Original Article

The influence of extraction temperature and time on antiradical activity and total phenolic extract of Ceciwis

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ABSTRACT

In Indonesia, cruciferous cabbage is known as Ceciwis (Brassica oleracea var. capitata alba), a vegetable that can be consumed as a diet. Ceciwis is a member of the Brassica family, which has been shown to have chemo-preventive action. The existence of antioxidant activity confirms its efficacy. The existence of antioxidant activity confirms its efficacy. Further research is required. Antioxidant activity will undoubtedly be affected by the extraction procedure and circumstances. The objective of this study was to examine antioxidant activity, DPPH radical scavenging, ABTS, and total phenol content in Ceciwis reflux extraction results as a function of temperature and extraction time. Ceciwis were washed after harvesting and then forced to submit to a reflux extraction process at temperatures ranging from 40 to 50 °C, 60 to 70 °C, and 80 °C, with extraction times, varies from 0.5 to 3.5 hours. The yield, total phenols, and radical scavenging of DPPH and ABTS were all examined in the condensed extract. The results of a four-response analysis revealed that ABTS radical scavenging was the most influential reaction to the temperature of the extract, accounting for 60.7%, followed by DPPH, yield, and total phenols, accounting for 50.7%, 26.16%, and 0.00%, respectively. The extraction period would have the greatest impact on the yield response and total phenols of 74.42 and 72.75%, respectively. The DPPH and ABTS reactions are unaffected by radical scavenging.

Keywords: Brassica oleracea var. capitata alba, Extraction, Radical scavenging, Phenol

Introduction

External sources of free radical generation include stress, ozone radiation, pollution, pesticides, and industrial chemicals, all of which can harm molecules, proteins, lipids, RNA, and DNA in the body [1, 2]. Free radicals can cause cellular oxidative damage, which diminishes the body's defense system and makes a significant contribution to diseases such as diabetes, hypercholesterolemia, cancer, arthritis, arteriosclerosis, degenerative neuro-diseases, and premature aging [1, 2]. As a result, antioxidants are required by our bodies to protect us from free radical damage.

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According to Halliwell in [3], antioxidants are substances that inhibit, prevent, and completely remove oxidative damage to target molecules. Antioxidants are inhibitors of oxidative processes even in small doses and have certain settings in the body. Plants contain antioxidants such as vitamin C, vitamin E, beta carotene, flavonoids, and anthocyanins [4].

Plant antioxidants are mostly phenolic or polyphenolic chemicals, such as flavonoids, sinamic acid derivatives, coumarins, tocopherols, and polyfunctional acids [5].

Moreover, ascorbic acid, often known as vitamin C, is a monosaccharide antioxidant present in animals and plants that breaks radical chain reactions by trapping peroxyls and other reactive radicals [6]. Vitamin C is found in numerous fruits and vegetables, particularly citrus fruits. Vitamin C is found in tomatoes, anung, potatoes, chili, and red peppers. Vitamin C is also found in the adas-adasan family (Apiaceae), the pumpkin family (Curcubitaceae), Brussels sprouts, and broccoli [7]. The quality of the extract is one of them influenced by the selection of methods used in the extraction process, wherein this research is chosen using the method of percolation is an extraction method by draining solvents continuously, which according to [8]

percolation can attract secondary metabolite compounds better than maceration.

Materials and Methods

The tools used in the study are UV-Vis spectrophotometry, rotary evaporators, a set of reflux tools, analytical balance sheets, micropipets, blenders, and glass tools. Information about the ingredients: ABTS, 1.1-Difenyl-2-Picrilhydrazil (DPPH), 70% ethanol, and vitamin C.

Methods of extraction

Sorting, washing, and cutting cabbage ceciwis are all part of the raw material treatment process. The extraction is carried out using a reflux technique with a 70% ethanol solvent. Using a solvent of up to 75 ml, up to 50 grams of cabbage ceciwis are refluxed. Reflux is performed at a temperature of 70°C for 6 hours. The extraction results are filtered using No. 41 Whatman filter paper.

Analysis of total phenolics

In 25 ml of 96% ethanol, 10 mg of the extract was dissolved. 2 ml of the solution were mixed in 50% with 5 ml of aquabidest and 0.5ml of Folin-Ciocalteau reagent and shaken with vorcies. After 5 minutes of refrigeration, 1 ml of 5% Na2CO3 was added, mixed, and the mixture was allowed to sit for 60 minutes. The solution absorbent is measured spectrophotometrically with a wavelength of 725 nm. The standard is gallic acid in various concentrations (10, 30, 50, and 70 g/ml).

Analysis of DPPH radical scavenging activity

A 40 ppm DPPH solution with a volume of 100 ml is made by weighing 0.004 g of DPPH dissolved in 70% ethanol. Taken up to 3 ml to observe its absorption at wavelengths of 450-600 nm. Weighing 0.01 g of ceciwis ethanol extract and dissolving it in 70% ethanol yields 1000 ppm of ethanol extract with maceration and soxhletation. The solution is diluted to 20 ppm, 40 ppm, 60 ppm, 80 ppm, and 100 ppm concentration levels. The solution was taken as much as 2 ml apiece and a solution of DPPH 40 ppm was added as much as 4 ml. The solution was incubated for 30 minutes and uptake was seen at the maximum wavelength. The same method is used to compare vitamin C. The following calculation is used to compute the fraction of antioxidants that scavenge free radicals:

$$\%\ Inhibtion = \frac{Abs.\,blank - Abs\ Sample}{Abs\ Blank} \times 100\% \qquad (1)$$

The equation of the linear regression line was calculated using the percent value of the scavenging to determine the effective radical scavenging by IC50.

Results and Discussion

Natural antioxidant compounds can be obtained from a natural material or plant by extraction using organic solvents. Extraction is done using ethanol solvents that are polar because they can dissolve antioxidant components that are a class of secondary metabolites in ceciwis.

The yield obtained using this reflux method is 30.52%, which is the greatest amount at 3 hours of extraction at 500°C with 250 ml of solvent. Because temperature is one of the factors that can affect the speed of extraction, the high yield is due to the addition of heat, which can help improve the extraction process. High temperatures can increase the desorption of active compounds from plants because cell destruction in the material increases due to high solvent temperatures. In addition to the addition of high temperatures, the solvent reflux method used will remain fresh when extraction occurs so as to avoid the occurrence of solvent saturation that can increase the solvent's ability to attract compounds [9].

The standard curve equation was used in this study to determine the amount of total phenol in the extract. The standard curve line y = 0.006x + 0.0901 with a value of R2 = 0.9939 was derived from the concentration of Gallic acid in the absorbance data.

From the equation, we obtained the highest total phenol level of 2304 mg GAE/g extract. According to [10], environmental factors such as soil composition, temperature, rainfall, and ultraviolet radiation can affect the concentration of phenol components, including flavonoids. In addition, solvents are an important factor in extracting phenolic components and flavonoids.

This analysis aims to determine what percentage of arrest or inhibition concentration (IC) 50 extracts are effective against DPPH radicals. IC stands for the 50 sample concentrations required to inhibit 50% of DPPH activity [10]. Based on the table above, the IC value 50 derived from a 66.751g/ml cabbage ceciwis extract is inversely proportional to 50 antioxidant activity. The antioxidant activity is potent the lower the IC 50 number is. These results indicate that cabbage ceciwis extract can be suggested as a powerful antioxidant with a concentration range of 50-100 g/ml.

The total levels of phenols and their total flavonoids influenced the difference in antioxidant activity obtained. Since phenol compounds and flavonoids made a significant contribution to antioxidant activity, the higher the levels, the better the antioxidants. Antioxidant activity was not always related to levels of phenol or flavonoid [11]. This can be attributed to a variety of factors, along with differences in active components in plants, synergistic or antagonistic effects between the active components contained, research conditions, and methods used, which can all have an impact on antioxidant activity in plants [12].

The current research applies design for data analysis by using particular software as shown in **Tables 2 and 3**.

Table 2 shows the temperature parameters tested and then analyzed using Minitab software with linear regression methods to see correlations between parameters and their effect on independent variables. The results of the analysis provide an equation for calculating the number of contributions made to variables depending on the linear regression approach. The

equations for the linear model contribution on each parameter are presented in **Table 1** below:

Table 1. Model contribution of the parameters Regression Equation

Yield	Yield = 133.9 + 1.096 DPPH - 1.594 ABTS
DPPH	DPPH = -101.2 + 0.737 Yield + 1.220 ABTS + 0.00400 Total Phenol
ABTS	ABTS = 84.4 - 0.582 Yield + 0.716 DPPH - 0.00291 Total Phenol
Total Phenol	Total Phenol = 7331 - 91.7 Yield + 56 DPPH - 69 ABTS

The linear equation then calculates the magnitude of each variable's contribution based on the variable data provided and which variables contribute to the effect on the free variable. **Table 1** shows temperature as an independent variable versus yield, total phenol, DPPH, and ABTS radical scavenging as dependent variables. The results of normal probability of the highest contribution correlation (a) temperature response and (b) time extraction response can be observed in **Figure 1**.

Table 2. Contribution analysis results with temperature parameters.

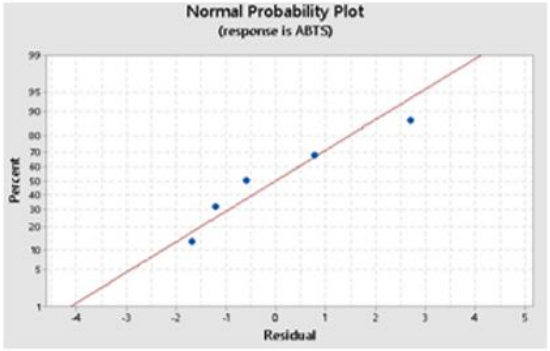
Independent variable		Dependent Variable			
Time (hour)	**Temperature (℃)**	**Yield (%)**	**DPPH (%)**	**ABTS (%)**	**Total phenol (mg GAE/100 gram)**
2	40	25,32	186,755	85,209	159,5
2	50	17,68	1,827,815	84,197	434,5
2	60	28,82	2,728,477	84,956	324,5
2	70	22,84	1,046,358	75,727	764,5
2	80	12,22	2,490,066	91,530	1479,5
Contribution correlation	R-sq (adj) (%)	26,16	50,74	60,700	0

Table 3. Contribution analysis results with temperature parameters.

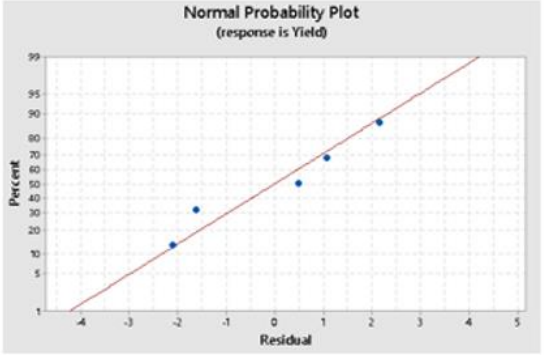
Independent variable		Dependent Variable			
Time (hour)	**Temperature (℃)**	**Yield (%)**	**DPPH (%)**	**ABTS (%)**	**Total phenol (mg GAE/100 gram)**
0,5	60	18,82	2,728,477	84,956	324,5
1	60	17,68	1,827,815	84,197	434,5
2	60	2,484,857	2,531,693	8,746,614	9,687,857
3	60	26,68	2,033,113	7,579,014	847
3,5	60	25,44	2,344,371	87,990	1699,5
Contribution correlation	R-sq(adj) (%)	74,42	0,00	0,00	72,75

The antioxidant activity of phenolic compounds is directly related to chemical structures such as glycosylation degree and number and position of hydroxyl groups associated with carboxyl functional groups. These compounds contribute significantly to antioxidant activity due to their ability to bind radicals and dissolve metals. Both free radicals and metal ions are poisonous to biological systems. In order to form stable intermediates, phenolic compounds could also donate hydrogen atoms or electrons to free radicals. These substances bind to free radicals, disintegrate oxidation products, and correct metal ions. Cabbage ceciwis had antioxidant properties. DPPH measured total antioxidant activity at 27.28477, 18,27815, 25,31693, 20,33113, and 23.44371%, while ABTS measured 84.956, 84, 197, 87,46614, 75.79014, and 87.990%.

Due to the obvious presence of phenolic compounds in cabbage ceciwis, as well as its antioxidant activity, it is a suitable agent for the development of functional foods that can be absorbed requently. Natural antioxidants, such as phenolic chemicals, are an essential element of the human defensive mechanism.



a)



b)

Figure 1. ABTS and Yield Response

When there is contact between solids and solvents, some solute will move into the solvent and a solution will be formed. The transfer of solute can occur due to differences in solute concentration in solution and solids [13]. The longer the extraction time, the more phenols are produced. The longer the extraction time, the concentration will increase because the quantity of extracted materials will also increase.

The difference in the results of antioxidants and phenols obtained can be seen in the above description. This occurs because increasing the temperature increases the solubility of the solvent. This is because increasing the temperature reduces the viscosity of the solution and enlarges the pores of the solid, facilitating diffusion into the solvent and thus increasing the leaching speed [13]. As a result, the percentage of potassium produced increases as the temperature rises, as does the extraction time; the longer the extraction period, the higher the percentage of phenol produced.

Conclusion

The yield, total phenols, and radical scavenging of DPPH and ABTS were all examined in the condensed extract. The results of a four-response analysis revealed that ABTS radical scavenging was the most influential reaction to the temperature of the extract, accounting for 60.7%, followed by DPPH, yield, and total phenols, accounting for 50.7%, 26.16%, and 0.00%, respectively. The extraction period would have the greatest impact on the yield response and total phenols of 74.42 and 72.75%, respectively. The DPPH and ABTS reactions are unaffected by radical scavenging.

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Conflict of interest: None

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Ethics statement: None

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