In Vitro Antibacterial Activity of Plant Extracts and Their Complexes Against Chicken-Derived Pathogenic Bacteria: Postprint

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Abstract

This study selected 10 plant extracts with strong antibacterial activity to investigate their in vitro antibacterial effects against chicken-derived pathogenic bacteria (avian Escherichia coli and avian Salmonella), and to screen three plant extracts with strong antibacterial activity against each pathogen, which were then combined at different ratios to obtain composite plant extracts with enhanced antibacterial activity. The study consisted of two experiments. Experiment 1: A total of 11 groups were established, including thyme essential oil, oregano essential oil, cinnamon essential oil, garlic oil, Scutellaria baicalensis flavonoids, bamboo leaf flavonoids, Astragalus polysaccharides, berberine, matrine, chlorogenic acid, and a chlortetracycline group (positive control), with six replicates per group. The Oxford cup method was employed to determine the antibacterial activity of the plant extracts against E. coli and Salmonella. Experiment 2: Based on the results of Experiment 1, three plant extracts with superior antibacterial activity against E. coli—cinnamon essential oil, matrine, and chlorogenic acid—were selected and combined pairwise or as a three-extract mixture at different concentration ratios. The Oxford cup method was used to determine the antibacterial activity of the composites against E. coli, with control groups of the three single plant extracts at corresponding concentrations established, totaling 20 groups with six replicates per group. Three plant extracts with superior antibacterial activity against Salmonella—bamboo leaf flavonoids, Scutellaria baicalensis flavonoids, and cinnamon essential oil—were selected, and the Oxford cup method was used to determine the antibacterial activity of composites at different concentration ratios and single plant extracts at corresponding concentrations (control groups) against Salmonella, totaling 17 groups with six replicates per group. Based on these results, the antibacterial effects of bamboo leaf flavonoids and cinnamon essential oil combined at different concentration ratios against Salmonella were investigated to screen for the optimal combination ratio, with 15 groups and six replicates per group. The results

demonstrated that five plant extracts exhibited antibacterial activity against E. coli, with the antibacterial activity in descending order being cinnamon essential oil, matrine, chlorogenic acid, thyme essential oil, and oregano essential oil. Eight plant extracts exhibited antibacterial activity against Salmonella, with the antibacterial activity in descending order being bamboo leaf flavonoids, Scutellaria baicalensis flavonoids, cinnamon essential oil, matrine, chlorogenic acid, oregano essential oil, thyme essential oil, and garlic oil. Compared with the cinnamon essential oil control group, pairwise or three-extract combinations of cinnamon essential oil, matrine, and chlorogenic acid could not significantly enhance the antibacterial activity against E. coli ($P > 0.05$). The combination of bamboo leaf flavonoids and cinnamon essential oil significantly enhanced the antibacterial activity against Salmonella, with the maximum antibacterial effect achieved at a concentration ratio of 25:150 (1:6) ($P < 0.05$). In conclusion, among the 10 plant extracts, the three with superior antibacterial activity against E. coli were cinnamon essential oil, matrine, and chlorogenic acid, but the antibacterial activity of the composite of these three plant extracts was not greater than that of cinnamon essential oil alone. The three plant extracts with superior antibacterial activity against Salmonella were bamboo leaf flavonoids, Scutellaria baicalensis flavonoids, and cinnamon essential oil. The combination of bamboo leaf flavonoids and cinnamon essential oil enhanced the antibacterial effect, and the antibacterial activity against Salmonella was maximal at a combination ratio of 1:6.

Full Text

Bacteriostatic Effects of Plant Extracts and Their Compounds on Chicken-Derived Pathogenic Bacteria In Vitro

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Abstract

This study investigated the in vitro bacteriostatic effects of ten plant extracts with reported antimicrobial activity against chicken-derived pathogenic bacteria (*Escherichia coli* and *Salmonella*), and evaluated compounded formulations to identify synergistic combinations. The research comprised two experiments. Experiment 1 included eleven groups: thyme oil, oregano essential oil, cinnamon essential oil, garlic oil, skullcap flavonoids, bamboo leaf flavonoids, astragalus polysaccharides, berberine, matrine, chlorogenic acid, and aureomycin (positive control), with six replicates per group. The Oxford cup method was used to determine inhibition zones.

Experiment 2 selected the three most effective extracts against each pathogen

for combination studies. For *E. coli*, cinnamon essential oil, matrine, and chlorogenic acid were combined at various ratios; for *Salmonella*, bamboo leaf flavonoids, skullcap flavonoids, and cinnamon essential oil were tested similarly. All combinations were evaluated against single-extract controls using the Oxford cup method, with 20 and 17 treatment groups respectively (six replicates each). Additional optimization focused on bamboo leaf flavonoids combined with cinnamon essential oil at 15 different ratios.

Results showed that five extracts inhibited *E. coli*, with efficacy decreasing in order: cinnamon essential oil > matrine > chlorogenic acid > thyme oil > oregano oil. Eight extracts inhibited *Salmonella*, with efficacy decreasing in order: bamboo leaf flavonoids > skullcap flavonoids > cinnamon essential oil > matrine > chlorogenic acid > oregano oil > thyme oil > garlic oil. Compounds of cinnamon essential oil, matrine, and chlorogenic acid did not significantly enhance anti-*E. coli* activity compared to cinnamon essential oil alone ($P > 0.05$). However, bamboo leaf flavonoids combined with cinnamon essential oil significantly improved anti-*Salmonella* activity ($P < 0.05$), with maximal effect at a 25:150 (1:6) concentration ratio. In conclusion, while cinnamon essential oil, matrine, and chlorogenic acid were most effective against *E. coli*, their combinations were not superior to cinnamon essential oil alone. For *Salmonella*, bamboo leaf flavonoids, skullcap flavonoids, and cinnamon essential oil were most effective, with the bamboo leaf flavonoids-cinnamon essential oil combination (1:6) showing the strongest synergistic bacteriostatic effect.

Keywords: plant extracts; *Escherichia coli*; *Salmonella*; bacteriostatic effects

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Introduction

Bacterial diseases account for 30–40% of chicken illnesses, with increasing incidence. Among these, colibacillosis and salmonellosis are the most severe, causing high morbidity and mortality that significantly impact the poultry industry [1]. Current control relies heavily on antibiotics, but long-term, large-scale antibiotic use has led to growing concerns about drug resistance and food safety. Therefore, developing feed additives with high antimicrobial activity, low toxicity, minimal residues, and broad-spectrum effects is urgently needed to control and prevent polymicrobial infections in broilers.

Plant extracts are active components extracted from plants using physical, chemical, or biological methods. They offer stable composition, defined components, and measurable content with minimal toxic side effects for animals and humans. Plant extracts possess antimicrobial and antioxidant functions that improve intestinal health and immune function. Their safety, efficacy, stability, and controllability have attracted widespread attention in animal production [2]. Commonly studied natural plant extracts include saponins, alkaloids, polysaccharides, tea polyphenols, flavonoids, and volatile oils (essential oils) [3]. Approved feed additives in China include saccharicterpenin, alfalfa extract, eucommia leaf

extract, soy isoflavones, and perilla seed extract. Beyond immunomodulation, plant extracts can directly affect pathogenic microorganisms. Studies show that plant volatile oils, flavonoids, and polysaccharides can inhibit chicken-derived pathogens in vitro [4–11]. Reported plant extracts with strong antimicrobial activity include thyme oil, oregano oil, cinnamon essential oil, garlic oil, skullcap flavonoids, bamboo leaf flavonoids, astragalus polysaccharides, berberine, and matrine.

While many studies report on individual plant extracts against various pathogens, inconsistent experimental conditions, extract sources, bacterial strains, and methodologies yield varying results. Systematic research on plant extracts against chicken-derived pathogens is limited, and studies on compounded plant extracts are particularly scarce. This study selected ten plant extracts with reported strong in vivo and in vitro antimicrobial activity to evaluate their effects against chicken-derived *E. coli* and *Salmonella*, identify the three most effective extracts for each pathogen, and develop superior compounded formulations. This work provides a scientific basis for subsequent in vivo efficacy trials and development of antibiotic alternatives to inhibit pathogen growth, improve immunity, and prevent disease in chickens.

1.1 Experimental Materials

Plant extracts: Thyme oil (100% purity) from Shanghai Huien International Trade Co., Ltd.; oregano oil (100% purity) from Ji' an Shenghai Natural Plant Oil Co., Ltd.; cinnamon essential oil (100% purity) from Ji'an Shenghai Natural Plant Oil Co., Ltd.; garlic oil (100% purity) from Shanghai Shuangyuan Garlic Oil Co., Ltd.; skullcap flavonoids (80% purity) from Shaanxi Fusen Biotechnology Co., Ltd.; bamboo leaf flavonoids (40% purity) from Zhejiang Shengshi Biotechnology Co., Ltd.; astragalus polysaccharides (50% purity) from Chengdu Jintaihe Medical Chemistry Technology Co., Ltd.; berberine (97% purity) from Shaanxi Senfu Biotechnology Co., Ltd.; matrine (98% purity) from Shaanxi Fusen Biotechnology Co., Ltd.; chlorogenic acid (60% purity) from Shandong Hebentang Biotechnology Co., Ltd.

Bacterial strains: Chicken *E. coli* (CVCC 1569) and *Salmonella pullorum* (CVCC 79301) were purchased from the China Veterinary Culture Collection Center.

Reagents: Peptone (biochemical reagent, Beijing Aoboxing Biotechnology Co., Ltd.), beef extract (biochemical reagent, Beijing Aoboxing Biotechnology Co., Ltd.), agar powder (biochemical reagent, Beijing Aoboxing Biotechnology Co., Ltd.), sodium chloride (analytical pure, Sinopharm Chemical Reagent Co., Ltd.), Mueller-Hinton (MH) medium (biochemical reagent, Beijing Aoboxing Biotechnology Co., Ltd.), and aureomycin hydrochloride [USP grade, Shanghai Aladdin Biochemical Technology Co., Ltd.].

Instruments: YT-CJ-1N clean bench (Beijing Yatai Kelong Instrument Technology Co., Ltd.), water-jacketed electric constant temperature incubator (Shanghai Yuejin Medical Instrument Factory), and vertical pressure steam sterilizer (Shanghai Boxun Industrial Co., Ltd.).

1.2.1 Bacteriostatic Effects of Plant Extracts on *E. coli* and *Salmonella*

Eleven groups were established: thyme oil, oregano essential oil, cinnamon essential oil, garlic oil, skullcap flavonoids, bamboo leaf flavonoids, astragalus polysaccharides, berberine, matrine, chlorogenic acid, and aureomycin (positive control), with six replicates per group. Extract concentrations were determined based on solubility and concentration-dependent antibacterial activity.

The Oxford cup method was used to measure inhibition zone diameters [7]. Bacterial suspensions were diluted to 10^6 CFU/mL with sterile saline, and 200 µL was evenly spread on MH agar plates. Oxford cups were placed vertically on the medium (four per 9 cm plate) and pressed gently to ensure contact. Three cups received 100 µL plant extract solution (technical replicates), while one received saline as a blank control. Plates were incubated at 37 °C for 24 h, and inhibition zone diameters were measured with vernier calipers.

1.2.2 Bacteriostatic Effects of Plant Extract Compounds on *E. coli* and *Salmonella*

Compounds against *E. coli*: A single-factor completely randomized design was employed. Based on individual extract efficacy, cinnamon essential oil, matrine, and chlorogenic acid were selected for combination studies. Since cinnamon essential oil showed significantly superior activity against *E. coli*, its concentration was fixed at 25 mg/mL (effective yet low concentration). Matrine and chlorogenic acid concentrations were set based on solubility (matrine saturation: 50 mg/mL; chlorogenic acid saturation: 200 mg/mL) and concentration-dependent effects (Table 1).

Treatment groups included: cinnamon essential oil (25 mg/mL), matrine (25 and 50 mg/mL), and chlorogenic acid (50, 100, and 200 mg/mL) as controls. Compound ratios were: cinnamon:matrine at 1:1 (25:25) and 1:2 (25:50); cinnamon:chlorogenic acid at 1:1 (25:25), 1:2 (25:50), 1:4 (25:100), and 1:8 (25:200); and cinnamon:matrine:chlorogenic acid at 1:1:1 (25:25:25), 1:1:2 (25:25:50), 1:1:4 (25:25:100), 1:1:8 (25:25:200), 1:2:1 (25:50:25), 1:2:2 (25:50:50), 1:2:4 (25:50:100), and 1:2:8 (25:50:200). Twenty groups total, six replicates each.

Compounds against *Salmonella*: A similar design was used. Bamboo leaf flavonoids, skullcap flavonoids, and cinnamon essential oil were selected. Bamboo leaf flavonoids showed superior activity, so its concentration was fixed at 25

mg/mL. Other concentrations were based on skullcap flavonoids solubility (50 mg/mL saturation) and dose-response effects (Table 2).

Control groups: bamboo leaf flavonoids (25 mg/mL), skullcap flavonoids (25 and 50 mg/mL), and cinnamon essential oil (25, 50, and 100 mg/mL). Compound ratios: bamboo leaf:skullcap at 1:1 (25:25) and 1:2 (25:50); bamboo leaf:cinnamon at 1:1 (25:25), 1:2 (25:50), and 1:4 (25:100); and bamboo leaf:skullcap:cinnamon at 1:1:1 (25:25:25), 1:1:2 (25:25:50), 1:1:4 (25:25:100), 1:2:1 (25:50:25), 1:2:2 (25:50:50), and 1:2:4 (25:50:100). Seventeen groups total, six replicates each.

Additional optimization of bamboo leaf flavonoids and cinnamon essential oil evaluated ratios from 1:4 to 1:10. Controls included bamboo leaf flavonoids (25 mg/mL) and cinnamon essential oil (100, 125, 150, 175, 200, 225, and 250 mg/mL). Compound ratios: 1:4 (25:100), 1:5 (25:125), 1:6 (25:150), 1:7 (25:175), 1:8 (25:200), 1:9 (25:225), and 1:10 (25:250). Fifteen groups total, six replicates each.

1.2.3 Preparation of Plant Extract and Antibiotic Solutions

Based on active ingredient content, extracts and aureomycin hydrochloride were diluted with Tween-80 or sterile water. Thyme oil [12], oregano oil [13], cinnamon oil [14], and garlic oil were diluted with 2% Tween-80. Skullcap flavonoids [15], bamboo leaf flavonoids [16], astragalus polysaccharides [17], berberine [18], chlorogenic acid [19], matrine [20], and aureomycin hydrochloride were diluted with sterile water.

1.2.4 Culture Medium Preparation

Mueller-Hinton (MH) medium was used for antimicrobial susceptibility testing [21–22]. Nutrient broth and nutrient agar were used for bacterial culture and enumeration [23].

1.2.5 Preparation of Test Bacterial Suspensions

Bacterial suspensions were prepared following the method of Cheng et al. [4].

1.2.6 Statistical Analysis

Data were analyzed using SAS 9.0 software. Analysis of variance was performed using the General Linear Model (GLM) procedure. Significant differences among means were determined using the Least Significant Difference (LSD) test. $P < 0.05$ was considered statistically significant.

Results

Table 1 shows inhibition zone diameters of ten plant extracts against *E. coli*. Cinnamon essential oil, matrine, chlorogenic acid, thyme oil, and oregano oil exhibited antibacterial activity, with zone diameters increasing with concentration. Garlic oil, bamboo leaf flavonoids, skullcap flavonoids, berberine, and astragalus polysaccharides showed no activity. At equivalent concentrations, efficacy ranked: cinnamon essential oil > matrine > chlorogenic acid > thyme oil > oregano oil.

Table 1. Inhibition zone diameters of plant extracts on *Escherichia coli* (mm)

Items	Different concentrations (mg/mL)
Thyme oil	[data]
Oregano essential oil	[data]
Chlorogenic acid	[data]
Cinnamon essential oil	[data]
Matrine	[data]
Aureomycin	[data]

No inhibition zones were observed for garlic oil, bamboo leaf flavonoids, skullcap flavonoids, berberine, or astragalus polysaccharides.

Table 2 shows inhibition zones against *Salmonella*. Bamboo leaf flavonoids, skullcap flavonoids, cinnamon essential oil, matrine, chlorogenic acid, oregano oil, thyme oil, and garlic oil showed activity, with zones increasing with concentration. Berberine and astragalus polysaccharides were inactive. Efficacy ranked: bamboo leaf flavonoids > skullcap flavonoids > cinnamon essential oil > matrine > chlorogenic acid > oregano oil > thyme oil > garlic oil.

Table 2. Inhibition zone diameters of plant extracts on *Salmonella* (mm)

Items	Different concentrations (mg/mL)
Thyme oil	[data]
Oregano essential oil	[data]
Garlic oil	[data]
Chlorogenic acid	[data]
Cinnamon essential oil	[data]
Skullcap flavonoids	[data]
Matrine	[data]
Bamboo leaf flavonoids	[data]

Items	Different concentrations (mg/mL)
Aureomycin	[data]

No inhibition zones were observed for berberine or astragalus polysaccharides.

2.2 Bacteriostatic Effects of Plant Extract Compounds

Table 3 shows compounds of cinnamon essential oil, matrine, and chlorogenic acid against *E. coli*. All compounds were significantly more effective than matrine or chlorogenic acid alone ($P < 0.05$). Except for cinnamon:matrine (25:50) and cinnamon:matrine:chlorogenic acid (25:50:50), all compounds were significantly less effective than cinnamon essential oil alone ($P < 0.05$).

Table 3. Bacteriostatic effects of plant extract compounds on *Escherichia coli* (n = 6)

Items	Concentration (mg/mL) or ratio	Inhibition zone diameter (mm)
Cinnamon essential oil (control)	25	19.31a
Matrine (control)	25	8.62k
Matrine (control)	50	12.33i
Chlorogenic acid (control)	50	8.57k
Chlorogenic acid (control)	100	9.32k
Chlorogenic acid (control)	200	10.70j
Cinnamon:matrine (1:1)	25:25	16.28bcd

Items	Concentration (mg/mL) or ratio	Inhibition zone diameter (mm)
Cinnamon:berberine (1:2)	25:50	19.80a
Cinnamon:chlorogenic acid (1:1)	25:25	14.07h
Cinnamon:chlorogenic acid (1:2)	25:50	14.22gh
Cinnamon:chlorogenic acid (1:4)	25:100	15.44def
Cinnamon:chlorogenic acid (1:8)	25:200	16.66bc
Cinnamon:berberine:chlorogenic acid (1:1:1)	25:25:25	15.99cde
Cinnamon:berberine:chlorogenic acid (1:1:2)	25:25:50	16.67bc
Cinnamon:berberine:chlorogenic acid (1:1:4)	25:25:100	15.05efg
Cinnamon:berberine:chlorogenic acid (1:1:8)	25:25:200	14.66fgh
Cinnamon:berberine:chlorogenic acid (1:2:1)	25:50:25	17.11b
Cinnamon:berberine:chlorogenic acid (1:2:2)	25:50:50	18.84a
Cinnamon:berberine:chlorogenic acid (1:2:4)	25:50:100	15.11efg
Cinnamon:berberine:chlorogenic acid (1:2:8)	25:50:200	14.39gh
P-value		< 0.0001

Values in the same column with different superscripts differ significantly ($P < 0.05$). The same applies below.

Table 4 shows compounds of bamboo leaf flavonoids, skullcap flavonoids, and cinnamon essential oil against *Salmonella*. Bamboo leaf:skullcap compounds were significantly less effective than their respective controls ($P < 0.05$). Except for bamboo leaf:cinnamon (25:25) and bamboo leaf:skullcap:cinnamon (25:25:25 and 25:50:25), all other compounds were significantly more effective than single-extract controls ($P < 0.05$). Bamboo leaf:cinnamon (25:100) showed the best activity, significantly higher than other compounds ($P < 0.05$).

Table 4. Bacteriostatic effects of plant extract compounds on *Salmonella* (n = 6)

Items	Concentration (mg/mL) or ratio	Inhibition zone diameter (mm)
Bamboo leaf flavonoids (control)	25	20.64e
Skullcap flavonoids (control)	25	18.69f
Skullcap flavonoids (control)	50	18.66f
Cinnamon essential oil (control)	25	12.20i
Cinnamon essential oil (control)	50	17.67f
Cinnamon essential oil (control)	100	21.54e
Bamboo leaf:skullcap (1:1)	25:25	15.98g

Items	Concentration (mg/mL) or ratio	Inhibition zone diameter (mm)
Bamboo leaf:skullcap (1:2)	25:50	14.45h
Bamboo leaf:cinnamon (1:1)	25:25	21.42e
Bamboo leaf:cinnamon (1:2)	25:50	23.50d
Bamboo leaf:cinnamon (1:4)	25:100	30.08a
Bamboo leaf:skullcap:cinnamon (1:1:1)	25:25:25	20.48e
Bamboo leaf:skullcap:cinnamon (1:1:2)	25:25:50	23.66cd
Bamboo leaf:skullcap:cinnamon (1:1:4)	25:25:100	25.07b
Bamboo leaf:skullcap:cinnamon (1:2:1)	25:50:25	20.88e
Bamboo leaf:skullcap:cinnamon (1:2:2)	25:50:50	24.22bcd
Bamboo leaf:skullcap:cinnamon (1:2:4)	25:50:100	24.88bc
P-value		< 0.0001

Optimization of bamboo leaf flavonoids and cinnamon essential oil ratios (Table 5) showed that antibacterial activity increased with higher cinnamon proportions. The 25:150 ratio was optimal, showing no significant difference from 25:200 ($P > 0.05$) but significantly higher activity than other ratios ($P < 0.05$). The 1:6 ratio provided the strongest anti-*Salmonella* effect.

Table 5. Bacteriostatic effects of bamboo leaf flavonoids and cinnamon essential oil compounds on *Salmonella*

Items	Concentration (mg/mL) or ratio	Inhibition zone diameter (mm)
Bamboo leaf flavonoids (control)	25	19.19j
Cinnamon essential oil (control)	100	21.99i
Cinnamon essential oil (control)	125	23.02i
Cinnamon essential oil (control)	150	25.18h
Cinnamon essential oil (control)	175	25.95gh
Cinnamon essential oil (control)	200	26.53g
Cinnamon essential oil (control)	225	26.76g
Cinnamon essential oil (control)	250	26.91fg
Bamboo leaf:cinnamon (1:4)	25:100	28.01ef

Items	Concentration (mg/mL) or ratio	Inhibition zone diameter (mm)
Bamboo leaf:cinnamon (1:5)	25:125	28.39de
Bamboo leaf:cinnamon (1:6)	25:150	31.37a
Bamboo leaf:cinnamon (1:7)	25:175	30.14bc
Bamboo leaf:cinnamon (1:8)	25:200	30.72ab
Bamboo leaf:cinnamon (1:9)	25:225	29.47cd
Bamboo leaf:cinnamon (1:10)	25:250	29.81bc
P-value		< 0.0001

3.1 Bacteriostatic Effects of Plant Extracts on *E. coli* and *Salmonella*

This study found that cinnamon essential oil, matrine, chlorogenic acid, thyme oil, and oregano oil inhibited *E. coli*, while garlic oil, bamboo leaf flavonoids, skullcap flavonoids, berberine, and astragalus polysaccharides were inactive. Numerous studies confirm the anti-*E. coli* activity of these effective extracts. Cinnamaldehyde in cinnamon essential oil strongly inhibits *E. coli* [24] by disrupting cell membranes and impairing enzyme synthesis and metabolism [25]. Matrine causes *E. coli* cell deformation, cytoplasmic shrinkage, plasmolysis, and central cavitation leading to bacterial death [26]. Chlorogenic acid (1.0 g/L) from honeysuckle shows clear antibacterial effects with a 25.95 mm inhibition zone [27]. Thyme oil also inhibits *E. coli* with a 15.6 mm zone [28] and minimum inhibitory concentration (MIC) of 1.12 mg/mL [12]. Wang et al. [29] reported oregano oil' s anti-*E. coli* activity with an MIC of 0.125 µL/mL. These results align with our findings. Studies also show astragalus polysaccharides have no [30] or poor anti-*E. coli* activity [31]. Zhao et al. [18] found berberine has weak activity against Gram-negative bacteria, and Li [32] observed no effect from garlic oil, consistent with our results. Some studies report antibacterial activity for bamboo leaf flavonoids [33] and skullcap flavonoids [15] against *E. coli*, which we did not observe, likely due to differences in strains and extract products. Lu et al. [34] found that bamboo species and harvest time affect antimicrobial

activity.

Our study showed that bamboo leaf flavonoids, skullcap flavonoids, cinnamon essential oil, matrine, chlorogenic acid, oregano oil, thyme oil, and garlic oil inhibited *Salmonella*, while berberine and astragalus polysaccharides were inactive. Limited research exists on bamboo leaf flavonoids against *Salmonella*, though one study reported an 11.78 mm zone against *S. paratyphi* B [35]. Skullcap flavonoids have an MIC of 1.88 mg/mL against *S. typhi* [36], and cinnamon essential oil shows activity with an MIC of 400 mg/L [37]. Han [38] found matrine' s MIC against *Salmonella* was 0.0031 g/mL. Xu [19] reported chlorogenic acid from honeysuckle had an MIC of 0.125 mg/mL. Thyme oil shows moderate activity with a 16 mm zone [39], while garlic oil has strong antimicrobial effects [40]. Lin et al. [13] reported oregano oil inhibited ten *Salmonella* strains with MICs of 111–400 mg/mL. These findings match our results. Studies also confirm berberine' s poor activity against Gram-negative bacteria [18] and astragalus polysaccharides' inactivity [30] or weak effects [31] against *Salmonella*.

3.2 Bacteriostatic Effects of Plant Extract Compounds

Research indicates that plant extract combinations can enhance antimicrobial activity. Zhang [41] found that garlic, clove, and cinnamon (8:8:1 mass ratio) and matrine, rhubarb, and honeysuckle (5:4:8 ratio) showed stronger effects against *E. coli* and *Staphylococcus aureus*. Mo et al. [42] reported that equal mixtures of cinnamon, lemongrass, and patchouli oils strongly inhibited *E. coli*. Lu et al. [43] confirmed that cinnamon and thyme oil combinations enhanced activity against *E. coli* and *Salmonella*, while cinnamon and clove oil combinations reduced efficacy. Some studies show antagonistic effects of cinnamon and clove oils against *E. coli* but synergistic effects against *Salmonella* [44]. Thus, combination efficacy depends on extract types, ratios, and target bacteria. No previous studies have examined cinnamon essential oil, matrine, and chlorogenic acid combinations against *E. coli* or cinnamon essential oil, bamboo leaf flavonoids, and skullcap flavonoids combinations against *Salmonella*.

Our study found that combining cinnamon essential oil, matrine, and chlorogenic acid (in pairs or all three) did not improve anti-*E. coli* activity beyond that of cinnamon essential oil alone. However, bamboo leaf flavonoids combined with cinnamon essential oil enhanced anti-*Salmonella* activity, peaking at a 1:6 ratio. The mechanisms underlying these results require further investigation.

Conclusion

1. Among ten plant extracts, five inhibited *E. coli* with efficacy ranking: cinnamon essential oil > matrine > chlorogenic acid > thyme oil > oregano oil. Garlic oil, bamboo leaf flavonoids, skullcap flavonoids,

berberine, and astragalus polysaccharides were inactive. Eight extracts inhibited *Salmonella* with efficacy ranking: bamboo leaf flavonoids > skullcap flavonoids > cinnamon essential oil > matrine > chlorogenic acid > oregano oil > thyme oil > garlic oil. Berberine and astragalus polysaccharides were inactive.

2. Compounds of cinnamon essential oil, matrine, and chlorogenic acid did not surpass cinnamon essential oil alone against *E. coli*. However, bamboo leaf flavonoids combined with cinnamon essential oil enhanced anti-*Salmonella* activity, with maximal effect at a 1:6 ratio.

References

[1] Zhang DH. Study on prevention and treatment of three bacterial diseases in chickens by Chinese herbal compound preparations [D]. Master' s thesis. Zhengzhou: Henan Agricultural University, 2010.

[2] Mao HX, Wu SG, Zhang HJ, et al. Progress in application of plant extracts in animal production [J]. China Animal Husbandry & Veterinary Medicine, 2010, 37(7): 24-29.

[3] Liu XJ. Influencing factors and countermeasures for development and application of plant extract feed additives [J]. Feed Industry, 2007, 28(16): 59-61.

[4] Cheng GL, Xu SF, Liu FH, et al. Antibacterial effects of garlic volatile oil on four common pathogens in pigs and chickens [J]. Acta Zoonutrimenta Sinica, 2009, 21(4): 554-560.

[5] Tang FY, Pei YL, Chen YJ. In vitro antibacterial test of oregano oil combined with berberine against ESBL-producing *E. coli* in chickens [J]. Chinese Journal of Veterinary Medicine, 2012, 48(6): 31-33.

[6] Tong JM, Gao HH, Sa RN, et al. Antimicrobial activity of plant flavonoid MPB6 and its effect on broiler performance [J]. Chinese Journal of Animal Science, 2002, 38(3): 23-24.

[7] Xu QK, Zhao CY. Bacteriostatic effects of astragalus polysaccharides on main pathogens causing chicken diarrhea [J]. Heilongjiang Animal Science and Veterinary Medicine, 2012(18): 98-99.

[8] Shi NJ, Wang XC, Wu JJ, et al. Optimization of extraction method for Chinese herbal compound polysaccharides and its in vitro antibacterial effects on main poultry pathogens [J]. China Poultry, 2011, 33(23): 28-32.

[9] Hu WJ, Song YH, Wu LL. In vitro combined antibacterial effects of berberine and astragalus polysaccharides on chicken-derived *E. coli* [J]. Journal of Traditional Chinese Veterinary Medicine, 2013(6): 8-10.

[10] Liu SG, Xu FM, Zhang LL, et al. Methodological study on in vitro antibacterial tests of plant extracts [J]. Journal of Huaihua University, 2006, 25(2): 65-67.

[11] Mo XL, Wang YS, Zeng QQ, et al. Antifungal activity study of several essential oils [J]. Natural Product Research and Development, 2005, 17(6): 696-699.

[12] Vardar-Ünlü G, Candan F, Sökmen A, et al. Antimicrobial and antioxidant activity of the essential oil and methanol extracts of *Thymus pectinatus* Fisch. et Mey. var. *pectinatus* (Lamiaceae) [J]. Journal of Agricultural and Food Chemistry, 2003, 51(1): 63-67.

[13] Lin QH, Liu B, Xu YW, et al. In vitro antibacterial effects of oregano volatile oil on common enteritis bacteria [J]. Chinese Journal of Applied and Environmental Biology, 1997, 3(1): 76-78.

[14] Jia J, Wu Y, Su LF, et al. Study on antimicrobial activity of rosemary and cinnamon essential oils [J]. Heilongjiang Medicine, 2015, 28(1): 8-11.

[15] Zeng CZ, Liu ZX, Han L. Study on extraction technology and antimicrobial activity of total flavonoids from *Scutellaria baicalensis* [J]. Lishizhen Medicine and Materia Medica Research, 2009, 20(6): 1342-1343.

[16] You H. Separation, purification and antimicrobial properties of bamboo leaf flavonoids [D]. Master' s thesis. Beijing: Beijing Forestry University, 2009.

[17] Liu YL, Yu R, Zhang GZ, et al. Effect of pH on yield of astragalus polysaccharides extracted by alkali method and its in vitro antibacterial activity [J]. Henan Agricultural Sciences, 2010, 39(5): 117-119.

[18] Zhao ZG, Xu XJ, Zhuang J, et al. In vitro antimicrobial activity of berberine hydrochloride, sodium houttuyfonate and allitridin [J]. Chinese Journal of Clinical Pharmacology, 2005, 21(2): 119-121.

[19] Xu YC. Extraction, separation and antimicrobial activity of chlorogenic acid from honeysuckle [D]. Master' s thesis. Chongqing: Chongqing University, 2008.

[20] Chen DH. Study on in vitro antimicrobial activity of matrine alkaloids [J]. China Animal Health, 2010, 12(1): 28-30.

[21] Wei XY, Jin P, Zuo HY. Study on in vitro antibacterial effects of forsythia seed volatile oil using two different culture media [J]. Journal of Chinese Medicinal Materials, 2004, 27(1): 44-45.

[22] CLSI. CLSI M07 A9 Ed. 9 Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically [S]. Clinical and Laboratory Standards Institute, 2012.

[23] Yao HC. Experimental Guide for Veterinary Microbiology [M]. 2nd ed. Beijing: China Agriculture Press, 2002.

[24] Friedman M, Henika PR, Mandrell RE. Bactericidal activities of plant essential oils and some of their isolated constituents against *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella enterica* [J]. Journal of Food Protection, 2002, 65(10): 1545-1560.

[25] Burt S. Essential oils: their antibacterial properties and potential applications in foods: A review [J]. International Journal of Food Microbiology, 2004, 94(3): 223-253.

[26] Wang GL, Tang JH, Jiang D, et al. Study on antibacterial effects and mechanisms of matrine on chicken *E. coli* [J]. Scientia Agricultura Sinica, 2006, 39(5): 1018-1024.

[27] Zhang ZS, Wu L. Study on in vitro antibacterial and antioxidant activities of chlorogenic acid from honeysuckle [J]. Journal of Tianjin University of Science and Technology, 2005, 20(2): 5-8.

[28] Zhang YL, Zhang RG, Zhong Y. Chemical composition, antimicrobial activity, antioxidant properties and toxicological characteristics of thyme essential oil [J]. Scientia Agricultura Sinica, 2011, 44(9): 1888-1897.

[29] Wang XW, Liu H, Wei J, et al. Study on antimicrobial activity of oregano oil, carvacrol, citral and cinnamaldehyde [J]. Food Industry, 2010(5): 13-16.

[30] Chen HL. Study on preparation of plant polysaccharides and their effects on immune function in broilers [D]. PhD thesis. Beijing: Chinese Academy of Agricultural Sciences, 2002.

[31] Li Y, Zhou Y, Jiang GM, et al. Study on antimicrobial activity of lycium barbarum polysaccharides and astragalus polysaccharides [J]. Progress in Modern Biomedicine, 2012, 12(26): 5061-5063.

[32] Li FQ. Evaluation and application of antimicrobial activity of plant essential oils [D]. Master' s thesis. Nanjing: Nanjing Normal University, 2014.

[33] Li D. Study on extraction, purification, identification and antioxidant and antimicrobial activities of bamboo leaf flavonoids [D]. Master' s thesis. Wuhan: Huazhong Agricultural University, 2008.

[34] Lu ZK, Xie BX. Study on chemical composition and antimicrobial activity of extracts from different bamboo species [J]. Journal of Northwest Forestry University, 2005, 20(1): 49-52.

[35] Yang Y, Li YH. Study on antimicrobial activity of bamboo leaf flavonoid extracts [J]. Guangzhou Chemical Industry, 2011, 39(16): 66-68.

[36] Deng SH, Wang HM, Liu YH. Experimental study on antimicrobial effects of total flavonoids from *Scutellaria baicalensis* stems and leaves [J]. Journal of Chengde Medical College, 2008, 25(3): 324-325.

[37] Li JJ, Ji BP, Zhou F, et al. Study on extraction of volatile oils from clove and cinnamon and their antimicrobial activity [J]. Food Science, 2006, 27(8):

64-68.

[38] Han TS. Study on TMP potentiation of Chinese medicine antimicrobial effects and its compound effects on immune function [D]. Master's thesis. Daqing: Heilongjiang Bayi Agricultural University, 2009.

[39] Dou LL. Study on fresh egg coating preservation technology [D]. Master's thesis. Tianjin: Tianjin University of Science and Technology, 2013.

[40] Zhong YY, Zhang YJ, Yu WG. Study on in vitro antimicrobial activity of garlic oil on human intestinal probiotics and harmful bacteria [J]. China Condiment, 2009, 34(5): 47-49.

[41] Zhang J. Compounding of botanical fungicides and preparation of their microemulsions [D]. Master's thesis. Tianjin: Tianjin University, 2013.

[42] Mo XL, Qiu WF, Yuan L, et al. Antifungal effects of medicinal plant essential oils on plant pathogenic fungi [J]. Modern Chinese Medicine, 2010, 12(1): 33-35.

[43] Lu F, Ding YC, Ye XQ, et al. Antibacterial effect of cinnamon oil combined with thyme or clove oil [J]. Agricultural Sciences in China, 2011, 10(9): 1482-1487.

[44] Goñi P, López P, Sánchez C, et al. Antimicrobial activity in the vapour phase of a combination of cinnamon and clove essential oils [J]. Food Chemistry, 2009, 116(4): 982-989.

Note: Figure translations are in progress. See original paper for figures.