Agrobiodivers Improv Nutr Health Life Qual, 9, 2025(1): 85–92

Agrobiodiversity for Improving Nutrition, Health and Life Quality

Research Article

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Antibacterial Properties of Extracts Derived from the Leaves of Black Locust (*Robinia pseudoacacia* L.)

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Article Details:
Received: 2025-02-18
Accepted: 2025-05-14
Available online: 2025-05-31

DOI: https://doi.org/10.15414/ainhlq.2025.0009

The growing resistance of bacterial pathogens to conventional antibiotics has intensified the search for alternative antimicrobial agents, particularly those derived from plants. *Robinia pseudoacacia* L. (black locust) is a plant known for its diverse phytochemical profile, including compounds with potential antibacterial activity. This study aimed to evaluate the antimicrobial effects of *R. pseudoacacia* extract against selected Gram-positive and Gram-negative bacterial strains of clinical relevance. The antibacterial activity was assessed using the Kirby-Bauer disc diffusion method on six bacterial strains: Gram-positive strains such as *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC®51299™) (resistant to vancomycin; sensitive to teicoplanin), *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC®29212™), *Staphylococcus aureus* subsp. *aureus* Rosenbach ATCC®29213™, *Staphylococcus aureus* NCTC12493™, and Gram-negative strains such as *Pseudomonas aeruginosa* (Schroeter) Migula ATCC®27853™, *Escherichia coli* (Migula) Castellani and Chalmers ATCC®25922™, and *Escherichia coli* (Migula) Castellani and Chalmers ATCC®35218™ strains. The extract showed significant inhibitory effects on *Enterococcus faecalis* ATCC®51299™ and ATCC®29212™, with statistically significant increases in inhibition zones of 40.3% and 63.6%, respectively ($p < 0.05$). In contrast, *Staphylococcus aureus* strains (ATCC®29213™ and NCTC12493™) and Gram-negative bacteria, including *Escherichia coli* and *Pseudomonas aeruginosa* showed minimal or non-significant responses to the extract. These results suggest that *R. pseudoacacia* extract contains bioactive compounds with selective antibacterial activity against *E. faecalis*, highlighting its potential as a natural source of antimicrobial agents. Further studies are required to identify the active compounds and elucidate their mechanisms of action.

Keywords: *Robinia pseudoacacia*, antimicrobial activity, *Enterococcus faecalis*, phytochemicals, Gram-positive bacteria, plant extract, antibiotic resistance

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Introduction

The rapid emergence of multidrug-resistant (MDR) bacterial strains poses a major global health challenge, compromising the efficacy of conventional antibiotics and increasing the burden of infectious diseases (Prestinaci et al., 2015). In light of this growing threat, there is renewed interest in exploring plant-derived compounds as potential alternatives or adjuncts to traditional antimicrobial therapies (Vaou et al., 2021). Medicinal plants are known to synthesise a wide range of secondary metabolites, including alkaloids, flavonoids, phenolic acids, and tannins, which may have antimicrobial properties through different mechanisms of action (Cowan, 1999; Roy et al., 2022).

Robinia pseudoacacia L., commonly known as black locust, is a leguminous tree native to North America that has been widely introduced to other continents for its ornamental, economic, and ecological value (Kato-Noguchi and Kato, 2024). It has been extensively used for timber, erosion control, and as a nitrogen-fixing species in afforestation programmes (Bolat et al., 2015). However, its high growth rate, effective clonal propagation through root suckers (ramets), prolific seed production from five to six years of age, and tolerance to diverse environmental conditions have led to its classification as an invasive species in many regions of the world (Global Invasive Species Database; Invasive Species Compendium). Its horizontal roots can extend over 60 metres and produce new shoots, allowing it to regenerate rapidly after disturbance such as clear-cutting (Vítková et al., 2015).

In addition to its invasive growth, *R. pseudoacacia* displays several adaptations, such as effective nitrogen allocation, high photosynthetic efficiency through RuBisCo and chlorophyll synthesis, and leaf movement to avoid photoinhibition (Arena et al., 2008). These traits contribute to its ecological dominance and ability to outcompete native flora. The tree also changes soil properties and alters microbial communities, including symbiotic fungi, leading to long-term shifts in plant community composition (Taniguchi et al., 2007, 2009; Sheng et al., 2017). Its dominance can reduce native biodiversity, alter canopy structure and microclimate, and cause significant changes in faunal assemblages (Sitzia et al., 2018).

While its invasiveness raises ecological concerns, *R. pseudoacacia* also contains a number of secondary metabolites with potential biological activity (Uzelac et al., 2023). These include robinin, a toxic flavonoid glycoside, and various allelochemicals that may contribute to its competitive advantage by inhibiting the germination and growth of neighbouring plant species (Kato-Noguchi and Kato, 2024). Black locust flowers are rich in phenolic compounds with well-documented health benefits, including epigallocatechin, ferulic acid, hyperoside, and rutin (also known as rutoside, quercetin-3-rutinoside, or soforin) (Lina et al., 2013; Savic Gajic et al., 2019). Epigallocatechin has antioxidant, anti-inflammatory, and anti-apoptotic effects and has shown therapeutic potential in the treatment of various renal diseases (Alam et al., 2024). Ferulic acid is widely used in dermatological products for its role in photoprotection, inhibition of melanogenesis, and promotion of wound healing (Zduńska et al., 2018). Hyperoside has shown remarkable anticancer activity against several types of malignancies, including lung, cervical, colon, and breast cancer, and also possesses anti-inflammatory, antibacterial, antiviral, antidepressant, and cardioprotective activities (Zhang et al., 2025). Rutin, a flavonoid compound, is recognised for its beneficial effects in the treatment of neurodegenerative diseases (Al-Dhabi et al., 2015; Enogieru et al., 2018). In addition, *R. pseudoacacia* flowers are a source of luteolin, gallic acid, and caffeic acid, all known for their antioxidant, anti-inflammatory, and antimicrobial properties (Imran et al., 2019; Al Zahrani et al., 2020). In traditional medicine, particularly throughout Asia, Black locust is used for its diuretic, anti-inflammatory, antispasmodic, and sedative properties, which are attributed to its diverse profile of specialized metabolites (Zhang et al., 2014). The presence of such bioactive compounds has stimulated interest in their potential pharmacological and antimicrobial applications.

Despite the ecological challenges posed by *R. pseudoacacia*, its phytochemical wealth provides an opportunity for the development of plant-based antimicrobial agents. In the face of increasing antibiotic resistance, the search for natural alternatives is gaining momentum. This study investigates the *in vitro* antimicrobial activity of *R. pseudoacacia* extract against both Gram-positive and Gram-negative bacteria, with a particular focus on clinically relevant strains such as *Enterococcus faecalis*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Understanding the antimicrobial potential of *R. pseudoacacia* may contribute not only to medical microbiology but also to the sustainable use of invasive plant species.

Materials and Methodology

Collection of Plant Material

Plant material was collected during the peak flowering season (May–June 2024) of *Robinia pseudoacacia* from the green infrastructure of the city of Chernihiv (51° 30′ N, 31° 18′ E), Ukraine. Inflorescences of species were collected to investigate their potential antibacterial and antioxidant properties. In addition, samples of bark, leaves, and flowers were collected for subsequent phytochemical and biochemical analyses under laboratory conditions.

Leaves of *R. pseudoacacia* were collected from the south-eastern outskirts of Chernihiv, specifically from the steppe slope on the south-eastern exposure of the pine terrace along the Desna River, within the Malyiv Yar tract of the Boldyn Mountains. Immediately after collection, the plant material was placed in sterile, breathable paper bags and transported to the laboratory under refrigerated conditions (4–6 °C).

Preparation of Leaf Extracts for Microbiological Analysis

Sampled leaves were carefully washed with distilled water to remove surface contaminants, then air dried on filter paper, weighed, and crushed using a mortar and pestle. Homogenisation was carried out in 96% ethanol at a leaf to solvent ratio of 1 : 10 (w/v) at room temperature. The resulting mixture was centrifuged at 3,000 × g for 5 minutes to separate the solid debris. The supernatants obtained were transferred to sterile ethanol-resistant bottles and stored at -20 °C. To prevent photodegradation of sensitive compounds, the containers were wrapped in laminated foil until further analyses were performed.

Evaluation of Antibacterial Activity Using the Disc Diffusion Method

The antibacterial properties of *Robinia pseudoacacia* leaf extracts were assessed *in vitro* using the Kirby-Bauer (1966) disc diffusion method. A detailed description of the evaluation of antibacterial activity using the disc diffusion method was noted in our previous studies (Buyun et al., 2021; Tkaczenko et al., 2024). This experiment involved testing Gram-positive strains such as *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC®51299™) (resistant to vancomycin; sensitive to teicoplanin), *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC®29212™), *Staphylococcus aureus* subsp. *aureus* Rosenbach ATCC®29213™, *Staphylococcus aureus* NCTC12493™, and Gram-negative strains such as *Pseudomonas aeruginosa* (Schroeter) Migula ATCC®27853™, *Escherichia coli* (Migula) Castellani and Chalmers ATCC®25922™, and *Escherichia coli* (Migula) Castellani and Chalmers ATCC®35218™ strains.

Bacterial strains were cultured on Mueller-Hinton (MH) agar plates to assess their susceptibility to plant extracts. Sterile filter paper discs (6 mm diameter) were impregnated with a defined volume of the test extract and carefully placed on the surface of each inoculated agar plate under aseptic conditions. The plates were incubated at 37 °C for 24 hours to allow bacterial growth and interaction with the extract. After incubation, the zones of growth inhibition around the extract-impregnated discs were observed and measured in millimetres using a precision caliper. The presence of a clear zone of inhibition was interpreted as evidence of antibacterial activity. As a negative control, filter paper discs moistened with 96% ethanol (the solvent used to prepare the extract) were placed on separate MH agar plates inoculated with the same bacterial strains. Each treatment was replicated eight times (n = 8) for statistical reliability. Photographic documentation of inhibition zones was performed using a standardised set-up to ensure consistency in comparative analysis.

The antimicrobial response of the bacterial strains to the extract was classified based on the diameter of the inhibition zone, according to the interpretation criteria proposed by Okoth et al. (2013): Susceptible (S): ≥15 mm, Intermediate (I): 10–15 mm, Resistant (R): ≤10 mm. These results provide a preliminary indication of the potential antibacterial efficacy of the tested extract against selected microbial strains.

Statistical Analysis

The diameters of the bacterial inhibition zones, expressed in millimetres, were measured for each replicate using a digital caliper. The values obtained were averaged and presented as mean ± standard error of the mean (S.E.M.) to take into account the variability within replicates. To evaluate the antibacterial efficacy of each extract, statistical analyses were performed separately for each sample, treating them as independent data sets. One-way analysis of variance (ANOVA) was used to determine statistically significant differences in mean inhibition zone diameters between bacterial strains exposed to different extracts. The ANOVA tests were performed using Statistica software, version 13.3 (TIBCO Software Inc., USA). All statistical procedures were carried out according to the methodology outlined by Zar (1999), ensuring compliance with the standard assumptions of normality and homogeneity of variances. A *p*-value less than 0.05 ($p < 0.05$) was considered statistically significant.

Results and Discussion

The mean inhibition zone diameters induced by ethanolic extracts derived from leaves of *R. pseudoacacia* against Gram-positive strains such as *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC®51299™) (resistant to vancomycin; sensitive to teicoplanin), *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC®29212™), *Staphylococcus aureus* subsp. *aureus* Rosenbach ATCC®29213™, *Staphylococcus aureus* NCTC12493™, and Gram-negative strains such as *Pseudomonas aeruginosa* (Schroeter) Migula ATCC®27853™, *Escherichia coli* (Migula) Castellani and Chalmers ATCC®25922™, and *Escherichia coli* (Migula) Castellani and Chalmers ATCC®35218™ strains were presented in Figure 1.

The application of *R. pseudoacacia* extract showed a selective antibacterial effect against the tested bacterial strains. In particular, the extract induced a statistically significant increase in the diameter of the growth inhibition zones in *Enterococcus faecalis* strains. For *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz ATCC®51299™, the zone of inhibition increased by 40.3% compared to the control (11.48 ±0.64 mm vs. 8.18 ±0.55 mm, $p < 0.05$). Similarly, for *E. faecalis* ATCC®29212™, the inhibition zone was 63.6% larger in the presence of the extract (12.32 ±0.71 mm vs. 7.53 ±0.6 mm, $p < 0.05$), indicating intermediate antimicrobial activity against these Gram-positive cocci.

In contrast, *Staphylococcus aureus* strains showed resistance to the *R. pseudoacacia* extract. Application of the extract to *S. aureus* subsp. *aureus* Rosenbach ATCC®29213™ and *S. aureus* NCTC12493™ resulted in only small and statistically non-significant increases in growth inhibition zones of 5.1 and 13.8%, respectively ($p > 0.05$). Specifically, the inhibition zones were 10.84 ±0.58 mm vs. 10.31 ±0.59 mm and 11.52 ±0.62 mm vs. 10.12 ±0.48 mm for the treated and control samples, respectively (Figure 1).

Similarly, Gram-negative bacterial strains showed minimal susceptibility to the extract. *Pseudomonas aeruginosa* (Schroeter) Migula ATCC®27853™, *Escherichia coli* (Migula) Castellani and Chalmers ATCC®25922™, and *E. coli* ATCC®35218™ showed resistance to the extract. In the case of *E. coli*

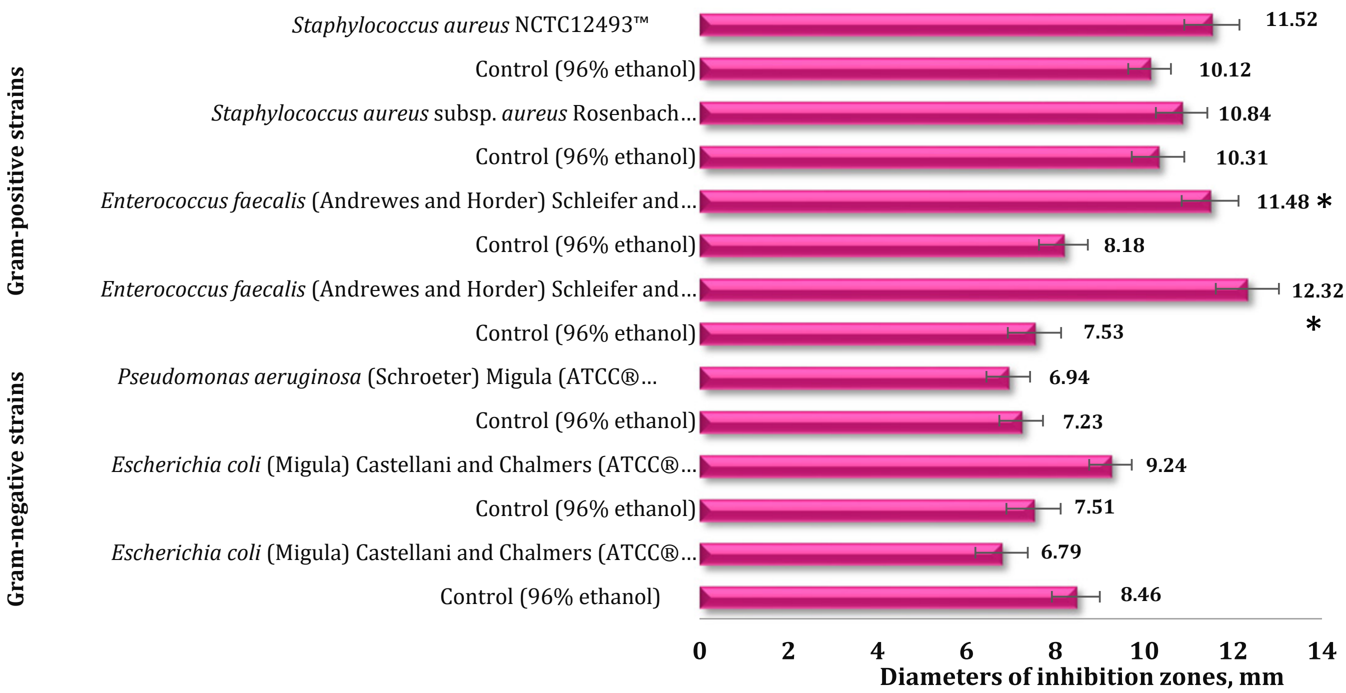

Figure 1 The mean inhibition zone diameters of *R. pseudoacacia* leaf extracts against Gram-positive and Gram-negative strains (M ±m, *n* = 8)
*– Changes were statistically significant compared to the 96% ethanol

ATCC®25922™, a 19.7% reduction in inhibition zone was observed (6.79 ±0.59 mm vs. 8.46 ±0.54 mm, p >0.05), while *E. coli* ATCC®35218™ showed a non-significant 23% increase (9.24 ±0.48 mm vs. 7.51 ±0.61 mm, p >0.05). *P. aeruginosa* ATCC®27853™ also showed resistance with inhibition zone diameters of 6.94 ±0.49 mm versus 7.23 ±0.49 mm in the control (Figure 1).

Taken together, these results demonstrate the selective antibacterial potential of *R. pseudoacacia* extract. The significant inhibition observed against *E. faecalis* strains highlights the presence of bioactive phytochemicals capable of disrupting bacterial physiology, possibly through mechanisms such as interference with peptidoglycan synthesis, disruption of membrane integrity, or inhibition of metabolic pathways (Khameneh et al., 2021). This is consistent with previous studies highlighting the efficacy of plant-derived compounds against Gram-positive cocci (Vaou et al., 2021).

Conversely, the limited or absent activity against *S. aureus* and Gram-negative bacteria suggests potential limitations in the spectrum of activity of the extract. The resistance observed in *S. aureus* may be related to well-characterised resistance mechanisms such as β-lactamase production, cell wall thickening, or the presence of efflux pumps that can neutralise or expel bioactive compounds (Lowy, 2003; Lade and Kim, 2023). For Gram-negative bacteria, the outer membrane is a formidable barrier that often prevents the uptake of hydrophobic compounds, including those derived from plant extracts (Delcour, 2009; Ghai, 2024). This structural feature is likely to contribute to the reduced susceptibility of *E. coli* and *P. aeruginosa* strains observed in this study.

The present study investigated the antibacterial activity of *R. pseudoacacia* extract against a panel of clinically relevant Gram-positive and Gram-negative bacterial strains. The results clearly demonstrate a strain-specific response, with marked susceptibility observed in *E. faecalis* strains, whereas *S. aureus* and Gram-negative bacteria showed limited or no significant response. These results support the hypothesis that the bioactive constituents of *R. pseudoacacia* possess selective antimicrobial properties, potentially making it a valuable natural alternative for targeting specific pathogens (Marinas et al., 2014). The significant increase in the growth inhibition zones of *E. faecalis* strains following treatment with the extract suggests the presence of phytochemicals capable of interfering with vital bacterial functions. This effect could be attributed to compounds such as flavonoids, tannins, or phenolic acids, which are known to interfere with bacterial cell wall synthesis, protein functions, or DNA replication (Vaou et al., 2021; De Rossi et al., 2025). The differential response between the two *E. faecalis* strains further suggests that the extract may target conserved cellular mechanisms that are highly susceptible in this genus.

In contrast, the extract showed minimal antimicrobial activity against *S. aureus*. The lack of statistically significant differences in inhibition zones suggests that *S. aureus* possesses intrinsic or acquired resistance mechanisms capable of neutralising the active components of *R. pseudoacacia*. These could include expression of efflux pumps, biofilm formation, or enzymatic degradation of plant-derived compounds (Sinha et al., 2024; Touaitia et al., 2025). In addition, differences in the cell wall structure of *S. aureus*, particularly the thick peptidoglycan layer, may contribute to reduced permeability and decreased susceptibility to phytochemicals (Wang et al., 2022; Nikolic and Mudgil, 2023).

Gram-negative bacteria, including *E. coli* and *P. aeruginosa*, showed resistance to the extract, with non-significant changes in growth inhibition zones. These results are consistent with previous reports that plant-derived compounds often show reduced efficacy against Gram-negative species (Vaou et al., 2022; Angelini, 2024). Research by Marinas et al. (2014) showed that catechin, rutin, resveratrol, and quercetin – phenolic compounds isolated from ethanolic extracts of black locust leaves – exhibited antimicrobial activity against both Gram-positive (*Staphylococcus aureus* Rosenbach, *Bacillus subtilis* Ehrenberg) and Gram-negative (*Pseudomonas aeruginosa* Schröter, *Klebsiella pneumoniae* Schroeter, and *Escherichia coli* Migula) bacterial strains. The outer membrane of these bacteria, which contains lipopolysaccharides and acts as a selective permeability barrier, probably hinders the diffusion of hydrophobic antimicrobial compounds (Zhang et al., 2013; Maher and Hassan, 2023). In addition, efflux mechanisms and enzymatic inactivation may further protect these organisms from the antimicrobial effects of plant extracts (Vaou et al., 2022; Gaurav et al., 2023).

The findings highlight the importance of bacterial cell structure and membrane composition in determining susceptibility to natural antimicrobial agents. While Gram-positive bacteria generally have a more permeable peptidoglycan layer, Gram-negative organisms have structural adaptations that confer significant resistance (Rajagopal and Walker, 2017). This suggests that the development of effective plant-based antimicrobials should take into account not only the chemical composition of the extract, but also the structural and biochemical defence systems of the target organism (Vaou et al., 2021).

Importantly, the observed antibacterial activity of *R. pseudoacacia* against *E. faecalis* is of particular clinical relevance. *E. faecalis* is an opportunistic pathogen commonly associated with nosocomial infections, including endocarditis, urinary tract infections, and root canal failure (Fiore et al., 2019). Its increasing resistance to conventional antibiotics highlights the need for alternative therapeutic strategies (Alaoui Mdarhri et al., 2022). The current findings suggest that *R. pseudoacacia* extract may serve as a promising candidate for further development into targeted antimicrobial treatments for *E. faecalis*-related infections.

Nevertheless, the limitations of the study should be acknowledged. The use of crude extract without further chemical characterisation prevents the identification of specific compounds responsible for the observed effects. Future studies should focus on phytochemical profiling, bioassay-guided fractionation, and elucidation of the mechanism of action to determine the precise mode of antibacterial activity. In addition, the cytotoxicity and pharmacokinetics of *R. pseudoacacia* extract need to be evaluated before its potential therapeutic application. These results provide valuable insight into the potential use of *R. pseudoacacia* extract as a natural antimicrobial agent, particularly against *E. faecalis*. However, the limited efficacy against other clinically relevant pathogens highlights the need for further phytochemical characterisation and optimisation of extract formulations to broaden the antimicrobial spectrum.

Conclusions

In conclusion, this study provides preliminary but promising evidence that *Robinia pseudoacacia* extract has significant antibacterial activity against *E. faecalis*, while its efficacy against *S. aureus* and Gram-negative bacteria remains limited. The differences in antibacterial susceptibility are likely to be due to structural and biochemical differences in the bacterial cell envelope, including the protective outer membrane in Gram-negative bacteria. The results suggest that *R. pseudoacacia* extract may contain bioactive compounds that selectively target Gram-positive bacteria, particularly *E. faecalis*. Given the clinical importance of *E. faecalis* and its resistance to conventional antibiotics, *R. pseudoacacia* extract warrants further investigation as a potential alternative antimicrobial agent. Future studies should aim to identify the active constituents of the extract, evaluate their mechanisms of action, and determine their safety and efficacy in clinical applications. These findings contribute to the growing body of literature on the selective antimicrobial potential of plant compounds and support further investigation of *R. pseudoacacia* as a natural antimicrobial agent.

Conflicts of Interest

The authors have no competing interests to declare.

Ethical Statement

This article does not include any studies that would require an ethical statement.

Funding

This study was funded by the statutory activities of the Institute of Biology at the Pomeranian University in Słupsk (Poland) and the International Visegrad Fund. The authors would like to express their sincere gratitude for this support.

References

Al Zahrani, N. A., El-Shishtawy, R. M., & Asiri, A. M. (2020). Recent developments of gallic acid derivatives and their hybrids in medicinal chemistry: A review. *European journal of medicinal chemistry*, 204, 112609. https://doi.org/10.1016/j.ejmech.2020.112609

Alam, M., Gulzar, M., Akhtar, M. S., Rashid, S., Zulfareen, Tanuja, Shamsi, A., & Hassan, M. I. (2024). Epigallocatechin-3-gallate therapeutic potential in human diseases: molecular mechanisms and clinical studies. *Molecular biomedicine*, 5(1), 73. https://doi.org/10.1186/s43556-024-00240-9

Alaoui Mdarhri, H., Benmessaoud, R., Yacoubi, H., Seffar, L., Guennouni Assimi, H., Hamam, M., Boussettine, R., Filali-Ansari, N., Lahlou, F. A., Diawara, I., Ennaji, M. M., & Kettani-Halabi, M. (2022). Alternatives therapeutic approaches to conventional antibiotics: Advantages, limitations and potential application in medicine. *Antibiotics* (Basel, Switzerland), 11(12), 1826. https://doi.org/10.3390/antibiotics11121826

Al-Dhabi, N. A., Arasu, M. V., Park, C. H., & Park, S. U. (2015). An up-to-date review of rutin and its biological and pharmacological activities. *EXCLI journal*, 14, 59–63. https://doi.org/10.17179/excli2014-663

Angelini P. (2024). Plant-derived antimicrobials and their crucial role in combating antimicrobial resistance. *Antibiotics* (Basel, Switzerland), 13(8), 746. https://doi.org/10.3390/antibiotics13080746

Arena, C., Vitale, L., & De Santo, A. V. (2008). Paraheliotropism in *Robinia pseudoacacia* L.: an efficient strategy to optimise photosynthetic performance under natural environmental conditions. *Plant biology* (Stuttgart, Germany), 10(2), 194–201. https://doi.org/10.1111/j.1438-8677.2008.00032.x

Bauer, A. W., Kirby, W. M., Sherris, J. C., & Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *American journal of clinical pathology*, 45(4), 493–496.

Bolat, I., Kara, O., Sensoy, H., & Yüksel, K. (2015). Influences of Black Locust (*Robinia pseudoacacia* L.) afforestation on soil microbial biomass and activity. *iForest – Biogeosciences and Forestry*, 9(1), 171–177. https://doi.org/10.3832/ifor1410-007

Buyun, L., Tkachenko, H., Kurhaluk, N., Gyrenko, O., Kovalska, L., & Góralczyk A. (2021). Evaluation of antibacterial activity of the ethanolic extracts derived from leaves of *Coelogyne brachyptera* Rchb. f. (Orchidaceae). *Agrobiodiversity for Improving Nutrition, Health, and Life Quality*, 5(1), 37–46. https://doi.org/10.15414/ainhlq.2021.0005

Cowan M. M. (1999). Plant products as antimicrobial agents. *Clinical microbiology reviews*, 12(4), 564–582. https://doi.org/10.1128/CMR.12.4.564

De Rossi, L., Rocchetti, G., Lucini, L., & Rebecchi, A. (2025). Antimicrobial potential of polyphenols: Mechanisms of action and microbial eesponses – A narrative review. *Antioxidants*, 14(2), 200. https://doi.org/10.3390/antiox14020200

Delcour A. H. (2009). Outer membrane permeability and antibiotic resistance. *Biochimica et biophysica acta*, 1794(5), 808–816. https://doi.org/10.1016/j.bbapap.2008.11.005

Enogieru, A. B., Haylett, W., Hiss, D. C., Bardien, S., & Ekpo, O. E. (2018). Rutin as a potent antioxidant: Implications for neurodegenerative disorders. *Oxidative medicine and cellular longevity*, 2018, 6241017. https://doi.org/10.1155/2018/6241017

Fiore, E., Van Tyne, D., & Gilmore, M. S. (2019). Pathogenicity of *Enterococci*. *Microbiology spectrum*, 7(4). https://doi.org/10.1128/microbiolspec.GPP3-0053-2018

Gaurav, A., Bakht, P., Saini, M., Pandey, S., & Pathania, R. (2023). Role of bacterial efflux pumps in antibiotic resistance, virulence, and strategies to discover novel efflux pump inhibitors. *Microbiology* (Reading, England), 169(5), 001333. https://doi.org/10.1099/mic.0.001333

Ghai, I. (2024). Electrophysiological Insights into antibiotic translocation and resistance: The impact of outer membrane proteins. *Membranes*, 14(7), 161. https://doi.org/10.3390/membranes14070161

Global Invasive Species Database, Species Profile: *Robinia pseudoacacia*. Available online: https://www.iucngisd.org/gisd/species.php?sc=572 (accessed on 14 April 2025).

Imran, M., Rauf, A., Abu-Izneid, T., Nadeem, M., Shariati, M. A., Khan, I. A., Imran, A., Orhan, I. E., Rizwan, M., Atif, M., Gondal, T. A., & Mubarak, M. S. (2019). Luteolin, a flavonoid, as an anticancer agent: A review. *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie*, 112, 108612. https://doi.org/10.1016/j.biopha.2019.108612

Invasive Species Compendium, *Robinia pseudoacacia*. Available online: https://doi.org/10.1079/cabicompendium.47698 (accessed on 14 April 2025).

Kato-Noguchi, H., & Kato, M. (2024). Invasive characteristics of *Robinia pseudoacacia* and its impacts on species diversity. *Diversity*, 16(12), 773. https://doi.org/10.3390/d16120773

Khameneh, B., Eskin, N. A. M., Iranshahy, M., & Fazly Bazzaz, B. S. (2021). Phytochemicals: A promising weapon in the arsenal against antibiotic-resistant bacteria. *Antibiotics* (Basel, Switzerland), 10(9), 1044. https://doi.org/10.3390/antibiotics10091044

Lade, H., & Kim, J. S. (2023). Molecular determinants of β-lactam resistance in methicillin-resistant *Staphylococcus aureus* (MRSA): An updated review. *Antibiotics* (Basel, Switzerland), 12(9), 1362. https://doi.org/10.3390/antibiotics12091362

Lina, C. D., Olah, N. K., Tru, P. E., Docea, A., Popescu, H., & Bubulica, M. V. (2013). Chromatographic analysis of the flavonoids from *Robinia pseudoacacia* species. *Current health sciences journal*, 39(4), 232–236

Lowy F. D. (2003). Antimicrobial resistance: the example of *Staphylococcus aureus*. *The Journal of clinical investigation*, 111(9), 1265–1273. https://doi.org/10.1172/JCI18535

Maher, C., & Hassan, K. A. (2023). The Gram-negative permeability barrier: tipping the balance of the in and the out. *mBio*, 14(6), e0120523. https://doi.org/10.1128/mbio.01205-23

Marinas, I., Oprea, E., Geana, E.-I., Chifiriuc, C., & Lazar, V. (2014). Antimicrobial and antioxidant activity of the vegetative and reproductive organs of *Robinia pseudoacacia*. *Journal of the* Serbian Chemical Society, 79, 1363–1378. https://doi.org/.2298/JSC140304049M

McEwen, S. A., & Collignon, P. J. (2018). Antimicrobial resistance: A one health perspective. *Microbiology spectrum*, 6(2). https://doi.org/10.1128/microbiolspec.ARBA-0009-2017

Nikolic, P., & Mudgil, P. (2023). The cell wall, cell membrane and virulence factors of *Staphylococcus aureus* and their role in antibiotic resistance. *Microorganisms*, 11(2), 259. https://doi.org/10.3390/microorganisms11020259

Okoth, D.A., Chenia, H.Y., & Koorbanally, N.A. (2013). Antibacterial and antioxidant activities of flavonoids from *Lannea alata* (Engl.) Engl. (Anacardiaceae). *Phytochemistry Letters*, 6, 476–481. https://doi.org/10.1016/j.phytol.2013.06.003

Prestinaci, F., Pezzotti, P., & Pantosti, A. (2015). Antimicrobial resistance: a global multifaceted phenomenon. *Pathogens and global health*, 109(7), 309–318. https://doi.org/10.1179/2047773215Y.0000000030

Rajagopal, M., & Walker, S. (2017). Envelope structures of gram-positive bacteria. *Current topics in microbiology and immunology*, 404, 1–44. https://doi.org/10.1007/82_2015_5021

Roy, A., Khan, A., Ahmad, I., Alghamdi, S., Rajab, B. S., Babalghith, A. O., Alshahrani, M. Y., Islam, S., & Islam, M. R. (2022). Flavonoids a bioactive compound from medicinal pants and Its therapeutic applications. *BioMed research international*, 2022, 5445291. https://doi.org/10.1155/2022/5445291

Savic Gajic, I., Savic, I., Boskov, I., Žerajić, S., Markovic, I., & Gajic, D. (2019). Optimization of ultrasound-assisted extraction of phenolic compounds from black locust (*Robinia*e *pseudoacacia*e) flowers and comparison with conventional methods. *Antioxidants* (Basel, Switzerland), 8(8), 248. https://doi.org/10.3390/antiox8080248

Sheng, M., Chen, X., Zhang, X., Hamel, C., Cui, X., Chen, J., Chen, H., & Tang, M. (2017). Changes in arbuscular mycorrhizal fungal attributes along a chronosequence of black locust (*Robinia pseudoacacia*) plantations can be attributed to the plantation-induced variation in soil properties. *The Science of the total environment*, 599–600, 273–283. https://doi.org/10.1016/j.scitotenv.2017.04.199

Sinha, S., Aggarwal, S., & Singh, D. V. (2024). Efflux pumps: gatekeepers of antibiotic resistance in *Staphylococcus aureus* biofilms. *Microbial cell* (Graz, Austria), 11, 368–377. https://doi.org/10.15698/mic2024.11.839

Sitzia, T., Campagnaro, T., Kotze, D. J., Nardi, S., & Ertani, A. (2018). The invasion of abandoned fields by a major alien tree filters understory plant traits in novel forest ecosystems. *Scientific reports*, 8(1), 8410. https://doi.org/10.1038/s41598-018-26493-3

Taniguchi, T., Kanzaki, N., Tamai, S., Yamanaka, N., & Futai, K. (2007). Does ectomycorrhizal fungal community structure vary along a Japanese black pine (*Pinus thunbergii*) to black locust (*Robinia pseudoacacia*) gradient? *The New phytologist*, 173(2), 322–334. https://doi.org/10.1111/j.1469-8137.2006.01910.x

Taniguchi, T., Kataoka, R., Tamai, S., Yamanaka, N., & Futai, K. (2009). Distribution of ectomycorrhizal and pathogenic fungi in soil along a vegetational change from Japanese black pine (*Pinus thunbergii*) to black locust (*Robinia pseudoacacia*). *Mycorrhiza*, 19(4), 231–238. https://doi.org/10.1007/s00572-008-0212-3

Tkaczenko, H., Lukash, O., Yakovenko, O., Opryshko, M., Maryniuk, M., Gyrenko, O., Buyun, L., & Kurhaluk, N. (2024). *In vitro* antibacterial efficacy of various natural rapeseed honey against some Gram-positive and Gram-negative bacterial strains. *Agrobiodiversity for Improving Nutrition, Health and Life Quality,* 8(1), 17–25. https://doi.org/10.15414/ainhlq.2024.0003

Touaitia, R., Mairi, A., Ibrahim, N. A., Basher, N. S., Idres, T., & Touati, A. (2025). *Staphylococcus aureus*: A review of the pathogenesis and virulence mechanisms. *Antibiotics*, 14(5), 470. https://doi.org/10.3390/antibiotics14050470

Uzelac, M., Sladonja, B., Šola, I., Dudaš, S., Bilić, J., Famuyide, I. M., McGaw, L. J., Eloff, J. N., Mikulic-Petkovsek, M., & Poljuha, D. (2023). Invasive alien species as a potential source of phytopharmaceuticals: Phenolic composition and antimicrobial and cytotoxic activity of *Robinia pseudoacacia* L. leaf and flower extracts. *Plants* (Basel, Switzerland), 12(14), 2715. https://doi.org/10.3390/plants12142715

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

Vítková, M., Tonika, J., & Müllerová, J. (2015). Black locust – successful invader of a wide range of soil conditions. *The Science of the total environment*, 505, 315–328. https://doi.org/10.1016/j.scitotenv.2014.09.104

Wang, M., Buist, G., & van Dijl, J. M. (2022). *Staphylococcus aureus* cell wall maintenance – the multifaceted roles of peptidoglycan hydrolases in bacterial growth, fitness, and virulence. *FEMS microbiology reviews*, 46(5), fuac025. https://doi.org/10.1093/femsre/fuac025

Zar, J. H. (1999). *Biostatistical Analysis*. 4th ed., Prentice Hall Inc., New Jersey.

Zduńska, K., Dana, A., Kolodziejczak, A., & Rotsztejn, H. (2018). Antioxidant properties of ferulic acid and its possible application. *Skin pharmacology and physiology*, 31(6), 332–336. https://doi.org/10.1159/000491755

Zhang, G., Meredith, T. C., & Kahne, D. (2013). On the essentiality of lipopolysaccharide to Gram-negative bacteria. *Current opinion in microbiology*, 16(6), 779–785. https://doi.org/10.1016/j.mib.2013.09.007

Zhang, W., Wang, R., Guo, R., Yi, Z., Wang, Y., Wang, H., Li, Y., Li, X., & Song, J. (2025). The multiple biological activities of hyperoside: from molecular mechanisms to therapeutic perspectives in neoplastic and non-neoplastic diseases. *Frontiers in pharmacology*, 16, 1538601. https://doi.org/10.3389/fphar.2025.1538601

Zhang, Y., Yang, S., Zhao, X., Yang, Y., Li, B., Zhu, F., & Zhu, R. (2014). Immune enhancement of Taishan *Robinia pseudoacacia* polysaccharide on recombinant *Proteus mirabilis* OmpA in chickens. *International immunopharmacology*, 22(1), 236–241. https://doi.org/10.1016/j.intimp.2014.06.036