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Optimization of Total Phenolic Content Analysis of Chayote (*Sechium edule*) using Ultrasonic-Assisted Extraction and Response Surface Methodology

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Abstract. Chayote (*Sechium edule*) is a plant from the Cucurbitaceae family that contains several vitamins such as vitamins A, B, and C and is often used for medicinal purposes. Based on phytochemical analysis, chayote extract contains bioactive compounds in the form of phenolics. Phenolic compounds are secondary metabolites and are used as cosmetic and medicinal ingredients due to their ability to reduce oxidative stress. This research uses UV-Vis spectrophotometry to determine the chayote extract's total phenolic content and antioxidant activity. Chayote extract was obtained using the Ultrasonic Assisted-Extraction (UAE) method with 96% ethanol solvent. The extraction variables used were time (15, 20, and 25 minutes), amplitude (45, 65, and 85%), and solvent ratio (1:5, 1:10, and 1:15). The determination of total phenolic content was based on the Folin-Ciocalteau method, while antioxidant activity was determined using DPPH. The determination of chayote extraction parameters for total phenolic content was carried out using the Response Surface Methodology with a Box-Behnken type. The optimal conditions obtained for chayote extraction were 1.327 mg GAE/g sample for total phenolic content and 29.45% for antioxidant activity under 20 minutes extraction time, 65% amplitude, and 1:10 solvent ratio.

Keywords: *chayote (Sechium edule), ultrasonic-assisted extraction, total phenolic content, antioxidant activity, response surface methodology*

1. Introduction

Free radicals are relatively unstable molecules that have one unpaired electron. This highly reactive property allows free radicals to achieve stability by pulling electrons from other molecules in the body. The ability to oxidize other substances can cause oxidative damage in the body [1]. Reactive oxygen species (ROS) is an example of free radicals with unpaired electrons that play a key role in the pathogenesis of various physiological conditions, such as

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cancer, aging, diabetes, atherosclerosis, inflammation, liver, cardiovascular, and kidney disorders [2], [3]. ROS can react by disrupting macromolecules such as nucleic acid, lipids, and proteins in the human body. If the damage caused by ROS cannot be stopped, it will lead to oxidative stress, which is an imbalance between free radicals and antioxidants in the body caused by an excess of free radicals and a lack of antioxidants. Continued oxidative stress causes oxidative damage to cells, tissues, to organs [1], [4]. Sources of ROS free radicals come from radiation, environmental pollutants, chemicals, spicy foods, fried foods, and physical stress. Therefore, people are now starting to change their eating habits by adding fresh and whole foods to their diets to increase antioxidant levels to counteract free radicals [5].

Antioxidants are chemical compounds that donate electrons to free radicals without electrons, thereby reducing the oxidative effects of free radicals. Antioxidants are mostly derived from compounds found in plants, fruits, and vegetables as natural exogenous sources, and have been clinically proven to be effective as antioxidants [6]. One vegetable that is a source of antioxidants is chayote. Chayote (*Sechium edule*) is a plant from the Cucurbitaceae family that grows in tropical regions such as Indonesia, Malaysia, and the Philippines. Based on data from the Central Statistics Agency (BPS) in 2022, chayote production in Indonesia reached 427,665 tons [7]. Chayote is often used for medicinal purposes. Some of the vitamins found in chayote include vitamin A, vitamin B, and vitamin C. Chayote is generally used to treat diseases such as lowering blood pressure, relieving urinary retention, reducing the burning sensation during urination, dissolving kidney stones, and arteriosclerosis by boiling the fruit [8]. In addition, based on phytochemical analysis of chayote extract, bioactive compounds in the form of phenolic compounds have been identified [9].

Phenolic compounds are abundant secondary metabolites in plants. Phenolic compounds have a common chemical structure consisting of an aromatic ring with one or more hydroxyl substituents that can be divided into several classes, and the group of phenolic compounds includes phenolic acids, flavonoids, tannins, and stilbenes [10], [11]. Phenolic compounds are utilized as pharmaceutical ingredients such as cosmetics and drugs because they can prevent oxidative damage to cells by reducing the risk of several types of cancer, inflammation, and neurodegenerative diseases [12].

In general, phenolic compounds in plants can be extracted by using extraction processes. The commonly used extraction methods are conventional methods such as maceration agitation with solvents and Soxhlet method, which is based on capturing compounds from solvents or matrices, with or without heat. However, these methods require a long time to reach the

maximum concentration of the desired compounds, as well as the use of high solvent and thermal due to the long processing time degradation [13]. Considering these weaknesses, environmentally friendly and economic methods are being sought, where solvent consumption and extraction time are minimized while the extraction yield is increased [14].

Ultrasonic-Assisted Extraction (UAE) is an extraction method that uses microwaves [14]. Ultrasonic-assisted extraction is widely used in the extraction process of plant components. This method allows for extraction of natural products in a shorter extraction time, high reproducibility, lower solvent consumption, and simpler procedures compared to conventional techniques [15]. This is consistent with research conducted by Silva Junior et al. [16] comparing the total phenolics obtained from conventional, microwave, and ultrasound extractions, which found that the maximum phenolic yield was obtained using the ultrasound method at 35.15 mg GAE/g FRC, while microwave and conventional extractions resulted in yields of 23.31 mg GAE/g FRC and 30.1 mg GAE/g FRC, respectively. In 2021, Tan Mei Chin et al. [17] compared conventional orbital shaker and ultrasound methods to obtain the total antioxidant activity in watermelon rind. The total antioxidant activity obtained by the orbital shaker and ultrasound methods was 499.8 mg and 561 mg, respectively. Other research has also been conducted by Jitrangsri et al. [18], comparing three methods, namely microwave, ultrasound, and solid-liquid extraction on beans, and obtaining total phenolics of 1.76 mg GAE/g, 2.41 mg GAE/g, and 1.68 mg GAE/g, respectively.

The total phenolic yield also depends on the type of solvent used during extraction, as well as the extracted metabolites which may vary [19]. The selection of the appropriate solvent is crucial as it can affect the quantity and rate of extracted metabolites. Solvents such as methanol, ethanol, propanol, acetone, methyl acetate, and combinations have been used to extract phenols with varying proportions of water [20]. Methanol is a polar solvent and is generally found to be more effective in extracting low molecular weight polyphenols [19]. However, methanol is also a toxic alcohol and exposure can be very dangerous and may cause morbidity and mortality if left untreated [21]. According to the Indonesian Department of Health [21], the only solvents that can be used for medicinal purposes are ethanol, water, and combinations of water and ethanol or ether. Ethanol is a polar solvent with lower polarity than methanol and is suitable for extracting polyphenols and safe for human consumption [19]. The polarity of ethanol depends on its concentration. The combination of ethanol and water can increase the polarity of ethanol, thereby increasing the extraction rate of higher polar metabolites [22]. Previous studies on the extraction of Thai pumpkin using various methods are

shown in Table 1 and previous studies on the Ultrasonic-Assisted Extraction (UAE) method for various materials are shown in Table 2.

Table 1. Previous studies on Chayote

No.	Method	Results	Reference
1.	Percolation	Extraction was performed with variations in time (2 – 4 hours), ethanol concentration (30 – 70%), and sample-solvent ratio (1:5 – 1:15). The optimal condition for total phenolic content was obtained with an extraction time of 2 hours, a sample-solvent ratio of 1:45.35, and 66.22% ethanol, yield 2.5 mg GAE/g of extract.	[23]
2.	UAE	The extraction was performed by varying the concentration of the ethanol solvent used. Based on the results, 70% ethanol solvent was able to recover phenolic compounds optimally, and a total of (188 ± 2 mg/100 g DW) was obtained.	[24]
3.	Percolation	Extraction was carried out for 105 minutes at room temperature using 70% ethanol as the solvent and circulated at a speed of 80 L/minute. The phenolic content obtained was 0.36±0.04 mg GAE/g extract.	[25]

Table 2. Previous Research on Total Phenolic Content in Various Materials Using Ultrasound-Assisted Extraction Method

No.	Material	Results	Reference
1.	Peach (*Prunus persica L.*) and Pumpkin (*Cucurbitaceae*)	The optimal conditions for total phenolic extraction from pumpkin were obtained using a temperature of 41.45°C, ultrasound amplitude of 44.60%, and time of 25.67 minutes, resulting in 44.09±1.090. The optimal conditions for free radical scavenging activity recovery were obtained using an extraction temperature of 40.99°C, ultrasound amplitude of 56.01%, and time of 25.71 minutes, resulting in 64.85±0.046. Additionally, the optimal conditions for peach fruit extract were obtained using an extraction temperature of 41.53°C, ultrasound amplitude of 43.99%, and time of 27.86 minutes, resulting in 54.82±0.581 for total phenolics. However, the optimal conditions for free radical scavenging activity were obtained using an extraction temperature of 41.60°C, ultrasound amplitude of 44.88%, and time of 27.49 minutes, resulting in 73.81±1.940.	[26]
2.	Petai Leaves (*Parkia speciosa Hassk*.)	The highest extract yield was obtained at an ethanol concentration of 40%, solid-liquid ratio of 1:30 (%w/v), 30	[27]

No.	Material	Results	Reference
		minutes, and temperature of 65°C, with a DPPH activity result of 92.53 ± 0.87% and an extract yield of 21.25 ± 2.38%.	
3.	Durian Peel (*Durio zibethinus Murr*.)	The best treatment was obtained with a ratio of 1:9 between durian peel and ethanol with an extraction time of 20 minutes. Extraction of durian peel under these conditions resulted in the highest yield of 12.77 ± 0.16%, antioxidant activity (IC50) of 38.33 ± 0.12 ppm, and total phenolic content of 63.30 ± 0.08 mg GAE/g.	[28]
4.	Guava leaves (*Syzygium samarangense*) dan Melinjo leaves (*Gnetum gnemon L.*)	The antioxidant activity obtained from water guava leaves under the operating conditions of solid:solvent ratio (1:10 g/mL), extraction time (30 minutes), and temperature (40ºC) was 86.17 ± 0.00%. Meanwhile, for melinjo leaves under the operating conditions of solid:solvent ratio (1:10 g/mL), extraction time (10 minutes), and temperature (50ºC), it was 55.29 ± 0.02%.	[29]
5.	Wheat Bran	The optimal condition for total phenolic extraction using ultrasound-assisted extraction (UAE) was obtained at an extraction time of 29 minutes, a temperature of 66°C, and a solid-to-liquid ratio of 1:45 (g/mL), which resulted in 245.74 mg GAE/100 g dw of total phenolic content.	[30]
6.	Dayak Onion *(Eleutherine palmifolia)*	The optimal condition was obtained using UAE method with an extraction time of 10 minutes, resulting in a total phenolic content of 6.17 ± 1.19 mg GAE/g.	[31]
7.	Pumpkin Seeds (*Cucurbita maxima*)	The optimum extraction result was obtained using UAE with a sample-solvent ratio of 1:10 mL g-1, amplitude of 150 Watt, and extraction time of 15 minutes. The total phenolic content obtained was 34.61 µg g-1 of oil, and the DPPH radical scavenging activity was 55.90%.	[32]
8.	Watermelon Seed and Rind (*Citrullus lanatus*)	The optimum extraction conditions for watermelon pulp (WMP) were obtained at a sonication temperature of 47.82 °C, sonication time of 31.63 min, and ethanol concentration of 42.84%, resulting in the maximum total phenolic content (7.944 mg GAE/100 mL) and antiradical activity (85.150%). On the other hand, for watermelon seeds (WMS), the optimal conditions were a sonication temperature of 50.32°C, sonication time of 37.60 min, and ethanol concentration of 39.18%, resulting in the maximum total phenolic content (32.152 mg GAE/100 mL) and antiradical activity (85.947%).	[33]

No.	Material	Results	Reference
9.	Bitter Melon (*Momordica charantia*)	The extraction, total phenolic content (TPC), phenolic content, and antioxidant activity were higher. The optimal conditions for UAE were 59% ethanol concentration and 277 W ultrasonic amplitude for 14 minutes. The yield, TPC, and antioxidant activity obtained were 33.42%, 18.73 mg GAE/g, and 66.93%, respectively.	[34]
10.	*Lavandula angustifolia* flowers	The optimal condition was obtained with ultrasound amplitude of 60%, temperature of 60°C, time of 17.5 minutes, liquid-solid ratio of 31.7 mL/g, and water content in NADES of 33.5%. The predicted phenolic content was 50.50 mg/g gallic acid equivalent, antioxidant capacity (AC)DPPH of 41.60 mg Trolox equivalent (TE)/g, ACABTS of 77.13 mg TE/g, and ACCUPRAC of 163.33 mg TE/g.	[35]

Based on the reference, research on bottle gourd using Ultrasonic-Assisted Extraction method considering the effect of time, amplitude, and solvent-to-material ratio to obtain optimal total phenolic and antioxidant activity has not been conducted yet. Therefore, our research aims to determine the optimal conditions for total phenolic and antioxidant activity in bottle gourd using Box-Behnken Design (BBD) model. Total phenolic content analysis will be conducted using Folin-Ciocalteu method and antioxidant activity will be analyzed using DPPH method. The procedure in this research will be carried out by extracting the components using ethanol as a universal solvent that can extract all components with different solubility. DPPH was selected as an antioxidant analyzer because the analysis procedure is easy, simple, fast, and the results obtained are reliable.

2. Materials and Method

2.1 Materials

The materials used in this research were chayote (*Sechium edule*) obtained from Sumenep Regency, Madura. Ethanol 96% solvent (Merck), Folin-Ciocalteu reagent (Merck), gallic acid (Merck), Na_2CO_3 (Merck), distilled water, DPPH (2,2-diphenyl-1-picrylhydrazyl) (Aldrich), filter paper, and aluminum foil.

2.2 Equipments

The equipment used in this research consists of an oven (Maspion MOT-600), blender (Philips HR-2115), 60 mesh sieve, analytical balance (Ohaus), sonicator (Vevor) with probe

type (80W, 20 kHz), UV-Vis spectrophotometer 725AP (Taisitelab), and commonly used glassware in chemical laboratories.

2.3 Methods

2.3.1 Sample Preparation

Chayote (*Sechium edule*) was peeled and then washed thoroughly. The flesh of the fruit was thinly sliced to expedite the drying process. Next, the chayote was dried in an oven at 50ºC until the moisture content was less than 10%. After that, it was ground using a blender and sifted through a 0.177 mm (80 mesh) sieve [36]. The material was weighed in predetermined ratios using Design Expert 11 software according to Table 3.

Table 3. Experimental Design Expert

Run	A: Time (Minutes)	B: Amplitude (%)	C: Ratio of Chayote to Solvent (g/mL)
1	15	65	1:15
2	20	65	1:10
3	20	45	1:15
4	15	65	1:5
5	20	65	1:10
6	20	45	1:5
7	25	85	1:10
8	15	85	1:10
9	20	85	1:5
10	20	65	1:10
11	20	65	1:10
12	25	45	1:10
13	15	45	1:10
14	25	65	1:5
15	25	65	1:15
16	20	65	1:10
17	20	85	1:15

2.3.2 Measurement of Moisture Content

The procedure for analyzing moisture content begins by heating a porcelain dish in an oven at a temperature of 105°C for 30 minutes and cooling it in a desiccator for 30 minutes. The porcelain dish is then weighed using an analytical balance. Next, 2 grams of chayote sample is placed into the dish and heated in the oven for 2 hours. The sample is cooled in the desiccator

for 1 hour, and the weight of the sample is determined. The moisture content is then calculated using equation [37].

$$\text{Moisture content} = \frac{(a+b)-c}{b}\ x\ 100\% \quad (1)$$

Keterangan:

a = weight of the dried porcelain dish (g).

b = weight of the sample (g).

c = weight of the porcelain dish + sample (g).

2.3.3 Ethanol Extraction of Chayote

Ethanol Extraction of Chayote using the Ultrasonic-Assisted Extraction (UAE) method by adding 96% concentration ethanol solvent into a beaker glass containing the raw material [38], [39]. According to the expert research design in Table 3. After the extraction process is completed, the filtrate and residue are separated using filter paper. The filtrate is stored in a vial bottle at room temperature [40].

2.3.4 Total Phenolic Content Assay

2.3.4.1 Preparation of Na_2CO_3 7% reagent

Sodium carbonate was weighed 3.5 grams and then dissolved in distilled water up to 50 mL [41].

2.3.4.2 Preparation of Gallic Acid Standard Solution

Gallic acid 100 ppm was weighed as much as 5 mg and dissolved in distilled water to a volume of 50 mL in a volumetric flask [41].

2.3.4.3 Measurement of standard gallic acid solution

Gallic acid at a concentration of 100 ppm was pipetted at 0.05, 0.1, 0.15, 0.2, and 0.25 mL to obtain concentrations of 1, 2, 3, 4, and 5 ppm. Each concentration was added with 0.1 mL of Folin-Ciocalteau reagent, shaken, and left for 5 minutes. Then, 1 mL of 7% Na_2CO_3 was added and shaken until homogenous. Aquadest was added up to 2.5 mL and left for 1 hour at room temperature. The absorbance value was measured at a wavelength of 755 nm and a calibration curve was created to determine the relationship between gallic acid concentration (μg/mL) and its absorbance value [41].

2.3.4.4 Measurement of Total Phenolic Content

The total phenolic content was measured using the Folin-Ciocalteu method with gallic acid as the standard phenol. A total of 0.05 mL of the sample solution was mixed with 0.1 mL of Folin-Ciocalteu phenol reagent and incubation for 5 minutes. Then, 1 mL of 7% Na_2CO_3 and distilled water was added up to 2.5 mL. After incubation at room temperature for 2 hours, the absorbance was measured at 755 nm. The analysis was performed with 2 replications. The phenolic content obtained is equivalent to mg gallic acid equivalent/gram of fresh sample [41].

2.3.5 The Antioxidant Activity Analysis Using DPPH

2.3.5.1 Preparing 0,1 mM DPPH Solution

4 mg of DPPH was weighed and dissolved in 96% ethanol up to a volume of 100 mL [42].

2.3.5.2 Measurement of DPPH Antioxidant Activity

The antioxidant activity was measured using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method. DPPH was used as a blank. A 0.25 mL sample extract was added to 1 mL of 0.1 mM DPPH. Then, ethanol was added to a volume of 2,5 mL. The solution was homogenized and incubated in the dark for 30 minutes. The absorbance value was measured at a wavelength of 517 nm. The measurement was repeated twice. The % inhibition was calculated using the following equation: [42].

$$\text{Inhibition (\%)} = \frac{A_{blank} - A_{sample}}{A_{blank}} \, x \, 100\% \qquad (2)$$

Where : A_{blank} = Absorbance blank

A_{sample} = Absorbance sample

2.4 Results and Discussion

2.4.1 Extraction of Total Phenolic Content from Chayote (*Sechium edule*)

The research was conducted from September 2022 to March 2023 in the Basic and Bioprocess Laboratory, Chemical Engineering Program, Department of Mechanical Engineering, Faculty of Engineering, Jember University. The main material used in this study was Chayote (*Sechium edule*), which was extracted, and analyzed for total phenolic content, and its relations with antioxidant activity was examined using the Ultrasound Assisted Extraction method. The first step was to determine the moisture content, as moisture content can affect the quality of the sample. Drying in this study was carried out using an oven, which can provide better quality and temperature control, resulting in a significant reduction in moisture content in a short time. The temperature used must be carefully considered because if

the temperature is too high, it can cause damage to the compounds contained in the sample and can reduce the quality of the final product [43]. High moisture content can have an effect on the extraction results, as a high moisture content can cause the extraction process to take longer as it needs to first evaporate the water content present in the material [44], [45]. The determination of water content in this research was carried out by the gravimetric method. Drying was done using an oven at a temperature of 105°C for 2 hours, and every 30 minutes the weighing was carried out. Drying was carried out until two successive weighings did not differ by more than 0.25%, and the water content obtained was in accordance with the requirement of not more than 10% [46] [47]. The moisture content value obtained from the Chayote material is 8.1%.

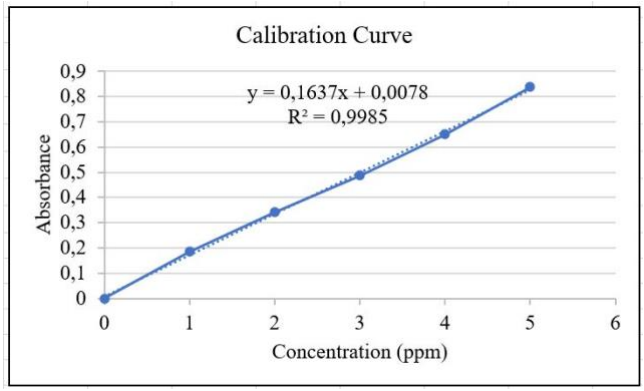
The variables that can affect the extraction process of chayote are extraction time, ultrasonic amplitude, and solvent-to-material ratio. Extraction time and temperature is a factor that affects the extraction rate. The longer the contact time between the material and the solvent, the higher the solubility of the extracted material, diffusion concentration, and the extraction rate. The extraction time also has a significant effect on the total phenolic content and antioxidant activity [48]. Longer extraction time can increase the concentration of extracted phenolic compounds from the material. However, excessive extraction time can be less effective for the extraction process and an optimal extraction time is needed in developing an effective extraction method with the highest analyte extracted from the material [49]. The other factor that can affect the extraction process is the ultrasonic amplitude. The cavitation effect is related to the increase in ultrasonic amplitude, which can increase the total phenolics in the extracted material [50] [51]. The ultrasonic amplitude can increase the efficiency of the extraction process by providing better mass transfer, which is achieved by breaking plant cells, increasing plant tissue permeability, improving analyte solubility and solvent penetration due to increased temperature and pressure, as well as increasing diffusion from the flow generated during ultrasonic process [52] [53].

In the determination of total phenolic content, gallic acid was used as the standard solution. The maximum absorption of gallic acid was found at a wavelength of 755 nm. The first step was to prepare a standard curve of gallic acid with concentrations of 1, 2, 3, 4, and 5 ppm. This curve can be useful in determining the total phenolic content in the sample through

the regression equation of the standard curve [54]. The results of the absorbance measurement of the standard solution of gallic acid can be seen in Table 4.

Figure 1. *Measurement Results of Absorbance of Gallic Acid Standard Solution at 755 nm Wavelength using UV-Vis Spectrophotometer*

From the standard gallic acid solution, a curve was obtained with a regression equation of



$y = 0{,}1637x + 0{,}0078$ and R^2 value of 0,9985. The R^2 value, which is close to 1, proves that the regression equation is linear [55]. The results of the total phenolic content of Chayote (*Sechium edule*) can be seen in Table 5.

Table 4. *Total Phenolic Content in chayote* (*Sechium edule*)

Run	A: Time (Minute)	B: Amplitude (%)	C: Ratio of Chayote to Solvent (g/mL)	Total Phenolic Content (mg GAE/g sample)
1	15	65	1:15	1.2196
2	20	65	1:10	1.3277
3	20	45	1:15	0.9333
4	15	65	1:5	0.8609
5	20	65	1:10	1.259
6	20	45	1:5	0.8815
7	25	85	1:10	0.9062
8	15	85	1:10	1.1368
9	20	85	1:5	0.8929
10	20	65	1:10	1.3033
11	20	65	1:10	1.2773
12	25	45	1:10	0.8818
13	15	45	1:10	0.8772
14	25	65	1:5	0.9311
15	25	65	1:15	1.0089
16	20	65	1:10	1.2972
17	20	85	1:15	1.2586

The Table 5 shows that the lowest total phenolic content obtained was 0.8609 mg GAE/g of sample and the highest total phenolic content obtained was 1.3277 mg GAE/g of sample. The lowest total phenolic content was obtained from the combination of extraction time of 15 minutes, ultrasound amplitude of 65%, and solvent-to-material ratio of 1:5. The highest total phenolic content was obtained from the combination of extraction time of 20 minutes, ultrasound amplitude of 65%, and solvent-to-material ratio of 1:10. This indicates that with the same amplitude and different extraction time and solvent-to-material ratio, there is an increase in the total phenolic content produced. Longer extraction time causes the cell walls of the material to break and release the soluble substances into the solvent, resulting in an increase in the yield up to the optimum point of the solvent [56]. The total phenolic content obtained in this study was higher compared to the previous study on chayote conducted by Fidrianny & Hartati (2016), which was 0.36 ± 0.04 mg GAE/g extract [25]. If compared to a previous study on chayote conducted by Rosidah et.al (2020), the total phenolic content obtained in this study is smaller, which is 2.5 mg GAE/g extract [23]. The difference in the total phenolic content obtained may occur due to different extraction conditions and other factors that affect the extraction process.

2.4.2 The Relations between Total Phenolic Content and Antioxidant Activity of Chayote (*Sechium edule*)

This study employed the DPPH radical scavenging method to analysis the antioxidant activity of the sample, as it is a simple and rapid method [54] The antioxidant activity of the samples caused a color change in the DPPH solution, which initially had a deep purple color, to pale yellow. The wavelength used in this antioxidant assay was 517 nm. The antioxidant activity of the chayote was expressed as the percentage inhibition, which is the difference in absorbance between the DPPH control and the sample measured using a UV-Vis spectrophotometer. The relations between total phenolic content and antioxidant activity in Chayote (*Sechium edule*) can be seen in Table.

Table 5 The relations between total phenolic content and antioxidant activity in Chayote

Run	Total Phenolic Content (mg GAE/g sample)	% Inhibition	Σ Antioxidant Moles
1	1.2196	27.943	2.7943×10^{-8}
2	1.3277	29.453	2.9453×10^{-8}
3	0.9333	26.699	2.6699×10^{-8}
4	0.8609	22.964	2.2964×10^{-8}

Run	Total Phenolic Content (mg GAE/g sample)	% Inhibition	Σ Antioxidant Moles
5	1.259	28.067	2.9067×10^{-8}
6	0.8815	24.427	2.4427×10^{-8}
7	0.9062	26.032	2.6032×10^{-8}
8	1.1368	27.852	2.7852×10^{-8}
9	0.8929	25.655	2.5655×10^{-8}
10	1.3033	29.089	2.9089×10^{-8}
11	1.2773	28.277	2.8277×10^{-8}
12	0.8818	24.879	2.4879×10^{-8}
13	0.8772	23.601	2.3601×10^{-8}
14	0.9311	26.274	2.6274×10^{-8}
15	1.0089	27.528	2.7528×10^{-8}
16	1.2972	28.944	2.8944×10^{-8}
17	1.2586	28.034	2.8034×10^{-8}

Table 6 shows that the lowest total phenolic content had the lowest antioxidant activity, which was 22.964%, while the highest total phenolic content had the highest antioxidant activity, which was 29.453%. This is consistent with the study conducted by Lushaini et al. (2015), which stated that there is a correlation between antioxidant activity and total phenolic content. The higher the total phenolic content, the higher the antioxidant activity [57].

2.4.3 ANOVA Analysis

Analysis of total phenolic content using the Response Surface Method (RSM) was conducted to demonstrate the influence of the variables used in the extraction process of chayote using the UAE method on the resulting content. Table 7 presents the ANOVA analysis results by Design Expert.

Table 6. The *Result Analysis of Variance* (ANOVA)

Source	*Sum of Squares*	*df*	*Mean Square*	*F-value*	*p-value*	
Model	0.5611	9	0.0623	108.08	< 0.0001	*significant*
A-Time	0.0168	1	0.0168	29.11	0.0010	
B-Amplitude	0.0482	1	0.0482	83.52	< 0.0001	
C-Ratio of Chayote to Solvent	0.0911	1	0.0911	158.00	< 0.0001	
AB	0.0138	1	0.0138	23.97	0.0018	

Source	*Sum of Squares*	*df*	*Mean Square*	*F-value*	*p-value*	
AC	0.0197	1	0.0197	34.23	0.0006	
BC	0.0246	1	0.0246	42.69	0.0003	
A^2	0.1138	1	0.1138	197.35	< 0.0001	
B^2	0.1334	1	0.1334	231.23	< 0.0001	
C^2	0.0641	1	0.0641	111.12	< 0.0001	
Residual	0.0040	7	0.0006			
Lack of Fit	0.0013	3	0.0004	0.6381	0.6290	*not significant*
Pure Error	0.0027	4	0.0007			
Cor Total	0.5651	16				

Based on the table, the F-value is inversely proportional to the p-value. The p-value is considered significant if it is ≤0.05. In the data, the p-value is <0.0001, which means that it is smaller than the probability set at 5% or 0.05. Therefore, it can be said that the research model of *Sechium edule* extract significantly affects the total phenolic content. The analysis results in Table 7 also show that the variables of extraction time, amplitude, and ratio of raw material to solvent have a significant effect on the total phenolic content with the following values in order: 0.001, <0.0001, and <0.0001 [58].

Furthermore, a variable can be considered significant if the lack of fit value with a p-value ≥ 0.05. Lack of fit is a discrepancy or deviation between experimental data and predicted model. The data in this study showed that the p-value for lack of fit is 0.6381, indicating insignificance and congruence of the response to the model [59].

The ANOVA analysis results shown in Table 8 also obtained an R^2 value of 99.29% or 0.9929, indicating that the model used is in accordance with the research results. The R^2 value can be considered appropriate if it is above 75% and approaching 1 indicates a better model [60], [61] The obtained adjusted R^2 value of 0.9837 indicates a strong relations between the variables of extraction time, amplitude, and ratio of raw material to solvent with the response of total phenolic content [62].

Table 7. *Fit Statistic*

R^2	***Adjusted R^2***	***Predicted R^2***	***Adeq Precision***
0,9929	0,9837	0,9554	23,5062

Mathematically, the equation model for total phenolic content as a response of extraction variables can be modeled by equation 3.

$$\text{Total phenolic content} = 1{,}29291 - 0{,}0458155A + 0{,}0776B + 0{,}106731C - 0{,}0587966AB - 0{,}0702505AC + 0{,}07845BC - 0{,}16442A^2 - 0{,}177974B^2 - 0{,}12337C^2 \quad (3)$$

The values of A, B, and C, respectively, represent the variables of extraction time, amplitude, and the ratio of raw material to solvent. Based on equation 3, all extraction variables statistically significantly affect the response of total phenolic content. The relationship between the model data (predicted) and the experimental data (actual) is presented in Figure 2.

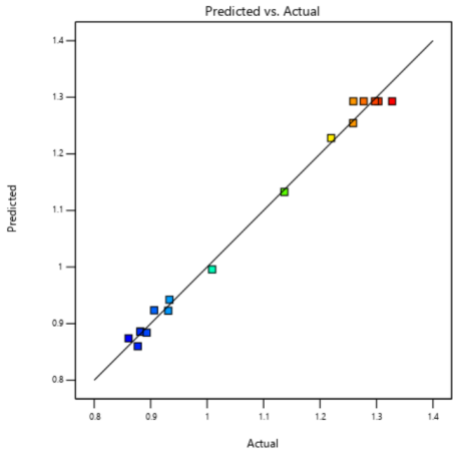


Figure 2. The graph shows the relations between model data and experimental data.

2.4.4 The Effect of Extraction Parameters on the Total Phenolic Content of Chayote (*Sechium edule*)

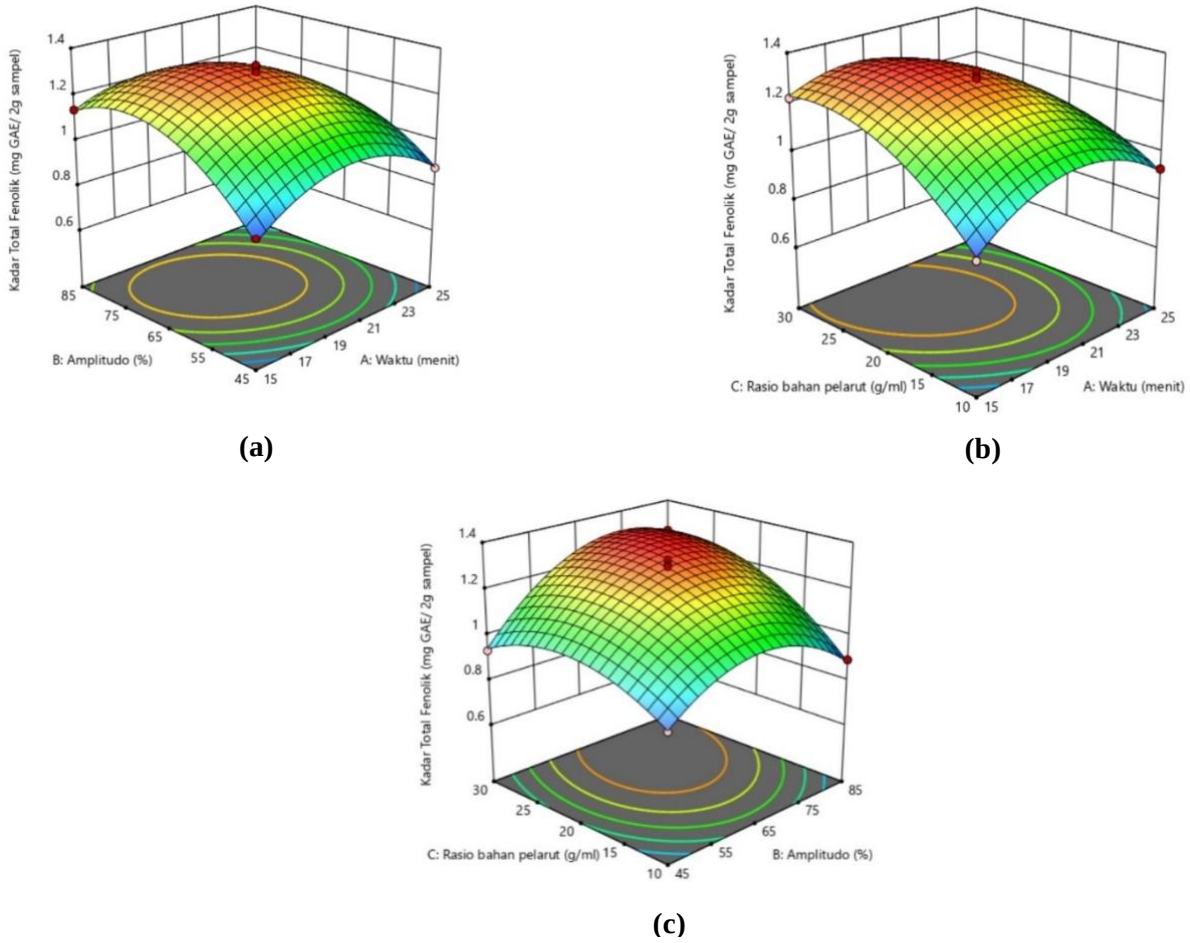


Figure 3. The relations between variables and total phenolic content are shown in (a) time (minutes) and amplitude (%); (b) time (minutes) and ratio of raw material to solvent; (c) ratio of raw material to solvent (g/ml) and amplitude (%)

Figure 3. This is a graph showing the effect of variables used in the extraction process (extraction time, solvent-to-sample ratio, and amplitude) on the total phenolic content produced from chayote. The total phenolic content is highly influenced by the extraction time, as shown in Figures 3(a) and 3(b), which indicate an increase in total phenolic content from 15-25 minutes of extraction time. At 15 minutes of extraction time with 45% amplitude, the phenolic content obtained was 0.87 mg GAE/gram of sample. At 20 minutes of extraction time, under the same conditions, the phenolic content increased to 0.93 mg GAE/gram of sample. However, at 25 minutes of extraction time, the total phenolic content decreased to 0.8818 mg GAE/gram of sample. This finding is consistent with the study conducted by Sekarsari et al. Sekarsari *et al* (2019), which also found that the optimal extraction time for obtaining the highest total phenolic content is 20 minutes. It was observed that if the extraction time is too long, it can decrease the phenolic content. This is because the longer the extraction time, the more energy is generated

from the sonicator, which leads to the degradation of phenolic compounds. Therefore, the appropriate extraction time is crucial in maintaining the quality of phenolic compounds in the extract [63].

In Figures 3(a) and 3(c), it can be seen that an increase in the amplitude used can affect the total phenolic content. In a 20-minute extraction time, an amplitude of 45% resulted in a total phenolic content of 0.9333 mg GAE/g sample. Meanwhile, an amplitude of 65% increased the total phenolic content to 1.3277 mg GAE/g sample in the same time period. However, at an amplitude of 85%, there was a decrease in phenolic content to 1.2586 mg GAE/g sample. In this study, the use of an amplitude of 65% resulted in the highest total phenolic content. This finding is consistent with the study conducted by Chemat et al. (2011) which stated that using an overly large amplitude in the UAE method can decrease the phenolic content in the extract [64].

Based on Figures 3(b) and 3(c), the ratio of raw material to solvent indicates that exceeding the optimum condition of the solvent can decrease the resulting phenolic content. In a ratio of raw material to solvent of 1:5 using an amplitude of 65%, the total phenolic content was 0.8609 mg GAE/g sample. On the other hand, a ratio of 1:10 with the same amplitude increased the total phenolic content to 1.3277 mg GAE/g sample. However, at a ratio of 1:15, a decrease in phenolic content to 1.2196 mg GAE/g sample was observed. This finding is in line with Zhang et al.'s (2018) study, which stated that the use of a solvent with too high a ratio of raw material can result in a longer extraction time [65]. The same study was also conducted by Buanasari (2019) on the extraction of active compounds from natural materials with a material to solvent ratio of 1:5-1:25. The highest content of bioactive compounds was obtained in the extract with a 1:10 ratio [66].

3. Conclusion

This research was conducted to determine the total phenolic content and antioxidant activity of sponge gourd extract using UV-Vis spectrophotometry. The chayote extract was obtained using Ultrasonic Assisted-Extraction (UAE) method with 96% ethanol as the solvent. The extraction variables used were time, amplitude, and solvent ratio. The results showed that chayote extract contained bioactive compounds in the form of phenolics, which had antioxidant activity and could reduce oxidative stress. The optimal conditions for chayote extraction were 20 minutes extraction time, 65% amplitude, and 1:10 solvent ratio, with a total phenolic content of 1.327 mg GAE/g sample and antioxidant activity of 29.45%. This research could be

beneficial in the development of natural drugs and cosmetic ingredients containing bioactive phenolic compounds.

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