

Optimisation Catechin Extract on Black Tea Waste (BTW) using Microwave Assisted Extraction (MAE) (Time Extraction and Ethanol Concentration as Parameters)

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Abstract. Remaining catechins from Black Tea processing can be found in significant amounts in tea waste. When using a 60% methanol organic solvent in the Microwave Assisted Extraction (MAE) method, as opposed to other methods, the extraction of black tea waste (BTW) results in a high polyphenol concentration. With a focus on yield extraction, antioxidant content, and catechin levels, the goal of this study is to identify the ideal alcohol solvent concentration and the impact of microwave heating duration on the extraction of BTW. The research uses a 2-factor Central Composite Design (CCD), which was created using Design Expert 9 Free Trial software. Factor I represents the microwave heating duration (4–8 minutes), while factor II represents the concentration of the alcohol solvent (60–80%). According to the results, the ideal conditions—a 62.74% alcohol solvent concentration and an 8-minute heating period—got a desirability of 74.20%. The expected yield was 54.8399% under these ideal circumstances, the antioxidant content (IC₅₀) was 7.50516 ppm, and the catechin level was 92.0558%. The yield, antioxidant content (IC₅₀), and catechin level of the verification results were 54.62%, 14.38847, and 94.74%, respectively, with response variations of 0.403%, 47.43%, and 2.833%.

1 Introduction

Humans are constantly exposed to oxidative stress during their lifespan from many factors such as environment, lifestyle and by products of metabolism. It is indicated by the production of reactive oxygen species (ROS) that induces cellular disruption [1-3]. Thus, the precaution of ROS creation can be useful to confront oxidative stress-related diseases. Antioxidant provides this prevention and tea plants, as a natural antioxidant, is considered as a good source of balancer with proven preclinical and epidemiological studies [2,3]. Hence, the demand of tea extract and encapsulated biomolecule, such as catechins from tea has increased in food and medicine industries as natural antioxidant and for other purposes.

Catechins are one of polyphenol group from tea leaves and important due to its antioxidant content which also found in medicinal plants. Black tea particularly provides the major source of catechin (*Camelia sinensis*) around 75% as polyphenol compound. Several health advantages provided by catechins such as free radical scavenger and retarding the

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degradation of extracellular by ultraviolet radiation and pollution [4,5]. Because of its benefits, the study of tea catechins is increasingly conducted in any purposes such as medical, pharmaceutical and food industry, even for cosmetic products [5].

In contemporary times, tea polyphenols have emerged as pivotal food additives, valued for their water solubility, which makes them suitable for application in foods with higher water content. Their utility extends to the inhibition of food component oxidation, thereby serving as a natural antioxidant for prolonged storage. Tea polyphenols are particularly effective in oil-in-water emulsions at a pH of 5.5, showcasing superiority over l-ascorbate in preserving the color of β -carotene. Additionally, they find application in chewing gums to combat halitosis and prevent dental caries, while L-theanine, with its umami taste, is employed for taste masking in various food products [6.]. Notably, tea antioxidants can contribute to the extended storage of model emulsions [7].

Heterocyclic aromatic amines (HA) identified as carcinogens generated during food processing, particularly in meat cooking, have drawn attention. Green tea marinade has shown promise in reducing HA content due to its free radical scavenging properties. Moreover, it alters the taste of pan-fried meat and serves as a non-alcoholic marinade [8]. Green tea extract treatments have been found to enhance the shelf life of fresh mutton, inhibiting lipolytic and proteolytic degradation responsible for spoilage, particularly at temperatures around 25°C [9]. Recent trends in the food industry include the incorporation of tea polyphenols in products such as biscuits and noodles for both value addition and stability improvement [10,11]. Another innovative concept involves integrating green tea extract into chitosan film for active food packaging, owing to its superior mechanical strength, antioxidant properties, and water vapor barrier capabilities [12]. Studies have hinted at the potential use of tea extract in preventing spoilage of pork sausages by increasing shelf life, attributed to its antimicrobial and antioxidant characteristics [13]. These applications underscore the recent significance of green tea as an antioxidant additive in the food industry.

To obtain its biochemical components, such as polyphenol, several methods of extraction have been conducted. Recently, microwave extraction method has proven in resulting high yield of extraction and stable antioxidant activity, especially in green tea [14]. Extraction of bioactive substances is the major step in tea consumption, while retaining the sensory properties of the beverage and maintaining the shelf life of the active components.

However, as the fresh prepared black tea is produced, tons of its waste are also produced and mostly burned or dumped into landfills as compost [15]. The previous research related to the utilisation of tea waste such as for beverage by Etoh [16], and feedlots by Krisnan [17] and Wahyuni [18]. Therefore, with the abundant availability, black tea (BT) waste from tea industry is considered to possess the same component when extracted.

Microwave Assisted Extraction (MAE) is a relatively new method for extraction by combining microwave energy and the conventional one, such as maceration. This study uses MAE due to its advantage in obtaining more yield extraction in a short amount of time [19]. The transmitted microwave energy is converted into thermal energy in a medium by ionic conduction and dipole rotation. When microwave passes through a biomass, gradual increase in temperature leads to vaporization of internal water which in turn to cell wall and plasma membrane disruption of the biomass. At this point. The permeability of solvent is increased to cell matrix then enhance the yield by dissolution of target molecule at the rise temperature [20,21]. Overall, the comparison of extraction methods is shown in table 1 below [21].

Table 1. The comparison of extraction methods

Extraction Method	Key Principles	Advantages	Limitation
Conventional	Maceration, Soxhlet, reflux, hydro distillation [22]	More time to extract	Thermal degradation, incomplete extraction, high resource requirements, environmental impact
Ultrasound Assisted Extraction (UAE)	Propagation ultrasound pressure waves, cavitation [23,24]	Increased efficacy at lower temperature	Time consuming, less extraction efficacy
Microwave Assisted Extraction (MAE)	Electromagnetic radiation, thermal energy conversion [20]	Rapid extraction, optimisation of various parameters	Possibility of structural changes, protein degradation
High pressure Processing (HPP)	Adiabatic heating, pressure increase [25-28]	Low temperature technique, freshness retention	Potential of degradation in polyphenols due to high temperature
Supercritical Fluid Extraction (SFE)	Use supercritical fluid CO ₂ [29-35]	Lesser time consumption, higher selectivity	Unavoidable loss of tea catechins, limitation in decaffeination efficiency
Subcritical Water Extraction (SWE)	Hot water extraction at high pressure [29,36]	Toxic organic solvent-free, better decaffeination efficiency	Limited temperature interval, moderate pressure requirement, less explored compared to others

Compared to the other methods, it is suggested that MAE only needs four minutes to extract polyphenol with more result than other method such as Ultrasonic which needs 90 minutes and reflux for 45 minutes [22].

There is only little research of black tea waste extraction that focus mainly on optimisation of catechin extraction, but information related to MAE method to extract bioactive compounds from various plants are available enough. Therefore, the aim of this study is to investigate the effect of extraction parameters on yield extraction, Antioxidant contents and catechin content from black tea waste (BTW) by MAE. The investigated factors were the concentration of organic solvent, such as ethanol (60%, 70%, and 80%) and extraction time of 4, 6, and 8 min.

Not only that the optimisation was obtained using Response Surface method as the statistical analysis. Response Surface Methodology (RSM) plays a crucial role in modelling and analyzing processes, particularly those influenced by multiple variables, with a primary objective of optimizing the desired response [23,24]. Within this methodology, the impacting variables are termed independent variables, while the outcomes are designated as dependent variables [25]. Therefore, the aim of this research is to discover the optimal formulation of catechins in black tea waste using Microwave Assisted Extraction (MAE) method and Response Surface Method analysis.

2 Materials and methods

2.1 Materials and chemical reagents

The Black Tea waste from *Camelia sinensis* was brought from National Plantation IX, L.td, Malang-Indonesia (PT. Perkebunan Nusantara IX). The material sample was dried to remove excessive water content and grinded to achieve smaller particle with diameter around 0.1 mm. The catechin standard and DPPH reagent were purchased in Alpha Chemical Malang (East Java, Indonesia), along with various ethanol with purity 60%, 70%, and 90%. All other chemicals were analytical grade.

2.2 Microwave Assisted Extraction (MAE) and analysis

This study uses the power of 450 W of Microwave (U-rulox Type UR-1807 made in China). A 50-gram BTW was grinded and mixed with 50 ml ethanol with various concentrations and various heating time based on the analysis for Central Composite Design (CCD) (Table 2). After that the samples were settled for an hour then filtered. The filtered specimen was then extracted using Rotary vacuum evaporator in 50-60°C for 45 minutes. The extraction was then used for further analysis. There were three analysis used in this study: (1) Yield analysis; (2) Catechin content; and (3) Antioxidant analysis using IC50 method.

2.2.1 Yield analysis

The extracted sample was weighed and compared to initial dried weight of the sample then calculate the presentation [26]. The calculation is shown as follows:

$$\text{Yield (\%)} = \frac{w0 \text{ (gr)}}{w1 \text{ (gr)}} \times 100\% \quad (1)$$

Where, $w0$ is the weight of extracted sample and $w1$ is the initial weight of the sample.

2.2.2 Catechin content analysis

The analysis of catechin used hot plate method, where the sample was diluted then heated for 5 minutes or until the temperature reach 52°C-75°C [27]. A 2 ml sample was taken and diluted in 50 mL ethyl acetate, then heated for 5 minutes or until the temperature reach 52°C-75°C then let it cool. The absorbance of the solution was measured with spectrophotometer at 517 nm. The value was then used to calculate the percentage of catechin using this formulation below.

$$\% \text{ Catechin} = \frac{Et \ 517}{Ec \ 517} \times \frac{Ws}{W} \times 100\% \quad (2)$$

Where, $Et \ 300$ is the absorbance of sample extraction at 300 nm, $Ec \ 300$ is the absorbance of catechin standard at 300 nm, Ws is the weight of catechin standard and W is the weight of dried BT waste.

2.2.3 Antioxidant analysis

DPPH (2,2-diphenyl-1-picrylhydrazyl) 0.004% was diluted in organic solvent, methanol and measured the absorbance in 517 nm [28]. The presentation of free radical capacity at 527 nm is measured using the following calculation:

$$\lambda_{Sample} = \lambda_{max} - \frac{A1+A2}{2}$$
$$\lambda_{Sample DPPH} = \lambda_{max} - \frac{A1+A2}{2}$$
$$Attenuation\ of\ DPPH\ (\%) = 1 - \frac{\lambda_{Sample}}{\lambda_{Sample\ DPPH}} \times 100\ \%$$

(3)

Where, A1 and A2 are the absorbance of sample at 517 nm before and after the peak maximum wavelength, respectively. The lower number of attenuations of DPPH show the higher free antiradical activity.

2.2.4 IC50 analysis

The extraction sample was diluted into organic solvent with five times dilution 10 ppm, 12.5 ppm, 15 ppm, 17.5 ppm and 20 ppm. The absorbance of each dilution was then measured with the following calculation for the inhibition presentation:

$$\left\{ \frac{Absorbance\ of\ DPPH - Absorbance\ of\ sample}{Absorbance\ of\ DPPH} \right\} \times 100\%$$

(4)

With the absorbance of 0.2 molar DPPH is 1.999

2.3 Experimental design and statistical analysis

RSM was applied to obtain the optimum catechin yield from BT waste (*Camelia sinensis*) using Central Composite Design with two independent variables such as the time of extraction using microwave (X1) and the concentration of organic solvent (X2). According to RSM with 2 factors, repetition was designed at the central pint of each variable (X=0) for five times. 4 minutes (X1=0) for time of extraction, and 70% of organic solvent (X2=0) for ethanol concentration. Therefore, the selected α value would be $k=2$ or $2^{k/4} = 2^{2/4} = 1,414$. The whole experimental design was composed of 13 experimentations as shown in table 2. Five replications (9-13) were performed in the design centre to analysis the error of squares. Design Expert 9 was used in this study with accuracy of the polynomial model equation of F-test and p-value ($p<0.05$).

Table 2. Experimental design

No	Variable Code		Original Variable		Respond		
	X1	X2	A= Alcohol (%)	B= time (minutes)	Yield	Catechin content	Antioxidant content
1	-1	-1	60	4	Y ₁	Y ₂	Y ₃
2	1	-1	80	4	Y ₁	Y ₂	Y ₃
3	-1	1	60	8	Y ₁	Y ₂	Y ₃
4	1	1	80	8	Y ₁	Y ₂	Y ₃

5	-1,414	0	55,86	6	Y ₁	Y ₂	Y ₃
6	1,414	0	84,14	6	Y ₁	Y ₂	Y ₃
7	0	-1414	70	3,17	Y ₁	Y ₂	Y ₃
8	0	1414	70	8,83	Y ₁	Y ₂	Y ₃
9	0	0	70	6	Y ₁	Y ₂	Y ₃
10	0	0	70	6	Y ₁	Y ₂	Y ₃
11	0	0	70	6	Y ₁	Y ₂	Y ₃
12	0	0	70	6	Y ₁	Y ₂	Y ₃
13	0	0	70	6	Y ₁	Y ₂	Y ₃

3. Results

3.1 Black Tea Waste (BTW) yield

The result of BTW yield’s response using Design Expert 9 can be seen in Table 3.

Table 3. Yield response from black tea waste

No	Variabel codes		Variabel name		Response Yield (%)
	X1	X2	B: concentration (%)	A: Time Extraction (minutes)	
1	-1	-1	60	4	44,48
2	1	1-	80	4	27,62
3	-1	1	60	8	46,46
4	1	1	80	8	47,48
5	-1,414	0	55,86	6	57,36
6	1,414	0	84,14	6	13,36
7	0	-1,414	70	3,17	20,94
8	0	1,414	70	8,83	41,30
9	0	0	70	6	41,21
10	0	0	70	6	40,01
11	0	0	70	6	42,34
12	0	0	70	6	49,88
13	0	0	70	6	43,24

Table 3 reveals that the maximum yield, reaching 57.36%, was achieved using alcohol with a purity of 55.86% and a 6-minute extraction time. Conversely, the minimum yield, at 13.36%, was observed with 84.14% alcohol and a 6-minute Microwave-Assisted Extraction (MAE) time. In contrast, control designs exhibited a consistent range of yield results, ranging from approximately 41-43%, except for the 12th sample, which recorded a yield of 49.88% with a 70% alcohol concentration in a 6-minute timeframe.

3.2 ANOVA of BTW’s yield response.

Result of Analysis Variant (ANOVA) of yield response from Black Tea Waste in table 4.

Table 4. Analysis Variant (ANOVA) of yield response from Black Tea Waste

Variations	F Value	P Value	Significancy
Model	7,86	0,0089	Significant
A-Time Extraction (Menit)	4,73	0,0546	Not significant

B- Alcohol concentration (%)	10,99	0,0078	<i>Significant</i>
Lack of Fit	7,04	0,0399	<i>Significant</i>
Std Dev	8,29		
R ²	0,6113		

Based on the ANOVA table, the linear model is favored, exhibiting a significant influence on the yield response with an F-value of 7.86 and a p-value less than 0.05, specifically 0.089%. The concentration of alcohol also holds significance for the model, with a p-value of 0.0078, indicating that varying alcohol concentrations affect the yield result. In contrast, the time extraction displayed an insignificant result with a p-value of 0.00546. The absence of interaction between factors is evident, aligning with the linear model recommendation and explaining why the quadratic factor remains undisclosed. The validation model's significance is indicated by a p-value of 0.0399, suggesting that the model is unreliable for predictions. The R2 value, representing the coefficient of determination, stands at 0.6113, signifying that 61.13% of the data supports the model. The positive correlation is highlighted by the Adj R-squared value of 0.5336. The second-order polynomial model equation for obtaining Black Tea Waste (BTW) yield, as determined by the Design Expert program, is provided below.

$$Y= 39,70 + 6,37X_1 - 9,71X_2 \tag{5}$$

The actual equation form of second-order polynomial equation is shown as follow.

$$Y= 88,57 + 3,19X_1 - 0,97X_2 \tag{6}$$

Where:

- Y = The Yield of Black Tea Waste
- X_1 = Time of Extraction (minutes)
- X_2 = Alcohol concentration (%)

3.3 Contour plot and surface curve response of BTW’s yield

Fig. 1 and Fig. 2 show a graphical from of yield optimisation from time extraction (minutes) and alcohol concentration (%).

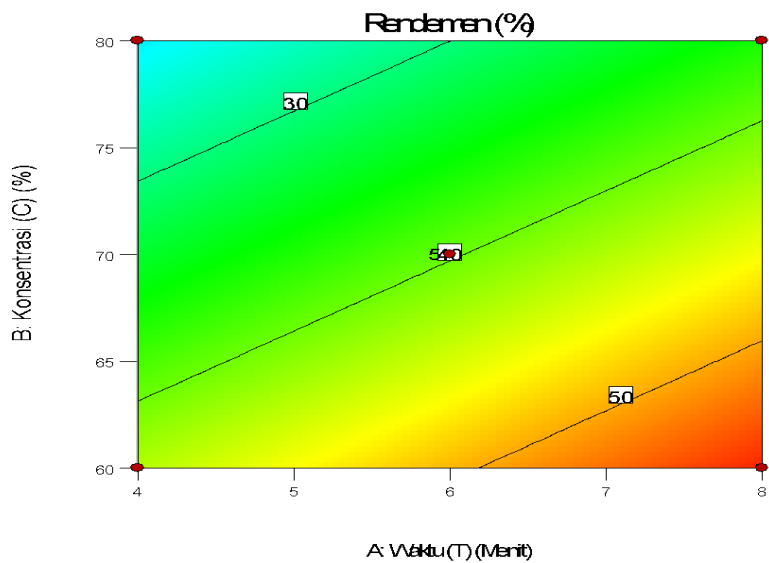


Fig. 1. Contour plot of yield from Black Tea Waste

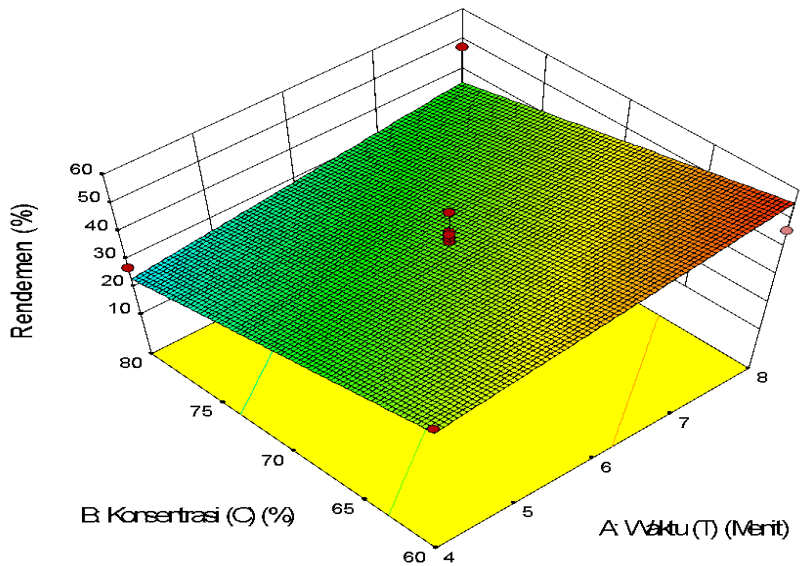


Fig. 2. Response surface curve of yield from Black Tea Waste

In Fig. 1, the X-axis represents the time extraction (in minutes), while the Y-axis denotes alcohol concentration (%). The line within the contour illustrates the yield response, with the outer line indicating the minimal response and the inner line representing the highest response. The red node at the center of the contour signifies the optimal value, which is 40% for the yield result. Additionally, Fig. 2 displays a surface curve that aligns with the optimization components of Fig. 1. Given the recommendation of a linear model, the yield

model's curve is relatively flat, with alcohol concentration (%) identified as the significant factor. Utilizing this curve involves examining the two corner spots, (X1) at 4 and (X2) at 60%, plugged into equation 2, resulting in a final optimal yield of 43.13%.

3.4 The response of antioxidant content (IC50)

The result of antioxidant content using IC50 parameter form Design Expert 9 is shown in Table 5.

Table 5. The result of antioxidant content (IC50) from black tea waste

No	Variabel codes		Original Variables		Respon Antioxidant IC50 (%)
	X1	X2	B: Alcohol (%)	A: heating time (minutes)	
1	-1	-1	60	4	8.42
2	1	1-	80	4	8.40
3	-1	1	60	8	8.39
4	1	1	80	8	8.44
5	-1,414	0	55,86	6	8.01
6	1,414	0	84,14	6	9.45
7	0	-1,414	70	3,17	8.52
8	0	1,414	70	8,83	6.13
9	0	0	70	6	8.53
10	0	0	70	6	8.51
11	0	0	70	6	8.35
12	0	0	70	6	8.32
13	0	0	70	6	8.39

Table 3 indicates that the lowest antioxidant activity is associated with an IC50 concentration of 9.45 ppm, achieved with an 84.14% alcohol concentration in 6 minutes. In contrast, the highest antioxidant activity is observed at 70% alcohol concentration in 8.83 minutes, resulting in an IC50 value of 6.13 ppm.

3.5 ANOVA of antioxidant content (IC50)

The best model to obtain antioxidant activity form BTW is quadratic model shown in Table 6.

Table 6. ANOVA of Antioxidant content (IC50) from black tea waste

Source of variation	F Value	P Value	Significance
Model	2,51	0,1310	Not significant
A-Time (Menit)	4,37	0,0749	Not significant
B-Alcohol Concentration (%)	1,64	0,240	Not significant
AB	3,77	0,9528	Not significant
A ²	4,56	0,0702	Not significant
B ²	1,25	0,3011	Not significant
Lack of Fit	78,46	0,0005	Significant
Std dev	0,57		
R ²	0,6420		

The table above reveals that the quadratic model is not statistically significant, evidenced by an F value of 2.51 and a p value of 0.1310 (> 0.05). Additionally, both factors, extraction

time and alcohol concentration, are not significant, with p values of 0.0749 and 0.240, respectively, exceeding the 0.05 threshold. Furthermore, the quadratic model's attempt to validate the interaction between factors remains insignificant. Despite this, the lack-of-fit value is significant at a p value of 0.0005 or 0.05%, rendering it unreliable for use as a predictive model. The R2 value (coefficient of determination) for this model is 0.6420, signifying that 64.20% of the antioxidant result is influenced by the included factors, while the remaining 35.8% is influenced by other excluded factors. The positive correlation of this model is indicated by an R value of 0.3862. The second-order polynomial equation for antioxidant content from Design Expert 9 is presented below.

$$Y= 8,44 -0,42X_1 + 0,26X_2 + 0,017X_1X_2 - 0,46 X_1^2 +0,24X_2^2 \tag{7}$$

The actual equation is as follow

$$Y= 15,933 + 1,112X_1 - 0,317X_2 + (8,75 \cdot 10^{-4})X_1X_2 - 0,115X_1^2 + (2,413 \cdot 10^{-3})X_2^2 \tag{8}$$

Where:

- Y = Antioxidant content from BT Waste
- X_1 = Time Extraction (minutes)
- X_2 = Alcohol concentration (%)

3.6 Contour plot and surface curve response of IC50

Fig. 3 and Fig. 4 show the interaction between the factors (time extraction and concentration of alcohol) to optimise the result of antioxidant content (IC50) in black tea waste.

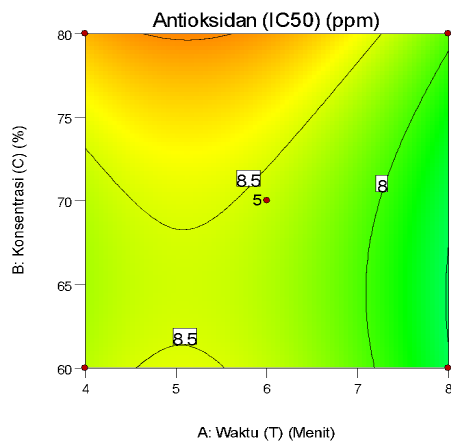


Fig. 3. Contour plot response of antioxidant content (IC50) in ppm from black tea waste

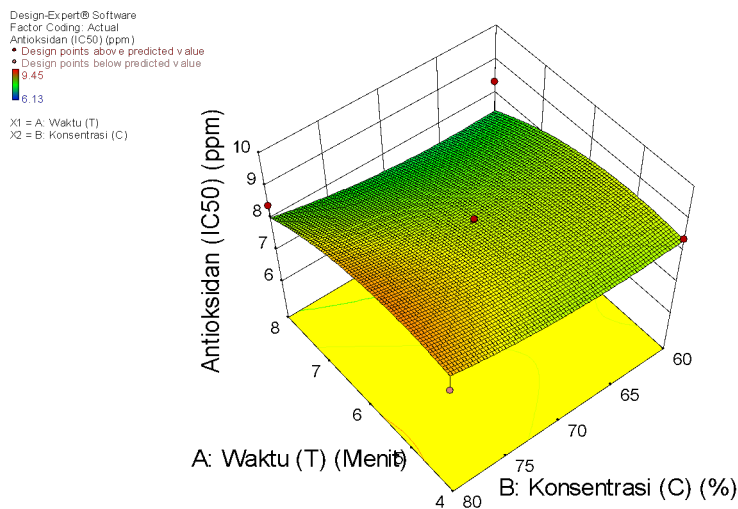


Fig. 4. The curve of surface response antioxidant content (IC50) in ppm from black tea waste

In the contour plot (Fig. 3), four lines illustrate the response of two factors, yet only three IC50 responses are displayed: 8.5 ppm, 8.5 ppm, and 8.0 ppm. The optimal response, depicted by the red node at 8.5 ppm, is achieved with an alcohol concentration between 70-75% in 6 minutes. The curved graph in Fig. 4 is a result of the quadratic model preference in the ANOVA table. From the curve, the optimum values (X1) and (X2) are 4 minutes and 60%, respectively. These values are substituted into equation (4), yielding a result of 8.46 ppm.

3.7 Contour plot and surface curve response of IC50

The result of catechin content from BT waste extraction is shown in Table 7.

Table 7. The result of catechin content from BT waste extraction

No	Variable code		Original Variables		Response of catechin (%)
	X ₁	X ₂	B: Alcohol (%)	A: heating time (minutes)	
1	-1	-1	60	4	90,16
2	1	-1	80	4	90,89
3	-1	1	60	8	91,89
4	1	1	80	8	91,84
5	-1,414	0	55,86	6	91,79
6	1,414	0	84,14	6	92,47
7	0	-1,414	70	3,17	90,47
8	0	1,414	70	8,83	91,74
9	0	0	70	6	92,32
10	0	0	70	6	92,42
11	0	0	70	6	92,47
12	0	0	70	6	92,37
13	0	0	70	6	92,68

Table 7 reveals that all samples exhibit a high range of catechin content, ranging from approximately 90-92%. Specifically, the highest catechin response is 92.68%, extracted

using 70% alcohol over 6 minutes, while the lowest response is obtained from 60% alcohol in 4 minutes. The significance of this model is elucidated in the subsequent analysis.

3.8 ANOVA of catechin content

The analysis of variant from catechin content extracted from black tea waste was explained in Table 8.

Table 8. Analysis of Variant (ANOVA) of catechin content

Source of variation	F Value	P Value	Significancy
Model	19,13	0,0006	<i>Significant</i>
A-Lama Pemansan	32,34	0,0007	<i>Significant</i>
B-Konsentrasi pelarut	4,35	0,0754	<i>Not Significant</i>
AB	1,96	0,2038	<i>Not Significant</i>
A ²	54,53	0,0002	<i>Significant</i>
B ²	6,39	0,0394	<i>Significant</i>
Residual			
<i>Lack of Fit</i>	8,00	0,0364	<i>significant</i>
Std Dev.	0,28		
R ²	0,9318		

Based on the table provided, the model for predicting catechin content demonstrated statistical significance. Additionally, both the time extraction factor (A) and its quadratic term (A2) were found to be statistically significant, with p-values of 0.0007 and 0.0002, respectively. However, the use of organic solvent (B) and the interaction between factors (AB) showed insignificance, with p-values of 0.0754 and 0.2038, respectively. The quadratic form of factor B (B2) exhibited significance in this model, with a p-value of 0.0394. Despite these findings, the model remains unreliable for predictive purposes, as indicated by the lack of fit with a p-value of 0.0364. The coefficient of determination (R2) is 0.9318, and the adjusted R value is 0.8831. The secondary-order polynomial equation for this model, as provided by Design Expert 9, is presented below:

$$Y = 92,45 + (0,56)X_1 + (0,21)X_2 - (0,78)X_1^2 - (0,27)X_2^2 - (0,20)X_1X_2. \tag{9}$$

The actual equation is as follow

$$Y = 65,16527 + 3,29963X_1 + (0,45230)X_2 - (0,19478)X_1^2 - (2,66625 \cdot 10^{-3})X_2^2 - (9,75 \cdot 10^{-3})X_1X_2 \dots\dots\dots (10)$$

Where:

Y

= Catechin conten extracted from BT waste

X₁

= Time extraction (minutes)

X₂

= Alcohol concentration(%)

3.9 Contour plot and surface response of catechin content

Fig. 5 and 6 shows the graphical information of catechin content.

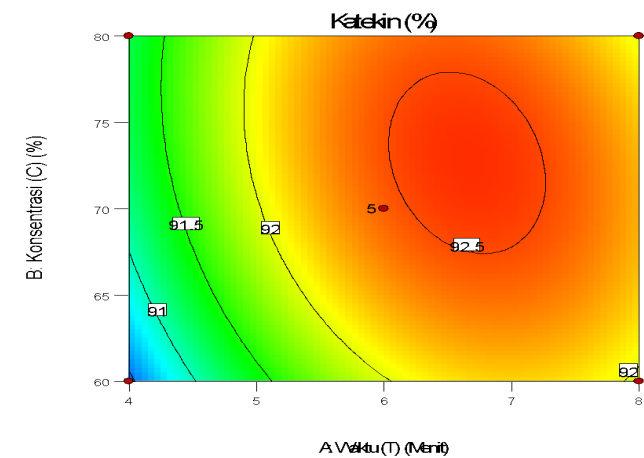


Fig. 5 Contour plot of catechin content extracted from black tea waste

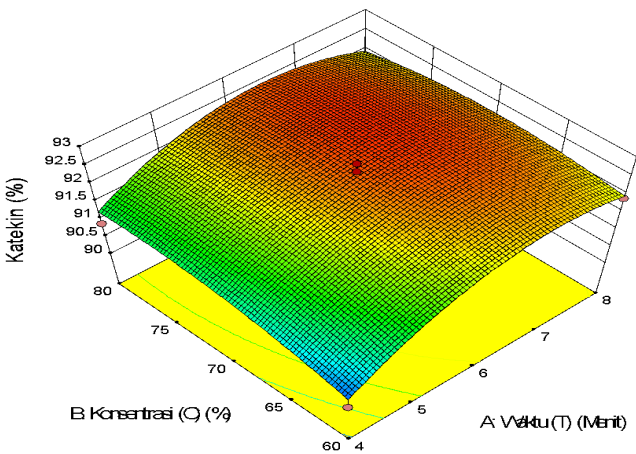


Fig. 6 The surface response of catechin content extracted from black tea waste

Fig. 5 displays a distinctive analysis compared to others. The contour plot suggests an optimal catechin content of approximately 92.5% (indicated by the node). Notably, the values along the outer line are close to the node, with 92% achieved with just under 70% alcohol concentration for over 6 minutes of extraction. The second outer line yields a result of 91.5% with around 5.2 minutes of extraction and slightly above 70% alcohol concentration. Lastly, a catechin content of 91% is attained within the range of 60-65% alcohol concentration in around 4 minutes.

Subsequently, Fig. 6 presents the optimal factors that align with the second-order polynomial equation (6). According to the curve, the optimal values for (X1) and (X2) are 4 minutes and 60%, respectively. When these values are inserted into equation (6), the anticipated result is 90.31476%

3.10 Prediction and verification results of the responses

The study set constraints on two parameters, namely the microwave extraction time and solvent concentration, to determine the optimal outcomes for yield, antioxidant activity, and catechin content in black tea waste. The comprehensive results obtained within these specified parameters are outlined in Table 9, including a comparison with findings from previous studies.

Table 9. Limit optimisation of response surface

Criteria	Name	Aims	Lower Limit		Upper Limit	
			Other studies	This study	Other studies	This study
Factor	Microwave extraction (minute)	<i>In Range</i>	4	4	8	8
Factor	Alcohol ratio (%)	<i>In Range</i>	60	60	90	84,14
Respond	Yield (%)	Maximum	20,31 ^{a)}	13,36	53,21 ^{a)}	57,36
Respond	Antioxidant (IC50) ppm	Minimum	8,06 ^{b)}	6,13	10,09 ^{b)}	9,45
Respond	Catechin content (%)	Maximum	90,00 ^{c)}	90,21	94,55 ^{c)}	92,68

Sources: ^{a)} Handayani [14] ^{b)} Filbert [48] ^{c)} Isnawati [49]

From the limitations set above, the optimum result from Design Expert 9 Free Trial is given to Table 10.

Table 10. Optimisation computational result from Design Expert 9 Free Trial

Parameters and Responses	Prediction Standard
Time Extraction in microwave (Min)	8
Ethanol ratio (%)	62,7458
Yield (%)	54,1157
Antioxidant content (IC50) ppm	7,48438
Catechin content	92,0847
Desirability	0,742
Information	<i>Selected</i>

Table 10 displays the standardized prediction for achieving the optimal response using 8 minutes of microwave heating and 62.75% ethanol concentration. The results obtained through the Design Expert 9 Free Trial program indicate optimal values of 54.12% for yield, 7.48 ppm for antioxidant content, and 92.08% for catechin content. The desirability number serves as an indicator of optimization accuracy, where a value closer to one signifies higher accuracy in optimization [24].

Data from Table 11 was verified to find the error. The verification result is shown in Table 9.

Table 11. Prediction values for optimal response

Responses	Prediction	SE Pred	95% PI low	95% PI high
Yield (%)	54,8399	9,46	33,76	75,92
Antioxidant (IC50) ppm	7,50516	0,71	5,83	9,18

Catechin content (%)	92,0558	0,56	90,74	93,37
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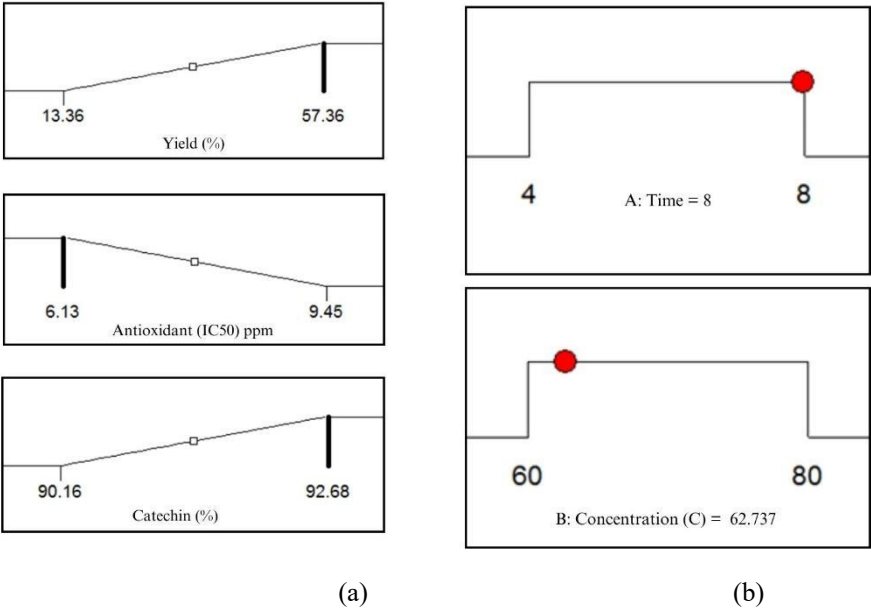
Table 11 illustrates that the lower response for yield is 33.76%, while the upper response may approach almost 76%, with an error of 9.46 being the highest among the responses. The lower predictions for antioxidant (IC50) and catechin content are 5.83 ppm and 90.74%, respectively. Finally, the highest predictions for these two responses could reach 9.18 ppm and 93.37%, respectively. These predictions were subsequently used to verify the results, and the verification outcomes of responses, based on Table 9, are presented in Table 12, utilizing the optimization parameters A (8 minutes) and B (62.75%) recommended by Design Expert 9.

Table 12. Verification results of responses

Responses	95% PI Low	Prediction	95% PI High	Verification results	difference	% Deviation
Yield (%)	33,76	54,8399	75,92	54,62	0,2199	0,403%
Antioxidant (IC50) ppm	5,83	7,50516	9,18	14,38847	6,88318	47,43 %
Catechin content (%)	90,74	92,0558	93,37	94,74	2,6842	2,833 %

From Table 12, it is evident that in the verification process of the RSM, the deviation for yield is 0.403%, while the antioxidant content (IC50) and catechin content are higher than predicted, with deviations of 47.21% and 2.833%, respectively. The verification results, especially in antioxidant content, exhibited significantly higher values, almost double the predicted values. Despite this considerable difference compared to the computational analysis, the results are still appropriate when compared to the standard (12 ppm). Similarly, the catechin content also demonstrated a higher result (94.47%) than the predicted value. The catechin levels in this study comply with the requirements of herbal pharmacopoeia, as the value exceeds or equals 90%.

□



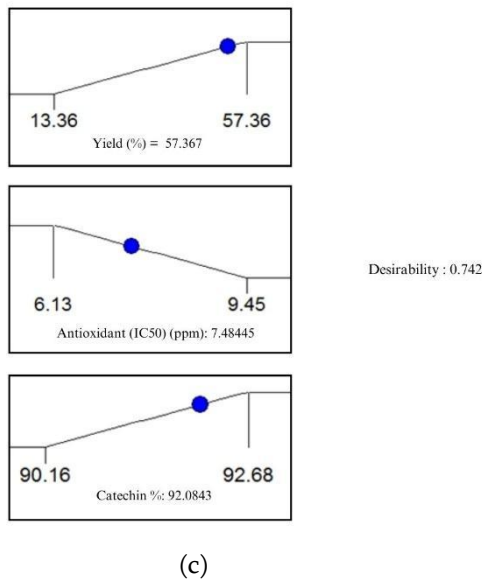


Fig. 7. (a) Optimisation results of yield (%), Antioxidant (IC50) ppm, and catechin content (%); (b) Optimum factors chosen by RSM model; (c) Results of each response and desirability of the model.

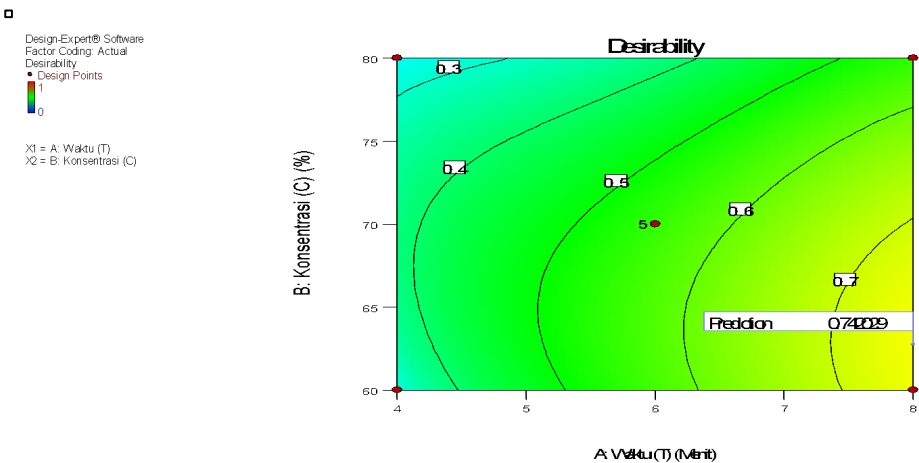


Fig. 8. Contour plot and response of model desirability.

4 Discussion

4.1 Yield extraction

The quantity of bioactive material dissolved and absorbed by the solvent is determined by the concentration of organic solvent, such as ethanol, combined with the extraction time. Specifically, the extraction time represents the duration during which the cell membrane is

open, allowing the components within black tea (BT) waste to be more readily attracted by the solvent. The application of microwave heat facilitates an increase in yield [14]. The microwave heat induces the evaporation of alcohol, leading to increased pressure on the cell wall, causing swelling. This pressure, in turn, pushes the cell wall from the inside, causing it to stretch and eventually break [29].

This study indicates that, to achieve yield from black tea waste, the influence of only two factors accounts for approximately 61%, leaving the remaining 38% possibly influenced by other unmentioned factors. This pattern holds true for every analysis, though the proportion may vary. Excluded factors, such as the temperature of the extraction process, should be noted. While an increase in temperature correlates linearly with an increase in yield, prolonged duration may result in the degradation of thermolabile bioactive compounds [50,51]. Additional factors that may impact yield include extended heating in a vacuum evaporator, encompassing temperature, pressure, and extraction time. Furthermore, the material's quality can influence the extraction yield [30]. Unprofessional handling processes, harvesting time, and post-harvest practices may also exert an influence on the yield of extraction.

This study reports varied yield results across different alcohol concentrations, contrary to the findings of Handayani [14] and Ramadhan and Phaza [31] in their studies on oleoresin extraction. A higher concentration of organic solvent typically results in a higher yield, attributed to the lower polarity in higher concentrations, where the added water is less than the solvent with lower concentration [32]. The yield analysis in this study was conducted over hours, especially during the extraction process using a vacuum evaporator.

4.2 Antioxidant (IC50)

The antioxidant content is quantified as IC50, representing the concentration of antioxidants required to reduce or inhibit 50% of free radicals. A medium antioxidant content is denoted by an IC50 value of 48.6 ppm. A lower IC50 value indicates a stronger antioxidant presence in the sample. For instance, Alpha-tocopherol at a concentration of 12 ppm exhibits a 96% inhibition of free radicals [33]. Despite the unreliability of this model prediction to yield significant values, this study highlights the robust antioxidant content in black tea waste.

Various factors, as noted by Tachakittirungrod [34], can influence antioxidant activity, including plant maturity, pre-treatment of materials that would otherwise be discarded, and the drying process of the material. Additionally, environmental conditions, such as climate and soil condition, play a role in the cultivation method, affecting the antioxidant activity, as stated by Thaipong [35].

Ongoing studies aim to enhance extraction rates, reduce extraction time and costs, and maximize processing throughput. However, the antioxidant components, frequently utilized as nutraceuticals or food-grade antioxidants, are easily degraded at increased temperatures and within body fluids. Kinetics studies are conducted to comprehend the production and degradation of these ingredients under varied parameters. Storage conditions, such as temperature, humidity, and the type of food, impact the stability of tea catechins [10]. Encapsulation technologies have recently shown promise in extending the shelf life of these tea antioxidant molecules for both food and pharmaceutical applications.

Efficiency in the extraction process is influenced by various conditions, including extraction parameters, setup, tea variety, and storage conditions. The advent of novel techniques aims to streamline the extraction process, minimize extraction costs and time, and maximize yield. Simultaneously, efforts are made to preserve the maximum antioxidant potential and sensory attributes, such as color, aroma, sweetness, while reducing proteins and pectin contents.

4.3 Catechin

Catechins, belonging to the flavonoid class of secondary metabolites, are naturally produced by plants, particularly tea. Recognized as polyphenol compounds due to their distinctive groups, catechins exhibit antioxidant activity attributed to phenol groups (A and B rings) and a dihydropyran group (C-Ring). In this study, catechin content was determined by measuring the absorbance of pure catechin, deducting it from the sample's absorbance, and then dividing the result by the absorbance corresponding to the catechin standard. High catechin levels, as indicated by Isnawati [36], signify greater antioxidant compounds to counteract free radicals. Catechins are considered equivalent to the standard when their content is $\geq 90\%$.

In studies involving the extraction of bioactive molecules using MAE, optimizing results involves considering factors like microwave power, frequency, duration, radiation amount, moisture content of plant samples, type of organic solvent, solid-liquid ratio, extraction process temperature, and pressure [20]. The sequence of factors begins with microwave irradiation time, intensity, solid-liquid ratio, and the number of irradiation cycles [37]. Polyphenol extraction in tea varies with parameters, such as in Tsubaki's study [38], where MAE with a 2-minute extraction and a temperature around 23°C not only extracted 60-70% of polyphenols but also almost 50% of polysaccharides and Cutin, a bio-polyester. High temperature is a limitation for polyphenol extraction, often causing degradation, especially in tea [20].

To prevent degradation and oxidation, selecting the right extraction temperature and MAE time, which is comparatively short compared to conventional techniques, is crucial [39]. Extending the extraction time has been shown to increase yield in some cases, as demonstrated by Dragović-Uzelac [40] in sage extracts. The composition of bioactive components in tea is influenced by factors like geographical distribution, climate conditions, cultivation processes, and leaf age [41]. Time efficiency, energy utilization during microwave extraction, and solvent choice also impact the outcome of bioactive components. Time plays a crucial role, correlating linearly with the outcome, despite the risk of compound degradation. Mass transfer rate from solid to liquid is significantly influenced by solvent substance concentration, leading to decreased mass transfer rate with increasing solute concentration [42].

The enhancement of extraction yield in MAE is attributed to its heating effect, caused by dipole rotation and ion conduction in the solute and solvent. These processes disrupt hydrogen bonds, facilitating solvent penetration into the matrix and component extraction [28]. Phenolic extraction, irrespective of the method, is influenced by factors such as solvent type and polarity, physical and chemical characteristics of samples, extraction time and temperature, sample-to-solvent ratio, and matrix characteristics, including particle size [39,47,40]. The efficiency of phenolic extraction by MAE is further affected by microwave power, controlling the set temperature [39,43].

The choice of extraction solvents depends on factors such as solvent penetration into the matrix, solubility of the target compound, interaction between the solvent and plant matrix, and the solvent's capability to heat up due to microwave energy absorption. Solvents with high dielectric constant and dielectric loss constant theoretically have a high capacity to absorb microwave energy [39,44]. Accordingly, water, ethanol, acetone, methanol, propan-2-ol, and acetonitrile are considered suitable solvents for the MAE of plant bioactive compounds based on their dielectric properties [45,46].

5 Conclusion

In this experimental study, catechin extraction from black tea waste was conducted using Microwave-Assisted Extraction (MAE), with the parameters of microwave extraction time

(A) and ethanol concentration (B) optimized through Response Surface Methodology (RSM). The RSM-predicted values indicated a yield of approximately 54.84%, an antioxidant content (IC₅₀) of 7.50 ppm, and a catechin content of 92.05%. Upon verification, the actual results were a yield of 52.62%, IC₅₀ of 14.38 ppm, and catechin content of 94.74%, with deviations of 0.403%, 47.43%, and 2.833%, respectively. While the results met pharmacopoeia standards, the model's reliability for obtaining each response was compromised due to a significant lack-of-fit value. Other limitations include the use of only two parameters and the absence of further analysis on the types of catechins in black tea waste, as well as an exploration of nutraceutical effects. These constraints stemmed from the limited access to certain laboratories and facilities. Future studies should extend their scope to identify the specific catechin types present in black tea waste (BTW).

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References

1. B.L. Tan, M.E. Norhaizan, W.P.P. Liew, H. S. Rahman. *Frontiers in pharmacology*. **9**, 1162 (2018)
2. D. Harman *Science of Aging Knowledge Environment*. **37**, cp14-cp. (2002)
3. A.M. Abraham, R.M. Alnemari, J. Br  bler, C.M. Keck. *Molecules*. **26**(3), 592 (2021)
4. Shi M, Nie Y, Zheng X-Q, Lu J-L, Liang Y-R, Ye J-H. *Molecules*. **21**(10),1345 (2016)
5. Bae J, Kim N, Shin Y, Kim S-Y, Kim Y-J. *Biomedical Dermatology*. **4**(1), 1-10 (2020)
6. Dufresne CJ, Farnworth ER. *The Journal of nutritional biochemistry*. **12**(7), 404-21. (2001)
7. A. Roedig-Penman, M.H. Gordon. *Journal of Agricultural and Food Chemistry*. **45**(11), 4267-70 (1997)
8. I. Quelhas, C. Petisca, O. Viegas, A. Melo, O. Pinho, I. Ferreira. *Food chemistry*. **122**(1), 98-104 (2010)
9. K. Kumudavally, H. Phanindrakumar, A. Tabassum, K. Radhakrishna, A. Bawa. *Food chemistry*. **107**(1), 426-33 (2008)
10. V.K. Ananingsih, A. Sharma, W. Zhou. *Food research international*. **50**(2), 469-79 (2013)
11. M. Li, J-H. Zhang, K-X. Zhu, W. Peng, S-K. Zhang, B. Wang, et al. *LWT-Food Science and Technology*. **46**(1), 23-8 (2012)
12. U. Siripatrawan, B.R. Harte. *Food hydrocolloids*. **24**(8), 770-5 (2010)
13. U. Siripatrawan, S. Noipha. *Food hydrocolloids*. **27**(1), 102-8 (2012)
14. D. Handayani, A. Mun'im, A.S. Ranti. *Majalah Obat Tradisional*. **19**(1), 29-35 (2014)
15. M. Kondo, K. Kita, H. Yokota. Effects of tea leaf waste of green tea, oolong tea, and black tea addition on sudangrass silage quality and in vitro gas production. *Journal of the Science of Food and Agriculture*. **84**(7), 721-7 (2004)
16. Etoh H, Ohtaki N, Kato H, Kulkarni A, Morita A. *Bioscience, biotechnology, and biochemistry*. 1003051882 (2010)
17. Krisnan R. *JITV*. **10**(1), 1-5 (2005)
18. Wahyuni TH. Pengaruh Pemberian Ampas Teh (*Camellia Sinensis*) Dalam Pakan Terhadap Analisis Usaha Domba Lokal Jantan Lepas Sapih Selama 3 Bulan Penggemukan: Universitas Sumatera Utara; (2009).
19. Delazar A, Nahar L, Hamedeyazdan S, Sarker SD. *Natural products isolation*. 89-115 (2012)

20. Zhang H-F, Yang X-H, Wang Y. Trends in Food Science & Technology. **22**(12), 672-88 (2011)
21. Banerjee S, Chatterjee J. Journal of food science and technology. **52**(6), 3158-68 (2015)
22. Pan X, Niu G, Liu H. Chemical Engineering and Processing: Process Intensification. **42**(2), 129-33 (2003)
23. Braima N, Maryam ANA, Odejobi OJ. Utilization of Response Surface Methodology (RSM) in the Optimization of Crude Oil Refinery Process, New Port-Harcourt Refinery. Nigeria (2016)
24. Montgomery DC. John wiley & sons; (2017)
25. Koç B, Kaymak-Ertekin F. GIDA-Journal of Food. **35**(1), 63-70 (2010)
26. Widyawati PS, Wijaya CH, Hardjosworo PS, Sajuthi D. Rekapangan Jurnal Teknologi Pangan. **5**(1), 1-14 (2011)
27. Annisa NM. Farm, Farmasi, Fakultas Matematika dan Ilmu Pengetahuan Alam, Universitas Sriwijaya, Inderalaya, Sumatera Selatan (2018).
28. Zuhra CF, Tarigan JB, Sihotang H. Jurnal Biologi Sumatera. **3**(1), 7-10 (2008)
29. Calinescu I, Ciuculescu C, Popescu M, Bajenaru S, Epure G, editors. Romanian International Conference on Chemistry and Chemical Engineering, (2001).
30. Nguyen HC, Nguyen HNT, Huang MY, Lin KH, Pham DC, Tran YB, et al. Journal of Food Processing and Preservation. **45**(3), e15221 (2021)
31. Eka Ramadhan A, Aprival Phaza H. Pengaruh konsentrasi etanol, suhu dan jumlah stage pada ekstraksi oleoresin jahe (*Zingiber Officinale* Rosc) secara batch: Jurusan Teknik Kimia UNDIP, (2010)
32. Shadmani A, Azhar I, Mazhar F, Hassan MM, Ahmed SW, Ahmad I, et al. Pakistan Journal of Pharmaceutical Sciences. **17**(1), 47-54 (2004)
33. Setiawan V, Phangestu S, Soetikno AG, Arianti A, Kohar I. Pharmaceutical Journal of Indonesia. **7**(1), 9-14 (2021)
34. Tachakittirungrod S, Okonogi S, Chowwanapoonpohn S. Food chemistry. **103**(2), 381-8 (2007)
35. Thaipong K, Boonprakob U, Cisneros-Zevallos L, Byrne DH. Southeast Asian journal of tropical medicine and public health. **36**, 254 (2005)
36. Isnawati A. (Karakterisasi dan Isolasi Ketekin dari 3 mutu Ekstrak Gambir) (2010)
37. Fu L, Xu B-T, Gan R-Y, Zhang Y, Xu X-R, Xia E-Q, et al. International journal of molecular sciences. **12**(4), 2112-24 (2011)
38. Tsubaki S, Iida H, Sakamoto M, Azuma J-i. Journal of agricultural and food chemistry. **56**(23), 11293-9 (2008)
39. Veggi PC, Martinez J, Meireles MAA. Springer; p. 15-52 (2012)
40. Dragović-Uzelac V, Elez Garofulić I, Jukić M, Penić M, Dent M. Food Technology and Biotechnology. **50**(3), 377-83 (2012)
41. Jang I-S, Ko Y-H, Moon Y-S, Sohn S-H. Asian-Australasian Journal of Animal Sciences. **27**(5), 749 (2014)
42. Jun X, Deji S, Ye L, Rui Z. Food Control. **22**(8) (2011)
43. Afoakwah A, Owusu J, Adomako C, Teye E. Global Journal of Bioscience and Biotechnology. **1**(2), 132-40 (2012)
44. Jain T, Jain V, Pandey R, Vyas A, Shukla S. Asian Journal of Research in Chemistry. **2**(1), 19-25 (2009)
45. Lovrić V, Putnik P, Bursać Kovačević D, Jukić M, Dragović-Uzelac V. Food technology and biotechnology. **55**(2), 243-50 (2017)
46. Khoddami A, Wilkes MA, Roberts TH. Molecules. **18**(2), 2328-75 (2013)
47. Dai J, Mumper RJ. Molecules. **15**(10) 7313-52 (2010)
48. Filbert F, Koleangan HS, Runtuwene MR, Kamu VS. Jurnal MIPA Unsrat Online. **3**(2), 149-54 (2014)

49. Isnawati A. Analisa Kualitatif dan Kuantitatif Senyawa Katekin dan Kuersetin pada 3 Mutu Ekstrak Gambir (Karakterisasi dan Isolasi Ketekin dari 3 mutu Ekstrak Gambir). (2010)
50. Wang L, Weller CL. Trends in Food Science & Technology. **17**(6), 300-12 (2006)
51. Eskilsson CS, Björklund E. Journal of chromatography A. **902**(1), 227-50 (2000)