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Antioxidant Activity of Ethanol Extract of Kepok Banana Peel (*Musa paradisiaca* L.) by Ferric Reducing Antioxidant Power Method

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ABSTRACT: Antioxidants are compounds capable of neutralizing the harmful effects of oxidants by donating electrons to unstable molecules, thereby preventing oxidative damage. These compounds play a vital role in protecting biological systems from oxidative stress, which is associated with aging and various chronic diseases. Kepok banana (*Musa paradisiaca* Linn.) peel is an underutilized natural resource rich in secondary metabolites such as saponins, flavonoids, alkaloids, tannins, and quinones, many of which are known for their strong antioxidant potential. The present study aimed to evaluate the antioxidant activity of the ethanol extract of kepok banana peel using the Ferric Reducing Antioxidant Power (FRAP) method. Extraction was performed by maceration with 96% ethanol as the solvent to obtain a concentrated extract. The FRAP assay was carried out by reacting the extract with FRAP reagents and measuring the absorbance using a UV–Vis spectrophotometer at a maximum wavelength of 710 nm. Quercetin was used as a standard antioxidant reference, producing a calibration curve with a linear regression equation of $y = 0.0203x + 0.0301$ and a correlation coefficient (r) of 0.9965, indicating excellent linearity. The results revealed that the ethanol extract of kepok banana peel exhibited high antioxidant capacity, with a value of 81.6939 mgQE/g extract. These findings suggest that kepok banana peel is a promising natural source of antioxidants and could potentially be developed into functional ingredients for pharmaceutical and nutraceutical applications.

KEYWORDS: Antioxidants; extraction; FRAP; spectrophotometry; nutraceutical.

1. INTRODUCTION

In recent years, public awareness of health in Indonesia has continued to increase, particularly regarding the impact of lifestyle and dietary habits on degenerative diseases. Changes in consumption patterns and exposure to free radicals can trigger oxidative stress, leading to cellular and tissue damage, autoimmune disorders, cholesterol deposition, atherosclerosis, and even cancer (Aminah et al., 2016; Arifin et al, 2025). To counteract these effects, antioxidants play a vital role in neutralizing oxidative damage.

Antioxidants are chemical compounds that, in specific concentrations, can slow or prevent oxidative degradation (Rahmi et al., 2021). They act by donating electrons to stabilize free radicals, thereby protecting the body from oxidative stress (Fawwaz, 2023). Bioactive compounds with antioxidant potential are not only found in leaves and fruits but are also abundant in fruit peels—plant parts often discarded as waste (Rahmi et al., 2021).

One such underutilized source is the kepok banana (*Musa paradisiaca* L.), whose peel contains various secondary metabolites including saponins, polyphenols, tannins, flavonoids, and terpenoids—compounds known for their antioxidant and antibacterial properties (Wahyuni et al., 2019). Despite its rich bioactive profile, banana peel remains largely unused, typically disposed of as organic waste or animal feed (Lumowa & Bardin, 2018).

Kepok banana peel has demonstrated several pharmacological properties. Its flavonoid content exhibits hypoglycemic activity by scavenging free radicals and reducing oxidative stress (Hasma & Winda, 2019). Moreover, previous studies have reported additional therapeutic benefits such as anti-inflammatory, wound-healing, anti-pruritic, and antidepressant effects due to its high levels of serotonin, vegetable oils, and fiber that improve digestion and boost immunity (Rifni & Novitasari, 2015).

To quantify antioxidant activity, several analytical techniques have been developed. However, each method detects different aspects of antioxidant mechanisms, resulting in varied interpretations of antioxidant potential. Among these, the Ferric Reducing Antioxidant Power (FRAP) assay has been recognized as a reliable, simple, and cost-effective method. The FRAP method, introduced by Benzie & Strain (1996), measures the reducing ability of antioxidants to convert Fe^{3+} to Fe^{2+} ions. This technique is rapid, reproducible, and exhibits good linearity with the molar concentration of antioxidants (Maryam et al., 2021).

Based on these considerations, this study aims to determine the antioxidant activity of the ethanol extract of kepok banana peel (*Musa paradisiaca* L.) using the Ferric Reducing Antioxidant Power (FRAP) method. The results of this study are expected to provide scientific evidence of the antioxidant potential of kepok banana peel as a natural source of bioactive compounds and to support its utilization in the development of herbal-based antioxidant formulations.

2. EXPERIMENTAL SECTION

2.1. Sample preparation and extraction

The sample used in this study was the peel of the kepok banana (*Musa paradisiaca* L.). Fresh banana peels were collected and thoroughly washed under running water to remove dirt and other impurities. The cleaned peels were then air-dried at room temperature to prevent decay or fungal growth. Once dried, the samples were cut into small pieces to facilitate the extraction process, followed by a dry sorting procedure prior to extraction using the maceration method (Rahmawati et al., 2024).

A total of 300 grams of dried kepok banana peel simplicia was placed into a maceration vessel, and 96% ethanol was added until the entire sample was completely submerged. The mixture was stirred thoroughly, and the vessel was tightly sealed. The maceration process was carried out for three days at room temperature in a dark place, with stirring every 24 hours. Afterward, the mixture was filtered, and the residue was subjected to remaceration using fresh solvent until the filtrate became clear. The combined filtrates were then filtered again using filter paper and a Buchner funnel to obtain a clear liquid extract. Finally, the liquid extract was concentrated using a rotary vacuum evaporator to produce a thick ethanol extract of kepok banana peel (Rahmi et al., 2021).

2.2. Preparation of Quercetin Solution

A quercetin standard solution was prepared by weighing 5 mg of quercetin and dissolving it in 96% ethanol within a 5 mL volumetric flask to obtain a concentration of 1000 µg/mL (1000 ppm). From this stock solution, 1 mL was pipetted and diluted to 10 mL with 96% ethanol to yield a 100 µg/mL (100 ppm) quercetin working solution (Ummum & Abidin, 2024). From the 100 ppm working solution, a concentration series was prepared by pipetting 0.75, 1.00, 1.25, 1.75, and 2.00 mL into separate 5 mL volumetric flasks. Each was diluted to the mark with 96% ethanol, shaken, and homogenized to obtain quercetin solutions with concentrations of 15, 20, 25, 30, and 35 ppm, respectively (Fawwaz, 2022).

2.3. Preparation of Reagents

The blank solution was prepared by pipetting 1 mL of 96% ethanol, adding 1 mL of phosphate buffer pH 6.6, and 1 mL of 1% potassium ferricyanide ($K_3Fe(CN)_6$). The mixture was vortexed for 5 minutes and incubated at 50 °C for 20 minutes. Subsequently, 1 mL of 10% trichloroacetic acid (TCA) was added, and the mixture was centrifuged at 3000 rpm for 10 minutes. From the supernatant, 1 mL was taken, mixed with 1 mL of distilled water and 0.5 mL of 0.1% $FeCl_3$, and incubated at room temperature for 5 minutes (Ummum & Abidin, 2024).

The 0.2 M phosphate buffer solution (pH 6.6) was prepared by mixing 50 mL of 0.2 M potassium dihydrogen phosphate with 16.4 mL of 0.2 N sodium hydroxide, then diluting with CO_2-free distilled water to a total volume of 200 mL. The 0.1% ferric chloride ($FeCl_3$) solution was prepared by dissolving 0.025 g of $FeCl_3$ in distilled water and making up the volume to 25 mL. The 10% TCA solution was prepared by dissolving 2.5 g of trichloroacetic acid in distilled water and diluting to 25 mL. Finally, the 1% potassium ferricyanide ($K_3Fe(CN)_6$) solution was prepared by dissolving 0.25 g of $K_3Fe(CN)_6$ in distilled water and making up to 25 mL (Arifin et al., 2025; Ainunnisa et al., 2025).

2.4. Qualitative Analysis

Phytochemical screening was conducted to identify the presence of secondary metabolites in the ethanol extract of kepok banana peel (*Musa paradisiaca* L.). Flavonoid content was tested by adding a few drops of concentrated HCl and approximately 10 mg of magnesium metal to the sample; the formation of a pink or magenta-red color within 3 minutes indicated the presence of flavonoids (Ummum & Abidin, 2024). Saponin content was examined by adding 5 mL of hot water to the sample, cooling, shaking vigorously, and then adding 2 N HCl; the formation of stable foam persisting for 30 minutes after shaking confirmed the presence of saponins (Hasma & Winda, 2019). Tannin content was tested by adding 5 mL of hot water to the sample, boiling it for 5 minutes, filtering, and then adding 3–4 drops of 1% $FeCl_3$; the appearance of a blackish-blue and/or blackish-green coloration indicated the presence of tannins (Hasma & Winda, 2019).

2.5. Quantitative Analysis of Antioxidant Activity Using the FRAP Method

2.5.1 Determination of Maximum Wavelength (λmax)

The maximum wavelength (λmax) was determined by measuring the absorbance of a 25 ppm quercetin standard solution. From this solution, 1 mL was taken and mixed with 1 mL of phosphate buffer (pH 6.6) and 1 mL of 1% potassium ferricyanide ($K_3Fe(CN)_6$). The mixture was vortexed for 5 minutes, then incubated at 50°C for 20 minutes. Subsequently, 1 mL of 10% trichloroacetic acid (TCA) was added, and the mixture was centrifuged at 3000 rpm for 10 minutes. From the resulting supernatant, 1 mL was taken and mixed with 1 mL of distilled water and 0.5 mL of 0.1% $FeCl_3$, then incubated at room temperature for 5 minutes. The absorbance was measured using a UV-Vis spectrophotometer within the wavelength range of 400–800 nm, and the maximum absorbance was obtained at 710 nm (Ummum & Abidin, 2024; Ainunnisa et al., 2025).

2.5.2 Preparation of the Quercetin Standard Curve

A series of quercetin standard solutions were prepared by diluting a 1000 ppm stock solution to 100 ppm, and further to concentrations of 15, 20, 25, 30, and 35 ppm. Each 1 mL aliquot of the standard solution was mixed with 1 mL of phosphate buffer (pH 6.6) and 1 mL of 1% potassium ferricyanide ($K_3Fe(CN)_6$). The mixture was vortexed for 5 minutes and incubated at 50°C for 20 minutes, followed by the addition of 1 mL of 10% TCA. The solution was centrifuged at 3000 rpm for 10 minutes, after which 1 mL of the supernatant was combined with 1 mL of distilled water and 0.5 mL of 0.1% $FeCl_3$, then incubated for 5 minutes at room temperature. Absorbance was measured at 710 nm to obtain the quercetin calibration curve (Ainunnisa et al., 2025).

2.5.3 Determination of Antioxidant Activity in Kepok Banana Peel Extract

A total of 5 mg of *Musa paradisiaca* L. peel extract was dissolved in 96% ethanol and made up to 5 mL in a volumetric flask to yield a concentration of 1000 ppm. From this, 1.5 mL was diluted to 5 mL with ethanol to obtain a 300 ppm solution. Then, 1 mL of this solution was mixed with 1 mL of 0.02 M phosphate buffer (pH 6.6) and 1 mL of 1% potassium ferricyanide ($K_3Fe(CN)_6$), followed by incubation at 50°C for 20 minutes. After incubation, 1 mL of 10% TCA was added, and the mixture was centrifuged at 3000 rpm for 10 minutes. Next, 1 mL of the upper layer was transferred to a test tube, and 1 mL of distilled water and 0.5 mL of 0.1% $FeCl_3$ were added. The mixture was incubated at room temperature for 5 minutes, and absorbance was measured at 710 nm using a UV-Vis spectrophotometer. All measurements were performed in triplicate under identical conditions (Ainunnisa et al., 2025).

3. RESULTS AND DISCUSSION

Antioxidants are compounds capable of neutralizing the harmful effects of oxidants by donating an electron to unstable oxidizing agents, thereby converting them into stable compounds (Balqis Hira, 2024). In general, antioxidants prevent oxidation reactions that may damage biological molecules, such as lipids, proteins, and DNA (Ayu et al., 2024; Fawwaz et al., 2025).

The present study aimed to determine the antioxidant activity of the ethanol extract of *Musa paradisiaca* L. (Kepok banana peel) using the FRAP method. The FRAP method quantifies the total antioxidant capacity of a sample by measuring its ability to reduce Fe^{3+} ions to Fe^{2+} (Ainunnisa et al., 2025).

Extraction is an essential step in isolating bioactive compounds from plant materials using appropriate solvents to attract secondary metabolites (Faisal Syamsu & Erwin Rachman, 2023). The choice of extraction technique depends on the chemical properties of the target compounds, the type of solvent, and the available laboratory equipment (Syamsul et al., 2020). In this study, the maceration method was employed because it is simple, cost-effective, and suitable for compounds that are sensitive to heat. Additionally, maceration allows efficient extraction without complex equipment (Faisal Syamsu & Erwin Rachman, 2023).

The process was continued with remaceration, which involves repeated solvent addition after the initial macerate is filtered, to maximize compound recovery (Saubatul Islamiah et al., 2021). The filtrate obtained was then concentrated using a rotary vacuum evaporator at 60°C—corresponding to the boiling point of ethanol—to remove the solvent. Further thickening was performed using a water bath at 50°C to accelerate solvent evaporation, yielding a dark brown viscous extract (Figure 8). The solvent used, 96% ethanol, was chosen for its polarity and ability to dissolve polar secondary metabolites such as flavonoids.

A total of 300 g of dried *Musa paradisiaca* L. peel was macerated in 96% ethanol, producing 5 g of extract, equivalent to a yield of 1.67% (**Table 1**). The yield represents the ratio between the dry weight of the extract obtained and the initial weight of the raw material (Sari, Syahrul & Iriani, 2021). A higher yield reflects greater extraction efficiency, indicating that more soluble compounds were successfully extracted by the solvent.

Table 1. Extraction yield of ethanol extract of *Musa paradisiaca* L. peel.

Sample	Solvent (mL)	Sample weight (g)	Extract yield (g)	Percentage (%)
Kepok banana peel	3 Liter	300 mg	5 g	1.67

Before conducting quantitative analysis, qualitative phytochemical screening was performed to identify the presence of major antioxidant-related compounds in the ethanol extract of *Musa paradisiaca* L. (Kepok banana peel). The presence of flavonoids was confirmed by adding concentrated HCl and magnesium metal to the extract, producing a pink to magenta-red coloration, which indicates a positive flavonoid reaction (Ummum & Abidin, 2024). The saponin content test was carried out by adding 2 N HCl after vigorous shaking; stable foam persisting for 30 minutes indicated a positive result for saponins (Hasma & Winda, 2019). Meanwhile, the tannin content test was conducted using 1% $FeCl_3$ reagent, and the appearance of a blackish-blue or blackish-green color confirmed the presence of tannins (Hasma & Winda, 2019). The results of these qualitative tests are shown in Figure 3.

For the quantitative analysis, quercetin was used as the standard compound due to its strong antioxidant activity and structural similarity to flavonoids commonly found in plant extracts. Quercetin functions as a secondary antioxidant that captures free radicals and inhibits chain reactions through its hydroxyl groups, which act as effective hydrogen or electron donors (Maryam, Baits & Nadia, 2016). The FRAP (Ferric Reducing Antioxidant Power) assay was used to assess the reducing potential of the sample, expressed as mg quercetin equivalent per gram extract (mgQE/g extract).

The analysis utilized a 0.2 M phosphate buffer (pH 6.6) to maintain pH stability and support the reduction of Fe^{3+} to Fe^{2+}—a redox process that serves as an indicator of antioxidant potential (Maesaroh, Kurnia & Al Anshori, 2018). The acidic environment in FRAP testing is crucial since it influences the reduction capacity of antioxidant compounds. In this reaction system, 1% potassium ferricyanide ($K_3Fe(CN)_6$) acted as the oxidizing agent, 10% trichloroacetic acid (TCA) was used to precipitate the potassium ferricyanide complex, and 0.1% $FeCl_3$ formed a green to blue (Prussian blue) complex, indicating successful reduction (Faisal Syamsu & Erwin Rachman, 2023).

The reducing power reflects the potential of an antioxidant compound to convert Fe^{3+} to Fe^{2+}. Compounds with strong reducing power can stabilize free radicals by donating electrons or hydrogen atoms, thereby preventing oxidative damage (Ummum & Abidin, 2024). The reaction mixture was vortexed for 5 minutes to ensure homogeneity, incubated at 50°C for 20 minutes to facilitate complete reaction with reagents, and centrifuged at 3000 rpm for 10 minutes to obtain a clear supernatant. Before measurement, the mixture was incubated again for 5 minutes to ensure reaction completion (Ummum & Abidin, 2024).

Prior to measuring antioxidant activity, the maximum wavelength (λmax) was determined to identify the optimal absorption range for both standard and sample solutions. The quercetin standard solution (25 ppm) was scanned in the 400–800 nm range, yielding a maximum absorbance at 710 nm (A = 0.518) (Sukma, 2018).

Absorbance values for quercetin standard solutions at concentrations of 15, 20, 25, 30, and 35 ppm are presented in **Table 2**. As shown, the absorbance increased proportionally with concentration, indicating a strong linear relationship. The regression equation obtained was $y = 0.0203x + 0.0301$ with a correlation coefficient (r) = 0.9965, demonstrating a highly linear relationship between concentration and absorbance. This standard curve was subsequently used to calculate the antioxidant activity of the ethanol extract of *Musa paradisiaca* L. The creation of a standard curve is essential to establish the relationship between concentration and absorbance (Fatimah, Aisyah & Nurani, 2018).

Table 2. Absorbance of Quercetin Standard Solution at Maximum Wavelength

Concentration (ppm)	Absorbance
15	0.332
20	0.453
25	0.521
30	0.632
35	0.750

The regression results of the concentration (x) with the absorbance value (y) of the quarcetin standard were then made into a curve until the value of $y = 0.0203x + 0.0301$ was obtained as in **Figure 1**. Then it was entered to calculate the FRAP antioxidant activity and the data obtained were as in **Table 3**.

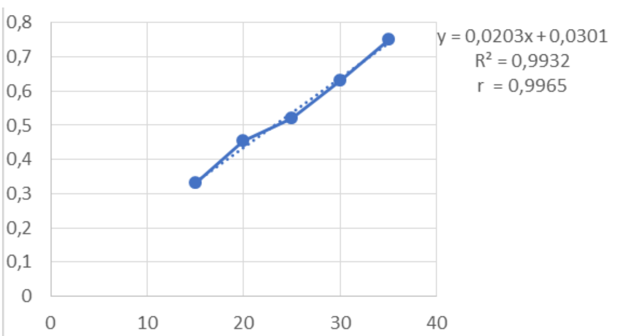

Figure 1. Standard curve of quercetin series

The average antioxidant activity of the ethanol extract of kepok banana peel (*Musa paradisiaca* L.) obtained from three replications was 81.6939 mg QE/g extract (as shown in **Table 3**). This result indicates that 1 gram of the ethanol extract of kepok banana peel is equivalent to 81.6939 mg of quercetin in antioxidant capacity. Conducting the experiment in triplicate was intended to ensure data accuracy and reliability, minimizing experimental error and improving the validity of the measured antioxidant activity.

Table 3. Results of Antioxidant Activity Measurement of Ethanol Extract of Kepok Banana Peel (*Musa paradisiaca* L.)

Replication	Sample weight (g)	Sample absorbance (y)	Antioxidant activity (mgQE/g extract)	Average antioxidant activity (mgQE/g extract)
1	0.0050	0.509	78.6291	81.6939
2	0.0050	0.536	83.0620	
3	0.0050	0.538	83.3906	

The results of this study demonstrated that the ethanol extract of kepok banana peel (*Musa paradisiaca* L.) exhibits significant antioxidant activity. This activity is primarily attributed to the presence of secondary metabolite compoundssuch as flavonoids, polyphenols, tannins, and alkaloids, which play crucial roles as natural antioxidants. Among these, flavonoids are particularly important due to their strong antioxidant properties. The antioxidant potential of flavonoids arises from their ability to donate hydrogen atoms to neutralize free radicals and their metal-chelating capacity, which helps inhibit oxidative reactions (Fajarwati et al., 2023).

In the FRAP assay, the intensity of the green coloration formed in the reaction mixture reflects the reducing power of the extract. A more intense green color corresponds to a higher absorbance value, indicating stronger antioxidant activity. Therefore, the observed increase in absorbance in the ethanol extract of kepok banana peel confirms the extract's high capability to act as an electron donor, effectively reducing oxidized intermediates and preventing oxidative damage. This finding supports the potential use of kepok banana peel extract as a natural antioxidant source in pharmaceutical or nutraceutical applications.

4. CONCLUSION

Based on the results of the antioxidant activity test of the ethanol extract of kepok banana peel (*Musa paradisiaca* L.) using the FRAP method with quercetin as the standard reference, it can be concluded that the extract possesses notable antioxidant potential. The ethanol extract of kepok banana peel demonstrated an antioxidant activity of 81.6939 mgQE/g extract, indicating that each gram of the extract has an antioxidant capacity equivalent to 81.6939 mg of quercetin. This result confirms that the kepok banana peel contains active secondary metabolites capable of acting as effective reducing agents and free radical scavengers, thereby supporting its potential application as a natural antioxidant source in pharmaceutical and nutraceutical formulations.

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