JOURNAL OF TROPICAL PHARMACY AND CHEMISTRY
https://jtpc.ff.unmul.ac.id
Volume 10.2

Research Article

Optimized Ethyl Acetate Extraction of Mangifera casturi Leaves: Impact of Temperature and Extraction Time on TPC, TFC, and Antioxidant Activity

Dharmastuti Cahya Fatmarahmi[1,4*], Dina Lorena[2], Dina Mutmainah[2], Rahmawati[3], Mifta Fajarwati[4], Sabtanti Harimurti[1]

[1]Department of Pharmaceutical Chemistry, School of pharmacy, Faculty of Medicine and Health Sciences, Universitas Muhammadiyah Yogyakarta, Yogyakarta, Indonesia
[2]Student of school of Pharmacy, Faculty of Medicine and Health Sciences, Universitas Muhammadiyah Yogyakarta, Indonesia
[3]Faculty of Pharmacy, Universitas Muhammadiyah Banjarmasin, Banjarmasin, Indonesia
[4]Halal Laboratory, Directorate of Innovation and Commercialization, Universitas Muhammadiyah Yogyakarta, Indonesia

*Corresponding author: dharmastuti.cahya@umy.ac.id

Abstract

Mangifera casturi is an endemic plant from South Kalimantan that has been traditionally used for its medicinal properties. This study aimed to determine the optimal extraction conditions of Mangifera casturi leaves ethyl acetate extract and evaluate its total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity. Extraction was performed using maceration assisted by an ultrasonic water bath at temperatures of 30, 40, and 50°C with extraction times of 30, 60, and 90 minutes. TPC was determined using the Folin-Ciocalteu method, TFC was measured using the $AlCl_3$ colorimetric method, and antioxidant activity was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. The optimal condition for TPC was achieved at 40°C for 90 minutes, yielding 89.09 ± 12.385 mg GAE/g extract. The optimal condition for TFC was obtained at 40°C for 30 minutes, yielding 86.68 ± 1.987 mg QE/g extract. The optimal antioxidant activity was obtained at 30°C for 90 minutes, with an IC_{50} value of 63.869 ± 0.431 ppm, indicating strong antioxidant activity. These findings suggest that extraction temperature and time significantly influence the phytochemical content and antioxidant potential of *M. casturi* leaves ethyl acetate extract, and that this plant represents a promising source of natural antioxidants.

Keywords: Casturi Mango, DPPH method, TPC, TFC, Optimized extraction

Accepted: 16 Juni 2026
Approved: 21 July 2026
Publication: 31 July 2026

Citation: D. C. Fatmarahmi, D. Lorena, D. Mutmainah, Rahmawati, M. Fajarwati, S. Harimurti "Optimized Ethyl Acetate Extraction of Mangifera casturi Leaves: Impact of Temperature and Extraction Time on TPC, TFC, and Antioxidant Activity" Journal of Tropical Pharmacy and Chemistry, vol. 10, no. 2, pp.80-89. July 2026, doi: 10.30872/jtpc.v10i2.411

Doi: 10.30872/jtpc.v10i2.411

1 Introduction

The increasing prevalence of degenerative diseases, including cancer, cardiovascular disorders, and premature aging, has become a major global health concern. These conditions are closely associated with the detrimental effects of free radicals generated within the body. To counteract oxidative cellular damage, effective antioxidants are required to neutralize reactive oxygen species and maintain cellular homeostasis [1]. This situation has stimulated extensive research on phenolic and flavonoid compounds, which have been scientifically recognized for their diverse pharmacological activities, particularly their potent antioxidant properties. In recent years, the healthcare sector, especially the pharmaceutical field, has increasingly emphasized the discovery of natural antioxidant sources that not only exhibit high efficacy but also ensure safety for long-term use as pharmaceutical ingredients or dietary supplements [2].

Indonesia possesses extraordinary biodiversity, representing a valuable source of natural resources with broad applications in pharmaceuticals, food products, and cosmetics. Numerous phytochemical investigations have been conducted on various plant species to identify bioactive constituents, both as a scientific basis for traditional medicinal use and as a foundation for novel drug development. Most bioactive compounds found in natural products belong to the secondary metabolite group, including alkaloids, terpenoids, phenolics, flavonoids, and other phytochemicals. Among these, phenolic and flavonoid compounds have attracted considerable scientific interest due to their biological activities[3]. One of the plant species native to Indonesia is Kasturi mango (*Mangifera casturi*), a rare endemic species originating from South Kalimantan. Previous studies have reported that the leaves of *Mangifera casturi* contain tannins, flavonoids, and triterpenoids [3], [4].

Kasturi mango represents one of the thousands of plant species in Indonesia with unique phytochemical potential. As a rare endemic plant from South Kalimantan, its endangered status highlights the urgency of both conservation and scientific investigation [5]. Although its fruit is well known locally, the utilization and scientific exploration of other plant parts, particularly the leaves, remain limited. In contrast, studies on other *Mangifera* species have demonstrated that mango leaves are rich sources of phenolic and flavonoid compounds and exhibit significant antioxidant activity [6].

The efficiency of extracting bioactive compounds from plant matrices is strongly influenced by several factors, including solvent selection, extraction method, temperature, and extraction duration. Ethyl acetate is a semi-polar solvent known for its effectiveness in extracting phenolic compounds and aglycone flavonoids, including methoxylated flavonoids, xanthones, and esterified phenolic acids, which are often not optimally extracted using polar solvents such as water or methanol alone [7], [8]. Several studies have reported that ethyl acetate fractions from various plant species exhibit higher antioxidant activity than fractions obtained using other solvents, which is generally attributed to their higher phenolic and flavonoid contents [9],[10].

Ultrasound-assisted extraction has been demonstrated to significantly enhance extraction efficiency compared with conventional maceration techniques. Extraction temperature and extraction time are considered critical parameters in ultrasound-assisted extraction, as they directly affect the recovery of phenolic and flavonoid compounds. Optimization studies on various medicinal plants have indicated that optimal extraction conditions generally occur within a temperature range of 30–60°C and extraction times of 30–90 minutes, depending on the plant material and solvent employed [11], [12], [13].

Despite growing interest in the pharmacological potential of *Mangifera casturi*, most existing studies have primarily focused on preliminary phytochemical screening and general biological activity evaluations without systematically investigating the influence of extraction conditions [14], [15]. Although extraction optimization has been explored in other *Mangifera* species, particularly *M. indica* and *M. pajang*, differences in phytochemical composition among species, plant matrices, extraction solvents, and extraction techniques may result in distinct extraction behavior and optimal conditions. Consequently, extraction conditions established for other *Mangifera* species cannot necessarily be directly extrapolated to *Mangifera casturi*. Furthermore, systematic studies investigating ultrasound-assisted extraction of *Mangifera casturi* leaves, particularly using ethyl acetate, remain scarce. Addressing this gap is important because extraction conditions strongly influence the recovery of bioactive constituents and, consequently, the measured

Doi: 10.30872/jtpc.v10i2.411

biological activity of plant extracts. Therefore, the present study systematically evaluated the effects of extraction temperature and duration on the ultrasound-assisted extraction of *Mangifera casturi* leaves using ethyl acetate, with total phenolic content (TPC), total flavonoid content (TFC), and DPPH radical-scavenging activity as response parameters. By establishing the temperature–time conditions associated with these responses, this study provides species-specific evidence for the extraction behavior of *Mangifera casturi* and extends previous optimization studies conducted on other members of the genus *Mangifera*.

2 Method

2.1 Tools and Materials

The instruments used in this study included a Shimadzu IRSpirit-X Fourier Transform Infrared (FTIR) spectrophotometer (Japan), an IKA RV-8 rotary evaporator (Germany), a Memmert UN260 oven (Germany), a Sartorius analytical balance (Germany), Socorex micropipettes (Switzerland), a Philips blender (The Netherlands), and standard laboratory glassware.

The materials used in this study included *Mangifera casturi* leaves, DPPH (2,2-diphenyl-1-picrylhydrazyl) (Sigma-Aldrich, USA), ethanol p.a. (Smart-Lab, Indonesia), methanol p.a. (Smart-Lab, Indonesia), Quercetin (Sigma-Aldrich, USA), gallic acid (Sigma-Aldrich, USA), ethyl acetate p.a. (Smart-Lab, Indonesia), Folin–Ciocalteu reagent (Sigma-Aldrich, USA), aluminum chloride ($AlCl_3$) (Sigma-Aldrich, USA), sodium hydroxide (NaOH) (Sigma-Aldrich, USA), and distilled water.

2.2 Research Produre

2.2.1 Plant Authentication

Mangifera casturi leaves were collected from North Banjarbaru District, Banjarbaru City, South Kalimantan, Indonesia. Plant authentication was carried out at the Laboratory of the Faculty of Mathematics and Natural Sciences, Universitas Lambung Mangkurat.

2.2.2 Preparation of *Mangifera casturi* Leaves Extract

Healthy *Mangifera casturi* leaves were washed, cut into small pieces, and dried in an oven at 60°C for three days. The dried leaves were sorted to remove damaged materials and then ground into powder. The powdered leaves were extracted using ethyl acetate at a sample-to-solvent ratio of 1:10 (w/v) in an ultrasonic water bath. Extraction optimization was performed at temperatures of 30, 40, and 50°C and extraction times of 30, 60, and 90 min, resulting in nine extraction conditions.

A 3 × 3 factorial experimental arrangement was used to evaluate three extraction temperatures (30, 40, and 50 °C) and three extraction durations (30, 60, and 90 min), resulting in nine temperature–time combinations, each performed in triplicate. The factorial design was selected to systematically assess the main and interactive effects of temperature and extraction time within predefined experimental conditions. Accordingly, the study focused on identifying the best-performing condition within the investigated range rather than estimating a mathematical optimum through response surface modeling.

2.2.3 Determination of Total Phenolic Content (TPC)

Total phenolic content was determined using the Folin–Ciocalteu method with gallic acid as the standard. Aliquots of gallic acid standard solution (10–70 µL) were mixed with 0.4 mL of Folin–Ciocalteu reagent and allowed to react for 8 min. Subsequently, 4.0 mL of 1% NaOH was added, and the volume was adjusted to 10 mL with distilled water. After incubation for 1 h, absorbance was measured at 750 nm using a UV–Vis spectrophotometer. A calibration curve was constructed from the gallic acid standards, and all measurements were performed in triplicate.

2.2.4 Determination of Total Flavonoid Content (TFC)

Total flavonoid content was determined using the aluminium chloride colorimetric method with quercetin as the reference standard. Quercetin standard solutions (100–600 µL) were reacted sequentially with distilled water, 2% NaOH, and 10% $AlCl_3$, followed by the addition of 1% NaOH and adjustment of the final volume to 10 mL. After incubation for 30 min, absorbance was measured at 430 nm. Sample solutions were treated similarly, and all analyses were conducted in triplicate.

Doi: 10.30872/jtpc.v10i2.411

2.2.5 DPPH Radical Scavenging Assay

Antioxidant activity was evaluated using the DPPH radical scavenging assay. Five concentrations of each extract were prepared and mixed with 1 mL of 0.4 mM DPPH solution in methanol. The mixtures were vortexed for 30 s, incubated in the dark for 30 min, and their absorbance was measured at 517 nm using a UV–Vis spectrophotometer. Quercetin was used as the positive control. Antioxidant activity was expressed as the IC_{50} value, and all measurements were performed in triplicate.

2.2.6 Statistical Analysis

All experiments were performed in triplicate, and the results are expressed as mean ± standard deviation (SD). The effects of extraction temperature and extraction time on total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity (IC_{50}) were evaluated using two-way analysis of variance (ANOVA), including the interaction between temperature and extraction time. When significant differences were detected, Tukey's honestly significant difference (HSD) post-hoc test was applied for multiple comparisons among extraction conditions. Differences were considered statistically significant at $p < 0.05$. In the tables, different superscript letters within the same response parameter indicate statistically significant differences among extraction conditions ($p < 0.05$), whereas values sharing the same superscript letter are not significantly different.

3 Results and Discussion

This study aimed to evaluate the effects of extraction temperature and time using ethyl acetate as the extraction solvent and an ultrasonic water bath as the extraction aid on total phenolic content (TPC), total flavonoid content (TFC), and free radical scavenging activity. *Mangifera casturi* leaves are known to contain various secondary metabolites, including phenolic compounds, flavonoids, tannins, and xanthones. These secondary metabolites possess considerable potential for development as natural sources of pharmaceutical raw materials [3], [16].

In natural product research, TPC, TFC, and antioxidant activity are commonly used to evaluate extract quality. The determination of TPC is important because phenolic compounds are among the major contributors to antioxidant activity, whereas TFC analysis is used to estimate flavonoid content, which has been reported to exhibit antioxidant, anti-inflammatory, and chemo preventive properties [16], [17]. Furthermore, antioxidant activity assessed using the DPPH radical scavenging assay and IC_{50} determination provides information regarding the actual ability of the extract to neutralize free radicals. Therefore, these three parameters were selected as indicators for optimizing the extraction conditions of *Mangifera casturi* leaves [18], [19].

The result of determination plant exhibited that the plant sample used in this study was officially authenticated as *Kasturi* (*Mangifera casturi* Kosterm.) under Certificate Number. 095/LB.LABDASAR/V/2025 by Universitas Lambung Mangkurat. The yield of extract percentage appeared in Table 1. Extraction condition using ethyl acetate extract at 50 °C as long as 90 minutes showed the highest extract yield of 30%. This result suggests that increasing extraction temperature and duration enhanced the penetration of ethyl acetate into the plant matrix and improved the diffusion of soluble compounds into the solvent. Furthermore, ultrasonic cavitation may have facilitated cell wall disruption, allowing greater release of intracellular metabolites. As a result, the combination of 50°C and 90 min provided the most efficient extraction condition, resulting in the highest extract yield among all treatments [20].

Doi: 10.30872/jtpc.v10i2.411

Table 1. Percentage Yield of Ethyl Acetate Extract from *Mangifera casturi* Leaves

Solvent	Temperature (°C)	Duration (minutes)	Yield (%)
Ethyl Acetate	30	30	24
	30	60	16.2
	30	90	19.8
	40	30	9.2
	40	60	15.4
	40	90	20.8
	50	30	12.8
	50	60	22.2
	50	90	30

Ethyl acetate was selected as the extraction solvent due to its intermediate polarity, with a polarity index of approximately 4.4, which enables the extraction of phenolic compounds, aglycone flavonoids, xanthones, and several terpenoid derivatives commonly found in the *Mangifer*a genus. Previous studies have reported that ethyl acetate effectively extracts major bioactive compounds from various *Mangifera* species, including mangiferin, quercetin, catechin, gallic acid, and other flavonoid derivatives that contribute significantly to antioxidant activity [21].

Ultrasound-assisted extraction was employed to reduce extraction time and enhance the recovery of bioactive compounds. The mechanism involves the formation and collapse of microbubbles (cavitation), which disrupt plant cell walls, increase the contact surface area between the solvent and plant material, and facilitate solvent penetration into plant tissues. Consequently, mass transfer is improved, leading to a more efficient extraction process. Several studies have demonstrated that ultrasonic-assisted extraction significantly shortens extraction time while increasing the yield of bioactive compounds, particularly phenolics, flavonoids, and other secondary metabolites [22].

The gallic acid calibration curve used for TPC determination demonstrated excellent linearity, with a regression equation of $y = 0.0181x + 0.0992$ and an R^2 value of 0.999 (Figure 1), indicating high reliability of the analytical method. Among all extraction conditions evaluated, extraction at 40°C for 90 min produced the highest total phenolic content (89.09 ± 12.39 mg GAE/g extract). This result suggests that the combination of moderate temperature and prolonged extraction time favored the release of phenolic compounds from the plant matrix while minimizing their thermal degradation. The complete TPC results of the ethyl acetate extracts of *Mangifera casturi* leaves are shown in Table 2. The quercetin calibration curve used for TFC determination showed satisfactory linearity, with a regression equation of $y = 0.0932x + 0.0395$ and an R^2 value of 0.995 (Figure 2), indicating the reliability of the analytical method. Among the extraction conditions evaluated, extraction at 40°C for 30 min resulted in the highest total flavonoid content (86.68 ± 1.99 mg QE/g extract). This finding suggests that flavonoid compounds were efficiently extracted at moderate temperature and shorter extraction time, whereas prolonged extraction may have promoted degradation or oxidation of certain flavonoid constituents. The complete TFC results are presented in Table 2. The antioxidant activity of the extracts was evaluated using the DPPH radical scavenging assay. The IC_{50} values were determined by preparing a series of extract concentrations and calculating the concentration required to inhibit 50% of DPPH radicals. Among all extraction conditions, the highest antioxidant activity was observed for the extract obtained at 30°C for 90 min, which exhibited the lowest IC_{50} value of 63.87 ± 0.43 ppm. Since lower IC_{50} values indicate stronger antioxidant activity, this result suggests that the extraction condition effectively recovered compounds with potent free radical scavenging capacity. The complete IC_{50} values for all extraction conditions are presented in Table 2.

Doi: 10.30872/jtpc.v10i2.411

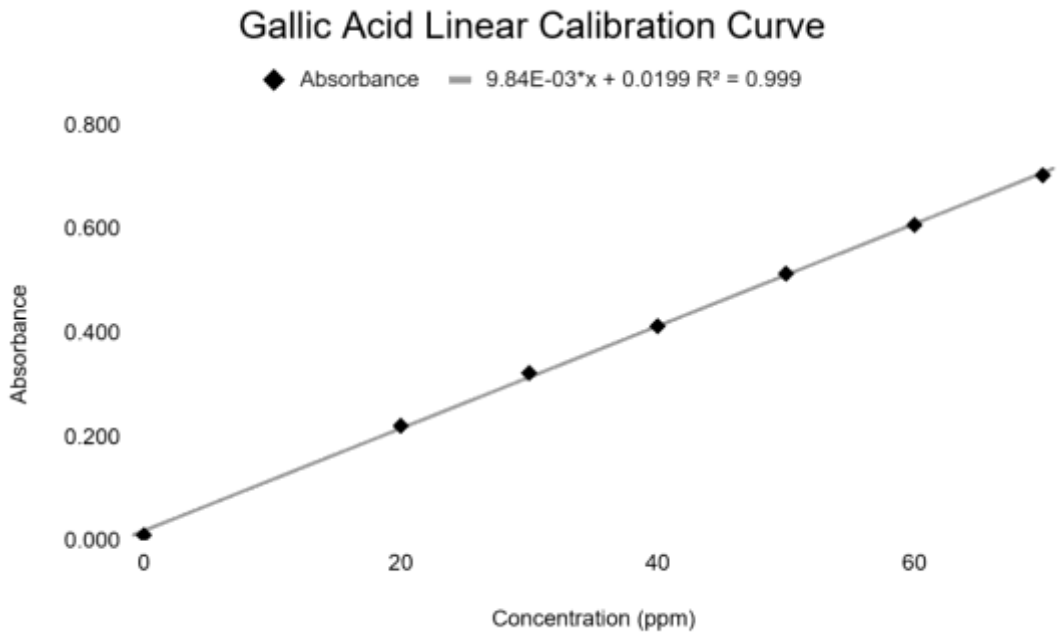

Figure 1 Gallic Acid Linear Calibration Curve

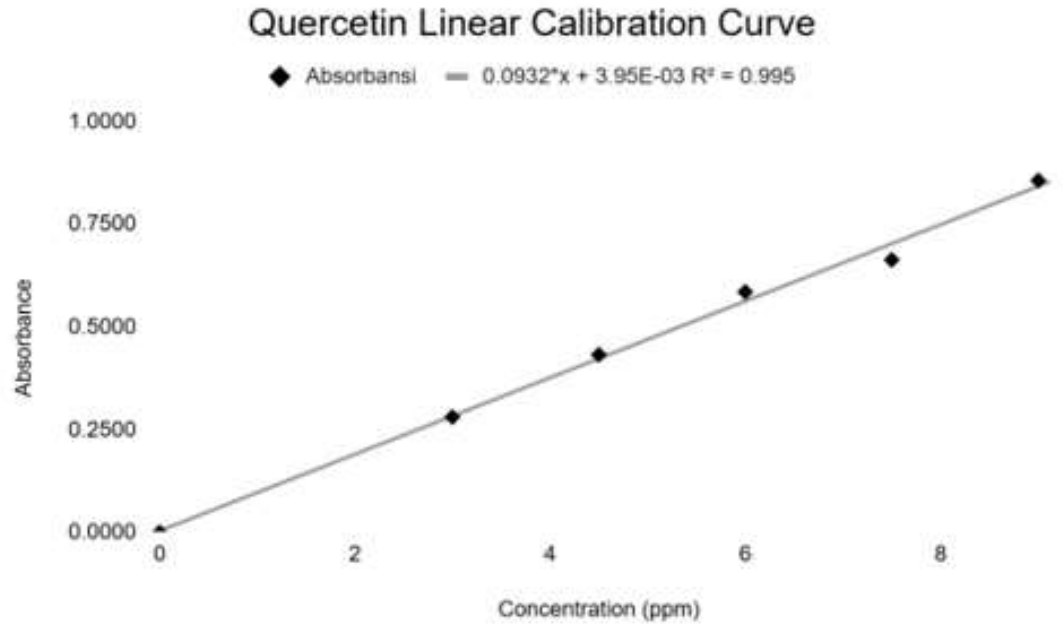

Figure 2 Quercetin Linear Calibration Curve

Table 2 TPC, TFC, and Antioxidant Activity (IC_{50}) of *Mangifera casturi* Leaf Extracts

Solvent	Temperature (°C)	Duration (minutes)	TPC± SD (mg GAE/g)	TFC± SD (mg QE/g)	IC_{50} ± SD (ppm)
Ethyl Acetate	30	30	85.77 ± 3.704^{ab}	40.62 ± 1.865^{ef}	119.92± 0.137^{b}
	30	60	72.42 ± 1.297^{cd}	35.93 ± 1.801^{f}	150.21± 3.457^{a}
	30	90	65.91 ± 0.869^{cde}	75.28 ± 4.883^{b}	63.87± 0.431^{g}
	40	30	68.43 ± 1.936^{cd}	86.68 ± 1.987^{a}	100.91± 0.417^{d}

Doi: 10.30872/jtpc.v10i2.411

	40	60	66.37 ± 2.524^{cde}	45.74 ± 1.654^{de}	67.42± 1.480^{g}
	40	90	89.09 ± 12.385^{a}	54.81 ± 4.923^{cd}	85.74± 0.503^{f}
	50	30	55.34 ± 0.527^{ef}	65.22 ± 0.318^{bc}	65.32± 0.854^{g}
	50	60	63.12 ± 2.100^{de}	42.35 ± 2.567^{ef}	114.41± 0.283^{c}
	50	90	52.09 ± 0.806^{f}	45.88 ± 7.009^{def}	105.27± 0.490^{e}

Values are expressed as mean ± SD (n = 3). Different superscript letters within the same parameter indicate significant differences among extraction conditions according to Tukey's HSD post-hoc test ($p < 0.05$).

The extraction yield did not follow the same pattern as the phytochemical content and antioxidant activity. The highest yield (30%) was obtained at 50 °C for 90 min, whereas the highest TPC and TFC and the lowest IC_{50} were observed under different temperature–time combinations. This indicates that a higher extraction yield does not necessarily correspond to greater recovery of phenolic and flavonoid compounds or stronger antioxidant activity. Extraction yield represents the total amount of soluble constituents recovered from the plant matrix, including both compounds contributing to the targeted bioactivity and other co-extracted constituents. Increasing temperature and extraction duration may enhance solvent penetration, mass transfer, and solubilization of a broader range of extractable compounds, thereby increasing the overall yield without proportionally enriching antioxidant constituents. Therefore, extraction efficiency should be evaluated not only on the basis of total extract yield but also on the phytochemical quality and biological activity of the resulting extract [23].

Extraction temperature is an important parameter in extraction optimization due to its influence on solvent diffusion and plant tissue permeability. An increase in temperature reduces solvent viscosity and enhances the diffusion coefficient, thereby facilitating the transfer of bioactive compounds from the plant matrix into the solvent. In the present study, the highest total flavonoid content (TFC) was obtained at 40°C for 30 min, whereas the highest total phenolic content (TPC) was achieved at 40°C for 90 min. These findings indicate that 40°C was sufficient to improve extraction efficiency without causing substantial degradation of bioactive compounds. Similar results have been reported in ultrasound-assisted extraction studies, where moderate temperatures (35–50°C) generally produced higher phenolic and flavonoid yields than lower or excessively high temperatures [24]

Extraction time was also evaluated because it determines the duration of contact between the solvent and plant material, thereby affecting the extent of bioactive compound transfer into the extraction medium. In this study, the longest extraction duration of 90 min, combined with controlled temperature and ultrasonic assistance, produced favourable TPC, TFC, and antioxidant activity results, while requiring considerably less time than conventional extraction methods, which typically require 24–72 h. However, excessively long extraction times may promote the degradation of bioactive compounds due to the combined effects of heat and ultrasound. Prolonged ultrasonic exposure can generate hydroxyl radicals and other reactive oxygen species through cavitation phenomena, potentially leading to the oxidation of certain phenolic and flavonoid compounds [25]. Therefore, extending extraction time does not necessarily result in higher concentrations of bioactive constituents. The results demonstrated that extraction for 90 min significantly improved TPC and antioxidant activity, whereas the highest TFC was already achieved after 30 min. This difference may be attributed to the relatively rapid extraction of flavonoid compounds from *Mangifera casturi* leaves, such that extending the extraction period did not provide a substantial additional benefit. This observation is consistent with previous studies reporting that increasing extraction time is not always directly proportional to the recovery of bioactive compounds [26].

The absence of a direct correspondence between TPC or TFC and IC_{50} suggests that antioxidant activity is determined not only by the total amount of phenolic or flavonoid compounds but also by their qualitative composition and chemical structure. Individual phenolic compounds differ considerably in their radical-scavenging capacity depending on structural features such as the number and position of

Doi: 10.30872/jtpc.v10i2.411

hydroxyl groups, conjugation, and substitution patterns [27]. Consequently, an extract with a higher total phenolic content may exhibit weaker antioxidant activity if its predominant constituents possess relatively lower radical-scavenging potency. Conversely, extracts containing smaller amounts of highly active phenolic compounds may demonstrate stronger antioxidant activity. This may explain why the extraction condition producing the highest TPC or TFC in the present study did not correspond to the condition producing the lowest IC_{50}. Therefore, extraction temperature and duration may influence not only the total recovery of phenolic constituents but also the relative composition of antioxidant compounds in the extract [28].

The TPC, TFC, and antioxidant activity results (Table 2) revealed that the extraction conditions yielding the highest TPC and TFC were not identical to those producing the strongest antioxidant activity. This finding suggests that antioxidant activity is not solely dependent on the total amounts of phenolic and flavonoid compounds but is also influenced by the composition and structural characteristics of individual constituents [29]. Antioxidant effectiveness is determined by factors such as the number and position of hydroxyl groups, the degree of conjugation within the molecular structure, and the presence of functional groups that affect electron- or hydrogen-donating capacity. Furthermore, synergistic or antagonistic interactions among secondary metabolites within the extract may contribute to the overall antioxidant response. Certain compounds present at relatively low concentrations may exhibit greater antioxidant potency than compounds occurring at higher levels [30]. Consequently, an increase in total phenolic or flavonoid content does not necessarily correspond to a lower IC_{50} value. However, TPC and TFC represent broad measures of phenolic and flavonoid contents and do not identify the individual constituents responsible for the observed antioxidant activity. Therefore, the specific compounds underlying the differences in antioxidant activity among extraction conditions remain to be elucidated. Further chemical characterization using HPLC, LC-MS/MS, or metabolomic approaches is warranted to identify and quantify individual antioxidant constituents and clarify their contribution to the antioxidant activity of *Mangifera casturi* leaf extracts.

4 Conclusion

In conclusion, extraction temperature and time significantly influenced the recovery of bioactive compounds and antioxidant activity of *Mangifera casturi* leaves extracted using an ultrasonic water bath. The best-performing conditions within the tested range varied according to the targeted response: 40 °C for 30 min produced the highest TFC, 40 °C for 90 min produced the highest TPC, and 30 °C for 90 min produced the lowest IC_{50} value. These findings demonstrate that no single extraction condition was universally superior and that the selection of extraction parameters should depend on the targeted phytochemical or antioxidant response. The results also indicate that antioxidant activity is not solely determined by total phenolic and flavonoid contents but may also depend on the composition and interactions of secondary metabolites in the extract. Therefore, ultrasound-assisted extraction provides a useful approach for recovering bioactive constituents from *Mangifera casturi* leaves, while the identified conditions may serve as a basis for future phytochemical characterization and further development of *Mangifera casturi* as a potential source of natural antioxidants.

5 Declaration

5.1 Acknowledgement

All authors thanked the Pharmacy Faculty Universitas Muhammadiyah Banjarmasin for supporting the raw materials and thanked the School of Pharmacy for providing the permission of laboratories to conduct this research.

5.2 Author Contribution

DC: Conceptual, ideation, resources, drafting. DL, DM, and MF: Research, data collecting, analysis data. RA: Supporting and processing raw material resources. TNT: Supervision, verification.

Doi: 10.30872/jtpc.v10i2.411

5.3 Ethics
This research did not need ethical approval.

5.4 Conflict of Interest
All authors declare there is no conflict of interest.

5.5 Funding Statement
The authors gratefully acknowledge the financial support provided by the Internal Research Grant of Universitas Muhammadiyah Yogyakarta under Grant No. 18/R-LRI/I/2025.

6 Bibliography
[1.] Hanin, N.N.F., & Pratiwi, R., 2017. Kandungan fenolik, flavonoid dan aktivitas antioksidan ekstrak daun paku laut (Acrostichum aureum L.) fertil dan steril di kawasan mangrove Kulon Progo, Yogyakarta, Journal of Tropical Biodiversity and Biotechnology, 2, (2), 51–57.

[2.] Rauf, A., Ahmad, Z., Formanowicz, D., Ribaudo, G., & Alomar, T.S., 2024. Antioxidant potential of polyphenolic and flavonoid compounds, Frontiers in Chemistry, 12, 1463755. https://doi.org/10.3389/fchem.2024.1463755

[3.] Marliani, L., Naimah, A., & Roni, A., 2016. Penetapan kadar fenolat total dan flavonoid total ekstrak etanol daun, kulit batang dan kulit buah kasturi (*Mangifera casturi*), Proceeding of Mulawarman Pharmaceuticals Conferences, 3, (2), 275–281. https://doi.org/10.25026/mpc.v3i2.121

[4.] Ramadhan, H., Rezky, D.P., & Susiani, E.F., 2021. Penetapan kandungan total fenolik-flavonoid pada fraksi etil asetat kulit batang kasturi (*Mangifera casturi* Kosterman), Jurnal Farmasi dan Ilmu Kefarmasian Indonesia, 8, (1), 58–67.

[5.] Gunawan, G., Muhamat, M., & Maghfiroh, A., 2024. Ethnobotany and conservation of Kasturi (*Mangifera casturi* Koesterm.) by Banjar Tribe in South Kalimantan, Indonesia, Biodiversitas, 25, (4), 1420–1426. https://doi.org/10.13057/biodiv/d250414

[6.] Pratama, M., Razak, R., & Rosalina, V.S., 2019. Analisis kadar tanin total ekstrak etanol bunga cengkeh (Syzygium aromaticum L.) menggunakan metode spektrofotometri UV-Vis, Jurnal Fitofarmaka Indonesia, 6, (2), 368–373.

[7.] [Kchaou, W., Abbès, F., Blecker, C., Attia, H., & Besbes, S., 2013. Effects of extraction solvents on phenolic contents and antioxidant activities of Tunisian date varieties (Phoenix dactylifera L.), Industrial Crops and Products, 45, 262–269. https://doi.org/10.1016/j.indcrop.2012.12.047

[8.] Palaiogiannis, D., Karavasiloglou, N., Ntourtoglou, G., & Irakli, M., 2023. Successive solvent extraction of polyphenols and antioxidant compounds using solvents of increasing polarity, Chemical Proceedings, 3, (3), 18. https://doi.org/10.3390/chemproc2023018018

[9.] Rafiee, Z., Jafari, S.M., Alami, M., & Khomeiri, M., 2011. Microwave-assisted extraction of phenolic compounds from olive leaves, Food Chemistry, 129, (3), 1150–1157. https://doi.org/10.1016/j.foodchem.2011.05.046

[10.] Assefa, A.D., Jeong, Y.J., Kim, D.J., Jeon, Y.A., Lee, J.E., Lee, H.S., et al., 2016. Antioxidant and antiplatelet activities of flavonoid-rich fractions of citrus fruits from Korea, 3 Biotech, 6, (1), 1–13. https://doi.org/10.1007/s13205-015-0348-4

[11.] Ghafoor, K., & Choi, Y.H., 2009. Optimization of ultrasound assisted extraction of phenolic compounds and antioxidants from grape peel through response surface methodology, Journal of the Korean Society for Applied Biological Chemistry, 52, (3), 295–300. https://doi.org/10.3839/jksabc.2009.050

[12.] Sanou, A., Dabiré, C., Dicko, M.H., et al., 2023. Modelling and optimisation of ultrasound-assisted extraction of phenolic compounds and antioxidant activity, Scientific Reports, 13, 1149. https://doi.org/10.1038/s41598-023-28183-4

[13.] Altemimi, A., Choudhary, R., Watson, D.G., & Lightfoot, D.A., 2016. Ultrasound assisted extraction of phenolic compounds from peaches and pumpkins, PLoS ONE, 11, (2), e0148758. https://doi.org/10.1371/journal.pone.0148758

[14.] Fuad, A.F., Alwi, A., Zulkifly, N.A.H., & Mohamed, N.A., 2020. Total phenolic, total flavonoid content and antioxidant activity of Mangifera sp. leaf extracts, Journal of Agrobiotechnology, 11, (1 Suppl.), 69–78. https://doi.org/10.37231/jab.2020.11.1S.236
[15.] Mulyani, F., Rahayu, Y.P., Daulay, A.S., & Nasution, H.M., 2023. Phytochemical screening and antioxidant activity test of ethanol extract of casturi mango leaves (*Mangifera casturi* Koesterm.) using the DPPH method, Journal of Pharmaceutical Sciences, 6, (Suppl. 1), 49–63.
[16.] Kumar, M., Saurabh, V., Tomar, M., Hasan, M., Changan, S., Sasi, M., et al., 2021. Mango (Mangifera indica L.) leaves: nutritional composition, phytochemical profile, and health-promoting bioactivities, Antioxidants, 10, (2), 299. https://doi.org/10.3390/antiox10020299
[17.] Panche, A.N., Diwan, A.D., & Chandra, S.R., 2016. Flavonoids: an overview, Journal of Nutritional Science, 5, e47. https://doi.org/10.1017/jns.2016.41
[18.] Dai, J., & Mumper, R.J., 2010. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties, Molecules, 15, (10), 7313–7352. https://doi.org/10.3390/molecules15107313
[19.] Shahidi, F., & Ambigaipalan, P., 2015. Phenolics and polyphenolics in foods, beverages and spices: antioxidant activity and health effects, Journal of Functional Foods, 18, 820–897. https://doi.org/10.1016/j.jff.2015.06.018
[20.] Medina-Torres, N., Ayora-Talavera, T., Espinosa-Andrews, H., Sánchez-Contreras, A., & Pacheco, N., 2017. Ultrasound Assisted Extraction for the Recovery of Phenolic Compounds from Vegetable Sources, Agronomy, 7, (3), 47. https://doi.org/10.3390/agronomy7030047
[21.] Muflihah, Y.M., Gollavelli, G., & Ling, Y.C., 2021. Correlation study of antioxidant activity with phenolic and flavonoid compounds in Indonesian Zingiberaceae herbs, Antioxidants, 10, (9), 1365. https://doi.org/10.3390/antiox10091365
[22.] Chemat, F., Rombaut, N., Sicaire, A.G., Meullemiestre, A., Fabiano-Tixier, A.S., & Abert-Vian, M., 2017. Ultrasound assisted extraction of food and natural products: mechanisms, techniques, combinations, protocols and applications, Ultrasonics Sonochemistry, 34, 540–560. https://doi.org/10.1016/j.ultsonch.2016.06.035
[23.] Alara, O.R., Abdurahman, N.H., & Ukaegbu, C.I., 2021. Extraction of phenolic compounds: a review, Current Research in Food Science, 4, 200–214. https://doi.org/10.1016/j.crfs.2021.03.011
[24.] Dai, J., & Mumper, R.J., 2010. Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties, Molecules, 15, (10), 7313–7352. https://doi.org/10.3390/molecules15107313
[25.] Maharani, S.N., & Rifkia, V., 2024. The effects of temperature and time of plant extraction using ultrasonic method on antioxidant activity: Systematic review, Journal of Research in Pharmacy and Pharmaceutical Sciences, 3, (1), 34–43. https://doi.org/10.33533/jrpps.v3i1.7434
[26.] Dorta, E., Lobo, M.G., & González, M., 2012. Reutilization of mango byproducts: Study of the effect of extraction solvent and temperature on their antioxidant properties, Journal of Food Science, 77, (1), C80–C88. https://doi.org/10.1111/j.1750-3841.2011.02477.x
[27.] Prasad, A.A., Hassan, M.A.H., Yang, B., Kong, K.W., Ramanan, R.N., Azlan, A., & Ismail, A., 2012. Selection of experimental domain using two-level factorial design to determine extract yield, antioxidant capacity, phenolics, and flavonoids from Mangifera pajang Kosterm., Separation Science and Technology, 47, (16), 2417–2423. https://doi.org/10.1080/01496395.2012.672511
[28.] Alwi, A., & Ahmad Fuad, A.F., 2020. Total phenolic, total flavonoids content and antioxidant activity of Mangifera sp. leaf extracts, Journal of Agrobiotechnology, 11, (1S). https://doi.org/10.37231/jab.2020.11.1S.236
[29.] Tinasy, N.A., & Wijayati, N., 2024. Effects of the extraction methods on the total phenolic content, the total flavonoid content, and the antioxidant activity of the mango (Mangifera indica L.) leaf

Doi: 10.30872/jtpc.v10i2.411

extracts, Indonesian Journal of Chemical Science, 13, (3). https://doi.org/10.15294/ijcs.v13i3.9557

[30.] Işık, M., Dikici, E., Altın, S., Alp, C., Kırboğa, K.K., Köksal, E., & Beydemir, Ş., 2025. Phenolic content, antioxidant capacity, and therapeutic potential of mango (Mangifera indica L.) leaves, Food Science & Nutrition, 13, (5), e70263. https://doi.org/10.1002/fsn3.70263