Jurnal Farmasi Galenika (*Galenika Journal of Pharmacy*) (e-Journal) 2023; 9 (2): 224 – 232
ISSN: 2442-8744 (electronic); 2442-7284 (printed)
Journal Homepage: https://bestjournal.untad.ac.id/index.php/Galenika
DOI: 10.22487/j24428744.2023.v9.i2.16106

Optimization of Extraction *Lannea coromandelica* for Antioxidant Activity using Analytical Factorial Design Approach

(*Optimisasi Ekstraksi Tanaman Lannea coromandelica Sebagai Antioksidan dengan Pendekatan Analisis design Faktorial*)

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Article Info:
Received: 1 November 2022
in revised form: 18 November 2022
Accepted: 12 May 2023
Available Online: 01 October 2023

Keywords:
Optimization
Antioxidant
Lannea coromandelica

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ABSTRACT

Background: The yield of active compound in a plant can be affected by the extraction method, such as variety of solvent and extraction technique used. **Objectives:** This study aimed to optimize the yield of *Lannea coromandelica* leaf extract using several types of solvent and extraction techniques and evaluate the antioxidant activity of the extracts. **Methods:** This study used a variety of solvent like distilled water, ethanol, n-hexane, and ethyl acetate with maceration, reflux, and sonication extraction techniques. The extract obtained was calculated for the percent yield and analyzed for their antioxidant activity using the DPPH method by UV -Vis Spectrophotometry with 523.5 nm maximum wavelength. The antioxidant activity was analyzed by factorial design analysis in Minitab 21 software. **Results:** The highest percentage yields was obtained from the extract with ethanol solvent, followed by distilled water, ethyl acetate and n-hexane. The highest extraction techniques was obtained from reflux and followed by maceration and sonication extracts. Analytical factorial design analysis showed that the linearity of solvent variation and extraction technique had a significant effect (P>0.05). From the analysis, it was found that there was a significant difference between the effect of the solvent on the antioxidant activities compared to the extraction technique. The extracts obtained have a very strong antioxidant activity against DPPH radicals. T **Conclusions:** *L. coromandelica* plant aquadest extract has potential to be developed in optimalization extraction method.

How to cite (APA 6th Style):
AR, M. A., Hikma, N., Yasir, B., Burhan, A., (2022). Optimization of Extraction *Lannea coromandelica* for Antioxidant Activity using Analytical Factorial Design Approach. *Jurnal Farmasi Galenika: Galenika Journal of Pharmacy (e-Journal), 9*(2), 224-232. doi:10.22487/j24428744.2023.v9.i2.16106

INTRODUCTION

Indonesia is the tropical country which known to have various types of plants with medicinal properties. Medicinal plants as raw materials needed in Indonesia. Medicinal plants used to cure various disease. It is gaining interest to apply as medicine because have low side effects and not harmful to the body. One of the plants that efficacious as medicinal plants is the *Lannea coromandelica* leaves. L. coromandelica is the plants that grows a lot in Indonesia, especially in South Sulawesi. Empirically, the leaves have potential as a neuromodulator and antidiabetic by increasing the work of pancreatic beta cells. The content in the leaves are alkaloids, steroids, tannins, and flavonoids as analgesics (Baso, 2022). Previous research by Muin (2021) also stated that the plant contains saponins and flavonoids. This plant has also been shown have an entioxidant effect (Azzahrah et al, 2019). Antioxidants are electron donor compounds that work by donating one electron to compounds that are radical then inhibited radical activities. Free radical instability can be stabilized by antioxidant by complementing the electron deficiency in free radical compounds (Gusungi et al, 2020).

To obtain the secondary metabolites, its influenced by extraction method. For example, in several previous studies, such as that studied by Nurhasnawati (2017), extracted guava leaves to measure IC50 value using a comparison of the extraction method between maceration and soxletation. The results indicated that the IC50 value in maceration method is 37.67 ppm and IC50 in the soxletation method is 47.80 ppm. Other researcher have also tested the antioxidant activity of lotus leaf using a variety of extraction methods. The research showed that the highest antioxidant activity is from maceration method than other method (Chandra et al, 2022). Based on the description above, the researchers wanted to do a test using a comparison of the extraction method on the antioxidant activity of L. coromandelica plants using factorial design approach analysis.

MATERIAL AND METHODS

Materials

The leaves *L. coromandelica* were collected from Takalar, South Sulawesi Province, Indonesia. The sample was identified/determined by BRIN (Badan Riset dan Inovasi Nasional), Cibinong, Jakarta. The sample were separated in an oven at around 40-50^{0}C for three days. The dried plant materials were then ground with a blender and passed through no. 18 mesh, then stored in a desicator. The other material such DPPH (*2,2 difenil-1-pikrihidrazil*), etanol p.a, ethanol 70%, ethyl acetate, kuarcetine, dan n-hexane.

Methods

Extraction

The dried plant powder was extracted by maceration, sonication, and reflux method using distilled water, ethyl acetate, ethanol 70%, and n-hexane by a ratio of 1:10 (sample: solvent). Each sample was weighed 100 g and then extracted using the predetermined method. In the maceration extraction method, the simplicia powder that has been weighed put into a maceration vessel and macerated using each solvent for 3 days protected from light while stirring occasionally (adiwibowo, 2020). In the reflux extraction method, simplicia powder is extracted for approximately 2 hours using a controlled temperature of 50ºc (utami et al., 2015). In the sonication extraction method, simplicia powder is put in a volumetric flask and extracted for 30 minutes in a sonicator (adiwibowo, 2020). Furthermore, each treatment was filtered to obtain a liquid extract of each solvent and then concentrated using a water bath with a controlled temperature of less than 60ºC. After that, the viscous extract obtained was weighed for further calculation of the percentage reduction (utami et al., 2015).

Antioxidant analysis

Testing of antioxidant activity was carried out by making a series of sample solution concentrations of 40 ppm, 80 ppm, 120 ppm, 160 ppm, and 200 ppm by pipetting a certain amount of stock solution, then adding 1 ml of dpph solution and then making up to 5 ml of ethanol p.a. Then homogenized and incubated for 30 minutes then the absorption was measured using uv-vis spectrophotometry at a wavelength of 513,5 nm (rusdi et al., 2018).

Design Factorial Analysis

Antioxidant activities from IC_{50} value was analized by Minitab 21 software. From the application, the data was analyzed by design factorial analysis.

RESULTS AND DISCUSSION

Optimization extraction method

The optimization of secondary metabolite content based on the percent yield value of the extraction method can be seen in table 1. The analysis was carried out by comparing the weight of the extract obtained from maceration, sonication, and reflux techniques to various solvents in the form of distilled water, hexane, ethyl acetate and 70% ethanol. The table shows the uniform weight distribution of extracts obtained from simplicia weights. In the table, it can be seen that the ethanol extract using maceration, sonication and reflux methods has a higher yield percentage than other solvents using the same method. This shows the ability of the ethanol solvent to attract a large number of compounds contained in the sample.

Table 1. Comparison of Extraction Results using Solvents with Varied Polarities and Percentage Yield of Extracts

Sample	Solvent	Weight simplicia (g)	Extraction technical	Weight extract (g)	Yield (%)
L. coromandelica leaves	Aquadest	100	Maseration	4.2	4.2
			Sonication	5.1	5.1
			Reflux	7.1	7.1
	n-Heksane	100	Maseration	2.4	2.4
			Sonication	1.2	1.2
			Reflux	2.2	2.2
	Ethyl acetate	100	Maseration	3.6	3.6
			Sonication	2.1	2.1
			Reflux	2.4	2.4
	Ethanol 70%	100	Maseration	18.7	18.7
			Sonication	6.4	6.4
			Reflux	30.8	30.8

Based on the results of statistical analysis using one-way anova (figure 1), it appears that there is an effect of the extraction technique and solvent used. The results of the analysis of the effect of the solvent showed that ethanol solvent had a higher ability to extract l. Coromandelica leaf samples, followed by aquadest, ethyl acetate, and hexane as solvents. This indicates that the compounds present in the sample of l. Coromandelica leaves have a tendency to be more polar (based on the principle like dissolves like). Compared to the influence of the solvent, the extraction technique also gives different results. The results of the analysis show that the reflux method of extraction gives a higher yield compared to maceration and sonication.

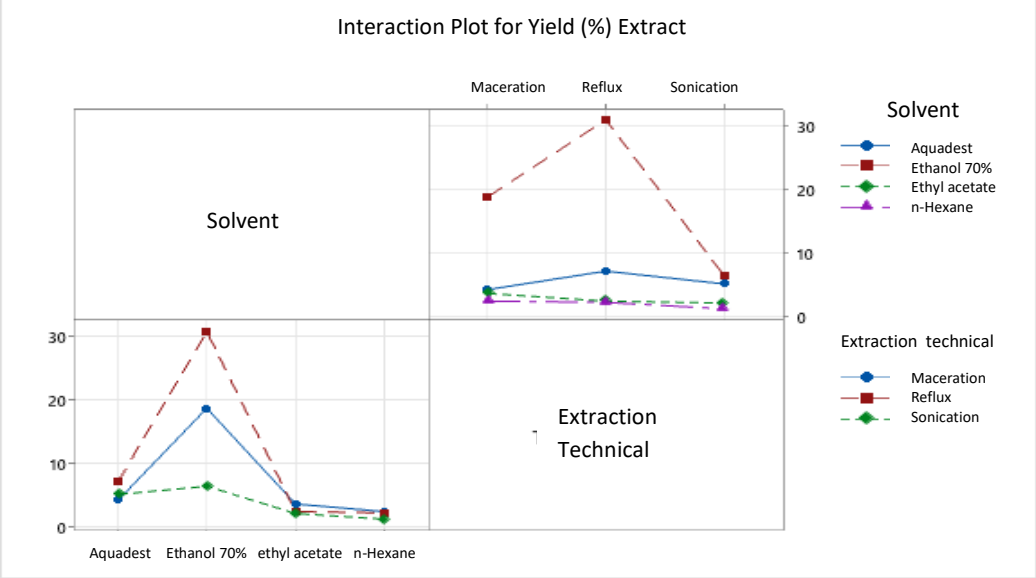

Figure 1. Corelation plot extraction methode (solvent and extraction technical) for yield (%) extract.

Antioxidant activity

Testing of antioxidant activity is one of the analyzes to determine the ability of a natural material to inhibit free radical molecules. In this study, an analysis of DPPH free radicals was carried out to determine the IC_{50} value using UV-Vis spectrophotometric instruments.

Table 2. Antioxidant activity *L. coromandelica* leaves in solvent variant and extraction technical

Sample	Solvent	Extraction technical	Concentration (µg/mL)	Average absorbance (nm)	Percent inhibition (%)	IC_{50} (µg/mL)
L. coromandelica leaves	Aquadest	Maceration	1	0.58	26.92	2.481
			1.5	0.53	32.91	
			2	0.48	40.16	
			2.5	0.42	47.7	
			3	0.36	55.03	
		Sonication	0.5	0.43	44.59	0.819
			1	0.4	52.99	
			1.5	0.31	61.06	
			2	0.24	69.21	
			2.5	0.19	76.05	
		Reflux	0.2	0.66	16.75	1.551
			0.4	0.59	25.50	
			0.6	0.55	31.11	
			0.8	0.42	47.78	
			1	0.36	54.77	
	n-Hexane	Maceration	5	0.67	15.45	38.868
			10	0.63	20.48	
			15	0.6	25.13	
			20	0.55	31.03	
			25	0.51	35.77	
		Sonication	5	0.65	18.34	29.197
			10	0.61	23.99	
			15	0.54	31.62	
			20	0.49	37.86	
			25	0.44	44.43	
		Reflux	5	0.55	15.25	35.669
			10	0.51	20.62	
			15	0.46	28.06	
			20	0.43	33.44	
			25	0.41	36.85	
	Ethyl acetate	Maceration	2	0.55	32.62	6.223
			4	0.47	40.95	
			6	0.40	49.20	
			8	0.34	57.16	

Sample	Solvent	Extraction technical	Concentration (µg/mL)	Average absorbance (nm)	Percent inhibition (%)	IC_{50} (µg/mL)
			10	0.27	65.49	
		Sonication	2	0.54	32.62	
			4	0.47	40.95	
			6	0.4	49.20	6.269
			8	0.34	57.16	
			10	0.28	64.66	
		Reflux	2	0.52	35.01	
			4	0.42	46.94	
			6	0.35	56.45	4.911
			8	0.28	64.82	
			10	0.23	71.52	
	Ethanol 70%	Maceration	1	0.79	18.16	
			2	0.65	33.37	
			3	0.49	49.16	3.317
			4	0.39	59.58	
			5	0.29	69.37	
		Sonication	1	0.75	22.78	
			2	0.64	34.57	
			3	0.49	50.02	3.124
			4	0.36	62.83	
			5	0.27	71.94	
		Reflux	1	0.66	31.79	
			2	0.45	53.58	
			3	0.31	68.45	1.948
			4	0.17	82.84	
			5	0.09	90.20	

The measurement results of antioxidant activity can be seen in table 2. The highest IC_{50} value was obtained using water solvent with sonication technique which reached 0.819 µg/ml compared to other methods. The lowest IC_{50} value was obtained using hexane solvent sonication technique with a value of 38,867 µg/ml. From the measurement results, *L. Coromandelica* leaves have antioxidant activity with a very strong category (IC_{50} value less than 50 g/ml).

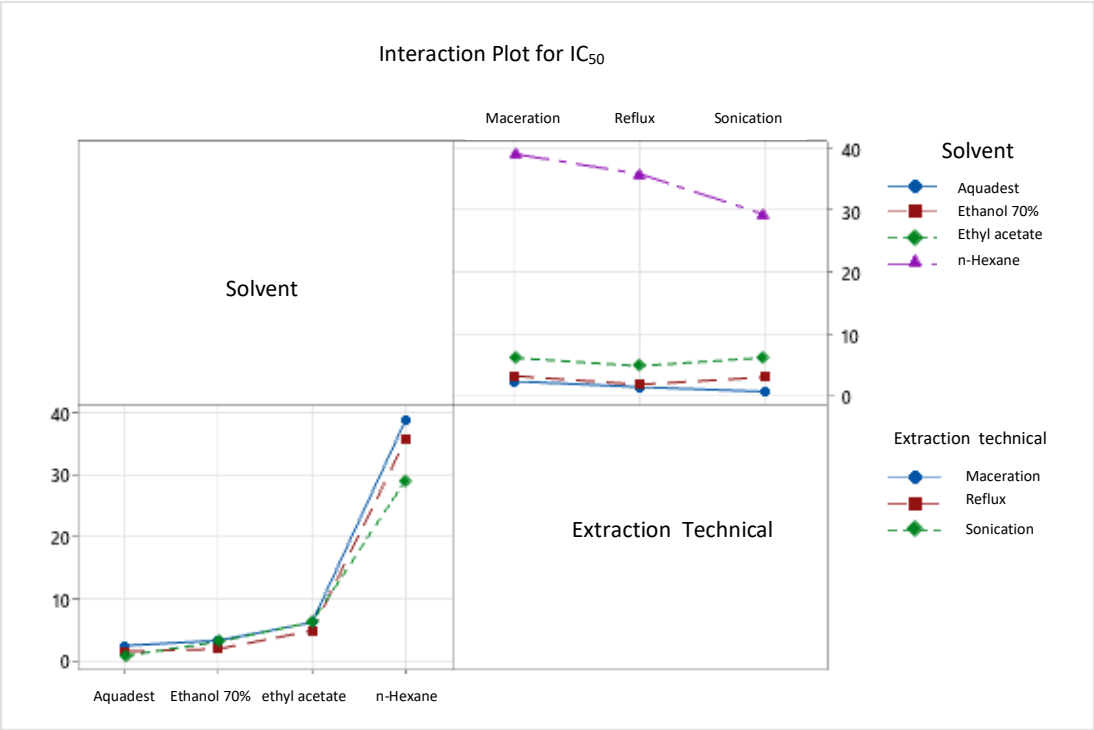

Figure 2. Interaction plot extraction methode (solvent and extraction method) for IC50.

The results of antioxidant measurements were then analyzed statistically one-way ANOVA. The results of the analysis showed the influence of solvents and extraction techniques. Figure 2 shows the effect of solvent and extraction technique on the value of free radical inhibition of DPPH. In the solvent variation, the highest IC_{50} value was obtained using distilled water, then ethanol, ethyl acetate and finally hexane. This can be caused by the presence of secondary metabolites from plants that have more polar activity which causes more polar solvents (aquadest, ethanol, and hexane) to have greater antioxidant activity than non-polar solvents (n-hexane). This is different from the extraction technique.

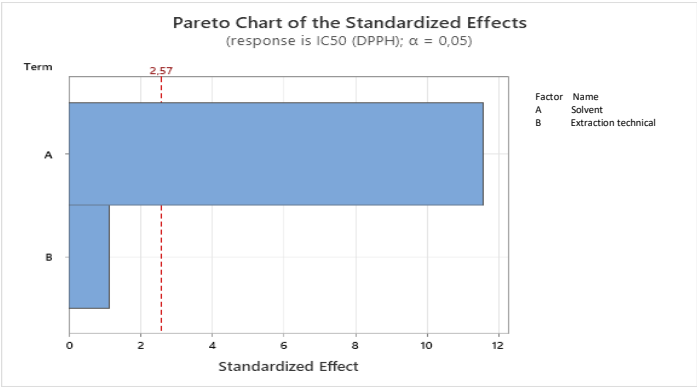

Figure 3. Analysis of the effect of solvents and extraction techniques on the IC50 value in L. Coromandelica extract with a factorial design approach

There was no significant difference in the effect of extraction techniques on antioxidant activity. This is confirmed again by analysis using a factorial design approach (figure 3). From this analysis, solvent variant was showed significant antioxidant value (over by 2.57) compared to extraction techniques. Analitical factorial design P-Value < 0.05 and R square 98.57% indicating that the resulting equation is a valid equation.

CONCLUSION

Optimization percent of yield of the extracts of *L. coromandelica* leaves with variation of solvent and extraction technical showed that the higest result result in term of solvent system was obtained from the extraction by aquadest and follwed by ethanol 70%, ethyl acetate and hexane. There is no significant different by extraction method. Optimization plots percent of the highest extracts of the P-value < 0.05. The factorial model gave 98.57% of R square indicating that the model fits the data relatively well.

ACKNOWLEDGEMENT

The authors would like to thank the STIFA of Makassar for all facilitation and RISTEK DIKTI for funding this research from Penelitian Dosen Pemula (PDP) 2022.

CONFLICT OF INTEREST

The authors declare no conflict of interest

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