Optimization of domestic microwave maceration extraction of phenolic compounds from Averrhoa bilimbi using statistical response surface methodology

by Wenny Irawaty

Optimization of domestic microwave maceration extraction of phenolic compounds from *Averrhoa bilimbi* using statistical response surface methodology

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Abstract

In this study, optimization of *Averrhoa bilimbi* extraction using Domestic Microwave Maceration Extraction (DMME) was performed using a statistical method called Response Surface Methodology (RSM). RSM could lead to some advantages, such as less number of experiments and cost savings. RSM was used to find condition from each variables (solid to liquid ratio, solvent concentration, and extraction time) that gives optimum response to the yield of phenolic. Yield of phenolic increased in smaller solid to liquid ratio, and gave its best at combination variables of 0% ethanol and 48% ethanol as solvent concentration. Based on the design of experiment, it was known that extraction time variable gave insignificant effect to the yield of phenolic as response. RSM result showed that the optimum DMME extraction condition was found at 1:15 solid-liquid ratio and 34% ethanol as extraction solvent, with 4.2762 mg GAE/g solid as the teoritical yield of phenolic. Validation of RSM model was carried out at the optimal condition and 90 seconds extraction time (the smallest value, to save energy). Yield of phenolic of the model validation was 4.2120 mg GAE/g solid. Error between teoritical yield of phenolic and the validation value was less than 5% (1.5% error value).

Introduction

Free radicals are responsible for many chronic diseases, such as cancer, tumor, and degenerative ailments. They are produced from external human body (i.e. pollution, food, cigarette smoking) or even from human metabolism (Pham-Huy *et al.*, 2008). When human cells use oxygen as energy, they produce reactive oxygen species (ROS) as by-products. Excess ROS can damage cell structures (Pham-Huy *et al.*, 2008). Antioxidants are needed to prevent cells damaging from free radicals (Young and Woodside, 2001). Nowadays, natural antioxidants from fruits or vegetables could be a great choice than the synthetics one, as many believe that natural products are healthier and safer.

Averrhoa bilimbi (belimbing wuluh in Indonesian) is a sour, elips shape fruit. Lack of use because of the sour flavor resulting in excess supply of the fruits, however, *Averrhoa bilimbi* could has greater value in antioxidant activity. Researches on *Averrhoa bilimbi* have been conducted, for examples by Hasanuzzman *et al.* (2013) who used methanol as solvent and 15 days soaking time at room temperature with occasional stirring. The total phenol content was pretty impressive, 65.16 mg of GAE/g extract. To date, there is no research conducted using a domestic microwave extraction for *Averrhoa bilimbi*. Microwave-assisted extraction was found to save time and amount of solvent (Mandal *et al.*, 2007). This present research employed Domestic Micowave Maceration Extraction (DMME) method for obtaining phenolic compounds from Averrhoa bilimbi fruit. Shorter extraction period and less ammount of solvent are the advantages of this method (compared to previous research which was held for 15 days period).

The optimization of phenolic extraction process from Averrhoa bilimbi using domestic microwave maceration extraction (DMME) method was performed using statistical analysis Response Surface Methodology (RSM) in response to total phenolic content of extracts. This statistical method could find series of variables that give the optimized response, whilst cost saving due to less number of experiments needed.

Materials and Methods

Materials

Averrhoa bilimbi fruits were collected from Setro, Surabaya, East Java, Indonesia. Folin-Ciocalteu reagent (Merck, Germany), gallic acid standard (Sigma–Aldrich, USA), and other chemicals were

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obtained from local distributors. All solvents and chemicals used were of analytical grade.

Phenolics extraction

The effects of solid to liquid ratio, solvent concentration, and extraction time using Domestic Microwave Maceration Extraction method on the yield of phenolics of *Averrhoa bilimbi* crude extracts were studied. Solid to liquid ratio (w/v) were carried out at 1:5, 1:10, 1:15 for 100 mL solvent volume (e.g. at 1:5 ratio, the extraction was conducted for 20 grams *Averrhoa bilimbi* powder in 100 mL solvent). Solvent concentration studied were 0% ethanol (distilled water), 48% ethanol, and 96% ethanol. Extraction time was varied from 90, 120, and 150 seconds. Fruits of *Averrhoa bilimbi* were cut into pieces, oven-dried at 50°C for 5 days. The dried fruits were ground into -20/+80 mesh in size and stored until further used. Ten grams of Averrhoa bilimbi powder was diluted with 100 mL of ethanol 96% in an erlenmeyer prior to extraction process in a domestic microwave using 20% power (Inextron WD9000SL23-2 2.450 MHz, 900W) for 120 seconds. Every 30 seconds, the erlenmeyer was taken out from microwave for two minutes which covers in shaking water bath (Memmert Type SV 1442) for 1 minute, and 30 minutes cooling time before shaking and before re-entering the microwave for further extraction process.

Total phenol content (TPC) determination

The total phenol content was estimated using a method previously described by Atanassova *et al.* (2011). Total phenolic content of extracts was determined with the Folin-Ciocalteau assay. One mL of plant extract was added to 9 mL of distilled deionised water in a 25 mL volumetric flask. After that, 1 mL Follin-Ciocalteu reagent was added to the mixture. After 5 minutes, 10 mL of 7% Na_2CO_3 was added. The solution diluted until 25 mL with distilled deionised water and incubated for 90 min. As a blank, 1 mL of plant extract was replaced with distilled deionised water with the same volume. Absorbance of both sample and blank were measured in Shimadzu UV-Vis 1700 spectrophotometer at wavelength 755 nm.

Response surface methodology analysis

Optimization of the experimental data using Response Surface Methodology was performed using Minitab 16 Statistical Software. Experiments were carried out based on Response Surface Methodology design of experiment. The actual factor was coded based on formulas below:

$$Ratio = \frac{x-10}{5} \tag{1}$$

$$Extraction\ time = \frac{x-120}{30} \tag{2}$$

$$Solvent\ concentration = \frac{x-48}{48} \tag{3}$$

Results and Discussion

Model fitting

A mathematical model was built to find the optimum variable condition for yield of phenolic. The model is given below.

$$Y= 3,99781 + 0,6097X_1 - 0,2431X_2 - 0,38206X_1^2 - 0,56706X_2^2 - 0,09675X_1X_2 \tag{4}$$

X_1 stands for solid to liquid ratio and X_2 represents solvent concentration. There is no extraction time variable in the above mathematical model because RSM does not recognize adequate effect of extraction time to the yield of phenolic. As seen on Table 1 for result analysis of variance for yield of phenolic, the Lack-of-Fit number give higher value than α given $(0,066 > α)$; α value for this experiment was set at 0,05. Thus, mathematical model given above could represent the experiment. Model adequacy was also evaluated using normal probability plot (Figure 1). Figure 1 shows that the residual was normally distributed. This conclusion was taken based on the dots that lie closed to the straight line.

Table 1. Analysis of variance for yield of phenolics

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	5	8,02078	8,02078	1,60416	14,78	0,000
Linear	2	4,30832	4,30832	2,15416	19,84	0,000
Ratio	1	3,71734	3,71734	3,71734	34,24	0,000
Concentration	1	0,59098	0,59098	0,59098	5,44	0,035
Square	2	3,63758	3,63758	1,81879	16,75	0,000
Ratio*ratio	1	2,60859	2,60859	0,46711	4,30	0,057
Concentration*Concentration	1	1,02899	1,02899	1,02899	9,48	0,008
Interaction	1	0,07488	0,07488	0,07488	0,69	0,420
Ratio*Concentration	1	0,07488	0,07488	0,07488	0,69	0,420
Residual Error	14	1,52001	1,52001	0,10857		
Lack-of-Fit	3	0,70990	0,70990	0,23663	3,21	0,066
Pure Error	11	0,81011	0,81011	0,07365		
Total	19	9,54079				

Effect of solid to liquid ratio on yield of phenolics

As seen on Figure 2, the smaller the value of solid to liquid ratio has resulted in higher yield of phenolic. Higher amount of solvent increased the yield of phenolic. This phenomena may occur due to the excessive swelling of materials (Guo *et al.*, 2001) which absorbed the microwave energy efficiently
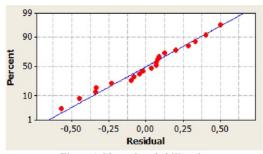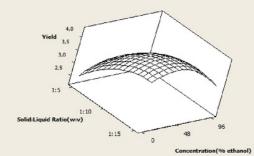
Figure 1. Normal probability plot

Figure 2. Surface plot of yield of phenolic vs solvent concentration and solid to liquid ratio

(i.e. less microwave energy absorbed by the material) (Yan *et al.*, 2009). Greater amount of solvent will also increase the concentration gradient between solvent and material, conduce longer time for saturated solvent (Li *et al.*, 2009 and Baiano *et al.*, 2014).

Effect of solvent concentration on yield of phenolics

Solvent concentration is one of the parameter studied in this experiment. Ethanol 0% (distilled water) gave higher value of phenolic yield than the 96% ethanol solvent concentration, yet the highest yield value was lied between 0% ethanol and 48% ethanol. Tannin in *Averrhoa bilimbi* was hydrolized become gallic acid in water (Markom *et al.*, 2007). It is thus important to further find out solvent mixture that give the optimum result.

Optimization and validation

Using Minitab 16 Statistical Software for Response Surface Methodology analysis, the optimized condition of the experiment was found at coded solvent concentration -0,2838 (i.e. 34% ethanol as solvent concentration) and coded solid-liquid ratio 1 (i.e. 1:15 solid to liquid ratio) (Figure 3). Validation of RSM model was carried out at 90 seconds extraction time, the lowest value of extraction time since the RSM result showed that extraction time variable was insignificant in response to the yield of phenolic. This validation process gave
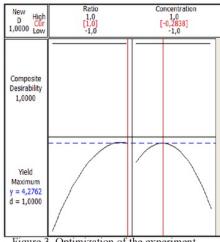

Figure 3. Optimization of the experiment

yield of phenolic value of 4.2120 mg GAE/g solid. Compared to the theoritical value (4.2762 mg GAE/g solid), error between validation and theoritical values was 1.5%.

Conclusions

Domestic Microwave Maceration Extraction (DMME) of *Averrhoa bilimbi* was optimized using a Response Surface Methodology statistical analysis. A mathematical model of two factor was developed in order to find an optimized extraction condition of *Averrhoa bilimbi*. An optimized DMME extraction condition were carried out at 34% of ethanol concentration as solvent, 1:15 of solid to liquid ratio, and 90 seconds extraction time. By using the optimized condition, the resulting *Averrhoa bilimbi* extract possesed 4.2120 mg GAE/g solid yield of phenolic, whilst the teoritical result was 4.2762 mg GAE/ g solid. The error value between validation and teoritical result was less than 5%.

Acknowledgement

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