



Determination of Total Phenolic and Flavonoid Contents in Carrot (*Daucus carota* L.) Root Extract by UV-Visible Spectrophotometry and Evaluation of Antibacterial Activity Against Gram-Positive and Gram-Negative Bacteria

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Revised: 05 August 2026 Accepted: 26 September 2026 Published: 30 September 2026 How to cite : Tanjung, S. W., Wahyuni, H. S., & Pertiwi, D. (2026). Determination of Total Phenolic and Flavonoid Contents in Carrot (<i>Daucus carota</i> L.) Root Extract by UV-Visible Spectrophotometry and Evaluation of Antibacterial Activity Against Gram-Positive and Gram-Negative Bacteria. <i>Contagion: Scientific Periodical Journal of Public Health and Coastal Health</i>, 8(3), 657–669.	<i>The increasing prevalence of antimicrobial resistance has become a major global health concern, prompting the research for plant-derived antibacterial agents. Carrot (<i>Daucus carota</i> L.) contains phenolic and flavonoid compounds that have been reported to possess antibacterial properties. However, studies integrating phytochemical quantification with antibacterial activity and membrane leakage mechanisms against both Gram-positive and Gram-negative bacteria remain limited. Carrot tubers were extracted by methanolic maceration. Total phenolic content was determined using the Folin–Ciocalteu method, while total flavonoid content was measured by the aluminum chloride colorimetric method using UV–Visible spectrophotometry. Antibacterial activity against <i>Staphylococcus aureus</i> and <i>Pseudomonas aeruginosa</i> was evaluated using the agar disc diffusion method at extract concentrations of 50, 100, 200, and 300 mg/mL. Membrane damage was assessed by measuring nucleic acid and protein leakage using UV–Visible spectrophotometry and potassium (K^+) and calcium (Ca^{2+}) ion leakage using Atomic Absorption Spectrophotometry (AAS). The extract exhibited total phenolic and total flavonoid contents of 142.2650 ± 5.7364 mg GAE/g extract and 11.2634 ± 0.1066 mg QE/g extract. Phytochemical screening confirmed the presence of flavonoids, glycosides, saponins, and triterpenoids/steroids. The extract inhibited the growth of <i>S. aureus</i> and <i>P. aeruginosa</i>, with inhibition zone diameters ranging from 8.93–11.52 mm and 8.68–10.37 mm, respectively. Statistical analysis demonstrated significant membrane leakage in <i>S. aureus</i> ($p \leq 0.05$), whereas no significant difference was observed in <i>P. aeruginosa</i> ($p > 0.05$). Increased extracellular K^+ and Ca^{2+} concentrations further supported membrane disruption as the antibacterial mechanism. The methanolic extract of <i>Daucus carota</i> L. contains substantial levels of phenolic and flavonoid compounds and exhibits antibacterial activity against both Gram-positive and Gram-negative bacteria. Its antibacterial effect appears to be associated with disruption of bacterial membrane integrity, resulting in the leakage of intracellular constituents, including nucleic acids, proteins, potassium ions, and calcium ions.</i> Keyword: <i>Daucus Carota L., Total Phenolic Content, Total Flavonoid Content, Antibacterial Activity, Staphylococcus Aureus, Pseudomonas Aeruginosa.</i>

INTRODUCTION

Antimicrobial resistance (AMR) has emerged as one of the most serious global public health challenges, diminishing the effectiveness of antibiotics and increasing morbidity, mortality, and healthcare costs. The World Health Organization has identified AMR as a priority health issue requiring the development of novel antimicrobial agents, particularly those derived from natural products. Consequently, medicinal plants have attracted growing

scientific interest as rich sources of bioactive secondary metabolites with broad-spectrum antimicrobial potential (Farhadi et al., 2019; World Health Organization, 2024).

Among medicinal plants, carrot (*Daucus carota* L.) is widely cultivated and consumed as a functional food due to its high nutritional value. In addition to being an important source of β -carotene, carrots contain phenolic acids, flavonoids, polyacetylenes, carotenoids, vitamins, and other phytochemicals that have been associated with antioxidant, anti-inflammatory, antimicrobial, and other pharmacological activities. Previous studies have demonstrated that carrot extracts exhibit inhibitory effects against several pathogenic microorganisms, highlighting their potential as natural antibacterial agents (Ismail et al., 2024).

Phenolic compounds and flavonoids are considered the principal bioactive constituents responsible for many of the biological activities of carrots. These compounds exert antibacterial effects through multiple mechanisms, including disruption of cytoplasmic membrane integrity, alteration of membrane permeability, inhibition of essential bacterial enzymes, interference with energy metabolism, suppression of biofilm formation, and inhibition of nucleic acid synthesis. The presence of multiple antibacterial targets makes phenolic and flavonoid compounds promising candidates for combating antibiotic-resistant bacteria (Farhadi et al., 2019; Xie et al., 2015).

Determination of total phenolic content (TPC) and total flavonoid content (TFC) is widely used to evaluate the phytochemical quality of medicinal plant extracts. The Folin–Ciocalteu and aluminum chloride colorimetric assays are commonly employed because of their simplicity, sensitivity, and reproducibility. Quantification of these compounds is important for elucidating the relationship between phytochemical composition and antibacterial activity (Ismail et al., 2024).

Staphylococcus aureus and *Pseudomonas aeruginosa* were selected as representative Gram-positive and Gram-negative bacteria, respectively, because both are clinically important pathogens frequently associated with healthcare-associated infections and antimicrobial resistance. While *S. aureus* is a common cause of skin, respiratory, and bloodstream infections, *P. aeruginosa* exhibits high intrinsic resistance due to its outer membrane, lipopolysaccharide barrier, multidrug efflux pumps, and biofilm-forming ability, making it particularly difficult to eradicate (Breijyeh et al., 2020).

Most previous studies on carrot have primarily focused on phytochemical composition, antioxidant activity, or antibacterial activity based on inhibition zone measurements. However, studies investigating the relationship between phenolic and

flavonoid contents and antibacterial mechanisms through membrane leakage, including nucleic acids, proteins, potassium ions, and calcium ions, remain limited. Therefore, this study aimed to determine the total phenolic and flavonoid contents of methanolic carrot extract and evaluate its antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Furthermore, membrane leakage assays were performed to elucidate the antibacterial mechanism of the extract (Ismail et al., 2024; Farhadi et al., 2019).

METHOD

This study employed an experimental laboratory design to determine the total phenolic content (TPC) and total flavonoid content (TFC) of *Daucus carota* L. extract and to evaluate its antibacterial activity against Gram-positive and Gram-negative bacteria. The antibacterial mechanism was further investigated by assessing bacterial membrane leakage through nucleic acid, protein, potassium (K^+), and calcium (Ca^{2+}) leakage assays.

Fresh carrot (*Daucus carota* L.) tubers were collected from Suka Village, Tigapanah District, Karo Regency, North Sumatra, Indonesia. Botanical identification was conducted at Herbarium Medanense (MEDA), Universitas Sumatera Utara, to confirm the taxonomic identity of the plant material before extraction.

Fresh carrot (*Daucus carota* L.) tubers were washed with running water, cut into small pieces, and dried under controlled conditions until a constant weight was obtained. The dried material was ground into powder and characterized according to the Indonesian Herbal Pharmacopoeia (Ministry of Health of the Republic of Indonesia, 2017). The powdered simplicia was extracted by maceration using methanol as the extraction solvent. The extraction process was carried out for three consecutive days with occasional stirring. The filtrate was concentrated under reduced pressure using a rotary evaporator at approximately 40°C to obtain a viscous methanolic extract, which was stored in an airtight container until further analysis (Ministry of Health of the Republic of Indonesia, 2017).

The powdered simplicia was characterized by determining: moisture content, water-soluble extractive value, ethanol-soluble extractive value, total ash, acid-insoluble ash. These parameters were evaluated according to the Indonesian Herbal Pharmacopoeia to ensure the quality of the crude drug before extraction. Qualitative phytochemical screening was performed to identify the presence of alkaloids, flavonoids, glycosides, saponins, tannins, and triterpenoids/steroids using standard phytochemical procedures described by Harborne (1998).

Total phenolic content (TPC) was determined using the Folin–Ciocalteu colorimetric assay with gallic acid as the reference standard (Singleton & Rossi, 1965; Blainski et al., 2013). Briefly, the extract solution was mixed with Folin–Ciocalteu reagent followed by sodium carbonate solution and incubated at room temperature. The absorbance was measured using a UV–Visible spectrophotometer (Shimadzu UV-1800, Japan) at the predetermined maximum wavelength. The phenolic content was calculated from the gallic acid calibration curve and expressed as milligrams of gallic acid equivalent per gram of extract (mg GAE/g extract). All analyses were performed in triplicate.

Total flavonoid content (TFC) was determined using the aluminum chloride (AlCl_3) colorimetric method with quercetin as the reference standard according to the method described by Chang et al. (2002). The extract solution was reacted with aluminum chloride reagent, incubated at room temperature, and the absorbance was measured using a UV–Visible spectrophotometer (Shimadzu UV-1800, Japan). Total flavonoid content was calculated from the quercetin calibration curve and expressed as milligrams of quercetin equivalent per gram of extract (mg QE/g extract). All measurements were performed in triplicate.

Antibacterial activity was evaluated against *Staphylococcus aureus* and *Pseudomonas aeruginosa* using the agar disc diffusion method. The bacterial culture belongs to the collection of the Microbiology Laboratory, Faculty of Pharmacy, Universitas Sumatera Utara, and was rejuvenated (subcultured) prior to use in the research. The bacterial suspension was adjusted to approximately 0.5 McFarland standard and inoculated onto Mueller–Hinton Agar (MHA). Sterile paper discs impregnated with carrot extract at concentrations of 50, 100, 200, and 300 mg/mL were placed on the agar surface. Chloramphenicol 30 μg was used as the positive control, while dimethyl sulfoxide (DMSO) served as the negative control. The plates were incubated at 37°C for 24 hours, after which the inhibition zone diameters were measured in millimeters. Each experiment was performed in triplicate.

To investigate the antibacterial mechanism, bacterial membrane damage was evaluated through leakage of intracellular constituents. To investigate the antibacterial mechanism of *Daucus carota* L. extract, bacterial membrane integrity was evaluated by measuring the leakage of intracellular constituents following previously reported membrane permeability assays.

Bacterial suspensions treated with carrot extract were centrifuged, and the resulting supernatants were analyzed using a UV–Visible spectrophotometer. Nucleic acid leakage was determined by measuring absorbance at 260 nm, whereas protein leakage was determined at

280 nm. Increased absorbance at both wavelengths indicated enhanced membrane permeability and leakage of intracellular constituents resulting from bacterial membrane damage (Alhadrami et al., 2021).

Leakage of intracellular potassium (K^+) and calcium (Ca^{2+}) ions was quantified using Atomic Absorption Spectrophotometry (AAS). The concentrations of released ions were expressed in ppm and used as indicators of membrane permeability following exposure to carrot extract (Miksusanti et al., 2009)

All experiments were performed in triplicate, and the results were expressed as mean $\pm$ standard deviation (SD). Data normality was evaluated using the Shapiro–Wilk test. Differences among treatment groups were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's Honestly Significant Difference (HSD) post hoc test. For membrane leakage data that were not normally distributed, the Mann–Whitney U test was applied. Statistical significance was established at $p < 0.05$.

RESULTS

Characteristics of Carrot Simplicia

The physicochemical characteristics of *Daucus carota* L. simplicia met the quality requirements for herbal raw materials. The moisture content, water-soluble extractive value, ethanol-soluble extractive value, total ash, and acid-insoluble ash were 9,99%, 43,52%, 27,91%, 8,45%, and 0,54%, respectively (Table 1).

Table 1.

Parameter	Result
Moisture Content	9,99%
Water-soluble extractive value	43,52%
Ethanol-soluble extractive value	27,91%
Total ash	8,45%
Acid-insoluble ash	0,54%

Phytochemical Screening

Phytochemical analysis revealed the presence of flavonoids, glycosides, saponins, and triterpenoids/steroids in the methanolic extract, whereas alkaloids and tannins were not detected (Table 2).

Table 2.

Plant Species	Phytochemical Screening Test Results					
Carrot root crude drug	Alkaloids	Flavonoids	Triterpenoids steroids	Glycosides	Saponins	Tannins
	-	+	+	+	+	-

Total Phenolic and Total Flavonoid Contents

The methanolic extract contained a relatively high amount of phenolic compounds with a total phenolic content of $142,2650 \pm 5,7364$ mg GAE/g extract (Table 3). The total flavonoid content was $11,2634 \pm 0,1066$ mg QE/g extract (Table 4).

Table 3. Measurement results of total phenolic content in carrot *Daucus carota* L. extract

Samples	Total Phenol Content (mg GAE/g extract)	Average (mg GAE/g extract)
Carrot extract P1	140,5228	$142,2650 \pm 5,7364$
Carrot extract P2	137,6015	
Carrot extract P3	148,6708	

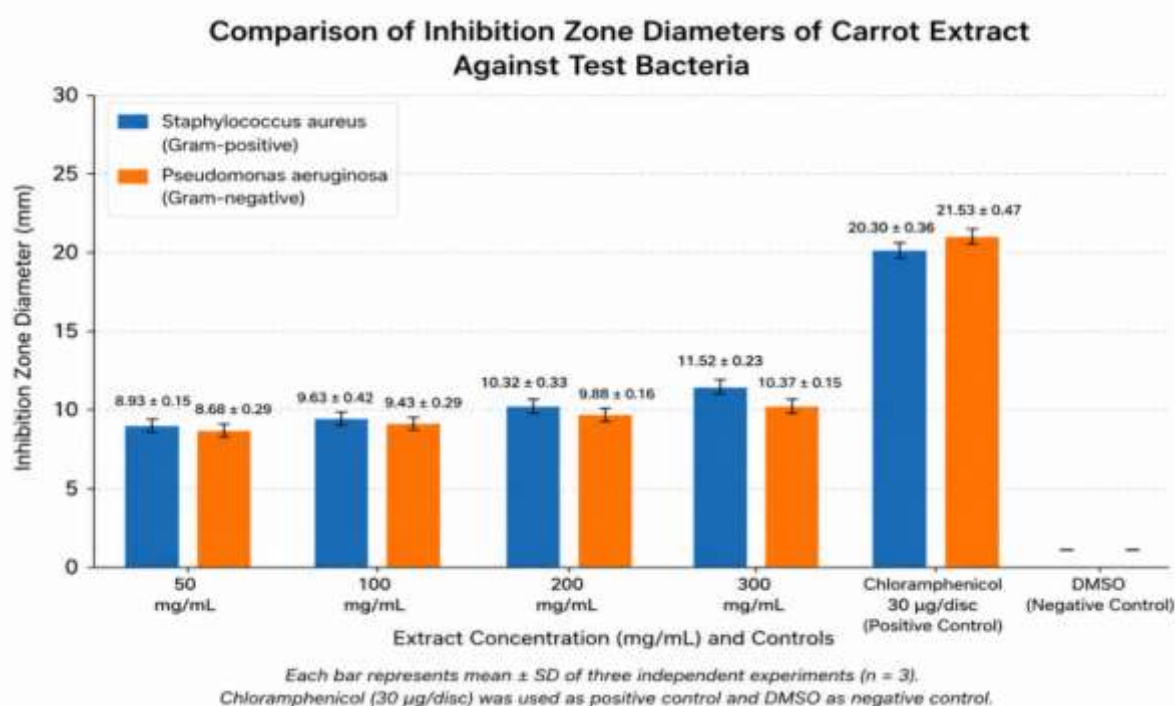
Table 4. Measurement results of total flavonoid content in carrot *Daucus carota* L. extract

Samples	Total Flavonoid Content (mg QE/g extract)	Average (mg QE/g extract)
Carrot extract P1	11,1405	$11,2634 \pm 0,1066$
Carrot extract P2	11.3194	
Carrot extract P3	11,3304	

Antibacterial Activity

Carrot extract inhibited the growth of both bacterial species in a concentration-dependent manner. The inhibition zone against *Staphylococcus aureus* ranged from 8.93 to 11.52 mm, whereas that against *Pseudomonas aeruginosa* ranged from 8.68 to 10.37 mm. One-way ANOVA showed significant differences among treatment groups ($p < 0.05$), and Tukey's HSD test confirmed significant differences between extract concentrations.

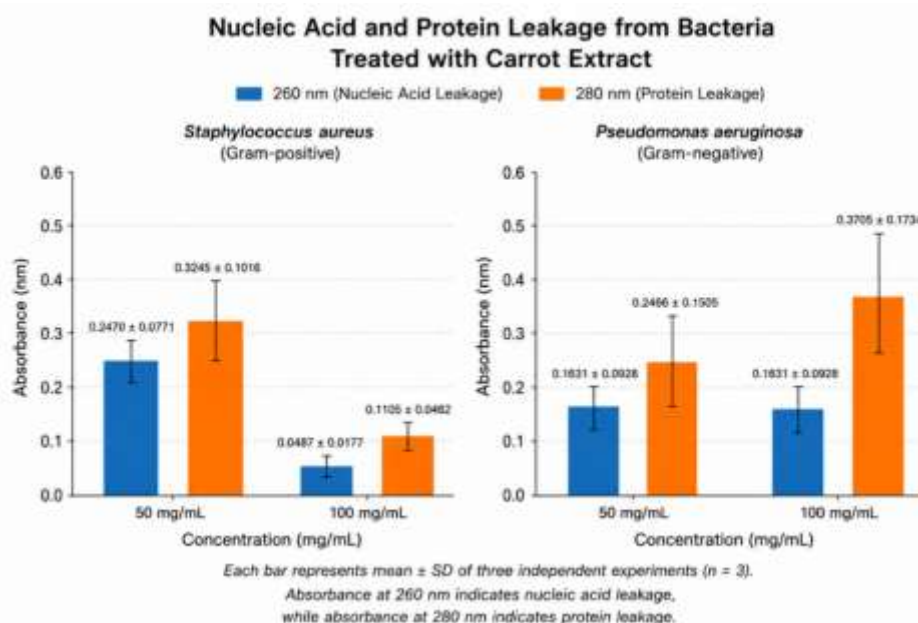
Table 5. Antibacterial activity test results of carrot (*Daucus carota* L.) extract



Nucleic Acid and Protein Leakage Assay

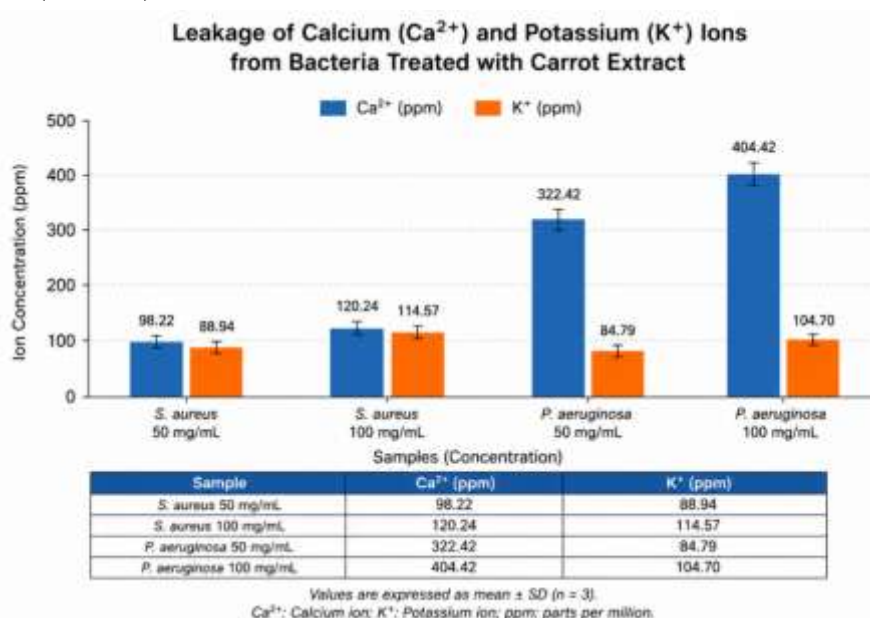
The antibacterial mechanism of carrot extract was evaluated by measuring nucleic acid and protein leakage using UV–Visible spectrophotometry at 260 nm and 280 nm, respectively. Increased absorbance at both wavelengths indicates bacterial membrane damage and leakage of intracellular components.

Table 6. Nucleic acid and Protein leakage from bacteria treated with Carrot extract



Membrane Leakage Assay

Exposure to carrot extract increased the leakage of intracellular constituents. Significant increases in nucleic acid and protein leakage were observed in *Staphylococcus aureus* ($p < 0.05$), whereas no significant differences were detected in *Pseudomonas aeruginosa* ($p > 0.05$). The extract also promoted the release of potassium (K^+) and calcium (Ca^{2+}) ions, with the highest Ca^{2+} concentration reaching 404.42 ppm in *Pseudomonas aeruginosa* (Table 7).



DISCUSSION

Discussion of *Simplicia* Characterization

The physicochemical characteristics of *Daucus carota* L. *simplicia* demonstrated that the raw material was suitable for phytochemical and antibacterial analyses. The moisture content (9.99%) complied with the recommended limit for herbal materials, indicating good storage stability by minimizing microbial growth and the degradation of bioactive compounds. The higher water-soluble extractive value (43.52%) compared with the ethanol-soluble extractive value (27.91%) suggests that carrot contains predominantly polar constituents, particularly phenolic compounds and flavonoids, which are efficiently extracted with polar solvents such as methanol (Afrellia., Puspitasari, 2023). Moreover, the low acid-insoluble ash content (0.54%) indicates minimal contamination with inorganic impurities, reflecting good processing quality. These findings are consistent with previous reports describing carrots as a rich source of polar phytochemicals and emphasizing that physicochemical standardization is essential for ensuring the quality, consistency, and reproducibility of herbal raw materials prior to biological evaluation .

Discussion of Phytochemical Screening

Phytochemical screening confirmed the presence of flavonoids, glycosides, saponins, and triterpenoids/steroids in the methanolic extract of *Daucus carota* L., whereas alkaloids and tannins were not detected. These findings indicate that carrot extract contains diverse bioactive secondary metabolites that may contribute to its antibacterial activity. Flavonoids and other phenolic constituents are recognized as the primary antibacterial compounds in carrots, acting through the disruption of bacterial membrane integrity, inhibition of nucleic acid synthesis, and interference with essential metabolic enzymes. In addition, saponins and triterpenoids may enhance membrane permeability and destabilize bacterial cell membranes, thereby enhancing the antibacterial effect. The absence of alkaloids and tannins may be attributed to variations in plant variety, environmental conditions, extraction solvent, and phytochemical detection methods. Overall, the phytochemical profile observed in this study is consistent with previous reports describing *Daucus carota* as a rich source of bioactive metabolites with antimicrobial potential (Ismail et al., 2024; Farhadi et al., 2019; Xie et al., 2015).

Discussion of Total Phenolic Content

The methanolic extract of *Daucus carota* L. contained a relatively high total phenolic content $142,2650 \pm 5,7364$ mg GAE/g extract, indicating that carrots are a rich source of phenolic compounds. This finding is consistent with the phytochemical screening results,

which confirmed the presence of flavonoids as one of the major phenolic constituents. Phenolic compounds are known to contribute to antibacterial activity through multiple mechanisms, including disruption of bacterial cell membranes, alteration of membrane permeability, inhibition of essential enzymes, and interference with cellular metabolism. Therefore, the relatively high phenolic content observed in this study may explain the concentration-dependent antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Previous studies have also reported that carrot contains abundant phenolic acids, including chlorogenic, caffeic, and ferulic acids, which contribute to their antimicrobial and antioxidant properties (Ismail et al., 2024; Dhalaria et al., 2022; Daglia, 2012).

Discussion of Total Flavonoid Content

The methanolic extract of *Daucus carota* L. contained $11,2634 \pm 0,1066$ mg QE/g extract of total flavonoids, confirming that carrot is a valuable source of flavonoid compounds. This finding is consistent with the phytochemical screening results, which identified flavonoids as one of the major secondary metabolites in the extract. Flavonoids are well recognized for their antibacterial activity through multiple mechanisms, including disruption of bacterial membrane integrity, inhibition of nucleic acid synthesis, interference with essential enzymes, and suppression of biofilm formation. Therefore, the flavonoid content observed in this study likely contributed to the concentration-dependent antibacterial activity of carrot extract against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Similar findings have been reported in previous studies demonstrating that flavonoid-rich carrot extracts possess significant antimicrobial activity against both Gram-positive and Gram-negative bacteria (Ismail et al., 2024; Farhadi et al., 2019; Xie et al., 2015).

Discussion of Antibacterial Activity

The methanolic extract of *Daucus carota* L. exhibited concentration-dependent antibacterial activity against both *Staphylococcus aureus* and *Pseudomonas aeruginosa*, as demonstrated by the increasing inhibition zone diameters with increasing extract concentration. Based on the classification proposed by Davis and Stout (1971), inhibition zones of 5–10 mm are categorized as moderate, whereas inhibition zones of 10–20 mm are classified as strong antibacterial activity. Accordingly, the extract exhibited moderate antibacterial activity at 50 and 100 mg/mL and strong activity at 200 and 300 mg/mL, indicating that higher extract concentrations enhanced antibacterial efficacy. Statistical analysis further confirmed that extract concentration significantly affected antibacterial activity ($p < 0.05$). The extract produced greater inhibition against *S. aureus* than *P.*

aeruginosa, which may be attributed to differences in bacterial cell envelope structure. Gram-positive bacteria possess a thick peptidoglycan layer without an outer membrane, facilitating the penetration of bioactive compounds, whereas the lipopolysaccharide-rich outer membrane of Gram-negative bacteria acts as an effective permeability barrier and contributes to higher intrinsic resistance. The antibacterial activity observed in this study is likely associated with the synergistic effects of phenolic and flavonoid compounds, which disrupt membrane integrity, inhibit essential enzymes, interfere with nucleic acid synthesis, and ultimately suppress bacterial growth. These findings are consistent with previous studies demonstrating the antibacterial potential of *Daucus carota* and other phenolic- and flavonoid-rich plant extracts against both Gram-positive and Gram-negative bacteria (Farhadi et al., 2019; Breijyeh et al., 2020; Ismail et al., 2024; Sabahi et al., 2024).

Discussion of Nucleic Acid and Protein Leakage

The nucleic acid and protein leakage assay demonstrated that the methanolic extract of *Daucus carota* L. altered bacterial membrane integrity, with a more pronounced effect on *Staphylococcus aureus* than *Pseudomonas aeruginosa*. In *S. aureus*, treatment with the extract significantly affected the release of nucleic acids and proteins, indicating disruption of cytoplasmic membrane permeability. In contrast, *P. aeruginosa* exhibited no significant changes in membrane leakage, suggesting that the lipopolysaccharide (LPS)-containing outer membrane limited the penetration of antibacterial compounds. The lower absorbance observed at 100 mg/mL in *S. aureus* may indicate that severe membrane damage reduced the amount of soluble intracellular components remaining in the supernatant, although this mechanism requires further investigation. These findings are consistent with the antibacterial activity observed in the agar diffusion assay and suggest that phenolic and flavonoid compounds in carrot extract exert their antibacterial effects by compromising membrane integrity, leading to leakage of intracellular macromolecules. Similar membrane-disrupting mechanisms have been reported for plant-derived phenolic compounds and flavonoids against both Gram-positive and Gram-negative bacteria (Farhadi et al., 2019; Wang et al., 2023; Alhadrami et al., 2024).

Discussion of K⁺ and Ca²⁺ Ion Leakage

The K⁺ and Ca²⁺ ion leakage assay further demonstrated that the antibacterial activity of *Daucus carota* L. extract is associated with disruption of bacterial membrane integrity. Treatment with increasing extract concentrations resulted in greater extracellular K⁺ and Ca²⁺ levels, indicating increased membrane permeability and loss of ionic homeostasis. Notably, *Pseudomonas aeruginosa* released higher levels of Ca²⁺ than *Staphylococcus aureus*, which

may be attributed to disruption of calcium-mediated stabilization of the lipopolysaccharide (LPS) layer in the outer membrane of Gram-negative bacteria. In contrast, comparable K^+ leakage observed in both bacterial species suggests that the extract also compromised cytoplasmic membrane integrity. These findings support the proposed antibacterial mechanism of carrot extract, in which phenolic and flavonoid compounds impair membrane function, resulting in ion leakage and inhibition of bacterial growth. Similar membrane-disrupting effects of plant-derived phenolic compounds have been reported in previous studies (Epand et al., 2019; Wang et al., 2023; Sabahi., 2024).

Collectively, the membrane leakage assays, including nucleic acid, protein, K^+ , and Ca^{2+} leakage, indicate that the antibacterial activity of *Daucus carota* L. extract is primarily mediated through disruption of bacterial membrane integrity, leading to leakage of intracellular constituents, loss of membrane homeostasis, and ultimately inhibition of bacterial growth.

CONCLUSION

The methanolic extract of *Daucus carota* L. exhibited appreciable total phenolic content of $142,2650 \pm 5,7364$ mg GAE/g extract and total flavonoid $11,2634 \pm 0,1066$ mg QE/g extract contents, which contributed to its concentration-dependent antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Based on the Davis and Stout classification, the extract demonstrated moderate antibacterial activity at lower concentrations and strong activity at higher concentrations. Membrane permeability assays further revealed leakage of nucleic acids, proteins, K^+ , and Ca^{2+} ions, indicating that disruption of bacterial membrane integrity is one of the principal antibacterial mechanisms of the extract. Overall, these findings suggest that methanolic carrot extract is a promising natural antibacterial agent and provide additional evidence that its antibacterial activity is closely associated with membrane damage mediated by phenolic and flavonoid compounds.

These findings provide a scientific basis for further studies on the isolation and characterization of the active antibacterial constituents of *Daucus carota* L., as well as in vivo evaluation to further validate its therapeutic potential.

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