ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by JK Welfare & Pharmascope Foundation

Journal Home Page: www.ijrps.com

Box-Behnken experimental design for extraction optimization of cytotoxic activity from *Curcuma aeruginosa* rhizome

Rani Khoiriyah[1], Made Artika I[1], Waras Nurcholis[*1,2]

[1]Department of Biochemistry, Bogor Agricultural University, Bogor-16680, West Java, Indonesia
[2]Tropical Biopharmaca Research Center, Bogor Agricultural University, Bogor -16128, West Java, Indonesia

Article History:

Received on: 10.09.2019
Revised on: 04.12.2019
Accepted on: 10.12.2019

Keywords:

Box-Behnken design,
Curcuma aeruginosa,
Cytotoxic activity,
Extraction yield,
Optimization

ABSTRACT

Check for updates

Curcuma aeruginosa is the common name of temu hitam in Indonesia, and the rhizome parts of the plant have several pharmacological activities. Generally, pharmacological activities are associated with bioactive content in the extract of medicinal plants. Several factors can influence the bioactive extraction from medicinal plants such as solvent types, extraction time, extraction technique, and liquid-to-solid ratio. In this research, the extraction factors for extraction yield and cytotoxic activity of *C. aeruginosa* rhizome were optimized using the Box-Behnken experimental design. Effect of ethanol concentration, the ratio of liquid to solid, and extraction time for the maceration process was studied. The cytotoxic activity was determined by the brine shrimp lethality test. The optimum value that maximizes the extraction yield was 70% ethanol, 300:15 ml/g liquid to solid ratio, and 1-day extraction time. The optimum value that maximizes the cytotoxic activity was 70% ethanol, 150:15 ml/g liquid to solid ratio, and 2-day extraction time. The predicted extraction yield and cytotoxic activity at these projected values are 14.78% and 78.26 mg/l, respectively. In this model, Adeq Precision (10.35 and 4.16), R-Squared (0.86 and 0.79), and F-value (7.92 and 2.04) is rational to fit the model for extraction yield and cytotoxic activity from *C. aeruginosa* rhizome.

*Corresponding Author

Name: Waras Nurcholis
Phone:
Email: wnurcholis@apps.ipb.ac.id

ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v11i2.2045

Production and Hosted by

IJRPS | www.ijrps.com

INTRODUCTION

Curcuma aeruginosa Roxb. (*C. aeruginosa*) is a medicinal plant that is generally known in Indonesia as temu hitam, and widely utilized in traditional medicine. The *C. aeruginosa* rhizome is used in folk medicine, including the treatment of rheumatic, asthma, enteritis, stomach pain, obesity, increase appetite, and obesity (Nurcholis *et al.*, 016a). Numerous works have reported the biological activity of *C. aeruginosa*, including its antimicrobial (Kamazeri *et al.*, 2012; Akarchariya *et al.*, 2017), anticancer (Fitria *et al.*, 2019), antioxidant (Nurcholis *et al.*, 016b, 2017), skin lightening, hairgrowth (Srivilai *et al.*, 2017), anti-dengue (Moektiwardoyo *et al.*, 2014), anti-androgenic (Suphrom *et al.*, 2012) and uterine relaxant (Thaina *et al.*, 2009) properties. Previous works have founded the presence of various metabolites such as germacrene, camphor (Akarchariya *et al.*, 2017), curcuminoid (Nurcholis *et al.*, 016a, 2019), cycloisolongifolene, 8,9-dihydro formyl, dihydrocostunolide (Kamazeri *et al.*, 2012), terpenoids (Simoh and Zainal, 2015), and sesquiterpenes (Takano *et al.*, 1995; Suphrom *et al.*, 2012; Awin *et al.*, 2019) in the *C. aeruginosa* extract. Several reports have reported that the metabolites content in medicinal plant extract associated with the pharmacological activities (Mosbah *et al.*, 2018; Chinnadurai *et al.*, 2019; Bistgani and Sefidkon, 2019). Thus, the development of rhizome extraction is needed to produce rhizome extract from *C. aeruginosa* with high pharmacological properties.

The extraction is the crucial step for the recovery of metabolite compounds from medicinal plants. Various factors can affect the extraction effectiveness and extract yields such as solvent types (Qomaliyah *et al.*, 2019), extraction time (Soós *et al.*, 2019), extraction technique (Hmidani *et al.*, 2019), and solvent-to-solid ratio (Sajid *et al.*, 2019). The evaluate interaction in its extraction factors is proportionately important to enhance the extract yield. Response surface methodology, called RSM, is one alternative statistical method useful for improving complex extraction procedures. Several works have successfully reported for optimizing the extraction yield using RSM in the medicinal plant (Belwal *et al.*, 2016; Pandey *et al.*, 2018). Therefore, the study aims to optimize the extraction parameters, i.e., solid-to-liquid ratio, extraction time, and ethanol concentration in *C. aeruginosa* rhizome using RSM. Also, extraction yield and cytotoxicity for potency pharmacology studied at maximum conditions are also investigated.

MATERIALS AND METHODS

Experimental

Plant material

The dry rhizome sample of *C. aeruginosa* was collected from Tropical Biopharmaca Research Center, Bogor Agricultural University, Indonesia, in February 2019.

Extraction process and determination of cytotoxic activity

The powdered rhizome sample (15 g) of *C. aeruginosa* were macerated with varying volume (75 – 300 ml) of ethanol at a varying concentration (50 – 96%) in 500 ml elementary flask. These samples were mixed and macerated with a shaker at 235 rpm for different time extraction (1 – 3 days). The solid-to-liquid proportion, ethanol concentration, and time extraction were based on an experimental design created with response surface methodology and Box-Behnken design using Design-Expert version 11 (State Ease, Inc.). After extraction, the solution was filtered using Whatman no. 5 filter paper and then evaporated at 50 °C using a rotary evaporator (BUCHI, R-250, Switzerland). Based on the extracted content, the extraction yield was determined (%, w/w). The extract directly applied to investigate the cytotoxic activity. Cytotoxic activity was performed according to the brine shrimp lethality test by (Meyer *et al.*, 1982).

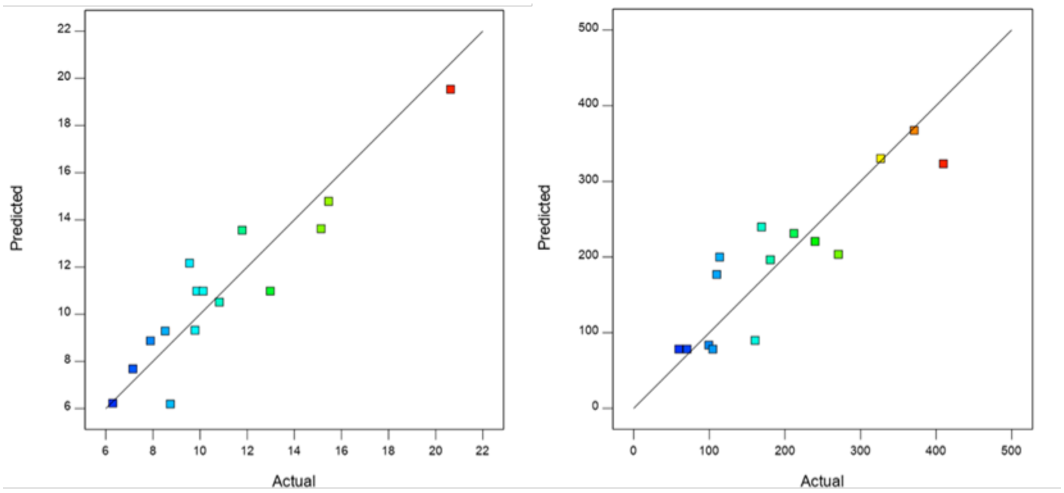
Experimental design

RSM and Box-Behnken design were used to evaluate the effect of extraction factors for obtaining the greatest extraction yield and cytotoxic activity with the maceration method from *C. aeruginosa* rhizome. The extraction parameter includes, ethanol concentration (%), liquid-to-solid ratio (ml/g), and time extraction (day). These variables were used for the optimization of extraction yield and cytotoxicity responses. The Box-Behnken experimental design consists of 15 experimental runs with the three replications at design center points of 150:15 ml/g liquid-to-solid ratio, 70% ethanol concentration, 2-day time extraction. (Table 1) showed the Box-Behnken experimental design and response of the extraction process. Optimization of the extraction process was performed using Design-Expert version 11.0 (Stat-Ease, Inc).

RESULTS AND DISCUSSION

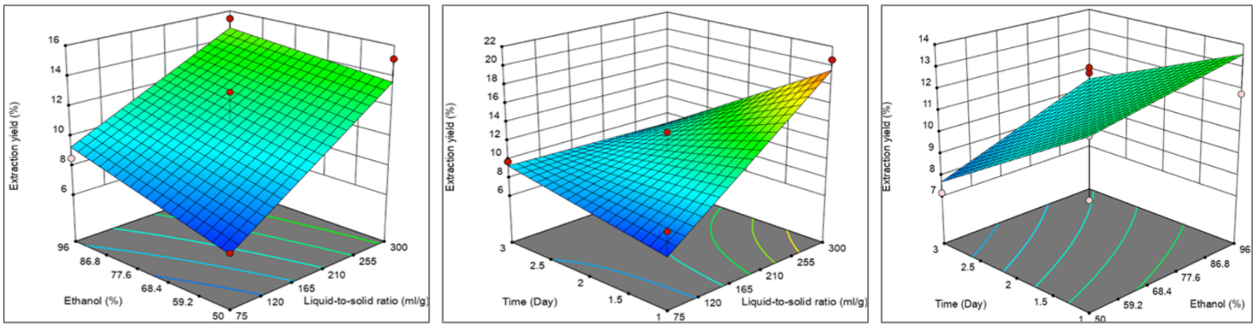
Model fitting

The two-factor interaction and quadratic were suggested as a model statistic for extraction yield and cytotoxicity responses, respectively (Table 2).In these models, the R^2 value was 0.8559 of extraction yield and 0.7859 of cytotoxic activity responses. This study showed an R^2 value of less than 0.9, which indicated that the low tolerability of the model (Koocheki *et al.*, 2009). (Table 3) presents the analysis of variance (ANOVA) employed in the 2FI and quadratic models for extraction yield and cytotoxic activity responses, respectively. The *F* values of 7.92 and 2.04 for extraction yield and cytotoxic activity, respectively, indicated the extraction yield model was significant but not significant for cytotoxicity response. The significant model indicated that the extraction parameters had a considerable influence on extraction yields but not effect cytotoxic activity (Tan *et al.*, 2016). This term, the extraction yield is significantly affected by the liquid-to-solid ratio (A), time extraction (C), and interaction between liquid-to-solid ratio and time extraction (AC). The second order of liquid-to-solid ratio was significantly affected by the cytotoxicity response. The Adeq Precision was 10.35 and 4.16 for extraction yield and cytotoxicity response, respectively. Because the Adeq Precision more than 4.0, this result indicated that the model was rationally acceptable to navigate the design opti-



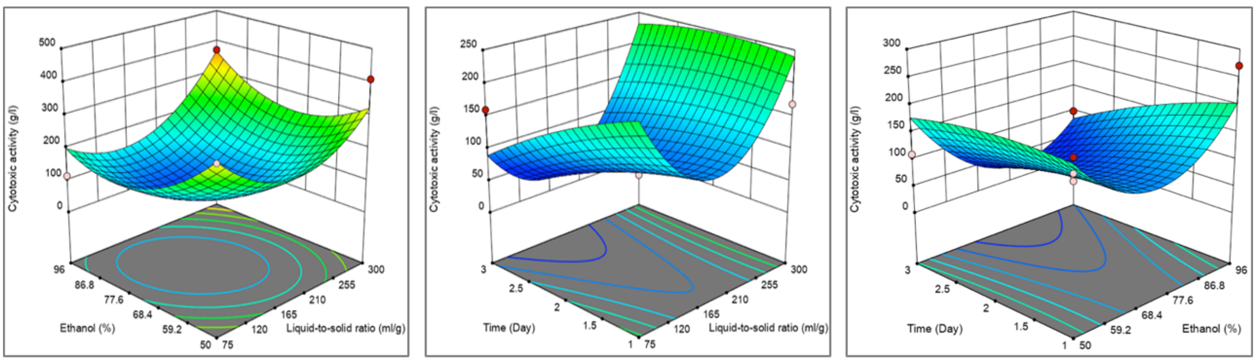
(a) (b)

Figure 1: Predicted value vs. actual value for (a) extraction yield and (b) cytotoxic activity



(a) (b) (C)

Figure 2: Response surface 3D plots displaying the influence of (a) ethanol vs. liquid-to-solid ratio, (b) time vs. liquid-to-solid ratio, and (c) time vs ethanol in the extraction parameters for extraction yield responses



(a) (b) (c)

Figure 3: Response surface 3D plots displaying the influence of (a) ethanol vs. liquid-to-solid ratio, (b) time vs. liquid-to-solid ratio, and (c) time vs ethanol in the extraction parameters for cytotoxic activity responses

Table 1: The extraction yield and cytotoxic activity responses with Box-Behnken experimental design from extraction process

Run	Liquid-to-solid ratio (ml/g)	Ethanol (%)	Time (Day)	Extraction yield (%)	Cytotoxic activity (mg/l)
1	150:15	70	2	10.13	59.62
2	150:15	70	2	9.86	70.58
3	75:15	96	2	8.52	113.65
4	75:15	70	1	8.73	239.90
5	150:15	50	1	9.55	180.72
6	150:15	50	3	7.15	109.77
7	150:15	96	1	11.78	270.67
8	300:15	70	3	7.89	211.93
9	150:15	70	2	12.98	104.58
10	300:15	70	1	20.63	169.08
11	150:15	96	3	10.82	99.27
12	300:15	50	2	15.14	409.61
13	75:15	70	3	9.79	160.52
14	75:15	50	2	6.29	326.62
15	300:15	96	2	15.46	371.00

Table 2: Model summary statistics for extraction yield and cytotoxic activity

Extraction yield (%)					
Source	SD	R^2	R^2Adj	R^2Pre	Comment
Linear	0.0133	0.3027	0.5016	0.1914	
2FI	1.89	0.8559	0.7479	0.3587	Suggested
Quadratic	0.5973	0.4088	0.7144	-0.2174	
Cubic	0.4088		0.7890		Aliased
Cytotoxic activity					
Source	SD	R^2	R^2Adj	R^2Pre	Comment
Linear	0.5859	0.0341	-0.0752	-0.5704	
2FI	0.8344	0.0253	-0.3352	-2.0595	
Quadratic	85.17	0.7859	0.4006	-2.3360	Suggested
Cubic	0.0451		0.9546		Aliased

mize (Shahinuzzaman *et al.*, 2019). (Figure 1) presents the predicted vs. actual value for extraction yield and cytotoxicity, respectively.

Extraction yield

As presented in (Figure 2), liquid-to-solid ratio, ethanol concentration and extraction time were understood in the ranges of 75:15 – 300:15 ml/g, 50 – 96%, and 1 – 3 days for extraction yield response. Extraction yield was ranged 6.29% to 20.63% with maximum yield on the solid-to-liquid ratio of 300:15 (ml/g), ethanol concentration of 70%, and time extraction of 2 days (Table 1). At the constant extraction time (2 days), the maximum extraction yield (15.46%) was found at the solid-to-liquid ratio of 300:15 (ml/g) and ethanol concentration of 96% (Figure 2a). (Figure 2b) illustrates the influence of liquid-to-solid ratio and extraction time on the extraction yields. The response surface 3D plot was generated with the ethanol concentration fixed at 73%. The highest extraction yield (20.63%) was obtained at a liquid-to-solid ratio of 300:15 (ml/g) at the 1-day maceration process. The variation of extraction yield with extraction time and ethanol concentration at constant liquid-to-solid ratio (187.5:15 ml/g) is presented in (Figure 2c). The highest extraction yield was approximately 12.98% at an ethanol concentration of 73% and 2-day extraction. This work shows that the extraction yield increased linearly with extraction variables of ethanol concentration and liquid-to-solid ratio.

Table 3: ANOVA for response surface 2FI model for extraction yield and quadratic model for cytotoxic activity

Extraction yield						
Source	Sum of Squares	df	Mean Square	F-value	p-value	Status
Model	169.39	6	28.23	7.92	0.0051	significant
A	83.13	1	83.13	23.32	0.0013	
B	8.92	1	8.92	2.50	0.1522	
C	28.36	1	28.36	7.96	0.0225	
AB	0.9063	1	0.9063	0.2543	0.6277	
AC	47.56	1	47.56	13.34	0.0065	
BC	0.5146	1	0.5146	0.1444	0.7139	
Residual	28.51	8	3.56			
Lack of Fit	22.55	6	3.76	1.26	0.5056	not significant
Pure Error	5.97	2	2.98			
Cor Total	197.91	14				
Cytotoxic activity						
Source	Sum of Squares	df	Mean Square	F-value	p-value	Status
Model	1.332E+05	9	14795.66	2.04	0.2238	not significant
A	12874.19	1	12874.19	1.77	0.2403	
B	3703.69	1	3703.69	0.5106	0.5069	
C	9721.55	1	9721.55	1.34	0.2993	
AB	7600.21	1	7600.21	1.05	0.3530	
AC	3735.39	1	3735.39	0.5149	0.5051	
BC	2522.45	1	2522.45	0.3477	0.5810	
A^2	61068.41	1	61068.41	8.42	0.0337	
B^2	35718.68	1	35718.68	4.92	0.0772	
C^2	488.87	1	488.87	0.0674	0.8055	
Residual	36271.00	5	7254.20			
Lack of Fit	35171.96	3	11723.99	21.33	0.0451	significant
Pure Error	1099.04	2	549.52			
Cor Total	1.694E+05	14				

A = Liquid-to-solid ratio (ml/g); B= Ethanol (%); C = Time (Day)

Cytotoxic activity

The effect of liquid-to-solid ratio, ethanol concentration, and extraction times on the cytotoxic activity was shown in (Figure 3). Cytotoxic activity (LC_{50} value) was ranged 59.62 mg/l to 409.61 mg/l. The highest cytotoxicity (lower value of LC_{50}) was obtained at a liquid-to-solid ratio of 150:15 (ml/g), 70% ethanol, and 2-days extraction time. Because LC_{50} < 1000 mg/l, all extracts were potency showed as the anticancer agent (Meyer et al., 1982). Cytotoxic activity under different ethanol concentrations and the liquid-to-solid ratio at a constant time of 2-days is seen in (Figure 3a). The lowest LC_{50} value, highest cytotoxicity (113.65 mg/l), was obtained at a liquid-to-solid ratio of 75:15 ml/g and 96% ethanol. (Figure 3b) represents the interaction between ethanol concentration and extraction times on cytotoxic activity. The maximum cytotoxicity (59.62 mg/l) was observed at extraction situations 187.5:15 (ml/g) of liquid-to-solid ratio and 2-days extraction at a fixed of ethanol concentration (73%). (Figure 3c) depicts the cytotoxic activity with respect to ethanol concentration and extraction time at the liquid-to-solid ratio of 187.5:15 ml/g. The highest cytotoxic activity (59.62 mg/l) was recorded at 73% ethanol and 2-days extraction time.

CONCLUSIONS

RSM with Box-Behnken experimental design was effectively used to optimize the extraction factors from *C. aeruginosa* rhizome. The ethanol concentration, liquid-to-solid ratio, and extraction time were important parameters affecting the extraction yield and cytotoxic activity. For the extraction yield (20.63%), the maximize combination of parameters was 70% ethanol, 300:15 ml/g liquid to solid ratio, and 1-day extraction time. In cytotoxic activity (59.62 mg/l), the maximize combination of parameters was 70% ethanol, 150:15 ml/g liquid-to-solid ratio and 2-day extraction time.

ACKNOWLEDGEMENT

Authors acknowledge financial support from IPB University and the Ministry of Research, Technology, and Higher Education of the Republic of Indonesia in terms of the PDUPT research grant (No 4182/IT3.L1/PN/2019).

REFERENCES

Akarchariya, N., Sirilun, S., Julsrigival, J., Chansakaowa, S. 2017. Chemical profiling and antimicrobial activity of essential oil from Curcuma aeruginosa Roxb., Curcuma glans K. Larsen & J. Mood and Curcuma cf. xanthorrhiza Roxb. collected in Thailand. *Asian Pacific Journal of Tropical Biomedicine*, 7(10):881–885.

Awin, T., Mediani, A., Maulidiani, Leong, S. W., Faudzi, S. M. M., Shaari, K., Abas, F. 2019. Phytochemical and bioactivity alterations of Curcuma species harvested at different growth stages by NMR-based metabolomics. *Journal of Food Composition and Analysis*, 77:66–76.

Belwal, T., Dhyani, P., Bhatt, I. D., Rawal, R. S., Pande, V. 2016. Optimization extraction conditions for improving phenolic content and antioxidant activity in Berberis asiatica fruits using response surface methodology (RSM). *Food Chemistry*, 207:115–124.

Bistgani, Z. E., Sefidkon, F. 2019. Review on ethnobotany, phytochemical, molecular and pharmacological activity of Thymus daenensis Celak. *Biocatalysis and Agricultural Biotechnology*.

Chinnadurai, V., Viswanathan, P., Kalimuthu, K., Vanitha, A., Ranjitha, V., Pugazhendhi, A. 2019. Comparative studies of phytochemical analysis and pharmacological activities of wild and micropropagated plant ethanol extracts of Manihot esculenta. *Biocatalysis and Agricultural Biotechnology*, 19.

Fitria, R., Seno, D. S. H., Priosoeryanto, B. P., Nurcholis, W. 2019. Volatile compound profiles and cytotoxicity in essential oils from rhizome of Curcuma aeruginosa and Curcuma zanthorrhiza. *Biodiversitas Journal of Biological Diversity*, 20(10):2943–2948.

Hmidani, A., Bouhlali, T., Khouya, T., Ramchoun, M., Filali-Zegzouti, Y., Benlyas, M., Alem, C. 2019. Effect of extraction methods on antioxidant and anticoagulant activities of Thymus atlanticus aerial part. *Scientific African*, 5.

Kamazeri, T. S. A. T., Samah, O. A., Taher, M., Susanti, D., Qaralleh, H. 2012. Antimicrobial activity and essential oils of Curcuma aeruginosa, Curcuma mangga, and Zingiber cassumunar from Malaysia. *Asian Pacific Journal of Tropical Medicine*, 5(3):202–209.

Koocheki, A., Taherian, A. R., Razavi, S. M. A., Bostan, A. 2009. Response surface methodology for optimization of extraction yield, viscosity, hue and emulsion stability of mucilage extracted from Lepidium perfoliatum seeds. *Food Hydrocolloids*, 23(8):2369–2379.

Meyer, B., Ferrigni, N., Putnam, J., Jacobsen, L., Nichols, D., Mclaughlin, J. 1982. Brine Shrimp: A Convenient General Bioassay for Active Plant Constituents. *Planta Medica*, 45(05):31–34.

Moektiwardoyo, W., Tjitraresmi, A., Susilawati, Y., Iskandar, Y., Halimah, E., Zahryanti, D. 2014. The Potential of Dewa Leaves (Gynura Pseudochina (L) D.C) and Temu Ireng Rhizomes (Curcuma aeruginosa Roxb.) as Medicinal Herbs for Dengue Fever Treatment. *Procedia Chemistry*, 13:134–141.

Mosbah, H., Louati, H., Boujbiha, M. A., Chahdoura, H., Snoussi, M., Flamini, G., Ascrizzi, R., Bouslema, A. 2018. Phytochemical characterization, antioxidant, antimicrobial and pharmacological activities of Feijoa sellowiana leaves growing in Tunisia. *Industrial Crops and Products*, 112:521–531.

Nurcholis, W., Khumaida, N., Syukur, M., Bintang, M. 2016a. Variability of curcuminoid content and lack of correlation with cytotoxicity in ethanolic extracts from 20 accessions of Curcuma aeruginosa RoxB. *Asian Pacific Journal of Tropical Disease*, 6(11):61152–61152.

Nurcholis, W., Khumaida, N., Syukur, M., Bintang, M. 2016b. Variability of Total Phenolic and Flavonoid Content and Antioxidant Activity Among 20 Curcuma aeruginosa Roxb. Accessions of Indonesia. *Asian Journal of Biochemistry*, 11(3):142–148.

Nurcholis, W., Khumaida, N., Syukur, M., Bintang, M. 2017. Evaluation of Free Radical Scavenging Activity in Ethanolic Extract from Promising Accessions of Curcuma aeruginosa RoxB. *Molekul*, 12(2):133–133.

Nurcholis, W., Khumaida, N., Syukur, M., Bintang, M. 2019. Variability of Curcumin, Demethoxycurcumin and Bisdemethoxycurcumin Contents in Ethanolic Extract from Ten Curcuma aeruginosa Roxb. Cultivated in West Java Indonesia. *Asian Journal of Chemistry*, 31(11):2461–2465.

Pandey, A., Belwal, T., Sekar, K. C., Bhatt, I. D., Rawal, R. S. 2018. Optimization of ultrasonic-assisted extraction (UAE) of phenolics and antioxidant compounds from rhizomes of Rheum moorcroftianum using response surface methodology (RSM). *Industrial Crops and Products*, 119:218–225.

Qomaliyah, E. N., Artika, I. M., Nurcholis, W. 2019. Optimization of extraction process for extract yields, total flavonoid content, radical scavenging activity and cytotoxicity of Curcuma aeruginosa RoxB. rhizome. *International Journal of Research in Pharmaceutical Sciences*, 10(3):1650–1659.

Sajid, M., Woźniak, M. K., Płotka-Wasylka, J. 2019. Ultrasound-assisted solvent extraction of porous membrane packed solid samples: A new approach for extraction of target analytes from solid samples. *Microchemical Journal*, 144:117–123.

Shahinuzzaman, M., Yaakob, Z., Sani, N. A., Akhtar, P., Islam, M. Z., Mimi, M. A., Shamsudin, S. A. 2019. Optimization of Extraction Parameters for Antioxidant and Total Phenolic Content of Ficus carica L. Latex from White Genoa Cultivar. *Asian Journal of Chemistry*, 31(8):1859–1865.

Simoh, S., Zainal, A. 2015. Chemical profiling of Curcuma aeruginosa Roxb. rhizome using different techniques of solvent extraction. *Asian Pacific Journal of Tropical Biomedicine*, 5(5):30378–30384.

Soós, A., Bódi, E., Várallyay, S., Molnár, S., Kovács, B. 2019. Mineral content of propolis tinctures in relation to the extraction time and the ethanol content of the extraction solvent. *LWT Food Science and Technology*, 111:719–726.

Srivilai, J., Phimnuan, P., Jaisabai, J., Luangtoomma, N., Waranuch, N., Khorana, N., Ingkaninan, K. 2017. Curcuma aeruginosa Roxb. essential oil slows hairgrowth and lightens skin in axillae; a randomised, double blinded trial. *Phytomedicine*, 25:29–38.

Suphrom, N., Pumthong, G., Khorana, N., Waranuch, N., Limpeanchob, N., Ingkaninan, K. 2012. Antiandrogenic effect of sesquiterpenes isolated from the rhizomes of Curcuma aeruginosa Roxb. *Fitoterapia*, 83(5):864–871.

Takano, I., Yasuda, I., Takeya, K., Itokawa, H. 1995. Guaiane sesquiterpene lactones from Curcuma aeruginosa. *Phytochemistry*, 40(4):425–432.

Tan, S. F., Masoumi, H. R. F., Karjiban, R. A., Stanslas, J., Kirby, B. P., Basri, M., Basri, H. B. 2016. Ultrasonic emulsification of parenteral valproic acid-loaded nanoemulsion with response surface methodology and evaluation of its stability. *Ultrasonics Sonochemistry*, 29:299–308.

Thaina, P., Tungcharoen, P., Wongnawa, M., Reanmongkol, W., Subhadhirasakul, S. 2009. Uterine relaxant effects of Curcuma aeruginosa Roxb. rhizome extracts. *Journal of Ethnopharmacology*, 121(3):433–443.