

## Research Article

# Polyphenol-Rich Extracts of Traditional Culinary Spices and Herbs and Their Antibacterial Activity in Minced Beef

Saeed Akhtar,<sup>1</sup> Muhammad Waseem,<sup>1</sup> Nazir Ahmad ,<sup>2</sup> Tariq Ismail,<sup>1</sup> Zulfiqar Ahmad ,<sup>3</sup> Muhammad Faisal Manzoor ,<sup>4</sup> and Azhari Siddeeg <sup>5</sup>

<sup>1</sup>Institute of Food Science and Nutrition, Bahauddin Zakariya University, Multan, Pakistan

<sup>2</sup>Institute of Home and Food Sciences, Government College University, Faisalabad, Pakistan

<sup>3</sup>University College of Agriculture and Environmental Sciences, The Islamia University of Bahawalpur, Bahawalpur, Pakistan

<sup>4</sup>School of Food Science and Engineering, South China University of Technology, Guangzhou, Guangdong 510640, China

<sup>5</sup>Department of Food Engineering and Technology, Faculty of Engineering and Technology, University Gezira, Wad Medani, Sudan

Correspondence should be addressed to Nazir Ahmad; [drnazirahmad@gcuf.edu.pk](mailto:drnazirahmad@gcuf.edu.pk); Muhammad Faisal Manzoor; [faisaluos26@gmail.com](mailto:faisaluos26@gmail.com); and Azhari Siddeeg; [azhari\\_siddeeg@uofg.edu.sd](mailto:azhari_siddeeg@uofg.edu.sd)

Received 30 July 2019; Accepted 26 November 2019; Published 16 December 2019

Guest Editor: Melvin J. Holmes

Copyright © 2019 Saeed Akhtar et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

This study was conducted to elucidate minced beef stabilization properties of hydroalcoholic extracts of commonly used culinary spices from Pakistan against meat oxidative stress and microbial spoilage. Hydroalcoholic extracts of six selected spices, namely, onion, ginger, turmeric, coriander, fennel, and mint, were evaluated to inhibit microbial growth in minced beef under refrigerated storage (4°C) of nine days. Maximum phenolic concentration, i.e., 70.8 mg GAE/100 g, and free radical scavenging activity (75.9%) were anticipated by hydromethanolic extracts of ginger. The results propose that the addition of hydroalcoholic extracts of ginger and coriander @ 6.0% anticipate significantly ( $p < 0.05$ ) higher inhibitory effects against *Staphylococcus aureus* and *Escherichia coli*. The results of this research conclude that the utilization of hydroalcoholic extracts may serve as a promising approach to preserve microbiological as well as the oxidative quality of minced beef and products of meat origin.

## 1. Introduction

Spices have a long history for culinary application as seasoning ingredients in various cultures, e.g., garlic, onion, cinnamon, anise, clove, and red pepper are preferred seasoning agents of Chinese culture while coriander and black pepper are likely consumed in the East Indian region [1]. Spice extracts and essential oils have been extensively explored for shelf stability of raw and processed chicken [2, 3], shallow and deep-fried meat [4], fermented meat [5], meat sausages [6], and dried cured meat [7].

Microbiological food safety in the meat distribution system can be achieved to a greater extent with natural ingredients of plants and animal origins like organic acids, plant extracts, and essential oils [8]. Antimicrobial features of spices are predominantly associated with phytochemicals

like flavonoids, flavones, isoflavones, and anthocyanins that anticipate significant free radicals and free metal ion binding properties in food systems [9, 10]. Active ingredients of spices have also been attributed to bringing about changes in cellular membrane permeability leading to intracellular matrix leakage and cell lysis [11]. Typical characteristics of spices defining their role as potential antimicrobial agents have been embedded in rendering bacteria to poorly synthesize microbial nucleotides, i.e., DNA and RNA, which could further halt microbial growth and proliferation [12].

Spices primarily provide a convenient and reasonable choice towards minimizing household and industrial use of synthetic additives and to add value to the consumer good. Spices could further substantially contribute to reducing the adverse effects of synthetic additives on product quality and consumer's health. The objectives of the present study entail

investigation into assessing the antioxidant potential of turmeric, onion, ginger, fennel, coriander, and mint extracts and their role as antimicrobials in inhibiting microbial growth in the minced beef model under refrigerated storage.

## 2. Material and Methods

**2.1. Procurement of Raw Materials and Chemicals.** Fresh ginger (*Zingiber officinale*) rhizomes, onion (*Allium cepa*) bulbs, turmeric (*Curcuma longa*) stems, coriander (*Coriandrum sativum*) seeds, fennel (*Foeniculum vulgare*) seeds, and peppermint (*Mentha piperita*) leaves were purchased from the local market of Multan, Pakistan. Samples were maintained at  $-18^{\circ}\text{C}$  until drying. All reagents unless specified including solvents, sodium acetate buffer (pH = 3.6), DPPH (2,2-diphenyl-1-picryl-hydroxyl) reagent, Folin-Ciocalteu phenol reagent (FCR), gallic acid, sodium carbonate, TPTZ [2, 4, 6-tri (2-pyridyl)-s-triazine], and ascorbic acid were analytical grade and purchased from Sigma-Aldrich Inc., USA.

**2.2. Development of Spice Powders.** Green spices were procured from the local vegetable market, washed with potable water, sorted, graded, and dehydrated in a cabinet dryer at  $70^{\circ}\text{C}$  to 15–17% moisture contents. Dehydrated spices were ground to 70 mm mesh size, sieved, and stored in airtight plastic containers at  $25^{\circ}\text{C}$  for further applications [13].

**2.3. Physicochemical Analysis of Spice Powders.** Fine powders of spices were analyzed for moisture, fat, ash, and protein contents in accordance with the procedure laid down in AOAC [14, 15]. Carbohydrate contents were estimated as nitrogen-free extract (NFE) using formula, i.e.,  $\text{NFE}\% = 100 - (\text{moisture} + \text{crude protein} + \text{total ash} + \text{crude fat} + \text{crude fiber})$ . Mineral contents (Na, Ca, and K) of spice powders were analyzed using a flame photometer in accordance with the method laid down by AOAC [14].

**2.4. Total Phenolic Contents.** Spice powders were soaked in hydromethanolic and ethanolic solvents (70:30) (solvent: distilled water) for 8 hours. Orbital shaking was performed at  $40^{\circ}\text{C}$  for 3 hours. The supernatant was filtered via filter paper no. 41 followed by rotary evaporation at  $40^{\circ}\text{C}$ . Extracts' concentrates were freeze-dried and stored at  $-18^{\circ}\text{C}$  for further assay. Total phenolic contents in spice extracts were determined by the method adopted by Manzoor et al. [16]. Plant extracts (i.e., 100 ppm) were prepared with solvents, i.e., methanol and ethanol. An aliquot of 0.5 mL was transferred to the test tubes followed by the addition of 10-fold diluted FCR. 2 mL of sodium carbonate (7.5%) was added, and samples were subjected to react for 30 min at  $25^{\circ}\text{C}$ . Absorbance was measured spectrophotometrically (UV-Vis 3000, ORI, Germany) at 760 nm using gallic acid standard (10–100 ppm), and results were expressed as mg GAE/100 g.

**2.5. 2, 2-Diphenyl-1-Picryl-Hydrazyl (DPPH) Assay.** Free radical scavenging activity of hydroalcoholic extracts was

determined using DPPH assay [17]. Different concentrations of spice extracts ranging from 50–100 ppm were prepared. Aliquots (50–100  $\mu\text{L}$ ) were pipetted in labeled test tubes, and the final volume was adjusted to 100  $\mu\text{L}$  with methanol. 5 mL of DPPH reagent (0.1 mM) was added to each test tube. The contents of test tubes were vortexed and incubated for 20 min at  $27^{\circ}\text{C}$ . Absorbance was measured spectrophotometrically at 517 nm. Free radical scavenging activity was calculated using the following formula:

$$\text{radical scavenging activity (\%)} = \frac{\text{control Abs.} - \text{sample Abs.}}{\text{control Abs.}} \times 100. \quad (1)$$

## 2.6. Microbiological Analysis

**2.6.1. Bacterial Cultures and Inocula Preparation.** Bacterial isolates from minced beef were spread onto specific microbial culture media including mannitol salt agar (*Staphylococcus aureus*), MacConkey agar (*Escherichia coli*), and SS agar (*Salmonella* spp.). Confirmed colonies of each test microorganisms were shifted to phosphate buffer saline and incubated at  $37^{\circ}\text{C}$  for 3–6 hours to achieve 0.5 McFarland turbidity standard.

**2.6.2. Antimicrobial Assay (Disc Diffusion Method).** Antimicrobial screening of spice extracts was performed in accordance with the method developed by Adetunde et al. [18]. Microbial cultures vis. *S. aureus*, *E. coli*, and *Salmonella* spp. were evenly spread on Muller Hinton Agar (MHA) plates. Sterilized discs were aseptically placed over the inoculated MHA media plates. Spice extracts (50  $\mu\text{L}$ ) of 150 ppm strength were loaded onto the discs. Solvent and standard drugs, i.e., gentamycin and penicillium (20–30  $\mu\text{g}$ ), were taken as negative and positive controls, respectively. MHA plates were subjected to incubation at  $37^{\circ}\text{C}$  for 24 hours, and zones of inhibitions (mm) were computed.

**2.6.3. Microbiological Inhibition Properties of Spice Extracts in Minced Beef.** Freshly purchased minced beef was decontaminated using sodium hypochlorite (20 ppm). Hundred-gram minced beef sample with no decontamination treatment was designated as a negative control. Twenty-five grams of minced beef was marinated with hydromethanolic extracts of spices including onion, turmeric, ginger, coriander, fennel, and mint at the rate of 1.5%, 3%, and 6%. Marinated samples were stomached for 2 min (Stomacher® 400 Circulator). Stomached samples were further inoculated with 100  $\mu\text{L}$  ( $1.5 \times 10^8$  CFU/mL ~ 0.5 McFarland turbidity standard) cultures of *S. aureus* and *E. coli*. Microbial spiked minced beef samples were stored at  $4 \pm 2^{\circ}\text{C}$ , and total counts of *S. aureus* and *E. coli* of minced beef samples were enumerated on 0, 3<sup>rd</sup>, 6<sup>th</sup>, and 9<sup>th</sup> day of storage. Results were expressed as  $\log_{10}$  CFU/g [19, 20].

**2.7. Statistical Analysis.** All experiments were performed twice, and the results were expressed as mean  $\pm$  SD. Data

were statistically analyzed with Statistics 8.1 software using a two-way analysis of variance (ANOVA) technique at  $p < 0.05$ . Means were compared using the least significant difference (LSD) test.

### 3. Results and Discussion

#### 3.1. Physicochemical Properties of Spices and Their Extracts.

Data on the nutritional composition of spices powder are presented in Tables 1 and 2. A significant difference in ash contents was detected in turmeric (6.5%) and mint powder (1.9%). Maximum fat contents were recorded in fennel while coriander depicted the highest concentration of protein. A significantly higher amount of carbohydrates was recorded in onion powder.

The appreciable concentration of sodium, calcium, and potassium was observed in coriander, fennel, and ginger powder, respectively (Table 2). Average spice consumption from various modes in the Indian subcontinent has been reported around 10 g that can anticipate ~1.2–8% of daily energy requirements [21]. In addition to create appeal and anticipate functional properties, compositional analysis of spices thus suggests their supplementary role in improving the nutritional value of the finished goods.

**3.2. Total Phenolic Contents and Antioxidant Activity.** The extracts' yield, total phenolic contents, and antioxidant potential of spices are presented in Table 3. Significant ( $p < 0.05$ ) effect of solvent and type of spices was revealed on phenolic recovery. The highest total phenolic contents with a mean value of 70.8 mg GAE/100 g were recovered from ginger followed by turmeric extracts, i.e., 70 mg GAE/100 g, while onion and fennel hydroethanolic extracts were bearing lower phenolics recovery rate, i.e., 36 mg GAE/100 g and 35 mg GAE/100 g, respectively. Relatively lower total phenolic contents were reported in spices by Kumari and Gupta [22] wherein the phenolic recovery rate was in a range between 20–78 mg GAE/100 g. Hydroalcoholic extraction of plant phenolics has variable recovery rates that depend on the type of solvent, combinations of solvents like water: alcohol ratio (70:30) and solvent/solid ratio, part of the plant, i.e., leaves, roots, seeds, fruit, flower, and bark, particle size or the surface area of the plant matter, and extraction conditions like pressure (30–250 bar), extraction time (3–4 hours), and extraction temperature, i.e., ~25°C [23].

Significantly higher antioxidant properties were observed in the ginger extract in comparison with extracts of other spices under investigation (Table 3). Hydroalcoholic extracts yielded higher free radical scavenging properties with ginger followed by turmeric, i.e., 75.9%, while hydroethanolic extracts presented higher DPPH radical scavenging activities for ginger (66.3%) and coriander (51.7%). Higher DPPH free radical scavenging properties of ginger and turmeric correlates with their higher phenolic contents as compared to the onion extract. DPPH free radical scavenging property of ginger extracts has been previously cited between 67–78% [22]. The considerably higher concentration of hydroxyl rich total phenolics and

synergistic role of spices extracts could be achieved by their application as additives in meat and meat-based products.

#### 3.3. Antimicrobial Screening of Spice and Herb Extracts.

The antimicrobial activity of spice extracts against various pathogenic microbes at 150 ppm concentration is presented in Table 4. Inhibition zones of various extracts against Gram-negative and Gram-positive bacteria including *E. coli*, *Salmonella* spp., and *S. aureus* were determined for methanolic and ethanolic extracts of ginger, turmeric, onion, coriander, fennel, and mint extracts at 150 ppm concentration (30–40 µg extracts disc). In comparison with gentamycin and penicillin discs, both methanolic and ethanolic extracts of onion generated larger zones of inhibition against the tested pathogens. Onion extracts generated wider inhibition zones, i.e., 17.1 mm, 16.5 mm, and 15.5 mm, for *E. coli*, *S. aureus*, and *Salmonella* spp., respectively. Comparatively lower antimicrobial activities against tested pathogens were reflected by hydroethanolic extracts of mint, fennel, and coriander extracts.

#### 3.4. Effect of Extracts' Supplementation on *E. coli* Counts in Minced Beef.

*E. coli* counts under refrigeration of minced beef treated with hydromethanolic extracts of spices were estimated in log<sub>10</sub> CFU/g during 0–9 days of storage. Interpretation of the data presented in Figure 1 suggests significant ( $p < 0.05$ ) reductions in *E. coli* counts of minced beef on treatment with varying levels of spice extracts at different storage intervals. In comparison to the negative control where *E. coli* counts were found to increase from 5.72 log<sub>10</sub> CFU/g to 6.29 log<sub>10</sub> CFU/g, coriander extracts' supplementation in *E. coli* inoculated minced beef presented peak inhibitory properties, i.e., from 4.8 log<sub>10</sub> CFU/g to 5 log<sub>10</sub> CFU/g, during 9 d refrigerated storage. Around 0.23 log<sub>10</sub> CFU/g increase in *E. coli* counts was observed in minced beef supplemented with 6% ginger extracts as compared to 0.56 log<sub>10</sub> CFU/g and 0.60 log<sub>10</sub> CFU/g for negative and positive control under similar study conditions. Fennel and mint extracts were also found equally efficacious in inhibiting the pathogenic load of *E. coli*. Furthermore, methanolic extracts of fennel, mint, and coriander increased the lag period in relation to the normal control. Comparable role of turmeric and onion extracts were noticed against *E. coli* inhibition. Pearson correlation ( $r=0.96$ ) shows that the extracts' amount and storage duration suggested higher *E. coli* inhibitory properties of spice extracts at an extended amount of supplementation.

Antimicrobial activities of spices have been attributed to flavonoids, saponins, glucosinolates, thiosulfinates, and saponins [24]. Ginger bioactive compounds that exhibit antimicrobial activity include ar-curcumin, caryophyllene,  $\beta$ -sesquiphellandrene,  $\alpha$ -farnesene, and zingiberene [25]. Coriander methanolic extracts have been already reported effective against human pathogens including *E. coli* and *Salmonella typhi* [26]. An earlier study carried out by Bali et al. [27] endorsed coriander application at the rate of 2–5% in beef sausages to attribute improved meat quality parameters under refrigerated storage for a period of 14

TABLE 1: Proximate composition of spice powders on dry weight basis (g/100 g).

Spices	Moisture	Ash	Fat	Protein	Fiber	Carbohydrates†
Onion	9.5 ± 1.3 <sup>b</sup>	3.4 ± 0.5 <sup>c</sup>	1.5 ± 0.7 <sup>c</sup>	2.5 ± 0.6 <sup>c</sup>	2.2 ± 1.59 <sup>e</sup>	80.9 ± 3.3 <sup>a</sup>
Ginger	10.0 ± 0.3 <sup>b</sup>	5.4 ± 0.4 <sup>b</sup>	1.9 ± 0.2 <sup>c</sup>	6.2 ± 0.6 <sup>b</sup>	6.0 ± 2.7 <sup>cd</sup>	70.5 ± 2.7 <sup>b</sup>
Turmeric	8.8 ± 0.7 <sup>b</sup>	6.5 ± 0.6 <sup>a</sup>	3.8 ± 0.2 <sup>b</sup>	4.0 ± 0.7 <sup>c</sup>	4.8 ± 1.0 <sup>de</sup>	72.2 ± 4.0 <sup>b</sup>
Coriander	9.2 ± 0.6 <sup>b</sup>	2.5 ± 0.1 <sup>cd</sup>	6.8 ± 0.3 <sup>a</sup>	13.2 ± 0.3 <sup>a</sup>	14.3 ± 0.9 <sup>b</sup>	63.1 ± 2.0 <sup>c</sup>
Fennel	9.4 ± 0.8 <sup>b</sup>	5.9 ± 0.4 <sup>b</sup>	7.1 ± 0.6 <sup>a</sup>	3.9 ± 0.2 <sup>c</sup>	20.3 ± 0.9 <sup>a</sup>	53.4 ± 0.9 <sup>c</sup>
Mint	12.3 ± 1.0 <sup>a</sup>	1.9 ± 0.9 <sup>d</sup>	1.3 ± 0.2 <sup>c</sup>	2.2 ± 0.2 <sup>c</sup>	8.4 ± 1.6 <sup>bc</sup>	74.0 ± 3.3 <sup>b</sup>

Mean ± SD; means bearing same letters in a column are statistically nonsignificant at  $p < 0.05$ . †Calculations on dry weight basis as 100 – (Ash + Protein + Fiber + Fat).

TABLE 2: Mineral composition of spice powders on dry weight basis (mg/kg<sup>-1</sup>).

Spices	Calcium	Potassium	Sodium
Onion	5.0 ± 0.0 <sup>d</sup>	135.0 ± 0.0 <sup>c</sup>	14.3 ± 0.6 <sup>c</sup>
Ginger	6.0 ± 0.0 <sup>d</sup>	229.7 ± 0.6 <sup>a</sup>	16.0 ± 0.0 <sup>c</sup>
Turmeric	6.3 ± 0.6 <sup>d</sup>	169.0 ± 0.0 <sup>b</sup>	16.3 ± 0.6 <sup>c</sup>
Coriander	18.3 ± 2.5 <sup>c</sup>	94.7 ± 4.9 <sup>e</sup>	95.3 ± 5.5 <sup>a</sup>
Fennel	32.0 ± 2.6 <sup>a</sup>	116.3 ± 5.0 <sup>d</sup>	42.7 ± 2.5 <sup>b</sup>
Mint	24.7 ± 1.5 <sup>b</sup>	89.7 ± 0.6 <sup>e</sup>	18.3 ± 0.6 <sup>c</sup>

Mean ± SD; means bearing same letters in a column are statistically nonsignificant at  $p < 0.05$ .

TABLE 3: Extracts' yield, total phenolic contents, and antioxidant potential of spices.

Spices	Solvent	Extracts yield (%)	TPC (mg GAE/100 g)	DPPH (%)
Onion	MeOH	9.5 ± 1.5 <sup>a</sup>	55.9 ± 3.2 <sup>bc</sup>	54.4 ± 3.8 <sup>e</sup>
	EtOH	5.9 ± 0.5 <sup>bc</sup>	36.0 ± 4.6 <sup>hi</sup>	43.1 ± 4.4 <sup>g</sup>
Ginger	MeOH	5.5 ± 1.6 <sup>bc</sup>	70.8 ± 3.3 <sup>a</sup>	75.9 ± 3.9 <sup>a</sup>
	EtOH	3.2 ± 0.9 <sup>ef</sup>	54.1 ± 3.4 <sup>bcd</sup>	66.3 ± 5.0 <sup>bc</sup>
Turmeric	MeOH	4.8 ± 0.1 <sup>cd</sup>	51.6 ± 1.6 <sup>cde</sup>	61.4 ± 1.9 <sup>cd</sup>
	EtOH	6.2 ± 1.2 <sup>b</sup>	42.7 ± 6.0 <sup>fgh</sup>	46.1 ± 4.0 <sup>fg</sup>
Coriander	MeOH	2.4 ± 0.3 <sup>f</sup>	69.8 ± 1.6 <sup>a</sup>	70.5 ± 2.5 <sup>ab</sup>
	EtOH	3.1 ± 0.0 <sup>ef</sup>	49.0 ± 0.5 <sup>def</sup>	51.7 ± 1.6 <sup>ef</sup>
Fennel	MeOH	5.4 ± 0.1 <sup>bc</sup>	46.3 ± 5.2 <sup>efg</sup>	56.2 ± 5.2 <sup>de</sup>
	EtOH	4.0 ± 0.0 <sup>de</sup>	34.8 ± 4.7 <sup>i</sup>	41.2 ± 1.2 <sup>g</sup>
Mint	MeOH	3.9 ± 0.1 <sup>de</sup>	59.1 ± 5.7 <sup>b</sup>	62.1 ± 3.2 <sup>c</sup>
	EtOH	3.0 ± 0.0 <sup>ef</sup>	40.6 ± 2.9 <sup>ghi</sup>	42.6 ± 1.2 <sup>g</sup>

Mean ± SD; means bearing same letters in a column are statistically nonsignificant at  $p < 0.05$ . MeOH = hydromethanolic extracts; EtOH = hydroethanolic extracts; TPC = total phenolic content; DPPH = diphenyl picrylhydrazyl.

days. The study further suggested coriander application to anticipate ~1.18 log inhibition of total bacterial count in comparison with normal control during extended refrigerated storage of 21 days. Relatively lesser antibacterial activity of ginger, turmeric, and onion extracts was observed in ground beef that may be associated with poor distribution of spice extracts in a beef matrix. A study conducted by Gupta and Ravishankar [28] revealed that antimicrobial activity of pure pastes of ginger, garlic, and turmeric against *E. coli* O157:H7 was found higher than that observed in beef, thus suggesting a partial reduction in bactericidal properties of extracts in food system. Ginger extracts have been also reported as proteolytic because they enhance the antimicrobial characteristics against Gram-negative and positive pathogens including *E. coli* and *L. monocytogenes* [29].

**3.5. Effect of Extracts' Supplementation on *S. aureus* Counts in Minced Beef.** In comparison with both the positive and negative controls, methanolic extracts of tested spices significantly ( $p < 0.05$ ) inhibited *S. aureus* growth in minced beef during 0–9 days of the study period. Data presented in Figure 2 showed that minced beef marinated with spice extracts at the rate of 1.5–6.0% offered better shelf stability and reduced pathogen growth. Least *S. aureus* count increment, i.e., 0.24 log<sub>10</sub> CFU/g, was observed on 9<sup>th</sup> day of storage in minced beef marinated with 6% ginger extracts, whereas positive control inoculated with *S. aureus* at same inoculation levels as of treatment groups was observed with 0.77 log<sub>10</sub> CFU/g increase in pathogen counts at the end of the study. Methanolic extracts of coriander and turmeric also delivered pronounced inhibition in *S. aureus* proliferation during 9 d storage with

TABLE 4: Antimicrobial activity of spice extracts against various pathogenic microbes at 150 ppm concentration.

Spices	Extracts	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Salmonella</i> spp.
Gentamycin	—	21.5 ± 0.7 <sup>a</sup>	22.0 ± 0.7 <sup>a</sup>	24.0 ± 0.0 <sup>a</sup>
Penicillin	—	19.5 ± 0.7 <sup>b</sup>	20.5 ± 1.4 <sup>a</sup>	23.5 ± 0.7 <sup>a</sup>
Onion	MeOH	17.1 ± 0.6 <sup>c</sup>	16.3 ± 1.1 <sup>bc</sup>	15.5 ± 3.5 <sup>b</sup>
	EtOH	14.5 ± 0.7 <sup>def</sup>	16.5 ± 0.7 <sup>bc</sup>	15.3 ± 0.4 <sup>b</sup>
Ginger	MeOH	11.5 ± 0.7 <sup>hi</sup>	14.5 ± 0.7 <sup>bcd</sup>	9.8 ± 0.4 <sup>f</sup>
	EtOH	13.5 ± 2.1 <sup>efg</sup>	16.0 ± 1.4 <sup>bc</sup>	11.0 ± 1.4 <sup>ef</sup>
Turmeric	MeOH	14.8 ± 0.4 <sup>def</sup>	15.5 ± 2.1 <sup>bc</sup>	14.0 ± 1.4 <sup>bcd</sup>
	EtOH	16.0 ± 1.4 <sup>cd</sup>	15.0 ± 1.4 <sup>bcd</sup>	15.5 ± 0.7 <sup>b</sup>
Coriander	MeOH	15.2 ± 0.2 <sup>de</sup>	15.5 ± 0.7 <sup>bc</sup>	14.3 ± 0.4 <sup>bcd</sup>
	EtOH	13.7 ± 0.2 <sup>efg</sup>	14.0 ± 0.0 <sup>cde</sup>	12.0 ± 0.0 <sup>def</sup>
Fennel	MeOH	13.2 ± 0.3 <sup>fgh</sup>	15.0 ± 0.0 <sup>bcd</sup>	14.8 ± 0.4 <sup>bc</sup>
	EtOH	12.3 ± 0.4 <sup>ghi</sup>	12.0 ± 1.4 <sup>e</sup>	13.1 ± 0.2 <sup>bcd</sup>
Mint	MeOH	16.0 ± 0.7 <sup>cd</sup>	16.6 ± 1.3 <sup>b</sup>	14.1 ± 0.4 <sup>bcd</sup>
	EtOH	11.0 ± 0.7 <sup>i</sup>	12.7 ± 1.4 <sup>de</sup>	12.7 ± 0.2 <sup>cde</sup>

Mean ± SD; means bearing same letters in a column are statistically nonsignificant at  $p < 0.05$ . MeOH = hydromethanolic extracts; EtOH = hydroethanolic extracts.

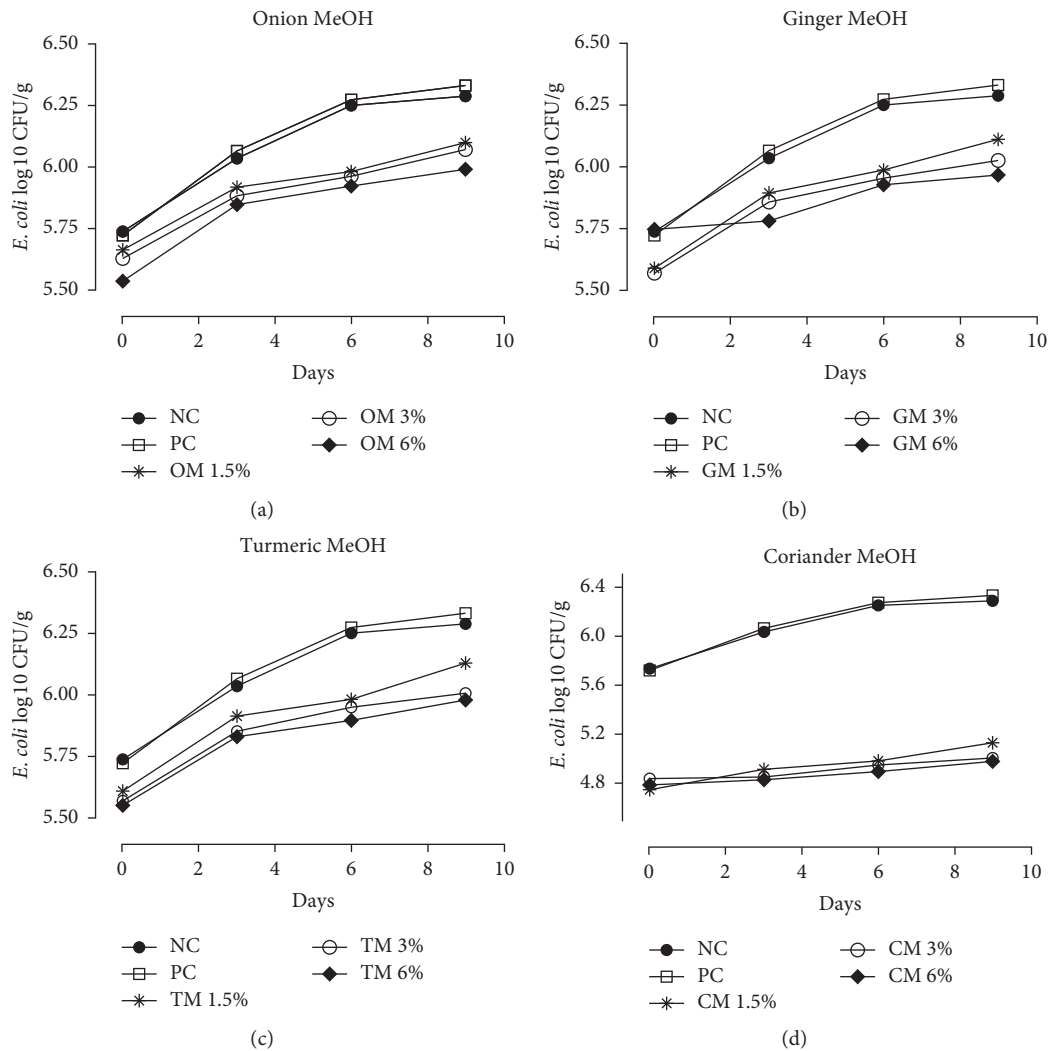


FIGURE 1: Continued.



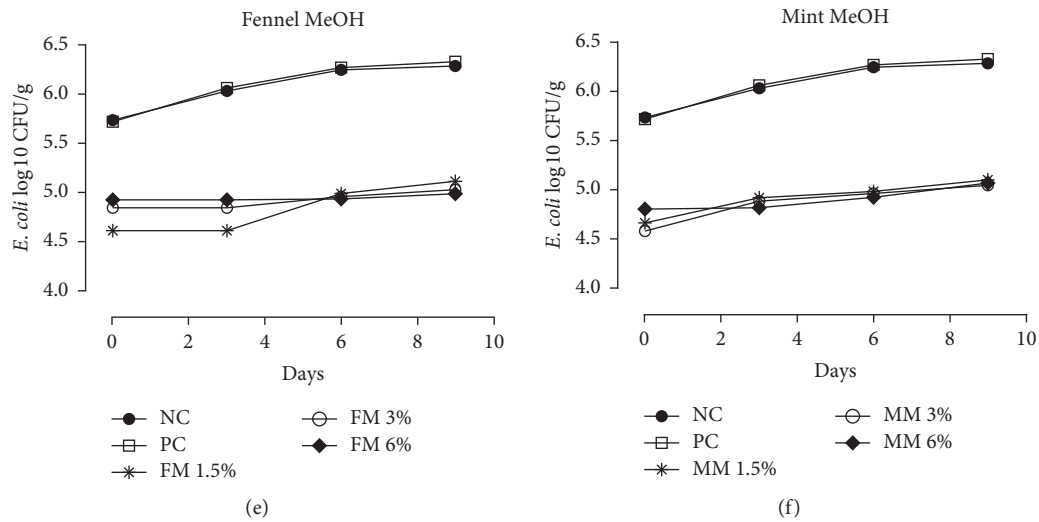


FIGURE 1: *E. coli* inhibitory activity of spice extracts in marinated minced beef under refrigerated (4°C) 9 d storage. NC: negative control, PC: positive control, OM: onion methanolic extracts, GM: ginger methanolic extracts, TM: turmeric methanolic extracts, CM: coriander methanolic extracts, FM: fennel methanolic extracts, and MM: mint methanolic extracts.

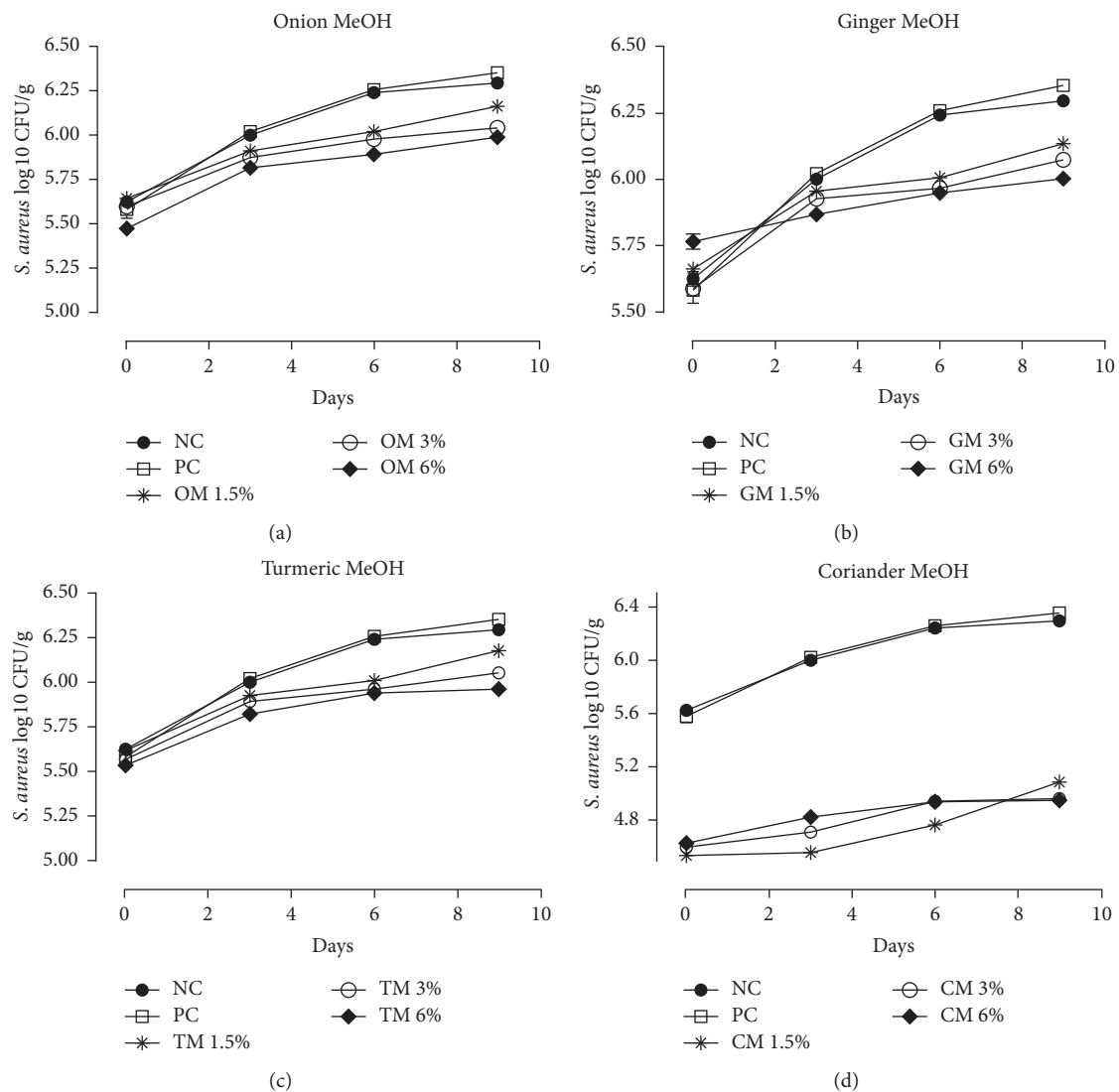


FIGURE 2: Continued.

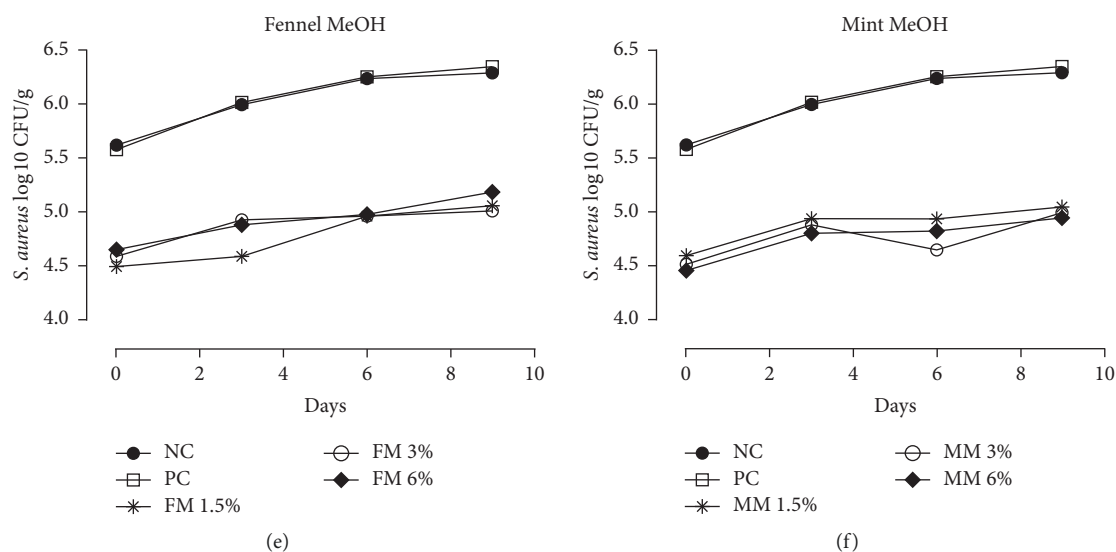


FIGURE 2: *S. aureus* inhibitory activity of spice extracts in marinated minced beef under refrigerated (4°C) 9 d storage. NC: negative control, PC: positive control, OM: onion methanolic extracts, GM: ginger methanolic extracts, TM: turmeric methanolic extracts, CM: coriander methanolic extracts, FM: fennel methanolic extracts, and MM: mint methanolic extracts.

up to 0.32 and 0.43 log<sub>10</sub> CFU/g upsurge for coriander and turmeric extracts, respectively. Fennel, mint, and onion extracts also exhibited significant ( $p < 0.05$ ) *S. aureus* inhibition in comparison with the normal and positive control (Figure 2).

Ginger extracts have been reported efficacious against both the Gram-positive and Gram-negative bacteria including *S. aureus* and *E. coli* [30]. In an earlier study wherein ginger extracts were applied as natural preservatives in frozen beef sausages, the extracts' application at the rate of 1.0% was found to increase product shelf stability by significantly inhibiting microbial growth and lipid oxidation [31]. The study in question declares the application of a relatively higher amount of ginger extracts, i.e., 6.0%, to deliver strong microbial inhibitory properties under refrigerated storage. Ginger extracts have also been reported to disrupt and extensively break muscle fibers [32]. This feature enables spice extracts to deliver higher microbicidal activities alongside meat tenderizing properties.

The complex composition of minced beef, i.e., carrying a higher amount of lipids, protein, water, and salts, makes it more resistant towards both the synthetic and natural antimicrobials. Hence, higher concentration and amount of spice extracts are desired to offer microbiological inhibitory properties in food in comparison with the microbial growth medium [10]. A study on 43 different spices used in meat-based cuisines of 36 countries concludes that the spices application as food cleanser and their utilization increases in cookeries [33]. Earlier work on spice application in meat-based products suggests palatability of cooked recipes at relatively higher doses of spices than recommended in recent study. Supplementing 10% extracts of myrtle, rosemary, lemon balm, nettle leaves, green tea, and ginger in beef patties, cooked pork meat, and stewed pork had been suggested as organoleptically acceptable [34, 35]. Variability of different spice extracts in presenting

microbicidal properties against both *E. coli* and *S. aureus* as observed in this study is associated with their varied antimicrobial property-bearing phytochemical profile and growth conditions. Findings from this recent study also suggest relatively higher amount of extract (i.e., 6.0%) supplementation as marinades in meat-based system to offer antimicrobial and other quality enhancement and preservative properties.

## 4. Conclusions

Development of natural ingredient-based blends as preservatives for the meat industry serves emerging challenges including microbiological and oxidative spoilage, pathogenicity, and risks of synthetic additive-associated toxicity. This research demonstrates culinary spices and herbs commonly used in South East Asia and Central Asia as a potential source of antibacterial compounds that might anticipate a broad range of functional and biological properties in meat and meat-based edible goods. This study defines the correlation among phenolic contents and antioxidant activity of spice extracts. However, unique antimicrobial response identified for spices bearing comparatively lower phenolic pool and antioxidant activity is expected to be an outcome of their particular chemical composition with strong bactericidal properties. The findings of this research work are suggestive of spice and herb extracts' application in beef as culinary agent up to a level of 6% to contribute as a viable strategy in preventing pathogen growth and proliferation under refrigerated storage.

## Data Availability

The dataset supporting the conclusions of this article is included within the article.

## Conflicts of Interest

The authors declare no conflicts of interest.

## References

- [1] F. Toldrá, Y. H. Hui, I. Astiasaran, J. Sebranek, and R. Talon, *Handbook of Fermented Meat and Poultry*, John Wiley & Sons, Hoboken, NJ, USA, 2014.
- [2] K. Radha Krishnan, S. Babuskin, P. A. S. Babu, M. Sivarajan, and M. Sukumar, "Evaluation and predictive modeling the effects of spice extracts on raw chicken meat stored at different temperatures," *Journal of Food Engineering*, vol. 166, pp. 29–37, 2015.
- [3] S. Sudarshan, N. Fairoze, S. W. Ruban, S. R. Badhe, and B. V. Raghunath, "Effect of aqueous extract and essential oils of ginger and garlic as decontaminant in chicken meat," *Research Journal of Poultry Sciences*, vol. 3, no. 3, pp. 58–61, 2010.
- [4] F. Lu, G. K. Kuhnle, and Q. Cheng, "The effect of common spices and meat type on the formation of heterocyclic amines and polycyclic aromatic hydrocarbons in deep-fried meatballs," *Food Control*, vol. 92, pp. 399–411, 2018.
- [5] S. Kittisakulnam, D. Saetae, and W. Suntornsuk, "Antioxidant and antibacterial activities of spices traditionally used in fermented meat products," *Journal of Food Processing and Preservation*, vol. 41, no. 4, Article ID e13004, 2017.
- [6] S. G. Dragoev, D. K. Balev, N. S. Nenov, K. P. Vassilev, and D. B. Vlahova-Vangelova, "Antioxidant capacity of essential oil spice extracts versus ground spices and addition of antioxidants in Bulgarian type dry-fermented sausages," *European Journal of Lipid Science and Technology*, vol. 118, no. 10, pp. 1450–1462, 2016.
- [7] J. García-Díez, J. Alheiro, V. Falco, M. J. Fraqueza, and L. Patarata, "Chemical characterization and antimicrobial properties of herbs and spices essential oils against pathogens and spoilage bacteria associated to dry-cured meat products," *Journal of Essential Oil Research*, vol. 29, no. 2, pp. 117–125, 2017.
- [8] B. K. Tiwari, V. P. Valdramidis, C. P. O'Donnell, K. Muthukumarappan, P. Bourke, and P. J. Cullen, "Application of natural antimicrobials for food preservation," *Journal of Agricultural and Food Chemistry*, vol. 57, no. 14, pp. 5987–6000, 2009.
- [9] M. F. Manzoor, N. Ahmad, Z. Ahmed et al., "Novel extraction techniques and pharmaceutical activities of luteolin and its derivatives," *Journal of Food Biochemistry*, vol. 43, no. 9, Article ID e12974, 2019.
- [10] L. A. Shelef, "Antimicrobial effects of spices," *Journal of Food Safety*, vol. 6, no. 1, pp. 29–44, 1984.
- [11] D. Trombetta, F. Castelli, M. G. Sarpietro et al., "Mechanisms of antibacterial action of three monoterpenes," *Antimicrobial Agents and Chemotherapy*, vol. 49, no. 6, pp. 2474–2478, 2005.
- [12] B. Shan, Y.-Z. Cai, J. D. Brooks, and H. Corke, "The in vitro antibacterial activity of dietary spice and medicinal herb extracts," *International Journal of Food Microbiology*, vol. 117, no. 1, pp. 112–119, 2007.
- [13] S. Varakumar, K. V. Umesh, and R. S. Singhal, "Enhanced extraction of oleoresin from ginger (*Zingiber officinale*) rhizome powder using enzyme-assisted three phase partitioning," *Food Chemistry*, vol. 216, pp. 27–36, 2017.
- [14] G. Latimer and W. Horwitz, *Official Methods of Analysis. Association of Official Analytical Chemists (AOAC) International*, AOAC, Rockville, MD, USA, 19th edition, 2012.
- [15] M. F. Manzoor, N. Ahmad, R. M. Aadil et al., "Impact of pulsed electric field on rheological, structural, and physico-chemical properties of almond milk," *Journal of Food Process Engineering*, vol. 42, no. 8, Article ID e13299, 2019.
- [16] M. F. Manzoor, X.-A. Zeng, A. Rahaman et al., "Combined impact of pulsed electric field and ultrasound on bioactive compounds and FT-IR analysis of almond extract," *Journal of Food Science and Technology*, vol. 56, no. 5, pp. 2355–2364, 2019.
- [17] Z. Ahmed, M. F. Manzoor, N. Begum et al., "Thermo-ultrasound-based sterilization approach for the quality improvement of wheat plantlets juice," *Processes*, vol. 7, no. 8, p. 518, 2019.
- [18] L. A. Adetunde, I. Sackey, E. O. Kombat, and N. Issah, "Antimicrobial activities of heated extracts of garlic (*Allium sativum*) and ginger (*Zingiber officinale*) on some selected pathogens," *Nature and Science*, vol. 12, no. 3, pp. 121–126, 2014.
- [19] J. Ahn, I. U. Grün, and A. Mustapha, "Antimicrobial and antioxidant activities of natural extracts in vitro and in ground beef," *Journal of Food Protection*, vol. 67, no. 1, pp. 148–155, 2004.
- [20] K. Radha Krishnan, S. Babuskin, P. A. S. Babu et al., "Antimicrobial and antioxidant effects of spice extracts on the shelf life extension of raw chicken meat," *International Journal of Food Microbiology*, vol. 171, pp. 32–40, 2014.
- [21] K. U. Pradeep, P. Geervani, and B. O. Eggum, "Common Indian spices: nutrient composition, consumption and contribution to dietary value," *Plant Foods for Human Nutrition*, vol. 44, no. 2, pp. 137–148, 1993.
- [22] S. Kumari and A. Gupta, "Nutritional composition of dehydrated ashwagandha, shatavari, and ginger root powder," *International Journal of Home Science*, vol. 2, no. 3, pp. 68–70, 2016.
- [23] T. Ismail, S. Akhtar, P. Sestili, M. Riaz, A. Ismail, and R. G. Labbe, "Antioxidant, antimicrobial and urease inhibitory activities of phenolics-rich pomegranate peel hydroalcoholic extracts," *Journal of Food Biochemistry*, vol. 40, no. 4, pp. 550–558, 2016.
- [24] M. M. Tajkarimi, S. A. Ibrahim, and D. O. Cliver, "Antimicrobial herb and spice compounds in food," *Food Control*, vol. 21, no. 9, pp. 1199–1218, 2010.
- [25] G. S. El-Baroty, H. A. El-Baky, R. S. Farag, and M. A. Saleh, "Characterization of antioxidant and antimicrobial compounds of cinnamon and ginger essential oils," *African Journal of Biochemistry Research*, vol. 4, no. 6, pp. 167–174, 2010.
- [26] B. Dash, S. Sultana, and N. Sultana, "Antibacterial activities of methanol and acetone extracts of fenugreek (*Trigonella foenum*) and coriander (*Coriandrum sativum*)," *Life Sciences and Medicine Research*, vol. 2011, pp. 1–8, 2011.
- [27] A. Bali, S. K. Das, A. Khan, D. Patra, S. Biswas, and D. Bhattachar, "A comparative study on the antioxidant and antimicrobial properties of garlic and coriander on chicken sausage," *International Journal of Meat Science*, vol. 1, no. 2, pp. 108–116, 2011.
- [28] S. Gupta and S. Ravishankar, "A comparison of the antimicrobial activity of garlic, ginger, carrot, and turmeric pastes against *Escherichia coli* O157:H7 in laboratory buffer and ground beef," *Foodborne Pathogens and Disease*, vol. 2, no. 4, pp. 330–340, 2005.



- [29] V. D. Pawar, B. D. Mule, and G. M. Machewad, "Effect of marination with ginger rhizome extract on properties of raw and cooked chevon," *Journal of Muscle Foods*, vol. 18, no. 4, pp. 349–369, 2007.
- [30] K. Islam, A. A. Rowsni, M. M. Khan, and M. S. Kabir, "Antimicrobial activity of ginger (*Zingiber officinale*) extracts against food-borne pathogenic bacteria," *International Journal of Science, Environment and Technology*, vol. 3, no. 3, pp. 867–871, 2014.
- [31] L. E. Sediek, A. M. Wafaa, D. H. Alkhalifah, and S. E. Farag, "Efficacy of ginger extract (*Zingiber officinale*) and gamma irradiation for quality and shelf-stability of processed frozen beef sausage," *Life Science Journal*, vol. 9, no. 2, pp. 448–461, 2012.
- [32] D. Ruitong, Y. Zhi, L. Yuan, L. Xingmin, and M. Lizhen, "Tenderizing and preserving yak meat by ginger extract (*Zingiber officinale* rose)," *Journal of Muscle Foods*, vol. 21, no. 4, pp. 757–768, 2010.
- [33] J. Billing and P. W. Sherman, "Antimicrobial functions of spices: why some like it hot," *The Quarterly Review of Biology*, vol. 73, no. 1, pp. 3–49, 1998.
- [34] Y. Cao, W. Gu, J. Zhang et al., "Effects of chitosan, aqueous extract of ginger, onion and garlic on quality and shelf life of stewed-pork during refrigerated storage," *Food Chemistry*, vol. 141, no. 3, pp. 1655–1660, 2013.
- [35] K. M. Wójciak, Z. J. Dolatowski, and A. Okoń, "The effect of water plant extracts addition on the oxidative stability of meat products," *Acta Scientiarum Polonorum Technologia Alimentaria*, vol. 10, no. 2, pp. 175–188, 2011.

