RJC

RASĀYAN *J. Chem.*
Vol. 18 | No. 2 |1022-1027| April - June | 2025
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

ANTIBACTERIAL ACTIVITY AND LC-MS IDENTIFICATION OF QUERCETIN IN THE ETHYL ACETATE FRACTION OF *Polygonum barbatum* L. LEAVES FROM CENTRAL KALIMANTAN, INDONESIA

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ABSTRACT

Mengkrengan (*Polygonum barbatum* L.) is a plant that is empirically used by the Dayak tribe in Kapuas Village, Central Kalimantan, Indonesia, for the treatment of typhoid fever. Secondary metabolites found in the leaves of mengkrengan include saponins, terpenoids, flavonoids, tannins, and alkaloids. The objective of this study was to determine the antibacterial activity and identify the metabolite compounds from the ethyl acetate fraction of mengkrengan (*Polygonum barbatum* L.) leaves originating from Central Kalimantan, Indonesia, using the *Liquid Chromatography – Mass Spectrometry* (LC-MS) method. The stages of the research began with the preparation of simplicia, the production of the ethyl acetate fraction, antibacterial activity testing at concentrations of 5%, 10%, and 15%, and the identification of metabolite compounds from the ethyl acetate fraction of mengkrengan leaves using LC-MS. The results obtained at concentrations of 5%, 10%, and 15% showed antibacterial activity with inhibition zones of 9 mm (moderate category), 12 mm (strong category), and 17 mm (strong category), respectively. LC-MS identification of compounds revealed a metabolite compound with the molecular formula C15H10O7 (MW 303.0511), which was identified as quercetin. The LC-MS chromatogram also showed the presence of six other unidentified compounds.

Keywords: Mengkrengan Leaves, *Polyganum barbatum* L, Quercetin, Antibacterial Activity, LC–MS (*Liquid Chromatography – Mass Spectrometry*), Typhoid Fever

RASĀYAN *J. Chem.*, Vol. 18, No. 2, 2025

INTRODUCTION

Mengkrengan (*Polygonum barbatum* L.) is used by the community of Kapuas Village, Central Kalimantan, Indonesia, to treat typhoid fever. The part of the plant utilized by the Kapuas Village community for typhoid treatment is the leaves. Previous studies have shown that the leaves of *Mengkrengan* contain metabolites such as saponins, terpenoids, flavonoids, tannins, and alkaloids.[2] Other studies have indicated that the bark of the *Mengkrengan* plant has pharmacological effects such as hypoglycemic, antibacterial, anti-HIV effects, and can be used as an antidote for diarrhea.[2] Additional bioactive properties, including anticancer activity, have been shown to potentially inhibit the proliferation, migration, and invasion of cancer cells and modulate signaling pathways associated with cancer cell growth.[4] Active compounds isolated from *Mengkrengan* leaves include dihydrobenzofuran derivatives and sesquiterpenes, which have potential antitumor activities. These compounds are capable of inducing apoptosis in cancer cells by disrupting the mitochondrial membrane potential.[5] Liquid Chromatography–Mass Spectrometry (LC-MS) combines the separation capabilities of liquid chromatography (LC) with the detection and identification capabilities of mass spectrometry (MS), enabling highly sensitive and specific analysis of various compounds in complex mixtures.[6] The aim of this study was to analyze the antibacterial activity and identify metabolite compounds from the ethyl

http://doi.org/10.31788/RJC.2025.1829262

acetate fraction of *Mengkrengan* leaves (*Polygonum barbatum* L.) from Central Kalimantan, Indonesia, using the LC-MS method.

EXPERIMENTAL

Materials

Beaker Glass (Pyrex®), Erlenmeyer Flask (Pyrex®), Test Tube, Test Tube Rack, Stirring Rod, Glass Jar, Dropper Pipet, Measuring Glass (Pyrex®), Analytical Balance (ACS®), Porcelain Crucible, Petri Dish, Aluminum Tray, Filter Paper, Oven (Binder®), Blender (Maspion®), Mesh Sieve 200, Buchner Funnel (Pyrex®), Aluminum Foil (Total Wrap®), Plastic Wrap (Total Wrap®), Vacuum, *Rotary Evaporator* (Biobase®), Waterbath (B-One®), Micropipette and Tips (Dragon lab®), Vernier Caliper, Syringe, and Liquid Chromatography-Mass Spectrometry (LC-MS) (Shimadzu®). The raw materials used are fresh Mengkrengan leaves, 70% Ethanol, Aquadest, 2N HCl, Ethyl acetate, n-Hexane, MHA (*Mueller Hinton Agar*), and *Salmonella thypi* ATCC 6539.

Extraction and Fractionation

Mengkrengan leaves were extracted according to the referenced study.[7]. The resulting extract was then fractionated using several solvents with different polarity levels, such as n-hexane, ethyl acetate, and distilled water. The obtained fractions were subsequently concentrated using a *rotary evaporator* at a temperature of 40°C.[8]

Antibacterial Activity Test

The antibacterial test of the ethyl acetate fraction from Mengkrengan leaves was carried out using the paper disc diffusion method. Sterilized MHA (*Mueller Hinton Agar*) media was inoculated with *Salmonella typhii* ATCC 6539 bacteria on the entire surface of the medium. A paper disc was applied with 18-20 µL of the ethyl acetate fraction from Mengkrengan leaves and then placed on the surface of the media inoculated with the test bacteria. The incubation was conducted for 24 to 48 hours at 37°C. A clear zone (inhibition zone) was observed to form around the paper disc, and the results were displayed as mean ± standard deviation.[9]

LC-MS (Liquid Chromatography-Mass Spectrometry) Test

In this study, we applied a quantitative LC-MS method to analyze the compounds present in the ethyl acetate fraction of Mengkrengan leaves. This analysis is based on the principles of separation, identification of compounds, molecular weight, and chemical structure. Stages of compound analysis using LC-MS:[10]

Sample Dilution

The ethyl acetate fraction from mengkrengan leaves was dissolved in 70% ethanol at a concentration of 100 ppm in a test tube. The solution was then vortexed until it became homogeneous. The formed solids were separated by centrifugation at 800 rpm for 10 minutes, and the resulting supernatant was used in the next step.

Protein Precipitation

A chemical solvent was used to separate the proteins in a precipitation technique. Three milliliters of acetonitrile acidified with 0.2% formic acid were combined with two milliliters of the diluted supernatant in a centrifuge tube. After 30 seconds of centrifugation at 8000 rpm, the supernatant was collected for further examination.

Purification with SPE (Solid Phase Extraction)

The conditioned sample was mixed with 1 ml of acetonitrile-water solution and loaded into a *Sep-Pak* C18 Cartridge (1 cc, 100 mg). A total of 0.5 ml of the eluate was collected, followed by loading another 1 ml of sample into the Sep-Pak C18 Cartridge and collecting an additional 0.5 ml of eluate. Next, a solution (0.5 ml of a solution with an 80:20 acetonitrile-water ratio was added to the Sep-Pak column, and the eluate (0.5 ml of the eluate) was collected again. A 0.25 ml portion of the purified solution was mixed with 200 ml of ammonium phosphate solution in a 50:50 acetonitrile-ethanol mixture in the Sep-Pak. Subsequently, 0.5 ml of the eluate was collected and mixed with 0.2 ml of a solution of acetonitrile and

buffer (25:75). The solution was then filtered using a 0.45 µm pore size *cellulose acetate membrane filter* and degassed, making it ready for injection into the LC-MS.

LC-MS Analysis Procedure

The active compound concentration was tested using the LC-MS method under the following conditions: autosampler positioned at 35°C and using a 1 µl injection volume; *Ultra High Pressure Liquid Chromatography* (UHPLC) operating with an isocratic mobile phase; a column measuring 2 mm x 150 mm x 3 µm; and MS/MS for further analysis.[10]

RESULTS AND DISCUSSION

Antibacterial Test of Ethyl Acetate Fraction of Mengkrengan Leaves (*Polygonum barbatum* L.)

The antibacterial activity of the ethyl acetate fraction of mengkrengan leaves against *Salmonella typhi* was tested at concentrations of 5%, 10%, and 15%. The results of the bacterial inhibition zone measurements were as follows:

Table-1: Inhibition Zone Results of the Ethyl Acetate Fraction from Mengkrengan Leaves

	Ethyl Acetate Fraction 5%	Ethyl Acetate Fraction 10%	Ethyl Acetate Fraction 15%	Positive Control	Negative Control
Average Inhibition Zone (mm) ± SD	9 ± 1,5	12 ± 2,2	17 ± 3,1	38	6
Inhibition Zone Category	Moderate	Strong	Strong	Very Strong	Weak

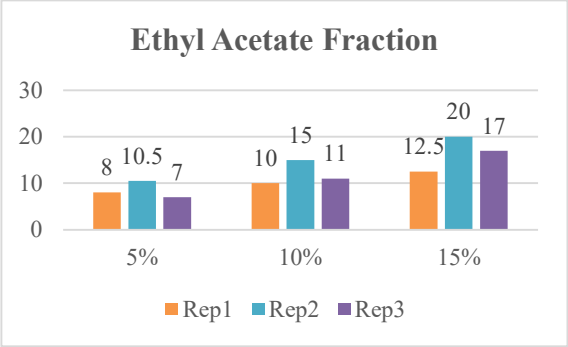

Fig.-1: Diagram of Bacterial Inhibition Zone Measurements of the Ethyl Acetate Fraction from Mengkrengan Leaves

LC-MS (Liquid Chromatography-Mass Spectrometry) Results

Fractionation of the extract from Mengkrengan (*Polygonum barbatum* L.) leaves was performed to further separate the chemical metabolite compounds contained in the solution, which have varying levels of polarity. The ethyl acetate fraction from the Mengkrengan leaves was chosen because ethyl acetate is a semi-polar solvent, making it capable of dissolving both polar and non-polar compounds. Chloramphenicol was used as a positive control in the antibacterial activity test, as it is the first-line treatment for typhoid fever caused by *Salmonella typhi.*[11] The antibacterial activity test used the disk diffusion method because it allows easy observation of inhibition zones.[12] As shown in Table-1, the diameters of the inhibition zone increased with each concentration. Among the three concentrations, the largest inhibition zone diameter was observed at 15%, measuring 17 mm ± 3.1 (categorized as strong inhibition).

This indicates that the ethyl acetate fraction at 15% concentration contained a higher level of antibacterial secondary metabolites compared to the 5% and 10% concentrations. Flavonoids are a group of secondary metabolites that can dissolve in ethyl acetate and are identified in *Polygonum* species, including quercetin, epicatechin, catechin, quercitrin, kaempferol, myricetin, and other flavonoids.[13] Figure-1 shows the results of the bacterial inhibition zone measurements at various concentrations of the ethyl acetate fraction from Mengkrengan leaves, illustrating a positive correlation between increasing concentration and antibacterial

activity. The higher the concentration, the larger the diameter of the inhibition zone (indicating the effectiveness of the fraction in inhibiting bacterial growth).

Previous studies have shown that quercetin is capable of inhibiting the growth of viruses, fungi, and both Gram-positive and Gram-negative bacteria.[14] Its antibacterial mechanism includes damaging bacterial cell membranes, altering membrane permeability, limiting biofilm formation, reducing virulence factor expression, inhibiting protein and nucleic acid production, and damaging mitochondria.[15]

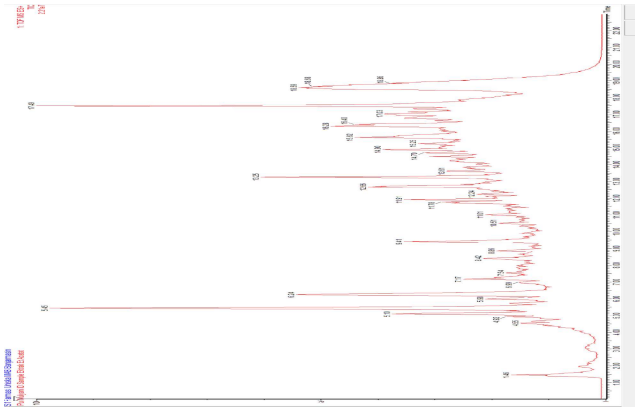

Fig.-2: Chromatogram Results of the Ethyl Acetate Fraction from Mengkrengan Leaves

Table-2: LC-MS Results of the Ethyl Acetate Fraction

No	Retention Time	Molecule Structure	Molecular Weight	Metabolite Compound
1	1.457	$C_8H_{15}NO$	142.1208	Tropine
2	4.529	$C_{15}H_{14}O_6$	291.0873	Epichatecin
3	4.902	$C_{27}H_{27}NO_{10}$	526.1710	*unknown compound*
4	5.098	$C_{11}H_{12}O_{10}$	303.0510	*unknown compound*
5	6.266	$C_{11}H_{16}O_3$	197.1182	*unknown compound*
6	7.187	$C_{15}H_{10}O_7$	303.0511	Quercetin
7	7.559	$C_{16}H_{12}O_7$	563.2490	Isorhamnetin
8	8.417	$C_{18}H_{26}O_2$	275.2018	Cinmethylin
9	8.960	$C_{23}H_{29}NO_2$	353.2306	*unknown compound*
10	9.409	$C_{11}H_{16}O_2$	181.1233	3-tert-Butyl-5-methylcatechol
11	11.890	$C_{21}H_{47}N_3O_{11}$	694.4000	*unknown compound*
12	12.656	$C_{22}H_{45}N_7O_7$	520.3413	*unknown compound*

Additional studies have shown that the antimicrobial activity of *Polygonum aviculare* can produce inhibition zones against Gram-negative bacteria (*E. coli, S. typhi, P. aeruginosa*, etc.) and Gram-positive bacteria (*S. aureus, B. subtilis, S. pyogenes*).[16,17] The antibacterial potential of the compounds found in the Mengkrengan (*Polygonum barbatum L.*) plant supports their use as a natural treatment for microbial infections. The combination of flavonoids, alkaloids, and other compounds has a synergistic effect in combating pathogenic bacteria. The analytical technique known as LC-MS Identification (*Liquid Chromatography-Mass Spectrometry*) combines the selectivity of mass spectrometric detection with the physical separation capabilities of liquid chromatography.[18] LC-MS data allows the determination of molecular weight, structure, identity, and quantity of specific sample components.[19] The LC-MS data from the plant extract revealed the presence of several compounds with unique characteristics based on retention time, molecular formula, and molecular weight, as shown in Fig.-2 and listed in Table-2. From Table-2, several compounds were successfully identified, including tropine, epicatechin, quercetin, isorhamnetin, and cinmethylin, while other compounds remained unidentified and require further analysis. The retention times of the compounds ranged from 1.457 minutes (very polar) to 12.656 minutes (non-polar), indicating a variation in polarity and molecular size within the extract. Compounds such as epicatechin and quercetin, belonging to the flavonoid group, exhibit excellent biological activities,

including antibacterial properties. For example, quercetin inhibits the growth of various bacteria by damaging cell membranes or inhibiting bacterial enzymes. Isorhamnetin, a derivative of quercetin, exhibits similar antioxidant and anti-inflammatory activities. In addition to flavonoids, alkaloids such as tropine play an important role in antibacterial activity. Alkaloids damage bacterial cell walls, inhibit protein synthesis, and damage bacterial DNA. In some (pre)-hypertensive patients, epicatechin, obtained from tea and cocoa, contributes to cardioprotective effects by improving endothelial function and reducing inflammation.[19] Other compounds, such as cinmethylin, which are better known as herbicides, have structures that allow the development of other biological activities, including antimicrobial activity.[20] Thus, the ethyl acetate fraction of mengkrengan leaves shows strong potential as a natural antibacterial agent, especially at higher concentrations, as supported by the presence of bioactive compounds identified through LC-MS. Further exploration of these compounds has the potential to develop environmentally friendly and safe natural antimicrobial agents for modern medical use.

CONCLUSION

From the tests conducted, it was found that the ethyl acetate fraction of mengkrengan (*Polygonum barbatum* L.) leaves at concentrations of 5%, 10%, and 15% produced inhibition zones of 7 mm (moderate category), 12 mm (strong category), and 17 mm (strong category), respectively. The compound identification results through LC-MS analysis indicated the presence of quercetin and six new compounds that have not yet been identified.

ACKNOWLEDGMENTS

The author would like to express gratitude to the Faculty of Pharmacy, Islamic University of Kalimantan, Muhammad Arsyad Albanjari, Banjarmasin, Indonesia, for providing the facilities and laboratory infrastructure for this research.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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REFERENCES

1. L.I. Syarif, A.R. Junita, M. Hatta, R. Dwiyanti, C. Kaelan, M. Sabir, R.A. Noviyanthi, M.R. Primaguna, N.I. Purnamasari, *Systematic Reviews in Pharmacy*, **11(6)**, 1171(2020), https://doi.org/10.31838/srp.2020.6.170.
2. V. Nithya, A. Bhaskar, *Inventi Journals*, **2021**, 1(2021), https://portal.issn.org/resource/ISSN/0976-3805.
3. B.B. Shen, J. Wang, Y.F. Wang, X.R. Zhang, H. Zhou, *Digital Chinese Medicine*, **1**, 19(2018), http://dx.doi.org/10.1016/S2589-3777(19)30005-9.
4. P.K. Chang, I.C. Yen, W.C. Tsai, S.Y. Lee, *Scientific Reports*, **13**, 1(2023), https://doi.org/10.1038/s41598-023-45630-1.
5. U. Farooq, T. Gulzar, R. Imran, A. Jabeen, H. Iqbal, *Biological Research*, **52**, 1(2019), https://doi.org/10.1186/s40659-018-0209-0.
6. T.R. Rao, *Journal of Drug Delivery and Therapeutics*, **14(6)**, 298(2024), https://doi.org/10.22270/jddt.v14i6.6669.

7. Q.W. Zhang, L.G. Lin, W.C. Ye, *Chinese Medicine*, **13**, 1(2018), https://doi.org/10.1186/s13020-018-0177-x.
8. H.M. Siregar, R.S. Purwantoro, P. Praptiwi, A. Agusta, *Nusantara Bioscience*, **10(3)**, 159(2018), https://doi.org/10.13057/nusbiosci/n100305.
9. M. T. Hidayat, M. P. Koentjoro, E. N. Prasetyo, *Bioscientist*, **11(1)**, 456(2023), https://doi.org/10.33394/bioscientist.v11i1.7544.
10. R.D. Martha, Y.D. Safitri, N.B. Lestari, H. Danar, H. Parbuntari, M.C. Maida, A. Muadifah, C. Huda, *CHEESA: Chemical Engineering Research Articles*, **7(1)**, 15(2024), https://doi.org/10.25273/cheesa.v7i1.13826.
11. A. Susatyo, *Tropical and Infectious Disease*, **6(3)**, 74(2016), https://doi.org/10.20473/ijtid.v6i3.3255.
12. I. Gajic, J. Kabic, D. Kekic, M. Jovicevic, M. Milenkovic, D.M. Culafic, A. Trudic, L. Ranin, N. Opavski, *Antibiotics*, **11**, 427(2022), https://doi.org/10.3390/antibiotics11040427.
13. S. Idoudi, A. Tourrette, J. Bouajila, M. Romdhane, W. Elfalleh, *Heliyon*, **10**, e28947(2024), https://doi.org/10.1016/j.heliyon.2024.e28947.
14. A.G. Veiko, E.O. Grabarek, S. Sekowski, A. Roszkowska, E.A. Lapsina, I. Dobrzynska, M. Zamaraeva, I.B. Zavondnik, *Molecules*, **28**, 1252(2023), https://doi.org/10.3390/molecules28031252.
15. T.L.A. Nguyen, D. Bhattacharya, *Molecules*, **27**, 2494(2022), https://doi.org/10.3390/molecules27082494.
16. H.M.H. Salama, N. Marraiki, *Saudi Journal of Biological Sciences*, **17**, 57(2010), https://doi.org/10.1016/j.sjbs.2009.12.009.
17. J. Gou, Y. Zou, J. Ahn, *Food Science and Biotechnology*, **20**, 283(2011), http://dx.doi.org/10.1007/s10068-011-0040-7.
18. G. Anusha, R. Sunayana, M. Ponnam, B.A. Kumar, *Asian Journal of Pharmaceutical Research and Development*, **7(4)**, 43(2019), https://doi.org/10.51601/ijhp.v3i4.309.
19. J.I. Dower, L.J. Geleijnse, P.L. Gijsbers, M.C. Zock, D. Kromhout, *The Journal of Nutrition*, **145(7)**, 1459(2015), https://doi.org/10.3945/jn.115.211888.
20. H. Xu, Q. Leng, W. Su, L. Sun, J. Cheng, R. Wu, *Agronomy*, **14**, 885(2024), https://doi.org/10.3390/agronomy14050885.

[RJC-9262/2025]