http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2025.369.375

Research Article
Antibacterial Potential of *Micromelum falcatum* Leaf Extracts Against Antibiotic-Resistant Human Pathogens

[1]Sutarat Khonkayan, [1]Phongkeat Nareephot, [1]Amphon Kunlauaong, [2]Rujirek Boongapim and [1]Surachai Rattanasuk

[1]Department of Science and Technology, Faculty of Liberal Arts and Science, Roi Et Rajabhat University, Selaphum, Roi Et, Thailand
[2]Department of General Science, Faculty of Education and Human Development, Roi Et Rajabhat University, Selaphum, Roi Et, Thailand

Abstract

Background and Objective: *Micromelum falcatum*, a therapeutic plant belonging to the Rutaceae family, has been predominantly utilized in Traditional Chinese Medicine for its efficacy against ailments such as colds and rheumatoid arthritis, in addition to its anti-inflammatory and antimicrobial properties. This research aims to evaluate the antibacterial activity of *M. falcatum* extracts against four human pathogenic bacteria. **Materials and Methods:** Extracts were obtained from dried *M. falcatum* leaves using solvents including methanol, ethanol, ethyl-acetate, dichloromethane and hexane. The antibacterial activity was assessed against three antibiotic-resistant bacterial strains, namely *Acinetobacter baumannii*, *Stenotrophomonas maltophilia* and multidrug-resistant *Klebsiella pneumoniae* (MDR-K), as well as the reference strain *Pseudomonas aeruginosa* TISTR 2370. The agar disc diffusion assay served as the primary screening method for antibacterial activity. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values were determined using a microbroth dilution and colorimetric assay. The results were analyzed using Duncan's Multiple Range Test (DMRT), which was used to determine significant mean differences at the 95% confidence level. **Results:** The hexane extracts demonstrated the most substantial inhibition zone diameter, measuring 10 mm, against *A. baumannii*, *S. maltophilia* and P. aeruginose TISTR 2370. The most minimal MIC (3.125 mg/mL) and MBC (6.25 mg/mL) values were observed in the methanolic and ethanolic extracts against *A. baumannii*, *S. maltophilia* and MDR-K, respectively. **Conclusion:** This constitutes the inaugural documentation of the antibacterial efficacy of *M. falcatum* extracts against antibiotic-resistant bacteria. The findings from this study present promising prospects for the creation of innovative antibiotic medications and suggest potential therapeutic uses in the management of diseases associated with the bacteria tested.

Key words: *Micromelum falcatum* extracts, antibacterial activity, *Acinetobacter baumannii*, *Stenotrophomonas maltophilia*, multidrug-resistant *Klebsiella pneumoniae* (MDR-K)

Citation: Khonkayan, S., P. Nareephot, A. Kunlauaong, R. Boongapim and S. Rattanasuk, 2025. Antibacterial potential of *Micromelum falcatum* leaf extracts against antibiotic-resistant human pathogens. Pak. J. Biol. Sci., 28: 369-375.

Corresponding Author: Surachai Rattanasuk, Department of Science and Technology, Faculty of Liberal Arts and Science, Roi Et Rajabhat University, Selaphum, Roi Et 45120, Thailand Tel: +6643556111

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The increasing prevalence of antibiotic-resistant bacteria has become a significant public health concern globally, posing severe challenges to the effective treatment of infectious diseases. The misuse and overuse of conventional antibiotics have accelerated bacterial evolution, leading to pathogens capable of resisting multiple antibiotic therapies. This situation underscores the critical need to explore alternative antimicrobial agents from natural sources, such as plant-derived compounds, to combat resistant strains[1,2]. Recent studies indicate promising antibacterial activities of plant essential oils, including *Micromelum integerrimum*, highlighting their potential as effective agents against antibiotic-resistant bacteria[3].

Multidrug-resistant (MDR) pathogens such as *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Stenotrophomonas maltophilia* and colistin-resistant *Pseudomonas aeruginosa* present significant challenges in clinical settings due to their resistance to multiple antibiotics[4]. *Acinetobacter baumannii* is known for causing nosocomial infections, including pneumonia and bloodstream infections, particularly in critically ill patients and has been designated a serious threat by the centers for disease control and prevention (CDC)[5]. Similarly, *K. pneumoniae* has developed resistance mechanisms, such as the production of *Klebsiella pneumoniae* carbapenems (KPC), leading to infections associated with high morbidity and mortality[6]. *Stenotrophomonas maltophilia*, another MDR pathogen, is associated with respiratory tract infections, especially in immunocompromised individuals[7]. The emergence of colistin-resistant *P. aeruginosa* strains further complicates treatment options, as colistin is often considered a last-resort antibiotic for MDR Gram-negative infections[8,9].

Micromelum falcatum (Lour.) Tan, a member of the *Micromelum* genus within the Rutaceae family, is a mangrove-associated medicinal plant known for its distinctively pungent flowers and fruits, which change in color from green to orange or red as they mature. Traditionally utilized in traditional Chinese medicine (TCM) for the treatment of colds and rheumatoid arthritis, this species is primarily distributed across Guangdong, Hainan, Guangxi and Yunnan Provinces in China, as well as in Vietnam, Laos, Cambodia and Thailand[10,11]. The *Micromelum* genus comprises approximately 10 species, many of which exhibit medicinal properties and have been extensively used in traditional medicine. For instance, *M. integerrimum* has been employed for alleviating blood stasis, pain relief and the treatment of stomachaches and rheumatic bone pain. Similarly, *M. falcatum* has been traditionally used for managing traumatic injuries, snakebites and rheumatism[12,13]. Four new compounds, microminutin B, microminutin C, micromarinate and secomicromelin, along with 17 known compounds, were isolated from the fruits of *Micromelum falcatum*. All isolated compounds were assessed for antifungal activity against *Pythium insidiosum* using the disc diffusion assay[13,14]. A chemical investigation of the stems and stem bark of *Micromelum falcatum* (Lour.) Tan. led to the isolation of two novel lactam derivatives 3-(hydroxy(10-hydroxyphenyl) methyl)-4-(16-hydroxyphenyl)-1-methylpyrrolidin-2-one and 3-(hydroxy(10-hydroxy-9-methoxyphenyl)methyl)-4-(16-hydroxyphenyl)-1-methylpyrrolidin-2-one along with five known compounds: Gallic acid, p-hydroxybenzoic acid, 4-hydroxybenzaldehyde, trans-4-hydroxycinnamic acid and m-hydroxybenzoic acid[10]. Additionally, two previously unreported quinoline Dione alkaloids, 3-hydroxy-1-methyl-3-(2-oxopropyl)quinoline-2,4(1H,3H)-dione and methyl 2-(3-hydroxy-1-methyl-2,4-dioxo-1,2,3,4-tetrahydroquinolin-3-yl)acetate, were isolated for the first time as natural products from the stem bark of *M. falcatum*[11].

Previous phytochemical studies on various parts of *M. minutum* have revealed the presence of bioactive compounds, including coumarins, triterpenes, alkaloids and phenylpropanoids[15]. In a related investigation, *M. integerrimum* was examined for the chemical constituents and antibacterial properties of its essential oil. Gas Chromatography-Mass Spectrometry (GC-MS) analysis revealed a total of 52 distinct chemical compounds, primarily comprising monoterpene hydrocarbons, oxygenated monoterpenes and sesquiterpenes. The major constituents identified were terpinolene, α-pinene, β-pinene and camphene. Furthermore, the essential oil exhibited broad-spectrum antimicrobial activity in a concentration-dependent manner, with inhibition zones ranging from 7 to 16 mm at a concentration of 100 µL. Notably, the oil demonstrated strong inhibitory activity against *Bacillus subtilis* MTCC 441 and *Bacillus spizizenii* ATCC 6633, comparable to the standard antibiotic neomycin B (22 mm)[3]. From the information presented above, it can be concluded that *M. falcatum* contains several phytochemical compounds with significant medicinal potential. This research aims to evaluate the antibacterial activity of *M. falcatum* extracts against four antibiotic-resistant human pathogenic bacteria. The findings from this study could support the development of natural therapeutic agents for treating infections caused by these resistant bacterial strains.

MATERIALS AND METHODS

Study area: The study was carried out between June and December, 2021 in the Microbiology Laboratory, Department of Science and Technology, Faculty of Liberal Arts and Science, Roi Et Rajabhat University, Roi Et, Thailand.

Plant sample collection and extraction: Three kilograms of *M. falcatum* leaves were collected from a natural forest. The leaf samples were washed twice with tap water, cut into small pieces and dried at 50°C using a hot air oven (POL-EKO-APARATURA, Wodzisław Śląski, Poland) for three days. The dried leaves were then ground into a fine powder using a blender (WF-20B THAIGRINDER, Thailand) and subjected to extraction with solvents of varying polarities, including methanol, ethanol, ethyl acetate, hexane and dichloromethane (Italamar (Thailand) Co., Ltd.). The extracted solutions were filtered and evaporated to dryness at 50°C for two days using a hot air oven. The extraction yield was determined as a percentage. Dimethyl sulfoxide (DMSO, Sigma) was added to each extract to achieve a final concentration of 500 mg/mL before further use[16,17]. The percentage yield of extracts was calculated as below equation[18]:

$$\text{Yield (\%)} = \frac{\text{Dry weight of plant extract}}{\text{Dry weight of plant material}} \times 100$$

Preparation for antibiotic-resistant bacterial strains: Three antibiotic-resistant bacterial strains, *Acinetobacter baumannii*, multidrug-resistant *Klebsiella pneumoniae* (MDR-K) and *Stenotrophomonas maltophilia*, were obtained from the Department of Clinical Microbiology, Roi Et Hospital, Roi Et, Thailand (Table 1). The reference strain, *Pseudomonas aeruginosa* TISTR 2370, was obtained from the Thailand Institute of Scientific and Technological Research culture collection (TISTR culture collection), Thailand. Each strain was streaked onto a nutrient agar (NA) plate and incubated at 37°C overnight. A single colony of each strain was subsequently inoculated into 5 mL of nutrient broth (NB) and incubated under shaking conditions (150 rpm, 37°C) for 18 hrs. Before use, bacterial suspensions were adjusted to an optical density (OD600) of 0.1 to standardize the concentration[19].

Agar disc diffusion assay: The disc diffusion assay was employed as a preliminary screening method to evaluate antibacterial activity. The concentration of pathogenic bacteria was adjusted to an optical density (OD_{600}) of 0.1. A 100 µL aliquot of each bacterial suspension was evenly spread onto nutrient agar (NA) plates. Sterile paper discs were placed on the surface of the inoculated plates and 10 µL of each extract was carefully applied to the discs. The NA plates were then incubated at 37°C for 18 hrs. The resulting inhibition zones were measured and recorded.

Broth microdilution and colorimetric assay: The minimum inhibitory concentration (MIC) refers to the lowest concentration of an extract that inhibits bacterial growth, while the minimum bactericidal concentration (MBC) denotes the lowest concentration of an extract required to eliminate bacteria. These values are critical parameters in microbiology and antimicrobial susceptibility testing, providing essential insights into the efficacy of antimicrobial agents.

In this study, MIC and MBC values were determined using the broth microdilution method combined with a colorimetric assay. Extracts that exhibited positive results in the disc diffusion assay were subjected to a two-fold serial dilution in a 96-well plate containing 100 µL of nutrient broth (NB). Subsequently, 100 µL of antibiotic-resistant bacterial suspension was introduced into each well, containing varying concentrations of *M. falcatum* leaf extract. The 96-well plates were then incubated overnight at 37°C. Following incubation, iodonitrotetrazolium chloride (INT) solution was added to each well and further incubated at 37°C for 30 min. The MIC was defined as the lowest concentration of *M. falcatum* leaf extract that inhibited the visible growth of antibiotic-resistant bacteria. The MBC was determined as the lowest concentration at which complete bacterial eradication occurred, indicated by the absence of color change after INT addition.

Table 1: Pathogenic bacteria used in the study

Bacterial strain	Description
Acinetobacter baumannii	Antibiotic-resistant strain, clinical isolate
Multidrug-resistant *Klebsiella pneumoniae*	Antibiotic-resistant strain, clinical isolate
Stenotrophomonas maltophilia	Antibiotic-resistant strain, clinical isolate
Pseudomonas aeruginosa TISTR 2370	Reference strain

Data analysis: The diameters of the inhibition zones were analyzed using SAS software version 5.0. The experimental design employed was a Completely Randomized Design (CRD) with three replicates per treatment, each replicate comprising three plates. Statistical differences among treatments were evaluated using One-way Analysis of Variance (ANOVA). Mean comparisons were subsequently conducted using Duncan's Multiple Range Test (DMRT). Differences were considered statistically significant at a p-value of less than 0.05.

RESULTS AND DISCUSSION

Antibacterial activity of *Micromelum falcatum* leaf extracts: Leaves of *M. falcatum* were sequentially extracted using solvents of varying polarity, including methanol, ethanol, ethyl acetate, hexane and dichloromethane. The highest extraction yield was obtained from the methanol extract (4.160%), followed by ethanol (2.953%), dichloromethane (2.100%), ethyl acetate (1.683%) and hexane (0.648%), respectively (Table 2).

The antibacterial activity of each extract was assessed using the agar disc diffusion assay against selected bacterial strains by measuring inhibition zone diameters to determine their relative effectiveness (Fig. 1a-b). Results demonstrated that the hexane extract exhibited the largest inhibition zones (10 mm) against *A. baumannii*, *S. maltophilia* and *P. aeruginosa* TISTR 2370. Both methanol and ethanol extracts produced inhibition zones measuring 7 mm against *A. baumannii*, *S. maltophilia* and MDR-K. The ethyl acetate extract showed a 7 mm inhibition zone exclusively against *S. maltophilia* and *P. aeruginosa* TISTR 2370. In contrast, the dichloromethane extract displayed antibacterial activity only against *S. maltophilia*, with an inhibition zone of 7 mm (Table 3). The result of this research, according to Gutierrez-Montiel *et al.*[20], showed that the antimicrobial activity of guava leaf crude extract against XDR *A. baumannii* and *P. aeruginosa* ATCC 27853 by the agar diffusion technique. The result indicated that guava leaf crude extract can inhibit the growth of XDR *A. baumannii* (9-12 mm) and *P. aeruginosa* ATCC 27853 (26.97 mm). In 2012, researchers reported the isolation of two novel coumarins, 7-methoxy-8-(2-hydroxymethyl-1-*O*-isovaleryl-4-butenyl)coumarin and 7-methoxy-8-(1-hydroxy-2-*O*-β-glucopyranosyl-3-methyl-4-butene-1-yl)coumarin, from *M. falcatum*. However, antibacterial assays revealed that these compounds exhibited no inhibitory activity against *Bacillus subtilis*, *Bacillus thuringiensis* and *Escherichia coli*[21]. Almalki *et al.*[22] reported that ethanolic *Chlorella vulgaris* extracts can inhibit the growth of *S. maltophilia* CSK1 by presenting the inhibition zone of 13±0.4 mm.

Table 2: Extraction yield of the *Micromelum falcatum* leaf extracts

Solvents	Yield (%)
Methanol	4.160
Ethanol	2.953
Ethyl acetate	1.683
n-hexane	0.648
Dichloromethane	2.1

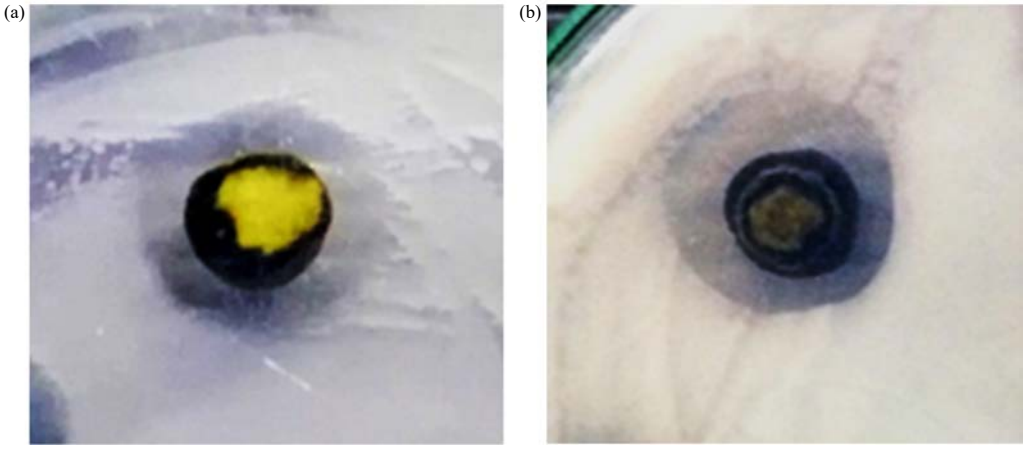

Fig. 1(a-b): Inhibition zone *Micromelum falcatum* leaf extracted with hexane against (a) multidrug-resistant *Klebsiella pneumoniae* and (b) *Stenotrophomonas maltophilia*

Table 3: Inhibition zone diameter of *Micromelum falcatum* leaf extracts against the tested pathogenic bacteria

Solvents	Bacteria	Zone of inhibition (mm)
Methanol	*Acinetobacter baumannii*	7[b]
	Stenotrophomonas maltophilia	7[b]
	Multidrug-resistant *Klebsiella pneumoniae*	7[b]
Ethanol	*Acinetobacter baumannii*	7[b]
	Stenotrophomonas maltophilia	7[b]
	Multidrug-resistant *Klebsiella pneumoniae*	7[b]
Ethyl acetate	*Stenotrophomonas maltophilia*	7[b]
	Pseudomonas aeruginosa TISTR2370	7[b]
n-hexane	*Acinetobacter baumannii*	10[a]
	Stenotrophomonas maltophilia	10[a]
	Pseudomonas aeruginosa TISTR2370	10[a]
Dichloromethane	*Stenotrophomonas maltophilia*	7[b]
p-value		0.0067
CV (%)		16.13

*Means (n = 3) in the column followed by the same common letter were not significantly different (DMRT, p>0.05)

Table 4: MIC value of *Micromelum falcatum* leaf extracts against pathogenic bacteria

Solvents	Bacteria	MIC (mg/mL)	MBC (mg/mL)
Methanol	*Acinetobacter baumannii*	3.125	6.25
	Stenotrophomonas maltophilia	3.125	6.25
	Multidrug-resistant *Klebsiella pneumoniae*	3.125	6.25
Ethanol	*Acinetobacter baumannii*	3.125	6.25
	Stenotrophomonas maltophilia	6.25	12.5
	Multidrug-resistant *Klebsiella pneumoniae*	6.25	12.5
Ethyl acetate	*Stenotrophomonas maltophilia*	6.25	12.5
	Pseudomonas aeruginosa TISTR2370	6.25	12.5
n-hexane	*Acinetobacter baumannii*	6.25	12.5
	Stenotrophomonas maltophilia	6.25	12.5
	Pseudomonas aeruginosa TISTR2370	6.25	12.5
Dichloromethane	*Stenotrophomonas maltophilia*	6.25	12.5

The minimum inhibitory concentration (MIC) refers to the lowest concentration of a leaf extract that effectively inhibits the visible growth of a microorganism, while the minimum bactericidal concentration (MBC) is defined as the lowest concentration required to achieve bacterial cell death. The findings indicate that the lowest MIC value (3.125 mg/mL) was observed in the methanolic leaf extract against *A. baumannii*, *S. maltophilia* and MDR-K, as well as in the ethanolic extract against *A. baumannii*. Similarly, the lowest MBC value (6.25 mg/mL) was recorded for the methanolic extract against *A. baumannii*, *S. maltophilia* and MDR-K and the ethanolic extract against *A. baumannii* (Table 4). The result is similar previous report by Mahmoud *et al.*[23]. They reported that the minimum bactericidal concentration (MBC) of *Syzygium aromaticum* aqueous and ethyl acetate extracts against *A. baumannii* isolates ranged from 0.17 to 0.25 mg/mL. In contrast, ethanol extracts exhibited significantly lower MBC values, ranging from 0.04 to 0.125 mg/mL, indicating higher antibacterial potency.

Anek *et al.*[24] reported that pyrogallol exhibited the highest antimicrobial activity, with a minimum inhibitory concentration (MIC) of 0.25 mg/mL and a minimum bactericidal concentration (MBC) ranging from 0.25 to 0.5 mg/mL against methicillin-susceptible *Staphylococcus aureus*, methicillin-resistant *S. aureus*, *Escherichia coli* ATCC 25922, colistin-resistant *E. coli* and colistin-resistant *Klebsiella pneumoniae*. Similarly, Hassan *et al.*[25] investigated the antimicrobial activities of ethanolic and methanolic extracts of *Rhazya stricta* leaves against 15 *K. pneumoniae* isolates using MIC and MBC assays. The MIC of the ethanolic extract ranged from 0.122 to 0.970 mg/mL, while the MBC varied between 0.224 and 1.9 mg/mL. In contrast, the methanolic extract exhibited MIC values ranging from 0.224 to 1.9 mg/mL, with MBC values between 0.448 and 3.9 mg/mL. This study was limited to *in vitro* assays using disc diffusion and MIC/MBC methods without identifying specific active compounds responsible for the antibacterial effects. Future research should focus on the isolation, structural elucidation and mechanism of action of the bioactive constituents in *M. falcatum* leaf extracts, as well as conducting in *vivo* evaluations to confirm their therapeutic potential.

CONCLUSION

This study evaluated the antibacterial efficacy of *M. falcatum* leaf extracts obtained using methanol, ethanol, ethyl acetate, hexane and dichloromethane against three antibiotic-resistant human pathogens and one reference strain. The extracts exhibited significant antibacterial activity against *A. baumannii*, MDR-K, *S. maltophilia* and *P. aeruginosa* TISTR 2370. Among the extraction solvents, methanol demonstrated the highest antibacterial potency from *M. falcatum* leaves.

SIGNIFICANCE STATEMENT

This study discovered the potent antibacterial activity of Micromelum falcatum leaf extracts that can be beneficial for treating infections caused by antibiotic-resistant human pathogens. The findings highlight the therapeutic potential of natural plant-based compounds in addressing the global challenge of antimicrobial resistance. By targeting resistant strains and demonstrating measurable inhibition, the study opens up possibilities for developing novel, plant-derived antimicrobial agents. The investigation into this underexplored species adds value to ethnobotanical knowledge and supports the integration of traditional medicinal plants into modern pharmacology. This study will help the researchers to uncover the critical areas of phytochemical-based antibacterial mechanisms that many researchers were not able to explore. Thus, a new theory on natural resistance modulation may be arrived at.

ACKNOWLEDGMENT

This research project is supported by the Science Research and Innovation Fund, Roi Et Rajabhat University. Grant No 182002.

REFERENCES

1. Raut, J.S. and S.M. Karuppayil, 2014. A status review on the medicinal properties of essential oils. Ind. Crops Prod., 62: 250-264.
2. Rattanasuk, S. and T. Phiwthong, 2021. A new potential source of anti-pathogenic bacterial substances from *Zamioculcas zamiifolia* (Lodd.) Engl. extracts. Pak. J. Biol. Sci., 24: 235-240.
3. Kotoky, R., M. Bordoloi, A. Yadav, K.J. Tamuli and S. Saikia *et al*., 2019. Chemical composition and antibacterial activity of essential oil from fruit of *Micromelum integerrimum (Buch.-Ham. ex DC.) Wight & Arn. ex M. Roem*. Nat. Prod. Res., 33: 3573-3576.
4. Peleg, A.Y., H. Seifert and D.L. Paterson, 2008. *Acinetobacter baumannii*: Emergence of a successful pathogen. Clin. Microbiol. Rev., 21: 538-582.
5. Whiteway, C., A. Breine, C. Philippe and C. van der Henst, 2022. *Acinetobacter baumannii*. Trends. Microbiol., 30: 199-200.
6. Wei, J., J. Huang, C. Zou, S. Shen and B.N. Kreiswirth *et al*., 2025. Diverse evolutionary trajectories of *Klebsiella pneumoniae* carbapenemase: Unraveling the impact of amino acid substitutions on β-lactam susceptibility and the role of avibactam in driving resistance. mSystems, Vol. 10. 10.1128/msystems.00184-25.
7. Brooke, J.S., 2021. Advances in the microbiology of *Stenotrophomonas maltophilia*. Clin. Microbiol. Rev., Vol. 34. 10.1128/cmr.00030-19.
8. El Chakhtoura, N.G., E. Saade, A. Iovleva, M. Yasmin, B. Wilson, F. Perez and R.A. Bonomo, 2018. Therapies for multidrug resistant and extensively drug-resistant non-fermenting gram-negative bacteria causing nosocomial infections: A perilous journey toward 'molecularly targeted' therapy. Expert Rev. Anti-Infect. Ther., 16: 89-110.
9. Abd El-Baky, R.M., S.M. Masoud, D.S. Mohamed, N.G.F.M. Waly and E.A. Shafik *et al*., 2020. Prevalence and some possible mechanisms of colistin resistance among multidrug-resistant and extensively drug-resistant *Pseudomonas aeruginosa*. Infect. Drug Resist., 13: 323-332.
10. Zan, K., Y. Gan, P. Xie, R. Zhang, S. Ma, Q. Lin and J. Zheng, 2021. Anti-inflammatory new cyathane diterpene glucosides from *Onychium japonicum*. Phytochem. Lett., 44: 206-209.
11. Zhang, J.H., J.J. Guo, Y.X. Yuan, Y.H. Fu and Y.C. Gu *et al*., 2016. Four new tetracyclic alkaloids with cis-decahydroquinoline motif from *Myrioneuron effusum*. Fitoterapia, 112: 217-221.
12. Jun, W., Q. Xiao, J. Xu, M.Y. Li, J.Y. Pana and M.H. Yang, 2008. Natural products from true mangrove flora: Source, chemistry and bioactivities. Nat. Prod. Rep., 25: 955-981.
13. Suthiwong, J., U. Sriphana, Y. Thongsri, P. Promsuwan, C. Prariyachatigul and C. Yenjai, 2014. Coumarinoids from the fruits of *Micromelum falcatum*. Fitoterapia, 94: 134-141.
14. Kouloura, E., E. Danika, S. Kim, M. Hoerlé, M. Cuendet, M. Halabalaki and L.A. Skaltsounis, 2014. Rapid identification of coumarins from *Micromelum falcatum* by UPLC-HRMS/MS and targeted isolation of three new derivatives. Molecules, 19: 15042-15057.
15. Fatema-Tuz Zohora, C.M. Hasan and M. Ahsan, 2019. Chemical constituents, cytotoxic activities and traditional uses of *Micromelum minutum* (Rutaceae): A review. Pharm. Pharmacol. Int. J., 7: 229-236.
16. Rattanasuk, S., P. Hamcumpai, M. Wongkamjan, R. Boongapim and A. Junsongdua, 2025. Potential antibacterial activity of *Oroxylum indicum* (L.) Kurz extracts against antibiotic-resistant bacteria isolated from Roi Et Hospital. Pak. J. Biol. Sci., 28: 88-94.

17. Armassa, N., D. Wongsorn, B. Saenmahaya and S. Rattanasu, 2025. Evaluation of the antibacterial potential of ethanolic *Cannabis sativa* L. (Hang Kra Rog Phu Phan ST1) extracts against human pathogenic bacteria. Pak. J. Biol. Sci., 28: 1-7.
18. Rattanasuk, S. and T. Phiwthong, 2020. Evaluation of the antibacterial activity of *Spathiphyllum wallisii* extracts against human pathogenic bacteria. Pak. J. Biol. Sci., 23: 1436-1441.
19. Armassa, N., D. Wongsorn, B. Saenmahaya, S. Rayan and S. Rattanasuk, 2024. *In vitro* antibacterial activity of ethanolic Tanao Si Kan Dang RD1 (*Cannabis sativa* L.) extracts against human antibiotic-resistant bacteria. Pak. J. Biol. Sci., 27: 119-124.
20. Gutierrez-Montiel, D., A.L. Guerrero-Barrera, F.Y. Ramírez-Castillo, F. Galindo-Guerrero and I.G. Ornelas-García *et al*., 2025. Guava leaf extract exhibits antimicrobial activity in extensively drug-resistant (XDR) *Acinetobacter baumannii*. Molecules, Vol. 30. 10.3390/molecules30010070.
21. Luo, X., W. He, H. Yin, Q. Li, Q. Liu, Y. Huang and S. Zhang, 2012. Two new coumarins from *Micromelum falcatum* with cytotoxicity and brine shrimp larvae toxicity. Molecules, 17: 6944-6952.
22. Almalki, M.A., A.Y.Z. Khalifa and Y.A. Alkhamis, 2022. *In vitro* antibiosis of *Chlorella vulgaris* extract against the phytopathogen, *Stenotrophomonas maltophilia*. J. Pure Appl. Microbiol., 16: 630-637.
23. Mahmoud, A., M.M. Afifi, F. El Shenawy, W. Salem and B.H. Elesawy, 2021. Syzygium aromaticum extracts as a potential antibacterial inhibitors against clinical isolates of *Acinetobacter baumannii*: An *in-silico*-supported *in-vitro* study. Antibiotics, Vol. 10. 10.3390/antibiotics10091062.
24. Anek, P., S. Kumpangcum, S. Roytrakul, C. Khanongnuch, C. Saenjum and K. Phannachet, 2024. Antibacterial activities of phenolic compounds in *Miang* extract: Growth inhibition and change in protein expression of extensively drug-resistant *Klebsiella pneumoniae*. Antibiotics, Vol. 13. 10.3390/antibiotics13060536.
25. Hassan, M.M., B. Albogami, T. Mwabvu, M.F. Awad and R.H. Kadi *et al*., 2023. The antibacterial activity of *Rhazya stricta* extracts against *Klebsiella pneumoniae* isolated from some soil invertebrates at high altitudes. Molecules, Vol. 28. 10.3390/molecules28083613.