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Optimization of *Graptophyllum pictum* leaves extraction using a simplex centroid design focused on extracting flavonoids with antioxidant activity

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ABSTRACT

Graptophyllum pictum (L.) Griff, which is called "daun ungu" in Indonesia, is a medicinal plant that contained flavonoids with antioxidant activity. The different extractor solvents (water, acetone, methanol, and ethanol) and their combinations were studied using a simplex centroid design through the Design Expert 13.0 program. From the optimized crude extract, it was investigated for total flavonoid content (TFC) using the colorimetric method ($AlCl_3$), as well as the antioxidant activity using 2,2-diphenyl-1-picryl hydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) methods. The optimum condition was obtained at a desirability value of 0.745 with a water solvent system at a proportion of 1.00 (100%) which resulted in a TFC of 10.464 mg QE/g DW, the antioxidant activity of DPPH 2.179 µmol TE/g DW, and FRAP 22.009 µmol TE/g DW. The method was verified and analyzed based on the percentage of residual standard error, which resulted in <5% so that the predicted and actual results were not significantly different. These results indicate that the simplex centroid design can optimize flavonoid compounds with potent antioxidant activity from *G. pictum* leaves.

INTRODUCTION

Graptophyllum pictum (L.) Griff, commonly knowns as "daun ungu" or purple leaf in Indonesia, is a well-known traditional Indonesian medicinal herb (Kusumawati *et al*., 2022). *Graptophyllum pictum* is endemic to Papua New Guinea, although it has now spread to several other countries, including Asia (such as Indonesia), Ghana, India, Mexico, and Bolivia (Makkiyah *et al*., 2021). Several studies reported that this plant leaves contained phytochemicals including glycosides, flavonoids, alkaloids, steroids, phenolic, carbohydrates, phenolics, anthraquinones, coumarins, and tannins (Jiangseubchatveera *et al*., 2017; Poh-Yen *et al*., 2018). The plant's leaves are beneficial in traditional medicine for treating rheumatism, hemorrhoid, scabies, swelling, hepatomegaly, constipation, ulcers, urinary infection, maturing boil process, and ear disease and smoothing skin, increasing fertility, and wound healing (Ibrahim *et al*., 2022). Furthermore, the plant leaves exhibit multiple pharmacological properties, including anticancer, antibacterial, antioxidant (Jiangseubchatveera *et al*., 2015), antihemorrhoid (Kusumawati *et al*., 2022), nephroprotective (Srinivasan *et al*., 2015), inhibitory a-glucosidase (Nurcholis *et al*., 2014), and photoprotective activities (Poh-Yen *et al*., 2018). However, standardization of the *G. pictum* extract is still necessary to demonstrate the phytochemical content and pharmacological activity.

Natural antioxidants, unique polyphenols, are coveted since they contribute positively to human health (Gasmi *et al*., 2022). Antioxidants are molecules that can protect cells from

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free radicals, which have been linked to stroke, heart disease, and cancer (Sharifi-Rad *et al*., 2020). Research related to polyphenolic compounds is growing, especially its application in the therapeutic field, including in the treatment of ischemic stroke (Liu *et al*., 2022), coronavirus (Park *et al*., 2021), fatty liver (Abenavoli *et al*., 2021), and heart diseases (Qi *et al*., 2021). The important polyphenol compound class in human health is flavonoids (Carey *et al*., 2021). Consequently, the optimal extraction technique that yields plant extracts with a high flavonoid content is crucial.

The solvents are one of the most important factors influencing the separation efficiency of flavonoids from plant sources. Although ethanol is the preferred solvent for extract extraction, acetone, methanol, water, and their mixtures are also employed (Lonni *et al*., 2012; Naczk and Shahidi, 2004). Extracts of flavonoid compounds from plants typically contain a wide variety of metabolites that are all soluble in the solvent used to extract them (Wang *et al*., 2022). In order to increase the selectivity of flavonoid extraction, it is common practice to use a combination of solvents (Fadil *et al*., 2022; Marliani *et al*., 2022). The variety of flavonoid compounds, interferences, and matrix effects contained in plant tissues provide obstacles to the standardization of extraction techniques. This is indicated by the fluctuation in the total flavonoid content (TFC) of *G. pictum* (2.02–28.21 mg QE/g extract) and the extraction yield, which is dependent on the solvent used in the extraction procedure (Jiangseubchatveera *et al*., 2017). Therefore, developing a flavonoid extraction technique in conjunction with mixture design is a strategy that increases the selectivity and optimization needed for the target analysis of plants. The most popular mixture designs are simplex centroid, simplex lattice, extreme vertex, and simplex axial designs. Time, solvent, and raw material consumption may all be minimized with the help of the mixture design (Alara *et al*., 2018; Icyer *et al*., 2016; Lonni *et al*., 2012). Evaluation of optimal conditions for extracting flavonoid compounds from a plant matrix has been performed using the simplex centroid design (Dos Santos *et al*., 2020; Fadil *et al*., 2022; Nurcholis *et al*., 2021; Wibisono *et al*., 2019).

To the authors' knowledge, no previous research has used simplex centroid design to optimize the solvent extraction of flavonoids and antioxidant activity from *G. pictum* leaves. Therefore, in this study, a simplex centroid design was utilized to determine the optimal solvent mixture for the extraction of flavonoid compounds from *G. pictum* leaves with the highest antioxidant activity. The TFC and antioxidant activity were considered to determine the optimal extractor.

MATERIAL AND METHODS

Plant material

Graptophyllum pictum leaves were obtained in June 2022 from the Tropical Biopharmaca Research Center, Institut Pertanian Bogor, Bogor, Indonesia. The leaves were washed and dried for three days in an oven at 50°C. The dried leaves were ground with a disk mill and sieved using an 80 mesh sieve for further use in the extraction process.

Simplex centroid design

Simplex centroid design uses Design Expert® 13.0 software (Stat-Ease Inc., Minneapolis, MN) for optimization. Solvents consisting of water, ethanol, acetone, and methanol were used to extract *G. pictum* leaves with a design based on the simplex centroid design generated by the Design Expert 13.0 program. Figure 1 shows a composition comprising 15 experiments with proportions, as shown in Table 1. Each component of the mixture is in the proportion range from 0% to 100%; then, an adequate model is adopted for each response.

Sample preparation and extraction

The extraction process was carried out by mixing 4 g of dry *G. pictum* leaves powder with 40 ml of solvent (1:10 w/v) according to the composition presented in Table 1. The mixture was sonicated for 30 minutes, then macerated for 3 hours in a water bath shaker (DAIHAN WiseBath) at 30°C. Then it was filtered and concentrated using a rotary evaporator (Hahnvapor HS-2005V by Hahnshin Scientific). The volume was sufficient (mixed, according to the composition in Table 1) to produce a filtrate with a concentration of 0.2 g/ml for further analysis.

Determination of TFC

The TFC was determined by the Pharmacopeia (1989) method with slight modification by Marliani *et al*. (2022) based on the colorimetric method using standard quercetin diluted with methanol at various concentrations of 25–500 ppm. The TFC was expressed as milligrams of quercetin equivalent per gram of dry weight (mg QE/g DW). In summary, 120 µl of distilled water was added to 96-well microplates, 10 µl of sample extract, 10 µl of 10% $AlCl_3$, 10 µl of glacial acetic acid, and 50 µl of proanalysis ethanol. The mixture was incubated for 30 minutes in the dark. The absorbance was measured at 415 nm using a nanospectrophotometer (SPECTROstar Nano, BMG LABTECH).

Determination of antioxidant activity

2,2-Diphenyl-1-picryl hydrazyl (DPPH) radical scavenging method

The radical scavenging activity of DPPH refers to the procedure of Benzie and Strain (1996) with slight modification by Marliani *et al*. (2022). In summary, 120 µl of sample extract was mixed with 80 µl of DPPH (125 M in ethanol) and then was incubated at room temperature in the dark. Absorbance measurement was done at a wavelength of 515 nm using a nanospectrophotometer (SPECTROstar Nano, BMG LABTECH). Trolox was a standard solution at various concentrations of 5–50 µM, so the radical scavenging activity was expressed as micromoles equivalent to Trolox per gram dry weight (µmol TE/g DW).

Ferric reducing antioxidant power (FRAP) method

The method is carried out using the procedure of Blois (1958) with slight modification by Marliani *et al*. (2022). FRAP solution was first prepared by mixing acetate buffer (pH 3.6), 10 M tripyridyl-s-triazine (in 40 mM HCl), 20 mM $FeCl_3$ with a ratio of 10:1:1 (v/v/v), and it was stored in a dark bottle. After that, the test was carried out by adding 50 µl of sample extract into 96 well-microplates and 260 µl of new FRAP solution, incubated for 30 minutes in a dark room. The absorbance was measured at 593 nm using a nanospectrophotometer (SPECTROstar Nano, BMG LABTECH). Trolox was used as a standard curve solution at

various concentrations of 100–600 µM. Antioxidant activity was expressed as micromoles equivalent to Trolox per gram dry weight (µmol TE/g DW).

Statistical analysis

Data analysis was performed with the IBM SPSS Statistics 22 program to analyze the data with one-way analysis of variance (ANOVA). The mean value was considered to be significantly different at $p < 0.05$, while the data for determining the point of optimization was analyzed using the Design Expert 13.0 program (Stat-Ease Inc., Minneapolis, MN). The selection of optimum condition results was chosen based on the highest desirability close to the value of 1.00 (Khalafyan *et al*., 2019).

Model verification

The chosen optimum conditions were verified by extracting the same *G. pictum* leaves and determining TFC, DPPH, and FRAP. The verification value is compared with the actual value as an evaluation of the level of accuracy through the percentage of residual standard error (%RSE), which is calculated for each response using the formula:

$$\% \text{ RSE} = \frac{(\text{Actual value} - \text{Predicted value})}{\text{Predictied value}} \times 100. \quad (1)$$

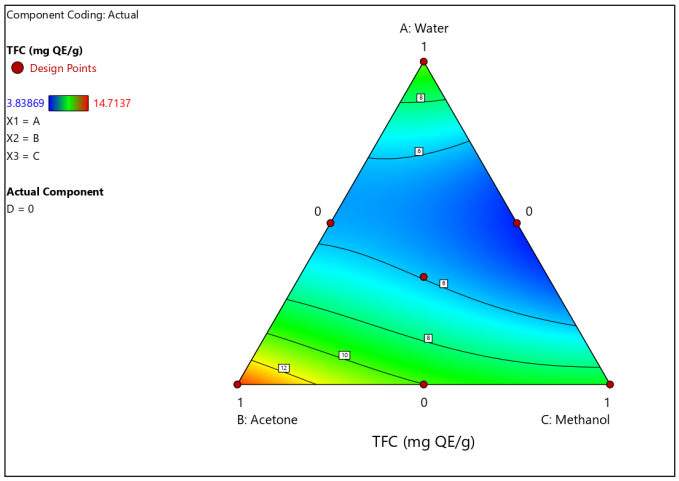

Figure 1. Contour plot for flavonoid extraction in (A) water, (B) acetone, (C) and methanol (D) using 0% ethanol, as predicted by the special cubic model.

RESULTS AND DISCUSSION

Sample extraction optimization

Simplex centroid is a part of the mixture design as an applied statistical method that allows the identification of synergistic effects of mixtures and predicts suitable models in their interactions. This method is an easy-to-manage design where the variables are ingredients or proportions. This study used four types of solvents with different levels of polarity, namely water, acetone, methanol, and ethanol. The ratio was produced based on the simplex centroid design, as shown in Table 1. Each proportion resulted in response to the TFC and antioxidant activity method DPPH and FRAP was significantly different in each treatment ($p < 0.05$) based on Duncan's test.

The purple leaves using the TFC test ranged from 3.839 to 14.741 mg QE/g DW, with the highest value being the water-ethanol mixture solvent. Meanwhile, according to the results of the DPPH test, it ranged from 0.456 to 2.208 µmol TE/g DW with the highest activity coming from water solvents, while according to the FRAP test, the antioxidant activity ranged from 9.521 to 27.597 µmol TE/g DW with the highest activity using acetone as solvent. In general, the type of solvent used in this study is a polar solvent; all solvents can dissolve the compounds in *G. pictum* leaves.

Fitting model

Fifteen mixed formulations were run according to the experiment, and each model's coefficient and the mathematical

Table 1. Proportion of extraction solvent for *G. pictum* based on simplex centroid design and observed response.

Run	Water (A)	Acetone (B)	MeOH (C)	EtOH (D)	TFC (mg QE/g DW)	DPPH (µmol TE/g DW)	FRAP (µmol TE/g DW)
1	0.00	0.00	50.00	50.00	5.738 ± 0.162^{ef}	0.965 ± 0.176^{bc}	12.979 ± 0.197^{fg}
2	50.00	0.00	50.00	0.00	4.214 ± 0.087^{g}	0.700 ± 0.069^{d}	11.354 ± 0.759^{gh}
3	0.00	0.00	0.00	100.00	14.714 ± 0.551^{a}	0.804 ± 0.041^{bcd}	16.979 ± 0.819^{d}
4	0.00	50.00	0.00	50.00	9.499 ± 0.346^{bc}	0.684 ± 0.041^{de}	19.549 ± 0.737^{c}
5	50.00	50.00	0.00	0.00	5.666 ± 0.148^{ef}	0.965 ± 0.107^{bc}	10.201 ± 0.304^{hi}
6	0.00	0.00	100.00	0.00	9.046 ± 0.565^{c}	0.684 ± 0.040^{de}	12.854 ± 0.583^{fg}
7	50.00	0.00	0.00	50.00	3.839 ± 0.132^{g}	0.784 ± 0.031^{cd}	10.938 ± 0.541^{hi}
8	0.00	33.33	33.33	33.33	9.963 ± 0.573^{bc}	0.731 ± 0.014^{d}	13.924 ± 0.850^{ef}
9	0.00	50.00	50.00	0.00	10.035 ± 0.764^{bc}	0.674 ± 0.048^{de}	14.904 ± 0.033^{e}
10	25.00	25.00	25.00	25.00	7.071 ± 0.283^{d}	1.013 ± 0.034^{b}	20.743 ± 0.759^{c}
11	100.00	0.00	0.00	0.00	**10.463 ± 0.101^{b}**	2.208 ± 0.080^{a}	22.779 ± 0.175^{b}
12	33.33	33.33	33.33	0.00	5.910 ± 0.039^{e}	0.957 ± 0.030^{bc}	11.313 ± 0.674^{gh}
13	33.33	33.33	0.00	3.33	5.613 ± 0.252^{ef}	0.456 ± 0.018^{f}	11.076 ± 0.457^{hi}
14	0.00	100.00	0.00	0.00	14.124 ± 0.153^{a}	0.487 ± 0.017^{ef}	27.597 ± 0.289^{a}
15	33.33	0.00	33.33	33.33	4.660 ± 0.120^{fg}	0.745 ± 0.009^{d}	9.521 ± 0.063^{i}

Values with different letters in the same column differ significantly at *p*-value <0.05.
TFC, DPPH, and FRAP are total flavonoid content, 2,2-diphenyl-picrylhydrazyl, and ferric-reducing antioxidant power, respectively.

model's ANOVA were adjusted according to the response function. The best models were selected for TFC, DPPH, and FRAP through ANOVA at 95% confidence intervals (Table 2). According to Rasera *et al*. (2019), the performance of the model can be determined by calculating the coefficient of R^2; i.e., the mathematical equation is usually considered reasonable if R^2 exceeds 70% because this coefficient indicates the suitability of the regression model, or the value of R^2 which is getting closer to 1.00 indicating a suitable model and when close to 0, it is an unsuitable model. In this study, the R^2 coefficients on the TFC, DPPH, and FRAP responses were 0.9999, 0.9128, and 0.7568, respectively. So it can be said that the results obtained can explain 99.91%, and 75% of the variability of the experimental data, respectively. In addition, when the *p*-value <0.05, the model is significant and can be applied to describe the relationship between the analyzed variables. The results of this experiment show that the TFC response has a significant model in the special cubic model (p = 0.0260), and DPPH with a quadratic model shows a significant model (p = 0.0334). In contrast, FRAP with a quadratic model provides an insignificant model (p = 0.2835).

Effect of the solvent system on the response variable

TFC

Different solvent systems have been used to extract polyphenolic compounds from plant materials. Among various phytochemicals, flavonoid compounds are one of the most abundant phytochemicals in plants with diverse biological activities, such as antioxidants (Roy *et al*., 2022). Several previous studies reported that water with a mixture of organic solvents such as ethanol, methanol, and acetone is a common solvent used in extracting bioactive compounds in various plant parts, especially leaves (Sun and Ho, 2005). Organic solvents can extract raw materials well without a mixture or combination of two or more types of solvents.

In determining the solvent system for TFC, a particular cubic model was chosen to describe the TFC obtained from *G. pictum* leaves; this model showed the effect of the interaction between water, acetone, methanol, and ethanol solvents. An actual equation is used to predict the response at a given level of each factor. This equation can define an increase in the value or synergistic effect of the activity of each solvent system used. Equation (2) is an equation that shows the interaction of the TFC response in this study.

$$TFC = 10.464A + 14.125B + 9.047C + 14.715D - 26.560AB - 22.213AC - 35.048AD - 6.253BC - 19.730BD - 24.619CD + 22.912ABC + 42.807ABD + 64.423ACD + 80.816BCD \quad (2)$$

where, A = water, B = acetone, C = methanol, D = ethanol.

The optimization point is seen based on the color contour; yellow to red indicates the higher response point, while blue indicates the lowest point. In the research results, Figure 1 shows the contour plots from the simplex centroid design optimization analysis results. The optimum condition for obtaining TFC was at the point of acetone (100% proportion) with a TFC concentration of 14.7137 mg QE/g DW. These results indicate that acetone can extract flavonoids optimally. Compared with water, ethanol, and methanol, acetone is a solvent with lower polarity, so the flavonoid content of the sample extract is dominated by flavonoid group compounds that are polar and tend to approach being nonpolar because flavonoids are influenced by their capacity to form

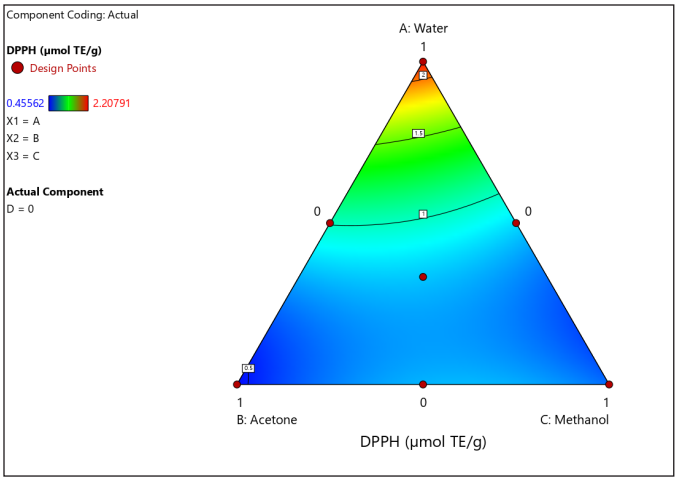

Figure 2. Contour plot for antioxidant DPPH extraction in (A) water, (B) acetone, (C) and methanol (D) using 0% ethanol, as predicted by the quadratic model.

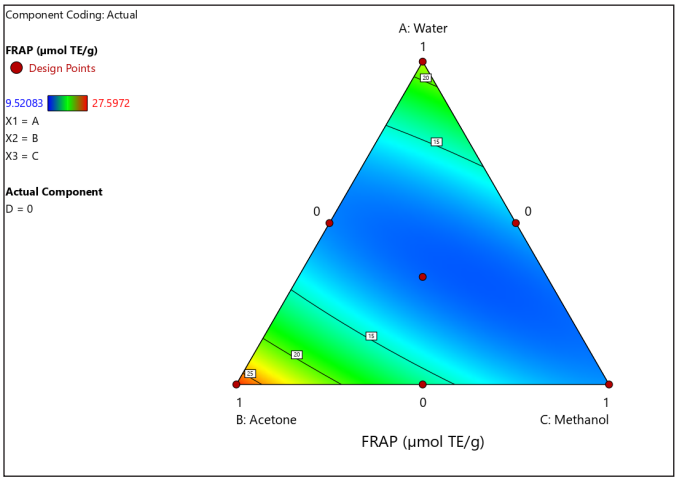

Figure 3. Contour plot for antioxidant FRAP extraction in (A) water, (B) acetone, (C) and methanol (D) using 0% ethanol, as predicted by the quadratic model.

Table 2. Evaluation of the selection of the optimization model on the response variables.

	TFC	DPPH	FRAP
	Special cubic	Quadratic	Quadratic
F	905.32	5.82	1.73
P	0.0260	0.0334	0.2835
R^2	0.9999	0.9128	0.7568
Adjusted R^2	0.9988	0.7559	0.3190
Std. dev.	0.1181	0.2018	4.40
Mean	8.04	0.8571	15.11
CV (%)	1.47	23.54	29.12

hydrogen bonds with the solvent. Previous studies reported fewer polar flavonoids such as flavonols, isoflavones, and flavanones extracted with solvents such as acetone, hexane, ethyl acetate, and chloroform. In addition, the optimal percentage also depends on the matrix characteristics of the extracted compounds and the extraction conditions, such as temperature and time (Grigonis *et al*., 2005). Extraction of *G. pictum* leaves using acetone has not previously been found; so far, previous studies have used solvents such as ethanol and methanol which have the potential to extract compounds in purple leaves, especially the flavonoid group. Therefore, we have got this new finding that the flavonoids extracted from purple leaves can be optimized using acetone as a solvent. The study by Kobus-Cisowka *et al*. (2020) showed that the flavonoid content of *Morus alba* fruit was higher in acetone extract, with the dominant flavonoid being rutin. Turmeric showed the highest flavonoid content using 80% acetone and the lowest using water extraction (Sepahpur *et al*., 2018).

Antioxidant activity

DPPH radical scavenging activity

High antioxidant capacity depends on the high levels of polyphenolic compounds in the extract; this is because polyphenol compounds, especially flavonoids, are believed to inhibit oxidative reactions in the body (Zhou and Yu, 2004). One of the antioxidant mechanisms is the inhibition of DPPH free radicals. This method reduces DPPH radicals by accepting electron radicals from antioxidants to form reduced DPPH. A spectrophotometer observes this change with a color change from purple to yellow at a wavelength of 515 nm (Moon and Shibamoto, 2009). The effect of the solvent system on the antioxidant capacity of the DPPH method provides a significant model ($p < 0.05$) (Table 2) with the contour plot presented in Figure 2, which shows that the extracts obtained the highest activity with water solvent in the proportion of 100%, which was 2.20791 µmol TE/g DW.

$$\text{DPPH: } 2.179A + 0.470B + 0.642C + 0.804D - 1.260AB - 2.279AC - 2.937AD + 0.844BC - 0.109BD \quad (3)$$

where, A = water, B = acetone, C = methanol, D = ethanol.

Equation (3) shows the factors coded to predict the response at a given level of each factor. In antioxidant activity, the highest coefficient value indicates better antioxidant activity using water (+2.179), followed by ethanol (+0.804), methanol (+0.642), and acetone (+0.470). In addition, if acetone is mixed with methanol, a synergistic effect occurs (+0.884). The most antagonistic effect was exerted by a mixture of water and ethanol (−2.937). These results are confirmed in Figure 3 that water with the highest activity of 2.20791 µmol TE/g DW is the effective solvent that can increase antioxidant activity. Based on previous research, *G. pictum* is often extracted using ethanol (Rustini and Ariati, 2018) because there have been no studies examining the effect of increasing antioxidant activity of various components of pure or mixed solvents. Polar water has a good working capacity as a solvent for extracting *G. pictum* leaves through its antioxidant activity; this could be due to the content of hydrophilic polyphenolic compounds in the extract (Wang *et al*., 2017). A previous study using the simplex centroid design in determining the extraction method reported that Mejhoul dates fruit has high antioxidant activity with a mixture of water-acetone and water-methanol (Jdaini *et al*., 2022). *Capsicum annum* L. showed high antioxidant activity with an aqueous extract (Salamatullah *et al*., 2022). *Justicia gendarussa* leaves have the most optimal DPPH antioxidant activity by extraction using a binary ethanol-hexane mixture (Marliani *et al*., 2022). Different soluble polyphenolic compounds can explain variations in DPPH radical scavenging activity due to other solvents' polarities.

FRAP

The antioxidant mechanism of the FRAP method is basically through the ability of antioxidant compounds to reduce Fe^{3+} ions to Fe^{2+}, a colorless change to blue as measured using a spectrophotometer at a wavelength of 593 nm. The strength of an antioxidant compound is analogous to its ability to reduce. The effect of the solvent system on the antioxidant activity of FRAP gave insignificant results ($p > 0.005$) in the selected model (Table 2); this was because the antioxidant activity values of each

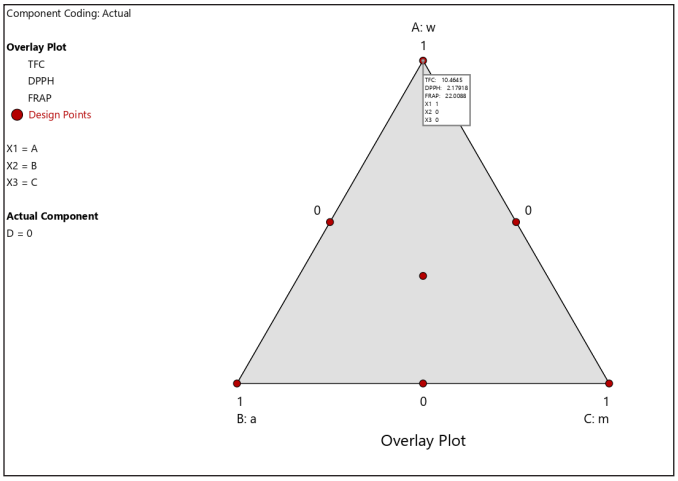

Figure 4. Overlay plot of the optimum point of response variable for TFC and antioxidant activity of DPPH and FRAP in (A) water, (B) acetone, (C) and methanol (D) using 0% ethanol.

Table 3. Optimum condition data and verification of predicted and actual values for optimized TFC parameters and antioxidant activity of radical scavenging of DPPH and FRAP.

	A	B	C	D	TFC	DPPH	FRAP	Desirability
Prediction	1.00	0	0	0	10.464	2.179	22.009	0.745
Verification	1.00	0	0	0	10.573	2.262	22.262	0.745
% RSE					1.042	3.809	1.149	

proportion of solvent components were not significantly different. The optimum point of this response is shown in Figure 3; the most optimum FRAP antioxidant activity was obtained from the acetone solvent with a proportion of 100% with an activity value of 27.5972 µmol TE/g DW.

$$\text{FRAP: } 22.008A + 26.863B + 12.182C + 16.438D - 50.308AB - 17.328AC - 29.599AD - 13.428AC - 5.454AD - 3.363CD \quad (4)$$

where, A = water, B = acetone, C = methanol, D = ethanol.

The highest coefficient value from the actual equation showed better antioxidant activity using acetone (+26.863), followed by water (+22.008), ethanol (+16.438), and methanol (+12.182). An antagonistic effect is produced when we have the binary water-acetone mixture (−50.308) or other binary mixtures. Due to its lower dielectric constant, acetone is a solvent with lower polarity than other solvents in this study. Solvents with large dielectric constants will be more polar; components of a compound from plants easily dissolve in solvents that are relatively the same in the level of polarity (Handayani, 2021). So far, the use of acetone in the extraction of *G. pictum* is still low; the optimization results using the simplex centroid design provide new findings. Previous studies are in line with the results of this study that *Tamarindus indica* acetone extract and *Detarium microcarpum* significantly provided the highest FRAP antioxidant value compared to the methanol extract (Lamien-Meda *et al*., 2008).

Optimum conditions of extraction parameters

Optimum conditions are selected based on the highest desirability value. The desirability value is a function that can have a level of confidence in finding the optimum point based on the analysis parameters used. The closer the desirability value is to 1, the higher the accuracy of the optimum condition is. The optimum solvent proportion for the extraction of *G. pictum* leaves is presented in Figure 4; the highest desirability value of 0.745 implies an accuracy of 74.5% from the results of the criteria used in 1.00 water (40 ml), with the results of the TFC of 10.464 mg QE/g DW, antioxidant activity DPPH 2.179 µmol TE/g DW and FRAP 22.009 µmol TE/g DW. After that, it was verified by extracting the leaves of *G. pictum* from the selected proportions (Table 3). The verification data were analyzed based on the RSE (%) as an evaluation of the accuracy level of the model from the resulting method by comparing the predicted value and the actual value obtained. According to Sulaiman *et al*. (2017), an adequate model is indicated by the percentage of RSE < 5%, which means that there is no significant difference between the actual value and the predicted value, so the model is reliable. Based on the calculation results, we obtained TFC, DPPH, and FRAP with, respectively, 1.042%, 3.809%, and 1.149%, which indicates that the model specified is very effective.

CONCLUSIONS

This study shows that the simplex centroid design is an effective tool to optimize the extraction conditions of *G. pictum* (L.) Griff leaves. The criteria of the selected model were statistically verified by ANOVA, showing a significant model, except for FRAP. The R^2 values for TFC, DPPH, and FRAP are 0.9999, 0.9128, and 0.7568, respectively, indicating that the developed model is very accurate. The optimum conditions for extracting *G. pictum* (L.) Griff leaves were 1.00 (40 ml) water with a desirability level of 0.745, resulting in TFC of 10.464 mg QE/g DW, the antioxidant activity of DPPH 2.179 µmol TE/g DW, and FRAP 22.009 µmol TE/g DW. This study is helpful in developing cosmetic and medicinal products containing *G. pictum* leaves.

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AUTHOR CONTRIBUTIONS

Concept and design, and drafting the article were carried out by Waras Nurcholis, Feda Anisah Makkiyah, Eldiza Puji Rahmi, and Tiwuk Susantiningsih. Acquisition of data, interpretation of data, statistical analysis, critical revision, supervision, and final approval were carried out by Waras Nurcholis, Nelly Marliani, and Rini Anggi Arista.

CONFLICTS OF INTEREST

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

ETHICAL APPROVALS

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

DATA AVAILABILITY

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

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REFERENCES

Abenavoli L, Larussa T, Corea A, Procopio AC, Boccuto L, Dallio M, Federico A, Luzza F. Dietary polyphenols and non-alcoholic fatty liver disease. Nutrients, 2021; 13:494; doi:10.3390/nu13020494.

Alara OR, Abdurahman NH, Ukaegbu CI, Azhari NH. *Vernonia cinerea* leaves as the source of phenolic compounds, antioxidants, and anti-diabetic activity using microwave-assisted extraction technique. Ind Crops Prod, 2018; 122:533–44; doi:10.1016/j.indcrop.2018.06.034.

Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: the FRAP assay. Anal Biochem, 1996; 239(1):70–6; doi:10.1006/abio.1996.0292.

Blois MS. Antioxidant determinations by the use of a stable free radical. Nature, 1958; 181:1199–200; doi:10.1038/1811199a0

Carey CC, Lucey A, Doyle L. Flavonoid containing polyphenol consumption and recovery from exercise-induced muscle damage: a systematic review and meta-analysis. Sport Med, 2021; 51:1293–316; doi:10.1007/s40279-021-01440-x.

Dos Santos C, Mizobucchi AL, Escaramboni B, Lopes BP, Angolini CFF, Eberlin MN, De Toledo KA, Núñez EG. Optimization of *Eugenia punicifolia* (Kunth) D. C. leaf extraction using a simplex centroid design focused on extracting phenolics with antioxidant and antiproliferative activities. BMC Chem, 2020; 14:34; doi:10.1186/s13065-020-00686-2.

Fadil M, Lebrazi S, Aboulghazi A, Guaouguaou F-E, Rais C, Slimani C, Es-safi NE. Multi-response optimization of extraction yield, total phenols-flavonoids contents, and antioxidant activity of extracts from moroccan *Lavandula stoechas* leaves: predictive modeling using simplex-centroid design. Biocatal Agric Biotechnol, 2022; 43:102430; doi:10.1016/j.bcab.2022.102430.

Gasmi A, Mujawdiya PK, Noor S, Lysiuk R, Darmohray R, Piscopo S, Lenchyk L, Antonyak H, Dehtiarova K, Shanaida M, Polishchuk A. Polyphenols in metabolic diseases. Molecules, 2022; 27:6280; doi:10.3390/molecules27196280.

Grigonis D, Venskutonis PR, Sivik B, Sand ahl M, Eskilsson CS. Comparison of different extraction techniques for isolation of antioxidants from sweet grass (*Hierochloë Odorata*). J Supercrit Fluids, 2005; 33:223–33; doi:10.1016/j.supflu.2004.08.006.

Handayani S. Aktivitas antioksidan ekstrak hipokotil *Bruguiera gymnorhiza* pada pelarut dan fase kematangan yang berbeda. AGROINTEK, 2021; 15(3):685–94; doi:10.21107/agrointek.v15i3.8477.

Ibrahim ND, Seow LJ, Sekar M, Izzati Mat Rani NN, Lum PT. Ten commonly available medicinal plants in Malaysia with potential sun protection factor and antioxidant properties—a review. Pharmacogn J, 2022; 14:444–55; doi:10.5530/pj.2022.14.57.

Icyer NC, Toker OS, Karasu S, Tornuk F, Bozkurt F, Arici M, Sagdic O. Combined design as a useful statistical approach to extract maximum amount of phenolic compounds from virgin olive oil waste. LWT, 2016; 70:24–32; doi:10.1016/j.lwt.2016.02.029.

Jdaini K, Alla F, Mensouri F, Parmar A, Elhoumaizi MA. Optimizing the extraction of phenolic antioxindats from date palm 2 fruit by simplex centroid solvent mixture design. Heliyon, 2022; doi: 10.2139/ssrn.4065295

Jiangseubchatveera N, Liawruangrath B, Liawruangrath S, Teerawutgulrag A, Santiarworn D, Korth J, Pyne SG. The chemical constituents and the cytotoxicity, antioxidant and antibacterial activities of the essential oil of *Graptophyllum pictum* (L.) Griff. J Essent Oil Bear Plants, 2015; 18:11–7; doi:10.1080/0972060X.2014.935036.

Jiangseubchatveera N, Liawruangrath S, Teerawutgulrag A, Santiarworn D, Pyne SG, Liawruangrath B. Phytochemical screening, phenolic and flavonoid contents, antioxidant and cytotoxic activities of *Graptophyllum pictum* (L.) Griff. Chiang Mai J Sci, 2017; 44:193–202.

Khalafyan AA, Temerdashev ZA, Yakuba YF, Guguchkina TI. Computer analysis of the sensory qualities of red wines as a method to optimize their blend formulation. Heliyon, 2019; 5:e01602; doi:10.1016/j.heliyon.2019.e01602.

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:10.1016/j.jep.2021.114765.

Kobus-Cisowska J, Szczepaniak O, Szymanowska-Powalowska D, Piechocka J, Szule P, Dziedzinski M. Antioxidant potential of various solvent extract from *Morus alba* fruits and its major polyphenols composition. Cienc Rural, 2020; 50(1); doi:10.1590/0103-8478cr20190371.

Lamien-Meda A, Lamien CE, Compaoré MMY, Meda RNT, Kiendrebeogo M, Zeba B, Millogo JF, Nacoulma OG. Polyphenol content and antioxidant activity of fourteen wild edible fruits from Burkina Faso. Molecules, 2008; 13(3):581–94; doi:10.3390/molecules13030581.

Liu S, Lin F, Wang J, Pan X, Sun L, Wu W. Polyphenols for the treatment of ischemic stroke: new applications and insights. Molecules, 2022; 27(13):4181.https://doi.org/10.3390/molecules27134181.

Lonni AASG, Longhini R, Lopes GC, de Mello JCP, Scarminio IS. Statistical mixture design selective extraction of compounds with antioxidant activity and total polyphenol content from *Trichilia catigua.* Anal Chim Acta, 2012; 719:57–60; doi:10.1016/j.aca.2011.12.053.

Makkiyah F, Rahmi EP, Revina R, Susantiningsih T, Setyaningsih Y. *Graptophyllum pictum* (L.) Griff. (Syn: *Justicia picta* Linn.) and its effectiveness: a well-known Indonesian plant. Pharmacogn J, 2021; 13:835–8; doi:10.5530/pj.2021.13.106.

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 Nat Sci, 2022; 21:e2022046; doi:10.12982/CMUJNS.2022.046.

Moon JK, Shibamoto T. Antioxidant assays for plant and food components. J Agric Food Chem, 2009; 57:1655–66; doi:10.1021/jf803537k.

Naczk M, Shahidi F. Extraction and analysis of phenolics in food. J Chromatogr A, 2004; 1054:95–111; doi:10.1016/j.chroma.2004.08.059.

Nurcholis W, Ma'rifah K, Artika MI, Aisyah SI, Priosoeryanto BP. Optimization of total flavonoid content from cardamom fruits using a simplex-centroid design, along with the evaluation of the antioxidant properties. Trop J Nat Prod Res, 2021; 5:1382–8; doi:10.26538/tjnpr/v5i8.10.

Nurcholis W, Artika I M, Seno DSH, Andrianto D, Aprianti A, Febrianti F, Inawati, Ratu AP, Arendra A. Phytochemical analysis, alfa-glucosidase inhibition activity *in vitro* and enzyme kinetics of ethyl acetate and hexane estracts of *Graptophylum pictum* (L.) Griff. Curr Biochemy, 2014; 1(2):58–65; doi:10.29244/cb.1.2.58-65.

Park J, Park R, Jang M, Park YI. Therapeutic potential of EGCG, a green tea polyphenol, for treatment of coronavirus diseases. Life, 2021; 11:197; doi:10.3390/life11030197.

Poh-Yen K, Lay-Jing S, Hanani F. *In vitro* evaluation of photoprotective potential of the different solvent extracts of *Graptophyllum pictum* leaves. J Appl Pharm Sci, 2018; 8:147–51; doi:10.7324/JAPS.2018.8122.

Qi Y, Li J, Nie Q, Gao M, Yang Q, Li Z, Li Q, Han S, Ding J, Li Y, Zhang J. Polyphenol-assisted facile assembly of bioactive nanoparticles for targeted therapy of heart diseases. Biomaterials, 2021; 275:120952; doi:10.1016/j.biomaterials.2021.120952.

Rasera GB, Hilkner MH, de Alencar SM, de Castro RJS. Biologically active compounds from white and black mustard grains: an optimization study for recovery and identification of phenolic antioxidants. Ind Crops Prod, 2019; 135:294–300; doi:10.1016/j.indcrop.2019.04.059.

Rustini NL, Ariati NK. Identification of active antioxidant compounds from Unge leaf ethanol extact (*Graptophyllum pictum* L. Griff). J Health Sci Medicine, 2018; 2(1):120952.

Roy A, Khan A, Ahmad I, Alghamdi S, Rajab BS, Babalghith AO, Alshahrani MY, Islam S, Islam M. Flavonoids a bioactive compound from medicinal plants and its therapeutic applications. Biomed Res Int, 2022; 2022:5445291; doi:10.1155/2022/5445291.

Salamatullah AM, Hayat K, Husain FM, Ahmed MA, Arzoo S, Althbiti MM, Alzahrani A, Al-Zaied BAM, Alyahya HK, Albade N, Nafiidi HA, Bourhia M. Effects of different solvents extractions on total polyphenol content, HPLC analysis, antioxidant capacity, and antimicrobial properties of pepers (red, yellow, and green (*Capsicum annum* L,). Evid Based Complement Alternat Med, 2022; 7372101:11; doi:10.1155/2022/7372101.

Sepahpur S, Selamat J, Manap MYA, Khatib A, Razis AFA. Comparative analysis of chemical composition antioxidant activity and quantitative characterization of some phenolis compounds in selected herbs and spices in different solvent extraction systems. Molecules, 2018; 23(402); doi:10.3390/molecules23020402.

Sharifi-Rad M, Anil Kumar N V, Zucca P, Varoni EM, Dini L, Panzarini E, Rajkovic J, Tsouh Fokou PV, Azzini E, Peluso I, Prakash Mishra A. Lifestyle, oxidative stress, and antioxidants: Back and forth in the pathophysiology of chronic diseases. Front Physiol, 2020; 11:694; doi:10.3389/fphys.2020.00694.

Srinivasan KK, Mathew JE, D'Silva KJA, Lobo R, Kumar N. Nephroprotective potential of *Graptophyllum pictum* against renal injury induced by gentamicin. Iran J Basic Med Sci, 2015; 18(4):412; doi:10.22038/IJBMS.2015.4292.

Sun T, Ho CT. Antioxidant activities of buckwheat extracts. Food Chem, 2005; 90(4):743–9; doi:10.1016/j.foodchem.2004.04.035.

Sulaiman ISC, Mahiran B, Hamid RM, Wei JC, Siti EA, Maznah I. Effects of temperature, time, and solvent-ratio on the extraction of phenolic compounds and the anti-radical activity of *Clinachantus nutans* Lindau leaves by response surface methodology. Chem Central J, 2017; 11(54); doi:10.1186/s13065-017-0285-1.

Pharmacopeia. State Pharmacopeia of USSR, Moscow. Medicina, 1989; 2:324–34.

Wang YY, Peng C, Zhang Y, Wang ZR, Chen YM, Dong J-F, Xiao ML, Li DL, Li W, Zou QJ, Zhang K. Optimization, identification and bioactivity of flavonoids extracted from Moringa oleifera leaves by deep eutectic solvent. Food Biosci, 2022; 47:101687; doi:10.1016/j.fbio.2022.101687.

Wang X, Pengfei L, Wang F, Fu B, He F, Zhao M, 2017. Influence of altitudinal and latitudinal variation on the composition and antioxidant activity of polyphenols in *Nicotiana tabacum L*. Leaf. Emirates Journal of Food and Agriculture, 29(5): 359-366. doi: 10.9755/ejfa.2016-09-1213.

Wibisono K, Aisyah SI, Suhesti S, Nurcholis W. Optimization of total flavonoids extraction and A-glucosidase inhibitory activity from *Plectranthus amboinicus* (Lour.) spreng. leaves using the simplex-centroid design. Molekul, 2019; 14; doi:10.20884/1.jm.2019.14.2.497.

Zhou K, Yu L. Effects of extraction solvent on wheat bran antioxidant activity estimation. LWT Food Sci Technol, 2004; 37(7):717–21. https://doi.org/10.1016/j.lwt.2004.02.008.

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