



Effect of Ultrasonication Extraction Time on Determination of Flavonoid Levels in Ciplukan Plants

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Abstract

Ciplukan contains bioactive substances in the form of flavonoids, saponins, alkaloids, polyphenols, vitamin C, stearic acid, palmitic acid. Bioactive substances from natural ingredients are known to function as immunomodulators that can increase the body's immune system, activate the body's natural defenses and restore immune system imbalances. Immunomodulators are important in studies related to the issue of the coronavirus disease 19 (Covid-19) pandemic. The purpose of this study was to determine the effect of the extraction time on the ultrasonic method on the flavonoid yield of ciplukan plants using methanol as a solvent. The method used is ultrasonication extraction with a variable extraction time. The analysis was carried out by qualitative analysis using a solution of quersetin to determine the concentration of flavonoids. The results of the study obtained the optimum flavonoid concentration at the extraction time of 15 minutes.

INTRODUCTION

Utilization of natural materials is currently a matter of much research to be developed into useful new materials. especially during the covid-19 pandemic, where most Indonesian people believe that the content or efficacy of natural ingredients can be active substances that ward off the virus. In addition, the use of natural materials is also in accordance with the mandate of the state, which in 2022 will focus one of them on the green economy, namely maximizing natural materials that can improve the Indonesian economy.

One of the plants that has the potential to be researched is ciplukan. This plant comes from America, but is now widely grown in tropical areas. Ciplukan can grow in areas with a height of 1 to 1550 meters above sea level. This plant consists of stems, shoots, roots, fruits and leaves. This plant is known by the Latin name *physalis angulate* L, and

in Indonesian it is known as Ciplukan, each region has a different name for this plant.

Ciplukan contains bioactive substances in the form of flavonoids, saponins, alkaloids, polyphenols, vitamin C, stearic acid, palmitic acid. Bioactive substances from natural ingredients are known to function as immunomodulators that can increase the body's immune system, activate the body's natural defenses and restore immune system imbalances. Immunomodulators are important in studies related to the issue of the coronavirus disease 19 (Covid-19) pandemic.

Studies related to the content of ciplukan with process technology that still tends to be simple so that the optimization of the potential content of other ciplukan plants cannot be utilized optimally. With considerable development potential as a source of herbal medicinal raw materials, it is necessary to conduct a comprehensive study related to the optimization of ciplukan plant flavonoid

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extraction starting from the roots, stems, leaves to the fruit as immunomodulators.

It is mentioned in (Zheng, Wen, Yuan, & Gao, 2016) that distillation or liquid extract with solvent is the traditional method used in the extraction of flavonoids. Where the extraction method in the process of extracting flavonoids from plants often requires a long extraction time, the solvent is required in large quantities, and the efficiency is low. Especially if the type of flavonoid is (Ridwanuloh F. , 2019) thermally unstable and easily degraded during the extraction process. Ultrasonic assisted extraction (UAE) or so-called ultrasonic extraction is an extraction method with the help of ultrasonic waves. Where ultrasonic waves have a frequency of 70kHz which means they have a frequency above the frequency of human hearing. In extracting natural ingredients with content such as antioxidants, sonication extraction method can produce higher yields in a relatively short time. The process that occurs in the extraction of organic compounds in plants and grains using organic solvents is to break down the cell walls with ultrasonic vibrations so that the content in the extraction raw materials can come out easily (Ashley, Andrews, Cavazos, & Demange, 2001). In this literature study, it is known that UAE or sonication extraction is carried out in 2 methods, namely direct sonication and indirect sonication. The indirect sonication method is carried out with water heating media or is called an ultrasonic water bath. In this method, the extracted material or solution is not in direct contact with the ultrasonic wave sensor. (Sharma & Janmeda, 2014). In the previous research method, it is known that the maceration extraction method has not been able to show optimal results in the amount of extract yield. In the Application of Ultrasonic Waves to Increase Extraction Yield and Effectiveness of Antioxidants and Mangosteen Peel in 2017, it is known that ultrasonic extraction technology has been widely used to increase yields up to 30% and reduce extraction time so that it will be more effective (Aminah, Tomayahu, & Abidin, 2011).

Flavonoids are known as polar substances. As is known in the principle like dissolves like, that the effectiveness of the solubility of a compound depends on the type of solvent. Polar solvents and have been widely used in the extraction process are water, acetone, methanol, ethanol. For this reason, in the research carried out, methanol was chosen as a solvent which has shown the effectiveness of the

solubility of flavonoid compounds. (Verdiana, Widarta, & Permana, 2018) (Kusmaningtyasa, Laily, & Putri, 2015)

Based on the literature review that has been carried out, this study aims to determine the effect of extraction time on the ultrasonic method on the flavonoid yield of ciplukan plants using methanol as a solvent.

MATERIALS AND METHODS

Materials

The materials used are all parts of the ciplukan plant; dried roots, stems, leaves and fruit. In addition, in the extraction process 90% methanol is used.

Equipments

In this study, ultrasonic waves assisted extraction using a water bath, rotary evaporator, white man filter paper, a blender used to chop ciplukan plant raw materials, and screening were used.

Preparation

The ciplukan plants that have been taken are then cleaned of dirt and soil attached to the roots. Then the dried ciplukan plant, cut into small pieces. After getting a smaller size, the ciplukan plant was mashed using a blender and sieved to get a uniform size.

Extraction Process

The extraction process was carried out by varying the ultrasonication extraction time starting from 5, 10, 15, 20, 25 and 30 minutes with a variable mass of 30 g of ciplukan sample. After getting the extraction process, then all the filtrate was filtered using a funnel and Whiteman filter paper. All the filtrate was combined and concentrated using a rotary evaporator until no more liquid dripped so that a concentrated extract of ciplukan was obtained.

Qualitative Analysis with Determination of Quercetin Wavelength

The maximum wavelength of quercetin was determined. Determination of the maximum wavelength of quercetin was carried out by running the quercetin solution at a wavelength of 380 - 500 nm. The results of the measurement of the maximum wavelength is at the point of 420 nm with

Table 1. Corelation Between Time of Extraction and Total Flavonoid Content

| Time (minute) | Concentration Flavonoid | Extract Weight (g) | Total Flavonoid Content (%) |
|---------------|-------------------------|--------------------|-----------------------------|
| 10 | 19.3125 | 0.93 | 20.7661 |
| 15 | 137.1250 | 1.83 | 74.9317 |
| 20 | 59.0875 | 2.24 | 26.3783 |
| 25 | 20.3500 | 1.6 | 12.7188 |
| 30 | 57.8750 | 2.12 | 27.2995 |

an absorbance of 0.1158. The maximum wavelength is used to measure the absorption of the ciplukan extract sample

Quarctine Standard Curve

Weighed as much as 25 mg of standard quercetin and dissolved in 25 mL of methanol. The stock solution was pipetted as much as 1 mL and the volume was made up to 10 mL with methanol to obtain a concentration of 100 ppm. From a standard solution of 100 ppm quercetin, then several concentrations were made, namely 2 ppm, 4 ppm, 6 ppm, 8 ppm, and 10 ppm. From each concentration of the standard solution of quercetin, 1 mL was pipetted. Then 1 mL of 2% AlCl₃ and 1 mL of 120 mM potassium acetate were added. Samples were incubated for one hour at room temperature. The absorbance was determined using the UV-Vis spectrophotometric method at a maximum wavelength of 420 nm.

Determination of total extract flavonoid content

Weighed 15 mg of extract, dissolved in 10 mL of methanol, in order to obtain a concentration of 1500 ppm. 1 mL of this solution was pipetted and then 1 mL of 2% AlCl₃ solution and 1 mL of 120 mM potassium acetate were added. Samples were incubated for one hour at room temperature. The absorbance was determined using the UV-Vis spectrophotometric method at a maximum wavelength of 420 nm.

RESULTS AND DISCUSSION

In this study, all parts of the ciplukan plant were used. This is intended to obtain the optimum flavonoid concentration. Flavonoids are found in almost all parts of plants including fruits, roots, leaves, and outer bark of stems. Flavonoids are natural compounds that have the potential as antioxidant active substances that can counteract

free radicals that play a role in the emergence of degenerative diseases through the mechanism of destroying the body's immune system, lipid and protein oxidation.

Quantitative analysis of total flavonoid compounds using UV-Vis spectrophotometry was carried out to determine how much total flavonoid content contained in the methanol extract of the ciplukan plant. The analysis of flavonoids was carried out using UV-Vis spectrophotometry because flavonoids contain a conjugated aromatic system so that they show strong absorption bands in the ultraviolet and visible spectrum regions. (Harborne, J.B 1987).

In this study, to determine the total flavonoid content in the sample, quercetin was used as a standard solution with a concentration series of 2, 4, 6, 8, and 10 ppm. Concentration series is used because the method used in determining the concentration is a method that uses a standard curve equation, to make a standard curve, several concentration series are first made to obtain a linear equation that can be used to calculate the percent concentration. Quercetin is used as a standard solution because quercetin is a flavonoid of the flavonol group which has a keto group at C-4 and has a hydroxyl group at the C-3 or C-5 atom which is neighboring of flavones and flavonols. (Azizah dan Faramayuda 2014, h. 48).

The maximum wavelength absorption measurement was carried out from a wavelength of 380 to 500 nm. The results of running show that the maximum wavelength of quercetin standard is at a wavelength of 420 nm. The maximum wavelength was used to measure the uptake of the ciplukan plant extract sample.

From these measurements, it can be concluded that the higher the concentration used, the higher the absorbance obtained. The standard yield of quercetin obtained was plotted between its concentration and absorbance, so that a linear

regression equation was obtained, namely $y = 0.0888x - 0.0788$ with an R^2 value of 0.9814. The quercetin calibration curve equation can be used as a comparison to determine the concentration of total flavonoid compounds in the sample extract.

Table 2. The maximum wavelength

| Concentration (ppm) | Absorbance |
|---------------------|------------|
| 2 | 0.1058 |
| 4 | 0.1710 |
| 6 | 0.2992 |
| 8 | 0.3419 |
| 10 | 0.4645 |

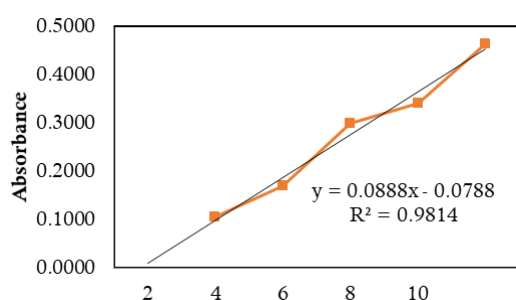


Figure 1. Quercetin standard curve.

It is stated in that research related to the ciplukan extraction process needs to be developed with more appropriate methods. This is done to obtain a higher content of flavonoid active substances that can be used as immunomodulators. Therefore, based on the results obtained above, it shows that with the ultrasonic extraction method using methanol as a solvent, a fairly good concentration has been obtained. (Kusumaningtyasa, Laily, & Limandha, 2015).

CONCLUSION

Based on the results of research that has carried out, it can be concluded that the levels of flavonoid The total extract of the ciplukan plant extract was at the extraction time of 15 minutes. Where the resulting concentration of total flavonoid levels is 74.9317% extract.

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