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# Optimization of Conditions for Flavonoids Extraction from Mangosteen (*Garcinia mangostana* L.)

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**ABSTRACT**

*Mangosteen (Garcinia mangostana L.) is one of tropical fruits used for medicinal purposes, especially the use of the peel of fruit. The purpose of this study was to determine the levels of flavonoids in mangosteen extracted using Respone Surface Methodology Central Composite Design (RSM CCD) with various concentrations of ethanol and duration of microwave heating or Microwave Assisted Extraction (MAE). The extracting conditions used were concentration of ethanol in the range of 45 % to 96 % and temperature in the range 5,8 $^{o}$C to 34,1 $^{o}$C. Test of total flavonoid content was conducted by colorimetri method ($AlCl_3$). The results showed that optimum extraction conditions for flavonoids were at alcohol concentration of 70% and heating time of 32.9 minutes which resulted in flavonoid content of 11.82 %. More over, under these conditions resulted total flavonoid content of 11.42%. Hence, the optimized extraction conditions were able to produce high flavonoid content.*

**Key words** : flavonoid, Mangosteen, MAE, RSM-CCD, optimum

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## INTRODUCTION

Mangosteen (*Garcinia mangostana* L.) is one of tropical fruits used for medicinal purposes, especially the peel of fruits. Mangosteen is one of the favorite fruits favored by the people of Indonesia. The peel of mangosteen is removed and it can be developed as drug candidates. The peel of mangosteen has been used as a laxative menstruation, thrush medication, fever, chelating agent (adstringen) and drug for treating dysentery [1-2]. The compounds contained in mangosteen rind includes flavonoids epicatechin, anthocyanins and xanton derivatives, among which α-mangostin, β-mangostin, γ-mangostin, and gartanin mangostanol are beneficial to health [3]. The mangosteen fruit is round and dark purple because they contain a lot of anthocyanin in the peel [4]. Besides anthocyanin, mangosteen also contains epicatechin [5], tannins, monoterpenes, saponins and quinone [6], xanthone [7].

Plants that contents flavonoids are widely used in traditional medicine. This is due to flavonoids have a wide range of activity against a variety of microorganisms [8]. Pharmacological studies on flavonoid compounds suggests that some flavonoid compounds show activity such as antifungal, diuretics, antihistamines, antihypertensives, insecticidal, bactericidal and antiviral [9], antioxidant and antimicrobial [2]. Flavonoids are the typical compounds of green plants and are among of the compounds actively studied for development of traditional medicine in Indonesia like 'Jamu'. It is important to note that from the spread of flavonoids in plants there is a strong tendency that taxonomically related plants produce similar types of flavonoids, hence based on information available from studied plants the flavonoids contained in related-plants can be predicted [10]. Flavonoids are a group of compounds that are often found among others in the daily diet. Compounds of this group is the substance in red, purple, blue and yellow that are found in plants. As many as 2% of all carbon plants is converted into flavonoids and their derivatives [10]. Sseveral studies have been conducted to find the optimum extraction conditions for flavonoids.

According to [11], optimum conditions for extraction of flavonoids can be achieved by varying four factors, namely the concentration of solvent, temperature, ratio of raw materials to solvents, and extraction time. Studies that have been described indicated that the use of organic solvents is one of the options need be considered although the cost of production still high. It is therefore, one of the efforts being made is to reduce the cost by optimization of conditions for flavonoids extraction process.

Different extraction conditions can result in different amount of flavonoids. In the present study, the concentration of ethanol and the duration of heating step were optimized in order to obtain optimum flavonoid levels from mangosteen.

## MATERIALS AND METHODS

### Materials

Materials used include: the peels of mangosteen (*Garcinia mangostana* L.) from Bogor, West Java, Indonesia, aluminum chloride, quercetin standards, Dragendrof reagent, Meyer reagents, Bouchard reagent, chloroform, acetic anhydride, sulfuric acid, ether, concentrated hydrochloric acid, magnesium powder, gelatin 1%, glacial acetic acid, ammonium hydroxide, amyl alcohol, methanol, ethanol.

### Methods

#### Preparation of Powdered Crude Mangosteen Peel

The peel of mangosteen fresh and mature (Figure 1) were obtained cleaned of dirt, then were washed with water and drained, then were sliced crosswise and dried under the sun for ± 3 days. Once dried and they were cleaned again from dirt, grounded and sieved to obtain crude mangosteen rind powder. The powder was stored containers and tightly closed.

**Figure 1. Mangosteen (*Garcinia mangostana* L.)**

#### Flavonoid Extraction

Crude powder of the peel of mangosteen was extracted with by using the help of microwave (microwave), with a ratio of 1:20 [12-13]. The peels of Mangosteen powder as much as 30 g, put in a 600 ml Erlenmeyer flask and added with different concentrations of ethanol, each Erlenmeyer covered with black material, and then the sample was extracted by soaking and stirring for 18 hours. After that, the sample was heated with the help of microwave (KRIS MICROWAVE OVEN). After the extract was filtered and the filtrate dried to form a powder. The yield obtained was calculated using the following formula: Yield (%) = (weight of extract/weight of sample) x 100%. Experimental design using RSMCCD [14].

**Table 1. Experimental design using RSMCCD**

| No | Variable | Level | | | | |
|---|---|---|---|---|---|---|
| | | -1 | +1 | 0 | -α | +α |
| 1 | Time | 10 | 30 | 20 | 5,8 | 34,1 |
| 2 | Concentration | 52 | 88 | 70 | 45 | 96 |

Extract was then hydrolyzed by adding 20 ml acetone and 2 ml of 25% hydrochloric acid, refluxed for 30 minutes and then filtered and the filtrate volume matched 100 ml with acetone and then inserted into the rotary evaporator.

**Qualitative Analysis of Chemical Compounds Content Mangosteen Extract peel.** Qualitative test for chemical compounds present in the peel extracts of mangosteen (*Garcinia mangostana* L.) was conducted for alkaloids, flavonoids, saponins, tannins, terpenoids and steroids [15].

**Alkaloid test**. Mangosteen peel extract as much as ± 0.1 g put into a test tube, then added 2 drops of ammonia and ± 5 ml of chloroform and then filtered. To the filtrate was added 1 ml of sulfuric acid 2 M. Acid fractions was taken and added differently with Dragendorf, Meyer, or Bouchardat reagent. The presence of alkaloids characterized by the formation of red precipitate with Dragendorf reagent, white precipitate with Meyer reagent, and a brown precipitate with Wagner reagent.

**Flavonoids test**. Extract as much as ± 0.1 g was poured into a test tube, then added with 5 ml distilled water and filtered. The filtrate obtained was added with magnesium powder, 1 ml of concentrated hydrochloric acid and 1 ml of amyl alcohol. The mixture was shaken and phase separation allowed to happen. Colors that form in the lining of amyl alcohol showed the presence of the flavonoid.

**Saponin test**. Extract as much as ± 0.1 g inserted into a test tube, then add 5 ml ± hot distilled water and filtered. The filtrate obtained strongly shaken and left to stand for 10 minutes. The formation of stable foam shows the compound saponin.

**Tanin test**. Extract as much as ± 0.1 g diluted with water and into the solution was added 10% ferric chloride reagent. The formation of a dark blue or dark green colour indicates a group of tannins [16]. Test for tannin group can also be done by adding a solution of 1% gelatin (1:1) into the extract, the positive results are indicated by formation of white precipitate.

**Triterpenoid and Steroid Test**. Extract as much as ± 0.1 g inserted into a test tube, then added 5 ml of ethanol ± shaken and filtered. Into the filtrate obtained was added 1 mL of diethyl ether, following homogenization, 1 ml of concentrated sulfuric acid and 1 ml of acetic acid anhydride were added. Red or purple color indicates presence of triterpenoids and green or blue showed the presence of steroids.

**Flavonoids test.**

**Preparation of Standard Solution Series Quercetin.** Weighted exactly 0.1 g of quercetin, put into 100 ml flask and diluted with methanol to mark boundaries (1000 ppm). To get the 100 ppm standard solution of quercetin, done by 10 ml quercetin standard solution 1000 ppm, put in a 100 ml flask and diluted with methanol to mark boundaries (100 ppm). Quercetin standard solution prepared in several concentrations, ie 10, 20, 30, 40, 50 and 60 ppm.

**Determination of Wavelength.** Determination of the maximum wavelength was carried out by measuring the absorption of quercetin standard solution of 10 ppm. As much as 10 ml of quercetin 10 ppm standard solution was mixed with 1 ml of 2% aluminum chloride solution (in glacial acetic acid 5%) in a 25 ml flask, then was added glacial acetic acid (5% in methanol) to mark boundaries. Furthermore, the solution incubated at $37^0$C for 30 minutes. After 30 minutes absorbance was measured at a wavelength of 380 nm-780 nm.

**Determination of Optimum Incubation Time.** Determination of the optimum incubation time is done by using a standard solution of quercetin 10 ppm, which is incorporated by 10 ml quercetin 10 ppm standard solution into 25 ml flask, add 1 ml of 2% aluminum chloride solution (in glacial acetic acid 5%) and matched to marks the boundary of glacial acetic acid (5% in methanol), and absorption was measured at the maximum wavelength at 10, 20, 30, 40, 50 and 60 minutes.

**Preparation of Calibration Curve Standard Solutions Quercetin.** Preparation of calibration curve by introducing 10 ml of standard solution of quercetin (10, 20, 30.40, 50 and 60 ppm) to a 25 ml flask, then added 1 ml of 2% aluminum chloride solution (in glacial acetic acid 5%) and glacial acetic acid (5% in methanol) to mark boundaries. Settling for optimum incubation time and absorbance measured at a wavelength of maximum, after which it made a calibration curve between the concentration of the solution to the uptake value obtained and sought curve equation.

**Determination of Content Flavonoids.** Determination of flavonoids levels are determine by adding 10 ml of ethanol extract of the peel of mangosteen and put in a 25 ml flask and add 1 ml of 2% aluminum chloride (in glacial acetic acid 5%) and matched to mark boundaries with glacial acetic acid 5% (in methanol). The solution was allowed to stand for optimum incubation time and measured at a wavelength of maximum absorption, the next level of flavonoid ethanol extract of mangosteen peel is determined by the equation of the calibration curve of the standard solution of quercetin. Then the total flavonoids calculated using the formula: flavonoid contents (%, w/w) = (value (ppm) x dilution factor x volume of titrant (ml)/1.000.000 x weight of sample (g)) x 100 %.

## RESULTS AND DISCUSSION

Preparation of ethanol extracts using various concentrations of ethanol and microwave heating time was aimed to obtain optimum conditions for yield and flavonoid content of 13 samples. The use of ethanol, to disolve the phenolic derivative compounds flavonoids, alkaloids, tannins and saponins [17-18]. An important factor is the selection of the extraction solvent. Solvents used for extraction should be able to attract the active component in the mixture. Important factos to consider in choosing solvent is selectivity, solvent properties, the ability to extract, non-toxic, easy to be evaporated and the price is relatively cheap [19-20]. Pretreatment before extraction depends on the properties of the compounds in the material to be extracted [8]. Pretreatment for solid materials can be done in several ways including by drying the raw material to a certain moisture content and milling to facilitate the extraction process to increase the contact between the materials and solvents [15]. Soaking materials can increase the permeability of the cell wall through three stages: (1) the entry of solvent into the plant cell wall and cell swelling, (2) compounds found in plant cell walls and going off into the solvent, (3) diffusion of compounds extracted by solvent out of the plant cell wall.

Extraction of total flavonoids from mangosteen peel done with ethanol, because of its semipolar allows all types of flavonoids extracted participate. In addition, [16] allowed only ethanol and water as solvent medicine. Ethanol also has a better energy absorption when compared to water. HCl was also added in the process of quantitative analysis to hydrolyze bonds contained in the flavonoid glycosides so expect free flavonoid extracted. The initial phase extraction is by maceration or immersion in the ethanol extract of the Erlenmeyer using a orbital shaker for 18 hours at a speed of 170-200 rpm and the entire surface is covered with a black cloth in order to extract the light generated is not exposed to the sun during the maceration process so as not to affect compound will be analyzed. Next phase extraction results into a microwave oven heated maceration with various heating times and different concentrations in 13 samples. This method is also called MAE (Microwave Assisted Exraction) is a method of extraction using microwave assistance that can destroy the cell so that the sample to be extracted out of the cell and mixed with a solvent and to increase contact between solvent and sample [12]. The extraction process in this way has the advantage that the volume used 10 times smaller than the volume used with a shorter time thus saving the cost of extraction [21-22]. This phase aims to further simplify the extraction process after macerated samples, these compounds are expected to be extracted perfectly desirable, then extract incorporated into the tool rotary evaporator to change the consistency of a thick extrect obtained in advance through the hydrolysis process which aims to separate compounds with compounds aglycone compounds. Hydrolysis process is appropriate according to [16] that established levels as aglycone flavonoids, which is expected to attract maximum aglycone compounds. The timing is intended to determine the incubation time required by a substance in order to react in order to obtain maximum absorption value stable. Determination optimum incubation time performed using a solution of quercetin 10 ppm at maximum wavelength of 412 nm and obtained optimum incubation time is aimed at the 20th minute, with no further decline in the value of absorption. Data resulting from the determination of the optimum incubation time can be seen in Appendix 14 and the curve can be seen in Figure 2.

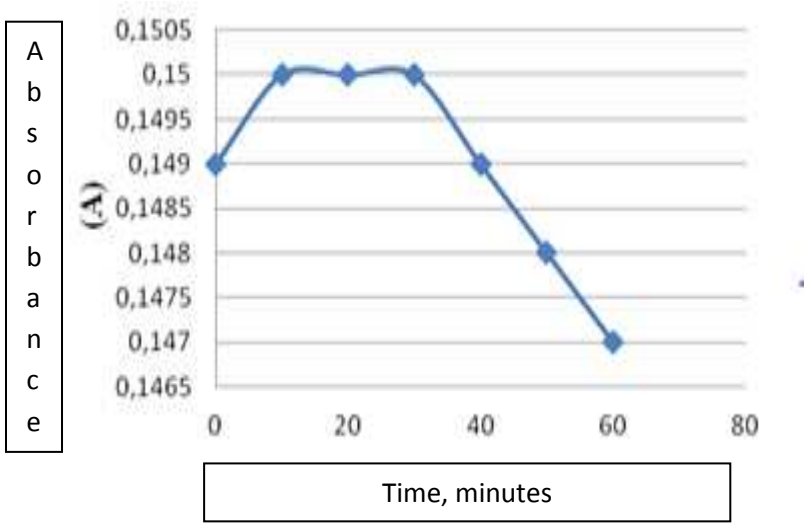


**Figure 2. Determination of optimum incubation for quercetin standard**

Preparation of standard curve based on the method of flavonoids $AlCl_3$ [16]. As standard used quercetin, a flavonoid compound identifier that has been commonly used. A calibration curve was made to determine the levels of a

compound of unknown concentration. Data absorbance measurements of quercetin standard solutions is shown in Appendix 14 and the calibration curve is shown in Figure 3.

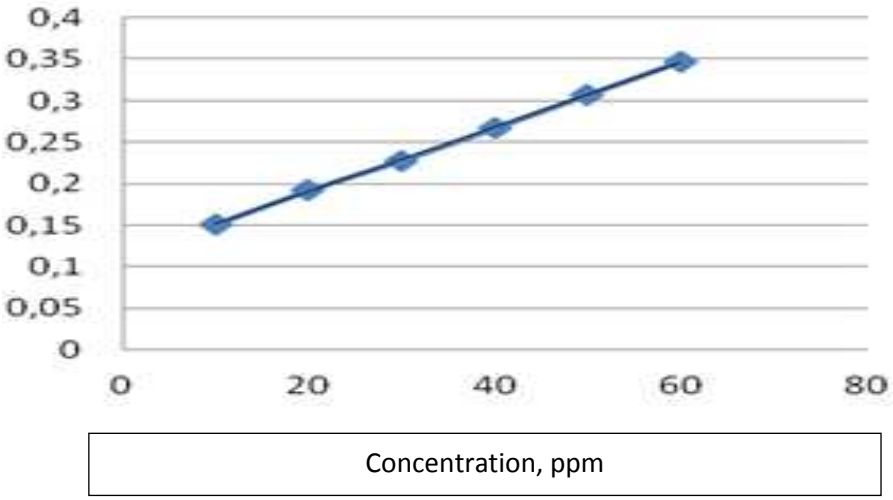


**Figure 3. Calibration curve of standard quercetin solution**

Based on the curve equation for the yield is y = 0.0039 x + 0.1123, with $R^2$ = 0.9997, this value indicates nearly linearity 1, it can be said that the absorbance is a function whose value is proportional to the concentration and following linear regression equation. The results of measurements of total flavonoids are given in Table 2.

**Table 2. The results of the measurements of various factors flavonid total heating time and the concentration of ethanol**

| No. | Heating duration, minutes | Ethanol, % | Flavonoids (%) |
|---|---|---|---|
| 1 | 10 | 88 | 6,19 |
| 2 | 30 | 88 | 6,88 |
| 3 | 10 | 52 | 5,96 |
| 4 | 30 | 52 | 7,56 |
| 5 | 5,8 | 70 | 9,01 |
| 6 | 34,1 | 70 | 12,68 |
| 7 | 20 | 45 | 4,66 |
| 8 | 20 | 96 | 3,06 |
| 9 | 20 | 70 | 11,15 |
| 10 | 20 | 70 | 11,00 |
| 11 | 20 | 70 | 11,38 |
| 12 | 20 | 70 | 11,53 |
| 13 | 20 | 70 | 11,08 |

The analysis aims to determine the class of phytochemical compounds contained in a plant, in this study the phytochemical analysis carried out on the powder and ethanol extract optimum the peel of mangosteen (*Garcinia mangostana* L.). Class of compounds being analyzed include alkaloids, flavonoids, saponins, tannins, triterpenoids and steroids. Tests carried out on the powder were done to determine the compound found in the peel of mangosteen (*Garcinia mangostana* L.) that has not undergone the process of extraction. Phytochemical analysis results can be seen in Table 3.

**Table 3. Phytochemical test of mangosteen *(Garcinia mangostana* L.)**

| Compound | Powder | Optimum ethanol extract 1 | Optimum ethanol extract 2 |
|---|---|---|---|
| Alkaloid | | | |
| • Meyer | + | + | + |
| • Dragendorf | + | + | + |
| • Bouchardat | + | + | + |
| Flavonoid | + | + | + |
| Tanin | + | + | + |
| Saponin | + | + | + |
| Steroid | - | - | - |
| Triterpenoid | + | + | + |

Phytochemical test of optimum ethanol extract showed the same results. According to [17-18], ethanol can attract chemical compounds such as tannins, flavonoids, saponins, alkaloids and glycosides because ethanol is a polar solvent that can attract chemical compounds are polar "like disolvent like". The results of the data analysis of the

levels of flavonoids long warm-up time and the concentration of ethanol using RSMCCD, to obtain the following equation: $Y = -49{,}4680 + 0{,}2422\ X_1 + 1{,}6512\ X_2 - 0{,}0037\ X_1^1 - 0{,}00119\ X_2^2$.

The results of these equations showed that the length of heating time ($X^1$) and concentration ($X^2$) significantly affect levels of flavonoids. It can be seen from the value of the coefficient indicates the value of $X^1$ and $X^2$ is greater than the value of the other coefficients. According to the results obtained from the analysis of variance P value on X1 (heating time) yield (0.0026) <P value (0.05), the heating time is very influential on the levels of flavonoids, as well as the P value on X2 (concentration solvent) yields (0.000) <P value (0.05), so that the concentration of the solvent is very influential on the outcome levels of flavonoids. While the test results for the model mismatch can be seen in the results of lack-of-fit obtained by the P value> 0.05 means the model matches the response is received. These results were obtained by the P value (0.016) <P value (0.05) (Table 4).

**Table 4. Analysis of Variance for flavonoid**

| Source | DF | Seq SS | Adj SS | Adj MS | F | P |
|---|---|---|---|---|---|---|
| Regression | 4 | 111,957 | 111,957 | 27,9892 | 85,86 | 0,000 |
| Linear | 2 | 8,063 | 99,879 | 49,9397 | 153,20 | 0,000 |
| Square | 2 | 103,894 | 103,894 | 51,9468 | 159,36 | 0,000 |
| Residual Error | 8 | 2,608 | 2,608 | 0,3260 | | |
| Lack-of-Fit | 4 | 2,414 | 2,414 | 0,6034 | 12,42 | 0,016 |
| Pure Error | 4 | 0,194 | 0,194 | 0,0486 | | |
| Total | 12 | 114,565 | | | | |

RSMCCD analysis has many advantages one of which describes the overall extraction yield levels of flavonoids, as shown Figure 4.

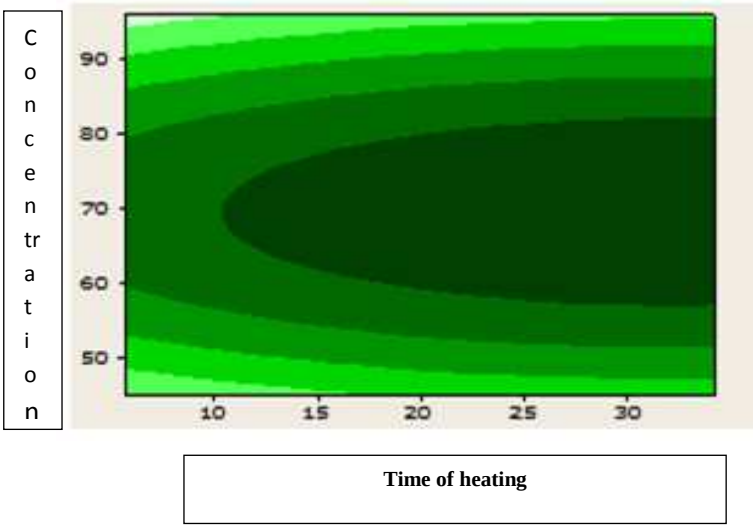


**Figure 4. *Countour Plot* of Flavonoids the peel of mangosteen**

The data showed that the higher the concentration and the longer extraction time much higher the levels of flavonoids is obtained. Treatment with 88 and 96% ethanol resulted in a smaller yield of flavonoids compared to 70 indicates that the number of polar solvent result in dissolution of different flavonoids. This is due to polarity of water is stronger than ethanol.

It proved that the fluid solventi used for the extraction process affects the levels of flavonoids in the peel of mangosteen. This difference is due to the flavonoid solubility and polarity properties of liquids extract. The greater polarity solvent fluid, the greater the levels of flavonoids were obtained from the extraction process. Flavonoids are compounds that are polar [10]. When the heating effect on the total amount of flavonoids. MAE method is characterized by rapid and uniform heating of the extract and extraction solvent and involves about 15-30 minutes so it is around 10 times smaller than the volume used by a traditional extraction techniques and significantly reduce the cost of extraction [21-22].

Based on the picture above that the Surface Plot has a maximum shape. This shows that the RSMCCD has a maximum value equal to each treatment the length of heating time ± 30 min with 70% ethanol concentration. Value indicates a decrease in the levels of flavonoid concentrations > 70%. The results of the analysis of levels of

flavonoids used test D-optimally. The results of the analysis of D-optimally allegedly obtained the results of the optimization levels of flavonoids is 11.8174% at 32.8 min with a concentration of 70% ethanol. Optimization aims to find the value of variables in the process that produces the best value on the terms of the conditions used. Completion of optimization focused on the selection of variables and the best among the whole process efficient quantitative methods including computers and software programs are appropriate computing and cost effective. In the process of making extract optimum is not much different as the manufacture of extracts previously Based RSMCCD, optimizations were performed using 70% ethanol. Corresponding properties of flavonoids that are polar, 70% ethanol can dissolve compounds that are polar or nonpolar. It aims to prove the allegations while the optimization results obtained from the analysis RSMCCD. Apparently the results are not too far away that significant levels of flavonoid 11.42 %.

## CONCLUSION

Obtained results of the analysis of the levels of flavonoids RSMCCD allegedly obtained results concluded that the levels of flavonoids optimization 11.8174% at 32.8 min with a concentration of 70% ethanol and verification of research results obtained by the total flavonoid content of 11.42% (w/w) . RSMCCD obtained from the analysis that the most influential variable is the concentration of ethanol, followed by microwave heating time variable.

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