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

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Abstract: *Tetrigona apicalis* were known as lebah penyengat and its can be produce *p*ropolis were extracted by metode shaking ultrasound-assisted extraction (SUAE), which produces perfect extraction, higher yield and active content, and a shorter time. The purpose of this research were fined the optimum conditions for extracted *its* by using the SUAE method in glycerol solvent. It were done to raw propolis with size reduced, then solvent was added and macerated using shaker. It was processed with a sonicator, than filtered. The solvent used 70% glycerol, and the results of the propolis extract are processed using Response Surface Methodology (RSM). The optimal conditions of its 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 4.238%. 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 0.9191. A quadratic equation model was obtained which states the relationship between propolis extract yield and the varied factors $X = 0.865 + 0.0091A - 0.0186B - 0.000006A^2 + 0.00370.B^2 + 0.00215AB$.

Keywords: Functional food; Propolis extraction; RSM; SUAE; *Tetrigona apicalis*.

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 (Bakkaloglu et al., 2021). 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 (Maroof & Gan, 2020; Popova et al., 2022). Propolis is a mixture of beeswax and resin collected by honey bees from plant shoots, leaves, and exudates (de Cordova, 2024). Propolis is a soft and sticky substance when heated, and becomes hard and brittle when frozen (Bobiş, 2022). Propolis is a by-product produced by worker bees from plant sources and their surrounding environment. The primary components of propolis comprise 50% resin and 30% wax and fatty acid compounds. The remaining constituents include essential oils, bee pollen and organic mineral compounds. The resin components in propolis contain flavonoids, phenolic compounds and various acids. Notably, propolis's flavonoids, such as chrysin, pinocembrin, akasetin, rutin and catechin, exhibit antibacterial, antifungal and antioxidant properties. Furthermore, phenolic compounds and acids within propolis serve as antibiotics (Yarlina, 2020).

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 (Hashemirad et al., 2024). Functional food is food or food components that provide the body with essential nutrients to maintain normal growth/development of the body (El Ghouizi et al.,

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2023; Triandita et al., 2020). 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 (Triandita et al., 2020).

Indonesia is rich in natural resources with medicinal potential. One such resource, particularly abundant in Kalimantan, is the product of stingless bees, also known as kelulut bees. Approximately 500 species of stingless bees have been identified, with 40 exhibiting potential for honey production (Kustiawan et al., 2023). This study focused on the *Tetrigona apicalis* species. Characteristically, stingless bees have a three-part body, comprising the head, thorax, and abdomen (Harjanto et al., 2020).

Raw propolis from farmers needs to be purified before human consumption because raw propolis still contains beeswax, resin, and other impurities (Reddy et al., 2025; Sadapotto et al., 2023). 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 (Freitas et al., 2022; Nichitoi et al., 2021; Touzani et al., 2021). Antioxidants are useful for delaying or inhibiting cell damage, especially through free radical properties (Ibroham et al., 2022). 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 (Ramandani et al., 2022). 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 (Pobiega et al., 2019). The purpose of this research is analyzed optimum conditions for extracted *Tetrigona apicalis* propolis using the SUAE method in glycerol solvent.

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 (Ramandani et al., 2022).

Method

Sample Preparation

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.

Materials

The materials used are raw propolis *Tetrigona apicalis* from the Taman Nasional Bukit Barisan Selatan forest managed by PT. Suhita Lebah Indonesia, 70% glycerol, filter paper and aluminum foil.

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.

Procedure

Propolis was weighed as much as 10 grams then 70% glycerol 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 (Pobiega et al., 2019). 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. The yield value can be calculated using the Formula 1.

$$\%\ \text{Yield} = \frac{\text{extract mass}}{\text{sample mass}} \times 100\% \quad (1)$$

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.

Table 2. Process Trial Design Based on CCD

Run Order	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

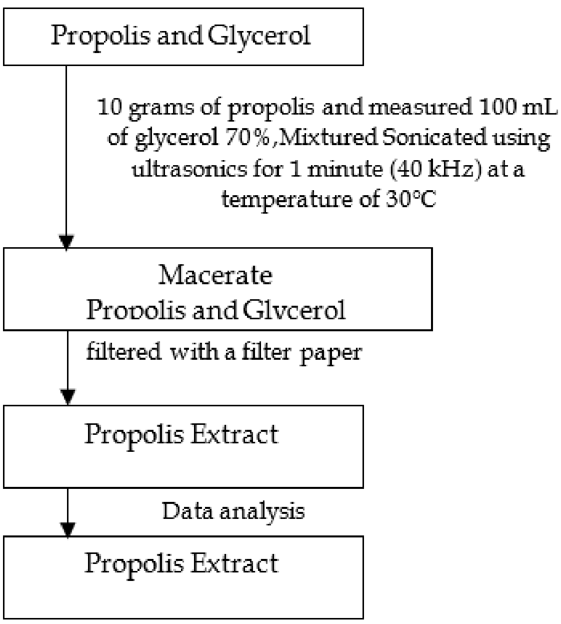

Figure 1. Procedure propolis extraction

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. Procedure propolis extraction as shown in Figure 1.

Result 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

Run	Stirring time (hours)	Ultrasonic process time (min)	Yield (%)
1	1.00	1.00	0.7883
2	24.00	1.00	1.2467
3	1.00	20.00	2.1017
4	24.00	20.00	3.5000
5	0.00	10.50	0.9967
6	28.76	10.50	1.7333
7	12.50	0.00	0.9867
8	12.50	23.94	3.0817
9	12.50	10.50	1.5200
10	12.50	10.50	1.6100
11	12.50	10.50	1.5533
12	12.50	10.50	1.7667
13	12.50	10.50	0.9067

Central Composite Design, RSM with Minitab Software Version 19

Table 3 shows the lowest yield, namely 0.7883% with a stirring time of 1 hour and an ultrasonic processing time of 1 minute. Meanwhile, the highest yield was 3.0817% 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 (Sudibyo et al., 2022). Table 4 shows the results of the Analysis of Variance (ANOVA) in the quadratic model for propolis extraction.

The P value of less than 0.0000 indicates that the model is significant for the variables being varied (Kwak, 2023). Lack of Fit analysis is recommended with not significant information from the software, Lack of Fit analysis produces a value of more than 0.05 (not significant) so the lack of fit model analysis is good to apply (Sudibyo et al., 2022).

Tabel 4. Analysis of Variance (ANOVA)

Source	F value	P value	Categories
Model	15.90	0.001	Sig
Linear	34.31	0.000	
stirring time (hour)	11.29	0.012	Sig
ultrasonic process time (min)	57.32	0.000	Sig
Square	4.24	0.062	
stirring time (hour)* stirring time (hour)	0.00	0.995	Not Sig
ultrasonic process time (min)* ultrasonic process time (min)	8.34	0.023	Sig
2-Way Interaction	2.38	0.167	
stirring time (hour)*ultrasonic process time (min)	2.38	0.167	Not Sig
Error			
Lack-of-Fit	0.66	0.616	Not Sig
Pure Error	15.90	-	
Total	34.31		
R^2			0.9191

The P value for varying stirring time (hours) is 0.012 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 (Shen et al., 2023).

Based on the summary statistics model, the R^2 value is 0.9191 and Adeq Precision is 15.90. 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 0.9191 shows that 91.91% of the data is correct or close to correct and only 8.09% of the data is wrong (Pawignya et al., 2019; Sudibyo et al., 2022). Based on the analysis, a quadratic equation model was obtained which states the relationship between propolis extract yield and the varied factors (Actual Factor).

$$X = 0.865 + 0.0091A - 0.0186B - 0.000006A^2 + 0.00370B^2 + 0.00215AB$$

Note :
A = stirring time (hour)
B = ultrasonic process time (min)
X = Response propolis extract with glycerol solvent

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 2 and 3.

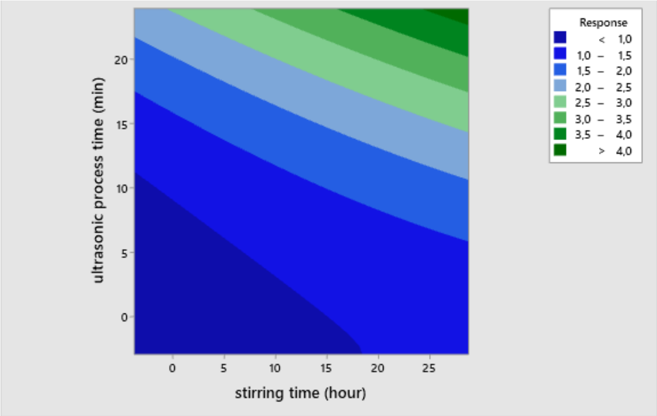

Figure 2. Contour plot of stirring time and ultrasonic process time

Figure 2 shows that the longer the stirring time and the ultrasonic processing time, the higher the response will be, namely >4, followed by a dark green color on the contour plot.

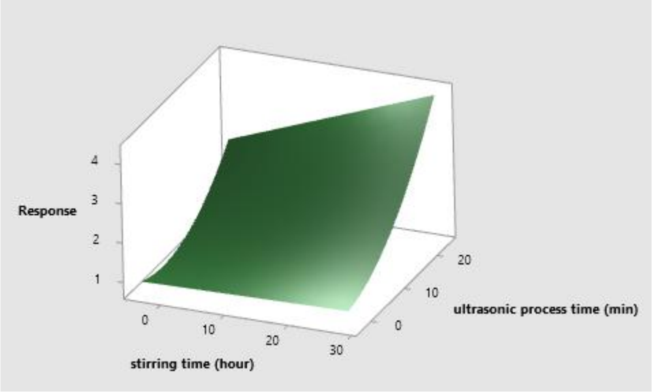

Figure 3. Surface plot of response, stirring time, and ultrasonic process time

Figure 3 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 (Christou et al., 2021; Pedisić et al., 2023).

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 4.2741%. The optimization value can be seen in Figure 4.

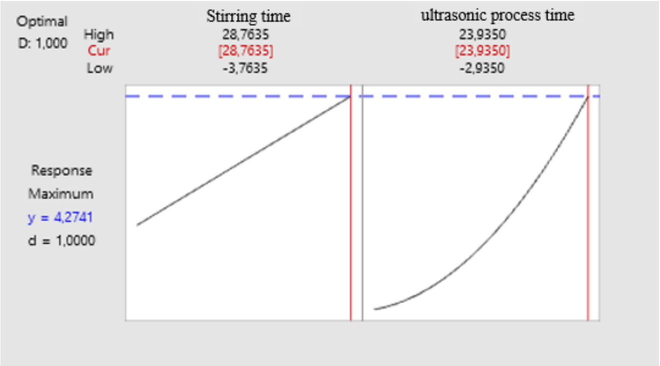

Figure 4. Process Optimization Graph

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

Parameters	Stirring time (hour)	Ultrasonic process time (min)	Response Yield (%)
Prediction*	28.7635	23.94	4.2741
Validation**	28.7635	23.94	4.238 ±0.0278
Error Value			0.8435

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 0.8453%. 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 (Pinard et al., 2020).

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 4.238%. 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 0.9191. 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%. A quadratic equation model was obtained which states the relationship between propolis extract yield and the varied factors $X = 0.865 + 0.0091A - 0.0186B - 0.000006A^2 + 0.00370B^2 + 0.00215AB$

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Author Contributions
Dwi Desmiyeni Putri was responsible for conceptualization. Syahdilla Anggiva Akhni Rarasati was technically conducted in the laboratory and responsible for writing the draft manuscript. Oktaf Rina was responsible for conceptualization, reviewing the use of terms, completing the manuscript and publication. Isnina was responsible for research methodology and provided raw materials.

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Conflicts of Interest
The authors declare no conflict of interest

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