BIOTROPIA ISSN: 0215-6334 | e-ISSN: 1907-770X
The Southeast Asian Journal of Tropical Biology
Vol. 34 No. 1, 2027: 90 - 95
DOI: 10.11598/btb.2027.34.1.2843

Research Paper

Check for updates

ANTIBACTERIAL ACTIVITY OF *Melaleuca cajuputi* HYDROSOL FROM ESSENTIAL OIL DISTILLATION AGAINST PATHOGENIC BACTERIA

Siti Khadijah Awang[1], Irdeana Marsha Baharuddin[1], Muhammad Iqbal Mashudi[1], Muhammad Hanif Abdullah[3] and Fazilah Ariffin[1,2*]

[1]Faculty of Science and Marine Environment, Universiti Malaysia Terengganu, Kuala Nerus 21030, Malaysia

[2]Biological Security and Sustainability Research Group, Universiti Malaysia Terengganu, Kuala Nerus 21030, Malaysia

[3]DTree Botanica, PT 35707A, Level 1, Jalan Bukit Tumbuh, Kuala Terengganu 21300, Malaysia

ARTICLE HIGLIGHTS

- *Melaleuca cajuputi* hydrosols inhibited the growth of selected pathogenic bacteria.
- Strong antibacterial activity was observed against Gram-positive bacteria, while no antibacterial effect was detected against Gram-negative bacteria (*Salmonella* sp. and *Escherichia coli*).
- Streptococcus pyogenes and Micrococcus sp. showed the largest antibacterial activity with the presence of inhibition zones of 13.00 ± 2.00 mm and 13.00 ± 1.73 mm.
- Hydrosols from *Melaleuca cajuputi* oil extraction demonstrate potential as sustainable natural antimicrobial agents.

Article Information

Received : 12 January 2026
Revised : 26 May 2026
Accepted : 8 June 2026
Available Online : 17 November 2026

***Corresponding author, e-mail:**
fazilah@umt.edu.my

OPEN ACCESS
Copyright (c) 2027@author(s).

ABSTRACT

Antibiotic resistance has become a major global concern and remains one of the most pressing threats to public health. In the search for alternative antimicrobial agents, plants have long been a valuable source of bioactive compounds. *Melaleuca cajuputi* is well known for its essential oils, which are rich in phytochemicals with antimicrobial potential. However, limited attention has been given to its hydrosols, which are typically considered by-products of essential oil distillation but may also possess biologically active constituents. This study investigated the antibacterial activity of hydrosols obtained from *Melaleuca cajuputi* oil extraction. The Kirby–Bauer disc diffusion method was employed to evaluate the hydrosol's effects against six pathogenic bacteria. The results showed clear inhibition zones for all tested Gram-positive bacteria, whereas no inhibitory effects were observed for Gram-negative bacteria, including *Salmonella* spp. and *Escherichia coli*. The highest activity was observed against *Streptococcus pyogenes* and *Micrococcus* sp., with inhibition zones of 13.00 ± 2.00 mm and 13.00 ± 1.73 mm, respectively, at a hydrosol concentration of 15% (v/v). These findings suggest that *Melaleuca cajuputi* hydrosols may contain bioactive compounds with potential for antibacterial properties. However, more studies involving chemical characterization and minimum inhibitory concentration (MIC) are required to substantiate its efficacy and elucidate its mechanism of action.

Keywords: bioactive compounds, disc diffusion, Gram-positive bacteria, inhibition zone, microbial susceptibility

INTRODUCTION

Antibiotic resistance has emerged as a major global health concern and is recognized as one of the leading threats to public safety. Antibiotic resistance can arise through bacterial mutations or horizontal gene transfer, allowing antibiotics to be inactivated or bypassed and enabling growth at high drug concentrations (Windels *et al.* 2019). As a result, many commonly used antibiotics have become ineffective against clinically important pathogens, including vancomycin-resistant *Enterococcus* species. Additionally, through mutation or environmental exposure, bacteria such as *Streptococcus pneumoniae* and *Mycobacterium tuberculosis* can develop antibiotic resistance, leading to persistent respiratory infections (Guitor & Wright 2018). The increasing prevalence of drug-resistant microorganisms has therefore intensified the search for alternative antimicrobial agents, particularly those derived from natural sources.

For centuries, plants have served as valuable sources of medicinal compounds. *Melaleuca cajuputi* (*M. cajuputi*), commonly known as "Pokok Gelam" or white samet, belongs to the family Myrtaceae and is widely distributed in tropical regions. Members of this family are characterized by leathery leaves containing abundant oil glands. *M. cajuputi* is well known for its rich chemical and phytochemical composition, including compounds with antibacterial activity. Previous studies have identified more than 25 bioactive constituents in *M. cajuputi*, including α-terpineol, limonene, and cineole, which exhibit antibacterial activity and may enhance immune responses (Ukit *et al.* 2019). Moreover, *M. cajuputi* has been reported to exhibit low toxicity and to contain bioactive compounds with anxiolytic, antidepressant, and antioxidant effects (Noor *et al.* 2021).

Despite extensive research on *M. cajuputi* essential oils, limited attention has been paid to its hydrosols, the aqueous co-products of the distillation process that may contain water-soluble bioactive compounds contributing to antibacterial activity (Hombach *et al.* 2015). Hydrosols may contain small amounts of water-soluble volatile and non-volatile compounds that could contribute to antibacterial activity. However, their potential applications remain poorly explored. In addition, improper disposal of hydrosols may lead to environmental contamination, as they may contain residual organic compounds or trace amounts of heavy metals (Riyankati *et al.* 2022). Therefore, a systematic scientific evaluation of hydrosols is necessary to assess their biological potential and promote their safe and sustainable utilization.

This study investigates the antibacterial activity of hydrosols derived from *M. cajuputi* oil extraction against selected pathogenic bacteria. Antibacterial susceptibility was evaluated using the Kirby–Bauer disc diffusion method. The findings of this study are expected to provide preliminary evidence on the potential of *M. cajuputi* hydrosols as natural antimicrobial agents that may contribute to alternative strategies to address antibiotic resistance.

MATERIALS AND METHODS

Study Design

This study was conducted as a laboratory experiment using the Kirby-Bauer disc diffusion method to evaluate the antibacterial activity of hydrosols derived from *Melaleuca cajuputi* oil. Hydrosol samples obtained from a commercial supplier were prepared in the laboratory, evaluated for antimicrobial susceptibility, and subjected to statistical analysis. The effects of different hydrosol concentrations were compared between positive and negative controls. All experiments were performed in triplicate.

Source of Hydrosol Samples

Hydrosols derived from *Melaleuca cajuputi* oil extraction were obtained from Dtreebotanica Sdn. Bhd. According to the supplier, the raw plant material originated from Lembah Bidong, Terengganu, Malaysia. The hydrosols were supplied as a by-product of essential oil distillation, in which water was used as the extraction solvent. All samples were stored in a dark environment before laboratory analysis to prevent degradation.

Preparation of Hydrosols

Hydrosols of *Melaleuca cajuputi* in liquid form were mixed with in methanol and kept in a sealed container for 24 hours. The mixture was then concentrated using a rotary evaporator at 40 °C until the methanol was completely removed and the hydrosol's viscosity increased. The concentrated hydrosols were dissolved in dimethyl sulfoxide (DMSO), a widely used solvent in microbiological assays for compound solubility, and is typically regarded as having minimal antimicrobial effects at low concentrations (Summer *et al.* 2022). Based

on supplier information, the hydrosols consisted of approximately 90% water and 10% oil by-products. For antibacterial testing, the hydrosols were diluted using 0.1% (v/v) DMSO to prepare three concentrations: 5%, 10%, and 15% (v/v). The DMSO concentration was maintained at a non-inhibitory level to ensure normal bacterial growth.

Preparation of Pathogenic Bacteria

Six Gram-positive bacterial strains, namely *Streptococcus pyogenes*, *Bacillus cereus*, *Enterococcus* sp., *Staphylococcus aureus*, *Micrococcus* sp., and *Streptococcus agalactiae*, together with two Gram-negative bacterial strains (*Salmonella* sp. and *Escherichia coli*), were used to evaluate the antibacterial activity of *Melaleuca cajuputi* hydrosols. All bacterial cultures were obtained from the Microbiology Laboratory, Universiti Malaysia Terengganu (UMT). Each bacterial strain was streaked onto nutrient agar plates and incubated at 37 °C for 24 hours to confirm purity and viability. All bacterial strains were handled in accordance with appropriate biosafety guidelines, and all experimental procedures were conducted in a certified Class II biosafety cabinet following standard aseptic and antiseptic techniques to minimize contamination and exposure risks. A single well-isolated colony was transferred into sterile 0.9% (w/v) saline solution. The bacterial suspension was standardized to a 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL) and confirmed by visual turbidity comparison and spectrophotometric measurement at 600 nm (OD_{600}). According to Hombach *et al.* (2015), standardization of bacterial culture to 0.5 McFarland turbidity, together with spectrophotometric measurement at 600 nm (OD_{600}), is a commonly used approach to ensure consistent inoculum density in antimicrobial testing.

Antibacterial Susceptibility Test (Disc Diffusion Assay)

The antibacterial activity of *Melaleuca cajuputi* hydrosols was assessed using the Kirby–Bauer disc diffusion method. Mueller–Hinton agar (Oxoid, UK) was prepared by dissolving the appropriate amount of agar powder (38 g/L) in distilled water in a Schott bottle and sterilized by autoclaving at 121 °C for 15 minutes. Bacterial suspensions standardized to the 0.5 McFarland standard were evenly spread onto the agar surface using sterile cotton swabs. Sterile 6 mm paper discs (Sigma-Aldrich) were impregnated with 20 µL of hydrosol samples at concentrations of 5%, 10%, and 15% (v/v), prepared using distilled water as the extraction solvent. The discs were then placed onto inoculated agar plates and incubated at 37 °C for 24 hours. After incubation, the diameters of inhibition zones were measured in millimeters. Commercial antibiotic discs were used, including streptomycin (10 µg, Oxoid, UK) as the positive control and optochin (5 µg, Oxoid, UK) for comparison, while 0.1% (v/v) DMSO prepared in distilled water served as the negative control.

Statistical Analysis

All experiments were conducted in triplicate, and the results were expressed as mean ± standard deviation (SD). The antibacterial activity of *Melaleuca cajuputi* hydrosols at concentrations of 5%, 10%, and 15% (v/v) was evaluated by measuring the inhibition zone diameter in millimeters. Statistical analysis was performed using one-way analysis of variance (ANOVA) with the Analysis ToolPak in Microsoft Excel to determine differences among hydrosol concentrations. The level of statistical significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

Antibacterial Activity of *Melaleuca cajuputi* Hydrosol

In this study, the antibacterial activity of *Melaleuca cajuputi* hydrosol was evaluated using the Kirby–Bauer disc diffusion method. The results are presented in Figure 1 and Table 1.

The results in Figure 1 showed that the hydrosols effectively inhibited all six Gram-positive bacteria tested, as indicated by clear inhibition zones. Streptomycin produced clear zones of inhibition against the tested bacterial strains. As an aminoglycoside antibiotic, streptomycin exhibits broad-spectrum antibacterial activity and is primarily effective against aerobic Gram-negative bacteria (Webster & Shepherd 2023). Meanwhile, optochin showed no inhibitory effect against any of the tested bacterial strains. According to Vohrnová *et al.* (2025), optochin is a quinine-derived compound that inhibits ATP synthase in bacterial cells and is commonly used to identify *Streptococcus pneumoniae*, with limited application as a broad-spectrum antibacterial agent. Therefore, in this study, no inhibition zones were observed for the Gram-negative bacteria (*Salmonella* sp.

and *Escherichia coli*); therefore, these results are not included. The lack of inhibition observed in Gram-negative bacteria may be attributed to structural differences between Gram-positive and Gram-negative bacteria. According to Breijyeh *et al.* (2020), Gram-negative bacteria can modify their outer membranes, for example, through porin mutations or changes in hydrophobicity, thereby contributing to their resistance to various antimicrobial compounds. The key distinction lies in their cell wall structures; Gram-positive bacteria possess a thick peptidoglycan layer (30–100 nm), whereas Gram-negative bacteria have a much thinner one, only a few nanometers thick (Rohde 2019). The outer membrane of Gram-negative bacteria, which contains lipopolysaccharides and phospholipids, acts as a selective barrier, reducing the penetration of hydrophilic antimicrobial agents.

In contrast, Gram-positive bacteria have cell walls rich in teichoic acids and peptidoglycans but low in lipid content, thereby facilitating the penetration of antibacterial compounds (Malanovic & Lohner 2016; Ukit *et al.* 2019). As expected, the negative control (0.1% DMSO) showed no inhibition zones, confirming that the solvent concentration used was too low to affect bacterial growth. DMSO at 0.1% is considered biologically safe, whereas higher concentrations can be cytotoxic (Gallardo *et al.* 2022).

Based on Table 1, the largest inhibition zones were observed against *Micrococcus* sp. and *Streptococcus pyogenes* (13.00 ± 1.73 mm and 13.00 ± 2.00 mm, respectively). The positive control, optochin, showed no zone of inhibition against any of the tested bacterial strains, whereas streptomycin produced clear zones of inhibition, thereby indicating the reliability of the antibacterial assay conducted in this study. Interestingly, the inhibition zone for *Micrococcus* sp. was slightly larger than that produced by the positive control (streptomycin), suggesting that the hydrosol exhibited moderate antibacterial activity comparable to that of a commercial antibiotic. According to Quinn *et al.* (2020), streptomycin exerts its antibacterial effect by binding to the 30S ribosomal subunit, thereby inhibiting protein synthesis and preventing bacterial growth. *Bacillus cereus* showed the smallest inhibition zone (8.70 ± 1.53 mm), possibly due to its resistance mechanisms. *B. cereus* can form desiccation-resistant endospores, allowing survival under harsh environmental conditions such as heat, acid, and UV exposure (Ehling-Schulz *et al.* 2019).

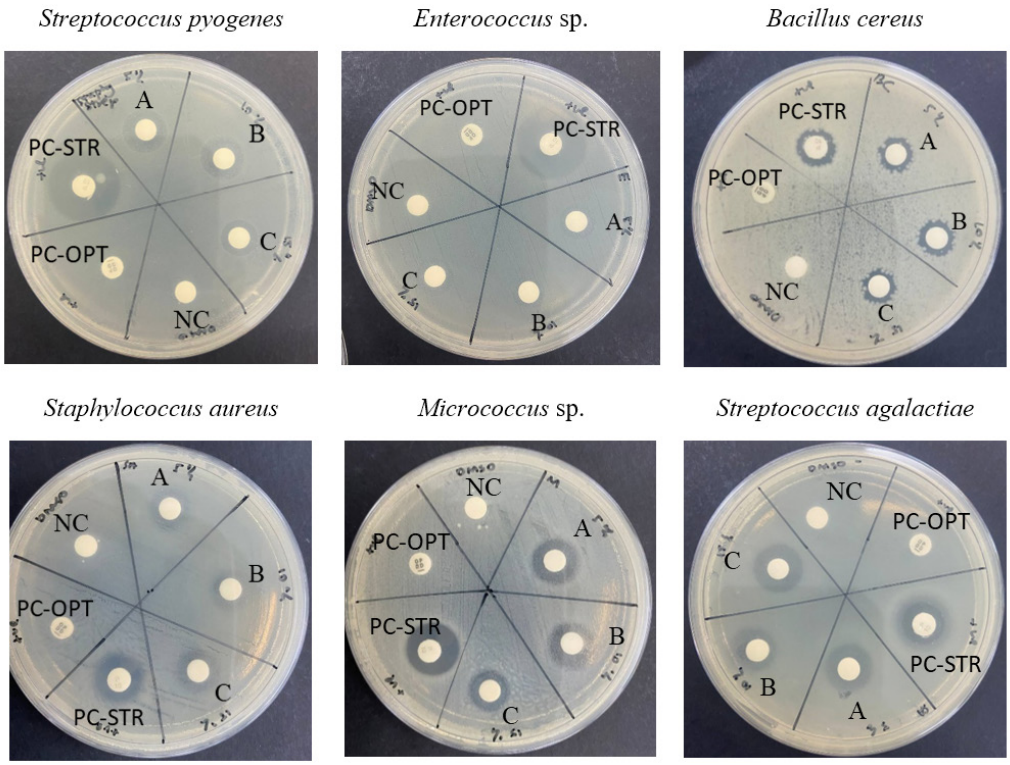


Figure 1 Representative Kirby–Bauer disc diffusion plates showing inhibition zones of *Melaleuca cajuputi* hydrosols against pathogenic bacteria at 5% (A), 10% (B), and 15% (C) (v/v), with optochin (PC-OPT) and streptomycin (PC-STR) as positive controls and 0.1% DMSO as the negative control (NC)

Table 1 Zone of inhibition of *Melaleuca cajuputi* hydrosol against selected bacteria at different hydrosol concentrations

Bacteria	Zone of inhibition by different treatments (mm)					
	5%	10%	15%	Streptomycin	Optochin	DMSO
Streptococcus pyogenes	12.00 ± 1.00	13.00 ± 1.73	13.00 ± 1.73	20.70 ± 1.15	0.00 ± 0.00	0.00 ± 0.00
Bacillus cereus	9.00 ± 0.00	9.30 ± 1.15	8.70 ± 1.53	12.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Enterococcus	10.70 ± 0.58	9.70 ± 0.58	9.70 ± 1.15	21.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Staphylococcus aureus	12.70 ± 1.53	12.70 ± 0.58	12.70 ± 1.15	12.70 ± 0.58	0.00 ± 0.00	0.00 ± 0.00
Micrococcus	12.30 ± 1.53	12.70 ± 1.15	13.00 ± 2.00	10.30 ± 0.58	0.00 ± 0.00	0.00 ± 0.00
Streptococcus agalactiae	11.00 ± 1.00	10.30 ± 2.89	11.70 ± 0.58	18.00 ± 3.61	0.00 ± 0.00	0.00 ± 0.00

Melaleuca cajuputi hydrosols effectively inhibited *S. pyogenes*, *B. cereus*, *Enterococcus*, *S. aureus*, *Micrococcus* sp., and *S. agalactiae*, with the strongest effects against *S. pyogenes*, *S. aureus*, and *Micrococcus* sp. The inhibition of these pathogens by hydrosols suggests that bioactive compounds in the extract, such as hydroxycinnamate esters, flavonoids, and tannins, may help suppress bacterial toxin production and growth (Farahmandfar *et al.* 2019; Lai *et al.* 2025). At 15% (v/v) hydrosol concentration, high antibacterial activity (inhibition zone >10 mm) was observed against *S. pyogenes*, *S. aureus*, *Micrococcus* sp., and *S. agalactiae*, while moderate activity (5–10 mm) was seen against *B. cereus* and *Enterococcus*. No weak activity (<5 mm) was detected. These findings indicate that *Melaleuca cajuputi* hydrosols have promising antibacterial potential, particularly against Gram-positive bacteria.

The observed antibacterial effects align with previous studies on *Melaleuca* species. For example, *Melaleuca alternifolia* hydrosols have been reported to contain monoterpenoids, such as terpinen-4-ol, which contribute to significant antimicrobial activity (Li *et al.* 2025). Similarly, GC-MS analysis by Riyankati *et al.* (2022) revealed that tea tree (*M. alternifolia*) hydrosols contain monoterpenoids, including terpineol and myrcene, which are also known for their antibacterial effects. Hydrosols, compared to essential oils, are simpler and more cost-effective to produce, and are generally safer for human applications (Taglienti *et al.* 2022). Overall, the results suggest that *M. cajuputi* hydrosols can serve as a natural and effective antibacterial agent against selected Gram-positive pathogens. As noted by Aćimović *et al.* (2020), hydrolates derived from spices and aromatic plants may be used as natural preservatives in food and feed products, providing antimicrobial protection and potential benefits for gut health. Additional studies focusing on chemical characterization and mechanistic elucidation are required to better understand the compounds that contribute to antibacterial activity.

CONCLUSION

In conclusion, this study demonstrated that hydrosols derived from *Melaleuca cajuputi* effectively inhibited the growth of pathogenic bacteria, as evidenced by the clear inhibition zones observed in the disc diffusion assay. While Gram-negative bacteria such as *Salmonella* and *Escherichia coli* showed no inhibition, this was likely due to the impermeability of their outer cell walls. In contrast, the hydrosols exhibited notable antibacterial activity against Gram-positive species, with the strongest inhibitory effects observed against *Micrococcus* sp. and *Streptococcus pyogenes*. Given the growing threat of antibiotic resistance, these findings highlight the potential of natural products such as hydrosols as alternative antibacterial agents to essential oils, offering advantages in safety, cost, and environmental sustainability. Therefore, further investigations involving chemical characterization, determination of minimum inhibitory concentration (MIC), and elucidation of the mechanism of action are necessary to better understand its antibacterial efficacy and potential applications.

ACKNOWLEDGMENTS

The authors would like to thank Universiti Malaysia Terengganu for providing the facilities and support for this study. Appreciation is also extended to Dtreebotanica Sdn. Bhd. for supplying the hydrosol samples.

REFERENCES

Aćimović M, Tešević V, Smiljanić K, Cvetković M, Stanković J, Kiprovski B, Sikora V. 2020. Hydrolates: By-products of essential oil distillation, chemical composition, biological activity, and potential uses. Adv Technol 9(2):54–70.

Breijyeh Z, Jubeh B, Karaman R. 2020. Resistance of Gram-negative bacteria to current antibacterial agents and approaches to resolve it. Molecules 25(6):1340.

Ehling-Schulz M, Lereclus D, Koehler TM. 2019. The *Bacillus cereus* group: *Bacillus* species with pathogenic potential. Microbiol Spectr 7(3): GPP3-0032-2018.

Farahmandfar R, Esmaeilzadeh Kenari R, Asnaashari M, Shahrampour D, Bakhshandeh T. 2019. Bioactive compounds, antioxidant and antimicrobial activities of *Arum maculatum* leaf extracts as affected by various solvents and extraction methods. Food Sci Nutr 7(2):465–475.

Gallardo-Villagrán M, Paulus L, Leger DY, Therrien B, Liagre B. 2022. Dimethyl sulfoxide: A bio-friendly or bio-hazard chemical? The effect of DMSO in human fibroblast-like synoviocytes. Molecules 27(14):4472.

Guitor AK, Wright GD. 2018. Antimicrobial resistance and respiratory infections. Chest 154(5):1202–1212.

Hombach M, Maurer FP, Pfiffner T, Böttger EC, Furrer R. 2015. Standardization of operator-dependent variables affecting precision and accuracy of the disk diffusion method for antibiotic susceptibility testing. J Clin Microbiol 53(12):3864–3869.

Lai HY, Lu YCC, Fan KH, Virgirinia RP, Prasetyaningrum N, Nugraeni Y, Huang HM. 2025. Investigating the biological activities of *Sapindus mukorossi* seed hydrosol and its potential as a skincare ingredient. Sci Rep 15(1):42801.

Li X, Li Z, Li R, Zhang Y, Fu W, Li L, Hao R. 2025. Hydrosol extracts from *Prunus* species: Optimization, antibacterial activity, and applications in food preservation. Food Biosci 107406.

Malanovic N, Lohner K. 2016. Antimicrobial peptides targeting Gram-positive bacteria. Pharmaceuticals 9(3):59.

Noor AA, Yusuf SM, Wahab WN, Adam MF, Sul'ain MD. 2021. Evaluation of composition, antioxidants and toxicity of *Melaleuca cajuputi* leaves. Adv Tradit Med 21(4):693–699.

Quinn GA, Banat AM, Abdelhameed AM, Banat IM. 2020. Streptomyces from traditional medicine: sources of new innovations in antibiotic discovery. J Med Microbiol 69(8):1040–1048.

Riyankati B, Hadi S, Handayani SS. 2022. Characterization and chemical composition analysis of tea tree (*Melaleuca alternifolia*) leaf hydrosols growing on Lombok Island. J Penelit Pendidik IPA 8(1):119–123.

Rohde M. 2019. The Gram-positive bacterial cell wall. Microbiol Spectr 7(3):10–1128.

Summer K, Browne J, Hollanders M, Benkendorff K. 2022. Out of control: The need for standardised solvent approaches and data reporting in antibiofilm assays incorporating dimethyl-sulfoxide (DMSO). Biofilm 4:100081.

Taglienti A, Donati L, Ferretti L, Tomassoli L, Sapienza F, Sabatino M, Di Massimo G, Fiorentino S, Vecchiarelli V, Nota P, Ragno R. 2022. In vivo antiphytoviral activity of essential oils and hydrosols from *Origanum vulgare*, *Thymus vulgaris*, and *Rosmarinus officinalis* to control *Zucchini yellow mosaic virus* and *Tomato leaf curl New Delhi virus* in *Cucurbita pepo* L. Front Microbiol 13:840893.

Ukit U, Widiana A, Rahmawati E, Hasby RM. 2019. Antibacterial activities test of cajuput leaf waste extract (*Melaleuca cajuputi* Powell) on pathogenic bacteria. J Phys Conf Ser 1402(3):033030.

Vohrnová S, Kozáková J, Honskus M. 2025. Comparison of *Streptococcus pneumoniae* isolates occurring in optochin-susceptible and optochin-resistant variants by analyzing whole-genome sequencing data. Microbiol Spectr 13(4), e01939-24.

Webster CM, Shepherd M. 2023. A mini-review: environmental and metabolic factors affecting aminoglycoside efficacy. World J Microbiol Biotechnol 39(1):7.

Windels EM, Michiels JE, Van den Bergh B, Fauvart M, Michiels J. 2019. Antibiotics: Combating tolerance to stop resistance. mBio 10(5), e02095-19.