# PERSPECTIVES ON THE COMBINED APPLICATION OF BACTERIOPHAGES AND PHYTOSEPTICS FOR OVERCOMING ANTIMICROBIAL RESISTANCE IN IN VITRO STUDIES

*Oleh KOLESNYK*[1] *– Marina KRYVTSOVA* [1] *– Zsolt Tibor HÖRCSIK*[2] *– Judit CSABAI*[2]

[1] Uzhhorod National University, 3 Narodna, Uzhhorod, 88000
[2] University of Nyíregyháza, 4400 Nyíregyháza, Sóstói str. 31/b
https://doi.org/10.71066/FTTMK.1.20

**Abstract**

Antimicrobial resistance is increasingly complex to treat with single antibiotic therapies. As new strains continue to develop, scientists look towards other solutions that have multiple modes of action. Among the greatest hopes are bacteriophages and plant-based antimicrobial agents (phytoseptics). This article reviews current results on the solo and combined actions of these two technologies. *In vitro* studies have revealed that bacteriophages exhibit target-specific efficacy against multiple clinical strains of bacteria, although their efficacy is often strain-specific. Plant extracts, in contrast, exhibited broader antimicrobial activity and additional anti-inflammatory and antioxidant properties. In combined use, multiple instances implied a synergistic interaction between phages and phytochemicals that augments therapeutic opportunity with reduced side effects. These findings bode well for the development of multifaceted treatment regimes that incorporate natural and biological agents to counter the rise in antibiotic resistance and refine clinical outcomes.

**Keywords:** bacteriophage therapy, phytoseptics, antimicrobial resistance, in vitro study

## Introduction

Antibiotic resistance remains one of the most pressing challenges in modern clinical microbiology and infectious disease medicine. Frequent and irrational use of antibacterial agents, including empirical prescriptions, contributes to the growing prevalence of resistant strains, reduces the effectiveness of standard therapies, and complicates the selection of optimal treatment strategies. At present, the most common causes of nosocomial and multidrug-resistant infections are represented by the group of ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp.) (Church – McKillip 2021).

In response to the significant global increase in pan-resistant strains, the World Health Organization (WHO) recognized antimicrobial resistance as a global threat in 2014 (Urban-Chmiel et al., 2022). According to preliminary projections, infectious diseases caused by antibiotic-resistant microorganisms may account for up to 10 million deaths annually by 2050. It is currently estimated that more than 70% of all microorganisms exhibit resistance to at least one commercially available antibiotic. Resistance emerges both at the hospital and community levels. Although the development of resistance is a gradual process, its dissemination is markedly accelerated by the irrational and improper use of antibiotics. The coronavirus disease (COVID-19) pandemic further intensified this issue, largely due to excessive antibiotic use (Nwobodo et al., 2022).

Numerous studies on microbial resistance demonstrate the limited efficacy of newly developed antibiotics. Consequently, current scientific efforts are increasingly focused on identifying alternative approaches to combat multidrug-resistant microorganisms (Çobanoğlu et al., 2010; et al., 2023). Equally important is the development of innovative strategies aimed at reducing the emergence of resistance or counteracting its further spread (Nwobodo et al., 2022). One of the promising directions in this context is the investigation of bacteriophage activity in combination with plant-derived extracts (phytoseptics).

## Results

*Antimicrobial Activity of Bacteriophages*

Bacteriophages have been known for more than a century. Their activity was first observed in 1896 by the British scientist Ernest Hanbury Hankin while studying the effect of water from the Ganges River on Vibrio cholerae. It was determined that the water destroyed cholera bacteria, most likely due to the presence of specific phages. During World War I, the British researcher Frederick Twort and the Franco-Canadian scientist Félix d'Hérelle independently identified lytic zones on bacterial cultures, which indicated phage activity. D'Hérelle further developed this phenomenon for the treatment of bacterial infections, marking the beginning of phage therapy (Skurnik et al., 2022).

With the rise of antibiotics in the mid-20th century, scientific and clinical interest in bacteriophages gradually declined. However, today, as multidrug-resistant strains become increasingly prevalent, phages are once again being applied in hospitals worldwide. Phage therapy is under active global investigation to develop effective combinations suitable for clinical use (Berger et al., 2024).

Studies of in vivo interactions between bacteriophages and antibiotics predominantly demonstrate a synergistic effect. At the same time, some reports describe antagonistic or neutral interactions between these antimicrobial agents. Recent scientific publications on the combined use of phages and antibiotics for treating patients with severe bacterial infections indicate improved clinical outcomes when both therapeutic modalities are applied concurrently (Łusiak-Szelachowska et al., 2022).

Extensive literature data highlight the effectiveness of bacteriophages in managing a variety of bacterial infections. They are most frequently employed in the treatment of gastrointestinal tract diseases caused by *Escherichia coli*, *Proteus* spp., *Vibrio cholerae*, *Shigella* spp., and *Salmonella* spp.. Phage therapy has also proven effective in cases of trophic ulcers, chronic wounds, diabetic foot syndrome, and cystic fibrosis. Recently, interest in phages has grown significantly in the field of traumatology, particularly for treating infections caused by antibiotic-resistant pathogens (Poniatovskyi et al., 2023).

Our own studies demonstrate high efficacy of monovalent bacteriophages against clinical isolates associated with surgical wound complications and pathogens involved in periodontal inflammatory processes. At the same time, it should be noted that phage susceptibility was strain-specific, underscoring the necessity of determining bacterial sensitivity to a given phage preparation prior to initiating treatment (Kolesnyk et al., 2024).

*Antimicrobial Activity of Medicinal Plant Extracts*

Another promising approach to overcoming antibiotic resistance is the study of plant-derived extracts. According to the World Health Organization (WHO), in nearly 80% of developing countries traditional medicine, which largely relies on plant-based remedies, is still widely used. Therefore, medicinal plants are considered a potential source of new drugs, as well as an almost unlimited reservoir of bioactive compounds. To date, more than 1,340 plants with confirmed antimicrobial activity have been identified [11].

Plant extracts exhibit antibacterial, antioxidant, and anti-inflammatory effects. The mechanisms of action of pharmacologically active ingredients of plant origin differ from those of most antibiotics. Some of these compounds not only demonstrate intrinsic antimicrobial properties but also possess the ability to modulate antibiotic resistance (e.g., a synergistic effect has been reported between Salvia spp. and *Matricaria recutita* in combination with oxacillin). Several in vitro studies have shown a reduction in the minimum inhibitory concentration (MIC) of antibiotics when administered together with plant-based preparations. This ability of plant extracts to modify microbial susceptibility to antibiotics is of particular importance in addressing resistance (Vaou et al., 2021).

Phytochemical analyses of capers (*Capparis* sp.) revealed the presence of various biologically active compounds, including spermidine, rutin, quercetin, tocopherol, and carotenoids, which are responsible for their antimicrobial, antioxidant, anti-inflammatory, and antiviral properties. Seed extracts of *Capparis decidua* have demonstrated antibacterial, antifungal, and antileishmanial activity, likely due to the presence of quaternary ammonium compounds and glucosinolates (Tlili et al., 2011).

In the scientific literature, the use of bearberry (*Arctostaphylos uva-ursi*) and cranberry juice (*Vaccinium macrocarpon*) has been reported for the treatment of urinary tract infections, while plants such as lemon balm (*Melissa officinalis*), garlic (*Allium sativum*), and tea tree (*Melaleuca alternifolia*) are described as agents with broad-spectrum antimicrobial activity (Joshi 2017).

High antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella typhi* has been demonstrated by extracts of common myrtle (*Myrtus communis*) and vervain (*Verbena officinalis*). Myrtus communis also showed strong activity against *Pseudomonas aeruginosa.* Carrot seed oil (*Daucus carota*) and tea tree oil (*Melaleuca alternifolia*) were found to be effective against *Helicobacter pylori* and *Mycoplasma pneumoniae*, respectively (Wangchuk et al., 2011).

Methanolic extracts of creeping wood sorrel (*Oxalis corniculata*), mugwort (*Artemisia vulgaris*), Indian bay leaf (*Cinnamomum tamala*), and crofton weed (*Ageratina adenophora*) exhibited inhibitory effects against *Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Citrobacter koseri* (Manandhar et al., 2019). Hydromethanolic extracts of *Berberis vulgaris*, *Cistus monspeliensis*, and *Punica granatum* have also been shown to possess significant antimicrobial activity against *Staphylococcus aureus*, *Enterococcus faecalis*, and *Enterobacter cloacae* (Bereksi et al., 2018).

Extracts of *Acmella oleracea* (L.) R. K. Jansen contains approximately 77 compounds, among which spilanthol, malic acid, and phenolic acid are the most common. These extracts also include flavonoids with notable antimicrobial and anti-inflammatory activity. Antimicrobial sensitivity testing of *Acmella* extracts demonstrated effectiveness against *Pseudomonas aeruginosa*, *Streptococcus mutans*, *Rhizopus arrhizus*, *Rhizopus stolonifer*, *Fusarium oxysporum*, and *Fusarium moniliforme*. Our studies further indicated their activity against *Candida krusei* (Csabai et al., 2024).

Research has also demonstrated pronounced antibiofilm activity of Achillea millefolium L. extracts, which are characterized by high tannin content and antioxidant properties, acting against biofilms formed by clinical biofilm-producing isolates. Their strong antioxidant activity, combined with the presence of tannins and flavonoids, supports the potential application of *Achillea millefolium* L. extracts as components of phytoseptic formulations with both antimicrobial and anti-inflammatory properties for the treatment of inflammatory processes in the oral mucosa and periodontal tissues (Kryvtsova et al., 2024).

Thyme essential oil has shown strong antibacterial activity against clinical isolates of Staphylococcus aureus. Moderate antimicrobial effects have also been reported for the essential oils of *Hyssopus officinalis*, *Menta piperita*, and *Coriandrum sativum* (Kryvtsova et al., 2017).

## Conclusion

No single strategy for combating infectious agents ensures complete effectiveness, as microorganisms are capable of rapidly adapting even to the most advanced therapeutic options. A promising solution lies in complex antibacterial therapy that integrates different mechanisms of antimicrobial action.

The combination of bacteriophages and phytoseptics may produce a synergistic effect, enhancing each other's activity while lowering the required doses, thereby reducing the risk of toxic side effects. Applying diverse approaches within one therapeutic framework also helps to prevent the selection of resistant strains. Plant-derived antiseptics add further value by providing not only antibacterial but also antioxidant and anti-inflammatory properties.

Thus, the integration of bacteriophages and phytoseptics offers promising prospects for the development of new therapeutic strategies aimed at slowing the spread of antibiotic resistance and improving the effectiveness of treatments for complex infections.

**Acknowledgements**

The authors gratefully acknowledge the support of the CEEPUS program, which enabled student and staff mobility and thereby made the joint research and publication possible.

**References**

Abdelsattar S. A. – Yakoup A. Y. – Khaled Y. – Safwat A. – El-Shibiny A.: 2023. The synergistic effect of using bacteriophages and chitosan nanoparticles against pathogenic bacteria as a novel therapeutic approach. *International Journal of Biological Macromolecules*, 228, 374–384. (https://doi.org/10.1016/j.ijbiomac.2022.12.246)

Bereksi M. – Hassaine H. – Bekhechi C. – Abdelouahid D.: 2018. Evaluation of antibacterial activity of some medicinal plants extracts commonly used in Algerian traditional medicine against some pathogenic bacteria. *Pharmacognosy Journal*, 10 (3), 507–512. (https://doi.org/10.5530/pj.2018.3.85)

Berger I. – Loewy Z. G.: 2024. Antimicrobial resistance and novel alternative approaches to conventional antibiotics. *Bacteria*, 3 (3), 171–182. (https://doi.org/10.3390/bacteria3030012)

Church N. A. – McKillip J. L.: 2021. Antibiotic resistance crisis: Challenges and imperatives. *Biologia*, 76 (5), 1535–1550. (https://doi.org/10.1007/s11756-021-00697-x)

Çobanoğlu G. – Sesal C. – Gökmen B. – Çakar S.: 2010. Evaluation of the antimicrobial properties of some lichens. South Western Journal of Horticulture, 1 (2), pp. 153–158.

Csabai J. – Kolesnyk A. – Hörcsik Z. – Kolesnyk O. – Dobránszki J. – Cziáky Z.: 2024. A comprehensive study to identify novel active components, amino acids, and antibiotic activity of *Acmella oleracea*. *Acta Biologica Szegediensis*, 15, 75–96.

Joshi R. K.: 2017. A perspective on the phytopharmaceuticals responsible for the therapeutic applications. In: Keservani R. K. – Sharma A. K. (eds.): *Recent Advances in Drug Delivery Technology*, IGI Global, pp. 208–228. (https://doi.org/10.4018/978-1-5225-1034-5.ch009)

Kolesnyk O. O. – Kryvtsova M. V. – Kharina A. V. – Mormil O. Y. – Savenko M. V.: 2024. Efficacy of bacteriophages against isolates of complicated gunshot wounds in in vitro experiments. *Intermedical Journal*, (2), 92–97. (https://doi.org/10.32782/2786-7684/2024-2-16)

Kryvtsova M. V. – Hoblyk Y. I. – Kolesnyk A. V. – Salamon I. – Miglias V. H. – Zayachuk I. P.: 2024. Peculiarities of medicinal plant raw materials: Phytochemical and antimicrobial properties of *Achillea millefolium* L. extracts. *Intermedical Journal*, (2), 100–108. (https://doi.org/10.32782/2786-7684/2024-2-17)

Kryvtsova M. V. – Livak O. H. – Balabanska B. V. – Marochka N. A. – Bilak O. M. – Hanych T. T.: 2017. Antibacterial action of essential oils on clinical isolates of opportunistic infection pathogens. *Scientific Bulletin of Uzhhorod University. Series Biology*, 42, 82–85.

Łusiak-Szelachowska M. – Międzybrodzki R. – Drulis-Kawa Z. – Cater K. – Knežević P. – Winogradow C. – et al.: 2022. Bacteriophages and antibiotic interactions in clinical practice: What we have learned so far. *Journal of Biomedical Science*, 29 (1), 23. (https://doi.org/10.1186/s12929-022-00806-1)

Manandhar S. – Luitel S. – Dahal R.: 2019. In vitro antimicrobial activity of some medicinal plants against human pathogenic bacteria. *Journal of Tropical Medicine*, 2019, 1–5. (https://doi.org/10.1155/2019/1895340)

Nwobodo D. C. – Ugwu M. C. – Anie C. O. – Al-Ouqaili M. T. S. – Ikem J. C. – Chigozie U. V. – Saki M.: 2022. Antibiotic resistance: The challenges and some emerging strategies for tackling a global menace. *Journal of Clinical Laboratory Analysis*, 36 (9), e24655. (https://doi.org/10.1002/jcla.24655)

Poniatovskyi V. A. – Shyrobokov V. P. – Bobyr V. V. – Kharina A. V.: 2023. Bacteriophages as a possible direction of fighting antibiotic-resistant strains of microorganisms. *Preventyvna medytsyna. Teoriia i praktyka*, 1 (1), 33–37.

Skurnik M.: 2022. Can bacteriophages replace antibiotics? *Antibiotics*, 11 (5), 575. (https://doi.org/10.3390/antibiotics11050575)

Tlili N. – Elfalleh W. – Saadaoui E. – Khaldi A. – Triki S. – Nasri N.: 2011. The caper (Capparis L.): Ethnopharmacology, phytochemical and pharmacological properties. *Fitoterapia*, 82 (2), 93–101. (https://doi.org/10.1016/j.fitote.2010.09.006)

Urban-Chmiel R. – Marek A. – Stępień-Pyśniak D. – Wieczorek K. – Dec M. – Nowaczek A. – Osek J.: 2022. Antibiotic resistance in bacteria—A review. *Antibiotics*, 11 (8), 1079. (https://doi.org/10.3390/antibiotics11081079)

Vaou N. – Stavropoulou E. – Voidarou C. – Tsigalou C. – Bezirtzoglou E.: 2021. Towards advances in medicinal plant antimicrobial activity: A review study on challenges and future perspectives. *Microorganisms*, 9 (10), 2041. (https://doi.org/10.3390/microorganisms9102041)

Wangchuk P. – Keller P. A. – Pyne S. G. – Taweechotipatr M. – Tonsomboon A. – Rattanajak R. – Kamchonwongpaisan S.: 2011. Evaluation of an ethnopharmacologically selected Bhutanese medicinal plants for their major classes of phytochemicals and biological activities. *Journal of Ethnopharmacology*, 137 (2), 730–742. (https://doi.org/10.1016/j.jep.2011.06.032)