Journal of Experimental Biology and Agricultural Sciences

http://www.jebas.org

ISSN No. 2320 – 8694

Extraction of Flavonoids from Parasitic plant *Macrosolen cochinchinensis* using Ultrasound-Assisted Extraction: An Optimization Approach

Yayu Mulsiani Evary*, Gemini Alam, Muhammad Raihan, Khusnul Khotimah

Département of Pharmacy Science and Technology, Pharmacy Faculty, Hasanuddin University, Makassar Indonesia, 90245.

Received – August 17, 2024; Revision – September 18, 2024; Accepted – September 23, 2024
Available Online – September 25, 2024

DOI: http://dx.doi.org/10.18006/2024.12(4).616.624

KEYWORDS

Macrosolen cochinchinensis

Optimization

Extraction

Flavonoids

ABSTRACT

The parasitic plant *Macrosolen cochinchinensis* (Lour.) VAN Tiegh, commonly found parasitizing mango trees, contains flavonoid compounds with potential anticancer properties. This study aims to optimize the extraction of flavonoids from *M. cochinchinensis* using the Ultrasonic Assisted Extraction (UAE) method. Three extraction parameters were investigated to determine the best conditions for maximizing extract yield and flavonoid concentration. The parameters considered for the UAE technique were different ethanol concentrations (30%, 70%, and 96%), extraction times (15, 30, and 45 minutes), and solvent-to-sample ratios (1:10, 1:20, and 1:30). The study used Response Surface Methodology (RSM) to identify the optimal extraction conditions. The analysis using RSM indicated that the highest extraction yield (10%) was achieved with a sample-to-solvent ratio of 1:30, 30% ethanol concentration, and an extraction time of 45 minutes. The highest flavonoid content (457.96 mg QE/g extract) was obtained with a solid-to-liquid ratio between 1:20 and 1:30, using 65 to 80% ethanol solvent and an extraction time of 45 minutes. These results suggest that these parameters extract flavonoid compounds from *M. cochinchinensis* leaves.

* Corresponding author

E-mail: yayuevary7@gmail.com (Yayu Mulsiani Evary)

Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

Production and Hosting by Horizon Publisher India [HPI] (http://www.horizonpublisherindia.in/).

1 Introduction

Mistletoe (*Macrosolen cochinchinensis*) is a hemiparasitic plant from the Loranthaceae Family known for its various bioactivities, including antioxidant, antidiabetic, anticancer, and antihyperglycemic properties (Mutiah et al. 2018; Shin and Lee 2018; Firmata Sari 2020; Kumal et al. 2021). This plant commonly parasitizes trees such as jackfruit (*Artocarpus heterophyllus*), sapodilla (*Manilkara zapota*), and mango (*Mangifera indica*) (Nigam 2022). Previous research focused on four fractions of *M. cochinchinensis* ethyl acetate extracted and isolated from the jackfruit host plant for their anticancer properties. The study revealed that the ethyl acetate extract of *M. cochinchinensis* possesses anticancer activity against the T47D breast cancer cell line, with an IC_{50} of 314.8 µg/mL (Indradmojo 2016). Although a relatively high concentration is required for the anticancer effect, the study suggested that the mechanism involves inhibiting cell cycle phases. The *M. cochinchinensis* extract also induced early apoptosis and disrupted the proliferation of cancer cells (Indradmojo 2016). Further research showed that a combination of *Eleutherine palmifolia* (L.) Merr and *M. cochinchinensis* (Lour.) inhibited cell cycle progression and increased HeLa cell apoptosis (Mutiah et al. 2018). Another study compared the total phenolic and flavonoid compounds in several *M. cochinchinensis* species and reported that this extract contains a total flavonoid content of 24.9 ± 2.3 mg QE/g and a phenolic content exceeding 30 mg GAE/g in the methanolic extract. Additionally, they found that *M. cochinchinensis* exhibits significant DPPH radical inhibition with an IC50 of 65.9 ± 2.8 µg/mL (Kumal et al. 2021).

The interactions between plant parasites and different host species can directly affect the performance of both the host and the parasite. *M. cochinchinensis* contains phytochemical compounds such as terpenoids and flavonoids. Specifically, the terpenoids found in *M. cochinchinensis* are identified as thymol, while the flavonoids are identified as rutin, quercetin, and quercitrin (Santosa et al. 2022).

Flavonoids are a group of secondary metabolites characterized by a three-ring structure responsible for many herbal medicines' colour and therapeutic effects (Iwashina 2000; Samanta et al. 2011). The extraction of flavonoids is challenging due to their wide range of medicinal properties. Several factors, such as the duration of extraction, type and pH of solvents, particle size of the materials, and the selection of extraction methods, may significantly affect the level of obtained flavonoids. Flavonoids possess physicochemical properties that range from polar to nonpolar; therefore, various types of solvents (e.g., water, acetone, methanol, ethanol, or their water mixtures) have been reported for their extraction (Sharma and Janmeda 2017; Chaves et al. 2020). One advanced extraction technique used to extract flavonoids is Ultrasound-assisted extraction. This technique allows researchers to extract flavonoids quickly and with relatively few solvents. Furthermore, it is known to produce higher yields by the capability of ultrasound to induce the breaking of cell walls, enabling the movement of phytochemical compounds from the cell to the solvents. The sound vibration and bubbling during extraction prevent the early saturation of soluble compounds in the solvent, allowing for more compounds to be extracted (Pico 2013; Chemat et al. 2017; Medina-Torres et al. 2017).

Several studies have investigated the best ways to extract flavonoids from plants. For example, Azahar et al. (2017) studied the extraction of flavonoids from *Curcuma zedoaria* using the

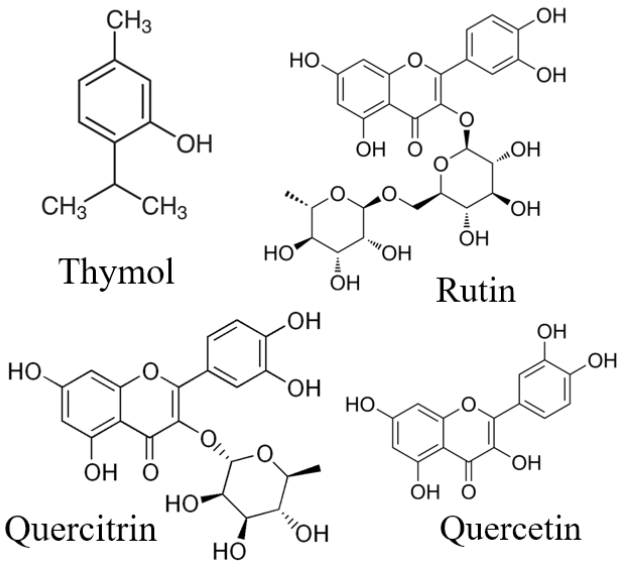

Figure 1 Some flavonoid derivatives reported to be found in *M. cochinchinensis* (Santosa et al. 2022)

reflux method to find the best time and temperature for the process. The results showed that the best conditions for flavonoid extraction were using 90% ethanol at a temperature of 75°C for 92 minutes (Azahar et al. 2017). Similarly, another study found that the best way to extract phenolic, flavonoid and antioxidant compounds from *Clitoria ternatea* was through maceration at 45°C, combined with agitation using 37% ethanol for 90 minutes (Jaafar et al. 2020). These studies demonstrate that the best extraction conditions depend on the plant species, likely due to plant flavonoid composition differences. Therefore, studying the ideal extraction process to maximize flavonoid yield from different plants is important. The present research aims to investigate the best extraction conditions for flavonoids from *M. cochinchinensis* (MC) using a response surface methodology to analyze three parameters: ethanol concentration, extraction time, and sample-to-solvent ratio in the solid state.

2 Materials and Methods

2.1 Plant material

The plant material was gathered from the mango tree, which was the host. The leaves of *M. cochinchinensis* (MC) were dried in an oven for three days, and the resulting dried material was then stored in a tightly sealed container.

2.2 Experimental Design

Respond Surface Methodology (RSM) using Design Expert 7.1.5 and a three-level factorial Box Behnken Design (BBD) was used to find the best extraction conditions for MC. Three independent variables (ethanol concentration, extraction time, and sample-to-solvent ratios) were tested to determine the total flavonoid content (TFC) in *M. cochinchinensis* plant extract. Graphical and numerical optimization techniques were applied to identify the ideal extraction condition. The complete design, with a rotatable alpha, included 20 experimental runs. Six replicate runs were performed at the centre points of the design to estimate the pure error. All experiments were randomly conducted to minimize the influence of unexplained variability in the observed responses due to systematic errors.

2.3 Extraction

The mistletoe leaves, weighing 10 grams, were ground and then extracted with 100 mL of ethanol using the ultrasound-assisted extraction method. Three different concentrations of ethanol (30%, 70%, and 96%) in water were used for the extraction, with three different extraction times (15, 30, and 45 minutes) and three different solid-to-liquid ratios (1:10, 1:20, and 1:30). The extracts were filtered through Whatman no. 40 filter paper. The filtrates were concentrated using a rotary evaporator until a thick extract was obtained (Pan et al. 2012).

2.4 Spectrophotometric determination of total flavonoids

The extracts' total flavonoid content (TFC) was determined using a modified spectrophotometric method with aluminum chloride reagent. The technique was calibrated against quercetin, which served as the reference standard. The TFC was quantified based on a calibration curve prepared by diluting a quercetin stock standard with ethanol p.a to obtain concentrations of 2, 4, 6, 8, and 10 µg/mL. The results were calculated using the calibration curves for quercetin, and the total flavonoids were expressed as milligrams of quercetin equivalents (QE) per 100 grams of extract based on duplicate analysis. The values were reported as means (N=3) ± standard deviations (S.D.).

2.5 Statistical analysis

All analyses were conducted twice, and the results were presented as means ± standard deviation. This study examined the significance of the quadratic model and the interactions between the independent variables using analysis of variance (ANOVA) in the RSM, with a significance level set at $p<0.05$. The experimental R2 assessed in the laboratory was compared to the predicted models.

3 Results and Discussion

3.1 Influence of Different Extraction condition on total yield

Three independent variables, sample-to-solvent ratio, extraction time, and ethanol concentration, were assessed and optimized using the Box-Behnken Design (BBD) to determine the best extraction conditions for maximizing yield and flavonoid content (Table 1). The yield of MC extracts ranged from 1.45% to 8.50%, while the flavonoid content varied from 161.18 mg/g to 430.79 mg/g QE. This study investigated the impact of three independent variables on the total yield and flavonoid content extracted from MC leaves: ethanol concentration, extraction time, and sample-to-solvent ratio. Maximizing extraction yield is essential to meet the standards of Indonesian traditional medicine. Furthermore, optimizing these factors is important for increasing flavonoid content, as different types of flavonoids offer various pharmacological benefits, including cancer prevention, diabetes management, and potent antioxidant properties (Mohan and Nandha Kumar 2014; Batra and Sharma 2013; Abotaleb et al. 2018; Manavi et al. 2021). The models assessed in this study were created using the Box-Behnken Design in MINITAB 18. This software generated 15 models to analyze the effects of ethanol concentration, extraction time, and sample-to-solvent ratio as independent variables. The results of these models are presented in Table 1.

The results in Table 1 indicate that the highest yield achieved was 8.50%. This was obtained under the following conditions: simplicial-

Table 1 The mass fraction of TFC extracted from MC using an Ultrasound-assisted extraction method under different conditions

No.	Sample code	X_1	X_2	X_3	Extract weight (g)	Average of Yield (%)	Average of Flavonoid content (mg/g)
1	A1	-1	1	0	0.66	6.75	341.26±1.45
	A2	-1	1	0	0.69		
2	B1	1	0	1	0.27	2.75	427.37 ± 8.21
	B2	1	0	1	0.28		
3	C1	0	0	-1	0.26	2.35	429.77 ± 11.59
	C2	0	0	-1	0.21		
4	D1	0	-1	-1	0.20	1.90	327.59 ± 23.68
	D2	0	-1	-1	0.18		
5	E1	0	0	-1	0.22	2.15	422.93 ± 8.70
	E2	0	0	-1	0.21		
6	F1	1	1	0	0.44	4.25	333.74 ± 0.48
	F2	1	1	-1	0.41		
7	G1	-1	0	-1	0.61	6.45	293.76 ± 33.83
	G2	-1	0	-1	0.68		
8	H1	0	1	-1	0.34	3.50	402.43 ± 7.73
	H2	0	1	-1	0.36		
9	I1	1	0	-1	0.36	3.35	396.62 ± 0.48
	I2	1	0	-1	0.31		
10	J1	0	-1	1	0.19	1.65	429.08 ± 26.09
	J2	0	-1	1	0.14		
11	K1	0	0	0	0.28	2.55	430.79 ± 10.15
	K2	0	0	0	0.23		
12	L1	0	-1	0	0.16	1.75	161.18 ± 15.46
	L2	0	-1	0	0.19		
13	M1	0	0	1	0.82	8.50	252.07 ± 12.56
	M2	0	0	1	0.88		
14	N1	0	1	1	0.56	5.60	419.86 ± 2.42
	N2	0	1	1	0.56		
15	O1	1	-1	-1	0.18	1.45	316.66 ± 7.25
	O2	1	-1	-1	0.11		

Independent Variables	Code Unit	Coded Variable Level		
		-1	0	1
Ethanol Concentration (%)	X_1	30	70	90
Ratio of simplicial to solvent	X_2	01:10	01:20	01:30
Time (minutes)	X_3	15	30	45

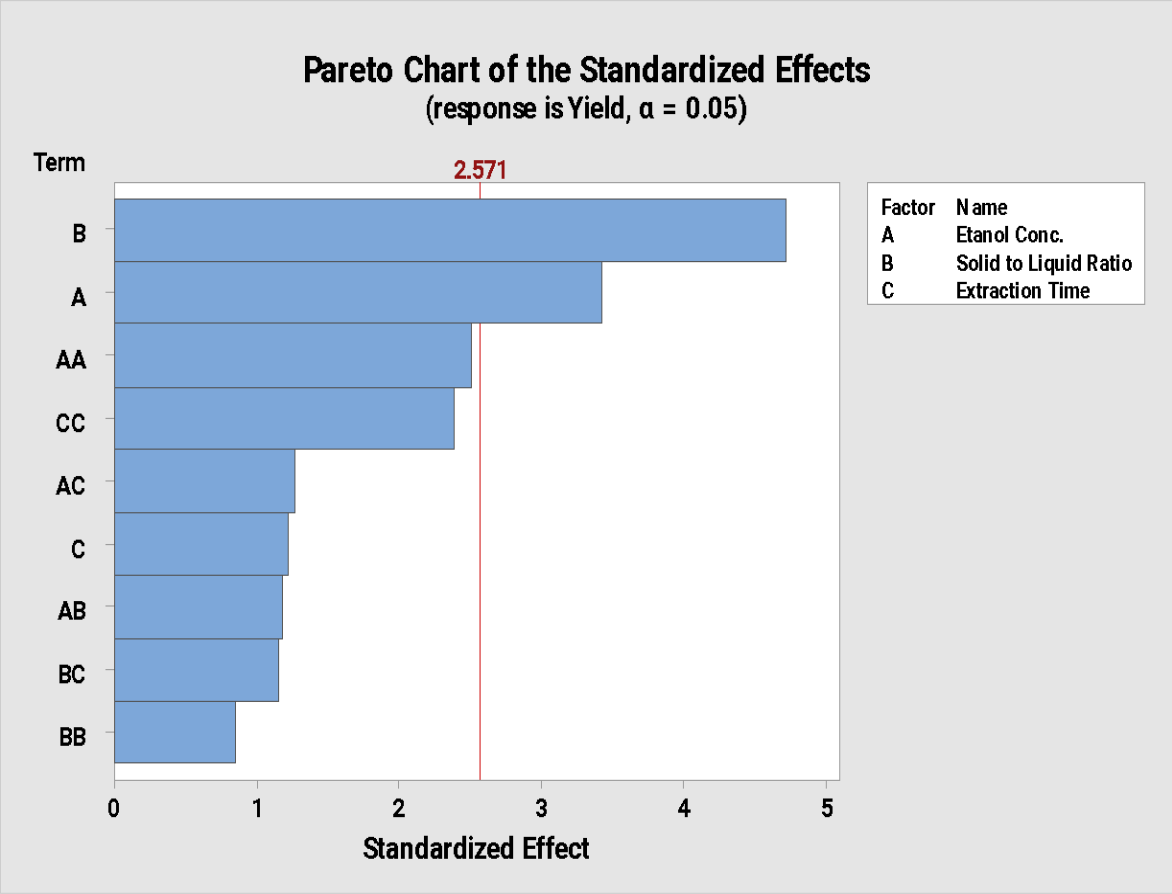

Figure 2 Pareto chart on the effect of ethanol concentration, extraction time, and simplicial: solvents ratio to % yield of MC leaves

solvent ratio of 1:20, 45-minute extraction time, and 30% ethanol concentration as the solvent. This suggests that the extract contains a significant number of hydrophilic compounds that are highly soluble in solvents with a higher water concentration. On the other hand, the lowest yield was 1.45%, obtained from the model with a simplicial-solvent ratio of 1:10, 30-minute extraction time, and 96% ethanol as the solvent. This result shows that a low solid-liquid ratio and a high ethanol concentration produced less extract.

The yield data was analyzed using the MINITAB application to assess the impact of the simplicial-solvent ratio, extraction time, and ethanol concentration on the yield percentage. The resulting Pareto chart is depicted in Figure 2.

Figure 2 indicates that the solid-liquid ratio and ethanol concentration parameters have a standardized effect value of 2.571, suggesting that they significantly influence the yield percentage from MC leaf extraction.

The relationship between the independent variables and the yield was shown in contour plots created by the model (Figure 3). The dark green colour on the plot indicates the interactions between the variables being tested. Figure 3a presents the impact of the solid-liquid ratio and ethanol concentration on the yield of MC extracts. It was observed that an increase in the solid-liquid ratio and the water content of the solvent (30% ethanol) led to a rise in yield, indicated by the dark green region. This result is consistent with Prasad et al. (2012), who found that a higher yield is achieved with a higher solid-to-liquid ratio and water concentration in ethanol. A solid-liquid ratio between 1:20 to 1:30 and 30% ethanol concentration resulted in a 6-7% yield range. Additionally, Figure 3b demonstrates the effect of extraction time and ethanol concentration on MC extract yield. It can be seen that a longer extraction time and higher water content of the solvent (30% ethanol) increased yield. Extraction times exceeding 40 minutes and using 30% ethanol as the solvent led to a 6-7% yield range. This finding aligns with other research that reported that more polar solvents and longer extraction times result in an optimal yield (Nawaz et al. 2018). Furthermore, Figure 3c shows the correlation between extraction time, solid-liquid ratio, and the yield of MC extract. This figure illustrates that increased extraction time and solid-liquid ratio led to a higher extract yield. The results confirm an increased contact period between the sample and solvents leads to more compounds being extracted.

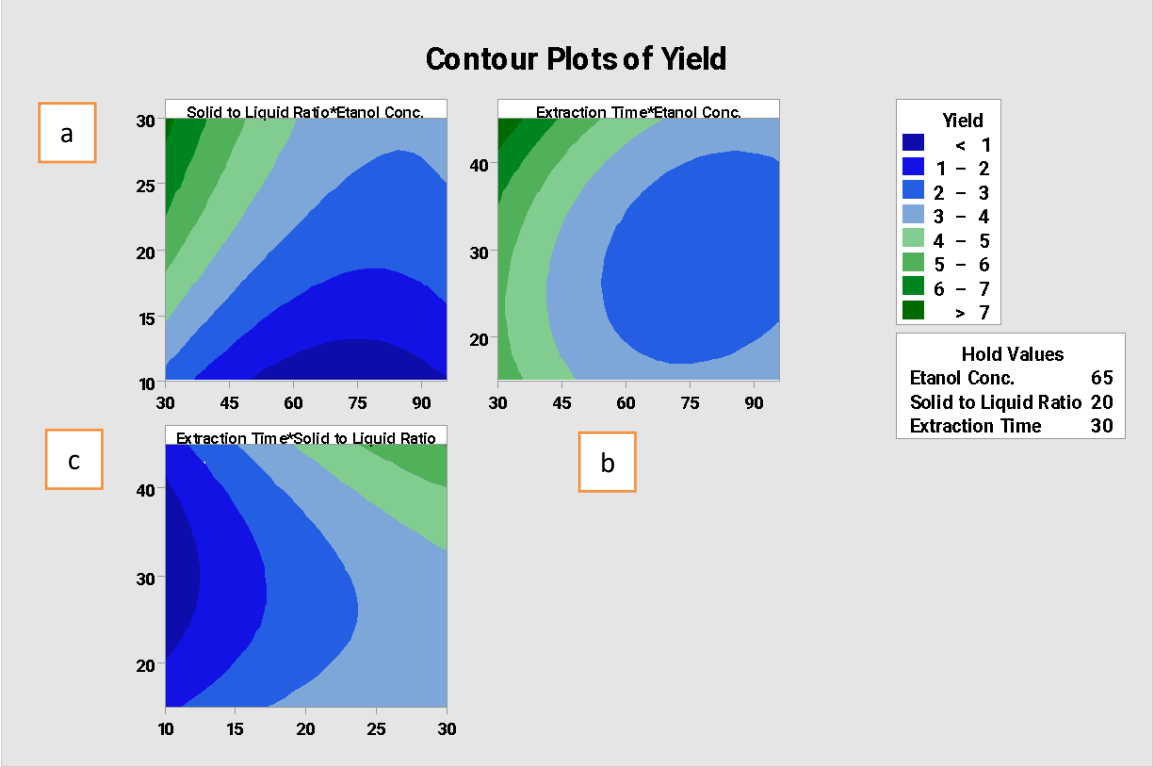

Figure 3 Counterplots of the correlation between the independent variables (including solid-liquid ratio, extraction time, and ethanol concentration) and MC extracts yield

3.2 Influence of different extraction conditions on total flavonoids

The ultrasonic-assisted extraction method successfully extracted a high concentration of flavonoids from MC. However, the flavonoid content varied in each extract due to different extraction conditions, such as ethanol concentration, extraction time, and solvent ratios. To improve extraction efficiency, in this study, different ethanol concentrations (30%, 70%, and 95%), extraction times (15, 30, and 45 minutes), and solvent ratios (1:10, 1:20, and 1:30) have been used. Furthermore, Response Surface Methodology (RSM) was used to evaluate the total flavonoids of MC and fitted all the independent variables into a second-order model equation.

Flavonoid content = -403 + 13.21 X1 + 33.67 X2 – 1.95X3 – 0.0814 X1X1 – 0.437 X2X2 + 0.0470 X3X3 – 0.1311 X1 X2 + 0.0426 X1 X3 – 0.140 X2 X3

Where X1 is the ethanol concentration, X2 is the solid-to-liquid ratio, and X3 is the extraction time.

The model was found to be significant, with a p-value of 0.01. The lack of fit value was 0.011 ($p < 0.05$), indicating significance. The significant models included X1, X2, X1X2, X1X1, and X2X2 with p-values < 0.05.

Furthermore, the concentration data for flavonoids was analyzed using the MINITAB application to assess the impact of solid-to-liquid ratio, extraction time, and ethanol concentration on flavonoid content. The Pareto chart obtained is depicted in Figure 4.

In Figure 4, it is evident that the interactions between ethanol concentration and solid-liquid ratio, and ethanol concentration and extraction time, as well as solid-liquid ratio and extraction time, substantially affect the flavonoid content extracted from MC. The reported value of 2.571 confirms the significant impact of these parameter interactions. However, it was observed that the extraction time does not significantly impact the flavonoid content.

The study investigated the relationship between the independent variables and the flavonoid content of MC extract using surface and contour plots generated by the model (Figure 5). The contour plot shapes (ellipse or round) indicated the significance of interactions between the variables being tested. Figure 5a illustrates the effect of solid-to-liquid ratio and ethanol concentration on the flavonoid content of MC extracts. The round shape in green indicates that solid-to-liquid ratios between 1:20 to 1:30 and ethanol concentrations between 50% to 90% resulted in a high flavonoid content (400 - 450 mg/g QE). This finding is consistent with Prasad et al. (2012), who reported that a 68% ethanol concentration and a liquid-to-solid ratio of 20.2 mL/g resulted in

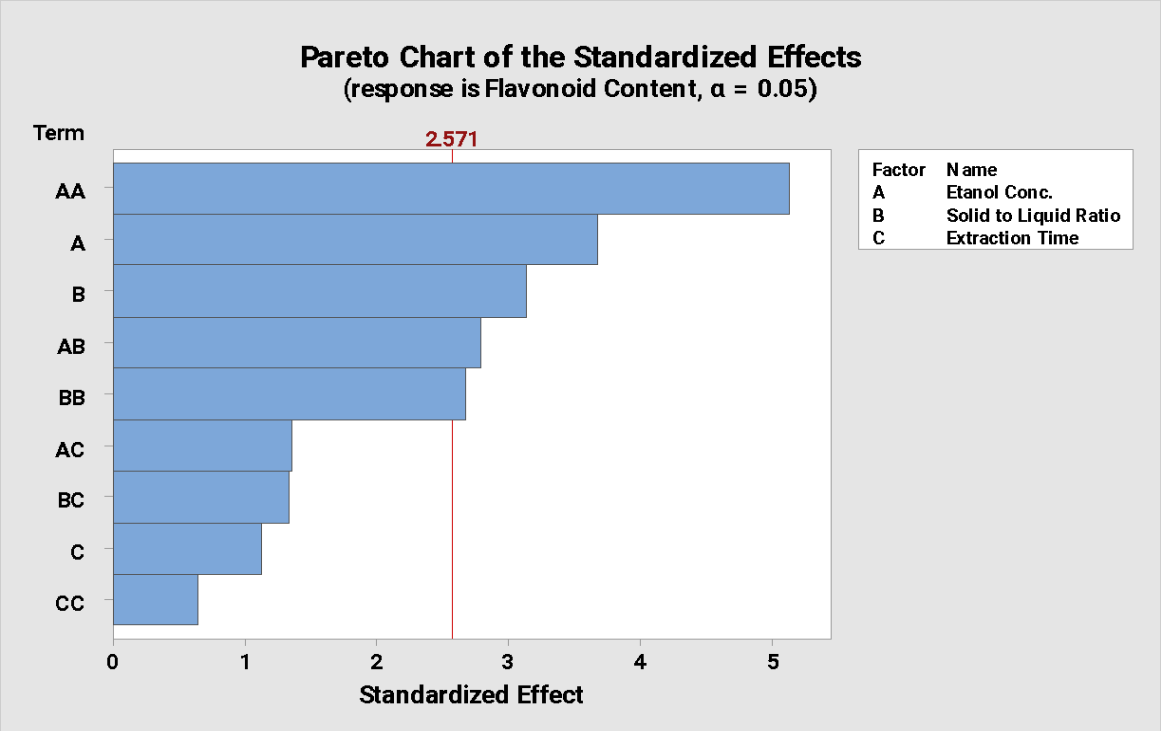

Figure 4 Pareto chart on the effect of ethanol concentration, extraction time, and solid-to-liquid ratio on flavonoid content of MC leaves

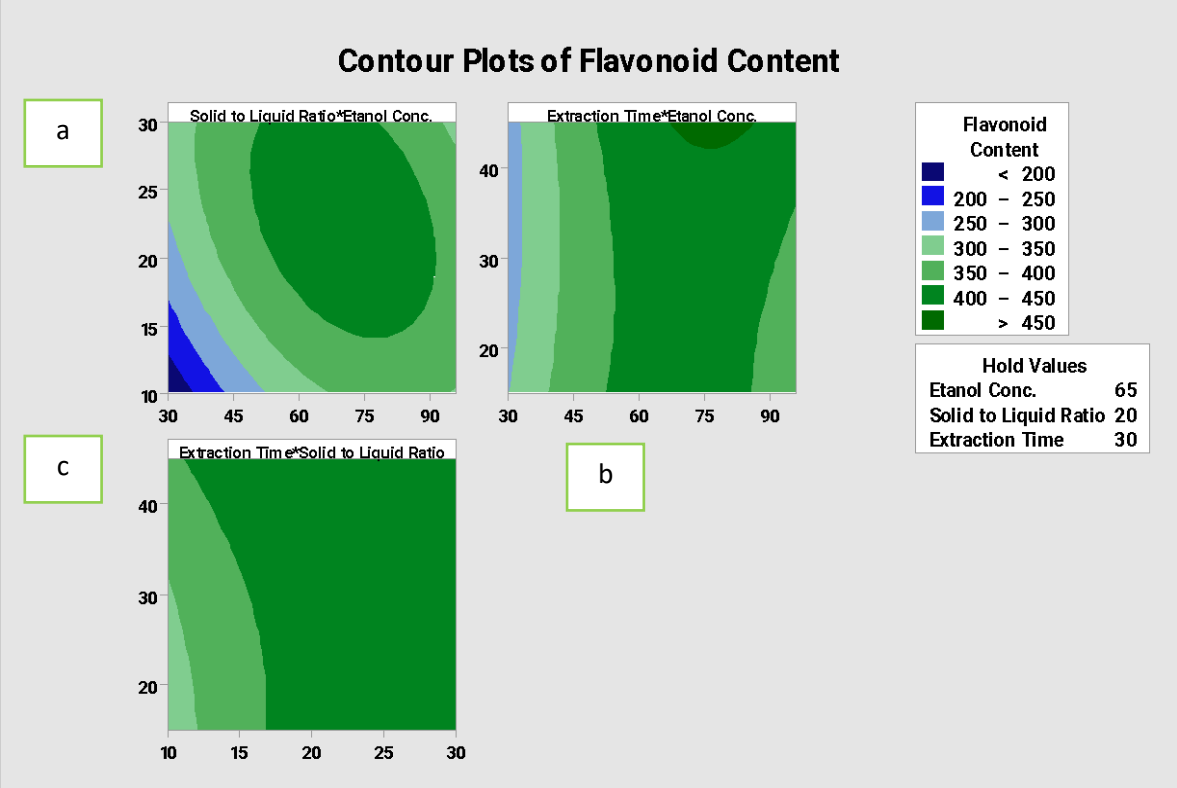

Figure 5 Counter plots of the correlation between the independent variables (including solid-to-liquid ratio, extraction time and ethanol concentration) and the flavonoid content of MC extracts

optimal flavonoid content. Additionally, Figure 5b shows the effect of extraction time and ethanol concentration on the flavonoid content of MC extract. This figure indicates that ethanol concentrations between 65% and 80% and extraction times exceeding 40 minutes resulted in a flavonoid content of more than 450 mg/g QE. This confirms that prolonged extraction time and a mixture of ethanol and water as the solvent increase the flavonoid content of the extract. This result is in line with Pan et al. (2012), who reported that 72% ethanol and 1.5 hours of extraction time resulted in the optimal extraction of flavonoid content from hawthorn seed extract. Figure 5c demonstrates the effect of extraction time and solid-to-liquid ratio on the flavonoid content of MC extract. This figure indicates that increasing the solid-to-liquid ratio (1:20 to 1:30) resulted in a higher flavonoid content (400-450 mg/g QE). The result confirms that increasing the solid-to-liquid ratio increases the amount of compound extracted. However, further confirmation experiments of these optimal parameters are still needed to understand the actual results better and achieve the optimum conditions for flavonoid extraction from MC leaves.

Conclusion

The study found that Ultrasound-assisted extraction (UAE) was effective and cost-effective in increasing the extract yield and flavonoid content from MC leaves. The optimal conditions for UAE of flavonoid content from MC leaves, as determined by Response Surface Methodology (RSM), were a solid-to-liquid ratio of 1:20 to 1:30, an ethanol concentration of 65-80%, and an extraction time of more than 45 minutes. This research also highlighted that MC can be considered one of the best sources of flavonoids.

Acknowledgement

The authors acknowledge the Faculty of Pharmacy for providing the facilities for this research.

Ethical Clearance

It is confirmed that no animal or human model was utilized in the study; therefore, no ethical clearance is necessary.

Conflict of interest

The authors declare no conflict of interest in this study.

References

Abotaleb, M., Samuel, S. M., Varghese, E., Varghese, S., Kubatka, P., Liskova, A., & Büsselberg, D. (2018). Flavonoids in Cancer and Apoptosis. *Cancers*, *11*(1), 28. https://doi.org/10.3390/cancers11010028

Azahar, N. F., Gani, S. S. A., & Mohd Mokhtar, N. F. (2017). Optimization of phenolics and flavonoids extraction conditions of Curcuma Zedoaria leaves using response surface methodology. *Chemistry Central journal*, *11*(1), 96. https://doi.org/10.1186/s13065-017-0324-y

Batra, P., & Sharma, A. K. (2013). Anticancer potential of flavonoids: recent trends and future perspectives. *3 Biotech*, *3*(6), 439–459. https://doi.org/10.1007/s13205-013-0117-5.

Chaves, J. O., de Souza, M. C., da Silva, L. C., Lachos-Perez, D., Torres-Mayanga, P. C., Machado, A. P. D. F., Forster-Carneiro, T., Vázquez-Espinosa, M., González-de-Peredo, A. V., Barbero, G. F., & Rostagno, M. A. (2020). Extraction of Flavonoids From Natural Sources Using Modern Techniques. *Frontiers in chemistry*, *8*, 507887. https://doi.org/10.3389/fchem.2020.507887.

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. A review. *Ultrasonics sonochemistry*, *34*, 540–560. https://doi.org/10.1016/j.ultsonch.2016.06.035.

Firmata Sari, A. A. (2020). *Isolasi Dan Karakterisasi Komponen Utama Dari Ekstrak Daun Benalu (Macrosolen cochinchinensis (Lour.) Van Tiegh) Dengan Inang Pohon Mangga (Mangifera Indica L.)*. [Doctoral thesis, Hasanuddin University, Makassar Indonesia].

Indradmojo, C. (2016) Aktivitas Antikanker Dan Mekanisme Farmakologi Ekstrak Dan Fraksi Benalu Nangka (MacrosolenCochinchinensis) Pada Sel Kanker Payudara T47D.

Iwashina, T. (2000). The Structure and Distribution of the Flavonoids in Plants. *Journal of Plant Research*, *113*(3), 287-299.

Jaafar, N. F., Ramli, M. E., & Mohd Salleh, R. (2020). Optimum Extraction Condition of *Clitorea ternatea* Flower on Antioxidant Activities, Total Phenolic, Total Flavonoid and Total Anthocyanin Contents. *Tropical life sciences research*, *31*(2), 1–17. https://doi.org/10.21315/tlsr2020.31.2.1

Kumal, K., Pant, D. R., Aryal, B., Tripathi, G. R., & Joshi, G. P. (2021). Phytochemical and Antioxidant Properties Of Traditionally Used Mistletoes In Nepal. *Scientific World*, *14*(14), 83–89. https://doi.org/10.3126/sw.v14i14.34999.

Manavi, S. P., Amiri, T., & Mozafaryan, M.J. (2021). Role of Flavonoids in Diabetes. *Journal of Reviews in Medical Sciences*, *1*(3), 149–61.

Medina-Torres, N., Ayora-Talavera, T., Espinosa-Andrews, H., Angeles 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

Mohan, S., & Nandhakumar, L. (2014). Role of Various Flavonoids: Hypotheses on Novel Approach to Treat Diabetes. *Journal of Medical Hypotheses and Ideas*, *8*(1), 1–6.

Mutiah, R., Anik, L., Arief, S., Annisa, R., Hakim, A., Anggraini, W., Susilowati, R. (2018) Activity of inhibit of the cell cycle and induct apoptosis in HeLa cancer cell with combination of Sabrang onion (*Eleutherine palmifolia* (L.) Merr) and Starfruit Mistletoe *Macrosolen cochinchinensis* (Lour.) Tiegh extracts. *Journal of Applied Pharmaceutical Science* 8(10), 122–28.

Nawaz, H., Shad, M. A., & Rauf, A. (2018). Optimization of extraction yield and antioxidant properties of Brassica oleracea Convar Capitata Var L. leaf extracts. *Food chemistry*, *242*, 182–187. https://doi.org/10.1016/j.foodchem.2017.09.041

Nigam, Y. P. (2022). The Bornean Mistletoes as Versatile Parasites: A Systematic Review. *Systematic Reviews in Pharmacy*, *13*(1), 42–47.

Pan, G., Yu, G., Zhu, C., & Qiao, J. (2012). Optimization of ultrasound-assisted extraction (UAE) of flavonoids compounds (FC) from hawthorn seed (HS). *Ultrasonics sonochemistry*, *19*(3), 486–490. https://doi.org/10.1016/j.ultsonch.2011.11.006

Pico, Y. (2013). Ultrasound-Assisted Extraction for Food and Environmental Samples. *TrAC Trends in Analytical Chemistry*, *43*, 84–99. DOI: https://doi.org/10.1016/j.trac.2012.12.005.

Prasad, K. N, Weng Kong, K., Ramakrishnan Nagasundara Ramanan, R.N., Azlan, A., & Ismail, A. (2012). Determination and Optimization of Flavonoid and Extract Yield from Brown Mango Using Response Surface Methodology. *Separation Science and Technology*, *47*(1), 73–80.

Samanta, A., Das, G., & Das, S.K. (2011). Roles of Flavonoids in Plants. *Carbon*, *100*(6), 12–35.

Santosa, D., Widyastuti, S. M., Rosyidah, U., & Silmia, B. (2022). Detection of Terpenoid and Flavonoid of Five Species of Mistletoes at *Stelechocarpus burahol* (Bl.) Hook. f. & Th and *Lagerstroemia speciosa* (L.) Pers. by Using Thin Layer Chromatography Method. In A. Linggawati, A. A. P. Anfa, S, A. Linatoc, C. K. Fusianto, D. Lawrie, et al. (Eds.) *7th International Conference on Biological Science (ICBS 2021)* (pp. 388–393). Atlantis Press. DOI: https://doi.org/10.2991/absr.k.220406.055.

Sharma, V., & Janmeda, P. (2017). Extraction, Isolation and Identification of Flavonoid from Euphorbia Neriifolia Leaves. *Arabian Journal of Chemistry*, *10*(4), 509–14.

Shin, H. W., & Lee, N. S. (2018). Understanding plastome evolution in Hemiparasitic Santalales: Complete chloroplast genomes of three species, Dendrotrophe varians, Helixanthera parasitica, and Macrosolen cochinchinensis. *PloS one*, *13*(7), e0200293. https://doi.org/10.1371/journal.pone.0200293