The bioactive compounds and antioxidant activity of ethanol and ethyl acetate extracts of Candi Banana (*Musa paradisiaca*)

R A Laeliocattleya, T Estiasih, G Griselda and J Muchlisyiah

Department of Agricultural Product Technology, Faculty of Agricultural Technology, Universitas Brawijaya

Email: deeochalina@gmail.com

**Abstract.** Banana has various benefits for health. One local variety of banana is *candi* banana (*Musa paradisiaca*). The aim of this research was to study the content of the bioactive compounds of phenolics, flavonoids, tannin, carotenoids and the antioxidant activity of extract ethanol and ethyl acetate of *candi* banana. Powdered *candi* banana was extracted using ethanol and ethyl acetate in an ultrasonic bath. The results showed that the content of phenolics, flavonoids, tannin and carotenoids in ethanol extract were 58.76 ± 3.19 mg/kg, 416.08 ± 18.79 mg/kg, 209.83 ± 15.87 mg/kg and 74.55 ± 4.31 mg/kg, respectively. The content of phenolics, flavonoids, tannin and carotenoids in ethyl acetate extract were 0.83 ± 0.12 mg/kg, 4.31 ± 0.66 mg/kg, 49.97 ± 2.43 mg/kg and 304.40 ± 16.62 mg/kg. While the antioxidant activity (IC$_{50}$) of ethanol extract and ethyl acetate were 3374.13 ± 123.46 ppm and 40318.19 ± 1014.90 ppm. This research showed that type of solvents of ethanol and ethyl acetate affected the content of bioactive compounds and antioxidant activity of *candi* banana. The antioxidant activity of ethanol extract was higher than that of ethyl acetate extract. It showed that ethanol was a better solvent than ethyl acetate to extract bioactive compounds in *candi* banana.

1. Introduction

Oxidative stress is among the major causative factors in induction of many chronic and degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immunosuppression, neurodegenerative diseases and others [1]. An antioxidant can be broadly defined as any substance that delays or inhibits oxidative stress [2]. The main characteristic of an antioxidant is its ability to trap free radicals. Antioxidant compounds like polyphenols and flavonoids scavenge free radicals and inhibit the oxidative mechanisms that lead to degenerative diseases [3]. Plants that consist of bioactive compounds considered as good antioxidant since ancient times. Banana has a great source of minerals, vitamins, carbohydrates, and bioactive compounds [4]. Indonesia as one of great producer of banana has many type varieties of banana. One of varieties is called *candi* banana (*Musa paradisiaca*). This variety of banana is rich in bioactive compounds, such as phenol, flavonoid, tannin and carotenoids but not many research explore the content of the bioactive compounds. Furthermore, this type of banana probably have specific antioxidant activity which is beneficial for health as a radical scavenger and it can help to prevent the degenerative diseases.
2. Materials and Methods

2.1. Materials and tools
Tools used in this research were rotary vacuum evaporator, UV-Vis spectrophotometer, automatic cabinet dryer, and column of chromatography. Materials used were ripe candi banana, aquadest, ethyl acetate, ethanol (96%), pro-analysis ethanol, methanol, gallic acid standard, tannic acid standard, quercetin, β-carotene, Folin Ciocalteu reagent (10%), Na₂CO₃ (2%), Na₂CO₃ (20%), NaNO₃ (5%), AlCl₃ (10%), NaOH 1 M, cotton, alumina oxide, Na₂SO₄, petroleum ether : acetone solution (10:1), petroleum ether : acetone solution (1:1), and DPPH 0.2 mM in methanol.

2.2. Methods

2.2.1. Sample preparation
The candi banana was peeled, washed and cut into 3 mm of thickness. The cuts were dried in an automatic cabinet dryer for 12 hours at 45°C. The dried sample was ground using dry blender for 2 minutes and sieved using 80 mesh of siever. The candi banana powder is ready for the next process.

2.2.2. Sample extraction
The candi banana powder of 10 g was added with 200 ml of 96% ethanol or ethyl acetate and put in an ultrasonic bath for 30 minutes at 30°C. After the extraction process, the mixture was filtered using fine filter paper. Filtrate was evaporated using rotary vacuum evaporator at 50 rpm and 40°C.

2.2.3. Total phenolic compounds (TPC) determination
TPC of candi banana was determined by Folin-Ciocalteu reagent as described [5] with modification. The absorbance of the mixture was measured using UV-Vis spectrophotometer at 759 nm. TPC is defined as mg of gallic acid equivalent per kg of sample (mg GAE/kg). The formula of TPC calculation is:

\[
TPC = \frac{C \times V \times FP}{W}
\]

Where: C = phenol concentration (mg/L), V = extract volume (L), FP = dilution factor, W = sample weight (kg)

2.2.4. Total flavonoid compounds (TFC) determination
TFC was determined based on [6] with modification. The absorbance of the mixture was measured using spectrophotometer at 510 nm. TFC is defined as mg quercetin equivalent per kg of sample (mg QE/kg). The formula for this calculation is as follows:

\[
TFC = \frac{C \times V \times FP}{W}
\]

Where: C = Flavonoid concentration (mg/L), V = extract volume (L), FP = dilution factor, W = sample weight (kg)

2.2.5. Total tannin compounds (TTC) determination
TTC was determined based on [7] with modification. The absorbance of the mixture was measured using UV-vis spectrophotometer at 765 nm. TTC is defined as mg tannic acid equivalent per kg of sample (mg TAE/kg). The formula used to measure this parameter is:

\[
TTC = \frac{C \times V \times FP}{W}
\]

Where: C = tannin concentration (mg/L), V = extract volume (L), FP = dilution factor, W = sample weight (kg)
2.2.6. Total carotenoids (TC) determination

TC was determined by column chromatography method as described by [8] with modification. Firstly, sample of 1 g was added with 8 mL of petroleum ether : acetone (1:1). The flask was covered with alumina foil and shaken for 10 minutes and then filtered using filter paper. The filtrate obtained was stored at room temperature. The residue was added with 8 mL of petroleum ether:acetone (1:1), covered with alumina foil and shaken for 10 minutes. This treatment was repeated twice. Then, the filtrate was diluted with petroleum ether:acetone (1:1) until total volume of 25 mL. After that, the filtrate was transferred to separator funnel and added with 10 mL of aquadest. The separator funnel was shaken and incubated until the mixture was separated into 2 phases. The lower part (water:acetone phase) was discarded and the upper part (ether phase) was transferred to a tube. Then, 0.5 g of Na$_2$SO$_4$ was added for each of 10 mL of ether phase’s volume. Then, the mixture was loaded into the column of chromatography. Petroleum ether:acetone (10:1) solvent was added to the mixture in the column until the mixture turned clear. Then the absorbance of the eluate was measured using UV-Vis Spectrophotometer at 450 nm. Total carotenoid is defined as weight of β-carotene (mg) equivalent per kg of sample. The formula for calculation is:

$$TC = \frac{C \times V \times FP}{W}$$  \hspace{1cm} (4)

Where: $C = \beta$-carotene concentration (mg/L), $V = \text{mixture volume (L)}$, $FP = \text{dilution factor}$, $W = \text{sample weight (kg)}$

2.2.7. IC$_{50}$ value of antioxidant activity determination

Antioxidant activity was determined according to [6] with modifications. Extract of candi banana was made into 5 different concentration (200, 400, 600, 800 and 1000 ppm). Extract of 3 mL was added with 3 mL of 0.2 mM DPPH. Then, the mixtures were vortexed and incubated for 30 minutes in dark room. After that, the absorbance of the mixture was measured using UV-Vis Spectrophotometer at 514 nm. Radical inhibition was calculated in the following formula:

$$\% \text{ DPPH Radical Inhibition} = \left( \frac{\text{Blank absorbance} - \text{Sample absorbance}}{\text{Blank absorbance}} \right) \times 100\%$$  \hspace{1cm} (5)

IC$_{50}$ value was extrapolated from the curve of Y-axis as inhibition percentage value and X-axis as concentration.

3. Results and Discussion

3.1. TPC

Table 1 shows that TPC of ethanol extract of candi banana was higher than that of ethyl acetate. Chemical characteristic and polarity of compounds affect the solubility of the compounds in a particular solvent. An appropriate solvent polarity results a better extraction yield [9]. Furthermore, it can be observed that phenolic compounds tend to be soluble in polar solvent (ethanol).

| Extract                | TPC (mg/kg)      |
|------------------------|------------------|
| Ethanol Extract        | 58.76 ± 3.19$^a$|
| Ethyl Acetate Extract  | 0.83 ± 0.12$^b$  |
3.2. **TFC**

Table 2 shows that ethanol extract of *candi* banana has higher TPC than that of ethyl acetate extract. Flavonoid is polyphenolic compound that is ubiquitous in nature, comprising a number of hydroxyl groups attached to aromatic ring structures that determine its antioxidative properties [10]. Flavonoid has similar characteristic with other polyphenols which is diluted in more polar solvent such as ethanol.

| Extract               | TFC (mg/kg)     |
|-----------------------|-----------------|
| Ethanol Extract       | 416.08 ± 18.79a |
| Ethyl Acetate Extract | 4.31 ± 0.66b    |

3.3. **TTC**

Tannin are plant polyphenols that can either bind or precipitate proteins. Table 3 shows that TTC of ethanol extract is higher than that of ethyl acetate.

| Extract               | TTC (mg/kg) |
|-----------------------|-------------|
| Ethanol Extract       | 209.83 ± 15.87a |
| Ethyl Acetate Extract | 49.97 ± 2.43b |

3.4. **TC**

Table 4 shows the TC in extract of *candi* banana is higher than that ethyl acetate extract. Carotenoids are lipid-soluble of C40 tetraterpenoids. The majority carotenoids are derived from a 40-carbon polyene chain, which could be considered the backbone of the molecule. These chains are either terminated by cyclic end-groups or complemented with oxygen-containing functional groups [11]. Because carotenoid has diene-conjugated group which is a hydrocarbon, so it was more dilute in nonpolar solvent of ethyl acetate than in polar solvent of ethanol [12].

| Extract               | TC (mg/kg)     |
|-----------------------|----------------|
| Ethanol Extract       | 74.55 ± 4.31a  |
| Ethyl Acetate Extract | 304.40 ± 16.62b |

3.5. **Antioxidant activity (IC<sub>50</sub>)**

Table 5 shows IC<sub>50</sub> value of ethanol and ethyl acetate extract of *candi* banana were 3374.13 ppm and 40318.19 ppm. Therefore, extract obtained using ethanol had higher antioxidant activity than that of ethyl acetate extract. Extract ethanol contains higher of phenolic, flavonoid and tannin than that of ethyl acetate extract. Phenolic compounds have been found as strong antioxidants in delaying the influence of free radicals and ROS, which are the main causes of chronic human diseases [13]. Phenolic compounds can also inactivate free radicals from lipid deterioration or preventing decomposition of hydroperoxides into free radicals [14].
4. Conclusion

This research showed that type of solvents of ethanol and ethyl acetate affects the amount of extracted bioactive compounds and antioxidant activity of *candi* banana. The antioxidant activity of ethanol extract is higher than that of ethyl acetate extract. It showed that ethanol was a better solvent than ethyl acetate to extract bioactive compounds in *candi* banana.

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**Table 5. IC$_{50}$ of Candi Banana extract obtained using ethanol and ethyl acetate**

| Extract                  | IC$_{50}$ (ppm)     |
|--------------------------|--------------------|
| Ethanol Extract          | 3374.13 ± 123.46   |
| Ethyl Acetate Extract    | 40318.19 ± 1014.90 |

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4. Conclusion

This research showed that type of solvents of ethanol and ethyl acetate affects the amount of extracted bioactive compounds and antioxidant activity of *candi* banana. The antioxidant activity of ethanol extract is higher than that of ethyl acetate extract. It showed that ethanol was a better solvent than ethyl acetate to extract bioactive compounds in *candi* banana.