Optimization of Phenolic in Extraction Kasturi Mango Leaves (*Mangifera casturi* Kosterm.) with Taguchi Method

Kasandra Budiarni[1*], *Irni* Furnawanthi Hindaningrum[1], *Roni* Kartiman[1], *Maily*[2], *Sophie* Anggita Raharjani[2], *Fauziah* Sri Pambayun[2], *Aufadil* Rahmadi Putra[2], *Herly* Angga Valentino[2], and *Dwi* Putri Sulastry[2]

[1]Research Center for Plant Conservation, Botanic Gardens, and Forestry, Nasional Research and Innovation Agency (BRIN), Cibinong, West Java, Indonesia
[2]Laboratory Plant Research & Raw Material Standardization, Plant Research and Extract Development, PT Martina Berto Tbk, East Jakarta, Indonesia

Abstract. Kasturi mango (Mangifera *casturi* Kosterm.) is one of specific germplasm in South Kalimantan which in the category threatened with extinction. This plant has active metabolites such as phenolic groups which potentially to be bioactive. The active substance in the extract is usually obtained using the extraction method. Extraction is a method widely applied to the separation of compounds from plants to be used as raw materials products. Selection of the appropriate solvent and method is important to get optimal compound. The purpose of this study was to optimize phenolic extraction from Kasturi mango leaves using Taguchi method. The research method used consisted of 9 treatments with factors of temperature, time, and ethanol concentration. The extraction process was carried out on wet and dry samples of Kasturi mango leaves using digestion method with 50% ethanol at 50 ºC for 60 minutes. The result of determining the optimal solid solvent ratio (SSR) was get on dry samples with SSR ratio 1:5 and phenolic content 16995.8 mg/L. For the results of optimization phenolic extraction of dry samples Kasturi mango leaves with Taguchi method, the highest phenolic content obtained was 19407.8 mg/L with use of 30% ethanol at 70 ºC for 60 minutes.

1 Introduction

Indonesia has high ecosystem diversity, including various climates, soil types, and other environmental factors. According to National Geographic Indonesia (2019), Indonesia's mainland biodiversity ranking is number two after Brazil. Biodiversity on earth can use as food, shelter, clothing, drugs, or just to enjoy its beauty. Most of the plants in Indonesia have effective benefits as raw materials for the drug industry, one of which is the Kasturi mango plant (Mangifera *casturi* Kosterm.).

* Corresponding author: kasandra.budiarni@brin.go.id

Kasturi mango (Mangifera *casturi* Kosterm.) is a plant from South Kalimantan. Kasturi mango was classified in the IUCN Red List Categories on November 30, 1994. A team assessment from the World Conservation Monitoring Center in 1998 decided that Mangifera *casturi* was in the extinct in situ category or Extinct in the Wild (EW). Kasturi mango plants have not been widely cultivated because the fruiting time is very long. All this time, people use only fruit for consumption because sweetness and distinctive aroma, while other plant parts such as stems, roots, leaves, seeds and fruit skins have not been used optimally [20].

Kasturi mango leave is known as traditional medicine because of its flavonoid and phenolic compounds as antioxidants [2]. Because there is a linear relationship between total phenolic levels and antioxidant activity [33]. Research [21] states the higher total phenolic content so the higher antioxidant activity. Total phenolic content can be determined by spectrophotometric method. This method is cheap, easy and acceptable method compared to the chromatographic method [18]. Colored complex compounds formed from reactions between reactants and phenolic compounds or flavonoids will be measured by absorbance using spectrophotometry to obtain total phenolic content or total flavonoid content [8].

Extraction method selection is important to determine success rate extraction results [14]. The proper method for several applications is Taguchi experiment which can improve quality [7, 32]. Taguchi method is one of which Off-line Quality Control methods proposed by Dr. Genichi Taguchi (1949). Taguchi method is a new methodology in engineering field to improve product quality and reducing costs to a minimum of product manufacturing processes [5]. Therefore, in this study, Kasturi mango leaves extraction has been optimized using the Taguchi method, so that extract phenolic content is expected to be useful as an alternative source of natural medicinal ingredients.

One of the studies was found that kasturi mango leaves contain phenolic compounds such as flavonoids, tannins and triterpenoids [10]. Meanwhile, this research used the Taguchi method to optimize the phenolic extraction of kasturi mango leaves. The advantages Taguchi method make an effective and efficient tool in experimental design and process optimization in various industrial and research fields.

In Taguchi method begins with designing extraction parameters of Kasturi mango leaves using an Orthogonal Array factorial design. Then, the Signal Noise Ratio (S/N) was determined to identify factors that affect experimental results [16]. Analysis of Variance (ANOVA) was carried out to determine effect on each input parameter from experimental results and interpret experimental data [6]. By Taguchi's experimental design will be known as extraction optimum conditions of Kasturi mango leaves so the extract has phenolic content high level.

2 Materials and Methods

2.1 Materials

Kasturi mango leaves (Mangifera *casturi* Kosterm.) were collected from the Bogor Botanical Gardens, ethanol p.a (J.T. Baker), gallic acid (Sigma), Folin-Ciocalteu (Merck), NaOH (Merck), and aquademin.

2.2 Methods

This research is experimental including sampling, extraction and phenolic testing. This research was conducted at the Raw Material, Development & Standardization Laboratory of PT Martina Berto Tbk.

2.2.1 Sample preparation

Mango leaves were cleaned with tissue/cloth and chopped/cut into smaller sizes. For dry samples, put them in the oven at 50 ºC until the moisture content is <10% M while wet samples can be directly extracted.

2.2.2 Determination of Solid Solvent Ratio (SSR) optimal

Wet and dry samples were weighed according to SSR of 1:5; 1:10; and 1:15. Then extracted in a water bath at 50 ºC with 50% ethanol for 60 minutes. Extraction results were filtered to produce a filtrate (extract). The extract was put into a dark glass bottle, then analyzed for phenolic content.

2.2.3 Measurement of Gallic Acid standard solution

Measurement of standard gallic acid solution was carried out by making a series concentration of 3.125; 6.25; 12.5; 25; 50; 100; 200 and 400 ppm. Pipette 20 µL of each solution, add Folin-Ciocalteu solution and homogenize. NaOH solution was added as much and incubated for one hour. The absorbance of the solution was measured at λ 730 nm with microplate reader [9]. Then obtained the gallic acid calibration curve and the linear equation $y = bx + a$.

2.2.4 Analysis of phenolic content

The extraction method is used digest extraction. Kasturi mango leaves were weighed according of optimal SSR. Then the sample was digested with temperature, time, and solvent according to Table 1. Extraction results were filtered to produce a filtrate (extract). The extract was analyzed for phenolic content with a microplate reader (with modification from references). Pipette 20 µL of extract, add Folin-Ciocalteu solution and homogenize. NaOH solution was added as much and incubated for one hour. The absorbance of the solution was measured at λ 730 nm with microplate reader. Each treatment was carried out in triplicate. Absorbance of Kasturi mango leaves extract is measured against standard curve of gallic acid, which will be converted to Gallic Acid Equivalents (GAE) [3, 19].

2.2.5 Design parameters

This research method uses Taguchi's experimental design. Table 1 shows the level values of each factor which are determined based on values commonly used in extraction process. Number of rows in the orthogonal array table determines number of experiments, while the number of columns determines forming factors [5].

Table 1. Control Factors (Control)

Factor	Level 1	Level 2	Level 3	Unit
Temperature	50	60	70	ºC
Time	30	60	90	(minute)
Concentration ethanol	30	50	70	(%)

The requirement for Orthogonal matrix selection is experimental value is equal to or more than the degrees of freedom. The orthogonal array used consists of 3 levels and 3 factors, so the degrees of freedom are 6, then choose the orthogonal array matrix. The corresponding orthogonal array notation is L_9 (3^3) according to Table 2.

Table 2. Ortogonal Array $L_9(3^3)$

No.	Factor A	Factor B	Factor C
1.	1	1	1
2.	1	2	2
3.	1	3	3
4.	2	1	2
5.	2	2	3
6.	2	3	1
7.	3	1	3
8.	3	2	1
9.	3	3	2

2.2.6 Analysis of Variance (ANOVA)

Analysis of Variance (ANOVA) is a method for finding optimal level settings to minimize variance deviations [12]. The data is tested and processed statistically using Minitab 19. Test results show that the probability/significance, if the probability/significant level is less than 0.05 (< 5%) so level of hypothesis is accepted/proven/there is an effect.
Captions should be typed in 9-point Times. They should be centred above the tables and flush left beneath the figures.

3 Results and Discussion

3.1 Sample preparation

Kasturi mango leaves that have been collected from the Bogor Botanical Gardens, then cleaned with tissue or cloth to remove grime attached. Mango leaves were chopped as small as using scissors or knife to facilitate drying. The purpose of chopping is to obtain small particle size. With small particle size, the surface area of simplicia can contact solvent larger and make solvent penetrate simplicia easily, so the extracted result will also be optimal. Drying was carried out in oven at 50 ºC until moisture content < 10 %M. The results drying during ± 6 hours reached 5.179% M. The purpose of drying is to reduce the water content. If simplicia still contains water, fungi and other microorganisms grow easily during storage [9].

3.2 Determination of Solid Solvent Ratio (SSR) optimal

The extraction method used the digestion method. Digestion is a kinetic maceration with temperature higher than room temperature which is generally at 40-50 ºC [24]. Solid Solvent Ratio (SSR) is ratio between solute and solvent. SSR treatments used consist of 1:5; 1:10; and 1:15. Extraction was carried out at the same temperature, time and concentration of ethanol, namely 50 ºC (measured with a thermometer) for 60 minutes (calculated with a timer) and extracted with 50% ethanol. In this study, evaporation was not carried out because this is preliminary research to determine optimal extraction conditions. Next, by using optimal conditions, a thick extract can be made that can be characterized.

Based on Table 3, the phenolic content in dry sample of Kasturi mango leaves was greater than wet sample. The wet sample still contains water, thus affecting the weighted sample. The highest phenolic content was obtained in dry samples with SSR 1:5, namely 16,955.8 mg/L (ppm). Based on data [20], the extract of Kasturi mango leaves with 96% ethanol has 18.44% of total phenolic compounds (highest levels), when compared to the bark and skin. Based on data [1] Mangifera contains potential bioactive and one of the producers of high antioxidants. Because phenolic and flavonoid compounds play a role in producing bioactive activity. The phenolic content in genus Mangifera includes gallic acid, α-tocopherol, 3-methylgalate, propyl gallate, and mangiferin [15, 27]. This genus also contains several types of flavonoids, including kaempferol 3-O-glucoside, quercetin 3-β-D glucoside, epicatechin catechins, daidzein and genistein [13, 15, 29].

Table 3. Determination of SSR Optimal

No.	SSR	Temperature (ºC)	Time (minute)	Concentration ethanol (%)	Phenolic Content (mg/L)	
					Wet sample	Dry sample
1.	1:5 (1)	50	60	50	5218,4	16955,8
2.	1:10 (1)	50	60	50	2092,2	7415,4
3.	1:15 (1)	50	60	50	1121,2	3419,2

3.3 Measurement of Gallic Acid standard solution

The phenolic content analysis method used the Folin-Ciocalteu colorimetric method, which has the principles of reduction and oxidation. The reaction shows in Figure 1 that the phenolic compound with the phosphotungstate-phosphomolybdate (Folin-Ciocalteu) will form a yellow color, and when added with a base (NaOH) it will form a blue color [17]. The darker blue color formed, phosphotungstate-phosphomolybdate complex more reduce. Gallic acid standard is a phenolic acid group compound that can react, so it is used as a standard curve. The standards used consist of 8 concentrations, namely 3.125; 6.25; 12.5; 25; 50; 100; 200; and 400 mg/L.

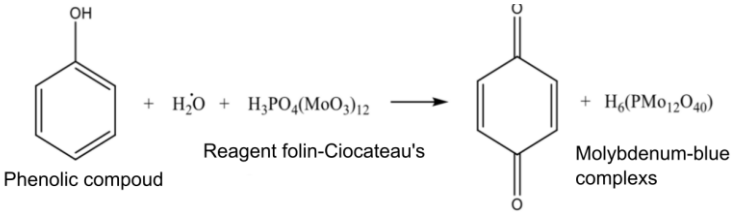

Fig. 1. Reaction of phenol with Folin – Ciocalteau's reagent

Standard series measurements are shown in Figure 2 so the relationship between concentration and absorbance can be identified. After measuring, we get a linear regression equation y = 0.0044x + 0.006 with a correlation coefficient r = 0.9996 and a coefficient of determination R2 = 0.9992. This means 99.92% of absorption is affected by concentration, while the rest is influenced by other factors such as temperature, light and so on. The concentration sample solution can be determined using linear regression equation [23].

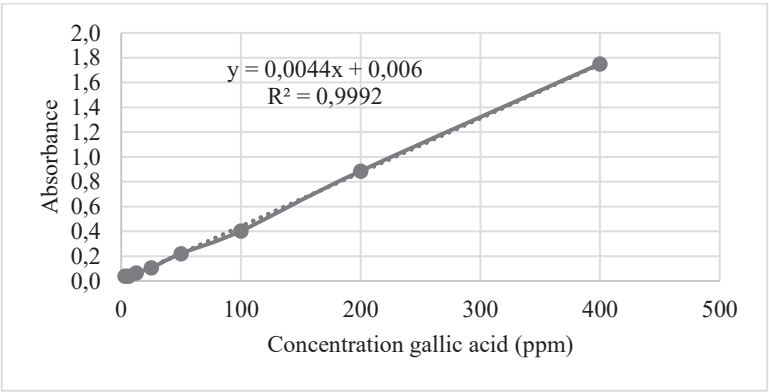

Fig. 2. Effect of Absorbance and Gallic Acid Standard Concentration

3.4 Experimental Implementation

Based on the determination of SSR optimal, the highest phenolic content was found in dry samples with SSR of 1:5. Then dry samples were extracted at various factors and levels using the Taguchi method according to Table 4. Temperature, time, and solvent concentration are the general parameters in the extraction process that can affect the resulting extract (phenolic content). The Taguchi method is one of the Off-line Quality Control methods proposed by Dr. Genichi Taguchi (1949). The Taguchi method is used to improve product and process quality, optimize product and process design, and can reduce costs and resources to a minimum [4]. The Taguchi method uses a special matrix called the Orthogonal Matrix or Orthogonal Array (OA). This standard matrix is a step to determine the minimum number of experiments. The most important part of the Orthogonal Matrix lies in selecting the combination of variable levels for each experiment [28]. Number of factors and levels will affect orthogonal matrix selection as the basis experiment. The orthogonal matrix in experimental design is based on the total degrees of freedom of the factor parameters and levels. Calculation total degrees of freedom use the following equation:

DoF total = (Number of levels – 1) × Number of factors (1)

Table 4. Experimental Data Results

No.	Factor						GAE (mg/L)
	A	B	C	Temperature (ºC)	Time (minute)	Concentration ethanol (%)	
1.	1	1	1	50	30	30	15674.2
2.	1	2	2	50	60	50	16955.8
3.	1	3	3	50	90	70	13414.1
4.	2	1	2	60	30	50	12731.1
5.	2	2	3	60	60	70	15866.2
6.	2	3	1	60	90	30	17612.4
7.	3	1	3	70	30	70	12296.7
8.	3	2	1	70	60	30	19407.8
9.	3	3	2	70	90	50	16452.0

This study uses three factors where each has three levels so the total degrees of freedom are six. The total degrees of freedom on the orthogonal matrix must be greater than or equal to the parameters of the degrees of freedom. The orthogonal matrix L_9 (3^3) is chosen as the experimental design. In Taguchi method, there are combination parameters that will be evaluated to get the optimal combination. Table 4 shows the experimental design and phenolic content results from the extraction process of the dry sample. From these data, the optimum factors were calculated with response characteristics of phenolic content average as shown in Table 5.

Tabel 5. Response Table for Means

Level	Temperature (ºC)	Time (minute)	Concentration ethanol (%)
1	15348	13567	17565
2	15403	17410	15380
3	16052	15826	13859
Delta	704	3843	3706
Rank	3	1	2

From Table 5, the effect of level on average phenolic content can be interpreted in graphical form in Figure 3.

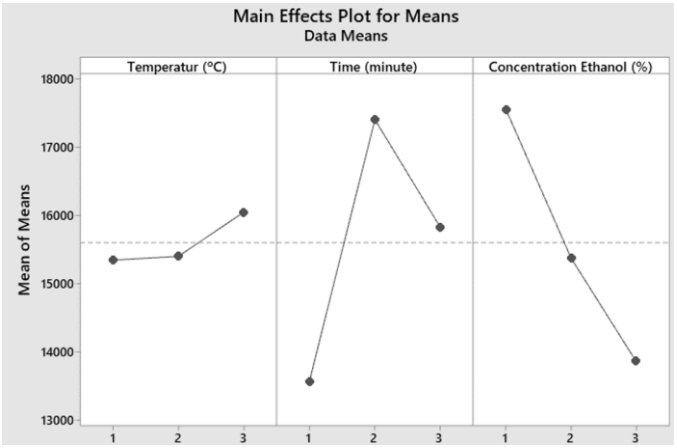

Fig. 3. Effect of Level on Average Response

3.5 Calculation of Signal-to-Noise Ratio

The Signal to noise ratio (SNR) is carried out to measure level of quality sensitivity from each controlled factor to the influence of uncontrolled external factors [26]. The Signal to Noise Ratio is a way to see distribution characteristics and the influence of factor characteristics in each experiment [5]. SNR value is obtained from the transformation results of several data loops so the value represents the presentation quality of variations [22]. Smaller is better, Nominal is the best, and Higher is better are three SNR characteristics according to Taguchi [30]. The extract result quality can be seen in high phenolic content. The higher phenolic content, the higher bioactive (antioxidant) potential of the extract. Therefore, the objective function that will be used in this research is larger is better. This can be seen in Table 6. From these data, the optimum factors were calculated with SNR response characteristics. The phenolic content of Kasturi mango leaves is higher, so better (the larger is the better) as in Table 7.

Tabel 6. SNR for Phenolic Response

No.	Factor						SNR
	A	B	C	Temperature (ºC)	Time (minute)	Concentration ethanol (%)	
1.	1	1	1	50	30	30	83.9037
2.	1	2	2	50	60	50	84.5864
3.	1	3	3	50	90	70	82.5512
4.	2	1	2	60	30	50	82.0973
5.	2	2	3	60	60	70	84.0094
6.	2	3	1	60	90	30	84.9164
7.	3	1	3	70	30	70	81.7958
8.	3	2	1	70	60	30	85.7595
9.	3	3	2	70	90	50	84.3244

Based on Table 7, the ranking order of factors is seen from the delta (difference in SNR between factors). The higher the SNR difference, the greater the influence of these factors' response. Based on calculation of GAE average, can be seen the optimal combination to increase the phenolic content is A3B2C1 (temperature factor at 70 ºC, time factor at 60 minutes, and concentration ethanol factor at 30 %). The higher extraction temperature used up to 70 ºC and the longer extraction time of up to 60 minutes will produce phenolic optimal. At the temperature and time that has reached the optimum point, namely 70 ºC and 60 minutes, phenolic content has decreased, so the diffusion process is no longer taking place. Ethanol concentration will also affect the polarity. The polarity compatibility between the solvent and with compound to be dissolved maximizes extraction process [32]. From Table 7, effect of level on the average SNR can be interpreted in graphical form in Figure 4.

Tabel 7. Response Table for Signal-to-Noise Ratios (Larger is better)

Level	Temperature (ºC)	Time (minute)	Concentration ethanol (%)
1	83.68	82.60	84.86
2	83.67	84.79	83.67
3	83.96	83.93	82.79
Delta	0.29	2.19	2.07
Rank	3	1	2

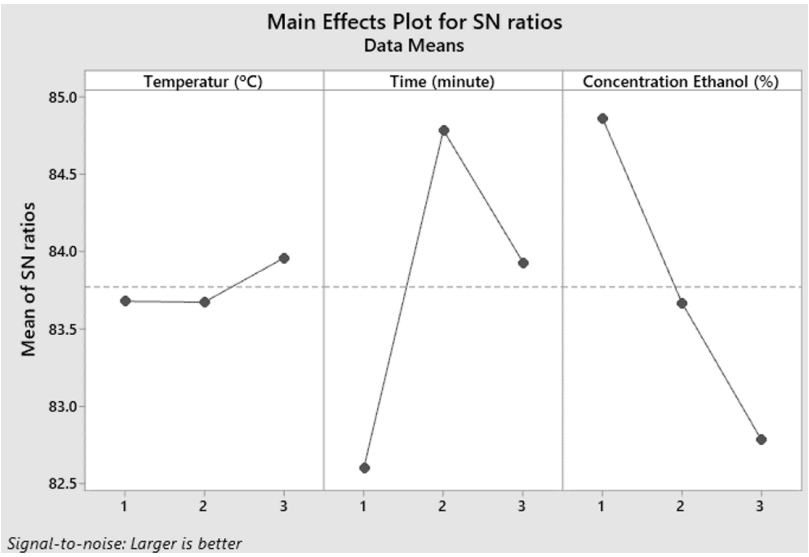

Fig. 4. Effect of Level on Average Response SNR (Larger is Better)

3.6 Analysis of Variance (ANOVA)

To find out more factors that have a significant effect on the compressive strength of phenolic content, it is necessary to do an analysis of variance (ANOVA). Analysis of variance is one of the techniques for analyzing and describing all variations on the parts studied. Analysis of variance aims to identify the influencing factors so that the right formula can be determined. Using a 95% confidence level (α:0.05), the results of ANOVA calculations with Minitab 19 are presented in Table 8.

Table 8. ANOVA

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Temperature (ºC)	2	920037	460019	0.93	0.519
Time (minute)	2	22376273	11188137	22.52	0.043
Concentration ethanol (%)	2	20820435	10410218	20.95	0.046
Error	2	993647	496824		
Total	8	45110393			
S	704.857				
R-sq	97.80%				

Table 8 shows significant factors that contributed to phenolic content, namely time factor (B) and concentration ethanol factor (C) as indicated by P-value <α 0.05. This is consistent with the rate average response and SNR which shows the factor B (time) in the first rank and

C (concentration ethanol) in the second rank, while the temperature factor has no significant effect. According to [11] states extraction time is longer making the quantity of extract increase. This is because materials can contact greater with solvent so the result will increase until the solution saturation point. In the study of [25] total phenol content of red galangal extraction using 96% ethanol would increase with a longer extraction time to optimum. The time factor used increases the penetration solvent into material so becomes solvent easier to pull out chemicals in material, while extraction time used shorter will make solvent difficult to penetrate wall material.

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