

Optimization of Synthesis Processes Asymmetric Curcumin Analogues From Cullilawan Oil using Response Surface Methodology

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Abstract: Asymmetric curcumin analogues are anticancer compounds that are synthesized from Cullilawan Oil with several stages of the process include: isolation safrole from Cullilawan Oil, safrole isomerization, oxidation, synthesis intermediate product and synthesis asymmetric curcumin analogues. The final stage of the process is the aldol condensation reactions using microwaves method that are affected by the processing time, power and concentration. The purpose of this study is the optimization of synthesis process conditions asymmetric curcumin analogues using response surface methodology and to determine the best model to obtain optimum process conditions. Design of experiments using a box-behnken design with 3 variables (power, time and concentration) and responses used is the yield that is associated with purity. The results showed cubic models give real effect to the value of $F = 7.33$ and p -value 0.0197 . Mathematical model equations derived to predict the relationship between power microwave (X_1), time (X_2) and the concentration (X_3) is $Y = 155,63 - 0,67X_1 - 185,53X_2 + 5,01X_3 + 0,70X_1X_2 - 0,004X_1X_3 - 0,26X_2X_3 + 44,27X_2^2 - 0,045X_3^2 - 0,165X_1X_2^2$. The optimum condition with a predicted yield of 97.47% is the microwave power to 140 watts, the processing time of 1 minute and 46.98 mmol concentrations.

Keywords: RSM, asymmetric curcumin analogues, microwaves method

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I. INTRODUCTION

Product asymmetrical curcumin analog (5-benzene [1,3] dioxol-5-yl-1-phenyl-penta-2,4-dien-1-one) is a product derived from cullilawan oil using several stages of the reaction process. The last stage in the synthesis process is a condensation reaction between intermediate products (3-benzo [1,3] dioxol-5-yl-propenal) with acetophenone in alkaline conditions (Kapelle, 2015a). Cytotoxicity test asymmetrical curcumin analog against breast cancer cells T47D shows that the products obtained using microwaves method have high inhibitory activity with IC_{50} value of 7.247 $\mu\text{g/ml}$ (Kapelle, 2015b). The yield of the synthesis reaction asymmetrical curcumin analogues with the process conditions 140 watts of power, a time of 2 minutes and a concentration of 40 mmol was 82.82% (Kapelle, 2015a). The low yield of the microwave method is influenced by the amount of power, time and concentration of reactants used so that the process needs to be optimized in order to obtain better results.

One method of optimization is Response Surface Methodology (RSM) which is a combination of the methods of statistical and mathematical techniques to optimize the independent variable (Lee, 2013; Ho, 2014). RSM consists of adjustments empirical model to data obtained experimentally (Amdounet al.2010) and is a statistical technique that is effective because it can optimize the complex procedures to investigate variables and interactions of variables simultaneously (Zhao, 2012; Wang, 2013). Use of RSM has been widely used to find the optimum conditions for some processes, such as the extraction of natural materials (Zou, 2011; Wang, 2014; Gomez, 2014), biological processes (Huo, 2014), chemical processes (Ebshish, 2014; Kalantari, 2014) and the environment (Dawood, 2013). RSM analysis techniques to note is the form of the equation, if the function of the order of one or two. The function of order two polynomial equation is used as follows: $Y = \beta_0 + \sum \beta_i X_i + \beta_{2X_2} + \dots + \beta_{kX_k} + \epsilon$. Where X_i is the independent variable that affects the response Y . Value β_0 , β_i , β_{ii} and β_{ij} is the regression coefficient (Vuonget al.2014).

A second order function with three independent variables then there are two design experiments that can be used is central composite design (CCD) (Lai et al.2013) or box-behnken design require fewer experimental units (Goldsmith et al.2014). One of the differences box-behnken design with central composite design is the box behnken no axial / star run so it is more efficient because fewer experimental units (Amdounet

al.2010). This research uses a box-behknen design with three independent variables. The parameter of the visits is a product yield of asymmetric curcumin analogues associated with purity by HPLC of data. Based on the description above, the purpose of this study is the optimization of synthesis process conditions asymmetric curcumin analogues using response surface methodology and to determine the best model to obtain optimum process conditions.

II. MATERIAL AND METHOD

2.1 Material

Cullilawan oils from Maluku-Indonesia, NaOH, KOH, KMnO₄, CH₃COOH, Na₂SO₄, H₂SO₄, Diethyl ether, acetofenone petroleum ether, Dichloromethane, Methanol, Acetaldehyde and Polisorbat (Tween 80).

2.1 Identify Subsections

2.2 Isolation safrole

137.42 g Cullilawan oils was added 40 g of NaOH in 300 mL of Aquades. The mixture was stirred to form two layers, and then the upper layer was separated. The bottom layer was extracted twice with 100 mL of petroleum ether and added to the top layer, then washed with distilled water until neutral and dried with Na₂SO₄ anhydrous. Petroleum ether was separated using evaporator and conducted distillation at reduced pressure.

2.3 Isomerization of safrole

Into a three-neck flask 500 mL size that has been equipped with a magnetic stirrer, thermometer, cooling tube, and blue silica gel. Added 71.56 g (0.44 mol) safrole and 50 g (0.89 mol) KOH. The mixture was refluxed at a temperature of 120 ° for 6 hours, and cooled then added 250 mL of aquades and then extracted with diethyl ether. Results dried with Na₂SO₄ and diethyl ether separated using evaporator. Purification was performed using distilled under reduced pressure.

2.4 Synthesis of piperonal

Into a 250 mL three-neck flask included 2.97g (0.02 mol) isosafrole, 100 mL aquades, 2 mL CH₃COOH, 15 ml H₂SO₄ 50%, 100 mg twin 80 and 100 mL dichloromethane. Further 9.79 g (0.062 mol) KMnO₄ was added about 500 mg every minute, the temperature is < 30 °C by placing in an ice bath. After KMnO₄ added, the flask is heated slowly at 40 °C until the purple color disappeared (15 minutes). The solution is cooled for a few minutes and precipitate MnO₂ filtered using silica gel. Separation of the resulting solution is then poured into a separating funnel and the layers separated. Water layer (upper layer) was extracted with dichloromethane (2 x 30 mL). All organic layers are combined, and then washed with 2 x 30 mL aquades. The organic layer was dried with Na₂SO₄, filtered and evaporated at the evaporator. The residue was added 20% NaOH solution and the mixture was stirred for 30 minutes. Furthermore, the mixture was extracted with dichloromethane, washed with aquades, dried with Na₂SO₄ anhydrous and evaporated back.

2.5 Synthesis of 3-Benzo[1,3]dioxol-5-yl-propenal

An intermediate product done by mixing 16 g (0.4 mol) NaOH, 100 ml aquades and 150 ml methanol. Stirred the mixture, next 8.8 g (0.2 mol) acetaldehyde a mixture is poured into. As many as 30 g (0.2 mol) piperonal poured into mix and stirred for 3 hours. The result then cooled and included in refrigerator for 12 hours. Solids results filtered with filters Buchner and washed with aquades until neutral. Crystals that formed in a recrystallization and analyzed.

2.6 Optimization of synthesis process conditions asymmetric curcumin analogues

A method of microwave by way of mixing 3.2 g (0.08 mol) NaOH, 20 ml aquades and 30 ml methanol. A mixture of stirred, next (0.02-0.06mol) acetofenone poured into the mix and stirred immediately. As many as 7.04 g (0.04 mol) an intermediate product is poured into a mixture of. Included a mixture of resources into the microwave on the level of (140-420) watt with the warming of (1-3) minutes. Then cooled and included in the refrigerator for 12 hours. Strained by the results of the solids Buchner and washed with aquades until the pH neutral. Crystals formed in a recrystallization and analyzed.

III. RESULTS

3.1 Isolation safrole

Safrole can be separated from cullilawan oils by using NaOH. Eugenol and other phenolic components will react with NaOH to form water-soluble salts and formed two layers that can be separated, safrole layer which is not soluble in water are at the top of the mixture. Safrole were then purified using fractionation distillation at pressure reduction. In Fraction 2 at temperatures 90-123 ° C / 1 mmHg obtained safrole with yield 19.30%. The properties of the resulting safrole is a clear liquid form, fragrant, insoluble in water but soluble in ethanol, chloroform and ether. Safrole analysis using gas chromatography obtained with a purity of 89.186% safrole. Infrared spectrum of safrole shows absorption bands in the region 3000-2800 cm⁻¹ which is the absorption Csp₃-H, this was confirmed by the appearance of absorption at 1442.7 cm⁻¹ for -CH₂- (methylene). Uptake range of C=C aromatic absorptions appeared at 1608.5 cm⁻¹ and is supported by absorption at 3150-3000

cm^{-1} which is the absorption band for $=\text{Csp}_2\text{-H}$ (aromatic). Absorption band at 1247 cm^{-1} region and 1041 cm^{-1} shows the range of C-O-C (ether) supported by each tape 916 cm^{-1} and 808 cm^{-1} . Analysis and interpretation safrole $^1\text{H-NMR}$ spectrum (fig.1) are as follows; $\delta = 3.2 \text{ ppm}$ (d, $-\text{CH}_2-$), $\delta = 5.0 \text{ ppm}$ (d $=\text{CH}_2$), $\delta = 5.5$ to 6.2 ppm (m, $=\text{CH}-$), $\delta = 5.9 \text{ ppm}$ (s, $-\text{O-CH}_2\text{-O-}$), $\delta = 6.8 \text{ ppm}$ (m, 3H Ar). Safrole analysis using mass spectrum gives the following description, (m / z): 39, 51, 63, 77, 91, 104, 119, 131, and 162 [$\text{C}_{10}\text{H}_{10}\text{O}_2$] $^+$ (base peak).

3.2 Isomerization of safrole

Isosafrole can be carried on without solvent system using KOH at $120 \text{ }^\circ\text{C}$ for 8 hours and obtained yield 77.56%. The properties of the resulting isosafrole is light yellow viscous liquid and fragrant. Analysis using gas chromatography obtained cis-isosafrole the 3rd peak with a retention time of 3.375 minutes (15.40%) and trans-isosafrole the peak-to-5 with a retention time of 3.700 minutes (69.34%). Infrared spectrum of isosafrole showed absorption at area $3000\text{-}2800 \text{ cm}^{-1}$ which is $\text{Csp}_3\text{-H}$ absorption. Absorption range of C=C aromatic appeared at 1608 cm^{-1} . Absorption band $\text{Csp}_2\text{-H}$ (aromatic) appears in the area $3150\text{-}3000 \text{ cm}^{-1}$, this conclusion is supported by the presence of sharp band with moderate strength at 1490 cm^{-1} . Absorption at 1247 to 1091 cm^{-1} shows the range of the C-O-C. Analysis and interpretation safrole $^1\text{H-NMR}$ spectrum are as follows; $\delta = 1.8 \text{ ppm}$ (d, $-\text{CH}_3$), $\delta = 5.9 \text{ ppm}$ (s, $-\text{O-CH}_2\text{-O-}$), $\delta = 6.3 \text{ ppm}$ (d, $-\text{CH=}$), $\delta = 6.7 - 6.9 \text{ ppm}$ (d, H Ar). Indications of changes in safrolebeisosafrol visible from signal loss ($-\text{CH}_2-$) in area 3.2 ppm safrole and formation methyl signal in area 1.8 ppm.

3.3 Synthesis of piperonal

Piperonal properties produced in the form of white crystals and fragrant, insoluble in water but soluble in methanol (mp $56\text{-}57 \text{ }^\circ\text{C}$). The results obtained by recrystallization using methanol piperonal to yield 65.63%. Infrared spectrum of piperonal obtained their range C=C aromatic appearing on uptake 1604 cm^{-1} is supported by absorption above 3000 cm^{-1} as absorption $\text{Csp}_2\text{-H}$ (aromatic). Absorption area between $3000\text{-}2800 \text{ cm}^{-1}$ which indicates the absorption $\text{Csp}_3\text{-H}$ are reinforced by the presence of absorption 1448 cm^{-1} and 1357 cm^{-1} for methylene group ($-\text{CH}_2-$). Aldehyde group is shown by the presence of a weak absorption in the area twins 2711 cm^{-1} and 2781 cm^{-1} which is very typical for aldehyde compound. This was confirmed by uptake 1689 cm^{-1} which shows the carbonyl group. Absorption band 1249 cm^{-1} , 1099 cm^{-1} and 1037 cm^{-1} shows the compound ether. Besides the loss of the double bond in isosafrole characterized by loss of absorption area at 962.4 cm^{-1} . Analysis and interpretation safrole $^1\text{H-NMR}$ spectrum are as follows; $\delta = 5.9 \text{ ppm}$ (d, $-\text{O-CH}_2\text{-O-}$), $\delta = 6.9 \text{ ppm}$ (d, 1H Ar), $\delta = 7.2 \text{ ppm}$ (d, 2H Ar), $\delta = 9.9 \text{ ppm}$ (d, CH=O). Hints of the data $^1\text{H-NMR}$ is a powerful clue oxidation of the double bond isosafrole is $\delta = 9.9 \text{ ppm}$ peak which is the aldehyde proton unprotected because the induction effect of the carbonyl oxygen atom which is electronegative.

3.4 Synthesis of 3-Benzo[1,3]dioxol-5-yl-propenal

An intermediate product 3-Benzo[1,3]dioxol-5-yl-propenal obtained from condensation piperonal reaction with acetaldehyde having yield 70.28 % .Time retention 12.43 minutes to an intermediate product with purity 23.59 % showed compound with the molecular weight of 176 g/mol. The product that has the highest concentration in the retention 9,58 minute is piperonal who had not participated in react. Synthesis of compounds 3-benzof[1,3]dioxol-5-yl-propenal based on Claisen-Schmidt reaction involving two stages reaction. The first stage is an addition reaction nucleophile stage. At this stage carboanion of acetaldehyde will attack the carbonyl group on piperonal. The results of an addition reaction nucleophile above will experience the transfer of protons from the molecule of water produces β -hydroxyketone. Reaction the second stage is dehydration compound β -hydroxyketone because compound β -hydroxyketonehave atoms H_α against the carbonyl group, so that in the course of an alkali atoms H_α easily off. It is speed up dehydration compound β - hydroxyketone produce products with stable because they have the double bond that conjugated aromatic within the ring. A product produced purity all produce low caused due comparative concentration vested equally among acetaldehyde with piperonal so that in the final outcome there are piperonal that is not be fit react.

3.5 Optimization of synthesis process conditions asymmetric curcumin analogues

Asymmetrical curcumin analogues results condensation reaction acetophenone with intermediate products in the form of orange crystals and data of the 17th treatment can be seen in Table 1.

Table 1.The data Optimization of synthesis process conditions

Run	X ₁ watt	X ₂ Minute	X ₃ mmol	Melting point $^\circ\text{C}$	Purity	Yield
1	420	1	40	122,5	98,808	59,62
2	420	3	40	107,1	94,880	49,65
3	140	2	60	121,7	99,534	51,90
4	280	2	40	122,6	98,619	55,11
5	280	2	40	122,0	98,581	63,73
6	280	2	40	119,9	96,386	71,85
7	280	2	40	122,7	99,521	72,67
8	140	1	40	121,5	99,643	95,60

9	140	3	40	103,7	92,398	65,14
10	140	2	20	123,9	100,00	30,75
11	280	3	60	120,6	99,762	44,04
12	280	1	60	121,4	99,719	69,76
13	280	2	40	118,6	99,216	51,56
14	420	2	20	120,3	96,859	33,70
15	280	1	20	121,0	98,412	30,88
16	280	3	20	112,7	66,352	26,42
17	420	2	60	116,0	98,694	90,43

IV. DISCUSSION

Methods processes using microwave radiation will give the effect of thermal and non-thermal effects in the system (Tellez et al.2011) so that the interaction of the same material can obtain different results. Data shows the relationship between purity with a melting point proportional. The melting point of the solid is the temperature at which the equilibrium between solid phase and liquid phase at a pressure of 1 atm is principally due to intermolecular bonding disconnected. The breakdown of molecules require different temperatures depending on the bond strengths and the presence of impurities.

Response surface methodology is an empirical method that can be used to evaluate the relationship between a group of variables of an experiment and the response is measured based on certain criteria. The variables tested were microwave power, time and concentration while the yield response is observed. Determining the appropriate empirical model in the optimization process of evaluation of the models include linear, quadratic, cubic and 2FI. Results of analysis of variance of the four models show that the model cubic significant effect, with a value of $F = 7.33$ and p -value 0.0197. The influence of each variable (power, time and concentration) showed significant effect on yield viewed from the p -value for the third response of <0.1 . The mathematical equation cubic models that can be used to predict the relationship between power microwave (X_1), time (X_2) and the concentration (X_3) as follows:

$$Y = 155,63 - 0,67X_1 - 185,53X_2 + 5,01X_3 + 0,70X_1X_2 - 0,004X_1X_3 - 0,26X_2X_3 + 44,27X_2^2 - 0,045X_3^2 - 0,165X_1X_2^2$$

The result of normality test error model (normality test) showed that the model errors are normally distributed and independent with relatively homogeneous diversity. Spreading graph model predictions with actual data provides relatively similar results (Figure 1). Response surface contour plot is a visual representation that states the relationship between variables was observed in the response. Effect of variable microwave power, time and concentration to yield asymmetriccurcumin analogues are presented in Figure 2-4.

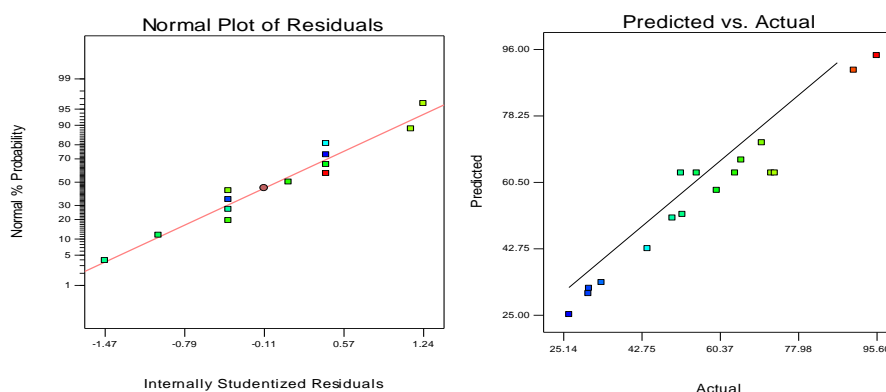


Figure 1. Contour plot (a) test of normality. (b) The model predictions vs. actual data yield

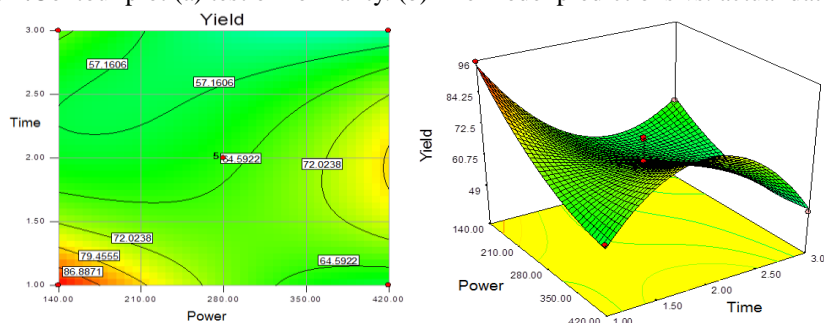


Figure 2. Contour plot (a) surface response (b) the relationship between power and time to yield product

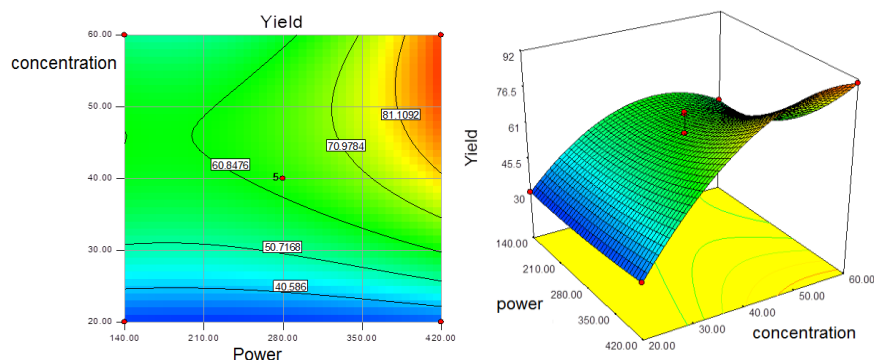


Figure 3. Contour plot (a) surface response (b) the relationship between power and concentration to yield product

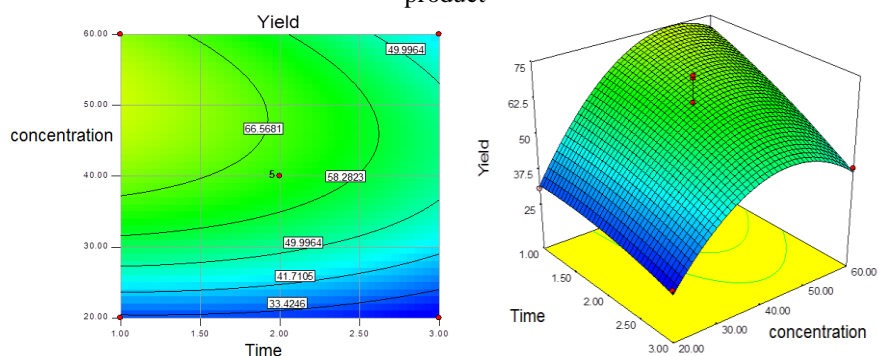


Figure 4. Contour plot (a) surface response (b) the relationship between time and concentration to yield product

Based on the objectives to be achieved to optimize the synthesis process conditions, so that the desired response is optimum. The optimum process conditions with a predicted yield of 97.47% on a 140 watts power, a time of 1 minute and 46.98 mmolconcentration. Validation is done to ensure the optimum conditions of the mathematical equations obtained by response surface methodology and the validation results obtained 96.97% yield (melting point 115.9 ° C). Value differences validation results with the results of model predictions is 0.5%.

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