Aktivitas antioksidan, kandungan fenolik total dan kandungan flavonoid total ekstrak etil asetat buah Mengkudu serta fraksi-fraksinya

Antioxidant activities, total phenolic and flavonoid contents of ethyl acetate extract of Mengkudu (Morinda citrifolia, L) fruit and its fractions*

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Abstract

This present study was carried out to evaluate antioxidant activities, total phenolic and total flavonoid contents of ethyl acetate extract of Mengkudu fruit and its fractions. Ethyl acetate extract was fractionated by column chromatography and yielded 15 fractions based on the identical TLC (thin layer chromatography) profile.

Antioxidant activities in ethyl acetate extract and its fractions were determined by radical scavenging assay using DPPH (2,2-diphenyl-1-picrylhydrazyl) radical. The total phenolic and total flavonoid contents were determined spectrophotometrically.

Among 15 fractions of ethyl acetate extract evaluated, fraction 8 (IC50 5.49 µg/mL) and fraction 7 (IC50 7.90 µg/mL) revealed antioxidant activities that higher than that of vitamin E (IC50 8.27 µg/mL). The total phenolic contents ranged from 5.94 ± 0.08 to 36.52 ± 0.35 g of gallic acid equivalent/100 gram dry material whereas the total flavonoid contents...
 ranged from 1.19 ± 0.02 to 17.65 ± 0.17 g of quercetin equivalent/100 gram dry material.

A linear positive relationship existed between the antioxidant activities and total phenolic contents of the tested ethyl acetate extract and its fractions: \( y = -1.220x + 44.022; r^2 = 0.67 \), while the correlation between antioxidant activities and total flavonoid contents revealed a linear regression: \( y = -2.202x + 35.82; r^2 = 0.4278 \).

**Key words:** antioxidant activity, *Morinda citrifolia*, L, Fraction

**Introduction**

Several epidemiological studies have revealed that certain dietary elements play a pivotal role in the prevention of chronic diseases such as heart disease and several types of human cancers. People who consume much plant-derived foods such as fruits, vegetables, and soybeans were found to have lower incidence of cancer (Tsai et al., 2005). Consumption of fruits and vegetables also has been associated with reduced risk of chronic disease (Adom and Liu, 2002).

Natural phenolic phytochemicals in fruits and vegetables have been receiving increased interest from consumers and researchers for the beneficial health effects on coronary heart disease and cancer mainly due to their antioxidant activities (Kim et al., 2002). A number of phenolic compounds mainly flavonoid and phenolic acids are among the antioxidant compounds which present in fruits and vegetables (Madhujith and Shahidi, 2005).

Antioxidant are compounds that can delay, inhibit, or prevent the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions (Javanmardi et al., 2003). There are at least four general sources of antioxidant: (1) enzymes, for example superoxide dismutase, glutathione peroxidase, and catalase; (2) large molecules (albumin, ceruloplasmin, ferritin); (3) small molecules (ascorbic acid, glutathione, uric acid, tocopherol, carotenoids, (poly)phenols); and (4) some hormones such as estrogen, angiotensin, melatonin, etc (Prior et al., 2005).

Synthetic antioxidant such as tert-butylhydroxy anisole, tert-butylhydroxy toluene, and tert-butyl hydroquinone (TBHQ) have been widely used to retard lipid oxidation, however, such synthetic antioxidants are not preferred due to toxicological concerns. For this reason, there have been increasing interest in the exploration of natural antioxidant especially from fruits and vegetables (Rababah et al., 2004). *Morinda citrifolia*, L has been used in folk remedies by Polynesians for over 2000 years and is reported to have a broad range of therapeutic effects, including antibacterial, antiviral, antifungal, antitumor, analgesic, hypotensive, anti-inflammatory, and immune enhancing effect (Wang et al., 2002).

The previous study showed that among three extracts evaluated, ethyl acetate extract revealed the most effective antioxidant with IC\(_{50}\) 46.7 µg/mL, whereas chloroform and methanol extracts have IC\(_{50}\) 227.7 and 888.6 µg/mL respectively (Abdul and Sugeng, 2005). Zin et al., (2006) has studied the antioxidant activities of crude extract from root, fruit, and leaf of Mengkudu and their fractions on a Sephadex LH-20 column with ethanol as eluate. However, the antioxidant activities of ethyl acetate extract and its fractions on a silica column as well as their correlation with total phenolic and total flavonoid contents have not been reported yet.

The objectives of this study were (i) to fractionate ethyl acetate extract of Mengkudu fruit (ii) to evaluate antioxidant activities by radical scavenging assay using DPPH (2,2-diphenyl-1-picrylhydrazyl) radical (iii) to determine total phenolic and total flavonoid contents, and (iv) to correlate antioxidant activities with total phenolic and total flavonoid contents of ethyl acetate extract and its fractions.

**Methodology**

**Materials and chemicals**

Mengkudu fruit was obtained from Bantul, Jogjakarta. DPPH (2,2-diphenyl-1-picrylhydrazyl) and vitamin E were purchased from Sigma (St.Louis, MO, USA). Folin-Ciocalteu’s phenol reagent, sodium carbonate, sodium nitrite, aluminium chloride, sodium hydroxide, silica gel GF\(_{254}\), silica gel, ethanol, petroleum ether, methanol,
chloroform, and ethyl acetate were purchased from Merck (Darmstadt, Germany), and bidestilled water (Ika Pharmindo).

**Preparation and fractionation of ethyl acetate extract of mengkudu fruit**

Ethyl acetate extract was prepared according to Abdul and Sugeng (2005). Ethyl acetate extract obtained was applied onto silica gel column (75 x 3.5 cm i.d.; silica gel 60, 70 – 230 mesh, Merck, Darmstadt, Germany). The column was then eluted with a mixture petroleum ether, chloroform, and methanol. Eluents of each 100 mL were collected and subjected to thin layer chromatography (TLC; aluminum sheet, silica gel GF254 Merck, Darmstadt, Germany).

After development in the solvent mixture of petroleum ether, chloroform, and methanol, the spots were visualized by ultraviolet (UV) light. Eluents with the similar TLC profile are collected and designated as fraction. Fifteen fractions obtained and ethyl acetate extract before fractionating were determined antioxidant activities as well as their total phenolic and total flavonoid contents.

**Determination of antioxidant activity by radical scavenging assay**

Antioxidant activity was determined by radical scavenging activity using DPPH radical according to Zou et al., (2004). Briefly, 50 µL of testing antioxidant solution with five different concentrations was added to 1,0 mL of DPPH 0,4 mM in methanol. The solution was diluted to the volume (5.0 mL) with ethanol, shaken vigorously, and kept for 30 min at room temperature in the dark. The absorbance at 517 nm was measured by spectrophotometer Genesys-20 against a blank of ethanol. A control containing ethanol instead of DPPH solution was also made. The experiment was done in triplicate.

The antioxidant activity of the samples was calculated according to the formula:

Percentage (% of antioxidant activity =
\[
\frac{\text{Abs.of control} - \text{Abs.of sample}}{\text{Abs.of control}} \times 100\% 
\]

The percentage of antioxidant activity was plotted against the sample concentration (µg/mL) to obtain IC\textsubscript{50}, defined as the concentration of the sample necessary to cause 50% scavenging of DPPH radical calculated by linier regression curve.

**Determination of total phenolic content**

Total phenolic contents of Ethyl acetate extract and its fractions were determined by using a modified colorimetric method according to Chun et al., (2003). A 200 µL portion of appropriately diluted extracts was added to 10-mL volumetric flask filled with 3 mL of bidestilled water (ddH\textsubscript{2}O). A reagent blank using ddH\textsubscript{2}O instead of sample was prepared. A 400 µL of Folin-Ciocalteu’s phenol reagent was added to the mixture and mixed. After 5 min, a 4 mL of 7 % Na\textsubscript{2}CO\textsubscript{3} solution was added with mixing. The solution was diluted to the volume (10 mL) with ddH\textsubscript{2}O, then, allowed to stand for 90 min, and the absorbance was measured at 750 nm versus the prepared blank. Total phenolic contents of ethyl acetate extract and its fractions were expressed as gram gallic acid equivalent/100 gram dry material. The sample was analyzed in triplicate.

**Results and Discussion**

Antioxidant activity measured by radical scavenging using DPPH (2,2-diphenyl-1-picrylhydrazyl) radical

Free radical scavenging is generally the accepted mechanism for antioxidants inhibiting lipid oxidation. The model of scavenging stable DPPH radical can be used to evaluate the antioxidant activities in a relatively short time, as compared to other methods, and it has been used extensively to predict the antioxidant activities of various chemicals (Kuo et al., 2002). The antioxidant activities of ethyl acetate extract and its fractions determined by radical scavenging activity using DPPH radical was shown in Figure 1.

Antioxidant activities were expressed by IC\textsubscript{50} defined as the concentration of the sample
necessary to cause 50% scavenging of DPPH radical calculated by linear regression curve. The smaller of IC$_{50}$ value, the more effective as antioxidant.

There are seven fractions having antioxidant activity that higher than that of ethyl acetate extract before fractionating. These are in the order: fraction 8 (IC$_{50}$ 5.49 µg/mL) > fraction 7 (IC$_{50}$ 7.90 µg/mL) > fraction 9 (IC$_{50}$ 11.63 µg/mL) > fraction 6 (IC$_{50}$ 16.44 µg/mL) > fraction 15 (IC$_{50}$ 17.19 µg/mL) > fraction 14 (IC$_{50}$ 19.73 µg/mL) > fraction 13 (IC$_{50}$ 31.13 µg/mL). Furthermore, there are two fractions with antioxidant activity that higher than that of vitamin E i.e. fraction 8 and fraction 7. Vitamin E known as antioxidant has IC$_{50}$ value 8.27 µg/mL. Fraction 1, 2, 3, and 4 have no antioxidant activities.
Determination of total phenolics content

Total phenolics of ethyl acetate extract and its fractions were expressed as gram gallic acid equivalent/100 gram dry material. The amount of total phenolic contents varied in ethyl acetate extract and its fractions and ranged from 5.94 ± 0.08 to 36.52 ± 0.35 gram gallic acid equivalent/100 gram dry material. The highest total phenolic contents were detected in “fraction 7”, “fraction 8”, and “fraction 6”, and the lowest in “fraction 12” and “fraction 13” (Figure 2).

The typical phenolic compounds that posses antioxidant activity have been characterized as phenolic acids and flavonoids (Kahkonen et. al., 1999). Phenolic acids have repeatedly been implicated as natural antioxidant in fruits, vegetables, and other plants. For example, caffeic acid, ferulic acid, and vanillic acid are widely distributed in the plant kingdom (Javanmardi et. al., 2003).

The correlation between IC₅₀ (Y) and total phenolic contents (X) of ethyl acetate extract and its fractions had a correlation coefficient of r² = 0.67, (Y = -1.220x + 44.022). This result suggests that 67% of the antioxidant activity of ethyl acetate extract and its fractions from the contribution of phenolic compounds. Also, it can be concluded that antioxidant activity of ethyl acetate extract and its fractions is not limited to phenolics. Activity may also come from the presence of other antioxidant secondary metabolites such as volatile oils, carotenoids, and vitamins, among others, that in this case contributed to 33% of the antioxidant activity (Javanmardi et. al., 2003).
The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers. They may also have a metal chelating potential (Kahlkonen, 1999).

**Determination of total flavonoid content**

Total flavonoid of ethyl acetate extract and its fractions were expressed as gram quercetin equivalent/100 gram dry material. The amount of total flavonoid contents varied and ranged from 1.19 ± 0.02 to 17.65 ± 0.17 gram quercetin equivalent/100 gram dry material. The highest total flavonoid contents were detected in “fraction 8”, and the lowest in ethyl acetate extract before fractionating (Figure 4).

The correlation between IC\textsubscript{50} (Y) and total flavonoid contents (X) of ethyl acetate extract and its fractions had a correlation coefficient of \( r^2 = 0.4278 \). This result suggests that 42.78% of the antioxidant activity of ethyl acetate extract and its fractions from the contribution of flavonoid compounds. Also, it can be concluded that antioxidant activity of ethyl acetate extract and its fractions is not limited to flavonoids.

**Conclusions**

Fractionation of ethyl acetate extract yielded 15 fractions. Among 15 fractions, fraction 8 (IC\textsubscript{50} 5.49 µg/mL) and fraction 7 (IC\textsubscript{50} 7.90 µg/mL) showed antioxidant activities that higher than that of vitamin E (IC\textsubscript{50} 8.27 µg/mL). The total phenolic contents ranged from 5.94 ± 0.08 to 36.52 ± 0.35 g of gallic acid equivalent/100 gram dry material, whereas the total flavonoid contents ranged from 1.19 ± 0.02 to 17.65 ± 0.17 g of quercetin equivalent/100 gram dry material. There was a linear positive relationship between the antioxidant activities and total phenolic contents of the tested ethyl acetate extract and its fractions \( y = -1.220x + 44.022 \) (\( r^2 = 0.67 \)) while the correlation between antioxidant activities and total flavonoid contents revealed a linear regression \( y = -2.202x + 35.82 \); \( r^2 = 0.4278 \).

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**References**


