



Effect of Extraction Time on Dried Form and Powder of Cardamom Seeds in Aqueous Solvent on Physical, Total Phenol, and Flavonoid Properties

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Abstract

Cardamom seed spice is a plant that has bioactive compounds that are thought to be able to act as a natural antioxidant, so it has the potential to be a natural food additive in functional food products. These bioactive compounds can be obtained by extraction method using distilled water as a solvent. However, there have not been many studies regarding the effect of the extraction time used. Therefore, this research aims to determine the effect of the extraction time of cardamom seeds in dried form and powder on the physical properties, total phenols and total flavonoids. The extraction method used is maceration with extraction time levels of 5 minutes, 7 minutes and 9 minutes using distilled water as a solvent. The extract obtained will be tested for physical, qualitative and quantitative properties of total phenols and total flavonoids. The research results show that cardamom seed extract contains phenolics and flavonoids compounds with different strengths and the extract color is brownish yellow. The resulting yield was 27.14% with the highest of total phenols at 168.80 µg/mL and total flavonoids at 38.56 µg/mL in the treatment of cardamom seed powder extraction time of 9 minutes. Cardamom seed powder extract prepared with an extraction time of 9 minutes is more capable of extracting bioactive compounds compared to other time levels of dried form and powder of cardamom seeds.

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Introduction

Cardamom (*Amomum cardamomum*) belongs to the *Zingiberaceae* family which is an aromatic spices plant native in Indonesia which is widely used and has health benefits because it contains various phytochemical compounds such as phenols, starch, tannins, terpenoids, flavonoids, proteins, and sterols, which are also known as perennial herbs (Moulai-Hacene et al., 2020; Juliana et al., 2022). Apart from that, cardamom is also an export commodity that is included in the 9 major groups of world spices which are usually bought and sold in the form of dried fruit or essential oil (Tambunan, 2017). In Indonesia, cardamom productivity in 2018 was recorded at 81,724,526 tons/year and relatively increased every year. Based on Directorate General of Horticulture, in 2018 Indonesia was able to export 7,842 tons/year. This number always increased since 2014. The growth in the export value of cardamom over the last few years indicates that cardamom has the potential to be developed (Nurhayati et al., 2019). Cardamom seeds can be used in the form of dried fruit or essential oil.

Cardamom essential oil can be used as an aromatic ingredient which is generally applied in the food and health sectors (Alam et al., 2021; Anugrah et al., 2018). Meanwhile, dried fruit is usually used as a food additive for flavoring dishes, bakery products, confectionaries, and traditional spice drinks which have the effect of increasing the body's immune system (Tambunan, 2017).

Many herbs and spices commonly used to flavor foods contain phenolic compounds that are reported to show good antioxidant activity. Consequently, identification of alternative natural and safe sources of food antioxidants from plants has been identified (Bhatti et al., 2010). According to Agoes (2010), cardamom seeds contain (3-7) % of essential oil compounds consisting of cineol, borneol, terpineol, terpineol acetate, and camphor which can be efficacious in thinning phlegm, relieving flatulence, warming, relieve pain, and aromatherapy stimulants. Apart from essential oils, extracts from cardamom seeds also contain bioactive compounds which can act as natural antioxidants. Extraction of bioactive compounds can be carried out

using the maceration method (Nuryanti et al., 2021). This extraction is based on the principle of mass transfer of substance compounds into the solvent, where the transfer begins to occur at the interfacial layer and then diffuses into the solvent (Hudaya, 2010). Apart from the extraction method, the type of solvent and the length of the time extraction process are also determining factors in extracting optimal bioactive compounds (Naufalin et al., 2021).

According to Juliana et al. (2022), extraction is important in recovering phenolic and flavonoid compounds, which is influenced by several factors such as solvent ratio (Sajid et al., 2019), type of solvent (Qomaliyah et al., 2019), and extraction time (Soos et al., 2019). Extraction time is one of the most important parameters that influences the extraction of bioactive compounds in cardamom seeds. This affects the mass transfer of bioactive compounds from the material to the solvent. The longer the extraction time for bioactive compounds means the longer the contact between the solvent and the material, so that mass deposition will occur from both of them by diffusion until there is an equilibrium concentration of the solution inside and outside extraction material. Many researchers agree that increasing the extraction time will increase the solubility of the solute and the diffusion coefficient, and affect the extraction efficiency of bioactive compounds (Spigno et al., 2007; Sayuti et al., 2017; Anthony et al., 2022). Therefore, studies about the extraction of the bioactive compounds of cardamom seeds at the right time need to be done to determine the optimal extraction process by comparing two different preparation forms of cardamom seeds. Thus, this research aims to determine the effect of the preparation form and the length of time extracting cardamom seeds using distilled water on the physical properties profile, total phenols and total flavonoids that act as a natural antioxidant so it has the potential to be a natural food additive which can later be applied to functional food products.

Materials and Methods

Material

The ingredients used are dried Javanese cardamom seeds obtained from farmers in Banyumas, Central Java-Indonesia that reaches full maturity with the moisture content around 8.09%. Apart from that, the chemicals used include: aqueous or distilled water, iron (III) chloride, ethanol, methanol, magnesium, hydrochloric acid, quercetin, aluminum (III) chloride, acetic acid, folin-ciocalteu, sodium bicarbonate, and gallic acid. All the chemical materials in this research using analytical grade (PA).

Research method

Experimental design

The research was done in the basic science and microbiology laboratory at the Institute of Technology Telkom Purwokerto (ITTP). The experimental design used a Completely Randomized Design with 12 treatment combinations and 5 replications. The factors

studied were the dried form and powder of cardamom seeds and the length of time extraction process at the level of 5, 7, and 9 minutes using the maceration method with distilled water or aqueous as a solvent. This solvent was selected because the materials obtained will be applied to a food product to avoid the toxicity of organic solvents. The variables observed included physical properties, qualitative profiles of phenolics and flavonoids, as well as total phenols and total flavonoids.

Preparation cardamom powder

Dried cardamom seeds obtained from local farmers in Banyumas, were converted into powder with these following characteristics: whole dried seed form and a moisture content of 8.09%. First, the dried form cardamom seeds grinded using grinder, after that the powder obtained was sifted using a 60 mesh sieve until a homogeneous particle size was obtained.

Extraction cardamom seeds form

The extraction process for dried form and powder of cardamom seeds is performed using maceration method. Dried form and powder of cardamom seed are soaked in distilled water for 2 hours in an erlenmeyer container before being placed in a shaker waterbath (Ryanata et al., 2014). The ratio between dried form and powder with the solvent are 1:10 (w/v). Then each treatment was subjected to an extraction process with time levels of (5,7, and 9) minutes using a shaker waterbath with 60°C water temperature. The extraction results were then filtered using filter paper to obtain extracts for each treatment.

Physical properties

The yield of the cardamom seed extract was determined using the following equation (Nurhaen et al., 2016):

$$\text{Yield (\%)} = \frac{\text{final sample volume (mL)}}{\text{(initial sample weight (g))}} \times 100$$

Qualitative phenolic and flavonoids properties

Qualitative testing of phenolic properties began with cardamom seed extract being put into a test tube, then added with 3 drops of FeCl₃ 3% reagent in 3 drops of ethanol solvent, in which the color change is observed. Positive results are indicated by the presence of green, red, blue, purple or black colors (Mukhriani et al., 2019). Whereas the properties of flavonoids was evaluated placing 1 g of cardamom seeds extract into a test tube and dissolved in 2 mL of 50% methanol. Then, magnesium metal powder and 4-5 drops of concentrated HCl were added. A positive result is indicated if a reddish brown or brick red solution is formed (Mukhriani et al., 2019).

Total phenol properties

The maximum wavelength of gallic acid was determined by measuring the gallic acid solution with a concentration of 30 ppm in the range 600-850 nm and the maximum wavelength with the highest absorbance

value was determined. Then, standard series solutions of gallic acid were prepared with concentrations of 10, 20, 30, 40 and 50 ppm which obtained by diluting the solution of 1000 ppm gallic acid. Subsequently, 1.5 mL of Folin-Ciocalteu reagent was added, shaken homogeneously and held for 3 minutes. Next, 1.2 mL of 7% Na₂CO₃ solution added to the sample, then shaken until homogeneous. The mixture was held for 60 minutes at room temperature and the absorbance at the maximum wavelength were easured. Determination of total phenolic in the sample was done by taking 0.3 mL of cardamom seed extract then adding 1.5 mL of Folin-Ciocalteu reagent, then shaking and leaving it for 3 minutes. After that, add 1.2 mL of 7% Na₂CO₃ solution, then shake until homogeneous. Leave it for 60 minutes at room temperature and the absorbance is measured at the maximum wavelength (Andriani and Murtisiwi, 2018).

Total flavonoids properties

Determination of the maximum wavelength was done by taking 10 mg of quercetin dissolved in 10 mL of ethanol (1000 ppm), then diluted to a concentration of 60 ppm. A total of 1 mL of 60 ppm quercetin solution was pipetted then 1 mL of 2% AlCl₃ and 8 mL of 5% acetic acid were added, and then incubated for 30 minutes. Absorbance was measured at a wavelength of 400-800 nm. Meanwhile, measurements of standard series solutions of gallic acid can be carried out by 10 mg of quercetin dissolved in 10 mL of ethanol (1000 ppm).

Then the concentration variations were made at 20, 40, 60, 80, and 100 ppm. A total of 1 mL of each concentration of quercetin solution was pipetted then 1 mL of 2% AlCl₃ and 8 mL of 5% acetic acid were added, then incubated for 30 minutes. Absorbance is measured at maximum wavelength. Determination of total flavonoids was done by taking 1 mL of cardamom seed extract then added with 1 mL of 2% AlCl₃ and 8 mL of 5% acetic acid, then incubated for 30 minutes. After that absorbance was measured at a previously determined maximum wavelength (Ipandi et al., 2016).

Data analysis

The data were analyzed using the two-way ANOVA method followed by DMRT if there were differences at the significance level $\alpha = 5\%$.

Results and Discussion

Physical properties

Extraction of bioactive compounds in cardamom seeds using distilled water as a solvent was done using the wet extraction method. The wet extraction method used to produce cardamom seed extract was carried out by maceration method using distilled water as a solvent by comparing two preparation forms of cardamom seeds, namely dried form and powder at extraction time levels of 5, 7 and 9 minutes. The physical properties and yield produced from dried form and powder cardamom seed extract at each different extraction time treatment are presented in Table 1.

Table 1. Physical properties of dried form and powder of cardamom seed extract with various treatments

Physical properties and yield	Extraction time 5 minutes		Extraction time 7 minutes		Extraction time 9 minutes	
	Dried form	Powder	Dried form	Powder	Dried form	Powder
Color	light brown	brownish yellow	light brown	brownish yellow	light brown	brownish yellow
Yield (%)	82.87	41.06	69.77	28.72	67.98	27.14
Average Yield (%)*	62.06 ^a		49.25 ^b		47.56 ^c	

*This data collected from the average of dried form and powder in each of variation time based on ANOVA analysis with significance level $\alpha = 5\%$.

The physical properties of color of cardamom seed extract in dried form and powder in this study produced a light brown and brownish yellow color as shown in Figure 1. In each treatment, the level of extraction time and the preparation forms used did not show any prominent color differences. This shows that the bioactive compounds which is brownish yellow in color is thought to come from the phenolic group which is easily soluble in distilled water and is not affected by the preparation form of processed cardamom seeds. This is comparable to research conducted by Noviantari et al. (2017), that the phenolic group can be extracted with solvents that have the appropriate polarity and level of fineness of the material.

Table 1 shows the profile that the yield of cardamom seed extract in powder preparation form at a time level of 9 minutes produces the lowest extract yield, that is 27.14% compared to the dried form in other treatments. Overall, the yield of extraction that obtained

from powder has much lower quantity over a yield from dried form. It occurs because the powder which has a greater ability to absorb solvents than the dried form due to differences in the surface area of the cardamom seed particles.

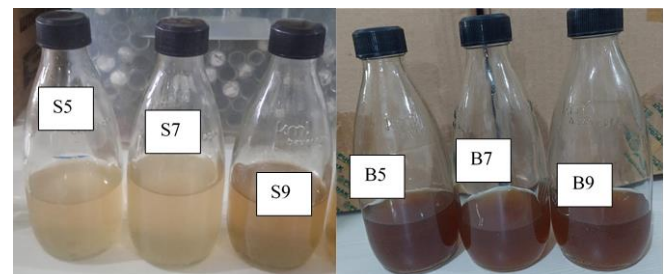


Figure 1. Cardamom seed extract from dried form (S) and powder (B) with variation of extraction time treatment

Poveda et al. (2019) stated that the yield of extraction obtained from CBS powder have lower yield extraction compared to dried form. This decrease could be mostly due to the water-holding capacity of the powder present in the CBS. This powder became more available when the surface-to-volume ratio of the CBS powder increased compared than dried form. Thus, the overall yield of the extract produced from the powder is less than the dried form of cardamom seeds. The results of the variance analysis of the average yield of cardamom seed extract which had a significant effect are presented in Figure 2.

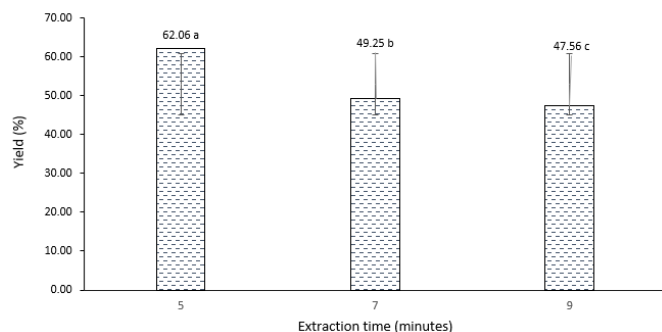


Figure 2. Average yield of cardamom seed extract with variation of extraction time treatment

*This data collected from the average of dried form and powder in each of variation time based on ANOVA analysis with significance level $\alpha = 5\%$.

Figure 2 shows the results of the variance analysis test of the average yield of cardamom seed extract in dried form and powder at time levels of (5, 7 and 9) minutes, which have significant differences. This data collected based on each variation time of extraction to giving information about the effect of extraction time on yield of extraction. These results show that the yield

of the extract produced from an extraction time of 9 minutes has a lower yield than an extraction time of 7 and 5 minutes respectively, that is 47.56%; 49.25%; and 62.06%. However, a low yield does not necessarily correlate positively with low bioactive compounds extracted. Bhatti et al. (2010) report that the plant that has to be extracted depends on nature, amount and especially the concentration of the solvent. Therefore, an efficient appropriate and adequate concentration of the solvent should be used to extract maximum bioactive compounds from any plant material. As the bioactive compounds are organic in nature and organic compounds mostly dissolve in organic solvents. Noviantari et al. (2017), also stated that smaller the powder particle size will expand the surface of the material, thereby increasing the contact between the powder particles and the solvent and causing the solvent to easily break down the cell walls of the material.

Qualitative phenolic and flavonoids properties

Phytochemical screening of phenolic compounds in distilled water extract of cardamom seeds in dried form and powder using FeCl_3 1% reagent gave positive results, which were indicated by the formation of a yellow-brown color. The phenolic test result is said to be positive if a reaction occurs between the phenolic compound and FeCl_3 to form a green, purple, brown-red, blue or black color (Supriningrum et al., 2020). Meanwhile, flavonoid compounds will give positive results which are characterized by the formation of a brick red color due to the reaction with magnesium metal and concentrated HCl (Mukhriani et al., 2019). Phytochemical screening of phenolic and flavonoid compounds in cardamom seed extract in dried form and powder at various levels of extraction time is presented in Table 2.

Table 2. Qualitative phenolic and flavonoids properties of cardamom seed extract with various treatments

Treatment	Qualitative phenolic	Color transform	Qualitative flavonoids	Color transform
Dried form 5 minutes	+	brownish yellow	+	bright yellow
Dried form 7 minutes	+	brownish yellow	+	bright yellow
Dried form 9 minutes	+	brownish yellow	+	bright yellow
Powder 5 minutes	+++	dark brown	++	reddish yellow
Powder 7 minutes	+++	dark brown	++	reddish yellow
Powder 9 minutes	+++	dark brown	++	reddish yellow

Note: -: "Negative"; +: "Positive weak"; ++: "Positive"; +++: "Positive strong"; ++++: Positive very strong

Table 2 shows that all treatments tested for phenolic and flavonoid content produced positive reactions with various levels of strength. Treatment of cardamom seed extract with the dried form at extraction time levels of (5, 7 and 9) minutes produced a positive reaction which was marked by a color change to yellow-brown for testing phenolic content and a bright yellow color for testing flavonoid content. Meanwhile, cardamom seed extract in powder also produces a stronger positive reaction, marked by a change in color to dark yellow-brown for phenolic testing and reddish

yellow color for testing flavonoid content. Thus, all cardamom seed extract treatments contain phenolic compounds and flavonoids at different levels which can be influenced by processing or preparation methods (Poveda et al., 2019).

Total phenol and total flavonoids properties

Cardamom seed contains secondary metabolites that are pharmacologically efficacious. Some secondary metabolites reported in cardamom are phenolic and flavonoids compounds, which are used as antioxidants

(Juliana et al., 2022; Ballard et al., 2018). These compounds can be isolated from cardamom seeds by extraction. Extraction is important in recovering phenolic and flavonoid compounds, which is influenced by several factors such as type of solvent (Qomaliyah et al., 2019) and extraction time (Soos et al., 2019; Juliana et al., 2022).

The results of the analysis of variance (ANOVA) of cardamom seed extract using dried form and powder with various extraction time showed significant interactions and significant differences ($p < 0.05$) in testing total phenols which are presented in Figure 3 and total flavonoids that can be seen in Figure 4.

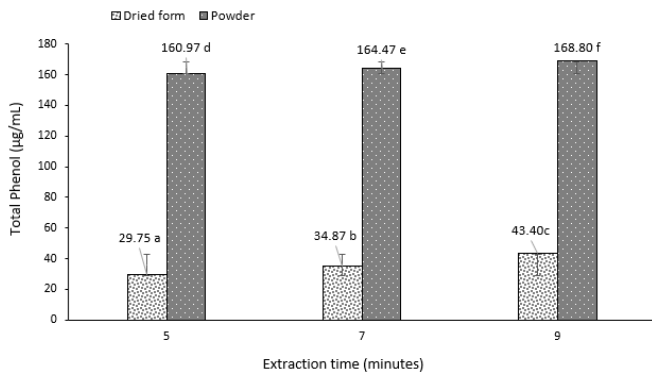


Figure 3. Total phenol compounds in cardamom seed extract with various treatment

The results showed that cardamom seed extract in powder at extraction time levels of (5, 7 and 9) minutes had a higher total phenol content compared to cardamom seed extract in dried form at all extraction process time levels. In the treatment of cardamom seed powder extract with 9 minutes extraction time had the highest total phenol content at 168.80 µg/mL, compared to cardamom seed powder extract with an extraction time of 5 minutes and 7 minutes at 160.97 µg/mL and 164.47 µg/mL. The high total phenol content in the cardamom seed powder extract treatment is also influenced by the long extraction time which allows the bioactive compounds in this natural material to be extracted more optimally than during other extraction processes. According to the statement by Juliana et al. (2022), that one of the factors that influences the success of the extraction process in extracting active compounds in natural ingredients is extraction time. The extraction time will have a positive correlation with the number of bioactive compounds extracted until it reaches the maximum level of bioactive compounds in the natural material that can be extracted.

Figure 4 also shows that cardamom seed extract in all treatments contains flavonoid compound at different levels. The treatment interaction between preparation form and extraction time of cardamom seed extract in testing flavonoid content had significant results ($p < 0.05$) and significantly different. Treatment of cardamom seed powder extract with an extraction time of 9 minutes produced the highest flavonoid content at 38.56 µg/mL compared to processing times of 5 minutes

and 7 minutes with result at 33.92 µg/mL and 36.04 µg/mL. These results are in accordance with research by Juliana et al. (2022), which shows the effect of solvent type, solvent ratio and extraction time has an increased response to the total flavonoid content of extracted cardamom. Kanthal et al. (2021), also stated that the overall extract extracted with different types of solvents such as distilled water, ethanol and ethyl acetate had adequate flavonoid content and efficient as a medicinal plant. This result is much higher than the flavonoid content in the dried form cardamom seed extract sample with extraction time levels of (5, 7, and 9) minutes, namely at 12.92 µg/mL; 17.62 µg/mL; and 19.37 µg/mL.

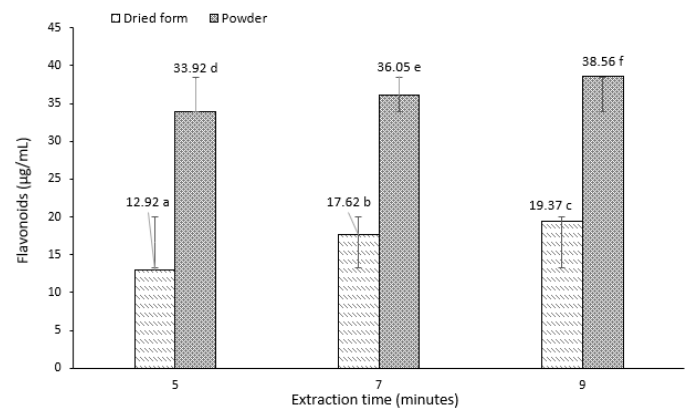


Figure 4. Flavonoids compounds in cardamom seed extract with various treatment

Conclusion

The use of distilled water as a solvent in the process of extracting bioactive compounds of cardamom seeds provides significant results were able to extract the bioactive compounds contained, especially phenolic and flavonoid compounds. The powder of cardamom seed extract is more effective in extracting the bioactive compounds contained in the material compared to the dried form which is characterized by higher levels of total phenols, and total flavonoids. The best treatment resulted in extracts from powder with an extraction process time of 9 minutes with yield, total phenols and total flavonoids respectively 27.14%; 168.80 µg/mL; and 38.56 µg/mL. Soon, additional research can be conducted regarding its antioxidant activity and stability during storage.

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