Journal of Applied Pharmaceutical Science Vol. 16(03), pp 292-300, March, 2026
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2026.282660
ISSN 2231-3354

Optimization of microwave-assisted extraction of phenolics, flavonoids, and antioxidant capacity from *Graptophyllum pictum* leaves using response surface methodology

Feda Anisah Makkiyah[1]*, Eldiza Puji Rahmi[2], Ayu Rana Esadini[2], Dhigna Luthfiyani Citra Pradana[2], Fahma Alifa Insani[3], Djarot Sasongko Hami Seno[3], Waras Nurcholis[3,4]*

[1]Department of Neurosurgery, Faculty of Medicine, Universitas Pembangunan Nasional Veteran Jakarta, Jakarta, Indonesia.
[2]Pharmacy Program, Faculty of Medicine, Universitas Pembangunan Nasional Veteran Jakarta, Jakarta, Indonesia.
[3]Department of Biochemistry, Faculty of Mathematics and Natural Sciences, IPB University, Dramaga, Bogor, Indonesia.
[4]Tropical Biopharmaca Research Center, IPB University, Taman Kencana, Bogor, Indonesia.

ARTICLE HISTORY

Received on: 09/09/2025
Accepted on: 29/11/2025
Available Online: 05/02/2026

Key words:
Graptophyllum pictum, microwave-assisted extraction, response surface methodology, phenolics, flavonoids, antioxidant activity

ABSTRACT

The present study aimed to optimize microwave-assisted extraction (MAE) conditions for enhancing the recovery of phenolics, flavonoids, and antioxidant activity from *Graptophyllum pictum* (L.) Griff. leaves. Response surface methodology (RSM) with an I-optimal design was used to evaluate the effects of solvent-to-solid ratio (10–15 ml/g) and pH (2–6) on total phenolic content (TPC), total flavonoid content (TFC), and antioxidant capacity assessed by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays. The experimental runs revealed significant variation among treatments ($p < 0.05$), with the highest TPC (2.81 ± 0.10 mg GAE/g DW) and DPPH capacity (2.11 ± 0.01 µmol TE/g DW) observed at a ratio of 15:1 and pH 5.92, while the highest TFC (6.50 ± 0.07 mg QE/g DW) and FRAP activity (13.09 ± 0.48 µmol TE/g DW) were recorded at a ratio of 13.25:1 and pH 2.8. The desirability function identified an optimal condition at a ratio of 15:1 and pH 6.0, yielding predicted values of 2.481 mg GAE/g DW (TPC), 6.159 mg QE/g DW (TFC), 2.337 µmol TE/g DW (DPPH), and 13.395 µmol TE/g DW (FRAP), with a desirability score of 0.940, indicating high overall optimization performance. Model validation yielded residual standard errors (RSE) of −6.55% (TPC), 0.54% (TFC), −6.12% (DPPH), and 6.47% (FRAP), all within ±10%, confirming predictive accuracy. These findings indicate that MAE coupled with RSM is a reliable and efficient strategy to maximize bioactive compound recovery from *G. pictum* leaves, supporting their potential in pharmaceutical and nutraceutical applications.

1. INTRODUCTION

Free radicals are highly reactive molecules that play a central role in the pathogenesis of various degenerative diseases such as cancer, cardiovascular disorders, and premature aging. Although the human body is equipped with endogenous antioxidant defense systems, these are often insufficient to neutralize excessive oxidative stress, necessitating the intake of exogenous antioxidants [1,2]. Synthetic antioxidants such as butylated hydroxytoluene and butylated hydroxyanisole have been widely used; however, their long-term use has raised safety concerns due to potential toxicity [3]. Consequently, the search for safer natural antioxidants has intensified.

Medicinal plants, particularly those found in tropical regions, have gained significant attention as alternative antioxidant sources. One such plant is *Graptophyllum pictum* (L.) Griff., commonly known as the purple leaf plant or "daun wungu" in Indonesia. Traditionally, the leaves of *G. pictum* have been used to treat ailments such as menstrual pain, hemorrhoids, and inflammation [4,5]. Phytochemical analyses have confirmed the presence of various bioactive compounds in its leaves, including flavonoids, phenolics, alkaloids, and steroids, which contribute to its pharmacological activities [5,6]. Despite these findings,

*Corresponding Author
Feda Anisah Makkiyah, Department of Neurosurgery, Faculty of Medicine, Universitas Pembangunan Nasional Veteran Jakarta, Jakarta, Indonesia.
E-mail: fedaanisah @ upnvj.ac.id; wnurcholis @ apps.ipb.ac.id

© 2026 Feda Anisah Makkiyah *et al*. This is an open access article distributed under the terms of the Creative Commons Attribution 4.0 International License (https://creativecommons.org/licenses/by/4.0/).

scientific reports specifically addressing the optimization of bioactive compound recovery from *G. pictum* remain limited.

The efficiency of extracting these bioactive compounds is strongly influenced by the extraction method. Conventional methods such as maceration and Soxhlet extraction often require longer durations and larger solvent volumes [7,8]. In contrast, microwave-assisted extraction (MAE) has emerged as a promising green technology that enhances extraction efficiency by facilitating dielectric heating, which induces rapid molecular rotation and ionic conduction within the plant matrix, leading to cell wall disruption and improved diffusion of phytochemicals [7,9]. MAE has been reported to improve the yield and quality of phenolic-rich extracts from several medicinal plants [10–13]. However, the potential of MAE for *G. pictum* has not yet been thoroughly explored.

The effectiveness of MAE is highly dependent on operational factors, notably solvent-to-solid ratio and pH, which influence compound solubility, cell wall permeability, and microwave energy absorption [14,15]. Inappropriate parameter settings may result in suboptimal recovery or degradation of sensitive phytochemicals. Solvent-to-solid ratio determines the availability of solvent molecules to penetrate plant tissues and solubilize target compounds, while the tested pH range (2.0–6.0) may not have substantially altered the ionization state of phenolics, possibly explaining its limited effect. Thus, optimization is necessary to maximize extraction efficiency while maintaining antioxidant integrity.

Response surface methodology (RSM) offers a powerful statistical framework for such optimization. By modeling the interaction effects of multiple factors, RSM minimizes the number of required experimental trials while enabling precise determination of optimal conditions [8]. Although RSM has been successfully applied to optimize MAE for other plant species, no systematic study has yet applied this approach to *G. pictum* leaves. In this study, a linear model was selected after model simplification and statistical validation, as higher-order terms (interaction and quadratic) were non-significant and did not improve model fit or predictive reliability.

Therefore, this study was designed to address this knowledge gap by optimizing the solvent-to-solid ratio and pH in the MAE of *G. pictum* leaves. The effects of these parameters on total phenolic content (TPC), total flavonoid content (TFC), and antioxidant capacity [measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays] were evaluated, and optimal conditions were determined using RSM. Although recent studies have investigated MAE optimization for *G. pictum* [16], this study extends the scope by modeling multiple antioxidant responses and verifying model accuracy using desirability function-based RSM. The reported desirability score of 0.940, coupled with residual errors within ±10%, demonstrates high predictive validity. The findings offer further insights for pharmaceutical and nutraceutical development.

2. MATERIALS AND METHODS

2.1. Materials and preparation of plant material

Dried leaves of *G. pictum* (L.) Griff. were obtained from the Tropical Biopharmaca Research Center, IPB University, Bogor, West Java, Indonesia. The leaves were blended, sieved through an 80-mesh sieve to obtain uniform particle size, and stored in airtight containers at room temperature until further use. The following reagents were used: 95% methanol, Folin–Ciocalteu reagent, 10% sodium carbonate (Na_2CO_3), gallic acid, methanol p.a., 10% aluminum chloride ($AlCl_3$), glacial acetic acid, quercetin, DPPH, Trolox, sodium acetate (CH_3COONa), acetic acid, hydrochloric acid (HCl), sodium hydroxide (NaOH), ferric chloride ($FeCl_3$), TPTZ (2,4,6-tripyridyl-s-triazine), and distilled water. All reagents used were of analytical grade and procured from Merck (Germany).

2.2. Optimization of extraction conditions

The optimization of extraction conditions was carried out using RSM to determine the optimal combination of solvent-to-solid ratio and pH for the efficient recovery of phenolic and flavonoid compounds from *G. pictum* leaves. The design was constructed using a quadratic I-optimal coordinate exchange model, which generated 12 randomized experimental runs without blocking. Although the design supported quadratic and interaction terms, model simplification was subsequently applied based on the statistical output from Design-Expert®. Although quadratic models are often used in extraction optimization, the linear model was retained in this study as it was statistically significant, exhibited acceptable fit parameters, and was recommended by the software due to the lack of significance of higher-order terms. The final models were reduced to linear forms, as higher-order terms (2FI and quadratic) were not significant, and only linear models satisfied both significance and lack-of-fit criteria [see Section 3.1 and Analysis of variance (ANOVA) results]. The experimental matrix included both model points and replicate points to assess reproducibility. For example, run 1 and run 10 were technical replicates at the same solvent-to-solid ratio (10 ml/g) and pH (3.6), and although some variation in responses (e.g., TPC) was observed, this is within expected limits of biological sample heterogeneity and analytical variability. This model was developed using Design-Expert® version 23.1.4 (64-bit), build date May 30, 2024 (Stat-Ease Inc., Minneapolis, MN), under a single-user subscription license.

Informed by preliminary experiments and corroborated by existing literature, the selection of independent variables emphasized the significance of the solvent-to-solid ratio and pH in enhancing the extraction of bioactive compounds from *G. pictum* [16]. Based on this, two numeric, continuous variables were selected: the solvent-to-solid ratio (ranging from 10 to 15 ml/g) and pH (ranging from 2.0 to 6.0). The optimization aimed to maximize four key response variables: TPC, TFC, and antioxidant capacities measured through DPPH and FRAP assays. All measurements were conducted in triplicate and expressed as mean ± SD.

In each experimental run, 5 g of dried and pulverized *G. pictum* leaves were combined with a methanol–water mixture (80:20, v/v) according to the designated solvent-to-solid ratio. The pH of the aqueous phase was adjusted using 1 M HCl or 1 M NaOH prior to methanol addition. The suspensions were then subjected to MAE in a household microwave oven (Panasonic NN-ST342M, 135 W) for three minutes. The extraction was

performed in open-vessel mode without temperature or pressure control. The time and power settings were fixed throughout the experiments based on preliminary trials and existing literature on short-duration MAE. After extraction, samples were cooled to ambient temperature, filtered through Whatman No. 1 paper, and adjusted to 10 ml with methanol. The filtrates were stored in amber vials at 4°C until further analysis.

An overview of the experimental factor settings and the corresponding measured responses is provided in Table 1, which summarizes the effect of each treatment combination on TPC, TFC, and antioxidant activities.

2.3. Model fitting and statistical analysis

The experimental data were subjected to statistical analysis using Design-Expert® software version 23.1.4 (Stat-Ease Inc., Minneapolis, MN) to model the relationship between the extraction conditions and the measured responses. A RSM approach was adopted using a linear model structure, following model hierarchy principles and goodness-of-fit criteria. The linear model was statistically significant for TPC and DPPH, whereas quadratic and interaction terms were not, supporting its use for initial screening and optimization within the tested parameter space.

ANOVA was employed to evaluate the adequacy of the model and to determine the significance of each factor and interaction. The regression model was evaluated based on the *F*-value, *p*-value, coefficient of determination (R^2), adjusted and predicted R^2, lack-of-fit tests, and adequate precision. The lack-of-fit test was used to assess whether the model adequately represents the data without significant unexplained variation.

Model coefficients, including intercept, linear, and interaction terms, were computed in coded units, and their statistical significance was examined. The models were also assessed for multicollinearity using variance inflation factors, with values below 10 considered acceptable. Adequate precision, defined as the signal-to-noise ratio, was used as an indicator of the model's reliability in navigating the design space, with values above four deemed desirable.

These statistical procedures ensured the selection of appropriate models for each response variable—TPC, TFC, and antioxidant activities (DPPH and FRAP). The validated models were used for further response surface analysis and optimization of the extraction conditions for *G. pictum* leaves.

2.4. Determination of bioactive compounds and antioxidant activities

The determination of TPC, TFC, and antioxidant activities (DPPH and FRAP) was conducted using spectrophotometric techniques adapted from previously published protocols [13].

TPC was measured using the Folin–Ciocalteu assay. In a 96-well microplate (Biologix), 20 µl of sample extract was added to 120 µl of 10% Folin–Ciocalteu reagent and incubated for 5 minutes. Subsequently, 80 µl of 10% sodium carbonate (Na_2CO_3) was added, and the mixture was kept in the dark at room temperature for 30 minutes. Absorbance was recorded at 750 nm using a nano spectrophotometer (SPECTROstarNano, BMG LABTECH). Gallic acid (0–300 ppm) was used to establish the standard calibration curve. Results were expressed as mg gallic acid equivalents per gram dry weight (mg GAE/g DW).

TFC was determined using the aluminum chloride colorimetric method. A total of 10 µl extract was mixed with 60 µl methanol, 10 µL of 10% aluminum chloride ($AlCl_3$), 10 µl glacial acetic acid, and 120 µl distilled water. After 30 minutes of incubation in the dark, absorbance was measured at 415 nm. Quercetin (0–900 ppm) was used as the standard, and results were expressed as mg quercetin equivalents per gram dry weight (mg QE/g DW).

Table 1. Experimental design matrix showing the combinations of solvent-to-solid ratio and pH (independent variables), along with measured responses of TPC (TPC, expressed as mg gallic acid equivalents per gram dry weight), TFC (TFC, expressed as mg quercetin equivalents per gram dry weight), and antioxidant capacities determined by DPPH and FRAP assays (expressed as µmol Trolox equivalents per gram dry weight). Values are presented as mean ± SD (n = 3). Replicated runs (e.g., Run 1 and Run 10) were included for assessing experimental consistency; see Section 2.2.

Independent variables			Responses			
Run	**Solvent-to-solid (ml/g)**	**pH**	**TPC**	**TFC**	**DPPH**	**FRAP**
1	10.00	3.60	0.51 ± 0.02	2.87 ± 0.11	0.39 ± 0.01	5.05 ± 0.07
2	12.97	6.00	1.89 ± 0.07	5.79 ± 0.25	1.79 ± 0.02	11.46 ± 0.49
3	10.87	6.00	1.40 ± 0.05	4.78 ± 0.18	1.72 ± 0.00	10.97 ± 0.18
4	13.25	2.80	1.85 ± 0.04	6.50 ± 0.07	1.84 ± 0.00	13.09 ± 0.48
5	15.00	4.20	1.68 ± 0.03	5.90 ± 0.24	1.94 ± 0.01	11.72 ± 0.33
6	12.90	4.20	1.96 ± 0.05	6.15 ± 0.11	1.94 ± 0.04	10.14 ± 0.15
7	11.65	2.00	0.72 ± 0.02	2.55 ± 0.06	0.34 ± 0.00	1.94 ± 0.01
8	15.00	2.50	2.13 ± 0.08	5.61 ± 0.08	1.82 ± 0.01	10.96 ± 0.38
9	11.00	2.00	1.95 ± 0.04	5.59 ± 0.11	1.48 ± 0.04	8.23 ± 0.07
10	10.00	3.60	1.40 ± 0.05	4.86 ± 0.18	1.68 ± 0.02	10.95 ± 0.22
11	12.90	4.20	2.11 ± 0.02	5.49 ± 0.19	2.00 ± 0.04	11.68 ± 0.06
12	15.00	5.92	2.81 ± 0.10	5.03 ± 0.02	2.11 ± 0.01	11.22 ± 0.21

The DPPH radical scavenging activity was evaluated by reacting 100 µl of sample with 100 µl of 125 µM DPPH solution in a 96-well plate. The reaction was incubated for 30 minutes at room temperature in the absence of light, and the absorbance was measured at 515 nm. Trolox (0–50 µM) served as the standard, and antioxidant activity was expressed as µmol Trolox equivalents per gram dry weight (µmol TE/g DW).

The FRAP assay was conducted by preparing a reagent mixture consisting of 300 mM acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl, and 20 mM $FeCl_3$ in a 10:1:1 ratio. A 10 µl sample was added to 300 µl of freshly prepared FRAP reagent, incubated at 37°C for 30 minutes in the dark, and measured at 593 nm. Antioxidant activity was calculated based on a Trolox calibration curve (0–600 µM) and expressed in µmol TE/g DW.

2.5. Validation of the model

RSM was employed to optimize the solid-to-liquid ratio and pH, using Design-Expert® version 23.1.4 (Stat-Ease Inc., Minneapolis, USA). The experimental design was based on an I-optimal design type and a coordinate exchange design approach, implemented as a randomized response surface study with 12 runs and no blocks. The optimal extraction conditions were identified based on the highest desirability value generated by the software. The desirability score of 0.940 reflects the model's ability to simultaneously approach the target values for TPC, TFC, DPPH, and FRAP. To verify the accuracy of the optimization, the experimental results obtained under the predicted optimal conditions were compared with model-predicted values, and the validity was assessed using the percentage residual standard error (%RSE) following the approach described by Marliani *et al*. [17].

3. RESULTS AND DISCUSSION

3.1. Fitting the Model

The regression analysis demonstrated that the linear models for TPC and DPPH were statistically significant (p = 0.0302 and p = 0.0329, respectively), while the models for TFC and FRAP were not (p = 0.1797 and p = 0.1138, respectively), as summarized in Table 2. The selection of linear models followed Design-Expert® software's fit summary recommendation, identifying linear as the highest non-aliased model with significant predictive capacity. The selection of linear models followed Design-Expert® software's fit summary recommendation. Although quadratic models are commonly used in extraction studies, they were excluded here due to non-significance, limited degrees of freedom, and the superior statistical performance of the linear model in this dataset. For both TPC and DPPH, the solvent-to-solid ratio exhibited a statistically significant positive effect (p = 0.0114 and p = 0.0374, respectively), whereas pH had no significant impact (p > 0.1). These results highlight solvent availability as a more influential factor than pH in enhancing the release of phenolic compounds and in increasing radical scavenging capacity, as measured by the DPPH assay. Comparable results have been reported for *Melastoma sanguineum*, where MAE combined with RSM identified the solvent-to-solid ratio as the most influential factor for phenolic recovery [18]. Similarly,

Table 2. Regression coefficients (3b2), SE, 95% confidence intervals (CI), p-values, and model statistics for linear regression models of TPC, TFC, DPPH, and FRAP.

Response	Term	3b2 coefficient	SE	95% CI low	95% CI high	p-value	R^2	Adjusted R^2	Predicted R^2	F (Model, p)	Lack of fit (p)	Model significance
TPC	Intercept	1.5600	0.1246	1.2800	1.8400	–	**0.5406**	**0.4385**	**0.2017**	**5.30 (0.0302)***	0.9484	Significant
	Solvent-to-solid	**0.5448**	0.1801	0.1374	0.9521	**0.0114**						
	pH	0.1384	0.1782	−0.2648	0.5416	0.4573						
TFC	Intercept	4.5600	0.3238	3.8300	5.3000	–	**0.3171**	**0.1654**	**−0.2168**	2.09 (0.1797)	0.8884	Not significant
	Solvent-to-solid	0.9014	0.4679	−0.1570	1.9600	0.0861						
	pH	0.1920	0.4630	−0.8555	1.2400	0.6881						
DPPH	Intercept	298.61	24.90	242.29	354.93	–	**0.5318**	**0.4278**	**0.1756**	**5.11 (0.0329)***	0.9631	Significant
	Solvent-to-solid	**87.75**	35.98	6.36	169.13	**0.0374**						
	pH	61.06	35.60	−19.47	141.60	0.1205						
FRAP	Intercept	9.7800	0.8092	7.9500	11.6100	–	**0.3830**	**0.2459**	**−0.0882**	2.79 (0.1138)	0.8348	Not significant
	Solvent-to-solid	1.8600	1.1700	−0.7881	4.5000	0.1467						
	pH	1.7600	1.1600	−0.8614	4.3700	0.1634						

*p < 0.05. Only linear models were reported, based on Design-Expert® recommendations and statistical significance criteria.
Bold values indicate statistical significance at p < 0.05.

optimization of MAE conditions for date seeds confirmed that solvent ratio significantly improved extraction yields of phenolic compounds, with validated regression models showing strong predictive ability [19].

The present study achieved moderate R^2 values for TPC (0.5406) and DPPH (0.5318), with adjusted R^2 values of 0.4385 and 0.4278, respectively, and predicted R^2 values below 0.21. Although these R^2 values reflect limited explanatory power, they remain within acceptable ranges for initial RSM models focused on two variables, as also observed in previous MAE studies [20].

In contrast, the models for TFC and FRAP were not statistically significant and exhibited low adjusted R^2 values (0.1654 and 0.2459, respectively), along with negative predicted R^2 values. These results suggest that additional extraction variables not included in the current design (e.g., time, temperature, or solvent polarity) may be required to improve the model's predictive capability for these responses. The experimental design employed in this study utilized a total of 12 randomized runs, optimized for two numeric factors—solvent-to-solid ratio and pH—based on a quadratic I-optimal coordinate exchange approach. While this approach reduced the number of runs and allowed for efficient initial screening, it inherently limited the design space. Previous studies have shown that additional variables, such as extraction time, microwave power, temperature, and solvent composition, significantly influence the recovery of phenolics and flavonoids. The exclusion of these parameters may explain the lack of significance observed in the TFC and FRAP models. Future designs should consider expanding the factorial scope to include such variables, potentially using central composite or Box-Behnken designs to improve model fit and predictive performance. This trend has also been observed in *Senna alata* (candle bush), where optimization of flavonoid extraction required more complex quadratic models to achieve significance [21].

Recent studies further support these findings. Optimization of MAE in mango peel demonstrated that integrating RSM with artificial neural networks (ANN) could better capture the complexity of antioxidant responses, particularly for FRAP [22]. Likewise, aqueous MAE of pomegranate and banana peels showed that while solvent ratio consistently influenced phenolic recovery, pH contributed less significantly to antioxidant capacity [23].

Taken together, the results reinforce that under the tested conditions, the solvent-to-solid ratio is the most influential parameter for phenolic extraction and antioxidant activity, while pH plays a secondary role. However, caution is warranted when interpreting non-significant models (TFC and FRAP), and future studies should implement multi-factorial designs to enhance robustness.

3.2. Influence of solvent-to-solid ratio and pH on TPC and TFC

The regression analysis revealed that the solvent-to-solid ratio exerted a significant positive effect ($p < 0.05$) on TPC, whereas the influence of pH was not statistically significant (Table 2). The fitted model for TPC showed an R^2 of 0.54, indicating a moderate level of prediction accuracy. The lack-of-fit test was not significant, confirming that the model adequately represented the experimental data. As depicted in the contour plot (Fig. 1a), TPC increased progressively with higher solvent-to-solid ratios, ranging from 10 to 15 ml/g, regardless of the pH level (Table 1). This trend suggests that solvent availability is a critical factor for enhancing the solubilization of phenolic compounds during MAE. Similar findings were reported for *M. sanguineum* and date seeds, where the solvent ratio was consistently identified as the most influential factor for phenolic recovery under MAE conditions [18,19].

For TFC, the model did not reach statistical significance, as also shown in Table 2, although the response surface indicated a gradual increase in flavonoid content with higher solvent-to-solid ratios (Fig. 1b). This suggests the two-variable linear model was insufficient to explain the response behavior and indicates the need to incorporate variables such as microwave power, extraction time, and solvent polarity. Previous work on *S. alata* demonstrated that flavonoid extraction often requires more

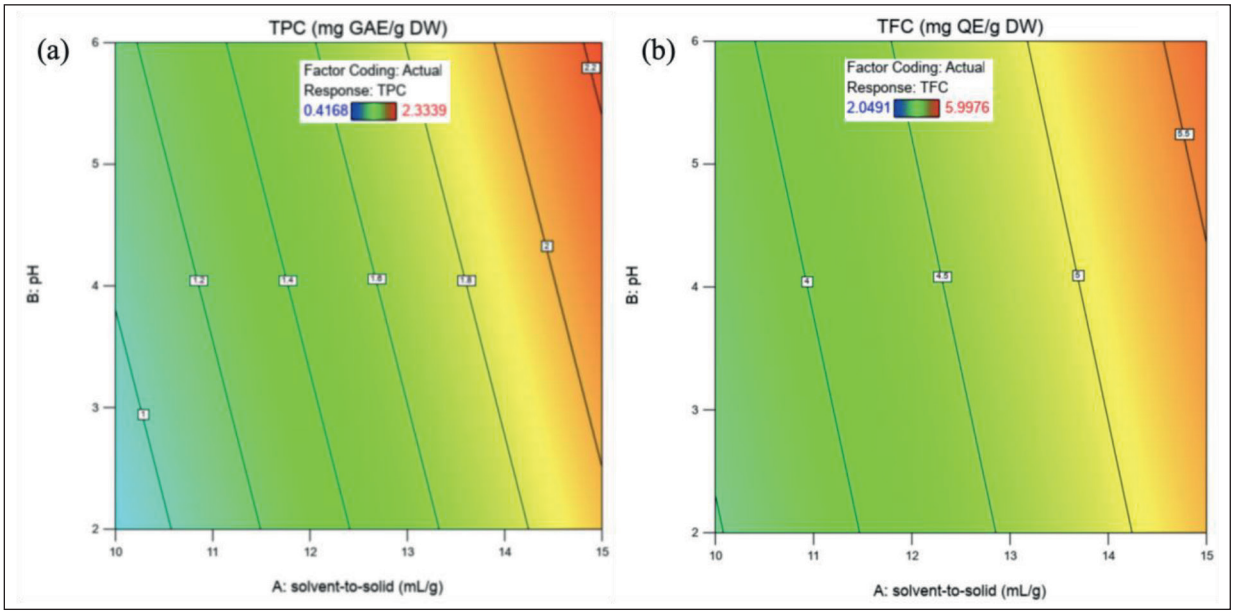


Figure 1. Contour plots showing the effect of solvent-to-solid ratio and pH on (a) TPC (mg GAE/g DW) and (b) TFC (mg QE/g DW).

complex quadratic models, highlighting that linear models may not fully capture the variability of flavonoid responses [21].

The positive association between solvent ratio and bioactive compound recovery aligns with previous reports on pomegranate, banana, and mango peels, where increased solvent accessibility facilitated greater solubilization of phenolics and flavonoids [22,23]. Conversely, the limited contribution of pH in this study is consistent with other optimization experiments, which showed that pH played only a marginal role compared to solvent ratio in determining antioxidant yields [20]. Thus, while solvent-to-solid ratio emerges as the dominant factor for both TPC and TFC, optimization of additional parameters may be required to maximize flavonoid extraction.

3.3. Influence of solvent-to-solid ratio and pH on DPPH and FRAP

The regression model for DPPH scavenging activity was statistically significant ($p < 0.05$), with solvent-to-solid ratio exerting a notable positive effect, while pH contributed less significantly (Table 2). The fitted model had an R^2 of 0.53, demonstrating moderate predictive capacity. As illustrated in the contour plots (Fig. 2a), DPPH activity increased with higher solvent-to-solid ratios, reaching maximum values near 15 ml/g. This trend indicates that greater solvent availability enhances the diffusion and stabilization of antioxidant compounds capable of donating hydrogen atoms to DPPH radicals. Comparable findings have been reported in studies of grape pomace and sweet potato peel, where the solvent-to-solid ratio was the primary driver of DPPH activity [20].

In contrast, the FRAP response model was not statistically significant ($p > 0.05$), and both solvent-to-solid ratio and pH showed no meaningful effect on the reducing power of extracts (Table 2). The response surface (Fig. 2b) revealed only slight variations across the tested conditions, suggesting that FRAP activity may depend more strongly on other extraction parameters not investigated here, such as extraction time, temperature, or solvent composition. Previous studies on mango peel and *Senna alata* extracts similarly reported weak model fits for FRAP under limited variable designs, reinforcing that additional factors are required to adequately explain the variability of ferric reducing capacity [21,22]. This further supports the limitation of using only two variables and linear modeling in the present work.

Overall, these results demonstrate that the solvent-to-solid ratio significantly influenced radical scavenging activity as measured by the DPPH assay, but was less predictive for FRAP. This indicates that different antioxidant assays capture distinct mechanisms of activity and may respond differently to extraction parameters, highlighting the need for multi-response optimization in MAE studies.

From a mechanistic perspective, the dominance of the solvent-to-solid ratio can be attributed to improved solvent availability, which facilitates mass transfer and prevents saturation effects, thereby enhancing the solubilization of phenolic and flavonoid compounds [24]. The limited role of pH within the tested range may be explained by the relative stability of hydroxyl groups under mildly acidic to near-neutral conditions, which does not strongly alter ionization or extractability.

The limitations observed for TFC and FRAP responses suggest that additional extraction parameters, such as solvent polarity, irradiation power, or extraction time, could exert greater influence than those tested here. Similar conclusions were drawn in previous optimization studies on *S. alata* and mango peel, where more complex quadratic or hybrid models (e.g., RSM-ANN) were required to adequately capture the variability of flavonoid and FRAP responses [21,22].

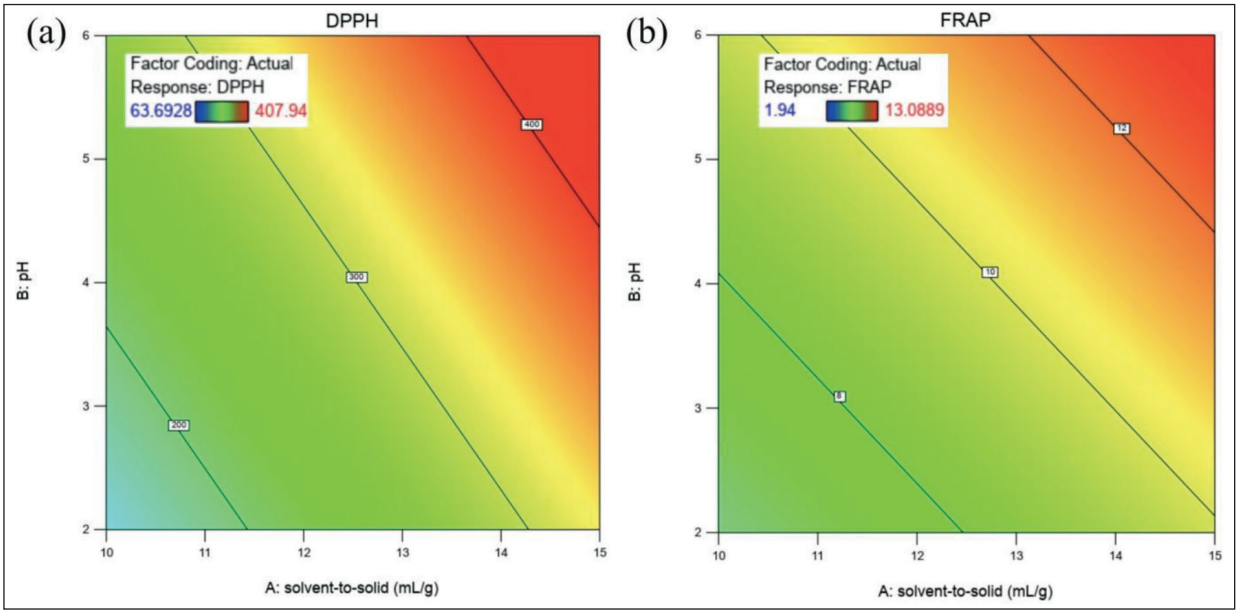


Figure 2. Contour plots showing the effect of solvent-to-solid ratio and pH on (a) DPPH radical scavenging activity (µmol TE/g DW) and (b) FRAP antioxidant capacity (µmol TE/g DW).

Table 3. Validation of predicted versus actual responses at optimal extraction conditions

Treatment	A (Ratio)	B (pH)	TPC (mg GAE/g DW)	TFC (mg QE/g DW)	DPPH (µmol TE/g DW)	FRAP (µmol TE/g DW)	Desirability
Predicted	15.000	6.000	2.481	6.159	2.337	13.395	0.940
Actual	15.000	6.000	2.319	6.192	2.194	14.262	
%RSE			−6.55%	0.54%	−6.12%	6.47%	

Note: A = solvent-to-solid ratio; B = pH; DPPH = 2,2-diphenyl-1-picrylhydrazyl; FRAP = ferric reducing antioxidant power; TPC = total phenolic content; TFC = total flavonoid content; RSE = residual standard error.

These findings align with prior reports on *M. sanguineum*, date seeds, pomegranate, and banana peels, which consistently highlighted solvent-to-solid ratio as the most critical factor under MAE, while pH played only a secondary role [18,19,23]. Importantly, this reinforces the practical implication that solvent ratio should be prioritized in scaling up extraction protocols for *G. pictum*. Comparable conclusions were also reported by Andres *et al*. [25] in a study on brewers' spent grain, where RSM demonstrated that solvent-to-solid ratio and extraction conditions significantly affected TPC and antioxidant capacity.

Future work should expand the design space to include additional process variables (e.g., solvent composition, temperature, and extraction duration) and evaluate the use of greener technologies such as ultrasound-assisted extraction or natural deep eutectic solvents. Validation of optimized conditions at pilot or industrial scale would further strengthen the applicability of these results in pharmaceutical and nutraceutical industries.

3.4. Optimization and verification results

The desirability function in Design-Expert identified the optimal extraction condition at a 15:1 solvent-to-solid ratio and pH 6.0, with predicted responses of 2.481 mg GAE/g DW for TPC, 6.159 mg QE/g DW for TFC, 2.337 µmol TE/g DW for DPPH, and 13.395 µmol TE/g DW for FRAP, corresponding to a desirability score of 0.940, which indicates high simultaneous optimization across multiple responses. Verification of these conditions yielded minimal %RSE, indicating strong agreement between predicted and actual values and confirming the robustness of the model (Table 3). The experimental results closely matched the predicted responses, with deviations within ±10%, supporting the reliability of the optimization framework.

These outcomes are consistent with previous reports, where desirability-based multi-response optimization confirmed the validity of RSM in predicting phenolic and antioxidant yields under MAE conditions [8,26]. The RSEs between predicted and actual values (within ±10%) further support model accuracy, as values below 10% are generally considered acceptable in bioactive extraction models. In particular, Nurcholis *et al*. [8] reported %RSE values of 4.47% for TPC and 7.08% for DPPH in *Justicia gendarussa*, demonstrating the high predictive accuracy of RSM models. Similarly, Azahar *et al*. [26] found near-perfect agreement between predicted and experimental phenolic and flavonoid yields in *Curcuma zedoaria* extracts, further validating the robustness of RSM for optimizing bioactive compound extraction.

Differences observed for FRAP and TFC across studies may be attributed to variation in solvent type, irradiation power, or plant matrix composition. Moreover, comparisons with other modeling approaches suggest that when %RSE values remain below 10%, RSM predictions can match or even surpass the accuracy of ANN models in estimating extraction yields and physicochemical properties [27–29]. Collectively, these results reinforce the applicability of RSM as a reliable strategy for optimizing MAE of phenolic compounds and antioxidant activity from *G. pictum* leaves.

4. CONCLUSION

This study successfully optimized MAE conditions to enhance the recovery of phenolic compounds, flavonoids, and antioxidant capacity from *G. pictum* leaves. Using RSM, the solvent-to-solid ratio was identified as the dominant factor, while pH played a secondary role. The optimal extraction condition was achieved at a 15:1 solvent-to-solid ratio and pH 6.0, with a desirability score of 0.940 and predicted values confirmed by experimental results within ±10% error. These findings confirm that MAE combined with RSM offers an efficient and practical approach for maximizing antioxidant-rich extracts from *G. pictum*. Future studies should consider using closed-vessel MAE systems with real-time feedback and incorporate chromatographic techniques such as HPLC or LC–MS/MS to characterize individual bioactive compounds.

5. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

6. FINANCIAL SUPPORT

This study was supported by internal research funding from Universitas Pembangunan Nasional Veteran Jakarta (UPNVJ).

7. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

8. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

9. DATA AVAILABILITY

All data generated and analyzed are included in this research article.

10. PUBLISHER'S NOTE

All claims expressed in this article are solely those of the authors and do not necessarily represent those of the publisher, the editors and the reviewers. This journal remains neutral with regard to jurisdictional claims in published institutional affiliation.

11. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declare that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

REFERENCES

1. Gulcin I. Antioxidants and antioxidant methods: an updated overview. Arch Toxicol. 2020;94:651–715. doi: https://doi.org/10.1007/s00204-020-02689-3
2. Mardhiyyah K, Yunita Intan Ryandini, Yopi Hermawan. Red and white galangal puree antioxidant activity and phytochemistry screening. Jurnal Jamu Indonesia. 2021;6(1):23–31. doi: https://doi.org/10.29244/jji.v6i1.174
3. Ren J, Li Z, Li X, Yang L, Bu Z, Wu Y, *et al.* Exploring the mechanisms of the antioxidants BHA, BHT, and TBHQ in hepatotoxicity, nephrotoxicity, and neurotoxicity from the perspective of network toxicology. Foods. 2025;14:1095. doi: https://doi.org/10.3390/foods14071095
4. Kusumawati I, Rullyansyah S, Rohmania, Rizka AF, Hestianah EP, Matsunami K. Histomorphometric study of ethanolic extract of *Graptophyllum pictum* (L.) Griff. leaves on croton oil-induced hemorrhoid mice: a Javanese traditional anti-hemorrhoid herb. J Ethnopharmacol. 2022;284:114765. doi: https://doi.org/10.1016/j.jep.2021.114765
5. Niluh Puspita Dewi, Miranti M, Magfirah M, Kurnia Kasim A, Dipayana IM. Determination of total levels of secondary metabolites and oral acute toxicity testing of purple leaf ethanol extract (PLEE) in wistar rats. J Public Health Pharm. 2024;4(2):123–31. doi: https://doi.org/10.56338/jphp.v4i2.5131
6. Arista RA, Jalloh MA, Insani FA, Balqis PA, Larasati C, Arief BFP, *et al.* Phytochemical screening, cytotoxicity test, and antibacterial activity test of wungu plant extract (*Graptophyllum pictum* L. Griff). Jurnal Jamu Indonesia. 2023;8(2):77–83. doi: https://doi.org/10.29244/jji.v8i2.330
7. Barão CE, Tanaka MRR, da Silva C, Madrona GS, Rosset M, Pimentel TC. Extraction of natural food ingredients by modern techniques. Extraction Processes in the Food Industry, Elsevier; 2024, pp. 299–343. doi: https://doi.org/10.1016/B978-0-12-819516-1.00004-1
8. Nurcholis W, Safithri M, Marliani N, Iqbal M. Response surface modeling to optimize sonication extraction with the maceration method for the phenolic content and antioxidant activity of *Justicia gendarussa* Burm f. J Appl Pharm Sci. 2023;13:181–7. doi: https://doi.org/10.7324/JAPS.2023.148030
9. Ferrara D, Beccaria M, Cordero CE, Purcaro G. Microwave-assisted extraction in closed vessel in food analysis. J Sep Sci. 2023;46:2300390. doi: https://doi.org/10.1002/jssc.202300390
10. Mikucka W, Zielinska M, Bulkowska K, Witonska I. Recovery of polyphenols from distillery stillage by microwave-assisted, ultrasound-assisted and conventional solid–liquid extraction. Sci Rep. 2022;12:3232. doi: https://doi.org/10.1038/s41598-022-07322-0
11. Addo PW, Sagili SUKR, Bilodeau SE, Gladu-Gallant FA, Mackenzie DA, Bates J, *et al.* Microwave- and Ultrasound-Assisted Extraction of Cannabinoids and Terpenes from Cannabis Using Response Surface Methodology. Molecules. 2022;27:8803 doi: https://doi.org/10.3390/molecules27248803
12. Ramayani SL, Fitria Rohmawati, Yasmine Savira Rahmadani. The effect of material to solvent ratio to the flavonoid content and free radical scavenging activity of extract of *Morinda citrifolia* L. leaves. Jurnal Jamu Indonesia. 2022;7:56–61. doi: https://doi.org/10.29244/jji.v7i2.245
13. Nurcholis W, Alfadzrin R, Izzati N, Arianti R, Vinnai BÁ, Sabri F, *et al.* Effects of methods and durations of extraction on total flavonoid and phenolic contents and antioxidant activity of Java cardamom (*Amomum compactum* Soland Ex Maton) fruit. Plants 2022;11:2221. doi: https://doi.org/10.3390/plants11172221
14. Mellinas AC, Jiménez A, Garrigós MC. Optimization of microwave-assisted extraction of cocoa bean shell waste and evaluation of its antioxidant, physicochemical and functional properties. LWT - Food Sci Technol. 2020;127:109361. doi: https://doi.org/10.1016/j.lwt.2020.109361
15. Özbek HN, Yanık DK, Fadıloğlu S, Göğüş F. Optimization of microwave-assisted extraction of bioactive compounds from pistachio (*Pistacia vera* L.) hull. Sep Sci Technol. 2020;55:289–99. doi: https://doi.org/10.1080/01496395.2019.1577444
16. R. Y. Galingging, F.A. Insani, D.S.H. Seno, W. Nurcholis. Optimized pH and solid-to-solvent ratio for enhanced polyphenol and antioxidant extraction from *Graptophyllum pictum* leaves using microwave-assisted extraction. RASAYAN J Chem. 2025;18:869–74. doi: https://doi.org/10.31788/RJC.2025.1829189
17. Marliani N, Artika IM, Nurcholis W. Optimization extraction for total phenolic, flavonoid contents, and antioxidant activity with different solvents and UPLC-MS/MS metabolite profiling of *Justicia gendarussa* Burm.f. Chiang Mai Univ J Natural Sci. 2022;21:e2022046. doi: https://doi.org/10.12982/CMUJNS.2022.046
18. Zhao CN, Zhang JJ, Li Y, Meng X, Li HB. Microwave-assisted extraction of phenolic compounds from *Melastoma sanguineum* fruit: optimization and identification. Molecules. 2018;23:2498. doi: https://doi.org/10.3390/molecules23102498
19. Khalfi A, Garrigós MC, Ramos M, Jiménez A. Optimization of the microwave-assisted extraction conditions for phenolic compounds from date seeds. Foods. 2024;13:3771. doi: https://doi.org/10.3390/foods13233771
20. Kadiri O, Gbadamosi SO, Akanbi CT. Extraction kinetics, modelling and optimization of phenolic antioxidants from sweet potato peel vis-a-vis RSM, ANN-GA and application in functional noodles. J Food Meas Characterization. 2019;13:3267–84. doi: https://doi.org/10.1007/s11694-019-00249-7
21. Bao Q, Le Thi HN, Thuy Nguyen Pham D. Optimization of polyphenols and flavonoids extraction from candle bush (*Senna alata* L.) leaves using response surface methodology. Nat Prod Commun. 2025;20:1–2. doi: https://doi.org/10.1177/1934578X251343482
22. Ramírez-Brewer D, Quintana SE, García-Zapateiro LA. Modeling and optimization of microwave-assisted extraction of total phenolics content from mango (*Mangifera indica*) peel using response surface methodology (RSM) and artificial neural networks (ANN). Food Chem X. 2024;22:101420. doi: https://doi.org/10.1016/j.fochx.2024.101420
23. Shijarath TR, Madhu G, Sahoo DK, Abdullah S. Microwave assisted aqueous extraction of phenolic compounds from pomegranate and banana peels: Process modelling and optimization. Food Hum. 2024;3:100456. doi: https://doi.org/10.1016/j.foohum.2024.100456
24. Oreopoulou A, Tsimogiannis D, Oreopoulou V. Extraction of polyphenols from aromatic and medicinal plants: An overview of the methods and the effect of extraction parameters. Polyphenols

in Plants, Elsevier; 2019, pp. 243–59. doi: https://doi.org/10.1016/B978-0-12-813768-0.00025-6.
25. Andres AI, Petron MJ, Lopez AM, Timon ML. Optimization of extraction conditions to improve phenolic content and *in vitro* antioxidant activity in craft brewers' spent grain using response surface methodology (RSM). Foods. 2020;9:1398. doi: https://doi.org/10.3390/foods9101398
26. Azahar NF, Gani SSA, Mohd Mokhtar NF. Optimization of phenolics and flavonoids extraction conditions of *Curcuma Zedoaria* leaves using response surface methodology. Chem Cent J. 2017;11:96. doi: https://doi.org/10.1186/s13065-017-0324-y
27. Mia M, Dhar NR. Response surface and neural network based predictive models of cutting temperature in hard turning. J Adv Res. 2016;7:1035–44. doi: https://doi.org/10.1016/j.jare.2016.05.004
28. Ray S, Haque M, Ahmed T, Nahin TT. Comparison of artificial neural network (ANN) and response surface methodology (RSM) in predicting the compressive and splitting tensile strength of concrete prepared with glass waste and tin (Sn) can fiber. J King Saud Univ - Eng Sci. 2023;35:185–99. doi: https://doi.org/10.1016/j.jksues.2021.03.006
29. Seetharaman S, Suresh S, Shivaranjani RS, Dhamodaran G, Js FJ, Ali Alharbi S, *et al.* Prediction, optimization, and validation of the combustion effects of diisopropyl ether-gasoline blends: a combined application of artificial neural network and response surface methodology. Energy. 2024;305:132185. doi: https://doi.org/10.1016/j.energy.2024.132185

How to cite this article:
Makkiyah FA, Rahmi EP, Esadini AR, Pradana DLC, Insani FA, Seno DSH, Nurcholis W. Optimization of microwave-assisted extraction of phenolics, flavonoids, and antioxidant capacity from *Graptophyllum pictum* leaves using response surface methodology. J Appl Pharm Sci. 2026;16(03):292-300. DOI: 10.7324/JAPS.2026.282660