

Research Article

Stability and efficacy determination of crude *Limnocharis flava* (L.) Buch extract gel in inhibiting pathogenic bacteria

Ekkwit Chaichamnong, Thanai Surasilp and Surachai Rattanasuk*

Department of Science and Technology, Faculty of Liberal Arts and Science, Roi Et Rajabhat University, Roi Et, Thailand

(*Corresponding author's e-mail: surachai_med@hotmail.com)

Received: 26 April, 2026; Revised: 28 May, 2026; Accepted: 6 June, 2026

Abstract

This study investigated the antibacterial efficacy of crude *Limnocharis flava* leaf extract gel against antibiotic-resistant clinical bacterial isolates. The gel was formulated with crude ethanolic *L. flava* leaf extract at 12.5 mg/mL, corresponding to the lowest minimum bactericidal concentration previously reported for the extract. Clinical isolates, including multidrug-resistant *Klebsiella pneumoniae* (MDR-K), colistin-resistant *Pseudomonas aeruginosa* (CRP), carbapenem-resistant Enterobacteriaceae (CRE), *Proteus mirabilis*, and *Burkholderia pseudomallei*, were used. Antibacterial activity was determined using the agar disc diffusion method. The crude *L. flava* extract gel inhibited all tested isolates, producing inhibition zones ranging from 8 to 10 mm. MDR-K was the most susceptible isolate, showing the largest inhibition zone of 10 mm, followed by CRE with 9 mm. CRP, *P. mirabilis*, and *B. pseudomallei* showed comparable inhibition zones of 8 mm. Statistical analysis demonstrated that the activity against MDR-K was significantly higher than that against CRP, *P. mirabilis*, and *B. pseudomallei* ($p < 0.05$), whereas the inhibition observed against CRE showed no significant difference from the other isolates. These results indicate that crude *L. flava* extract gel exhibits measurable antibacterial activity against clinically relevant antibiotic-resistant bacteria, particularly MDR-K. The moderate inhibition observed may be associated with bioactive phytochemicals in *L. flava* leaf extract, which may interfere with bacterial growth and viability. Therefore, crude *L. flava* extract gel represents a potential plant-based antibacterial formulation for further topical pharmaceutical development. Additional studies on phytochemical characterization, formulation stability, cytotoxicity, and antibacterial mechanisms are required.

Keywords: *Limnocharis flava*; antibacterial activity; crude *Limnocharis flava* extract gel; antibiotic-resistant bacteria

Introduction

The rapid emergence and spread of antimicrobial resistance (AMR) have become a critical global health concern, reducing the effectiveness of conventional antibiotics and increasing the risks of treatment failure, prolonged illness, and mortality. Multidrug-resistant (MDR) pathogens, particularly Gram-negative bacteria such as *Klebsiella pneumoniae* and *Acinetobacter baumannii*, are major clinical challenges because of their ability to acquire resistance to multiple antibiotic classes. Therefore, the search for novel, safe, and sustainable antimicrobial agents is urgently needed, and natural plant extracts represent a promising source for therapeutic development (Bukhari et al., 2025; Wongarmart et al., 2025). Several studies have demonstrated that plant-derived compounds can inhibit a broad range of pathogenic

microorganisms, supporting their potential as alternative or complementary antibacterial agents (Anggraeni et al., 2025; Azrifitria et al., 2022; Boongapim et al., 2025). In this context, plant-based antimicrobials incorporated into hydrogel or gel formulations have gained increasing attention as innovative approaches for controlling bacterial infections.

Natural products, particularly plant-derived compounds, contain diverse bioactive constituents with potential antibacterial activity. Phenolics, flavonoids, alkaloids, terpenoids, tannins, and saponins have been reported to exert antimicrobial effects through multiple mechanisms, including disruption of bacterial cell membranes, inhibition of enzyme activity, and interference with genetic material replication (Mehdipour et al., 2022). These multifaceted mechanisms may help reduce the likelihood of resistance development compared with single-target conventional antibiotics. In addition, the discovery of antimicrobial agents from plant resources aligns with global efforts to identify new antibacterial substances, especially given the economic and regulatory challenges associated with the development of synthetic drugs (Arip et al., 2022).

Limnocharis flava (L.) Buch., an edible aquatic plant widely distributed in Southeast Asia, is traditionally consumed as a vegetable and used in folk medicine. Preliminary phytochemical investigations have shown that *L. flava* contains bioactive compounds such as flavonoids, tannins, and saponins, which are associated with antioxidants, anti-inflammatory, and antimicrobial properties (Gan et al., 2022; Khameneh et al., 2021). However, despite its traditional use and biological potential, scientific evidence regarding the antibacterial efficacy of *L. flava*, particularly against pathogenic and drug-resistant bacteria, remains limited. *L. flava* extracts have demonstrated promising inhibitory effects against a range of pathogenic bacteria in preliminary *in vitro* studies, suggesting its potential as a source for novel antimicrobial agents (Rahman et al., 2021).

For practical application, plant extracts should be developed into stable and effective delivery systems. Gel-based formulations are suitable for topical use because they are easy to apply, enhance contact with the target area, improve formulation stability, and may support the controlled release of active compounds. Moreover, stability evaluation under stress conditions is essential to determine whether the antibacterial activity of plant-based formulations can be maintained during storage and use. Therefore, this study aimed to evaluate the antibacterial activity of crude *L. flava* extracts obtained using different solvents and to determine the stability and efficacy of the extract formulated as a gel against pathogenic and multidrug-resistant bacteria.

Materials and Methods

Preparation of crude plant extracts

Fresh plant material of *L. flava* (L.) Buch. was collected from Thamuang, Selaphum, Roi Et, Thailand. Fresh plant samples were washed thoroughly with clean water to remove dirt and contaminants. The samples were then cut into small pieces and dried in a hot-air oven at 50 °C for 3 days or until completely dry. The dried samples were ground into a fine powder using an herbal grinder. (Khonkayan et al., 2025; Rattanasuk et al., 2025; Sawisit et al., 2025). The powdered samples were separately extracted with 500 mL of ethanol. The flasks were tightly sealed and placed on a shaker at 150 rpm for 24 h. After extraction, the mixtures were filtered, and the filtrates were collected. The filtrates were dried in a hot air oven at 50 °C until completely dry.

Development of crude extract gel formulation

Pre-formulation study

Before developing the *L. flava* crude extract into a gel formulation, a pre-formulation study was conducted to evaluate the stability of the extract under stress conditions.

Forced degradation testing was performed to assess changes in extract properties under different environmental conditions according to the Thai FDA guideline.

For moisture hydrolysis, 500 mg of crude extract was placed in a test tube, mixed with three drops of distilled water, and heated in a hot-air oven at 80 °C for 3 h. The treated extract was then tested for MIC against the bacterial strains.

For acid hydrolysis, 500 mg of crude extract was mixed with three drops of 3 N HCl and heated at 80 °C for 3 h before MIC testing.

For alkaline hydrolysis, 500 mg of crude extract was mixed with three drops of 3 N NaOH and heated at 80 °C for 3 h. The pH was then neutralized with HCl before MIC testing.

For oxidative degradation, 500 mg of crude extract was mixed with three drops of 30% H₂O₂ and heated at 80 °C for 3 h prior to MIC testing.

The formulated gel product was packed in sterile glass containers and further evaluated for antibacterial efficacy and gel quality.

Preparation of Crude Extract Gel

The crude extract obtained from *L. flava* leaves was prepared at a concentration of 12.5 mg/mL, corresponding to the lowest minimum bactericidal concentration (MBC) of the ethanolic extract reported in a previous study (Wongarmart et al., 2025). The extract was dissolved in 1 mL of dimethyl sulfoxide (DMSO) to enhance the solubility of the major bioactive constituents. Subsequently, 4 mL of distilled water and 0.1 g of hydroxyethyl cellulose were added to prepare the crude *L. flava* extract gel formulation. The mixture was stirred continuously until a homogeneous formulation was obtained. The mixture was then heated at approximately 70 °C under continuous stirring until it changed into a semi-solid gel form. The prepared crude extract gel was allowed to cool and was stored in sterile containers for further stability and antibacterial efficacy testing

Bacterial strains

Clinical isolates, including multidrug-resistant *Klebsiella pneumoniae* (MDR-K), Colistin-resistant *Pseudomonas aeruginosa* (CRP), *Proteus mirabilis*, Carbapenem-resistant Enterobacteriaceae (CRE), and *Burkholderia pseudomallei* were confirmed and provided by the Department of Microbiology, Roi Et Hospital, Thailand.

Preparation of bacterial inoculum

Bacterial strains used in this study were cultured in nutrient broth (NB) and incubated in a shaking incubator at 37 °C and 150 rpm for 24 h. After incubation, the turbidity of each bacterial suspension was adjusted using a spectrophotometer at 600 nm (OD₆₀₀) to obtain an absorbance of 0.1. The standardized bacterial suspensions were then used for subsequent antibacterial assays. (Armassa et al., 2025; Boongapim et al., 2025).

Evaluation of antibacterial efficacy of gel formulation

Agar disc diffusion assay

The antibacterial activity of the crude *L. flava* extract gel was initially screened using the agar disc diffusion method. Nutrient agar plates were prepared, and 100 µL of each standardized bacterial suspension (OD₆₀₀ = 0.1) was spread evenly over the agar surface. Sterile paper discs with a diameter of 5 mm were placed on the inoculated agar plates. Crude *L. flava* extract gel was added onto the paper discs at a volume of 10 µL per disc. DMSO was used as the negative control. The plates were incubated at 37 °C for 24 h. After incubation, the diameter of the inhibition zone was measured in millimeters.

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) determination

The MIC and MBC of the crude extracts gel were determined using the broth microdilution method in 96-well microplates. Briefly, 100 µL of NB was added to each well. The 100 µL of crude *L. flava* extract gel was added to the first well and serially diluted two-

fold across wells 1–10. Subsequently, 100 μ L of each standardized bacterial suspension was added to wells 1–11. The microplates were incubated at 37 °C for 24 h. After incubation, 50 μ L of iodo-nitrotetrazolium chloride (INT) solution at a concentration of 4 mg/mL was added to each well, followed by further incubation at 37 °C for 1 h. Color changes were observed to determine bacterial growth. The MIC was defined as the lowest concentration of the crude *L. flava* extract gel that changed the INT color from dark violet to light violet or pink, indicating inhibition of bacterial growth. The MBC was defined as the lowest concentration at which no visible color change was observed, indicating complete bacterial elimination (Armassa et al., 2024; Khlongkhlaeo et al., 2024; Rattanasuk et al., 2023).

Statistical analysis

Antibacterial activity data were analyzed using SPSS software version 28. The experiment was arranged in a completely randomized design (CRD). Each treatment was performed in triplicate, and each replicate consisted of three plates. Statistical differences among treatments were evaluated using analysis of variance (ANOVA), followed by Duncan's Multiple Range Test (DMRT). Differences were considered statistically significant at $p < 0.05$.

Results and discussion

Inhibition zone diameter

The antibacterial activity of crude *L. flava* extract gel against selected clinical pathogenic bacteria was evaluated using the agar disc diffusion assay, and the results are presented in Table 1. The crude gel exhibited inhibitory activity against all tested bacterial strains, with inhibition zone diameters ranging from 8 to 10 mm. The gel demonstrated measurable antibacterial effects against a variety of clinically important Gram-negative pathogenic bacteria, including antibiotic-resistant isolates, indicating its potential broad-spectrum antimicrobial properties. These findings are consistent with previous reports demonstrating that plant-derived extracts possess inhibitory activity against multidrug-resistant bacterial pathogens (Kebede et al., 2021). Furthermore, the growing resistance of pathogenic bacteria, particularly *P. aeruginosa*, to conventional antibiotics has intensified the search for alternative antimicrobial agents from natural sources (Rita et al., 2025). The present findings therefore support the potential application of *L. flava* as a promising source of bioactive antibacterial compounds (Khare et al., 2021).

Among the tested pathogens, MDR-K exhibited the highest susceptibility to crude *L. flava* extract gel, with an inhibition zone diameter of 10 mm (Figure 1). This result suggests that the crude gel possesses notable antibacterial activity against MDR bacterial strains commonly associated with healthcare-associated infections. Similar findings have been reported for medicinal plant extracts exhibiting inhibitory activity against resistant nosocomial pathogens (Mlozi et al., 2023). In contrast, CRE demonstrated moderate susceptibility, with an inhibition zone of 9 mm, whereas CRP, *P. mirabilis*, and *B. pseudomallei* showed lower sensitivity, each producing inhibition zones of 8 mm. The variation in antibacterial efficacy observed among the tested bacterial species may be associated with differences in bacterial cell structure, membrane permeability, and intrinsic resistance mechanisms.

Statistical analysis revealed that the inhibition zone against MDR-K was significantly greater than those observed against CRP, *P. mirabilis*, and *B. pseudomallei* ($p < 0.05$), whereas the inhibitory effect against CRE was not significantly different from those of the remaining bacterial strains. These findings indicate differential antibacterial susceptibility among the tested pathogens and further highlight the promising activity of crude *L. flava* extract gel against MDR-K. Similar variations in bacterial susceptibility to plant extracts have previously been reported and are often attributed to differences in bacterial defense systems and phytochemical interactions (Nigussie et al., 2021). Previous studies have also demonstrated that medicinal plants containing alkaloids, flavonoids, tannins, terpenoids, and phenolic

compounds can exert broad-spectrum antibacterial activity through multiple mechanisms, including disruption of bacterial cell membranes, inhibition of enzymatic activity, and interference with microbial metabolic processes (Jagdale et al., 2024).

The relatively lower susceptibility observed in CRP, *P. mirabilis*, and *B. pseudomallei* may be attributed to the structural complexity of Gram-negative bacteria, particularly the presence of an outer lipopolysaccharide membrane and active efflux pump systems that reduce the intracellular accumulation of antimicrobial compounds (Saoudi et al., 2023; Stan et al., 2021). Such structural barriers are recognized as important contributors to antimicrobial resistance and frequently limit the efficacy of plant-derived antibacterial agents against Gram-negative pathogens (Mayianda et al., 2025). Nevertheless, despite exhibiting moderate inhibition zones, crude *L. flava* extract gel was capable of inhibiting several clinically important antibiotic-resistant bacteria, including MDR-K, CRE, and CRP. This finding highlights the potential of crude *L. flava* extract gel as a natural antibacterial formulation for pharmaceutical or topical applications. Further studies involving phytochemical profiling, purification of active constituents, and elucidation of antibacterial mechanisms are warranted to better understand its therapeutic potential and support the development of novel antimicrobial agents from *L. flava* (Ismail et al., 2022; Song et al., 2021).

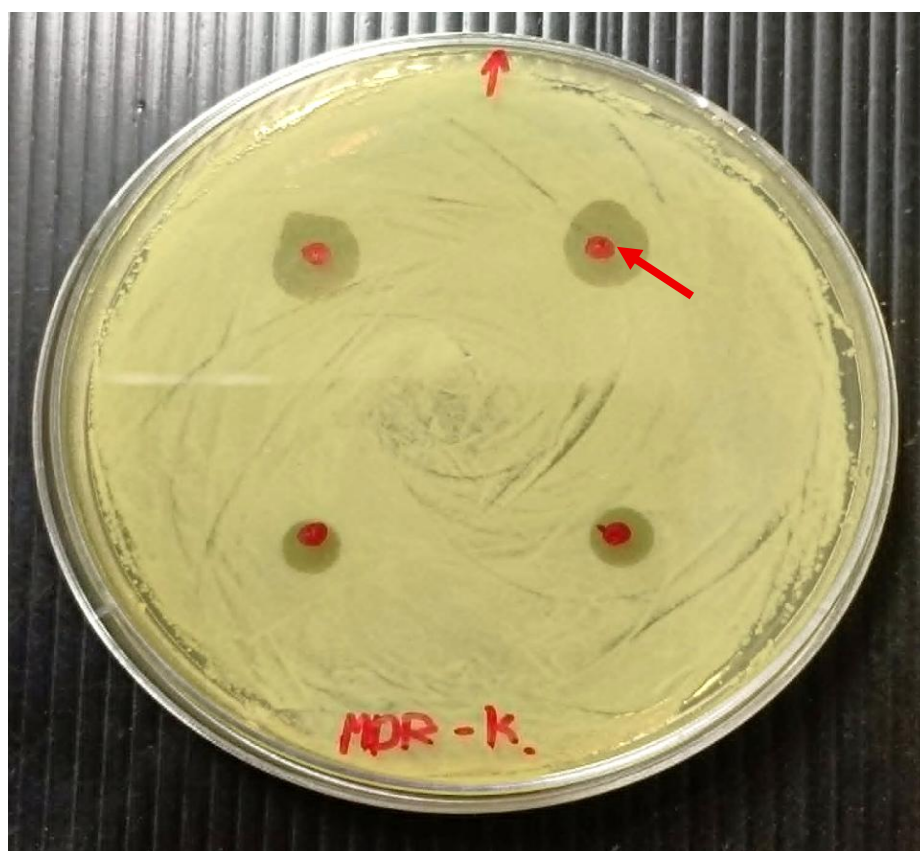


Figure 1 The inhibition zone of crude *L. flava* extract gel against MDR-K

Table 1 Inhibition zone diameter of *L. flava* extract gel

Bacterial strain	Inhibition zone diameter (mm)
Multidrug-resistant <i>Klebsiella pneumoniae</i> (MDR-K)	10a
Colistin-resistant <i>Pseudomonas aeruginosa</i> (CRP)	8b
<i>Proteus mirabilis</i>	8b
Carbapenem-resistant Enterobacteriaceae (CRE)	9ab
<i>Burkholderia pseudomallei</i>	8b

Values are means of three replicates. Means within the same column followed by the same superscript letter are not significantly different according to DMRT ($p < 0.05$).

Stability and antibacterial efficacy of the gel formulation

The MIC values of the gel formulation containing crude *L. flava* extract were evaluated under different stress conditions, including moisture hydrolysis (H_2O), oxidation (H_2O_2), acid hydrolysis (HCl), and alkaline hydrolysis (NaOH). The results showed a similar pattern across all tested conditions. MDR-K showed the lowest MIC value of 0.78 mg/mL, indicating the highest susceptibility to gel formulation. This value was significantly lower than those observed for the other bacterial strains ($p < 0.05$). CRE showed a moderate MIC value of 3.125 mg/mL, whereas CRP, *P. mirabilis*, and *B. pseudomallei* showed the highest MIC values of 6.25 mg/mL under all stress conditions (Table 2). These findings indicate that the crude *L. flava* extract gel formulation retained antibacterial activity after exposure to moisture, oxidative, acidic, and alkaline stress, suggesting good stability of the active antimicrobial constituents within the gel matrix.

Although the gel formulation-maintained activity under all stress conditions, bacterial susceptibility varied among strains. MDR-K was the most sensitive organism, while colistin-resistant *P. aeruginosa*, *P. mirabilis*, and *B. pseudomallei* required higher concentrations for growth inhibition. This strain-dependent response may reflect differences in bacterial cell envelope structure, intrinsic resistance mechanisms, and susceptibility to plant-derived bioactive compounds. The persistence of inhibitory activity after stress treatment is important for topical applications, as gel formulations may be exposed to different physiological and environmental conditions during storage and use.

Table 2 MIC values of crude *L. flava* extract gel under different stress conditions

Bacterial strain	H_2O	H_2O_2	HCl	NaOH
MDR- <i>Klebsiella pneumoniae</i> (MDR-K)	0.78 ^a	0.78 ^a	0.78 ^a	0.78 ^a
Colistin-resistant <i>Pseudomonas aeruginosa</i> (CRP)	6.25 ^c	6.25 ^c	6.25 ^c	6.25 ^c
<i>Proteus mirabilis</i>	6.25 ^c	6.25 ^c	6.25 ^c	6.25 ^c
Carbapenem-resistant Enterobacteriaceae (CRE)	3.125 ^b	3.125 ^b	3.125 ^b	3.125 ^b
<i>Burkholderia pseudomallei</i>	6.25 ^c	6.25 ^c	6.25 ^c	6.25 ^c

Values are expressed as mg/mL. Means within the same column followed by the same superscript letter are not significantly different according to DMRT ($p < 0.05$).

The MBC values of the gel formulation were 12.5 mg/mL for all tested bacterial strains under all stress conditions (Table 3). No significant differences were observed among stress conditions or bacterial strains ($p > 0.05$). In contrast to the MIC results, which differentiated bacterial susceptibility, the MBC results suggested that bactericidal activity occurred at a consistent concentration across all tested bacteria and stress conditions. This consistency supports the stability of the antimicrobial compounds in the gel formulation and indicates that the extract retained its ability to kill pathogenic bacteria after stress exposure (Elkhateeb et al., 2022; Olaitan & Nwadike, 2024; Rammali et al., 2024; Saleh & Abu-Dieyeh, 2021).

The difference between MIC and MBC values also provides useful information regarding the antibacterial profile of the gel. MIC reflects the lowest concentration required to inhibit

bacterial growth, while MBC indicates the lowest concentration required to kill bacterial cells (Dash et al., 2023; Sharma et al., 2024). In this study, the higher MBC values compared with MIC values for several strains suggest that lower concentrations of the gel mainly inhibited bacterial growth, whereas higher concentrations were required to achieve bactericidal activity (Maliehe et al., 2022). Overall, the stable inhibitory and bactericidal performance of the crude *L. flava* extract gel formulation under different stress conditions supports its potential as a natural topical antibacterial product, particularly for applications involving multidrug-resistant pathogens (Karimi et al., 2023).

Table 3 MBC values of crude *L. flava* extract gel under different stress conditions

Bacterial strain	H ₂ O	H ₂ O ₂	HCl	NaOH
MDR- <i>Klebsiella pneumoniae</i> (MDR-K)	12.5	12.5	12.5	12.5
Colistin-resistant <i>Pseudomonas aeruginosa</i> (CRP)	12.5	12.5	12.5	12.5
<i>Proteus mirabilis</i>	12.5	12.5	12.5	12.5
Carbapenem-resistant Enterobacteriaceae (CRE)	12.5	12.5	12.5	12.5
<i>Burkholderia pseudomallei</i>	12.5	12.5	12.5	12.5

Values are expressed as mg/mL. No significant differences were observed among treatments or bacterial strains according to DMRT ($p < 0.05$).

Conclusion

This study demonstrated that crude extracts of *L. flava* possess notable antibacterial activity against both Gram-positive and Gram-negative bacteria, including multidrug-resistant strains. The development of the crude extract into a gel formulation further confirmed its potential for practical application. The gel maintained its antibacterial efficacy under various stress conditions, including moisture, oxidative, acidic, and alkaline environments, indicating good stability. Moreover, the gel formulation demonstrated consistent bactericidal activity across all tested strains. These findings suggest that *L. flava* crude extract gel could serve as a promising natural antibacterial agent, especially for controlling drug-resistant pathogens. Future studies should focus on isolating active compounds, evaluating long-term stability, and assessing safety and efficacy *in vivo* models to support further pharmaceutical or topical applications.

Ethics Statement

This study was conducted in accordance with ethical standards and was approved by the Roi Et Rajabhat Ethics Committee under approval number 034/2568.

Acknowledgements

This research was financially supported by the Fundamental Fund for the fiscal year 2025 from the Science, Research and Innovation Promotion Fund, Roi Et Rajabhat University (Project Code: 203538).

References

- Anggraeni, A. D., Rahmasari, D., Nurfirdaus, A. R., Wulandari, O. T., Aprilia, A. D., Pratiwi, N. C., Ghofar, A., Salsabiella, E., Yen, H., & Khong. (2025). Formulation, stability, antibacterial activity, and het-cam irritation testing of Clinacanthus nutans ethanol fraction emulgel. *Medicinal Plants - International Journal of Phytomedicines and Related Industries*, 17(2), 313–322. <https://doi.org/10.5958/0975-6892.2025.00031.2>
- Arip, M., Selvaraja, M., R, M., Tan, L. F., Leong, M. Y., Tan, P. L., Yap, V. L., Chinnapan, S., Tat, N. C., Abdullah, M., K, D., & Jubair, N. (2022). Review on Plant-Based

- Management in Combating Antimicrobial Resistance - Mechanistic Perspective. *Frontiers in Pharmacology*, 13, 879495. <https://doi.org/10.3389/fphar.2022.879495>
- Armassa, N., Wongsorn, D., Saenmahayak, B., & Rattanasuk, S. (2025). Evaluation of the Antibacterial Potential of Ethanolic Cannabis sativa L. (Hang Kra Rog Phu Phan ST1) Extracts Against Human Pathogenic Bacteria. *Pakistan Journal of Biological Sciences*, 28(1), 1–7. <https://doi.org/10.3923/pjbs.2025.1.7>
- Armassa, N., Wongsorn, D., Saenmahayak, B., Rayan, S., & Rattanasuk, S. (2024). In vitro Antibacterial Activity of Ethanolic Tanao Si Kan Dang RD1 (Cannabis sativa L.) Extracts Against Human Antibiotic-Resistant Bacteria. *Pakistan Journal of Biological Sciences*, 27(3), 119–124. <https://doi.org/10.3923/pjbs.2024.119.124>
- Azrifitria, A., Purwaningsih, S., & Fatmala, A. R. (2022). In vitro antimicrobial activity and formulation of herbal anti-acne gel containing Rhizophora stylosa fruits extract. *Pharmaciana*, 12(3), 309–309. <https://doi.org/10.12928/pharmaciana.v12i3.21942>
- Boongapim, R., Katanan, S. C., Apisakulchat, T., Chaichamnong, E., & Rattanasuk, S. (2025). Antibacterial Efficacy of Garcinia mangostana Extracts Against Multidrug-Resistant and Clinically Significant Bacterial Pathogens. *Pakistan Journal of Biological Sciences*, 28(7), 421–428. <https://doi.org/10.3923/pjbs.2025.421.428>
- Bukhari, D. A., Alraddadi, F. A., Alqurashi, A. A., & Almutrafy, A. M. (2025). Evaluating the Synergistic and Antibacterial Effects of Freshwater Plants and Seagrass Extracts on Acinetobacter Baumannii. *Applied Ecology and Environmental Research*, 23(3), 4827–4841. https://doi.org/10.15666/aeer/2303_48274841
- Dash, P. P., Kumar, S., Mishra, A., & Srivastava, S. (2023). Antimicrobial activity of Haldina cordifolia (Roxb.) Ridsdale and Thevetia peruviana (Pers.) Schum. leaf extract against multidrug resistant microbes. *Annals of Phytomedicine An International Journal*, 12(1). <https://doi.org/10.54085/ap.2023.12.1.14>
- Elkhateeb, O. M., Badawy, M. E. I., Noreldin, A. E., Abou-Ahmed, H. M., El-Kammar, M. H., & Elkhenany, H. A. (2022). Comparative evaluation of propolis nanostructured lipid carriers and its crude extract for antioxidants, antimicrobial activity, and skin regeneration potential. *BMC Complement Med Ther*, 22(1), 256. <https://doi.org/10.1186/s12906-022-03737-4>
- Gan, R. Y., Li, H. B., Corke, H., & Yang, H. (2022). Editorial: Discovery of novel plant-derived compounds with antibacterial actions against antibiotic-resistant bacteria, volume II. *Frontiers in Microbiology*, 13, 1027679. <https://doi.org/10.3389/fmicb.2022.1027679>
- Ismail, N. A., Matawali, A., Kanak, F. A., C, L., How, S. E., Goh, L. P. W., & Gansau, J. A. (2022). Antimicrobial activities and phytochemical properties of Blumea balsamifera against pathogenic microorganisms. *Journal of Medicine and Life*, 15(8), 951–954. <https://doi.org/10.25122/jml-2021-0296>
- Jagdale, S., Gijare, A. J., Pardeshi, K. J., & Mandot, A. M. (2024). Development of Blumea lacera Gel Targeting Skin Disease. *Biosciences Biotechnology Research Asia*, 21(2), 547–553. <https://doi.org/10.13005/bbra/3246>
- Karimi, F., Azadi, A., Omidifar, N., Najafabady, N. M., Mohammadi, F., Kazemi, R., & Gholami, A. (2023). Pharmacotechnical aspects of a stable probiotic formulation toward multidrug-resistance antibacterial activity: design and quality control. *BMC Complement Med Ther*, 23(1), 391. <https://doi.org/10.1186/s12906-023-04224-0>
- Kebede, T., Gadisa, E., & Tufa, A. (2021). Antimicrobial activities evaluation and phytochemical screening of some selected medicinal plants: A possible alternative in the treatment of multidrug-resistant microbes. *PloS One*, 16(3). <https://doi.org/10.1371/journal.pone.0249253>

- 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)*, 10(9), 1044–1044. <https://doi.org/10.3390/antibiotics10091044>
- Khare, T., Anand, U., Dey, A., Assaraf, Y. G., Chen, Z. S., Liu, Z., & Kumar, V. (2021). Exploring Phytochemicals for Combating Antibiotic Resistance in Microbial Pathogens. *Frontiers in Pharmacology*, 12. <https://doi.org/10.3389/fphar.2021.720726>
- Khlongkhlaeo, A., Sookruksawong, S., & Rattanasuk, S. (2024). The Comparison of Antibacterial Efficacy, Cell Proliferation, and Wound Healing Properties in Extracts Derived from Leaves and Inflorescences of Hang Kra Rog Phu Phan ST1 (*Cannabis sativa* L.). *Trends in Sciences*, 21(12), 8446. <https://doi.org/10.48048/tis.2024.8446>
- Khonkayan, S., Nareephon, P., Kunlanaong, A., Boongapim, R., & Rattanasuk, S. (2025). Antibacterial Potential of *Micromelum falcatum* Leaf Extracts Against Antibiotic-Resistant Human Pathogens. *Pakistan Journal of Biological Sciences*, 28(6), 369–375. <https://doi.org/10.3923/pjbs.2025.369.375>
- Maliehe, T. S., Ngidi, L. S., Shandu, J. S. E., Poole, O. J., & Selepe, T. N. (2022). Antibacterial activity and chemical profile of the bioactive compounds from *Aloe polyphylla* Schönland. *Research Square (Research Square)*. <https://doi.org/10.21203/rs.3.rs-1801070/v1>
- Mayianda, J., Musyimi, D. M., & Muriira, N. (2025). Antimicrobial activities of crude extracts from selected medicinal climbers against *Candida albicans*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. *Archives of Ecotoxicology*, 7(3), 66–78. <https://doi.org/10.36547/ae.2025.7.3.66-78>
- Mehdipour, A., Ehsani, A., Samadi, N., Ehsani, M., & Sharifinejad, N. (2022). The antimicrobial and antibiofilm effects of three herbal extracts on *Streptococcus mutans* compared with Chlorhexidine 0.2% (in vitro study). *Journal of Medicine and Life*, 15(4), 526–532. <https://doi.org/10.25122/jml-2021-0189>
- Mlozi, S. H., Mmongoyo, J. A., & Chacha, M. (2023). In vitro evaluation of the herbal cream formulation from methanolic leaf extracts of *Tephrosia vogelii* Hook.f for topical application. *Clinical Phytoscience*, 9(1). <https://doi.org/10.1186/s40816-023-00357-4>
- Nigussie, D., Davey, G., Legesse, B. A., Fekadu, A., & Makonnen, E. (2021). Antibacterial activity of methanol extracts of the leaves of three medicinal plants against selected bacteria isolated from wounds of lymphoedema patients. *BMC Complementary Medicine and Therapies*, 21(1). <https://doi.org/10.1186/s12906-020-03183-0>
- Olaitan, M. O., & Nwadike, B. I. (2024). *Lawsonia inermis* (Henna) Extracts as a Sustainable Solution to Combat Multidrug-Resistant Gram-Negative Bacteria in Surface Water, Sewage, and Abattoir Leachate: A Quantitative Antimicrobial Analysis. *Research Square (Research Square)*. <https://doi.org/10.21203/rs.3.rs-5270522/v1>
- Rahman, M. M., Rahaman, M. S., Islam, M. R., Hossain, M. E., Mannan Mithi, F., Ahmed, M., Saldías, M., Akkol, E. K., & Sobarzo-Sánchez, E. (2021). Multifunctional Therapeutic Potential of Phytocomplexes and Natural Extracts for Antimicrobial Properties. *Antibiotics*, 10(9), 1076. <https://www.mdpi.com/2079-6382/10/9/1076>
- Rammali, S., Ciobica, A., El Aalaoui, M., Rahim, A., Kamal, F. Z., Dari, K., Khattabi, A., Romila, L., Novac, B., Petroaie, A., & Bencharki, B. (2024). Exploring the antimicrobial and antioxidant properties of *Lentzea flaviverrucosa* strain E25-2 isolated from Moroccan forest soil. *Frontiers in Microbiology*, 15, 1429035. <https://doi.org/10.3389/fmicb.2024.1429035>
- Rattanasuk, S., Hamcumpai, P., Wongkamjan, M., Boongapim, R., & Junsongduang, A. (2025). Potential Antibacterial Activity of *Oroxylum indicum* (L.) Kurz Extracts

- Against Antibiotic-Resistant Bacteria Isolated from Roi Et Hospital. *Pakistan Journal of Biological Sciences*, 28(2), 88–94. <https://doi.org/10.3923/pjbs.2025.88.94>
- Rattanasuk, S., Wechgama, K., Chumroenphat, T., Chaiyachet, O. A., & Charoensopharat, K. (2023). Potential Antibacterial Activity of Ethanolic *Curcuma longa* L. Rhizome Extract Against Antibiotic-Resistant Bacteria. *Pakistan Journal of Biological Sciences*, 26(3), 119–123. <https://doi.org/10.3923/pjbs.2023.119.123>
- Rita, N., Chidimma, O. P., Oluebebe, N. A., Chisom, O. G., & Kingsley, C. I. (2025). Solvent-dependent antimicrobial and phytochemical extracts of *Curcuma longa* against clinical pathogens. *Tropical Journal of Phytochemistry and Pharmaceutical Sciences*, 4(8). <https://doi.org/10.26538/tjpps/v4i8.3>
- Saleh, I., & Abu-Dieyeh, M. H. (2021). Novel *Prosopis juliflora* leaf ethanolic extract as natural antimicrobial agent against food spoiling microorganisms. *Scientific Reports*, 11(1), 7871. <https://doi.org/10.1038/s41598-021-86509-3>
- Saoudi, A., Bouacha, M., Amri, S., Benbouzid, H., Boufligha, K., Belhaoues, S., Boutarfa, K., Abdelkrim, M., Abdouni, I., & Bensouilah, M. (2023). The bactericidal effect of green macroalgae *Cladophora* sp. from freshwater toward Gram-negative and Gram-positive bacteria. *Malaysian Journal of Microbiology*. <https://doi.org/10.21161/mjm.220075>
- Sawisit, A., Payakapan, S., Rodpon, P., Charoenphakdee, A., Khamboonlhea, B., Pimthong, P., Jangsanam, C., Laongernthong, S., Wiengsima, W., & Rattanasuk, S. (2025). Comparative Antibacterial Activities of Extracts from Two Varieties of Bitter Gourd (*Momordica charantia*). *Pakistan Journal of Biological Sciences*, 28(4), 246–252. <https://doi.org/10.3923/pjbs.2025.246.252>
- Sharma, A., Rashid, M., Chauhan, P., Kaur, S., & Kaur, A. (2024). In vitro antibacterial and anti-biofilm potential of an endophytic *Schizophyllum commune*. *AMB Express*, 14(1), 10. <https://doi.org/10.1186/s13568-024-01663-x>
- Song, M., Liu, Y., Li, T., Liu, X., Hao, Z., Ding, S., Panichayupakaranant, P., Zhu, K., & Shen, J. (2021). Plant Natural Flavonoids Against Multidrug Resistant Pathogens. *Advanced Science*, 8(15). <https://doi.org/10.1002/advs.202100749>
- Stan, D., Enciu, A. M., Mateescu, A. L., Ion, A. C., Brezeanu, A. C., Stan, D., & Tănase, C. (2021). Natural Compounds With Antimicrobial and Antiviral Effect and Nanocarriers Used for Their Transportation. *Frontiers in Pharmacology*, 12. <https://doi.org/10.3389/fphar.2021.723233>
- Wongarmart, U., Reungbun, K., Tanampai, B., Nateesirikul, P., Parathang, R., Chaichamnong, E., Boongapim, R., & Rattanasuk, S. (2025). Antibacterial Activity of Leaf Extracts from *Blumea balsamifera*, *Morus alba* and *Limnocharis flava* Against Skin Pathogenic Bacteria. *Pakistan Journal of Biological Sciences*, 28(6), 376–382. <https://doi.org/10.3923/pjbs.2025.376.382>