Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2016, 8(21):35-39
(http://www.derpharmachemica.com/archive.html)

Extraction of Total Flavonoid Contents and Antibacterial Activities from *Curcuma aeruginosa* RoxB. Rhizome Using Two Level Half Factorial Design

I Made Artika[1], Uswatun Khasanah[1], Maria Bintang[1], Waras Nurcholis[1,2]*

[1]*Department of Biochemistry, Bogor Agricultural University, Bogor, Indonesia*

[2]*Tropical Biopharmaca Research Center, Bogor Agricultural University, Bogor, Indonesia*

ABSTRACT

The rhizome of Curcuma aeruginosa RoxB. was contain flavonoid compounds which potential as antibacterial activities. The objective was to determine factor extraction using maceration method by two level (low and high) half factorial design and followed independent factor: pH (2-6), temperature (30-80°C), ethanol concentration (20-80%), time (30-300 min), and liquid to solid ratio (10-100 mL/g). Total flavonoid content of extract was determined by colorimetric method with aluminum chloride, while antibacterial activity was screened using the disc-diffusion method against Escherichia coli and Staphylococcus aureus. Half factorial design was employed to determine the significant contribution of the factors extraction towards flavonoid content and antibacterial activities. The ethanol concentration ($p<0.01$) and liquid to solid ratio ($p<0.05$) are very significant contribution in extraction of rhizome C. aeruginosa to obtaining higher flavonoid contents (33.36 ± 29.81 mg QE/g extract). The highest of the zones of inhibition in C. aeruginosa extract against E. coli is 1.82 ± 2.40 mm that time extraction was very significant contribution ($p<0.01$), while antibacterial activity in S. aureus is significant contribution extraction by temperature and liquid to solid ratio ($p<0.05$) with highest value 8.75 ± 8.59 mm. Thus, the rhizome extract of C. aeruginosa can be proposed to be used as natural antibacterial.

Keywords: Half factorial design, Flavonoid, *Curcuma aeruginosa*, Antibacterial, Extraction

INTRODUCTION

Curcuma aeruginosa RoxB. is the rhizome medicinal plant that belongs to family Zingiberaceae. Recent years, *C. aeruginosa* has attracted great attention among researchers because of the potential use in medicines. Many pharmacological activities have been reported for *C. aeruginosa*, including antioxidant [1,2] antibacterial and anticandidal [3,4] cytotoxicity and anticancer [5,6] and antiinflammation [7]. Flavonoids are a large group of biological active compounds that potential use as antibacterial activities [8,9]. Extraction is the key step in the recovery active compound from plant materials. Many factors are significant during extraction of active compound, including solvent, solvent composition, pH, solvent to solid ratio, temperature and pressure [10]. Therefore, if all factors used in extraction then time consuming, expensive and results are not accurate since interactive factors are not known [11]. Two level factorial designs are used for estimating main as well as interactive effects during extraction [12]. Several literatures have been reported for factorial screening to screen significant factor for extraction of phenolic and flavonoid from palm [11], and folates in vegetables [13]. No one has done in research extraction to screen significant factor from rhizome of *C. aeruginosa* to get highest flavonoids content and antibacterial activities. Therefore, the objective in this study was to determine independent factors namely pH (2 and 6), temperature (30°C and 80°C), ethanol concentration (20% and 80%), time (30 and 300 min), and liquid to solid ratio (10 mL/g and 100 mL/g) on the recovery of flavonoid contents and antibacterial activity from rhizome of *C. aeruginosa*.

MATERIALS AND METHODS

The rhizomes of *C. aeruginosa* were collected from Bogor, Indonesia in June 2016. The *C. aeruginosa* was botanically

authenticated and voucher specimens were deposited in the Tropical Biopharmaca Research Center, Bogor Agricultural University, Indonesia. The prepare of the rhizome was conducted by the following procedure to get the powder with size of 100 mesh and moisture content <10% [14]. Extraction was carried out based on half factorial design 11 using two levels of each variable (high and low levels) and consisted of five independent factors were pH (2 and 6), temperature (30°C and 80°C), ethanol concentration (20% and 80%), time (30 min and 300 min), and liquid to solid ratio (10 mL/g and 100 mL/g) as shown in Table 1. Briefly, 20 g of the powder was placed in Erlenmeyer and selected based on the experimental design with pH, time, temperature, aqueous ethanol and liquid to solid ratio (Table 2). Extraction was carried out using a water bath shaker (Memmert, Germany). The extract was then filtered using Whatman paper No. 4 and finally concentrated by evaporation (BUCHI, R-250, and Switzerland) at 60ºC. Extract stored in freezer and then used for determining total flavonoids content and antibacterial activities.

Total flavonoid contents were determined by colorimetry using aluminum chloride [15] with modification. Briefly, 10 mg extract was dissolved in ethanol (Merck, German) (10 mg/mL). Then, 120 µL aquadest in the well of 96-well microplate added with 10 µL of extract, 60 µL of methanol, 10 µL of 10% (b/v) aluminium cloride, and 10 µL of 1 M potassium acetate. After 30 min incubation in room temperature, the absorbance of the reaction mixture was measured at 415 nm using microplate reader (Epoch BioTek, USA). The total flavonoid content was determined using the standar quercetin calibration curve (25 µg/mL to 200 µg/mL) and the results were expressed as mg per gram quercetin equivalents (mg/g QE).

Antibacterial activity of *C. aeruginosa* extracts was determined by the agar disc diffusion method against *Escherichia coli* and Staphylococcus aureus using nutrient agar and trypticase soy agar medium, respectively [16]. A 700 µL of freshly prepared inoculum was mixed with 700 mL of nutrien agar (*E. coli*) and trypticase soy agar (*S. Aureus*) and then 20 mL poured into a petri dish. A 20 µL extract samples (10 mg/mL) dropped on the paper discs (approximately 6 mm in diameter) and placed in agar petri dish. The agar petri dishs were incubated at 37ºC for 24 h. The diameters of inhibition zones were measured in millimetres. The inhibition zones of extracts were calculated by reducing total inhibition zone with paper disc diameter (6 mm) used.

Result for total flavonoid content and inhibition zone of antibacterial activity were expressed as means of three independent determinations. The resulting values and statistical analysis were processed using Design Expert 7.0 (Minneapolis, USA). Analysis of variance was employed to find statistical significance of the model.

RESULTS AND DISCUSSION

Results of total flavonoid contents and antibacterial activities from extract samples showed in Table 3. Total flavonoid contents of *C.*

Table 1: Factor levels used in two level half factorial design

Factor	Notation	Factor levels	
		Low (-)	High (+)
Ph	A	2	6
Ethanol concentration (%)	B	20	80
Temperature (°C)	C	30	80
Time (min)	D	30	300
Liquid to solid ratio (mL/ g)	E	10	100

Table 2: Design matrix of two level half factorial design

Run order	Factor				
	A (pH)	B (%)	C (°C)	D (min)	E (mL/ g)
1	2	80	30	30	10
2	2	20	30	300	10
3	6	80	80	30	10
4	6	20	30	300	100
5	2	80	30	300	100
6	6	80	30	30	100
7	6	20	80	300	10
8	2	20	80	300	100
9	2	20	80	30	10
10	6	80	30	300	10
11	2	20	30	30	100
12	2	80	80	30	100
13	6	80	80	300	100
14	2	80	80	300	10
15	6	20	80	30	100
16	6	20	30	30	10

A-pH; B-Ethanol concentration (%); C-Temperature (ºC); D-Time (min); E-Liquid to solid ratio (mL/g)

aeruginosa extracts ranged 2.67 ± 0.88 to 33.36 ± 29.81 mg QE/g extract. The zone of inhibition of *C. aeruginosa* extracts against *E. coli* and *S. aureus* ranged 0.00 mm to 1.82 mm and 0.145 mm to 8.75 mm, respectively. These results are consistent with the literature review indicates that flavonoids have antibacterial properties [17-18]. There are several types of flavonoids that potent as antibacterial activities, included betmidin [8], quercetin [9], balsacones [19], and carboxymethyl flavonoids [20]. The mechanism of flavonoids for antibacterial through by damaging cytoplasmic membrane, inhibiting energy metabolism and inhibiting synthesis of nucleic acids [21]. In addition to mechanism of antibacterial activities, flavonoids inhibit a number of bacterial virulence factors, including quorum-sensing signal receptors, enzymes and toxins [22].

For total flavonoid contents, ethanol concentration and liquid to solid ratio were found significant ($p<0.01$ and $p<0.05$, respectively) in obtaining higher total flavonoid contents of rhizome *C. aeruginosa* (Table 4). Effect of ethanol concentration was the major contributing factor of 19.19% (Table 5), followed by liquid to solid ratio (8.24%), while pH (3.49%), temperature (3.15%) and time (0.69%) have a lower contribution (Table 5). Similarly by comparison of the line plots shown in Figure 1, the effect of ethanol concentration was also shown by the significant gradient of the slope. This is possible because the proportion of ethanol concentration in the extraction medium of plant material can increases the contact surface area between the plant matrix and the solvent, thus resulting in increasing extraction efficiency [23]. The highest zone of inhibition of *C. aeruginosa* extract in *E. coli* was 1.82 ± 2.40 mm with extraction is strongly influenced by the time ($p<0.01$) (Table 4), while the highest of zone of inhibition against *S. aureus* was 8.75 ± 8.59 mm with significant factor extraction influenced by temperature ($p<0.05$) and liquid to solid ratio ($p<0.05$) (Table 4). In comparison with other factors, time has the greatest influence on antibacterial activity against *E. coli* (Percentage contribution, 21.38%) (Table 5 and Figure 1). This condition facilitates extraction efficiency as more bioactive compounds can be extracted under longer period[11]. However, temperature and liquid to solid ratio were the major contributor (7.07% and 9.56%, respectively) on antibacterial activity against *S. aureus* (Table 5 and Figure 1).

Table 3: Results analysis of total flavonoid contents and antibacterial activities of two level half factorial design (*Results analysis (n=3). Y1-Zone of inhibition in *E. coli* (mm); Y2-Zone of inhibition in *S. aureus* (mm); Y3-Total flavonoid contents (mg QE/g))

Run order	Results analysis*		
	Y1	Y2	Y3
1	0.48 ± 0.29	6.35 ± 0.35	12.47 ± 5.67
2	0.30 ± 0.13	0.18 ± 0.26	13.11 ± 3.39
3	0.68 ± 0.10	1.51 ± 0.45	17.08 ± 2.80
4	0.53 ± 0.03	0.54 ± 0.34	7.16 ± 2.04
5	0.15 ± 0.12	0.31 ± 0.15	22.08 ± 7.83
6	1.45 ± 0.003	0.17 ± 0.18	33.36 ± 29.81
7	0.40 ± 0.07	8.75 ± 8.59	6.07 ± 1.06
8	0.00 ± 0.00	0.28 ± 0.25	14.88 ± 6.37
9	1.82 ± 2.40	0.84 ± 0.19	7.70 ± 0.57
10	0.44 ± 0.28	1.15 ± 0.86	18.20 ± 2.97
11	0.57 ± 0.38	0.20 ± 0.13	24.76 ± 3.81
12	1.27 ± 0.23	0.49 ± 0.03	22.12 ± 3.37
13	0.30 ± 0.12	0.47 ± 0.49	12.93 ± 6.63
14	0.35 ± 0.21	4.47 ± 6.64	17.47 ± 3.75
15	0.83 ± 0.42	0.15 ± 0.11	5.73 ± 0.24
16	0.57 ± 0.22	0.79 ± 0.23	2.67 ± 0.88

Table 4: *P* value of results analysis of total flavonoid content and antibacterial activities of two level half factorial design against factors extraction (A-pH; B-Ethanol concentration (%); C-Temperature (ºC); D-Time (min); E-Liquid to solid ratio (mL/g); Y1-Zone of inhibition in *E. coli* (mm); Y2- Zone of inhibition in *S. aureus* (mm); Y3-Total flavonoid contents (mg QE/g))

Factors	*P*-value for results analysis		
	Y1	Y2	Y3
A	0.8570	0.3177	0.1181
B	0.9407	0.6609	0.0007
C	0.4403	0.0434	0.1374
D	0.0013	0.0742	0.4799
E	0.9640	0.0202	0.0192

Table 5: Percentage contribution of factors on results analysis variables (A-pH; B-Ethanol concentration (%); C-Temperature (°C); D-Time (min); E-Liquid to solid ratio (mL/g); Y1-Zone of inhibition in *E. coli* (mm); Y2-Zone of inhibition in *S. aureus* (mm); Y3-Total flavonoid contents (mg QE/g))

Factor	Percentage contribution (%)		
	Y1	Y2	Y3
A	0.06	1.65	3.49
B	0.01	0.31	19.19
C	1.04	7.07	3.15
D	21.38	5.45	0.69
E	0.00	9.56	8.24
AB	0.75	4.90	7.57
AC	5.82	0.40	0.31
AD	1.71	0.98	0.79
AE	3.10	1.60	1.14
BC	0.77	0.45	0.04
BD	0.00	0.61	0.77
BE	4.47	0.46	0.02
CD	2.87	4.28	0.46
CE	2.45	6.71	3.99
DE	0.93	4.39	6.81

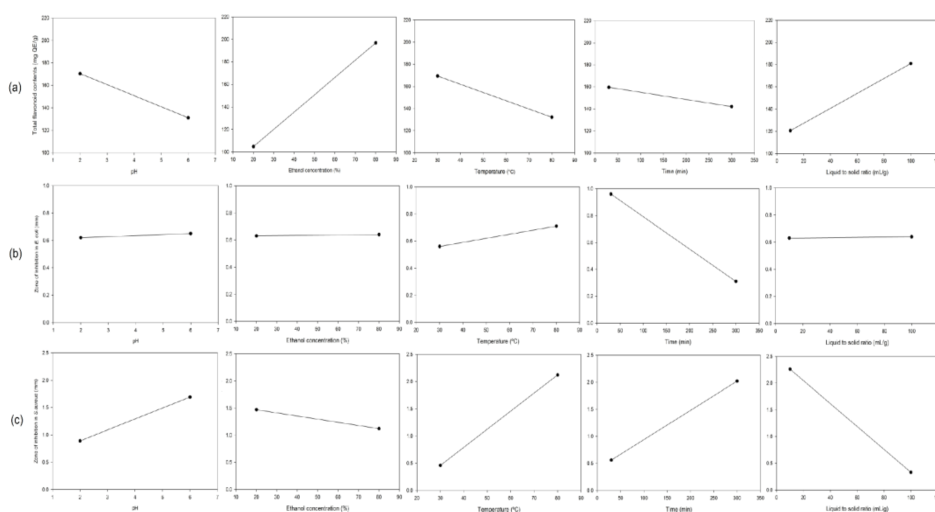

Figure 1: Effect of (A) pH, (B) ethanol concentration, (C) temperature, (D) time, and (E) liquid to solid ratio on the recovery of total flavonoid contents (a), diameter zone of inhibition against E. coli (b) and S. aureus (c)

CONCLUSION

Two level half factorial design was effectively employed to decide significant factors in contributing to high total flavonoid contents and antibacterial activity against *E. coli* and *S. aureus* from rhizome *C. aeruginosa*. From the results obtained, ethanol concentration and liquid to solid ratio were very significant ($p < 0.01$ and $p < 0.05$, respectively) in obtaining higher total flavonoid contents from the rhizome of *C. aeruginosa*. The efficacy of rhizome *C. aeruginosa* extract as antibacterial activity against *E. coli* influenced by time extraction ($p < 0.01$), while on *S. aureus* influenced by temperature ($p < 0.05$) and liquid to solid ratio ($p < 0.05$). The flavonoid types of the rhizome *C. aeruginosa* extracts that has efficacy as antibacterial activity needs to be investigated on further work.

ACKNOWLEDGEMENTS

This study was supported by the Ministry of Research, Technology and Higher Education of the Republic of Indonesia research grant on schema of Riset Unggulan Perguruan Tinggi-Penelitian Unggulan Divisi (503/IT3.11/PN/2016).

REFERENCES

[1] W. Nurcholis, N. Khumaida, M. Syukur, M. Bintang, *Asian. J. Biochem.,* **2016**, 11 (3), 142-148.

[2] M. George, S.J. Britto, *Int. Res. J. Pharm.,* **2015**, 6 (8), 573-579.

[3] S. Jose, T.D. Thomas, *Int. J. Green Pharm.,* **2014**, 8 (1), 65-71.

[4] T.S.A.T. Kamazeri, O.A. Samah, M. Taher, D. Susanti, H. Qaralleh, *Asian. Pac. J. Trop. Med.*, **2012**, 5(3), 202-209.

[5] S. Atun, R. Arianingrum, N. Aznam, S. Nurestri, A. Malek, *Int. J. Pharmacogn. Phytochem. Res.,* **2016**, 8(7), 1168-1172.

[6] W. Nurcholis, N. Khumaida, M. Syukur, M. Bintang, *Asian Pacific J. Trop. Dis.,* **2016**, 6(11), 887-891.

[7] I. Jantan, I.A.A. Rafi, Jalil, *J. Phytomedicine.,* **2005**, 12 (1-2), 88-92.

[8] D.A. Okoth, H.Y. Chenia, N.A. Koorbanally, *Phytochem. Lett.*, **2013**, 6(3), 476-481.

[9] V.G.N.V. Prasad, B.V. Krishna, P.L. Swamy, T.S. Rao, G.S. Rao, *Asian Pacific J. Trop. Dis.*, **2014**, 4, S326-S329.

[10] K.N. Prasad, E. Yang, C. Yi, M. Zhao, Y. Jiang, *Innov. Food Sci. Emerg. Technol.*, **2009**, 10(2), 155-159.

[11] W.H. Wong, W. X. Lee, R.N. Ramanan, L.H. Tee, K.W. Kong, C.M. Galanakis, J. Sun, K.N. Prasad, *Ind. Crops. Prod.,* **2015**, 63, 238-248.

[12] Z. Khiari, D.P. Makris, P. Kefalas, *Food. Bioprocess. Technol.,* **2009**, 2 (4), 337-343.

[13] E. Prado de Paiva, C. Azevedo Filho, S.G. Ferreira, T.L.M. Stamford, J.A. da Paixão, *J. Chromatogr.,* **2012**, 1260, 33-41.

[14] N. Waras, K. Nurul, S. Muhamad, B. Maria, I. Ardyani, I.D.A.A.C., *Int. J. Res. Ayurveda Pharm.,* **2015**, 6(5), 634-637.

[15] C.C. Chang, M.H. Yang, H.M. Wen, J.C. Chern, *J. Food Drug Anal.,* **2002**, 10(3), 178-182.

[16] Wuryanti, Murnah, *J. Sains Mat.,* **2009**, 17(3), 151-158.

[17] L.G. Bahrin, M.O. Apostu, L.M. Birsa, M. Stefan, *Bioorg. Med. Chem. Lett.,* **2014**, 24(10), 2315-2318.

[18] A.C. Thavaranjit, *Der Pharma Chem.* **2016**, 8(3), 218–221.

[19] F. Simard, C. Gauthier, J. Legault, S. Lavoie, V. Mshvildadze, A. Pichette, *Bioorg. Med. Chem.* **2016**, 24(18), 4188-4198.

[20] Z. Zou, P. Xu, C. Wu, W. Zhu, G. Zhu, X. He, G. Zhang, J. Hu, S. Liu, W. Zeng, K. Xu, G. Tan, *Fitoterapia.,* **2016**, 111, 124-129.

[21] A. Ahmad, M. Kaleem, Z. Ahmed, H. Shafiq, *Food Res. Int.,* **2015**, 77, 221-235.

[22] T.P.T Cushnie, A.J. Lamb, *Int. J. Antimicrob. Agents.,* **2011**, 38*(*2), 99-107.

[23] W. Xiao, L. Han, B. Shi, *Ind. Crops Prod.,* **2008**, 43(3), 671-681.