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Optimization of the Acid Catalyst Concentration for Synthesis of Anti-Cancer Agent Gamavuton-0 by Using Mathematical and Statistical Software

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Abstract

Cancer is a condition that occurs due to abnormal growth of cells in the body. One of the compounds that potentially developed as anticancer agent is Gamavuton-0 (GVT-0). GVT-0 is a curcumin's analog compound. This compound can be synthesized by a reaction of one mole of acetone and two moles of vanillin as starting materials using acid or base catalyst. The main purpose of this research is to determine the best concentration of the acid catalyst for the synthesis of GVT-0. Synthesis was carried out in the round bottle flask with heating. The dissolved of 4.14 gram of vanillin in ethanol was mixed with one milliliter of acidified acetone. The concentration of acid catalyst (HCl) that was used is 2 ppm, 4 ppm, 6 ppm, and 8 ppm of acetone, respectively. The heating was conducted for 1.5 hours and then let it cool in the refrigerator for overnight at 1-10 ^{0}C. After that, the purification process can be conducted. In this purification, the hot water maceration method was used. In the finding of the best concentration of acid catalyst, the mathematical and statistical Stratigraphic Centurion 15 software was used. The analysis that was used in this research is regression polynomial second order. The result of the analysis showed that the maximum GVT-0 produced was 2.66 gram at acid catalyst concentration was 5 ppm of acetone.

Keywords
GVT-0, HCL, catalyst, cancer, regression

1. Introduction

The number of cancer incidence increase in the recent years. National Cancer Institute predicts as much as 1,685,210 new cases of cancer will be diagnosed in the United States and 595,690 people will die because of the disease. Breast, lung cancer and prostate cancer are the most common cancers incident predicted (NCI, 2016). Cancer may be a hereditary diseases (Suryohudoyo, 2004) but incorrect lifestyle such as smoking and consuming instant foods and drinks often increase the incident of cancer significantly (Ministry of Health of Indonesia, 2015).

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Developing of anticancer drug is urgently conducted to help the patient to fight the cancer. One of the new compound that possible to be developed as anticancer is Gamavuton-0 (GVT-0) (Agrawal and Mishra, 2010). Nugroho et.al. (2009) reported that GVT-0 has a cytotoxic activity and anti-proliferation activity on the leukemia basophil cell in rat. Also, GVT-0 has been reported has a higher potency as anti-free radical compared to curcumin (Yuniarti, 2000). GVT-0 is one of the analogues of curcumin with better stability compare to curcumin without losing its anticancer effect. GVT-0 along with other curcumin analogues for several years has been widely studied on the pharmacological effectiveness. The efficacy as an anti-inflammatory agent has been reported to be higher than the curcumin and phenylbutazone (Sardjiman, 2000).

Synthesis of GVT-0 is done by modifying of the structure of the middle of (1,7-diphenyl-1,6-heptadien-3,5-dion) in curcumin into (1,5-diphenyl-1,4-pentadien-3-one) on GVT-0. GVT-0 has a shorter carbon chain bridge than curcumin, carbonyl group and without methylene group. Based on the disconnection analysis of the structure, GVT-0 can be synthesized by reacting two molecules of vanillin and one molecule of acetone. The reaction mechanism follows the reaction mechanism of Claisen-Schmidt condensation (Sardjiman, 2000).

The crucial problem during synthesis is commonly occur on the optimization process to obtain the best value of many parameter affecting the reaction, such as concentration of starting material, temperature, concentration of catalyst, duration of heating, and etc. Recently, many researchers use statistical and mathematical software to overcome the problem, since the conventional method commonly requires time for conducting the experiment and high cost for experimental materials. By utilizing the software, the number and the cost of the experiment will be reduced. In addition, the software can be used to analyze the effect of varying value of parameter studied toward the response by generating a mathematical model and statistical analysis (Harimurti et al, 2012).

Based on the advantages of utilizing of mathematical and statistical software for the optimization process, therefore in this present work the software will be used for determining the best acid catalyst concentration during synthesis of GVT-0.

2. Materials and Methods

2.1. Apparatus

The apparatus that was used for the synthesis of GVT-0 were Beaker glass (Pyrex), test tubes (Pyrex), Erlenmeyer (Pyrex), round-bottom flask (Pyrex), funnel, porcelain bowls, condenser, magnetic stirrer CIMAREC®, heat mantle KDM-1000®, filter paper, 1 and 5 ml pipette volume, pipette filler, white tip, pH universal indicator, micropipette Socorex Swiss, silica gel GF254 TLC plate, TLC chamber, and computers equipped with Portable software Statgraphics Centurion.

2.2. Materials

During experimental work, the materials that were used in the synthesis of GVT-0 were aniline technical grade (Brataco®), acetone for synthesis (Sigma Aldrick®), concentrated hydrochloric acid 37% (Merck®), glacial acetic acid for analysis (Brataco®), Na sulfate anhydrous for analysis (BRATACO ®), Chloroform for analysis (Merck®), ethanol for analysis (Sigma Aldrick®) and distilled water (Brataco®)

2.3. *Synthesis of Gamavuton-0 (GVT-0)*

Synthesis of GVT-0 was carried out in a round bottle flask with condenser by varying the acid catalyst concentration (2 ppm, 4 ppm, 6 ppm, and 8 ppm, respectively) while keeping constant of heating period (1.5 hours), heating level (4.5 scale) and ratio of vanillin and acetone (2:1). Vanillin that was used as much as 4.141 grams and acetone as much as 1 ml. Before reaction, Vanillin was dissolved in the ethanol 95% using a magnetic stirrer until dissolved. In the other hand, a designed value of concentrated HCl (acid catalyst) was added into acetone, separately. The HCL that was added was 20 µL, 40 µL, 60 µL and 80 µL for 10 ml of acetone, respectively. Thus the concentration of HCl was 2 ppm, 4 ppm, 6 ppm and 8 ppm, respectively. Further, 1 ml acetone was taken from the mixture of acetone-HCl and then added into dissolved vanillin in a round bottle flask that was prepared with condenser. Heating at scale of 4.5 was setup. Cooling was done by turning on the water cooling through condenser. The duration of reaction was stoped after 1.5 hours and the end of the reaction can be identified by forming a brownish yellow solution. The obtained solution was cooled in the refigerator at 1-10^{o}C for 12 hours.

2.4. *Purification of GVT-0*

The GVT-0 purification was carried out using maceration method. The yellowish crystal formed after cooling then dissolved using chloroform and then transferred into a separating funnel for settling up. The chloroform phase was further transferred into evaporating porcelain. Removing chloroform and trace water was done by adding Na-Sulfate anhydrate and then putting the evaporating porcelain on the boiling water bath until getting a greenish-black residue. After that, the residue was added glacial acetic acid and then added with distillated water until forming a greenish yellow crystalline compound. The crystal was purified one more time to get purer crystal by hot water maceration process at 70-80 ^{0}C. Last the brownish yellow crystal then filtered for drying and weighing.

2.5. *Purity analysis*

The purity analysis was performed using Thin Layer Chromatography (TLC). The purified GVT-O and vanillin were elucidated on the Silica gel GF 254 plate. Mobile phase that was used is chloroform and ethyl acetate at ratio 5:1 (Fahrurozi, 2008). The distance of elucidation was 8 cm. The detection of the spot was conducted using visible light and UV light at λ 254 and 366 nm, respectively. The retardation factor (Rf) of the spot was calculated using Equation (1) as follows:

$$Rf = \frac{mileage\ of\ sample}{mileage\ of\ mobile\ phase} \quad (1)$$

2.6. *Optimization of acid catalyst concentration*

Optimization of the acid catalyst concentration was performed by utilizing mathematical and statistical software named Statgraphics Centurion software. Regression polynomial second order of the data obtained was performed to determine

the correlation model between acid catalyst concentration and the GVT-0 produced as well as to determine the optimum catalyst concentration for the maximum of GVT-O produced. Quality of the fit data to the second order equation was expressed by determination of R^2. The statistical significance (P-value) was analyzed using analysis of variance (ANOVA). In this experiment, the P-value was designed not more than 10%. The P-value was designed at this high since the synthesis through very complicated process.

3. Results and Discussions

Vanillin and acetone as starting materials were selected based on the disconnection analysis of GVT-O structure. The GVT-0 can be divided into one molecule of acetone and two molecules of vanillin. Methyl group of Acetone will be connected to carbonyl group of vanillin to form a C = C bond through Claisen-Schmidt reaction mechanism. Acid or base are commonly used as the catalyst of the reaction (Fessenden and Fessenden, 1999; Tonnesen and Karlsen, 1985). Modified Samtisar method was used during the synthesis of GVT-O. Fahrurozi (2008) reported that the modification of the method Samtisar had done by changing the reaction procedure, i.e. the crystal of vanillin was firstly dissolved in the 95% of ethanol before reaction. The previous procedure was conducted by direct mixing of vanillin crystal into the acidified acetone.

Synthesis of GVT-0 was started by calculating the required starting materials (vanillin and acetone) for reaction. Theoretically, the GVT-0 is developed from two molecules of vanillin and one molecule of acetone (2:1). Based on the theoretical ratio of starting materials, the 4,141 grams of vanillin and 1 ml of acetone were used. In this synthesis, hydrochloric acid (HCl) 37% (concentrated) was used as the catalyst. The main purpose of acid catalyst in the reaction is for reducing the electronegativity of carbonyl group of acetone. Therefore, the possibility of the reaction is easier to be done. Acid catalyst is more favorable since the reaction process is more stable compare to the base catalyst (Fessenden and Fessenden, 1999). The yellow-green crystal of GVT-O obtained from the synthesis can be seen in Figure 1. This result is in accordance with the previous research that was conducted by Fahrurozi in 2008.

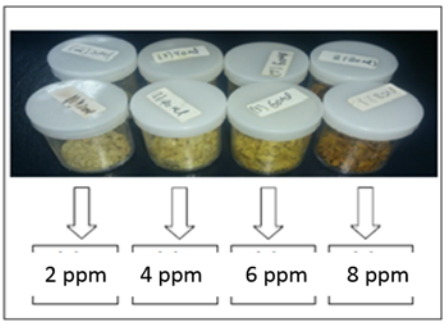


Figure 1. GVT-0 Crystal

Identification of the purity of GVT-O from the synthesis was analyzed using Thin Layer Chromatography (TLC). The TLC result can be seen in Figure 2. In the Figure 2 can be observe that the GVT-0 produced still impure, where the vanillin as the starting material still found. This may happen because of the purification process not completely achieved. The vanillin contamination can be identified from the spot on the TLC silica, which is containing two spots (GVT-0 and vanillin). The Rf of GVT-O was found to be 0.5. This Rf was the same with the Rf for GVT-O reported by Fahrurozi (2008).

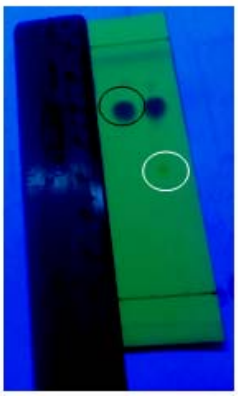


Figure 2. TLC of GVT-0 (yellow) and vanillin (purple) as the standard

The GVT-O obtained from the synthesis at varying HCl concentration was arranged in Table 1.

Table 1. Obtained GVT-0 from the synthesis

Sample	Vary of HCl concentration (ppm)	GVT-O (g)	Average (g)
1	2	0.49	0.50
2	2	0.51	
1	4	2.25	2.48
2	4	2.99	
1	6	2.54	2.39
2	6	2.25	
1	8	0.75	0.76
2	8	0.77	

Table 2 is the ANOVA result of the obtained data from the experiment. ANOVA was conducted to determine the variation of the data. The significant variation of the data can be evaluated from the P-value. The P-value indicates the significance of the relationship between independent parameter and the response variable (Daneshvar, 2007). Based on the ANOVA results, there is significant difference between the data that has been obtained. The significant difference can be identified from the P-value generated from the analysis, i.e. 0.0633 (P-value). This result is less than the acceptable quality level (AQL) of ANOVA that was set up at P-value of 0.1. That means there is a significant relationship between the variations of acid catalyst concentration and GVT-O produced. In the other word, by changing the acid concentration significantly change the GVT-0 produced.

Table 1. Result of analysis of variance (ANOVA)

Source	Sum of squares	df	Mean square	F-Ratio	P-value
Model	3.28037	2	1.64018	120.90	**0.0633**
Residual	0.0135668	1	00135		
Total(Corr.)	3.29394	3			

Regression analysis was chosen as a method for hypothesis testing based on its ability to measure of the strength of the relationship between the response variable and the predictor variables, determine the influence of one or several variables predictors of response variables, and was useful for predicting the effect of a variable or multiple response variables (Iriawan and Astuti, 2006).

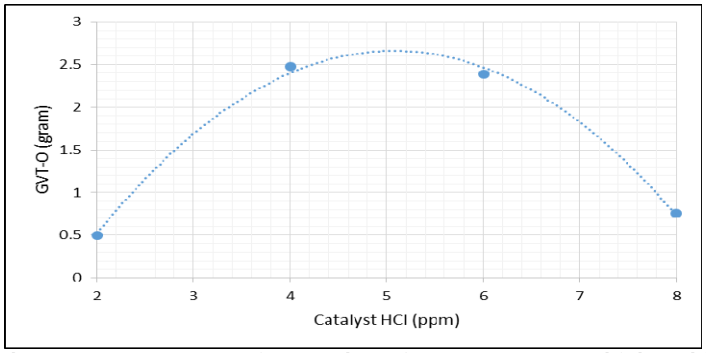


Figure 3. Plot of catalyst concentration vs GVT-O (R-squared = 99.5881 percent; R-squared (adjusted for df.) = 98.7644)

Correlation of the acid catalyst concentration (X) and the GVT-0 produced (Y) can be plotted as shown in Figure 3. The R-Squared value of the plot data was found to be 99.5881, means that the GVT-0 produced following the mathematical model generated very well. The analysis using regression polynomial second order gave an equation as expressed as Equation 2. The use of the equation is for predicting the GVT-O produced once the concentration of catalyst is known at the experiment set up as mentioned above. When the Equation 2 assumed to be $Y = aX^2+bX+c$, the highest value of Y will be reached when the value of $X = -b/2a$. Based on the Equation 2, calculation of the optimum value of X was found to be 5.08 ppm.

$$Y = -0.2256X^2 + 2.2908X - 3.1525 \quad (2)$$

Figure 3 shows that by increasing the catalyst concentration increase the GVT-O produced. At the optimum concentration of catalyst, the GVT-O produced was the highest one, this may due to at the optimum concentration the fastest reaction rate was occurred. Still increase the catalyst concentration at 6 ppm and 8 ppm, the rate of the reaction decreasing. The decrease of the rate of reaction can be detected by reducing the GVT-0 produced.

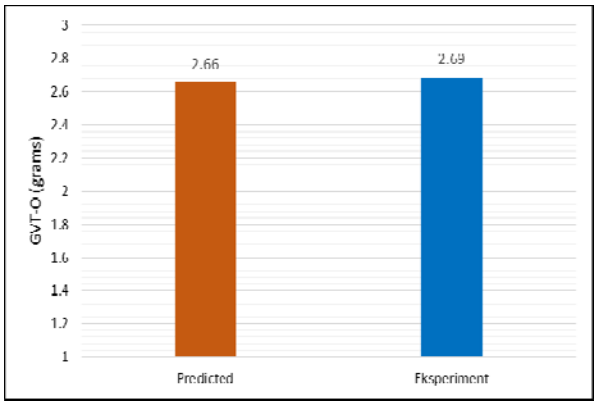
Equation 2 can be used to predict the theoretical amount of GVT-0 produced once the acid catalyst concentration is known. By using Equation 2, GVT-0 predicted at optimum acid catalyst concentration of 5.08 was found to be 2.66 grams. The calculation process can be conducted as follows:

$Y = -0.2256X^2 + 2.2908X - 3.1525$

$Y = -0.2256(5.08)^2 + 2.2908(5.08) - 3.1525$

Y = 2,66 gram (predicted)

Validation of the equation can be conducted by carrying out an experiment using a theoretical condition suggested. During the experiment using 5.08 ppm of acid catalyst concentration, the GVT-0 produced was 2.69 grams. The comparison between the predicted to the experimental results can be seen in Figure 4.



The difference between predicted and experiment of GVT-O produced can be calculated using Equation 3.

$$\text{The diference} = \frac{\text{experiment} - \text{predicted}}{\text{predicted}} X100\% \quad (3)$$

The difference of GVT-0 produced is 1.22%. This indicates that the equations obtained can be used to predict the number of GVT-0 desired when the acid catalyst concentration is known. This data will be very useful for further optimization process on finding the other optimum condition of parameter during the synthesis. Surely, this result will be very useful in proposing of production in the industry with larger scale.

Conclusion

Based on the experimental results, by using mathematical and statistic software the experiment was more efficient and the best acid catalyst concentration was found to be 5 ppm at the setup of experimental condition.

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