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Original Research Article

Antibacterial Activity of Tannin Extracts from Capparis spinosa Flowers, Citrus aurantium, and Citrus sinensis Against Bacterial Isolates Recovered from Poultry Meat

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Abstract: ***Background:*** Poultry meat is among the most popular sources of animal protein; however, the food product is very susceptible to bacterial contamination. Natural plant compounds like tannins are promising natural alternatives to synthetic antimicrobials, the researchers said. ***Objective:*** So, in this study, we employed tannin extracts of Capparis spinosa flower and Citrus aurantium and Citrus sinensis to assess the antibacterial activity against bacterial isolates (from poultry meat) as a background. ***Methods:*** Accordingly, 10 chicken meat samples were chosen for the purposes of testing and bacterial isolation using the VITEK 2 Compact system. Tannins were extracted using an aqueous method, while the antibacterial activity was determined through agar well diffusion at 100, 75, 50, 25 and 12.5 mg/mL. Ampicillin and sterile distilled water were used as the positive and negative control, respectively. ***Results:*** All the tests have showed significant antibacterial activity. The investigated cell-free filtrated EEP showed the most potent inhibition (22.8 mm) zone effect with Capparis spinosa followed by Citrus aurantium -17.9 and C.sinensis -15.6 (Table 3). The most inhibitive was on Providence alcalifaciens, however the least susceptible were Escherichia coli and Proteus mirabilis. The antibacterial effect was concentration-dependent. ***Conclusions:*** Our data showed a strong antibacterial activity of tannins, especially derived from Capparis spinosa. Thus, these extracts can have protective effects against the bacterial contamination of poultry meat and products; they may even act as natural antimicrobials that protect the quality of poultry while reducing health risks associated with microbial infections.

Keywords: Poultry meat, antibacterial activity, tannins, Capparis spinosa, Citrus aurantium, Citrus sinensis, and natural antimicrobials.

INTRODUCTION

Globally, poultry meat is a major source of animal protein and is cheap and widely available. Nevertheless, poultry products are often subject to microbiological contamination which may occur at point-to-slaughtering, transportation or storage. Such contamination introduces harmful microorganisms that can induce foodborne diseases in humans [1, 2, 19, 20].

Most foodborne pathogens in poultry are prominent contributors to contamination and the dissemination

of disease, drawing researchers' attention. Another component of the global threat was antibiotic-resistant gram-negative bacteria, which have become a major health concern internationally because they are resistant to multiple classes of antibiotic treatment and stem in part from the widespread use of antibiotics for medical and agricultural purposes [3, 4, 21, 22, 33].

In Iraq, the previous studies focused on different chicken meat products purchased from markets in cities and suburbs detectable of immure Salmonella and thermotolerant Campylobacter. These aspects also signal the need for surveillance of poultry enteritis associated pathogens in this region and an urgent search for natural alternatives to conventional antimicrobial[23, 24].

Due to these challenges, researchers have investigated natural plant sources as potential alternatives to existing synthetic antimicrobial agents. Plants are used in traditional medicine and they contain various bioactive compounds including phenolics, flavonoids, alkaloids, and tannins that exhibited an antimicrobial effect against microorganisms of different groups [5, 6]. One subset of plant polyphenols that has received considerable attention is tannins, which act as antimicrobial through the selective precipitation of proteins, inhibition of enzymes and damaging bacterial cell membranes [7, 8].

Tannins are a common plant secondary metabolite and are associated with several medicinal plants, therefore tannin content and antimicrobial activity have been studied in certain medicinal plant species [31,32]. *Capparis spinosa* is distributed in the Mediterranean and Middle East; its phytochemicals have antimicrobial, antioxidant, and anti-inflammatory properties. [9, 10, 25, 26, 27]. Citrus species, such as *Citrus aurantium* and *Citrus sinensis*, help keep pergamon pathogenic bacteria in check thanks to their polyphenol and tannin content. Previous studies have shown that extracts derived from citrus possess the ability to inhibit growth against multiple food-related pathogens, suggesting their efficacy as natural antimicrobials [11, 12, 28, 29].

To assess and compare the antibacterial activity of tannin extracts from *Capparis spinosa* flowers, *Citrus aurantium* and *Citrus sinensis* against bacteria isolated from poultry meat. The objective was to analyze the potential use of these extracts as natural antimicrobials.

Materials and Methods

Study Design and Sample Collection

This had been a cross-sectional study using poultry meat samples of 10 local markets in Boston. These were placed in sterile saline solution (0.85% NaCl) under aseptic conditions and further taken to the laboratory for microbiological analysis. Bacterial colonies were isolated via sensitive serial dilutions.

Isolation and Purification of Bacterial Isolates

Bacterial isolates were obtained by inoculating samples on selective and differential media (MacConkey, Salmonella–Shigella, Mannitol salt agar and nutrient agar). The plates were then incubated at 37°C for 24! and colonies with distinct morphologies were re-isolated by replica plating onto new nutrient agar plates.

Identification of Bacterial Isolates

Identification of purified bacterial isolates was performed according to the manufacturer's instructions, by means of automated identification using VITEK 2 Compact system (bioMérieux, France). Suspensions of organisms in sterile saline were adjusted to 0.5 McFarland standards and loaded into the system with GN/GP identification cards. The system's software analyzed the results to identify bacterial species based on biochemical reactions.

Preparation of Bacterial Inoculum

For the use of antibacterial tests, bacterial bands were prepared fresh before using by mixing in sterile saline solution. The turbidity of the suspensions was adjusted to one 0.5 McFarland unit (approximately 1.5×10^8 CFU/mL),in accordance with reference [13].

Preparation of Plant Material

The plant samples (flowers of Capparis spinosa, fruits of Citrus aurantium and fruits of Citrus sinensis) were collected washed with tap water to eliminate dust and other impurities after that distilled water. The materials were then dried to constant weight at 60 °C for 48 h in a hot air oven. The dried materials were milled into a suitable powder by grinder of laboratory and they were kept in sterile tightly sealed containers until use.

Extraction of Tannins

Tannins were extracted from the dried powders of plants by a water-based technique (with some modifications) [14, 31]. Each was soaked in distilled water (50 mL) and boiled for 30 min (about 0.5 g). The suspension was filtered with Whatman No. 1 filter paper and centrifuged at 2000 rpm for 20 min. Polyvinylpolypyrrolidone (PVPP) was added to the supernatant after which it was filtered out; this works as a great help to separate tannins because of its ability to bind so tightly with polyphenolic compounds [31, 32]. This mixture was left standing for full precipitation. The precipitate was filtered out and washed several times with ethanol, followed by

ether, to remove any remaining excess dye material. The tannin extracts required the prior drying of crude material applied in these new antibacterial tests.

Preparation of Extract Concentrations

The researchers went on to dilute crude tannins extracts with sterile distilled water for use as stock solutions. Five concentrations were made for performing antibacterial tests (100, 75, 50, 25 and 12.5 mg/mL).

Antibacterial Activity Assay

Antibacterial Activity of Tannin Extracts. The antibacterial activity was evaluated using the agar well diffusion method depicted by Balouiri et al. [15]. Bacterial suspensions were adjusted to 0.5 McFarland standard (approximately 5.0 × 109 CFU mL−1; cfu: colony-forming unit) for inoculating sterile nutrient agar plates by streaking. They used a sterile cork borer to make 6 mm wells in the agar, filling each well with 100 µL of extract at the appropriate concentration. After the extract was spread, plates were incubated at room temperature for 30 min and after it at 37°C for 24 h; Inhibition zones formed in mm (millimeters) with a ruler measured.

Controls

Ampicillin (10 µg) and sterile distilled water were used as the positive and negative controls, respectively.

All experiments were performed in triplicate, and all the results are expressed as means ± standard deviation (mean ± SD). Differences between treatments were determined for significance using one-way analysis of variance (ANOVA). P-value of <0.05 was considered statistically significant.

Ethical Considerations

This study aimed to analyze poultry meat offered for consumers. The experiments did not involve living animals. The study protocol was approved by the Institutional Ethics Committee of Tikrit University, College of Education for Pure Sciences.

RESULTS

By the agar well diffusion method, the antifungal activity of extract of *Capparis spinosa* flowers, *Citrus aurantium* and *Citrus sinensis* was tested against poultry meat isolates. These inhibition zones were measured in mm, and averages of 3 tests with standard deviations were reported.

Antibacterial tannins of *Capparis spinosa*. The antibacterial activity of the *Capparis spinosa* flower treatment is shown in Table 1, where a strong effect against most tested bacteria can be observed. The broadest inhibition zone was produced by *Providencia alcalifaciens* 100 mg/ml (34.4 ± 0,53 mm) followed by *Staphylococcus sciuri* 32.27 ± 0.64 mm and *Staphylococcus lentus* 29.67±0.58 mm. *Campylobacter jejuni* and *Klebsiella* spp. were inhibited moderately while, *Escherichia coli* and *Proteus mirabilis* among others had little to no effect at lower concentrations of extracts. The effectiveness of the extract decreased when the concentration was lowered from 100 mg/ml to 12.5 mg/ml.

Citrus aurantium have Antibacterial activity (Tannins). Moderate antibacterial activities of *Citrus aurantium* tannin extract were showed in Table 2. For *Campylobacter jejuni*, the maximum inhibition zone (29.93 ± 0.21 mm) was produced at 100 mg/ml. The same concentration produced a considerable inhibition by *Proteus mirabilis* (29.63 ± 0.72 mm) and *Klebsiella oxytoca* (25.1 ± 0.1 mm). More than other microorganisms, a smaller inhibition zone was detected with various concentrations against *Escherichia coli* and *Klebsiella pneumoniae*. Antibacterial activity was inversely dependent on the concentration.

Also, *Citrus sinensis* extract was assessed. Table 3 shows the results of various concentrations of *Citrus sinensis* tannin extract against the bacteria tested. *Proteus mirabilis* showed maximum inhibition zone at 100 mg/ml (28.03 ± 0.06 mm). The other pathogens affected at this concentration included *Providencia alcalifaciens* (27.07 ± 0.12 m) and *Staphylococcus sciuri* (22.93 ± 0.12 mm). Overall, the inhibitory effect of *Citrus sinensis* tannins was lower than for the other two extracts, especially against *Escherichia coli* and *Campylobacter jejuni*. As with the other extracts, lower concentration led to reduced antibacterial activity.

(A) Avg. inhibition zones of the studied plant extracts. Table 4 presents a summary of the average inhibition zones. The highest inhibition zone of 22.8 mm was in *Capparis spinosa* flower tannins. The second best result was using bitter orange (*Citrus aurantium*) (17.9 mm), which resulted in about 2.3 mm less than the above, while the third best was sweet orange (*Citrus sinensis*) which resulted in 15.6 mm; The result shows that *Capparis spinosa* has the highest potency among the bioactive compounds tested against bacteria isolated from poultry meat in this study.

Control results

Table 5 displays the results of control experiments. Ampicillin inhibited all tested bacterial isolates as positive controls. No inhibition zones were observed in the negative control (sterile distilled water). This means that the antibacterial activity was due to active compounds present in tannin extracts.

Table 1

Antibacterial activity of tannins from *Capparis spinosa* flowers against bacterial isolates

Bacterial species	100 mg/ml	75 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml
Staphylococcus lentus	29.67 ± 0.58	28.33 ± 0.58	25.83 ± 0.76	20.27 ± 0.25	19.5 ± 0.5
Staphylococcus sciuri	32.27 ± 0.64	26.23 ± 0.4	25.03 ± 0.25	22.07 ± 0.12	17.53 ± 0.5
Providencia alcalifaciens	34.4 ± 0.53	29.77 ± 0.4	28.0 ± 0.2	25.2 ± 0.2	17.3 ± 0.36
Campylobacter jejuni	28.07 ± 0.12	22.97 ± 0.15	23.1 ± 0.17	19.8 ± 0.35	17.73 ± 0.64
Klebsiella pneumoniae	21.87 ± 0.23	16.97 ± 0.25	16.27 ± 0.46	14.07 ± 0.12	12.87 ± 0.12
Proteus mirabilis	22.9 ± 0.17	20.07 ± 0.12	18.07 ± 0.12	16.07 ± 0.12	10.9 ± 0.17
Escherichia coli	23.9 ± 0.17	21.03 ± 0.06	17.93 ± 0.12	14.0 ± 0.3	10.97 ± 0.25
Klebsiella oxytoca	24.07 ± 0.12	19.0 ± 0.2	17.93 ± 0.12	17.7 ± 0.52	13.93 ± 0.12

Values are presented as the mean ± standard deviation (SD) of three replicates. Inhibition zones were measured in millimeters (mm), including the well diameter (6 mm).

Table 2

Tannin activity from *Citrus aurantium* on isolating bacteria

Bacterial species	100 mg/ml	75 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml
Staphylococcus lentus	21.93 ± 0.12	17.73 ± 0.87	13.93 ± 0.12	13.17 ± 0.15	11.07 ± 0.12
Staphylococcus sciuri	20.13 ± 0.12	16.3 ± 0.36	15.3 ± 0.36	12.07 ± 0.12	10.8 ± 0.35
Providencia alcalifaciens	22.07 ± 0.12	19.93 ± 0.12	21.1 ± 0.1	16.9 ± 0.17	11.07 ± 0.12
Campylobacter jejuni	29.93 ± 0.21	24.93 ± 0.12	18.1 ± 0.36	17.93 ± 0.12	13.93 ± 0.12
Klebsiella pneumoniae	17.13 ± 0.15	18.93 ± 0.12	13.97 ± 0.15	13.07 ± 0.12	11.1 ± 0.1
Proteus mirabilis	29.63 ± 0.72	22.07 ± 0.12	17.9 ± 0.17	15.03 ± 0.06	12.07 ± 0.12
Escherichia coli	15.97 ± 0.15	17.87 ± 0.23	15.07 ± 0.12	13.07 ± 0.12	10.03 ± 0.06
Klebsiella oxytoca	25.1 ± 0.1	23.9 ± 0.17	17.03 ± 0.06	20.07 ± 0.12	13.07 ± 0.12

Values are presented as the mean ± standard deviation (SD) of three replicates. Inhibition zones were measured in millimeters (mm), including the well diameter (6 mm).

Table 3

Antibacterial activity of tannins obtained from *Citrus sinensis* against bacterial isolates.

Bacterial species	100 mg/ml	75 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml
Staphylococcus lentus	19.03 ± 0.06	14.93 ± 0.12	14.2 ± 0.26	11.93 ± 0.12	10.07 ± 0.12
Staphylococcus sciuri	22.93 ± 0.12	16.07 ± 0.12	14.07 ± 0.12	12.03 ± 0.06	11.03 ± 0.06
Providencia alcalifaciens	27.07 ± 0.12	15.07 ± 0.12	14.07 ± 0.12	11.93 ± 0.12	9.83 ± 0.29
Campylobacter jejuni	19.1 ± 0.1	14.07 ± 0.12	13.07 ± 0.12	14.13 ± 0.23	11.03 ± 0.06
Klebsiella pneumoniae	20.93 ± 0.12	15.17 ± 0.21	14.07 ± 0.12	12.13 ± 0.23	10.93 ± 0.12
Proteus mirabilis	28.03 ± 0.06	25.87 ± 0.12	17.1 ± 0.17	17.8 ± 0.35	15.1 ± 0.1
Escherichia coli	16.1 ± 0.17	16.03 ± 0.06	15.07 ± 0.12	13.13 ± 0.23	10.07 ± 0.12
Klebsiella oxytoca	19.17 ± 0.21	20.17 ± 0.21	19.07 ± 0.12	17.17 ± 0.21	13.07 ± 0.12

Values are presented as the mean ± standard deviation (SD) of three replicates. Inhibition zones were measured in millimeters (mm), including the well diameter (6 mm).

Table 4

Antibacterial activity of the tested plant tannins — an overall comparison

Plant extract	Overall mean inhibition zone (mm)	Activity ranking
Capparis spinosa (flowers)	22.8	1
Citrus aurantium	17.9	2
Citrus sinensis	15.6	3

Table 5

Zone of Inhibition of Endo agar for Positive & Negative controls

Control	Observation
Ampicillin (positive control)	Strong inhibition zones observed
Deionized distilled water (negative control)	No inhibition observed

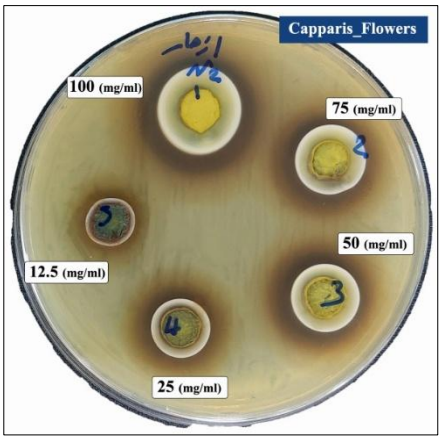

Figure 1. The agar well diffusion method was used to assess the antibacterial activity of tannins extract of *C. spinosa* flower against bacterial isolates.

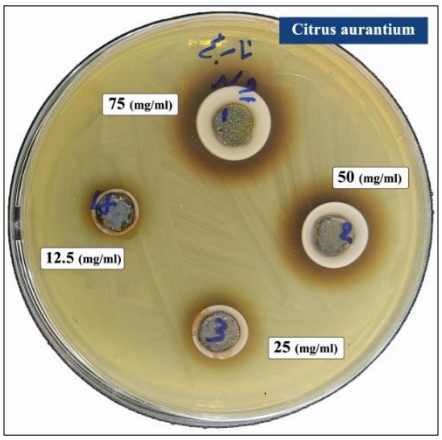

Figure 2. Tannin extract *C. aurantium* antibacterial activity at different concentrations.

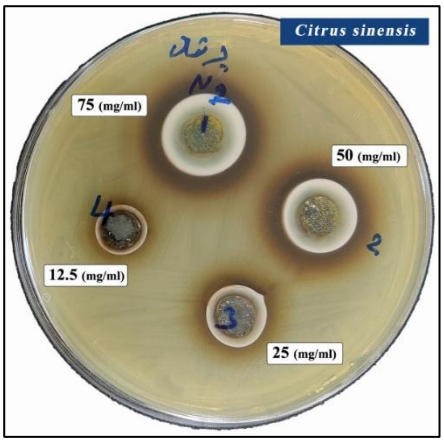

Figure 3. Antibacterial activity of tannin extract from *C. sinensis* against bacterial isolates..

DISCUSSION

These contained tannin extracts from Capparis spinosa flowers, Citrus aurantium and Citrus sinensis have been studied for their interfering activity upon growth of bacteria in poultry meat. In fact, all showcased an anti-bacterial effect; Capparis spinosa extract exhibited the strongest.

The elevated activity of Capparis spinosa flower tannins against microbes in agreement with previous studies that reported abundance of many functional phytochemicals present in the plant [9, 10, 25, 26, 27]. because it combats Gram-negative bacteria such as Providencia alcalifaciens and Staphylococcus spp — which can be seen as a possible synergy of bioactive compounds, such as flavonoids and phenolic acids that seem to act in a synergistic way with tannins ^2526. Tannins destroy bacteria in a multitude of mechanisms, such as aggregating microbial proteins, inhibiting specific enzymes and rupturing bacterial cell membrane [16, 17]. These findings are in agreement with previously published data that demonstrate the potent natural antimicrobial activity of Capparis spinosa against a wide range of pathogenic bacteria [9, 10, 27].

Citrus aurantium and Citrus sinensis had moderate antibacterial activity compared to Capparis spinosa making a case that may explain some of the antimicrobial potential displayed by some citrus species. One explanation may be due to the polyphenolic and flavonoid-rich content found in citrus fruits, in particular the phytochemicals hesperidin and naringin common among citrus fruits [11, 28, 29, 30]. In previous work, it has been observed that these compounds inhibit bacterial metabolism and membrane potential [12, 28, 30]. The differences in the type and quantity of tannins and other components contained within their peel or fruit may account for the variation in antibacterial potency.

One important result was that the higher the concentration of extract, the larger their zones were where bacteria could not grow. Such a dose-response pattern is typical of active compounds derived from plants. Considering that, it can be said that the higher number of active substances in a natural phenolic compound the better their antimicrobial action [18]. This has encouraged the use of PVP-PVC for the removal of polysphenolic compounds, and is a favorable approach to incorporate phenolic compounds in extract preparation [31, 32].

You will also see that these extracts acted differently in bacteria. Gram-positive species (Staphylococcus lentus and Staphylococcus sciuri) were generally more sensitive than Gram-negative bacteria (Escherichia coli and Klebsiella pneumoniae). That's a pattern we recognize — mostly because they have different structural cell walls. Gram-negative bacteria have an outer membrane that is composed of lipopolysaccharides to allow the diffusion of large or hydrophobic molecules (e.g., tannins) [16]. In a stunning counterpoint, the gram-negative organism Providencia alcalifaciens was inhibited with high potency by Capparis spinosa - implying that some tannins have evolved strategies to circumvent this hurdle. This needs more study. In this study, the VITEK 2 Compact system was used to identify bacterial isolates with great precision. It ensured that the activities noted on paper were aimed at sharply defined pathogens. Providencia alcalifaciens and Staphylococcus sciuri were found in poultry meat, which should be considered due to introdecement of significantly microbial diversity into consumer products. This underlines the need for effective control strategies. Along meat and poultry chains, however, contaminated pathways and resistance problems were reported as similar findings [19, 23, 24, 34, 35].

Finally, these findings serve as the basis for further research on natural antimicrobials derived from plants containing tannins like Capparis spinosa. It could reduce the use of synthetic antibiotics in poultry, a major driver of the risk of antimicrobial resistance.

Conclusion

Tannin extracts from Capparis spinosa flowers as well as Citrus aurantium and Citrus sinensis showed strong antibacterial activity against bacteria isolated from poultry meat in this study. Identification and molecular characterization indicated that largely inhibited species were obtained from Capparis spinosa and Providencia alcalifaciens: the largest inhibition zones (20 mm) were recorded against Staphylococcus spp.

These observations revealed that the antibacterial activity of the polymer was concentration-dependent. The inhibition of secreted MMP-2 and -9 activity was dose dependent. Some bacteria were more or less sensitive — possibly due to the structural differences between gram-positive (which have thick peptidoglycan cell walls) and gram-negative (which have thin layers of peptidoglycan separated by outer membranes) bacteria. The reliability of these results was confirmed by the identification of the isolates with an automated bacterial identification instrument, VITEK 2 Compact system.

These findings emphasize the efficacy of tannins from galangal, and more specifically, those isolated

from C. spinosa. The extract-tannins of spinach leaves consequently appeared to show significant antimicrobial effect and can be said to have a good inhibitory capacity especially towards Gram-positive organisms with C. spinosa tannins having the strongest property in this regard toward both types of Gram stains. They could reduce foodborne pathogens in poultry products, and they may be a natural alternative to synthetic antibiotics for inclusion in the food safety toolbox. Further research is needed for the most effective foods preserved by other-compounds showing minimum inhibitory concentrations, active compounds against the bacteria and efficacy per volume of the product approved based on qualitative tests from real preserved food.

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