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Antioxidant Activity and Tryptophan Content of Banana Peel

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Abstract

Agung Banana (*Musa paradisiaca* L. var Semeru) is a superior banana variety from Lumajang, East Java. This study aims to determine the total phenolic content, radical scavenging activity, tryptophan content of Agung banana peel powder, and banana peel ethanol extract (BPE). BPE's free radical scavenging activity was tested using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays. Total phenolic content was tested using the Folin-Ciocalteu reagent. The tryptophan content was analyzed using HPLC. The concentration of the sample that could scavenge 50% of DPPH free radicals (IC_{50}) of BPE was 684.69 ± 120.73 ppm, and the antioxidant activity index was 0.37 ± 0.07. The total phenolic content of BPE was 17.41 ± 0.14 gallic acid equivalents (GAE) mg/g. There was no significant correlation between total phenolic content (TPC) and IC_{50} value ($r = -0.269$, $p > 0.05$). The tryptophan content of BPE was 0.02 % w/w. DPPH assays of BPE showed that their antioxidant activity was weak. The phenolic content of the BPE did not significantly contribute to their antioxidant activities. The tryptophan content of BPE was not as high as expected.

Keywords: Antioxidant activity; Banana peel; Banana extract; Tryptophan;

1. Introduction

Banana contains polyamine and biogenic amines like serotonin, norepinephrine, or dopamine; phenolic substances with free radical scavenging activity; and anti-inflammation activity from the radical scavenging activity (Waalkes *et al.,* 1958; Bellik *et al.,* 2013; Pereira and Maraschin, 2015; Vu *et al.,* 2018; Lopes *et al.,* 2020). Serotonin is a potent antioxidant that can capture reactive oxygen species (ROS) and exhibit potent antioxidant activity (Gonçalves *et al.,* 2021). In addition, bananas are rich in phenolic compounds. The total phenolic content of banana peels is 4.95 - 47 mg GAE/g dry matter (1.5 - 3 times higher than banana pulp) (Sulaiman *et al.,* 2011). Banana peel has a radical scavenging activity and a much higher reducing capacity than other fruit peels. Several previous studies showed a strong relationship between the content of phenolic compounds and oxygen radical absorbance capacity, free radical scavenging ability, and iron reduction ability. More than 40 types of phenolic compounds are identified in bananas, and all of them can be classified into four subgroups: hydroxycinnamic acids, flavonols, flavan-3-ols, and catecholamines. Banana peel extracts (BPE) from various solvents show solid antioxidants and can be used as supplements (Vu *et al.,* 2018).

Furthermore, banana is rich in tryptophan (Islam *et al.,* 2016). Tryptophan is one of the essential amino acids which is a precursor to serotonin and can provide

antidepressant and antianxiety effects. The results of a systematic review show that tryptophan affects negative feelings and joy in healthy individuals, so tryptophan can be an effective therapy to reduce anxiety and increase positive feelings in healthy individuals. The results of 11 randomized controlled trials show that administering 0.1403 grams of tryptophan per day can increase the personal feeling of being healthy (Kikuchi *et al.,* 2021).

After the Covid-19 pandemic, the prevalence of depression increased (Rogers *et al.,* 2020). Covid-19 pathophysiology involves inflammation related to depression (Rogers *et al.,* 2020; Benedetti *et al.,* 2021). Repeated and prolonged physical and psychological stress can stimulate the hypothalamus-pituitary-adrenal (HPA) axis, which also results in high reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Steardo *et al.,* 2020). Preclinical and clinical study regarding oxidative stress and antioxidant effect has revealed that antioxidant can omit ROS and RNS through radical scavenging and suppression of oxidative stress pathways, thereby protecting against oxidative stress-induced nerve damage and leading to remission and functional restoration of depressive or anxiety symptoms (Xu *et al.,*, 2014). A decrease in the number of antioxidants and an increase in ROS and RNS will cause damage to membrane lipids and functional proteins, causing an autoimmune response to neoepitopes, which in turn causes depression (Köhler *et al.,* 2017).

Free radicals are molecules with unpaired electrons that readily participate as electron donors or acceptors to become stable. Free radicals are a natural product of cellular metabolism and are increased if there is a pathological process (i.e., inflammation). Free radicals can change the chemical composition of lipids, proteins, carbohydrates, and DNA. Enzymatic compounds and processes protect the body, but the most important is the consumption of antioxidants in food. An antioxidant is a molecule that is stable enough to donate an electron to a free radical (it will be oxidized), neutralize the free radical, and decrease its capacity to cause cellular damage. Many antioxidant compounds in food are polyphenolic compounds from plants, and many plants are rich in phenolic compounds. The antioxidant potential was tested by various in vitro techniques, and the most commonly used test to evaluate the antioxidant activity of herbal extracts was the 2,2-diphenyl-1-picrylhydrazyl (DPPH) test (Mendelson, 2019).

Agung Banana (Musa paradisiaca L. var Semeru) has unique properties. The size of the fruit is large (19 cm circumference and 40 cm length), with a weight of 10-30 kg/bunch. In addition, it has a thick peel (± 0.5 cm), allowing a more extended storage period (Hadisoewignyo *et al.,* 2017; Rakhmawati and Lestari, 2021). This banana is generally processed as banana chips. Banana production in Lumajang Regency reaches 47.40% of total fruit production. The high production of bananas produces waste in the form of banana peels. Banana peel weight reaches 38.8% of the fruit and causes storage costs to be incurred at a low selling price (Nurhayati *et al.,* 2021). Banana peel waste is used only as goat fodder, so other utilization efforts are needed, for example, for depression therapy.

This study explores the potential of banana peels by determining the total phenolic content, radical scavenging activity, phytochemicals content of banana peel powder, and banana peel ethanol extract.

2. Materials and Methods

2.1 Materials

The raw material used is Agung Banana peel from Lumajang, East Java, Indonesia. The banana peel was processed into powder, and some were extracted. The chemicals used for testing are analytical grade including DPPH (Sigma-Aldrich), Folin-Ciocalteau (Merck), gallic acid (Merck), ascorbic acid (Merck), methanol (Merck), and distilled water. The equipment used includes a spectrophotometer (Hitachi U-1800), HPLC column (Thermo Scientific ODS 2-hyersil), analytical balance (Ohaus), Oven (Memmert), and micropipette (Socorex), TLC chamber (Camag), TLC silica gel 60 F254 (Merck).

2.2 Banana Peel Powder Preparation

Banana peels were rinsed using tap water, distilled water, chopped, ground, and then dried using an oven at 70 ºC. Once dried, it was ground into a fine powder and sieved with a 40-mesh sieve. It was stored as dry powder prior to extraction.

2.3 Banana Peel Powder Extraction

Banana peel powder was extracted using the maceration method (Farooq *et al.,* 2022) with ethanol 50% as solvent (related to the solubility of tryptophan in alcohol and water) at room temperature. One thousand grams of banana peel powder were soaked for 24 hours in 2.5 liters of 50% ethanol and stirred occasionally. The suspension was filtered using filtered paper, and the residue was added with 1 liter of 50% ethanol. Maceration was repeated seven times with the same procedure. The extracted sample was then concentrated in a water bath until all solvents were evaporated.

2.4 Qualitative Analysis of Phytochemicals Analysis

The test was performed on BPE based on the previous method with the primary objective of detecting the presence of tryptophan (Raaman, 2006; Velumani, 2016).

2.5 Thin Layer Chromatography of BPE

BPE and tryptophan were weighed 10 mg each and suspended in 4 mL of 50% ethanol. The 2 µL of each suspension was spotted on a TLC plate (silica gel 60 F254). The mobile phase was ethanol: acetic acid: water 2:1:1. Once it was dried, it was eluted with the mobile phase until it reached near the silica plate's tip. The visualization reagent used to identify amino acids is 1% ninhydrin. The Rf number for each spot was then calculated.

2.6 Tryptophan Content Assays using HPLC

First, the protein content in the sample was determined using the Kjeldahl method. The Agung banana peel powder and BPE containing 6 mg of protein and HCl 6 N were added to a screw tube, and hydrolysis was performed in an oven (110 ºC, 24 hours). The sample was evaporated, and 10 mL HCl 0.01 N was added and filtered. Potassium borate buffer was added (1:1), 5 µL of the sample was taken, and 5 µL of o-phthalaldehyde Reagent was added before the sample was injected into the HPLC. The mobile phases were buffer A and B with gradient concentration and mobile phase rate of 1 mL/minute. Buffer A consists of 2 grams of Na Acetate pH 6.6, 0.5 g of Na-EDTA, 90 mL of methanol, and 15 mL of tetrahydrofuran dissolved in 1 liter of water. Meanwhile, buffer B consists of 95% methanol and water.

2.7 Rutin and Quercetin Content Assays using HPLC

A 1 gram of the sample was weighed, 10 mL of methanol was added, sonicated for 20 minutes, and filtered into a 50 mL flask. After collecting the filtrate, it was calibrated with methanol and filtered through a 0.45 µm Whatman filter paper. The solution was then injected with 20 µL at a wavelength of 355 mm.

2.8 DPPH Radical Scavenging Activities

The DPPH radical scavenging test was performed as described by Blois (1958) with modification. The BPE was made with a concentration of 2.500 ppm, prepared in six different concentrations. Ascorbic acid as control was also prepared with a concentration of 50 ppm, prepared in six different concentrations. For each sample, 0.75 mL of DPPH and 50% ethanol were added to 5 mL. The sample was incubated in the dark for 30 minutes. Absorbance was measured at a maximum wavelength of 515 nm. The percentage of DPPH radical scavenging power was calculated using equation 1, then the IC_{50} value was calculated.

$$\%\ inhibition = \frac{A_0 - A_s}{A_0}\ x\ 100\% \quad \ldots\ldots\ldots\ldots\ldots\ldots\ (1)$$

A_0 = absorbance of methanol + DPPH;
A_s = absorbance of sample

The antioxidant activity index was calculated by dividing the DPPH concentration by IC_{50}. The interpretation is as follows: poor activity < 0.05 < moderate < 1.0 < strong < 2.0 < very strong (Scherer and Godoy, 2009).

2.9 Total Phenolic Content Determination using Folin-Ciocalteau Reagent

The total phenolic content determination was performed as described by Widodo *et al.* (2019) with modification. First, a gallic acid calibration curve was made. Folin-Ciocalteau reagent (1:10) and 7.5% Na_2CO_3 solution were prepared, then a gallic acid calibration curve was made. The 50 mg gallic acid was weighed and then dissolved in methanol to obtain a final volume of 50 mL. A series of dilutions were made. From each concentration, 1 mL of solution was pipetted into the vial and 5 mL of Folin-Ciocalteau, then 4 mL of Na_2CO_3 was added. The solution was incubated for 1 hour at room temperature in dark conditions. The absorbance was measured at a wavelength of 740 nm, and a calibration curve was made. Second, the total phenol content of the Agung banana peel powder and BPE was determined. The 25 mg banana peel powder and BPE were weighed (3 replications), and 10 mL methanol was added. A 1 mL of solution was pipetted into the vial, and 5 mL of Folin-Ciocalteau reagent, then 4 mL of Na_2CO_3 was added. The solution was incubated for 1 hour at room temperature in dark conditions. The absorbance was measured at a wavelength of 740 nm.

2.10 Statistical analysis

Statistical analysis was performed using SPSS ver. 26 for Windows. A normality test of the data was performed using the Shapiro-Wilk test, then a comparison test using one-way ANOVA was performed when the data distribution was normal; otherwise, the Kruskal-Wallis test was performed. A correlation between total phenolic content value dan IC_{50} was performed using Pearson's correlation test when the data distribution was normal. Otherwise, Spearman's correlation test was performed.

3. Results and Discussion

3.1 Qualitative Analysis of Phytochemicals

The qualitative analysis of phytochemicals in BPE obtained positive results for the Hopkins-Cole test (for detecting aldehyde/ tryptophan), xanthoproteic acid (for detecting aromatic amino acid), and ninhydrin test (for detecting tryptophan) (Table 1).

3.2 Thin Layer Chromatography of BPE

The qualitative analysis confirmed the tryptophan's content in BPE, so a thin layer chromatography was performed. The chromatogram from BPE and tryptophan gave a similar pattern under the 254 nm and 366 nm UV light (Figure 1). The BPE spot showed a faint purplish stain compared with tryptophan, meaning the tryptophan content was not high. However, the R_f number was the same for both BPE and tryptophan spots, i.e., 0.86.

3.3 Tryptophan Content Assays Using HPLC

The tryptophan was also analyzed using HPLC. The tryptophan content in banana peel powder was higher than BPE (Table 2). In this study, the banana peel powder only obtained 0.10% (1 mg/g; 100 mg%) tryptophan meanwhile, and the BPE only contained 0.02% (0.2 mg/g; 20 mg%) tryptophan.

3.4 Rutin and Quercetin Content Assays using HPLC

Apart from tryptophan, rutin and quercetin were also thought to have antidepressant effects, so the levels of rutin and quercetin in BPE were also examined. As a result, neither rutin nor quercetin was detected (Table 3).

3.5 DPPH Radical Scavenging Activities

BPE had a weak antioxidant activity index (Table 4). The result of this study shows that ascorbic acid had 180 times more antioxidant potential than BPE. Ascorbic acid had a robust antioxidant activity index (> 2.0), and BPE had a weak antioxidant activity index (<0.05).

3.6 Total Phenolic Content Determination using Folin-Ciocalteau Reagent

A gallic acid calibration curve (y = 0.0104x + 0.0025) was used to calculate the banana peel's total phenolic content (TPC). TPC was expressed in gallic acid equivalent (GAE), which showed a higher result in BPE (Table 5). Total phenolic content was negatively correlated with the IC_{50} value of BPE ($r = -0.269$, $p > 0.05$). There was no significant correlation between TPC and IC_{50} value.

Table 1. Qualitative analysis of phytochemicals in banana peel ethanol extracts

Phytochemicals	**Reagent**	**Result**
Alkaloid	Dragendorff/Mayer/Wagner	-/-/-
Flavonoid	$AlCl_3$	+
	Amyl alcohol+chlorhydrate alcohol+Mg powder	+
Polyphenol	$FeCl_3$	+
Steroid and Terpenoid	Glacial acetic acid + conc. sulphuric acid	+
Saponin	Tube shuffling + HCl 2N	-
Tanin	Gelatine + NaCl 1%	-
Hopkins-Cole Test	Glacial acetic acid + conc. sulphuric acid	+
Xanthoproteic acid	HNO_3 + NaOH	+
Ninhydrin	Ninhydrin	+

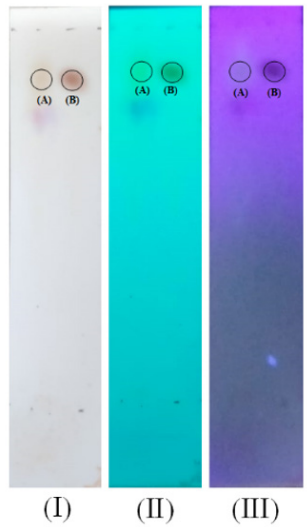

Figure 1. TLC chromatogram of banana peel extract (A) and tryptophan (B) under visible light (I), 254 nm UV light (II), and 366 nm UV light (II)

Table 2. Tryptophan content in the banana peel

Sample	Tryptophan (% w/w)
Banana Peel Powder	0.10
Banana Peel Ethanol Extract (BPE)	0.02

Table 3. Rutin and quercetin content in the banana peel extract

Phytochemical Content	(% w/w)
Rutin	Not detected
Quercetin	Not detected

Table 4. Antioxidant activity of banana peel by DPPH assay

Sample	IC_{50} (Mean ± SD; ppm)	Antioxidant Activity Index
Ascorbic Acid	3.62 ± 0.10^{a}	68.60 ± 1.96
Banana Peel Ethanol Extract (BPE)	684.69 ± 120.73^{b}	0.37 ± 0.07

*Different letters in the same column showed significant differences ($p < 0.05$)

Table 5. TPC in banana peel using Folin-Ciocalteu reagent

Sample	TPC (GAE mg/g)
Banana Peel Powder	3.42 ± 0.94^{a}
Banana Peel Ethanol Extract (BPE)	17.41 ± 0.14^{b}

*Different letters in the same column showed significant differences ($p < 0.05$)

The tryptophan content in the banana peel used in this experiment was low. A previous study on tryptophan content in banana (*Musa paradisiaca* L.) peel after 24 hours of hydrolysis obtained 538 mg/g protein of tryptophan (Muttaqin, 2018). However, another study reported a comparable amount of tryptophan, namely 50 mg% tryptophan in water and ethanol extract and 33.3 mg % in chloroform extract (Velumani, 2016). Tryptophan is slightly soluble in ethanol and water. In previous studies, it was known that the highest solubility of L-tryptophan was found in an ethanol mole fraction of 0.371 (equal to 67% ethanol) (Bowden *et al.,* 2018).

The different maturation stages of the banana can be a reason the tryptophan content was low. The banana peel used in this study was in stage 1 (green) according to Von Loesecke banana maturity scale (Von Loesecke, 1950). A previous study on tryptophan content in different varieties of bananas reported a higher tryptophan content in stage 5 (more yellow than green) and stage 7 (yellow/a few brown spots) of the maturation stage (Emaga *et al.,* 2007).

It is presumed that the tryptophan was also degraded before the analysis was performed. Due to its great susceptibility to oxidation, tryptophan is known to break down into several compounds during production, storage, and processing. This molecule degrades by various physical and chemical mechanisms, chiefly through oxidation or cleavage of the very reactive indole ring. Reactive oxygen species, including singlet oxygen, hydrogen peroxide, hydroxyl radicals, light and photosensitizers, metals, and heat, are the main causing agents (Bellmaine *et al.,* 2020). The storage factor is not a significant problem if the antioxidant content in banana peels is high. In this case, the banana peel ethanol extract's antioxidant properties were low, as reported in the DPPH assay result.

DPPH could accept an electron or hydrogen radical to become a stable molecule. It appeared as a deep violet color solution. As the electron paired off, the decolorization and absorbance decreased (Blois, 1958). The DPPH test can be interpreted from EC_{50} or IC_{50} value. It defined the concentration that caused the 50% loss of the DPPH activity (Mishra *et al.,* 2012; Irawan *et al.,* 2021). The lowest IC50 represents a higher antioxidant activity.

The Folin-Ciocalteau assay is a reaction based on the electron transfer that measures the reductive capacity of antioxidants to measure the total polyphenol content in the sample (Lamuela-Raventós, 2017). Phenolic compounds are natural antioxidants with a hydroxyl group on the benzene ring. Antioxidant phenolic compounds protect against chronic diseases induced by free radicals (Zeb, 2020). It is also a significant primary antioxidant but not the only contributor to the antioxidant effect. A non-phenolic antioxidant such as vitamin C, E, or beta-carotene also has antioxidant properties (Husain and Kumar, 2012). Besides, the banana peel has been known to

have polyphenols components and antioxidant activity (Chel-Guerrero *et al.,* 2022).

A previous study on a banana peel (*Musa paradisiaca* L.) showed that the total phenolic content was increased as the polarity of solvent increased (17.89 ± 0.16 for methanol extract 80%; 15.21 ± 0.09 for ethanol extract 80%, and 15.44 ± 0.19 mg GAE/g for acetone extract 80%) (Aboul-Enein *et al.,* 2016). However, the lowest IC_{50} value was found in the acetone extract. The IC_{50} values of 80% methanol extract, 80% ethanol extract, and 80% acetone extract were reported as 56.22 ± 1.25, 75.34 ± 4.77, and 55.45 ± 0.86 ppm. The IC_{50} value of BHT as standard was 4.73 ± 0.72 ppm (Aboul-Enein *et al.,* 2016). Another study reported the highest total phenolic content on the most nonpolar solvent, acetone (136.87 ± 5.69 GAE/g). The total phenol of the other aqueous and ethanol extract of *M. paradisiaca* peel was 83.32 ± 4.38 and 133.42 ± 8.18 mg GAE/g, respectively (Oluwatomide and Afolayan, 2020). The IC_{50} values of the *M. paradisiaca* peel extract were 60 ppm for acetone and ethanol extract and 40 ppm for aqueous extract. As comparators, the IC_{50} values of the gallic acid and rutin were 1 and 10 ppm, respectively (Oluwatomide and Afolayan, 2020). A lower IC_{50} values, 4.4 ppm, was reported in the ethanol extract of Musa paradisiaca forma typica peel (Ariani and Nurani, 2021). Another study also reported low phenolic content of M. paradisiaca peel (0,76 ± 0.04 mg GAE/g) (Fakai *et al.,* 2014).

Regarding the low antioxidant activity in the banana peel, a study on the antioxidant activity of fraction made from *M. paradisiaca* peel methanol extract showed that the polar fraction, namely the butanol fraction, had low antioxidant activity (1071.14 ppm) (Atun *et al.,* 2007). The semipolar fraction (ethyl acetate) had lower antioxidant activity (2347.40 ppm). The highest antioxidant activity was obtained from the chloroform fraction (693.15 ppm), compared with IC_{50} of ascorbic acid (83.87 ppm). The isolates obtained from that chloroform fraction were 5,6,7,4'-tetrahydroxy-3, 4-flavan-diol or 5,7,8,4'-tetrahydroxy-3, 4-flavan-diol and 2-cyclohexane-1-on-2,4, 4-trimethyl-3-O-2'-hydroxypropyl ether.

Another study on various banana pulps and peels revealed that the total phenolic content and antioxidant activity were significantly affected by sample preparation and solvent extraction (Sulaiman *et al.,* 2011). Chloroform was the best solvent for extracting antioxidant compounds from dried banana peels and pulp. For fresh banana pulp, methanol was the best solvent, and for fresh banana peels, water or chloroform was preferable (Sulaiman *et al.,* 2011). Another study comparing local bananas known as *Pisang Tanduk* peel extract from n-hexane, ethyl acetate, and ethanol reported that the lowest total phenolic content was from the n-hexane extract, and the highest was from the ethyl acetate extract. However, the IC_{50} value was lowest in the n-hexane extract, and the highest was from the ethanol extract (Fidrianny *et al.,* 2018).

Phenols have one or more hydroxyl groups as a polar part (hydroxyl groups) that are directly attached to a nonpolar part (aromatic ring) (Galanakis *et al.,* 2013). The extraction process's yield depends on the solvent's nature. Certain phenolic terpenes were extracted using nonpolar solvents (hexane, petroleum ether), and flavonoid aglycons and phenolic acid can be extracted using diethyl ether and ethyl acetate. A more polar solvent can extract flavonoid glycosides and higher molecular weight phenols. In addition, heat during banana processing can also have a positive or negative impact. The heat treatment can result in Maillard reaction products that increase antioxidant activity but can also cause depletion of natural antioxidants (Sulaiman *et al.,* 2011). Further exploration by various solvent extraction is needed to explore the antioxidant potency of banana peel.

4. Conclusion

It can be concluded that BPE had a weak antioxidant activity with no correlation with its total phenolic content. In addition, BPE had a low amount of tryptophan. It is doubted that BPE will have a positive effect as an antidepressant. A preclinical study is needed to be performed to prove it.

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