OPEN ACCESS

Microbiological & Immunological Communications

DOI: **doi.org/10.55627/mic.003.002.0989**

Research Article

Antibacterial Activity of Bacteriophages and Extracts of *Caesalpinia decapetala* and *Parrotiopsis jacquemontiana* Against *Pseudomonas aeruginosa* and *Staphylococcus aureus*

Yousuf Muhammad, Farooq Hina*, Shah Qadeer

Department of Microbiology Hazara University Mansehra, Pakistan.
Correspondence: hinafarooq23122001@gmail.com

Abstract

The emergence of antimicrobial resistance is one of the burning issues mankind faces. The discovery of new antibiotics has declined significantly, and existing antibiotics are often ineffective against many MDR bacterial strains. Aqueous extracts of *Caesalpinia decapetala* and *Parrotiopsis jacquemontiana* were prepared in this study. Phytochemical analysis and antibacterial activity of extracts at a concentration of 6000µg/ml were examined against two multi-drug-resistant (MDR) bacterial species, Pseudomonas aeruginosa and Staphylococcus aureus. Spot assay was done to assess the lytic activity of bacteriophages against the MDR strains. The synergistic effect of extracts and bacteriophages was examined using a mixture of specific concentrations of extract and bacteriophages. Phytochemical screening revealed the presence of different phytochemicals in the root and stem extract of the plants including phenols, phlobatanins, coumarins, glycosides, and terpenoids. The well-diffusion assay revealed that the stem extract of *C. decapetala* demonstrated the highest activity against *P. aeruginosa (PA2)* with a 13mm zone of inhibition (ZOI), followed by *P. jacquemontiana* with an 11.5mm ZOI. In contrast, the lowest activity (9.7mm ZOI) was exhibited by the root extract of *C. decapetala* against *S. aureus.* The synergistic study demonstrated that the stem extracts of both *P. jacquemontiana* and *C. decapetala* exhibit inhibitory activity against the bacteriophages. The combined application of plant extract and bacteriophage against selected MDR strains led to a 60-70% reduction in the zone of inhibition compared to their individual effects, suggesting a strong antagonistic interaction.

Keywords: Antimicrobial activity, bacteriophage therapy, synergistic effect, phytochemicals, *C. decapetala*, *P. jacquemontiana*.

1. Introduction

Antimicrobial resistance has become a significant concern in medical science, as existing antibiotics are becoming increasingly ineffective against many bacterial species, such as multi-true resistant (MDR) strains of *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Plant-derived products have been used for therapeutic purposes since ancient times. Its use is increasing exponentially with the emergence of antimicrobial resistance to chemically synthesized antimicrobial agents(Parveen et al. 2014). Plants contain secondary metabolites which can be used to treat various kinds of infectious diseases (Fauci, Touchette, and Folkers 2005; Rizwan et al. 2017). A study showed that *C. decapetala* has antiviral activity, suppressing the replication of a panel of influenza A and B viruses (Zhang et al. 2020). *P. jacquemontiana* also showed a

remarkable antibacterial and antifungal impact on a panel of microorganisms in the investigation. This might be owing to the high concentrations of phytochemicals like flavonoids, saponins, alkaloids, phenols, and tannins in plant extracts (Ali et al. 2018).

Phage therapy was used a decade before penicillin's discovery by Alexander Fleming. However, the discovery of penicillin shifted the research focus to antibiotics, leaving phage therapy largely unexplored in many countries (Ackermann and Ackermann 2011). It is interesting to know that bacteriophages are more abundant with an estimate of 10^{31} -10^{32} than other organisms including bacteria on earth and every 24 hours bacteriophages kill 20% - 40% of bacteria on the surface of marine water (Suttle 2007; N. Ullah 2017). Unfortunately, shortly after the discovery of penicillin, resistant strains began to emerge, highlighting the rapid adaptability of bacteria. Today, antimicrobial resistance has escalated into a critical global issue, threatening the effectiveness of existing treatments and public health (Bartlett, Gilbert, and Spellberg 2013). Bacterial resistance to antibiotics arises mainly from their misuse. Misuse occurs due to easy access to antibiotics in pharmacies, indiscriminate use in livestock for growth promotion, and prescriptions by physicians without lab confirmation. A U.S. study found that 30% of antibiotic prescriptions were unnecessary, underscoring this issue (Fleming-Dutra et al. 2016).

To combat the increasing frequency of antibiotic resistance we urgently need new classes of antibiotics as well as researchers should pay attention to generating suitable alternatives to antibiotics. There are many substitutes which include bacteriophage therapy, herbal medicines, CRISPR-Cas9-based therapy, and short antimicrobial peptides (Merril, Scholl, and Adhya 2003; Smith and Huggins 1983). Phage therapy has shown promise in treating antibiotic-resistant infections. However, the effectiveness of phage therapy is often limited by the emergence of phage-resistant bacteria. To overcome this challenge, researchers are increasingly investigating the potential of combining phage therapy with phototherapy as a synergistic approach. While phage-antibiotic synergism has been studied in the literature(Tayyarcan et al. 2019), there is currently limited research on the possible synergistic effects of plant extracts in combination with phage therapy.

To address this gap, our study aimed to investigate the potential synergism of the extract of *C. decapetala* and *P. jacquemontiana* against two common bacterial pathogens, *P. aeruginosa* and *S. aureus*. To evaluate the broad-spectrum activity, both Gram-positive and Gram-negative species were selected. Specifically, the study focused on extracting phytochemicals from the roots and stems of these plants using a cost-effective and eco-friendly aqueous extraction method, selected based on ethnomedicinal knowledge. Furthermore, the research explored the integration of phage therapy with phytotherapy as a novel approach to combating antibiotic resistance in these bacteria.

2. Material & Methods

2.1 Chemicals and Growth Media

The study used the following chemicals: methanol, chloroform, ethanol, Mayer reagent, NH_3 solution, H_2SO_4, ferric chloride, NaOH, and iodine, all sourced from Oxoid (UK). Culture media included Luria-Bertani broth, Muller Hinton agar, Luria-Bertani agar, and MacConkey agar, also from Oxoid (UK), for bacterial growth and antibacterial testing.

2.2 Multi-Drug Resistant (MDR) Bacterial Species

The selected MDR strains used in this study were acquired from the Department of Microbiology, Hazara University, and were

revived using a protocol reported in the literature with slight modifications. Shortly, an appropriate number of colonies were transferred from the stock culture using a sterile loop streaked onto Luria-Bertani plates and then incubated at 37 ◦C for 24 h(Stachurska et al. 2021).

2.3 Antibiotics Sensitivity Profile of MDR Strands

The antibiotic sensitivity profile of selected MDR strains was determined according to the protocol provided by the American Society for Microbiology(Hudzicki 2012). Commercially available antibiotic discs, including Cefepime (5μg), Cefixime (5 μg), Ertapenem (10 μg), Streptomycin (10 μg), and Fusidic acid (10μg), were used. Bacterial cultures from the logarithmic growth phase containing approximately 1.5 x 108 CFU/ml were evenly spread on Muller-Hinton (MH) agar plates. The antibiotic discs were then placed on the inoculated plates with a standard spacing of 24 mm between the discs and gently pressed onto the agar using forceps. Subsequently, the plates were incubated for 24 hours at 37℃.

2.4 Bacteriophage Isolation

Bacteriophages against MDR *S. aureus* (SA3320) and *P. aeruginosa* (PA2) were obtained from the Department of Microbiology and Molecular Genetics, University of Punjab, Lahore, Pakistan, where they were isolated and preserved following standard protocols. To ensure stability during transport and storage, the bacteriophages were kept in sodium magnesium (SM) buffer at near-freezing temperature.

For the revival, phage stocks were thawed at room temperature (Cotton and Desiccant 2021). Then, 10 μL of each bacteriophage and 10 μL of bacterial host culture were mixed in sterile Eppendorf tubes, left at room temperature for 10–15 minutes for adsorption, and diluted to 1 mL with sterile LB broth. The mixture was incubated at 37°C for 24 hours, then centrifuged at 12,000 rpm for 5 minutes to remove cell debris, and the supernatants were collected. This process was repeated three times to increase the dominant phage titer (Tayyarcan et al. 2019).

2.5 Assessment of Lytic Activity of Bacteriophages Using Spot Assay

To assess the lytic activity of bacteriophages against MDR strains (*S. aureus* 3320 and *P. aeruginosa* 2), bacterial cultures from the logarithmic growth phase were evenly spread on Luria-Bertani (LB) agar plates. Subsequently, 10μL of the respective bacteriophages 10^6 PFU/mL were inoculated onto the prepared plates, allowing the phage mixture to flow down by keeping the plates at a slant position for a few seconds. The plates were then incubated at 37℃ for 24 hours (Rahimzadeh et al. 2020).

2.6 Plant Extract Preparation

In this study, roots and stems of *C. decapetala* and *P. jacquemontiana*, collected from Village Chamla, District Buner, Khyber Pakhtunkhwa, Pakistan, were used. Plant identification was confirmed by experts at the Department of Botany, Hazara University, Mansehra. Following a protocol reported in a previous study (Bhattacharjee et al. 2006), dried plant powder was soaked in distilled water, agitated for 72 hours at 40℃, filtered, and then evaporated to obtain the extract, which was stored for later use.

2.7 Phytochemical Analysis

For phytochemical analysis, different colorimetric tests were conducted using the protocol reported in the literature(Wadood 2013) with slight modifications. Tests were performed to detect the presence or absence of chemical compounds, such as phlobatannins, phenol, terpenoids, glycoside, carbohydrates, and anthraquinone based on characteristic color changes.

Table 1. Antibiotic Sensitivity Profile of Selected MDR Strains (R indicates resistant S indicates susceptible).

Antibiotic Used	Dose(µg)	S. aureus (SA3320)	P. aeruginosa (PA2)
cefixime	5	R	R
ertapenem	10	R	S
streptomycin	10	S	R
cefixime	5	R	R
fusidic acid	10	R	R

2.8 Antibacterial Activity of Plant Extracts

The antibacterial activity of plant extracts was evaluated using the Agar well-diffusion method as described in the literature (Ramadan et al. 2012). Bacterial suspensions of MDR *S. aureus (SA3320)* and *P. aeruginosa (PA2)* with a turbidity of 0.5 MacFarland (approximately) in LB broth were used to inoculate 90-mm-diameter Mueller-Hinton MH agar petri plates. Wells were bored using a sterile Cork-borer (6mm) and filled with 30 µl of a 6000 µg/ml extract solution prepared by dissolving extract powder in 1% DMSO. One well in each plate was filled with DMSO as a control. The plates were then incubated at 37°C for 24 hours in ambient air. The inhibition zone diameters were measured to evaluate the antibacterial activities, and the experiments were conducted in triplicate.

2.9 Synergistic Effect of Extract and Bacteriophages

For the examination of the combined effect of bacteriophages and plant extracts, we prepared pure phages at a concentration of 106 PFU /mL and stem extracts of both plants at a concentration of 6000µg/ml in Eppendorf tubes, root extracts were not used for synergistic effect study because stem extracts showed better antibacterial activity compared to root extracts. We used bacterial suspensions of selected MDR strains with a turbidity of 0.5 MacFarland (approximately) in LB broth to inoculate 90-mm-diameter MH agar petri plates. Three wells were bored using a sterile Cork-borer (6mm) in each plate. Well "A" was filled with 30 µl of pure extract solution, well "B" with 30 µl of phage, and well "C" with a mixture of 15 µl of extract solution and 15 µl of phage suspension (total 30 µl). The plates were then incubated for 24 hours at 37℃. The diameter of the zones of inhibition was measured. This approach was adapted from previous literature on similar research work (Abdelsattar et al. 2021).

3. Results

3.1 Antibiotics Sensitivity Profile of MDR Strains

The antibiotic susceptibility profile of *P. aeruginosa (PA2)* and *S. aureus (SA3320)* revealed alarming resistance patterns. As shown in Figure 1, *P. aeruginosa (PA2)* is susceptible only to ertapenem and *S. aureus (SA3320)* only to streptomycin, with both strains resistant to all other tested antibiotics. Overall, the susceptibility profiles of *P. aeruginosa (PA2)* and *S. aureus (SA3320)* are listed in Table 1.

3.2 Spot Assay

The images of petri plates shown in Figure 2 demonstrated the clear plagues indicating successful lytic activity of the isolated bacteriophages against MDR strains of *P.aeruginosa PA2* and *S. aureus SA3320*. Both the bacterial strains exhibited susceptibility to their respective phages. Three different strains of bacteriophage were tested against *S. aureus SA3320.* Among them, only one exhibited

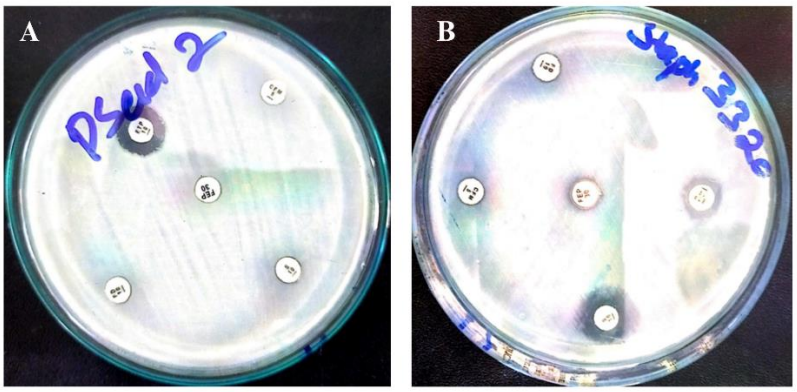

Figure 1 Antibiotic Susceptibility Test of MDR Strains (A) *P. aeruginosa* (PA2) (B) *S. aureus* (SA3320).

substantial lytic activity resulting in large clear plaque as shown in Figure 2 (A).

3.3 Phytochemical Analysis

Table 2 summarizes the result of phytochemical screening. From the findings, it can be observed that *P. jacquemontiana* extract contains phlobatannin, phenol, terpenoids, and anthraquinones. Similarly, the phytochemical screening of *C. decapetala* revealed the presence of fewer compounds including coumarins, phenols, glycosides, and terpenoids.

3.4 Antibacterial Assay of Plants Extracts

Figure 3 depicts the antibacterial activity of extracts. The findings indicated that stem extracts of both species exhibited notably stronger antibacterial activity compared to root extracts. Specifically, the stem extract of *C. decapetala* showed the highest antibacterial activity against *P. aeruginosa PA2* achieving a zone of inhibition of 13mm followed by the stem extract of *P. jacquemontiana*.

3.5 Combined Antibacterial Activity of Bacteriophages and Plant Extracts

Contrary to our initial hypothesis, our study revealed that the stem extracts of both *P. jacquemontiana C. decapetala* exhibit inhibitory activity against the bacteriophages of MDR *S. aureus(SA3320)* and *P. aeruginosa(PA2)* and the combine effect of phages and extracts against the bacteria was less than that of their individual activities. In Figure 4 it can be observed that the zone of inhibition is almost 60-70 percent less than the zone of inhibition shown by extracts and phages individually.

4. Discussion

Our findings shown in Figure 1 depict an alarming situation of antibiotic resistance in *P. aeruginosa (PA2)* and *S. aureus (SA3320)* which is consistent with previous literature (Van Nieuwenhuyse et al. 2022). These results highlight the importance of continuous monitoring of antibiotic susceptibility trends to guide empirical therapy and inform treatment procedures. The selected MDR strains were susceptible to tested phages highlighting the potential of phage therapy as a viable alternative to traditional antibiotics (Chan et al. 2016). The size of clear plaque observed in this study on the *S. aureus (SA3320)* plate was significantly larger compared to those previously reported in the literature (Rahimzadeh, Gill, and Rezai 2017). Similarly, the results indicated that among the three tested strains of bacteriophage, only one demonstrated significant lytic activity against *S. aureus SA3320*, as evidenced by the formation of large clear plaques Figure 2 (A). This finding suggests a selective interaction between the bacteriophage and the bacterial strain, highlighting the importance of phage specificity in therapeutic applications(Fujiki et al. 2023). Phage therapy has gained the attention of the scientific community as a

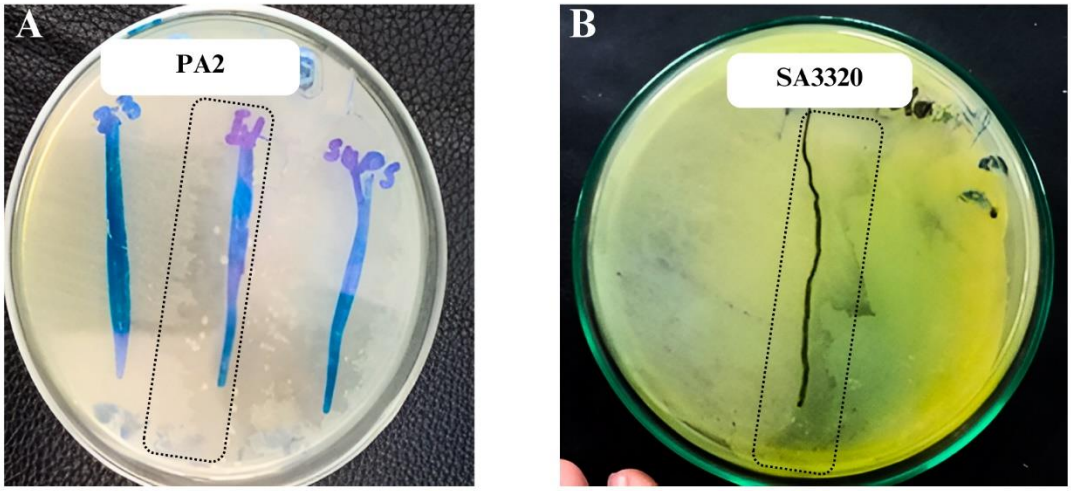

Figure 2. Bacteriophage Lytic Activity Against MDR Strains of (A) *Pseudomonas aeruginosa* PA2 and (B) *Staphylococcus aureus* SA3320.

Table 2. Phytochemical Analysis of *P. jacquemontiana and C. decapetala*. (+ indicates presence – indicates absence)

Phytochemicals	*P. jacquemontiana*	*C. decapetala*
Phlobatannins	+	-
Coumarins	-	+
Phenols	+	+
Terpenoids	+	+
Glycosides	-	+
Carbohydrates	-	-
Anthraquinones	+	-

promising alternative to combat MDR bacterial strains. And researchers are exploring various strategies to enhance the effectiveness of phage therapy, synergism of phage and phytochemicals is one of them (Gibbons, Moser, and Kaatz 2004).

The phytochemical analysis of the extract of *P. jacquemontiana* and *C. decapetala* revealed the presence of different chemical constituents which are considered medicinally important. It is observed that *P. jacquemontiana* extract contains phlorotannin, phenol, terpenoids, and anthraquinones were present. While coumarin, carbohydrates, and glycosides were not found these findings are consistent with previous studies conducted on phytochemical analysis of this plant However, unlike our study, coumarins were reported by (Ali et al. 2018).This variation highlights differences in environmental factors or extraction methods. Similarly, in *C. decapetala* fewer compounds coumarins, phenols, glycosides, and terpenoids

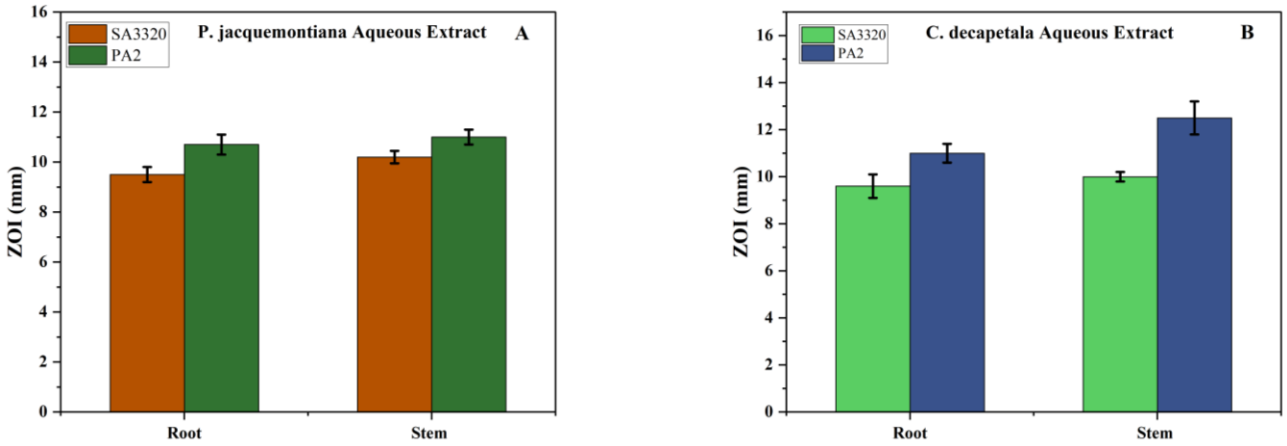

Figure 3. Antibacterial activity of Plant Extracts against selected MDR strains (A) P. *jacquemontiana (B) C. decapetala*

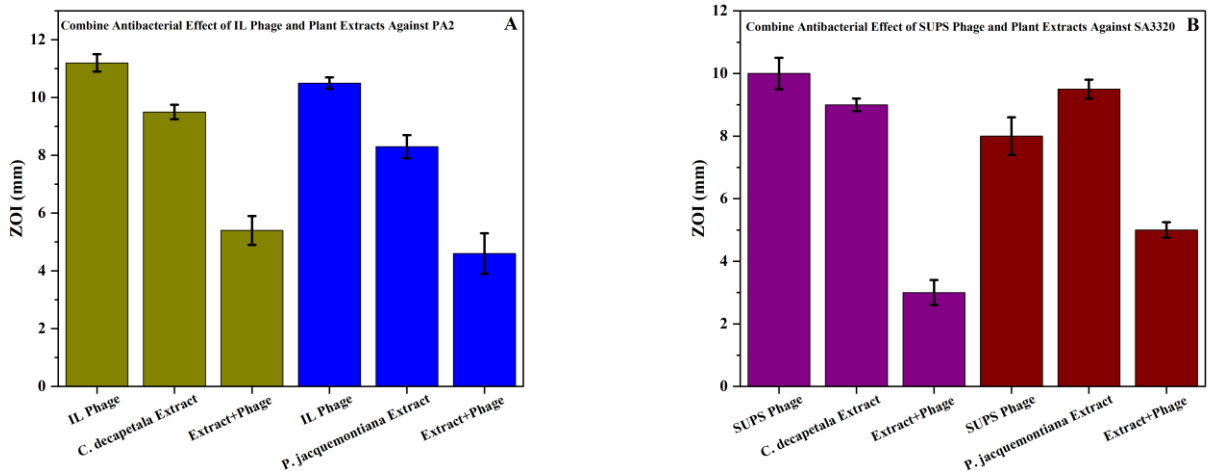

Figure 4. (A)Combined activity of phage and plant extracts against *P. aeruginosa* (B) Combined activity of phage and plant extracts against *S. aureus*.

were found. In contrast, a study on phytochemical analysis of *C. decapetala* by (Wei et al. 2013) identified a broader range of phytochemicals, including flavonoids such as quercetin and baicalein, as well as anthraquinones like emodin, utilizing gel column chromatography. The reduced number of chemical constituents in the case of our extract can be attributed to its aqueous nature as previous studies have reported aqueous extract exhibit a lower range of phytochemicals compared to other extracts (Parthasarathy et al. 2009).

As illustrated in Figure 3 stem extracts of both plants showed comparatively highest activity against the MDR strains compared to root extracts. This finding highlights the potential of stem extracts as a more effective source of bioactive compounds for combating MDR bacteria. Overall, *S. aureus (3320)* was found to be more susceptible to the plant extracts compared to *P. aeruginosa (PA2)*. The varying

susceptibility of different bacterial strains to plant extracts can be attributed to factors such as cell wall structure, resistance mechanisms, and the presence of specific phytochemicals in the extracts (Manilal et al. 2023). In contrast to previous studies where methanolic extracts demonstrated higher antibacterial activity, our study showed that aqueous extracts of *C. decapetala* and *P. jacquemontiana* exhibited potent activity against MDR bacterial strains (Ali et al. 2018; A. Ullah et al. 2023).

Bacteriophages and phytochemicals have been extensively studied as antibacterial agents. On the other hand, there are limited numbers of research on the interaction between these phytochemicals and bacteriophages when used in combination with bacteria. There are a number of explanations for synergism between phages and phytochemicals one of them is that the phytochemicals disturb the integrity and morphology of bacterial cells leading to enhanced rupturing of bacterial cells by phages and bursting out (Comeau et al. 2007). Although several studies reported in the literature have shown synergism between bacteriophages and other antimicrobial agents (Comeau et al. 2007; Ryan et al. 2012; Amal et al. 2016). However, our findings are surprising, as we anticipated that the plant extracts would enhance phage efficacy against the MDR strains. Instead, the presence of the stem extracts appears to interfere with the activity of the phages, potentially by binding to them or altering their ability to infect bacterial cells. This interaction raises important questions about the compatibility of phytochemicals and phage therapy, suggesting that phytochemicals present in these extracts inactivated the plague-forming activities of bacteriophages, and certain extracts may not only lack synergistic effects but could actively inhibit phage function. In literature, several studies have reported that these phytochemicals from natural sources have antiviral effects. In one of these studies, pomegranate peel extract and S. aureus phage showed antagonism (Tayyarcan et al. 2019). Similarly, in another study, cranberry juice showed inhibitory activity against phages (Su, Howell, and D'Souza 2010; Lipson et al. 2007). Further researches are needed to explore the interaction between these phytochemicals and phages.

5. Conclusions

In contrast to our initial hypothesis, the plant extracts showed inhibitory activity against bacteriophages, indicating a significant antagonistic interaction between these two agents. The combined application of plant extract and bacteriophage against MDR strains resulted in a 60-70% reduction in the zone of inhibition compared to their individual activities. These findings suggest that the concurrent use of plant extracts and bacteriophages may not be synergistic and need to explore their interaction in the future. The study has several limitations, including the lack of statistical analysis to validate antibacterial activity and the absence of advanced phytochemical techniques like HPLC or GC-MS for identifying active compounds. Additionally, molecular interactions between phytochemicals and bacteriophages were not explored. In the future, it is recommended to focus on advanced analysis, and molecular-level exploration of bacteriophage-phytochemical interactions to understand their synergy better and explore applications against multidrug-resistant bacteria.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Funding Statement

N/A

Ethics Approval

This study was approved by the Department of Microbiology at Hazara University Mansehra, Pakistan.

Consent Forms

NA

Data Availability

All the data related to this manuscript is available with the authors.

Author Contributions

MY; Conceptualization, Methodology Writing –original draft, Investigation, analysis, Data curation. HF**;** Conceptualization, methodology, review, and editing. QS**;** Data analysis, review, and editing.

Acknowledgments

We sincerely thank our respected teachers Dr Zeeshan Niaz and Dr Iqbal Alvi who helped us to complete this research at Hazara University Mansehra**.**

References

Abdelsattar, Abdallah S, Rana Nofal, Salsabil Makky, Anan Safwat, Amera Taha, Ayman El-Shibiny, Alan J Hibbitts, Sofia A Papadimitriou, and Carlos M Franco. 2021. "The Synergistic Effect of Biosynthesized Silver Nanoparticles and Phage ZCSE2 as a Novel Approach to Combat Multidrug-Resistant Salmonella Enterica." *Mdpi.ComAS Abdelsattar, R Nofal, S Makky, A Safwat, A Taha, A El-ShibinyAntibiotics, 2021•mdpi.Com*. https://doi.org/10.3390/antibiotics.

Ackermann, Hans-W., and Hans-W. Ackermann. 2011. "The First Phage Electron Micrographs." *Bacteriophage* 1 (4): 225–27. https://doi.org/10.4161/BACT.1.4.17280.

Ali, Saima, Muhammad Rashid Khan, Moniba Sajid, and Zartash Zahra. 2018. "Phytochemical Investigation and Antimicrobial Appraisal of Parrotiopsis Jacquemontiana (Decne) Rehder." *BMC Complementary and Alternative Medicine* 18 (1). https://doi.org/10.1186/S12906-018-2114-Z.

Amal, Amina, Mahmoud Nouraldin, Mohammad Baddour, Abdel Reem, Harfoush Hameed, Sara Abdelaziz, Mohamed Essa, et al. 2016. "Bacteriophage-Antibiotic Synergism to Control Planktonic and Biofilm Producing Clinical Isolates of Pseudomonas Aeruginosa." *Alexandria Journal of Medicine* 52 (2): 99–105. https://doi.org/10.1016/J.AJME.2015.05.002.

Bartlett, John G, David N Gilbert, and Brad Spellberg. 2013. "Seven Ways to Preserve the Miracle of Antibiotics." *Clinical Infectious Diseases* 56 (10): 1445–50. https://doi.org/10.1093/cid/cit070.

Bhattacharjee, Indranil, Soroj Kumar Chatterjee, Soumendranath Chatterjee, and Goutam Chandra. 2006. "Antibacterial Potentiality of Argemone Mexicana Solvent Extracts against Some Pathogenic Bacteria." *Memorias Do Instituto Oswaldo Cruz* 101 (6): 645–48. https://doi.org/10.1590/S0074-02762006000600011.

Chan, Benjamin K., Mark Sistrom, John E. Wertz, Kaitlyn E. Kortright, Deepak Narayan, and Paul E. Turner. 2016. "Phage Selection Restores Antibiotic Sensitivity in MDR Pseudomonas Aeruginosa." *Scientific Reports 2016 6:1* 6 (1): 1–8. https://doi.org/10.1038/srep26717.

Comeau, André M, Françoise Tétart, Sabrina N Trojet, Marie-Françoise Prère, and H M Krisch. 2007. "Phage-Antibiotic Synergy (PAS): β-Lactam and Quinolone Antibiotics Stimulate Virulent Phage Growth." *Plos One* 2 (8): e799–e799. https://doi.org/10.1371/journal.pone.0000799.

Cotton, Tip Insulator, and Cotton Desiccant. 2021. "Reviving Freeze-Dried Microorganisms Instructional Guide." www.atcc.org.

Fauci, Anthony S, Nancy A Touchette, and Gregory K Folkers. 2005. "Emerging Infectious Diseases: A 10-Year Perspective from the National Institute of Allergy and Infectious Diseases."

International Journal of Risk & Safety in Medicine 17 (3–4): 157–67. https://doi.org/10.3201/eid1104.041167.

Fleming-Dutra, Katherine E, Adam L Hersh, Daniel J Shapiro, Monina Bartoces, Eva A Enns, Thomas M File, Jonathan A Finkelstein, Jeffrey S Gerber, David Y Hyun, and Jeffrey A Linder. 2016. "Prevalence of Inappropriate Antibiotic Prescriptions among US Ambulatory Care Visits, 2010-2011." *Jama* 315 (17): 1864–73. https://doi.org/10.1001/jama.2016.4151.

Fujiki, Jumpei, Keisuke Nakamura, Tomohiro Nakamura, and Hidetomo Iwano. 2023. "Fitness Trade-Offs between Phage and Antibiotic Sensitivity in Phage-Resistant Variants: Molecular Action and Insights into Clinical Applications for Phage Therapy." *International Journal of Molecular Sciences 2023, Vol. 24, Page 15628* 24 (21): 15628. https://doi.org/10.3390/IJMS242115628.

Gibbons, Simon, Elisabeth Moser, and Glenn W. Kaatz. 2004. "Catechin Gallates Inhibit Multidrug Resistance (MDR) in Staphylococcus Aureus." *Planta Medica* 70 (12): 1240–42. https://doi.org/10.1055/S-2004-835860.

Hudzicki, Jan. 2012. "Kirby-Bauer Disk Diffusion Susceptibility Test Protocol Author Information." *American Society For Microbiology*, no. December 2009, 1–13. https://www.asm.org/Protocols/Kirby-Bauer-Disk-Diffusion-Susceptibility-Test-Pro.

Lipson, S. M., L. Sethi, P. Cohen, R. E. Gordon, I. P. Tan, A. Burdowski, and G. Stotzky. 2007. "Antiviral Effects on Bacteriophages and Rotavirus by Cranberry Juice." *Phytomedicine* 14 (1): 23–30. https://doi.org/10.1016/J.PHYMED.2006.11.009.

Manilal, Aseer, Kuzhunellil Raghavanpillai Sabu, Azene Tsefaye, Teklu Teshome, Addis Aklilu, Mohammed Seid, Gebre Kayta, Amanuel Albene Ayele, and Akbar Idhayadhulla. 2023. "Antibacterial Activity Against Multidrug-Resistant Clinical Isolates of Nine Plants from Chencha, Southern Ethiopia." *Infection and Drug Resistance* 16:2519. https://doi.org/10.2147/IDR.S402244.

Merril, Carl R, Dean Scholl, and Sankar L Adhya. 2003. "The Prospect for Bacteriophage Therapy in Western Medicine." *Nature Reviews Drug Discovery* 2 (6): 489–97. https://www.nature.com/articles/nrd1111.

Nieuwenhuyse, Brieuc Van, Dimitri Van der Linden, Olga Chatzis, Cédric Lood, Jeroen Wagemans, Rob Lavigne, Kaat Schroven, et al. 2022. "Bacteriophage-Antibiotic Combination Therapy against Extensively Drug-Resistant Pseudomonas Aeruginosa Infection to Allow Liver Transplantation in a Toddler." *Nature Communications 2022 13:1* 13 (1): 1–12. https://doi.org/10.1038/s41467-022-33294-w.

Parthasarathy, Suhanya, Juzaili Bin Azizi, Surash Ramanathan, Sabariah Ismail, Sreenivasan Sasidharan, Mohd I Mohd Said, and Sharif Mahsufi Mansor. 2009. "Evaluation of Antioxidant and Antibacterial Activities of Aqueous, Methanolic and Alkaloid Extracts from Mitragyna Speciosa (Rubiaceae Family) Leaves." *Molecules (Basel, Switzerland)* 14 (10): 3964–74. https://doi.org/10.3390/molecules14103964.

Parveen, Amna, Muhammad Sajid Hamid Akash, Kanwal Rehman, Qaisar Mahmood, and Muhammad Imran Qadir. 2014. "Analgesic, Anti-Inflammatory and Anti-Pyretic Activities of Caesalpinia Decapetala." *BioImpacts: BI* 4 (1): 43. https://doi.org/https://doi.org/10.5681/bi.2014.013.

Rahimzadeh, Golnar, Pooria Gill, and Mohammad Sadegh Rezai. 2017. "Characterization of Methicillin-Resistant Staphylococcus Aureus (MRSA) Phages From Sewage at a Tertiary Pediatric Hospital." *Archives of Pediatric Infectious Diseases 2017 5:1* 5 (1). https://doi.org/10.5812/PEDINFECT.39615.

Rahimzadeh, Golnar, Gregory Resch, Mohammad Sadegh Rezai, and Ebrahim Nemati Hevelaee. 2020. "Characterization and Lytic Activity of

Isolated Escherichia Coli Bacteriophages against Escherichia Coli in Vitro." *Iranian Journal of Medical Sciences* 45 (4): 298–303. https://doi.org/10.30476/IJMS.2019.45420.

Ramadan, A, G Soliman, Sawsan S Mahmoud, Salwa M Nofal, and Rehab F Abdel-Rahman. 2012. "Evaluation of the Safety and Antioxidant Activities of Crocus Sativus and Propolis Ethanolic Extracts." *Journal of Saudi Chemical Society* 16 (1): 13–21. https://doi.org/https://doi.org/10.1016/j.jscs.2010.10.012.

Rizwan, Muhammad, Shafaqat Ali, Muhammad Farooq Qayyum, Yong Sik Ok, Muhammad Adrees, Muhammad Ibrahim, Muhammad Zia-ur-Rehman, Mujahid Farid, and Farhat Abbas. 2017. "Effect of Metal and Metal Oxide Nanoparticles on Growth and Physiology of Globally Important Food Crops: A Critical Review." *Journal of Hazardous Materials* 322:2–16. https://doi.org/http://dx.doi.org/10.1016/j.jhazmat.2016.05.061.

Ryan, Elizabeth M., Mahmoud Y. Alkawareek, Ryan F. Donnelly, and Brendan F. Gilmore. 2012. "Synergistic Phage-Antibiotic Combinations for the Control of Escherichia Coli Biofilms in Vitro." *FEMS Immunology & Medical Microbiology* 65 (2): 395–98. https://doi.org/10.1111/J.1574-695X.2012.00977.X.

Smith, H Williams, and M B Huggins. 1983. "Effectiveness of Phages in Treating Experimental Escherichia Coli Diarrhoea in Calves, Piglets and Lambs." *Microbiology* 129 (8): 2659–75. https://doi.org/10.1099/00221287-129-8-2659.

Stachurska, Xymena, Marta Roszak, Joanna Jabłońska, Małgorzata Mizielińska, and Paweł Nawrotek. 2021. "Double-layer Agar (Dla) Modifications for the First Step of the Phage-antibiotic Synergy (Pas) Identification." *Antibiotics* 10 (11): 1306. https://doi.org/10.3390/ANTIBIOTICS10111306/S1.

Su, Xiaowei, Amy B. Howell, and Doris H. D'Souza. 2010. "The Effect of Cranberry Juice and Cranberry Proanthocyanidins on the Infectivity of Human Enteric Viral Surrogates." *Food Microbiology* 27 (4): 535–40. https://doi.org/10.1016/J.FM.2010.01.001.

Suttle, Curtis A. 2007. "Marine Viruses — Major Players in the Global Ecosystem." *Nature Reviews Microbiology 2007 5:10* 5 (10): 801–12. https://doi.org/10.1038/nrmicro1750.

Tayyarcan, Emine K., Esra Acar Soykut, Ozay Menteş Yılmaz, Ismail H. Boyaci, Maha Khaaladi, and Sami Fattouch. 2019. "Investigation of Different Interactions between Staphylococcus Aureus Phages and Pomegranate Peel, Grape Seed, and Black Cumin Extracts." *Journal of Food Safety* 39 (5): e12679. https://doi.org/10.1111/JFS.12679.

Ullah, Asad, Unays Siraj, Atif Muhammad, Muhammad Junaid, Hafsa Arif, Sidra Batool, Shakir Ullah, and Shahab Ullah. 2023. "Antimicrobial Activity of Parrotiopsis Jacquemontiana and Caesalpinia Decapetala Plant Extracts against Selected Pathogens." *Natural and Applied Sciences International Journal (NASIJ)* 4 (2): 78–93. https://doi.org/10.47264/IDEA.NASIJ/4.2.5.

Ullah, Nazif. 2017. "Medicinal Plants of Pakistan: Challenges and Opportunities." *International Journal of Complementary & Alternative Medicine* Volume 6 (Issue 4): 119–22. https://doi.org/10.15406/IJCAM.2017.06.00193.

Wadood, Abdul. 2013. "Phytochemical Analysis of Medicinal Plants Occurring in Local Area of Mardan." *Biochemistry & Analytical Biochemistry* 02 (04): 2–5. https://doi.org/10.4172/2161-1009.1000144.

Wei, Xiao Hua, Sheng Jie Yang, Na Liang, De Yu Hu, Lin Hong Jin, Wei Xue, and Song Yang. 2013. "Chemical Constituents of Caesalpinia Decapetala (Roth) Alston." *Molecules 2013, Vol.*

18, Pages 1325-1336 18 (1): 1325–36. https://doi.org/10.3390/MOLECULES18011325.

Zhang, Li, Jungang Chen, Chang Ke, Haiwei Zhang, Shoujun Zhang, Wei Tang, Chunlan Liu, et al. 2020. "Ethanol Extract of Caesalpinia Decapetala Inhibits Influenza Virus Infection In Vitro and In Vivo." *Viruses 2020, Vol. 12, Page 557* 12 (5): 557. https://doi.org/10.3390/V12050557.