Informatics in Biology, Health, and Food

Interdisciplinary Explorations in Life Science

Volume 3. Number 1. Jun. 2026 DOI: 10.22034/ibhf.2026.582146.1052

Original Article

Valorization of Red Onion Peel Waste: Antibacterial Activity of Its Ethanolic Extract Against MDR *Pseudomonas aeruginosa*

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Received: 2026/05/18 **Revised:** 2026/07/07 **Accepted:** 2026/07/09

Abstract

The increasing prevalence of multidrug-resistant (MDR) *Pseudomonas aeruginosa* highlights the need for alternative antimicrobial agents. This study investigated the antibacterial activity of ethanolic extract derived from red onion peel, an agricultural waste product, against clinical MDR *P. aeruginosa* isolates. Twelve clinical isolates were identified using standard microbiological and biochemical methods. Antibacterial activity was evaluated using agar well diffusion, broth microdilution, and OD_{600}-based spectrophotometric assays. The extract was tested at concentrations ranging from 100 to 0.195 mg/mL. The results demonstrated a strong concentration-dependent antibacterial effect. At 100 and 50 mg/mL, bacterial growth inhibition exceeded 90% across all isolates. At 25 and 12.5 mg/mL, a marked inter-strain variability in susceptibility was observed. Concentrations below 6.25 mg/mL showed negligible activity, comparable to the negative control ($p > 0.05$). The minimum inhibitory concentration (MIC) was defined using a modified IC90 (≥90% growth inhibition) approach due to the optical interference of crude plant extracts, with effective inhibitory activity observed mainly between 12.5 and 50 mg/mL. Overall, red onion peel ethanolic extract exhibited significant dose-dependent antibacterial activity against MDR *P. aeruginosa*, supporting its potential as a valorized bioactive waste-derived antimicrobial agent.

Keywords: Multidrug-resistant (MDR) *Pseudomonas aeruginosa, Allium cepa L.*

Cite this article: Khavari Hashemi L., Asakhi F., Vaseghi A. Valorization of Red Onion Peel Waste: Antibacterial Activity of Its Ethanolic Extract Against MDR *Pseudomonas aeruginosa*. *Informatics in Biology, Health, and Food*. 2026;3(1):63-75.

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Introduction

Antimicrobial resistance (AMR) has become one of the most pressing global health challenges of the modern era, threatening decades of progress achieved through the discovery and widespread use of antibiotics. The increasing prevalence of resistant bacterial pathogens compromises the effectiveness of currently available antimicrobial therapies and has significant consequences for patient outcomes, healthcare systems, and economic development. Recent estimates indicate that millions of deaths worldwide are associated with bacterial antimicrobial resistance each year, highlighting the urgent need for innovative strategies to combat resistant infections (1, 2). The development of new antibiotics has not kept pace with the rapid emergence of resistance, creating a growing gap between the availability of effective therapeutic agents and the clinical demand for their use. Consequently, there is increasing interest in exploring alternative antimicrobial compounds derived from natural and sustainable sources (3).

Among the bacterial pathogens responsible for healthcare-associated infections, *P.aeruginosa* remains one of the most problematic and clinically significant species. This opportunistic Gram-negative bacterium possesses remarkable metabolic versatility, enabling it to colonize diverse ecological niches and survive under harsh environmental conditions (4). In clinical settings, *P. aeruginosa* is frequently isolated from patients with ventilator-associated pneumonia, urinary tract infections, burn wound infections, surgical site infections, bloodstream infections, and chronic respiratory diseases. Individuals with compromised immune systems, prolonged hospitalization, invasive medical devices, or underlying chronic illnesses are particularly vulnerable to infection. The pathogen's ability to persist in hospital environments and contaminate medical equipment further contributes to its epidemiological importance (5-7).

The success of *P. aeruginosa* as a pathogen is largely attributed to its extensive repertoire of virulence factors and resistance mechanisms. It produces numerous extracellular enzymes and toxins, including elastases, proteases, phospholipases, pyocyanin, exotoxin A, and rhamnolipids, which contribute to host tissue damage and facilitate bacterial invasion (8). Furthermore, the organism employs sophisticated regulatory networks, including quorum sensing systems, that coordinate virulence gene expression and biofilm development. Biofilm formation represents a particularly important pathogenic strategy because bacterial cells embedded within biofilms exhibit substantially greater tolerance to antibiotics and host immune defenses than their planktonic counterparts. The extracellular polymeric matrix surrounding biofilm-associated cells restricts antimicrobial penetration and promotes the persistence of chronic infections (9, 10).

In addition to its virulence attributes, *P. aeruginosa* exhibits an extraordinary capacity to develop resistance to antimicrobial agents. Intrinsic resistance arises from the low permeability of its outer membrane, the presence of inducible chromosomal β-lactamases, and multiple multidrug efflux pump systems. Acquired resistance may result from horizontal transfer of resistance genes, chromosomal mutations, or adaptive responses triggered by environmental stress. Major mechanisms of resistance include carbapenemase production, extended-spectrum β-lactamase expression, target-site modification, reduced porin expression, enzymatic antibiotic inactivation, and overexpression of resistance-nodulation-division (RND) efflux pumps. The simultaneous presence of several resistance mechanisms frequently results in multidrug-resistant (MDR), extensively drug-resistant (XDR), or even pan-drug-resistant phenotypes, substantially limiting treatment options and increasing the likelihood of therapeutic failure (11-13).

The alarming increase in MDR *P. aeruginosa* has stimulated intensive efforts to identify novel antimicrobial compounds capable of overcoming conventional resistance mechanisms (14). Natural products remain one of the most important reservoirs of biologically active molecules and have historically contributed significantly to pharmaceutical development. Approximately half of currently available antimicrobial agents are directly

derived from or inspired by natural products (15, 16). Plant-derived metabolites, in particular, have attracted growing scientific interest because of their structural diversity, broad spectrum of biological activities, and relative safety. These compounds frequently possess multiple cellular targets, which may reduce the probability of resistance development compared with conventional antibiotics that act through a single mechanism (16, 17).

Plants synthesize a wide variety of secondary metabolites as part of their defense systems against microbial pathogens, herbivores, and environmental stresses. These compounds include phenolics, flavonoids, tannins, alkaloids, terpenoids, saponins, and sulfur-containing metabolites. Numerous studies have demonstrated that such phytochemicals exhibit antibacterial activity through diverse mechanisms, including disruption of bacterial membranes, inhibition of essential enzymes, interference with nucleic acid synthesis, induction of oxidative stress, suppression of quorum sensing, and inhibition of biofilm formation. Because bacterial resistance often develops against specific molecular targets, the multitarget nature of plant-derived compounds represents an attractive strategy for combating MDR pathogens (15, 18, 19).

Concurrently, increasing attention has been directed toward the sustainable utilization of agricultural and food-processing residues as alternative sources of valuable bioactive compounds. The global agri-food industry generates millions of tons of waste annually, creating environmental, economic, and logistical challenges. Traditional disposal methods, including landfilling and incineration, are associated with environmental pollution and the loss of potentially valuable biological resources. Consequently, the concept of waste valorization has emerged as a key component of the circular bioeconomy, promoting the conversion of agricultural by-products into high-value products such as pharmaceuticals, nutraceuticals, functional foods, cosmetics, and antimicrobial agents. Valorization strategies not only reduce environmental burdens but also contribute to resource efficiency and sustainable development (20, 21).

Among vegetable-derived agricultural wastes, onion peel has attracted considerable attention because of its abundance and rich phytochemical composition. Onion (*Allium cepa L.)* is one of the most widely cultivated and consumed vegetables worldwide, with annual production exceeding one hundred million tons (22). During processing and household preparation, the outer dry scales and peels are typically discarded, accounting for a significant proportion of onion waste. Despite being considered a low-value by-product, onion peel contains substantial amounts of bioactive compounds that are often present at higher concentrations than in the edible bulb. This characteristic makes onion peel an attractive candidate for valorization and bioactive compound recovery (23).

Red onion peel is particularly noteworthy because of its exceptionally high content of flavonoids and phenolic compounds. Quercetin and its glycosylated derivatives constitute the dominant flavonoid fraction and are largely responsible for the biological activities associated with onion peel extracts. In addition, red onion peels contain anthocyanins, phenolic acids, organosulfur compounds, dietary fibers, and various antioxidant molecules. These phytochemicals exhibit a broad range of biological properties, including antioxidant, anti-inflammatory, anticancer, cardioprotective, antiviral, and antimicrobial activities. The high concentration of these compounds in onion peel suggests that this agricultural waste may serve as a cost-effective and sustainable source of natural antimicrobial agents (24-26).

Several investigations have reported antibacterial effects of onion peel extracts against both Gram-positive and Gram-negative bacteria. Antimicrobial activity is generally attributed to synergistic interactions among multiple phytochemical constituents. Flavonoids can alter membrane permeability, destabilize bacterial cell walls, inhibit DNA gyrase activity, and interfere with energy metabolism. Phenolic acids may induce membrane damage and oxidative stress, while sulfur-containing compounds have been shown to affect essential metabolic processes and cellular redox balance. Furthermore, some onion-derived compounds have demonstrated

the ability to inhibit bacterial adhesion, suppress quorum sensing pathways, and reduce biofilm formation, properties that are particularly relevant for controlling *P. aeruginosa* infections (27-30).

The efficiency of recovering these bioactive compounds depends strongly on extraction methodology. Solvent selection is a critical factor influencing extraction yield and phytochemical composition. Ethanol is widely recognized as an effective extraction solvent because it combines relatively low toxicity, environmental compatibility, and excellent extraction efficiency for phenolic and flavonoid compounds (31). Ethanolic extraction has consistently been reported to produce extracts rich in quercetin, anthocyanins, and other bioactive metabolites responsible for antimicrobial activity. Moreover, ethanol is considered suitable for future pharmaceutical and food-related applications due to its regulatory acceptance and favorable safety profile (16, 22).

Despite growing evidence supporting the antimicrobial potential of onion peel extracts, relatively few studies have focused specifically on clinical MDR *P. aeruginosa* isolates, which represent one of the most challenging bacterial pathogens encountered in healthcare settings. Additionally, quantitative assessments of concentration-dependent antibacterial activity using spectrophotometric growth inhibition approaches remain limited (25, 32, 33). Given the increasing prevalence of MDR *P. aeruginosa* infections and the urgent demand for sustainable antimicrobial alternatives, further investigation of onion peel-derived bioactive compounds is warranted.

Therefore, the present study aimed to evaluate the antibacterial activity of ethanolic extract obtained from red onion peel waste against clinical MDR *P. aeruginosa* isolates. By integrating agar well diffusion, broth microdilution, and OD_{600}-based spectrophotometric analyses, this study sought to characterize the concentration-dependent antibacterial effects of the extract and assess the feasibility of utilizing red onion peel as a value-added agricultural by-product for the development of sustainable natural antimicrobial agents.

Materials and Methods

Plant Material and Extract Preparation

Red onion peel (ROP) waste (the dry, outer papery skins) was collected from local markets and restaurants in Mashhad, Iran. The peels were manually separated from any foreign materials and rotten parts. They were washed gently under running tap water to remove surface dust, followed by a final rinse with distilled water. The cleaned peels were shade-dried at room temperature until a constant weight was achieved and subsequently ground into a fine powder using a mechanical blender (Pars Khazar, Iran).

For phytochemical extraction from powdered plant samples, 500 g of the powdered sample was macerated using 70% ethanol as solvent in a 1:15 (w/v) ratio. The mixture was incubated for 48 h at room temperature (25 ± 2°C) under continuous agitation (80 rpm). Following extraction, the mixture was centrifuged at 3000 rpm for 15 min, and the supernatant was filtered through Whatman No. 1 filter paper to remove insoluble particles. The extract was concentrated under reduced pressure using a rotary evaporator at 40-50°C to remove the ethanol and obtain a concentrated aqueous extract. The remaining aqueous extract was then freeze-dried to yield a dry powdered ethanolic extract. The dried extract was stored in an airtight container at room temperature until further use.

Bacterial Isolates and Identification

A total of Twelve multidrug-resistant (MDR) *P. aeruginosa* isolates were obtained from various clinical specimens collected from hospitalized patients at Imam Reza Hospital, Mashhad, Iran. The isolates had been preserved at −70°C in nutrient broth containing 15–20% glycerol and were revived by streaking onto Nutrient Agar plates followed by incubation at 37°C for 24 h.

Identification of the isolates was performed using standard microbiological methods, including colony morphology, growth on Cetrimide agar, Gram staining, and biochemical characterization. The biochemical tests included oxidase and catalase activity, oxidative glucose

metabolism in oxidative–fermentative medium, citrate utilization, motility, pigment production, and growth at 42°C. Antimicrobial susceptibility testing was carried out using the Kirby–Bauer disk diffusion method against imipenem, levofloxacin, ofloxacin, cefepime, ceftriaxone, gentamicin, and piperacillin/tazobactam. Isolates resistant to at least three classes of antimicrobial agents were classified as MDR and selected for further analysis. *P. aeruginosa* ATCC 27853 was used as the quality control strain throughout the study.

Antibacterial activity of ROP extracts

For antimicrobial assays, the ROP ethanolic extract was dissolved in 100 µl ethanol (Sigma-Aldrich, Germany) and then diluted in distilled water to obtain a stock solution of 200 mg/mL. The solution was further used for agar well diffusion and minimum inhibitory concentration (MIC) assays. Bacterial inocula were prepared by suspending 3–5 freshly isolated colonies of *P. aeruginosa* in sterile 0.85% saline. The suspension was adjusted to 0.5 McFarland standard, corresponding to approximately 1.5 × 10^8 CFU/mL.

Agar Well Diffusion Assay

The antibacterial activity of the extract was initially evaluated using the agar well diffusion method on Mueller–Hinton agar (MHA). The standardized bacterial suspension was uniformly swabbed onto MHA plates and allowed to dry. Wells of 6 mm diameter were aseptically punched into the agar, and 100 µL of the extract at different concentrations (100%, 50%, and 25% of the stock solution) were added into each well. Cefixime (5 µg) was used as a control. Plates were incubated aerobically at 37°C for 18–24 h, and inhibition zone diameters were measured in millimeters. All experiments were performed in triplicate, and results were expressed as mean ± SD.

Minimum Inhibitory Concentration (MIC) Assay

The MIC of the extract was determined using the broth microdilution method in 96-well microplates according to Clinical and Laboratory Standards Institute (CLSI) guidelines, with minor modifications for natural products. Serial two-fold dilutions of the extract were prepared in Mueller–Hinton broth supplemented with 10% DMSO to enhance solubility, yielding a concentration range from 100 to 0.195 mg/mL.

Each well was inoculated with approximately 5 × 10^5 CFU/mL of the standardized bacterial suspension. Positive control wells contained bacterial suspension without extract, while negative control wells contained sterile broth only. The final volume in each well was adjusted to 200 µL. Microplates were incubated at 37°C for 18–24 h, and MIC was defined as the lowest concentration showing no visible bacterial growth.

Minimum Bactericidal Concentration (MBC)

For MBC determination, 10 µL aliquots from wells corresponding to the MIC and higher concentrations were subcultured onto Mueller–Hinton agar plates and incubated at 37°C for 24 h. The MBC was defined as the lowest concentration of the extract showing no bacterial colony growth. The nature of antibacterial activity was interpreted based on MIC/MBC relationship.

Growth Inhibition Based on Optical Density

The antibacterial effect of the red onion peel extract was evaluated based on optical density measurements at 600 nm (OD600) using a microplate reader (Bio-Rad, Hercules, CA, USA). Instead of time-dependent growth kinetics, a concentration-dependent inhibition profile was generated by measuring bacterial growth in the presence of different extract concentrations.

The percentage of growth inhibition was calculated relative to the untreated bacterial control using the following equation:

$$GI(\%) = \left(1 - \frac{OD_{\text{test}} - OD_{\text{blank}}}{OD_{\text{control}} - OD_{\text{blank}}}\right) \times 100$$

Where:
ODtest: Optical density of the sample with extract and bacteria.

ODcontrol: Optical density of the bacteria in medium (without extract).
ODblank: Optical density of the sterile medium (extract without bacteria).

Statistical Analysis

All experiments were performed in triplicate, and the results were expressed as the mean ± standard deviation (SD). Statistical analysis was performed using SPSS version 17.0 (Statistical Package for the Social Sciences). Differences between the treatment groups and controls were analyzed using nonparametric Wilcoxon test ($p < 0.05$).

Results

Identification of *P. aeruginosa* Isolates

The bacterial isolate recovered from clinical specimens was identified as *P. aeruginosa* based on standard phenotypic and microbiological characterization. On Cetrimide agar, the isolate produced characteristic colonies with blue–green pigmentation and a fruity odor (Figure 1). Microscopic examination following Gram staining revealed Gram-negative rod-shaped bacteria. Biochemical testing further confirmed the identity of the isolate, demonstrating positive reactions for oxidase and catalase activitie.

Antibacterial Activity of ROP Waste Extract

The antimicrobial activity of the red onion peel ethanolic extract was evaluated against MDR *P. aeruginosa* isolates using the agar well diffusion method and MIC assay.

Agar Well Diffusion Assay

Initial screening was performed using the agar well diffusion method at a stock concentration of 200 mg/mL, followed by serial dilutions (100%, 50%, and 25%). Figure 2 illustrates a representative well diffusion assay plate.

The extract exhibited a concentration-dependent inhibitory effect against the tested MDR isolates. At 100% concentration, the inhibition zone diameters ranged from 0.3 to 1.1 cm across different isolates. The highest inhibition zone was observed in isolates Pa.37, Pa.64, and Pa.14 (1.1 cm), whereas the lowest inhibition zone was recorded against Pa.119, Pa.86, and Pa.75 (0.3 cm). The reference strain *P. aeruginosa* PAO1 showed an inhibition zone of 0.7 cm.

At 50% concentration, antibacterial activity decreased markedly, with several isolates (e.g., Pa.25, Pa.110, Pa.119, Pa.86, Pa.149, Pa.75, Pa.121, Pa.12, and Pa.22) showing no detectable inhibition zone or only minimal inhibition (0.2–0.3 cm). At 25% concentration, inhibitory activity was further reduced, with only a limited number of isolates (Pa.37, Pa.71, Pa.64, and Pa.14) showing very small inhibition zones (0.6–0.7 cm), while most isolates exhibited no visible inhibition. These findings clearly indicate a strong concentration-dependent antibacterial effect of the extract.
In comparison, cefepime demonstrated substantially higher antibacterial activity against all isolates, with inhibition zone diameters ranging from 6 cm (Pa.84) to 33 cm (Pa.121 and Pa.22). Highly susceptible isolates included Pa.121 and Pa.22 (3.3 cm), Pa.87 (30 cm), Pa.86 (3 cm), and Pa.119, Pa.134, Pa.41, and Pa.12 (2.7–2.8 cm).

Minimum Inhibitory Concentration (MIC) Based on OD Measurement

The OD-based MIC of the red onion peel ethanolic extract was determined using a broth microdilution assay with spectrophotometric evaluation (OD_{600}). Due to the inherent color and complexity of crude plant extracts, which may interfere with visual endpoint determination, a modified OD-based approach was applied.

In this study, MIC was operationally defined as the lowest concentration of the extract that achieved ≥90% inhibition of bacterial growth compared with the untreated control (IC90 endpoint), consistent with previously reported approaches for plant-derived antimicrobial agents.

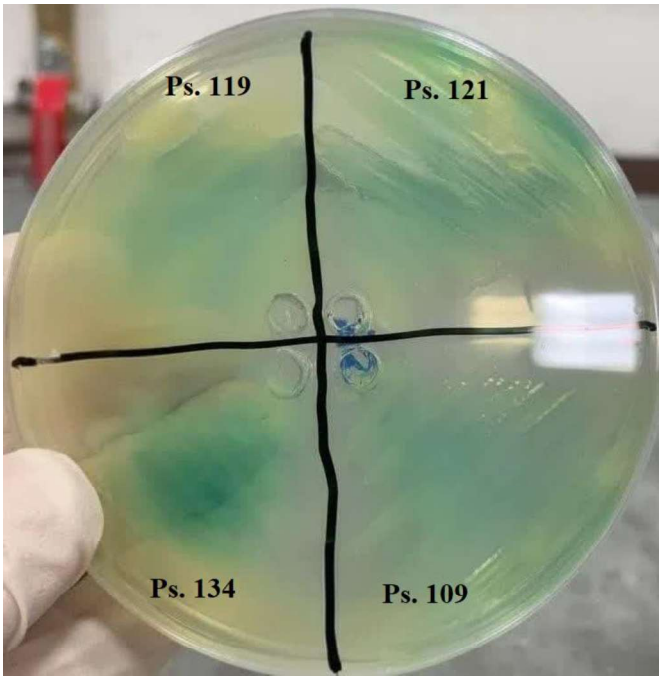

Figure 1. Growth of *P. aeruginosa* on Cetrimide agar showing characteristic colony morphology and blue–green pigment production after incubation.

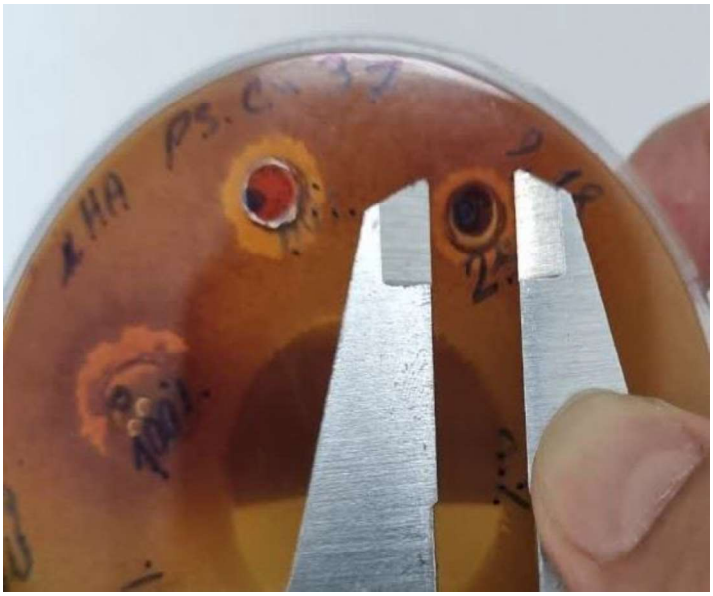

Figure 2. Agar well diffusion assay of red onion peel ethanolic extract against MDR *P. aeruginosa* isolates.

The results demonstrated a clear concentration-dependent inhibitory pattern against MDR *P. aeruginosa* isolates. At higher concentrations (up to 100 mg/mL), bacterial growth was almost completely suppressed, with OD values approaching baseline levels. As the extract concentration decreased, a progressive increase in OD600 values was observed, indicating reduced antibacterial activity.

Based on the IC90 criterion, concentrations between 12.5 and 50 mg/mL were identified as the effective inhibitory range, while concentrations below this threshold resulted in OD values comparable to the negative control, indicating loss of significant antibacterial activity (Table 1).

Dose-Dependent Growth Inhibition

Spectrophotometric analysis of 12 MDR *Pseudomonas aeruginosa* isolates revealed a strong concentration-dependent inhibitory effect of red onion peel ethanolic extract. At high concentrations (100 and 50 mg/mL), the extract exhibited potent antibacterial activity across all isolates, with OD_{600} values approaching baseline levels (mean OD ≈ 0.018–0.026), indicating near-complete growth inhibition.

At 25 mg/mL, a heterogeneous response was observed among isolates, ranging from strong inhibition (OD 0.009–0.097) to partial inhibition (OD 0.322–0.462), demonstrating significant inter-strain variability in susceptibility. A similar but more variable pattern was observed at 12.5 mg/mL, where OD values ranged from 0.037 to 0.564 (mean ≈ 0.236), indicating moderate inhibitory activity with strain-dependent differences.

A clear threshold effect was observed between 12.5 and 6.25 mg/mL. Below this concentration (6.25 mg/mL and lower), a sharp transition from partial inhibition to full bacterial growth occurred. OD values increased progressively (0.303–0.629) and were not significantly different from the negative control ($p > 0.05$), indicating loss of antibacterial activity at sub-inhibitory concentrations. Figure 3 illustrates the concentration-dependent inhibitory effect of the red onion peel ethanolic extract on *P. aeruginosa*, as reflected by OD_{600} measurements across a concentration range of 100 to 0.195 mg/mL

3.2.4 Growth Inhibition Based on Optical Density

At high concentrations (100 and 50 mg/mL), the red onion peel ethanolic extract exhibited strong antibacterial activity against all 12 MDR *P. aeruginosa* isolates. At 100 mg/mL, growth inhibition exceeded 90% across all isolates, reaching complete inhibition (100%) in isolate Ps. 109. Similarly, at 50 mg/mL, inhibition remained consistently high, ranging from 90.4% (Ps. 25) to 99.6% (Ps. 121), indicating near-complete suppression of bacterial growth within this concentration range.

Table 1. OD-based MIC and MBC values of red onion peel ethanolic extract against *P. aeruginosa* isolates.

Bacterial isolates	MIC (mg/mL)	MBC (mg/mL)
Ps. 121	50	100
Ps. 64	25	≥100
Ps. 134	12.5	≥100
Ps. 109	25	100
Ps. 41	50	≥100
Ps. 87	25	100
Ps. 14	50	≥100
Ps. 119	50	≥100
Ps. 12	50	≥100
Ps. 22	50	≥100
Ps. 75	25	≥100
Ps. 25	50	≥100

At 25 mg/mL, a pronounced transition in antibacterial response was observed, revealing clear inter-strain variability. Isolates could be broadly categorized into two groups: highly susceptible isolates, including Ps. 64 (88%), Ps. 87 (98%), Ps. 134 (92%), Ps. 109 (88.5%), and Ps. 119 (83.5%), which retained substantial growth inhibition (>80%); and less susceptible isolates, such as Ps. 121, Ps. 22, Ps. 25, and Ps. 14, which showed a marked reduction in inhibition, indicating reduced sensitivity to the extract.

At 12.5 mg/mL, the differences between susceptible and less susceptible isolates became more pronounced. Ps. 64 (84.8%) and Ps. 134 (92.7%) maintained relatively high inhibition levels, whereas Ps. 121 exhibited minimal inhibition (4.4%), and Ps. 22 (19.3%) and Ps. 25 (32.2%) showed weak antibacterial responses. This pattern highlights significant inter-strain variability in susceptibility to the extract.

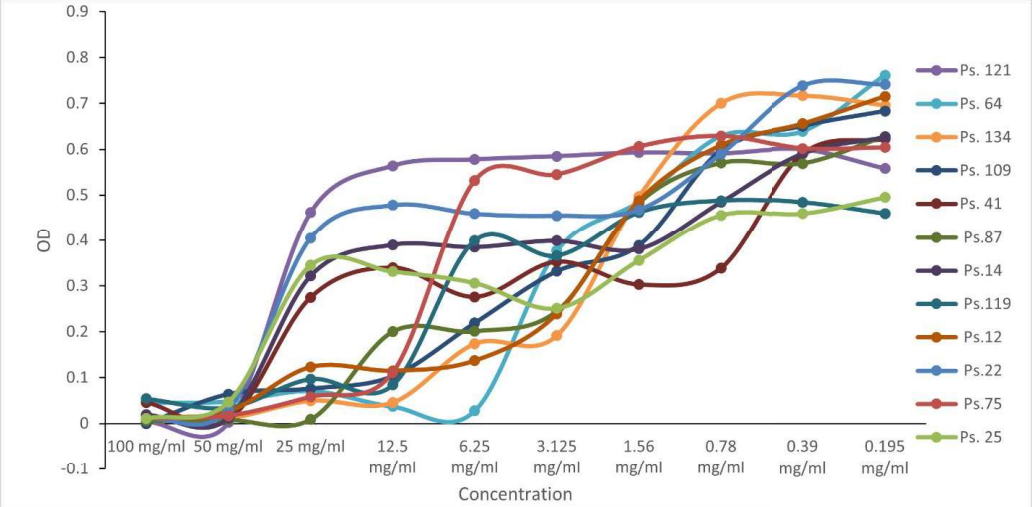

Figure 3. Dose–response relationship of red onion peel ethanolic extract on the growth of MDR *P.aeruginosa* based on OD_{600} measurements

At 6.25 mg/mL and lower concentrations, a progressive and consistent decline in inhibitory activity was observed across all isolates, confirming a clear concentration-dependent effect.

Discussion

The growing prevalence of antimicrobial resistance has renewed interest in natural products as potential alternatives or adjuncts to conventional antibiotics. Plant-derived compounds are particularly attractive because they possess diverse bioactive molecules capable of targeting multiple bacterial pathways simultaneously. Unlike many conventional antibiotics that act on a single cellular target, phytochemicals such as flavonoids, phenolic acids, and sulfur-containing compounds can disrupt membrane integrity, interfere with metabolic processes, induce oxidative stress, and inhibit quorum sensing and biofilm formation. This multitarget mode of action may reduce the likelihood of rapid resistance development and enhance activity against multidrug-resistant pathogens (15, 18).

The antibacterial activity of red onion peel is primarily attributed to its high concentration of bioactive phytochemicals, particularly flavonoids such as quercetin and quercetin glycosides, phenolic acids, anthocyanins, and sulfur-containing compounds. These metabolites have been shown to exert antimicrobial effects through multiple complementary mechanisms, including disruption of bacterial cell membrane integrity, alteration of membrane permeability, inhibition of essential enzymes, interference with nucleic acid synthesis, and induction of oxidative stress (34). The multitarget nature of these phytochemicals is particularly important in the context of multidrug-resistant bacteria, as it may reduce the probability of resistance development compared with conventional antibiotics that typically act on a single cellular target. Previous investigations have consistently demonstrated that onion peel extracts possess antibacterial activity against a broad range of Gram-positive and Gram-negative bacteria, with efficacy influenced by extraction method, phytochemical composition, and bacterial species (22, 25). Although crude onion peel extracts generally exhibit lower potency than purified antibiotics and often require relatively high concentrations to achieve effective inhibition, their antimicrobial activity highlights the potential of this agricultural by-product as a sustainable source of novel antibacterial compounds. Furthermore, the complex mixture of phytochemicals present in red onion peel may act synergistically, enhancing overall antibacterial efficacy and providing a

foundation for the development of new antimicrobial agents or antibiotic adjuvants against multidrug-resistant pathogens (34, 35).

The present study demonstrated that ethanolic extract of red onion peel exhibits significant in vitro antibacterial activity against MDR *P. aeruginosa* clinical isolates. The observed inhibitory effect was strongly concentration-dependent and consistent across both agar diffusion and OD_{600}-based assays. Importantly, measurable inhibition was achieved in all tested MDR isolates at higher extract concentrations, supporting the potential of red onion peel as a promising source of bioactive antimicrobial compounds derived from agricultural waste.

The clinical relevance of these findings is underscored by the fact that *P. aeruginosa* is recognized as a critical priority pathogen due to its intrinsic resistance to multiple antibiotic classes and its ability to persist in healthcare environments. Therapeutic options against MDR strains are increasingly limited, highlighting the urgent need for alternative or adjunct antimicrobial agents. In this context, the activity of onion peel extract suggests that plant-derived by-products may represent a valuable reservoir for future antimicrobial discovery.

A key strength of this study is the use of clinical MDR isolates rather than laboratory-adapted reference strains. Clinical isolates typically display enhanced resistance due to prolonged antibiotic exposure and adaptive evolution within hospital settings. Despite this, all isolates demonstrated susceptibility to the extract at higher concentrations, suggesting that onion-derived phytochemicals may target conserved bacterial structures or essential physiological pathways that are less susceptible to conventional resistance mechanisms.

The antibacterial effect observed in this study showed a clear dose-response relationship, with maximal inhibition at higher concentrations and progressive loss of activity upon dilution. This pattern suggests that the antimicrobial activity of onion peel extract is driven by cumulative phytochemical effects rather than a single active compound. Such multi-component activity may offer a therapeutic advantage, as simultaneous targeting of multiple bacterial pathways reduces the likelihood of rapid resistance development.

Biofilm formation represents a major barrier in the treatment of *P. aeruginosa* infections, as it significantly reduces antimicrobial penetration and enhances bacterial survival. Therefore, part of the observed inhibitory effect in this study may be related not only to direct bacteriostatic or bactericidal activity but also to interference with biofilm-associated pathways and bacterial communication systems.

The relatively high concentrations required to achieve effective inhibition (12.5–50 mg/mL) are typical for crude plant extracts. Unlike purified antibiotics, crude extracts contain complex mixtures of active, weakly active, and inactive compounds, which reduces the apparent potency of individual bioactive constituents. Nevertheless, crude extracts remain important as initial sources for bio-guided fractionation and isolation of more potent antimicrobial compounds with improved pharmacological properties.

From a translational perspective, red onion peel extract should not be considered a substitute for conventional antibiotics but rather a potential complementary agent or source of lead compounds for drug development. Its most promising application may be in combination therapy, where phytochemicals could enhance antibiotic efficacy, reduce required doses, or disrupt biofilm-associated tolerance. Such synergistic strategies are increasingly recognized as promising approaches for combating MDR Gram-negative pathogens.

The present findings are consistent with previous studies reporting antimicrobial activity of *Allium cepa* by-products. Kumar et al. demonstrated broad-spectrum antibacterial activity of onion peel extracts against both Gram-positive and Gram-negative bacteria, with variation depending on solvent type and concentration (24). Similarly, Shabir et al. reported that phenolic-rich onion peel fractions exhibit strong antimicrobial activity attributed to high flavonoid and quercetin content. These findings align with the present study, although the current work extends previous research by focusing specifically on MDR *P. aeruginosa* clinical isolates and employing both diffusion-based and quantitative OD_{600} assays (23).

Comparable dose-dependent antibacterial effects have been reported for quercetin-rich

plant extracts, where higher concentrations are required to achieve effective bacterial suppression (36). This is consistent with the present findings, where ≥90% inhibition was observed at higher concentrations. Furthermore, the intrinsic resistance mechanisms of *P. aeruginosa*, including low outer membrane permeability, efflux pumps, and biofilm formation, are well documented and explain the reduced susceptibility compared with other bacterial species (13).

The inter-strain variability observed in this study is also consistent with previous reports describing significant phenotypic and genotypic heterogeneity among clinical *P. aeruginosa* isolates, which directly influences antimicrobial susceptibility profiles. Differences in efflux activity and membrane composition are known to be key determinants of susceptibility to plant-derived compounds (37, 38).

In terms of MIC comparability, previous studies on onion-derived extracts have reported inhibitory concentrations ranging from 5 to 80 mg/mL depending on extraction method, plant part, and bacterial strain. The IC90-based effective range observed in the present study (12.5–50 mg/mL) is consistent with this range and provides a more stringent and clinically relevant estimation of inhibitory activity due to the use of MDR clinical isolates and OD-based quantification (23, 39, 40).

Conclusion

this study differs from many earlier reports in two major aspects: (i) the use of clinical MDR *P. aeruginosa* isolates instead of laboratory strains, and (ii) the integration of quantitative spectrophotometric analysis alongside diffusion assays. These methodological improvements enhance the reliability and translational relevance of the findings.

Overall, the present results support the growing evidence that onion peel is a promising and underutilized source of antimicrobial phytochemicals. However, the relatively high concentrations required for inhibition highlight the need for further optimization, including purification of active compounds, mechanistic studies, and evaluation of synergistic effects with conventional antibiotics.

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