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Antibacterial effect of germander extract inclusion with Aloe vera gel using raw chicken meat

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ORIGINAL ARTICLE

Abstract

The combined antibacterial effect of germander extract (*Teucrium polium* L.) and Aloe vera gel against common and pathogenic foodborne bacteria was evaluated using raw meat. This study involved five treatments, including (1) Control: Aloe gel with no additive, (2) Aloe gel + 0.2 g kg^{-1} of germander extract (GRL_1), (3) Aloe gel + 0.3 g kg^{-1} of (GRL_2), (4) Aloe gel + 0.5 g kg^{-1} of (GRL_3), and (5) 14 ppm of butylated hydroxyanisole (BHA), prepared and mixed into ground chicken meat. All treatments with antimicrobial additives showed a significant ($P < 0.05$) inhibitory effect. Overall, germander extract level 3 (GRL_3) exhibited the greatest antimicrobial influence among all the additives ($P < 0.05$). However, the antimicrobial effect of GRL_2 was comparable to that of synthetic BHA during storage. In conclusion, the incorporation of germander extract at level 3 with 5% Aloe gel is recommended for use in the meat industry.

Keywords: Aloe vera; Enterobacteriaceae; *Escherichia coli*; germander; Listeria; plant extracts; Salmonella

Introduction

In the last two decades, many research studies have focused on meat safety and quality using natural additives (Efenberger-Szmechtyk *et al.*, 2021; Pinto *et al.*, 2023). These additives are applied to advanced technology and safer applications without compromising the nutritive value and quality of meat (Quinto *et al.*, 2019). Even though the effectiveness of using natural plant extracts as antimicrobial agents has been studied extensively, more research is still required to determine the right amount and method of addition so that the results are comparable to those of synthetic antimicrobial alternatives (Pinto *et al.*, 2023). One such plant extract that has not been thoroughly studied for food preservation is germander extract. This plant (*T. polium* L.) is widely grown in arid Mediterranean regions such as Jordan (Common name Ja'adeh) and has been used as a medicinal plant to treat some human ailments, such as abdominal pain, indigestion, gastrointestinal disorders, and type 2 diabetes (Al Bahtiti, 2012; Bahramikia, 2022; Jaradat, 2015). In addition, the extract possesses good antioxidant, anticancer, anti-inflammatory, and antimicrobial properties. Several polyphenolic compounds were identified in their essential oil, including as 8-cedren-13-ol (24.8%), β-caryophyllene (8.7%), sabinene (5.2%), and germacrene D (6.8%) (Aburjai *et al.*, 2006; Al Bahtiti, 2012; Bahramikia, 2022). In addition, flavonoids and several other compounds (β-pinene, α-pinene, glucose and raffinose, and sesquiterpenoid compounds) were found using different extraction techniques. These bioactive compounds are accountable for their antioxidant and antimicrobial activities (Al-Hijazeen, 2024, 2025; Bahramikia, 2022; Jaradat, 2015). The antimicrobial effect is attributed to its unique composition of several polyphenolic compounds. These bioactive compounds affect bacterial cell membranes (by depolarizing the cell wall), interact with bacterial proteins, alter membrane permeability, impact energy metabolism,

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and may inhibit DNA synthesis. Nevertheless, there are always restrictions and challenges associated with adding these plant extracts, such as the loss of viability and activity during storage (Chen *et al*., 2024; Vaou *et al*., 2021). Studies are being conducted to improve and support these additives effectively and prolong or maximize their activity by utilizing different natural combinations (Al-Hijazeen and Ibrahim, 2024; Basavegowda and Baek, 2022). In addition, adding antimicrobial additives in combination may cause prospective synergistic effects (Atta *et al*., 2023). In general, the mechanism of antimicrobial effect was always linked to the polyphenol compounds existing inside these plant extracts. Aloe vera gel is considered a very interesting natural biofilm that can help accomplish this purpose and support the activity of antimicrobial additives (Kahramanoğlu *et al*., 2019). This gel is isolated and extracted from plant leaves of Aloe vera (*Aloe Barbadensis Miller*), which is widely found in Jordan (Al Bahtiti, 2012; Al-Hijazeen and Ibrahim, 2024; Jaradat, 2015). The gel was subjected to analysis, which revealed several compounds and ingredients with antimicrobial and antioxidant effects, such as phenolic acids, enzymes, minerals, saponins, flavonoids, and vitamins (Arbab *et al*., 2021; Kahramanoğlu *et al*., 2019; Kaur and Bains, 2024; Leitgeb *et al*., 2021). The natural edible coating property of Aloe gel, which not only maintains or stabilizes antimicrobial effectiveness but also maximizes it, is a good solution to the loss of antimicrobial activity during storage. No prior research evaluated the effect of combining germander extract with Aloe vera gel as an antimicrobial agent against common bacterial strains and foodborne bacterial pathogens. Total aerobics and Enterobacteriaceae are considered general microbiology tests that could be used for this evaluation. In addition, foodborne bacteria such as *Listeria monocytogenes*, *Escherichia coli O157:H7*, and *Salmonella enterica* are considered the most challenging in meat preservation (Bantawa *et al*., 2018; FDA, 2024; Sousa *et al*., 2024), and this technique may help to delay their growth during storage. Furthermore, the bitter taste of germander can be masked by using flavor modifiers, spices, or sweetness combined with Aloe gel. The objectives of this study were (1) to estimate the antimicrobial effects of various levels of germander extract combined with Aloe gel (2) to compare this effect with the commercial alternative of butylated hydroxyanisole (BHA) used in meat processing, and (3) to select the optimal level to recommend for use in the meat industry.

Materials and Methods

Meat sample preparation

Chicken meat was obtained from the National Poultry Co., Jordan, located 90 miles south of Amman, Karak Governate-Al-Qatrana. All bird carcasses were moved to the animal production laboratory (Animal Production Department; Agriculture College at Mutah University) and received at the cooling unit. The chicken carcasses were then mixed in ice-cold water at 4°C, placed in a plastic container, and refrigerated until use. The specialists in the meat lab cut and separated all legs or thigh meat at the deboning and cleaning stage. The chicken thigh meat was cleaned of skin, visible fat, and hard connective tissues. After cleaning and vacuuming (using oxygen impermeable bags), the meat was transferred to the freezer unit at –20°C until use. At the first stage of meat preparation, the meat was thawed and ground twice using 8- and 3-mm plates (Moulinex, Model DKA-1, France), respectively. Five different meat batches were formulated, including (1) Control: Aloe gel with no additive, (2) Aloe gel + 0.2 g kg^{-1} of germander extract (GRL_1), (3) Aloe gel + 0.3 g kg^{-1} of (GRL_2), (4) Aloe gel + 0.5 g kg^{-1} of (GRL_3), and 5) 14 ppm of BHA, prepared and mixed with raw ground (thigh) meat. The ground meat patties were inoculated and stored for 7 days at 10°C, packaged in oxygen-permeable bags for measuring the total viable counts of *L. monocytogenes*, *E. coli O157:H7*, and *S. enterica.* All treatment meat batches were formulated to have the same quantity of Aloe vera gel at the 5% level. This level was selected based on several previous studies that evaluated the antioxidant and antimicrobial effects of Aloe vera gel, especially as food preservation agent (Al-Hijazeen, 2024, 2025; Azahra *et al*., 2019; Kahramanoğlu *et al*., 2019; Rajkumar *et al*., 2015; Soltanizadeh and Ghiasi-Esfahani, 2015). Aloe vera gel was obtained as previously described by Al-Hijazeen and Ibrahim (2024), in which the fresh leaves were purchased from various local farmers in southern Jordan. In the meat facility, Aloe vera gel (parenchymatous internal colorless gel) was hand-collected, washed, cleaned with cold distilled water, and placed in oxygen-impermeable bags until use. Germander extract was obtained from a local certified plant (Green-Field Factory for Oils, Amman, Jordan), where the plant leaves and stems were collected from the wild in southern Jordan. The chemical composition of the germander extract was analyzed and reported in previous research studies, as discussed. The effect of adding germander extract combined with Aloe vera gel on meat quality and its sensorial characteristics has been partially evaluated in a previous study by Al-Hijazeen (2024). The germander extract (purified and dissolved in 5% organic oil) and BHA powder were solubilized in 10 mL of pure ethanol and mixed with 50 mL of crude Aloe vera gel. The ethanol was eliminated using the rotary evaporation technique (Rotary evaporator W; Heidolph Laborota 4001-Efficient), where the system unit was programmed at 70°C and 175 mbar vacuum pressure, before mixing. The mixture was then tested, and the results showed no presence of ethanol, and therefore no potential ethanol antimicrobial effect. All inclusion treatment additives were mixed into the

ground meat using a bowl mixer for 3 min. The ground thigh meat patties (25 g each) were prepared and enveloped individually inside oxygen-permeable bags. After the inoculation, meat patties were transferred to a refrigeration unit set at 10°C (example on failure temperature control) and stored for 7–8 days. This temperature (10°C) represented an inadequate or poor refrigeration system during storage, problems during transportation, handling, and so on. Microbial counting analysis was performed at various storage times to assess the bacterial survival and growth rate. In a separate experiment, the growth of total aerobics and ENT in noninoculated thigh meat samples (prepared using the same protocol to evaluate pathogenic bacteria) stored for 5 days at 10°C was evaluated.

pH determination

The pH values were measured according to the methodology by Sebranek *et al.* (2001). The ground meat samples (1.0 g of raw meat) were immersed in 9 mL of deionized distilled water (DDW), homogenized, and measured using a pH meter (PL-600, pH/mV/Temp Meter, Taipei Taiwan), which was calibrated and checked before use.

Water activity (aw)

All treatment meat batches (thigh meat) were checked for their water activity (*aw*) using a water activity instrument (Rotronic *HP23-AW-A-SET-40*, Portable Analyzer, Wilmington, North Carolina, NC, USA). Non-inoculated meat samples were inserted into the reusable equipment cups, where they were distributed to the upper line. However, meat sample measurements were performed individually following the calibration stage.

Preparation of bacterial pathogenic cultures

The bacterial culture was prepared at the Microbiology and Food Safety Laboratory (Mutah University/ Agriculture College) using four mixed strains for each pathogen, including *L. monocytogenes*, *S. enterica*, and *E. coli* O157:H7. The bacterial frozen stock, stored in 10% glycerol at –80°C, was transferred after thawing to a tryptic soy broth (TSB; *Sigma Aldrich*; T8907-500 g; General Purpose Powder) enriched with 0.6% yeast extract (TSBYE) at 35°C for 2 days. After two consecutive 24-h transfers of each culture using TSBYE (35°C) in sterilized tubes, the stock culture was prepared. This transfer will prepare healthier bacterial cells, which give homogenous culture strains. Each stock culture was grown separately in 10 mL of TSB enriched with 0.6% yeast extract (TSBYE- Biolab Zrt. 1141 *Budapest* Öv u. Headquarters No 43, *Hungary*) at 35°C for 24 h before meat inoculation. Different amounts of Nalidixic acid were gradually added to the TSBYE, which allowed the bacterial strain to adapt (NA; Antibiotic; M.W 232.24, 10 ug/mL, 25 ug/mL, and 50 ug/mL; *Santa Cruz Biotechnology*, Dallas, Texas State, USA). This adaptation process gives the bacterial pathogenic strains the ability to grow on the selective media containing Nalidixic acid at a level 50 µg/mL. In addition, it will differentiate the inoculated bacterial cells that can survive using this concentration. Aseptically, the specialist made a double (24 h) transfer of each Naldixic acid-resistant strain (NAR) culture. A mixed bacterial strain culture (NAR) was harvested and resuspended (in fresh saline) at 10^6 CFU/mL before meat inoculation according to the method of Al-Hijazeen (2018).

Inoculation of prepared raw meat

For each study, ground meat patties prepared from different treatments were inoculated separately with a mixture of 1) four serotypes of *Salmonella enterica* resistant to nalidixic-acid (NAR) (*S. typhimurium*, *S. newport*, *S. kentucky*, *S. oranienburg*), 2) a 4-strain mixture of *E. coli* O157:H7 (NAR) (ATCC 43894, ATCC 35150, ATCC 43895, WS 3062), and 3) a 4-strain mixture of *L. monocytogenes* (NAR) (H7596, Scott A, H7762, and H7962; all serotype 4b) for a starting cell concentration of approximately 10^4 colony forming units (CFU)/g for each pathogen. The final cell concentration in the ground chicken meat was evaluated in a primary study. The procedure of preparing these strains and their cell concentrations was well discussed by Al-Hijazeen (2018). In each pathogenic experiment, all inoculated meat samples were massaged for approximately 30 s from the outside of the bags to homogenize the bacterial colonies. Finally, the inoculated meat patties were stored at 10°C, and then the total apparent count was measured at different times.

Bacterial analysis and counting

All bacterial analyses, including meat sampling, meat homogenization using sterile stomacher bags, serial dilutions, and surface plating on the appropriate agar for the enumeration of pathogenic bacteria, were performed at the microbiology and food safety laboratory. Different selective agar media were prepared, including xylose lysine deoxycholate (XLD), sorbitol MacConkey agar (SMA), and modified Oxford agar (MOX), and were used to enumerate *S. enterica*, *E. coli* O157:H7, and *L. monocytogenes*, respectively. Finally, incubation (35°C) and bacterial colony counting (48 h) were performed according to the description and methodology of Al-Hijazeen (2018, 2022). Finally, using tryptic soy agar (TSA), the total aerobic count was evaluated by surface plating of

0.1 mL of the meat homogenate. The inoculated TSA was placed aseptically in the incubator set at 35°C for 48 h. The ENT viable count was evaluated using the agar overlay technique. Violet red bile agar (VRBA) poured over solidified TSA media was prepared according to the protocol described by Al-Hijazeen (2018), and the plates were incubated at 35°C for 24 h.

Statistical analysis

In these microbiological studies, a completely randomized design (CRD) was applied. For each independent experiment, two different samples were analyzed over two replications for each treatment. A general linear model (Proc. GLM, SAS program, version 9.3, SAS, 2012) was used for statistical analysis. Standard error of the means (SEM) and mean values were provided. The significance was defined at $P < 0.05$, and Tukey's test or Tukey's multiple comparison test was used to determine the presence of significant variations between mean values.

Results and Discussion

Water activity and pH value

The water content and acidity of raw meat are key determinants of the bacterial growth rate and survival. In the current study, there were no considerable variations ($P > 0.05$) among all treatments regarding meat acidity and its water activity (Table 1).

Similar results were obtained when testing chicken thigh meat mixed with plant essential oils such as oregano and rosemary (Al-Hijazeen, 2018 and 2022). In addition, adding Aloe vera gel to all treatments using the same level (5%) did not affect this univariate situation; however, it had been reported that Aloe gel could reduce the pH value of the meat system (Rajkumar *et al*., 2015; Soltanizadeh and Ghiasi-Esfahani, 2015). Furthermore, Soltanizadeh and Ghiasi-Esfahani (2015) concluded that adding Aloe vera gel at a level of 3–5% to low-meat beef burgers enhanced their water absorption and textural quality. So, all meat batches used for microbial analysis had the same initial water activity and pH values at the start of bacterial inoculation. This indicated that any significant differences in the bacterial growth response were due to the treatment effect alone.

Table 1. The water activity and acidity of the tested raw meat samples.

Treatments	Water activity (wa)[1]	(pH)[2]
Control	0.95[a]	5.55[a]
GRL_1	0.95[a]	5.57[a]
GRL_2	0.96[a]	5.57[a]
GRL_3	0.95[a]	5.58[a]
BHA	0.96[a]	5.56[a]
SEM	0.002	0.045

Treatments: Control; GRL_1; GRL_2; GRL_3; BHA: *butylated hydroxyanisole*.
[1](w_a): Water activity of raw meat samples.
[2](pH): Ultimate pH values measured after 24 h of slaughtering.

Total aerobics and Enterobacteriaceae counts

The total aerobic plate count (TAPC) is a general microbiology test used to show the degree of food safety in processing plants and their hygienic instructions. However, it just indicates the total bacterial count (without information regarding bacterial type or strains) grown on the different types of food. In addition, the total viable count of Enterobacteriaceae (ENT) gives general data regarding a large family of gram-negative bacteria (facultative anaerobes), which usually live inside the intestines (gut microbiota) of humans or animals. Thus, the presence of these bacteria indicates a high risk of contamination during animal slaughtering, food handling, and weak prevention treatments. In the current study, both the TAPC and ENT tests showed no significant differences among treatments on the first day of the experiment (Tables 2 and 3). Briefly, the addition of germander extract at level one (GRL_1) was very weak against total aerobics and ENT during storage time. In addition, the

Table 2. Total aerobic counts of ground thigh meat during storage at 10°C.

Treatments	0 day	3 days	5 days	SEM
	Log CFU/g meat			
Total aerobic				
Control	4.28^{az}	7.32^{ay}	9.73^{ax}	0.032
GRL_1	4.30^{az}	7.11^{ay}	9.25^{bx}	0.090
GRL_2	4.29^{az}	6.64^{by}	8.37^{cx}	0.033
GRL_3	4.29^{az}	5.39^{cy}	7.57^{dx}	0.068
BHA	4.30^{az}	6.60^{by}	8.29^{cx}	0.030
SEM	0.038	0.067	0.059	

[a–d]Different superscripts within a column indicate significant differences ($P < 0.05$).
[x–z]Different superscripts within a row indicate significant differences ($P < 0.05$).
Treatments: Control; GRL_1; GRL_2; GRL_3; BHA: *butylated hydroxyanisole*.
[x–z]Different superscripts within a row indicate significant differences ($P < 0.05$).
Treatments: Control; GRL_1; GRL_2; GRL_3; BHA: *butylated hydroxyanisole*.

Table 3. Number of viable *Enterobacteriaceae* in ground thigh meat stored at 10°C.

Treatments	0 day	3 days	5 days	SEM
	Log CFU/g meat			
Enterobacteriaceae				
Control	4.23^{az}	6.61^{ay}	7.52^{ax}	0.033
GRL_1	4.24^{az}	5.34^{by}	7.08^{bx}	0.098
GRL_2	4.24^{az}	5.08^{bcy}	6.28^{cx}	0.026
GRL_3	4.19^{az}	4.80^{cy}	5.58^{dx}	0.050
BHA	4.20^{az}	5.24^{by}	6.14^{cx}	0.042
SEM	0.028	0.074	0.057	

$^{a–d}$Different superscripts within a column indicate significant differences (P < 0.05).

effect of adding GRL_2 was comparable to that of using the synthetic BHA. However, GRL_3 showed the highest significant (P < 0.05) antibacterial effect regarding both TAPC and ENT final viable counts. These results aligned with the previous research findings, which focused on the antimicrobial effect of germander extract (Arbab *et al*., 2012; Bantawa *et al*., 2018; Kaur and Bains, 2024; Leitgeb *et al*., 2021). In addition, adding this plant extract in combination with Aloe gel seems to improve its antimicrobial properties. The antibacterial mechanism of germander extract (inclusion with Aloe gel) is generally linked to the presence of several polyphenol compounds existing inside natural plant additives (Aburjai *et al*., 2006; Bahramikia, 2022; Chen *et al*., 2024; Jaradat, 2015; Vaou *et al*., 2021). Different explanations were reported in previous research studies, such as its effect on bacterial membrane structure, permeability, internal biosynthesis, DNA synthesis and regulation, and internal enzymes and metabolism (Chen *et al*., 2024; Vaou *et al*., 2021).

Number of viable *Escherichia coli* O157:H7

Escherichia coli O157:H7 is considered a critical bacterial pathogen that can cause human illness after ingestion from contaminated foods (FDA, 2024; Takó *et al*., 2020). On Day 0 of storage, no considerable differences (P > 0.05) were found between any of the treatments based on the total viable counts of *E. coli* (Table 2).

On the second day, most treatment additives (GRL_2, GRL_3, and BHA) showed a significant anti-E. *coli* effect compared to the control meat samples. The antibacterial effect of plant-derived extracts against E. *coli* in food systems has been reported previously (Al-Hijazeen, 2018; Beya *et al*., 2021; Takó *et al*., 2020). Germander extract (GRE) and its essential oils also inhibited various foodborne pathogens (Alreshidi *et al*., 2020; Jaradat, 2015; Pinto *et al*., 2023). In addition, several research studies found that GRE has significant inhibitory effects, including retarding the *E. coli* growth rate in different microbial media (Alreshidi *et al*., 2020; Chioibas *et al*., 2019; Mandura *et al*., 2024). This antimicrobial effect was attributed to its polyphenolic compounds, which affect the cell membranes and internal biological activity of bacteria and thus their survival (Chen *et al*., 2024; Mandura *et al*., 2024; Pinto *et al*., 2023). *Escherichia coli* is a gram-negative bacterium with rod-shaped and facultative anaerobic properties (Lim *et al*., 2010), which increase its resistance to antimicrobial additives. However, in the current study, GRL_2 and GRL_3 exhibited the greatest (P < 0.05) anti-*E. coli* effect on Day 5. In addition, the antibacterial effect of GRL_1 was very weak on Day 5 compared to that of the other additives. The antimicrobial effect of synthetic BHA was still low at this point compared to that of GRE inclusion with Aloe vera gel. The antibacterial inhibitory effect was demonstrated on the last day of storage, when the control and GRL_1 differed significantly (P < 0.05) in the number of *E. coli*. The most intriguing result of this experiment was the effect of GRL_3, which was greater than that of all other additives. The presence of GRE incorporated with protective Aloe gel will also improve its antimicrobial activity during storage. This "hurdle technology," which tends to decrease the synthetic antibacterial additives, was successfully achieved in this combination with Aloe gel (Pinto *et al*., 2023). It also showed that the inclusion of BHA with Aloe vera gel had a smaller but still significant effect than the *T. polium* extract (GRE) on Day 7.

Number of viable *Listeria monocytogenes*

Listeria monocytogenes (a gram-positive bacterium) contamination in deli foods and prepared meats is still challenging, with several outbreaks reported (CDC, 2024a). In addition, Listera infection is rare but serious in contaminated foods, and it represents the third most common cause of death from foodborne illness in the United States (CDC, 2024b). The ability of this bacterium to survive in low temperatures, over a wide pH range, and in salty foods (low water activity) increases the risk and prolongs its survival (Matle *et al*., 2020; Osek *et al*., 2022). Thus, worldwide, many meat processing companies frequently conduct *L. monocytogenes* detection tests in their microbiology laboratories. The data analysis showed that no significant variations (P > 0.05) were observed between treatment additives regarding the total viable counts of Listeria on Day 0 of storage (Table 3). In addition, adding GRL_2, GRL_3, and BHA reduced the total *L. monocytogenes* count more efficiently on Day 2 compared to the other treatments.

Similarly, the Listeria growth response was affected by the treatment additive on Day 5 of storage, and GRL_3 was clearly superior. The storage period from Day 6–8 also proved that adding GRL_3 with 5% Aloe gel achieved the greatest considerable ($P < 0.05$) anti-Listeria effect compared to the other additives. However, the GRL_2 and BHA were comparable during the storage period. The experiment revealed that GRL_1 had a very weak antibacterial effect, and it could not be used as a natural alternative at this level. This was mostly due to the low dose of GRE that was used. The antibacterial mechanism of this oil would be similar to that discussed previously regarding plant-derived polyphenols (Pinto *et al*., 2023). These phenolic acids and flavonoids may synergistically inactivate *L. monocytogenes* via different mechanisms, affect bacterial cell membranes, and prevent surface adhesion to food (Zamuz *et al*., 2021). However, further investigation is required to understand the inhibitory effect of GRE combined with Aloe vera gel. Based on current data, there was no clear evidence of a synergistic effect between GR and Aloe gel; however, it can be reported that adding Aloe gel enhances the antimicrobial effect during storage.

Number of viable *Salmonella enterica*

Very little research has investigated the effect of combining germander extract on the survival and growth of foodborne pathogens, which consisted mainly of in vitro studies (Chioibas *et al*., 2019; Sailović *et al*., 2024; Yazdi *et al*., 2013; Zeidvand *et al*., 2024). For example, the disc diffusion method showed a potential antibacterial effect of methanol extracts of *T. polium* aerial parts against several strains of gram-negative and gram-positive bacteria. Usually, gram-negative bacteria such as *S. enterica* have greater resistance to antibacterial compounds due to differences in their cell structure compared to gram-positive bacteria (Alreshidi *et al*., 2020). As anticipated, on the first Day (0) of storage, there were no significant ($P > 0.05$) differences among treatments (Table 4).

On the other hand, the total viable counts of *S. enterica* were significantly reduced using GRL_3 and BHA compared to the other treatment additives after 2 days of refrigerated storage. The effect of adding GRL_1 was very weak on Day 2, and no significant ($P > 0.05$) differences were observed compared to the control treatment. On Day 5 of storage, GRL_3 and BHA maintained higher effectiveness than the other treatment additives. It was also clear that the antimicrobial activity of GRE increased considerably with the increase in the oil concentration

Table 4. Total colony counts of *Escherichia coli* O157:H7 in raw chicken thigh meat during storage at 10°C.

Treatments	0 day	2 days	5 days	7 days	SEM
	Log CFU/g meat				
Escherichia coli					
Control	3.82^{az}	5.44^{ay}	6.78^{ax}	8.79^{aw}	0.027
GRL_1	3.85^{az}	5.31^{ay}	6.43^{abx}	8.36^{bw}	0.081
GRL_2	3.78^{az}	4.56^{by}	6.13^{bcx}	7.37^{dw}	0.130
GRL_3	3.84^{az}	4.24^{by}	5.87^{cx}	6.82^{ew}	0.057
BHA	3.83^{az}	4.60^{by}	6.40^{abx}	7.54^{cw}	0.083
SEM	0.047	0.114	0.105	0.030	

$^{a–e}$Different letters within a column indicate significant differences ($P < 0.05$).
$^{x–z}$Different letters within a row indicate significant differences ($P < 0.05$).
Treatments: Control; GRL_1; GRL_2; GRL_3; BHA: *butylated hydroxyanisole*.

Table 5. Total colony counts *of Listeria monocytogenes* in raw chicken thigh meat during storage at 10°C.

Treatments	0 days	2 days	4 days	6 days	8 days	SEM
	Log CFU/g meat					
Listeria monocytogenes						
Control	4.28^{az}	5.23^{ay}	6.39^{ax}	8.23^{aw}	8.86^{av}	0.042
GRL_1	4.25^{az}	4.92^{by}	5.69^{bx}	7.89^{bw}	8.50^{bv}	0.046
GRL_2	4.26^{az}	4.63^{cy}	5.45^{bcx}	6.55^{cw}	7.18^{cv}	0.055
GRL_3	4.28^{az}	4.41^{dz}	4.78^{dy}	6.08^{ex}	6.54^{dw}	0.080
BHA	4.26^{az}	4.58^{cdy}	5.37^{cx}	6.34^{dw}	6.97^{cv}	0.038
SEM	0.059	0.048	0.068	0.041	0.052	

$^{a–e}$Different letters within a column indicate significant differences ($P < 0.05$), n = 4.
$^{x–z}$Different letters within a row indicate significant differences ($P < 0.05$).
Treatments: Control; GRL_1; GRL_2; GRL_3; BHA: *butylated hydroxyanisole*.

Table 6. Total colony counts *of Salmonella enterica* in raw chicken thigh meat during storage at 10°C.

Treatments	0 days	2 days	5 days	7 days	SEM
	Log CFU/g meat				
Salmonella enterica					
Control	3.95^{az}	5.57^{ay}	6.88^{ax}	8.35^{aw}	0.050
GRL_1	3.95^{az}	5.32^{aby}	6.54^{bx}	8.01^{bw}	0.023
GRL_2	3.93^{az}	5.10^{bcy}	6.15^{cx}	7.23^{cw}	0.061
GRL_3	3.94^{az}	4.67^{dy}	5.70^{dx}	6.81^{dw}	0.074
BHA	3.93^{az}	4.84^{cdy}	5.90^{dx}	6.94^{dw}	0.045
SEM	0.031	0.077	0.056	0.037	

$^{a–e}$Different letters within a column differ significantly ($P < 0.05$), n = 4. $^{x–z}$Different letters within a row differ significantly ($P < 0.05$). Treatments: Control; GRL_1; GRL_2; GRL_3; BHA: *butylated hydroxyanisole*.

(Mandura *et al*., 2024; Sailović *et al*., 2024; Zeidvand *et al*., 2024). On the last day of storage, GRL_3 exhibited the highest inhibitory effect of all the additives. The antibacterial activity of GRL_3 was also comparable to that of the synthetic BHA currently used. Finally, GRL_1 was observed as the least significant ($P < 0.05$) anti-Salmonella additive among the other treatments. The comparable effect of GRL_3 to that of synthetic BHA is attributed to the interaction (synergistic/additive effect) of the germander constituent with Aloe vera gel components. The polyphenols, terpenoids, enzymes, flavonoids, alkaloids, and other antioxidant components in this mixture worked effectively on the Salmonella cell structure and its biological activity. Furthermore, the mechanism of the inhibitory effect of these polyphenols is already discussed in the current study.

Conclusion

The novel discovery of the current study was the superior antimicrobial effect of germander extract at level 3, which was improved by the inclusion of Aloe vera gel compared to synthetic BHA. Overall, GRL_2 and GRL_3 showed the most impressive effects as natural replacements. However, the effect of GRL_1 was very weak for the general bacteria (TAPC and ENT) and all pathogens tested in this experiment. In addition, the effects of GRL_2 and BHA were comparable. However, the difference in the bacterial response is mostly due to the variation in their cell structures. Finally, we recommend that the GRL_3 had the highest antibacterial effect considering TAPC, ENT, and all tested pathogens. This GRL_3 could be an excellent natural alternative to synthetic BHA, especially with the enhancement effect of Aloe vera gel. Since there was no germander extract group, it is hard to determine whether the effect was synergistic or additive. However, more research is needed to determine the suitable industrial level regarding meat quality and its sensorial characteristics.

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Author Contribution

Al-Hijazeen Marwan: The corresponding author wrote all the manuscript parts, supervised work in the meat lab, designed the experiment, analyzed all statistical data, edited and made all the corrections, reviewed the process, and prepared the published final proof.

Conflict of Interest

The author has no relevant financial or non-financial interests in the current study to disclose.

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