Bioactive Compound Contents and Antioxidant Activity of Purified Red Sorghum Pericarp Extract by Membrane Ultrafiltration Process

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Abstract

Sorghum plants contain bioactive compounds such as phenolic compounds and proanthocyanidins. The presence of these compounds is extremely valuable for use as antioxidants in health care. This study aims to determine the total phenolic content, total proanthocyanidin content, and antioxidant activity of purified red sorghum extract using the ultrafiltration membrane method with different transmembrane pressure. At pressures of 8, 9, and 10 Bar, an ultrafiltration process was carried out using a polyethersulfone (PES) membrane with a molecular weight cut-off size of 10 kDa. The total phenolic content was determined using the Folin-Ciocalteu method and identified using UV-Vis spectrophotometry at 760 nm. Total proanthocyanidin content was measured using the acid-butanol method and then analyzed using UV-Vis spectrophotometry at 550 nm. The DPPH (2,2-diphenyl-2-picrylhydrazyl) method will be used to determine the antioxidant activity of purified red sorghum extract. The results of the measurements show that the higher the transmembrane pressure, the higher the concentration of phenolic and proanthocyanidins content, and that the purified red sorghum extract has a high antioxidant value (IC₅₀ = 66.852 ppm).

Keywords: Phenolic, proanthocyanidin, antioxidant, red sorghum pericarp, ultrafiltration

Introduction

Sorghum is a staple grain that is widely grown and consumed in developing countries (Keyata et al., 2020). Sorghum is the world’s fifth most important cereal crop, after wheat, rice, maize, and barley (Wu et al., 2018). Sorghum pericarp has contained many bioactive compounds depending on its genotypes such as phenolic acid, flavonoids, condensed tannin, or proanthocyanidin (Wu et al., 2017). Sorghum has gained popularity in recent years in the food and pharmaceutical industries due to its bioactive phenolic compounds, with the most abundant phenolic compounds (100-2300 mg gallic acid equivalent/100 g grain) when compared to the major cereals (wheat, maize, rice, oats, barley, rye, and millet, 9-1459 mg gallic acid equivalent/100 g grain) (Xiong et al., 2021). In general, colored sorghum genotypes have higher levels and more complex profiles of phenolic compounds than white sorghum genotypes (Rhodes et al., 2014; Xu et al., 2021).

Sorghum’s phenolic compounds are more diverse than other major grains. Sorghum contains almost all types of phenolic compounds, with phenolic acids and flavonoids predominating (Xiong et al., 2019; Xiong et al., 2020). Phenolics have been recognized for their superior antioxidant properties in the food, pharmaceutical, and cosmetics industries (Lafka et al., 2007; Víctor-Ortega et al., 2017). Phenolics was a group of polyphenols is a molecule that can prevent or slow down the oxidation of other compounds and become a reducing agent (Landete, 2012).

One form of polyphenolic compound observed in sorghum is condensed tannin known as proanthocyanidin that’s a spinoff of oligomeric flavonoid compounds that feature as secondary metabolites and are generally acquired in inexperience plants (Ou & Gu, 2014; Liu & White, 2012; Xu et al., 2021). Proanthocyanidins have turned out to be increasingly popular, as confirmed through the developing range of

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Antioxidants are chemical compounds that can counteract the effects of free radicals. These compounds function by donating electrons to achieve a stable form (Sarma et al., 2010). Natural antioxidants and synthetic antioxidants are the two types of antioxidants based on their source. Natural antioxidants are antioxidant compounds that occur naturally in the body as part of the body’s defense or immunity, whereas synthetic antioxidants are antioxidants that are chemically synthesized from various types of plants, one of which contains high levels of polyphenols (Hidayati et al., 2017; Tristantini et al., 2016).

The objective of this study was to evaluate the total concentration of polyphenol, proanthocyanidin, and antioxidant activity of purified red sorghum extract by ultrafiltration process using polyethersulfone (PES) membrane with 10 kDa of molecular weight cut-off (MWCO) at 8, 9, and 10 bar of pressure. The different transmembrane pressure (TMP) is used to compare the concentrations of polyphenol and proanthocyanidin at various pressures. Folin-Ciocalteau method using for total polyphenol content and the acid-butanol method using for total proanthocyanidin content. The antioxidant activity of purified red sorghum extract will be determined using DPPH (2,2-diphenyl-2-picrylhydrazyl) method.

**Methods**

**Materials**

In the Microwave-Assisted extraction, red sorghum pericarp was extracted using aquadest as a solvent with a ratio (1:100) at 70 ºC for 150 minutes. PES membranes with MWCOs of 10 kDa were used (Sartorius Stedim Biotech Germany). Folin-Ciocalteau reagent (Merek, Germany), sodium carbonate (Merek, Germany), 1-butanol (Merek, Germany), hydrochloric acid (37 percent purity, Merek, Germany), ammonium iron(III) sulfate dodecahydrate (Merek, Germany), DPPH reagent (Merek, Germany), Methanol (Merek, Germany), buffer phosphate pH 7 (Merek, Germany) and distilled water (aquadest) were used in the analysis.

**Ultrafiltration process**

The membrane separation was accomplished through the use of the dead-end filtration method with N2 gas as the driving pressure. Before continuing to the ultrafiltration stage, the extract was first passed through the microfiltration membrane using the 0.2 µm PES membrane at 1 bar of pressure. Aquadest was used to compact the PES membrane first. The ultrafiltration stage was carried out using two types of PES membranes with MWCOs of 10 kDa at pressures of 8, 9, and 10. The PES membrane had a surface area of 17.35 cm².

**Total phenolic content**

Total phenolic content was determined using a modified Folin-Ciocalteau method (Torun et al., 2015) in which 0.5 mL of sample extract was mixed with 2.5 mL of Folin-Ciocalteau reagent and 2 mL of Na2CO3 was added (7.5%). The mixture was then incubated at 50 ºC for 5 minutes before the absorbance was measured at 760 nm with a Uv-Vis spectrophotometer (Genesys 20). The outcome was expressed in milligrams of gallic acid.

**Total proanthocyanidins content**

The butanol-assay method was used to determine the total proanthocyanidin content (Gessner & Steiner, 2005). 6 mL of butanol-HCl (95:5) solution was mixed with 1 mL of sample extract and 0.2 mL of iron reagent (FeNH4(SO4).12H2O). The mixture was then incubated at 90 ºC for 50 minutes before the absorbance was measured at 550 nm with a UV-Vis spectrophotometer. The result was expressed in milligrams of catechin.
Antioxidant activity

The DPPH method was used to determine the antioxidant activity of purified red sorghum extract. 0.1 mL of sample was mixed with 3.9 mL of DPPH solution (0.00394-gram DPPH in 50 mL of methanol and pH 7 of buffer phosphate) (Afandy et al., 2021). The mixture was then incubated at room temperature and before the absorbance was measured at 515 nm using a UV-Vis spectrophotometer.

Results and Discussion

Total phenolic content

The phenolic content was determined using the Folin-Ciocalteu method. Folin-Ciocalteu is a reagent used for the quantification of polyphenolic compounds with improved sensitivity and reproducibility. The Folin-Ciocalteu reagent is a mixture of phosphotungstic acid and phosphomolybdic acid that reacts with phenolic and non-phenolic reducing agents to form a chromogen (Lamuela-Raventós, 2017). This method can be validated spectrophotometrically, as the oxo tungstate and oxo molybdate formed in this redox reaction exhibit a blue color proportional to the concentration of polyphenols under basic conditions (Lamuela-Raventós, 2017). In this test, a Na₂CO₃ solution is also added to act as an alkaline atmosphere so that the Folin-Ciocalteu reduction reaction with the OH groups of the phenolic compounds in the sample takes place. The total phenolic content of purified red sorghum pericarp extract is shown in Fig 1. The total concentration of phenolic after extraction with the microwave-assisted method is 5.0640 mg/L.

The concentration of phenolic content changes with the increase of TMP. Figure 3 shows, at the pressure of 8 bar, the concentration of phenolic increased from 0.0784 to 0.7391 mg/L in 120 minutes, at a pressure of 9 bar increased from 0.2023 to 0.8939 mg/L in 120 minutes, and at a pressure of 10 bar increase from 0.3365 to 1.0281 mg/L in 120 minutes. Figure 3 proves that expanding the transmembrane pressure produces a higher concentration of phenolic compounds. Because of the increase in transmembrane pressure, which causes an increase in permeate flow, the solute will easily pass through the membrane pores. It also demonstrates that as the driving force increases with increasing pressure, the particles are pushed through the membrane’s pores (Benitez et al., 2009). The concentration of phenolic will increase as time in this process because the equilibrium moves to the permeate and pass through the membrane (Benitez et al., 2009).

When the Folin-Ciocalteu reagent is combined with phenolic compounds, the color of the sample changes from dark orange to blue (Lamuela-Raventós, 2017). The intensity of the blue color is caused by the formation of oxo molybdate, which produces a blue color proportional to the concentration of polyphenols in alkaline conditions. In this reaction, the Folin-Ciocalteu reagent reduces the OH group on phenol compounds, resulting in the formation of phenolic compounds.

Total proanthocyanidin content

The total proanthocyanidin content was determined using the butanol-assay method. The acid-butanol method is a very specific method for the determination of total proanthocyanidins and is based on the oxidative depolymerization of the bonds between flavans under acidic conditions and at elevated temperatures to produce anthocyanidin compounds, which can be measured spectrophotometrically at a wavelength of 550 nm. The total concentration of proanthocyanidin after extraction from the microwave-assisted method is 0.7264 mg/L.

The concentration of proanthocyanidin content changes with the increase of TMP. Fig. 4 shows, at the pressure of 8 bar, the concentration of proanthocyanidin increased from 0.0927 to 0.1442 mg/L in 120 minutes, at the pressure of 9 bar
increased from 0.0999 to 0.1563 mg/L in 120 minutes, and at the pressure of 10 bar increase from 0.1099 to 0.1627 g/mL in 120 minutes. Figure 4 indicates that the concentration of proanthocyanidins will increase as the pressure and time in the membrane ultrafiltration process increase. This occurs because the equilibrium moves towards the permeate, letting proanthocyanidin compounds pass through the membrane and increasing the proanthocyanidin concentration in the permeate. The reaction of proanthocyanidin compounds in purified red sorghum pericarp extract with acid-butanol and FeNH₄(SO₄)₂·12H₂O reagents will result in an oxidation process by acid catalysts on proanthocyanidin compounds heated in a mixture of alcohol and acid solutions, breaking the bonds between flavans and producing anthocyanidin compounds.

![Figure 3. Total phenolic content of purified red sorghum pericarp extract with different TMP](image)

![Figure 4. Total proanthocyanidins content of purified red sorghum pericarp extract with different TMP](image)

**Antioxidant activity**

This stage was carried out to determine the antioxidant activity of proanthocyanidin compounds that had been separated using an ultrafiltration membrane. This test was performed on red sorghum pericarp extract samples that had passed membrane separation using a PES membrane with an MWCO size of 10 kDa. At a pressure of 10 bar, the sample with the highest concentration of phenolic and proanthocyanidin is used to measure antioxidant activity. The percentage of antioxidant activity is shown in Table 1.

The percentage antioxidant values obtained in Table 1 are then used to determine IC₅₀ values. The IC₅₀ value is the effective concentration required to reduce total DPPH by 50% (Tristantini...
et al., 2016). The data were then regressed using the extract concentration as the x-value and the antioxidant percentage as the y-value. Regression results from this data are shown in Figure 5. Based on the data obtained in Figure 5, the regression equation obtained is said to be $y = 0.7632x - 1.021$. In determining the IC 50 value, the y value in the regression equation is replaced with a value of 50, resulting in an x value of 66,852 ppm expressed as the IC$_{50}$ value. Antioxidant properties can be determined by their IC$_{50}$ value. Antioxidants with IC values below 50 ppm are said to be very strong, and IC values below 50 ppm are said to be strong. 50-100 ppm is moderate, between 100 and 150 ppm and 150 and 200 ppm are weak (Molyneux, 2004). Based on this description, proanthocyanidin compounds from sorghum seed extracts that have undergone a 10 kDa PES membrane separation process are classified as potent antioxidants (IC$_{50}$ between 50 and 100 ppm).

The presence of phenolic compounds contributes significantly to the antioxidant activity. The ability of phenolic compounds to capture DPPH free radicals is strongly influenced by the OH group (Nakiboglu et al., 2007). The chemical structure, as well as the number and position of hydroxy and methyl groups on the ring, determines the difference in phenolic antioxidant activity. The more substituted hydroxyl groups in a molecule, the greater its free radical scavenging ability because more hydrogen atoms can be donated (Lin et al., 2009). An antioxidant reaction mechanism occurs when there is a reaction capture of hydrogen by free electrons in the DPPH radical compound of an antioxidant compound, which is characterized by the presence of a discoloration ranging from purple to yellow (Setiawan et al., 2018).

<table>
<thead>
<tr>
<th>Control absorbance</th>
<th>Concentration (ppm)</th>
<th>Absorbance</th>
<th>% Antioxidants</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.832</td>
<td>2</td>
<td>0.828</td>
<td>0.481</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.813</td>
<td>2.284</td>
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<tr>
<td></td>
<td>6</td>
<td>0.805</td>
<td>3.245</td>
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<td>8</td>
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</tr>
<tr>
<td></td>
<td>10</td>
<td>0.776</td>
<td>6.731</td>
</tr>
</tbody>
</table>

Table 1. The antioxidants activity of purified red sorghum pericarp extract

![Figure 5. Relationship between concentration with antioxidants percentage](image)

Conclusions

The total phenolic content and total proanthocyanidin content will be affected by changes in transmembrane pressure. When the transmembrane pressure is increased, the concentration of total phenol content and total proanthocyanidin content of the purified red sorghum pericarp extract increases. The antioxidant activity of purified red sorghum extract revealed by the ultrafiltration membrane process was classified as a strong antioxidant (IC value 50 = 66,852 ppm).

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References


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