska et al., 2010).

Different plants are known of their maintenance ability of good human health since long time ago. In addition, the interest in natural products as food preservatives has greatly increased because of development of multiple antibiotic resistance pathogens that increases interest in the use of herbal extracts as antimicrobial agents (Abdallah and Koko, 2017).

Natural herbal extracts of medicinal purposes contain high biologically active compounds which have great bacterial inhibition properties. Natural plants spices and extracts of various plant parts have been used widely, especially in traditional cultures, as natural antimicrobials, antioxidants, and flavoring agents (George et al., 2009).

Black pepper (*Piper nigrum*) is one of the most public flavoring spices over the world. It is known as the king of flavors. In addition to its flavoring properties, it has many bio-active effects on the human body, such as its perceptible antibacterial action (Abdallah and Abdalla, 2018).

Cumin (*Cuminum cyminum*) is one of these herbs; it is an herbaceous annual plant used as a relish ingredient in many foods processing not only for its seasoning and flavoring effect, but also as natural preservative due to its inhibitory effect against many bacteria (Bahraminejad et al., 2010).

Both black pepper and cumin characterized by medicinal and health benefits; where cumin is used to reduce GIT inflammation, carminative effect, and suppress muscle spasms; moreover, it is also used in improving ingestion, jaundice, diarrhea and flatulence (Eikani et al., 2007). In addition, black pepper and its bioactive extract especially the chemical piperine that has numerous physical and antimicrobial actions. Singletary (2010) documented that black pepper may have health benefits, particularly in enhancing the function of GIT, nervous system benefits, and may influence body energy; also the author suggested that black pepper contains antioxidant constituents and possesses anti-inflammatory and antimicrobial properties.

Therefore, this study aimed to investigate the antibacterial effect of pepper and cumin extracts against *S. aureus, E. coli*, and *S. Typhi* in the artificially inoculated minced chicken meat samples.

* Corresponding author: *Suhair, N. Shehab Eldin, Directorate of Veterinary Medicine, Qalubiya governorate*

2. MATERIAL AND METHODS

2.1. Collection of samples
2.1.1. Meat samples
About 2000 g of fresh chicken meat samples were collected from poultry butchery located in Menoufiya Governorate, Egypt. Samples were minced and divided into equal portions (200g) and kept in separate plastic bags and refrigerated at 4 °C.

2.1.2. Herbal extracts
Black pepper and cumin extracts were obtained from Animal Health Research Institute, Shebin Elkom Lab.
2.1.3. Bacterial strains
Staphylococcus aureus, Escherichia coli and *Salmonella Typhi* strains were obtained from the Central Food Quality Lab., Faculty of Veterinary Medicine, Benha University.

2.2. Experimental application
2.2.1. Minced meat samples
After Ultraviolet sterilization, minced meat samples were divided into 4 groups, 1st untreated, 3 treated groups (200 g for each) and classified into:

2.2.2. Black pepper extract (Kaur *et al.*, 2017)
1st group: control group kept in 4 °C without any additives.
2nd group: inoculated with of *S. Typhi* 10^5 (CFU/g) + pepper extract (150 mg/ml).
3rd group: inoculated with of *S. aureus* (10^6 CFU/g) + pepper extract (150 mg/ml).
4th group: inoculated with of *E. coli* (10^4 CFU/g) + pepper extract (150 mg/ml).

2.2.3. Cumin extract
1st group: control group kept in 4 °C without any additives.
2nd group: inoculated with of *S. Typhi* (10^5 CFU/g) + cumin extract (150 mg/ml) (Sheikh *et al.*, 2010).
3rd group: inoculated with of *S. aureus* (10^6 CFU/g) + cumin extract (150 mg/ml) (Mostafa *et al.*, 2018).
4th group: inoculated with of *E. coli* (10^4 CFU/g) + cumin extract (150 mg/ml) (Sheikh *et al.*, 2010).
Both untreated and treated samples were packed in a separate plastic bags, stored at (4±1°C), and then examined bacteriologically after (2 hrs) as zero time and at predetermined interval (24 hrs) till decomposition of meat has occurred organoleptically after the 7th day of the experiment. The experiment was conducted in triplicate. Colonies of the examined samples in each group were counted and recorded after the preparation of samples according to APHA (2013) to obtain ten-fold serial dilution through mixing of 25 grams of each sample with 225 ml of 0.1% peptone water, the contents were homogenized at stomacher at 450 to 640 strokes/min for 2 minutes; 1ml of the homogenization was transferred into separate tubes containing 9 ml of sterile peptone water 0.1%, from which ten-fold serial dilutions were prepared.

2.3. S. aureus count was done according to ISO 6888-1 (1999), A1 (2003).

2.4. E. coli count was performed according to ISO 16649-2 (2001).
2.5. S. Typhi count was performed according to ISO 6579-1 (2017).

2.6. Statistical analysis:
The obtained results were statistically evaluated by application of Analysis of Variance (ANOVA) test according to Feldman et al. (2003).

3. RESULTS

Application of black pepper and cumin extracts as natural herbal preservative to control *S. aureus, E. coli* and *S. Typhi* in minced chicken meat samples showed significant inhibitory effect with reduction rate exceeded 99.0%.
Regarding the effect of herbal extracts on artificially inoculated *S. aureus* minced chicken meat sample, table (1) revealed that the mean value of examined strain count at zero time was $2.3x10^6±5.7x10^4$ CFU/g in control and treated groups. After seven days, *S. aureus* counts showed significant reduction with mean value of $5.5x10^4±3.0x10^3$ and $6.5x10^4±1.8x10^3$ (CFU/g) and reduction rate of 99.9% for both pepper and cumin treated groups, respectively. While *S. aureus* counts was $1.9x10^8±2.3x10^7$ CFU/g in the control group. Significant differences between the bacterial count/group were noticed at P≤0.05.
The inhibitory effect of tested herbal extracts on artificially inoculated *E. coli* strain into minced chicken meat sample was estimated. Results tabulated in table (2) showed that the mean count of *E. coli* at zero time was $2.2x10^4±8.8x10^4$ in control and treated groups. The mean value of *E. coli* after the 7th day of experiment in control group was $3.5x10^6±5.0x10^5$ CFU/g. While in treated groups, *E. coli* showed significant reduction with mean value of $4.7x10^2±$ 2.4x10 and $6.7x10^2±1.8x10$ CFU/g with reduction rate of 99.98% for both pepper and cumin treated groups, respectively. Significant differences between the bacterial counts were at P≤0.05.
Table (3) presented the inhibitory effect of cumin and pepper extracts on artificially inoculated *S. Typhi* minced chicken meat sample. The mean count at zero time was $2.2x10^5±1.2x10^4$ CFU/g in control and treated groups. At 7th day of the experiment, the mean value of *S. Typhi* was $3.8x10^7±1.7x10^6$ CFU/g in control group, but it was $7.5x10^2±0.8x10$ and $8.1x10^2±1.4x10$ CFU/g with reduction percent of 99.9% for both pepper and cumin treated groups, respectively. Significant differences between the bacterial counts were at P≤0.05.

4. DISCUSSION

Herbal spices have been used for centuries by many ancient cultures for improving the flavor and aroma of foods. In addition, they have been used as food preservative due to its antimicrobials properties (Pan et al., 2008). Referring to the global foodborne illness reports, bacterial food poisoning represents two third of foodborne disease outbreaks (Sodha et al., 2009). The main microorganism related to manipulation practices, Staphylococcus spp., *E. coli* and Salmonella spp. (Lukinmaa et al., 2004).
Referring to the present obtained results, usage of black pepper and cumin extracts showed promising inhibitory effects against foodborne tested *S. aureus*, *E. coli* and *S. Typhi*. In line with many previous studies that recorded great antibacterial effects against many foodborne bacteria.
Comparing with the previous studies, the inhibitory effect of black pepper effect could be compared with the records of Abdel Gadir et al. (2007) revealed inhibitory effect of

methanol extract of pepper against *E. coli* and *S. Typhi*, while had no activity against *S. aureus*. Moreover, petroleum ether extract had no activity against all tested species, while ethanolic extract revealed lower inhibitory activity. Sulieman and Allaahmed, (2012) reported a dramatic decrease in *S. aureus* count to $2.1x10^4$ CFU/g after treatment with 5% pepper for 96 hrs. Rani et al. (2013) documented that the inhibitory effect of black pepper extract was at maximum against Gram positive bacteria *S. aureus* and but at minimum against Gram negative bacteria *E. coli*. Nagy et al. (2015) recorded inhibitory effect of methanol extract of pepper against *E. coli* and *S. aureus*. Zou et al. (2015) showed inhibitory effect of chloroform extract of pepper against *E. coli* and *S. aureus*. Ibrahim et al. (2016) declared bactericidal effect of methanol extract of pepper against *E. coli* and *S. Typhi*, while lesser inhibition against *S. aureus*. Kaho et al. (2019) recorded low inhibitory effect of pepper against *E. coli*.

Regarding to the present inhibitory effect of cumin extract, it could be compared with the records of Dua et al. (2013), who mentioned that cumin extract was effective against both Gram-positive and -negative bacteria which was referred to causing of cell membrane damage and release of the intracellular nucleotides and proteinaceous material. Mostafa et al. (2018), reported that the effect of cumin extract (5-10 mg/ml) was only effective against *S. aureus*, while it was not effective against *E. coli* and *S. Typhi*. Abdul Jabbar (2013) recorded that cumin oil exhibited a strong antibacterial activity against *E. coli*, *S. aureus* isolated from food samples, Baljeet et al. (2015) reported lesser inhibition effect of cumin against *S. Typhi*.

According to the aforementioned studies, different black pepper and cumin extracts showed significant inhibitory effects against varied gram-positive and gram-negative bacteria, although some studies revealed that some extracts had no antibacterial activity, which could be attributed to differences in plant varieties, microbiological methods, solvents used and tested microorganisms. In general, the majority of these studies suggested that the black pepper and cumin extracts could be a potential candidate for developing new food preservative against wide ranges of pathogenic bacteria either food borne, food spoilage or clinical isolates.

Table 1 Statistical analytical results of the effect of some herbal extracts on artificially inoculated *Staphylococcus aureus* into mined chicken meat samples during different period of cold storage (4 °C).

Time	Control	Group A (pepper)		Group B (cumin)	
	Mean ± S.E.	Mean ± S.E.	R%	Mean ± S.E.	R%
Zero time	$2.3x10^6±5.7x10^{4d}$	$2.3x10^6±5.7x10^{4a}$	-	$2.3x10^6±5.7x10^{4a}$	-
2nd day	$4.6x10^6±1.5x10^{5cd}$	$1.3x10^6±5.7x10^{4b}$	71.74	$1.6x10^6±8.8x10^{4b}$	65.22
3rd day	$8.3x10^6±1.1x10^{5cd}$	$9.2x10^5±8.8x10^{3c}$	88.92	$9.8x10^5±5.7x10^{3c}$	88.19
4th day	$9.5x10^6±1.2x10^{5cd}$	$6.3x10^5±1.1x10^{4d}$	93.37	$6.7x10^5±8.8x10^{3d}$	92.94
5th day	$3.3x10^7±1.1x10^{6c}$	$2.3x10^5±1.4x10^{4e}$	99.30	$3.7x10^5±1.1x10^{4e}$	98.87
6th day	$6.2x10^7±8.8x10^{5b}$	$8.2x10^4±5.7x10^{2f}$	99.86	$9.6x10^4±2.5x10^{3f}$	99.84
7th day	$1.9x10^8±2.3x10^{7a}$	$5.5x10^4±3.0x10^{3f}$	99.99	$6.5x10^4±1.8x10^{3f}$	99.97

The values represent Mean ± SE of three experiments. Means within a column followed by different letters are highly significantly different ($P < 0.05$). Zero time: 2h after inoculation. R%: Reduction percent.

Table 2 Statistical analytical results of the effect of some herbal extracts on artificially inoculated *Escherichia coli* into mined chicken meat samples during different period of cold storage (4 °C).

Time	Control	Group A (pepper)		Group B (cumin)	
	Mean ± S.E.	Mean ± S.E.	R%	Mean ± S.E.	R%
Zero time	$2.2x10^{4c}±8.8x10^2$	$2.2x10^{4a}±8.8x10^4$	-	$2.2x10^{4a}±8.8x10^4$	-
2nd day	$5.4x10^{4c}±8.8x10^2$	$1.2x10^{4b}±1.2x10^3$	75.92	$1.7x10^{4b}±1.2x10^3$	68.52
3rd day	$8.3x10^{4c}±8.8x10^2$	$7.4x10^{3c}±1.2x10^2$	91.08	$8.5x10^{3c}±1.7x10^2$	89.76
4th day	$5.2x10^{5bc}±5.7x10^3$	$5.2x10^{3d}±8.8x10$	99.00	$6.8x10^{3d}±5.7x10$	98.69
5th day	$7.7x10^{5b}±8.8x10^3$	$1.4x10^{3e}±1.2x10^2$	99.81	$2.4x10^{3e}±2.3x10^2$	99.68
6th day	$9.6x10^{5b}±1.8x10^4$	$8.3x10^{2e}±0.5x10$	99.91	$9.4x10^{2ef}±1.1x10$	99.90
7th day	$3.5x10^{6a}±5.0x10^5$	$4.7x10^{2e}±2.4x10$	99.98	$6.7x10^{2f}±1.8x10$	99.98

The values represent Mean ± SE of three experiments. Means within a column followed by different letters are highly significantly different ($P < 0.05$). Zero time: 2h after inoculation. R%: Reduction percent.

Table 3 Statistical analytical results of the effect of some herbal extracts on artificially inoculated *Salmonella typhi* into mined chicken meat samples during different period of cold storage (4 °C).

Time	Control	Group A (pepper)		Group B (cumin)	
	Mean ± S.E.	Mean ± S.E.	R%	Mean ± S.E.	R%
Zero time	$2.2x10^5±1.2x10^{4e}$	$2.2x10^5±1.2x10^{4a}$	-	$2.2x10^5±1.2x10^{4a}$	-
2nd day	$4.5x10^5±2.4x10^{4de}$	$1.5x10^5±2.8x10^{4b}$	66.6	$1.7x10^5±1.2x10^{4b}$	62.2
3rd day	$8.5x10^5±1.7x10^{4de}$	$8.1x10^4±2.0x10^{3c}$	90.5	$8.6x10^4±1.4x10^{3c}$	89.9
4th day	$2.5x10^6±1.7x10^{5d}$	$5.6x10^4±1.4x10^{3cd}$	97.8	$6.3x10^4±1.4x10^{3d}$	97.5
5th day	$5.5x10^6±1.7x10^{5c}$	$2.2x10^4±8.8x10^{3de}$	99.6	$3.2x10^4±1.2x10^{3e}$	99.4
6th day	$9.6x10^6±1.4x10^{5b}$	$1.5x10^3±1.2x10^{2e}$	99.9	$3.7x10^3±6.6x10^{2f}$	99.9
7th day	$3.8x10^7±1.7x10^{6a}$	$7.5x10^2±0.8x10^e$	99.9	$8.1x10^2±1.4x10^f$	99.9

The values represent Mean ± SE of three experiments. Means within a column followed by different letters are highly significantly different ($P < 0.05$). Zero time: 2h after inoculation. R%: Reduction percent.

5. CONCULSION

From the present results, it can be concluded that herbal extracts of black pepper and cumin had potential inhibitory effects against foodborne bacterial contamination giving promising trend in replacing chemical preservatives with natural safe herbal extracts.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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