RASĀYAN *J. Chem.*
Vol. 18 | No. 2 |869-874| April - June | 2025
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

OPTIMIZED pH AND SOLID-TO-SOLVENT RATIO FOR ENHANCED POLYPHENOL AND ANTIOXIDANT EXTRACTION FROM *Graptophyllum pictum* LEAVES USING MICROWAVE-ASSISTED EXTRACTION

R. Y. Galingging[1], F.A. Insani[2], D.S.H. Seno[2] and W. Nurcholis[2,3,✉]
[1]Horticultural Research Center, National Research and Innovation Agency, Indonesia
[2] Department of Biochemistry, IPB University, Indonesia
[3]Tropical Biopharmaca Research Center, IPB University, Indonesia
✉Corresponding author: wnurcholis@apps.ipb.ac.id

ABSTRACT

This study investigated the optimization of pH and solid-to-solvent ratios for microwave-assisted extraction (MAE) of polyphenol-rich extracts from the leaves of *Graptophyllum pictum*. Dried leaves were subjected to methanol extraction under varying conditions to determine the influence of solid-to-solvent ratios (3:1, 5:1, 10:1, and 15:1) and pH levels (2, 4, 6, and 8) on total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity. Optimal extraction was achieved at a 10:1 solid-to-solvent ratio, yielding the highest TPC (1.051 mg GAE/g DW), TFC (2.471 mg QE/g DW), and DPPH radical-scavenging activity (0.509 µmol TE/g DW) values. However, the FRAP antioxidant capacity peaked at a 15:1 ratio (5.430 µmol TE/g DW). pH analysis revealed that acidic conditions (pH 2) enhanced the extraction of phenolic and flavonoid compounds, with maximum TPC (3.390 mg GAE/g DW) and TFC (5.511 mg QE/g DW) values. The antioxidant activity measured using the DPPH assay peaked at pH 6 (0.541 µmol TE/g DW). These findings highlight the importance of optimizing extraction parameters to maximize the yield of bioactive compounds, offering valuable insights for industrial applications.

Keywords: Extraction, Optimization, Polyphenols, Antioxidants, Flavonoid, Microwave-Assisted Extraction, *Graptophyllum Pictum*

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

INTRODUCTION

The pursuit of a healthy lifestyle has become increasingly challenging in contemporary society, as elevated exposure to deleterious substances such as free radicals and reactive molecules with unpaired electrons has resulted in an increase in diseases associated with oxidative stress.[1] Antioxidants, which neutralize free radicals, are essential for mitigating their deleterious effects.[2] Despite the widespread availability of synthetic antioxidants, concerns regarding their potential carcinogenicity at elevated doses have redirected attention toward natural antioxidants derived from plant sources.[3] *Graptophyllum pictum* L. Griff., commonly referred to as purple leaves or known as *daun ungu* or *daun wungu* in Indonesia, is a tropical species recognized for its medicinal properties. This plant, predominantly found in Indonesia, has been traditionally utilized to address various ailments, including hemorrhoids, diarrhea, and ulcers.[4] The leaves of *G. pictum* are characterized by their abundance of secondary metabolites, notably phenolics and flavonoids, which exhibit substantial antioxidant properties.[5] Phenolic compounds, particularly polyphenols such as flavonoids, are potent natural antioxidants that neutralize free radicals through the donation of hydrogen atoms.[6] Given the antioxidant potential of these compounds, there is substantial interest in optimizing methodologies to efficiently extract high-quality polyphenol-rich compounds from *G. pictum* leaves. Microwave-assisted extraction (MAE) has emerged as a promising technique. MAE employs electromagnetic waves in the microwave spectrum to extract biologically active compounds from plant sources.[7] Compared with conventional extraction methodologies, this approach offers advantages, including reduced extraction duration, diminished solvent consumption, and enhanced yields through the internal heating of both the solvent and plant material. [8] The effectiveness of MAE is greatly affected by various key factors, including the concentration of the solvent, acidity or alkalinity levels, heat, duration of extraction, and the proportion of solid material to the solvent used.[9] Although previous studies have

http://doi.org/10.31788/RJC.2025.1829189

investigated the antioxidant activity, total phenolic content, and flavonoid content of ethanol extracts derived from *G. pictum* leaves,[5,10] there is a paucity of research focusing on the optimization of MAE conditions, particularly regarding the influence of pH and solid-to-solvent ratio on the extraction of polyphenols and antioxidants from these leaves. Therefore, this study aimed to optimize the pH and solid-to-solvent ratio for MAE to enhance the yield of polyphenol-rich extracts from *G. pictum* leaves. By identifying the ideal extraction conditions, this study provides insights into maximizing bioactive compound recovery, contributing to the efficient utilization of this medicinal plant.

EXPERIMENTAL

Preparation of Plant Leaves

Dried *G. pictum* leaves were prepared using a method adapted from Makkiyah *et al.,*[10] with modifications. The foliage, obtained from the Tropical Biopharmaca Research Center of IPB University in Bogor, West Java, Indonesia, underwent initial dehydration in an oven for 24 h at 45°C. The dried leaves were then pulverized into a fine powder using a blender. To achieve a consistent particle size appropriate for the extraction procedure, the powdered material was passed through an 80-mesh sieve.

Single-Factor Extraction Experiment

A single-factor experimental approach was employed to evaluate the influence of the solid-to-liquid ratio and pH on the extraction process. For the experiment, one gram of dried leaves was extracted using 95% methanol. Four different solid-to-liquid ratios were tested: 3:1, 5:1, 10:1, and 15:1, along with four pH levels: 2, 4, 6, and 8. The extractions were first carried out to determine the optimal solid-to-liquid ratio, followed by experiments to determine the ideal pH. Each extraction was performed using MAE, [11] where the sample solution was heated at 135 W for 3 min in a microwave. After heating, the solution was allowed to cool gradually and filtered. The final extract volume was standardized to 10 mL after filtration. The samples were evaluated for total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity. The Folin-Ciocalteu technique was employed to determine TPC, with outcomes reported as mg GAE/g DW.[12] The $AlCl_3$ technique was employed to measure TFC, with the outcomes reported as mg QE/g DW.[10] The antioxidant potential was evaluated using two methods: the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging test and the FRAP (ferric reducing antioxidant power) analysis. The outcomes were reported in µmol TE/g dry weight (DW).[10]

Analysis Data

Statistical analysis was conducted using IBM SPSS Statistics 25, employing one-way ANOVA with a significance level of 0.05, followed by Tukey's HSD post hoc test (also at $\alpha = 0.05$). The findings are expressed as mean values with standard deviations (SD).

RESULTS AND DISCUSSION

Impact of Liquid-Solid Ratio on Polyphenol and Antioxidant Extraction Efficiency

Various methanol extracts of *G. pictum* leaves were analyzed using different liquid-to-solid ratios (3:1, 5:1, 10:1, and 15:1) to assess their TPC, TFC, and antioxidant capacity. As illustrated in Fig.-1, the maximum values for TPC (1.051 mg GAE/g DW), TFC (2.471 mg QE/g DW), and DPPH antioxidant capacity (0.509 µmol TE/g DW) were observed at a 10:1 ratio. However, the antioxidant capacity measured using the FRAP method reached its peak at a 15:1 ratio, attaining 5.430 µmol TE/g DW. These results indicate that an appropriate solid-to-solvent ratio is crucial for balancing the extraction efficiency of phenolic compounds and their associated antioxidant activities. The decrease in TPC and TFC at a 15:1 ratio indicates that exceeding the optimal ratio can result in the dilution of active compounds or extraction inefficiencies. The discrepancy in antioxidant activity observed between the DPPH and FRAP methods can be attributed to their distinct mechanisms of action: DPPH involves hydrogen atom donation, whereas FRAP is based on electron transfer. [13,14] Our findings align with previous studies demonstrating that the extraction efficiency varies depending on the solvent ratio and methodology employed for assessing antioxidant capacity. [15,16] This study provides further insight into the balance required to optimize phenolic compound extraction while maintaining antioxidant potential. Statistical analysis utilizing analysis of variance (ANOVA) and Tukey's honest significant difference (HSD) test ($p<0.05$) demonstrated statistically significant differences among the ratios, notably between 10:1 and 15:1 compared to 3:1.

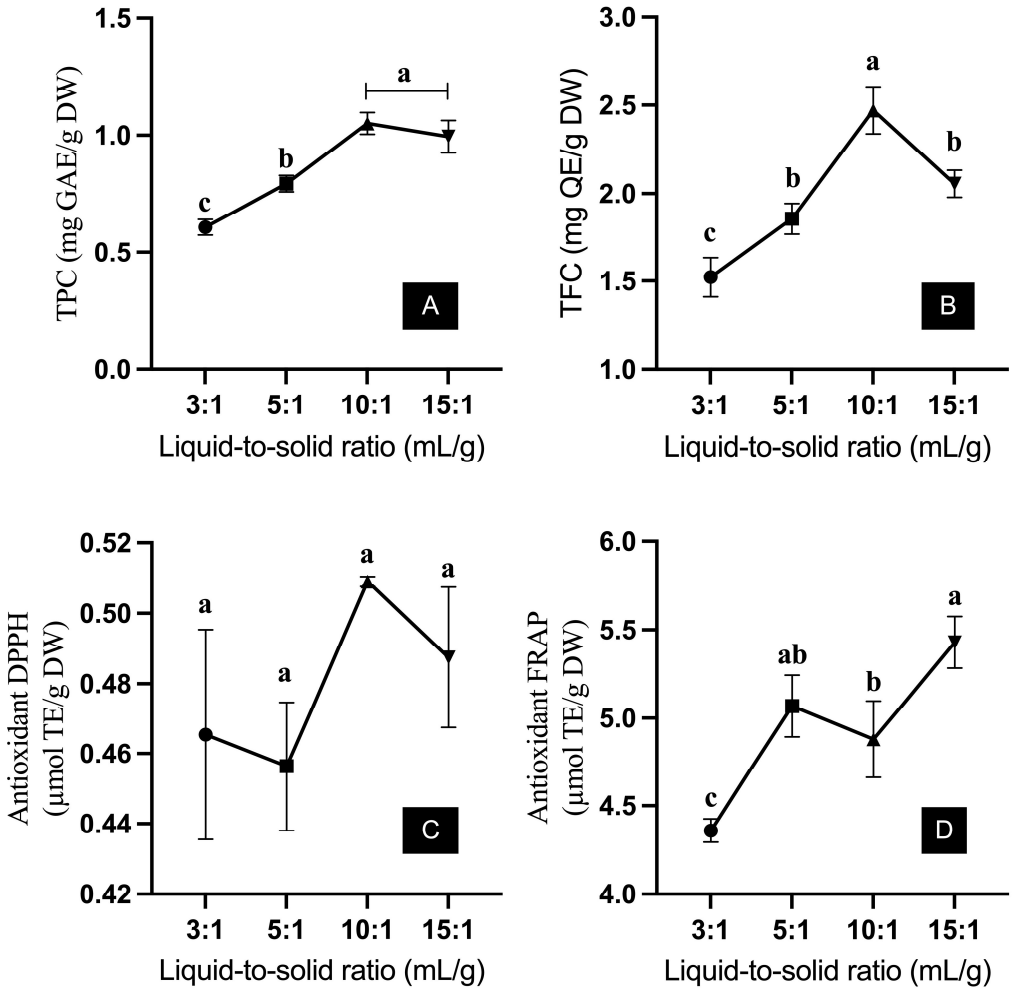

Fig.-1: Impact of Solvent-to-Sample Ratio on the Extraction Efficiency of (A) Total Phenolics (TPC), (B) Flavonoids (TFC), (C) DPPH Antioxidant Activity, and (D) FRAP Antioxidant Capacity. Statistically Significant differences are Denoted by Distinct Letters, as Determined by Tukey's HSD Test at a p-value of 0.05.

Impact of pH on Polyphenol and Antioxidant Extraction Efficiency

Fig.-2 illustrates the influence of pH on the extraction yield of total phenolic content (TPC), total flavonoid content (TFC), and antioxidant capacities (DPPH and FRAP) from *G. pictum* leaves. Analysis across pH levels of 2, 4, 6, and 8 demonstrated that pH significantly affected these parameters. The maximum TPC (3.390 mg GAE/g DW) and TFC (5.511 mg QE/g DW) were observed at pH 2, suggesting that acidic conditions facilitate the extraction of phenolic and flavonoid compounds from the plant material. This enhancement is attributed to the increased concentration of hydrogen ions, which stabilize these compounds and inhibit their degradation, as supported by previous studies.[17] The antioxidant capacity, as measured by the DPPH assay, reached its maximum at pH 6 (0.541 µmol TE/g DW), suggesting that a mildly acidic environment is optimal for DPPH radical scavenging activity. Conversely, the FRAP assay exhibited the highest antioxidant capacity at pH 2 (3.110 µmol TE/g DW), reflecting the method's enhanced sensitivity to acidic conditions, which facilitates the reduction of Fe^{3+} to Fe^{2+}.[18] These findings are consistent with existing literature, which indicates that acidic conditions generally enhance the extraction of phenolic and flavonoid compounds, although the optimal pH for antioxidant activity may vary depending on the assay employed. [19,20] Consequently, pH 2 is essential for maximizing both TPC and TFC, whereas pH 6 is critical for optimizing the DPPH antioxidant capacity.

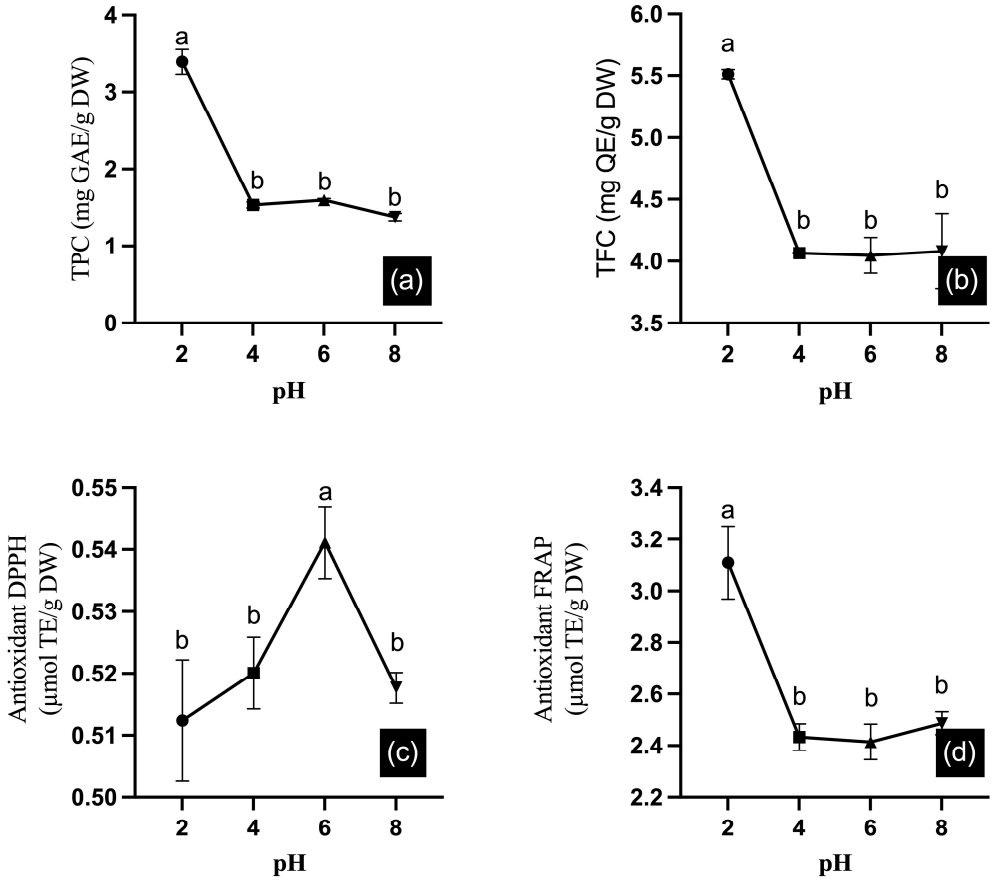

Fig.-2: The Influence of pH on the Extraction Yield of (a) Total Phenolic Content (TPC), (b) Flavonoid Content (TFC), (c) Antioxidant DPPH, and (d) Antioxidant FRAP. Values Followed by different Letters Indicate Statistically Significant Differences as Determined by Tukey's HSD Test at $p<0.05$.

Significance of the Findings

This study provides essential contributions to the optimization of microwave-assisted extraction (MAE) of bioactive compounds from *G. pictum* leaves. By establishing the ideal pH and solid-to-solvent ratio, this study enhanced the extraction efficiency and maximized the antioxidant yield. These optimized conditions can serve as guidelines for industrial-scale extraction processes aimed at obtaining high-quality polyphenol-rich extracts with enhanced bioactivity. Furthermore, this study underscores the importance of fine-tuning MAE parameters to achieve high antioxidant potency, contributing to the broader field of natural product extraction and its pharmaceutical and nutraceutical applications.

CONCLUSION

This study successfully optimized the pH and solid-to-solvent ratio for the microwave-assisted extraction (MAE) of polyphenol-rich extracts from *G. pictum* leaves. A solid-to-solvent ratio of 10:1 was determined to be optimal for extracting the highest total phenolic content (TPC) of 1.051 mg GAE/g DW, total flavonoid content (TFC) of 2.471 mg QE/g DW, and DPPH radical scavenging activity of 0.509 µmol TE/g DW, while a ratio of 15:1 maximized the antioxidant capacity in the FRAP assay, reaching 5.430 µmol TE/g DW. Acidic conditions (pH 2) enhanced the extraction efficiency, yielding the highest TPC (3.390 mg GAE/g DW) and TFC (5.511 mg QE/g DW). However, DPPH antioxidant activity was optimal at pH 6 (0.541 µmol TE/g DW), and FRAP activity peaked at pH 2 (3.110 µmol TE/g DW). These findings highlight the importance of controlling both pH and solid-to-solvent ratios to maximize bioactive compound yield and antioxidant capacity, providing valuable insights for the industrial application of MAE in producing high-quality polyphenol-rich extracts from *G. pictum* leaves.

ACKNOWLEDGMENTS

The authors extend their sincere gratitude to IPB University, particularly the Agricultural Biochemistry Laboratory at the Department of Biochemistry, Faculty of Mathematics and Natural Sciences, for their invaluable support and contributions to this research.

CONFLICT OF INTERESTS

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

All 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:

Ronny Y. Galingging https://orcid.org/0000-0002-7406-3368
Fahma A. Insani https://orcid.org/0009-0007-4380-1069
Djarot S.H. Seno https://orcid.org/0009-0006-4249-0310
Waras Nurcholis https://orcid.org/0000-0001-7047-5093

REFERENCES

1. D. Phaniendra, D. B. Jestadi, L. Periyasamy, *Indian Journal of Clinical Biochemistry*, **30**, 1126 (2015), https://doi.org/10.1007/s12291-014-0446-0
2. G. Martemucci, C. Costagliola, M. Mariano, L. D'Andrea, P. Napolitano, A. G. D'Alessandro, *Oxygen*, **2**, 4878 (2022), https://doi.org/10.3390/oxygen2020006
3. J. Pokorný, *European Journal of Lipid Science and Technology*, **109**, 629642 (2007), https://doi.org/10.1002/ejlt.200700064
4. P. R. W. Astana, U. Nisa, *Jurnal Ilmu Kefarmasian Indonesia*, **16**, 115123 (2018), https://doi.org/10.35814/jifi.v16i2.562
5. F. A. Makkiyah, E. P. Rahmi, F. R. Mahendra, F. Maulana, R. A. Arista, W. Nurcholis, *Journal of Applied Pharmaceutical Science*, **14**, 5563 (2024), https://doi.org/10.7324/JAPS.2024.153548
6. M. M. Rahman, S. Rahaman, M. R. Islam, F. Rahman, F. M. Mithi, T. Alqahtani, M. A. Almikhlafi, S. Q. Alghamdi, A. S. Alruwaili, S. Hossain, M. Ahmed, R. Das, T. F. Emran, M. S. Uddin, *Molecules*, **27**, 233 (2022), https://doi.org/10.3390/molecules27010233
7. Y. Zhang, P. Cai, G. Cheng, Y. Zhang, *Natural Product Communications*, **17**, 1934578X2110697 (2022), https://doi.org/10.1177/1934578X211069721
8. B. K. Behera, *Techniques for Downstream Process for Biologic Drugs and Vaccines*, **1**, 115142 (2023), https://doi.org/10.1016/B978-0-443-19157-2.00001-7
9. M. Bonfigli, E. Godoy, M. A. Reinheimer, N. J. Scenna, *Journal of Food Engineering*, **207**, 5672 (2017), https://doi.org/10.1016/j.jfoodeng.2017.03.011
10. F. A. Makkiyah, E. P. Rahmi, T. Susantiningsih, N. Marliani, R. A. Arista, W. Nurcholis, *Journal of Applied Pharmaceutical Science*, **13**, 214221(2023), https://doi.org/10.7324/JAPS.2023.117043
11. W. Nurcholis, R. Alfadzrin, N. Izzati, R. Arianti, B. Á. Vinnai, F. Sabri, et al., *Plants*, **11**, 2221 (2022), https://doi.org/10.3390/plants11172221
12. N. Marliani, I. M. Artika, W. Nurcholis, *Chiang Mai University Journal of Natural Sciences*, **21**, e2022046 (2022), https://doi.org/10.12982/CMUJNS.2022.046
13. A. Malik, A. R. Ahmad, A. Najib, *Jurnal Fitofarmaka Indonesia*, **4**, 238240 (2017), https://doi.org/10.33096/jffi.v4i2.267
14. P. Jayanthi, P. Lalitha, *International Journal of Pharmacy and Pharmaceutical Sciences*, **3**, 126128 (2011), https://www.innovareacademics.in/journal/ijpps/Vol3Suppl3/2155.pdf

15. N. P. Mauliza, N. Suriaini, C. M. Rosnelly, S. Satriana, M. D. Supardan, *Jurnal Rekayasa Kimia dan Lingkungan*, **17**, 114120 (2023), https://doi.org/10.23955/rkl.v17i2.26915
16. J. D. V. Arroy, H. Ruiz-Espinosa, J. J. Luna-Guevara, M. L. Luna-Guevara, P. Hernández-Carranza, R. Ávila-Sosa, et al., *Czech Journal of Food Sciences*, **35**, 456465 (2017), https://doi.org/10.17221/316/2016-CJFS
17. N. Kemit, D. G. M. Permana, P. Kencana, *Media Ilmiah Teknologi Pangan*, **6**, 3442 (2019), https://ojs.unud.ac.id/index.php/pangan/article/view/58906
18. S. Syarif, R. Kosman, N. Inayah, *As-Syifaa*, **7**, 2633 (2015), https://jurnal.farmasi.umi.ac.id/index.php/as-syifaa/article/view/18
19. F. E. Giuliana, M. Ardana, R. Rusli, *Proceedings of Mulawarman Pharmaceuticals Conferences*, **1**, 242251 (2015), https://doi.org/10.25026/mpc.v1i1.115
20. L. Wang, L. Shi, L. Fan, Y. Ding, S. Zhao, Y. Liu, C. Ma, *Industrial Crops and Products*, **42**, 587594 (2013), https://doi.org/10.1016/j.indcrop.2012.06.031

[RJC-9189/2024]