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Article

Optimization of *Tetrigona apicalis* Propolis Extract using Ethanol Solvent with Shaking Ultrasound Assisted Extraction Method

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Abstract: Indonesia as a country with a tropical climate, is famous for its natural wealth and has various kinds of flora and fauna that can be found and utilized. Several herbal products have antioxidant and antibacterial capabilities, one of which is propolis. Bee propolis is a functional food because of its nutrition and contains high levels of bioactive components that help protect health. Raw propolis from farmers needs to be purified before human consumption because raw propolis still contains beeswax, resin, and other impurities. The method used is shaking ultrasound-assisted extraction (SUAE), which produces perfect extraction, higher yield and active content, and a shorter time. The purpose of this research is analyzed optimum conditions for extracted Tetrigona apicalis propolis using the SUAE method in ethanol solvent. The propolis extraction process is that raw propolis is size reduced, then solvent is added and macerated using shaker. Next, the extract is processed with a sonicator, and the extract is filtered. The solvent used is 70% ethanol, and the results of the propolis extract are processed using Response Surface Methodology (RSM). The parameters that were optimized were variations in stirring time of 1-24 hours and ultrasonic process time of 1-20 minutes. The best conditions in the propolis extraction process from the yield response shown were a stirring time of 28.76 minutes and an ultrasonic process time of 23.94 minutes with a yield of 8.735%. Based on statistical analysis using software, stirring time and ultrasonic processing time are factors that have a significant influence on the yield of propolis extract produced. The model used is a quadratic model with R^2 0.9452. The yield value of the propolis extract predicted by the software is close to the percent yield value obtained based on experiments carried out with an error value of less than 5%.

Keywords: propolis extraction, response surface methodology, shaking ultrasound-assisted extraction.

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1. Introduction

Indonesia, as a country with a tropical climate, is famous for its natural wealth which has a variety of flora and fauna that can be found and used, for example, to treat various diseases caused by pathogenic microorganisms. Several efforts have been made in recent years regarding natural products to develop medicines and healthy foods [1]. One alternative that can be used is the honey-producing bee Tetrigona apicalis. The honey bee Tetrigona apicalis is not only useful for honey, but also for hives which produce propolis products. Propolis is a mixture of beeswax and resin collected by honey bees from plant shoots, leaves, and exudates. Propolis is a soft and sticky substance when heated, and becomes hard and brittle when frozen. Propolis consists of more than 300 different compounds including flavonoids, phenolics, lipophilic aldehydes, flavonoid-aglycones, and other compounds such as pollen, wax, vitamins, minerals, and so on [2].

Bee products such as honey, pollen, bee bread, royal jelly, and propolis play an important role as functional foods because of their nutritional content that helps protect health [3]. Functional food is food or food components that provide the body with essential nutrients to maintain normal growth/development of the body [4]. Bee products are said to be 'functional foods' by adding them to other food products to increase their nutritional value or used alone because of their natural nutritional content and high bioactive components [3].

Propolis is the result of the activity of honey bees, the production of which is directly proportional to the amount of honey produced by bee farmers. However, in its development, there is still very little utilization of the natural potential of functional foods, especially propolis, in Indonesia. So far, bee farmers have used very little propolis and there are still many farmers who have not used propolis as a functional food with high antioxidant activity. Therefore, people need to understand functional food and its benefits for improving health to avoid degenerative diseases [4].

Raw propolis from farmers needs to be purified before human consumption because raw propolis still contains beeswax, resin, and other impurities. Propolis is a product that contains antioxidants and antibacterials because it has properties as a bactericide and fungicide, antioxidant, antivirulence-inflammatory, and is used as an alternative medicine. Antioxidants are useful for delaying or inhibiting cell damage, especially through free radical properties [5]. This is a potential opportunity to produce propolis extract as a source of antioxidants and antibacterials, which can be implemented for bee farmers and the pharmaceutical industry.

The method used is shaking ultrasound-assisted extraction (SUAE) using ultrasonic energy (>20 kHz) for extraction using ultrasonics or an ultrasonic probe. SUAE is an extraction method that uses effective ultrasonic waves to produce fast solvent transfer, resulting in higher mass transfer and faster extraction times [6]. Extraction only by stirring or ultrasonic provides lower extraction yields and lower phenol and flavonoid contents compared to SUAE. SUAE provides a higher yield, phenol, flavonoid content, and antibacterial activity and the time used is shorter compared to extraction methods using only stirring or ultrasonic [7].

The research design used a central composite design and data analysis using response surface methodology (RSM). RSM is a statistical method for experimental design, process optimization, and mathematical modeling to predict the quantity and quality of products produced. RSM reviews 2 or more process variables that interact with each other simultaneously [6].

The purpose of this research is to analyze the optimum conditions for extracting Tetrigona apicalis propolis using the shaking ultrasound-assisted extraction (SUAE) method for ethanol solvent using RSM and analyzing the parameters (stirring time and ultrasonic process time) that influence the yield response of propolis extract using RSM.

2. Materials and Methods

The equipment used in this research are Shimadzu analytical balance, reagent bottles, ball mill, Thermo Scientific magnetic stirrer, 80 mesh sieve, Branson model 5800 ultrasonic batch, glass funnel, volumetric pipette, bulb, drip pipette, stopwatch, and Erlenmeyer. Meanwhile, the materials used are raw

propolis tetrigona apicalis from the Taman Nasional Bukit Barisan Selatan forest managed by PT. Suhita Lebah Indonesia, 70% ethanol, filter paper and aluminum foil.

Propolis Extraction

Raw propolis from the Taman Nasional Bukit Barisan Selatan forest (PT. Suhita Lebah Indonesia) was reduced in size using a ball mill until a size of 60-80 mesh was obtained.

Ethanol solvent (Running 1)

Propolis was weighed as much as 10 grams then 70% ethanol was added with a volume of 100 mL. Then the mixture of propolis and solvent was stirred using a magnetic stirrer (300 rpm) for 1 hour, after that the process was sonicated using ultrasonics for 1 minute (40 kHz) at a temperature of 30°C, after that, it was filtered with a filter paper to separate the beeswax and impurities. The filtrate obtained is then calculated for the yield. Runs 2 to 13 were carried out according to variations in stirring time and ultrasonic process time in the process experimental design in table 2.

Observation and Analysis

The research was carried out with two parameters, namely variations in stirring time and ultrasonic process time. Variations in operating factors in this study can be seen in Table 1.

Tabel 1. Variation of the operating factors

Factor	Variable	
	Min	Max
stirring time (hours)	1	24
Ultrasonic process time (min)	1	20

The experimental process design based on Central Composite Design with Minitab Version 19 software can be seen in Table 2.

Tabel 2. Process Trial Design Based on CCD

RunOrder	stirring time (hours)	Ultrasonic process time (min)
1	1.00	1.00
2	24.00	1.00
3	1.00	20.00
4	24.00	20.00
5	0.00	10.50
6	28.76	10.50
7	12.50	0.00
8	12.50	23.94
9	12.50	10.50
10	12.50	10.50
11	12.50	10.50
12	12.50	10.50
13	12.50	10.50

Source: Central Composite Design design with Minitab Version 19 software

The results obtained from the experimental results were processed using the Minitab version 19 application. The results of the data processing will be presented with the results of the ANOVA analysis and the optimization results for each solvent. The optimization results were validated by carrying out experiments again from the software data validation data. The experimental validation results are calculated and compared with the theoretical validation results to prove whether the experimental results obtained produce accurate data or not.

3. Results and Discussion

Yield of Propolis Extraction

Data from research on propolis extraction with variations in stirring time and ultrasonic process time as well as the response to the yield of propolis extract. The result data can be seen in Table 3.

Table 3. Yield Results from Propolis Extraction

RunOrder	stirring time (hours)	Ultrasonic process time (min)	Yield (%)
1	1.00	1.00	1.4667
2	24.00	1.00	1.5767
3	1.00	20.00	4.6033
4	24.00	20.00	5.2767
5	0.00	10.50	2.7333
6	28.76	10.50	5.0017
7	12.50	0.00	2.0917
8	12.50	23.94	5.8267
9	12.50	10.50	1.4767
10	12.50	10.50	1.4767
11	12.50	10.50	1.4767
12	12.50	10.50	1.4767
13	12.50	10.50	1.4767

Source: Central Composite Design, RSM with Minitab Software Version 19

Table 3 shows the lowest yield, namely 1.4667% with a stirring time of 1 hour and an ultrasonic processing time of 1 minute. Meanwhile, the highest yield was 5.8267% with a stirring time of 12.50 hours and an ultrasonic process time of 23.94 minutes. The results in Table 3 were then analyzed using Minitab Version 19 software to determine optimum conditions and equation models.

Statistical Analysis

Data processing was analyzed using Minitab Version 19 software with a quadratic model. Analysis of Variance (ANOVA) is an analysis technique used to identify the importance of the model obtained and its parameters [8]. Table 4 shows the results of the Analysis of Variance (ANOVA) in the quadratic model for propolis extraction.

Tabel 4. Analysis of Variance (ANOVA)

ANOVA for Response Surface Quadratic model Analysis of variance table [Partial sum of squares - Type III]						
Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F	
Model	33.9471	5	6.7894	24.14	0.000	Significant
Linear	20.3493	2	10.1746	36.18	0.000	
stirring time (hour)	1.9913	1	1.9913	7.08	0.032	Significant
ultrasonic process time (min)	18.3580	1	18.3580	65.28	0.000	Significant
Square	13.5185	2	6.7592	24.04	0.001	
stirring time (hour)* stirring time (hour)	7.3057	1	7.3057	25.98	0.001	Significant
ultrasonic process time (min)* ultrasonic process time (min)	7.9738	1	7.9738	28.35	0.001	Significant
2-Way Interaction	0.0793	1	0.0793	0.28	0.612	
stirring time (hour)*ultrasonic process time (min)	0.0793	1	0.0793	0.28	0.612	Not Significant
Error	1.9685	7	0.2812	-	-	
Lack-of-Fit	1.9685	3	0.6562	-	-	
Pure Error	0.0000	4	0.000	-	-	
Total	35.9156	12	-	-	-	

A Prob>F value of less than 0.0000 indicates that the model is significant for the variables being varied [9]. Lack of Fit analysis is recommended with insignificant information from the software, Lack of Fit analysis does not produce a Prob>F value and there is no information in the software. So the Lack of Fit model analysis was not carried out [8].

The Prob>F value for varying stirring time (hours) is 0.032 and ultrasonic process time (minutes) is 0.0000, which means that the stirring time and ultrasonic process time have a significant effect on the yield of the propolis extract produced. The shaking ultrasound assisted extraction method results in a process with faster extraction times, lower solvent consumption, increased extraction rates, and increased extract quality. Extraction of bioactive components from complex sample matrices is more efficient, economical, and environmentally friendly. Moreover, it not only reduces extraction time but also improves extract quality and yield in extracting bioactive components from samples [10].

Based on the summary statistics model, the R^2 value is 0.9452 and Adeq Precision is 24.14. Adeq Precision with a value <4 for the process carried out, shows that the quadratic model can be used to describe the relationship between the response and interaction variables. The R^2 model of 0.9452 shows that 94.52% of the data is correct or close to correct and only 5.48% of the data is wrong [11] [8]. Based on the analysis, a quadratic equation model was obtained which states the relationship between propolis extract yield and the varied factors.

Actual Factor:

Response = 1.948 – 0.1639 stirring time (hour) – 0.1058 ultrasonic process time (min) + 0.00775 stirring time (hour)* stirring time (hour) + 0.01186 ultrasonic process time (min)* ultrasonic process time (min) + 0.00129 stirring time (hour)*ultrasonic process time (min)

Effect of the Stirring Time and Ultrasonic Process Time

The relationship between stirring time and ultrasonic process time is presented in contour plots and surface plots presented in figures 1 and 2.

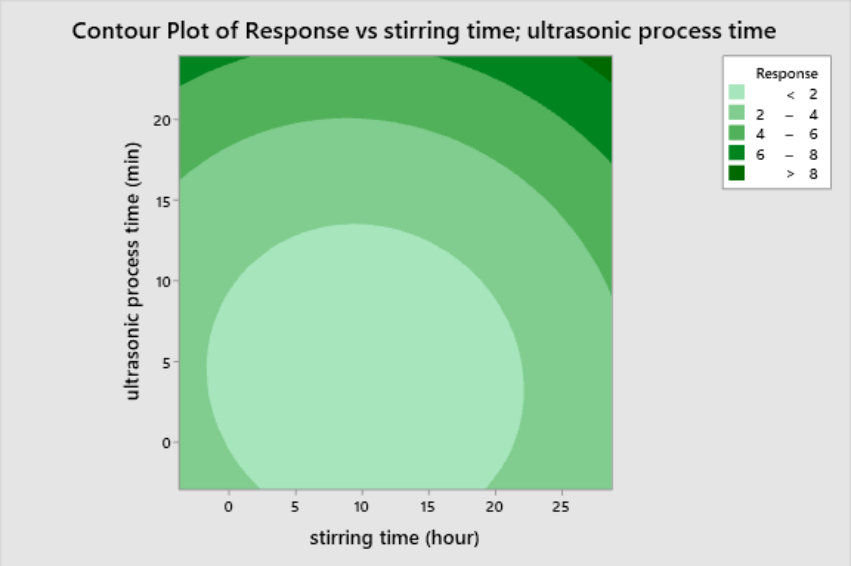

Figure 1. Contour plot of stirring time and ultrasonic process time

Figure 1 shows that the longer the stirring time and the ultrasonic processing time, the higher the response will be, namely >8, followed by a dark green color on the contour plot.

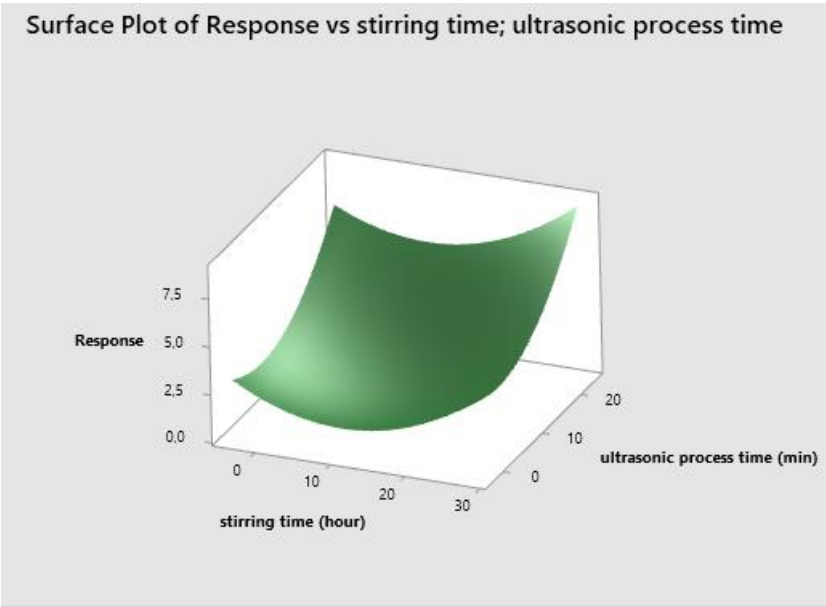

Figure 2. Surface plot of response, stirring time, and ultrasonic process time

Figure 2 shows that the longer the stirring time and the longer the ultrasonic process time, the higher the response produced. The influence between variables (stirring time and ultrasonic process time) determines the resulting product. The results of this research show that the longer the stirring time, the higher the resulting yield, and the length of time of the ultrasonic process also influences the resulting yield.

Optimization Process

Process optimization of the yield response of propolis extract was demonstrated through the Response Surface Methodology software, which was the highest, namely at a stirring time of 28.7635 hours and an ultrasonic process time of 23.94 minutes with a predicted yield of 8.797%. The optimization value can be seen in Figure 3.

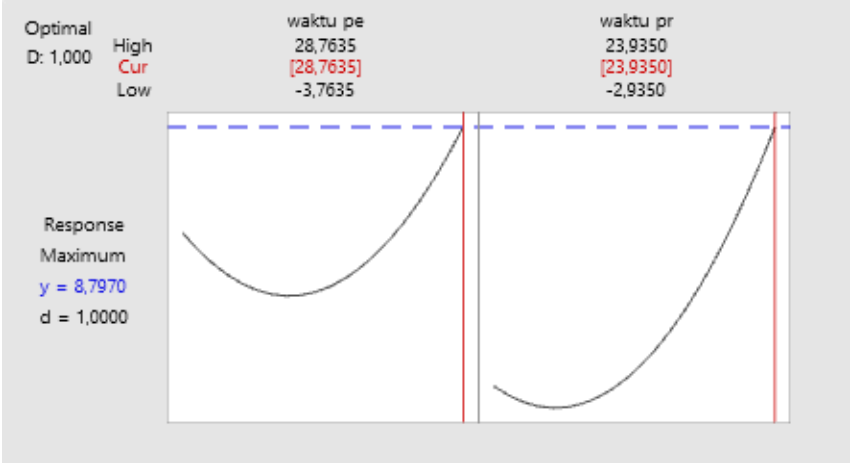

Figure 3. Process Optimization Graph

A comparison of the validation results with the calculation results provided by the program can be seen in Table 5.

Table 5. Comparison between Optimization Results and Validation Results

	stirring time (hour)	ultrasonic process time (min)	Response Yield (%)
Prediction*	28.7635	23.94	8.797
Validation**	28.7635	23.94	8.735 ± 0.0633
Error Value			0.062

Notes: *Results from Minitab Version 19 software

** Experimental data

From these data, it shows that the difference in the response value of the validation results for propolis extract and the predicted results is 0.062%. The results of this comparison show that the difference between the predicted and validated values is smaller than 5%, which means that the validated values are by the predicted values [9].

4. Conclusion

The best conditions in the propolis extraction process from the yield response shown were a stirring time of 28.76 minutes and an ultrasonic process time of 23.94 minutes with a yield of 8.735%. Based on statistical analysis using Minitab Version 19 software, stirring time and ultrasonic processing time are factors that have a

significant influence on the yield of propolis extract produced. The model used is a quadratic model with R^2 0.9452. The yield value of the propolis extract predicted by the software is close to the percent yield value obtained based on experiments carried out with an error value of less than 5%.

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