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Antibacterial Activity of Pomegranate Peel and Grapefruit Peel Tannin Extracts Against Spoilage and Psychrotrophic Bacteria for Preserving Frozen Chicken Meat

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Abstract: Poultry meat is considered as one of the important foods due to its high nutritional value and the preservation of frozen poultry meat from microbial spoilage is a great challenge, due to the ability of many species of bacteria to adapt and grow at low temperatures. There is a global drive to reduce chemical additives due to the long-term health damage they cause, with safe natural alternatives from agricultural waste. The objective of this study was to assess the in vitro inhibitory activity of hydrolysable tannin extracts from pomegranate peels (*Punica granatum*) and condensed tannin extracts from grapefruit peels (*Citrus paradisi*) at different concentrations (12.5, 25, 50, 75, 100 mg/mL) against four species of pathogenic and psychrophilic bacteria from chicken meat (*Campylobacter jejuni*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Escherichia coli*). The three-factor factorial experiment (2×4×5) was designed based on Completely Randomised Design (CRD) and Three-Way Analysis of Variance (Three Way ANOVA) was performed and Duncan's Multiple Range Test was applied at a significance level of ($p < 0.05$). The results showed a significant superiority of the grapefruit peel tannin extract with an average of 14.85 mm inhibition compared to the pomegranate peel extract (9.91 mm) ($p < 0.05$). In contrast, the extract of grapefruit peel at 100 mg/mL concentration showed the highest inhibition diameters against *P. mirabilis* with a mean of 21.83 mm, followed by *C. jejuni* with a mean of 21.00 mm. The inhibitory activity was also found to be directly proportional to the concentration (Dose-Dependent Manner). *C. jejuni* was also sensitive to grapefruit peel extract even at the low concentration of 12.5 mg/mL (15.27 mm), however it was resistant to pomegranate peel extract at the concentration of 25 mg/mL (0.00 mm). In contrast, both extracts showed complete resistance against *E. coli* at a concentration of 12.5 mg/mL. The study proves the high efficiency of grapefruit and pomegranate peel tannins as natural bio-additives for meat preservation. The concentration of 75 mg/mL of grapefruit peels can be used in active packaging technologies and microencapsulation to extend the shelf life of poultry meat.

Keywords Pomegranate peel tannins, Grapefruit peels, Meat preservation, Psychrotrophic bacteria, Frozen chicken, Antimicrobial activity, Biopreservatives.

1. Introduction

Poultry meat is considered one of the most consumed sources of animal protein globally; however, its high moisture content and essential nutrient profile make it an excellent medium for spoilage and bacterial contamination. Gram-negative enteric bacteria such as *Campylobacter jejuni*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis* constitute the main cause of most intestinal infection and food poisoning cases resulting

from poultry meat consumption [1,2]. What increases their risk is the ability of some of these bacteria to adapt and develop tolerance to low temperatures (Cryo-tolerance / Psychrotrophic behavior), as bacteria isolated from frozen meat develop cellular stress mechanisms enabling them to maintain their outer membrane integrity and pathogenic efficacy after exposure to freezing and prolonged cold storage [3].

The world has begun to reject chemical additives as preservatives for poultry meat products; conversely, bacteria have developed resistance to antibiotics such as ampicillin [4], which has driven food safety research toward exploring beneficial plant bioactive compounds as bio-preservatives derived from agricultural by-products [5].

Natural phenolic compounds, especially tannins from fruit peels, possess antibacterial and antioxidant properties [6]. Pomegranate (*Punica granatum*) peel extract is rich in hydrolysable tannins (HT) such as punicalagin and ellagic acid, which precipitate outer membrane proteins and inhibit cell permeability [7-9]. In contrast, the extract of the grapefruit (*Citrus paradisi*) peel is characterised by the presence of condensed tannins (CT), bioflavonoids (naringin and hesperidin), organic acids and active phenolic compounds that penetrate the phospholipid bilayer of the bacterial membrane, disrupting membrane potential and respiratory enzymes [10-12].

The research gap is the scarcity of comparative studies on the response of psychrotrophic meat-spoilage bacteria isolated from frozen chicken against different doses of pomegranate and grapefruit peel tannin extracts. Moreover, there is a lack of data describing the statistical and biochemical relationship of interaction between bacterial resistance patterns and the specific chemical nature of these fruit-derived tannin extracts.

The aim of this study was to extract tannin compounds from pomegranate (*Punica granatum*) and grapefruit (*Citrus paradisi*) peels, and to isolate spoilage and pathogenic psychrotrophic bacteria (*C. jejuni*, *K. pneumoniae*, *P. mirabilis*, and *E. coli*) from frozen chicken meat, and to identify them biochemically and by the VITEK-2 system. It also aims to evaluate and compare the in vitro antibacterial efficacy of different concentrations of these extracts to test their potential as natural preservatives for inhibiting bacterial spoilage by enteric and psychrotrophic microbes in a 2×4×5 factorial design.

2. Materials and Methods

Preparation of Peel Tannin Extracts

- **Preparation and Drying:** The fruit peels collected were washed thoroughly with water to remove the dust and impurities. Pomegranate and grapefruit peels were dried in a ventilated and shaded place at 25 °C or in drying ovens at 40 °C until a constant weight was obtained. The dried peels were ground to a fine, homogeneous powder so as to maximise the surface contact area with the solvent [7].
- **Extraction and Filtration:** The tannins were extracted from 100 g of peel powder with either 80% methanol or an aqueous solvent at a mass-to-volume ratio of 1:5 to 1:10 (v mL/g wet weight). Extraction was carried out under stirring (150 rpm) and mild heating at 60 °C in order to avoid heat sensitive components degradation [13,14]. The mixture was then filtered through Whatman No. 1 filter paper and subjected to centrifugation for 10–15 min at 4000 rpm to get an extract devoid of impurity.
- **Concentration and Storage:** The filtrate was put into the rotary evaporator at 40 °C to get the dry tannins. Then concentrations (12.5, 25, 50, 75, 100 mg/mL) were prepared dissolving tannins in deionised water and were kept in opaque containers at 20 °C until use.

Bacterial Isolation and Identification

Four bacterial strains (*C. jejuni*, *K. pneumoniae*, *P. mirabilis* and *E. coli*) were isolated from locally obtained frozen chicken meat samples by serial dilutions and plating onto selective media (MacConkey Agar, Psychrotrophic Count Agar and Campylobacter Selective Agar). Plates were incubated for 10 days at 7 °C, targeting psychrotrophic microorganisms. The isolates were identified by Gram staining, biochemical tests of reference and VITEK-2 system. Strains were refreshed on Nutrient Agar at 37 °C and bacterial suspension density was adjusted to match the 0.5 McFarland standard (equivalent to 1.5×10^8 CFU/mL) at an absorbance wavelength of 625 nm [4].

Agar Well Diffusion Assay

100 µL of the bacterial suspension were plated onto Muller-Hinton Agar (MHA) plates. Wells of 6 mm diameter were cut using a sterile cork borer and 100 µL of each concentration of pomegranate and grapefruit peel extracts were loaded separately into individual wells [15]. Plates were incubated at 37 °C for 24 h and inhibition zone diameters were measured in millimetres (mm) with a digital calliper.

Control Groups

- **Negative Control:** Deionised distilled water was used to ensure that there was no intrinsic solvent activity (0.00 mm) .
- **Positive Control:** Ampicillin antibiotic discs (10 µg) to verify the susceptibility and relative efficacy of the isolate.

Experimental Design and Statistical Analysis

The experiment was conducted using a $2 \times 4 \times 5$ factorial design within a Completely Randomized Design (CRD). Data were analyzed using Three-Way Analysis of Variance (Three-Way ANOVA) followed by Duncan's Multiple Range Test [16] to determine significant differences among treatments at a significance level of $p < 0.05$ using SAS software [17].

3. Results and Statistical Analysis

The Three-Way Analysis of Variance (Three-Way ANOVA) showed highly significant effects ($p < 0.05$) for the interaction of the three studied factors (Extract type $\times$ Bacteria type $\times$ Concentration).

Table 1. Inhibition zone diameters (mm) for the interaction of Extract $\times$ Bacteria $\times$ Concentration for frozen meat pathogens with Duncan's test letters ($p < 0.05$).

Bacteria Type	Concentration (mg/mL)	Pomegranate Peel Extract (Tannins)	Grapefruit Peel Extract (Tannins)
Campylobacter jejuni	100	13.90 $\pm$ 0.25 de	21.00 $\pm$ 0.20 a
	75	12.93 $\pm$ 0.21 ef	18.80 $\pm$ 0.30 b
	50	10.73 $\pm$ 0.25 h	19.00 $\pm$ 0.26 b
	25	0.00 $\pm$ 0.00 l	16.17 $\pm$ 0.25 c
	12.5	0.00 $\pm$ 0.00 l	15.27 $\pm$ 0.25 cd

Bacteria Type	Concentration (mg/mL)	Pomegranate Peel Extract (Tannins)	Grapefruit Peel Extract (Tannins)
Klebsiella pneumoniae	100	13.93 ± 0.29 de	17.07 ± 0.25 c
	75	12.83 ± 0.21 f	17.53 ± 0.25 bc
	50	11.93 ± 0.25 fg	14.30 ± 0.26 d
	25	10.93 ± 0.25 gh	12.07 ± 0.21 fg
	12.5	9.87 ± 0.25 hi	9.07 ± 0.25 i
Proteus mirabilis	100	12.93 ± 0.25 ef	21.83 ± 0.29 a
	75	13.87 ± 0.25 de	19.17 ± 0.25 b
	50	11.93 ± 0.25 fg	15.87 ± 0.25 cd
	25	9.77 ± 0.25 hi	15.17 ± 0.25 cd
	12.5	0.00 ± 0.00 l	12.17 ± 0.25 fg
Escherichia coli	100	14.90 ± 0.26 d	15.23 ± 0.25 cd
	75	12.73 ± 0.25 f	15.07 ± 0.25 cd
	50	12.13 ± 0.25 fg	13.20 ± 0.26 e
	25	9.90 ± 0.25 hi	11.93 ± 0.25 fg
	12.5	0.00 ± 0.00 l	0.00 ± 0.00 l

- Negative Control: Recorded 0.00 mm (fell within the statistical letter l).
- Positive Control (Ampicillin): Recorded inhibition zones ranging between 16.50–22.10 mm.
- Note: Different lowercase letters indicate statistically significant differences between treatments at a significance level of ($p < 0.05$).

Table 2. Main individual effects of the studied factors (Extract, Bacteria, Concentration).

Studied Factor	Categories / Concentrations	Overall Mean Inhibition Diameter (mm)	Significance Letter (p≤0.05)
Extract Type	Grapefruit peel extract	14.85 ± 0.18	A
	Pomegranate peel extract	9.91 ± 0.22	B
Bacteria Type	<i>Proteus mirabilis</i>	13.27 ± 0.21	a
	<i>Klebsiella pneumoniae</i>	12.95 ± 0.19	a
	<i>Campylobacter jejuni</i>	12.78 ± 0.24	a
	<i>Escherichia coli</i>	10.51 ± 0.20	b
Concentration (mg/mL)	100	16.35 ± 0.22	A
	75	15.37 ± 0.20	B
	50	13.64 ± 0.19	C
	25	10.74 ± 0.21	D
	12.5	5.79 ± 0.18	E

Scientific Discussion

The empirical results revealed that the biological advantage of the grapefruit peel tannins was significantly higher than that of the pomegranate peel extracts, with the overall mean inhibition zone of 14.85 mm compared to 9.91 mm of all the tested frozen-poultry bacterial isolates (p<0.05). Importantly, all treatments showed dose-dependent inhibition patterns. The high antibacterial activities of the grapefruit extract (maximum inhibition zones were observed for *P.mirabilis* (21.83 mm) and *C. jejuni* (21.00 mm)) could be ascribed to the copious presence of condensed tannin (CT) complexes that are naturally deposited in citrus flavedo together with bioflavonoids such as naringin and hesperidin and organic acids [10-12]. These bio-active components are effective in reducing the environmental pH and penetrate the lipopolysaccharide (LPS) outer bilayer of the Gram-negative bacterial membranes with high synergistic efficacy [18,19].

In contrast, pomegranate peels are rich in hydrolysable tannins (HT), mainly punicalagin and ellagic acid, which exhibit antimicrobial activity through cross-linking membrane proteins and removing essential mineral ions necessary for bacterial growth [7-9]. However, cryo-tolerant bacterial strains isolated from below zero storage conditions adapt their outer membrane integrity. Thus, the selective barrier of these adapted membranes prevents the entry of bulky hydrolysable tannin molecules at lower doses (\le

25 \text{ mg/mL}) which justifies the full resistance (0.00 mm) of *C. jejuni* to pomegranate extract at these thresholds [3,20].

Responses of microbial strains further demonstrate specific physiological susceptibilities of the isolates. Moreover, *C. jejuni* was highly sensitive to grapefruit tannins even at a low concentration of 12.5 mg/mL (15.27 mm), which was explained by its fragile membrane structure and high susceptibility to oxidative stress [18]. In contrast, *E. coli* showed the greatest resistance with the least mean zone of inhibition (10.51 mm). This tolerance is mainly due to the active multidrug efflux pump systems that extrude phenolic substances before damaging critical membrane [19,21].

4. Conclusions

Grapefruit and pomegranate peel tannin extracts proved to be highly efficient natural bioactive additives against bacteria in frozen chicken, with the grapefruit peel tannin extract demonstrating a significantly superior overall effect compared to pomegranate peel extract across all tested concentrations. The inhibitory activity increased in a dose-dependent manner with higher extract concentrations; specifically, *P. mirabilis* and *C. jejuni* were the most sensitive bacteria to lower concentrations of grapefruit peel extract, whereas *E. coli* exhibited the highest resistance.

REFERENCES

- [1] N. S. Al-Zoreky, "Antimicrobial activity of pomegranate (*Punica granatum*) fruit peel extract," *International Journal of Food Microbiology*, vol. 134, no. 3, pp. 244–248, 2009.
- [2] CLSI, *Performance Standards for Antimicrobial Susceptibility Testing*, 30th ed., CLSI supplement M100. Clinical and Laboratory Standards Institute, 2020.
- [3] S. S. Dahham, M. N. Ali, H. Tabassum, and M. Khan, "Studies on antibacterial and antifungal activity of pomegranate peel extract," *International Journal of Microbiology*, vol. 2015, pp. 1–7, 2015.
- [4] D. B. Duncan, "Multiple range and multiple F tests," *Biometrics*, vol. 11, no. 1, pp. 1–42, 1955.
- [5] EFSA, "The European Union One Health 2021 Zoonoses Report," *EFSA Journal*, vol. 20, no. 12, Art. no. e07666, 2022.
- [6] H. B. El-Nashi, A. A. Abdel Fattah, N. R. Abdel Rahman, and M. M. Abd El-Razik, "Quality characteristics and shelf life extension of refrigerated chicken meat treated with pomegranate peel extract," *Food Packaging and Shelf Life*, vol. 31, Art. no. 100798, 2022.
- [7] E. A. Elsherbiny, B. H. Amin, and Z. A. Baka, "Efficiency of pomegranate peel extract tannins as bio-preservative agent against foodborne pathogens in meat products," *Journal of Food Safety*, vol. 43, no. 2, Art. no. e13021, 2023.
- [8] R. Gyawali and S. A. Ibrahim, "Natural products as antimicrobial agents in food systems," *Critical Reviews in Food Science and Nutrition*, vol. 54, no. 11, pp. 1433–1444, 2014.
- [9] K. Kaderides, A. Kyriakoudi, I. Mourtzinis, and A. M. Goula, "Potential of pomegranate peel extract as a natural additive in foods," *Trends in Food Science & Technology*, vol. 115, pp. 275–290, 2021.
- [10] S. Kandasamy, J. Yoo, T. Y. Kang, and J. S. Ham, "Antimicrobial and antioxidant properties of citrus peel extracts and their application in active food packaging: A review," *Food Control*, vol. 124, Art. no. 107902, 2021.
- [11] A. Magri, M. Petriccione, and M. Gutierrez, "Agro-industrial by-products as source of bioactive compounds for active food packaging," *Trends in Food Science & Technology*, vol. 102, pp. 189–204, 2020.
- [12] M. Maheen, R. Sharma, and A. Kumar, "Efflux pump-mediated resistance in *Escherichia coli* and mitigation using plant polyphenols," *Microbial Pathogenesis*, vol. 158, Art. no. 105088, 2021.

- [13] T. Mehmood, M. R. Khan, M. A. Shabbir, and M. A. Zia, "Phytochemical profiling and HPLC quantification of citrus peel from different varieties," *Progress in Nutrition*, vol. 20, no. 1-S, pp. 279–288, 2018.
- [14] V. M. Miljković, I. Nešić, M. Z. Momčilović, J. Mrmošanin, I. Gajić, J. Zvezdanović, and M. Miljković, "Chemical profile, antioxidant and antimicrobial activity of red grapefruit (*Citrus paradisi* Macf.) from Serbian market," *Journal of Food and Nutrition Research*, vol. 11, no. 12, pp. 726–736, 2023.
- [15] H. Nikaido, "Molecular basis of bacterial outer membrane permeability revisited," *Microbiology and Molecular Biology Reviews*, vol. 67, no. 4, pp. 593–656, 2003.
- [16] M. Othman, H. S. Loh, C. Wiart, T. J. Khoo, K. H. Lim, and K. N. Ting, "Optimal methods for evaluating antimicrobial activities from plant extracts," *Journal of Microbiological Methods*, vol. 84, no. 2, pp. 224–226, 2011.
- [17] S. C. Ricke, D. K. Dittoe, and K. E. Richardson, "Formulating poultry diets for reducing foodborne pathogens," *Poultry Science*, vol. 94, no. 6, pp. 1400–1410, 2015.
- [18] M. Saleem, A. I. Durani, A. Asari, and M. Ahmed, "Investigation of antioxidant and antibacterial effects of citrus fruits peels extracts using different extracting agents: Phytochemical analysis with in silico studies," *Heliyon*, vol. 9, no. 3, Art. no. e15433, 2023.
- [19] SAS Institute Inc., *SAS/STAT® 9.22 User's Guide*. Cary, NC, USA: SAS Institute Inc., 2010.
- [20] M. Viuda-Martos, Y. Ruiz-Navajas, J. Fernández-López, and J. A. Pérez-Álvarez, "Antifungal and antibacterial activity of citrus essential oils," *Journal of Food Safety*, vol. 28, no. 2, pp. 214–225, 2008.
- [21] WHO, *Global Report on Epidemiology of Foodborne Diseases*. World Health Organization, 2023.